Biological Control Potential of Spore-forming Plant Growth-Promoting Rhizobacteria Suppressing *Meloidogyne incognita* on Cotton and *Heterodera glycines* on Soybean

by

Ni Xiang

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

Auburn, Alabama
December 10, 2016

Keywords: biological control, Plant Growth-Promoting Rhizobacteria (PGPR), *Meloidogyne incognita*, cotton, *Heterodera glycines*, soybean

Approved by

Kathy S. Lawrence, Chair, Professor of Entomology and Plant Pathology
Joseph W. Kloepper, Professor of Entomology and Plant Pathology
Edward J. Sikora, Professor of Entomology and Plant Pathology
David B. Weaver, Professor of Crop, Soil, and Environmental Sciences
Dennis P. Delaney, Extension Specialist of Crop, Soil, and Environmental Sciences

Abstract

The objective of this study was to screen a library of PGPR strains to determine activity to plant-parasitic nematodes with the ultimate goal of identifying new PGPR strains that could be developed into biological nematicide products. Initially a rapid assay was needed to distinguish between live and dead second stage juveniles (J2) of *H. glycines* and *M. incognita*. Once the assay was developed, PGPR strains were evaluated *in vitro* and selected for further evaluation in greenhouse, microplot, and field conditions.

Three sodium solutions, sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), and sodium hydroxide (NaOH) were evaluated to distinguish between viable live and dead *H. glycines* and *M. incognita* J2. The sodium solutions applied to the live J2 stimulated the J2 to twist their bodies in a curling shape and increased movement activity. Optimum movement of *H. glycines* was observed with the application of 1 µl of Na_2CO_3 (pH =10) added to the 100 µl suspension. *M. incognita* J2 responded best to 1 µl of NaOH (pH =10) added to the 100 µl suspension. Movement of the nematodes was observed immediately and for up to 30 minutes after application.

The 669 PGPR strains were evaluated for the potential of mortality to *M. incognita* J2 *in vitro* and for nematode management in greenhouse, microplot, and field trials. Results indicated that the mortality of *M. incognita* J2 by the PGPR strains ranged from 0.0% to 100% with an average of 39%. Among the PGPR strains examined, 33.5% caused more than 50% mortality of *M. incognita* J2. In subsequent trials, *B. velezensis* strain Bve2 reduced *M. incognita* eggs per gram of cotton root in the greenhouse trials at 45 days after planting (DAP). *Bacillus mojavensis* strain

Bmo3, *B. velezensis* strain Bve2, *B. subtilis* subsp. *subtilis* strain Bsssu3, and the Mixture 2 (Abamectin + Bve2 + Bal13) suppressed *M. incognita* eggs per gram of root in the microplot at 45 DAP. *Bacillus velezensis* strains Bve2 and Bve12 also increased seed cotton yield in the microplot and field trials. Overall, results indicate that *B. velezensis* strains Bve2 and Bve12, *B. mojavensis* strain Bmo3, and the Mixture 2 have potential to reduce *M. incognita* population density and to enhance growth of cotton when applied as in-furrow spray at planting.

The 670 PGPR strains were evaluated for the mortality of *H. glycines* J2 *in vitro* and for reducing nematode population density on soybean in greenhouse, microplot, and field trials. The major group causing mortality to *H. glycines in vitro* was the genus *Bacillus* that consisted of 91.6% of the total 670 PGPR strains evaluated. The subsequent greenhouse, microplot, and field trials indicated that *B. velezensis* strain Bve2 consistently reduced *H. glycines* cyst population density at 60 DAP. *Bacillus mojavensis* strain Bmo3 suppressed *H. glycines* cyst and total *H. glycines* population density under greenhouse conditions. *Bacillus safensis* strain Bsa27 and Mixture 1 (Bve2 + Bal13) reduced *H. glycines* cyst population density at 60 DAP in the field trials. *Bacillus subtilis* subsp. *subtilis* strains Bsssu2 and Bsssu3, and *B. velezensis* strain Bve12 increased early soybean growth including plant height and plant biomass in the greenhouse trials. *Bacillus altitudinis* strain Bal13 increased early plant growth on soybean in the greenhouse and microplot trials. Mixture 2 (Abamectin + Bve2 + Bal13) increased early plant growth in the microplot trials at 60 DAP, and also enhanced soybean yield at harvest in the field trials.

Acknowledgments

I would like to extend my gratitude to everyone that helped me complete this journey. I want to express my deepest appreciation to my major professor, Dr. Kathy S. Lawrence, for her guidance and encouragement throughout this project. She continually and convincingly conveyed a spirit of diligence in regard to research and teaching, and passion in regard to life. Without her patience and endless support, this dissertation would not have been possible. I would like to thank Dr. Joseph Kloepper for encouraging me to find my interest in biocontrol of plant-parasitic nematodes and his guidance on the subject. Thanks to my committee members Dr. Edward Sikora, Dr. David Weaver, and Dr. Dennis Delaney for their contribution of time and guidance of this project. Special thanks to Dr. Patricia Donald and Dr. Gary Lawrence, for their help, encouragement, and support throughout the project. Thanks to John McInroy for his technical support. Thank you to the graduate students and undergraduate students, Drew Schrimsher, David Bailey, Amber Smith, Caroline Land, Justin Luangkhot, Stephen Till, William Groover, Daniel Dodge, Meredith Hall, David Dyer, Andrew Sikkens, Presley Townsend, Yangxue Han, Casie Spenser, Bruce Faulk, Magnolia Wilson, and Cole White, for their support that helped make this project possible.

I would also like to extend a special thanks to my husband, Hongyu Chen, and my family for their encouragement and unconditional support during my whole Ph.D. process.

Finally, I want to thank my best friends Ke Liu, Jia Xu, and Eming Lee. I cannot finish my Ph.D. journey without their help and support.

Table of Contents

List of Tables

List of Figures

Chapter I. Biological control potential of spore-forming plant growth-promoting rhizobacteria suppressing *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean

Chapter II. Optimization of in vitro techniques for distinguishing between live and dead second stage juveniles of *Heterodera glycines* and *Meloidogyne incognita*

Chapter III. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton

Chapter IV: Biological control of *Heterodera glycines* by spore-forming plant growth-promoting rhizobacteria on soybean

List of Abbreviations

PGPR Plant Growth-Promoting Rhizobacteria

RKN Root-knot Nematode

SCN Soybean Cyst Nematode

PSRC Plant Science Research Center

PBU Planting Breeding Unit

PRRU Prattville Agricultural Research Unit

TVRC Tennessee Valley Research Center

TSA Tryptic Soy Agar

Chapter I. Biological control potential of spore-forming plant growth-promoting rhizobacteria suppressing *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean

1. Cotton and soybean

1.1 Cotton

1.1.1 Cotton history and production

Cotton (*Gossypium* spp.) is one of the most important textile fibers in the world and its development has been associated with human activity since before recorded history, with estimations of cotton cultivation at least 3000 years ago (Lee and Fang 2015). According to the evidence of biogeographical distribution of cotton types, four different species of *Gossypium* were independently domesticated in the old world and new world (Lee and Fang 2015). *Gossypium arboretum* L. and *G. herbaceum* L., both diploids (2n = 26), are native to the Old World (Lee and Fang 2015). *Gossypium barbedense* L. and *G. hirsutum* L., both tetraploids (2n = 52), evolved in the New World (Lee and Fang 2015). *Gossypium barbadense*, or extra-long-staple, Egyptian, and Pima cotton, and *G. hirsutum* or Upland cotton, are two species of cotton constitute all the current world fiber production (Lee and Fang 2015).

Currently 75 countries around the globe produce cotton; however, the United States, China, and India together produce nearly two-thirds of the world's cotton (USDA 2016a). In the United States, Upland cotton production was estimated at 12.46 million bales and extra-long staple production at 433,000 bales in 2015, with an average price of $0.61 / pound (NCC 2016b). In Alabama, Upland cotton production was estimated at 554,000 bales in 2015, which was estimated at a value of $169 million (NCC 2016b).

1.1.2 Cotton nematodes

The average annual cotton production losses due to plant diseases in the United States across the cotton belt over the last 20 years has been estimated at about 11% (NCC 2014). As the development of technology and improvements in host plant resistance over the last few years, some diseases have decreased, however, some diseases such as plant-parasitic nematodes have steadily increased in economic damage on cotton production (Starr et al. 2007). In 2014, cotton yield losses in the United States due to plant-parasitic nematodes were estimated at 870,000 bales, an estimated 5.5% of the total cotton production. Cotton yield losses due to *Meloidogyne* spp. were estimated at 494,000 bales (3.1% of total losses), *Rotylenchulus reniformis* losses were estimated at 333,000 bales (2.1% of total losses), and other nematode losses were estimated at 39,000 bales (0.2% of total losses) (Lawrence et al. 2015). *Meloidogyne incognita*, the only species in the genus *Meloidogyne* that is documented to parasitize and reproduce on cotton is found across the entire cotton belt in the United States and in many other regions of the world where the crop is grown (NCC 2016a). In Alabama, *M. incognita* and *R. reniformis* are also considered the predominant plant-parasitic nematodes on cotton (NCC 2016a; Gazaway and McLean 2003).

1.2 Soybean

1.2.1 Soybean history and production

Soybean (*Glycine max*), is the dominant oilseed crop in the United States and its domestication dates back to the Zhou Dynasty (1046-256 BCE) in the eastern half of northern China (Hymowitz 1990; USDA 2016b). From 2010 to 2014, 86.4% soybean production came from the Americas, 10.6% from Asia, 2.3% from Europe, and 0.7% from Africa (FAOSTAT 2015). Soybean was introduced to North America by Samuel Bowen in 1765 and the primary products are oil and meal (Hymowitz et al. 2015). In the United States, soybeans are planted in 31 states

and the top 10 states for soybean production in 2015 were Iowa, Illinois, Minnesota, North Dakota, Indiana, Nebraska, South Dakota, Ohio, Missouri, and Kansas (NASS 2016a). In Alabama, total soybean production was 20,090,000 bushel in 2015, which was estimated at $191 million based on an average of $9.49 per bushel (NASS 2016b).

1.2.2 Soybean nematodes

Soybean is susceptible to many plant-parasitic nematodes. Lewis et al. (1993) surveyed the plant-parasitic nematode distribution on soybean in South Carolina and found 11 different plant-parasitic nematode genera. *Helicotylenchus* and *Scutellonema* occurred in over 70% of the soybean soil samples, *Pratylenchus* and *Paratrichodorus* in more than 60%, *Meloidogyne* spp. in 27% and *Hoplolaimus columbus* in 14%, *Rotylenchulus reniformis* and *Belonolaimus* spp. in less than 10%, *Tylenchorhynchus* and *Mesocriconema* (*Criconemella*) in over 40%, and *Heterodera glycines* in 14% (mainly race 14 and race 3) (Lewis et al. 1993). Koenning and Barker (1998) surveyed the plant-parasitic nematodes on soybean in North Carolina from 1994 to 1996 and found six genera of plant-parasitic nematodes. Of those, *H. glycines* was detected in 71% of the fields, *M. incognita* was detected in 26% of the fields, *Helicotylenchus* spp. were detected in all fields, *Tylenchorhynchus* spp. were found in 62%, *Paratrichodorus* spp. in 56%, and *Pratylenchus* spp. in 72% of the fields (Koenning and Barker 1998). In the United States, *H. glycines* is considered the most economically damaging disease on soybean production, followed by *Phytophthora* root and stem rot and seedling diseases (Wrather et al. 2010). Soybean yield losses caused by *H. glycines* were estimated to be 25% to 38% of yield total losses in 28 U.S. states, which was more than any other soybean disease from 2006 to 2007 (Wrather and Koenning 2009).

2. *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean

2.1 *Meloidogyne incognita*

Meloidogyne incognita (Kofoid &White) Chitwood, commonly known as the southern root-knot nematode, is a sedentary endoparasitic plant-parasitic nematode and has a wide host range encompassing more than 3000 plant species (Abad et al. 2003). It is distributed in tropical and subtropical areas around the world (Sasser 1980) and *M. incognita* is considered the most damaging crop pathogen in the world (Trudgill and Blok 2001). *Meloidogyne incognita* was first identified on cotton in 1889 in the southern United States (Sasser 1954). The distribution of *M. incognita* has been found in all the cotton-producing states in the US and in many other regions of the world where cotton is grown (NCC 2016a). Based on a set of host differentials, *M. incognita* was differentiated into four races R1, R2, R3, and R4, but only R3 and R4 are able to reproduce on cotton (Taylor and Sasser 1978).

2.1.1 Disease symptom of *Meloidogyne incognita* on cotton

The most characteristic symptom of *M. incognita* on susceptible cotton varieties is the presence of galls on the lateral roots. Cotton plants are stunted or leaves are yellowing when infected by *M. incognita*. Symptoms often occur in patches or as irregular areas within fields. However, the symptom of *M. incognita* infection on cotton varies with the resistance of the cotton varieties. Brodie et al. (1960) found that *M. incognita* resistance in the seedling stage of Auburn 56 and in five breeding lines of cotton was associated with three kinds of host response: root necrosis, retarded gall development, and failure of the majority of nematodes to reach maturity.

2.1.2 Life cycle of *Meloidogyne incognita*

Meloidogyne incognita undergoes the first molt inside the egg (Fig. 1A) to develop from first-stage juveniles (J1) to second-stage juveniles (J2) (Fig. 1B) before hatching (Abad et al.

2009). Hatched, infective J2 then penetrate the host plant roots, usually close to the root tip, by using their stylet and releasing secretions containing cell-wall-degrading enzymes to enable the *M. incognita* J2 to enter the root cells (Abad et al. 2003). The J2 migrates intercellular and intracellularly through the cortical cells to the root tip where the active meristematic root tissue growth occurs (Abad et al. 2009). After migration, the J2 reaches the developing vascular root tissue. In order to obtain nutrients and sustain their subsequent sedentary parasitic stages, each J2 induces the differentiation of five to seven parenchymatic root cells into a multinucleate and hypertrophied feeding cells often referred to as giant cells (Abad et al. 2009). Giant cells grow very large in size. Root cells neighboring the giant-cells also enlarge and divide rapidly and resulting in gall formation presumably as a results of plant growth regulator diffusion. *Meloidogyne incognita* J2 feed from these giant cells and molt three additional times to reach the reproductive mature adult stage. Males molt back to the vermiform shape and migrate out of the plant to mate with females. Females (Fig. 1D) become pear-shaped, produce 200-1000 eggs, and release eggs on the root surface in a protective gelatinous matrix (Abad et al. 2009). The life cycle may be completed in as few as 20 days at an optimum temperature of 25 - 30 °C.

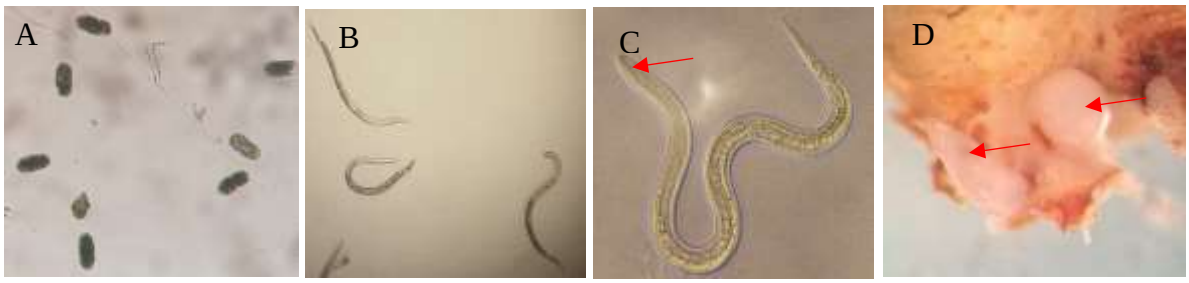

Figure 1. Life cycle of *M. incognita*. (A) *M. incognita* eggs under the microscope (×4); (B) *M. incognita* J2 under the microscope (×4); (C) *M. incognita* stylet of J2 under the microscope (×25); (D) *M. incognita* females attached to the roots (Photos by Ni Xiang).

2.2 *Heterodera glycines*

Heterodera glycines Ichinohe, soybean cyst nematode (SCN), is a sedentary endoparasitic plant-parasitic nematode. It was first reported in the United States in North Carolina in 1954 (Winstead et al. 1955) and now has been found in every soybean-producing state in the U.S. except New York and West Virginia probably due to their limited soybean production acreage (NASS 2016a). Most of the hosts of *H. glycines* are legumes such as soybean, adzuki bean, snapbean, and scarlet runner bean (Fujita et al. 1934; Ichinohe 1953; 1959). Anand and Gallo (1984) tested more than 9,000 soybean lines against one or more races of *H. glycines* and found that all those were hosts. Other legumes, including all cultivars of snapbean, mungbean, green pea, and common lespedeza that have been tested, were hosts (Riggs and Hamblen, 1962; 1966). They also tested representatives of 50 nonleguminous families and found most are not host (Riggs and Hamblen, 1962; 1966). However, 63 species representing 50 genera in 22 families were identified as *H. glycines* hosts with the Tubiflorae and Rosales families having the most species once tested and poor hosts or nonhosts are existed (Riggs 1992). The major economic host of *H. glycines* is soybean, although, bean, lespedeza, and tomato are other economically importance hosts (Riggs 1992). Many weed hosts (Venkatesh et al. 2000; Chen et al. 2006; Donald et al. 2007) are important because they may affect control practices for *H. glycines*. The host range of the plant-parasitic nematode provides important information for management of *H. glycines*.

Due to abundant genetic variability in *H. glycines* virulence, populations of *H. glycines* were characterized into 4 races by a race scheme developed by Golden et al. (1970) and then were expanded to 16 races by Riggs and Schmitt (1988). Modifications of the race test were developed as new virulence phenotypes were observed as new soybean varieties were released. In 2002, Niblack et al. revised the race scheme into 64 HG type test which was designed to contain all

published documents of plant resistance and make the HG scheme more useful to soybean breeders. The HG type scheme described the populations of *H. glycines* based on a set of indicator lines that represent seven sources of resistance (line 1 - PI 548402 (Peking), line 2 - PI 88788, line 3 - PI 90763, line 4 - PI 437654, line 5 - PI 209332, line 6 - PI 89772, and line 7 - PI 548316 (Cloud) which are used in U.S. breeding programs and differentiated according to their genes for resistant or tolerant soybean cultivars (Niblack 2002). Producers and seed companies use the race designation.

2.2.1 Disease symptom of *Heterodera glycines* on soybean

The classic symptoms associated with damage caused by *H. glycines* are stunting and chlorosis. Fewer seeds per pod and fewer pods per plant also are common symptoms of infected plants (Mueller 1984). Crop losses are a consequence of the interaction between *H. glycines* and soybean plants. Wang et al. (2003) reported that yield losses of approximately 15% can occur in the absence of obvious symptoms. The most important characteristic of the presence of *H. glycines* is the presence of white or yellow females or dark-brown cysts attached to the plant's roots.

2.2.2 Life cycle of *Heterodera glycines*

The life cycle of *H. glycines* is similar as most plant-parasitic nematodes. Upon fertilization of eggs (Fig. 2A), embryogenesis proceeds to the first-stage juvenile (J1), which molts to form second-stage juvenile (J2) (Fig. 2B) (Lauritis et al. 1983). J2 is exposed to the soil environment during the interval between hatching and penetration of a root. Upon contact with a susceptible host root, the J2 penetrates the root (Fig. 2C) and initiate the formation of a feeding site (syncytium) (Ross 1958). The syncytium consists of large, distinctive, metabolically active cell and is formed by incorporating of neighboring plant cells through cell wall dissolution and cell fusion, from which the J2 obtains nourishment. Postembryonic development continues with three

additional molts before adulthood is reached and reproduction occurs (Lauritis et al. 1983). Sexual differentiation is detectible during the third-stage. By the fourth-stage, males become vermiform and are coiled within the fourth-stage cuticle, while females continue to swell. Males come out of the fourth-stage cuticle, exit the roots, and seek sedentary mature females for reproduction (Koenning 2004).The mature lemon-shape female ruptures the root epidermis with her posterior end, exposing her vulva to rhizosphere to facilitate mating (Raski 1950).The female is white at this time and receptive to copulation. After insemination, females female (Fig. 2D) begin to produce 200 - 500 eggs (Fig. 2A). Some of the eggs are deposited in a gelatinous matrix and others retained inside her body (Niblack and Karr 1994). The female body wall eventually turns tans and becomes the cyst (Fig. 2D). The time to complete the life cycle of a cyst nematode will vary depending on the temperature. At 25 °C, it takes 21 days for *H. glycines* to develop from egg hatch to mature adult (Lauritis et al. 1983).

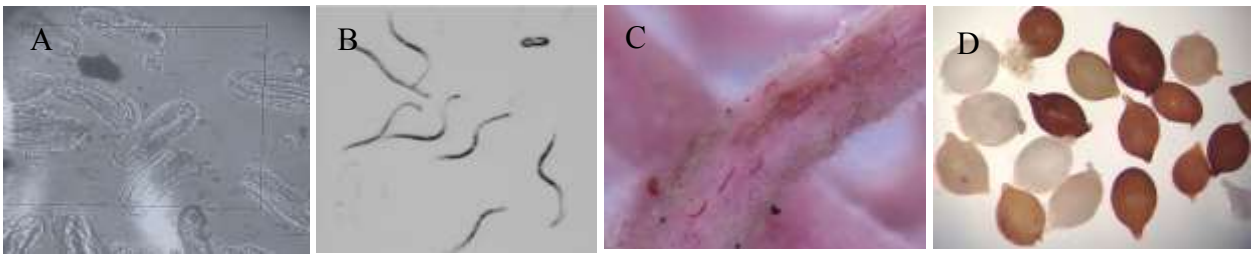

Figure 2. Life cycle of *Heterodera glycines*. (A) *H. glycines* eggs (×10); (B) *H. glycines* J2 (×4); (C) J2 infecting soybean roots (×4); (D) Young females (white) and soybean cysts (yellow or brown) (Photos by Ni Xiang).

3. Current management practices for *Meloidogyne incognita* and *Heterodera glycines*

Chemical control was the mainstay for reducing nematode population density in most economic crops in intensive production systems throughout much the 20th century (Nyczepir et al. 2009). However, environmental and human health concerns reduced the availability of such control options including the use of 1, 2-dibromo-3-chloropropane (DBCP) in 1979 (EPA 2007).

Many other nematicides suffered the similar fate. Some of the chemicals are broad-spectrum with nontarget effects beyond efficacy to the targeted nematodes. The nematicide used across most of the crop acreage was Aldicarb (Temik) (Bayer CropScience, Raleigh, NC), which will be completely banned for use in 2018 by EPA (Cone 2010). Numerous other nematicides have been tested and found to exhibit nematicidal properties (Xiang et al. 2013; Lawrence et al. 2016). The most recent one that has been released on the market is Fluopyram + Imidacloprid (Velum Total™) (Bayer Crop Science, Raleigh, NC). Lawrence et al. (2016) evaluated the nematicides Velum Total™ as in-furrow spray over the seed treatment Aeris (Thiodicarb + Imidacloprid, 0.75 mg ai/seed, Bayer Crop Science, Raleigh, NC) on cotton in the field trials on *M. incognita* and *R. reniformis*. Results indicated that the Velum Total plus Aeris reduced nematode population density similar to that of Temik 15G.

Cultural practices such as crop rotation in place of extended monocropping with annual crops or rotating crops with non-host crops or resistant cultivars is an economical method for nematode management. *Heterodera glycines*, exhibits a high level of specialization and has a host range which allows this nematode to be effectively managed by crop rotation. Niblack (2005) revised the "Rotate (non-host) - Rotate (resistant cultivar 1) - Rotate (resistant cultivar 2)" strategy to reduce *H. glycines* population density. *Meloidogyne incognita*, has a very large and broad host range. In India alone, 232 plant genera have been reported as hosts to *M. incognita* (Krishnappa 1985). When both nematodes *H. glycines* and *M. incognita* are present in the same field, crop rotation options are very limited for their management.

Planting a resistant cultivar is an effective tool for nematode management. Resistant cultivars contain resistance genes that are combined in the host through one or multiple breeding cycles and many of these genes are quantitative. Plant breeders have conducted much research on

host resistance in cotton and soybean nematodes such as *M. incognita* and *H. glycines*. Gutiérrez et al. (2010) found SSR (simple sequence repeat) markers closely associated with genes for resistance to *M. incognita* race 3 on chromosomes 11 and 14 of Upland cotton. Jenkins et al. (2012) developed new SSR markers for marker assisted selection of *M. incognita* resistant plants in cotton. This work will help commercial breeders rapidly develop *M. incognita* resistant cultivars by using these markers. Wang et al. (2012) did QTL analysis for transgressive resistance to *M. incognita* in interspecific cotton progeny derived from susceptible parents and results indicated that high levels of nematode resistance in cotton may be attained by pyramiding positive alleles using a QTL mapping approach. In 1970, a high level of *M. incognita* resistance was developed in the germplasm line Auburn 623 RNR. McPherson et al. (2004) evaluated the mode of inheritance of RKN resistance in M-315 RNR (a line with Auburn 623 RNR source of resistance) and in M78-RNR (a day-neutral version of the race stock line T78). These lines were crossed with M8, an RKN- susceptible cotton line, and results indicated that the Auburn 623 RNR source of RKN resistance should be easily transferable to commercial cultivars. Keim et al. (2013) evaluated five resistant lines with SNP haplotypes for RKN QTLs on A11 (RKN1) and A07 (RKN2) and five susceptible cultivars for RKN eggs/g root at 45 DAP and juveniles/500cc soil at harvest and found that resistant group had 50% less eggs/g root and 63% less juveniles/500cc compared to the susceptible group across all locations. Kadam et al. (2016) analyzed the phylogenetic diversity of the Rhg1 and Rhg4 loci in soybean and developed SNP markers for *H. glycines* resistant genes and QTL. Shi et al. (2015) identified SNPs and developed marker assay for high-throughput selection of soybean *H. glycines* nematode resistance. These studies are expected to accelerate *H. glycines* resistance breeding programs (Kadam et al. 2016; Shi et al. 2015). Cianzio et al. (2016) registered ‘AR11SDS’ soybean germplasm that is highly resistance to sudden death syndrome

death syndrome (SDS) caused by *Fusarium virguliforme*, resistant to *H. glycines* race 3, and moderately resistant to iron deficiency chlorosis (IDC). Carter et al. (2011) developed and released 'N7003CN' soybean with high yield and resistance to *H. glycines* race 2. When both nematodes are present in the same field, soybean cultivars with both *M. incognita* and *H. glycines* resistance genes should be considered.

3.1 Biological control of nematodes

Biological agents have also been used in the management of plant-parasitic nematodes. In recent years, biological control agents for plant-parasitic nematode management has attracted more attention, the market for biopesticides is growing, and the interest in microbial control research is increasing. Some biological control products in the market such as *B. firmus* (Bio-Nem-WP/BioSafe) (Agrogreen, Ashdod, Israel) (Keren-Zur et al. 2000), *B. amyloliquefaciens* strain IN937a and *B. subtilis* strain GB03 (BioYield) (Gusrafson LLC, Plano, TX) (Burkett-Cadena et al. 2008), *B. firmus* GB-126 (VOTIVO) (Bayer CropScience, Raleigh, NC) (Castillo et al. 2013), *Bacillus* spp. (Pathway Consortia) (Pathway Holdings, NY, USA) (Askary 2015) are playing a role in the management of plant-parasitic nematodes.

Biological control (or biocontrol), was described by Eilenger et al. (2001) as the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be. For plant pathologists, biological control is the direct or indirect manipulation of microorganisms for the purpose of reducing the inoculum density or inoculum potential of a plant disease (Nelson et al. 2004). Biological control of nematodes is defined as the reduction of nematode population density through the action of living organisms other than nematode-resistant plant cultivars, which occur naturally or through the manipulation of the environment or the introduction of antagonists (Stirling 1991).

Mechanisms of biological control acting through antagonistic microorganisms would have to act directly on the pathogen (antagonism) or through the intermediate agency of the host (Baker 1968). Two main groups of mechanisms can be concluded which are antagonism (antibiosis, competition for nutrients or niche exclusion, and siderophore-mediated suppression) and induced resistance (systemic acquired resistance or SAR and induced systemic resistance or ISR) of biological control of plant pathogens (Park 1960; Baker 1968; Kloepper et al. 1992; Hammerschmidt 1999).

The best stages of plant-parasitic nematodes to manage with biological control are the egg and second-stage juvenile stages. These life stages exist outside of the plant hosts in the water film of the soil particles which allows the antagonistic microorganisms have the opportunity to come in contact, infect, and parasitize the nematodes. If these two stages of the plant-parasitic nematodes are controlled, the life cycle of the nematodes will be terminated and result in reduce population density of the nematode and a successful management.

The main antagonists used for nematode biocontrol are fungi such as nematode-trapping fungi, endoparasitic fungi, cyst and egg parasites, bacteria such as *Pasteuria* as a hyperparasite of nematode, predatory and endomopathogenic nematodes and microarthropods, plant growth-promoting rhizobacteria and endophytes (Stirling 2014). Other antagonists (i.e., viruses, mites, collembola, turbellarians, oligochaetes, and protozoans) may reduce nematode populations but are limited on their efficacy. Two novel RNA viruses distantly related to known nodaviruses were found infecting *Caenorhabditis* (Félix et al. 2011). Bekal et al. (2014) found a novel flavivirus soybean cyst nematode virus 5 (SbCNV-5) in all nematode developmental stages of *H. glycines*.

