Beneficial Modulation of the Gut Microbiota Prevents Stress-Induced Adverse Effects

by

Henri Alexandre Giblot Ducray

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfilment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 6, 2019

Keywords: gut microbiota, probiotics, prebiotics,
stress, dysbiosis,

Approved by

Iryna Sorokulova, Chair, Professor of Anatomy, Physiology, and Pharmacology
Vitaly Vodyanoy, Alumni Professor of Anatomy, Physiology, and Pharmacology
Edward Morrison, Professor & Head of Anatomy, Physiology, and Pharmacology
Robert Judd, Professor of Anatomy, Physiology, and Pharmacology

Abstract

The gut microbiota plays a significant role in maintaining the homeostasis and overall health status of the host. Moreover, it has been shown that heat stress severely alters the stability of the gut microbiota. Thus, negative alterations of the gut microbiota, known as dysbiosis, have been linked to a plethora of disorders. One in particular, is the dysfunction of the intestinal barrier integrity, which results in increased intestinal permeability and translocation of toxic bacterial components, such as lipopolysaccharides (LPS), from the gut lumen into the circulation. Elevated intestinal permeability can cause tissue injury, multi-organ dysfunction, and even death. Therefore, the stability of the gut microbiota is key in maintaining homeostasis and, as a result, the ability of the host to tolerate stress. Various approaches that modulate the gut microbiota, such as probiotics and prebiotics, have been proposed to prevent dysbiosis and dysbiosis related disorders.

In this work, we examined the adverse effects generated by both environmental and metabolic heat stress on the morphology and function of the intestinal barrier. Then, we evaluated the efficacy of a *Bacillus subtilis* probiotic strain and *Saccharomyces cerevisiae* fermentate, as a prebiotic, in preventing the loss of the integral intestinal barrier function and maintaining the gut microbiota caused by heat stress. Animals were pre-treated by oral gavage with either a *Bacillus subtilis* probiotic strain, or *Saccharomyces cerevisiae* fermentate, or phosphate buffered saline (PBS) prior to exposure to heat stress. Morphological changes in the gastrointestinal tract gut of rats and the expression of intestinal tight junction proteins as a result of

heat stress were altered in stressed animals treated with PBS. There were significant disruptions in the morphological structure: decrease of villi height, total mucosal thickness, decreases in the number of Paneth and goblet cells, and reduced expression of tight junction proteins (Zonula occludence (ZO-1), occludin, claudin, junctional adhesion molecule A (JAM-A)). In addition, the serum of those experimental animals displayed significant elevation levels of pro-inflammatory cytokines, and markers of intestinal barrier dysfunction such as lipopolysaccharides (LPS). Culture-based and Tag-Encoded FLX Amplicon Pyrosequencing analysis of the gut microbiota demonstrated significant perturbations of the gut microbiota, with increases in pathobionts and reduction of beneficial species. Administration of probiotic and prebiotic prevented all registered disorders. Our results demonstrate the efficacy of *Bacillus subtilis* probiotic strain and *Saccharomyces cerevisiae* fermentate treatment in protecting the stability of the gut microbiota and preventing harmful effects of heat stress. We speculate that this approach can be utilized to treat and prevent the loss of the intestinal barrier observed in pathologies other than heat stress.

Acknowledgments

The author wishes to express their deepest gratitude to Dr. Iryna Sorokulova for the privilege of being mentored by her throughout their doctoral program. The author would also like to thank Dr. Vitaly Vodyanoy, Dr. Edward Morrison and Dr. Robert Judd for their support and guidance as committee members. Additionally, they are truly grateful to Ms. Ludmila Globa and Mr. Oleg Pustovyy for their many hours of training and assistance in the laboratory. The author is thankful for Dr. Melissa Singletary and Dr. Samantha Hagerty for their advice, support, and comradery. Additionally, the author is grateful to Dr. Karen Pettit for all the assistance with the proofreading. Lastly, the author wishes to thank their family for their love, patience and constant support, despite being located on the complete opposite side of the world.

Table of Contents

List of Tables

List of Figures

Chapter 1 – Introduction

Stress is universal and all living organisms will be exposed to some sort of stressor. Exposure to stress results in the disruption of an organism's homeostasis, various stimuli including environmental, physical, or psychological forces can act as stressors (Glaser and Kiecolt-Glaser 2005, Karl, Hatch et al. 2018). Regardless of the source, an adaptive response will be activated in order to restore homeostasis (Glaser and Kiecolt-Glaser 2005, Karl, Hatch et al. 2018). Evidence from a growing pool of stress research has demonstrated that consistent changes to the homeostasis will result in dysfunction and lead to pathological conditions. Stress has been shown to cause a wide spectrum of alterations, including the negative modulation of the immune system, gastrointestinal function, nervous system, and gut microbiota (Glaser and Kiecolt-Glaser 2005, Ulrich-Lai and Herman 2009, Baumann and Turpin 2010, Galley and Bailey 2014, Karl, Hatch et al. 2018).

Gut microbiota plays a significant role in maintaining stability of the intestinal barrier. The gut microbiota is composed of more than 10^{14} symbiotic organisms in the distal portions of the gastrointestinal tract (Sender, Fuchs et al. 2016). These organisms are part of a complex ecosystem comprising more than 3.3 million genes and corresponding to a large spectrum of enzymatic activities leading to molecular signals and metabolites that may directly influence host's health and well-being. It has been demonstrated that negative alterations of the gut microbiota, known as dysbiosis, has been linked to a plethora of disorders. Pathological changes in the gut microbiota

structure results in the disruption of the intestinal barrier function, elevated levels of foreign antigens in the plasma, inflammation, altered neural, endocrinal, and immune pathways. The intestinal barrier is a key component in maintaining the stability and overall health status of an organism. The intestinal barrier is so vital that it requires approximately forty percent of the body's total energy expenditure to perform its tasks (Bischoff, Barbara et al. 2014). Disruption of this barrier induces activation of the mucosal immune system and inflammation and can act as a trigger for the development of intestinal and systemic diseases. Elevated intestinal permeability can cause tissue injury, multi-organ dysfunction, and even death. Therefore, the stability of the gut microbiota is key in maintaining homeostasis and, as a result, the ability of the host to tolerate stress. Various approaches that modulate the gut microbiota, such as probiotics and prebiotics, have been proposed to prevent dysbiosis and dysbiosis-related disorders. Such methods beneficially modulate the gut microbiota, selecting beneficial microbiota and keeping the pathobionts under control. A prevalence and stability in beneficial gut microbes, results in the increased production of important metabolites. The major bacterial fermentation products are short chain fatty acids (SCFAs - acetate, propionate and butyrate), which have a wide spectrum of effects on the host. Other essential metabolites that are supplied by the gastrointestinal microbes include bile acids, vitamins, polyamines, lipids, glucose, and amino acids (Holmes, Kinross et al. 2012, Nicholson, Holmes et al. 2012). This work aims to evaluate the efficacy of a *Bacillus subtilis* probiotic strain and *Saccharomyces cerevisiae* fermentate, as a prebiotic, in preventing stress-related adverse effects.

Chapter 2 - Review of Literature

2.1. Stress and its Influence on Host's Homeostasis

Hans Selye, in the 1950's, was the first to introduce the concept of stress as the non-specific response of the body to any demand for change. Since then, there have been many attempts to redefine the meaning of stress, but the overall principal revolves around the idea of the body's response towards an unwanted change within an organism. Disruptions in homeostasis may be due to various stressors, such stimuli include environmental, physical, or psychological forces that elicit adaptive responses to restore homeostasis (Glaser and Kiecolt-Glaser 2005, Karl, Hatch et al. 2018).

2.1.1. Type of Stressors and the Stress Response

2.1.1.1. Environmental Stressors

Environmental stressors include extremes of pressure (high altitude), temperature (heat, and cold), pathogens, pollution (pesticides, herbicides, toxins, heavy metals, inadequate light, radiation, electromagnetic fields), and noise (Schulte 2014, Karl, Hatch et al. 2018).

2.1.1.2. Physical Stressors

Some examples of physical stressors include trauma (injury, infection, surgery), intense physical labour/over-exertion, illness (viral, bacterial, or fungal agents), fatigue, inadequate oxygen supply, hormonal and/or biochemical imbalances, diet (nutrient composition, food restriction, nutritional deficiencies, food allergies and

sensitivities), dehydration, substance abuse (alcoholism, smoking), medication, and musculoskeletal misalignments/imbalances (Collier, Renquist et al. 2017, Karl, Hatch et al. 2018).

2.1.1.3. Psychological Stressors

Psychological stressors can be divided into 3 categories: emotional stress (resentments, fears, frustration, sadness, anger, grief, etc.), cognitive stress (information overload, worry, guilt, self-criticism, anxiety, panic attacks, etc.), and perceptual stress (beliefs, roles, attitudes, etc.) (Galley, Nelson et al. 2014, Karl, Hatch et al. 2018).

2.1.1.4. Stress Response

Although stressors vary, the biological stress response is coordinated primarily by the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS), which includes the sympathetic (SNS) and parasympathetic (PNS) nervous systems (responsible for fight-or-flight and maintenance of homeostasis responses). Activation of the HPA axis and SNS stimulates the release of glucocorticoids (e.g. cortisol in humans and corticosterone in rodents), catecholamines (e.g. epinephrine, norepinephrine), and other hormones, which have varied effects throughout the body (Glaser and Kiecolt-Glaser 2005, Ulrich-Lai and Herman 2009, Baumann and Turpin 2010, Galley and Bailey 2014, Karl, Hatch et al. 2018).

The stress response is adaptive and acts to quickly restore homeostasis, but the response differs based on the source, the magnitude, and the duration of stress. Acute stress responses last from a few minutes after the beginning of the stress to a few days (Horowitz 2001). Persistence of stressors leads to chronic stress and alters the ability and efficiency of the response, leading to adaptive acclimatization. Severe or chronic stress can exceed the adaptive capacity of an organism causing reduced the

organism's performance and maladaptive responses to stress, leading to disease (Segerstrom and Miller 2004).

The mechanism of action that causes the release of glucocorticoids begins with the activation of the acute response to stress initiated by various receptors that respond to changes in homeostasis (Collier and Gebremedhin 2015). Following a change in homeostasis the receptors send afferent signals directed to the central nervous system, including the thalamus and hypothalamus, where signals are coordinated and processed. The hypothalamus activates efferent pathways to combat the effect of the stressor by stimulating the release of corticotropin-releasing hormone (CRH) that is conducted to the pituitary gland. CRH receptor expressing neuro-endocrine cells in the pituitary are activated leading to the release of adrenocorticotropic hormone (ACTH) into the circulation. This activates endocrine cells in the adrenal cortex and stimulates the release of adrenal glucocorticoid hormones, which leads to systemic effects in the body. The hypothalamus also stimulates the adrenal medulla to release epinephrine; this stimulates the autonomic nervous system to release catecholamines which alter metabolism and activate transcription factors involved in the stress response.

Glucocorticoids cause the release of glucose in the blood circulation, repress the immune system, and in the brain, facilitate information processing in limbic neuronal networks involved in emotion, cognition, and memory formation (Saaltink and Vreugdenhil 2014). Catecholamines prepare the body for the fight-or-flight systems by increasing the heart rate, blood pressure, blood glucose levels, and the activity of the sympathetic nervous system.

2.1.1.5. Stressors Influence on Homeostasis

As discussed, disruptions in homeostasis may be due to various types of stressors and cause the host's immediate response. The response will differ in pathway (HPA or ANS), magnitude, and duration. Evidence from a growing pool of stress research has demonstrated that consistent changes will result in dysfunction and lead to pathological conditions. Stress has been shown to cause a wide spectrum of alterations, including the negative modulation of the immune system, gastrointestinal function, nervous system, and more recently, the gut microbiota (Glaser and Kiecolt-Glaser 2005, Ulrich-Lai and Herman 2009, Baumann and Turpin 2010, Galley and Bailey 2014, Karl, Hatch et al. 2018).

Specifically, catecholamines and other neuroendocrine hormones directly modulate microbial growth (Lyte and Ernst, 1992), and are secreted by intestinal cells in the gastrointestinal tract in response to stress (Lyte, 2014). In addition, stress-induced changes in signalling via the vagus nerve and enteric nervous system alter gastrointestinal motility and reduce digestive activity, likely impacting the gut microbiota by modulating physical forces within the gastrointestinal tract and by altering substrate availability (Galley and Bailey, 2014).

2.1.2. Stress-Related Disorders

Stress results in varied biological effects, which are increasingly recognized. The gut microbiota is one of the main targets of stress. In turn, the gut microbiota influences the host stress response, implicating the gut microbiota as an important component of host health (Berg, Muller et al. 1999, Karl, Hatch et al. 2018). Even though it is difficult to demonstrate specific commensal bacterial species in health and disease, there is emerging evidence for certain gut microbial species being involved in disease aetiology (Borg, Bjorkman et al. 1999, Lingwood 1999, Pillai and Nelson

2008, Possemiers, Grootaert et al. 2009, Fujimoto, Imaeda et al. 2013). In addition, in many cases, reduced microbiota diversity can be correlated to compromised health, implicating this more generic microbiota-related parameter in health and disease (Ismail, Oppedisano et al. 2012, Cozen, Yu et al. 2013, Storro, Avershina et al. 2013).

Modification of the gut microbiota by stressors has been demonstrated to cause diverse pathological disorders. Negative alterations of the gut microbiota, which leads to the bacterial imbalance and impairment of the gut microbiota, is referred to as dysbiosis. Dysbiosis can affect health status of the organism and disrupt the processes of metabolism, immunity, and other processes. The environment, diet, stressors, and medications are all capable of altering the composition of the gut microbiota (Govender, Choonara et al. 2014, Karl, Hatch et al. 2018, Zhu, Grandhi et al. 2018). Alterations to the microbiota have significant impacts on the host-microbial interactions (Nicholson et al, 2012).

Evolution of the host and gut microbiota has resulted in an extremely mutualistic bi-directional relationship. Studies have shown that the host provides a favourable environment and nutrients that shapes the gut microbiota, and, in turn, the gut microbiota modulates the host's immunity, metabolism, and neural and endocrinal pathways (Hooper and Gordon 2001, O'Mahony, Clarke et al. 2015, Cani 2016).

Dysbiosis has been associated with transient health pathologies including gastrointestinal permeability and inflammation and increased susceptibility to illness and infection. Further, numerous chronic diseases such as obesity and associated cardiometabolic syndromes among others have also been linked to dysbiosis. These associations inspire extensive interest in identifying factors causing dysbiosis, and in developing strategies aiming to mitigate changes in the gut microbiota to maintain homeostasis. Germ-free animal studies have been an essential instrument in

demonstrating and assessing the importance of the gut microbiota to host health. The absence of gut microbiota has been shown to lead to malformation, defects in the lymphoid structures such as the spleen and Peyer's patches, abnormal numbers of immune cell types and expression of cytokine profiles, changes in the mucosal barrier function (Pollard and Sharon 1970, Bartizal, Wostmann et al. 1984, Williams, Probert et al. 2006, Yamamoto, Yamaguchi et al. 2012, Chen, Song et al. 2018, Kermanshahi, Shakouri et al. 2018, Wu, Zheng et al. 2018).

Dysbiosis has been associated with transient and chronic pathologies. The pathological conditions observed are not limited to the gastrointestinal tract, dysbiosis has systemic consequences throughout the host. Table 2.1.2.1. lists several of the diverse disease states associated with the negative changes of the intestinal gut microbiota. These pathologies have obvious changes to the gut microbiota structure and are often observed with elevated levels of foreign antigens in the plasma, disruption of the intestinal barrier function, inflammation, altered neural, endocrinal, and immune pathways, and/or altered metabolism. One of the most recognisable and dangerous consequence of dysbiosis is the commonly perceived elevated levels of lipopolysaccharides (LPS), a toxic component of Gram-negative bacteria. High levels of this endotoxin are strongly associated with inflammation and chronic diseases. Increased translocation of LPS from the lumen into the circulation, leading to systemic inflammation, which could result in tissue injury, multi-organ dysfunction, and death by causing systemic stimulation of inflammatory responses. Endotoxins (LP are integral components of the outer membrane of Gram-negative bacteria, which is primarily responsible for most of the toxic properties of bacteria. LPS is known to be highly proinflammatory and induce inflammation via the Toll-like receptors 4 (TLR4) (Bengmark 2013). Not only do endotoxins translocate into the circulation, but

viruses, live bacteria, and bacterial debris can also translocate, and remain intracellularly in various cell types, where they can lead to the enhancement of inflammation (Bouwman, Diepersloot et al. 2009, Hanses, Kopp et al. 2011, Kim, Ryu et al. 2011, Na and Nam 2012, Patel, Hinojosa et al. 2018). Components of the intestinal barrier, such as the gut-associated lymphoid tissue, which is the largest collection of lymphoid tissue in the body, provides a dynamic immunological barrier throughout the gastrointestinal tract. Changes in the activity of immune cells, epithelial cells, and in the secretion of anti-microbial peptides and other secretory factors within the intestinal barrier can directly alter the gut microbiota composition and function and in turn, the microbiota can alter the activity and composition of the cells and molecules present (Liu, Li et al. 2012, Bischoff, Barbara et al. 2014, Andrade, Araujo et al. 2015, Viggiano, Ianiro et al. 2015, Sundman, Chen et al. 2017, Wells, Brummer et al. 2017).

Table 2.1.2.1. Conditions Associated with Dysbiosis.

Condition	References
Allergy	(Beloglazov, Znamenska et al. 2012, Storro, Avershina et al. 2013, West, Dzidic et al. 2017, Pascal, Perez-Gordo et al. 2018)
Autism	(Emanuele, Orsi et al. 2010, Critchfield, van Hemert et al. 2011, de Theije, Wopereis et al. 2014, Hughes, Rose et al. 2018, Tabouy, Getselter et al. 2018)
Autoimmune Diseases	(Rabin and Levinson 2008, Fasano 2012, Mu, Kirby et al. 2017, Abdelhamid and Luo 2018)
Mental Health Conditions	(Jenkins, Harte et al. 2009, DellaGioia and Hannestad 2010, Collins, Kassam et al. 2013, Zaborin, Smith et al. 2014, Park, Brietzke et al. 2018)
Cancer	(Hsu, Chan et al. 2011, Rafter 2011, Zhu, Luo et al. 2011, Zhang, Du et al. 2012, Moreno-Indias, Sanchez-Alcoholado et al. 2016, Meng, Bai et al. 2018, Wardill, Secombe et al. 2018)
Cardio Vascular Diseases	(Risley, Jerrard-Dunne et al. 2003, Wrigley, Lip et al. 2011, Jackson, Verdi et al. 2018, Tunapong, Apaijai et al. 2018)
Chronic Fatigue Syndrome	(Tsigos and Chrousos 2002, Maes, Coucke et al. 2007, Maes and Leunis 2008, Maes, Kubera et al. 2011)
Chronic Kidney Disease	(McIntyre, Harrison et al. 2011, Twombley and Seikaly 2011, Krug, Schulzke et al. 2014, Guldris, Parra et al. 2017, Lau, Savoj et al. 2018, Lehto and Groop 2018)
Chronic Obstructive Pulmonary Diseases	(Urs and Heidemann 2004, Bengoechea and Ito 2011, Maes, Kubera et al. 2011)
Cognitive Impairment	(Wilson and Morley 2003, Davidson, Cooke et al. 2018)
Fibromyalgia	(Tsigos and Chrousos 2002, Maes and Leunis 2008)
Human Immunodeficiency Virus	(Hummelen, Hemsworth et al. 2010, Dohgu, Fleegal-DeMotta et al. 2011, Hemmerling and Cohen 2011, Lagenaur, Sanders-Beer et al. 2011)
Inflammatory Bowel Diseases	(Wasilewski, Zielinska et al. 2015, Chu, Khosravi et al. 2016, Konig, Wells et al. 2016, Fink 2017, White, Van den Bogaerde et al. 2018)
Infections	(Terrier, Simonet et al. 2014, Galley, Mackos et al. 2017, Mackos, Maltz et al. 2017)

Irritable Bowel Syndrome	(Haller, Antoine et al. 2010, Agrawal and Whorwell 2011, Ringel and Ringel-Kulka 2011, Whelan 2011, Gecse, Roka et al. 2012, Mercer, Brinich et al. 2012, Rogers and Mousa 2012, Simren, Barbara et al. 2013, Riddle, Welsh et al. 2016, Chen, Kim et al. 2018, El-Salhy and Mazzawi 2018, Rodino-Janeiro, Vicario et al. 2018)
Liver Cirrhosis	(Liu, Duan et al. 2004, Cesaro, Tiso et al. 2011, Pereg, Kotliroff et al. 2011, Scalera, Cesaro et al. 2012, Fooladi, Hosseini et al. 2013, Malaguarnera, Giordano et al. 2014, Usami, Miyoshi et al. 2015, Tilg, Cani et al. 2016)
Macular Degeneration	(Leung, Barnstable et al. 2009)
Metabolic Diseases	(Beyan, Goodier et al. 2006, Nymark, Pussinen et al. 2009, Caesar, Fak et al. 2010, Andreasen, Kelly et al. 2011, Lassenius, Pietilainen et al. 2011, Everard, Geurts et al. 2014, Yoo and Kim 2016, Molinaro, Caesar et al. 2017, Ferrocino, Ponzo et al. 2018, Neyrinck, Hiel et al. 2018, Pascale, Marchesi et al. 2018)
Encephalopathy	(Munakata, Arakawa et al. 2010, Agrawal, Sharma et al. 2011, Bengmark, Di Cocco et al. 2011, McGee, Bakens et al. 2011, Mittal, Sharma et al. 2011, Shukla, Shukla et al. 2011, Bajaj, Ridlon et al. 2012)
Neurodegenerative Diseases	(Fassbender 2004, Jaeger, Dohgu et al. 2009, Zhang, Miller et al. 2009, Zhang, Han et al. 2018)
Osteoarthritis	(Schwager, Hoeller et al. 2011)
Paradontosis	(Shaddox, Wiedey et al. 2011, Teughels, Loozen et al. 2011)
Parkinson's Disease	(Lange, Buja et al. 2006, Lama, Avagliano et al. 2018, Rajoka, Zhao et al. 2018)
Rheumatoid Disease	(Schwab, Brown et al. 1993, Lange 2004, Mandel, Eichas et al. 2010, Pineda, Thompson et al. 2010, Pineda, Thompson et al. 2011)
Stress	(Huang, Stewart et al. 2011, Davidson, Cooke et al. 2018, Karl, Hatch et al. 2018, Koenig, Bredehoft et al. 2018, Partrick, Chassaing et al. 2018, Vodicka, Ergang et al. 2018, Wilson 2018)
Uveitis	(Leung, Barnstable et al. 2009, Misiuk-Hojlo, Miedzybrodzki et al. 2011)

2.1.3. Approaches for Mitigation of Stress-Related Complications.

As seen by the numerous pathological conditions associated with dysbiosis, it can be said that stressors negatively impact the gut microbiota community structure and activity (Mackos, Maltz et al. 2017). Therefore, the gut microbiota is key in the ability of the host to tolerate stress. The gut microbiota and its metabolites have an essential role in the regulation of the intestinal barrier function, immunity, metabolism, and neural and endocrinal routes (Sanchez, Delgado et al. 2017, Wiley, Dinan et al. 2017, Robertson, Goethel et al. 2018). The gut microbiota, comprising the densest microbial community, and most diverse, within a host, demonstrates resilience to distress and attempts to maintain long-term stability (Sekirov, Russell et al. 2010, Huttenhower, Gevers et al. 2012, Ceapa, Wopereis et al. 2013, Tojo, Suarez et al. 2014, Cani and Everard 2016, Suchodolski and Jergens 2016, Wells, Brummer et al. 2017). Thus, modulation of the gut microbiota could be a potential avenue to maintain the homeostasis and general health status of the host. There are several tactics to modulate the gut microbiota, such approaches include specific diets, probiotics, prebiotics, synbiotics, postbiotics, antibiotics, faecal transplantation, and activated charcoal (Figure 2.1.3.1.).

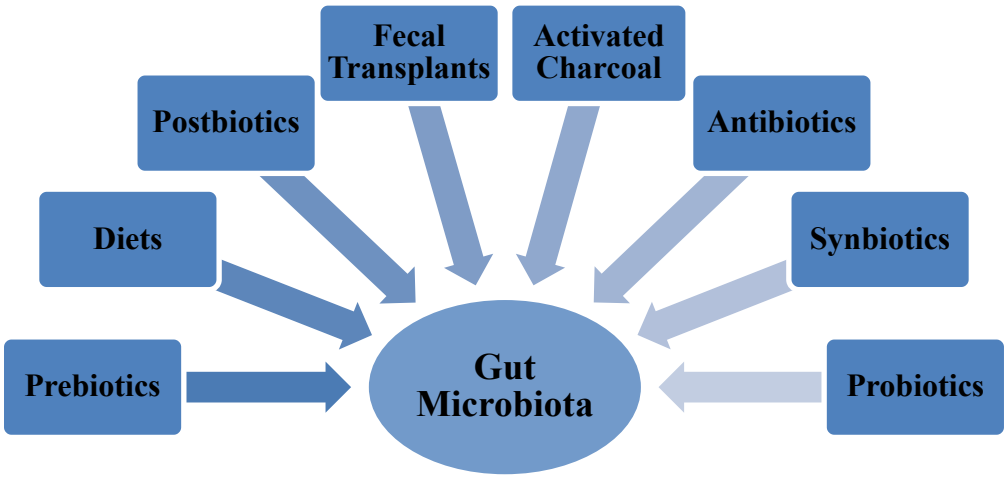


Figure 2.1.3.1. Approaches for the Mitigation of Stress-Related Complications. The many options to beneficially alter the gut microbiota in order to restore homeostasis and the health status of the individual and mitigate stress-related complications (White, Van den Bogaerde et al. 2018).

2.1.3.1. Specific Diets

It is clear that the diet can have a significant impact on the composition and functionality of the gut microbiota and thus influence the health status of the host (Scott, Duncan et al. 2011, Ottman, Smidt et al. 2012). There have been strong correlations observed between the diet and state of health (Gordon 2008, Videhult and West 2016, Anand and Mande 2018, Ericsson, Gagliardi et al. 2018, Moreno-Perez, Bressa et al. 2018). Diet is the most powerful influence on gut microbial communities and studies have shown that specific bacterial clusters are generated based on diet (Ley, Hamady et al. 2008, Muegge, Kuczynski et al. 2011, Wu, Chen et al. 2011). The significant impact of dietary composition is due to nutrient intake directly affecting the types of substrates available to the gut microbes present, and to the innumerable effects of the different nutrients absorbed and the microbial metabolites produced on the host.

Specific diets are based on the selection of foods that are typically considered to be healthy, including fibre-rich foods (vegetables, fruits, whole grains, nuts, seeds,

legumes), polyphenols (coffee, tea, red wine, dark chocolate) and water. The most recommended diet is the Mediterranean diet as it is typically rich in fibre. The greatest difference between the Mediterranean diet and the Western diet is the sources and proportion of dietary fat. Western diets are high in saturated fats and refined carbohydrates, whereas the Mediterranean diet has a greater proportion of monounsaturated fats. The importance of a healthy diet has been observed in various studies. Several studies showed the negative effect of a Western diet. For example, the volunteers of the study were asked to live for a month on a Western diet and this group demonstrated a significant increase in plasma levels of endotoxin activity (LPS in serum) when compared to those consuming "a prudent-style diet", who, in turn, demonstrated a 31% reduction LPS levels (Pendyala, Walker et al. 2012). Other specific diets exist to modulate the gut microbiota, but in general, these diets aim for increasing beneficial bacteria and inhibit the excessive abundance of pathobionts.

2.1.3.2. Probiotics

The definition of probiotics has changed throughout the years, but probiotics are selected live microorganisms, which when administered in sufficient amounts present a health benefit to the host (Salminen, Nybom et al. 2010, Bermudez-Humaran, Aubry et al. 2013). The concept of probiotics was first proposed by Elie Metchnikoff, who made reference to the properties of fermented milk (containing lactic acid bacteria) that the native long living Bulgarian populations were using and he linked the microorganism to increased well-being (Ceapa, Wopereis et al. 2013). The most commonly used microorganisms as probiotics are *Bifidobacterium* and *Lactobacillus* species, but other microbes such as *Enterococcus, Lactococcus, Bacillus, Escherichia*, and *Saccharomyces* have also been utilized (Bermudez-Humaran, Aubry et al. 2013, Govender, Choonara et al. 2014, Marchesi, Adams et al.

2016). Probiotics have been used clinically to treat various conditions for example to suppress diarrhoea, alleviate lactose intolerance and postoperative complications, participate in anti-microbial and anti-colorectal cancer activities, reduce irritable bowel symptoms, and prevent inflammatory bowel disease (Govender, Choonara et al. 2014). Beneficial probiotic bacteria contribute to enhance the intestinal barrier function, reduce the inflammatory response, prevent the overgrowth of pathobionts, and ameliorate gut permeability (Ohland and MacNaughton 2010, Ait-Belgnaoui, Colom et al. 2014, Marchesi, Adams et al. 2016).

2.1.3.3. Prebiotics

Prebiotics are a non-digestible compounds that, through metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host (Gibson, Hutkins et al. 2017). The compounds are non-digestible oligosaccharides, such as fructooligosaccharides, galactooligosaccharides, lactulose, and inulin, and have the potential to stimulate growth and activity of beneficial gut bacteria, particularly *Lactobacillus* and *Bifidobacterium* spp. (Bouhnik, Raskine et al. 2004, Vanhoutte, De Preter et al. 2006, Slavin 2013). Other oligosaccharides such as isomalto-oligosaccharides, mannan oligosaccharides, pectins, resistant starches, xylooligosaccharides, arabinoxylans, and human and bovine milk oligosaccharides, have also been studied for their prebiotic properties (Krumbeck, Maldonado-Gomez et al. 2016).

The benefits of promoting the growth of specific bacteria, such as *Lactobacillus* and *Bifidobacterium* spp., are due to the fact that these bacteria have the capacity to perform both saccharolytic metabolism and proteolytic activities, leading to enhanced levels of short-chain fatty acids and reduced colonic pH. This results in

changes in the gastrointestinal tract environment and this has been linked to an improved protection against potential pathogens, reduction of diarrhoea, improved digestion and absorption and immune-stimulation (Cummings and Macfarlane 1991, Gibson 1998, Fioramonti, Theodorou et al. 2003, Veereman 2007, de Vrese and Schrezenmeir 2008, Roberfroid, Gibson et al. 2010, Chen and Quigley 2014, Gibson, Hutkins et al. 2017).

2.1.3.4. Synbiotics

Synbiotics refer to food ingredients or dietary supplements combining probiotics and prebiotics in a form of synergism (Pandey, Naik et al. 2015). The concept behind synbiotics is to improve the survival and implantation of probiotic strains in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare. Synbiotics have three different mechanism of action. First, the synbiotic may be complementary, where each component acts independently for its potential additive beneficial effect on host health (Krumbeck, Walter et al. 2018). Another mechanism is that the chosen prebiotic substrate is specifically intended to support the growth of the chosen probiotic (Kolida and Gibson 2011, Krumbeck, Walter et al. 2018). Lastly, the prebiotic component could be necessarily not fermented by the chosen probiotic strain and thus could theoretically support other members of the gut microbiota. The probiotic strain would gain no advantage by being combined into a symbiotic, and additionally may not have the capacity of fermenting the substrate at all (Krumbeck, Walter et al. 2018). The most commonly studied synbiotics are combinations of probiotic strains of *Bifidobacterium* and *Lactobacillus*, with prebiotic compounds such fructooligosaccharides, inulin, or galactooligosaccharides (Gurry 2017, Markowiak and Slizewska 2017, Krumbeck,

Walter et al. 2018, Whittemore, Stokes et al. 2018). Studies of synbiotic products are still preliminary, with more high-quality evidence from clinical research studying the benefits needed.

2.1.3.5. Postbiotics

Postbiotics are another potential therapeutic approach for the mitigation of stress-related complications to the gut microbiota. These compounds are microbial fermentation end products, also referred to as metabolites, and are entirely associated with the gut microbiota. Examples of metabolites include organic acids, bacteriocins, carbonic substances, and enzymes. The quantity, type, and activity of the metabolites depend on the microbial species present within the gut microbiota and their activity. In addition, microbial activity is dependent of the substrate (prebiotic) available in the gastrointestinal tract (Figure 2.1.3.5.1.). Therefore, the connection between substrate, bacteria, and metabolite is very important.

Figure 2.1.3.5.1. Postbiotics.
The connection between prebiotics, probiotics, and postbiotics. Adapted image from (Patel and Denning 2013).

Examples of beneficial metabolites produced by the gut microbiota populations include amino acid derivatives and short-chained fatty acids (Macfarlane, Quigley et al. 1998, Fernando, Brennan et al. 2010, Fond, Boukouaci et al. 2015, Antonissen, Eeckhaut et al. 2016, Whittemore, Stokes et al. 2018). On the other hand, other postbiotic compounds have been observed in dysbiotic animals, and elevation of

those compounds can be harmful to the host. Examples of such molecules include p-cresol, indolepyruvate, indolyl-3-acrylolylglycine, n-acetylserine, and urinary concentrations of dimethylamine, hippurate, and phenyacetylglutamine (Yap, Angley et al. 2010, Hsiao, McBride et al. 2013, Persico and Napolioni 2013, Chen, Venkat et al. 2017). Therefore, targeting for specific postbiotics requires specific tailoring of prebiotics and probiotics to prevent the bacterial synthesis of harmful metabolites, while simultaneously producing beneficial products. Recent characterisation of certain postbiotics, which were sufficient to elicit the desired response in animals, is a potential avenue for treatment of adverse effects by circumventing the substrate requirements needed for bacterial activity and the necessity of specific bacteria to be present, even in dysbiotic conditions.

2.1.3.6. Antibiotics

The application of antibiotics as a means to remediate dysbiosis is somewhat counterintuitive. Antibiotics are either bacteriolytic or bacteriostatic in their mechanism of action and have effects on the susceptible intestinal bacteria. However, these drugs may also suppress the growth of commensal and beneficial bacteria present in the gastrointestinal tract, in addition to the pathobionts. It has been demonstrated that the application of broad-spectrum antibiotics can affect the abundances of 30% of the bacteria in the gut community, causing rapid and significant drops in taxonomic richness, diversity, and consistency, thus leading to dysbiotic conditions (Dethlefsen, Huse et al. 2008, Dethlefsen and Relman 2011). Moreover, pathogenic bacteria are known to have a greater ability to rebound once antibiotic treatment has been discontinued. Usually, the administration of antibiotics will dramatically destroy gastrointestinal homeostasis and this results in changes that affect approximately 90% of the critical functions performed by the gut microbiota,

affecting metabolism, immunity, and trophic structure (Dethlefsen, Huse et al. 2008, Antunes, Han et al. 2011, Dethlefsen and Relman 2011).

The gut microbiota has been shown to exhibit a certain degree of resilience and have the capacity of returning to its original composition or somewhat similar condition after antibiotic treatment has been discontinued. However, in reality, the gut microbiota will be more likely not recover to original conditions. In fact, antibiotic-induced dysbiosis is maintained long after the prescription has run its course; changes last for long periods of time, spanning months, and even years (De La Cochetiere, Durand et al. 2005, Jernberg, Lofmark et al. 2007, Dethlefsen, Huse et al. 2008, Dethlefsen and Relman 2011).

Antibiotic treatment has been linked to various disease states such as infections, atopic, inflammatory, and autoimmune diseases (Francino 2015). Due to the association of antibiotics and various diseases, antibiotics are not considered as a long-term therapy option for the remediation of dysbiosis. The microbiota imbalances caused by antibiotics can negatively affect health in numerous ways and for long periods of time. Strategies, like the co-administration of probiotics, have been proposed to minimize the negative consequences of antibiotics when their administration is required. Use of probiotic bacteria aimed at impeding dysbiosis or at re-establishing the gut microbiota after antibiotic treatment is a promising approach.

2.1.3.7. Faecal Transplants

Faecal microbiota transplantation is the therapeutic remediation strategy employed to restore normal intestinal flora balance through the introduction of gut bacteria from a healthy donor into a patient (Gupta, Allen-Vercoe et al. 2016, Bibbo, Ianiro et al. 2017). The faecal transplantation can occur by the transfer a faecal sample via a nasogastric tube, nasoduodenal tube, rectal enema, or biopsy channel of a

colonoscope (Aroniadis and Brandt 2013, Gupta, Allen-Vercoe et al. 2016, Bibbo, Ianiro et al. 2017). Faecal transplants, unlike probiotics, aim at introducing a complete and stable bacteria communities into the gastrointestinal tract to repair or replace the altered native microbiota and, thus, confer a health benefit.

Studies utilizing faecal microbiota transplantation to treat patients with certain diseases have indicated recovery to similar microbiota composition as healthy donors (Suskind, Brittnacher et al. 2015). The usage of faecal transplantation has attracted much attention in recent years due to the high efficacy, as high as 90% efficacy, in treating patients with recurrent *Clostridium difficile* infections (Bakken, Borody et al. 2011, Kassam, Lee et al. 2013, Goldenberg, Batra et al. 2018). Other studies have shown preliminary efficacy of faecal transplantation to treat autism spectrum disorder, gastrointestinal disorders, inflammatory bowel disease, Parkinson's disease, obesity, and metabolic syndromes (Aroniadis and Brandt 2013, Hartstra, Bouter et al. 2015, Rosenfeld 2015, Rossen, Fuentes et al. 2015, Marotz and Zarrinpar 2016, Oprita, Bratu et al. 2016, Kang, Adams et al. 2017). However, other studies have shown poor success rates in treating inflammatory bowel disease due to genetic, immunological, environmental, and microbial factors (Frank, Amand et al. 2007, Dave, Papadakis et al. 2014, Moayyedi, Surette et al. 2015). The introduction of a new microbial community alone may not be enough to yield beneficial results. A risk of faecal transplantation is the introduction of opportunistic pathogens. Since there is no standardization of the faecal sample, there is an existence of variation in the metabolic and microbial content in the stool sample among individuals and within individuals over time (Caporaso, Lauber et al. 2011, Ursell, Metcalf et al. 2012). The variation of the bacterial community and the consistency of the faecal samples utilized for

restoration is a major factor limiting the usage of the faecal transplantation method to remediate gut dysbiosis.

2.1.3.8. Activated Charcoal

Activated charcoal has also been used in the treatment of dysbiosis associated pathologies. Activated charcoal or carbon is a standard treatment in many acute oral toxicities as the chemical structure of the compound allows for toxins present to bind in the upper gastrointestinal tract and thereby prevent the absorption of the toxin across the intestinal barrier in lower portions of the gastrointestinal tract (Lau, Savoj et al. 2018). Toxins may either be produced by gut microbiota as a result of dysbiosis or may be ingested by host, either situation will lead to detrimental effects. The literature indicates that the gut microbiota have a capacity to metabolise compounds that can be classified in five core enzymatic families (azoreductases, nitroreductases, β-glucuronidases, sulfatases and β-lyases) (Claus, Guillou et al. 2016). The large enzymatic profile of the gut bacteria clearly is involved in the metabolism of over 430 environmental contaminants (Claus, Guillou et al. 2016). This is evidence that bacteria-dependent metabolism of pollutants modulates the toxicity for the host, therefore dysbiosis alters the metabolic capacity to breakdown toxins. Examples of bacterial-derived toxins are indoxyl sulphate, p-cresyl sulphate, and trimethylamine-N-oxide (Ito, Higuchi et al. 2013, Chen, Venkat et al. 2017, Lau, Savoj et al. 2018). These toxins induce breakdown of the gut epithelial barrier which in turn facilitates translocation of toxins into the systemic circulation. In addition to the removal of toxins, a few studies suggest activated charcoal suppresses the growth of antibiotic-resistant intestinal bacteria (Khoder, Tsapis et al. 2010, Grall, Massias et al. 2013).

Commonly utilized activated charcoals preparations, AST-120 or DAV131, have been shown in animal models to partially restore expression of intestinal tight

junction proteins, reduce monocyte activation, and lower the amount of inflammatory markers in the serum of the animals such as lipopolysaccharides, IL-6, and TNF-α (Ito, Higuchi et al. 2013, Vaziri, Yuan et al. 2013). Other studies in a small cohort of patients have reported lower plasma levels of bacterial-derived toxins in animals treated with an activated charcoal preparation (Shibahara and Shibahara 2010, Nakamura, Sato et al. 2011, Bolati, Shimizu et al. 2012). However, similar to antibiotic treatments, any beneficial effects of activated charcoal intervention will be temporary, and disease symptomology will return upon termination of this therapy due to the nature of the charcoal treatment.

2.2. Gut Microbiota in Health and Disease

Complex microbial congregations composed of diverse microbial species with distinct functions are referred to as the microbiota (Dominguez-Bello, Costello et al. 2010). These communities are found on virtually all environmentally exposed surfaces of the human body. Each section of the body, such as the mouth, hair, nose, ears, vagina, lungs, skin, eyes, etc., has its own unique microbiota.

2.2.1. Structure of the Gut Microbiota

The density and composition of the gut microbiota changes throughout the gastrointestinal tract, with different populations present based on the function at the various locations (Hillman, Lu et al. 2017).

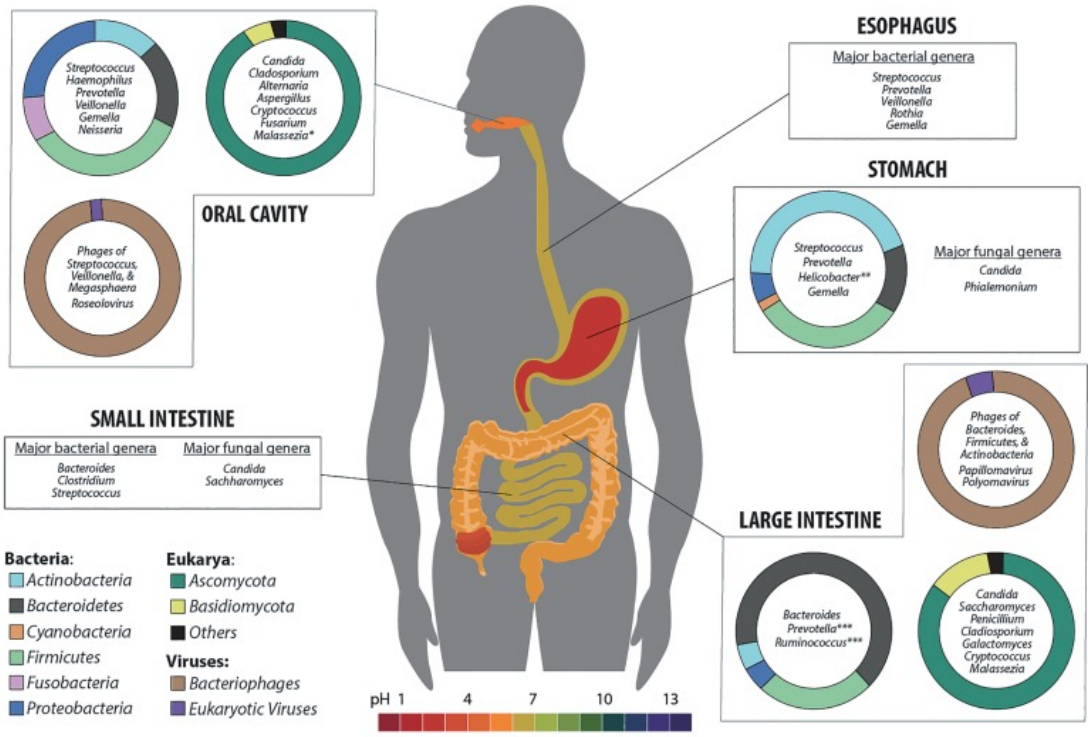


Figure 2.2.1.1. Microorganism Composition Along the Gastrointestinal Tract.

The microbiota has varying composition of bacteria, eukarya, and viruses among the different physiological niches of the gastrointestinal tract. The data shows phylum composition data with the most common genera in each location. The gastrointestinal tract colour correlates to the pH scale below the human schematic (Hillman, Lu et al. 2017).

The characterization of the gastrointestinal microbiota has been undertaken by researchers for decades now in an attempt to identify, enumerate, and differentiate the microbes involved in this elaborate ecosystem (Luckey 1972, Savage 1977). The gut microbiota profile is analysed by culture, and culture-independent techniques (Usai-Satta 2009, Sekirov, Russell et al. 2010, Simren, Barbara et al. 2013, Xiao, Feng et al. 2015). Recent evidence has revealed that the composition of mucosa-associated bacterial communities in the colon is significantly different from that in faeces (Figure 2.2.1.2**)** (Gevers, Kugathasan et al. 2014, Hillman, Lu et al. 2017). However, the majority of studies analyse the gut microbiota by collecting fresh faecal samples from the distal bowel as the majority of the microbes in the gastrointestinal tract reside

within this segment, as the distal small intestine and colon have more than 10^{14} symbiotic organisms in their microbiota (Serino, Luche et al. 2009, Sekirov, Russell et al. 2010).

Both, culture independent and dependent analyses are performed as other studies have showed that culture based-methods complement independent methods by overcoming bias inherent in sequencing approaches. Such independent approaches include biomarker sequencing (using 16S rRNA gene), metagenomics (generation of draft genomes), metatranscriptomics (gene expression), metaproteomics (protein expression), and metabolomics (metabolic potential) (Lynch and Pedersen 2016). In addition, the majority of intestinal bacteria are widely considered to be unculturable, only approximately 1% of the gut bacteria have been successfully cultured (Nocker, Burr et al. 2007, Vartoukian, Palmer et al. 2010). While there are benefits of using culture-based methods, approximately 90% of the gut microbiota are strict anaerobes that have not yet been characterized by culture-based methods due to indeterminate culture requirements (Nocker, Burr et al. 2007).

Advancements in culture independent techniques have allowed for the documentation in the diversity of the gut microbiota (Dahllof 2002). The strength of the associations between faecal samples, species richness, and the bacterial community composition in gut emphasizes the importance of assessment in metagenome-wide studies (Vandeputte, Falony et al. 2016). Studies have led to a general agreement on the predominant phyla in the human gut, however variations occur at the species level (Hayashi, Sakamoto et al. 2002, Eckburg, Bik et al. 2005, Lay, Rigottier-Gois et al. 2005, Gill, Pop et al. 2006, Zoetendal, Rajilic-Stojanovic et al. 2008).

An advantage of metagenomic approach is that it provides a view of

community structure (species richness and community composition) of not only bacteria, but also includes fungi, archaea, and viral genomes (Hugenholtz and Tyson 2008). In addition to providing a view of the community structure, this approach can also exhibit the metabolic potential of the gut microbiota (Hugenholtz and Tyson 2008, Heintz-Buschart and Wilmes 2018). In-depth genetic characterization of the microbiota has recently demonstrated that human beings can be divided into three different clusters based on the composition of their microbiota (Arumugam, Raes et al. 2014). Each of these three enterotypes are identifiable by the variation in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3)(Arumugam, Raes et al. 2014). Another conclusion regarding the structure of the gut microbiota is that microbial communities are more similar in subjects that are 1) genetically related, 2) of similar age, or 3) share common diets (Mitsuoka 1992, Flint, Duncan et al. 2007, Woodmansey 2007, Turnbaugh and Gordon 2009). In fact, studies have shown that there is a predominance of certain bacterial phyla through the life stages and perturbations of the host (Figure 2.2.1.3.) (Zhang, DiBaise et al. 2009, Biagi, Nylund et al. 2010, Koenig, Spor et al. 2011, Monira, Nakamura et al. 2011, Schwartz, Friedberg et al. 2012). The mode of birth, the usage of antibiotics, and the health status all have significant impacts on the gut microbial composition. Colonization of the gut microbiota evolves continuously after birth, and by the first year, the infant gut is dominated by bacteria from the phyla *Bacteroidetes* and *Firmicutes* (Walker 2014). It was thought that the microbiota resembles adult-like composition by the age of three (Cheng, Ringel-Kulka et al. 2016). However, recent studies reveal changes to the healthy infant gut continue after the age of three, and even at the age of five the gut microbiota is still distinguishable from that of adults in composition and in diversity

(Ringel-Kulka, Cheng et al. 2013, Cheng, Ringel-Kulka et al. 2016).

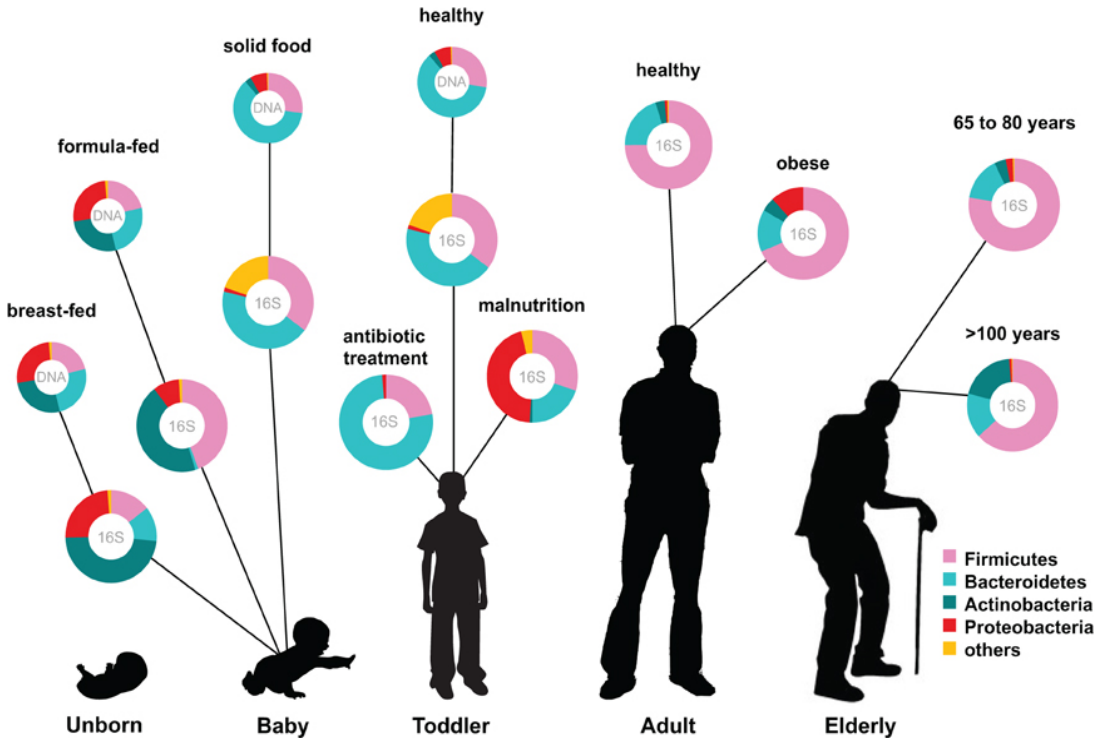


Figure 2.2.1.3. Microbiota: Through Life Stages and Perturbations.

A generalized overview of the relative abundance of key phyla in the gut microbiota composition in different stages of life. Measured by either 16S RNA or metagenomic approaches (DNA). Data arriving from: Babies breast- and formula-fed, baby solid food, toddler antibiotic treatment, toddler healthy or malnourished, adult, elderly, and centenarian healthy, and adult obese.(Ottman, Smidt et al. 2012)

The structure of the gut microbiota includes predominantly bacteria, with lesser quantities of archaea, eukarya, and viruses. As mentioned, these microorganisms are fundamental members of their biomes and participate in complex associations with their environment and with other members within their ecosystem. These organisms are part of a complex ecosystem comprising more than 3.3 million genes and corresponding to a large spectrum of enzymatic activities leading to molecular signals and metabolites that may directly influence health. This genomic pool is claimed to be at least 150 times larger than the eukaryotic human nuclear genome (Zoetendal, Rajilic-Stojanovic et al. 2008). According to Sekirov, Russell et

al. (2010) for each human cell present in the body, there are ten bacterial cells (Sekirov, Russell et al. 2010). However more recent estimates indicate the ratio between bacteria to human cells is closer to 1.3:1 (Sender, Fuchs et al. 2016).

Although there is no consensus on what constitutes a healthy or dysbiotic gut microbiota, there is some agreement on several characteristics and differences (Lloyd-Price, Abu-Ali et al. 2016). A healthy gut microbiota is considered to be a community in which beneficial microbes are predominant, while dysbiosis may be characterized by a dominance of one or a few harmful microbes (Karl, Hatch et al. 2018). Although the health effects of most gut commensals are varied or unclear, there are several taxa generally considered beneficial and several generally considered harmful. Other researchers consider a healthy gut to contain a microbiota that is extremely diverse in composition and genetic content (Blaser and Falkow 2009, Kriss, Hazleton et al. 2018). One reason may be because low-diversity microbiota lack core or "keystone" microbes or the microbial genes required to maintain a healthy ecosystem (Karl, Hatch et al. 2018). Linked with diversity as a healthy attribute is the ability of the gut microbiota to resist perturbations or to recover a healthy state following stressors (Lloyd-Price, Abu-Ali et al. 2016).

Even though, the bacteria represent the majority of the gut microbiota, other microbes, which are also a portion of the gut microbiota, may play important roles in the ecosystem (Mills, Shanahan et al. 2013). Therefore, researchers need to start shifting their approach to include all interactions in efforts to elucidate the roles of all components (archaeome, mycobiome, and virome) of the microbiota. Transkingdom commensal relationships among components of the microbiota have been observed to form from infancy and several co-occurring relationships have also been identified (Grimaudo, Nesbitt et al. 1996, Grimaudo and Nesbitt 1997, Breitbart, Hewson et al.

2003, Breitbart, Haynes et al. 2008, LaTuga, Ellis et al. 2011, Hoffmann, Dollive et al. 2013, Wright, Burns et al. 2013, Cavalcanti, Nobbs et al. 2016, Wampach, Heintz-Buschart et al. 2017).

Having covered how the different portions of the gastrointestinal tract contain different microbial compositions, the various means of analysing the gut microbiota and their benefits and disadvantages, and a generalized overview of the predominant phyla present through different life stages and health, the specific microbes of the large intestine can be discussed, beginning with the bacteria.

2.2.1.1. Bacteria

Bacteria in the gastrointestinal tract have received the most attention over their years in their composition, function, and link to health compared to the other microbes with the gastrointestinal tract. Most of the introduction relating to dysbiosis associated pathologies have been linked to changes in the bacterial composition as seen in previous segments of the dissertation. Over five hundred different species of bacteria that have been identified within the intestinal microflora (Serino, Luche et al. 2009, Govender, Choonara et al. 2014). In one gram of faeces, there are approximately 10^{11} bacteria present. Some of the bacteria present are permanently fixed in the intestine, while others are transient and only pass through (Saavedra and Tschernia 2002). Bacteria are the most abundant microorganisms in the gastrointestinal tract beside bacteriophages. In fact, more than 99% of the genes in the human gut microbiota are of bacterial origin (Suhr and Hallen-Adams 2015). The number of different bacterial species within the human gut microbiota is controversial with many authors referring to previous estimates of 400-500 species based on culture studies, while more recent estimates are reaching into the thousands (Jacobs, Gaudier et al. 2009). However, most agree that the gut microbiota comprises of five

predominant phyla and approximately 1000 species consistently found in the large intestine (Rajilic-Stojanovic and de Vos 2014).

Genetic analyses have correlated with culture based methods and have identified about five of the about fifty bacterial phyla in the lower gastrointestinal tract (Frank and Pace 2008). *Bacteroidetes* (23%) and *Firmicutes* (65-80%) are the predominant microbial phyla in the human gut (Eckburg, Bik et al. 2005, Gill, Pop et al. 2006, Tap, Mondot et al. 2009). Together, *Bacteroidetes* and *Firmicutes* account for at least 70% of the composition (Zhernakova, Kurilshikov et al. 2016). *Actinobacteria* (about 3%), *Proteobacteria* (1%) and *Verrumicrobia* (0.1%) exist only in smaller amounts (Frank and Pace 2008, Tap, Mondot et al. 2009, Sekirov, Russell et al. 2010). *Actinobacteria* and *Firmicutes*, are almost exclusively Gram-positive, while *Bacteroidetes* and *Proteobacteria* are mainly Gram-negative (Hakansson and Molin 2011). Gram-negative bacteria and anaerobes are dominant species in the intestinal lumen, numbers are estimated to be 100 to 1000 times higher than aerobic ones (Neish 2009). The gut microbiota is mostly characterized by strict anaerobes (70–90%), which are predominate over facultative anaerobes and aerobes (10–30%) (Frank and Pace 2008).

Table 2.2.1.1. Predominate Gut Bacteria.

Phyla	Important Families	Main Fermentation Products
Bacteroidetes (G-)	*Bacteroides* *Prevotellae* *Prophyromonadaceae* *Rikenellaceae*	Acetate, Propionate, Succinate from carbohydrates
Firmicutes (G+)	*Clostridiaceae* *Lactobacillaceae* *Leuconostocaceae* *Bacillaceae* *Streptococcaceae* *Eubacteriaceae* *Staphylococcaceae* *Peptococcaceae* *Peptostreptococcaceae*	Acetate, Formate, L- And D-lactate, Butyrate, Succinate, Propionate from Carbohydrates Bcfas, Indoles, Sulphides, Phenols, Amines, NH_3, H_2, CO_2, CH_4 from proteins & amino-acids
Actinobacteria (G+)	*Bifidobacteriaceae* *Actinomycetaceae* *Coriobacteriaceae* *Corynebacteriaceae* *Propionibacteriaceae* *Micrococcaceae*	Lactate, Acetate, Formate from carbohydrates
Proteobacteria (G-)	*Enterobacteraceae* *Oxalobacteriaceae* *Pseudomonadaceae* *Desulfovibrionaceae* *Helicobacteraceae*	Lactate, Acetate, Succinate, Formates from carbohydrates Sulphide from Sulphate, H_2S, Mercaptans
Euryachaeota (G-/G+)	*Methanobacteriaceae*	Ch_4
Fusobacteria (G-)	*Fusobacteriaceae*	Acetate, Butyrate, NH_3, Formate, Lactate
Verrucomicrobia (G-)	*Verrucomicrobiaceae*	Mucin Degradation
Lentisphaerae (G-)	*Victivallaceae*	Acetate, Ethanol, H_2

Table 2.2.1.1. depicts the predominate gut bacteria within the intestines with the major bacterial families and their main metabolic products (Jacobs, Gaudier et al. 2009). The order of the table is arranged with the most prevalent phylum at the top descending to the bottom in prevalence. As observed, some of the bacteria are gram positive and negative, and each family has the capacity to produce certain metabolites (Jacobs, Gaudier et al. 2009). The variation in metabolites and bacteria is why a

diverse gut microbiota is considered to be healthy as the composition and genetic content is varied. The other bacterial genetic material uncovered by genetic analyses requires more studies need to be performed to determine their function and metabolic profile in the gut.

2.2.1.2. Eukarya

The next microorganisms that reside within the gastrointestinal tract are eukarya. Even though they represent a small portion of the microbiota, they may play important roles in the ecosystem (Iliev, Funari et al. 2012). Therefore, researchers should modify their approach to include eukaryotic interactions in efforts to elucidate the roles of all components of the microbiota. The most dominate eukaryotes reported in the gut are fungi and protozoa (Rajilic-Stojanovic, Smidt et al. 2007, Nam, Chang et al. 2008, Scanlan and Marchesi 2008, Chabe, Lokmer et al. 2017).

Only a few of the intestinal protozoa have a role in disease in humans (Hamad, Raoult et al. 2016). *Giardia intestinalis, Cryptosporidium parvum, Cyclospora cayetanensis* and *Isospora belli, Blastocystis spp*., *Entamoeba histolytica* and *Balantidium coli* are the most abundant protozoa parasitizing the human gastrointestinal tract (Hamad, Raoult et al. 2016). *Blastocystis spp*. are the most prevalent eukaryotic microbe colonizing the human gut and are common constituents of the human gut microbial community (Gentekaki, Curtis et al. 2017). The pathogenicity of *Blastocystis spp*. is controversial, but these microbes *are* related to algae and some plant pathogens, and in organisms have been linked to various gastrointestinal diseases, including diarrhoea, abdominal pain, nausea, and irritable bowel syndrome (Gentekaki, Curtis et al. 2017).

Fungi, another eukaryotic component of the gut microbiota, are another relatively unexplored area of research. The mycobiome, which is the collection of

fungi in the gut microbiota, are considered to comprise 0.02%–0.03% of the gut microbiota, making them approximately 3,300-fold less prevalent than Bacteria (Ott, Kuhbacher et al. 2008). Approximately 390 fungal species have been cultured throughout the body, and in the digestive tract, 335 species and 158 genera exist (Gouba and Drancourt 2015). Among these, 221 species are found only in the intestines (Gouba and Drancourt 2015). The fungal DNA accounts for around 0.02% of the entire mucosa-associated microbiota (Ott, Kuhbacher et al. 2008). Fungal diversity in the human gut is also lower than that of bacteria (Scanlan, Shanahan et al. 2006, Ott, Kuhbacher et al. 2008) However, more taxa are being found as the number of individuals being studied using next generation sequencing is increasing (Scanlan, Shanahan et al. 2006, Cui, Morris et al. 2013, Rodriguez, Perez et al. 2015, Suhr, Banjara et al. 2016).

A review of 36 fungal gut microbiota studies revealed that there have been at least 267 distinct fungi identified in the human gut, while another study reported 221 (Gouba and Drancourt 2015, Suhr and Hallen-Adams 2015). These species belong to yeast and filamentous fungi taxa, of the *Ascomycota*, *Basidiomycota*, and *Zygomycota* phyla (Gouba and Drancourt 2015). Most of the fungal species detected appear to be either transient or environmental fungi that cannot colonize the gut and are not constantly found among individuals. A previous study indicated that the fungal community is unstable and that only 20% of the initially identified fungi can be detected after 4 months has passed (Hallen-Adams, Kachman et al. 2015). The roles of eukaryotic organisms have not been examined in as much detail to date. However, they are known to play important roles in microbiota dynamics and host physiology/immunity related to health and disease (Nam, Chang et al. 2008, Scanlan and Marchesi 2008, Iliev, Funari et al. 2012, Wheeler, Limon et al. 2016, Gentekaki,

Curtis et al. 2017). In fact, the richness and diversity of fungal species is higher in mucosal biopsies from patients suffering with gastrointestinal diseases, in direct contrast to bacteria (Ott, Kuhbacher et al. 2008, Li, Wang et al. 2014, Mukhopadhya, Hansen et al. 2015).

Yeasts represent between 40-63.4% of the fungal taxa from the gut and 10 of the 12 most commonly detected fungi are yeasts (Suhr and Hallen-Adams 2015). *Candida* is the most common fungal genus found in culture dependent and independent studies whereas the other taxa identified have been variable (Scanlan, Shanahan et al. 2006). The variability in taxa reported in various studies and the increasing number of fungi being identified maybe due to the fact that most fungi are transient residents (Gouba, Raoult et al. 2014). Under certain conditions some of the transient and resident fungi under certain conditions, may flourish and become pathogenic. Such taxa include *Candida*, *Aspergillus*, *Fusarium*, and *Cryptococcus* (Ott, Kuhbacher et al. 2008, Cui, Morris et al. 2013, Wang, Yang et al. 2014, Hoarau, Mukherjee et al. 2016, Noble, Gianetti et al. 2017). Hoffman et al. (2013) has described correlations found between fungi-bacteria and fungi-archaea. *Candida* and *Saccharomyces* commonly co-occur with bacterial taxa *Faecalibacterium*, *Bacteroides*, *Lachnospiraceae*, and *Ruminococcaceae*, and these fungal genera are also positively associated with the archaeon *Methanobrevibacter* and negatively associated with the archaeon *Nitrososphaera* (Suhr and Hallen-Adams 2015). Further analysis showed that *Candida* was negatively associated with *Bacteroides* (Suhr and Hallen-Adams 2015). This only underlines the imperative importance to take into account smaller fractions of the microbiota.

2.2.1.2. Archaea

More studies are needed in order to clarify the interaction between *Archaea*

and other components of the microbiota and between *Archaea* and the host, beyond methanogenesis. Better comprehension of the archaeome may contribute to our understanding of their fitness and function in the gut microbiome in health and diseases. When *Archaea* were first discovered, they were all believed to all be extremophiles of one sort or another, thriving either in high temperature, high salinity, low or high pH, with absolutely no oxygen or combinations environments, and that due to those physiological restrictions made them poor competitors to survive in the gastrointestinal tract. However, studies have demonstrated that *Archaea* display a wide range of metabolic capabilities, including anaerobic and aerobic respiration, fermentation, chemoautotrophy, heterotrophy, and photoheterotrophy (Eme and Doolittle 2015). These microbes are exclusively responsible for major metabolic pathways of significant importance. They perform methanogenesis, which generates approximately 85% of the methane on the planet (Eme and Doolittle 2015). They also uniquely perform anaerobic methane oxidation, a syntrophic association between sulphate-reducing bacteria and anaerobic methane oxidizing *Archaea* (Eme and Doolittle 2015). Thirdly, they contribute to the nitrogen cycle, through aerobic ammonia oxidation (Eme and Doolittle 2015). *Archaea* are emerging organisms in the complex human microbiomes due to these metabolic potentials. In fact, *Archaea* have demonstrated to have a niche where they partake in a mutualistic interaction between protozoa and methanogens in the gastrointestinal tract to digest cellulose (Chaban, Ng et al. 2006, Janssen and Kirs 2008). Methanogenic *Archaea* may also improve polysaccharide fermentation by cellulolytic microorganisms by preventing the accumulation of H^+ and other reaction end-products (Samuel, Hansen et al. 2007). Moreover, correlations were observed between the number of methanogens and

cellulolytic microorganisms in ruminants (Morvan, Bonnemoy et al. 1996).

When quantifying the presence of *Archaea* in the gastrointestinal tract, these microbes were found to represent 12% of the total anaerobes in the distal colon and only 0.003% in the proximal colon, and even smaller amounts were also discovered in the oral cavity (Pochart, Lemann et al. 1993, Eme and Doolittle 2015, Borrel, McCann et al. 2017, Hillman, Lu et al. 2017, Koskinen, Pausan et al. 2017, Nkamga, Henrissat et al. 2017, Moissl-Eichinger, Pausan et al. 2018). Eight Archaeal species have been isolated in the human gut microbiota, all of which belong to the *Euryarchaeota* phylum (Rajilic-Stojanovic and de Vos 2014). Those *Archaea* isolated from humans are strict anaerobes; such species include *Methanobrevibacter smithii, Methanobrevibacter oralis, Methanosphaera stadtmanae (Dridi 2012)*. The microbes include non-methanogenic and methanogenic species, with the methanogenic species representing up to 10^{10} cells per gram of faeces and having a significant role in the gut microbiota ecosystem (Miller and Wolin 1986, Gaci, Borrel et al. 2014).

Unlike bacteria and eukaryotic microbes, *Archaea* do not establish themselves into the gastrointestinal tract from the diet, instead it is assumed that they come from the environment, but their precise ecological niches and routes of acquisition remain unknown. Studies have demonstrated that *Archaea* are not detected in children under 27 months age, but numbers increase with age up to 60% in children, regardless of diet, after a certain period of time (Rutili, Canzi et al. 1996). The only link between *Archaea* and diseases is the role of *Archaea* to counteract or enhance the production of metabolites observed in diseases such as trimethylamine in cardiovascular diseases, short-chain fatty acids in obesity, and methane in constipation (Lurie-Weinberger and Gophna 2015, Nkamga, Henrissat et al. 2017). Better comprehension of *Archaea* is still necessary to understand its role in health and diseases.

2.2.1.3. Viruses

Viruses in the human microbiome have also been under studied although the information is available is limited (Reyes, Semenkovich et al. 2012). The majority of data available are related primarily to disease and do not address the commensal aspect of the virome (Cadwell 2015, Columpsi, Sacchi et al. 2016, Chatterjee and Duerkop 2018). As the virome represents a significant portion of the microbiota, they surely play a dynamic role in host physiology/immunity related to health and disease (Mills, Shanahan et al. 2013, Dalmasso, Hill et al. 2014, Stefanaki, Peppa et al. 2017). Viral communities are mainly comprised of bacteria-infecting phage families, (approximately 90%), while eukaryotic viruses are less abundant, (around 10%) (Reyes, Semenkovich et al. 2012, Rascovan, Duraisamy et al. 2016).

Metagenomic studies have revealed bacteriophages as one of the most abundant components of the human microbiota (Reyes, Haynes et al. 2010, Minot, Sinha et al. 2011, Foulongne, Sauvage et al. 2012, Pride, Salzman et al. 2012, Reyes, Semenkovich et al. 2012, Oh, Byrd et al. 2014, Dickson and Huffnagle 2015, Santiago-Rodriguez, Ly et al. 2015, Oh, Byrd et al. 2016, Galley, Mackos et al. 2017). In fact, viruses associated with the human gut microbiota have been calculated to be equivalent to 10^9 virus-like particles per gram of faeces (Zhang, Breitbart et al. 2006, Minot, Bryson et al. 2013). From studies examining the virome, it has become clear that bacteriophages undoubtedly influence the dominant microbial populations in many ecosystems including the human intestine.

Bacteriophages are the most abundant replicating entities on the planet and thrive wherever bacteria exist. Commensal bacteria regulate various aspects of host immunity, yet there are no clear understanding of the mechanisms of the virome modulating the gut microbiota (Mazmanian, Liu et al. 2005, Ivanov, Frutos et al.

2008, Arpaia, Campbell et al. 2013, Fung, Olson et al. 2017, Novince, Whittow et al. 2017, Schnupf, Gaboriau-Routhiau et al. 2017). Considering that bacteriophages influence the assembly of microbial communities and modulate bacterial diversity in various ecosystems, by altering the ratio of symbionts to pathobionts, enabling pathobionts to persist, it could be said that bacteriophages have a role in disease (Barr, Auro et al. 2013, Koskella and Meaden 2013, Maslov and Sneppen 2017). *Herpesviridae, Papillomaviridae, Polyomaviridae, Adenoviridae* are the most commonly found viral taxa found in the gastrointestinal tract of humans (Hillman, Lu et al. 2017).

Bacteriophages have been implicated in diseases associated with bacterial dysbiosis, however it is unclear if they play a direct role in pathogeny (Chatterjee and Duerkop 2018). An increasing body of data suggests that bacteriophages engage in interactions with immune cells and modulate different aspects of host immune response. Several studies propose a role for phages in promoting immune tolerance by downregulating T cell proliferation, reducing of antibody production, and preventing of allogenic transplant rejection in animal models (McVay, Velasquez et al. 2007). Therefore, the impact of bacteriophages must extend beyond their effect on their bacterial hosts and on human health (De Paepe, Leclerc et al. 2014, Norman, Handley et al. 2015, Manrique, Bolduc et al. 2016, Wahida, Ritter et al. 2016). For example, the diversity and composition of intestinal bacteriophages is significantly different between healthy individuals and patients with inflammatory bowel diseases (Wagner, Maksimovic et al. 2013, Norman, Handley et al. 2015). In addition, DNA or RNA viruses, including *Rotaviruses*, *Caliciviruses*, *Astroviruses* or *Adenoviruses*, have been associated with gastroenteritis (Klein, Boster et al. 2006). Moreover, several human viral pathogens such as *Enteroviruses*, as well as viruses that are transmitted via the

faecal-oral route and are excreted by the gut can be found in stool samples.

2.2.1.4. Structure of Gut Microbiota Amongst Animal Models

Animal models are commonly used to analyse the effect of the gut microbiota in health and diseases. Parameters like genetics, age, and diet, are easily controlled in laboratory animals, but the structure and function of the gastrointestinal tract in the animal models should also be considered, as they are different to humans (Hillman, Lu et al. 2017). Although some similarities exist in the anatomy of the gastrointestinal tract between humans and animal models, differences in anatomical structures and pH at different locations along the gastrointestinal tract exists. This may contribute to differences in the microbiota found in humans versus animal models (Booijink, Zoetendal et al. 2007, Nguyen, Vieira-Silva et al. 2015). So far, studies have demonstrated that the human gut microbiota are dominated by two phyla: *Firmicutes* and *Bacteroidetes*, which also dominate the gastrointestinal tract of commonly used animal models (Ley, Hamady et al. 2008, Huttenhower, Gevers et al. 2012, Nash, Auchtung et al. 2017). At lower taxonomic levels, some differences have been reported in microbiota compositions in the gut between humans and animal models as seen in Table 2.2.3.1. (Hillman, Lu et al. 2017). Microbial activity also differs along the gastrointestinal tract, with the most relevant being fermentation occurring in the ceca of most animal models, but not in humans. The differences in form and function of the gastrointestinal tract and the microbes present associated with the different animal models being used need to be taken into consideration.

Table 2.2.3.1. Major Phyla of the Gut Microbiota in Humans and Animals Models.

	Human	Mouse	Rat	Pig
Bacteria	*Firmicutes* *Bacteroidetes* *Actinobacteria* *Proteobacteria*	*Firmicutes* *Bacteroidetes*	*Firmicutes* *Bacteroidetes*	*Firmicutes* *Bacteroidetes*
Archaea	*Methanobrevibacter* *Nitrososphaera*	*Methanobrevibacter*	*Methanobrevibacter*	*Methanobrevibacter* *Methanosphaera*
Viruses	*Herpesviridae* *Papillomaviridae* *Polyomaviridae* *Adenoviridae*	*Variable*	*Variable*	*Picornaviridae* *Astroviridae* *Coronaviridae* *Caliciviridae*
Eukarya	*Candida* *Malassezia* *Saccharomyces* *Cladosporium*	*Ascomycota* *Basidiomycota* *Chytridiomycota* *Zygomycota*	*Ascomycota* *Basidiomycota* *Chytridiomycota* *Zygomycota*	*Kazachstania* *Candida* *Galactomyces* *Issatchenkia*

(Hillman, Lu et al. 2017)

2.2.2. Functions of the Gut Microbiota

The gut microbiota has many roles in its symbiotic relations with the host. As mentioned earlier, these microorganisms are part of a complex ecosystem comprising more than 3.3 million genes and corresponding to a large spectrum of enzymatic activities leading to molecular signals and metabolites that may directly influence health. As already stated this genomic pool is claimed to be at least 150 times larger than the eukaryotic human nuclear genome (Zoetendal, Rajilic-Stojanovic et al. 2008). This gives the microbiota a large capacity to produce and metabolize compounds that will affect the host. The effects of the gut microbiota can broadly be classified in 3 main categories (Figure 2.2.2.1.). The microbial metabolites produced are involved in immunity, metabolism, and have some trophic roles that related to

structure. Metabolic functions such as fermentation of indigestible fibres resulting in the production of short-chain fatty acids (SCFA), and production of vitamins, and bile acid transformation, trophic activity such as stimulation of angiogenesis, effect on intestinal motility, effect on intestinal morphology, and immune activity of resistance to pathogen colonization and improving the intestinal barrier function are all some compounds derived from microbial products (Round and Mazmanian 2009).

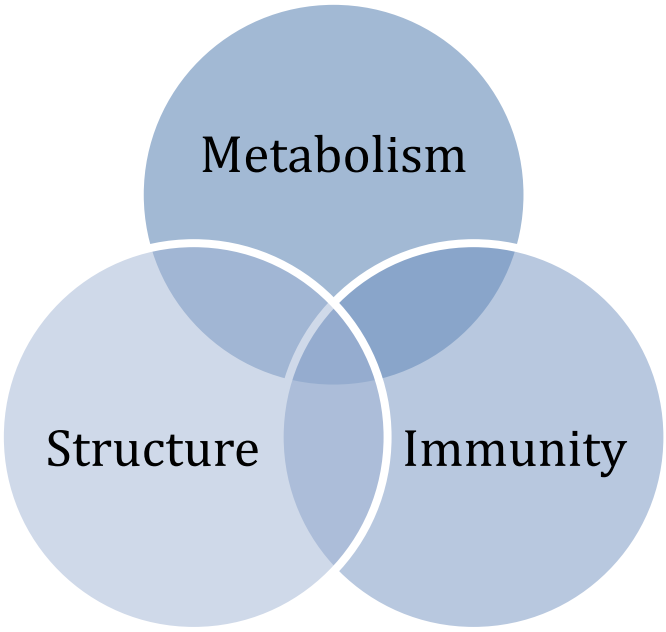


Figure 2.2.2.1. Functions of the Gut Microbiota.

A generalized overview of the three main functions of the gut microbiota. They are involved in immunity, metabolism, and have some trophic roles that related to structure.

2.2.3. Gut Microbiota Metabolites

A healthy gut contains a microbiota that is extremely diverse in composition and genetic content (Blaser and Falkow 2009, Kriss, Hazleton et al. 2018). Again, this is due to the fact that a diverse gut microbiota has a wider range of metabolites that can be produced. The major bacterial fermentation products are SCFAs (acetate,

propionate and butyrate), and their production tends to have a wide spectrum of effects. Other essential metabolites that are supplied by the gastrointestinal microbes include bile acids, vitamins, polyamines, lipids, glucose, and lysine (Holmes, Kinross et al. 2012, Nicholson, Holmes et al. 2012). Microbial fermentation in the colon also results in the production of end products. These end products are necessary for modulation of gut motility, immunity, detoxification of toxins, mental health, and maturation of intestinal mucosa (Sekirov, Russell et al. 2010, Ulluwishewa, Anderson et al. 2011, Furness, Rivera et al. 2013). Table 2.2.3.1. demonstrates the main metabolites produced by the gut microbiota and their function within the host. Data regarding the metabolites and their effects on the host have been discovered from the utilization of germ-free and conventional animals that demonstrated that the gut microbiota are essential for a variety functions.

Table 2.2.3.1. Gut Bacteria Metabolites.

Class	Examples	Functions
Anti-Microbial Peptides	Subtilin, Alveicin, Glycinecin, Divercin, Leucoccin, Microbisporicin	Anti-microbial
Bile Acids	Secondary Bile Acids	Anti-microbial Host Metabolism Lipid Absorption
Choline Metabolites	Trimethylamine	Lipid Metabolism
Gastrointestinal Hormones	Ghrelin, Leptin, Glucagon-Like-Peptide-1, PYY	Host Metabolism Appetite Regulation GI Motility/Secretion
HPA hormones	Cortisol	Stress Response Host Metabolism Anti-Inflammatory Wound Healing
Indole Derivatives	Indole, Indoleacetylglycine, Indoxyl Sulfate, Indole-3-Propionate	Anti-microbial Anti-Inflammatory Modulation of Intestinal Barrier
Lipids	LPS, Glycerol, Acylglycerols, Sphingomyelin, Cholesterol, Triglycerides	Modulation of Intestinal Barrier Gut-Brain Axis Inflammation Immune Regulation
Neurotransmitters	Serotonin, Dopamine, Noradrenaline, Gamma-Aminobutyric Acid	Mood Emotion Cognition Reward (CNS) Motility/Secretion (ENS)
Phenolic, Benzoyl, & Phenyl Derivatives	Methyl, Glucuronide Sulfate Derivatives	Detoxification of Xenobiotics Anti- Inflammatory Anti-microbial
Polyamines	Agmatine, Cadaverine, Putrescine, Spermidine, Spermine	Anti-Inflammatory Cell Proliferation
Precursors to Neuroactive Compounds	Tryptophan Kynurenine L-Dopa	Precursor To: 5-HT Kynurenic Acid, Quinolinic Acid Dopamine
SCFAs	Acetate Butyrate Propionate	Energy Source Host Metabolism Signalling Molecules
Vitamins	Vitamin B9, Thiamine, Vitamin B2, Biotin, Niacin,	Exogenous Supply of Vitamins Immune Regulation

2.2.3.1. Short-Chain Fatty Acids

The main end products of non-digestible carbohydrates fermentation by the gut microbiota are short-chain fatty acids (SCFAs): acetate, propionate, butyrate (Cummings, Pomare et al. 1987, Ohira, Tsutsui et al. 2017). Acetate, propionate, and butyrate are present in an approximate molar ratio of 60:20:20 in the colon and stool (den Besten, van Eunen et al. 2013). The types and amount of SCFAs produced by gut microorganisms are determined by the composition of the gut microbiota and the metabolic interactions between microbial species, but also by the amount, type, and balance of the main dietary macro- and micronutrients (De Filippo, Cavalieri et al. 2010, Jumpertz, Le et al. 2011, Hold 2014, Mach, Berri et al. 2015).

SCFAs affect a range of host processes including energy utilization, host–microbe signalling, and control of colonic pH, with consequent effects on microbiota composition, intestinal gut motility, gut permeability, and epithelial cell proliferation (Musso, Gambino et al. 2011). In addition, SCFAs have several beneficial effects on host health as they are the preferred energy source for colonocytes, promote epithelial integrity, affect the thickness of the mucus layer, support epithelial cell survival, regulate expression of tight junction proteins, regulate colonic mobility and blood flow and can influence colon pH, which has a direct impact on the uptake and absorption of nutrients and electrolytes (Tazoe, Otomo et al. 2008, Suzuki 2013, Puertollano, Kolida et al. 2014, Tan, McKenzie et al. 2014, Jonkers 2016). Another aspect of SCFAs is that they have been shown to exhibit anti-inflammatory properties by modulating immune cell chemotaxis, reactive oxygen species, and the release of cytokines (Tan, McKenzie et al. 2014). Fermentation and SCFA production also inhibit the growth of pathogenic organisms by reducing luminal and faecal pH. Low pH helps to reduce peptide degradation and the resultant formation of toxic

compounds such as ammonia, amines, and phenolic compounds, and decreases the activity of undesirable bacterial enzymes (Slavin 2013). Out of the SCFAs, butyrate in particular, is widely regarded as health promoting, as it is a major energy source for intestinal epithelial cells, affecting cell proliferation, cell differentiation, mucus secretion, and barrier function; and has anti-inflammatory and antioxidative potential (Hamer, Jonkers et al. 2008). The brain, muscles, and tissues metabolize acetate systemically, whereas propionate is cleared by the liver and may lower the hepatic production of cholesterol by interfering with its synthesis (Slavin 2013). Species of *Clostridia, Firmicutes, Eubacterium, Roseburia, Faecalibacterium*, and *Coprococcus* have been shown to be the main microbes responsible for the production of SCFs (Feng, Ao et al. 2018).

2.2.3.2. Neurotransmitters

Gut microbes are also capable of synthesizing neuroactive compounds that are identical to those produced by humans. Such compounds include serotonin, dopamine, histamine, and gamma-aminobutyric acid from amino acid precursors (Lyte 2014). These compounds are capable of impacting cognition and behaviour via the gut-brain axis (Dinan and Cryan 2012). Through the gut-brain axis, the microbes have an influence on memory, mood, and cognition and are clinically and therapeutically relevant to a range of disorders. *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Bacillus*, *Saccharomyces*, *Candida*, *Streptococcus*, and *Enterococcus* microbes have been shown to be able to produce neuro-transmitting metabolites that affect mood, emotion, cognition, CNS, motility, secretion, and behaviour (Hyland and Cryan 2010, O'Mahony, Hyland et al. 2011, Cryan and Dinan 2012, Dinan and Cryan 2012, Dinan, Stanton et al. 2013, O'Mahony, Clarke et al. 2015).

2.2.3.3. Neuroactive Compounds

Several microbial metabolites have displayed neuroactive properties such as gaseous molecules, SCFAs, amines, and precursor molecules (Mazzoli and Pessione 2016). Such gaseous molecules include carbon monoxide, hydrogen sulphide, and nitric oxide (Bienenstock and Collins 2010). SCFAs as discussed earlier are acetate, propionate, butyrate, valerate, and caproic acid (Engelstoft, Egerod et al. 2008, Nicholson, Holmes et al. 2012). The amines found to have neuroactive effects are putrescine, spermidine, spermine and cadaverine (Bienenstock and Collins 2010). In addition, other precursor molecules include tryptophan, kynurenine, L-Dopa and others have also been observed (Lyte 2010, O'Mahony, Clarke et al. 2015). Various species of *Clostridia, Firmicutes, Eubacterium, Roseburia, Faecalibacterium, Lactobacillus, Bifidobacterium, Escherichia, Bacillus, Saccharomyces, Candida, Streptococcus, Enterococcu*s, and *Coprococcus* are capable of producing numerous neuroactive molecules (O'Mahony, Clarke et al. 2015, Usami, Miyoshi et al. 2015).These neuroactive molecules can cross the intestinal barrier through multiple routes and exert extra-intestinal effects (Keita and Soderholm 2010, Psichas, Reimann et al. 2015, Said, Kaji et al. 2015).

2.2.3.4. Bile Acids

Bile acids are secreted in response to ingestion of fat. In the liver, the bile acids are produced, will then be secreted into the intestine, where they have the potential of being transformed by the gut microbiota. The gut microbes modify bile acids, forming primarily secondary bile acids that act as signalling molecules in multiple metabolic pathways, and which may be health-promoting or health-degrading (Devkota and Chang 2013, Tran, Grice et al. 2015, Wahlstrom, Sayin et al. 2016). Other primary, tertiary, sulphated, conjugated bile acids, etc maybe be

produced as well, but in lower concentrations. In a healthy individual, the majority of the bile acids are transformed into secondary bile acids, with low amounts of primary, conjugated, and sulphated bile acids. Bile acids have anti-microbial effects against some gut commensals, but may also enrich for bile-acid metabolizing pathobionts, which are potentially harmful sulphate-reducing bacteria (Lorenzo-Zuniga, Bartoli et al. 2003, Caesar, Fak et al. 2010, Devkota and Chang 2015). Additionally, high levels of conjugated relative to deconjugated bile acids can stress the gut microbiota and promote dysbiosis by increasing gastrointestinal permeability (Tran, Grice et al. 2015). In individuals with dysbiosis, there is a significant increase in primary and sulphated bile acids, and reduction in secondary bile acids. Increases in primary and sulphated bile acids have been linked to cause inflammation to the gut epithelium. Therefore, the gut microbiota is essential to maintain the balance in the bile acid pools. *Lactobacillus, Bifidobacterium, Enterobacter, Bacteroides*, and *Clostridium* bacterial species have been shown to be important in the regulation of bile acids in the gastrointestinal tract (Groh, Schade et al. 1993, Swann, Want et al. 2011, Ridlon, Harris et al. 2016).

2.2.3.5. Choline Metabolites

The gut microbiota also has an impact on the amount of choline metabolites. The choline metabolites include acetylcholine, an important neurotransmitter, betaine, an oxidative intermediate of choline, and glycerophosphocholine, which is like betaine in cells affecting osmosis (Artegoitia, Middleton et al. 2014). The choline-containing metabolites include phosphatidylcholine, sphingomyelin, and lysophosphatidylcholine, which are all critical structural components of membranes (Artegoitia, Middleton et al. 2014). Choline can be obtained from the diet or from endogenous biosynthesis that predominantly occurs in the liver. The gut microbiota

affects the levels of choline metabolites and can affect the amount of free choline available that interacts with trimethylamine. One of the choline metabolites of concern is trimethylamine, involved in lipid metabolism. Trimethylamine will be oxidized in the liver and affect lipid absorption and cholesterol homeostasis and modulate glucose and lipid metabolism by decreasing the total bile acid pool size. Elevated trimethylamine N-oxide also increases the risk of cardiovascular disease and exacerbates the effects of high choline and betanine levels on the risk of major adverse cardiovascular events (Chen, Venkat et al. 2017, Chhibber-Goel, Singhal et al. 2017, Lau, Savoj et al. 2018). *Faecalibacterium prausnitzii* and *Bifidobacterium* have been linked with choline metabolites (Martin, Sprenger et al. 2010, Wang, Klipfell et al. 2011).

2.2.3.6. HPA Hormones

The gut microbiota also influences and modulate the Hypothalamic-Pituitary-Adrenal axis (HPA) hormones, especially the release of cortisol. Although the modulation is indirect, the microbes produced metabolites that will affect the stress response, the metabolism, the anti-inflammation response, and wound healing capability of the host. Gut pathogens such as *Escherichia coli,* are recognised to activate the HPA axis if they permeate across the gastrointestinal barrier. However, germ-free animal studies have shown exaggerated HPA responses to stress, which was normalised with a single colonization by certain bacterial species. Stress causes the release of cortisol that will lead to the dysfunction of the gut microbiota, resulting in increased gut permeability, affecting the intestinal barrier function. The translocation of luminal antigens into the circulation causes the release of pro-inflammatory cytokines, which will cause the reduction of tryptophan. The altered 5-hydroxytryptophan availability and pro-inflammatory markers causes the elevated

release of cortisol. *Lactobacillus* and *Bifidobacterium* bacteria are the microbes that have been associated with this indirect modulation of the HPA axis (Dinan and Cryan 2012).

2.2.3.7. Vitamins

Vitamins (vitamin B9, thiamine, vitamin B2, biotin, vitamin B12, niacin, pyridoxine, vitamin K, vitamin B1, vitamin B5, vitamin B8, folate, riboflavin) are essential micronutrients necessary for normal development and health. There is an abundance of vitamins within body, however, some have to be obtained through diet or by the transformation and synthesis of other substances. In addition, vitamins can also be supplied by intestinal bacteria (Koenig, Spor et al. 2011, Ly, Litonjua et al. 2011, Said 2011, LeBlanc, Milani et al. 2013, Ramakrishna 2013, Li, Quan et al. 2018). It has been shown that members of the gut microbiota are able to synthesize vitamin K as well as most of the water-soluble B vitamins, such as biotin, cobalamin, folates, nicotinic acid, panthotenic acid, pyridoxine, riboflavin and thiamine (LeBlanc, Milani et al. 2013). Vitamins produced by microorganisms are mainly absorbed in the colon, whereas vitamins derived from diets and hosts are taken up in upper sections of the gastrointestinal tract such as the proximal small intestine (Li, Quan et al. 2018). Bifidobacterium are they main bacterial species associated with vitamin synthesis and they have been shown to help provide endogenous sources of vitamins, strengthen the immune function, and exert epigenetic effects to regulate cell proliferation (Koenig, Spor et al. 2011, Said 2011). *Bacillus Subtilis*, *Escherichia coli*, *Bacteroidetes, Fusobacteria, Proteobacteria*, and *Actinobacteria* are other bacterial taxa associated with vitamin synthesis (Feng, Ao et al. 2018).

2.2.3.8. Polyamines

Polyamines such as agmatine, cadaverine, putrescine, spermidine, and spermine, have been found to be mainly involved in intracellular effects related to cell proliferation and death mechanisms (Sanchez-Jimenez, Ruiz-Perez et al. 2013). Within the gastrointestinal tract, epithelial cell development is dependent on the available supply of polyamines to the dividing cells in the crypts, and moreover, polyamines also regulate intestinal epithelial cell apoptosis (Seiler and Raul 2007, Timmons, Chang et al. 2012). Polyamines also have an important role in regulating the intestinal barrier function (Timmons, Chang et al. 2012). Other amines, putrescine, spermidine, spermine, and cadaverine, have been found to have neuroactive effects (Bienenstock and Collins 2010). Such neuroactive effects, to act either as a co-transmitter or a direct neuromodulator, have been shown to be involved in mood control, appetite/satiety circuits, and attention deficit/hyperactive disorders (Shimazu and Miklya 2004, Burchett and Hicks 2006, Mazzoli and Pessione 2016). *Campylobacter jejuni*, *Clostridium saccharolyticum*, *Faecalibacterium prausnitzii*, *Bifidobacterium* are bacterial taxa associated with polyamines (Cardona, Andres-Lacueva et al. 2013, Duenas, Munoz-Gonzalez et al. 2015, Feng, Ao et al. 2018).

2.2.3.9. Lipids

Lipids, which include LPS, glycerol, acylglycerols, sphingomyelin, cholesterol, triglycerides, result from microbial metabolism. These lipids are part of a variety of signalling molecules produced by the gut microbiota that have potent effects on hepatic lipid and bile metabolism and on cholesterol transport, energy expenditure, and insulin sensitivity in tissues (Ghazalpour, Cespedes et al. 2016). It is also possible that the gut bacteria generate intermediate precursors that are further metabolized to exert direct effects on lipid levels. Rose*buria, Lactobacillus,*

Bifidobacterium, Klebsiella, Enterobacter, Citrobacter, and *Clostridium* taxonomic species have shown to be involved in lipid metabolites in the gastrointestinal tract (Feng, Ao et al. 2018). One way they alter lipid metabolism is through their ability to hydrolyse bile salts through the production of bile salt hydrolases (Ramakrishna 2013). This interferes with the bile salt reabsorption cycle, resulting in faecal bile salt loss and secondary reduction of serum cholesterol due to diversion of cholesterol to bile acid synthesis (Ramakrishna 2013). The other way lipid metabolism is altered is through the inhibitory effect of propionic acid on enzymatic activity in the liver leading to reduction in cholesterol synthesis (Ramakrishna 2013).

2.2.3.10. Indole Derivatives

Indole is produced by gut microbiota from tryptophan, through the tryptophanase enzyme (Jaglin, Rhimi et al. 2018). Indole following absorption, is further metabolized by xenobiotic metabolizing enzymes into oxidized and conjugated derivatives (Lee, Wood et al. 2015). Indole derivatives include indole, indoleacetylglycine, indoxyl sulfate, indole-3-propionate, 6-sulfate, oxindole and isatin. *Clostridium sporogenes* and *Escherichia coli* have been associated with indole and its derivatives (Lee, Wood et al. 2015, Feng, Ao et al. 2018, Jaglin, Rhimi et al. 2018). Elevated amount of indole derivatives can have significant impacts on motor activity and act as neuro-depressants (Jaglin, Rhimi et al. 2018). Lee et al has demonstrated that indole has a major role in signalling within the gut microbiota, interacting heavily with the gastrointestinal epithelium in addition to amongst themselves (Lee, Wood et al. 2015). In the gastrointestinal tract, indole derivatives can enhance colon integrity, modulate expression of proinflammatory and anti-inflammatory genes, and be implicated in brain-gut axis (Feng, Ao et al. 2018, Jaglin, Rhimi et al. 2018, Whittemore, Stokes et al. 2018).

2.2.3.11. Phenolic, Benzoyl, & Phenyl Derivatives

Phenolic, benzoyl, and phenyl derivatives are ubiquitous compounds, which have poor bioavailability in the small intestine, but are transformed by the gut microbiota into a variety of bioavailable and active compounds (Manach, Williamson et al. 2005, Espin, Gonzalez-Sarrias et al. 2017). Some of the compounds produced can be either beneficial or detrimental depending on the microbes present (Ramakrishna 2013). *Escherichia, Salmonella, Pseudomonas, Clostridium*, and *Bacteroides* are the most common bacterial species associated with the production of phenolic, benzoyl, and phenyl derivatives (Feng, Ao et al. 2018). Beneficial polyphenol metabolites may have prebiotic, anti-inflammatory, anti-oxidative, anti-carcinogenic, and anti-microbial properties, although other metabolites may be toxic to the host (Tuohy, Conterno et al. 2012, Ramakrishna 2013, Espin, Gonzalez-Sarrias et al. 2017, Guldris, Parra et al. 2017). Another negative impact of toxic phenols is the effect it has on the microbial conversion of polyphenols that affects other colonic pathways and processes like SCFA production and hydrogen disposal (Ramakrishna 2013).

2.2.3.12. Anti-Microbial Peptides

Anti-microbial peptides (AMPs), also known as host defence peptides, are short in size and are generally positively charged peptides produced in various organisms. Most AMPs have the ability to kill microbial pathogens directly, whereas others act indirectly by modulating the host defence systems. Many AMPs display either direct and rapid anti-microbial activity by causing disruption of the physical integrity of the microbial membrane and/or act by translocating across the membrane into the cytoplasm of bacteria to act on intracellular targets (Hancock and Sahl 2006).

Several databases exist for natural AMPs, covering more than 2000 peptides discovered (Wang 2015). Bacteriocins are a subset of AMPs and are produced by bacteria. Genetic analyses have shown some genes encode for anti-microbial peptides: anti-bacterial (3273), Gram-positive (2684), Gram-negative (2482), Anti-fungal (1563), anti-viral (286), anti-parasitic (111) (Huang, Chang et al. 2017). As seen from various studies, many of AMPs exhibit an extraordinarily broad range of anti-microbial activity covering both Gram-positive and Gram-negative bacteria as well as fungi, viruses, and unicellular protozoa (Hancock and Diamond, 2000; Reddy et al., 2004; Marr et al., 2006). Over 3271 anti-microbial peptides have been discovered, but some examples of bacterial produced bacteriocin include acidocin, actagardine, bisin, carnocin, carnocyclin, divercin, duramycin, erwiniocin, glycinecin, halocin, haloduracin, klebicin, lacticin, leucoccin, mutacin, nisin, pediocin, pentocin, plantaricin, sakacin, and subtilin. Most of the studied bacterial species, commensal and pathogenic, have been associated with the production of anti-microbial peptides (Mahlapuu, Hakansson et al. 2016, Murray, Pearson et al. 2016, Huang, Chang et al. 2017).

2.2.4. Microbiota-Gut-Brain Axis and Stress

As previously stated, stressors can be varied in nature and the biological stress response is coordinated primarily by the HPA axis and SNS. Stressor-induced activation of the HPA axis and SNS stimulates the release of glucocorticoids, catecholamines, and other hormones (Ulrich-Lai and Herman 2009). This, in turn, influences the gut microbiota, which affects the host stress response, implicating the gut microbiota as an important component of host health (Berg, Muller et al. 1999, Karl, Hatch et al. 2018). The gut microbiota and the human body are in constant communication, creating the microbiota-gut-brain axis.

The microbiota-gut-brain axis connects the central nervous system and the gut microbiota by means of the nervous system, the endocrine system, and the immune system, which has constructed a bidirectional communication system (Collins, Kassam et al. 2013, Lyte 2014, O'Mahony, Clarke et al. 2015, Foster, Rinaman et al. 2017, Sundman, Chen et al. 2017, Wiley, Dinan et al. 2017, Arneth 2018, Liang, Wu et al. 2018, Liu, Liong et al. 2018, Luo, Zeng et al. 2018). Regarding the nervous component of the microbiota-gut-brain axis, the enteric nervous system (ENS) runs the entire length of the gastrointestinal tract and contains nearly 500 million neurons, as many as are in the spinal cord (Freestone and Lyte 2010). Parts of the ENS innervates the intestinal villi, which interacts with the gut lumen and the gut microbiota. Changes in the nervous system activity influence the gut bacteria and lead to major shifts in microbe composition. Afferent spinal and vagal sensory neurons carry information back from the intestinal end to the brain stem, which in turn engages the hypothalamus and limbic system. Similarly, descending projections from the limbic system, activated by stressors, influences the neural activity of the intestines. The immune component of the microbiota-gut-brain axis consists of the gut associated lymphoid tissue, which comprises 70% of the body's immune system and can be conceptualised as the largest immune organ in the body. It is well established that stress alters the immune capability (Glaser and Kiecolt-Glaser 2005). The immune structure is highly dependent on the gut microbiota and on the hosts interactions. The final component are the bacterial products that act as neuropeptides such as peptide YY, neuropeptide Y, cholecystokinin, serotonin, gamma-aminobutyric acid, and glucagon-like peptide-1 and -2, influencing the endocrine system. These neuropeptides enter the bloodstream and/or directly influence the enteric nervous system. This emphasises the importance of the gut microbiota in their

regulatory role via the immune system, HPA axis, tryptophan metabolism, metabolites produced of bacteria, and vagus nerve pathway (Sudo, Chida et al. 2004, Bravo, Forsythe et al. 2011, Tan, McKenzie et al. 2014, Erny, de Angelis et al. 2015, O'Mahony, Clarke et al. 2015).

Under stress, the specific stress hormones, catecholamines and other neuroendocrine hormones directly modulate microbial growth, and are secreted by intestinal cells in the gastrointestinal tract in response to stress (Lyte 2014). In addition, stress-induced changes in signalling via the vagus nerve and ENS alter gastrointestinal motility and reduce digestive activity, likely impacting the gut microbiota (Galley and Bailey 2014). A growing body of evidence suggests that host responses to stress may be mediated in part by affecting the gut microbiota. Such examples of microbial changes include the decrease of beneficial bacteria, such as *Lactobacillus, Bifidobacterium, Firmicutes*, increases of pathobionts, including *Bacteroides, Clostridium, Escherichia*, and *Enterobacter*, increase in total aerobic bacteria, and changes to important ratios such as anaerobic to aerobic bacteria and *Firmicutes* to *Bacteroidetes* (Bailey and Coe 1999, Bailey, Dowd et al. 2010, Bailey, Dowd et al. 2011, Galley and Bailey 2014, Karl, Hatch et al. 2018). Changes in the gut microbiota is critical as it influences the HPA axis programming in early life and stress reactivity over the life span (Moloney, Desbonnet et al. 2014). In addition, Foster (2014) et al demonstrated that the development of the stress response system coincides with colonization of the gut microbiota in the gastrointestinal tract (Foster and Neufeld 2014).

In fact, alterations in the gut microbiota are known to immediately affect the intestinal barrier function, inducing increased translocation of bacterial antigens and dramatically alter the host immune reaction, leading to a chronic inflammatory state

and impaired metabolic function (Burcelin, Garidou et al. 2012). These effects ultimately degrade the intestinal barrier thereby increasing paracellular permeability within the intestinal epithelium, creating a vicious cycle (van Wijck, Lenaerts et al. 2012). Figure 2.2.4.1. is a schematic demonstrating the cyclic relationship of how a dysfunction in the intestinal epithelium barrier causes the diffusion of pro-inflammatory materials, which would then innervate the HPA axis and cause it to release factors that will amplify the stress response that creates a cyclic reaction. Furthermore, changes in oxygen levels and metabolic activity within gastrointestinal microenvironments can also impact the gut microbiota (Albenberg, Esipova et al. 2014).

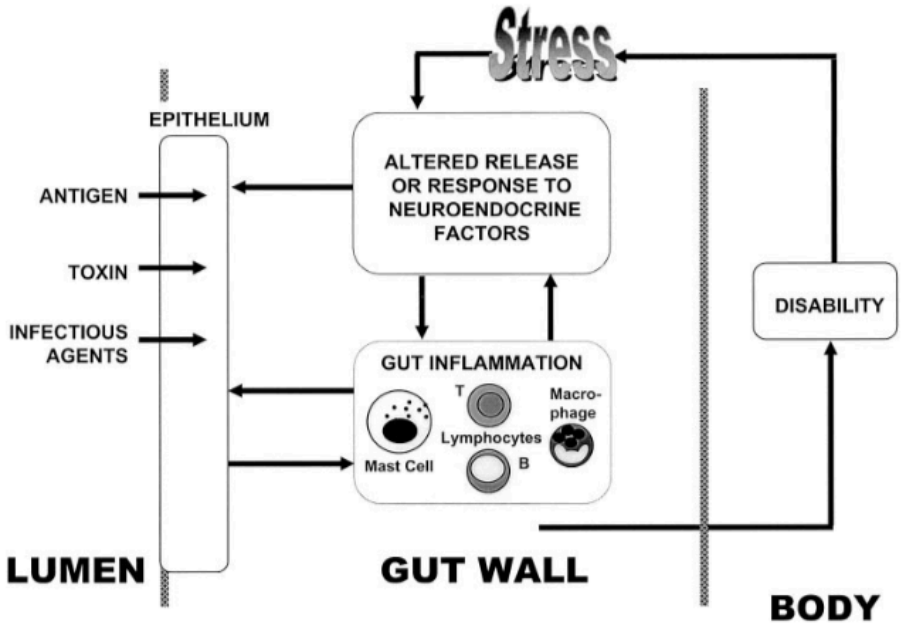


Figure 2.2.4.1. Impact of Stress-Induced Intestinal Barrier Dysfunction.

Stress leads to altered release or response to neuroendocrine factors in the intestinal barrier. Such factors have influence on the epithelium, inducing barrier dysfunction and uptake of proinflammatory material from the gastrointestinal lumen. The resultant inflammation causes worsens condition and increases stress, which further amplifies the dysfunction. (Soderholm and Perdue 2001)

One effect of stress is the alteration of intestinal permeability, which is associated with low-grade inflammation and can be functionally linked to changes to the gut microbiota. In many of these cases, it is the increased presence of circulating bacterial endotoxins, LPS, which are the fundamental factor in the inflammatory cascade. Elevated translocation, especially of LPS, a toxic component of gram-negative bacteria, can cause tissue damage, systemic inflammation, and even lead to organ failure (Leon and Helwig 2010).

Alternatively, other studies have suggested that the gut microbiota can produce neuroactive substances as a result of stress, which would alter processes within the host (Carabotti, Scirocco et al. 2016). This alternative hypothesis could signify a critical and relevant role of gut microbiota in the pathophysiology of many disorders (Kelly, Kennedy et al. 2015). However, most pathogenicity of any registered gut-related disorder involves the changes to the intestinal barrier function, which is why the next segment will concentrate on it.

2.2.4.1. Intestinal Barrier Function

The intestinal barrier function selectively controls absorption and secretion of solutes, nutrients and water, and also selectively excludes bacteria, bacterial agents, and infectious agents. The intestinal barrier is so vital that it requires approximately forty percent of the body's total energy expenditure to perform its tasks (Bischoff, Barbara et al. 2014). The intestinal barrier meets the exterior world across a surface suggested be approximately 8000 m^2, which is equivalent to the size of a soccer field. There are several components to the intestinal barrier, first and probably the most important, is the gut microbiota. Germ-free animal studies have been an essential instrument in demonstrating and assessing the importance of the gut microbiota to host health. For example, the absence of gut microbiota has been shown to lead to

malformation and defects in the lymphoid structures such as the spleen and Peyer's patches, abnormal numbers of immune cell types and expression of cytokine profiles, and decreased expression of tight junction proteins, which are all important factors in the intestinal barrier (Pollard and Sharon 1970, Bartizal, Wostmann et al. 1984, Williams, Probert et al. 2006, Yamamoto, Yamaguchi et al. 2012, Chelakkot, Ghim et al. 2018, Chen, Song et al. 2018, Kermanshahi, Shakouri et al. 2018, Wu, Zheng et al. 2018).

The gut microbiota is a major element in the development and the maintenance of the intestinal barrier and act as the first component and modulator of the intestinal barrier. The second component of the intestinal barrier is a chemical barrier with the presence of mucus layers, which are produced by goblet cells. The next layer consists of the epithelial cells (enteroendocrine cells, enterocytes, Paneth cells, and goblet cells) that form a physical barrier and are assisted by immune cells through their presence in the lamina propria, creating an immunological barrier (Duerkop, Vaishnava et al. 2009, Zhang, Hornef et al. 2015). Figure 2.2.4.1.1. depicts the various components of the intestinal barrier.

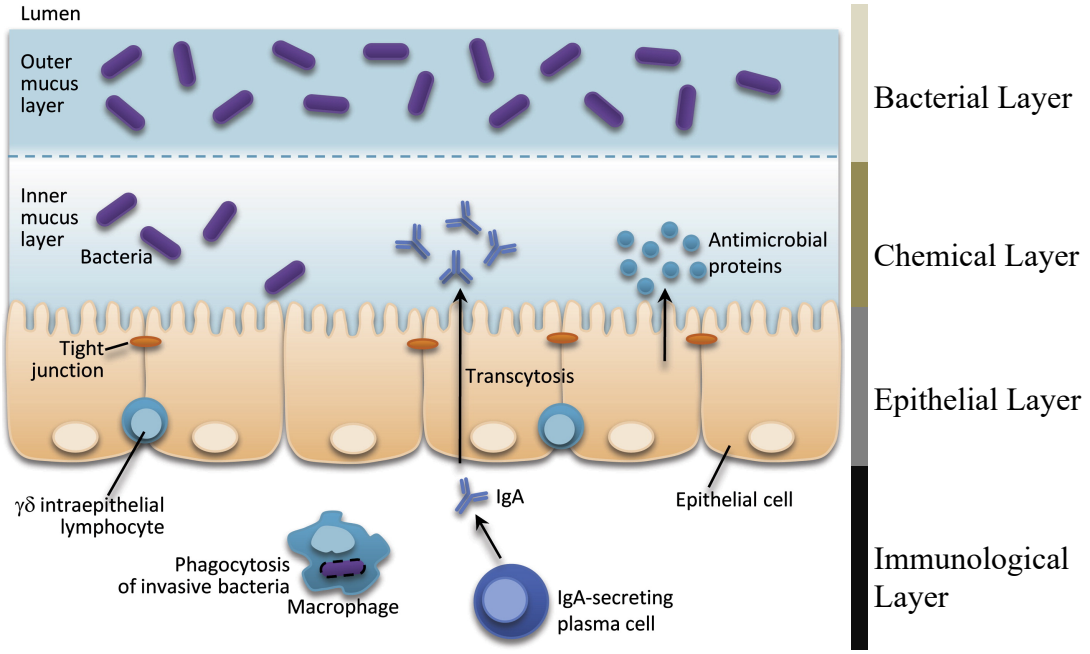


Figure 2.2.4.1.1. Components of the Intestinal Barrier.

The intestinal barrier acts as the line of defence in the gastrointestinal tract. There are several components to the barrier. One being the gut microbiota in the lumen and in the inner and outer mucus layer within the gastrointestinal tract. The second component of the intestinal barrier is a chemical layer, comprised of secreted metabolites from epithelia. The next layer consists of the epithelial cells (enteroendocrine cells, enterocytes, Paneth cells, and goblet cells) that form a physical barrier and are assisted by immune cells through their presence in the lamina propria, creating an immunological layer. Image adapted from Duerkop et al, 2009. (Duerkop, Vaishnava et al. 2009)

2.2.4.1.1. Bacterial Layer

Many microbial communities live in highly competitive surroundings, in which the fight for resources determines their survival and genetic persistence. The gut microbiota is composed of beneficial and pathogenic bacteria; both competing for space and nutrients. This constant fight and outcompeting of some are referred to as competitive exclusion. This prevents the colonization of opportunistic pathogens by producing anti-microbial compounds and by competing for nutrients and receptors (Grenham, Clarke et al. 2011). Under specific conditions, pathogenic bacteria can

attach to epithelial cells and cause pathogenicity; beneficial bacteria compete with them by preventing their attachment to epithelia. Such strains like *Escherichia coli* and *Salmonella* spp. need to attach to epithelium to cause pathogenicity (Mack, Michail et al. 1999, Villalobos, Leiva et al. 2018). Beneficial bacteria compete with the pathobionts by producing anti-microbial compounds, thus protecting their establishment and maintaining their niche in the gut (Guglielmetti, Taverniti et al. 2010, Konig, Wells et al. 2016, Novince, Whittow et al. 2017, Meng, Bai et al. 2018).

2.2.4.1.2. Chemical Layer

The chemical layer consists of secreted molecules that create a meshwork of molecules that have anti-microbial activity. Goblet cells will secret mucin, trefoil peptides, and phospholipids that act as a physical barrier between the gut microbiota and epithelium. Additionally, it stands in between enzymes, toxins, dehydration and abrasion (Van Tassell and Miller 2011). The mucus layer is integral to the intestinal barrier composed of mucin, a family of heavy molecular weight proteins that display extensive glycosylation and are constitutively secreted in the gastrointestinal tract, small and large intestinal epithelium (Corfield 2015). The majority of the bacteria will be kept at bay in the outer mucus layer, with few microbes in the inner mucus layer.

Immunoglobulins (IgAs) will be secreted by B-lymphocytes and epithelial cells that phagocytize microbes or block the penetration of microbes. They play an important role in trapping pathogens/pathogenic material (neutralisation) in the mucus layer through its ability to bind mucins (Biesbrock, Reddy et al. 1991, Micetic-Turk and Sikic-Pogacar 2011). Some membrane bound receptors, like FcRN, can assist in the transport of IgAs across the membrane. Paneth cells and enterocytes will secrete anti-microbial peptides like defensins and lysozymes, and lectins, and these have

direct anti-microbial activity. These proteins are stored in granules and released both constitutively and in response to bacterial threats to prevent microbial invasion (Salzman, Hung et al. 2010). In addition, the anti-microbial proteins disseminate into the mucus layer in order to reinforce the intestinal barrier function (Vaishnava, Behrendt et al. 2008, Grootjans, Hodin et al. 2011, Zhang, Hornef et al. 2015). Lastly, Paneth cells provide critical stem cell niche factors, including EGF, Wnt3 and Notch (Sato, van Es et al. 2011, Sailaja, He et al. 2016). Anti-microbial protease inhibitors secreted from epithelial cells and leukocytes also have direct anti-microbial activity (Hancock and Sahl 2006, Schneider and Balskus 2018). Table 2.2.4.1.2.1. has a summary of the different compounds secreted in the chemical layer.

Table 2.2.4.1.2.1. Compounds Secreted in Chemical Layer.

Chemical	Source	Function
Mucin	Goblet cells	Major macromolecular component of hydrophilic hydrated mucus: physical barrier, hydration, lubrication, retention of anti-microbial molecules
Trefoil peptides	Goblet cells	Co-secreted with MUC2, possible modulator of mucin polymerization, stimulator of wound repair.
Phospholipids (phosphatidylcholine)	Enterocytes	Hydrophobic element of mucus probably interspersed in striated layers with hydrated mucus: lubrication and barrier function
Immunoglobulins (IgAs), Secretory component, & FcRN (Receptors)	B lymphocytes Epithelial cells	Anti-microbial: opsonization of microbes, blocking microbial penetration of mucus SC and FcRN facilitate transport across epithelium. Glycosylated SC protects Fc region of IgA dimer in external environment.
Anti-microbial Peptides (defensins, PLAP2, cathelicidins, lysozyme)	Paneth cells Enterocytes	Peptides with direct anti-microbial activity
Lectins (RegIII-α & -γ, collectins)	Paneth cells Enterocytes	Direct anti-microbial activity
Anti-microbial protease inhibitors (SLPI, elafin)	Epithelial cells Leukocytes	Direct anti-microbial activity

2.2.4.1.3. Epithelial Layer

The epithelial layer contains all of the cells involved in producing the chemical layer, which includes M cells, Paneth cells, goblet cells, enterocytes, regenerative cells, and enteroendocrine cells. Many studies performed in rodent models have demonstrated that the Paneth cell distribution in the gastrointestinal tract is proportional to the establishment of an appropriate commensal gut microbiota and host protection from pathobionts (Brandl, Plitas et al. 2007, Salzman, Hung et al. 2010, Riba, Olier et al. 2017). Moreover, the activity and the anti-microbial peptide

repertoire of the Paneth cells are both dependent on the gut microbiota profile (Rhee, Sethupathi et al. 2004, Shanahan, Tanabe et al. 2011, Gulati, Shanahan et al. 2012). Even though some anti-microbial peptides are dependent on microbiota colonization, others, such as cryptdin, lysozyme and PLA2, are expressed independently of microbiota colonization (O'Neil, Porter et al. 1999, Putsep, Axelsson et al. 2000, Hooper and Gordon 2001, Cash and Hooper 2005). In mice, Paneth cells appear 2 weeks after birth and they are sensitive to environmental factors, this coincides with increased bacterial colonization of the gastrointestinal tract (Bry, Falk et al. 1994, van Es, Jay et al. 2005, Menard, Forster et al. 2008, Rodriguez, Eloi et al. 2012, Raetz, Hwang et al. 2013). Since Paneth cell activity, the distribution, and the profile of proteins produced are dependent on the colonization and profile of the gut microbiota it is a reasonable assumption that other epithelia cells such as includes M cells, goblet cells, enterocytes, regenerative cells, and enteroendocrine cells, are also dependent on the colonization and profile of the gut microbiota in the gastrointestinal tract.

In addition to the epithelia cells, the integrity of the intestinal barrier at the level of the epithelium is held in place by various types of proteins, including tight junction proteins, hemidesmosones, and junctional complexes. Zonula occludence (ZO-1), occludin, claudin, junctional adhesion molecule A (JAM-A) are some of the junctional proteins involved in maintaining the intestinal integrity (Ulluwishewa, Anderson et al. 2011, Krug, Schulzke et al. 2014, Chelakkot, Ghim et al. 2018). These proteins connect the epithelial cells to each other, creating a degree of tightness that allows for the selective permeability across the intestinal barrier (Ulluwishewa, Anderson et al. 2011). The loss of these proteins or the redistribution of them across the cells would increase the permeability of the intestinal barrier, allowing bacteria, their products, nutrients, toxins, or any other compounds to translocate into circulation

(Gonzalez-Mariscal, Betanzos et al. 2003, Lutgendorff, Akkermans et al. 2008, Groschwitz and Hogan 2009, Ulluwishewa, Anderson et al. 2011, Lin, Tan et al. 2017, Chelakkot, Ghim et al. 2018). The tight junctions, believed to be static structures, have come to be known as dynamic protein complexes and ready to adapt to a variety of developmental, physiological, and pathological circumstances, and regulated by several molecules including the interesting endogenous modulators named zonulins (Wang, Uzzau et al. 2000, Groschwitz and Hogan 2009, Fasano 2011, Raleigh, Boe et al. 2011, Shigetomi and Ikenouchi 2018).

Dysfunction of the tight junction proteins occurs in three general manners: 1) reduced paracellular transport of solutes; 2) increased paracellular transport of water and some solutes; 3) increased permeability to macromolecules (Krug, Schulzke et al. 2014). The gut microbiota and their metabolites have been shown to modulate the expression of tight junction proteins and to regulate the intestinal barrier function (Serino, Luche et al. 2009, Sekirov, Russell et al. 2010, Ulluwishewa, Anderson et al. 2011, Krug, Schulzke et al. 2014, Al-Asmakh and Hedin 2015, Zhang, Hornef et al. 2015, Thevaranjan, Puchta et al. 2017).

2.2.4.1.4. Immunological Layer

The Immunological layer is the last component of the intestinal barrier. This layer is composed of cells involved in the innate and adaptive immune system, containing cells are capable of producing anti-microbial compounds, act in signalling the recruitment of other cells against antigens, and phagocytize/destroy pathogens. The largest collection of lymphoid tissue in the body, the gut-associated lymphoid tissue (GALT) provides a dynamic barrier throughout the gastrointestinal tract. Changes in the activity of immune cells, epithelial cells, and in the secretion of anti-

microbial peptides and other secretory factors within this immunological barrier can directly alter gut microbiota composition and function (Hooper et al., 2012). The influence of the gut microbiota on the immune system extends beyond the intestinal lamina propria as it has also been shown to influence T cell proliferation (Wiley, Dinan et al. 2017). Most cells in the physical layer have a role in the immunological barrier. Enterocytes actively participate in innate immunity, by acting as immune sensors of microbial pathogens and commensal organisms (Nagler-Anderson 2001, Lambert 2009). The signalling loop that mediates the epithelial response to antigens is based on sensing of structural motifs, known as pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) on epithelial cells (Medzhitov and Janeway 2002).

PAMPs are expressed by both commensal and pathogenic gut microbes, and include LPS, lipoprotein and peptidoglycans (Koch and Nusrat 2012). Once PAMPs have interacted with PRRs, the release of cytokines will occur in the gastrointestinal tract. Pro-inflammatory cytokines include interleukin-1 (IL-1), IL-12, and IL-18, tumour necrosis factor (TNF), interferon gamma (IFN-gamma), and granulocyte-macrophage colony stimulating factor. Anti-inflammatory cytokines include IL-1ra, IL-4, IL-6, IL-10, IL-11, IL-13, and transforming growth factor beta (TGF-β). Paneth cells are involved in the production of anti-microbial peptides, goblet cells in the production of mucin, and M cells act as an antigen sampling system (Burger-van Paassen, Vincent et al. 2009, Bevins and Salzman 2011, Liu, Li et al. 2012, Sailaja, He et al. 2016, Trevizan, Vicentino-Vieira et al. 2016, Novince, Whittow et al. 2017, Lyte, Villageliu et al. 2018).

Each component is integral in maintaining the intestinal barrier function, but the gut microbiota is the most important member influencing the chemical, physical,

and immunological layers. Therefore, the stability of the gut microbiota is key in maintaining homeostasis and, as a result, the ability of the host to tolerate stress.

2.3. Probiotics in Stabilization of the Gut Microbiota

2.3.1. Definition of Probiotics

The term *probiotic* is derived from Latin (pro) and Greek (bios) meaning "for life" (Hamilton-Miller, Gibson et al. 2003, Ozen and Dinleyici 2015). The definition of probiotics has changed throughout the years. Initially the definition was any active substances that are essential for a healthy development of life (Kollath 1953). This was modified to live microbial food supplements which beneficially affects the host animal by improving its intestinal microbial balance (Fuller 1989). As of 2001, the current definition of probiotics is that they are selected live microorganisms, which when administered in sufficient amounts present a health benefit to the host (Salminen, Nybom et al. 2010, Bermudez-Humaran, Aubry et al. 2013).

The concept of probiotics was first proposed by Elie Metchnikoff in the early 20th century, who made reference to the properties of fermented milk (containing lactic acid bacteria) that increased the longevity of Bulgarian populations and he linked the microorganism to increased well-being (Ceapa, Wopereis et al. 2013). The use of microbials unintentionally originated centuries ago when people first noted the beneficial health effects of eating fermented foods. Modern probiotic-containing foods and products are the direct derivatives of these early fermented foods, and pharmacological supplements and medication are now available on the market. In the United States, probiotics can be labelled in several different ways depending on their intended usage. They can be marketed as foods, medical foods, dietary supplements or drugs. Each of these categories has unique requirements in terms of formulation,

scientific documentation, and/or Food and Drug Administration (FDA) approval. In most cases, probiotics are classified as either a dietary supplement (e.g., products in pill form) or as a food substance (e.g., yogurt).

As already stated, probiotics are selected live microorganisms, which when administered in sufficient amounts present a health benefit to the host (Isolauri, Sutas et al. 2001, de Vrese and Schrezenmeir 2008, Diop, Guillou et al. 2008, Bermudez-Humaran, Aubry et al. 2013). Probiotics differ from prebiotics, as prebiotics are selectively fermented ingredients that affect the composition or the activity of the microbiota (Marchesi, Adams et al. 2016, Yoo and Kim 2016, Bron, Kleerebezem et al. 2017, Markowiak and Slizewska 2017, Liu, Liong et al. 2018, Park, Brietzke et al. 2018). Probiotics themselves alter the composition and change the production of metabolites, interactions with the host, and interactions between bacteria (Isolauri, Sutas et al. 2001, Salminen, Nybom et al. 2010, Gibson, Hutkins et al. 2017).

There are certain criteria required to be an effective probiotic. First, the specificity of the probiotic strain is crucial (Shanahan, Dinan et al. 2012, Gosalbez and Ramon 2015). The species or genera are of lesser importance in comparison to the strain. Some strains within the same species might not express the beneficial outcome observed and desired in another strain within the same species (Shanahan, Dinan et al. 2012, Gosalbez and Ramon 2015).

Second, the probiotic must be resistant to gastric juices (hydrochloric acid, potassium chloride and sodium chloride), intestinal enzymes (amylase, pepsin, trypsin, pancreatic lipase), and bile salts. The acidity of the stomach is around a pH of 1-2, the intestines (duodenum, jejunum, ileum) 6-7, and the colon 5-7 (Bezkorovainy 2001, Govender, Choonara et al. 2014). The majority of microbes reside within the colon, therefore effective transportation and survival to the colon is essential (Bik

2009, Lamiki, Tsuchiya et al. 2010, Sekirov, Russell et al. 2010, Saad, Delattre et al. 2013). The two main species of probiotics that are utilized are *Lactobacillus* and *Bifidobacterium,* although other genera are used (Bezkorovainy 2001, Govender, Choonara et al. 2014). These species are able to survive the acidity and intestinal enzymes and are better in comparison to other species (Govender, Choonara et al. 2014). Naturally occuring probitoics do not demonstrate issues with bile salts, as they might develop tolerance over time due to constant exposure (Govender, Choonara et al. 2014).

The viability of probiotics that reach the inetstine and cause health benefits have been shown to have at least a colony forming unit (CFU) of 10^8 to 10^9 (Aureli, Capurso et al. 2011, Govender, Choonara et al. 2014). However, some studies have shown that nonviable bacteria might produce effects similar to the effects seen in viable bacteria (Galdeano and Perdigon 2004). The mechanims of actions of nonviable batceria remain unknown, but it could be hypothesied that antigens from nonviable bacteria interact with receptors within the intestines and mediate a response (Galdeano and Perdigon 2004).

The survival rates of some probiotic strains after transiting through the bile salts and acidity of the stomach are estimated to be at 20 to 40% (Bezkorovainy 2001, Giraffa 2012, Wright, Burns et al. 2013). The impact of ingested probiotics on the colonic environment is essentially attributed to the faecal persistence of the ingested strains. They colonize the gut temporarily and disappear once the consumption stops. *Lactobacillus* or *Bifidobacterium* probiotic strains can be recovered at a level ranging from 10^7 to 10^9 cells per gram of faecal matter corresponding to less than 0.1% of the faecal microbiota (Aureli, Capurso et al. 2011, Gerritsen, Smidt et al. 2011). Studies have also demonstrated that the survival of probiotic bacteria can be increased by the

assistance of stress-inducible proteins, thus microbes expressing high levels of stress-inducible proteins are considered effective probiotics (Papadimitriou, Zoumpopoulou et al. 2015). Pharmaceutics have been able to improve the viability of acidic intolorant strains by designing capsules, beads, tablets, among others to carry the probiotic through the stomach.

Another criteria for an effective probiotic is that the bacterium should be able to adhere and colonize against the intestinal wall. Some species such as *Lactobacillus, Bifidobacterium, Escherichia*, require attachment to the intestinal wall to execute their effects (Govender, Choonara et al. 2014). Even though some microbes are transient and others permanent in the gastrointetsinal tract, adhering to and colonizing the intestinal epithelium has various impacts, such as competitive exclusion, modulation of immunity, and modulation of the intestinal barrier function.

Studies show that adhesion to the intestinal epithelium is the first step in modulation of the immune system (Galdeano and Perdigon 2004). Microbes interact with dendritic cells and epithelial cells, recruiting monocytes, macrophages, and lymphocytes, and influence the production of anti-microbial peptides (Bermudez-Brito, Plaza-Diaz et al. 2012). Good adhesion also enhances the survival of the bacteria within the gastrointestinal tract (Galdeano and Perdigon 2004). Adhesion to the gastrointestinal tract allows for competitive exclusion of pathogenic strains from interacting with the intestinal mucosal cells. Studies have shown that some pathogenic species such as *Salmonella* spp. or *Escherichia* spp. need to have direct contact with epithelium in order to cause pathogenesis (Davis 1976).

Modulation of the intestinal barrier is a trait the probiotic should have as well. As mentioned in 2.2.4.1. Intestinal Barrier Function, the gut microbiota is the most important member influencing the chemical, physical, and immunological layer of the

intestinal barrier. Therefore, the stability of the gut microbiota is key in maintaining homeostasis and being able to adhere and colonize will solidify its effects in the gastrointestinal tract. Other desirable traits of a probiotic could include anti-cancer activity and the capacity for the removal of heavy metals (Kumar, Kumar et al. 2010, Salminen, Nybom et al. 2010, Azcarate-Peril, Sikes et al. 2011, Monachese, Burton et al. 2012, Uccello, Malaguarnera et al. 2012, Pace, Pace et al. 2015, Pandey, Naik et al. 2015).

However, most importantly, the microbes used as probiotics should be non-pathogenic, not limited to being found as a natural component of the human gastrointestinal tract, and safe for consumption. Safety can be assessed through animal model studies, clinical trials, and observations of adverse reactions. The human gut microbiota has been analysed through plating faecal samples on selective and differential media, and more recently through 16S rRNA sequencing. Therefore, a suitable probiotic can be chosen from the identified options.

2.3.2. Probiotic Microorganisms

The microorganisms most commonly used as probiotics are *Bifidobacterium* and *Lactobacillus* species, but other microbes such as *Enterococcus, Lactococcus, Bacillus, Escherichia*, and *Saccharomyces* have also been utilized (Bermudez-Humaran, Aubry et al. 2013, Govender, Choonara et al. 2014, Marchesi, Adams et al. 2016). Bacteriophages could also be used as probiotics (Hume 2011, Goldenberg, Batra et al. 2018, Vitetta, Vitetta et al. 2018). The major probiotic strains, *Lactobacillus* and *Bifidobacterium*, will be briefly reviewed on in this section, in addition to *Bacillus*, the probiotic taxa utilized as a probiotic in our studies.

2.3.2.1. Lactobacillus and Bifidobacterium

Lactobacillus, a lactic producing bacterium, is one of the two most popular probiotic species that are usually non-pathogenic and readily available on the market. *Lactobacillus* are commonly found in foods (yogurt, cheese, sauerkraut, pickles, beer, cider, kimchi, cocoa, kefir, and other fermented foods, as well as animal feeds) and it is known for a diversity in its application to maintain human well-being. *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus fermentum, Lactobacillus reuteri,* and *Lactobacillus plantarum* are the most common *Lactobacillus* species utilized as probiotic (Hakansson and Molin 2011). The other frequently used bacterial taxa are *Bifidobacterium*. These microbes have been shown to aid digestion by fermenting complex carbohydrates and generating essential metabolites (Govender, Choonara et al. 2014). *Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium infantis,* and *Bifidobacterium breve* are specific taxa utilized in probiotics (Hakansson and Molin 2011).

Between them *Lactobacillus* and *Bifidobacterium* have a range of effects on human health through a range of effects including: detoxification of xenobiotics, biosynthesis of vitamins, metabolic effects of fermentation of indigestible dietary fibre, positive influences on transit of luminal contents by peristalsis, competition with pathogenic microbes for nutrients and binding sites on mucosal epithelial cells, to reduction in the levels of intestinal endotoxin, improvement of or full restoration of mucosal barrier function, and modulation of the host's immune response (Bentley and Meganathan 1982, Buchman, Killip et al. 1999, Griffiths, Duffy et al. 2004, Mazmanian, Liu et al. 2005, Wang, Xiao et al. 2006, Candela, Perna et al. 2008,

Nilsson, Ostman et al. 2008, Turroni, Vitali et al. 2010, Matsumoto and Kurihara 2011, Matsumoto, Ishige et al. 2012, Maurice, Haiser et al. 2013, Ramakrishna 2013).

2.3.2.2. Bacillus Subtilis

Bacillus subtilis, also known as the hay bacillus or grass bacillus, can be ubiquitously found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus *Bacillus*, *Bacillus subtilis* is rod-shaped, and has the ability to produce endospores, allowing it to tolerate extreme environmental conditions. In addition to being ubiquitously found in the environment (air, water, soil, food, etc.) and constantly being exposed to humans, *Bacillus subtilis* species are documented for producing a wide spectrum of anti-microbial compounds, immunomodulation, modulation of the intestinal barrier, competitive exclusion of intestinal pathogens and documented for supporting and promoting the stability of the gut microbiota (Cutting 2011, Amuguni and Tzipori 2012, Moore, Globa et al. 2013, Horosheva, Vodyanoy et al. 2014, Moore, Globa et al. 2014, Bi, Zhao et al. 2015, Jager, Shields et al. 2016, Sorokulova, Globa et al. 2016, Deng, Wu et al. 2017, Gadde, Oh et al. 2017, Gepner, Hoffman et al. 2017, Wang, Du et al. 2017, Guo, Dong et al. 2018, Ianiro, Rizzatti et al. 2018, Wang, Yan et al. 2018).