Bringing a biocontrol product to market and demonstrating that it is effective is a complex process. This process involves innumerable steps beyond identification of the biocontrol agent.

Thus many issues must be addressed during product registration. Stirling (2014) outlines the steps as follows: 1. Identifying the potential useful biocontrol agents such as collecting, identifying large number of isolates, initial *in vitro* screening against target nematodes, and screening the isolates in the greenhouse and microplot with field soil trials. 2. Technical and commercial issues associated with registration addressed such as target market, mass-production of the agents and formulation, technology transfer and protection of intellectual property, and registration. 3. Efficacy of the registered product such as establishment and reproduction or competition in the soil, efficacy demonstrated in different soil types in the field trials, mechanism understood, and use guidelines determined and recommendations made available.

3.1.1 Techniques applied to determine efficacy toward plant-parasitic nematodes during *in vitro* screening of potential biological control agents

Most of the chemical or biological control product development for the management of plant-parasitic nematodes begins with the initial screening of the biological control agents *in vitro*. The *in vitro* screening of large number of samples can save time and money and determine the best candidates for advancement to greenhouse and field trials. However, distinguishing between live and dead plant-parasitic nematodes when they are exposed to the chemicals or biological compounds is a challenge. Multiple methods have been tried to distinguish between live and dead nematode eggs and juveniles. Different stains have been tried on different kinds of nematodes. Shepherd (1961) found that new blue R can stain the body contents of dead *Tylenchida* while live nematodes remain unstained. Chaudhuri et al. (1966) stained dead free-living nematodes with eosin-Y while live nematodes remained unstained. Ogiga and Estey (1974) found that meldola blue and nile blue A are superior and more dependable for distinguishing dead from living nematodes on the specimens of *Dorylaimus*, *Helicotylenchus*, *Mononchus*, *Panagrolaimus*,

Pratylenchus, *Rhabditis*, *Tylenchorhynchus*, and *Xiphinema* species but not *Heterodera* and *Meloidogyne* species. Meyer et al. (1988) tested seven different stains on the eggs of *H. glycines* and found that chrysoidin, eosin-Y, new blue R, and nile blue A were useful in differentiating dead from live eggs while acridine orange, eosin-Y, fluorescein, and fluorescein diacetate differentially stained live and dead eggs when viewed with fluorescence optics. These staining methods are time-consuming often requiring microscope capability and none of them distinguished between live and dead juveniles of *H. glycines* and *M. incognita*. Bird (1979) found that an enzymatically induced fluorescence method using fluorescein diacetate (FDA) can successfully assess the viability of nematodes under UV light. Sample preparation was lengthy for multiple samples. Schroeder and MacGuidwin (2007) used fluorescein isothiocyanate (FITC) to distinguish live *H. glycines* and found that nematodes incubated in FITC remained active with fluorescence even after two weeks at room temperature, however, not all the nematodes acquired fluorescence quickly or had uniform response. Grego et al. (2013) found that CellTracker Green labeling (CTG) method was able to distinguish live nematodes from dead anoxia-impacted nematodes. However, all these techniques require lengthy sample preparation and fluorescence microscopes which will not facilitate screening large numbers of samples. Some studies also tried tactile methods. Faske and Starr (2006) distinguished live from dead nematodes by touching each nematode with a small probe when testing the sensitivity of *M. incognita* and *R. reniformis* to Abamectin (Syngenta, Greensboro, NC). This method is slow and not feasible if many samples or chemicals need to be tested. Quick techniques for distinguishing between live and dead plant-parasitic nematodes are needed. Xiang and Lawrence (2016) developed a rapid technique that can successfully distinguish between live and dead J2 of *M. incognita* and *H. glycines*. This is a useful technique for high throughput screening.

3.1.2 Fungal antagonists for plant-parasitic nematodes

Fungi and bacteria are the most widely tested microorganisms for biocontrol activity on plant-parasitic nematodes. Chen and Dickson (2004) divided fungal antagonists of nematodes into five groups: 1) trapping (predacious) fungi, 2) endoparasites of vermiform nematodes, 3) parasites of sedentary females and eggs, 4) fungi producing antibiotic substances and 5) vesicular – arbuscular mycorrhizal fungi.

3.1.2.1 Nematode - trapping fungi

Nematode-trapping fungi, are commonly found in agricultural soils and capture nematodes or other microorganisms or microscopic animals with trapping structures such as adhesive networks, adhesive knobs, constricting rings, non-constricting rings, and adhesive branches (Stirling 2014). Stirling (2014) reported that the fungi that use nematodes as a nutrient source are widely distributed across the fungal kingdom. Those are: 1) Ascomycota including Hypocreales (*Drechmeria*, *Harposporium*, *Hirsutella*, *Fusarium*, *Pochonia*, *Purpureocillium*) and Orbiliales (*Arthrobotrys*, *Brachyphoris*, *Dactylella*, *Dactylellina*, *Drechslerella*, *Duddingtonia*, *Gamsylella*, *Monacrosporium*, *Orbilia*), 2) Basidiomycota including *Nematoctonus* and *Hohenbuehelia*, 3) Blastocladiomycota including *Catenaria*, 4) Zoopagomycotina including *Cystopage*, *Stylopage*, and *Rhopalomyces*, and 5) Entomophthoromycota including *Meristacrum* (Stirling 2014). For example, *Drechslerella dactyloides* and *D. brochopaga* were evaluated against *Rotylenchulus reniformis in vitro* and in greenhouse conditions (Castillo et al. 2010). *Monascrosporium drechsleri* was reported to attack *H. glycines* J2 (Liu and Chen 2000).

3.1.2.2 Endoparasites of vermiform nematodes

Fungal endoparasites of vermiform nematodes include encysting species, species forming adhesive conidia, species with conidia that may be ingested, and species with gun cells (Chen and

Dickson 2004). *Stylopage*, *Catenaria*, *Nematoctonus*, *Hohenbuehelia*, *Pleeurotus*, *Drechmeria*, *Harposporium*, *Hirsutella*. *Catenaria anguillulae*, a saprophytic fungus, is capable of colonizing nematodes, rotifers, and tardigrades (Stirling 2014). The zoospores of *C. anguillulae* encyst and germ tubes either enter the body through orifices or penetrate directly through the cuticle to initiate a new infection (Stirling 2014). Some studies considered *C. anguillulae* as a facultative endoparasite of nematodes (Vaish and Singh 2002) and indicated that *C. anguillulae* regulated the population of *M. graminicola* on rice (Singh et al. 2007). *Catenaria auxiliaris*, attacks saccate females of endoparasites rather than vermiform nematodes (Stirling 2014). Tribe (1977) reported that *C. auxiliaris* completely destroyed young females of *H. schachtii*. However, its infection occurs at a later stage of development; females were destroyed but eggs were unharmed. Recently, *C. auxiliaris* was found to parasitize the *R. reniformis* in Alabama (Castillo and Lawrence 2013). However, *C. auxiliaris* has never been cultured and is considered to be an obligate parasite. *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* were found to parasitize the J2 of *H. glycines* by Chen and Liu (2005), *and H. rhossiliensis* was negatively correlated with fungal inoculation level and positively correlated with the final nematode population densities in greenhouse trials.

3.1.2.3 Parasites of sedentary females and eggs

Parasites of sedentary females and eggs are associated with *M. incognita*, *Heterodera* spp., and *R. reniformis*. About 245 fungal species have been reported associated with females, cysts, and eggs of soybean cyst nematode from Brazil, Canada, China, Colombia, and the USA (Chen 2004). Eight genera of fungi including *Exophiala*, *Fusarium*, *Gliocladium*, *Neocosmospora*, *Paecilomyces*, *Phoma*, *Stagonospora*, *and Pochonia* were commonly found from females and cysts of soybean cyst nematode (Chen 2004). *Paecilomyces lilacinus* strain 251 was commonly found to be an egg-parasite fungus that can reduce egg numbers of *M. javanica* and *R. reniformis*

on tomato plants (Freitas et al. 1995; Kiewnick and Sikora 2006; Walters and Barker 1994; Castillo et al. 2013).

3.1.2.4 Fungi producing antibiotic substances

Some fungi produce substances toxic to plant-parasitic nematodes or substances that inhibit or suppress egg hatching. *Paecilomyces*, *Pochonia*, *Fusarium*, *Aspergillus*, *Trichoderma*, *Myrothecium*, and *Penicillium* were found to produce toxins to vermiform nematode species and their eggs (Chen and Dickson 2004). More fungal genera were listed for their nematicidal metabolites and nematode-toxic abilities by Li and Zhang (2014). The toxic compounds are mainly from the fungi in Ascomycota and Basidiomycota. These toxic compounds belong to diverse chemical groups including alkaloid, quinone, isoepoxydon, pyran, furan, peptide, macrolide, terpenoid, fatty acid, diketopiperazine, aphthalene and simple aromatics Li and Zhang (2014).

3.1.2.5 Vesicular - arbuscular mycorrhizal fungi

The response of vesicular-arbuscular mycorrhizal (VAM) fungi varies. Some reports indicated that VAM fungi have had little or no effect on population density of *H. glycines* (Chen 2004). However, arbuscular mycorrhizal fungi (AMF) were reported to affect the *Meloidogyne* spp. infection. Vos et al. (2012a) found that the penetration of *M. incognita* J2 was significantly lower in mycorrhizal colonized roots, as well as the numbers of third and fourth-stage juveniles and females accumulated in mycorrhizal colonized roots, than in control roots. They also found that AMF can induce systemic resistance in tomato plants against the sedentary nematode *M. incognita* and the migratory nematode *Pratylenchus penetrans* (Vos et al. 2012b).

3.1.3 Bacterial antagonists of plant-parasitic nematodes

A few bacterial species have been identified by their biocontrol potential on plant-parasitic nematodes. *Pasteuria* spp. and plant-growth promoting rhizobacteria (PGPR) received the most

attention in recent years. *Pasteuria* spp. are a group of obligatory parasitic, endospore- and mycelium-forming bacteria (Chen 2004). Some *Pasteuria* spp. are species specific. The endospores of *Pasteuria penetrans* were found more infective to *Meloidogyne* spp. than any other species (Mankau and Prasad 1977; Slana and Sayre 1981). Later, host specificity of four isolates of *P. penetrans* within 15 *Meloidogyne* spp. were examined by Stirling and specific endospores attachment was observed and the attachment specificity occurred at a sub-species level as well (Stirling 1985). Some species of *Pasteuria* were found to parasitize cyst nematodes (*Heterodera* and *Globodera* spp.). *Pasteuria nishizawae* was reported to reduce *H. glycines* on soybean in Japan (Nishizawa 1987). The attachment tests with the endospore of this isolate of *Pasteuria* indicated that the endospores only attached to *H. glycines, H. trifolii*, *G. rostochiensis* and several other unidentified populations of *Heterodera*, but did not attach to root-knot nematodes or other plant-parasitic nematodes (Sayre et al. 1991).

4. Plant growth-promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR), are a group of beneficial bacteria that increase the nutrient uptake, growth, and yield of plants, and often exhibit biological control activity against plant pathogens (Kloepper and Schroth 1978; Liu 2016). It was first found that the rhizobacteria significantly promoted plant growth as shown by the substantial increases in fresh matter yield obtained with inoculated radishes (Kloepper and Schroch 1978; Antoun 2013). Further information indicated that PGPR are a very small portion of rhizobacteria (2 - 5%) that can promote plant growth directly through as biofertilizers, or as rhizoremediators, or phytostimulators, and stress controllers, or indirectly through as inhibitor of plant pathogens including fungi, bacteria, viruses, and nematodes (Lugtenberg and Kamilova 2009; Antoun 2013).

PGPR are found among both gram-negative and gram-positive bacteria. However, predominantly most are gram-negative bacteria, such as fluorescent and nonfluorescent pseudomonads, *Burkholderia*, *Arthrobacter*, *Serratia*, *Achromobacter*, *Rhizobium* spp. capable of nitrogen fixation, *Azospirillum* spp., *Azotobacter* spp., and Diazotrophs spp. (Antoun 2013).

Fewer gram-positive bacteria are documented. Isolates of *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Paenibacillus*, *Sarcina*, *Bacillus*, and *Pseudomonas* were reported as PGPR (Antoun 2013; Kloepper et al. 2004). Among all the bacterial genera which were identified as PGPR, *Bacillus* and *Pseudomonas* spp. are two predominant genera investigated (Podile and Kishore 2007).

4.1 PGPR as biocontrol agents

PGPR play a very important role in protection of plant health. The direct effect by PGPR on plant health is promoting plant growth in the absence of plant pathogens through actions such as biofertilizers. Indirect protection occurs through reducing plant diseases caused by pathogens (Lugtenberg and Kamilova 2009; Kumar 2011). The biocontrol mechanisms of PGPR are commonly knowns as antibiosis, lytic enzyme production, and ISR (Kumar 2011).

4.2 Mechanisms of *Bacillus* PGPR against plant pathogens

Bacillus spp. are one of the intensively studied groups of PGPR. The principal mechanisms of growth promotion of *Bacillus* includes production of growth stimulating phytohormones, solubilization, and mobilization of phosphate, siderophore production, antibiosis, production of antibiotics, inhibition of plant ethylene synthesis, and induction of plant systemic resistance to pathogens (Kloepper et al. 2004; Kumar 2011). Ongena and Jacques (2008) illustrated the mechanisms of *Bacillus* lipopeptides on biological control of plant disease including rhizosphere competence, direct inhibition of phytopathogens, and host plant immunization.

4.2.1 Induced Systemic Resistance (ISR) by *Bacillus* spp.

A few studies of ISR of *Bacillus* spp. on plant parasitic nematodes are found. Kloepper et al. (2004) summarized the ISR by *Bacillus* spp. specifically *B. amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides,* and *B. sphaericus* eliciting significant reductions in the incidence or severity of various diseases on a diversity of hosts. Kempster et al. (2001) investigated the chemical and biological induction of resistance to the clover cyst nematode (*Heterodera trifolii*) in white clover (*Trifolium repens*). They found that *Pseudomonas*-like spp. and *B. cereus* induced a response on white clover as measured by reduced fecundity of the nematodes, increased the proportions of distorted females and of females with fewer eggs compared to water-treated controls, which is similar to that resulting from the chemical induction (Kempster et al. 2001). Schrimsher (2013) found that *B. firmus* strain GB-126 has a systemic effect on *H. glycines* and a decrease in *H. glycines* population density was observed when GB-126 was present in the split-root assay in the greenhouse.

4.2.2 Antagonism by *Bacillus* PGPR

The mode of action of antagonistic bacteria for the biocontrol of sedentary and migratory endoparasitic nematodes includes obligate parasitism, reduction in penetration, growth inhibition due to competition for nutrients and antibiosis associated with bioactive metabolites (Mendoza, et al. 2008). Mendoza et al. (2008) found that significant rates of paralysis and mortality were detected after incubation of three plant parasitic nematode species *Radopholus similis, M. incognita,* and *Ditylenchus dipsaci* in low concentrations of the pure culture filtrates of *Bacillus firmus* following removal of the bacterial cells. The production of bioactive compounds or secondary metabolites by the bacteria was responsible for nematode paralysis and mortality (Mendoza et al. 2008).

4.3 *Bacillus* PGPR against *Meloidogyne incognita* and *Heterodera glycines*

Increasing environment concerns and growing interest in microbial control have led to studies of biological control of *M. incognita* and *H. glycines*. Kloepper et al. (1992) found that the rhizosphere bacteria *B. megaterium*, *B. pumilus*, and *Bacillus* spp. were antagonistic to both *H. glycines* and *M. incognita*. Twelve species of *Bacillus* have been documented for *M. incognita* management, including *B. amyloliquefaciens* (Burkett-Cadena et al. 2008), *B. cereus* (Siddiqui and Mahmood 1999), *B. circulans* (Ambo et al. 2010), *B. coagulans* (Ambo et al. 2010), *B. firmus* (Castillo et al. 2013; Terefe et al. 2009), *B. licheniformis* (Siddiqui and Husain 1991; Siddiqui and Mahmood 1992), *B. megaterium* (Kloepper et al. 1992; Padgham and Sikora 2007; Mendoza et al. 2008), *B. penetrans* (Brown and Smart 1985; Brown et al. 1985), *B. polyinyxa* (Khan and Akram 2000), *B. sphaericus* (Krechel et al. 2002), *B. subtilis* (Raupach and Kloepper 1998; Kavitha et al. 2007), and *B. thuringiensis* (Devidas and Rehberger 1992; Zuckerman et al. 1993; Mohammed et al. 2008). These *Bacillus* strains indicated different mechanisms of antagonistic activity on *M. incognita* including ISR and antagonism.

Some studies reported that specific strains of *Bacillus* spp. can suppress the population of *H. glycines in vitro* and in greenhouse experiments. Sharma (1995) evaluated the efficiency of toxins from pure cultures of *B. sphaericus* (Bs 2362), *B. thuringiensis* var. *israelensis* (Bti-H-14), and *B. thuringiensis* var. *kurstaki* (Btk-HD-1) against *H. glycines* in a greenhouse pot experiment and none of the toxins significantly reduced the final nematode population density in relation to the untreated control. Sharma and Gomes (1996) evaluated the effect of those toxins again on oviposition and juvenile hatching of *H. glycines* race 3 in the greenhouse and found the number of hatched juveniles treated with Bs 2362 was significantly less than the control in one experiment. Tian and Riggs (2000) reported that among the 20 isolates that suppressed (≥ 50%) *H. glycines* in

the initial greenhouse screening test, four were *Pseudomonas* spp., two *Bacillus* spp. (*B. cereus* and *B. pumilus*), three *Paenibacillus* spp., and one *Streptomyces* spp.

4.4 Commercial *Bacillus* products for plant-parasitic nematodes management

There are some biological control products available on the market for the management of plant-parasitic nematodes. BioYield, a combination of *B. amyloliquefaciens* strain IN937a and *B. subtilis* strain GB03, was developed by Gustafson for management of soil-borne pathogens and suppression of *M. incognita* population density on tomato plants (Kloepper et al. 2004; Burkett-Cadena et al. 2008). BioNem-WP, a *B. firmus* product developed by AgrGreen, was reported effective against *M. incognita*, *M. hapla*, *Heterodera* spp., *Tylenchulus semipenetrans*, *Xiphinema index*, and *Ditylenchus dipsaci* (Keren-Zur et al. 2000). VOTiVO, *Bacillus firmus* GB-126, is marketed by Bayer CropScience for the control of *M. incognita*, *Ditylenchus dipsaci*, *Rotylenchulus reniformis* as seed treatments for corn, cotton, sorghum, soybean, and sugarbeet (Castillo et al. 2013). Pathway Consortia, mixed multiple PGPR strains of *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. coagulans*, *P. fluorescens*, *Streptomyces* spp., and *Trichoderma* spp., is a biocontrol product formulated in liquid, granular, and thixotropic forms for the management of *Meloidogyne* spp. and *R. reniformis* (Castillo 2012; Askary 2015).

5. Conclusion and future prospects

Over the past decade, we have seen a significantly increasing market for biopesticides and an increase in number of microbial control studies directed at plant-parasitic nematodes. The world's biggest agricultural companies are trying to expand their business in crop protection especially in biological control products. BASF acquired the U.S. crop-technology company specializing in biological products, Becker Underwood; Bayer CropScience acquired the biological companies Agraquest and Prophyta which were the leading supplier of microbial crop

protection products; and Syngenta acquired Pasteuria Bioscience which specialized in *Pasteuria* biologicals specifically for nematode management (Wilson and Jackson 2013).

Currently, biological control agents are not replacing nematicides. They are integrated with other management methods such as chemicals, cultural practices, and different organic amendments, or other biological control organisms, and are expected to reduce the dependence on nematicides. Researchers reported that combining multiple biological control practices such as combing the application of a biocontrol agent *Paecilomyces lilacinus* with various practices such as soil solarization or the application of the biological *B. firmus* or the chemical oxamyl (Vydate) (DuPont, Wilmington, DE) are effective for root-knot nematode management (Anastasiadis et al. 2008). Castillo et al. (2013) combined *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 for reniform nematode management in cotton and indicated an effective reduction in reniform population. Biological control agents are expected to play an important role in the market for Integrated Pest Management in the future.

Biological control studies on plant-parasitic nematodes have switched from the survey and empirical tests to quantitative experimentation and basic research on the modes of action, host specificity, and epidemiology of selected organisms in the past 20 years (Kerry 1997). With the development of molecular biology, biotechnology, and bioinformatics, new techniques and more available omics data will be available to explore the mode of actions of the biological control products and study the mechanisms of microbe-nematode interactions (Li et al. 2015). Simple microbe-nematode interaction is important, however, multiple predator-prey interactions should not be ignored while both the nematode and microbes live in the complex soil ecosystem. These new techniques and studies will provide more guidance for the development of more effective strategies for biological control of plant-parasitic nematodes.

6. Reference

Abad, P., Castagnone-Sereno, P., Rosso, M. N., Engler, J. D. A., and Favery, B. 2009. Invasion, feeding and development. P. 163 - 176. In Root-knot nematode. Eds. Perry, R. N., Moens, M., and Starr, J. L. CAB International. Wallingford, OX, UK.

Abad, P., Favery, B., Rosso, M. N., and Castagnone-Sereno, P. 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Molecular Plant Pathology. 4:217 - 224.

Ambo, P. B. N., Ethiopia, E. A., Serfoji, P., Rajeshkumar, S., and Selvaraj, T. 2010. Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby. by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*. Journal of Agricultural Technology. 6:37 - 45.

Anand, S. C., and Gallo, K. M. 1984. Identification of additional soybean germplasm with resistance to race 3 of soybean cyst nematode. Plant Disease. 68:593 - 595.

Anastasiadis, I. A., Giannakou, I. O., Prophetou-Athanasiadou, D. A., and Gowen, S. R. 2008. The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. Crop Protection. 27:352 - 361.

Antoun, H. 2013. Plant-growth-promoting rhizobacteria. p. 353 – 355. In Brenner's Encyclopedia of Genetics, 2nd Edition. Eds. Maloy, S., and Hughes, K. Academic Press, San Diego. Doi:10.1016/B978-0-12-374984-0.01169-4.

Askary, T. H. 2015. Limitation, research needs, and future prospects. P. 446 - 454. In Biocontrol agents of phytonematodes. Eds. Askary, T. H., and Martinelli, P. R. P. CAB International. Wallingford, OX, UK.

Baker, R. 1968. Mechanisms of biological control of soil-borne pathogens. Annual Review of Phytopathology. 6:263 - 294.

Bekal, S., Domier, L. L., Gonfa, B., McCoppin, N. K., Lambert, K. N., and Bhalerao, K. 2014. A novel flavivirus in the soybean cyst nematode. Journal of General Virology. 95:1272 - 1280.

Bird, A. F. 1979. A method of distinguishing between living and dead nematodes by enzymatically induced fluorescence. Journal of Nematology. 11:103 - 105.

Brodie, B. B., Brinkerhoff, L. A., and Struble, F. B. 1960. Resistance to root-knot nematode, *Meloidogyne incognita acrita* in up-land cotton seedlings. Phytopathology. 50:673 - 677.

Brown, S. M., Kepner, J. L., and Smart, J. G. C. 1985. Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita*. Soil Biology and Biochemistry. 17:483 - 486.

Brown, S. M., and Smart, J. G. C. 1985. Root penetration by *Meloidogyne incognita* juveniles infected with *Bacillus penetrans*. Journal of Nematology. 17:123 - 126.

Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K. S., Van Santen, E., and Kloepper, J. W. 2008. Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biological Control. 47:55 - 59.

Carter, T. E., Koenning, S. R., Burton, J. W., Rzewnicki, P. E., Villagarcia, M. R., Bowman, D. T., and Arelli, P. R. 2011. Registration of 'N7003CN' maturity-group-VII soybean with high yield and resistance to race 2 (HG Type 1.2.5.7-) soybean cyst nematode. Journal of Plant Registrations. 5:309 - 317.

Castillo, J. D. 2012. Biocontrol studies of *Rotylenchulus reniformis* in cotton crops in Alabama, Auburn University. PhD Dissertation. https://etd.auburn.edu/handle/10415/3318.

Castillo, J. D., and Lawrence, K. S. 2013. First report of *Catenaria auxiliaris* parasitizing the reniform nematode *Rotylenchulus reniformis* on cotton in Alabama. Plant Disease. 97:967 - 976.

Castillo, J. D., Lawrence, K. S., and Kloepper, J. W. 2013. Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 on cotton. Plant Disease. 97:967 - 76.

Castillo, J. D., Lawrence, K. S., Kloepper, J. W., and Santen, E. V. 2010. Evaluation of *Drechslerella dactyloides*, *Drechslerella brochopaga*, and *Paecilomyces lilacinus* for the biocontrol of *Rotylenchulus reniformis*. Nematropica. 40:71 - 85.

Chaudhuri, N., Dick, R. I., Engelbrecht, R. S., and Austin, J. H. 1966. Staining of free-living nematodes by eosin-Y dye. Nematologica. 12:337 - 344.

Chen, S. Y. 2004. Management with biological methods. P. 207 - 242. In Biology and management of soybean cyst nematode. 2nd edition. Eds. Schmitt, D. P., Wrather, J. A., and Riggs, R. D. Schmitt & Associates of Marceline. Marceline, Missouri, USA.

Chen, S. Y., and Dickson, D. W. 2004. Biological control with fungal antagonists. Pp. Nematology: Advances and Perspectives Vol II. Eds. Chen, Z. X., Chen, S. Y., and Dickson, D. W. Tsinghua University Press and CAB International. Beijing, China and Wallingford, OX, UK.

Chen, S. Y., and Liu, X. Z. 2005. Control of the soybean cyst nematode by the fungi *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* in greenhouse studies. Biological Control. 32:208 - 219.

Chen, S. Y, Wyse, D. L., Johnson, G. A., Porter, P. M., Stetina, S. R., Miller, D. R., Betts, K. J., Klossner, L. D., and Harr, M. J. 2006. Effect of cover crops alfalfa, red clover, and perennial

ryegrass on soybean cyst nematode population and soybean and corn yields in Minnesota. Crop Science. 46:1890 - 1897.

Cianzio, S. R., Lundeen, P., Gebhart, G., Rivera-Velez, N., Bhattacharyya, M. K., and Swaminathan, S. 2016. Registration of AR11SDS soybean germplasm resistant to sudden death syndrome, soybean cyst nematode, and with moderate Iron deficiency chlorosis scores. Journal of Plant Registrations. 10:177 - 188.

Cone, M. 2010. Insecticide to be banned-three decades after tainted melons sickened 2,000 people. Environmental Health News. Available: http://www.environmentalhealthnews.org/ehs/ news/ aldicarb-phaseout.

Devidas, P., and Rehberger, L. A. 1992. The effects of exotoxin (thuringiensin) from *Bacillus thuringiensis* on *Meloidogyne incognita* and *Caenorhabditis elegans*. Plant and Soil. 145:115 - 120.

Donald, P. A., Hayes, R., and Walker, E. 2007. Potential for soybean cyst nematode reproduction on winter weeds and cover crops in Tennessee. Plant Health Progress. Doi: 10.1094/PHP-2007-0226-01-RS.

Eilenberg, J., Hajek, A., and Lomer, C. 2001. Suggestions for unifying the terminology in biological control. BioControl. 46:387 - 400.

Faske, T. R., and Starr, J. L. 2006. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Abamectin. Journal of Nematology. 38:240 - 244.

Félix, M. A., Ashe, A., Piffaretti, J., Wu, G., Nuez, I., Bélicard, T., Jiang, Y. F., Zhao, G. Y., Franz, C. J., Goldstein, L. D., Sanroman, M., Miska, E. A., and Wang, D. 2011. Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to nodaviruses. PLoS Biol. 9(1): e1000586.

Freitas, L. G., Ferraz, S., and Muchovej, J. J. 1995. Effectiveness of different isolates of *Paecilomyces lilacinus* and isolate of *Cylindrocarpon destructans* on the control of *Meloidogyne javanica*. Nematropica. 25:109 - 115.

Fujita, K., and Miura, O. 1934. On the parasitism of *Heterodera schachtii* Schmidt on beans. Trans. Snapporo Natl. Hist. Soc. 13:359 - 364.

Gazaway, W. S., and McLean, K. S. 2003. Plant pathology & nematology: a survey of plant-parasitic nematodes associated with cotton in Alabama. The Journal of Cotton Science. 7:1 - 7.

Golden, A. M., Epps, J. M., Riggs, R. D., Duclos, L. A., Fox, J. A., and Bernard, R. L. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Disease Reporter. 54:544 - 546.

Grego, M., Stachowitsch, M., Troch, M. D., and Riedel, B. 2013. CellTracker Green labeling vs. rose Bengal staining: CTG wins by points in distinguishing living from dead anoxia-impacted copepods and nematodes. Biogeosciences. 10:4565 - 4575.

Gutiérrez, O. A., Jenkins, J. N., McCarty, J. C., Wubben, M. J., Hayes, R. W., and Callahan, F. E. 2010. SSR markers closely associated with genes for resistance to root-knot nematode on chromosomes 11 and 14 of Upland cotton. Theoretical and Applied Genetics. 121:1323 - 1337.