2.3.3. Mechanisms of Action

Probiotics have been used clinically to treat various conditions for example to suppress diarrhoea, alleviate lactose intolerance, postoperative complications, mental disorders, and allergies, participate in anti-microbial and anti-colorectal cancer activities, reduce irritable bowel symptoms, and prevent inflammatory bowel disease (Govender, Choonara et al. 2014, Abdelhamid and Luo 2018, Anand and Mande 2018, do Carmo, dos Santos et al. 2018, Lau, Savoj et al. 2018, Liang, Wu et al. 2018, Meng, Bai et al. 2018, Morris, Fernandes et al. 2018, Pascal, Perez-Gordo et al. 2018,

Rodino-Janeiro, Vicario et al. 2018, Saltzman, Palacios et al. 2018, Sanders, Merenstein et al. 2018, Tabouy, Getselter et al. 2018, Tunapong, Apaijai et al. 2018). Beneficial probiotic bacteria contribute to enhance the intestinal barrier function, reduce the inflammatory response, prevent the overgrowth of pathobionts, and ameliorate the gut permeability (Ohland and MacNaughton 2010, Ait-Belgnaoui, Colom et al. 2014, Marchesi, Adams et al. 2016).

Probiotics have certain mechanisms of action by which they affect the host (Bermudez-Brito et al, 2012). Figure 2.3.3.1. illustrates some of the various mechanism probiotics act by. These mechanisms include 1) interference with pathogenic bacteria, 2) improvement of the intestinal barrier function of the epithelial lining, 3) immunomodulation, and 4) influence on other organs of the body through the immune system and neurotransmitter production (McFarland 2009, Bermudez-Brito, Plaza-Diaz et al. 2012, Sanchez, Delgado et al. 2017). These mechanisms each have specific biological effects, and each will target a specific component within the host.

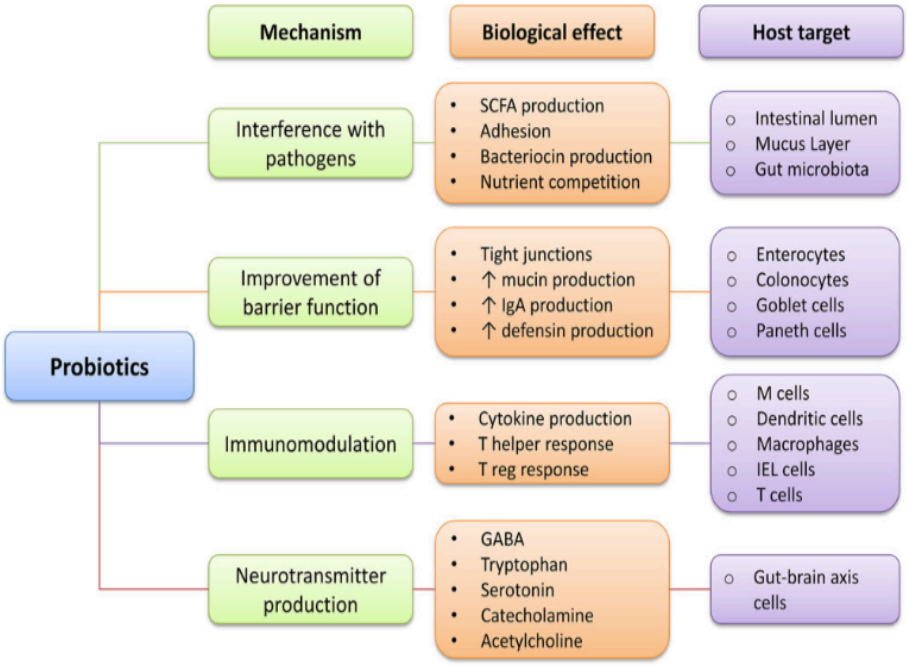


Figure 2.3.3.1. Mechanism of Action of Probiotics

The main mechanisms of action of probiotics. Mechanisms, biological effect, and host cells responsible for the interaction (Sanchez, Delgado et al. 2017).

2.3.3.1. Interference with Pathogens

Probiotics interfere with pathogens by their adhesion to intestinal mucosa, allowing for increased survival and possibility of the probiotic to interact with the host. In addition, through their establishment in the gastrointestinal mucosa, the probiotics perform competitive exclusion and inhibit the adhesion of pathogenic microorganisms, creating a hostile microecology for the pathobionts. Other ways probiotics obstruct is through their production metabolites, elimination of bacterial receptors, depletion of essential nutrients, and production of anti-microbial substances such as bacteriocins, antibiotics, and de-conjugated bile acids (McFarland 2009, Bermudez-Brito, Plaza-Diaz et al. 2012, Sanchez, Delgado et al. 2017).

2.3.3.2. Improvement of Intestinal Barrier Function

The enhancement of the intestinal barrier occurs through modulation of tight

junction proteins, production of mucus to increase the barrier, and increased IgA production by targeting the epithelia (McFarland 2009, Bermudez-Brito, Plaza-Diaz et al. 2012, Sanchez, Delgado et al. 2017). This intestinal barrier function is enforced by the ability of probiotics to influence mucin expression and mucus secretion from the goblet cells and by pathogen neutralisation by IgA in the mucus layer through the epithelial cells (Hardy, Harris et al. 2013). Probiotic bacteria can induce anti-microbial peptides presence by stimulating the secretion of anti-microbial peptides out from epithelial and Paneth cells (Hardy, Harris et al. 2013).

2.3.3.3. Immunomodulation

Probiotic bacteria can modulate the activity of many cells of the immune system, including innate system natural killer cells, dendritic cells, macrophages, epithelial cells and granulocytes, as well as recruiting monocytes, macrophages, and lymphocytes. Thus, probiotic bacteria have the potential to modulate any part of the immune system in the context of acute responses to intracellular or extracellular pathogens or chronic responses observed in dysregulated immunopathological conditions.

2.3.3.4. Neurotransmitter Production

Gut microbes are also capable of synthesizing neuroactive compounds that are identical to those produced by humans. Such compounds include serotonin, dopamine, histamine, and gamma-aminobutyric acid from amino acid precursors (Lyte 2014). Through the gut-brain axis, the microbes have an influence memory, mood, and cognition and are clinically and therapeutically relevant to a range of disorders. microbes have been shown to be able to produce neuro-transmitting metabolites that affect mood, emotion, cognition, CNS, motility, secretion, and

behaviour (Hyland and Cryan 2010, O'Mahony, Hyland et al. 2011, Cryan and Dinan 2012, Dinan and Cryan 2012, Dinan, Stanton et al. 2013, O'Mahony, Clarke et al. 2015).

2.3.4. Correction of Dysbiosis with Probiotics

Probiotics have such a wide range of mechanims to influence the homeostasis and the health status, which indicates their use as a potential theurapeutic means of preventing and remediating gastrointetsinal dysbiosis. Probiotics alter the compostion and the activity of the gut microbiota, which ultimately alters metabolism, immunity, and the trophic functions of the host. This highlights the fact that gut health and microbial health impact the health of the organism. In fact, clinical trials and animal studies have have begun using probiotics as means of correcting dysbiosis.

The environment, diet, stressors, and medications are all capable of altering the composition of the gut microbiota (Govender, Choonara et al. 2014, Karl, Hatch et al. 2018, Zhu, Grandhi et al. 2018). Alterations to the microbiota have significant impacts on the host-microbial interactions (Nicholson et al, 2012). As mentioned in section 2.1.2., dysbiosis has been associated with transient and chronic pathologies. These pathological conditions observed are not limited to the gastrointestinal tract, dysbiosis has systemic consequences throughout the host. Scientific evidence supports the important roles that probiotics can play in the digestive system, having significant effects in alleviating the symptoms of several diseases. This section demonstrates successful scientific evidence of beneficial gut microbiota modulation from probiotics for specific intestinal and extraintestinal diseases (Table 2.3.4.1.).

The results of probiotic treatment on dysbiosis related pathologies are dependent on the probiotic utilized. If one probiotic strain used was able to mitigate the adverse effects observed, there is no guarantee that another strain within the same

taxa would have the same effect. This explains the mixed scientific evidence on probiotic usage. As mentioned, the specificity of the probiotic strain is crucial (Shanahan, Dinan et al. 2012, Gosalbez and Ramon 2015). The species or genera are of lesser importance in comparison to the strain. Some strains within the same species might not express the beneficial outcome observed and desired in another strain within the same species (Shanahan, Dinan et al. 2012, Gosalbez and Ramon 2015).

Table 2.3.4.1. Correction of Dysbiosis with Probiotics

Pathological Condition	Reference	Pathological Condition	Reference
Antibiotic-Associated Diarrhoea	(Guarino, Guandalini et al. 2015, Blaabjerg, Artzi et al. 2017, Alrubaian, Alonazy et al. 2018, Ianiro, Rizzatti et al. 2018, MacPherson, Mathieu et al. 2018, Mantegazza, Molinari et al. 2018, Whittemore, Stokes et al. 2018, Zhang, Zhu et al. 2018)	**Lactose Intolerance**	(de Vrese and Schrezenmeir 2008, Chen and Walker 2011, Grover, Rashmi et al. 2012)
Colorectal Cancer	(Zhang, Du et al. 2012, Ambalam, Raman et al. 2016, Jacouton, Chain et al. 2017, Ding, Tang et al. 2018, Mota, Walter et al. 2018, Rossi, Mirbagheri et al. 2018, Wardill, Secombe et al. 2018, Zhang, Han et al. 2018)	**Postoperative Infections**	(Whelan and Myers 2010, Zhou, Yin et al. 2010, Jeppsson, Mangell et al. 2011, Lundell 2011, Zhang, Du et al. 2012)
Infectious Diarrhoea	(Girardin and Seidman 2011, Hom 2011, Preidis, Hill et al. 2011, Quigley 2012, Pace, Pace et al. 2015, Shoaib, Dachang et al. 2015, Dinleyici, Martinez-Martinez et al. 2018, do Carmo, dos Santos et al. 2018, The, de Sessions et al. 2018, Xu, Gu et al. 2018)	**Helicobacter Pylori Infection**	(Bercik, Verdu et al. 2009, Lionetti, Indrio et al. 2010, Song, Park et al. 2010, Wolvers, Antoine et al. 2010, Yasar, Abut et al. 2010, Chapman, Gibson et al. 2011, Cousin, Mater et al. 2011, Girardin and Seidman 2011, Taverniti and Guglielmetti 2011, Wilhelm, Johnson et al. 2011)
Inflammatory Bowel Disease	(Mercer, Brinich et al. 2012, von Schillde, Hormannsperger et al. 2012, Alvarez, Badia et al. 2016, Sanchez, Delgado et al. 2017, Celiberto, Pinto et al. 2018, Kho and Lal 2018, Rodriguez-Nogales, Algieri et al. 2018, Rodriguez-Nogales, Algieri et al. 2018, Wasilewska and Wroblewska 2018, Whittemore, Stokes et al. 2018)	**Hepatic Encephalopathy**	(McGee, Bakens et al. 2011, Pereg, Kotliroff et al. 2011, Fooladi, Hosseini et al. 2013, Patel and DuPont 2015, Fukui 2017, Mancini, Campagna et al. 2018)
Irritable Bowel Syndrome	(Cordina, Shaikh et al. 2011, Girardin and Seidman 2011, Ringel and Ringel-Kulka 2011, Mercer, Brinich et al. 2012, Quigley 2012, Rogers and Mousa 2012, Meijerink, Mercenier et al. 2013, Da Silva, Robbe-Masselot et al. 2014, Pace, Pace et al. 2015, Bron, Kleerebezem et al. 2017, Barbara, Cremon et al. 2018, Daliri, Tango et al. 2018)	**Necrotizing Enterocolitis**	(Deshpande, Rao et al. 2010, Guthmann, Kluthe et al. 2010, Deshpande, Rao et al. 2011, Frost and Caplan 2011, Girardin and Seidman 2011, Sari, Dizdar et al. 2011, Aceti, Gori et al. 2015, Fleming, Hall et al. 2015, Goncalves, Soares et al. 2015, Houghteling and Walker 2015, Johnson-Henry, Abrahamsson et al. 2016, Corpino 2017, Millar, Seale et al. 2017, Athalye-Jape, Rao et al. 2018, Biasucci 2018)
Learning and Memory	(Liang, Zhou et al. 2017, Beilharz, Kaakoush et al. 2018, Wallis, Ball et al. 2018)	**Sepsis**	(Lutgendorff, Akkermans et al. 2008, Liu, Shen et al. 2010, Garland, Tobin et al. 2011, Sari, Dizdar et al. 2011, Correia, Liboredo et al. 2012, Didari, Solki et al. 2014, Fijan 2014, Corpino 2017, Deshpande, Jape et al. 2017)

Metabolic Diseases	(Iacono, Raso et al. 2011, Mencarelli, Distrutti et al. 2011, Xu, Wan et al. 2012, Engen, Green et al. 2015, Xu, Hong et al. 2017, Biasucci 2018, Kim, Yun et al. 2018, Saltzman, Palacios et al. 2018, Vespasiani-Gentilucci, Gallo et al. 2018, Whittemore, Stokes et al. 2018)	**Autism**	(Critchfield, van Hemert et al. 2011, Williams, Hornig et al. 2011, Hsiao, McBride et al. 2013, Petrof, Claud et al. 2013, Buffington, Di Prisco et al. 2016, Santocchi, Guiducci et al. 2016, Kho and Lal 2018, Kim, Yun et al. 2018, Tabouy, Getselter et al. 2018, Yang, Tian et al. 2018)
Allergies	(Boyle, Ismail et al. 2011, Gourbeyre, Denery et al. 2011, Ly, Litonjua et al. 2011, Nogueira and Goncalves 2011, Gruber 2012, Brussow 2016, Mennini, Dahdah et al. 2017, West, Dzidic et al. 2017, Anand and Mande 2018, Canani, De Filippis et al. 2018)	**Stress**	(Moore, Pascoe et al. 2013, Ait-Belgnaoui, Colom et al. 2014, Moloney, Desbonnet et al. 2014, Song, Xiao et al. 2014, Davis, Doerr et al. 2016, Gill, Allerton et al. 2016, Gadde, Oh et al. 2017, McKean, Naug et al. 2017, Romijn, Rucklidge et al. 2017, Bonfili, Cecarini et al. 2018, Hill, Sugrue et al. 2018, Mohanty, Mishra et al. 2018, Robles-Vera, Toral et al. 2018, Toral, Romero et al. 2018, Wang, Yan et al. 2018)
Neurodegenerative Diseases	(Mallipattu, He et al. 2012, Dore, Multon et al. 2017, Skonieczna-Zydecka, Loniewski et al. 2017, Sundman, Chen et al. 2017, Westfall, Lomis et al. 2017, Fornai, van den Wijngaard et al. 2018, Zhang, Han et al. 2018)	**Rheumatoid Disease**	(Pineda, Thompson et al. 2011, de Oliveira, Leite et al. 2017, Schorpion and Kolasinski 2017)
Urinary Tract Infections	(Coudeyras and Forestier 2010, Alrubaian, Alonazy et al. 2018)	**Human Immuno-deficiency Virus**	(Ortiz, Klase et al. 2016, Ceccarelli, Brenchley et al. 2017, Scheri, Fard et al. 2017)
Ventilator-Associated Pneumonia	(Carlet 2010, Siempos, Ntaidou et al. 2010, Siempos, Ntaidou et al. 2010, van Silvestri, van Saene et al. 2010, Bailey and Yeung 2011, Clavel and Pichon 2011, Jacobi, Schulz et al. 2011, Oudhuis, Bergmans et al. 2011, Schultz and Haas 2011, Morrow, Gogineni et al. 2012, Morrow, Gogineni et al. 2012)	**Pancreatitis**	(Lutgendorff, Trulsson et al. 2008, Whelan and Myers 2010, Wolvers, Antoine et al. 2010, Sharma, Srivastava et al. 2011, Pagliari, Saviano et al. 2018)

2.4. Modulation of the Gut Microbiota with Prebiotics

2.4.1. Definition of Prebiotics

Prebiotics are a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host (Roberfroid, Gibson et al. 2010, Slavin 2013, Hutkins, Krumbeck et al. 2016, Gibson, Hutkins et al. 2017). These compounds are not hydrolysed and absorbed in the upper gastrointestinal tract and thus reach the large intestine unchanged, where the beneficial colonic bacteria may interact with them. Within the large intestine, unabsorbed and undigested carbohydrates are then fermented by the gut microbiota, resulting in the production of major metabolites, such as SCFAs and lactic acid, which have multiple effects within the host as described previously.

As per the definition, three criteria are necessary for a compound to be labelled as a prebiotic. Firstly, it must possess the ability to resist host digestion, for example gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption. Secondly, it can only be fermented by intestinal microorganisms. Lastly, the compound must selectively stimulate the growth and/or activity of intestinal bacteria associated with health and well-being.

Prebiotics, also known as non-digestible oligosaccharides, are obtained entirely from the diet in various foods, especially fruits, vegetables, and grains (Roberfroid, Gibson et al. 2010, Slavin 2013, Hutkins, Krumbeck et al. 2016). The daily consumption in typical US and European diets, containing non-digestible oligosaccharides, has been estimated to be several grams per day (Slavin 2013). Some known prebiotics (inulin) have low digestibility and are associated with impaired gastrointestinal tolerance, especially when consumed in large quantities, while other

prebiotic fibres (wheat dextrin, polydextrose) exhibit high gastrointestinal tolerability (Pasman, Wils et al. 2006, Grabitske and Slavin 2008, Grabitske and Slavin 2009, Lied, Lillestol et al. 2011). These compounds have the potential to stimulate growth and activity of selective and beneficial gut bacteria, particularly *Lactobacillus* and *Bifidobacterium* spp. (Bouhnik, Raskine et al. 2004, Vanhoutte, De Preter et al. 2006, Slavin 2013).

The benefits of selectively promoting the growth of specific bacteria, such as *Lactobacillus* and *Bifidobacterium* spp., are due to the fact that these bacteria have the capacity to perform both saccharolytic metabolism and proteolytic activities, leading to enhanced levels of essential metabolites, such lactic acid, acetate, and lactate. These metabolic activities result in changes to the microbiota's ecosystem and this has been linked to an improved protection against potential pathogens, reduction of diarrhoea, improved digestion and absorption and immune-stimulation (Cummings and Macfarlane 1991, Gibson 1998, Fioramonti, Theodorou et al. 2003, Veereman 2007, de Vrese and Schrezenmeir 2008, Roberfroid, Gibson et al. 2010, Chen and Quigley 2014, Gibson, Hutkins et al. 2017).

2.4.2. Prebiotic Compounds

The most extensively studied prebiotics are inulin, fructo-oligosaccharides, and galacto-oligosaccharides which, are nondigestible in the small intestine and are anaerobically fermented by especially beneficial bacteria, *Bifidobacterium* and *Lactobacillus* spp., in the colon (Yazawa, Imai et al. 1978, Mitsuoka, Hidaka et al. 1987, Bakker-Zierikzee, Alles et al. 2005, Scholtens, Alles et al. 2006, Bouhnik, Raskine et al. 2007, Wierdsma, Van Bodegraven et al. 2009, Mei, Carey et al. 2011, Sulek, Vigsnaes et al. 2014, Duenas, Munoz-Gonzalez et al. 2015, Alizadeh, Akbari et al. 2016, Kleniewska, Hoffmann et al. 2016, Krumbeck, Maldonado-Gomez et al.

2016, Barrera-Bugueno, Realini et al. 2017, Jafarpour, Shekarforoush et al. 2017, Jinno, Toshimitsu et al. 2017, Valdes-Varela, Ruas-Madiedo et al. 2017, Collins, McMillan et al. 2018, Zhou, Jiang et al. 2018). Other oligosaccharides such as including isomaltooligosaccharides, mannan oligosaccharides, pectins, resistant starches, xylooligosaccharides, arabinoxylans, and human and bovine milk oligosaccharides, have also been studied for their prebiotic properties (Krumbeck, Maldonado-Gomez et al. 2016). These prebiotic compounds originate from the diet in various foods, especially fruits, vegetables, and grains (Roberfroid, Gibson et al. 2010, Slavin 2013, De Preter, Machiels et al. 2015, Duda-Chodak, Tarko et al. 2015, Hutkins, Krumbeck et al. 2016).

Table 2.4.2. Prebiotic Compounds

Prebiotic	Origin
Lactulose	Lactose
Fructooligosaccharides	Hydrolysis of Inulin
Galactooligosaccharides	Lactose or Lactulose
Xylo-oligosaccharides	Fruits, Vegetables, Milk, Honey
Ismomalto-oligosaccharides	Sake, Soy Sauce, Honey
Arabinoxylanoligosaccharides	Fibre portion of Cereal Grain
Inulin	Chicory Root, Wheat, Barley, Onion, Garlic
Polyphenols	Fruits, Vegetables

Table 2.4.2. Prebiotic Compounds is a list of major nondigestible carbohydrates defined as prebiotics. The list also includes the origin of where the prebiotics are derived from. Certain nondigestible carbohydrates such as galacto-oligosaccharides, fructo-oligosaccharides, xylo-oligosaccharides, etc., have recognised beneficial effects on the gut microbiota, and until recently other compounds, such as plant polyphenols, and mixed nondigestible-polyphenol sources such as whole grains are emerging as beneficial modulators of the gut microbiota (Costabile, Klinder et al. 2008, Rastall 2010, Martinez, Lattimer et al. 2013, Duenas, Munoz-Gonzalez et al. 2015, Gibson, Hutkins et al. 2017, Vanegas, Meydani et al. 2017).

2.4.2.1. Saccharomyces cerevisiae Fermentate

A *Saccharomyces cerevisiae* fermentate, having prebiotic activity, was utilized in our studies. A fermentate is a nutritious whole food product made using a unique, specialized fermentation process. In this fermentation process, *Saccharomyces cerevisiae* are used to help produce the beneficial nutrients and metabolites found in food fermentate. Yeast species of the *Saccharomyces* genus including *Saccharomyces cerevisiae* and the probiotic *Saccharomyces boulardii* have been shown to have beneficial effects on the immune system when consumed directly (Martins, Vieira et al. 2013, Salim, Kang et al. 2013). *Saccharomyces cerevisiae*, also known as baker and brewer's yeast, has been used for a variety of purposes outside of baking, such as for brewing beer, for fermenting wine, and as a nutritional supplement. This microbe has a unique characteristic where it can grow in both aerobic and anaerobic conditions, and depending which condition, will produce a specific set of metabolites. Thus, this yeast fermentate combines the fermentation process of anaerobic and aerobic into one, resulting in a product that is high in yeast metabolites, including

vitamins, polyphenols, sterols, and phospholipids. Bioactive components include the nutrient/vitamin profile, cell wall components, and stress-induced defence metabolites (Jensen, Carter et al. 2015). Beta-glucans and polyphenols are compounds, both known to have prebiotic effects (Manach, Williamson et al. 2005, Cuervo, Arboleya et al. 2012, Tuohy, Conterno et al. 2012, Cardona, Andres-Lacueva et al. 2013, Duda-Chodak, Tarko et al. 2015, Duenas, Munoz-Gonzalez et al. 2015, Marchesi, Adams et al. 2016, Wang, Du et al. 2017, Bettermann, Hartman et al. 2018, Gong, Cao et al. 2018, Goulart, Lovatto et al. 2018, Shen, Gao et al. 2018, Teng and Kim 2018). In addition, beta-glucans are known immunomodulators, mediating the innate immune activating properties, such as natural killer cell and macrophage activation, classifying them in nature to be considered as pro-inflammatory (Cassone, Marconi et al. 1987, Ostroff, Mandeville et al. 2002, Pelizon, Kaneno et al. 2003, Plat and Mensink 2005, Goodridge, Reyes et al. 2011).

Saccharomyces cerevisiae fermentate has undergone vigorous studies, which includes randomized, double-blind, placebo-controlled clinical trials with over 300 adult participants and *in vivo* animal studies (Moyad, Robinson et al. 2010, Jensen, Redman et al. 2011, Evans, Reeves et al. 2012, Schauss, Glavits et al. 2012, Possemiers, Pinheiro et al. 2013, Jensen, Carter et al. 2015, Pinheiro, Robinson et al. 2017). From the studies, the fermentate has demonstrated the potential to beneficially modulate the immune responses without excessive suppression or stimulation of overall immune activity. Several mechanisms of action were suggested from the clinical trials and *in vitro* studies, such as enhanced salivary IgA production, NK cell activation, and increased antioxidant capacity (Jensen, Hart et al. 2007, Jensen, Patterson et al. 2008, Jensen, Patterson et al. 2008, Jensen, Redman et al. 2011, Schauss, Glavits et al. 2012, Jensen, Carter et al. 2015). In addition, other aspects

associated with the *Saccharomyces cerevisiae* fermentate include the beneficial effects the compound has on gastrointestinal health, modulating the intestinal barrier function and the immune system and protecting the intestinal barrier under heat stress (Jensen, Patterson et al. 2008, Possemiers, Pinheiro et al. 2013).

2.4.3. Mechanisms of Action

As mentioned, prebiotics are non-digestible compounds that, through metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host. Therefore, the mechanism of action involves the change of the composition of the gut microbiota and/or increasing the production of beneficial metabolites. Nondigestible oligosaccharides are major drivers of the colonic microbiota composition by promoting the saccharolytic activity of the microbiota. As such, nondigestible oligosaccharides have been largely used to selectively promote microbiota enrichment for *Lactobacillus* and/or *Bifidobacterium* and to stimulate the production of certain types of short chain fatty acids (Sim, Cox et al. 2012).

These prebiotic compounds offer an exhaustive array of molecules with different lengths, solubility and sugar composition, and form a diverse source of substrates to alter the gut microbiota and its activities. Moreover, the fermentation of non-digestible dietary carbohydrate results in the production of SCFAs, that have significant positive impacts on intestinal epithelial cell function, including maintenance of metabolism, proliferation, differentiation and promotion of a low (pH5) of the gut environment, favouring beneficial microbes with a concomitant reduction in pathogen bacterial growth and viability (Nepelska, Cultrone et al. 2012, Ohigashi, Sudo et al. 2013, Karl, Hatch et al. 2018).

Prebiotic treatment has been found to alter the gut microbiota, improve

metabolic function, and decrease gut permeability, and metabolic endotoxemia (Wiley, Dinan et al. 2017). The usage of certain prebiotics leads to specific changes in the gut microbiota profile at the species level (Sim, Cox et al. 2012). However, through the stability and improvement of the beneficial microbiota, these microbes can also produce metabolites that influence and maintain the immune system's function and also produce the metabolites that modulate in the gut-brain axis. Therefore, the main mechanism of action is providing the beneficial microbes with a diverse range of oligosaccharides, which enables the beneficial components to flourish and produce the metabolites essential to maintain health.

2.4.4. Beneficial Utilization of Prebiotics

It cannot be emphasised enough that alterations to the microbiota have significant impacts on the host-microbial interactions (Nicholson et al, 2012). As mentioned in section 2.1.2., dysbiosis has been associated with transient and chronic pathologies. These pathological conditions observed are not limited to the gastrointestinal tract, dysbiosis has systemic consequences throughout the host. Prebiotics, just like probiotics, can play a part in maintaining the stability in the gastrointestinal. In addition, the application of prebiotics for the treatment of certain pathologies have led to alleviation of some of the symptoms observed in several diseases. This section demonstrates successful scientific evidence of beneficial gut microbiota modulation from prebiotics for specific intestinal and extraintestinal diseases (Table 2.4.4.1.).

Table 2.4.4.1. Correction of Dysbiosis with Prebiotics

Pathological Condition	Reference	Pathological Condition	Reference
Mental Health	(Sarkar, Lehto et al. 2016, Brenner, Stearns-Yoder et al. 2017, Burokas, Arboleya et al. 2017, Chen, Yang et al. 2017, de Cossio, Fourrier et al. 2017, Kang and Cai 2017, Mancuso and Santangelo 2018)	**Allergy**	(Tang, Lahtinen et al. 2010, Gruber 2012, Forsberg, West et al. 2016)
Metabolic Diseases	(De Preter, Joossens et al. 2013, Aliasgharzadeh, Khalili et al. 2015, Salazar, Dewulf et al. 2015, de Cossio, Fourrier et al. 2017, Gonai, Shigehisa et al. 2017, Porras, Nistal et al. 2017, Sanchez-Tapia, Aguilar-Lopez et al. 2017, Chen, Li et al. 2018, Goulart, Lovatto et al. 2018, Neyrinck, Hiel et al. 2018, Saltzman, Palacios et al. 2018, Vespasiani-Gentilucci, Gallo et al. 2018, Westfall, Lomis et al. 2018, Zhou, Jiang et al. 2018)	**Inflammatory Bowel Disease**	(Rioux, Madsen et al. 2005, Packey and Sartor 2009, Grimoud, Durand et al. 2010, Romeo, Nova et al. 2010, Quigley 2012, Hardy, Harris et al. 2013, Tao, Duan et al. 2016, Wong, Harris et al. 2016, Costantini, Molinari et al. 2017, Norton, Czuber-Dochan et al. 2017, Jing, Li et al. 2018, Liu, Zhang et al. 2018)
Bone Demineralization	(de Vrese and Schrezenmeir 2008, Roberfroid, Gibson et al. 2010, Dias, Ferreira et al. 2016, Whisner and Castillo 2018)	**Irritable Bowel Syndrome**	(Szilagyi 2004, Spiller 2008, Roberfroid, Gibson et al. 2010, Thomas, Greer et al. 2010, Thomas, Greer et al. 2010, Vandenplas, Veereman-Wauters et al. 2011, Quigley 2012, Saad, Delattre et al. 2013, Martinez, Bedani et al. 2015, Staudacher and Whelan 2016, Chen, Xiao et al. 2017, Wang, Xin et al. 2017)
Infections	(Sazawal, Dhingra et al. 2010, Siempos, Ntaidou et al. 2010, Al-Waili, Salom et al. 2011, Shimizu, Ogura et al. 2013, Chen, Reiter et al. 2017, Kasatpibal, Whitney et al. 2017, Collins, McMillan et al. 2018, Deusch, Serrano-Villar et al. 2018)	**Necrotizing Enterocolitis**	(Szajewska 2010, Thomas, Greer et al. 2010, Vandenplas, Veereman-Wauters et al. 2011, Morrow, Gogineni et al. 2012, Bertelsen, Jensen et al. 2016, Johnson-Henry, Abrahamsson et al. 2016, Slattery, MacFabe et al. 2016)
Infectious Diarrhoea	(Whelan 2007, de Vrese and Schrezenmeir 2008, Sazawal, Dhingra et al. 2010, Thomas, Greer et al. 2010, Vandenplas, Veereman-Wauters et al. 2011, Saad, Delattre et al. 2013, Greenway, Wang et al. 2014, Chen, Xiao et al. 2017, Azagra-Boronat, Massot-Cladera et al. 2018, Graness, Swidsinski et al. 2018)	**Stress**	(Krishna, Divyashri et al. 2015, Al-Sagan and Abudabos 2017, Almeida, Curimbaba et al. 2017, Brenner, Stearns-Yoder et al. 2017, Panzella, Perez-Burillo et al. 2017, Zhang, Mao et al. 2017, Eutamene, Placide et al. 2018, Guerreiro, Oliva-Teles et al. 2018, Guo, Tang et al. 2018, Gurry, Gibbons et al. 2018, Khan, Khundmiri et al. 2018, Kim, Lee et al. 2018, Mohanty, Mishra et al. 2018, Morshedi, Agh et al. 2018, Mota, Walter et al. 2018, Tan, Chen et al. 2018, Nedaei, Noori et al. 2019)

Colorectal Cancer	(Grimoud, Durand et al. 2010, Zhu, Luo et al. 2011, Hardy, Harris et al. 2013, Wong, Harris et al. 2016, Mota, Walter et al. 2018, Rossi, Mirbagheri et al. 2018)	**Antibiotic-Associated Diarrhoea**	(Zoppi, Cinquetti et al. 2001, Hume 2011, Al-Sagan and Abudabos 2017, Kasatpibal, Whitney et al. 2017, Watkins, Stanton et al. 2017, Graness, Swidsinski et al. 2018, Khan, Khundmiri et al. 2018, Roychowdhury, Cadnum et al. 2018)
Rheumatoid Disease	(Abhari, Shekarforoush et al. 2016, Steves, Bird et al. 2016, Kamal, Kaddam et al. 2018)	**Autism**	(Weston, Fogal et al. 2015, Berding and Donovan 2016, Slattery, MacFabe et al. 2016, Grimaldi, Cela et al. 2017, Watkins, Stanton et al. 2017, Grimaldi, Gibson et al. 2018, Sherwin, Dinan et al. 2018)
Neurodegenerative Diseases	(Miragem and de Bittencourt 2017, Mancuso and Santangelo 2018, Sherwin, Dinan et al. 2018)	**Constipation**	(Waitzberg, Pereira et al. 2012, Michela, Pacchetti et al. 2015, Rondon, Valer et al. 2015, Barichella, Pacchetti et al. 2016, Cereda, Pacchetti et al. 2016, Buddington, Kapadia et al. 2017, Closa-Monasterolo, Ferre et al. 2017, Jinno, Toshimitsu et al. 2017, Pinheiro, Robinson et al. 2017, Prasad and Abraham 2017, Rasmussen, Hamaker et al. 2017, Vandeputte, Falony et al. 2017, Ceresola, Ferrarese et al. 2018, Dahl and Mendoza 2018, Nooshkam, Babazadeh et al. 2018, Shahramian, Kalvandi et al. 2018, Singh, Singh et al. 2018)

Chapter 3 - Objectives and Specific Aims

The main objective of this dissertation is to evaluate the efficacy of a probiotic and prebiotic as a novel approach in preventing the adverse effects of heat stress, whether the stressor is a result of metabolic or environmental heat stress.

In our approach, a *Bacillus subtilis* probiotic strain and a *Saccharomyces cerevisiae* fermentate, which has prebiotic activity, were utilized in *in vitro* studies to demonstrate their efficacy in modulating the gut microbiota and the intestinal barrier function.

Specific Aim 1

Characterize the Effects of Stress on the Intestinal Barrier Function

To accomplish this aim, heat stress animal models (environmental chamber and treadmill) were utilized. Morphological changes in the gastrointestinal tract of rats and the expression of intestinal tight junction proteins as a result of heat stress were characterized. In addition, the serum of the experimental animals was analysed for the levels of pro- and anti-inflammatory cytokines, and markers of intestinal barrier permeability such as lipopolysaccharides (LPS).

Specific Aim 2

Demonstrate the Effects of Stress on the Gut Microbiota Profile

Bacteriological and genetic analyses of the gut microbiota were performed, analysing the change in gut microbial profile after exposure to one of the heat stress animal models.

Specific Aim 3

Evaluate the Efficacy of Probiotic and Prebiotic in Preventing of Stress-Related Adverse Effects

Animals were administered with the *Bacillus subtilis* probiotic strain and a *Saccharomyces cerevisiae* fermentate before one of the heat stress models and the protective effect of the probiotic and prebiotic on the gut morphology, intestinal barrier function, and the composition of the gut microbiota were characterized.

Chapter 4 - Mitigation of Heat Stress-Related Complications by a Yeast Fermentate Product[1]

Abstract

Heat stress results in a multitude of biological and physiological responses which can become lethal if not properly managed. It has been shown that heat stress causes significant adverse effects in both human and animals. Different approaches have been proposed to mitigate the adverse effects caused by heat stress, among which are special diet and probiotics. We characterized the effect of the yeast fermentate EpiCor (EH) on the prevention of heat stress-related complications in rats. We found that in- creasing the body temperature of animals from 37.1 ± 0.2°C to 40.6 ± 0.2°C by exposure to heat (45°C for 25 min) resulted in significant morphological changes in the intestine. Villi height and total mucosal thickness decreased in heat-stressed rats pre-treated with PBS in comparison with control animals not exposed to the heat. Oral treatment of rats with EH before heat stress prevented the traumatic effects of heat on the intestine. Changes in intestinal morphology of heat-stressed rats, pre-treated with PBS re- sulted in significant elevation of lipopolysaccharides (LPS)

[1]This chapter was published: Giblot Ducray, H. A., Globa, L., Pustovyy, O., Reeves, S., Robinson, L., Vodyanoy, V. Sorokulova, I. (2016). "Mitigation of heat stress-related complications by a yeast fermentate product." Journal of Thermal Biology **60**: 26-32.

level in the serum of these animals. Pre- treatment with EH was effective in the prevention of LPS release into the bloodstream of heat-stressed rats. Our study revealed that elevation of body temperature also resulted in a significant increase of the concentration of vesicles released by erythrocytes in rats, pre-treated with PBS. This is an indication of a pathological impact of heat on the erythrocyte structure. Treatment of rats with EH completely protected their erythrocytes from this heat-induced pathology. Finally, exposure to heat stress conditions resulted in a significant increase of white blood cells in rats. In the group of animals pre-treated with EH before heat stress, the white blood cell count remained the same as in non-heated controls. These results showed the protective effect of the EH product in the prevention of complications, caused by heat stress.

4.1. Introduction

Heat stress results in a multitude of pathological and physiological responses, which can become lethal if not properly managed. It was shown that heat stress causes significant morphological changes in the gut (Bouchama, Roberts et al. 2005, Chang, Sang et al. 2013). Data to support these observations were obtained in human as well as in animal studies. For example, it has been found that heat exposure in pigs caused marked injury to the tips of the intestinal villi, inducing epithelial cell shedding, exposing the intestinal mucosa lamina propria, as well as shortening the villus height and crypt depth in the small intestine (Yu, Yin et al. 2010). These morphological changes clearly alter the integrity of the gastrointestinal tract, which serves as a first line of defence to protect the host from the internal environment of the gut containing bacteria and endotoxins in the form of lipopolysaccharides (LPS) from Gram-negative bacteria. Dysfunction of this protective barrier results in increased intestinal permeability and diffusion of toxic bacterial components from the gut lumen into the blood. The immune system serves to actively remove LPS from the circulatory system through the reticuloendothelial system of the liver, high-density lipoproteins and anti-LPS antibodies. The precise mechanism of injury and death from heat stress are proposed to arise from endotoxaemia, which develops when the rate of LPS clearance is not consistent with the rate of LPS translocation from the gut (Lim and Mackinnon 2006). This situation triggers a systemic inflammatory response that then leads to disseminated intravascular coagulation, necrosis of organ tissues, and multi-organ failure. It was previously reported that elevation of the body temperature during heat stress causes an increase of the shedding of erythrocyte membrane vesicles (Moore, Sorokulova et al. 2013, Moore, Globa et al. 2014). Vesicles constitute a heterogenic population of cell-derived microscopic size particles that participate in a wide range

of physiological and pathological processes. More recently, it was shown that the aging erythrocyte is characterized by changes in the plasma membrane, particularly in vesiculation of the cell membrane. This process is termed as eryptosis or programmed erythrocyte death. So, heat causes a deadly impact on the erythrocyte structure.

Different approaches are proposed for mitigation of heat stress adverse effects, among which are special diet and probiotics. But there is no data about the efficacy of prebiotics in mitigation of stress-induced complications. EpiCor (EH) is a yeast (*Saccharomyces cerevisiae*) fermentate. The fermentation produces a product that is high in yeast metabolites, including vitamins, polyphenols, sterols, phospholipids and polysaccharides such as beta-glucan. Previous studies showed that EH exhibits strong prebiotic properties (Possemiers, Verhelst et al. 2011). This product possess significant anti-inflammatory activity, selectively enhanced butyrate production in vitro (Jensen, Patterson et al. 2008, Possemiers, Pinheiro et al. 2013) and supports mucosal defence in clinical trials (Jensen, Patterson et al. 2008). We hypothesize that this yeast fermentate could be effective in the prevention of heat stress adverse effects. The main objective of this work was to study the efficacy of the prebiotic-like product EH, in the prevention of complications related to heat stress in rats.

4.2. Materials and Methods

4.2.1. Ethics Statement

All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee. The study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

4.2.2. Animals

Adult male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) weighing 250–300 grams were used in this study. The animals were housed two per cage under specific pathogen- free conditions and were acclimatized for 2 days at a temperature (20 ± 1 °C), humidity (50 ± 5%) and lighting (12-hour day/12-hour night) with free access to water and standard food (2018 Teklad Global 18% Protein Rodent Diet, Harlan, Indianapolis, IN, USA).

4.2.3. Yeast Fermentate

The yeast fermentate product EpiCor (EH) was provided by the manufacturer (Embria Health Sciences, Ankeny, IA, USA) in a powder form. For oral treatment of rats, suspension of the yeast fermentate in PBS was prepared at the rate 7 milligram per kilogram of animal weight in 1 millilitre of phosphate buffered saline (PBS).

4.2.4. Experimental Design

A total of 32 rats were used in this study. One group (16 rats) was fed a standard diet (basal diet) and the other group (16 rats) received the same diet, but they were treated by oral gavage with 1 millilitre of yeast fermentate suspension. Animals were treated once a day in the morning, every day for 14 days prior to the start of heat stress (Fig. 4.1). At the same time control rats received 1 millilitre of PBS by oral gavage. On day 15, rats in each group were subdivided (8 rats in each group), these groups were: 1) control (PBS/25 °C, 25 minutes), 2) EH (yeast fermentate/25 °C, 25 minutes), 3) stress (PBS/ 45 °C, 25 minutes), and 4) EH + stress (yeast fermentate/45 °C, 25 minutes).

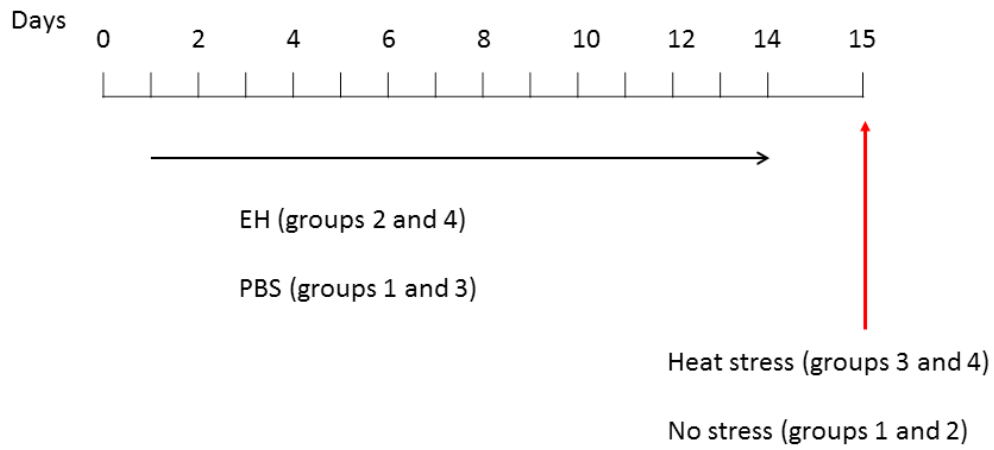


Figure 4.2.4.1. Experimental Design.

Animals from group 3 and 4 were exposed to 45 °C heat stress, relative humidity 55% for 25 minutes in a pre-heated climatic chamber (Environmental Chamber 6020-1, Caron, OH, USA). These conditions were previously described to achieve acute heat stress in rats (Sachidhanandam, Low et al. 2002, Moore, Sorokulova et al. 2013, Moore, Globa et al. 2014). Control animals (groups 1 and 2) were exposed to identical conditions as the heat stressed animals, but at 25 °C. The animals had access to food and water during and after heating. Rectal temperature was measured for each rat before and immediately after heat stress using an electronic digital thermometer, inserted to a depth of 4 centimetres (Moore, Sorokulova et al. 2013). Four hours after the heat stress experiments, rats were anesthetized with isoflurane (2–4%) and euthanized by rapid decapitation. Trunk blood was collected from each rat into sterile apirogenic tubes to obtain serum. Samples of blood were immediately taken for microscopic examination. Samples of small intestine from each rat were taken for morphological analysis.

4.2.5. Blood Serum Preparation

Blood in the tubes was allowed to clot for 30 minutes in a refrigerator. Tubes were centrifuged at 20 °C, 7000g for 10 minutes. Serum was collected and stored in 50µL aliquots at -20 °C until assay.

4.2.6. Histological Analysis

4.2.6.1. Sample Preparation

Samples of small intestine (0.5-2 centimetres in length) were immediately fixed in Bouin's solution (Electron Microscopy Sciences, Hatfield, PA, USA) for 10-15 minutes. Tissue samples were cut to proper size (5-7 millimetres in length) and transferred to fixative to make sure all tissues were completely immersed in fixative. The volume of fixative was 20-30 times the tissue volume. After 48 hours of fixation at room temperature, the excess fixative was washed out in 70% alcohol until most of the yellow colour was removed. Washed samples were put into tissue embedding cassettes (VWR, Radnor, PA, USA) and kept in 70% alcohol until processing in the Automated tissue processor (Tissue-Tek VIP, Miles/Sakura, Torrance, CA, USA). After processing, samples were embedded in paraffin blocks using embedding centre (Tissue-Tek TEC, Sakura, Torrance, CA, USA). Embedded tissue was sectioned at 6 millimetres using the microtome (Reichert-Jung 2040 Autocut, Leica Biosystems Nussloch GmbH, Heidelberger Straße 17-19, 69226 Nussloch, Germany).

4.2.6.2. Sample Staining

Hematoxylin–eosin (H&E) staining was performed according to the standard protocol (Stevens 1990), using Mayer's hematoxylin and Eosin Y (Electron Microscopy Sciences, Hatfield, PA). Briefly, sections were deparaffinized in Hemo-De x 3 changes for 8 minutes, 5 minutes and 5 minutes, cleared in 100% ETOH x 2

changes - 2 minutes each, followed by 95% ETOH x 2 - 2 minutes each and 80% ETOH - 2 minutes. Sections were rinsed with distilled water and stained with hematoxylin for 2–3 minutes. Samples were washed in running tap water for about 5 minutes, put in 80% ETOH for 2 minutes and stained in eosin for 1-2 minutes. After staining, sections were put in 70% ETOH - 2 minutes, 95% ETOH x 2 - 2 minutes each, 100% ETOH x 2 - 2 minutes each, Hemo-De x 2 changes until clear (5 minutes, 10 minutes or longer). Mounting of the samples was performed using Eukitt Mounting Medium (Electron Microscopy Sciences, Hatfield, PA).

4.2.6.3. Measurements

Intestinal villi height and total mucosal thickness were measured for each sample using a high resolution microscope system (Vainrub, Pustovyy et al. 2006). Only stained sections in which the mucosal villi were cut along their longitudinal axis were analysed. The villi height was measured as a distance from the apex of the villus to the base of the crypt; total mucosal thickness - from the top of the villus to the muscularis mucosae. Twenty measurements of each parameter in each sample were taken and expressed in micrometres. An average of these measurements was expressed as a mean villi height and total mucosal thickness for one treatment group.

4.2.7. Lipopolysaccharide Assay

Serum concentration of LPS was analysed by the Pierce LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific, Rockford, IL) using the Limulus Amebocyte Lysate (LAL) assay according to the manufacturer's recommendations. The sensitivity of the assay was 0.1 EU milliliters^{-1} (0.01 nanograms endotoxin per millilitres). Sterile pyrogen-free plastic and glassware were used throughout the assay.

4.2.8. Cortisol Assay

Cortisol concentration in serum of animals in all groups was analysed by Rat Cortisol ELISA Kit (NeoBioLab, Cambridge, MA) according to the manufacturer's instruction. The sensitivity of this assay was 1.0 nanogram milliliter^{-1}. Cortisol ELISA Assay Kit is a competitive immunoenzymatic colorimetric method for quantitative determination of cortisol concentration in serum.

4.2.9. IL-10 Cytokine Assay

Serum level of IL-10 cytokine was measured using the commercial rat cytokine-specific sandwich ELISA kit for IL-10 (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. Optical density values were measured at 450 nanometres using a microplate reader (Bio-Tek, Winooski, VT). The cytokine content of each sample was determined by comparison of mean experimental values with curve generated using standards, supplied with the kit. The minimum detectable dose of IL-10 kits was <5 picograms milliliter^{-1}.

4.2.10. High Resolution Light Microscopy of the Blood

The images of fresh blood were recorded with a super-resolution light microscope system described in Vainrub et al. (Vainrub, Pustovyy et al. 2006). Test images were calibrated using a Richardson slide (Richardson 1988). A small droplet (7 microliter) of freshly drawn blood was placed on a glass slide, cover slipped, and positioned on the microscope stage with oil contacts between condenser and objective lenses. Ten image frames of 72 x 53.3 micrometers^2 in each sample were videotaped, and concentrations of white blood cells and vesicles count, and diameter were measured by Image-Pro Plus software (Media Cybernetics), providing high-resolution direct- view optical images in real time. The samples were observed in an aqueous

environment and required no freezing, dehydration, staining, shadowing, marking or any other manipulation. At least 20 image frames were analysed for each animal. Each frame contained between 50 and 200 vesicles (depending on conditions).

4.2.11. Statistics

All results were presented as a mean and standard deviation. The differences between groups were analysed by the one-way ANOVA, followed by the Bonferroni test. The significance level was set at 0.05 to define statistical significance. Statistical calculations and graph plotting were carried out using Microcal Origin version 9.0 (Northhampton, MA) and 2010 Microsoft Excel.

4.3. Results

4.3.1. Body Temperature

Exposure of rats to high temperature resulted in a significant increase of body temperature. The mean body temperature (°C) of rats before and immediately after heat stress was 37.4 ± 0.2 and 40.6 ± 0.2 ($p < 0.05$) respectively in group 4 (EH) and 37.1 ± 0.2 and 40.4 ± 0.4 ($p < 0.05$) respectively in group 3 (PBS). No central nervous system abnormalities such as convulsions, or coma in animals during or after heat stress, as well as after 4 hours recovery were noticed. Body temperature of rats in control groups (groups 1 and 2) was stable during the experiment.

4.3.2. Morphometric Analysis of the Intestine

Villi height and total mucosal thickness in control rats, not exposed to heat were 612.8 + 8.3 micrometres and 739.9 ± 8.0 micrometres respectively (group 1 – PBS/25 °C) and 613.4 ± 7.1 micrometres and 740.6 ± 7.7 micrometres respectively (group 2 – EH/25 °C) (Fig. 4.3.2.1. A and B; Fig. 4.3.2.2). Exposure of rats pre-treated with PBS to heat stress conditions (group 3 – PBS/45 °C) significantly

decreased villi height and total mucosal thickness – 394.1 ± 7.5 micrometres and 526.5 ± 8.7 micrometres accordingly. Treatment of rats with EH before heat stress prevented the damage effect of heat on intestinal morphology. In this group of rats (group 4 – EH/45 °C) parameters for villi height and total mucosal thickness were similar to the control rats.

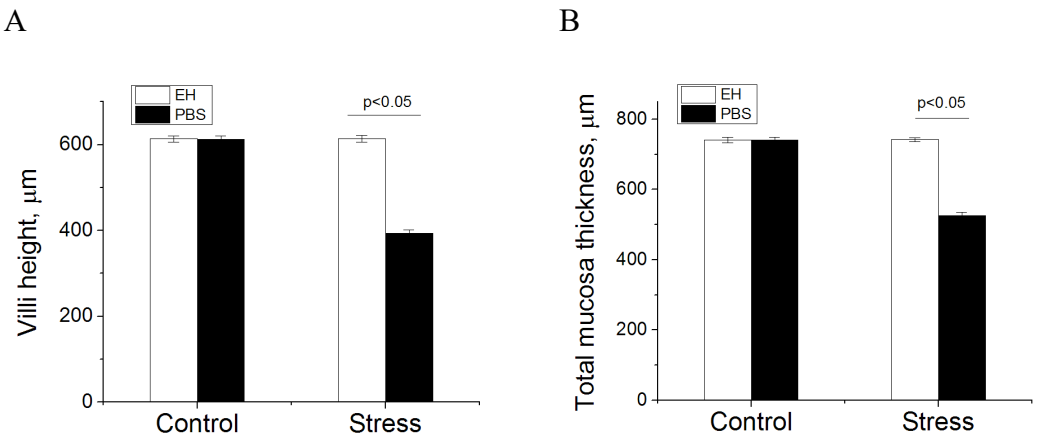
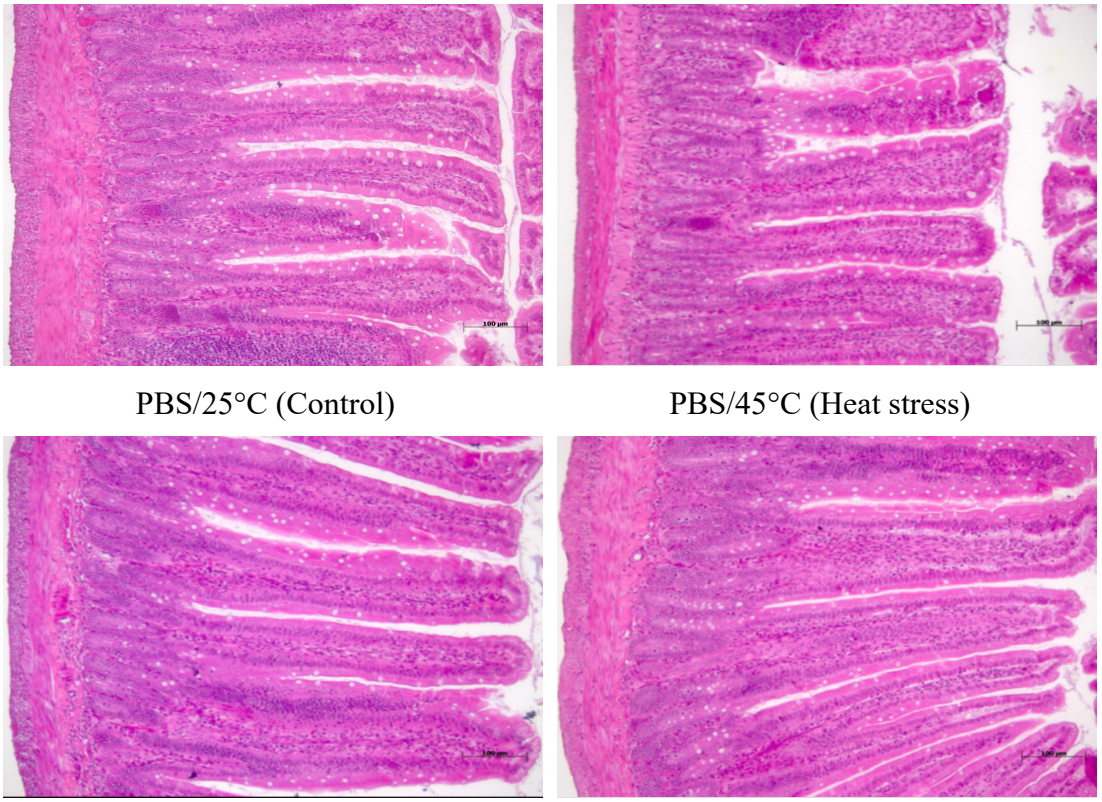


Figure 4.3.2.1. Intestinal villi height (A) and total mucosa thickness (B) of rats from different experimental groups. EH – groups of rats, treated with EpiCor; PBS – groups of rats, treated with PBS.



PBS/25°C (Control) PBS/45°C (Heat stress)

EH/25°C (Control) Eh/45°C (Heat stress)

Figure 4.3.2.2. Histological images of small intestine stained with hematoxylin and eosin. Rats were pre-treated with PBS or EpiCor (EH) by oral gavage every day for 14 days before exposure to 45 °C (Heat stress) or to 25 °C (Control). The villi height is a distance from the apex of the villus to the base of the crypt; total mucosal thickness – from the top of the villus to the muscularis mucosae. Bar-100 µm.

4.3.3. Microscopic Evaluation of the Blood

The concentration of free vesicles in the blood of control rats was (1.70 ± 0.07) x 10^9 particles millilietrs^{-1} (PBS treated) and (1.80 ± 0.07) x 10^9 vesicles millilters^{-1} (EH treated) (Fig. 4.3.3.1. A). Significant increase of vesicle concentration was found in heat-stressed rats, pre-treated with PBS (Figs. 4.3.3.1. A and 4.3.3.2). No change of free vesicle concentration was found in rats treated with EH and exposed to heat stress conditions (Fig. 4.3.3.1. A). The diameter of vesicles also significantly increased only in the blood of rats treated with PBS before heat stress (Fig. 4.3.3.1. B). Pre-treatment with EH prevented the formation of large vesicles in

the blood. We also found a significant increase of white blood cell (WBC) concentration in the blood of rats exposed to heat after treatment with PBS (Fig. 4.3.3.3). No change in the number of WBC (in comparison with control groups) was found in the blood of heat-stressed rats pre-treated with EH.

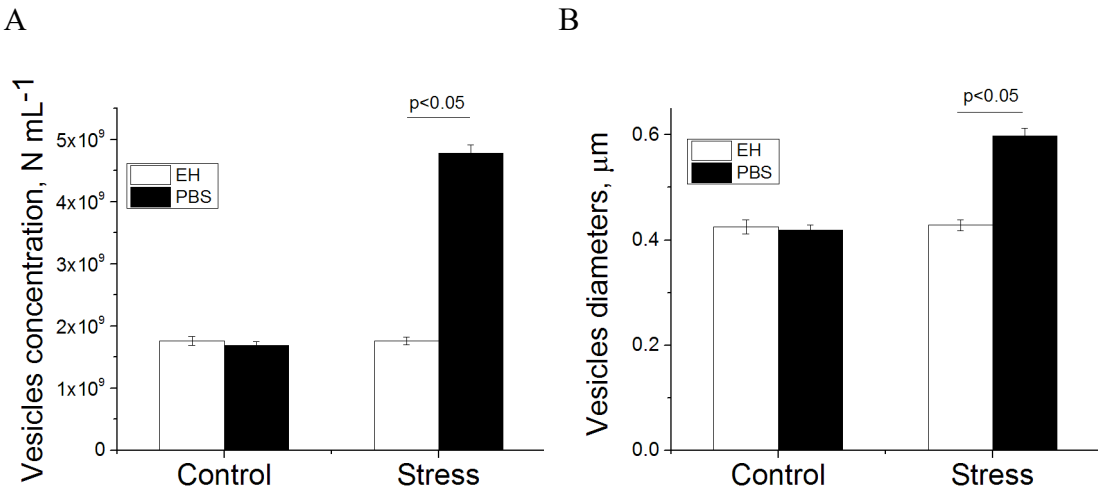
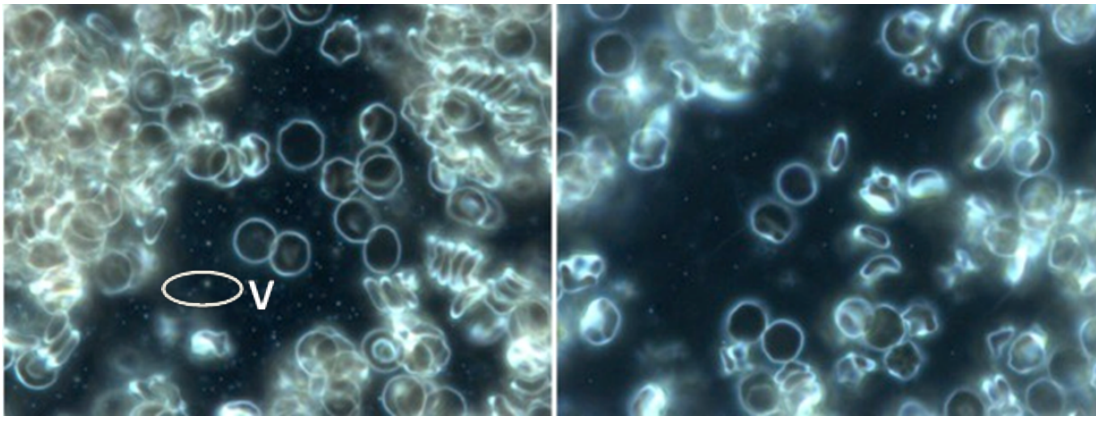
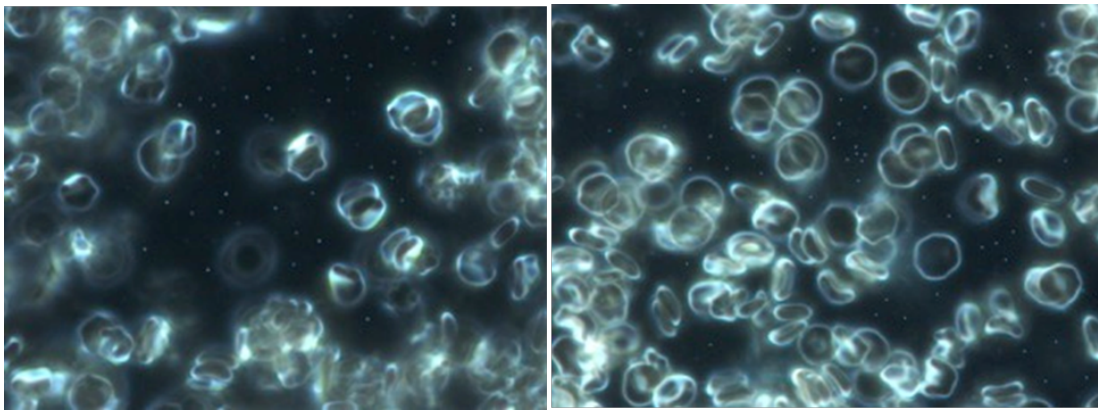


Figure 4.3.3.1. Vesicles concentration (A) and vesicles diameter (B) in rats of different experimental groups.



A B



C D

Figure 4.3.3.2. Microscopic images of free vesicles in the blood of rats. A: A dark field frame of the blood video image of rat, pre-treated with PBS and exposed to 45 °C; B: A dark field frame of the blood video image of rat, pre-treated with PBS exposed to 25 °C; C: A dark field frame of the blood video image of rat, pre-treated with EH and exposed to 45 °C; D: A dark field frame of the blood video image of rat, pre-treated with EH and exposed to 25 °C; Rat erythrocytes are ~6 μm in diameter, and therefore, they serve as natural scale bars. V is a free vesicle.

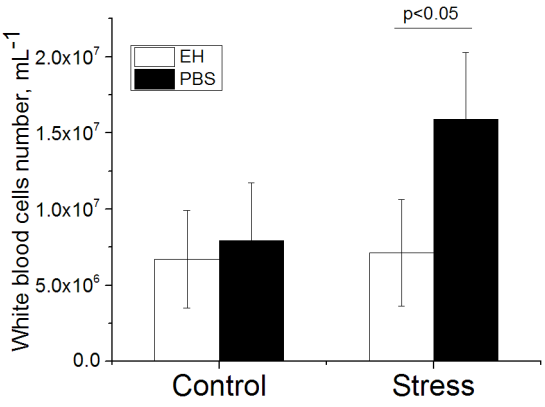


Figure 4.3.3.3. Concentration of white blood cells in the blood of rats from the different experimental groups.

4.3.4. Serum LPS Concentration

Levels of LPS significantly increased in serum of heat-stressed animals, which received PBS before heat exposure in comparison with EH-treated heated rats and rats in both control groups (Fig. 4.3.4.1). Concentration of LPS in the serum of rats pre-treated with EH before heat stress was not different from the LPS level in serum of animals from control groups.

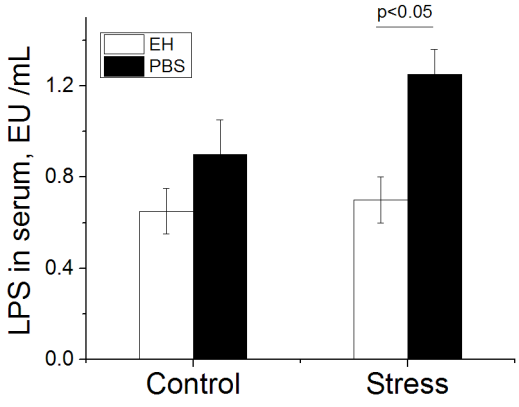


Figure 4.3.4.1. Concentration of LPS in serum of animals from different experimental groups.

4.3.5. Serum Cortisol Level

The serum cortisol level was stable in the rats of the different groups. Cortisol level in heat stressed rats pre-treated with PBS in comparison with control PBS-treated rats was 23.30 ± 1.48 nanograms millliters^{-1} and 20.30 ± 1.48 nanograms millliters^{-1} respectively. The concentration of serum cortisol in rats pre-treated with EH before exposure to heat stress conditions was comparable with the level registered in the serum of control EH-treated rats (19.30 ± 1.47 nanograms millliters^{-1} and 22.80 ± 1.48 nanograms millliters^{-1} respectively).

4.3.6. IL-10 in Serum

No significant change in the level of serum IL-10 of rats in different groups was found (3.40 ± 0.14–6.82 ± 0.38 picograms milliliter^{-1} in heat stressed and control EH groups respectively and 4.60 ± 0.34– 4.04 ± 0.18 picograms milliliter^{-1} in stressed and control PBS groups respectively).

4.4. Discussion

This study was designed to evaluate the efficacy of yeast fermentate EH in the prevention of heat stress-induced adverse effects in rats. The rat model of acute heat stress described by Sachidhanandam et al. was used in our study (Sachidhanandam, Low et al. 2002). We explored this model in our previous experiments (Moore, Sorokulova et al. 2013, Moore, Globa et al. 2014). The conditions of the model (45 °C for 25 minutes) resulted in increase of body temperature of rats to 40.3 ± 0.2 °C. The standard deviation of body temperature after heat stress indicated small variability between animals. No central nervous system abnormalities such as convulsions, or coma in animals during or after heat stress, as well as after 4 hours recovery were noticed. So, we can assume that this model of heat stress is a humane

model because the exposure to heat is very brief and does not result in mortality of animals but leads to the manifestation of the acute heat stress symptoms. Thus, it was found that the increase of the body temperature of animals exposed to heat stress conditions resulted in significant morphological changes in the intestine. Villi height and total mucosal thickness decreased in heat-stressed rats pre-treated with PBS in comparison with control animals not exposed to the heat. Treatment of rats with EH before heat stress prevented the traumatic effect of heat on the intestine because the results of measurements of villi height and total mucosal thickness in this group of animals were the same as in the control groups. We can speculate that mucosal immune protection by EH reported previously (Jensen, Patterson et al. 2008) can contribute to the gut integrity.

Changes in intestinal morphology of heat-stressed rats, pre-treated with PBS, resulted in significant elevation of LPS level in the serum of these animals. These findings are in accordance with our previous data (Moore, Globa et al. 2014) and with the results of other authors (Lambert, Gisolfi et al. 2002, Lambert 2004). LPS derived from the gut was shown to be critical in the development of various pathological conditions in humans and animals. Thus, LPS was identified as one of the triggering factors of metabolic diseases, chronic low-grade inflammation (Cani, Amar et al. 2007) and liver disorders (Minemura and Shimizu 2015). LPS plays an important role in the development of a distinct depressive-like behavioural syndrome in animals (O'Connor, Lawson et al. 2009) and depression in humans (Yirmiya 1996, Maes, Kubera et al. 2008). Elevated levels of LPS in blood (endotoxemia) were documented in patients with heat-stroke, ultramarathon runners (Ryan, Matthes et al. 1994), during exhaustive physical exercise (Ashton, Young et al. 2003), and in patients with severe

forms of autism (Emanuele, Orsi et al. 2010). It was found that endotoxemia is a result of the altered intestinal barrier function and increased gastrointestinal permeability. Normal gut microbiota plays a critical role in the maintaining of the gut barrier function (Hooper and Gordon 2001, Natividad and Verdu 2013). In this study, pre-treatment with EH prevented LPS release into circulation in rats exposed to heat. We can speculate that beneficial modulation of gut microbiota and anti-inflammatory activity of EH, demonstrated previously (Possemiers, Pinheiro et al. 2013), contribute to the protective effect of this yeast fermentate.

In our previous study, we showed that heat stress results in an increase of the shedding of erythrocyte membrane vesicles (Moore, Pascoe et al. 2013, Moore, Sorokulova et al. 2013). Vesiculation of the cell membrane is related to the aging of erythrocyte (Bosman, Lasonder et al. 2008, Ghashghaeinia, Cluitmans et al. 2012) and is termed as eryptosis or programmed erythrocyte death (Lang and Lang 2015). This study revealed that the elevation of the body temperature resulted in a significant increase of the concentration of vesicles in the blood of rats, compared to PBS-treated rats before heat exposure. This is an indication of a pathological impact of heat on the erythrocyte structure of this group of animals. Treatment of rats with EH completely protected their erythrocytes from the pathology, caused by heat stress. Our data are in accordance with previously published results about beneficial effects of EH on erythrocyte health (Jensen, Patterson et al. 2008).

A significant shift to a higher vesicle diameter was also indicated as an adverse effect of heat stress (Moore, Sorokulova et al. 2013). The results of this study showed that treatment with EH prevented rats from this adverse effect of heat stress. Exposure to heat stress conditions resulted in a significant increase of white blood cell

(WBC) counts in rats, pre-treated with PBS. This result is in accordance with the findings of other authors, showed elevated levels of WBC in stressed animals (Peli, Scagliarini et al. 2013, Matur, Akyazi et al. 2016). In the group of animals treated with EH before heat stress, the white blood cell count remained the same as in control groups.

We did not find significant changes in serum IL-10 levels in different groups of rats. These results confirmed previously obtained data that consumption of EH does not alter the level of serum IL-10 (Jensen, Patterson et al. 2008).

The level of cortisol was consistent in all groups of animals. Literature data show that cortisol level can be stable during stress (Radahmadi, Shadan et al. 2006) or even can be decreased during 2–4 hours after heat stress (McMorris, Swain et al. 2006).

Our previous results showed that the thermodynamic data for rat environmental hyperthermia and human exercise-induced temperature increase are consistent with each other and agree well with thermodynamic literature results (Vodyanoy 2015). Future directions of this study will help to understand the feasibility of our approach not only in environmental heat stress but also in heat stress, related to physical activity.

4.5. Conclusion

Our study showed a significant protective effect of EH against heat stress-related adverse effects in a rat model. Pre-treatment of animals with EH before exposure to heat protected gut morphology, prevented increase of LPS in blood and pathological impact of heat stress on blood erythrocytes. Further study of EH efficacy during heat stress will lead to a better understanding of the mechanisms of this

protection, and to develop new approaches for the prevention of heat stress-induced adverse effects in the gut.

4.6. Conflict of Interest

No conflict of interest declared.

4.7. Acknowledgement

This work was sponsored by Embria Health Sciences LLC (OSP#2007-14).

Chapter 5 - Yeast Fermentate Product Can Improve Intestinal Barrier Integrity During Heat Stress by Modulation of the Gut Microbiota

Abstract

Stress significantly affects the intestinal barrier integrity, causing increased gut permeability and systemic inflammation. Stability of the gut microbiota is essential for regulating the gut barrier function and keeping the homeostasis of the organism. The main objectives of this study were (1) to evaluate efficacy of a Saccharomyces cerevisiae fermentate prebiotic (EH) in protecting the intestinal barrier integrity in rats during heat stress; (2) to analyse the impact of heat stress and the preventative effect of EH treatment on the structure of the gut microbiota. Exposure of animals to heat stress conditions (25 min at 45°C and relative humidity 55%) resulted in inhibition of tight junction (TJ) proteins expression in intestine and decrease count of Paneth and goblet cells. Oral treatment of rats with EH before stress significantly prevented these adverse effects. Culture-based bacteriological analysis of the gut microbiota revealed dysbiotic changes only in rats pre-treated with PBS prior to stress exposure. We found a decrease in the anaerobic to aerobic bacteria ratio in these animals and a significant increase of pathobionts: haemolytic bacteria, *Escherichia* spp., and *Staphylococcus* spp.. High-throughput 16S rRNA gene sequencing detected significant changes of the gut microbiota at the genus taxonomic level. Exposure of rats to heat stress conditions resulted in the substantial decrease of beneficial bacteria (*Allobaculum,*

Bifidobacterium) and increase of pathogenic bacteria (*Alistipes, Bacteroides, Bilophila, Johnsonella, Oscillibacter, Tannerella, Staphylococcus*) in comparison with the control group. Essential impact of the EH on the gut microbiota during heat stress is manifested in the elevated numbers of beneficial bacteria (*Eubacterium, Lactococcu*s) and butyrate-producing bacteria (*Oscillospira, Roseburia, Vallitalea*). We assume that effect of EH in prevention of heat stress-related complications is associated with the beneficial modulation of the gut microbiota.

5.1. Introduction

Heat stress, as other types of stress, seriously impacts gastrointestinal physiology, which result in intestinal ulceration, development of irritable bowel syndrome, and inflammatory bowel disease. It was shown that stress significantly affects intestinal barrier function resulted in gut permeability and systemic inflammation. One of the mechanisms connecting stress and gastrointestinal diseases is stress-induced effects on mucosal barrier function. Intestinal barrier function is the ability to control uptake across the mucosa and to protect the inner environment from potentially harmful compounds present in the intestinal lumen. This barrier is achieved by the intracellular junctional complexes: tight junctions (TJ), adherens junctions (AJ), gap junctions, and desmosomes. The TJ are the apical-most junctional complex, responsible for sealing the intercellular space. They act as a primary barrier to the diffusion of solutes through the intercellular space. The main types of transmembrane proteins in TJ are occludin and claudins, which link adjacent enterocytes (Ohland and MacNaughton 2010). Zonula occludens (ZO) proteins are important intracellular tight junction proteins, that link the transmembrane tight junction proteins: claudins, occludin and junctional adhesion molecules (JAM) to the actomyosin cytoskeleton (Grootjans, Thuijls et al. 2010). Disruption of the intestinal TJ barrier induces activation of the mucosal immune system and inflammation and can act as a trigger for the development of intestinal and systemic diseases (Suzuki 2013). Various factors may cause destabilization of TJ proteins: enteric pathogens and their toxins, anti-inflammatory drugs, alcohol (Groschwitz and Hogan 2009). Heat stress was shown to disrupt intestinal barrier function (Hall, Buettner et al. 2001) and to change the expression of TJ proteins (Xiao, Tang et al. 2013). Usually, the effect of heat stress on TJ proteins is assessed in vitro in epithelial cell monolayers. Recently,

Pearce et al. (Pearce, Mani et al. 2013) showed changes in the TJ proteins composition in pigs, exposed to heat stress, but authors did not propose approaches to prevent/reduce this adverse effect of heat stress.

It was found that Paneth and goblet cells are critical for maintenance of intestinal barrier (Vaishnava, Behrendt et al. 2008, Bevins and Salzman 2011, Johansson and Hansson 2016). Goblet cells are responsible for production of mucins, forming the basic skeleton of mucus layer, which serves as a first line of innate defence. Paneth cells produce different anti-microbial compounds essential for control intestinal barrier and limit bacterial penetration to host tissues. Keeping the integrity of the intestinal barrier is a key for intestinal homeostasis and overall for the health status of the host. It was shown that microbiota and its metabolites can regulate the gut barrier function (Jakobsson, Rodriguez-Pineiro et al. 2015, Kelly, Kennedy et al. 2015).

Exposure to various types of stress results in significant changes in the composition of the gut microbiota and associated complications (Bailey, Dowd et al. 2011). Prebiotics and probiotics have been proposed as a promising approach to normalize microbiota and, as a result to improve intestinal barrier function (Russo, Linsalata et al. 2012, Wilms, Gerritsen et al. 2016). Our previous study showed that fermentate of Saccharomyces cerevisiae was very effective in prevention of heat stress-related complications in rats (traumatic changes of the gut morphology, elevation of serum lipopolysaccharides, pathology of erythrocytes) (Giblot Ducray, Globa et al. 2016). These beneficial effects of yeast fermentate are due to prebiotic activity of this product, previously confirmed in vitro (Possemiers, Pinheiro et al. 2013) and in clinical trials (Pinheiro, Robinson et al. 2017). We hypothesize that EH can protect the gut microbiota and improve intestinal barrier function during heat

stress conditions, thus preventing adverse effects of heat. The main objectives of this study were (1) to evaluate efficacy of yeast fermentate prebiotic (EH) in protection of intestinal barrier integrity during heat stress; (2) to analyse the impact of heat stress and preventive treatment with EH on the structure of the gut microbiota.

5.2. Materials and Methods

5.2.1. Animals

Adult male Sprague–Dawley rats weighing 250–300 grams were purchased from Harlan Laboratories (Indianapolis, IN, USA). Animals were housed under specific pathogen free conditions with a 12-h light/dark cycle at (20 ± 1°C) and were provided with food and water ad libitum. All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee.

5.2.2. Saccharomyces cerevisiae Fermentate

The powder form of S. cerevisiae fermentate (EH) was provided by the manufacturer (Embria Health Sciences, Ankeny, IA, USA). Before oral treatment of rats, the yeast fermentate was diluted in phosphate buffered saline (PBS) at the rate 7 mg per kg of animal weight in 1 mL of PBS.

5.2.3. Antibodies

Primary rabbit polyclonal antibodies against zonula occludence (ZO-1) (#40-2200), occludin (#40-4700), mouse anti-claudin-1-monoclonal antibody (#37-4900) and beta-Actin Loading Control antibody (# MA5-15739) were from ThermoFisher Scientific (Waltham, MA, USA), rabbit polyclonal antibodies against JAM-A (#ab125886) were from Abcam (Cambridge, MA, USA). IRDye 800CW goat anti-rabbit (#926-32211) and IRDye 800CW goat anti-mouse (#926-32210) secondary antibody were from LiCor (Lincoln, NE).

5.2.4. Experimental Design

Animal model of heat stress was successfully used in our previous study (Ducray, Globa et al. 2016). Briefly, two groups of rats (16 rats in each group) were treated by oral gavage with 1 mL of yeast fermentate prebiotic (EH group) or with 1 mL of PBS (PBS group) once a day for 14 days (Fig.1). On day 15, rats in each group were subdivided (8 rats in each group): PC- control (PBS/room temperature), EC- control prebiotic (EH /room temperature), PS- PBS+stress (PBS/45°C), and ES- prebiotic+stress (EH/45°C). Animals from group PS and ES were exposed for 25 min to heat stress conditions (45°C, relative humidity 55%) in a climatic chamber (Environmental Chamber 6020-1, Caron, OH, USA). Control animals (groups PC and EC) were kept at room temperature. Rectal temperature was measured in each rat before and immediately after the experiment. Animals were allowed to stand four hours at room temperature after the experiment, because it was showed that maximal effect of stress on epithelial function was four hours after exposure to stress conditions (Soderholm, Yang et al. 2002, Zareie, Johnson-Henry et al. 2006). After the recommended four hours, rats were anesthetized with isoflurane (2-4%) and euthanized by rapid decapitation. Samples of small intestine from each rat were taken for morphological analysis and Western blot. Faecal matter from the colon was immediately collected and placed into anaerobic broth for culture-based microbiological analysis. For 16S rRNA sequencing of the gut microbiota, faecal samples were stored at -80°C until analysis.

5.2.5. Histological Analysis

Samples of the small intestine were prepared as it was previously described (Giblot Ducray, Globa et al. 2016). Briefly, samples were fixed in Bouin's Fixative

(Electron Microscopy Sciences, Hatfield, PA, USA), embedded in paraffin, sectioned at 6 micrometres, and slide mounted.

5.2.6. Counting of Goblet Cells

Four sections from each rat were stained as previously described (Trevizan, Vicentino-Vieira et al. 2016). Briefly, sections were subjected to a series of deparaffinization, stained with Alcian Blue (Electron Microscopy Sciences, Hatfield, PA) for 15 min, washed with tap and distilled water, treated with 0.5% periodic acid (Electron Microscopy Sciences, Hatfield, PA), washed with distilled water for 2 min, stained with Schiff's Reagent (Electron Microscopy Sciences, Hatfield, PA) for 20 min, washed with tap water for 5 min, stained with haematoxylin (1 min), washed with tap water for 2 min, dehydrated, cleared in HemoDi (Fisher Scientific, Pittsburgh, PA), and mounted in Eukitt Mounting Mediun (Electron Microscopy Sciences, Hatfield, PA). Eight images from each section were taken with a digital camera (Pro series 3CCD camera) coupled to an optical microscope (Olympus BX50) at 20x objective. The number of goblet cells presented in 0.96 mm^2 in the mucosa of each animal were quantified using ImagePro Plus software (Media Cybernetics, Rockville, MD).

5.2.7. Paneth Cells Counting

Phloxine-tartrazine technique were used to analyse Paneth cells, as previously reported (Di Sabatino, Miceli et al. 2008). Briefly, sections were treated with alum hematoxylin (5 minutes), washed with tap water (5 min), stained in phloxine B-calcium carbonate (Electron Microscopy Sciences, Hatfield, PA) for 20 minutes, rinsed in tap water, blot dried, stained saturated solution of tartrazine saturated cellosolve (Electron Microscopy Sciences, Hatfield, PA) for 10 min, rinsed in 95% alcohol, dehydrated in absolute alcohol, cleared in HemoDi (Fisher Scientific,

Pittsburgh, PA), and mounted in Eukitt Mounting Mediun (Electron Microscopy Sciences, Hatfield, PA). The amount of Paneth cells was counted for each sample using a high-resolution microscope system (Vainrub, Pustovyy et al. 2006). Four sections from each rat were analysed.

5.2.8. SDS-PAGE and Western Blotting

Intestinal tissues were snap-frozen in liquid nitrogen and stored at -80°C until study. Tissues were homogenized using T-PER Reagent with Protease Inhibitor Cocktail (Thermo Scientific, Rockford, IL). Samples were centrifuged at 15,000 x g for 30 min at 4°C and supernatants were collected. A protein assay (Bio-Rad) was conducted to determine the protein concentration for each sample. An equal amount of proteins (50 μg) were separated by SDS–PAGE (10%) and transferred to nitrocellulose membranes. The membranes were blocked for 1 hr in Odyssey blocking buffer (LiCor, Lincoln, NE) and incubated overnight at 4°C with primary antibodies against β-actin, claudin, occluding, ZO-1, and JAM-A proteins, respectively. The membranes were washed with PBS/0.1% Tween-20 three times and incubated with goat anti-rabbits IRDye 800CW secondary antibodies for one hour, then washed with PBS/0.1% Tween-20 four times. Membranes were imaged by LiCor Odyssey scanner and blots were analysed by Image Studio 2.0 analytical software (LiCor, Lincoln, NE). The procedure was repeated at least four times for each protein. Bands were standardized to the density of actin and were represented as a ratio of each protein to actin.

5.2.9. Analysis of the Gut Microbiota

5.2.9.1. Culture-Based Microbiological Study

Determination of the gut microbiota was performed according to methods described previously (Sudo, Chida et al. 2004, Nishino, Mikami et al. 2013). Faecal matter was aseptically removed from the colon of each rat, placed into sterile preweighed tubes containing anaerobic broth, reweighed, and vortexed until homogenous. Serial tenfold dilutions from 10^{-1} to 10^{-7} were prepared and from the appropriate dilution, a 0.1 ml aliquot was then spread onto replicates of four plates with different media: Anaerobic Basal Agar (Alfa Aesar, Tewksbury, MA, USA) for total anaerobic bacteria; Brain Heart Infusion Agar (Hardy Diagnostic, Santa Maria, CA, USA) for total aerobes; Blood agar (Hardy Diagnostic, Santa Maria, CA, USA) for haemolytic bacteria; Violet Red Bile Agar (Hardy Diagnostic, Santa Maria, CA, USA) for *Enterobacteriaceae*; Bifidobacterium agar (HiMedia Laboratories, West Chester, PA, USA) for *Bifidobacterium*; Difco Lactobacilli MRS agar (Becton Dickinson, Sparks, MD, USA) for *Lactobacillus*; BBL Mannitol Salt agar (Becton Dickinson, Sparks, MD, USA) for *Staphylococcus*; Brucella agar with hemin and vitamin K1 (HiMedia Laboratories, West Chester, PA, USA) for *Bacteroides*; Reinforced Clostridial Medium (Hardy Diagnostic, Santa Maria, CA, USA) for *Clostridium*; Sabouraud agar (HiMedia Laboratories, West Chester, PA, USA) for yeasts. For isolation of anaerobic bacteria plates were placed in an anaerobic chamber in a microaerophilic environment generated by a GasPak EZ Anaerobe Container System (Becton Dickinson and Co, Sparks, MD, USA). All plates were incubated at 37°C and colonies were counted after incubation of 24 hr for aerobes and 48 hr for anaerobes. The number of colony-forming units (CFU) per gram of faecal matter was calculated. Bacterial cultures and yeasts were identified by morphology of colonies,

microscopical analysis of cells' morphology, Gram staining, formation of spores, and aerobic and anaerobic growth, as it was recommended elsewhere (Benno and Mitsuoka 1992; Sudo, Chida et al. 2004).

5.2.10. High-throughput 16S rRNA Sequencing of the Gut Microbiota

5.2.10.1. DNA Extraction and bTEFAP

Faecal samples were submitted to MR DNA (Shallowater, TX, USA) for DNA isolation and sequencing. Genomic DNA was isolated from samples using a QIAamp DNA stool mini kit (Qiagen, Germantown, MD) following the manufacturer's instructions. The purified DNA was eluted from the spin filter using 50uL of solution C6 and stored at -20°C until PCR amplification.

Amplicon sequencing using next generation technology (bTEFAP) was originally described by Dowd et al. (Dowd, Callaway et al. 2008). The 16s rRNA V1-V3 primers, 27F AGRGTTTGATCMTGGCTCAG and 519R GTNTTACNGCGGCKGCTG, were utilized to evaluate the microbial ecology of each sample on the MiSeq with methods via the bTEFAP DNA analysis service. Each sample underwent a single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) were used under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute; after which a final elongation step at 72°C for 5 minutes was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing the Illumina MiSeq chemistry following manufacturer's protocols.

The Q25 sequence data derived from the sequencing process was processed using a proprietary analysis pipeline (MR DNA, Shallowater, TX). Sequences were

depleted of barcodes and primers then short sequences < 200bp were removed, sequences with ambiguous base calls removed, and sequences with homopolymer runs exceeding 6bp removed. Sequences were then denoised and chimeras removed. Operational taxonomic units (OTUs) were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity). OTUs were then taxonomically classified using BLASTn against a curated NCBI database.

5.2.11. Statistics

All results were presented as a mean and standard deviation. The difference between groups were analysed by the one-way ANOVA, followed by the Bonferroni test. The significance level was set at 0.05 to define statistical significance. Statistical calculations and graph plotting were carried out using Microcal Origin version 9.0 (Northhampton, MA) and 2010 Microsoft Excel. Statistical analysis of sequence results was performed using a variety of computer packages including XLstat, NCSS 2007, "R", and NCSS 2010. Significance reported for any analysis is defined as $p<0.05$.

5.5.3. Results

5.3.1. Body Temperature

Body temperature of rats exposed to heat stress conditions (PS and ES groups) significantly increased. The mean body temperature of groups was 37.7 ± 0.7°C before and 40.5 ± 0.6°C immediately after stress, $p<0.05$. No change in body temperature of control rats (PC and EC groups) was found (Before 37.9 ± 0.7°C and after 37.8 ± 0.5°C, $p>0.05$)

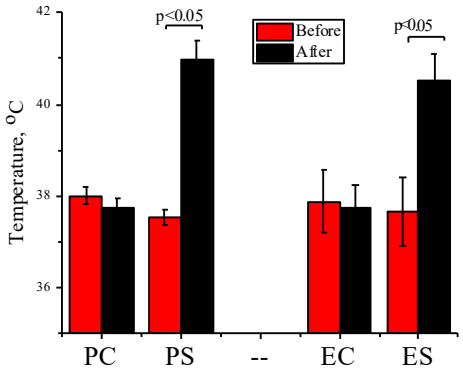


Figure 5.3.1.1. Core Body Temperature.

Rectal temperature recordings for each rat were taken before and immediately after environmental heat stress using an electronic digital thermometer, inserted to a depth of 4 centimetres.

PC- (PBS/no stress), EC- (Yeast fermentate/no stress), PS- (PBS/heat stress), and ES- (Yeast fermentate /heat stress).

5.3.2. Tight Junction Proteins Expression

Expression of tight junction proteins (occludin, claudin, ZO-1, and JAM-A) in the intestine of all rats was analysed by Western blot. Expression of all tested proteins was significantly depressed in animals from PS group in comparison with other groups ($p<0.05$) (Fig 5.3.2.1. A-E). Pre-treatment with EH before exposure to heat stress (group ES) resulted in significantly increased level of all proteins in comparison with PS group ($p<0.05$), though lower in comparison with EC group.

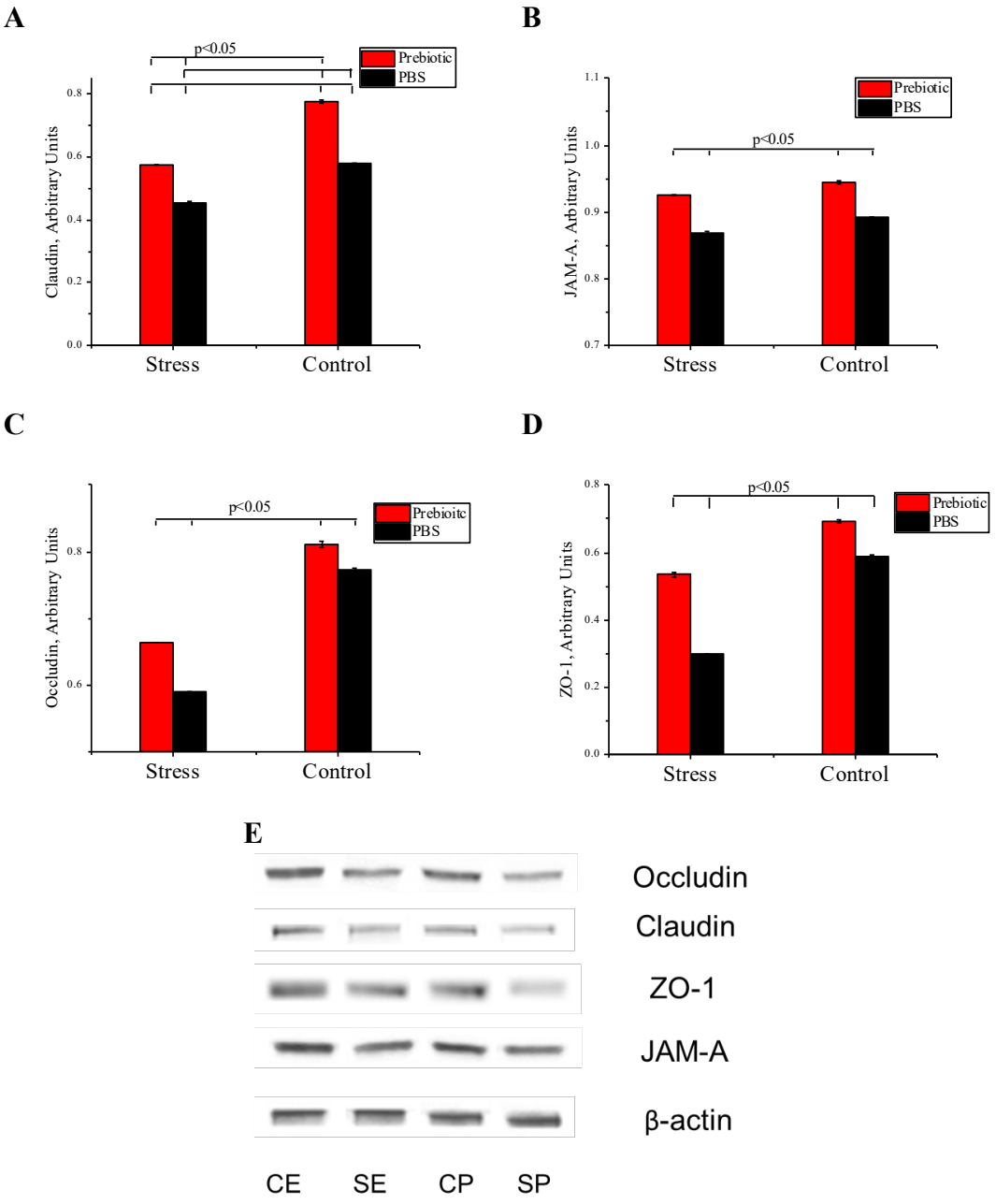


Figure 5.3.2.1. Tight Junction Proteins Expression.

A-E: Expression of tight junction proteins (Claudin, JAM-A, Occludin, and ZO-1) by Western Blot. Rats were pre-treated with PBS (■) or yeast fermentate prebiotic (■) by oral gavage before exposure to exposure to environmental heat stress (Stress) or no exposure (Control).

PC- (PBS/no stress), EC- (Yeast fermentate/no stress), PS- (PBS/heat stress), and ES- (Yeast fermentate /heat stress).

5.3.3. Paneth Cells Number

The number of Paneth cells in rats, exposed to heat stress (groups PS and ES), was significantly lower in comparison with control groups (PC and EC). Supplementation of rats with EH prior to heat stress (ES group) prevented the loss of Paneth cells in comparison with rats, pre-treated with PBS (PS group) (1.61 ± 0.07 and 1.12 ± 0.07 accordingly, $p<0.05$) (Fig. 5.3.3.1.).

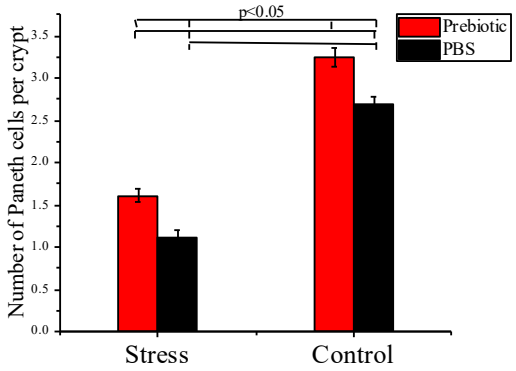


Figure 5.3.3.1. Paneth Cells Count in Small Intestine.

Paneth cell count in rats from different experimental groups. Each group consisted of four rats. Quantification of Paneth cells was performed for four sections for each rat using a high resolution microscope system. A: Rats were pre-treated with PBS (■) or yeast fermentate prebiotic (■) by oral gavage before exposure to exposure to environmental heat stress (Stress) or no exposure (Control).

PC- (PBS/no stress), EC- (Yeast fermentate/no stress), PS- (PBS/heat stress), and ES- (Yeast fermentate /heat stress).

5.3.4. Goblet Cells Count

The number of goblet cells was significantly decreased in rats from PS group in comparison with control rats (group PC) (448.8 ± 8.4 and 940.8 ± 8.4, accordingly, $p<0.05$). Goblet cell count in intestine of heat stressed rats pre-treated with EH (group ES) was lower than in non-stressed rats from EC group (883.8 ± 7.8 and 1251 ± 6.6 accordingly, $p<0.05$), but significantly higher than in animals, pre-treated with PBS

before exposure to heat stress (PS group) (448.8 ± 8.4) (Fig. 5.3.4.1.). Treatment of control rats with EH (EC group) resulted in significant elevation of goblet cells in comparison with control PC group (1251 ± 6.6 and 940.8 ± 8.4, respectively, $p<0.05$).

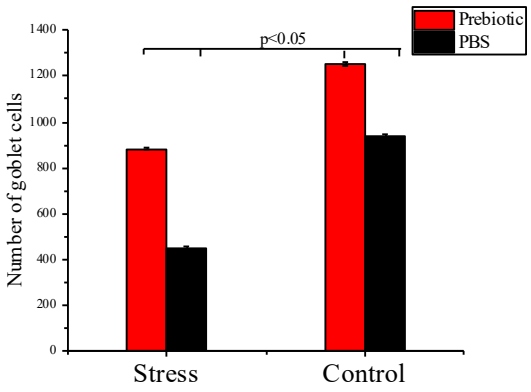


Figure 5.3.4.1. Goblet Cells Quantification in Small Intestine.

Goblet cells count in rats from different experimental groups. Each group consisted of four rats. Six images from each section were taken with a digital camera (Olympus BX50) coupled to an optical microscope with an objective of 20x. The number of goblet cells present in a 0.96 mm^2 field of vision in the mucosa of each animal were quantified by using ImagePro Plus software (Media Cybernetics). A: Rats were pre-treated with PBS (■) or yeast fermentate prebiotic (■) by oral gavage before exposure to environmental heat stress (Stress) or no exposure (Control).

PC- (PBS/no stress), EC- (Yeast fermentate/no stress), PS- (PBS/heat stress), and ES- (Yeast fermentate /heat stress).

5.3.5. Culture-Based Analysis of the Gut Microbiota

Analysis of the gut microbial community in rats from different experimental groups revealed the significant decrease in the anaerobic to aerobic bacteria ratio in rats from PS group in comparison with all other groups. No difference in this ratio was found in rats, treated with EH (Fig. 5.3.5.1. A). Significant elevation of *Escherichia* spp. (Fig. 5.3.5.1. B), haemolytic bacteria (Fig. 5.3.5.1. C), and *Staphylococcus* spp. (Fig. 5.3.5.1. D) was found in rats from PS group. The number of *Staphylococcus* spp. and haemolytic bacteria was significantly higher in animals from

ES group in comparison with PC group, but significantly lower than in animals from PS group. No difference in *Bifidobacterium* spp. number was observed in groups of animals, pre-treated with PBS (PC, PS), but treatment with EH resulted in significant elevation of these bacteria (groups ES, EC) (Fig. 5.3.5.1. E). The highest number of *Lactobacillus* spp. was revealed in rats pre-treated with PBS before exposure to heat stress conditions (Fig. 5.3.5.1. F). Treatment with EH did not affect *Lactobacillus* spp. number.

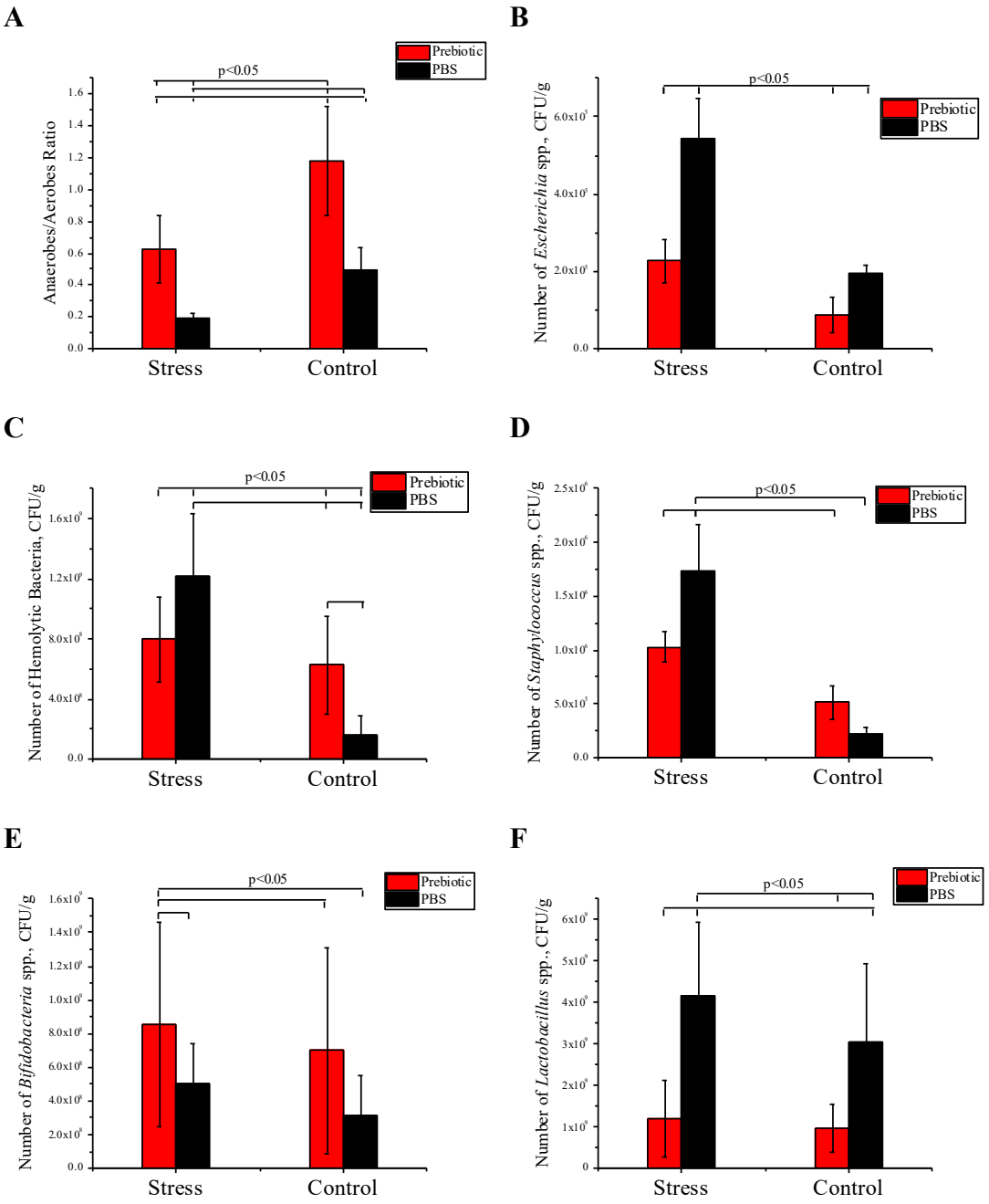


Figure 5.3.5.1. Bacteriological Analyses of the Gut Microbiota.

Bacteriological analysis of rats pre-treated with PBS (■) or yeast fermentate prebiotic (■) by oral gavage before exposure to environmental heat stress (Stress) or no exposure (Control). Faecal samples were analysed for anaerobic to aerobic bacteria ratio (A), *Escherichia* spp. (B), haemolytic bacteria (C), *Staphylococcus* spp. (D), *Bifidobacterium* spp. (E), and *Lactobacillus spp.* (F). Values are expressed as means of colony-forming unit (CFU) per gram of faecal matter.

PC- (PBS/no stress), EC- (Yeast fermentate/no stress), PS- (PBS/heat stress), and ES- (Yeast fermentate /heat stress).

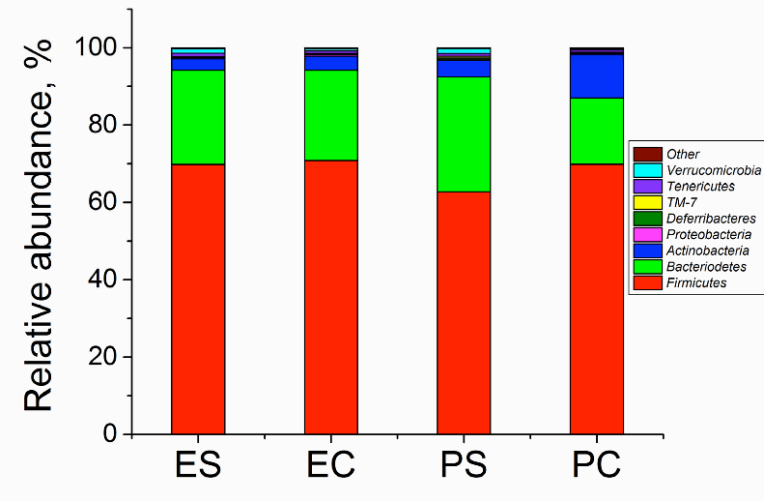
5.3.6. 16S rRNA Sequencing of the Gut Microbiota

After stringent quality sequence curation, a total of 1565513 sequences were parsed and 1382946 were then clustered. 1382796 sequences identified within the Bacteria and Archaea domains were utilized for final microbiota analyses. The average reads per sample was 60121. Ten different phyla were identified. The most abundant phyla in the gut microbiota of rats from different experimental groups were *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* (Fig. 5.3.6.1. A). *Firmicutes* was a dominant phylum (68.3%), following by *Bacteroidetes* (23.6%) and *Actinobacteria* (5.5%). Significantly higher number of *Actinobacteria* was found in PC group (11.3±1.8%, $p<0.05$), while *Bacteroidetes* were prevalent in PS group (29.7±4.8%, $p<0.05$).

At the genus level the most significant changes were found in PS group in comparison with control PC group (Figure 5.3.6.1. B; Table 5.3.6.1.). A total of 14 genera were affected by heat stress. Some genera considerably increased (*Alistipes, Bacteroides, Tannerella, Acetanaerobacterium, Oscillibacter, Johnsonella, Akkermansia, Staphylococcus*), whereas others (*Bifidobacterium, Allobaculum, Enterorhabdus, Pedobacter, Holdemanella*) significantly decreased. *Bilophila* was absent in rats from PC group but were detected in PS rats. Treatment of rats with the yeast fermentate before exposure to heat stress (ES group) resulted in fewer changes in the gut microbiota structure. Only 9 genera were significantly changed: relative abundance of *Bifidobacterium* and *Allobaculum* were declined, while *Eubacterium, Bacteroides, Oscillospira, Roseburia, Johnsonella, Vallitalea, Acetanaerobacterium*, and *Lactococcus* substantially increased. *Akkermansia* and *Staphylococcus* were significantly higher only in rats from PS group in comparison with animals from PC

group. Minor changes in the gut microbiota was found in EC group of rats in comparison with PC group- only *Bifidobacterium* significantly decreased.

A



B

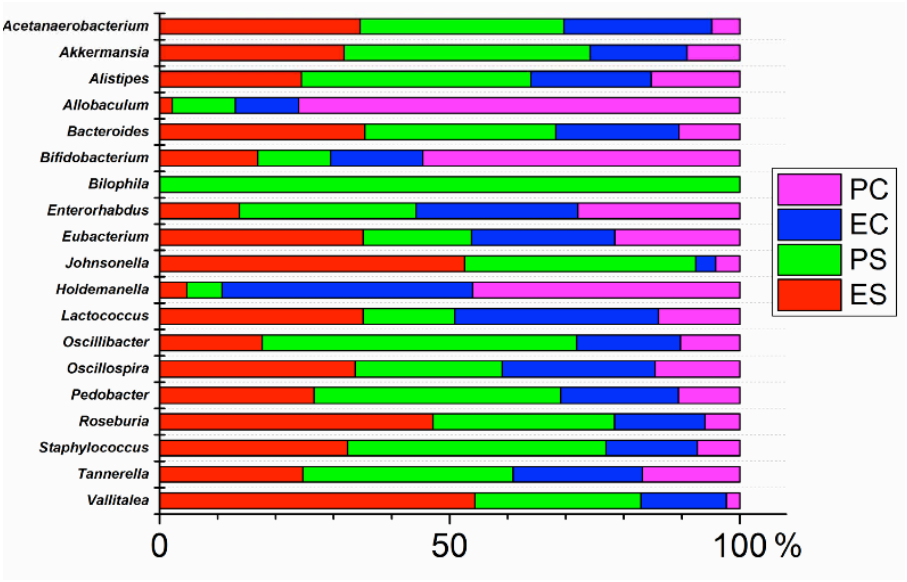


Figure 5.3.6.1. Results of 16s rRNA Sequencing of the Gut Microbiota.

16s rRNA sequencing of the gut microbiota of rats. A: Composition of the gut microbiota of rats from different experimental groups at the phylum level. All phyla present in abundance of <0.1% are included as other. B: Microbial composition of the gut microbiota in different groups are presented as a percent of abundance.

PC- (PBS/no stress), EC- (Yeast fermentate/no stress), PS- (PBS/heat stress), and ES- (Yeast fermentate /heat stress).

Table 5.3.6.1. Changes in the Gut Microbiota Genera.

	PC		PS		PS vs PC	P value
	Mean	SEM	Mean	SEM	Changes, %	
Acetanaerobacterium	0.0035	9.1E-4	0.02518	0.00606	619.46034	0.00532
Akkermansia	0.31695	0.11	1.49	0.44	370.10246	0.02198
Alistipes	2.81	0.55	7.29768	1.5	159.7039	0.01828
Allobaculum	4.76	1.2	0.57	0.4	-88.02521	0.01135
Bacteroides	0.88	0.12831	2.77	0.53325	214.77273	0.00629
Bifidobacterium	10.62	0.69	2.44	0.87	-77.02448	0.00151
Bilophila	0	0	0.0016	0.0007	PS*	
Enterorhabdus	0.05369	0.01019	0.02654	0.00345	-50.56175	0.03017
Johnsonella	7.3E-4	3.3E-4	0.008	0.0036	995.89041	0.04045
Holdemanella	3.55446	0.82404	0.47546	0.21575	-86.62364	0.00473
Oscillibacter	0.007	0.00257	0.03517	0.01076	402.44497	0.02741
Pedobacter	0.06	0.00213	0.02414	0.00524	-59.77354	0.00936
Tannerella	0.09	0.01782	0.21375	0.02428	137.50294	0.00342
Staphylococcus	0.35861	0.08092	2.2036	0.71853	514.48697	0.03409

	PC		ES		ES vs PC	P value
	Mean	SEM	Mean	SEM	Changes, %	
Acetanaerobacterium	0.0035	0.0009	0.02482	0.00811	615.99307	0.02581
Allobaculum	4.7639	1.2169	0.21861	0.06911	-95.41103	0.02570
Bacteroides	0.8846	0.1283	2.98014	0.48125	236.87424	0.00181
Bifidobacterium	10.62	0.6909	3.29	1.77	-69.02072	0.00256
Eubacterium	4.0526	0.5370	6.59424	0.60384	62.71752	0.01042
Johnsonella	0.0007	0.0003	0.00924	0.00141	1156.98303	0.00015
Lactococcus	0.00503	0.00158	0.10448	0.04153	214.11531	0.04366
Oscillospira	0.9808	0.1301	2.25787	0.31569	130.20434	0.00384
Roseburia	0.0268	0.0049	0.20908	0.04712	679.21596	0.00323
Vallitalea	4.88E-4	3.09E-4	0.01137	0.00379	2231.24214	0.01690

	PC		EC		EC vs PC	P value
	Mean	SEM	Mean	SEM	Changes, %	
Bifidobacterium	10.62043	0.69099	3.08779	0.97784	-70.92597	0.00012

*Genus was found only in this group

5.5.4. Discussion

This study aimed to evaluate the efficacy of a *Saccharomyces cerevisiae* fermentate (EH) in preventing the disruption of the intestinal barrier function through modulating of the gut microbiota during heat stress. Exposure of rats, pre-treated with PBS, to heat stress conditions resulted in significant decrease of occludin, claudin, ZO-1, and JAM-A expression. Decreased expression of TJ proteins during heat stress was found in Caco-2 cells (Gupta, Chauhan et al. 2017) and in animal studies (Wu, Liu et al. 2018). Inhibition of these proteins expression indicates the disturbance of the TJ barrier functions and accompanied by intestinal permeability (He, Liu et al. 2016). Our results showed that oral administration of EH to rats before heat stress significantly enhanced TJ proteins expression. In previous studies this fermentate demonstrated prebiotic activity by protecting against inflammation (Possemiers, Pinheiro et al. 2013) and improving gastrointestinal discomfort in patients (Pinheiro, Robinson et al. 2017). The findings of other authors revealed a positive role of prebiotics in supporting the normal intestinal barrier function. Thus, the dietary use of inulin-enriched pasta by healthy volunteers protected intestinal barrier functioning during physical exercise (Russo, Linsalata et al. 2012). Cani et al. (Cani, Possemiers et al. 2009) found that oligofructose enriched diet contribute to the improvement of gut barrier function in obese mice by up-regulation of TJ proteins expression.

We observed that heat stress resulted in significant decrease of Paneth and goblet cells in the intestine of rats. Paneth and goblet cells are essential components of the intestinal epithelium and contribute to the barrier function of epithelium (Furness, Rivera et al. 2013). Depletion of these cells may lead to the development of an epithelial barrier defect (Estienne, Claustre et al. 2010). Reduction of Paneth and goblet cells was shown to increase sensitivity of mice to TNF-induced toxicity,

accompanied by increased hypothermia, lethality, and intestinal permeability (Van Hauwermeiren, Vandenbroucke et al. 2015). Decrease of these cells induced by different stress conditions, such as neonatal maternal separation (Bessette, Henry et al. 2016), and chronic and heat stress (Deng, Dong et al. 2012, Gao, Cao et al. 2018). Our results revealed that pre-treatment of rats with EH before exposure to heat stress prevented decline of Paneth and goblet cells. The beneficial effect of beta-glucans, a component of S. cerevisiae cell wall, as a dietary supplement for stabilization of the number of goblet cells in chickens was demonstrated by Zhao et al. (Zhao, Goldflus et al. 2009). Prebiotic inulin in combination with rutin reduced inflammatory status and endoplasmic reticulum stress in Paneth cells (Guo, Tang et al. 2018). Paneth and goblet cells are indispensable for maintaining homeostasis with enteric microbes (Baurhoo, Phillip et al. 2007, Vaishnava, Behrendt et al. 2008), as they promote the removal of microbes from the mucosal surface (Chairatana and Nolan 2017). Reduction in number or defects in activity of these cells lead to microbiota dysbiosis (Baurhoo, Phillip et al. 2007, Riba, Olier et al. 2017).

Our data showed significant changes in the gut microbiota only in rats from PS group with substantial depletion of Paneth and goblet cells. Thus, culture-based bacteriological analysis of the gut microbiota revealed decrease in anaerobic to aerobic bacteria ratio in these animals. It is well known that most microorganisms in the distal small intestine and colon are anaerobes (Weng and Walker 2013), which numerously exceed aerobic bacteria in the gut (Maity, Adak et al. 2012). The predominance of aerobic bacteria in the gut microbiota has been found in the patients with colon cancer (Vargo, Moskovitz et al. 1980), necrotizing fasciitis (Saini, Gupta et al. 2004), in malnutrition (Million, Alou et al. 2016), and in severely burned patients (Chen, Zhang et al. 1998), indicating disbalance of the intestinal microbiota.

We also found significant increase of haemolytic bacteria, *Escherichia* spp., and *Staphylococcus* spp. in rats of PS group. Elevated number of bacteria with haemolytic activity indicates the microbiota disorder (Popova, Kaftyreva et al. 2017), as these bacteria can be a potentiator of intestinal inflammation and epithelial dysfunction in the gut (Wiegand, Zakrzewski et al. 2017). Imbalance in quantitative composition of *Escherichia* spp. and *Staphylococcus* spp. also specifies dysbiotic changes of the gut microbiota (Popova, Kaftyreva et al. 2017, Itani, Moubareck et al. 2018). The number of *Lactobacillus* spp. was significantly higher in rats of PS group in comparison with other groups of animals. The effect of stress on *Lactobacillus* spp. in the gut is estimated differently by researchers. Some of them observed an increase of *Lactobacillus* spp. during chronic stress (Wong, Inserra et al. 2016), while others reported about the depletion of these bacteria in stressed animals (Marin, Goertz et al. 2017). Treatment with EH did not change the relative abundance of *Lactobacillus* spp.. The same results were obtained with EH in clinical trial (Pinheiro, Robinson et al. 2017). We did not find the difference in *Bifidobacterium* spp. number in groups of rats pre-treated with PBS (PS and PC groups). But administration of EH significantly increased the number of *Bifidobacterium*. Positive effect of EH on *Bifidobacterium* was previously observed in vitro study (Possemiers, Pinheiro et al. 2013). Stimulation of *Bifidobacterium* in the gut of elderly people by prebiotic supplementation found in clinical trials (Guigoz, Rochat et al. 2002).

High-throughput 16S rRNA gene sequencing revealed that in all groups of rats *Firmicutes* was a dominant phylum, what is in accordance with the data of other authors (Golubeva, Crampton et al. 2015, Byerley, Samuelson et al. 2017). Significant changes of the gut microbiota in different groups were found at the genus taxonomic level. Exposure of rats to heat stress conditions (PS group) resulted in substantial

decrease of beneficial bacteria (*Allobaculum, Bifidobacterium*) in comparison with control (PC) group. Beneficial effects of these bacteria were shown in many studies. Thus, *Allobaculum* was associated with prevention of obesity and insulin resistance (Everard, Lazarevic et al. 2014), *Bifidobacterium* are known as a normal component of the gut microbiota and as probiotics for human and animal consumption (Russell, Ross et al. 2011). *Enterorhabdus* and *Pedobacter* were also decreased in PS group of rats. *Enterorhabdus* was shown to be associated with autism spectrum disorder in a murine model (de Theije, Wopereis et al. 2014) and with a genetic variant of the human leukocyte antigen complex that has been related to inflammatory diseases (Opstelten, Plassais et al. 2016). *Pedobacter*, heparinase-producing bacteria, are a normal component of the gut microbiota of healthy fish (Wang, Sun et al. 2018) and of the medicinal leech (Ott, Rickards et al. 2015). Significant increase of pathogenic bacteria (*Alistipes, Bacteroides, Bilophila, Johnsonella, Oscillibacter, Tannerella, Staphylococcus*) was found in PS group. This result corresponds to our data from the culture-based analysis of the gut microbiota, testifying that elevation of pathogenic bacteria was observed only in rats from PS group. *Alistipes*, *Bacteroides*, and *Bilophila* were overrepresented in the carcinoma patients (Feng, Liang et al. 2015). *Bilophila* is one of the most common anaerobic bacteria recovered from patients with perforated and gangrenous appendicitis (Baron 1997). It was shown, that increased number of *Bilophila* induces systemic inflammation and contributes to the commencement of the chronic diseases (Feng, Long et al. 2017). *Johnsonella* was highly associated with tumour site (Pushalkar, Ji et al. 2012) and with chronic obstructive pulmonary disease (Wu, Chen et al. 2017), *Tannerella* was found to be a predisposing factor in atherosclerosis progression (Lee, Jun et al. 2014). Our data show that stress results in significant increase of *Oscillibacter*, which is known as a

potential opportunistic pathogen, positively correlated with gut permeability (Lam, Ha et al. 2012). We hypothesize that *Oscillibacter* bacteria could be related to the disturbance in the TJ proteins expression, observed in PS group. Two genera (*Acetanaerobacterium* and *Akkermansia*) were elevated after heat stress. There is some evidence of beneficial effects of *Acetanaerobacterium*, associated with the high production of enterolactone (Hullar, Lancaster et al. 2015), which may protect against hormone-dependent cancers and cardiovascular diseases (Kilkkinen, Stumpf et al. 2001). *Akkermansia muciniphila* is a mucin-degrading bacterium, considered by some authors as an important member of the gut microbiota for control of physiological and homeostatic functions during obesity and type 2 diabetes (Everard, Belzer et al. 2013). Conversely, other studies showed that increased abundance of *A. muciniphila* is related to hypertension (Tain, Lee et al. 2018) and can impair intestinal barrier function after using mucin by these bacteria as a nutrient (Desai, Seekatz et al. 2016). Depletion of the mucus layer by enriched *A. muciniphila* was associated with higher susceptibility to a gastrointestinal pathogen. Analysis of the gut microbiota in PS group indicate that disturbance in the microbial community is mostly due to the increase of pathogenic bacteria. Our results revealed that *Akkermansia* number was considerably higher only in PS group, where intestinal barrier function was disrupted. Previously, we showed that exposure of rats to heat stress conditions significantly decreases the total thickness of intestinal mucosa (Giblot Ducray, Globa et al. 2016). Treatment with EH before stress (group ES) prevented the increase of *Akkermansia* and destruction of intestinal barrier. These results are consistent with data from Desai et al. (Desai, Seekatz et al. 2016), who found that abundance of *A. muciniphila* increased rapidly in the absence of prebiotic. We found significant decrease of two genera (*Allobaculum, Bifidobacterium*) and increase of *Acetanaerobacterium*,

Bacteroides, Johnsonella in the gut microbiota of rats from ES group vs PC group. Same trend was present in the PS group, which indicates the specific effect of stress on these groups of bacteria. Essential impact of the EH on microbiota during heat stress is manifested in elevated number of beneficial bacteria (*Eubacterium*, *Lactococcus, Oscillospira, Roseburia, Vallitalea*). *Roseburia, Eubacterium*, and *Oscillospira* are butyrate-producing bacterial genera, positively correlated with antioxidant activities and negatively correlated with inflammation (Gophna, Konikoff et al. 2017, Wang, Xie et al. 2018). Our results are consistent with previously in vitro study of Possemiers et al. (Possemiers, Pinheiro et al. 2013), who showed that EH induces butyrate production and possess anti-inflammatory activity. Butyrate is recognized as an essential host energy source (Donohoe, Garge et al. 2011), which can protect the mucus layer from injury (van der Beek, Dejong et al. 2017). Positive contribution of *Lactococcus* and *Vallitalea* to the change of microbiota noticed by other authors in humans and animals (Borrelli, Coretti et al. 2017, Mao, Cubillos-Ruiz et al. 2018, Savage, Lee-Sarwar et al. 2018).

We did not find significant changes of the gut microbiota in EC group, except for the decreased abundance of *Bifidobacterium*. Data about lower number of *Bifidobacterium* in PS, ES, and EC groups are in contrast with culture-based results. Other authors also reported that species, isolated from culture, did not generally correspond with the most abundant genera in the gut microbiome analysis (Koeller, Herlemann et al. 2018). For example, increased *Bifidobacterium* abundance was detectable only with an in vitro culture method, and not pyrosequencing (Li, Finegold et al. 2014). It was shown that the abundance of *Bifidobacterium* in humans and animals is underestimated with 16S rRNA gene-based approach (Hooda, Boler et al. 2012).

5.5.5. Conclusion

Overall, our results demonstrated the substantial effect of prebiotic EH in the prevention of heat stress-related complications. We assume, that this effect is associated with the beneficial modulation of the gut microbiota by the prebiotic.

Chapter 6 - *Bacillus subtilis* Probiotic Strain Mitigates Heat-Related Adverse Effects of Metabolic Stress

Abstract

Heat-related adverse effects of metabolic stress have significant impacts to the intestinal barrier integrity, resulting in increased gut permeability and systemic inflammation. The stability of the gut microbiota plays a key role in maintaining the intestinal barrier function. Thus, modulation of the gut microbiota can regulate the intestinal barrier and prevent its loss of function. The main objective of this study was to investigate whether a *Bacillus subtilis* BSB3 probiotic bacterium can mitigate heat-related adverse effects of metabolic stress. Exposure of animals to heat stress conditions (forced running) resulted in significant morphological changes (reduced expression of tight junction proteins, reduced number of Paneth and goblet cells, and reduced villi height and total mucosal thickness) and changes in the gut microbiota. Culture-based analysis of the gut microbiota revealed dysbiotic changes only in rats pre-treated with PBS prior to forced running. Those animals had decreases in anaerobic to aerobic bacteria and *Firmicutes* to *Bacteroidetes* ratios and significant increases in pathobionts: haemolytic bacteria, *Candida* spp., and *Bacteroides* spp.. High-throughput 16S rRNA gene sequencing detected significant changes in the gut microbiota at the genus taxonomic level with increases in pathobionts and decreases in beneficial bacteria. Oral treatment of rats with the probiotic prior to forced running significantly prevented all registered morphological and microbial adverse effects. We

assume that effect of *Bacillus subtilis* BSB3 in prevention of heat stress-related complications is associated with the beneficial modulation of the gut microbiota.

6.1. Introduction

Stress causes severe adverse effects and such consequences have been reported in the gastrointestinal tract (Holdeman, Good et al. 1976, Ducluzeau, Ladire et al. 1984, Lizko 1987, Bailey and Coe 1999, Everson and Toth 2000, Bailey, Lubach et al. 2004, Knowles, Nelson et al. 2008, Gareau, Wine et al. 2010, Sekirov, Russell et al. 2010). Recently, strenuous and intensive exercise have been shown to act as a stressor on the gastrointestinal tract (ter Steege and Kolkman 2012, van Wijck, Lenaerts et al. 2012, Bermon, Petriz et al. 2015, Hsu, Chiu et al. 2015, Clark and Mach 2016, Grootjans, Lenaerts et al. 2016, Davidson, Cooke et al. 2018). The impact of intensive exercise on splanchnic blood flow, intestinal ischemia, cytokine activity, gastrointestinal motility, nutrient absorption, and intestinal permeability have been studied (Lambert 2009, ter Steege and Kolkman 2012, Horner, Schubert et al. 2015, Peake, Della Gatta et al. 2015, Costa, Snipe et al. 2017). Few have taken into consideration the effect of exercise-related hyperthermia on health (Gill, Teixeira et al. 2015, Dokladny, Zuhl et al. 2016, Costa, Snipe et al. 2017).

Temperature is one of the most severe stressors affecting health. Elevated body temperature has been linked to various gastrointestinal disturbances (Pals, Chang et al. 1997, Lambert 2004, Yeh, Law et al. 2013, Dokladny, Zuhl et al. 2016, Mach and Fuster-Botella 2017). During heat stress the redistribution of blood flow to vital organs, muscles, and extremities occurs in order to reduce the impact of body temperature elevation (Bradbury and Ruckley 1996, Brandt 2000, Ceausu, Hostiuc et al. 2010). Intestinal ischemia is a frequently observed occurrence and is part of the normal physiological response to metabolic heat stress (Perko, Nielsen et al. 1998, van Wijck, Lenaerts et al. 2011).

Exercise-induced hyperthermia has been linked to the loss of the intestinal

barrier, the first protective barrier between lumenal content and circulation. Dysfunction of this barrier results in increased intestinal permeability and diffusion of bacterial endotoxins into the circulation, which can lead to multiorgan injuries (Lambert 2004, Bischoff, Barbara et al. 2014, King, Leon et al. 2015, Zhang, Hornef et al. 2015). An elevated level of lipopolysaccharides (LPS) accompanies various pathological conditions and serves as a marker for the dysfunction of the intestinal barrier (Sekirov, Russell et al. 2010, Gill, Allerton et al. 2016, Chelakkot, Ghim et al. 2018). The loss of the barrier is due to either physical breaks in the epithelium, damage to multi-protein complexes of the tight-junction proteins and/or the promotion of the dysfunction to tight-junction regulatory proteins (van der Flier and Clevers 2009, Zuhl, Schneider et al. 2014, Mach and Fuster-Botella 2017).

The gut microbiota and their metabolites are an essential factor that regulates the intestinal barrier function (Sekirov, Russell et al. 2010, de Vos and de Vos 2012, Viggiano, Ianiro et al. 2015, Karl, Margolis et al. 2017). Stress compromises and disrupts the stability of gut microbiota, which impacts the ability of the organism to tolerate stress (Berg, Muller et al. 1999, Karl, Margolis et al. 2017, Chelakkot, Ghim et al. 2018). The gut microbiota is a major element in the development and the maintenance of the intestinal barrier and acts as a component and modulator of the intestinal barrier function. The gut microbiota regulates the expression of tight junction proteins, the development and activity of epithelial cells, and the immunological components of the intestinal barrier (Sekirov, Russell et al. 2010, Ulluwishewa, Anderson et al. 2011, Al-Asmakh and Hedin 2015, Zhang, Hornef et al. 2015, Thevaranjan, Puchta et al. 2017, Chelakkot, Ghim et al. 2018). Beneficial probiotic bacteria contribute to enhance the gastrointestinal barrier function and ameliorate the gut permeability (Ohland and MacNaughton 2010, Ait-Belgnaoui,

Colom et al. 2014). Thus, restoration of the gut microbiota can present a novel approach for prevention of stress-related adverse effects. Prebiotics, probiotics, synbiotics, special diets, and faecal transplants have been proposed as promising approaches to normalize the gut microbiota, preventing the adverse impacts observed on health.

Bacillus bacteria are ideal probiotic candidates as they have been shown to have the capacity to improve exertional performance, positively modulate the intestinal microbiota, inhibit pathogen colonization, modulate the intestinal barrier function, prevent cell damage, and enhance immunity in the gastrointestinal tracts of treated animals (La Ragione and Woodward 2003, Lee, Lee et al. 2010, Ohland and MacNaughton 2010, Wolfenden, Pumford et al. 2011, Sen, Ingale et al. 2012, Park and Kim 2014, Gill, Allerton et al. 2016, Jager, Shields et al. 2016, Gepner, Hoffman et al. 2017). Previously, the efficacy of *Bacillus subtilis* BSB3 in the prevention of stress-related complications has been shown in an environmental heat stress model (Moore, Globa et al. 2014). Therefore, this study was undertaken to investigate whether *Bacillus subtilis* BSB3 can mitigate heat stress-related adverse effects of metabolic stress.

6.2. Materials and Methods

6.2.1. Ethics Statement

All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee. The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

6.2.2. Animals

Adult male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) weighing 250–300 grams were used in this study. The animals were housed two per cage under specific pathogen-free conditions and were acclimatized for 2 days prior to experimentation at a temperature of 20 ± 1°C and standard lighting (12-hour day/12-hour night) with free access to water and standard food (2018 Teklad Global 18% Protein Rodent Diet, Harlan, Indianapolis, IN, USA)

6.2.3. Bacillus subtilis

Bacillus subtilis BSB3 were cultivated on Difco sporulation medium (DSM) (Difco Nutrient Broth, Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 5 days. Bacteria were harvested by flooding the surface of the culture with sterile phosphate buffered saline (PBS) followed by scraping with a sterile cell spreader. The bacterial suspension was diluted in PBS to achieve $1x10^8$ CFU per millilitre.

6.2.4. Experimental Design

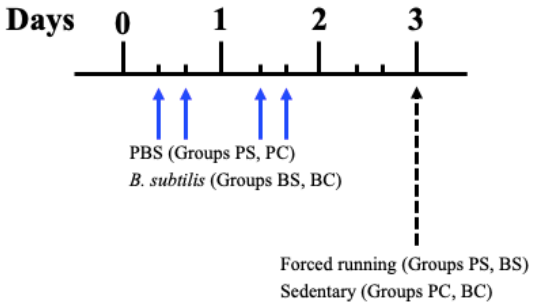


Fig. 6.2.4.1. Experimental Design.

Animals (n = 16) were treated by oral gavage either with *Bacillus subtilis* BSB3 strain (10^8 CFU in 1 millilitre of PBS per rat) (*B. subtilis*; n = 8) or 1 millilitre of PBS (PBS; n = 8). Pre-treatment of animals with PBS or probiotic occurred twice a day for 2 days prior to forced running. Animals in each group were subdivided (4 animals in each group). PS and BS groups were subjected to forced running on a treadmill until exhaustion, while the PC and BC groups were exposed to identical conditions without the forced running.