Hammerschmidt, R. 1999. Induced disease resistance: how do induced plants stop pathogens? Physiological and Molecular Plant Pathology. 55:77 - 84.

Hymowitz, T. 1990. Soybeans: the success story. P. 159 - 163. In Advances in new crops. Eds. Janick, J., and Simon, J. E. Timber Press, Portland, OR, USA.

Hymowitz, T., Nelson, R. L., Sinclair, J. B., and Hartman, G. L. 2015. P. 1 - 4. In compendium soybean diseases and pests. Eds. Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., Davis, J. A., and Steffey, K. L. The American Phytopathological Society. St. Paul, Minnesota, USA.

Ichinohe, M. 1953. On the parasitism of soybean cyst nematode, *Heterodera glycines*. Hokkaido Natl. Agric. Exp. Stn. Res. Bull. 64:113 - 124.

Ichinohe, M. 1959. Studies on the soybean cyst nematode, *Heterodera glycines* and its injury to soybean plants in Japan. Plant Disease Rep. Suppl. 260:239 - 248.

Jenkins, J. N., McCarty, J. C., Wubben, M. J., and Hayes, R. 2012. SSR markers for marker assisted selection of root-knot nematode (*Meloidogyne incognita*) resistant plants in cotton (*Gossypium hirsutum* L). Euphytica. 183:49 - 54.

Kadam, S., Vuong, T. D., Qiu, D., Meinhardt, C. G., Song, L., Deshmukh, R., Patil, G., Wan, J. R., Valliyodan, B., Scaboo, A. M., Shannon, J. G., and Nyuyen, H. T. 2016. Genomic-assisted phylogenetic analysis and marker development for next generation soybean cyst nematode resistance breeding. Plant Science. 242:342 - 350.

Kavitha, J., Jonathan, E. I., and Umamaheswari, R. 2007. Field application of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride* for the control of *Meloidogyne incognita* (Kofoid and White) Chitwood on sugarbeet. Journal of Biological Control. 21:211 - 215.

Keim, D.L., and N. Dighe. 2013. Breeding for high levels of root knot nematode resistance in a commercial cotton breeding program. Proceedings of the 2013 Beltwide Cotton Conferences. 1:407. National Cotton Council of America, Memphis, TN.

Kempster, V. N., Davies, K. A., and Scott, E. S. 2001. Chemical and biological induction of resistance to the clover cyst nematode (*Heterodera trifolii*) in white clover (*Trifolium repens*). Nematology. 3:35 - 43.

Keren-Zur, M., Antonov, J., Bercovitz, A., Feldman, K., Husid, A., Kenan, G., Markov, N., and Rebhun, M., 2000. *Bacillus firmus* formulations for the safe control of root-knot nematodes. In: Proceedings of the Brighton Crop Protection Conference on Pests and Diseases. Vol. 2A. 47 - 52.

Kerry, B. 1997. Biological control of nematodes: prospects and opportunities. Plant nematode problems and their control in the near east region. Rome, Italy: Food and Agriculture Organization of the United Nations: FAO Plant Production and Protection. 144:79 – 92.

Khan, M. R., and Akram, M. 2000. Effects of certain antagonistic fungi and rhizobacteria on wilt disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. Nematologia Mediterranea. 28:139 - 144.

Kiewnick, S., and Sikora, R. A. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control. 38:179 -187.

Kloepper, J. W., and Schroth, M. N. 1978. Plant growth-promoting rhizobacteria on radishes. Proceedings of the 4th international conference on plant pathogenic bacteria. 2:879 - 882.

Kloepper, J. W., Ryu, C. M., and Zhang, S. A. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology. 94:1259 - 1266.

Kloepper, J. W., Rodríguez-Kábana, R., McInroy, J. A., and Young, R. W. 1992. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: identification by fatty acid analysis and frequency of biological control activity. Plant and Soil. 139:75 - 84.

Koenning, S. R. 2004. Population biology. p.73 - 88. In Biology and Management of Soybean Cyst Nematode. 2nd Edition. Eds. Schmitt, D. P., Wrather, J. A., and Riggs, R. D. Schmitt & Associates of Marceline. Marceline, Missouri, USA.

Koenning, S. R., and Barker, K. R. 1998. Survey of *Heterodera glycines* races and other plant-parasitic nematodes on soybean in North Carolina. Journal of Nematology. 30:569 - 576.

Krechel, A., Faupel, A., Hallmann, J., Ulrich, A., and Berg, G. 2002. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Canadian Journal of Microbiology. 48:772 - 786.

Krishnappa, K. 1985. Nematology in developing countries: India - IMP Region VIII. P. 379 - 398. In An advanced treatise on *Meloidogyne*. Vol. I. Biology and Control. Eds. Sasser, J. N., and Carter, C. C. Raleigh, North Carolina State Graphics.

Kumar, A., Prakash, A., and Johri, B. N. 2011. P. 37 - 59. *Bacillus* as PGPR in crop ecosystem. In Bacteria in agrobiology: crop ecosystems. Springer. London.

Lauritis, J. A., Rebois, R. V., and Graney, L. S. 1983. Development of *Heterodera glycines* Ichinohe on soybean, *Glycine max* (L.) Merr., under gnotobiotic conditions. Journal of Nematology. 15:272 - 281.

Lawrence, K. S., Lawrence, G. W., Faske, T., Kemerait, B., Overstreet, C., Wheeler, T., Young, H., and Mehl, H. 2016. Beltwide nematode research and education committee 2015 nematode research report cotton varietal and nematicide responses in nematode soils. 1:737 – 740.

Lawrence, K., Olsen, M., Faske, T., Hutmacher, R., Muller, J., Mario, J., Kemerait, R., Overstreet, C., Price, P., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R., Young, H., Woodward, J., and Mehl, H. 2015. Cotton disease loss estimate committee report,

2014. Proceedings of the 2014 Beltwide Cotton Conference. 1:188 - 190. National Cotton Council of America, Memphis, TN.

Lee, J. A., and Fang, D. D. 2015. Cotton as a world crop: origin, history, and current status. p. 1 - 24. In Cotton. eds. Fang, D. D., and Percy, R. G. Madison, WI, American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc.

Lewis, S. A., Drye, C. E., Saunders, J. A., Shipe, E. R., and Halbrendt, J. M. 1993. Plant-parasitic nematodes on soybean in South Carolina. Journal of Nematology. 25:890 - 894.

Li, G. H., and Zhang, K. Q. 2014. Nematode-toxic fungi and their nematicidal metabolites. P. 313 - 375. In Nematode-trapping fungi. Eds. Zhang, K. Q. and Hyde, K. D. Springer. London.

Li, J., Zou, C. G., Xu, J. P., Ji, X. L., Niu, X. M., Yang, J. K., Huang, X. W., and Zhang, K. Q. 2015. Molecular mechanisms of nematode-nematophagous microbe interactions: basis for biological control of plant-parasitic nematodes. Annual Review of Phytopathology. 53:67 - 95.

Liu, K., Garrett, C., Fadamiro, H., and Kloepper, J. W. 2016. Induction of systemic resistance in Chinese cabbage against black rot by plant growth-promoting rhizobacteria. Biological Control. 99:8 - 13.

Liu, X. Z., and Chen, S. Y. 2000. Parasitism of *Heterodera glycines* by *Hirsutella* spp. in Minnesota soybean fields. Biological Control. 19:161 - 166.

Lugtenberg, B., and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology. 63:541 - 556.

Mankau, R., and Prasad, N. 1977. Infectivity of *Bacillus penetrans* in plant-parasitic nematodes. Journal of Nematology. 9:40 - 45.

McPherson, M. G., Jenkins, J. N., Watson, C. E., and McCarty, Jr., J.C. 2004. Inheritance of root knot nematode resistance in M-315 RNR and M78-RNR cotton. The Journal of Cotton Science. 8:154 - 161.

Mendoza, A. R., Kiewnick, S., and Sikora, R. A. 2008. *In vitro* activity of *Bacillus firmus* against the burrowing nematode *Radopholus similis*, the root-knot nematode *Meloidogyne incognita* and the stem nematode *Ditylenchus dipsaci*. Biocontrol Science and Technology. 18:377 - 389.

Meyer, S. L. F., Sayre, R. M., and Huettel, R. N. 1988. Comparisons of selected stains for distinguishing between live and dead eggs of the plant-parasitic nematode *Heterodera glycines*. Proc. Helminthol. Soc. Wash. 55:132 - 139.

Mohammed, S. H., El Saedy, M. A., Enan, M. R., Ibrahim, N. E., Ghareeb, A., and Moustafa, S. A. 2008. Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita*. Journal of Cell and Molecular Biology. 7:57 - 66.

Mueller, J. D. 1984. The effect of aldicarb and resistance on yield components of soybean and population dynamics of soybean cyst nematode. PhD. Dissertation. University of Illinois.

NASS. National Agricultural Statistics Service | USDA. 2016a. Crop Production 2015 Summary. 46. http://www.usda.gov/nass/PUBS/TODAYRPT/cropan16.pdf.

NASS. National Agricultural Statistics Service | USDA. 2016b. Agricultural Prices. Soybean Prices Received by Month, US. https://www.nass.usda.gov/Charts_and_Maps/Agricultural_Prices/pricesb.php.

NCC. National Cotton Council. 2014. Disease Database. http://www.cotton.org/search.cfm.

NCC. National Cotton Council. 2016a. Population Distributions and Densities Maps. http://www.cotton.org/tech/pest/nematode/distributions.cfm.

NCC. National Cotton Council. 2016b. Economic. Crop Information. Cotton Crop Database. http://www.cotton.org/econ/cropinfo/cropdata/state_data.cfm.

Nelson, E., Kageyama, K., Dijk, K. V., and Windstam, S. 2004. Biological control of soilborne diseases: important concepts from a model system. In Proc. XXVI IHC – Managing Soil-Borne Pathogens. Ed. Vanachter, A. Acta Hort. 635, ISHS 2004. Can. Int. Dev. Agency (CIDA).

Niblack, T. L. 2005. Soybean cyst nematode management reconsidered. Plant Disease. 89: 1020 - 1026.

Niblack, T. L., and Karr, A. L. 1994. Source of antimicrobial activity in the gelatinous matrix of *Heterodera glycines*. Journal of Nematology. 26:561 (Abstract).

Niblack, T., Arelli, P., Noel, G. R., Opperman, C. H., Orf, J. H., Schmitt, D. P., Shannon, J. G., and Tylka, G. L. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. Journal of Nematology. 34:279 - 288.

Nishizawa, T. 1987. A decline phenomenon in a population of the upland cyst nematode, *Heterodera elachista*, caused by a bacterial parasite, *Pasteuria penetrans*. Journal of Nematology. 19: 546 (Abstract).

Nyczepir, A. P., and Thomas, S. H. 2009. Current and future management strategies in intensive crop production systems. P. 412 - 435. In Root-knot nematode. Eds. Perry, R. N., Moens, M., and Starr, J. L. CAB International. Wallingford, OX, UK.

Ogiga, I. R., and Estey, R. H. 1974. The use of Meldola Blue and Nile Blue A, for distinguishing dead from living nematodes. Nematologica. 20:271 - 276.

Ongena, M., and Jacques, P. 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends in Microbiology. 16:115 - 125.

Padgham, J. L., and Sikora, R. A. 2007. Biological control potential and modes of action of *Bacillus megaterium* against *Meloidogyne graminicola* on rice. Crop Protection. 26:971 - 977.

Park, D. 1960. Antagonism-the background to soil fungi. P. 148 – 159. In The ecology of soil fungi. Eds. Parkinson, D., and Waid, J. S. Livepool University Press. UK.

Podile, A. R., and Kishore, G. K. 2007. Plant growth-promoting rhizobacteria. P. 195 - 230. In Plant-Associated Bacteria. Ed. Gnanamanickam, S. S. Springer, London.

Raski, D. J. 1950. The life history and morphology of the sugar-beet nematodes, *Heterodera schachtii* Schmidt. Phytopathology. 40:135 - 152.

Raupach, G. S., and Kloepper, J. W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology. 88:1158 - 1164.

Riggs, R. D. 1992. Host range. P. 107 - 114. In Biology and management of the soybean cyst nematode. Eds. Riggs, R. D., and Wrather, J. A. The American Phytopathological Society, St. Paul, Minnesota, USA.

Riggs, R. D., and Hamblen, M. L. 1962. Soybean cyst nematode host studies in the Leguminosae. Ark. Agric. Exp. Stn. Rep. Series 110. Fayetteville, AR. 17p.

Riggs, R. D., and Hamblen, M. L. 1966. Further studies on the host range of the soybean cyst nematode. Ark. Agric. Exp. Stn. Bull.718. Fayetteville, AR. 19p.

Riggs, R. D, and Schmitt, D. P. 1988. Complete characterization of the race scheme for *Heterodera glycines*. Journal of Nematology. 20:392 - 395.

Ross, J. P. 1958. Host-parasite relationship of the soybean cyst nematode in resistant soybean roots. Phytopathology. 48:578 - 579.

Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.). Bull. *Md agric. Exp. Stn*. A-77:1 - 30.

Sasser, J. N. 1980. Root-Knot Nematodes: A globe. Plant Disease. 64:36 - 41.

Sayre, R. M., Wergin, W. P., Schmidt, J. M., and Starr, M. P. 1991. *Pasteuria nishizawae* sp. Nov., a mycelial and endospore-forming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera*. Research in Microbiology. 142:551 - 564.

Schrimsher, D. W. 2013. The studies of plant host resistance to the reniform nematode in upland cotton and the effects of *Bacillus firmus* GB-126 on plant-parasitic nematodes, Auburn University. M.S. Thesis. http://hdl.handle.net/10415/3562.

Schroeder, N., and MacGuidwin, A. 2007. Incorporation of a fluorescent compound by live *Heterodera glycines*. Journal of Nematology. 39:43 - 49.

Sharma, R. D. 1995. Efficiency of *Bacillus s*pp. toxins to control *Heterodera glycines* on soybean. Nematologia Brasileira. 19:72 - 80.

Sharma, R. D., and Gomes, A. C. 1996. Effect of *Bacillus* spp. toxins on oviposition and juvenile hatching of *Heterodera glycines*. Nematologia Brasileira. 20:53 - 62.

Shepherd, A. M. 1961. New Blue R, a stain that differentiates between living and dead nematodes. Nematologica. 8:201 - 208.

Shi, Z., Liu, S. M., Noe, J., Arelli, P., Meksem, K., and Li, Z. L. 2015. SNP identification and marker assay development for high-throughput selection of soybean cyst nematode resistance. BMC genomics. 16:1.

Siddiqui, Z. A., and Husain, S. I. 1991. Studies on the biological control of root-knot nematode. Current Nematology. 2:5 - 6.

Siddiqui, Z. A., and Mahmood, I. 1992. Biological control of root-rot disease complex of chickpea caused by *Meloidogyne incognita* race 3 and *Macrophomina phaseolina*. Nematology Mediterranean. 20:199 - 202.

Siddiqui, Z. A, and Mahmood, I. 1999. Role of bacteria in the management of plant parasitic nematodes: a review. Bioresource Technology. 69:167 - 179.

Singh, K. P., Jaiswal, R. K., and Kumar, N. 2007. *Catenaria anguillulae* Sorokin: a natural biocontrol agent of *Meloidogyne graminicola* causing root knot disease of rice (*Oryza sativa* L.). World Journal of Microbiology and Biotechnology. 23:291 - 294.

Slana, L. J., and Sayre, R. M. 1981. A method for measuring incidence of *Bacillus penetrans* spore attachment to the second-stage-larvae of *Meloidogyne* spp. Journal of Nematology. 13: 461.

Starr, J. L., Koenning, S. R., Kirkpatrick, T. L., Robinson, A. F., Roberts, P. A., and Nichols, R. L. 2007. The future of nematode management in cotton. Journal of Nematology. 39:283 - 294.

Stirling, G. R. 1985. Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne*. Nematologica. 31:203 - 209.

Stirling, G. R. 1991. Biological control of plant parasitic nematodes. Wallingford. CAB International. Wallingford, OX, UK.

Stirling, G. R. 2014. Biological control of plant parasitic nematodes, 2nd Edition. Wallingford. CAB International. Wallingford, OX, UK.

Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University Graphics. Raleigh, NC, USA.

Terefe, M., Tefera, T., and Sakhuja, P. K. 2009. Effect of a formulation of *Bacillus firmus* on root-knot nematode *Meloidogyne incognita* infestation and the growth of tomato plants in the greenhouse and nursery. Journal of Invertebrate Pathology. 100:94 - 99.

Tian, H., and Riggs, R. D. 2000. Effects of rhizobacteria on soybean cyst nematode, *Heterodera glycines*. Journal of Nematology. 32:377 - 388.

Tribe, H. T. 1977. A parasite of white cysts of *Heterodera*: *Catenaria auxiliaris*. Transactions of the British Mycological Society. 69:367 - 376.

Trudgill, D. L., Bala, G., Blok, V. C., Daudi, A., Davies, K. G., Fargette, M., Gowen, S. R., Madulu, J. D., Mateille, T., Mwageni, W., Netscher, C., Phillips, M. S., Abdoussalam, S., Trivino, G. C., and Voyoulallou, E. 2000. The importance of tropical root-knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent. Nematology. 2:823 - 845.

Vaish, S. S., and K. Singh, K. P. 2002. Distribution of *Catenaria anguillulae* Sorokin, a facultative endoparasite of nematodes in soils from different locations of India. World Journal of Microbiology and Biotechnology. 18:65 - 67.

Venkatesh, R., Harrison, S. K., and Riedel, R. M. 2000. Weed hosts of soybean cyst nematode (*Heterodera glycines*) in Ohio. Weed Technology. 14:156 - 160.

Vos, C. M., Geerinckx, K., Mkandawire, R., Panis, B., Waele, D. D., and Elsen, A. 2012a. Arbuscular mycorrhizal fungi affect both penetration and further life stage development of root-knot nematodes in tomato. Mycorrhiza. 22:157 - 163.

Vos, C. M., Tesfahun, A. N. Panis, B., Waele, D. D., and Elsen, A. 2012b. Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode *Meloidogyne incognita* and the migratory nematode *Pratylenchus penetrans*. Applied Soil Ecology. 61:1 - 6.

USDA. 2016a. United States Department of Agriculture Economics Research Service: Cotton & Wool. http://www.ers.usda.gov/topics/crops/cotton-wool.aspx.

USDA. 2016b. United States Department of Agriculture Economics Research Service: Soybeans & Oil Crops. http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/ background.aspx.

Walters, S. A., and Barker, K. R. 1994. Efficacy of *Paecilomyces lilacinus* in suppressing *Rotylenchulus reniformis* on tomato. Journal of Nematology. 26:600 - 605.

Wang, J., Niblack, T. L., Tremaine, J. N., Wiebold, W. J., Tylka, G. L.,Marrett, C. R., Noel, G. R., Myers, O., and Schmidt, M. E. 2003. The soybean cyst nematode reduces soybean yield without causing obvious symptoms. Plant Disease. 87:623 - 628.

Wang, C. L., Ulloa, M., Mullens, T. R., Yu, J. Z., and Roberts, P. A. 2012. QTL analysis for transgressive resistance to root-knot nematode in interspecific cotton (*Gossypium* spp.) progeny derived from susceptible parents. PLoS One. 7(4):e34874.

Wilson, M. J., and Jackson, T. A. 2013. Progress in the commercialization of bionematicides. BioControl. 58:715 - 722.

Winstead, N. N., Skotland, C. B., and Sasser, J. N. 1955. Soybean cyst nematode in North Carolina. Plant Disease Reporter. 39:9 - 11.

Wrather, J. A., and Koenning, S. R. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. Plant Health Progress. Doi:10.1094/PHP-2009-0401-01-RS.

Wrather, J. A., Shannon, G., Balardin, R., Carregal, L., Escobar, R., Gupta, G. K., Ma, Z., Morel, W., Ploper, D., and Tenuta, A. 2010. Effect of diseases on soybean yield in the top eight producing countries in 2006. Plant Health Progress. Doi: 10.1094/PHP-2010-0125-01-RS.

Xiang, N., and Lawrence, K. S. 2016. Optimization of *in vitro* techniques for distinguishing between live and dead second stage juveniles of *Heterodera glycines* and *Meloidogyne incognita*. PLoS One. 11(5):e0154818.

Xiang, N., Lawrence, K. S., Schrimsher, D., and Nightengale, S. 2013. Evaluation of Poncho Votivo, Aeris, Temik, and UFS0 738 on cotton for root knot management in Alabama, 2013. Plant Disease Management Report.7: N005. Doi: 10.1094/PDMR07.

Zuckerman, B. M., Dicklow, M. B., and Acosta, N. 1993. A strain of *Bacillus thuringiensis* for the control of plant-parasitic nematodes. Biocontrol Science and Technology. 3:41 - 46.

Chapter II. Optimization of *in vitro* techniques for distinguishing between live and dead second stage juveniles of *Heterodera glycines* and *Meloidogyne incognita*

Abstract

Heterodera glycines (Soybean Cyst nematode, or SCN) and *Meloidogyne incognita* (Root-Knot nematode, or RKN) are two damaging plant-parasitic nematodes on important field crops. Developing a quick method to distinguish between live and dead SCN and RKN second stage juveniles (J2) is vital for high throughput screening of pesticides or biological compounds against SCN and RKN. The *in vitro* assays were conducted in 96-well plates to determine the optimum chemical stimulus to distinguish between live and dead SCN and RKN J2. Sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), and sodium hydroxide (NaOH) were evaluated to see if these compounds can help distinguish between viable and dead J2. Results indicated that live SCN J2 responded equally ($P \leq 0.05$) to 1 µl Na_2CO_3 and 10 µl $NaHCO_3$ in 100 µl of water at pH = 10. Live SCN J2 responded by twisting their bodies in a curling shape and increasing rate of movements within 2 minutes of exposure. The twisting activity continued for up to 30 minutes. Live RKN J2 responded by increasing activity with the application of 1 µl NaOH in 100 µl of water at pH = 10 also in the 2 minutes to 30 minutes time frame. Furthermore, in growth chamber tests to confirm the infectivity of live SCN. The live SCN as determined by exposure to 1 µl of Na_2CO_3 indicated 60.5% of the SCN J2 were alive and of those, 29.5% were infective and entered the soybean roots. The 1 µl of NaOH stimulus revealed that 75.2% RKN J2 were alive and of those, 14.9% were infective and entered soybean roots. These results confirmed that 1 µl of Na_2CO_3 added to 100 µl suspension of SCN J2 and 1 µl of NaOH added to 100 µl suspension of RKN J2 are the effective stimuli for rapidly distinguishing between live and dead SCN and RKN J2 *in vitro*. SCN and RKN J2 responded differently to different compounds.

1. Introduction

Soybean Cyst nematode (SCN), *Heterodera glycines* Ichinohe 1952 and Root-Knot nematode (RKN), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 are two plant-parasitic nematodes that cause extensive economic damage to soybean and cotton every year in the U.S. Initial screening of new chemical and biological compounds for management of these pathogens begins with *in vitro* screening of large numbers of samples to determine the best candidates for advanced screening to greenhouse and field trials. However, distinguishing live from dead J2 with *in vitro* screening is a challenge. Multiple methods have been tried to distinguish between live and dead nematodes of both eggs and juveniles. Shepherd (1961) found that new blue R can stain the body contents of dead Tylenchida while live nematodes remain unstained. Chaudhuri et al. (1966) stained dead free-living nematode with eosin-Y while live nematodes remained unstained. Ogiga and Estey (1974) found that meldola blue and nile blue A are superior and more dependable for distinguishing dead from living nematodes on the specimens of *Dorylaimus*, *Helicotylenchus*, *Mononchus*, *Panagrolaimus*, *Pratylenchus*, *Rhabditis*, *Tylenchorhynchus*, and *Xiphinema* species but not species of *Heterodera* and *Meloidogyne*. Meyer et al. (1988) tested seven different stains on the eggs of *H. glycines* and found that chrysoidin, eosin-Y, new blue R, and nile blue A were useful in differentiating dead from live eggs while acridine orange, eosin-Y, fluorescein, and fluorescein diacetate differentially stained live and dead eggs when with fluorescence optics. These staining methods mentioned previously are time-consuming and did not work on live juveniles of SCN or RKN. Faske and Starr (2006) tested the sensitivity of *M. incognita* and *Rotylenchulus reniformis* to abamectin with concentrations of 21.5, 2.15, 0.22, 0.022, and 0 μg of abamectin/ml *in vitro* in BPI (Bureau of Plant Industries) watch dishes. They distinguished live from dead nematodes by touching each nematode with a small

probe (Faske and Starr 2006). This method is too slow and not feasible if multiple samples or chemicals need to be tested. Bird (1979) found that an enzymatically induced fluorescence method using fluorescein diacetate (FDA) can successfully assess the viability of nematodes under UV light, however, preparation was lengthy for multiple samples. Schroeder and MacGuidwin (2007) used fluorescein isothiocyanate (FITC) to distinguish live *H. glycines* and found that nematodes incubated in FITC remained active with fluorescence even after two weeks at room temperature, however, not all the nematodes acquired fluorescence quickly or had uniform response. Grego et al. (2013) found that CellTracker Green labeling (CTG) method was able to distinguish live nematodes from dead anoxia-impacted nematodes. However, all these techniques require expensive florescent microscopes, specialized training, and lengthy sample preparation which will not facilitate screening large numbers of samples.

Many researchers studied the chemoreception and behavior of free living and plant parasitic nematodes. These studies provide a new aspect of using chemical stimuli to distinguish live plant parasitic nematodes from dead individuals based on their physiological characteristics. Lee and Atkinson (1976) reported that nematodes may respond to stimuli or environmental changes through a sense organ or the nervous system. The metabolism of the nematode and the behavioral responses of a nematode may be undirected movement under particular stimulation (kinesis) or directed movement with respect to the source of the stimulation (taxis) (Lee and Atkinson 1976). They also reported that the bacterial feeder *Caenorhabiditis* is attracted to cyclic nucleotides, certain anions and cations, and basic pH, and that the *Caenorhabiditis* is not attracted to acid pH. They observed the response to hydrate carbon dioxide at concentrations normally found in soils is dependent on the buffer that was used (Lee and Atkinson 1976). Sambongi et al. (2000) also proved that *C. elegans* is not attracted to an acidic environment (pH lower that ~4.0) formed

by organic or inorganic acids which was dependent on multiple amphid chemo-sensory neurons, and inhibited by a mutation of capsaicin in receptor homologue, and by the addition of amiloride and ruthenium red (inhibitors of proton-gated Na^+ channels and capsaicin receptors, respectively). Riddle and Bird (1985) tested the responses of *R. reniformis*, *Anguina agrostis* and *M. javanica* to chemical attractants and found that *R. reniformis* was attracted to salts and the attractiveness was: $Cl^- > Na^+ > C_2H_3O_2^- > Mg^{2+}$, NH_4^+, SO_4^{2-}, but *M. javanica* J2 were not attracted to the salts. Perry (1996) indicated that the sensilla amphids are conserved in a wide range of plant parasitic nematodes including J2 and adult males of RKN, SCN, and *Globodera rostochiensis*, and adults of *Pratylenchus* species, and the chemoreception of nematodes involved with the amphidial secretions in nematode species and amphidial secretions were dissimilar and more specialized in different nematodes. These reports indicated that plant parasitic nematodes may be not attracted to a lower pH environment but may respond to a higher pH environment, hydrate carbon dioxide at certain concentrations, and some chemical stimuli. The chemicals sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) are commonly used as buffers in many research areas at pH's of 9-10 (Kannappan and Palani 2007; Zhai et al. 2014), and Na^+ were previously found to be an attractant for nematodes (Lee and Atkinson 1976; Riddle and Bird 1985). Chen and Dickson (2000) also found that live juveniles of *H. glycines* were able to respond to sodium hydroxide (NaOH) and sodium hypochlorite (NaOCl) by changing the body shape to curl and forming a hook-shape within 30 seconds, and the curled body shape lasted more than 10 minutes. This response was used to determine live from dead J2 of *H. glycines*. Chen et al. (2000) used NaOH to detect live *H. glycines* J2 treated with various fungal culture filtrates. Carbon dioxide (CO_2) also plays a possible role in attraction of plant parasitic nematodes beyond root exudates and electric potential (Dropkin 1966). Dropkin (1966) also reported that exposure to high concentrations of

CO_2 stopped movement of *Heterodera* spp. in a few minutes, and the nematodes recovered promptly upon restoration of oxygen after six hours of exposure to high CO_2. The literature suggests that pH, Na^+, and CO_2 or HCO_3^- or CO_3^{2-} may play a role in plant parasitic nematode response. The study of the response of plant parasitic nematodes *H. glycines* and *M. incognita* to the chemicals Na_2CO_3, $NaHCO_3$, and NaOH at various pHs will give detailed information about the potential roles of these stimuli for rapidly detecting live or dead SCN and RKN *in vitro*.