Animals (n = 16) were treated by oral gavage either with *Bacillus subtilis* BSB3 strain (10^8 CFU in 1 millilitre of PBS per rat) (*B. subtilis*; n = 8) or 1 millilitre of PBS (PBS; n = 8) (Fig. 6.2.4.1). Pre-treatment of animals with PBS or probiotic occurred twice a day with 6-hour dose intervals for 2 days prior to the forced running. Treated animals (PBS and *B. subtilis*) were further subdivided into groups undergoing forced running (PS, BS, n = 4 in each group) and groups remaining sedentary (PC, BC, n = 4 in each group). Forced running protocol was adapted according to Scopel et al (Scopel, Fochesatto et al. 2006). Briefly, animals started running on a treadmill (Exer-3/6 Treadmill, Columbus Instruments, Columbus, Ohio, USA) at 5 meters per minute followed by gradual increases in speed of 2 meters every minute until exhaustion. Rectal temperature was measured for each rat before and immediately after forced running using an electronic digital thermometer, inserted to a depth of 4 centimetres (Moore, Sorokulova et al. 2013). Four hours after the forced running experiments, rats were anesthetized with isoflurane (2–4%) and euthanized by rapid decapitation. Trunk blood was collected from each rat into sterile apirogenic tubes to obtain serum. Sections of jejunum from each rat were taken for morphological analysis. Faecal pellets were aseptically obtained from each animal and placed into pre-weighed sterile tubes containing anaerobic broth for culture-based microbiological analysis. For 16S rRNA sequencing of the gut microbiota faecal samples were placed into sterile tubes and stored at -80°C until the experiment.

6.2.5. Blood Serum Preparation

Blood collected in sterile tubes was allowed to clot for 30 minutes at room temperature. Then, tubes were centrifuged at 20°C, 7000 *g* for 10 minutes. Serum was

collected and stored in 50 microliters aliquots at -20°C until assay.

6.2.6. Histological Analysis

6.2.6.1. Sample Preparation

Small intestinal samples (0.5-2 centimetres in length) were completely immersed in fixative, Bouin's solution (Electron Microscopy Sciences, Hatfield, PA, USA), immediately after harvesting. After 48 hours of fixation at room temperature, the excess fixative was washed out in 70% ethanol (ETOH). Washed samples were placed into tissue embedding cassettes (VWR, Radnor, PA, USA) and kept in 70% ETOH until processing in the Automated tissue processor (Tissue-Tek VIP, Miles/Sakura, Torrance, CA, USA). After processing, samples were embedded in paraffin blocks using embedding centre (Tissue-Tek TEC, Sakura, Torrance, CA, USA). Embedded tissues were sectioned at 6 millimetres using a microtome (Reichert-Jung 2040 Autocut, Leica Biosystems Nussloch GmbH, Heidelberger Straße 17-19, 69226 Nussloch, Germany) and then mounted on slides until staining.

6.2.6.2. Sample Staining

Hematoxylin–eosin (H&E) staining was performed according to the standard protocol (Stevens 1990), using Gill's hematoxylin and Eosin Y (Electron Microscopy Sciences, Hatfield, PA). Sections were deparaffinized in Hemo-Di x 3 changes for 8 minutes, 5 minutes and 5 minutes, cleared in 100% ETOH x 2 changes - 2 minutes each, followed by 95% ETOH x 2 - 2 minutes each and 80% ETOH - 2 minutes. Hydrated sections were rinsed with distilled water and stained with hematoxylin for 2–3 minutes. Samples were washed in running tap water for about 5 minutes, put in 80% ETOH for 2 minutes and stained in eosin for 1-2 minutes. After staining, sections were put in 70% ETOH - 2 minutes, 95% ETOH x 2 - 2 minutes each, 100%

ETOH x 2 - 2 minutes each, and Hemo-Di x 2 changes -5 minutes, 10 minutes or longer. The mounting of the samples was performed using Eukitt Mounting Medium (Electron Microscopy Sciences, Hatfield, PA).

6.2.6.3. Measurements

Intestinal villi height and total mucosal thickness were measured for each sample using a high resolution microscope system (Vainrub, Pustovyy et al. 2006). Only stained sections in which the mucosal villi were cut along their longitudinal axis were analysed. The villi height was measured as a distance from the apex of the villus to the base of the crypt, while total mucosal thickness was measured from the top of the villus to the muscularis mucosae. Twenty measurements of each parameter in each sample were taken and expressed in micrometres (μm). An average of these measurements was expressed as a mean villi height and mean total mucosal thickness for one treatment group.

6.2.6.4. Goblet Cells Quantification

Goblet cells were enumerated using the protocol proposed by Trevizan et al. (Trevizan, Vicentino-Vieira et al. 2016). Small intestinal samples from each rat were stained with Alcian Blue for 3-5 minutes, washed with tap and distilled water, treated with 0.5% periodic acid, washed with distilled water for 2 minutes, stained with Schiff's Reagent for 20 minutes, washed with tap water for 5 minutes, stained with Gill's hematoxylin for less than 1 minute, washed with tap water for 2 minutes, dehydrated, cleared in Hemo-Di, and then mounted in mounting medium. Six images from each section were taken with a digital camera (Olympus BX50) coupled to an optical microscope with an objective of 20x. Goblet cells present in a 0.96 mm^2 field

of vision in the mucosa of each animal were quantified by using ImagePro Plus software (Media Cybernetics).

6.2.6.5. Paneth Cells Quantification

A Phloxine-tartrazine technique was utilized to analyse Paneth cells (Di Sabatino, Miceli et al. 2008). Sections were stained with Gill's hematoxylin for 1 minute, washed with tap water, stained in phloxine B-calcium carbonate for 20 minutes, rinsed in tap water, blotted dry, stained with saturated solution of tartrazine for 10 minutes, rinsed in 95% ETOH, dehydrated in 100% ETOH, cleared in Hemo-Di, and then mounted in mounting medium. Quantification of Paneth cells was performed for four sections for each rat using a high resolution microscope system (Vainrub, Pustovyy et al. 2006).

6.2.7. Lipopolysaccharide (LPS) Assay

LPS serum concentrations were analysed by the Pierce LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific, Rockford, IL, USA) using the Limulus Amebocyte Lysate (LAL) assay according to the manufacturer's recommendations. The sensitivity of the assay was 0.1 EU milliliters^{-1} (0.01 nanograms endotoxin per millilitres). Sterile pyrogen-free plastic and glassware were used throughout the assay.

6.2.8. Tight Junction Proteins Expression

6.2.8.1. Antibodies

Primary rabbit polyclonal antibodies against zonula occludens (ZO-1)(#61-7300, Thermo Fisher Scientific, Waltham, MA, USA), occludin (#40-4700, Thermo Fisher Scientific, Waltham, MA, USA), claudin (#37-4900, Thermo Fisher Scientific, Waltham, MA, USA), and JAM-A (#ab125886, Abcam, Cambridge, MA, USA) were

used in this study. Alexa Fluor 555 goat anti-rabbit (#A32727) and Alexa Fluor 488 goat anti-mouse (#A32723) secondary antibodies were acquired from ThermoFisher Scientific (Waltham, MA, USA).

6.2.8.2. Immunofluorescence

Slides were deparaffinized in Hemo-Di x 2 changes for 5 minutes, then hydrated with 100%, 95%, and 70% ETOH and distilled water. Sections were blocked for 1 hour in Odyssey blocking buffer (LiCor, Lincoln, CA, USA) and then incubated for 2 hours at 4°C with primary antibodies against claudin (1:15 dilution), occludin (1:10 dilution), ZO-1 (1:10 dilution), and JAM-A (1:100 dilution) proteins. Sections were washed with PBS and incubated with secondary antibodies (1:100 dilution) for 1 hour, then washed with PBS/0.01% Tween. Sections were mounted with VectaShield Mounting Media (Vector Laboratories, Burlingame, CA, USA). Fluorescence images from each section were obtained with a digital camera (Olympus BX50) coupled to an optical microscope.

6.2.9. Gut Microbiota Analysis

6.2.9.1. Cultured-Based Analysis

Obtained faecal samples were homogenized, and serial dilutions were performed. Aliquots of 100 microliters of appropriate duplicate dilutions were plated on various media types: Anaerobic Basal Broth (HiMedia Laboratories Pvt Ltd, Nashik, MH, USA) with Agar (Alfa Aesar, Ward Hill, MA, USA) for total anaerobic bacteria; Brain Heart agar (Hardy Diagnostics, Santa Maria, CA, USA) for total aerobic bacteria; BD BBL Prepared Plated Media: Trypticase Soy Agar (TSA II) with Sheep Blood (Fisher Scientific, Hampton, NH, USA) for haemolytic bacteria; *Bifidobacterium* Selective Agar (HiMedia Laboratories Pvt Ltd, Nashik, MH, USA)

for *Bifibacteria* spp.; Lactobacilli MRS Broth (Difco Nutrient Broth, Becton, Dickinson and Company, Sparks, MD, USA) with Agar (Alfa Aesar, Ward Hill, MA, USA) for *Lactobacillus* spp.; Brucella Agar w/Hemin and vitamin K1 (HiMedia Laboratories Pvt Ltd, Nashik, MH, USA) for *Bacteroides* spp.: Reinforced Clostridial Medium (Hardy Diagnostics, Santa Maria, CA, USA) for *Clostridia* spp.; Sabouraud Dextrose HiVeg Agar for (HiMedia Laboratories Pvt Ltd, Nashik, MH, USA) for *Candida* spp.. Anaerobic incubation at 37°C for 48 hours was performed using BD GasPak EZ anaerobe container system (Becton, Dickinson and Company, Sparks, MD, USA). Aerobic bacteria were incubated at 37°C for 24 hours. Colonies were counted after incubation and the colony-forming unit (CFU) per gram of faecal matter was determined.

6.2.9.2. Genetic Analysis

Faecal samples were submitted to MR DNA (Shallowater, TX, USA) for DNA isolation and sequencing. Analysis of the 16S rDNA was performed according to the published procedure (Dowd, Callaway et al. 2008). Total genomic DNA was extracted from faecal samples using a QIAamp stool DNA (Qiagen, Germantown, MD, USA) according the manufacture's instruction. DNA samples were quantified using a Nanodrop spectrophotometer (Nyxor Biotech, Paris, France). 16S universal Eubacterial primers (530F, 5'-GTG CCA GCM GCN GCG G, and 1100R, 5'-GGG TTN CGN TCG TTG) were utilized to amplify the 600 base pair regions of the 16S rRNA genes. HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used for PCR under the following conditions: 94°C for 3 minutes followed by 32 cycles of 94°C for 30 seconds; 60°C for 40 seconds and 72°C for 1 minute; and a final elongation step at 72°C for 5 minutes. Following PCR, each stochastic replicate was combined and all amplicon products from different samples were mixed in equal

concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing the Illumina HiSeq chemistry following manufacturer's protocols. The sequence data was processed using a proprietary analysis pipeline (MR DNA, Shallowater, TX, USA). Sequences were removed when sequences were smaller than 200 base pairs, contained ambiguous base calls, or contained homopolymer runs exceeding 6 base pairs. Operational taxonomic units were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity) (Dowd, Callaway et al. 2008, Dowd, Sun et al. 2008, Edgar 2010, Eren, Zozaya et al. 2011, Swanson, Dowd et al. 2011). Operational taxonomic units were taxonomically classified using BLASTn against a curated National Center for Biotechnology Information database.

6.2.10. Statistics

All results were presented as a mean and standard deviation. The differences between groups were analysed by the one-way ANOVA, followed by the Fisher test. The proprietary analysis pipeline contained code performing XLstat, NCSS 2007, "R" and NCSS 2010 was used for genetic analyses of the gut microbiota. Alpha and beta diversity analyses were also conducted as described previously (Dowd, Callaway et al. 2008, Dowd, Sun et al. 2008, Edgar 2010, Eren, Zozaya et al. 2011, Swanson, Dowd et al. 2011) using Qiime. The significance level was set at $p < 0.05$ to define statistical significance. The relative abundance of bacteria detected below 1% was classified together as others. Statistical calculations and graph plotting were carried out using Microcal Origin version 9.0 (Northampton, MA, USA) and 2010 Microsoft Excel.

6.3. Results

6.3.1. Body Temperature

Forced running caused a significant increase in core body temperature in all animals that underwent treadmill treatment (Fig. 6.3.1.). The core body temperature recordings of animals pre-treated with PBS (PS) before and after forced running were 36.28°C ± 0.57 and 39.18°C ± 0.36, respectively ($p<0.05$). Probiotic treated animals that underwent forced running (BS) had before and after recordings of 37.63°C ± 0.51 and 39.48°C ± 0.22, respectively ($p<0.05$). No central nervous system abnormalities such as convulsions, or coma in animals during or after forced running, were observed in the animals. Body temperature of rats in control groups remained constant throughout experiment (PC- Before 37.2°C ± 0.58 and After 37.6°C ± 0.72; BC- Before 37.53°C ± 0.298 and After 37.2°C ± 0.14).

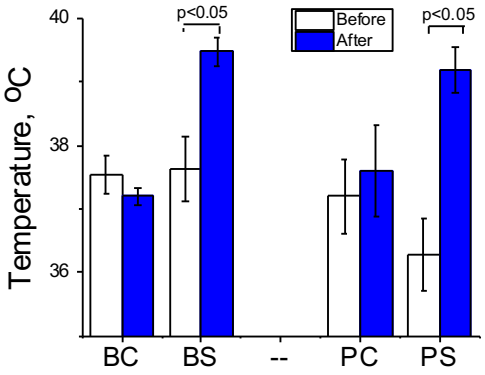


Fig. 6.3.1. Core Body Temperature.

Rectal temperature recordings for each rat were taken before and immediately after forced running using an electronic digital thermometer, inserted to a depth of 4 centimetres.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

6.3.2. Morphological Analysis of the Intestine

Morphometric analysis of small intestine showed that forced running caused the significant reduction of the villi height and total mucosal thickness in animals pre-treated with PBS (PS) in comparison with control (PC) animals (Fig. 6.3.2.1. A and B). Thus, villi height in animals from PS and PC groups was 435.57 ± 34.64 μm and 531.21 ± 26.11 μm and total mucosal thickness was 622.67 ± 15.27 μm and 715.83 ± 35.72 μm accordingly. Pre-treatment of animals with probiotic prior to forced running (BS group) also resulted in significant reductions of the villi height in comparison with control groups (PC and BC) (Fig. 6.3.2.1. A), but in significant increase in comparison with animals from PS group (531.21 ± 26.11 μm vs 435.57 ± 34.64 μm; $p<0.05$). No significant reduction of the total mucosal thickness was observed in the BS group in comparison with control groups (Fig. 6.3.2.1. B).

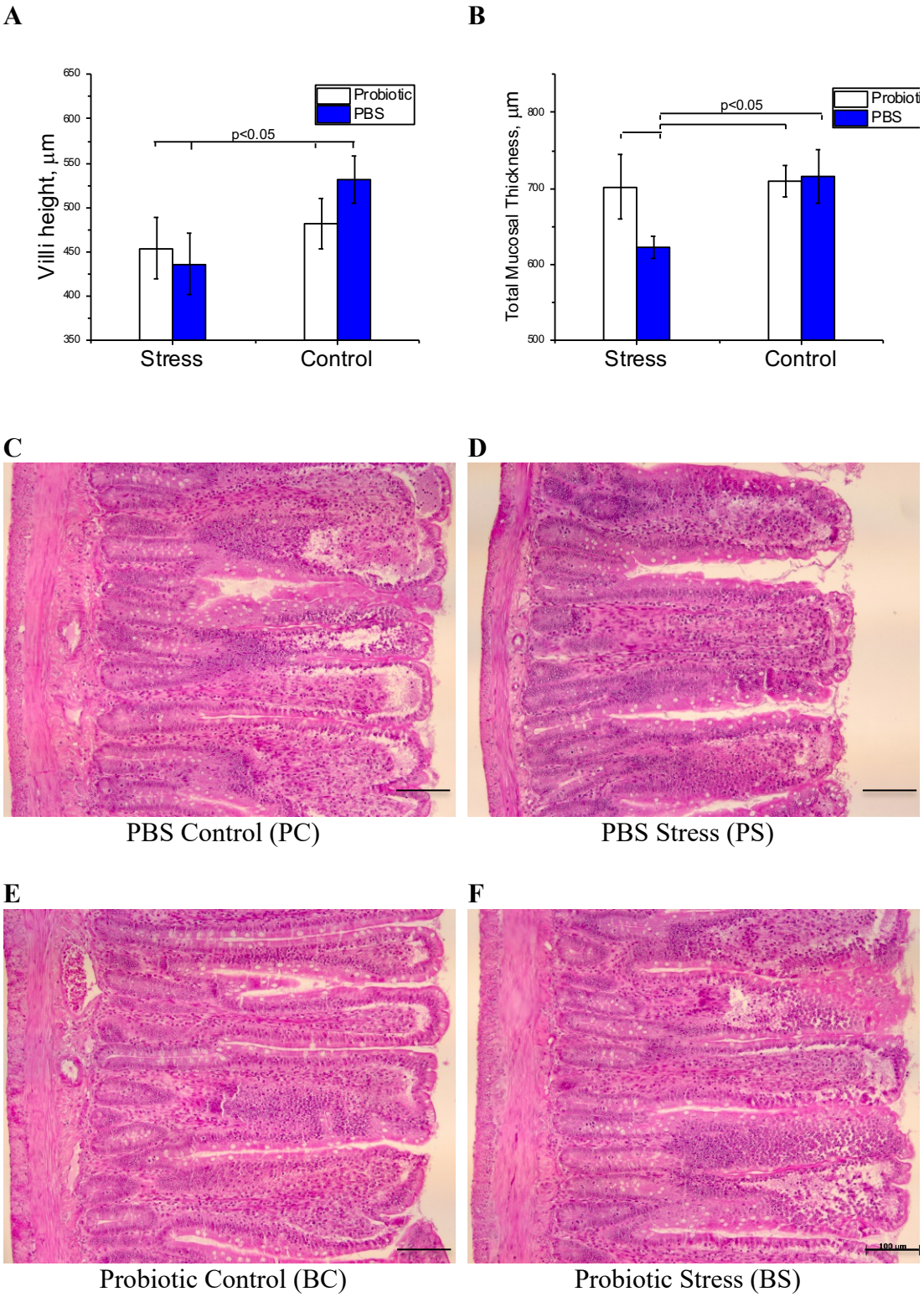


Fig. 6.3.2.1. Villi Height and Total Mucosal Thickness of Small Intestine.

Intestinal villi height (A) and total mucosal thickness (B) in rats from different experimental groups. Animals were pre-treated with PBS (■) or *Bacillus subtilis* BSB3 probiotic (□) by oral gavage before exposure to forced running (Stress) or no exercise (Control). Each group consisted of four rats. Twenty measurements of each

parameter in each sample were taken. C - F: Histological images of the small intestine stained with haematoxylin and eosin. Bar – 100 μm.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

6.3.3. Goblet Cells Quantification

Pre-treatment of animals with probiotic prior to forced running (BS) resulted in significant increase of goblet cells number in comparison with animals pre-treated with PBS (PS and PC groups). Thus, number of goblet cells in BS and PS animals was 178.63 ± 36.82 and 152.29 ± 34.79 respectively, $p<0.05$ (Fig. 6.3.3.1. A and C)

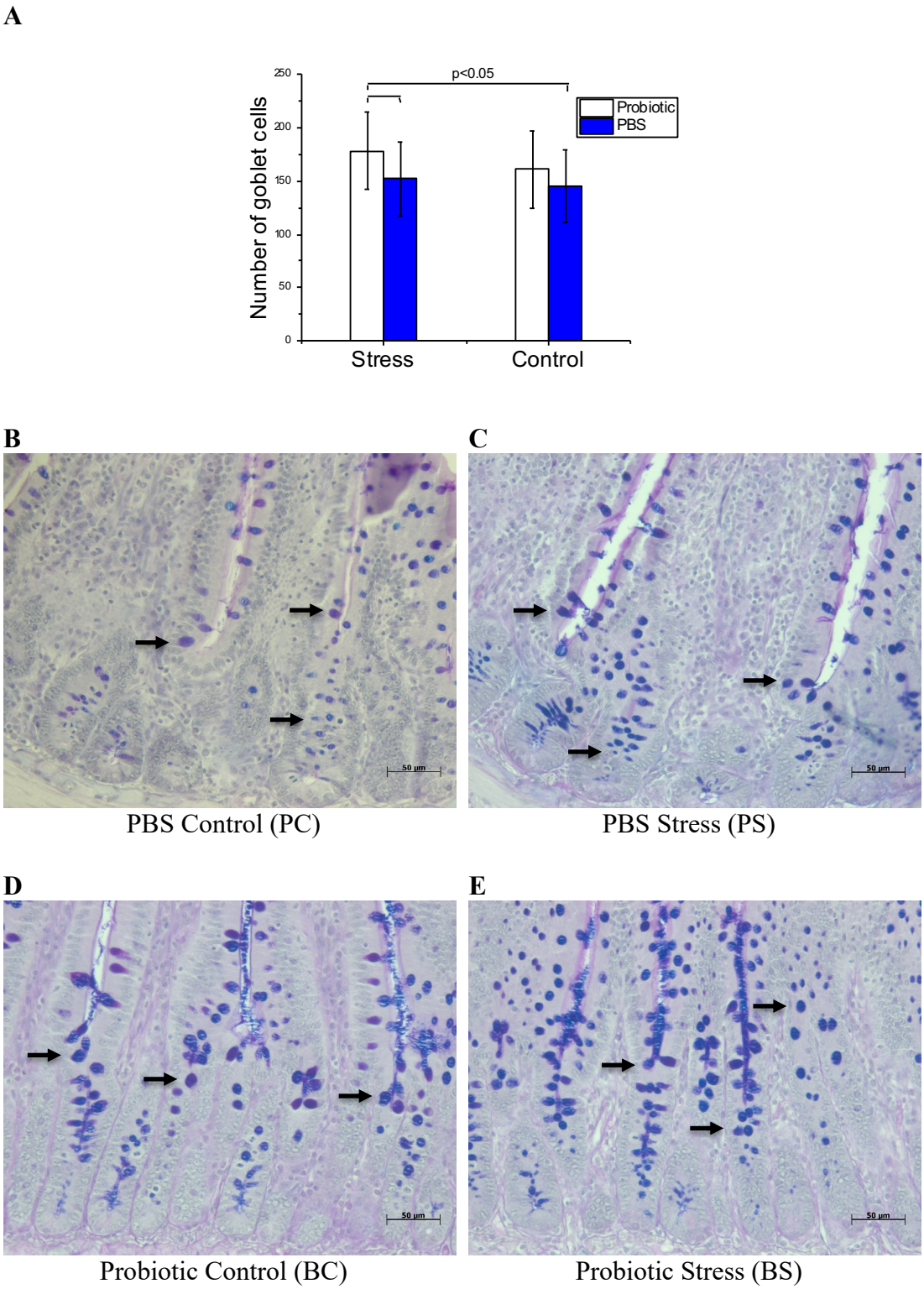


Fig. 6.3.3.1. Goblet Cells Quantification in Small Intestine.

Goblet cells count in rats from different experimental groups. Each group consisted of four rats. Six images from each section were taken with a digital camera (Olympus BX50) coupled to an optical microscope with an objective of 20x. The number of goblet cells present in a 0.96 mm^2 field of vision in the mucosa of each

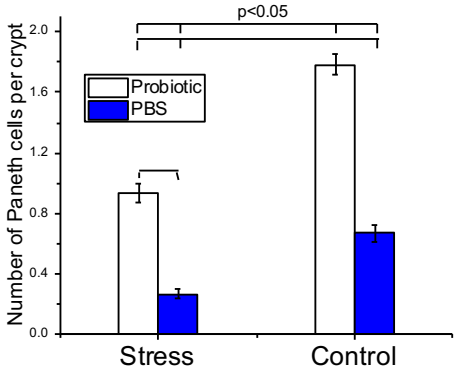
animal were quantified by using ImagePro Plus software (Media Cybernetics). A: Rats were pre-treated with PBS (■) or *Bacillus subtilis* BSB3 probiotic (□) by oral gavage before exposure to forced running (Stress) or no exercise (Control). B - E: Histological images of the small intestine. Arrows show goblet cells stained with Alcian Blue. Bar – 50 µm.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

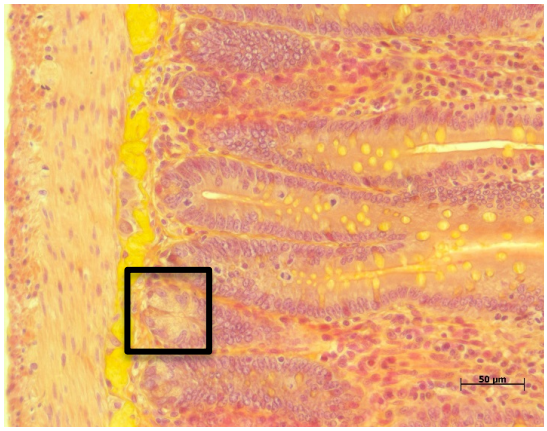
6.3.4. Paneth Cells Quantification

Forced running resulted in significant reductions in the number of Paneth cells in rats pre-treated with PBS (PS) and probiotic (BS) in comparison with control groups (BC and PC) (Fig. 6.3.4.1.). The average number of Paneth cells found in the crypts for PS and BS groups were 0.27 ± 0.03 and 0.93 ± 0.06, respectively (Fig. 6.3.4.1. A and C). Supplementation of rats with probiotic before forced running (BS group) resulted in significantly higher counts of Paneth cells in comparison with rats pre-treated with PBS (PS group). Pre-treatment of control animals with probiotic (BC) resulted in significantly higher numbers of Paneth cells (1.78 ± 0.07) when compared with animals from PC group (0.67 ± 0.06) (Fig. 6.3.4.1. A and B).

A

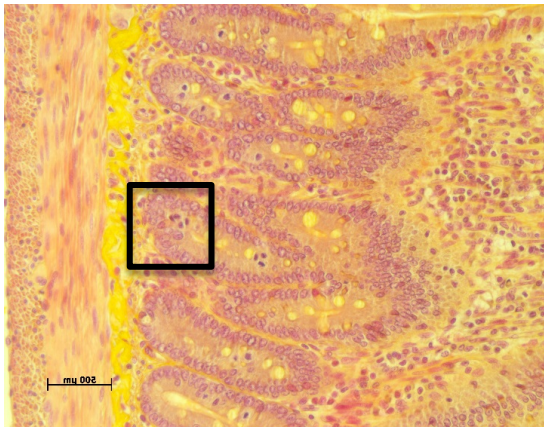


B



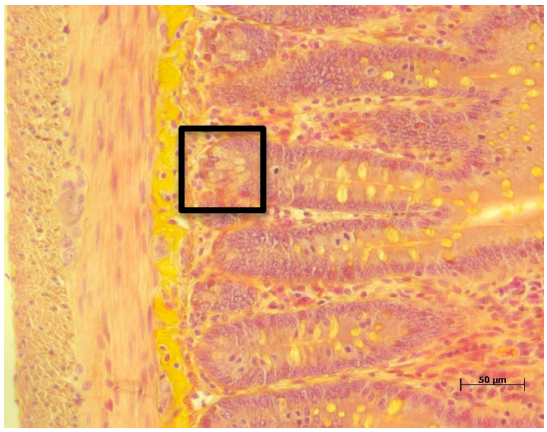
PBS Control (PC)

C



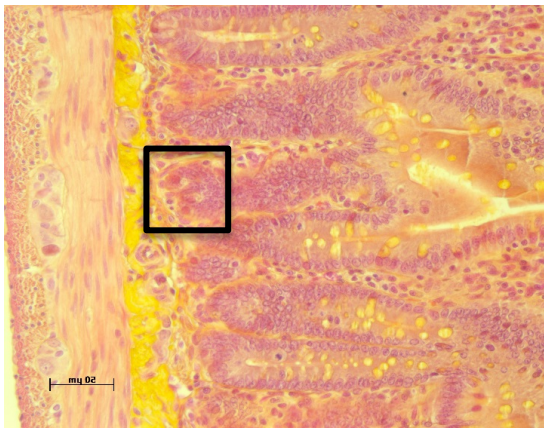
PBS Stress (PS)

D



Probiotic Control (BC)

E



Probiotic Stress (BS)

Fig. 6.3.4.1. Paneth Cells Count in Small Intestine.

Paneth cell count in rats from different experimental groups. Each group consisted of four rats. Quantification of Paneth cells was performed for four sections for each rat using a high resolution microscope system. A: Rats were pre-treated with PBS (■) or *Bacillus subtilis* BSB3 probiotic (□) by oral gavage before exposure to forced

running (Stress) or no exercise (Control). B - E: Histological images of the small intestine. Squares show Paneth cells stained with Phloxine-tartrazine. Bar – 50 µm. PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

6.3.5. Serum LPS Concentration

LPS serum concentrations significantly increased in animals gavaged with PBS prior to forced running (PS- 0.85 ± 0.14) in comparison with probiotic-treated stressed animals (BS- 0.55 ± 0.14) and animals in both control groups (BC- 0.49 ± 0.14; PC- 0.52 ± 0.14) (Fig. 6.3.4.1. A). Administration of probiotic before stress resulted in LPS serum concentrations similar to those of the control groups.

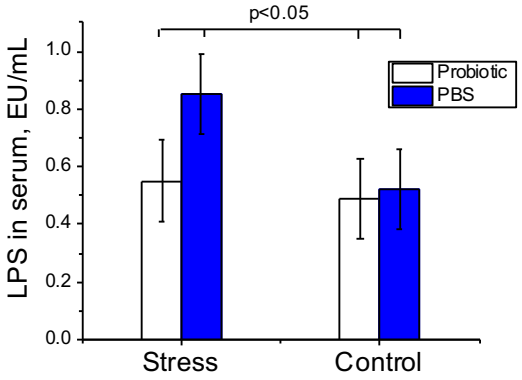


Fig. 6.3.5.1. Serum LPS Concentration.

LPS level in serum of rats pre-treated with PBS (■) or *Bacillus subtilis* BSB3 probiotic (□) by oral gavage before exposure to forced running (Stress) or no exercise (Control).

6.3.6. Tight Junction Proteins Expression

Expression of tight junction proteins (claudin (Fig. 6.3.6.1.), occludin (Fig. 6.3.6.2.), ZO-1 (Fig. 6.3.6.3.), and JAM-A (Fig. 6.3.6.4.)) were analysed in all groups by immunofluorescent staining. Expression of all measured tight junction proteins in pre-treated animals that underwent forced running (PS) were significantly reduced in

comparison with control animals orally gavaged with PBS (PC). Pre-treatment of rats with probiotic prior to forced running (BS) resulted in the significant increase in expression of all proteins in comparison with PS group, though lower than the control group pre-treated with the probiotic (BC). BC group showed higher protein expression of measured tight junction proteins than the sedentary animals pre-treated with PBS (PC).

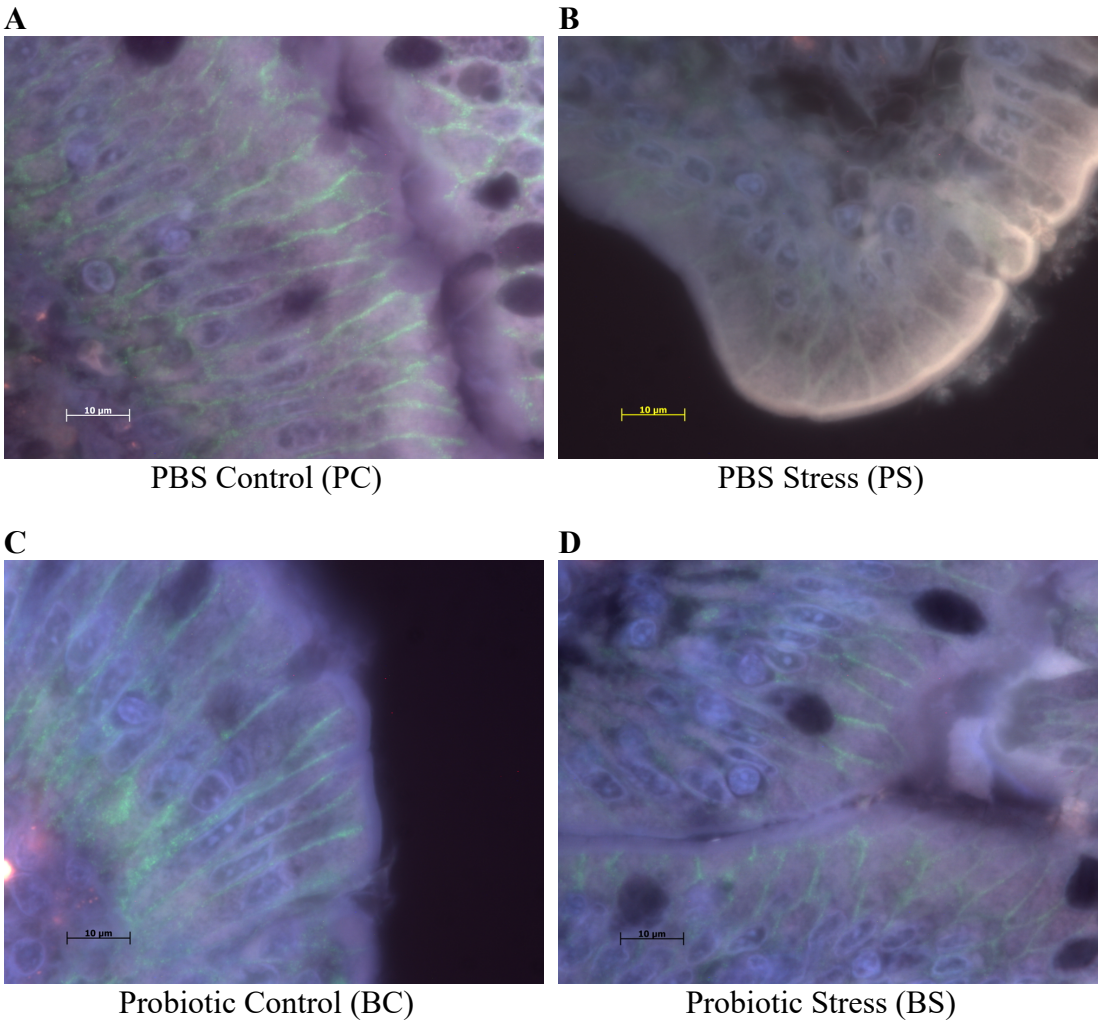


Fig. 6.3.6.1. Claudin Immunofluorescent Staining.

Fluorescence images of rats pre-treated with PBS or *Bacillus subtilis* BSB3 probiotic by oral gavage before exposure to forced running (Stress) or no exercise (Control). Images from each section were obtained with a digital camera (Olympus BX50) coupled to an optical microscope at 100X with a dilution of primary claudin antibody of 1:15 and secondary antibody at 1:100. Bar – 10 µm.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

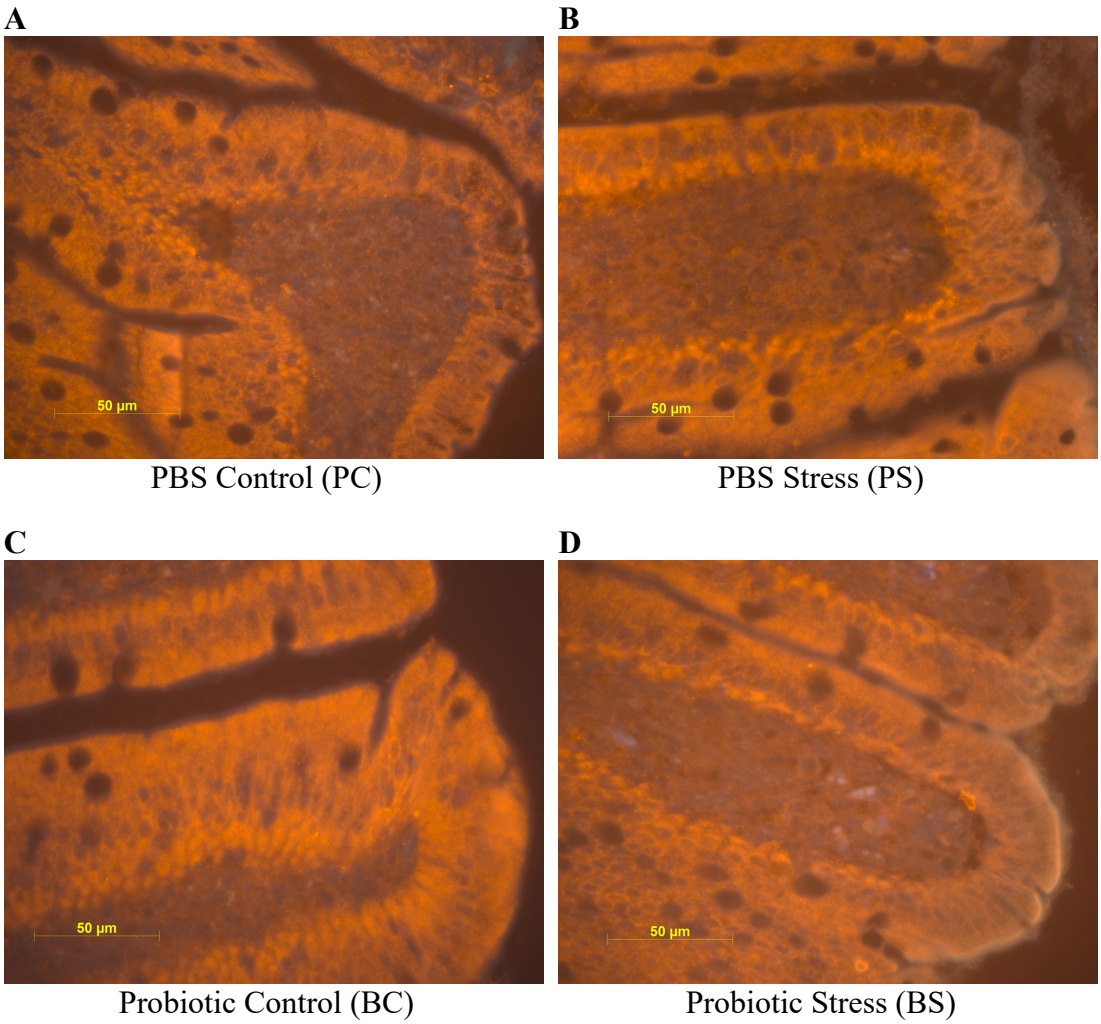


Fig. 6.3.6.2. Occludin Immunofluorescent Staining.

Fluorescence images of rats pre-treated with PBS or *Bacillus subtilis* BSB3 probiotic by oral gavage before exposure to forced running (Stress) or no exercise (Control). Images from each section were obtained with a digital camera (Olympus BX50) coupled to an optical microscope at 40X with a dilution of primary occludin antibody of 1:10 and secondary antibody at 1:100. Bar – 50 µm.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

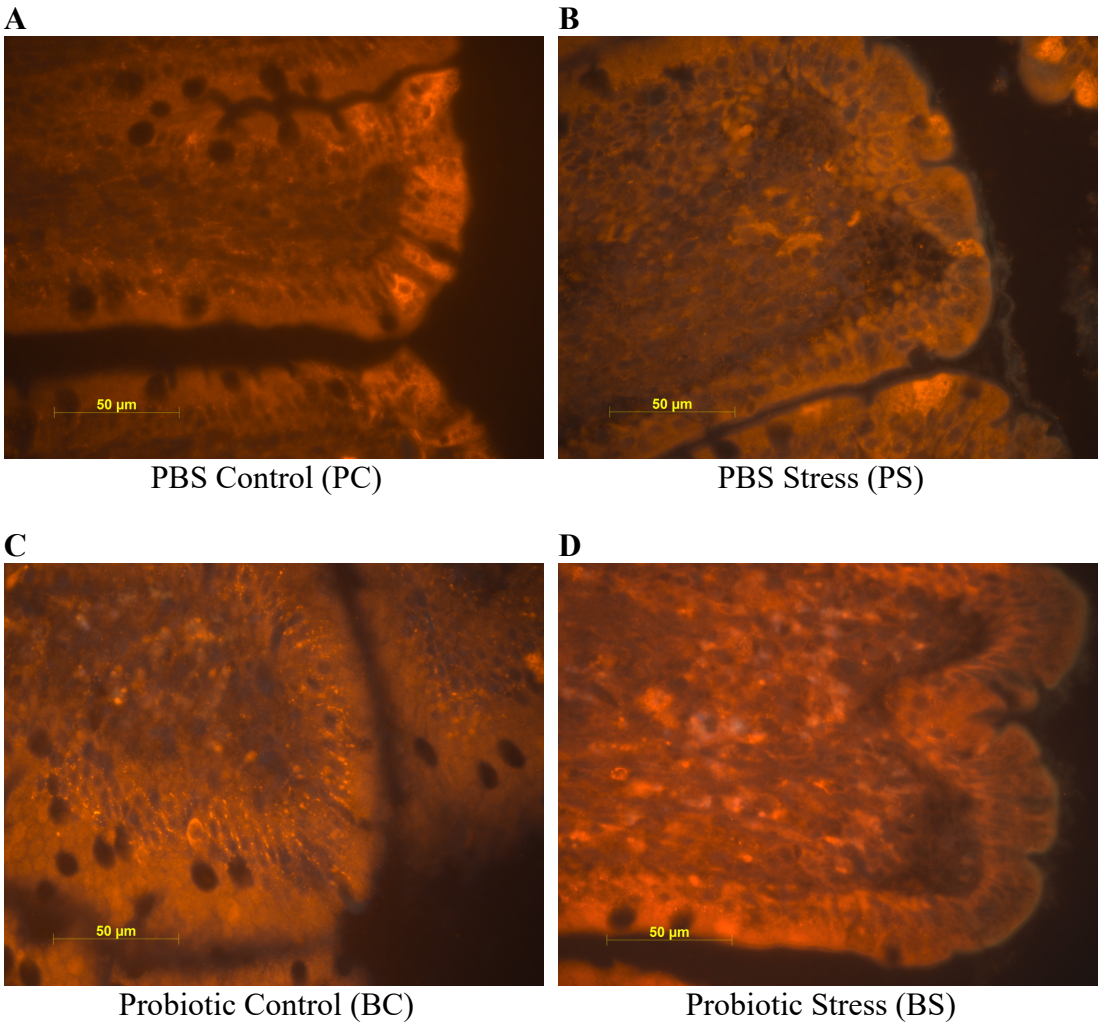


Fig. 6.3.6.3. ZO-1 Immunofluorescent Staining.

Fluorescence images of rats pre-treated with PBS or *Bacillus subtilis* BSB3 probiotic by oral gavage before exposure to forced running (Stress) or no exercise (Control). Images from each section were obtained with a digital camera (Olympus BX50) coupled to an optical microscope at 40X with a dilution of primary ZO-1 antibody of 1:10 and secondary antibody at 1:100. Bar – 50 µm.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

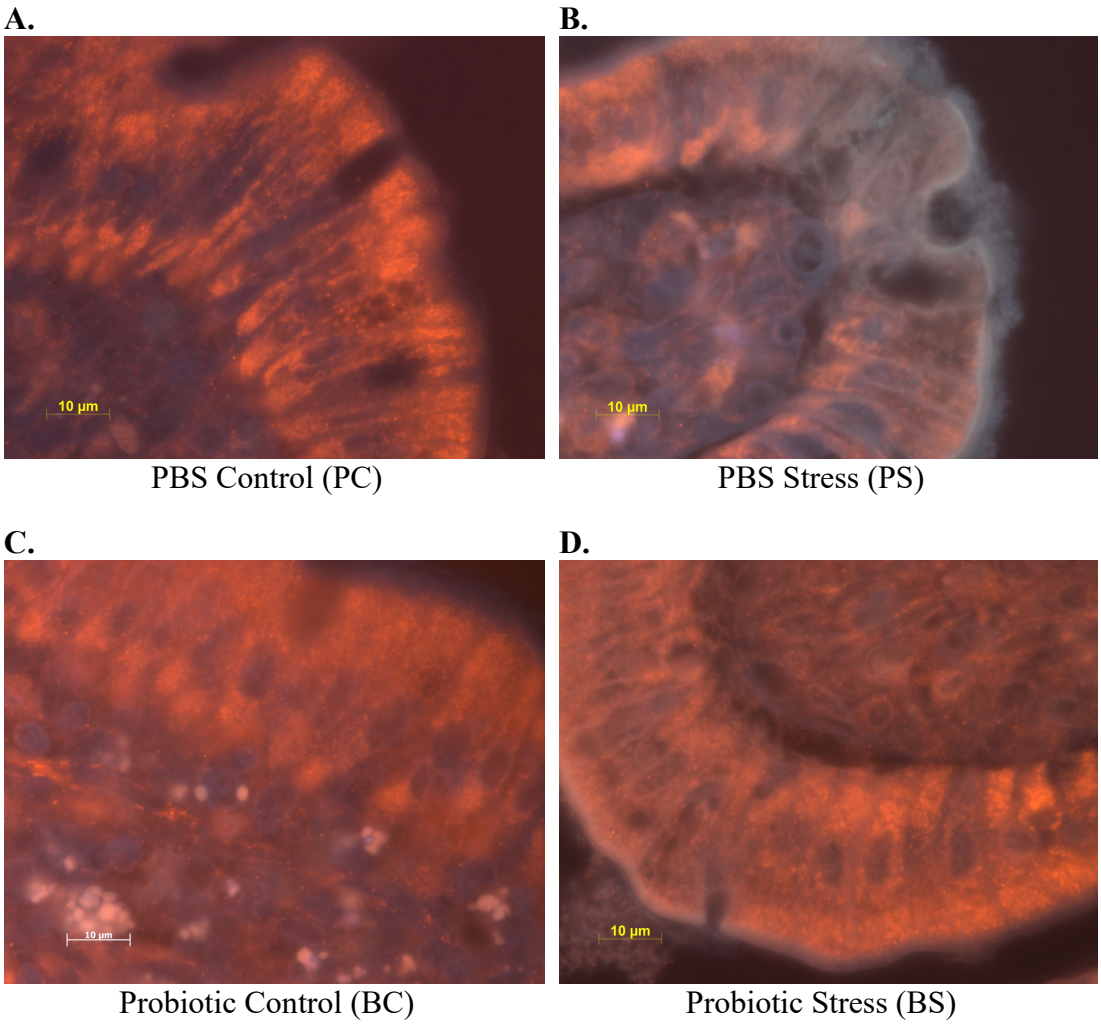


Fig. 6.3.6.4. JAM-A Immunofluorescent Staining.

Fluorescence images of rats pre-treated with PBS or *Bacillus subtilis* BSB3 probiotic by oral gavage before exposure to forced running (Stress) or no exercise (Control). Images from each section were obtained with a digital camera (Olympus BX50) coupled to an optical microscope at 100X with a dilution of primary JAM-A antibody of 1:100 and secondary antibody at 1:100. Bar – 10 µm.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

6.3.7. Culture-Based Analysis of the Gut Microbiota

Culture-based evaluations of the gut microbiota in rats revealed significant changes in the microbial community between treatment groups (Fig. 6.3.7.1.). Pre-treatment of animals with probiotic resulted in significant decreases in total bacterial count (anaerobic and aerobic bacteria combined) in comparison with animals pre-treated with PBS (Fig. 6.3.7.1. A). Probiotic pre-treated groups also resulted in a significantly higher anaerobic to aerobic bacteria ratio when compared with animals pre-treated with PBS (Fig. 6.3.7.1. B). Animals orally gavaged with PBS (PS and PC) significantly decreased the ratio of *Firmicutes* to *Bacteroidetes* in comparison to animals pre-treated with probiotic (BS and BC) (Fig. 6.3.7.1. C). Significant elevation of haemolytic bacteria, *Candida* spp., and *Bacteroides* spp. was found in rats from PS group (Fig. 6.3.7.1. D-F). Pre-treatment of animals with probiotic before forced running (BS) prevented the significant increase of these microorganisms. The number of *Lactobacillus* spp. and *Bifidobacterium* spp. significantly increased only in forced running animals pre-treated with PBS (Fig. 6.3.7.1. G-H).

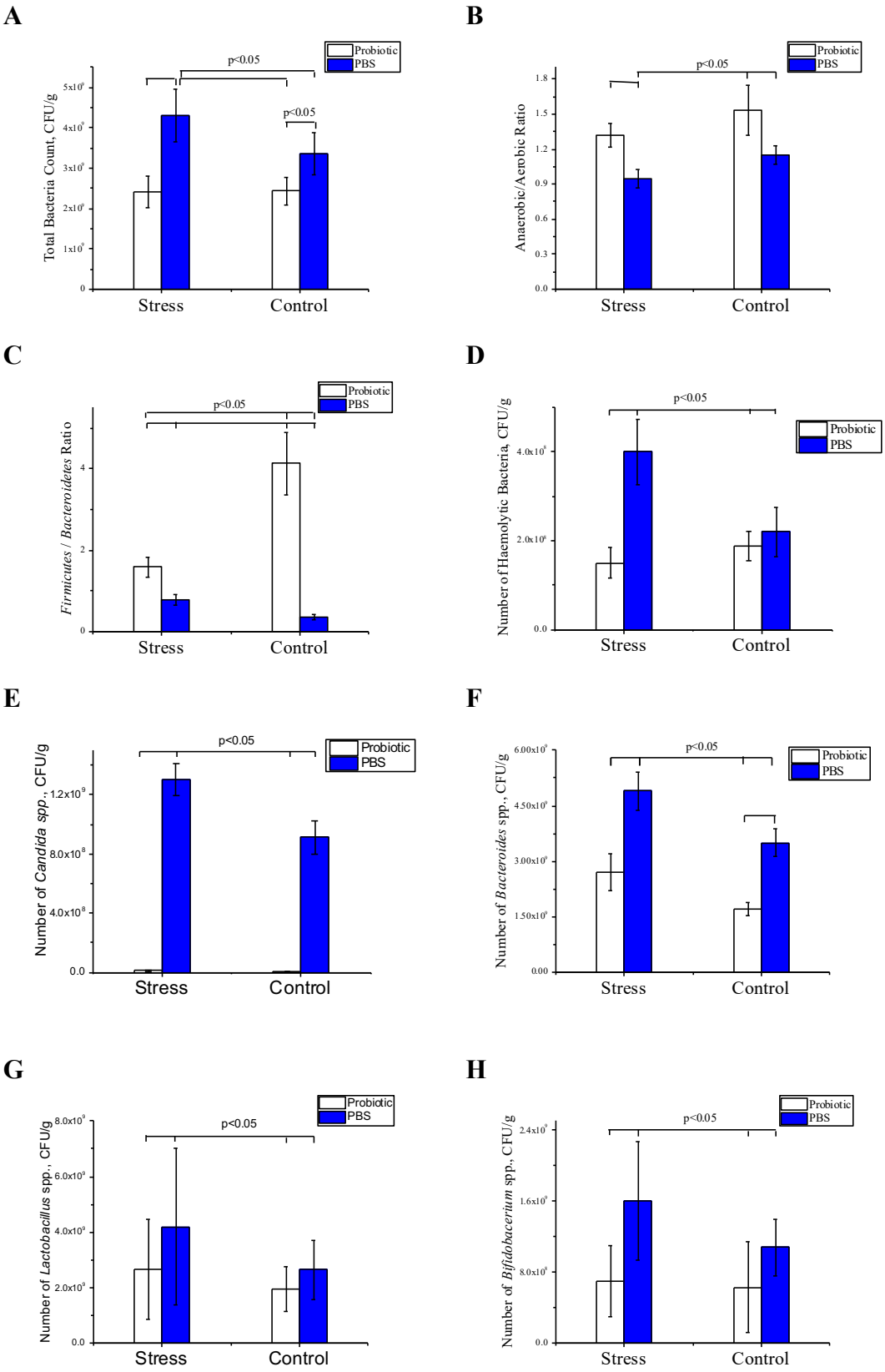


Fig. 6.3.7.1. Bacteriological Analyses of the Gut Microbiota.

Bacteriological analysis of rats pre-treated with PBS (■) or *Bacillus subtilis* BSB3 probiotic (□) by oral gavage before exposure to forced running (Stress) or no exercise (Control). Faecal samples were analysed for total bacterial count (anaerobic and aerobic) (A), anaerobic to aerobic bacteria ratio (B), *Firmicutes* to *Bacteroidetes* ratio (C), haemolytic bacteria (D), *Candida* spp. (E), *Bacteroides* spp. (F), *Lactobacillus spp.* (G), and *Bifidobacterium* spp. (H). Values are expressed as means of colony-forming unit (CFU) per gram of faecal matter.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

6.3.8. 16S rRNA Sequencing of the Gut Microbiota

16S rRNA sequencing of the gut microbiota detected eleven bacterial phyla to be present amongst all the experimental groups (Fig.6.3.8.1. A). Of the eleven phyla detected, three were found to be dominant in all groups: *Firm*icutes, *Bacteroidetes*, and *Actinobacteria* (Fig.6.3.8.1. A). No significant differences were found at the taxonomic level of phylum. *Firmicutes* to *Bacteroidetes* ratios showed that animals pre-treated with probiotic (BS) were significantly different to animals pre-treated with PBS (PS and PC), and sedentary animals fed probiotic (BC) (Fig.6.3.8.1. B).

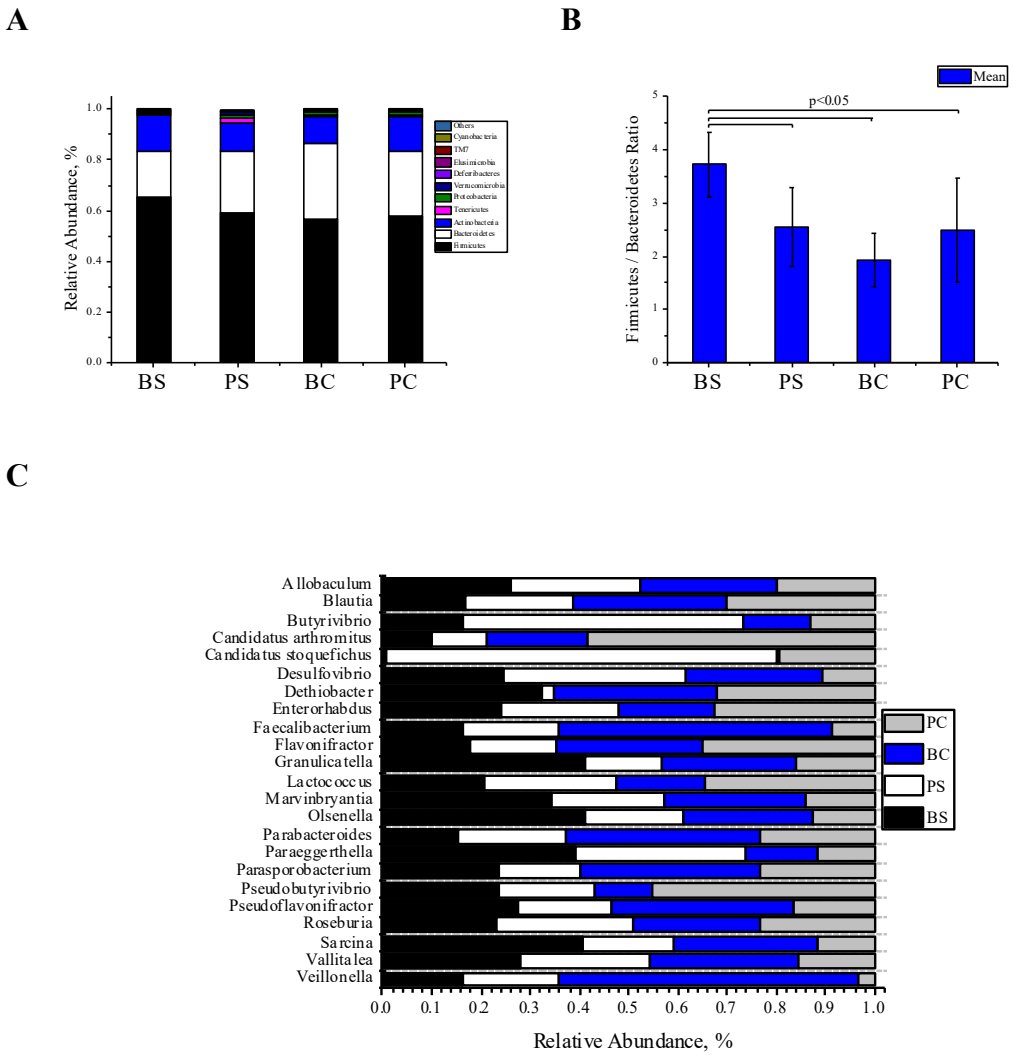


Fig. 6.3.8.1. Genetic Analyses of the Gut Microbiota.

Analysis of the 16S rRNA isolated from colonic faecal samples from all animals pre-treated with PBS or *Bacillus subtilis* BSB3 probiotic by oral gavage before exposure to forced running (Stress) or no exercise (Control). A: The sequenced data was utilized to profile the gut microbiota composition amongst the different experimental groups at the phylum taxonomic level. B: *Firm*icutes to *Bacteroidetes* ratio. C: Relative abundances of genera that were significantly different amongst experimental groups.

PC- (PBS/no stress), BC- (*B. subtilis*/no stress), PS- (PBS/forced running), and BS- (*B. subtilis*/forced running).

Significant changes in the gut microbiota of rats from different experimental groups was found at the genus level (Figure 6.3.8.1 C). The most significant changes were found in the animals that underwent forced running and were pre-treated with

PBS (PS) in comparison with the control PC group. A total of eight genera were significantly affected by forced running in the PS group, six of which belonged to the phylum *Firm*icutes, one to *Proteobacteria*, and 1 to *Actinobacteria* (Table 6.3.8.1). PS animals demonstrated significant increases in some specific genera (*Enterorhabdus, Sarcina, Vallitalea*), whereas others (*Blautia, Candidatus arthromitus, Desulfovibrio*) decreased significantly. *Dethiobacter* was only found in the PC group and *Veillonella* was only observed in PS animals. Ten genera were significantly changed in BS animals in comparison with PC animals. Nine of the genera belonged to the phylum *Firmicutes*, and one to *Actinobacteria* (Table 6.3.8.2). Significant increases were seen in *Allobaculum, Faecalibacterium, Granulicatella, Marvinbryantia, Olsenella*. Forced running of animals pre-treated with probiotic (BS) resulted in significant decreases of *Blautia, Candidatus arthromitus, Flavonifractor,* and *Lactococcus*. *Candidatus stoquefichus* was only observed in the PC group. BC group demonstrated nine significant genera changes in comparison with the control animals pre-treated with PBS (PC) (Table 6.3.8.3).

Tables 6.3.8.1-3. Changes in the Gut Microbiota Genera.

Table 6.3.8.1.

	PS		PC		PS vs PC		
Groups	**Mean**	**SEM**	**Mean**	**SEM**	**changes (%)**	**P-value**	**Phyla**
Enterorhabdus	0.0164	0.0020	0.0083	0.0005	97.2746	0.0176	*Actinobacteria*
Blautia	0.2503	0.0509	0.5306	0.0832	-52.8138	0.0453	*Firmicutes*
Candidatus arthromitus	0.0061	0.0038	0.0575	0.0119	-89.4505	0.0147	*Firmicutes*
Dethiobacter			0.0045	0.0037	PC		*Firmicutes*
Sarcina	0.0095	0.0017	0.0046	0.0008	107.1559	0.0342	*Firmicutes*
Vallitalea	0.0642	0.0088	0.0321	0.0059	100.2080	0.0246	*Firmicutes*
Veillonella	0.0022	0.0015			PS		*Firmicutes*
Desulfovibrio	0.0460	0.0070	0.0813	0.0099	-43.3854	0.0433	*Proteobacteria*

Table 6.3.8.2.

	BS		PC		BS vs PC		
Groups	**Mean**	**SEM**	**Mean**	**SEM**	**changes (%)**	**P-value**	**Phyla**
Olsenella	0.1998	0.0501	0.0104	0.0024	1830.1272	0.0241	*Actinobacteria*
Allobaculum	0.4302	0.0326	0.0289	0.0092	1390.7571	0.0002	*Firmicutes*
Blautia	0.2507	0.0586	0.5306	0.0832	-52.7467	0.0359	*Firmicutes*
Candidatus arthromitus	0.0181	0.0075	0.0575	0.0119	-68.5834	0.0487	*Firmicutes*
Candidatus stoquefichus			0.0054	0.0042	PC		*Firmicutes*
Faecalibacterium	0.0331	0.0038	0.0143	0.0056	131.3225	0.0411	*Firmicutes*
Flavonifractor	0.0224	0.0072	0.0469	0.0051	-52.3085	0.0499	*Firmicutes*
Granulicatella	0.0034	0.0001	0.0013	0.0005	155.7745	0.0128	*Firmicutes*
Lactococcus	0.5489	0.1569	2.1196	0.4366	-74.1042	0.0276	*Firmicutes*
Marvinbryantia	0.0515	0.0143	0.0168	0.0046	205.7799	0.0461	*Firmicutes*

Table 6.3.8.3.

	BC		PC		BC vs PC		
Groups	**Mean**	**SEM**	**Mean**	**SEM**	**changes (%)**	**P-value**	**Phyla**
Enterorhabdus	0.0118	0.0012	0.0083	0.0005	42.2445	0.0491	*Actinobacteria*
Olsenella	0.1729	0.0281	0.0104	0.0024	1569.6716	0.0045	*Actinobacteria*
Paraeggerthella	0.0045	0.0011	0.0010	0.0002	331.9448	0.0429	*Actinobacteria*
Parabacteroides	1.6252	0.2691	0.6280	0.0839	158.7955	0.0241	*Bacteroidetes*
Butyrivibrio	0.0035	0.0004	0.0066	0.0002	-47.7124	0.0022	*Firmicutes*
Parasporobacterium	0.1613	0.0326	0.4358	0.0763	-62.9872	0.0297	*Firmicutes*
Pseudobutyrivibrio	0.0022	0.0009	0.0233	0.0067	-90.7917	0.0353	*Firmicutes*
Pseudoflavonifractor	1.8413	0.2473	0.6962	0.1204	164.4928	0.0061	*Firmicutes*
Roseburia	0.0440	0.0033	0.0952	0.0133	-53.7748	0.0201	*Firmicutes*

6.4. Discussion

This study was undertaken to investigate the efficacy of a *Bacillus subtilis* BSB3 probiotic in mitigation of heat-related adverse effects of metabolic stress. Forced and intensive exercise can lead to exertional heatstroke (hyperthermia), which has been linked to the loss of the intestinal barrier and multiorgan injuries (Lambert 2004, King, Leon et al. 2015). In our study, increases in core body temperature was observed in all animals that underwent forced running (PS, BS), resulting in heat stress. The resultant heat stress caused significant morphological changes, especially in animals pre-treated with PBS (PS). Specifically, these animals exhibited a significant reduction in villi height and total mucosal thickness. In addition, these animals had a significant reduction in the number of goblet and Paneth cells present in the small intestine.

The morphological changes observed from our results due to stress are consistent with other studies (van Wijck, Lenaerts et al. 2012, Grootjans, Lenaerts et al. 2016). Intensive exercise can cause intestinal ischemia in an attempt to combat the increase in internal body temperature (Hall, Buettner et al. 2001, Derikx, Matthijsen et al. 2008). Intestinal villi damage can be observed after only 15 minutes of ischemia, but become more pronounced at 30 minutes (Grootjans, Thuijls et al. 2011). Degradation of villi occurs from the villus tips down due to the greater susceptibility to ischemia and distance from oxygen supply (Chiu, McArdle et al. 1970, Blikslager, Roberts et al. 1997, Grootjans, Lenaerts et al. 2016).

Administration of *Bacillus subtilis* BSB3 probiotic before forced running (BS group) prevented most of significant morphological changes observed in the PS animals. Forced running resulted in the significant decrease in the villi height in BS animals, which is an adverse effect of intestinal ischemia associated with exertional

stress (Hall, Buettner et al. 2001, Derikx, Matthijsen et al. 2008). The total mucosal thickness in BS group was higher than the PS group and was comparable to those of the control groups (PC, BC). The number of goblet and Paneth cells in BS group were also unchanged or slightly reduced in comparison with PC and BC groups, but significantly higher than PS. Hence, probiotic pre-treatment prior to forced running prevented the loss of goblet and Paneth cells and reduction of villi height and total mucosal thickness.

The loss and damage to epithelial cells (enterocytes, Paneth cells, and goblet cells) reduces the effectiveness of the intestinal barrier. The intestinal barrier is a vital component in maintaining homeostasis, requiring approximately forty percent of the body's total energy expenditure (Bischoff, Barbara et al. 2014).

The loss of the intestinal barrier, results in increased gut permeability and the increased translocation of luminal antigens into the circulation. Our studies demonstrated that only PS animals, which had significant morphological alterations, had significant elevation in the level of lipopolysaccharides (LPS) in their serum. LPS, a marker for the intestinal permeability (Bischoff, Barbara et al. 2014). Elevation of LPS are a clear indication of gastrointestinal barrier dysfunction and increased permeability (Derikx, van Waardenburg et al. 2008, Bischoff, Barbara et al. 2014). Serum concentration of LPS in BS animals were equivalent to the BC and PC groups. Pre-treatment with the probiotic prior to forced running (BS) had a protective effect, maintaining the intestinal barrier function and preventing the significant translocation of luminal antigens, such as LPS.

The analysis of tight junction proteins expression showed that only PS animals had a significant reduction in the expression of claudin, occludin, ZO-1, and JAM-A tight junction proteins. Tight junction proteins are the first line of proteins involved in

maintaining the integrity of the epithelium (Ulluwishewa, Anderson et al. 2011, Krug, Schulzke et al. 2014). These proteins connect the epithelial cells to each other and selectively control the permeability of molecules across the intestinal mucosa (Ulluwishewa, Anderson et al. 2011). The loss of tight junction proteins results in the increased permeability of the intestinal barrier, allowing bacteria, their products, nutrients, toxins, or any other compounds (Bischoff, Barbara et al. 2014, Latorre, Adhikari et al. 2018). Increased intestinal permeability and morphological damage signifies due to the loss of tight junction proteins have been observed in strenuous exercise (Zuhl, Schneider et al. 2014). Pre-treatment with the probiotic prior to stress (BS) prevented the significant reduction in expression of these proteins in comparison with PS group. In fact, sedentary animals, which received the probiotic (BC), had significantly higher expression of tight junction proteins than sedentary animals pre-treated with PBS.

The ability to tolerate stress and the composition of the gut microbiota are interconnected (Berg, Muller et al. 1999). Intensive exercise, such as endurance marathons, impact the gut microbiota stability and researchers have confirmed a link between exercise intensity and gut dysbiosis (Hsu, Chiu et al. 2015, Clark and Mach 2016, Mach and Fuster-Botella 2017). Culture-based results of our data demonstrated that PS animals displayed signs of gut dysbiosis. These animals had significantly lower anaerobic to aerobic bacteria ratio. Anaerobic bacteria should be prevalent in the gastrointestinal tract and is a sign of a healthy gut microbiota (Quigley 2013). PS group had significant increases in total bacteria count, especially in pathobionts, specifically haemolytic bacteria, *Candida* spp., and *Bacteroides* spp.. Elevated number of haemolytic bacteria and *Candida* spp. overgrowth indicates gut microbiota disorder (Hoarau, Mukherjee et al. 2016, Hall and Noverr 2017, Popova, Kaftyreva et

al. 2017, Yang, Inamine et al. 2017). *Bacteroides* are Gram-negative bacteria that contain LPS on their outer membrane are known to elicit pro-inflammatory cascades (Gnauck, Lentle et al. 2016). The number of *Lactobacillus* spp. and *Bifidobacterium* spp. only significantly increased in PBS pre-treated animals after forced running (PS). Researchers have reported differently on the effect of stress on *Lactobacillus* spp. and *Bifidobacterium* spp. abundance in the gut. *Bifidobacterium* and *Lactobacillus* spp. are short chain fatty-acid (SCFA) producing microorganisms that are known to enhance intestinal barrier function (Hamer, Jonkers et al. 2008, Lambert, Myslicki et al. 2015). Some studies have shown that these microorganisms increase in order to counter act the effects of exercise-associated dysfunction (Lambert, Myslicki et al. 2015, Mika, Rumian et al. 2016).

Probiotic administration to animals prior to forced running (BS) prevented the significant elevation of commensal pathogenic bacteria as seen in PS animals. The number of haemolytic bacteria and *Bacteroides* spp. in BS group were similar to the control groups (BC and PC). *Candida* spp. were almost completely depleted due to probiotic administration as *Bacillus* bacteria have been shown to have strong antagonistic activity against *Candida* spp. (Fickers, Guez et al. 2009). Probiotic pre-treatment to animals maintained the number of *Lactobacillus* spp. and *Bifidobacterium* spp. in both probiotic pre-treated groups (BC and BS), signifying the protective effect of *Bacillus subtilis* probiotic, being effective in keeping the bacterial number constant. Probiotic pre-treatment increased the prevalence of anaerobic bacteria to aerobic bacteria in both BC and BS groups in comparison with PC and PS groups, as anaerobic bacteria should be prevalent in the gut microbiota (Quigley 2013). Administration of *Bacillus* probiotic showed beneficial effects on the gut

microbiota profile, resulting in healthier gut status in comparison with PBS treated animals.

High-throughput 16S rRNA gene sequencing revealed that in all groups of rats, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were predominant. Predominance of *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* is consistent with other studies examining the rat gut microbiota that underwent strenuous exercise (Mach and Fuster-Botella 2017). Relative abundance percentages demonstrated significant prevalence of *Firm*icutes in comparison with *Bacteroidetes* in all experimental groups. This is contrary to culture- base analysis of the gut microbiota, but culture methods have limitations in comparison with 16S rRNA gene sequencing. *Firmicutes* to *Bacteroidetes* ratios in 16S rRNA gene sequencing showed that only animals pre-treated with probiotic (BS) were significantly to animals pre-treated with PBS (PS and PC), and sedentary animals fed probiotic (BC). Changes in the *Firmicutes* to *Bacteroidetes* ratio has been shown to be proportional to the total distance covered by the animals in exercise (Codella, Luzi et al. 2018).

Significant changes of the gut microbiota in different groups were found at the genus taxonomic level. At the genus level, analysis of the genera found that the prevalent changes observed were also within the phylum of *Firmicutes*. This correlates with other studies that have shown exercise-induced changes in the microbial genera especially in certain members of the *Firmicutes* (Mach and Fuster-Botella 2017). PS animals when compared with PC had significant decreases (*Blautia, Candidatus arthromitus*) or absence (*Dethiobacter*) of genera considered beneficial. *Blautia* is considered an important component of a healthy gut associated with improved metabolism and immunity (Jenq, Taur et al. 2015, Jia, Lin et al. 2018, Suzuki, Ito et al. 2018, Cantu-Jungles, do Nascimento et al. 2019, Zheng, Yuan et al.

2019). *Candidatus arthromitus,* also known as segmented filamentous bacteria, is essential for immune regulation (Thompson, Mikaelyan et al. 2013, Hedblom, Reiland et al. 2018, Million, Tomas et al. 2018). *Dethiobacter* has been proposed as potential probiotic candidate for controlling enteric infections (Zhang, Zhu et al. 2018). PS group had significant increases of pathogenic bacteria (*Enterorhabdus, Sarcina*) in comparison with PC group. *Enterorhabdus* is linked to inflammation and obesity, causing increased insulin resistance and steatosis (Clavel, Duck et al. 2010, de Theije, Wopereis et al. 2014, Opstelten, Plassais et al. 2016, Wang, Tang et al. 2016, Wegner, Just et al. 2017). Healthy individuals show decreased levels of *Enterorhabdus* in their gut microbiota (Yang, Summanen et al. 2015). *Sarcina,* an opportunistic pathogenic increased in PS group (Turroni, Rampelli et al. 2016, Sun, Yang et al. 2017). Increases in pathobionts has been shown to occur with strenuous exercise (Karl, Margolis et al. 2017). *Desulfovibrio* decreased in PS animals when compared with PC animals. This genus is considered pathogenic, causing inflammation and has been linked to diabetes (Shang, Kumar et al. 2018, Xiao, Fu et al. 2018, Zheng, Yuan et al. 2019). The decrease in *Desulfovibrio* spp. was observed in PS group. It has been shown that stress can affect the growth of this pathogenic microbe (Qin, Ji et al. 2019). *Veillonella* was only found in our PS animals. *Veillonella* are lactate fermenting bacteria and have been shown to be beneficial (Cha, Lee et al. 2018, Wang, Chen et al. 2019). During metabolic heat stress, lactic acid fermentation occurs in order to maintain energy needs of the host. This process breaks down glucose into two lactate molecules. This could explain for the increase of *Veillonella* in the gut microbiota observed in the PS animals. *Veillonella* has also been linked to D-lactic acidosis, a metabolic disorder that may occur in individuals with short bowel syndrome when lactate-producing bacteria in the colon overproduce D-lactate (Bulik-

Sullivan, Roy et al. 2018). Supplementation of probiotic before forced running (BS) significantly altered ten genera in comparison with PC group. Six of the altered genera were beneficial. Pre-treatment of the probiotic prior to stress caused the increase of beneficial bacteria (*Allobaculum, Faecalibacterium, Olsenella)* in comparison with PC animals. *Allobaculum* is associated with the prevention of obesity and insulin resistance (Everard, Lazarevic et al. 2014, Bai, Zhu et al. 2018, Chen, You et al. 2018). *Faecalibacterium* is another beneficial bacterium shown to be health promoting by their production of butyrate (Al-Bayati, Fasaei et al. 2018, Coretti, Paparo et al. 2018, Rizzello, Ricci et al. 2018). *Olsenella* has been shown to have anti-inflammatory properties and has the capacity to degrade lactic acid (Kraatz, Wallace et al. 2011, Andoh, Nishida et al. 2016, Wang, Jiang et al. 2018). Strenuous exercise resulted in the reduction of beneficial commensal bacteria (*Blautia, Candidatus arthromitus, Lactococcus)* in BS rats when compared with PC rats. As mentioned previously, *Blautia* is considered an important component of a healthy gut (Jenq, Taur et al. 2015, Jia, Lin et al. 2018, Suzuki, Ito et al. 2018, Cantu-Jungles, do Nascimento et al. 2019, Zheng, Yuan et al. 2019), *Candidatus arthromitus* is essential for immune regulation (Thompson, Mikaelyan et al. 2013, Hedblom, Reiland et al. 2018, Million, Tomas et al. 2018), and *Lactococcus* is also essential for immune regulation (Grangett, Muller-Alouf et al. 2001, Borrero, Jimenez et al. 2011, Wells 2011, Hasan, Jang et al. 2018). Pathogenic genera (*Granulicatella, Marvinbryantia*) significantly increased in BS group. *Granulicatella* is associated with diarrhoea and causes strong TLR expression (Pop, Walker et al. 2014, Dong, Wang et al. 2017, Thakur, Changotra et al. 2018). *Marvinbryantia* has pro-inflammatory properties and increased after forced running in our results (Treangen, Wagner et al. 2018, Wang, Qin et al. 2018) *Flavonifractor*, a pathogenic genus, decreased in our studies and also

in other studies in which the animals received a probiotic (Toscano, De Grandi et al. 2017, Liu, Lin et al. 2018, Armstrong, Alipour et al. 2019, Coello, Hansen et al. 2019). *Candidatus stoquefichus,* a neurodegenerative connected genus, was only detected in our PC group (Gerhardt and Mohajeri 2018). Administration of *Bacillus* probiotic prior to forced running had protective and beneficial effects on the gut microbiota. Probiotic pre-treatment (BS) resulted in the increase of more beneficial genera in comparison with sedentary animals that received PBS (PC). Forced running only resulted in the increase of two pathogenic bacteria in BS group. Decreases of beneficial bacteria (*Blautia, Candidatus arthromitus*) observed in heat stressed animals might be due to the fact that these genera are susceptible to stressors as both PS and BS had decreases in those genera. Sedentary animals pre-treated with probiotic (BC) had the most significant increases in beneficial bacteria in comparison with PC. A total of four genera increased. Specifically, *Olsenella, Parabacteroides, Paraeggerthella,* and *Pseudoflavonifractor* increased in BC animals. *Butyrivibrio, Pseudobutyrivibrio,* and *Roseburia* in BC animals exhibited significant decreases in prevalence when compared with PC in our results. *Butyrivibrio, Pseudobutyrivibrio,* and *Roseburia* are all butyrate-producing bacteria, being able to modulate the intestinal mucosa (Scott, Duncan et al. 2011, Scher, Ubeda et al. 2015, Noriega, Sanchez-Gonzalez et al. 2016, De Weirdt, Hernandez-Sanabria et al. 2017, Picchianti-Diamanti, Rosado et al. 2017, O'Hara, Kelly et al. 2018). Jacquier et al showed that a *Bacillus subtilis* probiotic strain favour butyrate-producing bacteria (Jacquier, Nelson et al. 2019). However, it has been shown that butyrate surplus in the gastrointestinal tract can affect the growth of some bacteria. *Butyrivibrio* spp. for example, have been shown to decrease due to increases in butyrate availability (O'Hara, Kelly et al. 2018). In BC animals, two pathogenic bacteria were observed to change in relative

abundance when compared to PC. Enterorhabdus, a genus associated with gut dysbiosis, significantly increased (Yang, Summanen et al. 2015). *Parasporobacterium,* a bacterium linked to Down's Syndrome and Irritable Bowel Disease, decreased in abundance (Rigsbee, Agans et al. 2012, Luo, Peng et al. 2013, Biagi, Candela et al. 2014). Pre-treatment of probiotic *Bacillus subtilis* BSB3 exerted significant positive effects on the gut microbiota. Probiotic supplementation aided in stabilizing the gut microbiota, increasing the number of beneficial bacteria, and decreasing the number of pathogenic bacteria.

6.5. Conclusion

In conclusion, the results demonstrate that the adverse effects of exercise (morphological changes in intestine, elevated levels of LPS, reduced tight junction protein expression, and gut dysbiosis) were observed only in stressed rats not protected with *Bacillus subtilis* BSB3 probiotic. Administration of the probiotic to animals prior to exposure to forced running prevented all the registered parameters. This is evidence for the high efficacy of the *Bacillus subtilis* BSB3 probiotic in preventing the adverse effects of heat stress. Other studies have shown that some probiotic combinations of *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* and other multi-species treatments were unsuccessful at altering exercise-induced adverse effects on the intestinal mucosa (Costa, Snipe et al. 2017). Therefore, positive modulation of the gut bacteria with this *Bacillus* probiotic could be an effective method in preventing the adverse effects during metabolic stress.

Chapter 7 – Conclusion

1. Exposure of animals to both environmental and metabolic heat stress conditions resulted in significant adverse effects, especially to the intestinal barrier morphology and function.
2. Environmental and metabolic heat stress resulted in the similar morphological alterations in the gastrointestinal tract. Villi height and total mucosal thickness significantly decreased in the heat stressed animals in comparison with control. In addition, those animals had significant reductions in the number of Paneth and goblet cells within the intestines. The expression of intestinal tight junction proteins (zonula occludence (ZO-1), occludin, claudin, junctional adhesion molecule A (JAM-A)) also were significantly reduced in those animals, thus disrupting the degree of tightness between the epithelial cells.
3. Intestinal morphological changes observed in environmental and metabolic heat-stressed animals resulted in a significant increase of gastrointestinal permeability, confirmed by the elevated levels of serum lipopolysaccharides (LPS).
4. Environmental heat stress resulted in the significant vesiculation of erythrocytes and the elevation of leukocytes in the blood of the stressed animals.
5. Acute environmental heat stress had no effect on serum cortisol and interleukin (IL-10) levels in the animals.

6. The gut microbiota profile altered in the environmental and metabolic heat-stressed animals in comparison with the control animals. Culture-based and Tag-Encoded FLX Amplicon Pyrosequencing analysis of the gut microbiota demonstrated significant perturbations of the gut microbiota, with increases in pathobionts and reduction of beneficial genera.
7. Oral administration with a *Bacillus subtilis* probiotic strain and *Saccharomyces cerevisiae* fermentate, as prebiotic, prior to exposure to environmental and metabolic heat stress prevented all registered adverse effects observed in the stressed animals pre-treated with PBS. Thus, our results demonstrated the efficacy of treatment with *Bacillus subtilis* probiotic strain and *Saccharomyces cerevisiae* fermentate in prevention of the stress-induced adverse effects.
8. Effect of *Bacillus subtilis* probiotic strain and *Saccharomyces cerevisiae* fermentate, as prebiotic, in the protection of animals against stress-related complications is associated with beneficial modulation of the gut microbiota.

References

Abdelhamid, L. and X. M. Luo (2018). "Retinoic Acid, Leaky Gut, and Autoimmune Diseases." Nutrients **10**(8).

Abhari, K., et al. (2016). "The effects of orally administered Bacillus coagulans and inulin on prevention and progression of rheumatoid arthritis in rats." Food & Nutrition Research **60**.

Aceti, A., et al. (2015). "Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis." Italian Journal of Pediatrics **41**.

Agrawal, A., et al. (2011). "Secondary prophylaxis of hepatic encephalopathy in cirrhosis: an open label, randomized controlled trial of lactulose, probiotics and no-therapy." Journal of Gastroenterology and Hepatology **26**: 7-7.

Agrawal, A. and P. Whorwell (2011). "Treatment of bloating and distension - role of probiotics." Alimentary Pharmacology & Therapeutics **34**(5): 580-581.

Ait-Belgnaoui, A., et al. (2014). "Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice." Neurogastroenterology and Motility **26**(4): 510-520.

Al-Asmakh, M. and L. Hedin (2015). "Microbiota and the control of blood-tissue barriers." Tissue Barriers **3**(3).

Al-Bayati, L., et al. (2018). "Longitudinal Analyses of Gut-Associated Bacterial Microbiota in Ulcerative Colitis Patients." Archives of Iranian Medicine **21**(12): 578-584.

Al-Sagan, A. A. and A. M. Abudabos (2017). "Effect of a prebiotic, probiotic and symbiotic on performance of broilers under Clostridium Perfringens challenge." Thai Journal of Veterinary Medicine **47**(2): 257-264.

Al-Waili, N. S., et al. (2011). "Honey and Microbial Infections: A Review Supporting the Use of Honey for Microbial Control." Journal of Medicinal Food **14**(10): 1079-1096.

Albenberg, L., et al. (2014). "Correlation Between Intraluminal Oxygen Gradient and Radial Partitioning of Intestinal Microbiota." Gastroenterology **147**(5): 1055-+.

Aliasgharzadeh, A., et al. (2015). "A Combination of Prebiotic Inulin and Oligofructose Improve Some of Cardiovascular Disease Risk Factors in Women with Type 2 Diabetes: A Randomized Controlled Clinical Trial." Advanced Pharmaceutical Bulletin **5**(4): 507-514.

Alizadeh, A., et al. (2016). "The piglet as a model for studying dietary components in infant diets: effects of galacto-oligosaccharides on intestinal functions." British Journal of Nutrition **115**(4): 605-618.

Almeida, L. D., et al. (2017). "Dietary intervention with green dwarf banana flour (Musa sp AAA) modulates oxidative stress and colonic SCFAs production in the TNBS model of intestinal inflammation." Journal of Functional Foods **38**: 497-504.

Alrubaian, M. B., et al. (2018). "USE OF PROBIOTICS AND ITS EFFECTS ON OVERALL HEALTH; A REVIEW OF RECENT LITERATURE." Indo American Journal of Pharmaceutical Sciences **5**(11): 11910-11916.

Alvarez, C. S., et al. (2016). "Outer Membrane Vesicles and Soluble Factors Released by Probiotic Escherichia coil Nissle 1917 and Commensal ECOR63 Enhance Barrier Function by Regulating Expression of Tight Junction Proteins in Intestinal Epithelial Cells." Frontiers in Microbiology **7**.

Ambalam, P., et al. (2016). "Probiotics, prebiotics and colorectal cancer prevention." Best Practice & Research in Clinical Gastroenterology **30**(1): 119-131.

Amuguni, H. and S. Tzipori (2012). "Bacillus subtilis A temperature resistant and needle free delivery system of immunogens." Human Vaccines & Immunotherapeutics **8**(7).

Anand, S. and S. S. Mande (2018). "Diet, Microbiota and Gut-Lung Connection." Frontiers in Microbiology **9**.

Andoh, A., et al. (2016). "Comparison of the gut microbial community between obese and lean peoples using 16S gene sequencing in a Japanese population." Journal of Clinical Biochemistry and Nutrition **59**(1): 65-70.

Andrade, M. E. R., et al. (2015). "The role of immunomodulators on intestinal barrier homeostasis in experimental models." Clinical Nutrition **34**(6): 1080-1087.

Andreasen, A. S., et al. (2011). "Type 2 Diabetes Is Associated with Altered NF-kappa B DNA Binding Activity, JNK Phosphorylation, and AMPK Phosphorylation in Skeletal Muscle after LPS." Plos One **6**(9).

Antonissen, G., et al. (2016). "Microbial shifts associated with necrotic enteritis." Avian Pathology **45**(3): 308-312.

Antunes, L. C. M., et al. (2011). "Effect of Antibiotic Treatment on the Intestinal Metabolome." Antimicrobial Agents and Chemotherapy **55**(4): 1494-1503.

Armstrong, H., et al. (2019). "Host immunoglobulin G selectively identifies pathobionts in pediatric inflammatory bowel diseases." Microbiome **7**.

Arneth, B. M. (2018). "Gut-brain axis biochemical signalling from the gastrointestinal tract to the central nervous system: gut dysbiosis and altered brain function." Postgraduate Medical Journal **94**(1114): 446-452.

Aroniadis, O. C. and L. J. Brandt (2013). "Fecal microbiota transplantation: past, present and future." Current Opinion in Gastroenterology **29**(1): 79-84.

Arpaia, N., et al. (2013). "Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation." Nature **504**(7480): 451-+.

Artegoitia, V. M., et al. (2014). "Choline and Choline Metabolite Patterns and Associations in Blood and Milk during Lactation in Dairy Cows." Plos One **9**(8).

Arumugam, M., et al. (2014). "Enterotypes of the human gut microbiome (vol 473, pg 174, 2011)." Nature **506**(7489).

Ashton, T., et al. (2003). "Exercise-induced endotoxemia: The effect of ascorbic acid supplementation." Free Radical Biology and Medicine **35**(3): 284-291.

Athalye-Jape, G., et al. (2018). "Effects of probiotics on experimental necrotizing enterocolitis: a systematic review and meta-analysis." Pediatric Research **83**(1): 16-22.

Aureli, P., et al. (2011). "Probiotics and health: An evidence-based review." Pharmacological Research **63**(5): 366-376.

Azagra-Boronat, I., et al. (2018). "Supplementation With 2 '-FL and scGOS/lcFOS Ameliorates Rotavirus-Induced Diarrhea in Suckling Rats." Frontiers in Cellular and Infection Microbiology **8**.

Azcarate-Peril, M. A., et al. (2011). "The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer?" American Journal of Physiology-Gastrointestinal and Liver Physiology **301**(3): G401-G424.

Bai, J., et al. (2018). "Modulation of gut microbiota and gut-generated metabolites by bitter melon results in improvement in the metabolic status in high fat diet-induced obese rats." Journal of Functional Foods **41**: 127-134.

Bailey, J. L. and S. Y. Yeung (2011). "Probiotics for Disease Prevention: A Focus on Ventilator-Associated Pneumonia." Annals of Pharmacotherapy **45**(11): 1425-1432.

Bailey, M. T. and C. L. Coe (1999). "Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys." Developmental Psychobiology **35**(2): 146-155.

Bailey, M. T., et al. (2011). "Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation." Brain Behavior and Immunity **25**(3): 397-407.

Bailey, M. T., et al. (2010). "Stressor Exposure Disrupts Commensal Microbial Populations in the Intestines and Leads to Increased Colonization by Citrobacter rodentium." Infection and Immunity **78**(4): 1509-1519.

Bailey, M. T., et al. (2004). "Prenatal stress alters bacterial colonization of the gut in infant monkeys." Journal of Pediatric Gastroenterology and Nutrition **38**(4): 414-421.

Bajaj, J. S., et al. (2012). "Linkage of gut microbiome with cognition in hepatic encephalopathy." American Journal of Physiology-Gastrointestinal and Liver Physiology **302**(1): G168-G175.

Bakken, J. S., et al. (2011). "Treating Clostridium difficile Infection With Fecal Microbiota Transplantation." Clinical Gastroenterology and Hepatology **9**(12): 1044-1049.

Bakker-Zierikzee, A. M., et al. (2005). "Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life." British Journal of Nutrition **94**(5): 783-790.

Barbara, G., et al. (2018). "Probiotics in irritable bowel syndrome: Where are we?" Neurogastroenterology and Motility **30**(12).

Barichella, M., et al. (2016). "Probiotics and prebiotic fiber for constipation associated with Parkinson disease: An RCT." Neurology **87**(12): 1274-1280.

Baron, E. J. (1997). "Bilophila wadsworthia: a unique gram-negative anaerobic rod." Anaerobe **3**(2-3): 83-86.

Barr, J. J., et al. (2013). "Bacteriophage adhering to mucus provide a non-host-derived immunity." Proceedings of the National Academy of Sciences of the United States of America **110**(26): 10771-10776.

Barrera-Bugueno, C., et al. (2017). "ANXIOGENIC EFFECTS OF A LACTOBACILLUS, INULIN AND THE SYNBIOTIC ON HEALTHY JUVENILE RATS." Neuroscience **359**: 18-29.

Bartizal, K. F., et al. (1984). "Distribution and effects of a defined six-member murine derived microflora in gnotobiotic gerbils." Applied and Environmental Microbiology **2**: 96-100.

Baumann, N. and J. C. Turpin (2010). "Neurochemistry of Stress. An Overview." Neurochemical Research **35**(12): 1875-1879.

Baurhoo, B., et al. (2007). "Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens." Poult Sci **86**(6): 1070-1078.

Beilharz, J. E., et al. (2018). "Cafeteria diet and probiotic therapy: cross talk among memory, neuroplasticity, serotonin receptors and gut microbiota in the rat." Molecular Psychiatry **23**(2): 351-361.

Beloglazov, V. A., et al. (2012). "Impact on Anti-Endotoxin Immunity of Allergen Specific Immunotherapy and Probiotics in Asthma Patients." Journal of Allergy and Clinical Immunology **129**(2): Ab72-Ab72.

Bengmark, S. (2013). "Gut microbiota, immune development and function." Pharmacological Research **69**(1): 87-113.

Bengmark, S., et al. (2011). "BIO-ECOLOGICAL CONTROL OF CHRONIC LIVER DISEASE AND ENCEPHALOPATHY." Minerva Medica **102**(4): 309-319.

Bengoechea, J. A. and K. Ito (2011). "Chronic Obstructive Pulmonary Disease Th1 Cells Display Impaired Response to Endotoxin." American Journal of Respiratory and Critical Care Medicine **183**(2): 148-150.

Bentley, R. and R. Meganathan (1982). "BIOSYNTHESIS OF VITAMIN-K (MENAQUINONE) IN BACTERIA." Microbiological Reviews **46**(3): 241-280.

Bercik, P., et al. (2009). "Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of Helicobacter pylori infection." American Journal of Physiology-Regulatory Integrative and Comparative Physiology **296**(3): R587-R594.

Berding, K. and S. M. Donovan (2016). "Microbiome and nutrition in autism spectrum disorder: current knowledge and research needs." Nutrition Reviews **74**(12): 723-736.

Berg, A., et al. (1999). "The gastrointestinal system - An essential target organ of the athlete's health and physical performance." Exercise Immunology Review **5**: 78-95.

Bermon, S., et al. (2015). "The microbiota: An exercise immunology perspective." Exercise Immunology Review **21**: 70-79.

Bermudez-Brito, M., et al. (2012). "Probiotic Mechanisms of Action." Annals of Nutrition and Metabolism **61**(2): 160-174.

Bermudez-Humaran, L. G., et al. (2013). "Engineering lactococci and lactobacilli for human health." Current Opinion in Microbiology **16**(3): 278-283.

Bertelsen, R. J., et al. (2016). "Use of probiotics and prebiotics in infant feeding." Best Practice & Research in Clinical Gastroenterology **30**(1): 39-48.

Bessette, C., et al. (2016). "Oral administration of a casein matrix containing beta-casofensin protects the intestinal barrier in two preclinical models of gut diseases." Journal of Functional Foods **27**: 223-235.

Bettermann, E. L., et al. (2018). "Higher Mediterranean Diet Quality Scores and Lower Body Mass Index Are Associated with a Less-Oxidized Plasma Glutathione and Cysteine Redox Status in Adults." Journal of Nutrition **148**(2): 245-253.

Bevins, C. L. and N. H. Salzman (2011). "Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis." Nature Reviews Microbiology **9**(5): 356-368.

Beyan, H., et al. (2006). "Altered monocyte cyclooxygenase response to lipopolysaccharide in type 1 diabetes." Diabetes **55**(12): 3439-3445.

Bezkorovainy, A. (2001). "Probiotics: determinants of survival and growth in the gut." American Journal of Clinical Nutrition **73**(2): 399S-405S.

Bi, H., et al. (2015). "Improvement of the nutritional quality and fibrinolytic enzyme activity of soybean meal by fermentation of *Bacillus subtilis*." Journal of Food Processing and Preservation **39**(6): 1235-1242.

Biagi, E., et al. (2014). "Gut Microbiome in Down Syndrome." Plos One **9**(11).

Biagi, E., et al. (2010). "Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians." Plos One **5**(5).

Biasucci, G. (2018). "Gut perturbation and probiotics in neonatology." Journal of Pediatric and Neonatal Individualized Medicine **7**(2).

Bibbo, S., et al. (2017). "Fecal microbiota transplantation: past, present and future perspectives." Minerva Gastroenterologica E Dietologica **63**(4): 420-430.

Bienenstock, J. and S. Collins (2010). "99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Psycho-neuroimmunology and the intestinal microbiota: clinical observations and basic mechanisms." Clinical and Experimental Immunology **160**(1): 85-91.

Biesbrock, A. R., et al. (1991). "INTERACTION OF A SALIVARY MUCIN-SECRETORY IMMUNOGLOBULIN-A COMPLEX WITH MUCOSAL PATHOGENS." Infection and Immunity **59**(10): 3492-3497.

Bik, E. M. (2009). "Composition and function of the human-associated microbiota." Nutrition Reviews **67**(11): S164-S171.

Bischoff, S. C., et al. (2014). "Intestinal permeability - a new target for disease prevention and therapy." Bmc Gastroenterology **14**.

Blaabjerg, S., et al. (2017). "Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Outpatients-A Systematic Review and Meta-Analysis." Antibiotics-Basel **6**(4).

Blaser, M. J. and S. Falkow (2009). "ESSAY What are the consequences of the disappearing human microbiota?" Nature Reviews Microbiology **7**(12): 887-894.

Blikslager, A. T., et al. (1997). "Is reperfusion injury an important cause of mucosal damage after porcine intestinal ischemia?" Surgery **121**(5): 526-534.

Bolati, D., et al. (2012). "AST-120 Ameliorates Epithelial-to-Mesenchymal Transition and Interstitial Fibrosis in the Kidneys of Chronic Kidney Disease Rats." Journal of Renal Nutrition **22**(1): 176-180.

Bonfili, L., et al. (2018). "SLAB51 Probiotic Formulation Activates SIRT1 Pathway Promoting Antioxidant and Neuroprotective Effects in an AD Mouse Model." Molecular Neurobiology **55**(10): 7987-8000.

Booijink, C., et al. (2007). "Microbial communities in the human small intestine: coupling diversity to metagenomics." Future Microbiology **2**(3): 285-295.

Borg, S., et al. (1999). "Novel Salmonella typhimurium properties in host-parasite interactions." Immunology Letters **68**(2-3): 247-249.

Borrel, G., et al. (2017). "Genomics and metagenomics of trimethylamine-utilizing Archaea in the human gut microbiome." Isme Journal **11**(9): 2059-2074.

Borrelli, L., et al. (2017). "Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens." Scientific Reports **7**.

Borrero, J., et al. (2011). "Use of the usp45 lactococcal secretion signal sequence to drive the secretion and functional expression of enterococcal bacteriocins in Lactococcus lactis." Applied Microbiology and Biotechnology **89**(1): 131-143.

Bosman, G., et al. (2008). "The proteome of red cell membranes and vesicles during storage in blood bank conditions." Transfusion **48**(5): 827-835.

Bouchama, A., et al. (2005). "Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke." Journal of Applied Physiology **98**(2): 697-705.

Bouhnik, Y., et al. (2007). "Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans." Nutrition Research **27**(4): 187-193.

Bouhnik, Y., et al. (2004). "The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study." American Journal of Clinical Nutrition **80**(6): 1658-1664.

Bouwman, J. J. M., et al. (2009). "Intracellular Infections Enhance Interleukin-6 and Plasminogen Activator Inhibitor 1 Production by Cocultivated Human Adipocytes and THP-1 Monocytes." Clinical and Vaccine Immunology **16**(8): 1222-1227.

Boyle, R. J., et al. (2011). "Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial." Allergy **66**(4): 509-516.

Bradbury, A. W. and C. V. Ruckley (1996). "Mesenteric ischaemia: A multidisciplinary approach - Reply." British Journal of Surgery **83**(5): 710-711.

Brandl, K., et al. (2007). "MyD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal Listeria monocytogenes infection." J Exp Med **204**(8): 1891-1900.

Brandt, L. (2000). "American Gastroenterological Association Medical Position Statement: Guidelines on Intestinal Ischemia. (vol 118, pg 951, 2000)." Gastroenterology **119**(1): 280-280.

Bravo, J. A., et al. (2011). "Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve." Proceedings of the National Academy of Sciences of the United States of America **108**(38): 16050-16055.

Breitbart, M., et al. (2008). "Viral diversity and dynamics in an infant gut." Research in Microbiology **159**(5): 367-373.

Breitbart, M., et al. (2003). "Metagenomic analyses of an uncultured viral community from human feces." Journal of Bacteriology **185**(20): 6220-6223.

Brenner, L. A., et al. (2017). "Growing literature but limited evidence: A systematic review regarding prebiotic and probiotic interventions for those with traumatic brain injury and/or posttraumatic stress disorder." Brain Behavior and Immunity **65**: 57-67.

Bron, P. A., et al. (2017). "Can probiotics modulate human disease by impacting intestinal barrier function?" British Journal of Nutrition **117**(1): 93-107.

Brussow, H. (2016). "Turning the inside out: the microbiology of atopic dermatitis." Environmental Microbiology **18**(7): 2089-2102.

Bry, L., et al. (1994). "PANETH CELL-DIFFERENTIATION IN THE DEVELOPING INTESTINE OF NORMAL AND TRANSGENIC MICE." Proceedings of the National Academy of Sciences of the United States of America **91**(22): 10335-10339.

Buchman, A. L., et al. (1999). "Short-term vitamin E supplementation before marathon running: A placebo-controlled trial." Nutrition **15**(4): 278-283.

Buddington, R. K., et al. (2017). "Oligofructose Provides Laxation for Irregularity Associated with Low Fiber Intake." Nutrients **9**(12).

Buffington, S. A., et al. (2016). "Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring." Cell **165**(7): 1762-1775.

Bulik-Sullivan, E. C., et al. (2018). "Intestinal Microbial and Metabolic Alterations Following Successful Fecal Microbiota Transplant for D-Lactic Acidosis." Journal of Pediatric Gastroenterology and Nutrition **67**(4): 483-487.

Burcelin, R., et al. (2012). "Immuno-microbiota cross and talk: The new paradigm of metabolic diseases." Seminars in Immunology **24**(1): 67-74.

Burchett, S. A. and T. P. Hicks (2006). "The mysterious trace amines: Protean neuromodulators of synaptic transmission in mammalian brain." Progress in Neurobiology **79**(5-6): 223-246.

Burger-van Paassen, N., et al. (2009). "The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection." Biochemical Journal **420**: 211-219.

Burokas, A., et al. (2017). "Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice." Biological Psychiatry **82**(7): 472-487.

Byerley, L. O., et al. (2017). "Changes in the gut microbial communities following addition of walnuts to the diet." Journal of Nutritional Biochemistry **48**: 94-102.

Cadwell, K. (2015). "Expanding the Role of the Virome: Commensalism in the Gut." Journal of Virology **89**(4): 1951-1953.

Caesar, R., et al. (2010). "Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism." Journal of Internal Medicine **268**(4): 320-328.

Canani, R. B., et al. (2018). "Gut microbiota composition and butyrate production in children affected by non-IgE-mediated cow's milk allergy." Scientific Reports **8**.

Candela, M., et al. (2008). "Interaction of probiotic Lactobacillus and Bifidobacterium strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production." International Journal of Food Microbiology **125**(3): 286-292.

Cani, P. D. (2016). "GUT MICROBIOTA Changes in gut microbes and host metabolism: squaring the circle?" Nature Reviews Gastroenterology & Hepatology **13**(10): 563-+.

Cani, P. D., et al. (2007). "Metabolic endotoxemia initiates obesity and insulin resistance." Diabetes **56**(7): 1761-1772.

Cani, P. D. and A. Everard (2016). "Talking microbes: When gut bacteria interact with diet and host organs." Molecular Nutrition & Food Research **60**(1): 58-66.

Cani, P. D., et al. (2009). "Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability." Gut **58**(8): 1091-1103.

Cantu-Jungles, T. M., et al. (2019). "Soluble xyloglucan generates bigger bacterial community shifts than pectic polymers during in vitro fecal fermentation." Carbohydrate Polymers **206**: 389-395.

Caporaso, J. G., et al. (2011). "Moving pictures of the human microbiome." Genome Biology **12**(5).

Carabotti, M., et al. (2016). "The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems (vol 28, pg 203, 2015)." Annals of Gastroenterology **29**(2).

Cardona, F., et al. (2013). "Benefits of polyphenols on gut microbiota and implications in human health." Journal of Nutritional Biochemistry **24**(8): 1415-1422.

Carlet, J. (2010). "Anti-, pre-, or ... probiotics to prevent ventilator-associated pneumonia in the intensive care unit?" Critical Care Medicine **38**(3): 1009-1010.

Cash, H. L. and L. V. Hooper (2005). "Commensal bacteria shape intestinal immune system development." Asm News **71**(2): 77-+.

Cassone, A., et al. (1987). "CELL-WALL OF CANDIDA-ALBICANS AND HOST RESPONSE." Crc Critical Reviews in Microbiology **15**(1): 87-95.

Cavalcanti, I. M. G., et al. (2016). "Interkingdom cooperation between Candida albicans, Streptococcus oralis and Actinomyces oris modulates early biofilm development on denture material." Pathogens and Disease **74**(3).

Ceapa, C., et al. (2013). "Influence of fermented milk products, prebiotics and probiotics on microbiota composition and health." Best Practice & Research in Clinical Gastroenterology **27**(1): 139-155.

Ceausu, M., et al. (2010). "Morphological diagnosis of hyperthermia-related deaths." Romanian Journal of Legal Medicine **18**(4): 239-246.

Ceccarelli, G., et al. (2017). "Impact of High-Dose Multi-Strain Probiotic Supplementation on Neurocognitive Performance and Central Nervous System Immune Activation of HIV-1 Infected Individuals." Nutrients **9**(11).

Celiberto, L. S., et al. (2018). "Isolation and Characterization of Potentially Probiotic Bacterial Strains from Mice: Proof of Concept for Personalized Probiotics." Nutrients **10**(11).

Cereda, E., et al. (2016). "Double-blind, placebo-controlled trial of a fermented milk containing multiple probiotic strains and prebiotic fiber for constipation associated with Parkinson's disease." Movement Disorders **31**: S99-S99.

Ceresola, E. R., et al. (2018). "Targeting patients' microbiota with probiotics and natural fibers in adults and children with constipation." European Review for Medical and Pharmacological Sciences **22**(20): 7045-7057.

Cesaro, C., et al. (2011). "Gut microbiota and probiotics in chronic liver diseases." Digestive and Liver Disease **43**(6): 431-438.

Cha, K. H., et al. (2018). "Effects of fermented milk treatment on microbial population and metabolomic outcomes in a three-stage semi-continuous culture system." Food Chemistry **263**: 216-224.

Chaban, B., et al. (2006). "Archaeal habitats - from the extreme to the ordinary." Canadian Journal of Microbiology **52**(2): 73-116.

Chabe, M., et al. (2017). "Gut Protozoa: Friends or Foes of the Human Gut Microbiota?" Trends in Parasitology **33**(12): 925-934.

Chairatana, P. and E. M. Nolan (2017). "Defensins, lectins, mucins, and secretory immunoglobulin A: microbe-binding biomolecules that contribute to mucosal immunity in the human gut." Critical Reviews in Biochemistry and Molecular Biology **52**(1): 45-56.

Chang, B., et al. (2013). "The protective effect of VSL#3 on intestinal permeability in a rat model of alcoholic intestinal injury." Bmc Gastroenterology **13**.

Chapman, C. M. C., et al. (2011). "Health benefits of probiotics: are mixtures more effective than single strains?" European Journal of Nutrition **50**(1): 1-17.

Chatterjee, A. and B. A. Duerkop (2018). "Beyond Bacteria: Bacteriophage-Eukaryotic Host Interactions Reveal Emerging Paradigms of Health and Disease." Frontiers in Microbiology **9**.

Chelakkot, C., et al. (2018). "Mechanisms regulating intestinal barrier integrity and its pathological implications." Experimental and Molecular Medicine **50**.

Chen, B. R., et al. (2018). "Prevalence and predictors of small intestinal bacterial overgrowth in irritable bowel syndrome: a systematic review and meta-analysis." Journal of Gastroenterology **53**(7): 807-818.

Chen, C., et al. (2018). "Modulation of gut microbiota by mulberry fruit polysaccharide treatment of obese diabetic db/db mice." Food & Function **9**(7): 3732-3742.

Chen, C. C. and W. A. Walker (2011). "Clinical applications of probiotics in gastrointestinal disorders in children." National Medical Journal of India **24**(3): 153-160.

Chen, D. L., et al. (2017). "Prebiotic Effect of Fructooligosaccharides from Morinda officinalis on Alzheimer's Disease in Rodent Models by Targeting the Microbiota-Gut-Brain Axis." Frontiers in Aging Neuroscience **9**.

Chen, J., et al. (1998). "A preliminary clinical study of bifidobacteria preparation on the treatment of diarrhea in severely burned patients." Chinese Medical Journal **111**(4): 381-382.

Chen, P. Y., et al. (2017). "Prebiotic Oligosaccharides Potentiate Host Protective Responses against L. Monocytogenes Infection." Pathogens **6**(4).

Chen, S. Y., et al. (2018). "Ophiopogonin D alleviates high-fat diet-induced metabolic syndrome and changes the structure of gut microbiota in mice." Faseb Journal **32**(3): 1139-1153.

Chen, W. C. and E. M. M. Quigley (2014). "Probiotics, prebiotics & synbiotics in small intestinal bacterial overgrowth: Opening up a new therapeutic horizon!" Indian Journal of Medical Research **140**: 582-584.

Chen, X. Y., et al. (2018). "Moderate Dietary Protein Restriction Optimized Gut Microbiota and Mucosal Barrier in Growing Pig Model." Frontiers in Cellular and Infection Microbiology **8**.

Chen, Y., et al. (2017). "Wuji Wan Formula Ameliorates Diarrhea and Disordered Colonic Motility in Post-inflammation Irritable Bowel Syndrome Rats by Modulating the Gut Microbiota." Frontiers in Microbiology **8**.

Chen, Z. L., et al. (2017). "Brain-Heart Interaction Cardiac Complications After Stroke." Circulation Research **121**(4): 451-468.

Cheng, J., et al. (2016). "Discordant temporal development of bacterial phyla and the emergence of core in the fecal microbiota of young children." Isme Journal **10**(4): 1002-1014.

Chhibber-Goel, J., et al. (2017). "The Metabolite Trimethylamine-N-Oxide is an Emergent Biomarker of Human Health." Current Medicinal Chemistry **24**(36): 3942-3953.

Chiu, C. J., et al. (1970). "INTESTINAL MUCOSAL LESION IN LOW-FLOW STATES .1. A MORPHOLOGICAL, HEMODYNAMIC, AND METABOLIC REAPPRAISAL." Archives of Surgery **101**(4): 478-&.

Chu, H. T., et al. (2016). "Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease." Science **352**(6289): 1116-1120.

Clark, A. and N. Mach (2016). "Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes." Journal of the International Society of Sports Nutrition **13**.

Claus, S. P., et al. (2016). "The gut microbiota: a major player in the toxicity of environmental pollutants?" Npj Biofilms and Microbiomes **2**.

Clavel, M. and N. Pichon (2011). "Probiotics as preventive measures against ventilator-associated pneumonia." Journal Des Anti-Infectieux **13**(4): 254-258.

Clavel, T., et al. (2010). "Enterorhabdus caecimuris sp. nov., a member of the family Coriobacteriaceae isolated from a mouse model of spontaneous colitis, and emended description of the genus Enterorhabdus Clavel et al. 2009." Int J Syst Evol Microbiol **60**(Pt 7): 1527-1531.

Closa-Monasterolo, R., et al. (2017). "The use of inulin-type fructans improves stool consistency in constipated children. A randomised clinical trial: pilot study." International Journal of Food Sciences and Nutrition **68**(5): 587-594.

Codella, R., et al. (2018). "Exercise has the guts: How physical activity may positively modulate gut microbiota in chronic and immune-based diseases." Digestive and Liver Disease **50**(4): 331-341.

Coello, K., et al. (2019). "Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives." Brain Behavior and Immunity **75**: 112-118.

Collier, R. J. and K. G. Gebremedhin (2015). "Thermal Biology of Domestic Animals." Annual Review of Animal Biosciences, Vol 3 **3**: 513-532.

Collier, R. J., et al. (2017). "A 100-Year Review: Stress physiology including heat stress." Journal of Dairy Science **100**(12): 10367-10380.

Collins, S. L., et al. (2018). "Promising Prebiotic Candidate Established by Evaluation of Lactitol, Lactulose, Raffinose, and Oligofructose for Maintenance of a Lactobacillus-Dominated Vaginal Microbiota." Applied and Environmental Microbiology **84**(5).

Collins, S. M., et al. (2013). "The adoptive transfer of behavioral phenotype via the intestinal microbiota: experimental evidence and clinical implications." Current Opinion in Microbiology **16**(3): 240-245.

Columpsi, P., et al. (2016). "Beyond the Gut Bacterial Microbiota: The Gut Virome." Journal of Medical Virology **88**(9): 1467-1472.

Cordina, C., et al. (2011). "Probiotics in the management of gastrointestinal disease: Analysis of the attitudes and prescribing practices of gastroenterologists and surgeons." Journal of Digestive Diseases **12**(6): 489-496.

Coretti, L., et al. (2018). "Gut Microbiota Features in Young Children With Autism Spectrum Disorders." Frontiers in Microbiology **9**.

Corfield, A. P. (2015). "Mucins: A biologically relevant glycan barrier in mucosal protection." Biochimica Et Biophysica Acta-General Subjects **1850**(1): 236-252.

Corpino, M. (2017). "Microbiota and probiotics." Journal of Pediatric and Neonatal Individualized Medicine **6**(2).

Correia, M. I. T. D., et al. (2012). "The role of probiotics in gastrointestinal surgery." Nutrition **28**(3): 230-234.

Costa, R. J. S., et al. (2017). "Systematic review: exercise-induced gastrointestinal syndrome-implications for health and intestinal disease." Alimentary Pharmacology & Therapeutics **46**(3): 246-265.

Costabile, A., et al. (2008). "Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study." British Journal of Nutrition **99**(1): 110-120.

Costantini, L., et al. (2017). "Impact of Omega-3 Fatty Acids on the Gut Microbiota." International Journal of Molecular Sciences **18**(12).

Coudeyras, S. and C. Forestier (2010). "Microbiota and probiotics: impact on human health." Canadian Journal of Microbiology **56**(8): 611-650.

Cousin, F. J., et al. (2011). "Dairy propionibacteria as human probiotics: A review of recent evidence." Dairy Science & Technology **91**(1): 1-26.

Cozen, W., et al. (2013). "Fecal microbiota diversity in survivors of adolescent/young adult Hodgkin lymphoma: a study of twins." British Journal of Cancer **108**(5): 1163-1167.

Critchfield, J. W., et al. (2011). "The Potential Role of Probiotics in the Management of Childhood Autism Spectrum Disorders." Gastroenterology Research and Practice.

Cryan, J. F. and T. G. Dinan (2012). "Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour." Nature Reviews Neuroscience **13**(10): 701-712.

Cuervo, A., et al. (2012). "Microbiota modulation by diet in humans Prebiotics, fibres and other compounds." Agro Food Industry Hi-Tech **23**(6): XXIII-XXVI.

Cui, L. J., et al. (2013). "The human mycobiome in health and disease." Genome Medicine **5**.

Cummings, J. H. and G. T. Macfarlane (1991). "THE CONTROL AND CONSEQUENCES OF BACTERIAL FERMENTATION IN THE HUMAN COLON." Journal of Applied Bacteriology **70**(6): 443-459.

Cummings, J. H., et al. (1987). "SHORT CHAIN FATTY-ACIDS IN HUMAN LARGE-INTESTINE, PORTAL, HEPATIC AND VENOUS-BLOOD." Gut **28**(10): 1221-1227.

Cutting, S. M. (2011). "*Bacillus* probiotics." Food Microbiology **28**(2): 214-220.

Da Silva, S., et al. (2014). "Stress disrupts intestinal mucus barrier in rats via mucin O-glycosylation shift: prevention by a probiotic treatment." American Journal of Physiology-Gastrointestinal and Liver Physiology **307**(4): G420-G429.

Dahl, W. J. and D. R. Mendoza (2018). "Is Fibre an Effective Strategy to Improve Laxation in Long-Term Care Residents?" Canadian Journal of Dietetic Practice and Research **79**(1): 35-41.

Dahllof, I. (2002). "Molecular community analysis of microbial diversity." Current Opinion in Biotechnology **13**(3): 213-217.

Daliri, E. B. M., et al. (2018). "Human microbiome restoration and safety." International Journal of Medical Microbiology **308**(5): 487-497.

Dalmasso, M., et al. (2014). "Exploiting gut bacteriophages for human health." Trends in Microbiology **22**(7): 399-405.

Dave, M., et al. (2014). "Immunology of Inflammatory Bowel Disease and Molecular Targets for Biologics." Gastroenterology Clinics of North America **43**(3): 405-+.

Davidson, G. L., et al. (2018). "The gut microbiome as a driver of individual variation in cognition and functional behaviour." Philosophical Transactions of the Royal Society B-Biological Sciences **373**(1756).

Davis, C. P. (1976). "PRESERVATION OF GASTROINTESTINAL BACTERIA AND THEIR MICROENVIRONMENTAL ASSOCIATIONS IN RATS BY FREEZING." Applied and Environmental Microbiology **31**(2): 304-312.

Davis, D. J., et al. (2016). "Lactobacillus plantarum attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish." Scientific Reports **6**.

de Cossio, L. F., et al. (2017). "Impact of prebiotics on metabolic and behavioral alterations in a mouse model of metabolic syndrome." Brain Behavior and Immunity **64**: 33-49.

De Filippo, C., et al. (2010). "Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa." Proceedings of the National Academy of Sciences of the United States of America **107**(33): 14691-14696.

De La Cochetiere, M. F., et al. (2005). "Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge." J Clin Microbiol **43**(11): 5588-5592.

de Oliveira, G. L. V., et al. (2017). "Intestinal dysbiosis and probiotic applications in autoimmune diseases." Immunology **152**(1): 1-12.

De Paepe, M., et al. (2014). "Bacteriophages: an underestimated role in human and animal health?" Frontiers in Cellular and Infection Microbiology **4**.

De Preter, V., et al. (2013). "Metabolic Profiling of the Impact of Oligofructose-Enriched Inulin in Crohn's Disease Patients: A Double-Blinded Randomized Controlled Trial." Clinical and Translational Gastroenterology **4**.

De Preter, V., et al. (2015). "Faecal metabolite profiling identifies medium-chain fatty acids as discriminating compounds in IBD." Gut **64**(3): 447-+.

de Theije, C. G. M., et al. (2014). "Altered gut microbiota and activity in a murine model of autism spectrum disorders." Brain Behavior and Immunity **37**: 197-206.

de Vos, W. M. and E. A. J. de Vos (2012). "Role of the intestinal microbiome in health and disease: from correlation to causation." Nutrition Reviews **70**: S45-S56.

de Vrese, M. and J. Schrezenmeir (2008). "Probiotics, Prebiotics, and Synbiotics." Food Biotechnology **111**: 1-66.

De Weirdt, R., et al. (2017). "Mucosa-associated biohydrogenating microbes protect the simulated colon microbiome from stress associated with high concentrations of poly-unsaturated fat." Environmental Microbiology **19**(2): 722-739.

DellaGioia, N. and J. Hannestad (2010). "A critical review of human endotoxin administration as an experimental paradigm of depression." Neuroscience and Biobehavioral Reviews **34**(1): 130-143.

den Besten, G., et al. (2013). "The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism." Journal of Lipid Research **54**(9): 2325-2340.

Deng, B., et al. (2017). "Probiotics and Probiotic Metabolic Product Improved Intestinal Function and Ameliorated LPS-Induced Injury in Rats." Current Microbiology **74**(11): 1306-1315.

Deng, W., et al. (2012). "The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens." Poultry Science **91**(3): 575-582.

Derikx, J. P. M., et al. (2008). "Rapid Reversal of Human Intestinal Ischemia-Reperfusion Induced Damage by Shedding of Injured Enterocytes and Reepithelialisation." Plos One **3**(10).

Derikx, J. P. M., et al. (2008). "New Insight in Loss of Gut Barrier during Major Non-Abdominal Surgery." Plos One **3**(12).

Desai, M. S., et al. (2016). "A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility." Cell **167**(5): 1339-+.

Deshpande, G., et al. (2017). "Benefits of probiotics in preterm neonates in low-income and medium-income countries: a systematic review of randomised controlled trials." Bmj Open **7**(12).

Deshpande, G., et al. (2010). "Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates." Pediatrics **125**(5): 921-930.

Deshpande, G. C., et al. (2011). "Evidence-based guidelines for use of probiotics in preterm neonates." Bmc Medicine **9**.

Dethlefsen, L., et al. (2008). "The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing." PLoS Biol **6**(11): e280.

Dethlefsen, L. and D. A. Relman (2011). "Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation." Proc Natl Acad Sci U S A **108 Suppl 1**: 4554-4561.

Deusch, S., et al. (2018). "Effects of HIV, antiretroviral therapy and prebiotics on the active fraction of the gut microbiota." Aids **32**(10): 1229-1237.

Devkota, S. and E. B. Chang (2013). "Diet-induced expansion of pathobionts in experimental colitis: implications for tailored therapies." Gut Microbes **4**(2): 172-174.

Devkota, S. and E. B. Chang (2015). "Interactions between Diet, Bile Acid Metabolism, Gut Microbiota, and Inflammatory Bowel Diseases." Digestive Diseases **33**(3): 351-356.

Di Sabatino, A., et al. (2008). "Distribution, proliferation, and function of Paneth cells in uncomplicated and complicated adult celiac disease." American Journal of Clinical Pathology **130**(1): 34-42.

Dias, N. S., et al. (2016). "Jerusalem artichoke (Helianthus tuberosus, L.) maintains high inulin, tuber yield, and antioxidant capacity under moderately-saline irrigation waters." Industrial Crops and Products **94**: 1009-1024.

Dickson, R. P. and G. B. Huffnagle (2015). "The Lung Microbiome: New Principles for Respiratory Bacteriology in Health and Disease." Plos Pathogens **11**(7).

Didari, T., et al. (2014). "A systematic review of the safety of probiotics." Expert Opinion on Drug Safety **13**(2): 227-239.

Dinan, T. G. and J. F. Cryan (2012). "Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology." Psychoneuroendocrinology **37**(9): 1369-1378.

Dinan, T. G., et al. (2013). "Psychobiotics: A Novel Class of Psychotropic." Biological Psychiatry **74**(10): 720-726.

Ding, C. B., et al. (2018). "Intestinal microbiota: a novel perspective in colorectal cancer biotherapeutics." Oncotargets and Therapy **11**: 4797-4810.

Dinleyici, E. C., et al. (2018). "Time Series Analysis of the Microbiota of Children Suffering From Acute Infectious Diarrhea and Their Recovery After Treatment." Frontiers in Microbiology **9**.

Diop, L., et al. (2008). "Probiotic food supplement reduces stress-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial." Nutrition Research **28**(1): 1-5.

do Carmo, M. S., et al. (2018). "Probiotics, mechanisms of action, and clinical perspectives for diarrhea management in children." Food & Function **9**(10): 5074-5095.

Dohgu, S., et al. (2011). "Lipopolysaccharide-enhanced transcellular transport of HIV-1 across the blood-brain barrier is mediated by luminal microvessel IL-6 and GM-CSF." Journal of Neuroinflammation **8**.

Dokladny, K., et al. (2016). "Intestinal epithelial barrier function and tight junction proteins with heat and exercise." Journal of Applied Physiology **120**(6): 692-701.

Dominguez-Bello, M. G., et al. (2010). "Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns." Proceedings of the National Academy of Sciences of the United States of America **107**(26): 11971-11975.

Dong, L. N., et al. (2017). "Faecal and mucosal microbiota in patients with functional gastrointestinal disorders: Correlation with toll-like receptor 2/toll-like receptor 4 expression." World Journal of Gastroenterology **23**(36): 6665-6673.

Donohoe, D. R., et al. (2011). "The Microbiome and Butyrate Regulate Energy Metabolism and Autophagy in the Mammalian Colon." Cell Metabolism **13**(5): 517-526.

Dore, J., et al. (2017). "The human gut microbiome as source of innovation for health: Which physiological and therapeutic outcomes could we expect?" Therapie **72**(1): 21-38.

Dowd, S. E., et al. (2008). "Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)." BMC Microbiol **8**: 125.

Dowd, S. E., et al. (2008). "Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)." Bmc Microbiology **8**.

Dowd, S. E., et al. (2008). "Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs." Foodborne Pathog Dis **5**(4): 459-472.

Dridi, B. (2012). "Laboratory tools for detection of archaea in humans." Clinical Microbiology and Infection **18**(9): 825-833.

Ducluzeau, R., et al. (1984). "EFFECT OF BRAN INGESTION ON THE MICROBIAL FECAL FLORAS OF HUMAN DONORS AND OF RECIPIENT GNOTOBIOTIC MICE, AND ON THE BARRIER EFFECTS EXERTED BY THESE FLORAS AGAINST VARIOUS POTENTIALLY PATHOGENIC MICROBIAL STRAINS." Annales De Microbiologie **A135**(2): 303-318.

Duda-Chodak, A., et al. (2015). "Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review." European Journal of Nutrition **54**(3): 325-341.

Duenas, M., et al. (2015). "A Survey of Modulation of Gut Microbiota by Dietary Polyphenols." Biomed Research International.

Duerkop, B. A., et al. (2009). "Immune Responses to the Microbiota at the Intestinal Mucosal Surface." Immunity **31**(3): 368-376.

Eckburg, P. B., et al. (2005). "Diversity of the human intestinal microbial flora." Science **308**(5728): 1635-1638.

Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST." Bioinformatics **26**(19): 2460-2461.

El-Salhy, M. and T. Mazzawi (2018). "Fecal microbiota transplantation for managing irritable bowel syndrome." Expert Review of Gastroenterology & Hepatology **12**(5): 439-445.

Emanuele, E., et al. (2010). "Low-grade endotoxemia in patients with severe autism." Neuroscience Letters **471**(3): 162-165.

Eme, L. and W. F. Doolittle (2015). "Archaea." Current Biology **25**(19): R851-R855.

Engelstoft, M. S., et al. (2008). "A Gut Feeling for Obesity: 7TM Sensors on Enteroendocrine Cells." Cell Metabolism **8**(6): 447-449.

Engen, P. A., et al. (2015). "The Gastrointestinal Microbiome Alcohol Effects on the Composition of Intestinal Microbiota." Alcohol Research-Current Reviews **37**(2): 223-236.

Eren, A. M., et al. (2011). "Exploring the diversity of Gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation." PLoS One **6**(10): e26732.

Ericsson, A. C., et al. (2018). "The influence of caging, bedding, and diet on the composition of the microbiota in different regions of the mouse gut." Scientific Reports **8**: 13.

Erny, D., et al. (2015). "Host microbiota constantly control maturation and function of microglia in the CNS." Nature Neuroscience **18**(7): 965-+.

Espin, J. C., et al. (2017). "The gut microbiota: A key factor in the therapeutic effects of (poly) phenols." Biochemical Pharmacology **139**: 82-93.

Estienne, M., et al. (2010). "Maternal deprivation alters epithelial secretory cell lineages in rat duodenum: role of CRF-related peptides." Gut **59**(6): 744-751.

Eutamene, H., et al. (2018). "PROTECTIVE EFFECT OF MUCOPROTECTANTS AND PREBIOTIC COMBINATION ON GUT BARRIER IMPAIRMENT AND VISCERAL HYPERSENSITIVITY INDUCED BY AN ACUTE STRESS IN RAT." Gastroenterology **154**(6): S916-S916.

Evans, M., et al. (2012). "A Dried Yeast Fermentate Prevents and Reduces Inflammation in Two Separate Experimental Immune Models." Evidence-Based Complementary and Alternative Medicine.

Everard, A., et al. (2013). "Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity." Proceedings of the National Academy of Sciences of the United States of America **110**(22): 9066-9071.

Everard, A., et al. (2014). "Intestinal epithelial MyD88 is a sensor switching host metabolism towards obesity according to nutritional status." Nature Communications **5**.

Everard, A., et al. (2014). "Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity." Isme Journal **8**(10): 2116-2130.

Everson, C. A. and L. A. Toth (2000). "Systemic bacterial invasion induced by sleep deprivation." American Journal of Physiology-Regulatory Integrative and Comparative Physiology **278**(4): R905-R916.

Fasano, A. (2011). "Zonulin and Its Regulation of Intestinal Barrier Function: The Biological Door to Inflammation, Autoimmunity, and Cancer." Physiological Reviews **91**(1): 151-175.

Fasano, A. (2012). "Leaky Gut and Autoimmune Diseases." Clinical Reviews in Allergy & Immunology **42**(1): 71-78.

Fassbender, K. (2004). "The LPS receptor CD14 links Alzheimer's disease with innate immunity." Journal of Neuroimmunology **154**(1-2): 165-165.

Feng, Q., et al. (2015). "Gut microbiome development along the colorectal adenoma-carcinoma sequence." Nature Communications **6**.

Feng, W. W., et al. (2018). "Gut Microbiota, Short-Chain Fatty Acids, and Herbal Medicines." Frontiers in Pharmacology **9**.

Feng, Z., et al. (2017). "A human stool-derived Bilophila wadsworthia strain caused systemic inflammation in specific-pathogen-free mice." Gut Pathogens **9**.

Fernando, W. M. A. D. B., et al. (2010). "Enhancement of short chain fatty acid formation by pure cultures of probiotics on rice fibre." International Journal of Food Science and Technology **45**(4): 690-696.

Ferrocino, I., et al. (2018). "Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM)." Scientific Reports **8**.

Fickers, P., et al. (2009). "High-level biosynthesis of the anteiso-C(17) isoform of the antibiotic mycosubtilin in Bacillus subtilis and characterization of its candidacidal activity." Appl Environ Microbiol **75**(13): 4636-4640.

Fijan, S. (2014). "Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature." International Journal of Environmental Research and Public Health **11**(5): 4745-4767.

Fink, G. (2017). "Selye's general adaptation syndrome: stress-induced gastro-duodenal ulceration and inflammatory bowel disease." Journal of Endocrinology **232**(3): F1-F5.

Fioramonti, J., et al. (2003). "Probiotics: what are they? What are their effects on gut physiology?" Best Practice & Research in Clinical Gastroenterology **17**(5): 711-724.

Fleming, P., et al. (2015). "Probiotics and necrotizing enterocolitis." Pediatric Surgery International **31**(12): 1111-1118.

Flint, H. J., et al. (2007). "Interactions and competition within the microbial community of the human colon: links between diet and health." Environmental Microbiology **9**(5): 1101-1111.

Fond, G., et al. (2015). "The "psychomicrobiotic": Targeting microbiota in major psychiatric disorders: A systematic review." Pathologie Biologie **63**(1): 35-42.

Fooladi, A. A. I., et al. (2013). "Probiotic as a Novel Treatment Strategy Against Liver Disease." Hepatitis Monthly **13**(2).

Fornai, M., et al. (2018). "Neuronal regulation of intestinal immune functions in health and disease." Neurogastroenterology and Motility **30**(12).

Forsberg, A., et al. (2016). "Pre- and probiotics for allergy prevention: time to revisit recommendations?" Clinical and Experimental Allergy **46**(12): 1506-1521.

Foster, J. and K. A. Neufeld (2014). "Gut-brain axis: How the microbiome influences anxiety and depression." International Journal of Neuropsychopharmacology **17**: 27-27.

Foster, J. A., et al. (2017). "Stress & the gut-brain axis: Regulation by the microbiome." Neurobiology of Stress **7**: 124-136.

Foulongne, V., et al. (2012). "Human Skin Microbiota: High Diversity of DNA Viruses Identified on the Human Skin by High Throughput Sequencing." Plos One **7**(6).

Francino, M. P. (2015). "Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances." Front Microbiol **6**: 1543.

Frank, D. N., et al. (2007). "Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases." Proceedings of the National Academy of Sciences of the United States of America **104**(34): 13780-13785.

Frank, D. N. and N. R. Pace (2008). "Gastrointestinal microbiology enters the metagenomics era." Current Opinion in Gastroenterology **24**(1): 4-10.

Freestone, P. and M. Lyte (2010). "Stress and microbial endocrinology: prospects for ruminant nutrition." Animal **4**(7): 1248-1257.

Frost, B. L. and M. S. Caplan (2011). "Probiotics and prevention of neonatal necrotizing enterocolitis." Current Opinion in Pediatrics **23**(2): 151-155.

Fujimoto, T., et al. (2013). "Decreased abundance of Faecalibacterium prausnitzii in the gut microbiota of Crohn's disease." Journal of Gastroenterology and Hepatology **28**(4): 613-619.

Fukui, H. (2017). "Gut Microbiome-based Therapeutics in Liver Cirrhosis: Basic Consideration for the Next Step." Journal of Clinical and Translational Hepatology **5**(3): 249-260.