The goal of this research was to develop a method to rapidly determine live and dead J2 of SCN and RKN *in vitro*. The specific objectives were: i) to determine the optimum pH that can stimulate a physical response of SCN and RKN J2; and ii) to evaluate the optimum chemical stimuli that elicit a physical response using 20 µl of Na_2CO_3, $NaHCO_3$, and NaOH for SCN and RKN J2; iii) to evaluate the optimum concentration using the optimum chemical stimuli; iv) to confirm the infectivity and viability of J2 after exposure to the optimum stimulus.

2. Materials and Methods

2.1 Nematode and sodium solution

SCN and RKN J2: SCN eggs were obtained by grinding soybean cysts which were extracted from the 60-d-old soybean stock cultures maintained in 500 cm^3 polystyrene pots in the greenhouse. Soybean roots were washed through nested 850-µm-pore and 250-µm-pore sieves and cysts were collected from the 250-µm-pore sieve (Riggs and Schmitt 1988). SCN eggs were grinded from the cysts using a pestle and mortar. The standard gravitational sieving followed by sucrose centrifugation (Jenkins 1964) and collected on nested 75-µm-pore over 25 -µm-pore sieves used to obtain the SCN eggs. RKN eggs were extracted from the 45-d-old corn stock cultures maintained in 500 cm^3 polystyrene pots in the greenhouse. Corn roots were rinsed free of the soil, immersed in 0.625% NaOCl solution and shaken at 120 rpm on a rotary shaker for 4 minutes

(Hussey and Barker 1973). RKN eggs were cleaned by sucrose centrifugation and collected as described above. SCN and RKN eggs were hatched separately in a modified Baermann funnel which was placed on a Slide Warmer (Model 77) (Marshall Scientific, Brentwood, NH) at 28 ˚C and 31 ˚C, respectively (Xiang et al. 2014). Hatching occurred after 4 to 7 days depending on the season. J2 were collected on a 25-µm-pore sieve, placed in 1.5 ml tubes, centrifuged at 10,000 rpm for 1 minute, washed with distilled sterile water and centrifuged again. Two separate 1.5 ml tubes were prepared with live J2. One tube with live J2 suspension was held at room temperature while the second tube was heated at 65 ˚C for 5 minutes to kill the J2. Both live and dead J2 suspensions were adjusted to 30 to 40 J2 in 100 µl of water and pipetted into the 96-well plates for the study.

Sodium solution: Solutions of 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH (VWR, Suwanee, GA) were prepared individually by dissolving 21.1, 16.8, or 8.0 g of the compounds, respectively in 200 ml of distilled sterile water. The pH values of these sodium solutions were adjusted to 4, 7, and 10, respectively. 1% acetic acid (CH_3COOH) was used for pH 4.

2.2 Experiment 1: Determine the optimum pH of $NaHCO_3$ for SCN J2 responses

Since pH may be an important factor that causes responses in live nematode J2, thus we tested pH values of 4 with 1% CH_3COOH, and 7, and 10 with 1N $NaHCO_3$ on SCN J2. The experiment was established in 96-well plates. Ten µl of either live, dead or a 50/50 mixture of live and dead SCN J2 suspension containing 30 to 40 J2 and 90 µl of distilled sterile water were pipetted in each well. A 20 µl of 1% CH_3COOH at pH 4 and $NaHCO_3$ at pH 7 or 10 were added to the wells. The experiment was arranged in a RCBD with four replications and the trial was repeated twice.

The J2 were observed at 2, 5, 15, and 30 minutes after exposure under a compound microscope (Nikon TS100) to determine the numbers of live and dead SCN J2 and rated using a 1

- 4 scale within 30 minutes of exposure. A rating scale was divided as follows based on the movements and body shapes: 1 - no movement of the J2; 2 - J2 twitched slowly; 3 - J2 moved with normal body shape; 4 - J2 twitched quickly with curling body shape. Only 2 and 30 minutes data were presented in the results. Percentages of live J2 were calculated as (live numbers of J2 / Total number of J2) × 100. Rating scales were recorded.

2.3 Experiment 2: Select the optimum chemical stimulus for SCN and RKN J2

The *in vitro* test to determine the best stimulus for physical movement of SCN and RKN J2 responses was conducted. The 20 µl of chemicals 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH at optimum pH selected in experiment 1 were tested in 96-well plates. Distilled sterile water was used as a control. Each well received a 10 µl suspension containing 30 to 40 J2 in a total of 100 µl distilled sterile water. The experiments were arranged in a RCBD with four replications and the trial was repeated twice. Percentages of live J2 were calculated as (live numbers of J2 / Total number of J2) × 100. The J2 were rated using 1 - 4 scales as described above.

2.4 Experiment 3: Select the optimum concentration for the chemical stimuli

An *in vitro* test to determine the optimum concentration of the optimum chemical stimulus for live and dead SCN and RKN J2 responses was conducted. The concentrations selected were 1 µl and 10 µl of the chemical at the optimum pH selected in experiments 1 and 2. The test was conducted in 96-well plates *in vitro* as descried previously. Percentages of live J2 were calculated as (live numbers of J2 / Total number of J2) × 100. The J2 were rated at 1 - 4 scales and recorded.

2.5 Experiment 4: Confirm infectivity and viability after exposure to selected chemical stimuli

Determination if the live J2 were truly alive and infective and the dead J2 were immobile and not infective was confirmed using soybean plants grown in growth chambers. The selected

sodium stimuli 1 µl 1N Na_2CO_3 and 10 µl 1N $NaHCO_3$ in a total of 100 µl water at pH = 10 tested on SCN and 1 µl 1N NaOH in 100 µl of water at pH = 10 tested on RKN were confirmed in growth chamber evaluations using 50 ml conical tubes filled with pasteurized soil. Two seeds of 'Hutcheson' soybean (susceptible to both SCN and RKN) were planted and thinned to one seedling in each tube. Six-day-old plants were inoculated with live or dead SCN or RKN J2. The SCN and RKN J2 treatments were standardized to 1000 J2 / ml and added to the respective tubes. The actual number of live J2 as determined by the sodium stimuli were calculated as (live numbers J2 / total numbers of J2) × 100. Controls were SCN and RKN live and dead J2 that did not receive sodium stimuli but viability determined by direct observations under the microscope. Plants were incubated at 28 ℃ for SCN and 30 ℃ for RKN in the growth chamber with a 12 hour light and dark phase and watered twice daily as needed for 21 days. Soybean roots were removed, weighed, and stained with acid fuchsin at 21 days after inoculation (DAI). The J2 in the roots were enumerated using a dissection microscope (Nikon SMZ800) at 10X. Percentages of J2 enumerated in the roots were calculated as (numbers of J2 in the roots/number of live J2 at inoculation) ×100. The experiments were arranged in a RCBD with five replications and the trial was repeated twice.

2.6 Data analysis

Data on percentages of live J2 increased in *in vitro* tests and percentages of live J2 inoculated and entering the soybean roots were analyzed in SAS 9.4 software (SAS Institute, Cary, NC) using Glimmix procedure. Student panel graphs were generated to test the normality of the residuals for the percentages of live J2 increased in *in vitro* tests and percentages of live J2 inoculated and entered the soybean roots in growth chamber tests. Treatment LS-means were compared by Tukey-Kramer's method at the significant level of $\alpha \leq 0.05$. Data from two repeated

trials were analyzed separately to determine any interactions over time prior to pooling if there was no interaction.

3. Results

3.1 Results of experiment 1: Optimum pH of $NaHCO_3$ for SCN J2 responses

Live SCN J2 responded differently to the solutions with pH 4 of 1% CH_3COOH and pH 7 and 10 of 1N $NaHCO_3$. The pH = 4 solution increased the movement of live SCN J2 by 7.8 % at 2 minutes indicating the nematodes were alive ($P \leq 0.05$) (Table 1). However, SCN J2 movement decreased significantly ($P \leq 0.05$) from the 5 to 30 minute time period. The pH = 7 did not stimulate SCN J2 movement at 2 minutes through the 30 minute time periods (Table 1). The pH = 10 solutions stimulated an increase of the SCN J2 movement by 10.7 % at 2 minutes which was similar to that observed by pH = 4. However, pH = 10 continued to stimulate SCN J2 movement through the 30 minute observation period (Table 1). Dead J2 did not respond to any pH test solutions and remained motionless (Table 1). These results indicated that pH = 10 is the optimum pH value to cause SCN J2 responses such as changing body shape which can determine if individuals were alive or dead. The pH = 10 could be used in the following trials.

Table 1. Percentages of live SCN J2 under different pH values over time in 30 minutes.

pH value	SCN J2	Before exposure	2 mins exposure	30 mins exposure	% live J2 changed at 2 mins	Rating at 2 mins	% live J2 changed at 30 mins	Rating at 30 mins
		Live/Total J2 (%)	Live/Total J2 (%)	Live/Total J2 (%)				
pH =4 1% CH_3COOH	Live	21/26 (80.7)	23/26 (88.5)	2/26 (7.7)	7.8 a	4	-73.7 c*	2
	Live/Dead	10/25 (40.0)	11/25 (44.0)	3/25 (12.0)	4.0 a	4	-32.0 b	2
	Dead	0/18 (0.0)	0/18 (0.0)	0/18 (0.0)	0.0 a	1	0.0 a	1
pH =7 1N $NaHCO_3$	Live	20/24 (83.3)	20/24 (83.3)	13/24 (54.2)	0.0 a	3	-29.1 b	2
	Live/Dead	11/24 (45.8)	11/24 (45.8)	11/24 (45.8)	0.0 a	3	0.0 a	3
	Dead	0/21 (0.0)	0/21 (0.0)	0/21 (0.0)	0.0 a	1	0.0 a	1
pH =10 1N $NaHCO_3$	Live	22/28 (78.6)	25/28 (89.3)	25/28 (89.3)	10.7 a	4	10.7 a	4
	Live/Dead	11/29 (37.9)	13/29 (44.8)	13/29 (44.8)	6.9 a	4	6.9 a	4
	Dead	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)	0.0 a	1	0.0 a	1

Numbers in the parentheses are the percentages of live J2 out of total number of J2.
*LS-MEANS with the same letter are not significantly different according to Tukey-Kramer's method ($P \leq 0.05$).

3.2 Results of experiment 2: Select the optimum chemical stimulus for SCN and RKN J2

SCN J2: The three chemicals 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH at pH=10 tested at 20 µl caused different responses on live SCN J2 at different time points which visibly distinguished live from dead J2. The three chemicals were equally effective ($P \leq 0.05$) at distinguishing live from dead SCN J2 from 0 to 2 minutes time period using 20 µl. With the extending of exposure time, 20 µl Na_2CO_3 appeared toxic to the SCN J2 and a significant decrease ($P \leq 0.05$) in movement of the nematodes was observed at 30 minutes (Fig 1). The 20 µl $NaHCO_3$ slightly decreased the movement of the nematode with increase time (Fig 1).

RKN J2: The 1N Na_2CO_3 was highly toxic to RKN J2 which caused a significant decrease in movement ($P \leq 0.05$) within 2 minutes exposure to the chemical (Fig 2). The $NaHCO_3$ and NaOH stimulated the movements of RKN J2 at 2 minutes (Fig 2) with distinctive curling or hooked body shapes. However, the $NaHCO_3$ caused significant decreasing movement of RKN J2 after 2 minutes exposure ($P \leq 0.05$). The NaOH also slightly decreased the movement of RKN J2 from 2 minutes to 15 minutes period of time.

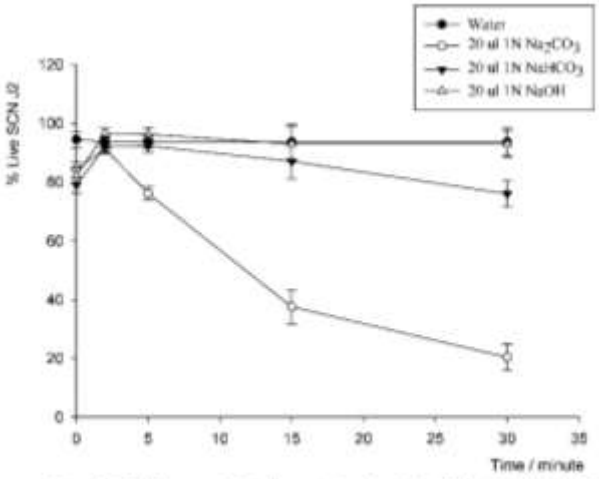

Figure 1. SCN J2 responded to three sodium chemicals, 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH at 20 µl application.

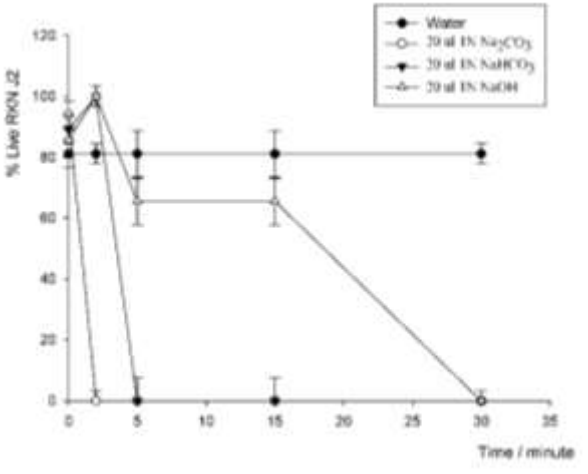

Figure 2. RKN J2 responded to three sodium chemicals, 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH at 20 µl application.

3.3 Results of experiment 3: Select the optimum concentration for the chemical stimuli

SCN J2: The optimum concentration for SCN J2 tested was from 1 µl and 10 µl of 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH at pH = 10. The 1 µl of Na_2CO_3, $NaHCO_3$, and NaOH stimulated movement of SCN J2 within 2 minutes exposure, but only 1 µl of Na_2CO_3 and 10 µl $NaHCO_3$ caused live SCN J2 to rapidly curl and twist into a hook shape (Fig 3A-B) after 2 minutes exposure which easily distinguished live from dead individuals (Table 2, Fig 3C-D). However, the 10 µl volume caused J2 to float and therefore compounded counting. The NaOH did not cause the live SCN J2 to curl and twist (Fig 3E). The 1 µl of Na_2CO_3 was optimum for distinguishing between live and dead SCN J2 in 30 minutes and was tested in the growth chamber.

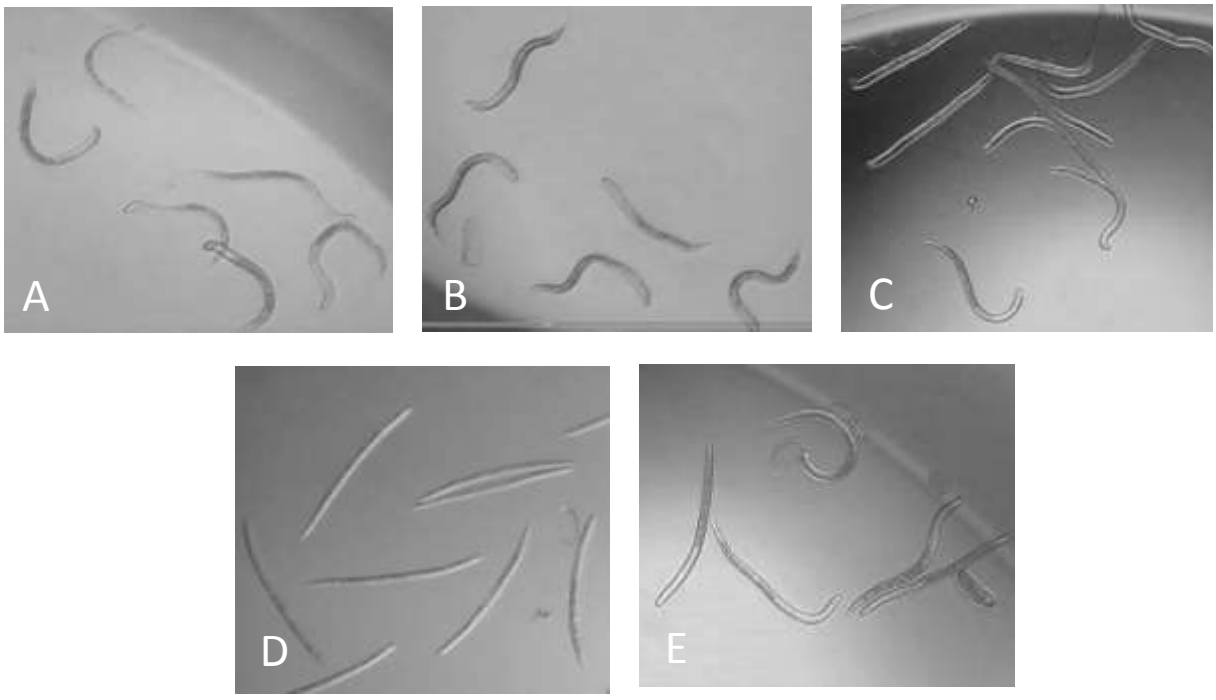

Figure 3 (A, B, C, D, and E). Responses of SCN J2 to test agents at 30 minutes. SCN J2 were exposed to 1 µl 1N Na_2CO_3 at 30 minutes (A); SCN J2 were exposed to 10 µl $NaHCO_3$ at 30minutes (B); SCN J2 in water at 30 minutes (C); Dead SCN J2 didn't response to any test agents (D); SCN J2 were exposed to 10 µl NaOH at 30 minutes (E).

Table 2. Response of SCN J2 to 1 or 10 µl of 1N Na_2CO_3, 1N $NaHCO_3$, and 1N NaOH solutions at pH = 10.

Sodium stimuli	Volume/µl	Before exposure	2 mins exposure	30 mins exposure	% changed live SCN J2 at 2 mins	Rating scale at 2 mins	% changed live SCN J2 at 30 mins	Rating scale at 30 mins
		Live / Total J2 (%)	Live / Total J2 (%)	Live / Total J2 (%)				
1N Na_2CO_3	1	25/28 (89.3)	26/28 (92.9)	26/28 (92.9)	3.6 abc	4	3.6 ab*	4
	10	23/28 (82.1)	26/28 (92.9)	13/28 (46.4)	10.8 ab	4	-35.7 b	1
1N $NaHCO_3$	1	28/36 (77.8)	29/36 (80.6)	28/36 (77.8)	2.8 bc	3	0.0 ab	3
	10	26/30 (86.7)	28/30 (93.3)	29/30 (96.7)	6.6 abc	3	10.0 ab	3
1N NaOH	1	27/34 (79.4)	29/34 (85.3)	24/34 (70.6)	5.9 abc	3	-8.8 b	3
	10	19/24 (79.2)	22/24 (91.7)	22/24 (91.7)	12.5 a	4	12.5 a	2
Water Control		37/39 (94.9)	37/39 (94.9)	37/39 (94.9)	0.0 c	3	0.0 ab	3

Numbers in the parentheses are the percentages of live number J2 out of total number of J2.
*LS-MEANS with the same letter are not significantly different according to Tukey-Kramer's method ($P \leq 0.05$).

RKN J2: The optimum concentration for RKN J2 was selected from 1 µl and 10 µl of 1N NaOH at pH = 10. The 1µl NaOH caused significant increasing movement of RKN J2 at 30 minutes ($P \leq 0.05$) with distinctive curled and twisted body shapes (Table 3, Fig 4A-B). The 10 µl of NaOH was toxic to the RKN J2 at 30 minutes and the 10 µl volume caused floating which is not recommended for *in vitro* screening (Table 3). The 1 µl of NaOH was chosen and tested in the growth chamber.

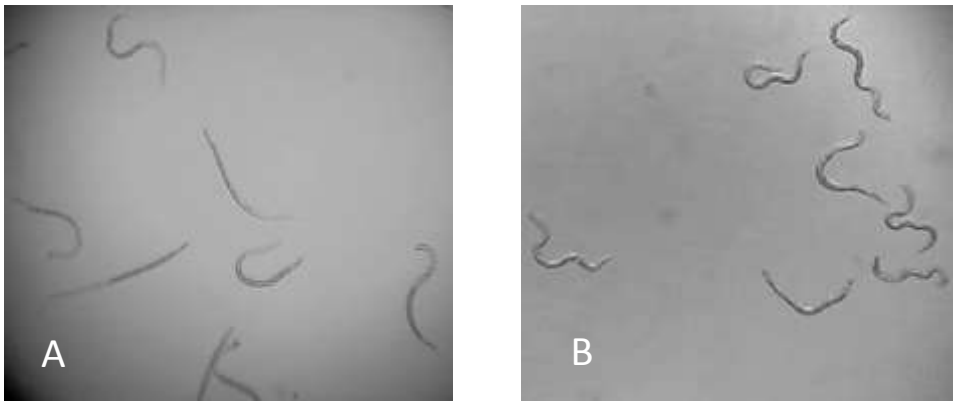

Figure 4. Responses of RKN J2 to water and 1 µl of 1N NaOH at 30 minutes. RKN J2 responded to water with normal annulation at 30 minutes (A); RKN J2 exposed in 1µl of 1N NaOH with curling shape at 30 minutes (B).

Table 3. Response of RKN J2 to different concentrations of 1N NaOH at pH = 10.

Sodium stimuli	Volume/µl	Before exposure	2 mins exposure	30 mins exposure	% changed live RKN J2 at 2 mins	Rating scale at 2 mins	%changed live RKN J2 at 30 mins	Rating scale at 30 mins
		Live / Total J2 (%)	Live / Total J2 (%)	Live /Total J2 (%)				
1N NaOH	1	25/32 (78.1)	32/32 (100.0)	32/32 (100.0)	21.9 a	4	21.9 a*	4
	10	25/32 (78.1)	32/32 (100.0)	22/32 (68.2)	21.9 a	2	-9.9 c	2
Water Control		39/50 (78.0)	39/50 (78.1)	39/50 (78.1)	0.0 a	3	0.0 bc	3

Numbers in the parentheses are the percentages of live number J2 out of total number of J2.
*LS-MEANS with the same letter are not significantly different according to Tukey-Kramer's method ($P \leq 0.05$).

3.4 Results of experiment 4: Confirm infectivity and viability after exposure to selected chemical stimuli

SCN in soybean

Results indicated that the 1µl 1N Na_2CO_3 in 100 µl of solution at pH=10 indicated that 60.5 % of the SCN J2 were alive and of these 29.5 % entered the soybean roots (Table 4, Fig 5A). All of the SCN J2 which were determined to be dead were not infective as indicated by their inability to enter the roots and thus none were observed within the root tissue (Table 4, Fig 5B). Soybean root fresh weights were similar among all the treatments (Table 4). The 1µl 1N Na_2CO_3 was the best indicator of live SCN J2 and J2 were infective entering soybean roots and beginning their life cycle.

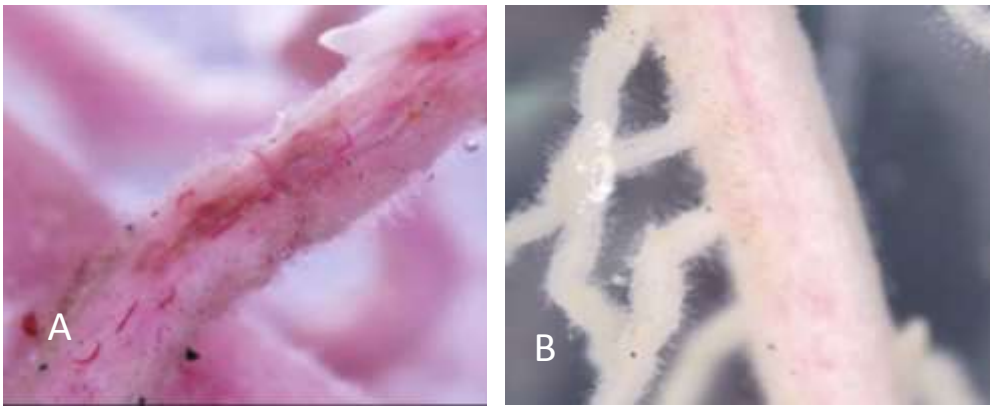

Figure 5. Stained SCN J2 in the root tissues from a live SCN treatment were observed at 21 DAI (A) and the stained root tissues from the dead SCN treatments with no SCN J2 or females (B).

Table 4. SCN J2 infection of soybean roots after exposed to 1 µl 1N Na_2CO_3 live and dead determination.

SCN J2	Sodium stimuli	Volume/µl	Percent live J2 inoculated	Percent Females and J2 in roots at 21 DAI	Root fresh weight at 21 DAI/g
Live	1N Na_2CO_3	1	60.5 a	29.5 a	1.5 a*
	Water Control		57.6 a	17.0 ab	1.4 a
Dead	1N Na_2CO_3	1	1.1 b	0.0 b	1.9 a
	Water Control		0.0 b	0.0 b	1.7 a

Percentage of live SCN J2 inoculated with soybean roots and SCN J2 penetrated in the roots at 21 (DAI).
*LS-means with the same letter are not significantly different according to Tukey-Kramer's method ($P \leq 0.05$).

RKN in soybean

Growth chamber results indicated that 1µl of 1N NaOH in 100 µl of solution at pH=10 indicated 75.2 % of RKN J2 were alive and 14.9 % J2 entered the roots (Table 5, Fig 6A). RKN J2 and females were recorded at 21DAI. Dead RKN J2 were confirmed dead and were not infective as measured by their absence in the roots (Table 5, Fig 6B). The root fresh weights were similar among all the treatments (Table 5). Thus, 1 µl 1N NaOH at pH = 10 is the best indicator for live RKN J2 and J2 were infective in soybean roots.

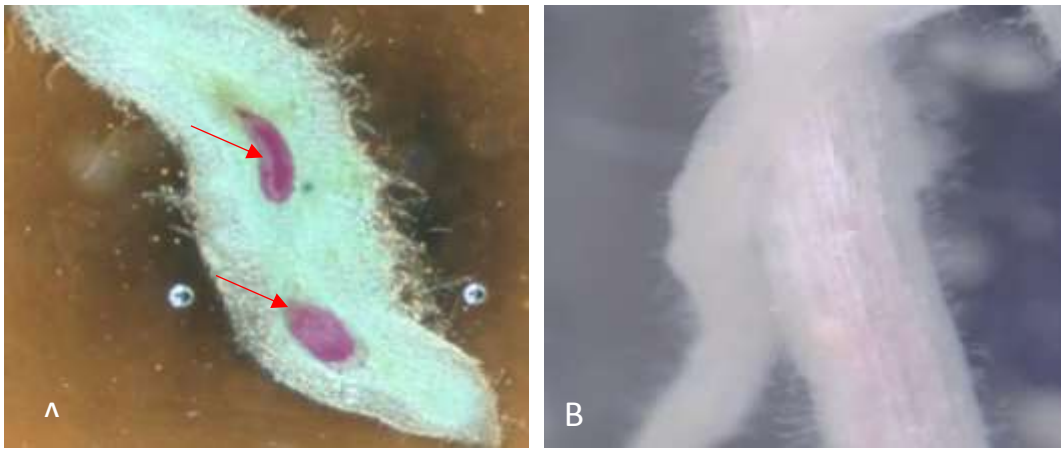

Figure 6. Stained RKN females in the root tissues from live treatment were observed at 21 DAI (A) and the stained root tissues from the dead RKN treatments with no RKN J2 or

Table 5. RKN J2 infection of soybean roots after 1 µl 1N NaOH live and dead determination.

RKN J2	Sodium stimuli	Volume/µl	Percent live J2 inoculated	Percent females and J2 in roots at 21 DAI	Root fresh weight at 21 DAI / g
Live	1N NaOH	1	75.2 a	14.9 a	1.4 a*
	Water control		66.5 a	8.0 ab	1.3 a
Dead	1N NaOH	1	1.7 b	0.0 b	1.4 a
	Water Control		1.9 b	0.0 b	1.5 a

Percentage of live RKN J2 inoculated in the roots and RKN J2 and females penetrated in the roots at 21DAI.
*LS-means with the same letter are not significantly different according to Tukey-Kramer's method ($P \leq 0.05$).

4. Discussion

The pH test indicated that live SCN J2 responded to higher pH levels. The pH = 10 can successfully distinguish between live and dead SCN J2 in 30 minutes *in vitro*. Chen and Dickson

(2000), previously reported pH about 12.3 effectively stimulated SCN J2 but theorized the response was because of toxic action of NaOH. Sambongi et al. (2000) also proved that *C. elegans* is not attracted to an acidic environment with pH lower than 4.0 formed by organic or inorganic acids. Our experiments demonstrated that high pH=10 effectively stimulated SCN J2, but low pH did not cause a response. The same response has also been found on RKN J2 (data not shown). We showed that SCN and RKN are not attracted to low pH but respond to high pH suggesting that the pH value plays an important role in stimulating nematode.

Results for selecting the optimum stimuli *in vitro* and in growth chamber revealed that SCN J2 responded to 1 µl 1N Na_2CO_3 and RKN J2 responded to 1 µl 1N NaOH in 100 µl of water at pH=10. These indicated 1 µl 1N Na_2CO_3 and 1 µl 1N NaOH in 100 µl of water at high pH are the best indicators to distinguishing between live and dead SCN and RKN J2 *in vitro*, respectively. Nehrke and Melvin (2002) found that the NHX-4, one of the nine putative homologs of *C. elegans* and the ubiquitous nematode Na^+-H^+ exchanger, mediates Na^+-dependent pH recovery after intracellular acidification. In our study, adding Na^+ and altering the pH of the environment may contribute to the stimulation of SCN and RKN J2 through the Na^+-H^+ exchanger, but more research is needed to understand this phenomena. Perry (1996) mentioned the role and functioning of the anterior chemosensory organs of plant parasitic nematode and found that the amphidial secretions were involved in the chemoreception and the behavioral of nematode responses to semiochemicals. In addition, amphids, which are the largest and most complex of the anterior sensilla, is conserved in many plant parasitic nematodes including J2 and adults males of *M. incognita* and *H. glycines* (Perry 1996; Baldwin and Hirschmann 1973; Wergin and Endo 1976). This information indicated that the response of SCN and RKN J2 to the high pH and Na_2CO_3 or NaOH are possibly involved

with chemosensory organs and amphidial secretions which play an important role in chemoreception (Perry 1996).