Fuller, R. (1989). "PROBIOTICS IN MAN AND ANIMALS." Journal of Applied Bacteriology **66**(5): 365-378.

Fung, T. C., et al. (2017). "Interactions between the microbiota, immune and nervous systems in health and disease." Nature Neuroscience **20**(2): 145-155.

Furness, J. B., et al. (2013). "The gut as a sensory organ." Nature Reviews Gastroenterology & Hepatology **10**(12): 729-740.

Gaci, N., et al. (2014). "Archaea and the human gut: new beginning of an old story." World J Gastroenterol **20**(43): 16062-16078.

Gadde, U. D., et al. (2017). "Dietary Bacillus subtilis-based direct-fed microbials alleviate LPS-induced intestinal immunological stress and improve intestinal barrier gene expression in commercial broiler chickens." Research in Veterinary Science **114**: 236-243.

Galdeano, C. M. and G. Perdigon (2004). "Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation." Journal of Applied Microbiology **97**(4): 673-681.

Galley, J. D. and M. T. Bailey (2014). "Impact of stressor exposure on the interplay between commensal microbiota and host inflammation." Gut Microbes **5**(3): 390-396.

Galley, J. D., et al. (2017). "Stressor exposure has prolonged effects on colonic microbial community structure in Citrobacter rodentium-challenged mice." Sci Rep **7**: 45012.

Galley, J. D., et al. (2014). "Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota." Bmc Microbiology **14**.

Gao, X. H., et al. (2018). "Chronic stress promotes colitis by disturbing the gut microbiota and triggering immune system response." Proceedings of the National Academy of Sciences of the United States of America **115**(13): E2960-E2969.

Gareau, M. G., et al. (2010). "Psychological Stress and Changes in the Intestinal Microflora Impact Memory in Mice." Faseb Journal **24**.

Garland, S. M., et al. (2011). "The ProPrems trial: investigating the effects of probiotics on late onset sepsis in very preterm infants." Bmc Infectious Diseases **11**.

Gecse, K., et al. (2012). "Leaky Gut in Patients with Diarrhea-Predominant Irritable Bowel Syndrome and Inactive Ulcerative Colitis." Digestion **85**(1): 40-46.

Gentekaki, E., et al. (2017). "Extreme genome diversity in the hyper-prevalent parasitic eukaryote Blastocystis." Plos Biology **15**(9).

Gepner, Y., et al. (2017). "Combined effect of Bacillus coagulans GBI-30, 6086 and HMB supplementation on muscle integrity and cytokine response during intense military training." Journal of Applied Physiology **123**(1): 11-18.

Gerhardt, S. and M. H. Mohajeri (2018). "Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases." Nutrients **10**(6).

Gerritsen, J., et al. (2011). "Intestinal microbiota in human health and disease: the impact of probiotics." Genes and Nutrition **6**(3): 209-240.

Gevers, D., et al. (2014). "The Treatment-Naive Microbiome in New-Onset Crohn's Disease." Cell Host & Microbe **15**(3): 382-392.

Ghashghaeinia, M., et al. (2012). "The impact of erythrocyte age on eryptosis." British Journal of Haematology **157**(5): 606-614.

Ghazalpour, A., et al. (2016). "Expanding role of gut microbiota in lipid metabolism." Current Opinion in Lipidology **27**(2): 141-147.

Giblot Ducray, H. A., et al. (2016). "Mitigation of heat stress-related complications by a yeast fermentate product." Journal of Thermal Biology **60**: 26-32.

Gibson, G. R. (1998). "Dietary modulation of the human gut microflora using prebiotics." British Journal of Nutrition **80**(4): S209-S212.

Gibson, G. R., et al. (2017). "The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics." Nature Reviews Gastroenterology & Hepatology **14**(8): 491-502.

Gill, S. K., et al. (2016). "Does Short-Term High Dose Probiotic Supplementation Containing Lactobacillus casei Attenuate Exertional-Heat Stress Induced Endotoxaemia and Cytokinaemia?" International Journal of Sport Nutrition and Exercise Metabolism **26**(3): 268-275.

Gill, S. K., et al. (2015). "Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition." Exercise Immunology Review **21**: 114-128.

Gill, S. R., et al. (2006). "Metagenomic analysis of the human distal gut microbiome." Science **312**(5778): 1355-1359.

Giraffa, G. (2012). "Selection and design of lactic acid bacteria probiotic cultures." Engineering in Life Sciences **12**(4): 391-398.

Girardin, M. and E. G. Seidman (2011). "Indications for the Use of Probiotics in Gastrointestinal Diseases." Digestive Diseases **29**(6): 574-587.

Glaser, R. and J. K. Kiecolt-Glaser (2005). "Science and society - Stress-induced immune dysfunction: implications for health." Nature Reviews Immunology **5**(3): 243-251.

Gnauck, A., et al. (2016). "The Characteristics and Function of Bacterial Lipopolysaccharides and Their Endotoxic Potential in Humans." International Reviews of Immunology **35**(3): 189-218.

Goldenberg, S. D., et al. (2018). "Comparison of Different Strategies for Providing Fecal Microbiota Transplantation to Treat Patients with Recurrent Clostridium difficile Infection in Two English Hospitals: A Review." Infectious Diseases and Therapy **7**(1): 71-86.

Golubeva, A. V., et al. (2015). "Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood." Psychoneuroendocrinology **60**: 58-74.

Gonai, M., et al. (2017). "Galacto-oligosaccharides ameliorate dysbiotic Bifidobacteriaceae decline in Japanese patients with type 2 diabetes." Beneficial Microbes **8**(5): 705-716.

Goncalves, F. L. L., et al. (2015). "Evaluation of the expression of I-FABP and L-FABP in a necrotizing enterocolitis model after the use of Lactobacillus acidophilus." Journal of Pediatric Surgery **50**(4): 543-549.

Gong, L. X., et al. (2018). "Whole Tibetan Hull-Less Barley Exhibit Stronger Effect on Promoting Growth of Genus Bifidobacterium than Refined Barley In Vitro." Journal of Food Science **83**(4): 1116-1124.

Gonzalez-Mariscal, L., et al. (2003). "Tight junction proteins." Progress in Biophysics & Molecular Biology **81**(1): 1-44.

Goodridge, H. S., et al. (2011). "Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'." Nature **472**(7344): 471-U541.

Gophna, U., et al. (2017). "Oscillospira and related bacteria - From metagenomic species to metabolic features." Environmental Microbiology **19**(3): 835-841.

Gordon, S. M. (2008). "Elie Metchnikoff: Father of natural immunity." European Journal of Immunology **38**(12): 3257-3264.

Gosalbez, L. and D. Ramon (2015). "Probiotics in transition: novel strategies." Trends in Biotechnology **33**(4): 195-196.

Gouba, N. and M. Drancourt (2015). "Digestive tract mycobiota: A source of infection." Medecine Et Maladies Infectieuses **45**(1-2): 9-16.

Gouba, N., et al. (2014). "Eukaryote Culturomics of the Gut Reveals New Species." Plos One **9**(9).

Goulart, F. R., et al. (2018). "Effect of dietary fiber concentrates on growth performance, gut morphology and hepatic metabolic intermediates in jundia (Rhamdia quelen)." Arquivo Brasileiro De Medicina Veterinaria E Zootecnia **70**(5): 1633-1640.

Gourbeyre, P., et al. (2011). "Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions." Journal of Leukocyte Biology **89**(5): 685-695.

Govender, M., et al. (2014). "A Review of the Advancements in Probiotic Delivery: Conventional vs. Non-conventional Formulations for Intestinal Flora Supplementation." Aaps Pharmscitech **15**(1): 29-43.

Grabitske, H. and J. Slavin (2009). "Gastrointestinal Effects of Low-Digestible Carbohydrates." Critical Reviews in Food Science and Nutrition **49**(4): 327-360.

Grabitske, H. A. and J. L. Slavin (2008). "Low-Digestible Carbohydrates in Practice." Journal of the American Dietetic Association **108**(10): 1677-1681.

Grall, N., et al. (2013). "Oral DAV131, a Charcoal-Based Adsorbent, Inhibits Intestinal Colonization by beta-Lactam-Resistant Klebsiella pneumoniae in Cefotaxime-Treated Mice." Antimicrobial Agents and Chemotherapy **57**(11): 5423-5425.

Graness, N., et al. (2018). "Analysis of the fecal microbiome in horses in association with antibiotic and prebiotic treatment." Berliner Und Munchener Tierarztliche Wochenschrift **131**(5-6): 224-238.

Grangett, C., et al. (2001). "Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant Lactobacillus plantarum." Infection and Immunity **69**(3): 1547-1553.

Greenway, F., et al. (2014). "A novel cobiotic containing a prebiotic and an antioxidant augments the glucose control and gastrointestinal tolerability of metformin: a case report." Beneficial Microbes **5**(1): 29-32.

Grenham, S., et al. (2011). "Brain-gut-microbe communication in health and disease." Frontiers in Physiology **2**.

Griffiths, E. A., et al. (2004). "In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in balb/c mice." Digestive Diseases and Sciences **49**(4): 579-589.

Grimaldi, R., et al. (2017). "In vitro fermentation of B-GOS: impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children." Fems Microbiology Ecology **93**(2).

Grimaldi, R., et al. (2018). "A prebiotic intervention study in children with autism spectrum disorders (ASDs)." Microbiome **6**.

Grimaudo, N. J. and W. E. Nesbitt (1997). "Coaggregation of Candida albicans with oral Fusobacterium species." Oral Microbiology and Immunology **12**(3): 168-173.

Grimaudo, N. J., et al. (1996). "Coaggregation of Candida albicans with oral Actinomyces species." Oral Microbiology and Immunology **11**(1): 59-61.

Grimoud, J., et al. (2010). "In vitro screening of probiotics and synbiotics according to anti-inflammatory and anti-proliferative effects." International Journal of Food Microbiology **144**(1): 42-50.

Groh, H., et al. (1993). "STEROID-METABOLISM WITH INTESTINAL MICROORGANISMS." Journal of Basic Microbiology **33**(1): 59-72.

Grootjans, J., et al. (2011). "Level of Activation of the Unfolded Protein Response Correlates With Paneth Cell Apoptosis in Human Small Intestine Exposed to Ischemia/Reperfusion." Gastroenterology **140**(2): 529-U248.

Grootjans, J., et al. (2016). "Life and death at the mucosal-luminal interface: New perspectives on human intestinal ischemia-reperfusion." World Journal of Gastroenterology **22**(9): 2760-2770.

Grootjans, J., et al. (2011). "Rapid lamina propria retraction and zipper-like constriction of the epithelium preserves the epithelial lining in human small intestine exposed to ischaemia-reperfusion." Journal of Pathology **224**(3): 411-419.

Grootjans, J., et al. (2010). "Non-invasive assessment of barrier integrity and function of the human gut." World Journal of Gastrointestinal Surgery **2**(3): 61-69.

Groschwitz, K. R. and S. P. Hogan (2009). "Intestinal barrier function: Molecular regulation and disease pathogenesis." Journal of Allergy and Clinical Immunology **124**(1): 3-20.

Grover, S., et al. (2012). "Probiotics for human health - new innovations and emerging trends." Gut Pathogens **4**.

Gruber, C. (2012). "Probiotics and prebiotics in allergy prevention and treatment: future prospects." Expert Review of Clinical Immunology **8**(1): 17-19.

Guarino, A., et al. (2015). "Probiotics for Prevention and Treatment of Diarrhea." Journal of Clinical Gastroenterology **49**: S37-S45.

Guerreiro, I., et al. (2018). "Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture." Reviews in Aquaculture **10**(4): 800-832.

Guglielmetti, S., et al. (2010). "Oral Bacteria as Potential Probiotics for the Pharyngeal Mucosa." Applied and Environmental Microbiology **76**(12): 3948-3958.

Guigoz, Y., et al. (2002). "Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people." Nutrition Research **22**(1-2): 13-25.

Gulati, A. S., et al. (2012). "Mouse background strain profoundly influences Paneth cell function and intestinal microbial composition." PLoS One **7**(2): e32403.

Guldris, S. C., et al. (2017). "Gut microbiota in chronic kidney disease." Nefrologia **37**(1): 9-19.

Guo, J. R., et al. (2018). "High-throughput sequencing reveals the effect of Bacillus subtilis CGMCC 1.921 on the cecal microbiota and gene expression in ileum mucosa of laying hens." Poultry Science **97**(7): 2543-2556.

Guo, X. L., et al. (2018). "Rutin and Its Combination With Inulin Attenuate Gut Dysbiosis, the Inflammatory Status and Endoplasmic Reticulum Stress in Paneth Cells of Obese Mice Induced by High-Fat Diet." Frontiers in Microbiology **9**.

Gupta, A., et al. (2017). "Heat stress modulated gastrointestinal barrier dysfunction: role of tight junctions and heat shock proteins." Scandinavian Journal of Gastroenterology **52**(12): 1315-1319.

Gupta, S., et al. (2016). "Fecal microbiota transplantation: in perspective." Therapeutic Advances in Gastroenterology **9**(2): 229-239.

Gurry, T. (2017). "Synbiotic approaches to human health and well-being." Microbial Biotechnology **10**(5): 1070-1073.

Gurry, T., et al. (2018). "Predictability and persistence of prebiotic dietary supplementation in a healthy human cohort." Scientific Reports **8**.

Guthmann, F., et al. (2010). "Probiotics for Prevention of Necrotising Enterocolitis: An Updated Meta-analysis." Klinische Padiatrie **222**(5): 284-290.

Hakansson, A. and G. Molin (2011). "Gut Microbiota and Inflammation." Nutrients **3**(6): 637-682.

Hall, D. M., et al. (2001). "Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia." American Journal of Physiology-Heart and Circulatory Physiology **280**(2): H509-H521.

Hall, R. A. and M. C. Noverr (2017). "Fungal interactions with the human host: exploring the spectrum of symbiosis." Current Opinion in Microbiology **40**: 58-64.

Hallen-Adams, H. E., et al. (2015). "Fungi inhabiting the healthy human gastrointestinal tract: a diverse and dynamic community." Fungal Ecology **15**: 9-17.

Haller, D., et al. (2010). "Guidance for substantiating the evidence for beneficial effects of probiotics: Probiotics in chronic inflammatory bowel disease and the functional disorder irritable bowel syndrome (vol 140, pg 690S, 2010)." Journal of Nutrition **140**(6): 1189-1189.

Hamad, I., et al. (2016). "Repertory of eukaryotes (eukaryome) in the human gastrointestinal tract: taxonomy and detection methods." Parasite Immunology **38**(1): 12-36.

Hamer, H. M., et al. (2008). "Review article: the role of butyrate on colonic function." Alimentary Pharmacology & Therapeutics **27**(2): 104-119.

Hamilton-Miller, J. M. T., et al. (2003). "Some insights into the derivation and early uses of the word 'probiotic'." British Journal of Nutrition **90**(4): 845-845.

Hancock, R. E. W. and H. G. Sahl (2006). "Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies." Nature Biotechnology **24**(12): 1551-1557.

Hanses, F., et al. (2011). "Intracellular Survival of Staphylococcus aureus in Adipocyte-Like Differentiated 3T3-L1 Cells Is Glucose Dependent and Alters Cytokine, Chemokine, and Adipokine Secretion." Endocrinology **152**(11): 4148-4157.

Hardy, H., et al. (2013). "Probiotics, Prebiotics and Immunomodulation of Gut Mucosal Defences: Homeostasis and Immunopathology." Nutrients **5**(6): 1869-1912.

Hartstra, A. V., et al. (2015). "Insights Into the Role of the Microbiome in Obesity and Type 2 Diabetes." Diabetes Care **38**(1): 159-165.

Hasan, M. T., et al. (2018). "Effects of Lactococcus lactis subsp lactis I2 with beta-Glucooligosaccharides on Growth, Innate Immunity and Streptococcosis Resistance in Olive Flounder (Paralichthys olivaceus)." Journal of Microbiology and Biotechnology **28**(9): 1433-1442.

Hayashi, H., et al. (2002). "Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods." Microbiology and Immunology **46**(8): 535-548.

He, S. S., et al. (2016). "Protective Effects of Ferulic Acid against Heat Stress-Induced Intestinal Epithelial Barrier Dysfunction In Vitro and In Vivo." Plos One **11**(2).

Hedblom, G. A., et al. (2018). "Segmented Filamentous Bacteria - Metabolism Meets Immunity." Frontiers in Microbiology **9**.

Heintz-Buschart, A. and P. Wilmes (2018). "Human Gut Microbiome: Function Matters." Trends in Microbiology **26**(7): 563-574.

Hemmerling, A. and C. R. Cohen (2011). "Probiotics: The Potential for a Live Microbicide to Prevent HIV." Jaids-Journal of Acquired Immune Deficiency Syndromes **56**(3): E98-E101.

Hill, D., et al. (2018). "The Lactobacillus casei Group: History and Health Related Applications." Frontiers in Microbiology **9**.

Hillman, E. T., et al. (2017). "Microbial Ecology along the Gastrointestinal Tract." Microbes and Environments **32**(4): 300-313.

Hoarau, G., et al. (2016). "Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease." Mbio **7**(5).

Hoffmann, C., et al. (2013). "Archaea and Fungi of the Human Gut Microbiome: Correlations with Diet and Bacterial Residents." Plos One **8**(6).

Hold, G. L. (2014). "The gut microbiota, dietary extremes and exercise." Gut **63**(12): 1838-+.

Holdeman, L. V., et al. (1976). "HUMAN FECAL FLORA - VARIATION IN BACTERIAL COMPOSITION WITHIN INDIVIDUALS AND A POSSIBLE EFFECT OF EMOTIONAL STRESS." Applied and Environmental Microbiology **31**(3): 359-375.

Holmes, E., et al. (2012). "Therapeutic Modulation of Microbiota-Host Metabolic Interactions." Science Translational Medicine **4**(137).

Hom, J. (2011). "Do Probiotics Reduce the Duration and Symptoms of Acute Infectious Diarrhea?" Annals of Emergency Medicine **58**(5): 445-446.

Hooda, S., et al. (2012). "454 Pyrosequencing Reveals a Shift in Fecal Microbiota of Healthy Adult Men Consuming Polydextrose or Soluble Corn Fiber." Journal of Nutrition **142**(7): 1259-1265.

Hooper, L. V. and J. I. Gordon (2001). "Commensal host-bacterial relationships in the gut." Science **292**(5519): 1115-1118.

Horner, K. M., et al. (2015). "Acute Exercise and Gastric Emptying: A Meta-Analysis and Implications for Appetite Control." Sports Medicine **45**(5): 659-678.

Horosheva, T. V., et al. (2014). "Efficacy of *Bacillus* probiotics in prevention of antibiotic-associated diarrhoea: a randomized, double-blind, placebo-controlled clinical trial." JMM Case Report **1**: doi:10.1099/jmmcr.1090.004036.

Horowitz, M. (2001). "Heat acclimation: phenotypic plasticity and cues to the underlying molecular mechanisms." Journal of Thermal Biology **26**(4-5): 357-363.

Houghteling, P. D. and W. A. Walker (2015). "From Birth to "Immunohealth," Allergies and Enterocolitis." Journal of Clinical Gastroenterology **49**: S7-S12.

Hsiao, E. Y., et al. (2013). "Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders." Cell **155**(7): 1451-1463.

Hsu, R. Y. C., et al. (2011). "LPS-Induced TLR4 Signaling in Human Colorectal Cancer Cells Increases beta 1 Integrin-Mediated Cell Adhesion and Liver Metastasis." Cancer Research **71**(5): 1989-1998.

Hsu, Y. J., et al. (2015). "EFFECT OF INTESTINAL MICROBIOTA ON EXERCISE PERFORMANCE IN MICE." Journal of Strength and Conditioning Research **29**(2): 552-558.

Huang, C. J., et al. (2011). "LPS-stimulated tumor necrosis factor-alpha and interleukin-6 mRNA and cytokine responses following acute psychological stress." Psychoneuroendocrinology **36**(10): 1553-1561.

Huang, K. Y., et al. (2017). "Identification of natural antimicrobial peptides from bacteria through metagenomic and metatranscriptomic analysis of high-throughput transcriptome data of Taiwanese oolong teas." Bmc Systems Biology **11**.

Hugenholtz, P. and G. W. Tyson (2008). "Microbiology - Metagenomics." Nature **455**(7212): 481-483.

Hughes, H. K., et al. (2018). "The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders." Current Neurology and Neuroscience Reports **18**(11).

Hullar, M. A. J., et al. (2015). "Enterolignan-Producing Phenotypes Are Associated with Increased Gut Microbial Diversity and Altered Composition in Premenopausal Women in the United States." Cancer Epidemiology Biomarkers & Prevention **24**(3): 546-554.

Hume, M. E. (2011). "Food Safety Symposium: Potential Impact of Reduced Antibiotic Use and the Roles of Prebiotics, Probiotics, and Other Alternatives in Antibiotic-Free Broiler Production." Poultry Science **90**(11): 2663-2669.

Hummelen, R., et al. (2010). "Micronutrients, N-Acetyl Cysteine, Probiotics and Prebiotics, a Review of Effectiveness in Reducing HIV Progression." Nutrients **2**(6): 626-651.

Hutkins, R. W., et al. (2016). "Prebiotics: why definitions matter." Current Opinion in Biotechnology **37**: 1-7.

Huttenhower, C., et al. (2012). "Structure, function and diversity of the healthy human microbiome." Nature **486**(7402): 207-214.

Hyland, N. P. and J. F. Cryan (2010). "A gut feeling about GABA: focus on GABA(B) receptors." Frontiers in Pharmacology **1**.

Iacono, A., et al. (2011). "Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms." Journal of Nutritional Biochemistry **22**(8): 699-711.

Ianiro, G., et al. (2018). "Bacillus clausii for the Treatment of Acute Diarrhea in Children: A Systematic Review and Meta-Analysis of Randomized Controlled Trials." Nutrients **10**(8).

Iliev, I. D., et al. (2012). "Interactions Between Commensal Fungi and the C-Type Lectin Receptor Dectin-1 Influence Colitis." Science **336**(6086): 1314-1317.

Ismail, I. H., et al. (2012). "Reduced gut microbial diversity in early life is associated with later development of eczema but not atopy in high-risk infants." Pediatr Allergy Immunol **23**(7): 674-681.

Isolauri, E., et al. (2001). "Probiotics: effects on immunity." American Journal of Clinical Nutrition **73**(2): 444S-450S.

Itani, T., et al. (2018). "Preterm infants with necrotising enterocolitis demonstrate an unbalanced gut microbiota." Acta Paediatrica **107**(1): 40-47.

Ito, S., et al. (2013). "Reduction of indoxyl sulfate by AST-120 attenuates monocyte inflammation related to chronic kidney disease." Journal of Leukocyte Biology **93**(6): 837-845.

Ivanov, II, et al. (2008). "Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine." Cell Host & Microbe **4**(4): 337-349.

Jackson, M. A., et al. (2018). "Gut microbiota associations with common diseases and prescription medications in a population-based cohort." Nature Communications **9**.

Jacobi, C. A., et al. (2011). "Treating critically ill patients with probiotics: Beneficial or dangerous?" Gut Pathogens **3**.

Jacobs, D. M., et al. (2009). "Non-Digestible Food Ingredients, Colonic Microbiota and the Impact on Gut Health and Immunity: A Role for Metabolomics." Current Drug Metabolism **10**(1): 41-54.

Jacouton, E., et al. (2017). "Probiotic strain Lactobacillus casei BL23 Prevents colitis-associated colorectal cancer." Frontiers in Immunology **8**.

Jacquier, V., et al. (2019). "Bacillus subtilis 29784 induces a shift in broiler gut microbiome toward butyrate-producing bacteria and improves intestinal histomorphology and animal performance." Poult Sci.

Jaeger, L. B., et al. (2009). "Lipopolysaccharide alters the blood-brain barrier transport of amyloid beta protein: A mechanism for inflammation in the progression of Alzheimer's disease." Brain Behavior and Immunity **23**(4): 507-517.

Jafarpour, D., et al. (2017). "Protective effects of synbiotic diets of Bacillus coagulans, Lactobacillus plantarum and inulin against acute cadmium toxicity in rats." Bmc Complementary and Alternative Medicine **17**.

Jager, R., et al. (2016). "Probiotic Bacillus coagulans GBI-30, 6086 reduces exercise-induced muscle damage and increases recovery." Peerj **4**.

Jaglin, M., et al. (2018). "Indole, a Signaling Molecule Produced by the Gut Microbiota, Negatively Impacts Emotional Behaviors in Rats." Frontiers in Neuroscience **12**.

Jakobsson, H. E., et al. (2015). "The composition of the gut microbiota shapes the colon mucus barrier." Embo Reports **16**(2): 164-177.

Janssen, P. H. and M. Kirs (2008). "Structure of the archaeal community of the rumen." Applied and Environmental Microbiology **74**(12): 3619-3625.

Jenkins, T. A., et al. (2009). "Neonatal lipopolysaccharide induces pathological changes in parvalbumin immunoreactivity in the hippocampus of the rat." Behavioural Brain Research **205**(2): 355-359.

Jenq, R. R., et al. (2015). "Intestinal Blautia Is Associated with Reduced Death from Graft-versus-Host Disease." Biol Blood Marrow Transplant **21**(8): 1373-1383.

Jensen, G., et al. (2008). A double-blind, placebo-controlled, randomized pilot study: consumption of a high-metabolite immunogen from yeast culture has beneficial effects on erythrocyte health and mucosal immune protection in healthy subjects. The Open Nutrition Journal. **2:** 68-75.

Jensen, G. S., et al. (2015). "Anti-Inflammatory Properties of a Dried Fermentate In Vitro and In Vivo." Journal of Medicinal Food **18**(3): 378-384.

Jensen, G. S., et al. (2007). "An antiinflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes in vitro." Nutrition Research **27**(6): 327-335.

Jensen, G. S., et al. (2008). "Yeast culture has anti-inflammatory effects and specifically activates NK cells." Comparative Immunology Microbiology and Infectious Diseases **31**(6): 487-500.

Jensen, G. S., et al. (2011). Antioxidant Bioavailability and Rapid Immune-Modulating Effects After Consumption of a Single Acute Dose of a High-Metabolite Yeast Immunogen: Results of a Placebo-Controlled Double-Blinded Crossover Pilot Study. J Med Food. 140 Huguenot Street, 3rd FloorNew Rochelle, NY 10801USA. **14:** 1002-1010.

Jeppsson, B., et al. (2011). "Use of Probiotics as Prophylaxis for Postoperative Infections." Nutrients **3**(5): 604-612.

Jernberg, C., et al. (2007). "Long-term ecological impacts of antibiotic administration on the human intestinal microbiota." Isme j **1**(1): 56-66.

Jia, N., et al. (2018). "Amelioration of hepatic steatosis is associated with modulation of gut microbiota and suppression of hepatic miR-34a in Gynostemma pentaphylla (Thunb.) Makino treated mice." Nutrition & Metabolism **15**: 13.

Jing, Y. P., et al. (2018). "Absorption of Codonopsis pilosula Saponins by Coexisting Polysaccharides Alleviates Gut Microbial Dysbiosis with Dextran Sulfate Sodium-Induced Colitis in Model Mice." Biomed Research International.

Jinno, S., et al. (2017). "Maternal Prebiotic Ingestion Increased the Number of Fecal Bifidobacteria in Pregnant Women but Not in Their Neonates Aged One Month." Nutrients **9**(3).

Johansson, M. E. V. and G. C. Hansson (2016). "Immunological aspects of intestinal mucus and mucins." Nature Reviews Immunology **16**(10): 639-649.

Johnson-Henry, K. C., et al. (2016). "Probiotics, Prebiotics, and Synbiotics for the Prevention of Necrotizing Enterocolitis." Advances in Nutrition **7**(5): 928-937.

Jonkers, D. (2016). "Microbial perturbations and modulation in conditions associated with malnutrition and malabsorption." Best Practice & Research Clinical Gastroenterology **30**(2): 161-172.

Jumpertz, R., et al. (2011). "Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans." American Journal of Clinical Nutrition **94**(1): 58-65.

Kamal, E., et al. (2018). "Gum Arabic Fibers Decreased Inflammatory Markers and Disease Severity Score among Rheumatoid Arthritis Patients, Phase II Trial." International Journal of Rheumatology.

Kang, D. W., et al. (2017). "Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study." Microbiome **5**.

Kang, Y. B. and Y. Cai (2017). "Gut microbiota and depression: from pathogenesis to new therapeutic strategies." Reviews in Medical Microbiology **28**(2): 56-62.

Karl, J. P., et al. (2018). "Effects of Psychological, Environmental and Physical Stressors on the Gut Microbiota." Frontiers in Microbiology **9**.

Karl, J. P., et al. (2017). "Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress." American Journal of Physiology-Gastrointestinal and Liver Physiology **312**(6): G559-G571.

Kasatpibal, N., et al. (2017). "Effectiveness of Probiotic, Prebiotic, and Synbiotic Therapies in Reducing Postoperative Complications: A Systematic Review and Network Meta-analysis." Clinical Infectious Diseases **64**: S153-S160.

Kassam, Z., et al. (2013). "Fecal Microbiota Transplantation for Clostridium difficile Infection: Systematic Review and Meta-Analysis." American Journal of Gastroenterology **108**(4): 500-508.

Keita, A. V. and J. D. Soderholm (2010). "The intestinal barrier and its regulation by neuroimmune factors." Neurogastroenterology and Motility **22**(7): 718-733.

Kelly, J. R., et al. (2015). "Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders." Frontiers in Cellular Neuroscience **9**.

Kermanshahi, H., et al. (2018). "Effects of non-starch polysaccharides in semi-purified diets on performance, serum metabolites, gastrointestinal morphology, and microbial population of male broiler chickens." Livestock Science **214**: 93-97.

Khan, M. S. A., et al. (2018). "Fruit-Derived Polysaccharides and Terpenoids: Recent Update on the Gastroprotective Effects and Mechanisms." Frontiers in Pharmacology **9**.

Kho, Z. Y. and S. K. Lal (2018). "The Human Gut Microbiome - A Potential Controller of Wellness and Disease." Frontiers in Microbiology **9**.

Khoder, M., et al. (2010). "Removal of residual colonic ciprofloxacin in the rat by activated charcoal entrapped within zinc-pectinate beads." European Journal of Pharmaceutical Sciences **41**(2): 281-288.

Kilkkinen, A., et al. (2001). "Determinants of serum enterolactone concentration." American Journal of Clinical Nutrition **73**(6): 1094-1100.

Kim, J. S., et al. (2011). "Differential immune response of adipocytes to virulent and attenuated Mycobacterium tuberculosis." Microbes and Infection **13**(14-15): 1242-1251.

Kim, N., et al. (2018). "Mind-altering with the gut: Modulation of the gut-brain axis with probiotics." Journal of Microbiology **56**(3): 172-182.

Kim, W. S., et al. (2018). "A new way of producing pediocin in Pediococcus acidilactici through intracellular stimulation by internalized inulin nanoparticles." Scientific Reports **8**.

King, M. A., et al. (2015). "Biomarkers of multiorgan injury in a preclinical model of exertional heat stroke." Journal of Applied Physiology **118**(10): 1207-1220.

Klein, E. J., et al. (2006). "Diarrhea etiology in a children's hospital emergency department: A prospective cohort study." Clinical Infectious Diseases **43**(7): 807-813.

Kleniewska, P., et al. (2016). "The Influence of Probiotic Lactobacillus casei in Combination with Prebiotic Inulin on the Antioxidant Capacity of Human Plasma." Oxidative Medicine and Cellular Longevity.

Knowles, S. R., et al. (2008). "Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: A possible mechanism underlying susceptibility to illness." Biological Psychology **77**(2): 132-137.

Koch, S. and A. Nusrat (2012). "The Life and Death of Epithelia During Inflammation: Lessons Learned from the Gut." Annual Review of Pathology: Mechanisms of Disease, Vol 7 **7**: 35-60.

Koeller, K., et al. (2018). "Microbiome and Culture Based Analysis of Chronic Rhinosinusitis Compared to Healthy Sinus Mucosa." Frontiers in Microbiology **9**.

Koenig, J. E., et al. (2011). "Succession of microbial consortia in the developing infant gut microbiome." Proceedings of the National Academy of Sciences of the United States of America **108**: 4578-4585.

Koenig, S., et al. (2018). "Age Dependent Hypothalamic and Pituitary Responses to Novel Environment Stress or Lipopolysaccharide in Rats." Frontiers in Behavioral Neuroscience **12**.

Kolida, S. and G. R. Gibson (2011). "Synbiotics in Health and Disease." Annual Review of Food Science and Technology, Vol 2 **2**: 373-393.

Kollath, W. (1953). "[The increase of the diseases of civilization and their prevention]." Munch Med Wochenschr **95**(47): 1260-1262.

Konig, J., et al. (2016). "Human Intestinal Barrier Function in Health and Disease." Clinical and Translational Gastroenterology **7**.

Koskella, B. and S. Meaden (2013). "Understanding Bacteriophage Specificity in Natural Microbial Communities." Viruses-Basel **5**(3): 806-823.

Koskinen, K., et al. (2017). "First Insights into the Diverse Human Archaeome: Specific Detection of Archaea in the Gastrointestinal Tract, Lung, and Nose and on Skin." Mbio **8**(6): 17.

Kraatz, M., et al. (2011). "Olsenella umbonata sp. nov., a microaerotolerant anaerobic lactic acid bacterium from the sheep rumen and pig jejunum, and emended descriptions of Olsenella, Olsenella uli and Olsenella profusa." Int J Syst Evol Microbiol **61**(Pt 4): 795-803.

Krishna, G., et al. (2015). "A Combination Supplement of Fructo- and Xylo-Oligosaccharides Significantly Abrogates Oxidative Impairments and Neurotoxicity in Maternal/Fetal Milieu Following Gestational Exposure to Acrylamide in Rat." Neurochemical Research **40**(9): 1904-1918.

Kriss, M., et al. (2018). "Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery." Current Opinion in Microbiology **44**: 34-40.

Krug, S. M., et al. (2014). "Tight junction, selective permeability, and related diseases." Seminars in Cell & Developmental Biology **36**: 166-176.

Krumbeck, J. A., et al. (2016). "Prebiotics and synbiotics: dietary strategies for improving gut health." Current Opinion in Gastroenterology **32**(2): 110-119.

Krumbeck, J. A., et al. (2018). "Synbiotics for Improved Human Health: Recent Developments, Challenges, and Opportunities." Annual Review of Food Science and Technology, Vol 9 **9**: 451-479.

Kumar, M., et al. (2010). "Cancer-preventing attributes of probiotics: an update." International Journal of Food Sciences and Nutrition **61**(5): 473-496.

La Ragione, R. M. and M. J. Woodward (2003). "Competitive exclusion by Bacillus subtilis spores of Salmonella enterica serotype Enteritidis and Clostridium perfringens in young chickens." Veterinary Microbiology **94**(3): 245-256.

Lagenaur, L. A., et al. (2011). "Prevention of vaginal SHIV transmission in macaques by a live recombinant Lactobacillus." Mucosal Immunology **4**(6): 648-657.

Lam, Y. Y., et al. (2012). "Increased Gut Permeability and Microbiota Change Associate with Mesenteric Fat Inflammation and Metabolic Dysfunction in Diet-Induced Obese Mice." Plos One **7**(3).

Lama, A., et al. (2018). "BUTYRATE MODULATES GUT-BRAIN AXIS IN ANTIBIOTIC-INDUCED INTESTINAL INJURY ASSOCIATED TO PARKINSON'S DISEASE IN MICE." Basic & Clinical Pharmacology & Toxicology **123**: 46-46.

Lambert, G. P. (2004). "Role of gastrointestinal permeability in exertional heatstroke." Exercise and Sport Sciences Reviews **32**(4): 185-190.

Lambert, G. P. (2009). "Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects." Journal of Animal Science **87**(14): E101-E108.

Lambert, G. P., et al. (2002). "Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress." Journal of Applied Physiology **92**(4): 1750-1761.

Lambert, J. E., et al. (2015). "Exercise training modifies gut microbiota in normal and diabetic mice." Applied Physiology Nutrition and Metabolism **40**(7): 749-752.

Lamiki, P., et al. (2010). "Probiotics in Diverticular Disease of the Colon: an Open Label Study." Journal of Gastrointestinal and Liver Diseases **19**(1): 31-36.

Lang, E. and F. Lang (2015). "Mechanisms and pathophysiological significance of eryptosis, the suicidal erythrocyte death." Seminars in Cell & Developmental Biology **39**: 35-42.

Lange, J. H. (2004). "Endotoxin as a factor for joint pain and rheumatoid arthritis." Clinical Rheumatology **23**(6): 566-566.

Lange, J. H., et al. (2006). "Endotoxin, a possible agent in the causation of Parkinson's disease." Journal of Occupational and Environmental Medicine **48**(7): 655-655.

Lassenius, M. I., et al. (2011). "Bacterial Endotoxin Activity in Human Serum Is Associated With Dyslipidemia, Insulin Resistance, Obesity, and Chronic Inflammation." Diabetes Care **34**(8): 1809-1815.

Latorre, J. D., et al. (2018). "Evaluation of the Epithelial Barrier Function and Ileal Microbiome in an Established Necrotic Enteritis Challenge Model in Broiler Chickens." Frontiers in Veterinary Science **5**.

LaTuga, M. S., et al. (2011). "Beyond Bacteria: A Study of the Enteric Microbial Consortium in Extremely Low Birth Weight Infants." Plos One **6**(12).

Lau, W. L., et al. (2018). "Altered microbiome in chronic kidney disease: systemic effects of gut-derived uremic toxins." Clinical Science **132**(5): 509-522.

Lay, C., et al. (2005). "Colonic microbiota signatures across five northern European countries." Applied and Environmental Microbiology **71**(7): 4153-4155.

LeBlanc, J. G., et al. (2013). "Bacteria as vitamin suppliers to their host: a gut microbiota perspective." Current Opinion in Biotechnology **24**(2): 160-168.

Lee, H. R., et al. (2014). "Tannerella forsythia BspA increases the risk factors for atherosclerosis in ApoE(-/-) mice." Oral Diseases **20**(8): 803-808.

Lee, J. H., et al. (2015). "Roles of lndole as an Interspecies and Interkingdom Signaling Molecule." Trends in Microbiology **23**(11): 707-718.

Lee, K. W., et al. (2010). "Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens." Poultry Science **89**(2): 203-216.

Lehto, M. and P. H. Groop (2018). "The Gut-Kidney Axis: Putative Interconnections Between Gastrointestinal and Renal Disorders." Frontiers in Endocrinology **9**.

Leon, L. R. and B. G. Helwig (2010). "Heat stroke: Role of the systemic inflammatory response." Journal of Applied Physiology **109**(6): 1980-1988.

Leung, K. W., et al. (2009). "Bacterial endotoxin activates retinal pigment epithelial cells and induces their degeneration through IL-6 and IL-8 autocrine signaling." Molecular Immunology **46**(7): 1374-1386.

Ley, R. E., et al. (2008). "Evolution of mammals and their gut microbes." Science **320**(5883): 1647-1651.

Li, Q. R., et al. (2014). "Dysbiosis of Gut Fungal Microbiota is Associated With Mucosal Inflammation in Crohn's Disease." Journal of Clinical Gastroenterology **48**(6): 513-523.

Li, Z. D., et al. (2018). "Effects of Metabolites Derived From Gut Microbiota and Hosts on Pathogens." Frontiers in Cellular and Infection Microbiology **8**: 12.

Li, Z. P., et al. (2014). "Xylooligosaccharide increases bifidobacteria but not lactobacilli demonstrating potential for obesity prevention and treatment." Faseb Journal **28**(1).

Liang, L., et al. (2017). "Effects of gut microbiota disturbance induced in early life on the expression of extrasynaptic GABA-A receptor alpha 5 and delta subunits in the hippocampus of adult rats." Brain Research Bulletin **135**: 113-119.

Liang, S., et al. (2018). "Gut-Brain Psychology: Rethinking Psychology From the Microbiota-Gut-Brain Axis." Frontiers in Integrative Neuroscience **12**.

Lied, G. A., et al. (2011). "Perceived food hypersensitivity: A review of 10 years of interdisciplinary research at a reference center." Scandinavian Journal of Gastroenterology **46**(10): 1169-1178.

Lim, C. L. and L. T. Mackinnon (2006). "The roles of exercise-induced immune system disturbances in the pathology of heat stroke - The dual pathway model of heat stroke." Sports Medicine **36**(1): 39-64.

Lin, Z. L., et al. (2017). "Lipid-rich enteral nutrition controls intestinal inflammation, improves intestinal motility and mucosal barrier damage in a rat model of intestinal ischemia/reperfusion injury." Journal of Surgical Research **213**: 75-83.

Lingwood, C. A. (1999). "Glycolipid receptors for verotoxin and Helicobacter pylori: role in pathology." Biochimica Et Biophysica Acta-Molecular Basis of Disease **1455**(2-3): 375-386.

Lionetti, E., et al. (2010). "Role of Probiotics in Pediatric Patients with Helicobacter pylori Infection: A Comprehensive Review of the Literature." Helicobacter **15**(2): 79-87.

Liu, L. Y., et al. (2018). "Cecal microbiome profile altered by Salmonella enterica, serovar Enteritidis inoculation in chicken." Gut Pathogens **10**.

Liu, Q., et al. (2004). "Synbiotic modulation of gut flora: Effect on minimal hepatic encephalopathy in patients with cirrhosis." Hepatology **39**(5): 1441-1449.

Liu, W., et al. (2018). "Quinoa whole grain diet compromises the changes of gut microbiota and colonic colitis induced by dextran Sulfate sodium in C57BL/6 mice." Scientific Reports **8**.

Liu, X., et al. (2012). "Reduction of intestinal mucosal immune function in heat-stressed rats and bacterial translocation." International Journal of Hyperthermia **28**(8): 756-765.

Liu, Y. W., et al. (2018). "New perspectives of Lactobacillus plantarum as a probiotic: The gut-heart-brain axis." Journal of Microbiology **56**(9): 601-613.

Liu, Z. H., et al. (2010). "Protective effects of Lactobacillus plantarum against epithelial barrier dysfunction of human colon cell line NCM460." World Journal of Gastroenterology **16**(45): 5759-5765.

Lizko, N. N. (1987). "Stress and Intestinal Microflora." Nahrung-Food **31**(5-6): 443-447.

Lloyd-Price, J., et al. (2016). "The healthy human microbiome." Genome Medicine **8**.

Lorenzo-Zuniga, V., et al. (2003). "Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats." Hepatology **37**(3): 551-557.

Luckey, T. D. (1972). "INTRODUCTION TO INTESTINAL MICROECOLOGY." American Journal of Clinical Nutrition **25**(12): 1292-1294.

Lundell, L. (2011). "Use of Probiotics in Abdominal Surgery." Digestive Diseases **29**(6): 570-573.

Luo, Y. H., et al. (2013). "Broilers fed dietary vitamins harbor higher diversity of cecal bacteria and higher ratio of Clostridium, Faecalibacterium, and Lactobacillus than broilers with no dietary vitamins revealed by 16S rRNA gene clone libraries." Poultry Science **92**(9): 2358-2366.

Luo, Y. Y., et al. (2018). "Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus." Translational Psychiatry **8**.

Lurie-Weinberger, M. N. and U. Gophna (2015). "Archaea in and on the Human Body: Health Implications and Future Directions." Plos Pathogens **11**(6).

Lutgendorff, F., et al. (2008). "The role of microbiota and probiotics in stress-induced gastrointestinal damage." Current Molecular Medicine **8**(4): 282-298.

Lutgendorff, F., et al. (2008). "Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis." American Journal of Physiology-Gastrointestinal and Liver Physiology **295**(5): G1111-G1121.

Ly, N. P., et al. (2011). "Gut microbiota, probiotics, and vitamin D: Interrelated exposures influencing allergy, asthma, and obesity?" Journal of Allergy and Clinical Immunology **127**(5): 1087-1094.

Lynch, S. V. and O. Pedersen (2016). "The Human Intestinal Microbiome in Health and Disease." New England Journal of Medicine **375**(24): 2369-2379.

Lyte, M. (2010). "Microbial endocrinology as a basis for improved L-DOPA bioavailability in Parkinson's patients treated for Helicobacter pylori." Medical Hypotheses **74**(5): 895-897.

Lyte, M. (2014). Microbial Endocrinology and the Microbiota-Gut-Brain Axis. Microbial Endocrinology: the Microbiota-Gut-Brain Axis in Health and Disease. M. Lyte and J. F. Cryan. **817:** 3-24.

Lyte, M. (2014). "The effect of stress on microbial growth." Animal Health Research Reviews **15**(2): 172-174.

Lyte, M. (2014). "The gut microbiota and neuroendocrine function." International Journal of Neuropsychopharmacology **17**: 27-27.

Lyte, M., et al. (2018). "Microbial endocrinology-Why the integration of microbes, epithelial cells, and neurochemical signals in the digestive tract matters to ruminant health." Journal of Dairy Science **101**(6): 5619-5628.

Macfarlane, S., et al. (1998). "Polysaccharide degradation by human intestinal bacteria during growth under multi-substrate limiting conditions in a three-stage continuous culture system." Fems Microbiology Ecology **26**(3): 231-243.

Mach, N., et al. (2015). "Early-life establishment of the swine gut microbiome and impact on host phenotypes." Environmental Microbiology Reports **7**(3): 554-569.

Mach, N. and D. Fuster-Botella (2017). "Endurance exercise and gut microbiota: A review." Journal of Sport and Health Science **6**(2): 179-197.

Mack, D. R., et al. (1999). "Probiotics inhibit enteropathogenic E-coli adherence in vitro by inducing intestinal mucin gene expression." American Journal of Physiology-Gastrointestinal and Liver Physiology **276**(4): G941-G950.

Mackos, A. R., et al. (2017). "The role of the commensal microbiota in adaptive and maladaptive stressor-induced immunomodulation." Hormones and Behavior **88**: 70-78.

MacPherson, C. W., et al. (2018). "Gut Bacterial Microbiota and its Resistome Rapidly Recover to Basal State Levels after Shortterm Amoxicillin-Clavulanic Acid Treatment in Healthy Adults." Scientific Reports **8**: 14.

Maes, M., et al. (2007). "Normalization of the increased translocation of endotoxin from gram negative enterobacteria (leaky gut) is accompanied by a remission of chronic fatigue syndrome." Neuroendocrinology Letters **28**(6): 739-744.

Maes, M., et al. (2008). "The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression." Neuroendocrinology Letters **29**(1): 117-124.

Maes, M., et al. (2011). "Depression's multiple comorbidities explained by (neuro)inflammatory and oxidative & nitrosative stress pathways." Neuroendocrinology Letters **32**(1): 7-24.

Maes, M. and J. C. Leunis (2008). "Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria." Neuroendocrinology Letters **29**(6): 902-910.

Mahlapuu, M., et al. (2016). "Antimicrobial Peptides : An Emerging Category of Therapeutic Agents." Frontiers in Cellular and Infection Microbiology **6**.

Maity, C., et al. (2012). "Study of the cultivable microflora of the large intestine of the rat under varied environmental hyperbaric pressures." Journal of Microbiology Immunology and Infection **45**(4): 281-286.

Malaguarnera, G., et al. (2014). "Gut microbiota in alcoholic liver disease: Pathogenetic role and therapeutic perspectives." World Journal of Gastroenterology **20**(44): 16639-16648.

Mallipattu, S. K., et al. (2012). "Role of Advanced Glycation Endproducts and Potential Therapeutic Interventions in Dialysis Patients." Seminars in Dialysis **25**(5): 529-538.

Manach, C., et al. (2005). "Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies." American Journal of Clinical Nutrition **81**(1): 230S-242S.

Mancini, A., et al. (2018). "Gut : liver : brain axis: the microbial challenge in the hepatic encephalopathy." Food & Function **9**(3): 1373-1388.

Mancuso, C. and R. Santangelo (2018). "Alzheimer's disease and gut microbiota modifications: The long way between preclinical studies and clinical evidence." Pharmacological Research **129**: 329-336.

Mandel, D. R., et al. (2010). "Bacillus coagulans: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial." Bmc Complementary and Alternative Medicine **10**.

Manrique, P., et al. (2016). "Healthy human gut phageome." Proceedings of the National Academy of Sciences of the United States of America **113**(37): 10400-10405.

Mantegazza, C., et al. (2018). "Probiotics and antibiotic-associated diarrhea in children: A review and new evidence on Lactobacillus rhamnosus GG during and after antibiotic treatment." Pharmacological Research **128**: 63-72.

Mao, N., et al. (2018). "Probiotic strains detect and suppress cholera in mice." Science Translational Medicine **10**(445).

Marchesi, J. R., et al. (2016). "The gut microbiota and host health: a new clinical frontier." Gut **65**(2): 330-339.

Marin, I. A., et al. (2017). "Microbiota alteration is associated with the development of stress-induced despair behavior." Scientific Reports **7**.

Markowiak, P. and K. Slizewska (2017). "Effects of Probiotics, Prebiotics, and Synbiotics on Human Health." Nutrients **9**(9).

Marotz, C. A. and A. Zarrinpar (2016). "Treating Obesity and Metabolic Syndrome with Fecal Microbiota Transplantation." Yale J Biol Med **89**(3): 383-388.

Martin, F. P. J., et al. (2010). "Dietary Modulation of Gut Functional Ecology Studied by Fecal Metabonomics." Journal of Proteome Research **9**(10): 5284-5295.

Martinez, I., et al. (2013). "Gut microbiome composition is linked to whole grain-induced immunological improvements." Isme Journal **7**(2): 269-280.

Martinez, R. C. R., et al. (2015). "Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: an update for current perspectives and future challenges." British Journal of Nutrition **114**(12): 1993-2015.

Martins, F. S., et al. (2013). "Inhibition of tissue inflammation and bacterial translocation as one of the protective mechanisms of Saccharomyces boulardii against Salmonella infection in mice." Microbes and Infection **15**(4): 270-279.

Maslov, S. and K. Sneppen (2017). "Population cycles and species diversity in dynamic Kill-the-Winner model of microbial ecosystems." Scientific Reports **7**.

Matsumoto, M., et al. (2012). "Promotion of Intestinal Peristalsis by Bifidobacterium spp. Capable of Hydrolysing Sennosides in Mice." Plos One **7**(2).

Matsumoto, M. and S. Kurihara (2011). "Probiotics-induced increase of large intestinal luminal polyamine concentration may promote longevity." Medical Hypotheses **77**(4): 469-472.

Matur, E., et al. (2016). "The effects of environmental enrichment and transport stress on the weights of lymphoid organs, cell-mediated immune response, heterophil functions and antibody production in laying hens." Animal Science Journal **87**(2): 284-292.

Maurice, C. F., et al. (2013). "Xenobiotics Shape the Physiology and Gene Expression of the Active Human Gut Microbiome." Cell **152**(1-2): 39-50.

Mazmanian, S. K., et al. (2005). "An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system." Cell **122**(1): 107-118.

Mazzoli, R. and E. Pessione (2016). "The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling." Frontiers in Microbiology **7**.

McFarland, L. V. (2009). "Evidence-based review of probiotics for antibiotic-associated diarrhea and Clostridium difficile infections." Anaerobe **15**(6): 274-280.

McGee, R. G., et al. (2011). "Probiotics for patients with hepatic encephalopathy." Cochrane Database of Systematic Reviews(11).

McIntyre, C. W., et al. (2011). "Circulating Endotoxemia: A Novel Factor in Systemic Inflammation and Cardiovascular Disease in Chronic Kidney Disease." Clinical Journal of the American Society of Nephrology **6**(1): 133-141.

McKean, J., et al. (2017). "Probiotics and Subclinical Psychological Symptoms in Healthy Participants: A Systematic Review and Meta-Analysis." Journal of Alternative and Complementary Medicine **23**(4): 249-258.

McMorris, T., et al. (2006). "Heat stress, plasma concentrations of adrenaline, noradrenaline, 5-hydroxytryptamine and cortisol, mood state and cognitive performance." International Journal of Psychophysiology **61**(2): 204-215.

McVay, C. S., et al. (2007). "Phage therapy of Pseudomonas aeruginosa infection in a mouse burn wound model." Antimicrobial Agents and Chemotherapy **51**(6): 1934-1938.

Medzhitov, R. and C. A. Janeway (2002). "Decoding the patterns of self and nonself by the innate immune system." Science **296**(5566): 298-300.

Mei, G. Y., et al. (2011). "Utilization of different types of dietary fibres by protential probiotics." Canadian Journal of Microbiology **57**(10): 857-865.

Meijerink, M., et al. (2013). "Challenges in translational research on probiotic lactobacilli: from in vitro assays to clinical trials." Beneficial Microbes **4**(1): 83-100.

Menard, S., et al. (2008). "Developmental switch of intestinal antimicrobial peptide expression." Journal of Experimental Medicine **205**(1): 183-193.

Mencarelli, A., et al. (2011). "Probiotics Modulate Intestinal Expression of Nuclear Receptor and Provide Counter-Regulatory Signals to Inflammation-Driven Adipose Tissue Activation." Plos One **6**(7).

Meng, C. T., et al. (2018). "Human Gut Microbiota and Gastrointestinal Cancer." Genomics Proteomics & Bioinformatics **16**(1): 33-49.

Mennini, M., et al. (2017). "Probiotics in Asthma and Allergy Prevention." Frontiers in Pediatrics **5**.

Mercer, M. B., et al. (2012). "How Patients View Probiotics Findings From a Multicenter Study of Patients With Inflammatory Bowel Disease and Irritable Bowel Syndrome." Journal of Clinical Gastroenterology **46**(2): 138-144.

Micetic-Turk, D. and M. Sikic-Pogacar (2011). "Clinical use of probiotics in pediatrics." Zdravniski Vestnik-Slovenian Medical Journal **80**(12): 933-943.

Michela, B., et al. (2015). "Double blind, placebo-controlled trial of a fermented milk containing multiple probiotics strains and prebiotic fiber for constipation associated with parkinson's disease." Journal of the Neurological Sciences **357**: E260-E260.

Mika, A., et al. (2016). "Exercise and Prebiotics Produce Stress Resistance: Converging Impacts on Stress-Protective and Butyrate-Producing Gut Bacteria." Gut Microbiome and Behavior **131**: 165-191.

Millar, M., et al. (2017). "The Microbiome of Infants Recruited to a Randomised Placebo-controlled Probiotic Trial (PiPS Trial)." Ebiomedicine **20**: 255-262.

Miller, T. L. and M. J. Wolin (1986). "METHANOGENS IN HUMAN AND ANIMAL INTESTINAL TRACTS." Systematic and Applied Microbiology **7**(2-3): 223-229.

Million, M., et al. (2016). "Increased Gut Redox and Depletion of Anaerobic and Methanogenic Prokaryotes in Severe Acute Malnutrition." Scientific Reports **6**.

Million, M., et al. (2018). "New insights in gut microbiota and mucosal immunity of the small intestine." Human Microbiome Journal **7-8**: 23-32.

Mills, S., et al. (2013). "Movers and shakers: influence of bacteriophages in shaping the mammalian gut microbiota." Gut Microbes **4**(1): 4-16.

Minemura, M. and Y. Shimizu (2015). "Gut microbiota and liver diseases." World Journal of Gastroenterology **21**(6): 1691-1702.

Minot, S., et al. (2013). "Rapid evolution of the human gut virome." Proceedings of the National Academy of Sciences of the United States of America **110**(30): 12450-12455.

Minot, S., et al. (2011). "The human gut virome: Inter-individual variation and dynamic response to diet." Genome Research **21**(10): 1616-1625.

Miragem, A. A. and P. I. H. de Bittencourt (2017). "Nitric oxide-heat shock protein axis in menopausal hot flushes: neglected metabolic issues of chronic inflammatory diseases associated with deranged heat shock response." Human Reproduction Update **23**(5): 600-628.

Misiuk-Hojlo, M., et al. (2011). "Elevated levels of anti-endotoxin antibodies in patients with bilateral idiopathic acute anterior uveitis." Acta Ophthalmologica **89**(3): e283-e288.

Mitsuoka, T. (1992). "INTESTINAL FLORA AND AGING." Nutrition Reviews **50**(12): 438-446.

Mitsuoka, T., et al. (1987). "EFFECT OF FRUCTO-OLIGOSACCHARIDES ON INTESTINAL MICROFLORA." Nahrung-Food **31**(5-6): 427-436.

Mittal, V. V., et al. (2011). "A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy." European Journal of Gastroenterology & Hepatology **23**(8): 725-732.

Moayyedi, P., et al. (2015). "Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial." Gastroenterology **149**(1): 102-+.

Mohanty, D., et al. (2018). "Prebiotics and synbiotics: Recent concepts in nutrition." Food Bioscience **26**: 152-160.

Moissl-Eichinger, C., et al. (2018). "Archaea Are Interactive Components of Complex Microbiomes." Trends in Microbiology **26**(1): 70-85.

Molinaro, A., et al. (2017). "Host-microbiota interaction induces bi-phasic inflammation and glucose intolerance in mice." Molecular Metabolism **6**(11): 1371-1380.

Moloney, R. D., et al. (2014). "The microbiome: stress, health and disease." Mammalian Genome **25**(1-2): 49-74.

Monachese, M., et al. (2012). "Bioremediation and Tolerance of Humans to Heavy Metals through Microbial Processes: a Potential Role for Probiotics?" Applied and Environmental Microbiology **78**(18): 6397-6404.

Monira, S., et al. (2011). "Gut microbiota of healthy and malnourished children in Bangladesh." Frontiers in Microbiology **2**.

Moore, T., et al. (2013). "*Antagonis*tic Activity of Bacillus Bacteria against Food-Borne Pathogens." Journal of Probiotics and Health **1**(3): 110. doi: 110.4172/2329-8901.1000110.

Moore, T., et al. (2014). "Oral administration of Bacillus subtilis strain BSB3 can prevent heat stress-related adverse effects in rats." Journal of Applied Microbiology **117**(5): 1463-1471.

Moore, T., et al. (2013). Novel Approach for Assessment and Mitigation of Heat-Stress Effects**:** 1 PDF.

Moore, T., et al. (2013). "Microscopic Evaluation of Vesicles Shed by Erythrocytes at Elevated Temperatures." Microscopy Research and Technique **76**(11): 1163-1170.

Moore, T., et al. (2013). "Microscopic evaluation of vesicles shed by rat erythrocytes at elevated temperatures." Journal of Thermal Biology **38**(8): 487-492.

Moreno-Indias, I., et al. (2016). "Insulin resistance is associated with specific gut microbiota in appendix samples from morbidly obese patients." American Journal of Translational Research **8**(12): 5672-5684.

Moreno-Perez, D., et al. (2018). "Effect of a Protein Supplement on the Gut Microbiota of Endurance Athletes: A Randomized, Controlled, Double-Blind Pilot Study." Nutrients **10**(3).

Morris, G., et al. (2018). "Leaky brain in neurological and psychiatric disorders: Drivers and consequences." Australian and New Zealand Journal of Psychiatry **52**(10): 924-948.

Morrow, L. E., et al. (2012). "Probiotic, prebiotic, and synbiotic use in critically ill patients." Current Opinion in Critical Care **18**(2): 186-191.

Morrow, L. E., et al. (2012). "Probiotics in the Intensive Care Unit." Nutrition in Clinical Practice **27**(2): 235-241.

Morrow, L. E., et al. (2012). "Synbiotics and probiotics in the critically ill after the PROPATRIA trial." Current Opinion in Clinical Nutrition and Metabolic Care **15**(2): 147-150.

Morshedi, V., et al. (2018). "Effects of dietary xylooligosaccharide on growth and feeding performance, body composition and physiological responses of sobaity seabream (Sparidentex hasta) juvenile." Aquaculture Nutrition **24**(6): 1796-1803.

Morvan, B., et al. (1996). "Quantitative determination of H-2-utilizing acetogenic and sulfate-reducing bacteria and methanogenic archaea from digestive tract of different mammals." Current Microbiology **32**(3): 129-133.

Mota, J. F., et al. (2018). "Insights Into the Relationship Between Gut Microbiota and Colorectal Cancer." Current Colorectal Cancer Reports **14**(6): 251-265.

Moyad, M. A., et al. (2010). "Immunogenic Yeast-Based Fermentate for Cold/Flu-like Symptoms in Nonvaccinated Individuals." Journal of Alternative and Complementary Medicine **16**(2): 213-218.

Mu, Q. H., et al. (2017). "Leaky Gut As a Danger Signal for Autoimmune Diseases." Frontiers in Immunology **8**.

Muegge, B. D., et al. (2011). "Diet Drives Convergence in Gut Microbiome Functions Across Mammalian Phylogeny and Within Humans." Science **332**(6032): 970-974.

Mukhopadhya, I., et al. (2015). "The fungal microbiota of de-novo paediatric inflammatory bowel disease." Microbes and Infection **17**(4): 304-310.

Munakata, S., et al. (2010). "A case of D-lactic acid encephalopathy associated with use of probiotics." Brain & Development **32**(8): 691-694.

Murray, B., et al. (2016). "Mechanism of Four de Novo Designed Antimicrobial Peptides." Journal of Biological Chemistry **291**(49): 25706-25715.

Musso, G., et al. (2011). "Interactions Between Gut Microbiota and Host Metabolism Predisposing to Obesity and Diabetes." Annual Review of Medicine, Vol 62, 2011 **62**: 361-380.

Na, H. N. and J. H. Nam (2012). "Adenovirus 36 as an Obesity Agent Maintains the Obesity State by Increasing MCP-1 and Inducing Inflammation." Journal of Infectious Diseases **205**(6): 914-922.

Nagler-Anderson, C. (2001). "Man the barrier! Strategic defences in the intestinal mucosa." Nature Reviews Immunology **1**(1): 59-67.

Nakamura, T., et al. (2011). "Oral adsorbent AST-120 ameliorates tubular injury in chronic renal failure patients by reducing proteinuria and oxidative stress generation." Metabolism-Clinical and Experimental **60**(2): 260-264.

Nam, Y. D., et al. (2008). "Bacterial, Archaeal, and Eukaryal Diversity in the Intestines of Korean People." Journal of Microbiology **46**(5): 491-501.

Nash, A. K., et al. (2017). "The gut mycobiome of the Human Microbiome Project healthy cohort." Microbiome **5**.

Natividad, J. M. M. and E. F. Verdu (2013). "Modulation of intestinal barrier by intestinal microbiota: Pathological and therapeutic implications." Pharmacological Research **69**(1): 42-51.

Nedaei, S., et al. (2019). "Effects of dietary galactooligosaccharide enriched commercial prebiotic on growth performance, innate immune response, stress resistance, intestinal microbiota and digestive enzyme activity in Narrow clawed crayfish (Astacus leptodactylus Eschscholtz, 1823)." Aquaculture **499**: 80-89.

Neish, A. S. (2009). "Microbes in Gastrointestinal Health and Disease." Gastroenterology **136**(1): 65-80.

Nepelska, M., et al. (2012). "Butyrate Produced by Commensal Bacteria Potentiates Phorbol Esters Induced AP-1 Response in Human Intestinal Epithelial Cells." Plos One **7**(12).

Neyrinck, A. M., et al. (2018). "Wheat-derived arabinoxylan oligosaccharides with bifidogenic properties abolishes metabolic disorders induced by western diet in mice." Nutrition & Diabetes **8**.

Nguyen, T. L. A., et al. (2015). "How informative is the mouse for human gut microbiota research?" Disease Models & Mechanisms **8**(1): 1-16.

Nicholson, J. K., et al. (2012). "Host-Gut Microbiota Metabolic Interactions." Science **336**(6086): 1262-1267.

Nilsson, A. C., et al. (2008). "Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers,

and increases satiety after a subsequent standardized breakfast." J Nutr **138**(4): 732-739.

Nishino, R., et al. (2013). "Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods." Neurogastroenterology and Motility **25**(6).

Nkamga, V. D., et al. (2017). Archaea: Essential inhabitants of the human digestive microbiota. Human Microbiome Journal. **3:** 1-8.

Noble, S. M., et al. (2017). "Candida albicans cell-type switching and functional plasticity in the mammalian host." Nature Reviews Microbiology **15**(2): 96-108.

Nocker, A., et al. (2007). "Genotypic microbial community profiling: A critical technical review." Microbial Ecology **54**(2): 276-289.

Nogueira, J. C. R. and M. D. R. Goncalves (2011). "Probiotics in allergic rhinitis." Brazilian Journal of Otorhinolaryngology **77**(1): 129-134.

Nooshkam, M., et al. (2018). "Lactulose: Properties, techno-functional food applications, and food grade delivery system." Trends in Food Science & Technology **80**: 23-34.

Noriega, B. S., et al. (2016). "Understanding the Impact of Omega-3 Rich Diet on the Gut Microbiota." Case Rep Med **2016**: 3089303.

Norman, J. M., et al. (2015). "Disease-Specific Alterations in the Enteric Virome in Inflammatory Bowel Disease." Cell **160**(3): 447-460.

Norton, C., et al. (2017). "Systematic review: interventions for abdominal pain management in inflammatory bowel disease." Alimentary Pharmacology & Therapeutics **46**(2): 115-125.

Novince, C. M., et al. (2017). "Commensal Gut Microbiota Immunomodulatory Actions in Bone Marrow and Liver have Catabolic Effects on Skeletal Homeostasis in Health." Scientific Reports **7**.

Nymark, M., et al. (2009). "Serum Lipopolysaccharide Activity Is Associated With the Progression of Kidney Disease in Finnish Patients With Type 1 Diabetes." Diabetes Care **32**(9): 1689-1693.

O'Connor, J. C., et al. (2009). "Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice." Molecular Psychiatry **14**(5): 511-522.

O'Hara, E., et al. (2018). "Effect of a butyrate-fortified milk replacer on gastrointestinal microbiota and products of fermentation in artificially reared dairy calves at weaning." Scientific Reports **8**.

O'Mahony, S. M., et al. (2015). "Serotonin, tryptophan metabolism and the brain-gut-microbiome axis." Behavioural Brain Research **277**: 32-48.

O'Mahony, S. M., et al. (2011). "Maternal separation as a model of brain-gut axis dysfunction." Psychopharmacology **214**(1): 71-88.

O'Neil, D. A., et al. (1999). "Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium." Journal of Immunology **163**(12): 6718-6724.

Oh, J., et al. (2014). "Biogeography and individuality shape function in the human skin metagenome." Nature **514**(7520): 59-+.

Oh, J., et al. (2016). "Temporal Stability of the Human Skin Microbiome." Cell **165**(4): 854-866.

Ohigashi, S., et al. (2013). "Changes of the Intestinal Microbiota, Short Chain Fatty Acids, and Fecal pH in Patients with Colorectal Cancer." Digestive Diseases and Sciences **58**(6): 1717-1726.

Ohira, H., et al. (2017). "Are Short Chain Fatty Acids in Gut Microbiota Defensive Players for Inflammation and Atherosclerosis?" Journal of Atherosclerosis and Thrombosis **24**(7): 660-672.

Ohland, C. L. and W. K. MacNaughton (2010). "Probiotic bacteria and intestinal epithelial barrier function." American Journal of Physiology-Gastrointestinal and Liver Physiology **298**(6): G807-G819.

Oprita, R., et al. (2016). "Fecal transplantation - the new, inexpensive, safe, and rapidly effective approach in the treatment of gastrointestinal tract diseases." J Med Life **9**(2): 160-162.

Opstelten, J. L., et al. (2016). "Gut Microbial Diversity Is Reduced in Smokers with Crohn's Disease." Inflammatory Bowel Diseases **22**(9): 2070-2077.

Ortiz, A. M., et al. (2016). "IL-21 and probiotic therapy improve Th17 frequencies, microbial translocation, and microbiome in ARV-treated, SIV-infected macaques." Mucosal Immunology **9**(2): 458-467.

Ostroff, G. R., et al. (2002). "Anthrax-protective effects of yeast beta 1,3-glucans." Abstracts of Papers of the American Chemical Society **224**: U274-U274.

Ott, B. M., et al. (2015). "Characterization of shed medicinal leech mucus reveals a diverse microbiota." Frontiers in Microbiology **5**.

Ott, S. J., et al. (2008). "Fungi and inflammatory bowel diseases: Alterations of composition and diversity." Scandinavian Journal of Gastroenterology **43**(7): 831-841.

Ottman, N., et al. (2012). "The function of our microbiota: who is out there and what do they do?" Frontiers in Cellular and Infection Microbiology **2**.

Oudhuis, G. J., et al. (2011). "Probiotics for prevention of nosocomial infections: efficacy and adverse effects." Current Opinion in Critical Care **17**(5): 487-492.

Ozen, M. and E. C. Dinleyici (2015). "The history of probiotics: the untold story." Beneficial Microbes **6**(2): 159-165.

Pace, F., et al. (2015). "Probiotics in digestive diseases: focus on Lactobacillus GG." Minerva Gastroenterologica E Dietologica **61**(4): 273-292.

Packey, C. D. and R. B. Sartor (2009). "Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases." Current Opinion in Infectious Diseases **22**(3): 292-301.

Pagliari, D., et al. (2018). "Gut Microbiota-Immune System Crosstalk and Pancreatic Disorders." Mediators of Inflammation.

Pals, K. L., et al. (1997). "Effect of running intensity on intestinal permeability." Journal of Applied Physiology **82**(2): 571-576.

Pandey, K. R., et al. (2015). "Probiotics, prebiotics and synbiotics- a review." Journal of Food Science and Technology-Mysore **52**(12): 7577-7587.

Panzella, L., et al. (2017). "High Antioxidant Action and Prebiotic Activity of Hydrolyzed Spent Coffee Grounds (HSCG) in a Simulated Digestion-Fermentation Model: Toward the Development of a Novel Food Supplement." Journal of Agricultural and Food Chemistry **65**(31): 6452-6459.

Papadimitriou, K., et al. (2015). "Discovering pro biotic microorganisms: in vitro, in vivo, genetic and omics approaches." Frontiers in Microbiology **6**.

Park, C., et al. (2018). "Probiotics for the treatment of depressive symptoms: An anti-inflammatory mechanism?" Brain Behavior and Immunity **73**: 115-124.

Park, J. H. and I. H. Kim (2014). "Supplemental effect of probiotic Bacillus subtilis B2A on productivity, organ weight, intestinal Salmonella microflora, and breast meat quality of growing broiler chicks." Poultry Science **93**(8): 2054-2059.

Partrick, K. A., et al. (2018). "Acute and repeated exposure to social stress reduces gut microbiota diversity in Syrian hamsters (vol 345, pg 39, 2018)." Behavioural Brain Research **348**: 277-277.

Pascal, M., et al. (2018). "Microbiome and Allergic Diseases." Frontiers in Immunology **9**: 9.

Pascale, A., et al. (2018). "Microbiota and metabolic diseases." Endocrine **61**(3): 357-371.

Pasman, W., et al. (2006). "Long-term gastrointestinal tolerance of NUTRIOSE (R) FB in healthy men." European Journal of Clinical Nutrition **60**(8): 1024-1034.

Patel, N. B., et al. (2018). "Acceleration of the formation of biofilms on contact lens surfaces in the presence of neutrophil-derived cellular debris is conserved across multiple genera." Molecular Vision **24**: 94-104.

Patel, R. and H. L. DuPont (2015). "New Approaches for Bacteriotherapy: Prebiotics, New-Generation Probiotics, and Synbiotics." Clinical Infectious Diseases **60**: S108-S121.

Patel, R. M. and P. W. Denning (2013). "Therapeutic Use of Prebiotics, Probiotics, and Postbiotics to Prevent Necrotizing Enterocolitis What is the Current Evidence?" Clinics in Perinatology **40**(1): 11-+.

Peake, J. M., et al. (2015). "Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects." Exercise Immunology Review **21**: 8-25.

Pearce, S. C., et al. (2013). "Heat Stress Reduces Intestinal Barrier Integrity and Favors Intestinal Glucose Transport in Growing Pigs." Plos One **8**(8).

Peli, A., et al. (2013). "Influence of heat stress on the immunity in growing beef cattle." Large Animal Review **19**(5): 215-218.

Pelizon, A. C., et al. (2003). "Down-modulation of lymphoproliferation and interferon-gamma production by beta-glucan derived from Saccharomyces cerevisiae." Mem Inst Oswaldo Cruz **98**(8): 1083-1087.

Pendyala, S., et al. (2012). "A High-Fat Diet Is Associated With Endotoxemia That Originates From the Gut." Gastroenterology **142**(5): 1100-+.

Pereg, D., et al. (2011). "Probiotics for patients with compensated liver cirrhosis: A double-blind placebo-controlled study." Nutrition **27**(2): 177-181.

Perko, M. J., et al. (1998). "Mesenteric, coeliac and splanchnic blood flow in humans during exercise." Journal of Physiology-London **513**(3): 907-913.

Persico, A. M. and V. Napolioni (2013). "Urinary p-cresol in autism spectrum disorder." Neurotoxicology and Teratology **36**: 82-90.

Petrof, E. O., et al. (2013). "Microbial ecosystems therapeutics: a new paradigm in medicine?" Beneficial Microbes **4**(1): 53-65.

Picchianti-Diamanti, A., et al. (2017). "Infectious Agents and Inflammation: The Role of Microbiota in Autoimmune Arthritis." Front Microbiol **8**: 2696.

Pillai, A. and R. Nelson (2008). "Probiotics for treatment of Clostridium difficile-associated colitis in adults." Cochrane Database of Systematic Reviews(1).

Pineda, M., et al. (2010). "A Randomized Double Blinded Placebo Controlled Study of Probiotics in Active Rheumatoid Arthritis (RA)." Journal of Rheumatology **37**(6): 1305-1305.

Pineda, M. D., et al. (2011). "A randomized, double-blinded, placebo-controlled pilot study of probiotics in active rheumatoid arthritis." Medical Science Monitor **17**(6): Cr347-Cr354.

Pinheiro, I., et al. (2017). "A yeast fermentate improves gastrointestinal discomfort and constipation by modulation of the gut microbiome: results from a randomized double-blind placebo-controlled pilot trial." Bmc Complementary and Alternative Medicine **17**.

Plat, J. and R. P. Mensink (2005). "Food components and immune function." Current Opinion in Lipidology **16**(1): 31-37.

Pochart, P., et al. (1993). "PYXIGRAPHIC SAMPLING TO ENUMERATE METHANOGENS AND ANAEROBES IN THE RIGHT COLON OF HEALTHY HUMANS." Gastroenterology **105**(5): 1281-1285.

Pollard, M. and N. Sharon (1970). "Responses of the Peyer's Patches in Germ-Free Mice to Antigenic Stimulation." Infect Immun **2**(1): 96-100.

Pop, M., et al. (2014). "Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition." Genome Biology **15**(6).

Popova, A. Y., et al. (2017). "COMPARATIVE CHARACTERISTICS OF INTESTINE MICROBIOME OF REPUBLIC OF GUINEA AND RUSSIAN FEDERATION RESIDENTS." Infektsiya I Immunitet **7**(4): 375-382.

Porras, D., et al. (2017). "Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation." Free Radical Biology and Medicine **102**: 188-202.

Possemiers, S., et al. (2009). "The Intestinal Environment in Health and Disease - Recent Insights on the Potential of Intestinal Bacteria to Influence Human Health." Current Pharmaceutical Design **15**(18): 2051-2065.

Possemiers, S., et al. (2013). "A Dried Yeast Fermentate Selectively Modulates both the Luminal and Mucosal Gut Microbiota and Protects against Inflammation, As Studied in an Integrated in Vitro Approach." Journal of Agricultural and Food Chemistry **61**(39): 9380-9392.

Possemiers, S., et al. (2011). Integrated in vitro study of prebiotic properties of a yeast-based food supplement and protection against gut in fl ammation. Microorganisms in human nutrition. Exploring new pathways for health**:** 10.

Prasad, V. G. M. and P. Abraham (2017). "Management of chronic constipation in patients with diabetes mellitus." Indian Journal of Gastroenterology **36**(1): 11-22.

Preidis, G. A., et al. (2011). "Probiotics, Enteric and Diarrheal Diseases, and Global Health." Gastroenterology **140**(1): 8-+.

Pride, D. T., et al. (2012). "Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome." Isme Journal **6**(5): 915-926.

Psichas, A., et al. (2015). "Gut chemosensing mechanisms." Journal of Clinical Investigation **125**(3): 908-917.

Puertollano, E., et al. (2014). "Biological significance of short-chain fatty acid metabolism by the intestinal microbiome." Current Opinion in Clinical Nutrition and Metabolic Care **17**(2): 139-144.

Pushalkar, S., et al. (2012). "Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma." Bmc Microbiology **12**.

Putsep, K., et al. (2000). "Germ-free and colonized mice generate the same products from enteric prodefensins." Journal of Biological Chemistry **275**(51): 40478-40482.

Qin, X. C., et al. (2019). "Response of treatment performance and microbial community structure to the temporary suspension of an industrial anaerobic bioreactor." Science of the Total Environment **646**: 229-237.

Quigley, E. M. (2013). "Gut bacteria in health and disease." Gastroenterol Hepatol (N Y) **9**(9): 560-569.

Quigley, E. M. M. (2012). "Prebiotics and Probiotics: Their Role in the Management of Gastrointestinal Disorders in Adults." Nutrition in Clinical Practice **27**(2): 195-200.

Rabin, R. L. and A. I. Levinson (2008). "The nexus between atopic disease and autoimmunity: a review of the epidemiological and mechanistic literature." Clinical and Experimental Immunology **153**(1): 19-30.

Radahmadi, M., et al. (2006). "Effects of stress on exacerbation of diabetes mellitus, serum glucose and cortisol levels and body weight in rats." Pathophysiology **13**(1): 51-55.

Raetz, M., et al. (2013). "Parasite-induced TH1 cells and intestinal dysbiosis cooperate in IFN-gamma-dependent elimination of Paneth cells." Nat Immunol **14**(2): 136-142.

Rafter, J. (2011). "Probiotics and Colon Cancer Prevention." European Journal of Cancer **47**: S21-S21.

Rajilic-Stojanovic, M. and W. M. de Vos (2014). "The first 1000 cultured species of the human gastrointestinal microbiota." Fems Microbiology Reviews **38**(5): 996-1047.

Rajilic-Stojanovic, M., et al. (2007). "Diversity of the human gastrointestinal tract microbiota revisited." Environmental Microbiology **9**(9): 2125-2136.

Rajoka, M. S. R., et al. (2018). "Origination, change, and modulation of geriatric disease-related gut microbiota during life." Applied Microbiology and Biotechnology **102**(19): 8275-8289.

Raleigh, D. R., et al. (2011). "Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function." Journal of Cell Biology **193**(3): 565-582.

Ramakrishna, B. S. (2013). "Role of the gut microbiota in human nutrition and metabolism." Journal of Gastroenterology and Hepatology **28**: 9-17.

Rascovan, N., et al. (2016). "Metagenomics and the Human Virome in Asymptomatic Individuals." Annual Review of Microbiology, Vol 70 **70**: 125-141.

Rasmussen, H. E., et al. (2017). "Starch-entrapped microsphere fibers improve bowel habit but do not exhibit prebiotic capacity in those with unsatisfactory bowel habits: a phase I, randomized, double-blind, controlled human trial." Nutrition Research **44**: 27-37.

Rastall, R. A. (2010). Functional Oligosaccharides: Application and Manufacture. Annual Review of Food Science and Technology, Vol 1. M. P. Doyle and T. R. Klaenhammer. **1:** 305-339.

Reyes, A., et al. (2010). "Viruses in the faecal microbiota of monozygotic twins and their mothers." Nature **466**(7304): 334-U381.

Reyes, A., et al. (2012). "Going viral: next-generation sequencing applied to phage populations in the human gut." Nature Reviews Microbiology **10**(9): 607-617.

Rhee, K. J., et al. (2004). "Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire." Journal of Immunology **172**(2): 1118-1124.

Riba, A., et al. (2017). "Paneth Cell Defects Induce Microbiota Dysbiosis in Mice and Promote Visceral Hypersensitivity." Gastroenterology **153**(6): 1594-+.

Richardson, T. M. (1988). "Test slides: Diatoms to divisions-What are you looking at?." Proc Roy Microsc Soc **22**: 3-9.

Riddle, M. S., et al. (2016). "The Epidemiology of Irritable Bowel Syndrome in the US Military: Findings from the Millennium Cohort Study." American Journal of Gastroenterology **111**(1): 93-104.

Ridlon, J. M., et al. (2016). "Consequences of bile salt biotransformations by intestinal bacteria." Gut Microbes **7**(1): 22-39.

Rigsbee, L., et al. (2012). "Quantitative Profiling of Gut Microbiota of Children With Diarrhea-Predominant Irritable Bowel Syndrome." American Journal of Gastroenterology **107**(11): 1740-1751.

Ringel, Y. and T. Ringel-Kulka (2011). "The Rationale and Clinical Effectiveness of Probiotics in Irritable Bowel Syndrome." Journal of Clinical Gastroenterology **45**: S145-S148.

Ringel-Kulka, T., et al. (2013). "Intestinal Microbiota in Healthy US Young Children and Adults-A High Throughput Microarray Analysis." Plos One **8**(5).

Rioux, K. P., et al. (2005). "The role of enteric microflora in inflammatory bowel disease: Human and animal studies with probiotics and prebiotics." Gastroenterology Clinics of North America **34**(3): 465-+.

Risley, P., et al. (2003). "Promoter polymorphism in the endotoxin receptor (CD14) is associated with increased carotid atherosclerosis only in smokers - The Carotid Atherosclerosis Progression Study (CAPS)." Stroke **34**(3): 600-604.

Rizzello, F., et al. (2018). "Dietary geraniol ameliorates intestinal dysbiosis and relieves symptoms in irritable bowel syndrome patients: a pilot study." Bmc Complementary and Alternative Medicine **18**.

Roberfroid, M., et al. (2010). "Prebiotic effects: metabolic and health benefits." British Journal of Nutrition **104**: S1-S63.

Robertson, S. J., et al. (2018). "Innate Immune Influences on the Gut Microbiome: Lessons from Mouse Models." Trends in Immunology **39**(12): 992-1004.

Robles-Vera, I., et al. (2018). "The Probiotic Lactobacillus fermentum Prevents Dysbiosis and Vascular Oxidative Stress in Rats with Hypertension Induced by Chronic Nitric Oxide Blockade." Molecular Nutrition & Food Research **62**(19).

Rodino-Janeiro, B. K., et al. (2018). "A Review of Microbiota and Irritable Bowel Syndrome: Future in Therapies." Advances in Therapy **35**(3): 289-310.

Rodriguez, M. M., et al. (2015). "Obesity changes the human gut mycobiome." Scientific Reports **5**.

Rodriguez, N. R. M., et al. (2012). "Expansion of Paneth Cell Population in Response to Enteric Salmonella enterica Serovar Typhimurium Infection." Infection and Immunity **80**(1): 266-275.

Rodriguez-Nogales, A., et al. (2018). "The Administration of Escherichia coli Nissle 1917 Ameliorates Development of DSS-Induced Colitis in Mice." Frontiers in Pharmacology **9**.

Rodriguez-Nogales, A., et al. (2018). "Intestinal anti-inflammatory effect of the probiotic Saccharomyces boulardii in DSS-induced colitis in mice: Impact on microRNAs expression and gut microbiota composition." Journal of Nutritional Biochemistry **61**: 129-139.

Rogers, N. J. and S. A. Mousa (2012). "The Shortcomings of Clinical Trials Assessing the Efficacy of Probiotics in Irritable Bowel Syndrome." Journal of Alternative and Complementary Medicine **18**(2): 112-119.

Romeo, J., et al. (2010). "Immunomodulatory effect of fibres, probiotics and synbiotics in different life-stages." Nutricion Hospitalaria **25**(3): 341-349.

Romijn, A. R., et al. (2017). "A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression." Australian and New Zealand Journal of Psychiatry **51**(8): 810-821.

Rondon, Y. J., et al. (2015). "Effectiveness of a yuogurt made with prebiotics and probiotics on people with constipation." Medula **24**(2): 78-U88.

Rosenfeld, C. S. (2015). "Microbiome Disturbances and Autism Spectrum Disorders." Drug Metabolism and Disposition **43**(10): 1557-1571.

Rossen, N. G., et al. (2015). "Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis." Gastroenterology **149**(1): 110-+.

Rossi, M., et al. (2018). "Nutraceuticals in colorectal cancer: A mechanistic approach." European Journal of Pharmacology **833**: 396-402.

Round, J. L. and S. K. Mazmanian (2009). "The gut microbiota shapes intestinal immune responses during health and disease (vol 9, pg 313, 2009)." Nature Reviews Immunology **9**(8): 600-600.

Roychowdhury, S., et al. (2018). "Faecalibacterium prausnitzii and a Prebiotic Protect Intestinal Health in a Mouse Model of Antibiotic and Clostridium difficile Exposure." Journal of Parenteral and Enteral Nutrition **42**(7): 1156-1167.

Russell, D. A., et al. (2011). "Metabolic activities and probiotic potential of bifidobacteria." International Journal of Food Microbiology **149**(1): 88-105.

Russo, F., et al. (2012). "Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers." Nutrition Research **32**(12): 940-946.

Rutili, A., et al. (1996). "Intestinal methanogenic bacteria in children of different ages." Microbiologica **19**(3): 227-234.

Ryan, A. J., et al. (1994). "Heat stress does not sensitize rats to the toxic effects of bacterial lipopolysaccharide." Med Sci Sports Exerc **26**(6): 687-694.

Saad, N., et al. (2013). "An overview of the last advances in probiotic and prebiotic field." Lwt-Food Science and Technology **50**(1): 1-16.

Saaltink, D. J. and E. Vreugdenhil (2014). Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? Cell Mol Life Sci. Basel. **71:** 2499-2515.

Saavedra, J. M. and A. Tschernia (2002). "Human studies with probiotics and prebiotics: clinical implications." British Journal of Nutrition **87**: S241-S246.

Sachidhanandam, S. B., et al. (2002). "Naltrexone attenuates plasma nitric oxide release following acute heat stress." European Journal of Pharmacology **450**(2): 163-167.

Said, H., et al. (2015). "Gastroduodenal mucosal defense mechanisms." Current Opinion in Gastroenterology **31**(6): 486-491.

Said, H. M. (2011). "Intestinal absorption of water-soluble vitamins in health and disease." Biochemical Journal **437**: 357-372.

Sailaja, B. S., et al. (2016). "The regulatory niche of intestinal stem cells." Journal of Physiology-London **594**(17): 4827-4836.

Saini, S., et al. (2004). "Surgical infections: a microbiological study." Braz J Infect Dis **8**(2): 118-125.

Salazar, N., et al. (2015). "Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women." Clinical Nutrition **34**(3): 501-507.

Salim, H. M., et al. (2013). "Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens." Poultry Science **92**(8): 2084-2090.

Salminen, S., et al. (2010). "Interaction of probiotics and pathogens-benefits to human health?" Current Opinion in Biotechnology **21**(2): 157-167.

Saltzman, E. T., et al. (2018). "Intestinal Microbiome Shifts, Dysbiosis, Inflammation, and Non-alcoholic Fatty Liver Disease." Frontiers in Microbiology **9**.

Salzman, N. H., et al. (2010). "Enteric defensins are essential regulators of intestinal microbial ecology." Nature Immunology **11**(1): 76-U71.

Samuel, B. S., et al. (2007). "Genomic and metabolic adaptations of Methanobrevibacter smithii to the human gut." Proceedings of the National Academy of Sciences of the United States of America **104**(25): 10643-10648.

Sanchez, B., et al. (2017). "Probiotics, gut microbiota, and their influence on host health and disease." Molecular Nutrition & Food Research **61**(1).

Sanchez-Jimenez, F., et al. (2013). "Pharmacological potential of biogenic amine-polyamine interactions beyond neurotransmission." British Journal of Pharmacology **170**(1): 4-16.

Sanchez-Tapia, M., et al. (2017). "Nopal (Opuntia ficus indica) protects from metabolic endotoxemia by modifying gut microbiota in obese rats fed high fat/sucrose diet." Scientific Reports **7**.

Sanders, M. E., et al. (2018). "Probiotics for human use." Nutrition Bulletin **43**(3): 212-225.

Santiago-Rodriguez, T. M., et al. (2015). "The human urine virome in association with urinary tract infections." Frontiers in Microbiology **6**.

Santocchi, E., et al. (2016). "Gut to brain interaction in Autism Spectrum Disorders: a randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters." Bmc Psychiatry **16**.

Sari, F. N., et al. (2011). "Oral probiotics: Lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial." European Journal of Clinical Nutrition **65**(4): 434-439.

Sarkar, A., et al. (2016). "Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals." Trends in Neurosciences **39**(11): 763-781.

Sato, T., et al. (2011). "Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts." Nature **469**(7330): 415-+.

Savage, D. C. (1977). "MICROBIAL ECOLOGY OF GASTROINTESTINAL-TRACT." Annual Review of Microbiology **31**: 107-133.

Savage, J. H., et al. (2018). "A prospective microbiome-wide association study of food sensitization and food allergy in early childhood." Allergy **73**(1): 145-152.

Sazawal, S., et al. (2010). "Prebiotic and Probiotic Fortified Milk in Prevention of Morbidities among Children: Community-Based, Randomized, Double-Blind, Controlled Trial." Plos One **5**(8).

Scalera, A., et al. (2012). "Gut microbiota, probiotics and liver diseases." Agro Food Industry Hi-Tech **23**(1): 36-38.

Scanlan, P. D. and J. R. Marchesi (2008). "Micro-eukaryotic diversity of the human distal gut microbiota: qualitative assessment using culture-dependent and -independent analysis of faeces." Isme Journal **2**(12): 1183-1193.

Scanlan, P. D., et al. (2006). "Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn's disease." Journal of Clinical Microbiology **44**(11): 3980-3988.

Schauss, A. G., et al. (2012). "Safety Evaluation of a Proprietary Food-Grade, Dried Fermentate Preparation of Saccharomyces cerevisiae." International Journal of Toxicology **31**(1): 34-45.

Scher, J. U., et al. (2015). "Decreased Bacterial Diversity Characterizes the Altered Gut Microbiota in Patients With Psoriatic Arthritis, Resembling Dysbiosis in Inflammatory Bowel Disease." Arthritis & Rheumatology **67**(1): 128-139.

Scheri, G. C., et al. (2017). "Modulation of Tryptophan/Serotonin Pathway by Probiotic Supplementation in Human Immunodeficiency Virus-Positive Patients: Preliminary Results of a New Study Approach." International Journal of Tryptophan Research **10**.

Schneider, B. A. and E. P. Balskus (2018). "Discovery of small molecule protease inhibitors by investigating a widespread human gut bacterial biosynthetic pathway." Tetrahedron **74**(26): 3215-3230.

Schnupf, P., et al. (2017). "Segmented filamentous bacteria, Th17 inducers and helpers in a hostile world." Current Opinion in Microbiology **35**: 100-109.

Scholtens, P., et al. (2006). "Bifidogenic effects of solid weaning foods with added prebiotic oligosaccharides: A randomised controlled clinical trial." Journal of Pediatric Gastroenterology and Nutrition **42**(5): 553-559.

Schorpion, A. and S. L. Kolasinski (2017). "Can Probiotic Supplements Improve Outcomes in Rheumatoid Arthritis?" Current Rheumatology Reports **19**(11).

Schulte, P. M. (2014). "What is environmental stress? Insights from fish living in a variable environment." Journal of Experimental Biology **217**(1): 23-34.

Schultz, M. J. and L. E. Haas (2011). "Antibiotics or probiotics as preventive measures against ventilator-associated pneumonia: a literature review." Critical Care **15**(1).

Schwab, J. H., et al. (1993). "Superantigen Can Reactivate Bacterial-Cell Wall-Induced Arthritis." Journal of Immunology **150**(9): 4151-4159.

Schwager, J., et al. (2011). "Rose hip and its constituent galactolipids confer cartilage protection by modulating cytokine, and chemokine expression." Bmc Complementary and Alternative Medicine **11**.

Schwartz, S., et al. (2012). "A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response." Genome Biology **13**(4).

Scopel, D., et al. (2006). "Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation." Brain Research Bulletin **71**(1-3): 155-159.

Scott, K. P., et al. (2011). "Nutritional influences on the gut microbiota and the consequences for gastrointestinal health." Biochemical Society Transactions **39**: 1073-1078.

Segerstrom, S. C. and G. E. Miller (2004). "Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry." Psychological Bulletin **130**(4): 601-630.

Seiler, N. and F. Raul (2007). "Polyamines and the intestinal tract." Critical Reviews in Clinical Laboratory Sciences **44**(4): 365-411.

Sekirov, I., et al. (2010). "Gut Microbiota in Health and Disease." Physiological Reviews **90**(3): 859-904.

Sen, S., et al. (2012). "Effect of supplementation of Bacillus subtilis LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology." Research in Veterinary Science **93**(1): 264-268.

Sender, R., et al. (2016). "Revised Estimates for the Number of Human and Bacteria Cells in the Body." Plos Biology **14**(8).

Serino, M., et al. (2009). "Intestinal microflora and metabolic diseases." Diabetes & Metabolism **35**(4): 262-272.

Shaddox, L. M., et al. (2011). "Local Inflammatory Markers and Systemic Endotoxin in Aggressive Periodontitis." Journal of Dental Research **90**(9): 1140-1144.

Shahramian, I., et al. (2018). "The effects of prebiotic supplementation on weight gain, diarrhoea, constipation, fever and respiratory tract infections in the first year of life." Journal of Paediatrics and Child Health **54**(8): 875-880.

Shanahan, F., et al. (2012). "Probiotics in Transition." Clinical Gastroenterology and Hepatology **10**(11): 1220-1224.

Shanahan, M. T., et al. (2011). "Strain-specific polymorphisms in Paneth cell alpha-defensins of C57BL/6 mice and evidence of vestigial myeloid alpha-defensin pseudogenes." Infect Immun **79**(1): 459-473.

Shang, Y., et al. (2018). "Effect of Dietary Fructooligosaccharide (FOS) Supplementation on Ileal Microbiota in Broiler Chickens." Poultry Science **97**(10): 3622-3634.

Sharma, B., et al. (2011). "Role of Probiotics on Gut Permeability and Endotoxemia in Patients With Acute Pancreatitis A Double-Blind Randomized Controlled Trial." Journal of Clinical Gastroenterology **45**(5): 442-448.

Shen, H., et al. (2018). "Ginseng polysaccharides enhanced ginsenoside Rb1 and microbial metabolites exposure through enhancing intestinal absorption and affecting gut microbial metabolism." Journal of Ethnopharmacology **216**: 47-56.

Sherwin, E., et al. (2018). "Recent developments in understanding the role of the gut microbiota in brain health and disease." Annals of the New York Academy of Sciences **1420**(1): 5-25.

Shibahara, H. and N. Shibahara (2010). "Cardiorenal protective effect of the oral uremic toxin absorbent AST-120 in chronic heart disease patients with moderate CKD." Journal of Nephrology **23**(5): 535-540.

Shigetomi, K. and J. Ikenouchi (2018). "Regulation of the epithelial barrier by post-translational modifications of tight junction membrane proteins." Journal of Biochemistry **163**(4): 265-272.

Shimazu, S. and I. Miklya (2004). "Pharmacological studies with endogenous enhancer substances: beta-phenylethylamine, tryptamine, and their synthetic derivatives." Progress in Neuro-Psychopharmacology & Biological Psychiatry **28**(3): 421-427.

Shimizu, K., et al. (2013). "Probiotic/Synbiotic Therapy for Treating Critically Ill Patients from a Gut Microbiota Perspective." Digestive Diseases and Sciences **58**(1): 23-32.

Shoaib, A., et al. (2015). "Determining the role of a probiotic in the restoration of intestinal microbial balance by molecular and cultural techniques." Genetics and Molecular Research **14**(1): 1526-1537.

Shukla, S., et al. (2011). "Meta-analysis: the effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy." Alimentary Pharmacology & Therapeutics **33**(6): 662-671.

Siempos, I. I., et al. (2010). "Impact of the administration of probiotics on the incidence of ventilator-associated pneumonia: A meta-analysis of randomized controlled trials." Critical Care Medicine **38**(3): 954-962.

Siempos, I. I., et al. (2010). "Probiotics to prevent ventilator-associated pneumonia: No robust evidence from randomized controlled trials Reply." Critical Care Medicine **38**(7): 1617-1617.

Sim, K., et al. (2012). "Improved Detection of Bifidobacteria with Optimised 16S rRNA-Gene Based Pyrosequencing." Plos One **7**(3).

Simren, M., et al. (2013). "Intestinal microbiota in functional bowel disorders: a Rome foundation report." Gut **62**(1): 159-176.

Singh, S., et al. (2018). "Mannans: An overview of properties and application in food products'." International Journal of Biological Macromolecules **119**: 79-95.

Skonieczna-Zydecka, K., et al. (2017). "Intestinal microbiota and nutrients as determinants of nervous system function. Part I. Gastrointestinal microbiota." Aktualnosci Neurologiczne **17**(4): 181-188.

Slattery, J., et al. (2016). "The Significance of the Enteric Microbiome on the Development of Childhood Disease: A Review of Prebiotic and Probiotic Therapies in Disorders of Childhood." Clinical Medicine Insights-Pediatrics **10**: 91-107.

Slavin, J. (2013). "Fiber and Prebiotics: Mechanisms and Health Benefits." Nutrients **5**(4): 1417-1435.

Soderholm, J. D. and M. H. Perdue (2001). "Stress and the gastrointestinal tract II. Stress and intestinal barrier function." American Journal of Physiology-Gastrointestinal and Liver Physiology **280**(1): G7-G13.

Soderholm, J. D., et al. (2002). "Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine." Gastroenterology **123**(4): 1099-1108.

Song, J., et al. (2014). "Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress." Poultry Science **93**(3): 581-588.

Song, M. J., et al. (2010). "The Effect of Probiotics and Mucoprotective Agents on PPI-Based Triple Therapy for Eradication of Helicobacter pylori." Helicobacter **15**(3): 206-213.

Sorokulova, I., et al. (2016). "Prevention of Heat Stress Adverse Effects in Rats by Bacillus subtilis Strain." Jove-Journal of Visualized Experiments(113).

Spiller, R. (2008). "Review article: probiotics and prebiotics in irritable bowel syndrome." Alimentary Pharmacology & Therapeutics **28**(4): 385-396.

Staudacher, H. M. and K. Whelan (2016). "Altered gastrointestinal microbiota in irritable bowel syndrome and its modification by diet: probiotics, prebiotics and the low FODMAP diet." Proceedings of the Nutrition Society **75**(3): 306-318.

Stefanaki, C., et al. (2017). "Examining the gut bacteriome, virome, and mycobiome in glucose metabolism disorders: Are we on the right track?" Metabolism-Clinical and Experimental **73**: 52-66.

Stevens, A. (1990). The haematoxylins. Theory and Practice of Histological Techniques. J. D. Bancrorft, A. Stevens and D. R. Turner. Edinburg, London, Melbourne and New York, Churchill Livingstone**:** 107-118.

Steves, C. J., et al. (2016). "The Microbiome and Musculoskeletal Conditions of Aging: A Review of Evidence for Impact and Potential Therapeutics." Journal of Bone and Mineral Research **31**(2): 261-269.

Storro, O., et al. (2013). "Diversity of intestinal microbiota in infancy and the risk of allergic disease in childhood." Current Opinion in Allergy and Clinical Immunology **13**(3): 257-262.

Suchodolski, J. S. and A. E. Jergens (2016). "Recent Advances and Understanding of Using Probiotic-Based Interventions to Restore Homeostasis of the Microbiome for the Prevention/Therapy of Bacterial Diseases." Microbiology Spectrum **4**(2).

Sudo, N., et al. (2004). "Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice." Journal of Physiology-London **558**(1): 263-275.

Suhr, M. J., et al. (2016). "Sequence-based methods for detecting and evaluating the human gut mycobiome." Letters in Applied Microbiology **62**(3): 209-215.

Suhr, M. J. and H. E. Hallen-Adams (2015). "The human gut mycobiome: pitfalls and potentials-a mycologist's perspective." Mycologia **107**(6): 1057-1073.

Sulek, K., et al. (2014). "A combined metabolomic and phylogenetic study reveals putatively prebiotic effects of high molecular weight arabino-oligosaccharides when assessed by in vitro fermentation in bacterial communities derived from humans." Anaerobe **28**: 68-77.

Sun, L. Y., et al. (2017). "Gut microbiota of liver transplantation recipients." Scientific Reports **7**.

Sundman, M. H., et al. (2017). "The bidirectional gut-brain-microbiota axis as a potential nexus between traumatic brain injury, inflammation, and disease." Brain Behavior and Immunity **66**: 31-44.

Suskind, D. L., et al. (2015). "Fecal Microbial Transplant Effect on Clinical Outcomes and Fecal Microbiome in Active Crohn's Disease." Inflammatory Bowel Diseases **21**(3): 556-563.

Suzuki, A., et al. (2018). "Quantification of hydrogen production by intestinal bacteria that are specifically dysregulated in Parkinson's disease." Plos One **13**(12): 14.

Suzuki, T. (2013). "Regulation of intestinal epithelial permeability by tight junctions." Cellular and Molecular Life Sciences **70**(4): 631-659.

Swann, J. R., et al. (2011). "Systemic gut microbial modulation of bile acid metabolism in host tissue compartments." Proceedings of the National Academy of Sciences of the United States of America **108**: 4523-4530.

Swanson, K. S., et al. (2011). "Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice." ISME J **5**(4): 639-649.

Szajewska, H. (2010). "Probiotics and prebiotics in preterm infants: Where are we? Where are we going?" Early Human Development **86**(1): S81-S86.

Szilagyi, A. (2004). "Redefining lactose as a conditional prebiotic." Canadian Journal of Gastroenterology **18**(3): 163-167.

Tabouy, L., et al. (2018). "Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders." Brain Behavior and Immunity **73**: 310-319.

Tain, Y. L., et al. (2018). "Resveratrol Prevents the Development of Hypertension Programmed by Maternal Plus Post-Weaning High-Fructose Consumption through Modulation of Oxidative Stress, Nutrient-Sensing Signals, and Gut Microbiota." Molecular Nutrition & Food Research **62**(15).

Tan, H. D., et al. (2018). "Pectin Oligosaccharides Ameliorate Colon Cancer by Regulating Oxidative Stress-and Inflammation-Activated Signaling Pathways." Frontiers in Immunology **9**.

Tan, J., et al. (2014). "The Role of Short-Chain Fatty Acids in Health and Disease." Advances in Immunology, Vol 121 **121**: 91-119.

Tang, M. L. K., et al. (2010). "Probiotics and prebiotics: clinical effects in allergic disease." Current Opinion in Pediatrics **22**(5): 626-634.

Tao, J. H., et al. (2016). "Simultaneous determination of six short-chain fatty acids in colonic contents of colitis mice after oral administration of polysaccharides from Chrysanthemum morifolium Ramat by gas chromatography with flame ionization detector." Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences **1029**: 88-94.

Tap, J., et al. (2009). "Towards The Healthy Human Intestinal Microbiota Phylogenetic Core?" Microbial Ecology **57**(3): 580-581.

Taverniti, V. and S. Guglielmetti (2011). "The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept)." Genes and Nutrition **6**(3): 261-274.

Tazoe, H., et al. (2008). "ROLES OF SHORT-CHAIN FATTY ACIDS RECEPTORS, GPR41 AND GPR43 ON COLONIC FUNCTIONS." Journal of Physiology and Pharmacology **59**: 251-262.

Teng, P. Y. and W. K. Kim (2018). "Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers." Frontiers in Veterinary Science **5**.

ter Steege, R. W. F. and J. J. Kolkman (2012). "Review article: the pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow." Alimentary Pharmacology & Therapeutics **35**(5): 516-528.

Terrier, M. C. Z., et al. (2014). "Recurrent Clostridium difficile infections: The importance of the intestinal microbiota." World Journal of Gastroenterology **20**(23): 7416-7423.

Teughels, W., et al. (2011). "Do probiotics offer opportunities to manipulate the periodontal oral microbiota?" Journal of Clinical Periodontology **38**: 159-177.

Thakur, N., et al. (2018). "Elucidation of bacterial species during childhood diarrhea through 16S rRNA Illumina Miseq approach." Meta Gene **16**: 234-240.

The, H. C., et al. (2018). "Assessing gut microbiota perturbations during the early phase of infectious diarrhea in Vietnamese children." Gut Microbes **9**(1): 38-54.

Thevaranjan, N., et al. (2017). "Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction." Cell Host & Microbe **21**(4): 455-+.

Thomas, D. W., et al. (2010). "Clinical Report-Probiotics and Prebiotics in Pediatrics." Pediatrics **126**(6): 1217-1231.

Thomas, D. W., et al. (2010). "Clinical Report-Probiotics and Prebiotics in Pediatrics." Pediatrics **126**(6): 1217-1231.

Thompson, C. L., et al. (2013). Immune-modulating gut symbionts are not "Candidatus Arthromitus". Mucosal Immunol. United States. **6:** 200-201.

Tilg, H., et al. (2016). "Gut microbiome and liver diseases." Gut **65**(12): 2035-2044.

Timmons, J., et al. (2012). "Polyamines and Gut Mucosal Homeostasis." J Gastrointest Dig Syst **2**(Suppl 7).

Tojo, R., et al. (2014). "Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis." World J Gastroenterol **20**(41): 15163-15176.

Toral, M., et al. (2018). "Lactobacillus fermentum Improves Tacrolimus-Induced Hypertension by Restoring Vascular Redox State and Improving eNOS Coupling." Molecular Nutrition & Food Research **62**(14).

Toscano, M., et al. (2017). "Ability of Lactobacillus kefiri LKF01 (DSM32079) to colonize the intestinal environment and modify the gut microbiota composition of healthy individuals." Digestive and Liver Disease **49**(3): 261-267.

Tran, C. D., et al. (2015). "Gut permeability, its interaction with gut microflora and effects on metabolic health are mediated by the lymphatics system, liver and bile acid." Future Microbiology **10**(8): 1339-1353.

Treangen, T. J., et al. (2018). "Traumatic Brain Injury in Mice Induces Acute Bacterial Dysbiosis Within the Fecal Microbiome." Frontiers in Immunology **9**.

Trevizan, A. R., et al. (2016). "Kinetics of acute infection with Toxoplasma gondii and histopathological changes in the duodenum of rats." Experimental Parasitology **165**: 22-29.

Tsigos, C. and G. P. Chrousos (2002). "Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress." Journal of Psychosomatic Research **53**(4): 865-871.

Tunapong, W., et al. (2018). "Chronic treatment with prebiotics, probiotics and synbiotics attenuated cardiac dysfunction by improving cardiac mitochondrial dysfunction in male obese insulin-resistant rats." European Journal of Nutrition **57**(6): 2091-2104.

Tuohy, K. M., et al. (2012). "Up-regulating the Human Intestinal Microbiome Using Whole Plant Foods, Polyphenols, and/or Fiber." Journal of Agricultural and Food Chemistry **60**(36): 8776-8782.

Turnbaugh, P. J. and J. I. Gordon (2009). "The core gut microbiome, energy balance and obesity." Journal of Physiology-London **587**(17): 4153-4158.

Turroni, S., et al. (2016). "Enterocyte-Associated Microbiome of the Hadza Hunter-Gatherers." Frontiers in Microbiology **7**.

Turroni, S., et al. (2010). "Antibiotics and probiotics in chronic pouchitis: A comparative proteomic approach." World Journal of Gastroenterology **16**(1): 30-41.

Twombley, K. E. and M. G. Seikaly (2011). "New Paradigms for the Use of Prebiotics, Probiotics, and Synbiotics in Renal Disease." Dialysis & Transplantation **40**(5): 200-204.

Uccello, M., et al. (2012). "Potential role of probiotics on colorectal cancer prevention." Bmc Surgery **12**.

Ulluwishewa, D., et al. (2011). "Regulation of Tight Junction Permeability by Intestinal Bacteria and Dietary Components." Journal of Nutrition **141**(5): 769-776.

Ulrich-Lai, Y. M. and J. P. Herman (2009). "Neural regulation of endocrine and autonomic stress responses." Nature Reviews Neuroscience **10**(6): 397-409.

Urs, J. and S. M. Heidemann (2004). "Heat stress decreases pulmonary MCP-1 production in endotoxemia." Cytokine **26**(6): 243-246.

Ursell, L. K., et al. (2012). "Defining the human microbiome." Nutrition Reviews **70**: S38-S44.

Usai-Satta, P. (2009). "Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference (vol 29, pg 1, 2009)." Alimentary Pharmacology & Therapeutics **31**(1): 166-166.

Usami, M., et al. (2015). "Gut microbiota and host metabolism in liver cirrhosis." World Journal of Gastroenterology **21**(41): 11597-11608.

Vainrub, A., et al. (2006). "Resolution of 90 nm (lambda/5) in an optical transmission microscope with an annular condenser." Opt Lett. **31**(19): 2855-2857.

Vaishnava, S., et al. (2008). "Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface." Proceedings of the National Academy of Sciences of the United States of America **105**(52): 20858-20863.

Valdes-Varela, L., et al. (2017). "In vitro fermentation of different fructo-oligosaccharides by Bifidobacterium strains for the selection of synbiotic combinations." International Journal of Food Microbiology **242**: 19-23.

van der Beek, C. M., et al. (2017). "Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing." Nutrition Reviews **75**(4): 286-305.

van der Flier, L. G. and H. Clevers (2009). "Stem Cells, Self-Renewal, and Differentiation in the Intestinal Epithelium." Annual Review of Physiology **71**: 241-260.

van Es, J. H., et al. (2005). "Wnt signalling induces maturation of Paneth cells in intestinal crypts." Nature Cell Biology **7**(4): 381-U337.

Van Hauwermeiren, F., et al. (2015). "TNFR1-induced lethal inflammation is mediated by goblet and Paneth cell dysfunction." Mucosal Immunology **8**(4): 828-840.

van Silvestri, L., et al. (2010). "Probiotics to prevent ventilator-associated pneumonia: No robust evidence from randomized controlled trials." Critical Care Medicine **38**(7): 1616-1617.

Van Tassell, M. L. and M. J. Miller (2011). "Lactobacillus Adhesion to Mucus." Nutrients **3**(5): 613-636.

van Wijck, K., et al. (2012). "Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: strategies for evaluation and prevention." American Journal of Physiology-Gastrointestinal and Liver Physiology **303**(2): G155-G168.

van Wijck, K., et al. (2011). "Exercise-Induced Splanchnic Hypoperfusion Results in Gut Dysfunction in Healthy Men." Plos One **6**(7).

Vandenplas, Y., et al. (2011). "Probiotics and prebiotics in prevention and treatment of diseases in infants and children." Jornal De Pediatria **87**(4): 292-300.

Vandeputte, D., et al. (2016). "Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates." Gut **65**(1): 57-62.

Vandeputte, D., et al. (2017). "Prebiotic inulin-type fructans induce specific changes in the human gut microbiota." Gut **66**(11): 1968-1974.

Vanegas, S. M., et al. (2017). "Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults." American Journal of Clinical Nutrition **105**(3): 635-650.

Vanhoutte, T., et al. (2006). "Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and Saccharomyces boulardii." Applied and Environmental Microbiology **72**(9): 5990-5997.

Vargo, D., et al. (1980). "Faecal bacterial flora in cancer of the colon." Gut **21**(8): 701-705.

Vartoukian, S. R., et al. (2010). "Strategies for culture of 'unculturable' bacteria." Fems Microbiology Letters **309**(1): 1-7.

Vaziri, N. D., et al. (2013). "Oral Activated Charcoal Adsorbent (AST-120) Ameliorates Chronic Kidney Disease-Induced Intestinal Epithelial Barrier Disruption." American Journal of Nephrology **37**(6): 518-525.

Veereman, G. (2007). "Pediatric applications of inulin and oligofructose." Journal of Nutrition **137**(11): 2585S-2589S.

Vespasiani-Gentilucci, U., et al. (2018). "The role of intestinal microbiota in the pathogenesis of NAFLD: starting points for intervention." Archives of Medical Science **14**(3): 701-706.

Videhult, F. K. and C. E. West (2016). "Nutrition, gut microbiota and child health outcomes." Current Opinion in Clinical Nutrition and Metabolic Care **19**(3): 208-213.

Viggiano, D., et al. (2015). "Gut barrier in health and disease: focus on childhood." European Review for Medical and Pharmacological Sciences **19**(6): 1077-1085.

Villalobos, V., et al. (2018). "Inhibiting Pathogen Surface Adherence by Multilayer Polyelectrolyte Films Functionalized with Glucofuranose Derivatives." Acs Applied Materials & Interfaces **10**(33): 28147-28158.

Vitetta, L., et al. (2018). "Immunological Tolerance and Function: Associations Between Intestinal Bacteria, Probiotics, Prebiotics, and Phages." Frontiers in Immunology **9**.

Vodicka, M., et al. (2018). "Microbiota affects the expression of genes involved in HPA axis regulation and local metabolism of glucocorticoids in chronic psychosocial stress." Brain Behavior and Immunity **73**: 615-624.

Vodyanoy, V. (2015). "Thermodynamic evaluation of vesicles shed by erythrocytes at elevated temperatures." Colloids Surf B Biointerfaces **133**: 231-238.

von Schillde, M. A., et al. (2012). "Lactocepin Secreted By Lactobacillus Exerts Anti-Inflammatory Effects By Selectively Degrading Proinflammatory Chemokines." Cell Host & Microbe **11**(4): 387-396.

Wagner, J., et al. (2013). "Bacteriophages in Gut Samples From Pediatric Crohn's Disease Patients: Metagenomic Analysis Using 454 Pyrosequencing." Inflammatory Bowel Diseases **19**(8): 1598-1608.

Wahida, A., et al. (2016). "The Janus-Face of Bacteriophages across Human Body Habitats." Plos Pathogens **12**(6).

Wahlstrom, A., et al. (2016). "Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism." Cell Metabolism **24**(1): 41-50.

Waitzberg, D. L., et al. (2012). "Microbiota benefits after inulin and partially hydrolized guar gum supplementation - a randomized clinical trial in constipated women." Nutricion Hospitalaria **27**(1): 123-129.

Walker, A. (2014). "Intestinal Colonization and Programming of the Intestinal Immune Response." Journal of Clinical Gastroenterology **48**: S8-S11.

Wallis, A., et al. (2018). "Open-label pilot for treatment targeting gut dysbiosis in myalgic encephalomyelitis/chronic fatigue syndrome: neuropsychological symptoms and sex comparisons." Journal of Translational Medicine **16**.

Wampach, L., et al. (2017). "Colonization and Succession within the Human Gut Microbiome by Archaea, Bacteria, and Microeukaryotes during the First Year of Life." Frontiers in Microbiology **8**.

Wang, C., et al. (2018). "Intestinal microbiota of healthy and unhealthy Atlantic salmon Salmo salar L. in a recirculating aquaculture system." Journal of Oceanology and Limnology **36**(2): 414-426.

Wang, G. (2015). "Improved methods for classification, prediction, and design of antimicrobial peptides." Methods Mol Biol **1268**: 43-66.

Wang, J. J., et al. (2016). "The structural alteration of gut microbiota in low-birth-weight mice undergoing accelerated postnatal growth." Scientific Reports **6**.

Wang, J. W., et al. (2018). "Alfalfa-containing diets alter luminal microbiota structure and short chain fatty acid sensing in the caecal mucosa of pigs." Journal of Animal Science and Biotechnology **9**.

Wang, W. C., et al. (2018). "Supplementation of Bacillus subtilis-based probiotic reduces heat stress-related behaviors and inflammatory response in broiler chickens." Journal of Animal Science **96**(5): 1654-1666.

Wang, W. D., et al. (2017). "Isomalto-oligosaccharides ameliorate visceral hyperalgesia with repair damage of ileal epithelial ultrastructure in rats." Plos One **12**(4).

Wang, W. L., et al. (2000). "Human zonulin, a potential modulator of intestinal tight junctions." Journal of Cell Science **113**(24): 4435-4440.

Wang, Y., et al. (2017). "Effects of Dietary Bacillus licheniformis on Gut Physical Barrier, Immunity, and Reproductive Hormones of Laying Hens." Probiotics and Antimicrobial Proteins **9**(3): 292-299.

Wang, Y. B., et al. (2018). "Probiotics-fermented Massa Medicata Fermentata ameliorates weaning stress in piglets related to improving intestinal homeostasis." Applied Microbiology and Biotechnology **102**(24): 10713-10727.

Wang, Y. D., et al. (2019). "Simulated digestion and fermentation in vitro with human gut microbiota of polysaccharides from Coralline pilulifera." Lwt-Food Science and Technology **100**: 167-174.

Wang, Z. K., et al. (2014). "Review article: fungal microbiota and digestive diseases." Alimentary Pharmacology & Therapeutics **39**(8): 751-766.

Wang, Z. N., et al. (2011). "Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease." Nature **472**(7341): 57-U82.

Wang, Z. T., et al. (2006). "The role of bifidobacteria thermal injury in rats." Journal of Trauma-Injury Infection and Critical Care **61**(3): 650-657.

Wang, Z. X., et al. (2018). "Evaluation of the nutrition and function of cow and goat milk based on intestinal microbiota by metagenomic analysis." Food & Function **9**(4): 2320-2327.

Wardill, H., et al. (2018). "Pre-treatment blautia abundance regulates chemo-toxicity in colorectal cancer patients: A pilot study." Neurogastroenterology and Motility **30**: 1.

Wasilewska, E. and B. Wroblewska (2018). "Effectiveness and safety of probiotic preparations in clinical treatment of inflammatory bowel disease." Postepy Higieny I Medycyny Doswiadczalnej **72**: 159-174.

Wasilewski, A., et al. (2015). "Beneficial Effects of Probiotics, Prebiotics, Synbiotics, and Psychobiotics in Inflammatory Bowel Disease." Inflammatory Bowel Diseases **21**(7): 1674-1682.

Watkins, C., et al. (2017). "Microbial Therapeutics Designed for Infant Health." Frontiers in Nutrition **4**.

Wegner, K., et al. (2017). "Rapid analysis of bile acids in different biological matrices using LC-ESI-MS/MS for the investigation of bile acid transformation by mammalian gut bacteria." Analytical and Bioanalytical Chemistry **409**(5): 1231-1245.

Wells, J. (2011). Mucosal Vaccination and Therapy with Genetically Modified Lactic Acid Bacteria. Annual Review of Food Science and Technology, Vol 2. M. P. Doyle and T. R. Klaenhammer. **2:** 423-445.

Wells, J. M., et al. (2017). "Homeostasis of the gut barrier and potential biomarkers." American Journal of Physiology-Gastrointestinal and Liver Physiology **312**(3): G171-G193.

Weng, M. and W. A. Walker (2013). "The role of gut microbiota in programming the immune phenotype." Journal of Developmental Origins of Health and Disease **4**(3): 203-214.

West, C. E., et al. (2017). "Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention." Allergology International **66**(4): 529-538.

Westfall, S., et al. (2017). "Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis." Cellular and Molecular Life Sciences **74**(20): 3769-3787.

Westfall, S., et al. (2018). "A polyphenol-rich prebiotic in combination with a novel probiotic formulation alleviates markers of obesity and diabetes in Drosophil." Journal of Functional Foods **48**: 374-386.

Weston, B., et al. (2015). "An agent-based modeling framework for evaluating hypotheses on risks for developing autism: Effects of the gut microbial environment." Medical Hypotheses **84**(4): 395-401.

Wheeler, M. L., et al. (2016). "Immunological Consequences of Intestinal Fungal Dysbiosis." Cell Host & Microbe **19**(6): 865-873.

Whelan, K. (2007). "Enteral-tube-feeding diarrhoea: manipulating the colonic microbiota with probiotics and prebiotics." Proceedings of the Nutrition Society **66**(3): 299-306.

Whelan, K. (2011). "Probiotics and prebiotics in the management of irritable bowel syndrome: a review of recent clinical trials and systematic reviews." Current Opinion in Clinical Nutrition and Metabolic Care **14**(6): 581-587.

Whelan, K. and C. E. Myers (2010). "Safety of probiotics in patients receiving nutritional support: a systematic review of case reports, randomized controlled trials, and nonrandomized trials." American Journal of Clinical Nutrition **91**(3): 687-703.

Whisner, C. M. and L. F. Castillo (2018). "Prebiotics, Bone and Mineral Metabolism." Calcified Tissue International **102**(4): 443-479.

White, L. S., et al. (2018). "The gut microbiota: cause and cure of gut diseases." Medical Journal of Australia **209**(7): 312-317.

Whittemore, J. C., et al. (2018). "Short and long-term effects of a synbiotic on clinical signs, the fecal microbiome, and metabolomic profiles in healthy research cats receiving clindamycin: a randomized, controlled trial." Peerj **6**: 32.

Wiegand, S., et al. (2017). "Zinc treatment is efficient against Escherichia coli alpha-haemolysin-induced intestinal leakage in mice." Scientific Reports **7**.

Wierdsma, N. J., et al. (2009). "Fructo-oligosaccharides and fibre in enteral nutrition has a beneficial influence on microbiota and gastrointestinal quality of life." Scandinavian Journal of Gastroenterology **44**(7): 804-812.

Wiley, N. C., et al. (2017). "The microbiota-gut-brain axis as a key regulator of neural function and the stress response: Implications for human and animal health." Journal of Animal Science **95**(7): 3225-3246.

Wilhelm, S. M., et al. (2011). "Treating Bugs with Bugs: The Role of Probiotics as Adjunctive Therapy for Helicobacter pylori." Annals of Pharmacotherapy **45**(7-8): 960-966.

Williams, A. M., et al. (2006). "Effects of microflora on the neonatal development of gut mucosal T cells and myeloid cells in the mouse." Immunology **119**(4): 470-478.

Williams, B. L., et al. (2011). "Impaired Carbohydrate Digestion and Transport and Mucosal Dysbiosis in the Intestines of Children with Autism and Gastrointestinal Disturbances." Plos One **6**(9).

Wilms, E., et al. (2016). "Effects of Supplementation of the Synbiotic Ecologic (R) 825/FOS P6 on Intestinal Barrier Function in Healthy Humans: A Randomized Controlled Trial." Plos One **11**(12).

Wilson, M. M. G. and J. E. Morley (2003). "Impaired cognitive function and mental performance in mild dehydration." European Journal of Clinical Nutrition **57**: S24-S29.

Wilson, P. B. (2018). "Perceived life stress and anxiety correlate with chronic gastrointestinal symptoms in runners." Journal of Sports Sciences **36**(15): 1713-1719.

Wolfenden, R. E., et al. (2011). "Evaluation of selected direct-fed microbial candidates on live performance and Salmonella reduction in commercial turkey brooding houses." Poultry Science **90**(11): 2627-2631.

Wolvers, D., et al. (2010). "Guidance for Substantiating the Evidence for Beneficial Effects of Probiotics: Prevention and Management of Infections by Probiotics." Journal of Nutrition **140**(3): 698S-712S.

Wong, C., et al. (2016). "Potential Benefits of Dietary Fibre Intervention in Inflammatory Bowel Disease." International Journal of Molecular Sciences **17**(6).

Wong, M. L., et al. (2016). "Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition." Molecular Psychiatry **21**(6): 797-805.

Woodmansey, E. J. (2007). "Intestinal bacteria and ageing." Journal of Applied Microbiology **102**(5): 1178-1186.

Wright, C. J., et al. (2013). "Microbial interactions in building of communities." Molecular Oral Microbiology **28**(2): 83-101.

Wrigley, B. J., et al. (2011). "The role of monocytes and inflammation in the pathophysiology of heart failure." European Journal of Heart Failure **13**(11): 1161-1171.

Wu, G. D., et al. (2011). "Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes." Science **334**(6052): 105-108.

Wu, Q. J., et al. (2018). "Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers." Poultry Science **97**(8): 2675-2683.

Wu, Q. J., et al. (2018). "Effect of dietary oridonin supplementation on growth performance, gut health, and immune response of broilers infected with Salmonella pullorum." Irish Veterinary Journal **71**.

Wu, X. W., et al. (2017). "16S rDNA analysis of periodontal plaque in chronic obstructive pulmonary disease and periodontitis patients." Journal of Oral Microbiology **9**.

Xiao, G. Z., et al. (2013). "Eicosapentaenoic Acid Enhances Heat Stress-Impaired Intestinal Epithelial Barrier Function in Caco-2 Cells." Plos One **8**(9).

Xiao, L., et al. (2015). "A catalog of the mouse gut metagenome." Nature Biotechnology **33**(10): 1103-+.

Xiao, M., et al. (2018). "Protective effects of Paederia scandens extract on rheumatoid arthritis mouse model by modulating gut microbiota." Journal of Ethnopharmacology **226**: 97-104.

Xu, P. F., et al. (2017). "DBZ is a putative PPAR gamma agonist that prevents high fat diet-induced obesity, insulin resistance and gut dysbiosis." Biochimica Et Biophysica Acta-General Subjects **1861**(11): 2690-2701.

Xu, Q. M., et al. (2018). "Protective Effect of Pediococcus pentosaceus L105 Against Clostridium difficile Infection in a Mouse Model." Frontiers in Microbiology **9**.

Xu, R. Y., et al. (2012). "Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat nonalcoholic fatty liver disease model." Journal of Clinical Biochemistry and Nutrition **50**(1): 72-77.

Yamamoto, M., et al. (2012). "A microarray analysis of gnotobiotic mice indicating that microbial exposure during the neonatal period plays an essential role in immune system development." Bmc Genomics **13**.

Yang, A. M., et al. (2017). "Intestinal fungi contribute to development of alcoholic liver disease." Journal of Clinical Investigation **127**(7): 2829-2841.

Yang, J. P., et al. (2015). "Xylooligosaccharide supplementation alters gut bacteria in both healthy and prediabetic adults: a pilot study." Frontiers in Physiology **6**.

Yang, Y. S., et al. (2018). "Targeting gut microbiome: A novel and potential therapy for autism." Life Sciences **194**: 111-119.

Yap, I. K. S., et al. (2010). "Urinary Metabolic Phenotyping Differentiates Children with Autism from Their Unaffected Siblings and Age-Matched Controls." Journal of Proteome Research **9**(6): 2996-3004.

Yasar, B., et al. (2010). "Efficacy of probiotics in Helicobacter pylori eradication therapy." Turkish Journal of Gastroenterology **21**(3): 212-217.

Yazawa, K., et al. (1978). "OLIGOSACCHARIDES AND POLYSACCHARIDES SPECIFICALLY UTILIZABLE BY BIFIDOBACTERIA." Chemical & Pharmaceutical Bulletin **26**(11): 3306-3311.

Yeh, Y. J., et al. (2013). "Gastrointestinal response and endotoxemia during intense exercise in hot and cool environments." European Journal of Applied Physiology **113**(6): 1575-1583.

Yirmiya, R. (1996). "Endotoxin produces a depressive-like episode in rats." Brain Research **711**(1-2): 163-174.

Yoo, J. Y. and S. S. Kim (2016). "Probiotics and Prebiotics: Present Status and Future Perspectives on Metabolic Disorders." Nutrients **8**(3).

Yu, J., et al. (2010). "Effect of heat stress on the porcine small intestine: A morphological and gene expression study." Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **156**(1): 119-128.

Zaborin, A., et al. (2014). "Membership and Behavior of Ultra-Low-Diversity Pathogen Communities Present in the Gut of Humans during Prolonged Critical Illness." Mbio **5**(5): 14.

Zareie, M., et al. (2006). "Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress." Gut **55**(11): 1553-1560.

Zhang, H. S., et al. (2009). "Human gut microbiota in obesity and after gastric bypass." Proceedings of the National Academy of Sciences of the United States of America **106**(7): 2365-2370.

Zhang, J. W., et al. (2012). "Preoperative Probiotics Decrease Postoperative Infectious Complications of Colorectal Cancer." American Journal of the Medical Sciences **343**(3): 199-205.

Zhang, K. Y., et al. (2015). "The intestinal epithelium as guardian of gut barrier integrity." Cellular Microbiology **17**(11): 1561-1569.

Zhang, N., et al. (2017). "Neoagarotetraose protects mice against intense exercise-induced fatigue damage by modulating gut microbial composition and function." Molecular Nutrition & Food Research **61**(8).

Zhang, R., et al. (2009). "Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS)." Journal of Neuroimmunology **206**(1-2): 121-124.

Zhang, T., et al. (2006). "RNA viral community in human feces: Prevalence of plant pathogenic viruses." Plos Biology **4**(1): 108-118.

Zhang, T. L., et al. (2018). "Comparative Epidemiological Investigation of Alzheimer's Disease and Colorectal Cancer: The Possible Role of Gastrointestinal Conditions in the Pathogenesis of AD." Frontiers in Aging Neuroscience **10**.

Zhang, W., et al. (2018). "Lactobacillus rhamnosus GG Affects Microbiots and Suppresses Autophagy in the Intestines of Pigs Challenged with Salmonella Infantis." Frontiers in Microbiology **8**.

Zhang, W. D., et al. (2018). "Bacteroides fragilis Protects Against Antibiotic-Associated Diarrhea in Rats by Modulating Intestinal Defenses." Frontiers in Immunology **9**.

Zhao, X., et al. (2009). "Purified Cell Wall of Saccharomyces cerevisiae Increases Protection Against Intestinal Pathogens in Broiler Chickens." International Journal of Poultry Science **8**(2).

Zheng, J. P., et al. (2019). "N-Acetylcysteine alleviates gut dysbiosis and glucose metabolic disorder in high-fat diet-fed mice." Journal of Diabetes **11**(1): 32-45.

Zhernakova, A., et al. (2016). "Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity." Science **352**(6285): 565-569.

Zhou, F., et al. (2018). "Lycium barbarum Polysaccharide (LBP): A Novel Prebiotics Candidate for Bifidobacterium and Lactobacillus." Frontiers in Microbiology **9**.

Zhou, H. J., et al. (2010). "Administration of Probiotics Reduces Bacterial Trans location after Intestinal Transplantation in Rats." Transplantation Proceedings **42**(10): 4643-4647.

Zhu, C. S., et al. (2018). A Review of Traumatic Brain Injury and the Gut Microbiome: Insights into Novel Mechanisms of Secondary Brain Injury and Promising Targets for Neuroprotection. Brain Sci. **8**.

Zhu, Y. M., et al. (2011). "Gut microbiota and probiotics in colon tumorigenesis." Cancer Letters **309**(2): 119-127.

Zoetendal, E. G., et al. (2008). "High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota." Gut **57**(11): 1605-1615.

Zoppi, G., et al. (2001). "Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone." Current Therapeutic Research-Clinical and Experimental **62**(5): 418-435.

Zuhl, M., et al. (2014). "Exercise regulation of intestinal tight junction proteins." British Journal of Sports Medicine **48**(12): 980-+.