Overall, this sodium technique is very accurate at determining live and dead nematodes when applied *in vitro* to test the efficacy of nematicides or biocontrol agents and can be used for high throughput screening. The application of stimuli is a simple screening method not requiring special training for sample preparation, or advanced equipment necessary for FDA, FITC, and CTG labeling methods (Bird 1979; Schroeder and MacGuidwin 2007; Grego et al. 2013). The quick consistent responses of the live nematodes to the sodium stimuli indicates efficacy of the tested agents. Other techniques (Bird 1979; Schroeder and MacGuidwin 2007; Grego et al. 2013) using dyes or labeling materials cannot guarantee all the nematode will be labelled the same in a short time period. Health and safety are also concerns when using fluorescent materials such as FDA, FTIC, and CTG, as well as availability of fluorescence microscopes. The application of 1 µl Na_2CO_3 or NaOH can not only distinguish between live and dead nematodes, but also are relatively safe. Beyond SCN and RKN J2, Lesion nematode J2 and adults also responded to the 1 µl Na_2CO_3 at pH = 10 (data not published).

In summary, results from this research clearly demonstrate that applying 1 µl 1N Na_2CO_3 in 100 µl SCN solution at pH = 10 and 1 µl 1N NaOH in 100 µl RKN solution at pH = 10 can be practical and economical method for high throughput screening chemical or biological agents of SCN or RKN *in vitro*. Using this method we screened 700 bacterial strains for efficacy to SCN and RKN in three months.

5. Reference

Baldwin, J. G., and Hirschmann, H. 1973. Fine structure of the cephalic sense organs in *Meloidogyne incognita* males. Journal of Nematology. 5:285 - 302.

Bird, A. F. 1979. A method of distinguishing between living and dead nematodes by enzymatically induced fluorescence. Journal of Nematology. 11:103 - 105.

Chaudhuri, N., Dick, R. I., Engelbrecht, R. S., and Austin, J. H. 1966. Staining of free-living nematodes by eosin-Y dye. Nematologica. 12:337 - 344.

Chen, S. Y., and Dickson, D. W. 2000. A technique for determining live second-stage juveniles of *Heterodera glycines*. Journal of Nematology. 32:117 - 121.

Chen, S. Y., Dickson, D. W., and Mitchell, D. J. 2000. Viability of *Heterodera glycines* exposed to fungal filtrates. Journal of Nematology. 32:190 - 197.

Dropkin, V. H. 1996. Physiology of nematodes of the soil. Annals of the New York Academy of Sciences. 139:39 - 52.

Faske, T. R., and Starr, J. L. 2006. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Abamectin. Journal of Nematology. 38:240 - 244.

Grego, M., Stachowitsch, M., Troch, M. D., and Riedel, B. 2013. CellTracker Green labelling vs. rose Bengal staining: CTG wins by points in distinguishing living from dead anoxia-impacted copepods and nematodes. Biogeosciences. 10:4565 - 4575.

Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter. 57:1025 - 1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter. 48:692.

Kannappan, A., and Palani, R. 2007. Acoustical behaviour of glycerine, dextrose and sucrose in Na_2CO_3 and $NaHCO_3$ buffer solution. Ind. J. Pure Appl. Phys. 45:573 - 579.

Lee, D. L., and Atkinson, H. J. 1976. Physiology of nematodes. 2nd ed. London and Basingstoke: The Macmillan Press LTD;

Meyer, S. L. F., Sayre, R. M., Huettel, R. N. 1988. Comparisons of selected stains for distinguishing between live and dead eggs of the plant-parasitic nematode *Heterodera glycines*. Proc. Helminthol. Soc. Wash. 55:132 - 139.

Nehrke, K., and Melvin, J. E. 2002. The NHX family of Na^+-H^+ exchangers in *Caenorhabditis elegans*. Journal of Biological Chemistry. 277:29036 - 29044.

Ogiga, I. R., and Estey, R. H. 1974. The use of Meldola Blue and Nile Blue A, for distinguishing dead from living nematodes. Nematologica. 20:271 - 276.

Perry, R. N. 1996. Chemoreception in plant parasitic nematodes. Annu. Rev. Phytopathol. 34:181 - 199.

Riddle, D. L., and Bird, A. F. 1985. Responses of the plant parasitic nematodes *Rotylenchulus reniformis*, *Anguina agrostis* and *Meloidogyne javanica* to chemical attractants. Parasitology. 91:185 - 195.

Riggs, R. D., and Schmitt, D. P. 1988. Complete characterization of the race scheme for *Heterodera glycines*. Journal of Nematology. 20:392 - 395.

Sambongi, Y., Takeda, K., Wakabayashi, T., Ueda, I., Wada, Y., and Futai, M. 2000. *Caenorhabditis elegans* senses protons through amphid chemosensory neurons: proton signals elicit avoidance behavior. Neuroreport. 11:2229 - 2232.

Schroeder, N., and MacGuidwin, A. 2007. Incorporation of a fluorescent compound by live *Heterodera glycines*. Journal of Nematology. 39:43 - 49.

Shepherd, A. M. 1961. New Blue R, a stain that differentiates between living and dead nematodes. Nematologica. 8:201 - 208.

Wergin, W. P., Endo, B. Y.1976. Ultrastructure of a neurosensory organ in a root knot nematode. J. Ultrastruct Res. 56:258 - 276.

Xiang, N., Lawrence, K. S., Kloepper, J. W., and Mcinroy, J. A. 2014. *In vitro* screening of biological control agents on *Meloidogyne incognita*. Proceedings of the 2014 Beltwide Cotton Conference. 1: 258-260.

Zhai, L., Xue, Y. F., Song, Y. H., Xian, M. J., Yin, L., Zhong, N.Q., Xia, G. X., and Ma, Y. H. 2014. Overexpression of *Aa*Pal, a peptidoglycan-associated lipoprotein from *Alkalomonas amylolytica*, improves salt and alkaline tolerance of *Escherichia coli* and *Arabidopsis thaliana*. Biotechnology Letters. 36:601 - 607.

Chapter III. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton

Abstract

In the past decade, increased attention has been placed on biological control of plant-parasitic nematodes using various fungi and bacteria. The objectives of this study were to evaluate the potential of 669 plant growth-promoting rhizobacteria (PGPR) strains for mortality to *Meloidogyne incognita* J2 *in vitro* and for nematode management in greenhouse, microplot, and field trials. Results indicated that the mortality of *M. incognita* J2 by the PGPR strains ranged from 0.0% to 100% with an average of 39%. Among the PGPR strains examined, 33.5% caused more than 50% mortality of *M. incognita* J2. Of those, 28.1% were *B. simplex*, 11.6% *B. aryabhattai*, 10.7% *B. toyonensis*, 6.3% *B. cereus*, 5.8% *B. mycoides*, 5.8% *B. safensis*, 4.9% *B. altitudinis*, 4.9% *B. velezensis*, 3.1% *B. subtilis subsp. inaquosorum*, 2.2% *B. weihenstephanensis*, 2.2% *Paenibacillus amylolyticus*, 1.8% *B. methylotrophicus*, 1.8% *Brevibacterium epidermidis*, 9.8% were multiple other genera. In subsequent trials, *B. velezensis* strain Bve2 reduced *M. incognita* eggs per gram of cotton root in the greenhouse trials at 45 days after planting (DAP). *Bacillus mojavensis* strain Bmo3, *B. velezensis* strain Bve2, *B. subtilis* subsp. *subtilis* strain Bsssu3, and Mixture 2 (Abamectin + Bve2 + Bal13) suppressed *M. incognita* eggs per gram of root in the microplot at 45 DAP. *Bacillus velezensis* strains Bve12 and Bve2 also increased seed cotton yield in the microplot and field trials. Overall, results indicate that *B. velezensis* strains Bve12 and Bve2, *B. mojavensis* strain Bmo3, and the Mixture 2 (Abamectin + Bve2 + Bal13) have potential to reduce *M. incognita* population density and to enhance growth of cotton when applied as in-furrow spray at planting.

1. Introduction

Meloidogyne incognita (Kofoid & White) Chitwood, the southern root-knot nematode, is one of the most important plant-parasitic nematodes affecting cotton production in the U.S. (Creech et al. 1995; Robinson 2007). In 2015, cotton yield in the U.S. was estimated to be 7.9 million bales, but losses due to *M. incognita* were estimated at 215,500 bales, which was equivalent to 1.35% of total production (Lawrence et al. 2016). Due to environmental and health concerns with the use of chemical nematicides for nematode management, many alternative strategies such as biological agents for plant-parasitic nematode control have been investigated (Burkett-Cadena et al. 2008; Kiewnick and Sikora 2006). Plant growth-promoting rhizobacteria (PGPR) promote plant growth and elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts (Kloepper et al. 2004). Strains of PGPR which also exhibit nematicidal activity and/or elicit induced systemic plant resistance to plant-parasitic nematodes could be potential alternatives to chemical nematicides.

Many studies have reported antagonistic activity of various strains of *Bacillus* spp. against plant-parasitic nematodes. Twelve species of *Bacillus* have been documented for *M. incognita* management, including *B. amyloliquefaciens* (synonymous as *B. velezensis*) (Burkett-Cadena et al. 2008), *B. cereus* (Siddiqui and Mahmood 1999), *B. circulans* (Ambo et al. 2010), *B. coagulans* (Ambo et al. 2010; Serfoji et al. 2010), *B. firmus* (Terefe et al. 2009; Mendoza et al. 2008), *B. licheniformis* (Siddiqui and Husain 1991; Siddiqui and Mahmood 1992), *B. megaterium* (Kloepper et al. 1992), *B. penetrans* (Brown et al. 1985; Brown and Smart 1985), *B. polymyxa* (Khan and Akram 2000), *B. sphaericus* (Krechel et al. 2002), *B. subtilis* (Siddiqui and Mahmood 1999; Kavitha et al. 2007), and *B. thuringiensis* (Devidas and Rehberger 1992; Zuckerman et al. 1993; Mohammed et al. 2008). Among these *Bacillus* species, some have been developed into

commercial products for controlling plant disease and nematodes. BioNem-WP/BioSafe, a *B. firmus* product developed by AgroGreen, was reported effective against *M. incognita*, *M. hapla*, *Heterodera* spp., *Tylenchulus semipenetrans*, *Xiphinema index*, and *Ditylenchus dipsaci* (Keren-Zur et al. 2000). BioYield, a combination of *B. velezensis* strain IN937a and *B. subtilis* strain GB03, was developed by Gustafson in a flowable formulation for management of soil-borne pathogens and suppression of *M. incognita* population density on tomato (Kloepper et al. 2004; Burkett-Cadena et al. 2008). Nemix, a *Bacillus* spp. product developed by AgriLife/Chr. Hansen, was reported for control of root-knot nematodes on vegetables and fruit trees (Hallmann et al. 2009). VOTiVO, *Bacillus firmus* GB-126, is marketed by Bayer CropScience as a seed treatment for the control of plant-parasitic nematodes on corn, cotton, sorghum, soybean, and sugar beet (Wilson and Jackson 2013). Pathway Consortia, a product containing *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. coagulans*, *Pseudomonas fluorescens*, *Streptomyces* spp., and *Trichoderma* spp. developed by Pathway Holdings, was reported for the management of plant-parasitic nematodes (Askary 2015).

Mode of action for biocontrol of plant-parasitic nematodes of some *Bacillus* strains have been studied. Sayre (1980) and Stirling (1984) reported *B. penetrans* (synonymous as *Pasteuria penetrans*) (Charles et al. 2005) was an obligate parasite of *Meloidogyne* spp. The *P. penetrans* spores attached to the cuticle of the J2 in the soil prior to entering the roots. The germ tube of the spores penetrated the cuticle and reproduced inside the nematode body consuming the nematode (Sayre 1980; Stirling 2014). Mendoza et al. (2008) studied *in vitro* activity of *B. firmus* against burrowing nematode *Radopholus similis*, root-knot nematode *M. incognita*, and stem nematode *Ditylenchus dipsaci* and detected rates of mortality of these nematodes and significant reduction of *M. incognita* hatching after incubation with a low concentration of pure culture filtrates

(Mendoza et al. 2008). The mode of action for the observed nematode paralysis and mortality was attributed to secondary metabolites produced by the bacteria (Mendoza et al. 2008).

Induced systemic resistance (ISR) of some *Bacillus* strains has been documented. Sikora (1988) found that *Bacillus subtilis* can induce protection against *M. incognita* in cotton. Kloepper et al. (2004) reported that specific strains of *B. velezensis*, *B. cereus*, *B. mycoides*, *B. pasteurii*, *B. pumilus*, *B. sphaericus*, and *B. subtilis* can elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts through ISR. Schrimsher (2013) studied the ISR of *B. firmus* GB-126 against *Heterodera glycines* and *M. incognita* in split-root experiments in the greenhouse and found that ISR was evident in the *H. glycines* split-root assay but not in the *M. incognita* split-root assay. Collectively, these studies indicate that *Bacillus* spp. are promising candidates for nematode disease management through diverse modes of action.

The overall goal of this research was to investigate selected PGPR strains for their potential biological control of *M. incognita* on cotton. The specific objectives were to assess the potential of PGPR strains for mortality of *M. incognita* J2 *in vitro* and evaluate the efficacy of PGPR strains for reduction of *M. incognita* population density and plant growth promotion on cotton in greenhouse and microplot trials, and in field production systems.

2. Materials and Methods

2.1 PGPR strains

A total of 669 PGPR strains (Appendix 1) were included in *in vitro* studies (Appendix 1). PGPR strains were originally isolated, identified, and stored by J. W. Kloepper at Auburn University, Auburn, AL. Among these strains, 91.8% were *Bacillu*s spp. including 208 strains of *B. simplex*, 70 strains of *B. toyonensis*, 53 strains of *B. aryabhattai*, 51 strains of *B. cereus*, 44 strains of *B. mycoides*, 41 strains of *B. velezensis*, 35 strains of *B. safensis*, 21 strains of *B.*

altitudinis, 21 strains of *B. weihenstephanensis*, 15 strains of *B. subtilis* subsp. *inaquosorum*, 13 strains of *B. methylotrophicus*, six strains of *B. pumilus*, five strains of *B. psychrosaccharolyticus*, four strains of *B. mojavensis*, four strains of *B. subtilis* subsp. *subtilis*, four strains of *B. thuringiensis*, three strains of *B. siamensis*, three strains of *B. tequilensis*, and 13 strains of other *Bacillus* spp. The remaining 8.2% of the collection, ten strains were *Sporosarcina globispora*, seven strains were *Brevibacterium epidermidis*, nine strains were *Paenibacillus amylolyticus*, four strains were *Paenibacillus lautus*, and 25 strains were from multiple genera. The PGPR strains stored in 30% glycerol at -80 ˚C were transferred to tryptic soy agar (TSA) (VWR, Radnor, PA) plates, and incubated at 35˚C for 24 hours. The 21 strains that had no significant growth on TSA medium were eliminated from the study (Appendix 3). Vegetative cells of each strain were suspended in 5 ml of sterile distilled water in 25 ml glass tubes, the concentration was adjusted to 1×10^7 CFU/ml.

2.2 Nematode inoculum

Meloidogyne incognita, originally isolated from an infested field at the Plant Breeding Unit (PBU) at E.V. Smith Research Center of Auburn University and maintained on corn plants "Mycogen 2H723" (Dow AgroScience, Indianapolis, IN) in 500 cm^3 poly styrene pots in the greenhouse, was used as inoculum in the experiments. Eggs were extracted from corn roots by placing the root system in a 0.625 % NaOCl solution for 4 min using a rotary shaker at 120 rpm (Hussey and Barker 1973). Eggs were rinsed with tap water, collected on a 25-µm-pore sieve, then processed by sucrose centrifugation-flotation at 240 g for 1 minute (Jenkins 1964). For *in vitro* tests, *M. incognita* eggs were placed in a modified Baermann funnel (Castillo et al. 2013) on a slide warmer (Model 77) (Marshall Scientific, Brentwood, NH) and incubated at 31°C for 5 to 7 days to obtain second stage juveniles (J2) (Xiang 2014). The J2 were collected on a 25-µm-pore

sieve, transferred to 1.5 ml micro centrifuged tubes, centrifuged at 5,000 g for 1 minute, rinsed with sterile distilled water, and centrifuged at 5,000 g for 1 minute. The J2 suspensions were adjusted to 30 to 40 J2 per 10 µl of water (Xiang 2014). For trials conducted in the greenhouse and microplot, eggs were enumerated at 40 × magnification using an inverted TS100 Nikon microscope and standardized to 2,000 eggs per cone-tainer or 50,000 eggs per microplot.

2.3 Tests *in vitro*

Tests *in vitro* were conducted to assess mortality of *M. incognita* J2 by PGPR strains. PGPR vegetative cell suspensions and *M. incognita* J2 inocula were prepared as mentioned previously. Ten µl nematode suspension containing 30 to 40 *M. incognita* J2 were added in each well of a 100 µl 96-well plate. Ninety µl of each PGPR bacterial vegetative cell suspension were transferred into each test well of the 96-well plate. Clothianidin plus *B. firmus* I-1582 (Poncho/VOTiVO) (Bayer CropScience, Raleigh, NC) at 0.7 µl/well (0.424 mg ai/seed) and 1 granule/well of Aldicarb (Temik 15G) (Bayer CropScience, Raleigh, NC) were used as chemical standards. Sterile distilled water was used as the untreated control. Each plate was sealed with parafilm and incubated at room temperature (22.2 to 25.5 °C) for 48 hours. Numbers of live *M. incognita* J2 were counted and recorded at experiment initiation and 48 hours after exposure to the bacterial strains. Viability of *M. incognita* J2 was determined using the sodium hydroxide technique developed by Xiang and Lawrence (2016). Mortality percentage of *M. incognita* J2 was calculated using the following equation: [(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] ×100. Each bacterial treatment was replicated four times and the *in vitro* screening experiment was repeated.

2.4 Plant materials

Cotton (*Gossypium hirsutum*) variety "FM1944 GLB2" (Bayer CropScience, Raleigh, NC) known to be susceptible to *M. incognita* (Lawrence et al. 2015) was used for the greenhouse,

microplot, and field experiments.

2.5 Trials in the greenhouse

Seventy-two PGPR strains were selected from the *in vitro* screening for initial evaluation in the greenhouse for their efficacy to reduce nematode population density and promote cotton plant growth. Confidential agreements were signed during this research study and only nine *Bacillus* strains were available for further testing. These nine strains included one strain of *B. mojavensis* (Bmo3), two strains of *B. safensis* (Bsa25 and Bsa26), two strains of *B. subtilis* subsp. *subtilis* (Bsssu2 and Bsssu3), and four strains of *B. velezensis* (Bve2, Bve12, Bve37, and Bve40). All experiments were conducted at the Plant Science Research Center (PSRC) greenhouse located at Auburn University, Auburn, AL. Experiments were performed in 150 cm^3 plastic cone-tainers (Stuewe & Sons Inc., Tangent, Oregon) filled with a soil sand mix (60:40 v/v). The soil was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from PBU located at E.V. Smith Research Center of Auburn University, located near Tallassee, AL. Soil was steam pasteurized at 180 °C for 90 minutes, cooled for 24 hours, then the steam pasteurizing process was repeated prior to use. Two cotton seeds were planted 1.3 cm deep in each cone-tainer. One ml of bacterial cell suspension (1×10^7 CFU/ml) was added to each seed at planting. For the nematicide controls, cotton seeds were treated with each compound following agricultural industry recommendations: 0.424 mg ai/seed of Clothianidin plus *B. firmus* I-1582, or 0.15 mg ai/seed of Abamectin (Syngenta, Greensboro, NC), or 1 granula/seed of Aldicarb was applied at planting. All seeds for Clothianidin plus *B. firmus* I-1582 treatment were treated with a Gustafson table-top seed treater (Bayer CropScience, Research Triangle Park, NC), mixed for 3 min in the 454-gm stainless steel bucket and allow to air-dry before packaging (Schrimsher et al. 2014). One ml of tap water was added to the untreated control seeds. One ml of water containing 2,000 *M. incognita* eggs was pipetted into

each cone-tainer at planting. Experiments were arranged in a randomized complete block design (RCBD). Each treatment had five replications and the experiment was repeated. Cotton seedlings were thinned to one per cone-tainer after emergence. Plants were watered as needed. Supplemental light of 1000 watt halide bulbs producing 110,000 lumens was supplied to maintain day length of 14 hours per day. Greenhouse temperatures ranged from 21°C to 35 °C. Experiments were terminated at 45 days after planting (DAP). Plant and nematode measurements were recorded. Plant measurements included Plant height (PH), biomass (Bio) including shoot and root fresh weights (SFW+RFW). Nematode measurement were *Meloidogyne incognita* eggs per gram of root (Eggs/gr).

2.6 Trials in the microplots

Six PGPR strains and two mixtures of PGPR strains were evaluated for nematode population development, early plant growth promotion, and yield enhancement on cotton. The strains included *B. altitudinis* strain Bal13, *B. mojavensis* strain Bmo3, *B. subtilis* subsp. *subtilis* strains Bsssu2 and Bsssu3, and *B. velezensis* strains Bve2 and Bve2. Mixtures were formed from the best performing strains based on greenhouse studies. The two mixtures were Mixture 1 (Bve2 + Bal13) and Mixture 2 (seeds treated with Abamectin + Bve2 + Bal13). The experiments were conducted at the PSRC. Experiments were established in 26.5 liter pots filled with a Kalmia loamy soil collected from PBU where *M. incognita* and *H. glycine* had not been detected. Experiments were arranged in a RCBD with 6 replications for each treatment and the experiment was repeated. Five cotton seeds were hand-planted at a 1.3 cm depth in a linear pattern to simulate a linear row foot in the field (Schrimsher et al. 2014). One ml bacterial suspension (1×10^7 CFU/ml) was applied to each seed at planting. Five ml containing 50,000 *M. incognita* eggs as inoculum were pipetted into each pot at planting. Cotton seeds treated with Clothianidin plus *B. firmus* I-1582,

and Abamectin as previously described were used as standards. The untreated control included 1 ml of tap water per seed. Each microplot received 30 ml per minute of water by an automated drip irrigation system adjusted throughout the season to run for 15 - 45 minutes twice a day, for a total of 450 - 1350 ml of water per microplot per day. At 48 DAP, one representative cotton plant from each microplot was removed for PH and Bio measurements. The *M. incognita* eggs were extracted from the root system as previously described and enumerated. At plant maturity, 142 DAP, seed cotton was handpicked, and yield was recorded as grams of seed cotton per microplot.

2.7 Trials in the field

The same strains and mixtures assessed in the microplot trials were evaluated in field trials for their effect on early-season nematode population development, plant growth promotion, and yield enhancement in cotton. The experiments were established at PBU and at Prattville Agricultural Research Unit (PARU) in a Sandy clay loam soil (64% sand, 10% silt, and 26% clay), Prattville, AL. Both fields were naturally infested with *M. incognita* and numbers of J2 were just at the detection level of the extraction technique as previously described. The experiment was arranged in a RCBD with 5 replications for each treatment. The field plots were planted in two-row plots, 7 m long with 0.9 m row spacing. Blocks were separated by a 6 m alley. One hundred cotton seeds were planted in each row with an Almaco plot planter (Almaco, Iowa). The PGPR strains were standardized to 1×10^7 CFU/seed and applied as in-furrow sprays at 32.5 liter per hectare at planting. Two industry standards were used: seeds treated with Clothianidin plus *B. firmus* I-1582, or Abamectin as described previously. Tap water applied as an in-furrow spray was the untreated control at 32.5 L/ha. At 40 DAP, four random representative cotton plants were removed from each plot. The same plant growth parameters evaluated in the microplots were also evaluated in the field. *Meloidogyne incognita* population density was determined by extracting

eggs from four root systems per plot. Cotton was harvested mechanically with a cotton picker (Deere & Company, Moline, IL) at plant maturity which was near 150 DAP and seed cotton yield was recorded.

2.8 Statistical analysis

Data collected from *in vitro*, greenhouse, microplot, and field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Dependent variables included J2 mortality, plant height (PH), biomass (Bio), *M. incognita* eggs per gram of root (Eggs/gr), and yield. Fixed effects were PGPR strains or nematicides treatments and the random effects included replication, test repeat, and location. Student panels were generated to determine the normality of the residuals. The data of PH, Bio, or Eggs/gr required a log-normal distribution transformation to satisfy the normal assumptions. LS-means were compared between the treatments, chemical standards Clothianidin plus *B. firmus* I-1582, Abamectin, Aldicarb and the untreated control by Dunnett's method at significant level of $P \leq 0.05$ or $P \leq 0.10$. The LS-means are presented in the tables and adjusted *P* values are presented for statistical differences.

3. Results

3.1 Tests *in vitro*

The mortality percentage of *M. incognita* J2 ranged from 0.0% to 100% for the PGPR strains (669) with an average of 39% (Appendix 1). Data presented are results of 216 PGPR strains causing significant higher mortality percentage of *M. incognita* J2 than untreated control (Table 1). Of those 216 strains, 63 strains were *B. simplex*, 26 were *B. aryabhattai*, 24 strains were *B. toyonensis*, 14 were *B. cereus*, 13 were *B. safensis*, 13 were *B. mycoides*, 11 were *B. velezensis*, 11 were *B. altitudinis*, seven were *B. subtilis* subsp. *inaquosorum*, five were *B. weihenstephanensis*, five were *Paenibacillus amylolyticus*, four were *B. methylotrophicus*, four were *Brevibacterium*

epidermidis, two were *B. mojavensis*, two were *B. pumilus*, two were *B. subtilis* subsp. *subtilis*, and the remaining 18 strains were *Arthrobacter defluvii*, *B. psychrosaccharolyticus*, *B. tequilensis*, *B. thuringiensis*, *Brevibacterium iodinum*, *Fictibacillus solisalsi*, *Lysinibacillus macroides*, *Paenibacillus lautus*, *P. tundrae*, *P. xylanexedens*, *Solibacillus isronensis*, *Sporosarcina globispora*, and indistinguishable species of *Bacillus* spp. Among all PGPR strains, 19.1% produced a significantly greater level of mortality percentage than the biological standard Clothianidin plus *B. firmus* I-1582 ($P \leq 0.05$), and 34.5% resulted in statistically similar mortality percentage to Aldicarb ($P \leq 0.05$) (Table 1). Among all the strains, *Bacillus* spp., was the major genera initiating greater mortality percentage when compared with the other genera.

3.2 Trials in the greenhouse

In evaluations conducted in the greenhouse, nine *Bacillus* PGPR strains reduced nematode eggs/gr at 45 DAP at levels statistically equivalent to the standard Clothianidin plus *B. firmus* I-1582, which is the biological standard currently available to cotton producers. *Bacillus velezensis* strain Bve2 suppressed *M. incognita* eggs/gr at a level statistically equivalent to the Abamectin control ($P \leq 0.1$) (Table 2). None of the tested *Bacillus* strains reduced *M. incognita* eggs/gr similarly to the chemical standard Aldicarb. Strains *B. mojavensis* Bmo3, *B. safensis* Bsa25, *B. subtilis* subsp. *subtilis* Bsssu3, and *B. velezensis* Bve2 (Fig. 1) and Bve40 significantly increased plant biomass compared with the standard Clothianidin plus *B. firmus* I-1582 at 45 DAP ($P \leq 0.10$) (Table 2). Strain Bsssu3 (Fig. 2) significantly increased plant height compared to Aldicarb ($P \leq 0.10$) (Table 2).

3.3 Trials in microplots

In the microplot studies, *M. incognita* eggs/gr were reduced by *B. mojavensis* strain Bmo3, *B. subtilis* subsp. *subtilis* strain Bsssu3, *B. velezensis* strain Bve2, and Mixture 2 (Abamectin +

Bve2 + Bal13) at 48 DAP compared with the untreated control ($P \leq 0.10$) (Table 3). The *M. incognita* eggs/gr were statically similar to those recovered from Clothianidin plus *B. firmus* I-158 and the Abamectin standards ($P \leq 0.05$). At harvest, the *B. velezensis* strain Bve12 treatment resulted in the highest seed cotton yield followed by the Mixture 2 and *B. velezensis* strain Bve2. These yields were statistically similar to the Clothianidin plus *B. firmus* I-158 and the Abamectin standards (Table 3).

3.4 Trials in the field

The *B. mojavensis* strain Bmo3 and Mixture 2 (Abamectin + Bve2 + Bal13) significantly reduced *M. incognita* eggs/gr on cotton at 40 DAP compared with untreated control ($P \leq 0.10$) which was similar to Clothianidin plus *B. firmus* I-1582 and Abamectin standards ($P \leq 0.10$) (Table 4). The *B. velezensis* strains Bve2 (Fig. 4) and Bve12 (Fig. 3) significantly increased seed cotton yield compared with untreated control which was similar to Abamectin ($P \leq 0.10$) (Table 4).

4. Discussion

The results indicated that among all the PGPR strains, 33% caused significantly greater level of mortality of *M. incognita* J2 than the untreated control and 35% caused statistically similar mortality to the level caused by Aldicarb ($P \leq 0.05$). *Bacillus* spp. was the primary genera causing mortality of *M. incognita* J2 in the *in vitro* tests. Further greenhouse, microplot, and field trials confirmed that specific strains of the *Bacillus* PGPR suppressed the population density of *M. incognita* in the greenhouse, microplot, and field evaluation systems, and increased seed cotton yield.

In vitro screening of the PGPR strains indicated that *Bacillus* spp. caused greater mortality of *M. incognita* J2 *in vitro* than other genera. Some strains of specific *Bacillus* species were previously reported to have nematicidal activity against plant-parasitic nematodes on different host

plants. Kloepper et al. (1992) reported that *B. megaterium* strain 1758 and *B. pumilus* strain 163 significantly reduced galls of *M. incognita* and cysts of *H. glycines* on soybean. Payne (1993) stated in a Bt patent that some strains of *B. thuringiensis* had nematicidal activity against nematodes including plant-parasitic nematodes *M. incognita* and *Aphelenchus avenae*. Siddiqui et al. (2001) reported that a *B. subtilis* strain isolated from the rhizosphere of *Helianthus annuus* had nematicidal activity on *M. javanica* in mungbean. Burkett-Cadena et al. (2008) found that *B. subtilis* strain GB03 and *B. velezensis* strain GB99 (BioYield, Gustafson LLC, Plano TX, USA) induced significant reductions in *M. incognita* eggs/gr, juvenile nematodes per cm^3 of soil, and galls per plant on tomato. *Bacillus firmus*, the active ingredient of BioNem-WP (AgroGreen, Israel) was reported to control root-knot nematode on vegetables (Hallmann et al. 2009). In our study, we also found the specific strains of the species *B. pumilus*, *B. thuringiensis*, *B. subtilis*, *B. velezensis*, and *B. firmus* had nematicidal activity on *M. incognita* in our tests. In our trials, 17 different *Bacillus* species and subspecies including *B. altitudinis*, *B. aryabhattai*, *B. cereus*, *B. galliciensis*, *B. lentus*, *B. methylotrophicus*, *B. mojavensis*, *B. mycoides*, *B. psychrosaccharolyticus*, *B. safensis*, *B. siamensis*, *B. simplex*, *B. subtilis* subsp. *inaquosorum*, *B. subtilis* subsp. *subtilis*, *B. tequilensis*, *B. toyonensis*, *B. weihenstephanensis*, were found to have antagonistic activity against *M. incognita*. This is the first documentation of antagonistic activity by these *Bacillus* species to *M. incognita*.

The results from the greenhouse, microplot, and field experiments indicated that Bve2 (*B. velezensis*), Bmo3 (*B. mojavensis*), and Mixture 2 (Abamectin + Bve2 + Bal13) were relatively consistent in reduction of *M. incognita* eggs/gr, and *B. velezensis* strains Bve2 and Bve12 increased early plant growth and enhanced cotton yield. Many reports have shown that specific strains of PGPR or mixture of PGPR strains can promote plant growth, reduce plant disease, and enhance

yield with multiple hosts under greenhouse, microplot, or field conditions (Wei et al. 1996; Raupach and Kloepper 1998; Jetiyanon and Kloepper 2002; Yan et al. 2002; Castillo et al. 2013; Liu et al. 2016). Castillo et al. (2013) evaluated PGPR *B. firmus* GB-126 combined with *Paecilomyces lilacinus* 251 in commercial formulations in the greenhouse, microplot, and field trials for the management of *Rotylenchulus reniformis* in cotton and reported that *R. reniformis* population density was decreased when exposed to *B. firmus* and *P. lilacinus* in the greenhouse, in the microplot at mid-season, and in the field at harvest. Liu et al. (2016) found that specific PGPR strains Bve12 and Bve15 (*B. velezensis*), and Bmo3 (*B. mojavensis*), strain mixture-1 (Bve12 + Bmo3 + *Lysinibacillus macrolides* strain Lma1 + Bve15) and mixture-2 (mixture-1 + *B. safensis* strain Bsa27 + *B. pumilus* strain Bpu6 + *B. velezensis* strain Bve40) used in our studies also reduced black rot on Chinese cabbage caused by *Xanthomonas campestris* pv. *campestris* and increased marketable yield. Our studies provided additional information to their studies that PGPR strains and mixture of PGPR strains can promote early-season plant growth, increased yield, and reduce nematode numbers.

Mode of action of some PGPR strains have been studied. Mendoza et al. (2008) reported that mortality of sedentary and migratory endoparasitic nematodes *M. incognita, Radopholus similis*, and *Ditylenchus dipsaci* by *B. firmus* in *in vitro* test were closely associated with the production of bioactive secondary metabolites by the bacteria. Huang et al. (2010) demonstrated that PGPR strain *B. megaterium* YMF 3.25 significantly inhibited hatching of nematode eggs and reduced infection of *M. incognita* through production of nematicidal volatiles. They also confirmed that the nematicidal volatiles produced by the bacterium were mainly benzeneacetaldehyde, 2-nonanone, decanal, 2-undecanone, and dimethyl disulphide, which were active against juveniles and eggs at the concentration of 0.5 mmol, and that six other compounds

also contributed to the nematicidal efficacy (Huang et al. 2010). Peng et al. (2011) tested three *B. thuringiensis* nematicidal crystal proteins Cry6Aa, Cry5Ba, and Cry55Aa against *M. incognita* and found that the combination of Cry6Aa and Cry55Aa caused significant synergistic toxicity against *M. incognita*. These reports indicated that the mode of action of the *Bacillus* PGPR strains with nematicidal activity is likely related to the production of bioactive secondary metabolites. Further research is needed to address the mode of actions of the PGPR strains with nematicidal activity on *M. incognita*.

ISR elicited by *Bacillus* spp. against plant-parasitic nematodes is another important mode of action. Kloepper et al. (2004) summarized the published results and reported that specific strains of the species *B. velezensis*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* elicit significant reductions in the incidence or severity of various diseases including root-knot nematode. The bacterial strains *B. sphaericus* B43 and *Rhizobium etli* G12 were reported to induce systemic resistance (ISR) towards *M. incognita* on tomato as expressed in reduced juvenile penetration in the responder roots (Hauschild et al. 2000; Schäfer et al. 2006; Sikora et al. 2007). *Bacillus mojavensis* strain Bmo3 and *B. velezensis* strain Bve12 which were previously found to induce systemic resistance to black rot disease on Chinese cabbage and increased yield (Liu et al. 2016), were also found to reduce *M. incognita* population density and increase yield on cotton in our study. It is possible that the reduced *M. incognita* population density observed in our cotton trials could have resulted from induction of ISR by the PGPR strains Bmo3, Bve2, and Mixture 2 (Abamectin + Bve2 + Bal13), but further work is needed to test this.

In summary, *B. mojavensis* strain Bmo3, *B. velezensis* strains Bve2 and Bve12, and Mixture 2 (Abamectin +Bve2 +Bal13) are promising biological control agents which should be further evaluated for potential use against plant-parasitic nematodes. These biological strains could

potentially be alternatives to chemical nematicides or combined with chemical nematicides for the management of *M. incognita*. Future studies need to investigate biocontrol mechanisms of these strains on *M. incognita* in cotton.

5. Reference

Ambo, P. B. N., Ethiopia, E. A., Serfoji, P., Rajeshkumar, S., and Selvaraj, T. 2010. Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby. by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*. Journal of Agricultural Technology. 6:37 - 45.

Askary, T. H. 2015. Biocontrol agents of phytonematodes: limitation, research needs and future prospects. p. 450. In Biocontrol agents of phytonematodes. Eds. Askary, T. H., and Martinelli, P. R. P. CAB International. Wallingford.

Brown, S. M., Kepner, J. L., and Smart Jr, G. C. 1985. Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita*. Soil Biology and Biochemistry. 17:483 - 486.

Brown, S. M., and Smart Jr., G. C. 1985. Root penetration by *Meloidogyne incognita* juveniles infected with *Bacillus penetrans*. Journal of Nematology. 17:123 - 126.

Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K. S., Van Santen, E., and Kloepper, J. W. 2008. Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biological Control. 47:55 - 59.

Castillo, J. D., Lawrence, K. S., and Kloepper, J. W. 2013. Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 on cotton. Plant Disease. 97:967 - 976.

Charles, L., Carbonne, I., Davies, K. G., Bird, D., Burke, M., Kerry, B. R. and Opperman, C. H. 2005. Phylogenetic analysis of *Pasteuria penetrans* using multiple genetic loci. Journal of Bacteriology. 187:5700 - 5708.

Creech, R. G., Jenkins, J. N., Tang, B., Lawrence, G. W., and McCarty, J. C. 1995. Cotton resistance to root-knot nematode: I. Penetration and reproduction. Crop Science. 35:365 - 368.

Devidas, P., and Rehberger, L. A. 1992. The effects of exotoxin (thuringiensin) from *Bacillus thuringiensis* on *Meloidogyne incognita* and *Caenorhabditis elegans*. Plant and Soil. 145:115 - 120.

Hallmann, J., Davies, K. G., and Sikora, R. A. 2009. Biological control using microbial pathogens, endophytes and antagonists. p. 380 - 411. In Root-knot nematode. Eds. Perry, R. N., Moens, M., and Starr, J. L. CAB International. Wallingford.

Hauschild, R., Hallmann, J., and Sikora, R. A. 2000. *Fusarium oxysporum* and *Meloidogyne incognita* on tomato can be controlled by antagonistic rhizobacteria. Communications in Agricultural and Applied Biological Science. 65:527 - 528.

Huang, Y., Xu, C. K., Ma, L., Zhang, K. Q., Duan, C. Q., and Mo, M. H. 2010. Characterization of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. European Journal of Plant Pathology. 126:417 - 422.

Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter. 57:1025 - 1028.

Jenkins, W. 1964. A rapid centrifugal-floatition technique for separating nematodes from soil. Plant Disease Report. 48:692.

Jetiyanon, K., and Kloepper, J. W. 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biological Control. 24:285 - 291.

Kavitha, J., Jonathan, E. I., and Umamaheswari, R. 2007. Field application of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride* for the control of *Meloidogyne incognita* (Kofoid and White) Chitwood on sugarbeet. Journal of Biological Control. 21:211 - 215.

Keren-Zur, M., Antonov, J., Bercovitz, A., Feldman, K., Husid, A., Kenan, G., Markov, N., and Rebhun, M., 2000. *Bacillus firmus* formulations for the safe control of root-knot nematodes. In: Proceedings of the Brighton Crop Protection Conference on Pests and Diseases. Vol. 2A. 47 - 52.

Khan, M. R., and Akram, M. 2000. Effects of certain antagonistic fungi and rhizobacteria on wilt disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. Nematologia Mediterranea. 28:139 - 144.

Kiewnick, S., and Sikora, R. A. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control. 38:179 - 187.

Kloepper, J. W., Rodríguez-Kábana, R., McInroy, J. A., and Young, R. W. 1992. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: identification by fatty acid analysis and frequency of biological control activity. Plant and Soil. 139:75 - 84.

Kloepper, J. W., Ryu, C. M., and Zhang, S. A. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology. 94:1259 - 1266.

Krechel, A., Faupel, A., Hallmann, J., Ulrich, A., and Berg, G. 2002. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Canadian Journal of Microbiology. 48:772 - 786.

Lawrence, K. S., Lawrence, G. W., Faske, T., Overstreet, C., Wheeler, T., Young, H., Kemerait, B., and Mehl, H. 2016. Beltwide nematode research and education committee 2015 nematode research report cotton varietal and nematicide responses in nematode soils. 2016 Beltwide Cotton Conferences. Vol 1:113 - 115. National Cotton Council of America, New Orleans, LA.

Lawrence, K., Olsen, M., Faske, T., Hutmacher, R., Muller, J., Mario, J., Kemerait, R., Overstreet, C., Price, P., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R., Young, H., Woodward, J., and Mehl, H. 2015. Cotton disease loss estimate committee report. Proceedings of the 2014 Beltwide Cotton Conference. Vol 1: 188 - 190. National Cotton Council of America, Memphis, TN.

Liu, K., Garrett, C., Fadamiro, H., and Kloepper, J. W. 2016. Induction of systemic resistance in Chinese cabbage against black rot by plant growth-promoting rhizobacteria. Biological Control. 99:8 - 13.

Mendoza, A. R., Kiewnick, S., and Sikora, R. A. 2008. *In vitro* activity of *Bacillus firmus* against the burrowing nematode *Radopholus similis*, the root-knot nematode *Meloidogyne incognita* and the stem nematode *Ditylenchus dipsaci*. Biocontrol Science and Technology. 18:377 - 389.

Mohammed, S. H., El Saedy, M. A., Enan, M. R., Ibrahim, N. E., Ghareeb, A., and Moustafa, S. A. 2008. Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita*. Journal of Cell and Molecular Biology. 7:57 - 66.

Payne, J. M. 1993. Isolates of *Bacillus thuringiensis* that are active against nematodes. U. S. Pat. No. 5,151,363. Dec 14, 1993.

Peng, D. H., Chai, L. J., Wang, F. S., Zhang, F. J., Ruan, L. F., and Sun, M. 2011. Synergistic activity between *Bacillus thuringiensis* Cry6Aa and Cry55Aa toxins against *Meloidogyne incognita*. Microbial Biotechnology. 4:794 - 798.

Raupach, G. S., and Kloepper, J. W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology. 88:1158 - 1164.

Robinson, A. F. 2007. Reniform in US cotton: when, where, why, and some remedies 1. Annual Review Phytopathology. 45:263 - 288.

Sayre, R. M. 1980. Biocontrol: *Bacillus penetrans* and related parasites of nematodes. Journal of Nematology. 12:260.

Schäfer, K., Silva Fabry, C., and Sikora, R. A. 2006. Molecular investigations of rhizobacteria-induced systemic resistance towards the root-knot nematode *Meloidogyne incognita* in tomato. Multitrophic interactions in soil. *IOBC/WPRS* Bulletin. 29:135 - 140.

Schrimsher, D. W. 2013. The studies of plant host resistance to the reniform nematode in upland cotton and the effects of *Bacillus firmus* GB-126 on plant-parasitic nematode. Auburn University. M.S. Thesis. http://hdl.handle.net/10415/3562.

Schrimsher, D. W., Lawrence, K. S., Sikkens, R. B., and Weaver, D. B. 2014. Nematicides enhance growth and yield of *Rotylenchulus reniformis* resistant cotton genotypes. Journal of Nematology. 46: 365 - 375.

Serfoji, P., Rajeshkumar, S., and Selvaraj, T. 2010. Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby. by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*. Journal of Agricultural Technology. 6: 37 - 45.

Siddiqui, A. I., Ehetshamul-Haque, S., and Shahid Shaukat, S. 2001. Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. Journal of Phytopathology. 149:337-346.

Siddiqui, Z. A., and Husain, S. I., 1991. Studies on the biological control of root-knot nematode. Current Nematology. 2: 5 - 6.

Siddiqui, Z.A., and Mahmood, I., 1992. Biological control of root-rot disease complex of chickpea caused by *Meloidogyne incognita* race 3 and *Macrophomina phaseolina*. Nematology Mediterranean. 20: 199 - 202.

Siddiqui, Z. A., and Mahmood, I. 1999. Role of bacteria in the management of plant parasitic nematodes: a review. Bioresource Technology. 69: 167 - 179.

Sikora, R. A. 1988. Interrelationship between plant health promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Communications in Agricultural and Applied Biological Sciences. 53:867 - 878.

Sikora, R. A., Schäfer, K., and Dababat, A. A. 2007. Modes of action associated with microbially induced *in planta* suppression of plant-parasitic nematodes. Australasian Plant Pathology. 36: 124 - 134.

Stirling, G. R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology. 74: 55 - 60.

Stirling, G. R. 2014. Obligate parasites of nematodes: viruses and bacteria in the genus *Pasteuria*. p. 196 - 198. In Biological control of plant-parasitic nematodes. 2nd ed. Ed. Stirling, G. R. CAB International, Wallingford.

Terefe, M., Tefera, T., and Sakhuja, P. K. 2009. Effect of a formulation of *Bacillus firmus* on root-knot nematode *Meloidogyne incognita* infestation and the growth of tomato plants in the greenhouse and nursery. Journal of Invertebrate Pathology. 100:94 - 99.

Wei, G., Kloepper, J. W., and Tuzun, S. 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology. 86:221 - 224.

Wilson, M. J., and Jackson, T. A. 2013. Progress in the commercialization of bionematicides. BioControl. 58:715 - 722.

Xiang, N., Lawrence, K. S., Kloepper, J. W., and Mcinroy, J. A. 2014. *In vitro* screening of biological control agents on *Meloidogyne incognita*. Proceedings of the 2014 Beltwide Cotton Conference. Vol 1:258-260. National Cotton Council of America, Memphis, TN.

Xiang, N., and Lawrence, K. S. 2016. Optimization of *in vitro* techniques for distinguishing between live and dead second stage juveniles of *Heterodera glycines* and *Meloidogyne incognita*. Plos One. 11: e0154818.

Yan, Z. N., Reddy, M. S., Ryu, C. M., McInroy, J. A., Wilson, M., and Kloepper, J. W. 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology. 92:1329 - 1333.

Zuckerman, B. M., Dicklow, M. B., and Acosta, N. 1993. A strain of *Bacillus thuringiensis* for the control of plant-parasitic nematodes. Biocontrol Science and Technology. 3:41 - 46.

Table 1. Effect of 216 PGPR strains on *Meloidogyne incognita* J2 mortality percentage significantly higher than untreated control[a].

Code	Scientific name	*Meloidogyne incognita* J2 mortality (%)[b]	Dunnett's *P* vs[c] ($P \leq 0.05$)		
			Clothianidin + *B. firmus*	Aldicarb[d]	Water
Ad1	*Arthrobacter defluvii*	53.5	0.8886	0.0681	0.0150
Bal2	*Bacillus altitudinis*	71.1	0.0535	0.9365	<.0001
Bal3	*Bacillus altitudinis*	96.4	<.0001	1.0000	<.0001
Bal4	*Bacillus altitudinis*	97.4	<.0001	1.0000	<.0001
Bal5	*Bacillus altitudinis*	100.0	<.0001	1.0000	<.0001
Bal11	*Bacillus altitudinis*	59.7	0.9047	0.6812	0.0483
Bal12	*Bacillus altitudinis*	52.5	0.9375	0.0531	0.0201
Bal13	*Bacillus altitudinis*	75.9	0.1480	1.0000	0.0009
Bal14	*Bacillus altitudinis*	87.7	0.0003	1.0000	<.0001
Bal15	*Bacillus altitudinis*	94.5	<.0001	1.0000	<.0001
Bal16	*Bacillus altitudinis*	61.3	0.3699	0.3157	0.0013
Bal17	*Bacillus altitudinis*	84.4	0.0010	1.0000	<.0001
Bar6	*Bacillus aryabhattai*	78.2	0.0077	1.0000	<.0001
Bar7	*Bacillus aryabhattai*	75.1	0.0193	0.9992	<.0001
Bar8	*Bacillus aryabhattai*	88.1	0.0003	1.0000	<.0001
Bar9	*Bacillus aryabhattai*	79.4	0.0054	1.0000	<.0001
Bar14	*Bacillus aryabhattai*	63.3	0.2694	0.4263	0.0006
Bar15	*Bacillus aryabhattai*	64.6	0.2131	0.5124	0.0004
Bar16	*Bacillus aryabhattai*	67.8	0.4568	0.9930	0.0078
Bar17	*Bacillus aryabhattai*	60.3	0.4294	0.2671	0.0018
Bar19	*Bacillus aryabhattai*	87.6	0.0003	1.0000	<.0001
Bar20	*Bacillus aryabhattai*	86.8	0.0004	1.0000	<.0001
Bar21	*Bacillus aryabhattai*	90.8	<.0001	1.0000	<.0001
Bar22	*Bacillus aryabhattai*	88.3	0.0002	1.0000	<.0001
Bar24	*Bacillus aryabhattai*	55.8	0.7464	0.1116	0.0078
Bar25	*Bacillus aryabhattai*	54.1	0.8589	0.0768	0.0129
Bar27	*Bacillus aryabhattai*	100.0	<.0001	1.0000	<.0001
Bar28	*Bacillus aryabhattai*	95.4	<.0001	1.0000	<.0001
Bar29	*Bacillus aryabhattai*	96.7	<.0001	1.0000	<.0001
Bar31	*Bacillus aryabhattai*	68.5	0.0971	0.7917	<.0001
Bar32	*Bacillus aryabhattai*	62.8	0.2907	0.3990	0.0007
Bar33	*Bacillus aryabhattai*	83.5	0.0014	1.0000	<.0001
Bar41	*Bacillus aryabhattai*	97.6	0.0011	1.0000	<.0001
Bar46	*Bacillus aryabhattai*	84.2	0.0315	1.0000	<.0001
Bar47	*Bacillus aryabhattai*	57.8	0.5981	0.1692	0.0041
Bar49	*Bacillus aryabhattai*	66.7	0.1423	0.6615	0.0002
Bce4	*Bacillus cereus*	71.1	0.0538	0.9355	<.0001
Bce6	*Bacillus cereus*	73.7	0.0275	0.9940	<.0001
Bce7	*Bacillus cereus*	56.8	0.6688	0.1395	0.0056
Bce8	*Bacillus cereus*	94.3	<.0001	1.0000	<.0001
Bce14	*Bacillus cereus*	79.3	0.0056	1.0000	<.0001
Bce15	*Bacillus cereus*	64.6	0.2121	0.5141	0.0004
Bce37	*Bacillus cereus*	51.0	0.9798	0.0369	0.0298
Bce38	*Bacillus cereus*	61.7	0.3489	0.3354	0.0011
Bce41	*Bacillus cereus*	73.3	0.0312	0.9895	<.0001
Bce42	*Bacillus cereus*	94.2	<.0001	1.0000	<.0001
Bce44	*Bacillus cereus*	79.1	0.0059	1.0000	<.0001
Bce45	*Bacillus cereus*	70.9	0.0564	0.9272	<.0001
Bce46	*Bacillus cereus*	94.3	<.0001	1.0000	<.0001
Bce47	*Bacillus cereus*	50.3	0.9898	0.0310	0.0355
Bmt2	*Bacillus methylotrophicus*	76.7	0.0120	1.0000	<.0001
Bmt5	*Bacillus methylotrophicus*	90.7	<.0001	1.0000	<.0001
Bmt7	*Bacillus methylotrophicus*	82.3	0.0021	1.0000	<.0001
Bmt9	*Bacillus methylotrophicus*	68.1	0.1058	0.7640	<.0001
Bmo2	*Bacillus mojavensis*	49.9	0.9940	0.0275	0.0398
Bmo3	*Bacillus mojavensis*	70.6	0.3255	0.9998	0.0039
Bmo4	*Bacillus mojavensis*	66.8	0.1402	0.6670	0.0002
Bmy1	*Bacillus mycoides*	75.9	0.0154	0.9998	<.0001
Bmy16	*Bacillus mycoides*	55.3	0.7762	0.1019	0.0089
Bmy17	*Bacillus mycoides*	71.1	0.0531	0.9375	<.0001
Bmy18	*Bacillus mycoides*	85.4	0.0007	1.0000	<.0001
Bmy20	*Bacillus mycoides*	67.8	0.1122	0.7446	0.0001
Bmy25	*Bacillus mycoides*	54.4	0.8406	0.0822	0.0118
Bmy26	*Bacillus mycoides*	70.0	0.0693	0.8845	<.0001
Bmy30	*Bacillus mycoides*	94.9	<.0001	1.0000	<.0001

Bmy34	*Bacillus mycoides*	54.3	0.8453	0.0808	0.0121
Bmy36	*Bacillus mycoides*	58.9	0.5226	0.2073	0.0029
Bps4	*Bacillus psychrosaccharolyticus*	75.1	0.0193	0.9992	<.0001
Bpu5	*Bacillus pumilus*	79.1	0.0059	1.0000	<.0001
Bpu6	*Bacillus pumilus*	60.0	0.8922	0.6990	0.0454
Bsa1	*Bacillus safensis*	96.8	<.0001	1.0000	<.0001
Bsa4	*Bacillus safensis*	66.0	0.1643	0.6089	0.0002
Bsa6	*Bacillus safensis*	92.9	<.0001	1.0000	<.0001
Bsa7	*Bacillus safensis*	87.9	0.0003	1.0000	<.0001
Bsa8	*Bacillus safensis*	100.0	<.0001	1.0000	<.0001
Bsa9	*Bacillus safensis*	53.7	0.8804	0.0705	0.0144
Bsa12	*Bacillus safensis*	90.2	0.0001	1.0000	<.0001
Bsa26	*Bacillus safensis*	64.6	0.6376	0.9319	0.0167
Bsa28	*Bacillus safensis*	56.9	0.6651	0.1409	0.0055
Bsa31	*Bacillus safensis*	64.9	0.2007	0.5346	0.0003
Bsa34	*Bacillus safensis*	64.4	0.2220	0.4972	0.0004
Bsa35	*Bacillus safensis*	96.5	<.0001	1.0000	<.0001
Bsp2	*Bacillus simplex*	82.0	0.0023	1.0000	<.0001
Bsp13	*Bacillus simplex*	65.1	0.1942	0.5468	0.0003
Bsp24	*Bacillus simplex*	76.2	0.0139	0.9999	<.0001
Bsp32	*Bacillus simplex*	75.7	0.0161	0.9998	<.0001
Bsp33	*Bacillus simplex*	65.5	0.1801	0.5749	0.0003
Bsp35	*Bacillus simplex*	81.6	0.0027	1.0000	<.0001
Bsp36	*Bacillus simplex*	68.4	0.0986	0.7865	<.0001
Bsp42	*Bacillus simplex*	79.0	0.0061	1.0000	<.0001
Bsp44	*Bacillus simplex*	66.0	0.1643	0.6089	0.0002
Bsp45	*Bacillus simplex*	58.0	0.5856	0.1750	0.0039
Bsp46	*Bacillus simplex*	55.8	0.7429	0.1128	0.0077
Bsp47	*Bacillus simplex*	74.9	0.0199	0.9990	<.0001
Bsp48	*Bacillus simplex*	71.0	0.0172	0.7796	<.0001
Bsp50	*Bacillus simplex*	83.0	0.0017	1.0000	<.0001
Bsp51	*Bacillus simplex*	75.5	0.0170	0.9996	<.0001
Bsp52	*Bacillus simplex*	76.9	0.0116	1.0000	<.0001
Bsp53	*Bacillus simplex*	82.7	0.0426	1.0000	0.0001
Bsp54	*Bacillus simplex*	83.1	0.0016	1.0000	<.0001
Bsp55	*Bacillus simplex*	81.1	0.0031	1.0000	<.0001
Bsp56	*Bacillus simplex*	75.3	0.0179	0.9995	<.0001
Bsp57	*Bacillus simplex*	83.2	0.0016	1.0000	<.0001
Bsp58	*Bacillus simplex*	88.3	0.0002	1.0000	<.0001
Bsp59	*Bacillus simplex*	83.7	0.0013	1.0000	<.0001
Bsp60	*Bacillus simplex*	64.3	0.2240	0.4939	0.0004
Bsp61	*Bacillus simplex*	84.8	0.0009	1.0000	<.0001
Bsp62	*Bacillus simplex*	69.5	0.0773	0.8574	<.0001
Bsp63	*Bacillus simplex*	76.4	0.0132	1.0000	<.0001
Bsp64	*Bacillus simplex*	89.2	<.0001	1.0000	<.0001
Bsp66	*Bacillus simplex*	70.9	0.0568	0.9261	<.0001
Bsp69	*Bacillus simplex*	51.2	0.9764	0.0385	0.0285
Bsp79	*Bacillus simplex*	76.8	0.0117	1.0000	<.0001
Bsp81	*Bacillus simplex*	86.1	0.0006	1.0000	<.0001
Bsp82	*Bacillus simplex*	73.5	0.0292	0.9921	<.0001
Bsp84	*Bacillus simplex*	99.6	0.0006	1.0000	<.0001
Bsp87	*Bacillus simplex*	81.3	0.0568	1.0000	0.0002
Bsp88	*Bacillus simplex*	91.5	<.0001	1.0000	<.0001
Bsp89	*Bacillus simplex*	84.3	0.0010	1.0000	<.0001
Bsp91	*Bacillus simplex*	64.4	0.2220	0.4972	0.0004
Bsp92	*Bacillus simplex*	90.2	0.0001	1.0000	<.0001
Bsp93	*Bacillus simplex*	56.1	0.7196	0.1208	0.0070
Bsp94	*Bacillus simplex*	81.3	0.0029	1.0000	<.0001
Bsp96	*Bacillus simplex*	95.5	<.0001	1.0000	<.0001
Bsp101	*Bacillus simplex*	98.0	0.0010	1.0000	<.0001
Bsp114	*Bacillus simplex*	99.9	0.0006	1.0000	<.0001
Bsp115	*Bacillus simplex*	99.9	0.0006	1.0000	<.0001
Bsp116	*Bacillus simplex*	99.1	0.0007	1.0000	<.0001
Bsp118	*Bacillus simplex*	69.3	0.0818	0.8422	<.0001
Bsp123	*Bacillus simplex*	83.7	0.0350	1.0000	<.0001
Bsp124	*Bacillus simplex*	67.5	0.1195	0.7232	0.0001
Bsp126	*Bacillus simplex*	76.2	0.0142	0.9999	<.0001
Bsp131	*Bacillus simplex*	88.2	0.0003	1.0000	<.0001
Bsp134	*Bacillus simplex*	62.9	0.2847	0.4064	0.0007
Bsp135	*Bacillus simplex*	71.4	0.0497	0.9477	<.0001

Bsp143	*Bacillus simplex*	98.2	0.0009	1.0000	<.0001
Bsp154	*Bacillus simplex*	99.9	0.0006	1.0000	<.0001
Bsp187	*Bacillus simplex*	67.7	0.1146	0.7375	0.0001
Bsp195	*Bacillus simplex*	90.0	0.0001	1.0000	<.0001
Bsp197	*Bacillus simplex*	64.3	0.2240	0.4939	0.0004
Bsp198	*Bacillus simplex*	80.6	0.0037	1.0000	<.0001
Bsp199	*Bacillus simplex*	52.8	0.9251	0.0571	0.0185
Bsp200	*Bacillus simplex*	71.9	0.0437	0.9643	<.0001
Bssin8	*Bacillus subtilis* subsp. *inaquosorum*	88.4	0.0002	1.0000	<.0001
Bssin9	*Bacillus subtilis* subsp. *inaquosorum*	94.6	<.0001	1.0000	<.0001
Bssin10	*Bacillus subtilis* subsp. *inaquosorum*	94.6	<.0001	1.0000	<.0001
Bssin11	*Bacillus subtilis* subsp. *inaquosorum*	54.1	0.8544	0.0781	0.0126
Bssin12	*Bacillus subtilis* subsp. *inaquosorum*	94.3	<.0001	1.0000	<.0001
Bssin14	*Bacillus subtilis* subsp. *inaquosorum*	94.5	<.0001	1.0000	<.0001
Bssin15	*Bacillus subtilis* subsp. *inaquosorum*	90.6	<.0001	1.0000	<.0001
Bsssu2	*Bacillus subtilis* subsp. *subtilis*	84.4	0.0302	1.0000	<.0001
Bsssu3	*Bacillus subtilis* subsp. *subtilis*	82.4	0.0457	1.0000	0.0001
Bte2	*Bacillus tequilensis*	93.5	<.0001	1.0000	<.0001
Bth2	*Bacillus thuringiensis*	58.8	0.5312	0.2026	0.0030
Bto18	*Bacillus toyonensis*	87.5	0.0003	1.0000	<.0001
Bto21	*Bacillus toyonensis*	63.5	0.2603	0.4387	0.0006
Bto22	*Bacillus toyonensis*	82.9	0.0017	1.0000	<.0001
Bto23	*Bacillus toyonensis*	73.3	0.0310	0.9898	<.0001
Bto24	*Bacillus toyonensis*	76.8	0.0118	1.0000	<.0001
Bto34	*Bacillus toyonensis*	74.0	0.0258	0.9957	<.0001
Bto36	*Bacillus toyonensis*	93.1	<.0001	1.0000	<.0001
Bto40	*Bacillus toyonensis*	98.5	<.0001	1.0000	<.0001
Bto45	*Bacillus toyonensis*	82.1	0.0022	1.0000	<.0001
Bto46	*Bacillus toyonensis*	64.2	0.2271	0.4889	0.0004
Bto49	*Bacillus toyonensis*	66.2	0.1572	0.6252	0.0002
Bto51	*Bacillus toyonensis*	87.2	0.0004	1.0000	<.0001
Bto52	*Bacillus toyonensis*	89.4	0.0002	1.0000	<.0001
Bto53	*Bacillus toyonensis*	75.6	0.0167	0.9997	<.0001
Bto54	*Bacillus toyonensis*	81.3	0.0029	1.0000	<.0001
Bto55	*Bacillus toyonensis*	91.3	<.0001	1.0000	<.0001
Bto57	*Bacillus toyonensis*	87.0	0.0004	1.0000	<.0001
Bto58	*Bacillus toyonensis*	81.0	0.0032	1.0000	<.0001
Bto59	*Bacillus toyonensis*	68.3	0.1019	0.7762	<.0001
Bto61	*Bacillus toyonensis*	91.3	<.0001	1.0000	<.0001
Bto63	*Bacillus toyonensis*	84.5	0.0010	1.0000	<.0001
Bto64	*Bacillus toyonensis*	66.6	0.1445	0.6560	0.0002
Bto65	*Bacillus toyonensis*	86.6	0.0004	1.0000	<.0001
Bto66	*Bacillus toyonensis*	72.3	0.0398	0.9737	<.0001
Bve2	*Bacillus velezensis*	72.8	0.2386	1.0000	0.0021
Bve4	*Bacillus velezensis*	54.1	0.8559	0.0777	0.0127
Bve5	*Bacillus velezensis*	54.7	0.8216	0.0879	0.0108
Bve12	*Bacillus velezensis*	81.1	0.0591	1.0000	0.0002
Bve13	*Bacillus velezensis*	61.9	0.7951	0.8106	0.0303
Bve14	*Bacillus velezensis*	89.3	0.0099	1.0000	<.0001
Bve21	*Bacillus velezensis*	52.4	0.9423	0.0516	0.0208
Bve28	*Bacillus velezensis*	60.9	0.3960	0.2932	0.0015
Bve34	*Bacillus velezensis*	58.9	0.7050	0.3464	0.0108
Bve37	*Bacillus velezensis*	76.5	0.1352	1.0000	0.0007
Bve40	*Bacillus velezensis*	76.5	0.1341	1.0000	0.0007
Bwe2	*Bacillus weihenstephanensis*	83.6	0.0013	1.0000	<.0001
Bwe5	*Bacillus weihenstephanensis*	57.8	0.5999	0.1684	0.0042
Bwe10	*Bacillus weihenstephanensis*	94.3	<.0001	1.0000	<.0001
Bwe15	*Bacillus weihenstephanensis*	75.4	0.0174	0.9996	<.0001
Bwe16	*Bacillus weihenstephanensis*	81.8	0.0025	1.0000	<.0001
Brep1	*Brevibacterium epidermidis*	83.8	0.0013	1.0000	<.0001
Brep5	*Brevibacterium epidermidis*	54.9	0.8084	0.0919	0.0102
Brep6	*Brevibacterium epidermidis*	67.8	0.1122	0.7446	0.0001
Brep7	*Brevibacterium epidermidis*	52.2	0.9486	0.0494	0.0218
Brio1	*Brevibacterium iodinum*	87.8	0.0003	1.0000	<.0001
Fso1	*Fictibacillus solisalsi*	70.3	0.3385	0.9997	0.0042
Lma1	*Lysinibacillus macroides*	64.8	0.6287	0.9368	0.0161
Paam2	*Paenibacillus amylolyticus*	58.0	0.5838	0.1758	0.0039
Paam3	*Paenibacillus amylolyticus*	58.3	0.5608	0.1871	0.0035
Paam6	*Paenibacillus amylolyticus*	70.8	0.0574	0.9240	<.0001
Paam7	*Paenibacillus amylolyticus*	82.8	0.0018	1.0000	<.0001

Paam8	*Paenibacillus amylolyticus*	95.2	<.0001	1.0000	<.0001
Pala4	*Paenibacillus lautus*	70.4	0.0628	0.9065	<.0001
Patu1	*Paenibacillus tundrae*	79.6	0.0050	1.0000	<.0001
Paxy2	*Paenibacillus xylanexedens*	90.4	0.0001	1.0000	<.0001
Spg8	*Sporosarcina globispora*	84.4	0.0010	1.0000	<.0001
Uid4	*Bacillus aerophilus/stratosphericus*[e]	89.1	0.0002	1.0000	<.0001
Uid6	*Bacillus aerophilus/stratosphericus*[e]	88.2	0.0002	1.0000	<.0001
Uid7	*Bacillus aerophilus/stratosphericus*[e]	54.8	0.8134	0.0904	0.0104
Uid8	*Bacillus altitudinis/stratosphericus/aerophilus*[e]	98.9	<.0001	1.0000	<.0001
Uid9	*Bacillus altitudinis/stratosphericus/aerophilus*[e]	96.2	<.0001	1.0000	<.0001
Uid10	*Bacillus altitudinis/stratosphericus/aerophilus*[e]	96.8	<.0001	1.0000	<.0001
Control	**Active ingredient**[d]				
Poncho/Votivo	Clothianidin and *B. firmus* I-1582	24.4	…	<.0001	1.0000
Temik	Aldicarb	99.2	<.0001	…	<.0001
Untreated control	Sterile distilled water	2.0	1.0000	<.0001	…

[a]*In vitro* tests were performed in 96-well plates. Data of 216 PGPR strains indicating significant higher mortality on *Meloidogyne incognita* J2 than untreated control were presented in the table. All the PGPR strains had 4 replications and controls were based on 17 repeats. Data collected were analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.05$. *P* value less than 0.05 indicate a significant effect. LS-means and adjusted *P* values were presented in the table.

[b]Mortality percentage was determined by the following equation: [(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] ×100.

[c]Dunnett's option was used in the LS-means statement to assess the differences between bacterial strains and the Poncho/Votivo, Temik, and the untreated control.

[d]Active ingredients for Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Temik is Aldicarb, and untreated control is sterile distilled water.

[e]Indistinguishable species and unidentified strains.

Table 2. Efficacy of nine *Bacillus* PGPR strains on plant height, biomass, and *M. incognita* eggs/gr on cotton under greenhouse conditions at 45 DAP[a].

Treatment	Scientific name	45 DAP[b]					45 DAP					45 DAP			
			Dunnett's *P* vs. ($P \leq 0.10$)					Dunnett's *P* vs. ($P \leq 0.10$)				Dunnett's *P* vs. ($P \leq 0.10$)			
		PH[c]	Clothianidin + *B. firmus*	Abamectin	Aldicarb	Water	Bio[d]	Clothianidin + *B. firmus*	Abamectin	Aldicarb	Water	Eggs/gr[e]	Clothianidin + *B. firmus*	Abamectin	Aldicarb	Water
Bmo3	*B. mojavensis*	11.5	0.1391	0.0327	0.2625	0.9944	4.5	0.0854	0.9656	0.9929	0.9995	15791	0.8793	0.0178	<.0001	0.9316
Bsa25	*B. safensis*	9.6	0.0055	0.0009	1.0000	0.9506	6.3	0.0212	0.4940	0.6013	0.7049	14311	0.6254	0.0127	0.0001	1.0000
Bsa26	*B. safensis*	10.6	0.0165	0.0024	0.8205	1.0000	4.4	0.1320	0.9945	0.9996	1.0000	14789	0.6830	0.0087	<.0001	0.9978
Bsssu2	*B. subtilis* subsp. *subtilis*	11.4	0.1168	0.0262	0.3030	0.9982	3.4	0.6943	1.0000	0.9998	0.9903	14821	0.8849	0.0183	<.0001	0.9268
Bsssu3	*B. subtilis* subsp. *subtilis*	12.4	0.5813	0.2230	0.0413	0.5017	4.5	0.0523	0.8865	0.9573	0.9905	18163	0.8353	0.0145	<.0001	0.9605
Bve12	*B. velezensis*	11.5	0.1404	0.0331	0.2603	0.9940	4.2	0.1542	0.9979	0.9999	1.0000	15474	0.9701	0.0323	0.0002	0.7773
Bve37	*B. velezensis*	9.0	0.0010	0.0001	1.0000	0.6093	3.9	0.4968	1.0000	1.0000	1.0000	21514	0.9613	0.0545	0.0009	0.9733
Bve40	*B. velezensis*	10.8	0.0731	0.0185	0.8411	1.0000	5.8	0.0190	0.4675	0.5729	0.6718	27339	0.2840	0.0026	<.0001	1.0000
Bve2	*B. velezensis*	11.5	0.1252	0.0286	0.2865	0.9971	4.5	0.0737	0.9470	0.9864	0.9986	7825	1.0000	0.2167	0.0043	0.1225
Control	**Active ingredient**															
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	14.1	…	0.9996	0.0023	0.0405	2.3	…	0.6274	0.5187	0.3105	9702	…	0.3217	0.0181	0.3400
Avicta	Abamectin	14.7	0.9996	…	0.0004	0.0070	3.2	0.6274	…	1.0000	1.0000	1815	0.3517	…	0.8740	0.0013
Temik	Aldicarb	9.3	0.0023	0.0004	…	0.8122	4.0	0.5187	1.0000	…	1.0000	456	0.0181	0.8401	…	<.0001
Untreated control	Water	10.8	0.0341	0.0061	0.7270	…	3.8	0.2441	1.0000	1.0000	…	15254	0.2687	0.0011	<.0001	…

[a] Greenhouse trials were performed in plastic cone-tainers with mixed pasteurized soil and sand (60:40, v/v) for 45 days. Data collected were repeated twice and analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.10$. Adjusted *P* values less than 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing the data according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

[b] DAP = days after planting.

[c] PH = plant height (cm) at 45 DAP.

[d] Bio =cotton plant biomass including shoot fresh weight (g) and root fresh weight (g) at 45 DAP.

[e] Eggs/gr = *M. incognita* eggs per gram of root at 45 DAP.

Table 3. Effect of six PGPR strains and two mixtures on cotton plant height, biomass, *Meloidogyne incognita* eggs/gr at 48 DAP, and cotton yield in the microplots at 142 DAP[a].

Treatment	Scientific name	48 DAP[b]				48 DAP				48 DAP				142 DAP			
			Dunnett's *P* vs. ($P \leq 0.05$)				Dunnett's *P* vs. ($P \leq 0.05$)				Dunnett's *P* vs. ($P \leq 0.05$)				Dunnett's *P* vs. ($P \leq 0.05$)		
		PH[c]	Clothianidin + *B. firmus*	Abamectin	Water	Bio[d]	Clothianidin + *B. firmus*	Abamectin	Water	Eggs/gr[e]	Clothianidin + *B. firmus*	Abamectin	Water	Yield[f]	Clothianidin + *B. firmus*	Abamectin	Water
Bal13	*B. altitudinis*	46.5	1.0000	0.9932	0.9874	95.1	1.0000	1.0000	0.9965	872	0.9933	0.1524	0.5332	185	0.9994	1.0000	1.0000
Bmo3	*B. mojavensis*	51.2	0.9988	0.6473	0.5630	108.0	0.8148	0.8738	0.6335	212	0.9983	0.8894	0.0459	199	1.0000	0.9980	0.9975
Bsssu2	*B. subtilis* subsp. *subtilis*	38.7	0.6070	0.9974	1.0000	58.5	1.0000	0.9997	1.0000	409	1.0000	0.4690	0.1835	178	0.9949	1.0000	1.0000
Bsssu3	*B. subtilis subsp. subtilis*	42.5	0.9646	1.0000	1.0000	65.3	0.9978	0.9928	1.0000	299	0.9730	0.9779	0.0231	190	0.9999	0.9999	0.9999
Bve12	*B. velezensis*	43.0	0.9810	1.0000	1.0000	67.4	1.0000	0.9999	1.0000	1357	0.7459	0.0354	0.9340	231	0.9992	0.7555	0.7434
Bve2	*B. velezensis*	48.5	1.0000	0.9196	0.7918	85.4	0.9977	0.9995	0.9700	163	0.9928	0.9398	0.0340	215	1.0000	0.9494	0.9438
Mixture 1[g]		43.8	0.9950	1.0000	0.9996	72.5	0.9996	0.9981	1.0000	913	1.0000	0.3256	0.2837	197	1.0000	0.9991	0.9988
Mixture 2[g]		46.3	1.0000	0.9950	0.9687	65.9	1.0000	0.9999	1.0000	361	0.9884	0.9549	0.0301	229	0.9997	0.7915	0.7799
Control	**Active ingredient**																
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	47.8	…	0.9575	0.9003	66.9	…	1.0000	1.0000	436	…	0.5022	0.1667	208	…	0.9829	0.9802
Avicta	Abamectin	42.3	0.9575	…	1.0000	81.3	1.0000	…	1.0000	69	0.5022	…	0.0022	173	0.9829	…	1.0000
Untreated control	Water	42.2	0.9494	1.0000	…	93.3	1.0000	1.0000	…	1551	0.1667	0.0022	…	172	0.9802	1.0000	…

[a] Microplot trials were performed in 26.5 liter pots with a kalmia loamy sand soil. The microplot trial was repeated and analyzed in SAS 9.4 using PROC GLIMMIX procedure at a significant level of 0.05.
Adjusted *P* values less than 0.05 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

[b] DAP = days after planting.

[c] PH = plant height (cm) at 48 DAP.

[d] Bio = cotton plant biomass including shoot fresh weight (g) and root fresh weight (g) at 48 DAP.

[e] Eggs/gr = *M. incognita* eggs per gram of root at 48 DAP.

[f] Yield = grams of seed cotton yield handpicked at harvest.

[g] Mixture 1 = strain Bve2+ strain Bal13; Mixture 2 = Abamectin + strain Bve2 + strain Bal13.

Table 4. Efficacy of six PGPR strains and two mixtures on plant height, plant biomass, and nematode population density at 40 DAP, and yield of cotton in a field production system at 150 DAP[a].

Treatment	Scientific name	PH[c]	40 DAP [b] Dunnett's *P* vs. ($P \leq 0.05$)			Bio[d]	40 DAP Dunnett's *P* vs. ($P \leq 0.05$)			Eggs/gr[e]	40 DAP Dunnett's *P* vs. ($P \leq 0.05$)			Yield[f]	150 DAP Dunnett's *P* vs. ($P \leq 0.10$)		
			Clothianidin + *B. firmus*	Abamectin	Water		Clothianidin + *B. firmus*	Abamectin	Water		Clothianidin + *B. firmus*	Abamectin	Water		Clothianidin + *B. firmus*	Abamectin	Water
Bal13	*B. altitudinis*	19.9	0.8818	0.9129	0.9793	58.9	0.9973	0.9999	1.0000	1747	0.9961	0.9998	0.6523	3902	1.0000	0.5795	0.4826
Bmo3	*B. mojavensis*	25.4	0.9997	0.9992	0.9879	81.8	0.9997	0.9952	0.9853	349	0.1023	0.6717	0.0126	4235	0.9974	0.9823	0.1278
Bsssu2	*B. subtilis* subsp. *subtilis*	22.6	1.0000	1.0000	1.0000	60.3	0.9989	1.0000	1.0000	1358	0.8676	1.0000	0.3360	4089	1.0000	0.8462	0.2211
Bsssu3	*B. subtilis subsp. subtilis*	23.6	1.0000	1.0000	1.0000	67.0	1.0000	1.0000	1.0000	2569	0.9998	0.5922	1.0000	4204	0.9993	0.9622	0.1313
Bve12	*B. velezensis*	20.7	0.9681	0.9807	0.9983	50.0	0.9152	0.9749	0.9905	2805	0.9867	0.3755	1.0000	4396	0.9398	0.9999	0.0499
Bve2	*B. velezensis*	23.0	1.0000	1.0000	1.0000	65.5	1.0000	1.0000	1.0000	1416	0.8906	1.0000	0.3486	4415	0.9096	1.0000	0.0528
Mixture 1[g]		20.5	0.9507	0.9681	0.9960	51.1	0.9351	0.9833	0.9944	2191	1.0000	0.9172	0.9706	4091	1.0000	0.8702	0.2403
Mixture 2[g]		23.0	1.0000	1.0000	1.0000	77.9	1.0000	0.9997	0.9981	699	0.9364	0.9438	0.0454	3582	0.9337	0.1532	0.9356
Control	**Active ingredient**																
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	23.8	…	1.0000	1.0000	71.9	…	1.0000	1.0000	2232	…	0.8926	0.9807	3993	…	0.7291	0.3523
Avicta	Abamectin	23.5	1.0000	…	1.0000	67.8	1.0000	…	1.0000	1419	0.8926	…	0.3509	4563	0.7276	…	0.0199
Untreated control	Water	22.7	1.0000	1.0000	…	65.4	1.0000	1.0000	…	2889	0.9809	0.3521	…	3186	0.3503	0.0198	…

[a] Field trials were performed in two naturally infested fields in AL. Data were combined and analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.05$ for PH, Bio, and Eggs/gr and at a significant level of $\alpha \leq 0.10$ for cotton yield. Adjusted *P* values less than 0.05 or 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

[b] DAP = days after planting.

[c] PH = plant height (cm) at 40 DAP.

[d] Bio = cotton plant biomass including shoot fresh weight (g) + root fresh weight (g) at 40 DAP.

[e] Eggs/gr = *M. incognita* eggs per gram of root at 40 DAP.

[f] Cotton yield = seed cotton yield in kilogram/hectare at 150 DAP.

[g] Mixture 1= strain Bve2 + strain Bal13; Mixture 2 = Abamectin + strain Bve2 + strain Bal13.

Figure 1. Cotton roots from greenhouse trials at 45 DAP. Untreated control (Left) and treatment with strain *B. velezensis* Bve2

Figure 2. Cotton plant height was increased from greenhouse trials at 45 DAP. Untreated control (Left) and treatment with strain *B.* subtilis subsp. subtilis Bsssu3 (Right).

Figure 3. Cotton plants in Plant Breeding Unit (PBU) at 40 DAP. Untreated control (Left) and treatment with strain *B. velezensis* Bve12 (Right).

Figure 4. Cotton plants in PBU at 90 DAP. Untreated control (Left) and treatment with strain *B. velezensis* Bve2 (Right).

Chapter IV. Biological control of *Heterodera glycines* by spore-forming plant growth-promoting rhizobacteria on soybean

Abstract

Heterodera glycines, the soybean cyst nematode, is the most economically important plant-parasitic nematode on soybean production in the U.S. The objectives of this study were to evaluate the potential of plant growth-promoting rhizobacteria (PGPR) strains for mortality of *H. glycines* J2 *in vitro* and for reducing nematode population density on soybean in greenhouse, microplot, and field trials. The major group causing mortality to *H. glycines in vitro* was the genus *Bacillus* that consisted of 91.6% of the total 670 PGPR strains evaluated. The subsequent greenhouse, microplot, and field trials indicated that *B. velezensis* strain Bve2 consistently reduced *H. glycines* cyst population density at 60 DAP. *Bacillus mojavensis* strain Bmo3 suppressed *H. glycines* cyst and total *H. glycines* population density under greenhouse conditions. *Bacillus safensis* strain Bsa27 and Mixture 1 (Bve2 + Bal13) reduced *H. glycines* cyst population density at 60 DAP in the field trials. *Bacillus subtilis* subsp. *subtilis* strains Bsssu2 and Bsssu3, and *B. velezensis* strain Bve12 increased early soybean growth including plant height and plant biomass in the greenhouse trials. *Bacillus altitudinis* strain Bal13 increased early plant growth on soybean in the greenhouse and microplot trials. Mixture 2 (Abamectin + Bve2 + Bal13) increased early plant growth in the microplot trials at 60 DAP, and also enhanced soybean yield at harvest in the field trials. These results demonstrated that individual PGPR strains and mixtures can reduce *H. glycines* population density in the greenhouse, microplot, and field conditions, and increased yield on soybean.

1. Introduction

Heterodera glycines Ichinohe, the soybean cyst nematode, was first reported in the United States in North Carolina in 1954 (Winstead et al. 1955). Now *H. glycines* has been found in every soybean-producing state in the U.S. except New York and West Virginia, due to their small soybean acreage and limited soybean production (NASS 2016). In the United States, *H. glycines* was the most important disease in soybean production, followed by *Phytophthora* root and stem rot and seedling diseases over the past 10 years (Wrather and Koenning 2009). Soybean yield losses caused by *H. glycines* were estimated to be 25% to 38% of total yield losses in 28 U.S. states, which is more than any other disease from 2006 to 2009 (Wrather et al. 2010).

The removal of chemical nematicides such as Aldicarb (Temik) (Bayer CropScience, Raleigh, NC) has driven the investigation of alternative strategies for integrated pest management of plant-parasitic nematodes. Biological control agents previously assessed for the management of *H. glycines* were nematophagous fungi, endoparasitic fungi, female and egg-parasitic fungi, fungi producing antibiotic substances, vesicular-arbuscular mycorrhizal (VAM) fungi, *Pasteuria* spp., chitinolytic bacteria, and plant-growth-regulatory bacteria (Chen 2004). *Monacrosporium drechsleri*, an example of nematophagous fungi, has been found to attack J2 of *H. glycines* (Liu and Chen 2000). *Hirsutella rhossiliensis* and *H. minnesotensis* are two endoparasitic fungi found to parasitize vermiform stages of *H. glycines* (Liu and Chen 2000), and both were found highly effective against *H. glycines* through paratisizing J2 in the soil when applied at planting or two weeks prior to planting in the greenhouse (Chen and Liu 2005). The fungal genera *Exophiala*, *Fusarium*, *Gliocladium*, *Neocosmospora*, *Paecilomyces*, *Phoma*, *Stagonospora*, and *Pochonia* were commonly recovered from females and cysts of *H. glycines* (Chen 2004). Isolates from those fungi could be female and/or egg-parasitic fungi. Some fungi were found to produce antibiotic

substances which inhibit eggs hatch or juvenile mobility. For example, an isolate of the fungus *Chaetomium globosum*, was found to produce a low molecular weight compound, flavipin, which inhibited *in vitro* egg hatch and juvenile mobility of *Meloidogyne incognita* and hatch of *H. glycines* (Nitao et al. 2002). VAM fungi were also reported to decrease numbers of *H. glycines*. Tylka et al. (1991) found that numbers of *H. glycines* in roots and soil were decreased by VAM fungi by as much as 73% at the highest *H. glycines* inoculum level through 49 days after planting in the greenhouse experiments.

Bacteria are another large group that offered potential in reducing *H. glycines* population density. *Pasteuria* spp. was first reported to attack *H. elachista* in Japan in 1987 (Nishizawa 1987) and was later found to attack *H. glycines* in North America in 1994 (Noel and Stanger 1994). Four chitinolytic bacterial strains were found to reduce numbers of *H. glycines* through the interaction with the chitin substrate mixed in the soil in the greenhouse (Tian et al. 2000). Thirty-six of 201 rhizobacteria strains were also found to reduce numbers of soybean cysts, eggs, and J2 in the initial greenhouse tests (Tian and Riggs 2000). Among 20 strains that suppressed (≥ 50%) *H. glycines* in the initial greenhouse screening test, four were *Pseudomonas* spp., two *Bacillus* spp. (*B. cereus* and *B. pumilus*), three *Paenibacillus* spp., and one *Streptomyces* spp. (Tian and Riggs 2000). Plant-growth-regulatory bacteria, especially plant-growth promoting rhizobacteria (PGPR), were found to have potential for the control of *H. glycines*. Kloepper et al. (1992) found that *B. megaterium*, *B. pumilus*, and *Bacillus* spp. were antagonistic to *H. glycines* and *M. incognita*. Sharma (1995) evaluated the efficiency of toxins from pure cultures of *B. sphaericus* (Bs 2362), *B. thuringiensis var. israelensis* (Bti-H-14), and *B. thuringiensis var. kurstaki* (Btk-HD-1) against *H. glycines* in a greenhouse pot experiment. However, none of the toxins significantly reduced the final nematode population density in relation to the untreated control. Sharma and Gomes (1996) evaluated the

effect of those toxins again on oviposition and J2 hatching of *H. glycines* race 3 in the greenhouse and found the number of hatched J2 treated with Bs 2362 was significantly less than the control in one experiment.

Among these antagonists, rhizobacteria, especially *Bacillus* PGPR, can promote plant growth and elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts (Kloepper et al. 2004), and also elicit nematicidal activity or induced systemic resistance to plant-parasitic nematodes. Many of these species produce endospores which help the bacteria survive in a wide range of environmental conditions and have long-shelf life giving them an advantage as a commercial product. Some *Bacillus* strains have been developed into commercial products for plant disease and plant-parasitic nematode management, such as BioNem-WP/BioSafe (*B. firmus*) (AgroGreen, Israel) (Keren-Zur et al. 2000), BioYield (combination of *B. amyloliquefaciens* strain IN937a and *B. subtilis* strain GB03) (Gustafson LLC, USA) (Kloepper et al. 2004; Burkett-Cadena et al. 2008), Nemix (*Bacillus* spp.) (AgriLife/Chr Hansen, Brazil) (Hallmann et al. 2009), VOTiVO (*B. firmus* GB-126) (Bayer CropScience, Germany) (Wilson and Jackson 2013), and Pathway Consortia (mixture of *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. coagulans*, *Pseudomonas fluorescens*, *Streptomyces* spp., and *Trichoderma* spp.) (Pathway Holdings, USA) (Askary 2015).

More research on beneficial PGPR strains as biocontrol agents for plant-parasitic nematodes management is needed. The overall objective of this project was to evaluate PGPR strains for biological control potential of *H. glycines* on soybean. The specific objectives were to assess the potential of PGPR strains for *H. glycines* J2 mortality percentage *in vitro* using high throughput screening and select strains to further test for *H. glycines* population density reduction and enhanced plant growth in the greenhouse, microplot, and field production systems.

2. Materials and Methods

2.1 PGPR strains

A total of 670 PGPR strains (Appendix 2) were included in an *in vitro* study. These strains were originally isolated, identified, and maintained by J. W. Kloepper at Auburn University, Auburn, AL. Among these strains, 91.6% were *Bacillu*s spp. including 208 strains of *B. simplex*, 70 strains of *B. toyonensis*, 53 strains of *B. aryabhattai*, 51 strains of *B. cereus*, 44 strains of *B. mycoides*, 41 strains of *B. velezensis*, 35 strains of *B. safensis*, 21 strains of *B. altitudinis*, 21 strains of *B. weihenstephanensis*, 15 strains of *B. subtilis* subsp. *inaquosorum*, 13 strains of *B. methylotrophicus*, six strains of *B. pumilus*, five strains of *B. psychrosaccharolyticus*, four strains of each *B. mojavensis*, *B. subtilis* subsp. *subtilis*, and *B. thuringiensis*, three strains of *B. siamensis* and *B. tequilensis*, and 13 strains of other *Bacillus* spp. For the remaining 8.4% of the collection of the strains, ten were *Sporosarcina globispora*, nine were *Paenibacillus amylolyticus*, seven were *Brevibacterium epidermidis*, four were *Paenibacillus lautus*, three were unknown species, and 23 were from multiple other genera. The PGPR strains, stored in 30% glycerol at -80 ˚C, were transferred to tryptic soy agar (TSA) (VWR, Radnor, PA) plates, and incubated at 35˚C for 24 hours. The 21 strains that had no significant growth on TSA plates were eliminated from the study (Appendix 3). Vegetative cells of each strain were suspended in 5 ml of sterile distilled water in glass tubes. The concentration of bacterial vegetative cell suspensions was adjusted to 1×10^7 CFU/ml.

2.2 Nematode inoculum

The *H. glycines* used as inoculum *in vitro*, in the greenhouse and microplot experiments were from a culture maintained in the greenhouse since 2000. Eggs for the experiments were extracted from a 60-day-old soybean (“Asgrow 5935”, Monsanto, St. Louis, MO) stock culture

maintained in 500 cm^3 polystyrene pots. Soil was gently washed from the soybean roots and cysts and females were dislodged from the roots (Riggs and Schmitt 1991). Water with the cyst and female suspension was poured through nested 850-µm-pore and 250-µm-pore sieves to separate trash from cysts and females (Riggs and Schmitt 1991). Cysts and females were ground with a mortar and pestle to release the eggs. Eggs were washed with water and collected on a 25-µm-pore sieve and the suspension was centrifuged at 240 g for 1 minute using the sucrose centrifugation-flotation method (Jenkins 1964). For *in vitro* tests, *H. glycines* eggs were placed in a modified Baermann funnel (Castillo et al. 2013) on a Slide Warmer (Model 77) (Marshall Scientific, Brentwood, NH) and incubated at 31°C for 5 to 7 days to obtain the J2 (Xiang et al. 2014). The J2 were collected on a 25-µm-pore sieve, transferred to 1.5 ml micro centrifuge tubes, centrifuged at 5,000 g for 1 minute, rinsed with sterile distilled water, and centrifuged at 5,000 g for 1 minute. The J2 suspensions were adjusted to 30 to 40 J2 per 10 µl of water (Xiang et al. 2014). For greenhouse and microplot trials, eggs were enumerated at × 40 magnification with an inverted TS100 Nikon microscope and standardized to 2,000 eggs per cone-tainer for tests in the greenhouse or 50,000 eggs per pot for tests in the microplot.

2.3 Tests *in vitro*

In vitro tests were conducted to assess mortality percentage of *H. glycines* J2 by PGPR strains. The PGPR vegetative cell suspensions and *H. glycines* J2 inocula were prepared as described previously. Ten µl of nematode suspension containing 30 to 40 *H. glycines* J2 were added in each well of a 100 µl 96-well plate. Ninety µl of each PGPR vegetative cell suspension was transferred into each test well of the 96-well plate. Clothianidin plus *B. firmus* I-1582 (Poncho/Votivo) (Bayer CropScience, Raleigh, NC) at a 0.7 µl / well (0.424 mg ai/seed), 100 million international unit (MIU) /well of *Pasteuria nishizawae* (Clariva) (Syngenta Greensboro,

NC), and 1 granule/well of Aldicarb (Temik 15G) (Bayer CropScience, Raleigh, NC) were used as industry standards, and sterile distilled water was the untreated control. Each plate was sealed with parafilm (VWR, Radnor, PA) and incubated at room temperature for 48 hours. Numbers of live *H. glycines* J2 were enumerated and recorded at experiment initiation and 48 hours after exposure to the treatments. Viability of *H. glycines* J2 was determined using the sodium technique developed by Xiang and Lawrence (2016) for high throughput screening of biological or chemical agents on plant-parasitic nematodes. Mortality percentage of *H. glycines* J2 were calculated as the following equation: [(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] × 100. Each bacterial treatment had four replications and the experiment was repeated.

2.4 Plant material

The soybean (*Glycine max*) variety "Asgrow 5935" (Monsanto, St. Louis, MO) as reported by Monsanto to be susceptible to *H. glycines* was used for all the experiments.

2.5 Trials in the greenhouse

Seventy two PGPR strains from the *in vitro* screenings with high J2 mortality were selected for initial evaluation in the greenhouse for their efficacy to reduce nematode population density and promote soybean plant growth. Confidential agreements were signed during this research study and only ten PGPR strains were available for further testing. These included *B. altitudinis* strains Bal11 and Bal13, *B. mojavensis* strain Bmo3, *B. safensis* strains Bsa26 and Bsa27, *B. subtilis* subsp. *subtilis* strains Bsssu2 and Bsssu3, *B. velezensis* strains Bve2 and Bve12, and *Fictibacillus solisalsi* strain Fso1. All the tests were conducted in the Plant Science Research Center (PSRC) greenhouse at Auburn University, Auburn, AL. Experiments were performed in 150 cm^3 plastic cone-tainers (Stuewe & Sons Inc., Tangent, Oregon) filled with a soil : sand mix (60:40 v/v). The soil was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from

Plant Breeding Unit (PBU) located at E.V. Smith Research Center of Auburn University near Tallassee, AL. Soil was steam pasteurized at 180 °C for 60 minutes to 120 minutes and cooled for 24 hours. Steam pasteurizing process was repeated prior to use. Two soybean seeds were planted 2.5 cm deep in each cone-tainer. One ml of bacterial cell suspension (1×10^7 CFU/ml) was inoculated on each seed at planting. For the nematicide controls, soybean seeds were treated with each compound following industrial recommendations: 0.13 mg a.i./seed of Clothianidin plus *B. firmus* I-1582 (Poncho/Votivo), or 0.15 mg a.i./seed of Abamectin (Avicta) (Syngenta, Greensboro, NC), or 10,000 million international unit (MIU) /ml of *Pasteuria nishizawae* (Clariva) (Syngenta Greensboro, NC) prior to planting. All seeds were treated with a Gustafson table-top seed treater (Bayer CropScience, Research Triangle Park, NC), mixed for 3 min in the 454-gm stainless steel bucket and allow to air dry before packaging (Schrimsher et al. 2014). One ml of tap water added to the seeds was used as the untreated control. One ml containing 2,000 *H. glycines* eggs was pipetted into each cone-tainer at planting. Experiments were arranged in a randomized complete block design (RCBD). Each treatment had five replications and the entire experiment was repeated twice. Soybean seedlings were thinned to one per cone-tainer after emergence. Plants were watered as needed. Supplemental light of 1000 watts halide bulbs producing 110,000 lumens was supplied to maintain the day length of 14 hours per day. Greenhouse temperature was ranged from 21°C to 35 °C. Experiments were terminated at 60 DAP. Plant and nematode measurements were recorded. Plant measurements included Plant height (PH) and Biomass including shoot and root fresh weights (SFW/RFW). *Heterodera glycines* cyst and vermiform stage numbers were recorded. The *H. glycines* cysts were extracted from the soybean roots as described previously in inoculum preparation. Water suspension containing 150 cm^3 of soil from cone-tainers was poured through nested 75-µm and 25-µm-pore sieve to extract vermiform stages (juveniles and males).

Vermiform stages were collected on the 75-µm-pore sieve and centrifuged using sucrose centrifugation-flotation method (Jenkins 1964).

2.6 Trials in the microplots

The performance of five strains and two strain mixtures were evaluated for nematode population density, early growth promotion, and yield enhancement of soybean in the microplots. The strains included a strain of *B. altitudinis* (Bal13), a strain of *B. safensis* (Bsa27), a strain of *B. subtilis* subsp. *subtilis* (Bsssu2), two strains of *B. velezensis* (Bve12 and Bve2), and two mixtures Mixture 1 (Bve2 + Bal13) and Mixture 2 (seeds treated with Abamectin + Bve2 + Bal13). Mixtures were formed from the best performing strains based on greenhouse studies. The experiments were conducted at the PSRC. Experiments were established in 26.5 liter pots filled with a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from PBU. Nematodes were extracted from the non-pasteurized soil and *H. glycines* population density was below the detection level of the extraction method as previously described. Experiments were arranged in a RCBD with 6 replications for each treatment and the experiment was repeated twice. Ten soybean seeds were hand-planted at 2.5 cm in depth in a linear pattern to simulate a linear row foot in the field (Schrimsher et al. 2014). One ml bacterial suspension (1×10^7 CFU/ml) was applied to each seed at planting. Five ml containing 50,000 *H. glycines* eggs were pipetted randomly in each pot at planting. Soybean seeds treated with Clothianidin plus *B. firmus* I-1582, Abamectin, and *P. nishizawae* as previously described were used as standards. The untreated control received 1 ml of tap water per seed. Each microplot received 30 ml per minute of water by an automatic drip irrigation system adjusted throughout the season to run for 15 - 45 minutes twice a day, for a total of 450 - 1350 ml of water per microplot per day. At 60 DAP, one representative soybean plant was dug from each microplot for PH and Biomass (SFW + RFW) measurements and nematode

extraction as previously described. Cysts were extracted from the roots. Vermiform stages were extracted from 100 cm^3 of soil surrounding the roots. Total nematode numbers including cysts and vermiforms were recorded. At plant maturity, approximately 160 DAP, soybeans were harvested and yield was recorded as grams of soybean seed per plot.

2.7 Trials in the field

The same strains and mixtures assessed in the microplot trials were evaluated in field trials for their effect on early-season nematode population density, plant growth promotion, and yield enhancement in soybean. The experiments were established at the research stations of E.V. Smith in a Wickham fine sandy loam soil (70% sand, 16% silt, and 18% clay), Tallassee, AL and Tennessee Valley Research and Extension Center (TVREC) in a Decatur silt loam soil (24% sand, 49% silt, and 28% clay), Belle Mina, AL. Both were artificially infested fields with soybean cysts added every year since 2011. The experiments were arranged in a RCBD with 5 replications for each treatment. The field trials were arranged in two-row plots that were 7 m long with 0.9 m row spacing. Blocks were separated by a 6 m alley. One hundred and seventy five soybean seeds were planted in each row with an Almaco plot planter (Almaco, Iowa). The PGPR treatments were applied as in-furrow spray standardized to 1×10^7 CFU/seed and applied at 32.5 liter per hectare at planting. Seeds treated with Clothianidin plus *B. firmus* I-1582, Abamectin, and *P. nishizawae* as previously described were included as industry standard controls. Tap water applied in-furrow was used as untreated control. At 60 DAP, four random soybean plants were removed from each plot. The same plant growth parameters evaluated in the microplots were evaluated in the field. *Heterodera glycines* population density was determined by extracting soybean cysts and females from the roots, and vermiform stages from the soil as described previously. Soybeans were

harvested mechanically with a Almaco plot harvester (Almaco, Iowa) at plant maturity approximately 160 DAP and yield recorded and adjusted to 13% moisture content.

2.8 Statistical analysis

Data collected from *in vitro*, greenhouse, microplot, and field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Dependent variables included J2 mortality, plant height (PH), biomass (Bio), cyst, vermiform stage (VS), total SCN, and yield. Fixed effects were PGPR strains or nematicides treatments and the random effects included replication, repeat in time, and location. Student panels were generated to determine the normality of the residuals. A log-normal distribution transformation was required for the PH, Bio, cyst, VS, total SCN, and yield data to satisfy the normal assumptions. LS-means were compared between the treatments, chemical standards Clothianidin plus *B. firmus* I-1582, Abamectin, *P. nishizawae* and the untreated control by Dunnett's method at significant level of $P \leq 0.05$ or $P \leq 0.10$. The LS-means are presented in the tables with adjusted *P* values for statistical differences.

3. Results

3.1 Test *in vitro*

The mortality percentage of *H. glycines* J2 ranged from 0.0% to 99.9% with the PGPR strains tested with an average of 16.0% (Appendix 2). Data presented were results of LS-means greater than 50% mortality percentage of *H. glycines* J2 (Table 1). Among the 670 PGPR strains tested, 7.9% of the strains caused greater than 50.0% mortality percentage of *H. glycines* J2. Of those 7.9%, 24 were *B. simplex*, five were *B. altitudinis*, five were *B. toyonensis*, three were *B. aryabhattai*, three were *B. safensis*, two were *B. mycoides*, two were *B. subtilis subsp. subtilis*, and the remaining were *B. lentus*, *B. methylotrophicus*, *B. mojavensis*, *B. pumilus*, *B. weihenstephanensis*, *Fictibacillus solisalsi*, *Paenibacillus taichungensis*, and *P. xylanexedens*.

Among all the PGPR strains tested, 6.7% caused significantly greater level of mortality percentage than the biological standard Clothianidin plus *B. firmus* I-1582 ($P \leq 0.05$); 7.8% caused significantly greater level of mortality percentage than the level caused by *P. nishizawae* ($P \leq 0.05$); 5.5% caused statistically similar mortality percentage to the level caused by Aldicarb ($P \leq 0.05$); and 13.1% caused significantly greater mortality percentage than the level caused by untreated control ($P \leq 0.05$) (Table 1). Among all the strains, 91.6% were *Bacillus* spp. strains, which was the major genera with greater mortality percentage than any other single genera.

3.2 Greenhouse trial

In the greenhouse trials, strains *B. mojavensis* Bmo3 and *B. velezensis* Bve2 suppressed *H. glycines* cyst population density at 60 DAP at levels statistically equivalent to Abamectin ($P \leq 0.10$) (Table 2). Strains *B. mojavensis* Bmo3, *B. subtilis* subsp. *subtilis* Bsssu2, *B. velezensis* Bve2, and *Fictibacillus solisalsi* Fso1 suppressed total *H. glycines* including cysts and vermiform stages at 60 DAP at levels statistically equivalent to Abamectin ($P \leq 0.10$) (Table 2). All ten *Bacillus* PGPR strains significantly increased the soybean plant height compared to the standard Clothianidin plus *B. firmus* I-1582 at 60 DAP ($P \leq 0.05$) (Table 3). Strains *B. altitudinis* Bal13 (Fig. 1-2), *B. subtilis* subsp. *subtilis* Bsssu2 and Bsssu3, and *B. velezensis* Bve12 significantly increased plant biomass (SFW + RFW) compared to the standard Clothianidin plus *B. firmus* I-1582 at 60 DAP ($P \leq 0.05$) (Table 3).

3.3 Microplot trial

Five *Bacillus* PGPR strains and two mixtures were evaluated in the microplot for early plant growth promotion, reduction of *H. glycines* population density, and yield enhancement. Results indicated that the *B. velezensis* strain Bve2 significantly reduced *H. glycines* cyst numbers compared to the biological standard *P. nishizawae* at 60 DAP ($P \leq 0.10$) (Table 4). *Bacillus*

altitudinis strain Bal13 and Mixture 2 significantly increased plant height compared to all the industrial standards ($P \leq 0.10$) (Table 5). *Bacillus altitudinis* strain Bal13, *B. safensis* strain Bsa27, and Mixture 2 significantly increased plant biomass (SFW + RFW) compared to the untreated control at 60 DAP ($P \leq 0.10$) (Table 5). Number of *H. glycines* vermiform stage (data not show) at 60 DAP and soybean yield (Table 5) at harvest were similar among all the PGPR strains and the industrial standards.

3.4 Field trial

In the field trials, strains *B. safensis* Bsa27, *B. velezensis* Bve2, and Mixture 1 significantly reduced *H. glycines* cyst numbers compared to untreated control at 60 DAP ($P \leq 0.10$) (Table 6). Strain Mixture 2 (Fig. 3) significantly increased soybean yield compared to the untreated control at 160 DAP ($P \leq 0.10$) (Table 6). Plant height, biomass, *H. glycines* vermiform stages, and total *H. glycines* were similar among all the PGPR strains and industrial standards (data not show).

4. Discussion

In vitro screening of the 670 PGPR strains indicated that 13 *Bacillus* species including *B. altitudinis, B. aryabhattai, B. lentus, B. methylotrophicus, B. mojavensis, B. mycoides, B. pumilus, B. safensis, B. simplex, B. subtilis* subsp. *subtilis, B. toyonensis, B. velezensis, B. weihenstephanensis*, and species of *Fictibacillus* and *Paenibacillus* caused greater than 50% mortality of *H. glycines* J2 *in vitro*. Strains of *B. altitudinis, B. aryabhattai, B. lentus, B. methylotrophicus, B. mojavensis, B. mycoides, B. safensis, B. simplex, B. toyonensis, B. velezensis, B. weihenstephanensis*, and strains of *Fictibacillus* were first documented in this study for antagonistic activity against *H. glycines*. Previously, some bacterial species have been documented to be antagonistic to *H. glycines*. *Bacillus megaterium* (Kloepper et al. 1992), *B. pumilus* (Kloepper et al. 1992; Tian and Riggs 2000), *B. sphaericus* (Sharma 1995; Sharma and Gomes 1996), *B.*

cereus (Tian and Riggs 2000), *Paenibacillus* spp. (Tian and Riggs 2000) were reported for their nematicidal activity on reduction of *H. glycines* population density in greenhouse trials. None of these studies has done the high throughput *in vitro* screening of biological agents to *H. glycines*. Our study is the first documentation of high throughput *in vitro* screening of biological control agents on efficacy to *H. glycines*.

Bacillus velezensis strain Bve2 consistently reduced *H. glycines* cyst numbers at 60 DAP in the greenhouse, microplot, and field trials. *Bacillus mojavensis* strain Bmo3 suppressed *H. glycines* cyst and total *H. glycines* population density under greenhouse conditions. *Bacillus safensis* strain Bsa27 and Mixture 1 (Bve2 + Bal13) reduced *H. glycines* cyst numbers at 60 DAP in the field trials. Individual strains of Bmo3 and Bve2 and Mixture 2 (Abamectin + Bve2 + Bal13) were previously found to reduce *M. incognita* eggs/g root on cotton plants in the greenhouse, microplot, and field studies (Ni Xiang, data unpublished). This study expanded the documented nematicidal activity of the strains Bmo3 and Bve2 on *H. glycines*. Some studies have documented individual or mixtures of PGPR strains and/or nematicides or other agents on reduction of plant-parasitic nematode population density. Burkett-Cadena et al. (2008) reported that the combination of *B. amyloliquefaciens* (sym. *B. velezensis*) strain GB99 and *B. subtilis* strain GB03 (BioYield, Gustafson LLC, USA) significantly reduced *Meloidogyne* spp. eggs per gram root, juvenile nematodes per cm^3 of soil, and galls per plant on tomato. Castillo et al. (2013) found that individuals strains of *B. firmus* GB-126 (Votivo, Bayer CropScience, Germany) and *Paecilomyces lilacinus* 251 (PL 251, Biological Control Products, South African), or the combination of *B. firmus* GB-126 and *P. lilacinus* reduced *Rotylenchulus reniformis* population density in the greenhouse, microplot, and field trials. Our results are in agreement with their studies that

individual PGPR strains and mixtures have biological control potential on plant-parasitic nematodes.

Bacillus subtilis subsp. *subtilis* strains Bsssu2 and Bsssu3, and *B. velezensis* strain Bve12 increased early soybean growth including plant height and plant biomass in the greenhouse trials. *Bacillus altitudinis* strain Bal13 increased early plant growth on soybean in the greenhouse and microplot trials. Mixture 2 (Abamectin + Bve2 + Bal13) increased early plant growth in the microplot trials at 60 DAP, and also enhanced soybean yield at harvest in the field trials. Some studies have reported that individual or mixtures of PGPR strains can promote plant growth and increase yield on multiple plant hosts. Raupach and Kloepper (2000) found seven PGPR seed treatments including single-strain treatments and mixtures of *B. pumilus* strain INR7, *Curtobacterium flaccumfaciens* strain ME1, and *B. subtilis* strain GB03 significantly promoted plant growth on cucumber in the field studies when methyl bromide was absent. The individual *B. subtilis* strain GB03 and mixture of *B. pumilus* strain INR7 plus *C. flaccumfaciens* strain ME1 promoted growth significantly on cucumber (Raupach and Kloepper 2000). Liu et al. (2016) found individual PGPR strains Bsa27 (AP7) and Bpu6 (AP18) promoted plant growth on Chinese cabbage and one strain mixture containing PGPR strains Bve12 (AP136) (*B. velezensis*), Bmo3 (AP209) (*B. mojavensis*), Lma1 (AP282) (*Lysinibacillus macroides*), Bve15 (AP305) (*B. velezensis*), Bsa27 (AP7) (*B. safensis*), Bpu6 (AP18) (*B. pumilus*), and Bve40 (AP218) (*B. velezensis*) increased shoot and root dry weights in the greenhouse test. They found that those individual strains and mixtures increased marketable yield of Chinese cabbage in the field (Liu et al. 2016). Our study is in an agreement with previous research that individual or mixtures of PGPR strains can promote plant growth under greenhouse or field conditions and that some PGPR strains can reduce plant-parasitic nematode population density.

Overall, this study indicated that *B. velezensis* strain Bve2, *B. mojavensis* strain Bmo3, and Mixture 1 (Bve2 + Bal13) have the potential to manage *H. glycines* on soybean. *Bacillus altitudinis* strain Bal13 and Mixture 2 (Abamectin +Bve2 + Bal13) have the ability to enhance soybean yield under field conditions. In the future, the formulation of these effective PGPR strains and mixtures should be further evaluated for the integrated management of *H. glycines* on soybean.

5. References

Askary, T. H. 2015. Limitation, research needs, and future prospects. p. 446 - 454. In Biocontrol agents of phytonematodes. Eds. Askary, T. H., and Martinelli, P. R. P. CAB International. Wallingford, OX, UK.

Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K. S., Van Santen, E., and Kloepper, J. W. 2008. Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biological Control. 47:55 - 59.

Castillo, J. D., Lawrence, K. S., and Kloepper, J. W. 2013. Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 on cotton. Plant Disease. 97:967 - 976.

Chen, S. Y. 2004. Management with biological methods. p. 207 - 242. In Biology and management of soybean cyst nematode. 2nd Edition. Eds. Schmitt, D. P., Wrather, J. A., and Riggs, R. D. Schmitt & Associates of Marceline. Marceline, Missouri, USA.

Chen, S. Y., and Liu, X. Z. 2005. Control of the soybean cyst nematode by the fungi *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* in greenhouse studies. Biological Control. 32:208 - 219.

Hallmann, J., Davies, K. G., and Sikora, R. A. 2009. Biological control using microbial pathogens, endophytes and antagonists. p. 380 - 411. In Root-knot nematode. Eds. Perry, R. N., Moens, M., and Starr, J. L. CAB International. Wallingford, UK.

Jenkins, W. 1964. A rapid centrifugal-floatition technique for separating nematodes from soil. Plant Disease Report. 48: 692.

Keren-Zur, M., Antonov, J., Bercovitz, A., Feldman, K., Husid, A., Kenan, G., Markov, N., and Rebhun, M., 2000. *Bacillus firmus* formulations for the safe control of root-knot nematodes. In: Proceedings of the Brighton Crop Protection Conference on Pests and Diseases. Vol. 2A. 47 - 52.

Kloepper, J. W., Rodríguez-Kábana, R., McInroy, J. A., and Young, R. W. 1992. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: identification by fatty acid analysis and frequency of biological control activity. Plant and Soil. 139:75 - 84.

Kloepper, J. W., Ryu, C. M., and Zhang, S. A. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology. 94:1259 - 1266.

Liu, K., Garrett, C., Fadamiro, H., and Kloepper, J. W. 2016. Induction of systemic resistance in Chinese cabbage against black rot by plant growth-promoting rhizobacteria. Biological Control. 99:8 - 13.

Liu, X. Z., and Chen, S. Y. 2000. Parasitism of *Heterodera glycines* by *Hirsutella* spp. in Minnesota soybean fields. Biological Control.19:161 - 166.

NASS. National Agricultural Statistics Service | USDA. 2016. Crop Production 2015 Summary. 46. http://www.usda.gov/nass/PUBS/TODAYRPT/cropan16.pdf.

Nishizawa, T. 1987. A decline phenomenon in a population of upland rice cyst nematode, *Heterodera elachista*, caused by bacterial parasite, *Pasteuria penetrans*. Journal of Nematology.19:546.

Nitao, J. K., Meyer, S. L. F., Oliver, J. E., Schmidt, W. F., and Chitwood, D. J. 2002. Isolation of flavipin, a fungus compound antagonistic to plant-parasitic nematodes. Nematology. 4:55 - 63.

Noel, G. R., and Stanger, B. A. 1994. First report of *Pasteuria* sp. attacking *Heterodera glycines* in North America. Journal of Nematology. 26:612 - 615.

Raupach, G. S., and Kloepper, J. W. 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Disease. 84:1073 - 1075.

Riggs, R. D., and Schmitt, D. P. 1991. Optimization of the *Heterodera glycines* race test procedure. Journal of Nematology. 23:149 - 154.

Schrimsher, D. W., Lawrence, K. S., Sikkens, R. B., and Weaver, D. B. 2014. Nematicides enhance growth and yield of *Rotylenchulus reniformis* resistant cotton genotypes. Journal of Nematology. 46: 365 - 375.

Sharma, R. D. 1995. Efficiency of *Bacillus* spp. toxins to control *Heterodera glycines* on soybean. Nematologia Brasileira. 19:72 - 80.

Sharma, R. D., and Gomes, A. C. 1996. Effect of *Bacillus* spp. toxins on oviposition and juvenile hatching of *Heterodera glycines*. Nematologia Brasileira. 20:53 - 62.

Tian, H., and Riggs, R. D. 2000. Effects of rhizobacteria on soybean cyst nematode, *Heterodera glycines*. Journal of Nematology. 32:377 - 388.

Tian, H. L., Riggs, R. D., and Crippen, D. L. 2000. Control of soybean cyst nematode by chitinolytic bacteria with chitin substrate. Journal of Nematology. 32:370 - 376.

Tylka, G. L., Hussey, R. S., and Roncadori, R. W. 1991. Interactions of vesicular-arbuscular mycorrhizal fungi, phosphorus, and *Heterodera glycines* on soybean. Journal of Nematology. 23:122 - 133.

Wilson, M. J., and Jackson, T. A. 2013. Progress in the commercialization of bionematicides. BioControl. 58:715 - 722.

Winstead, N. N., Skotland, C. B., and Sasser, J. N. 1955. Soybean cyst nematode in North Carolina. Plant Disease Reporter. 39:9 - 11.

Wrather, A., Shannon, G., Balardin, R., Carregal, L., Escobar, R., Gupta, G. K., Ma, Z., Morel, W., Ploper, D., and Tenuta, A. 2010. Effect of diseases on soybean yield in the top eight producing countries in 2006. Plant Health Progress. Doi: 10.1094/PHP-2010-0125-01-RS.

Wrather, J. A., and Koenning, S. R. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. Plant Health Progress. Doi:10.1094/PHP-2009-0401-01-RS.

Xiang, N., and Lawrence, K. S. 2016. Optimization of *in vitro* techniques for distinguishing between live and dead second stage juveniles of *Heterodera glycines* and *Meloidogyne incognita*. Plos One. 11: e0154818.

Xiang, N., Lawrence, K. S., Kloepper, J. W., and Mcinroy, J. A. 2014. *In vitro* screening of biological control agents on *Meloidogyne incognita*. Proceedings of the 2014 Beltwide Cotton Conference. Vol 1:258-260. National Cotton Council of America, Memphis, TN.

Table 1. Effect of 53 PGPR strains on *Heterodera glycines* J2 LS-means were more than 50% mortality as compared to the industry standard biologicals Poncho/Votivo, Clariva, and chemical Temik as well as an untreated control[a].

Code	Scientific name	*Heterodera glycines* J2 mortality (%)[b]	Dunnett's *P* vs[d] ($P \leq 0.05$)			
			Clothianidin + *B. firmus*[c]	*P. nishizawae*	Aldicarb	Water
Bal9	*Bacillus altitudinis*	51.7	0.1099	0.0206	<.0001	<.0001
Bal11	*Bacillus altitudinis*	64.0	0.0236	0.0045	0.1725	<.0001
Bal12	*Bacillus altitudinis*	54.7	0.0408	0.0059	0.0002	<.0001
Bal13	*Bacillus altitudinis*	81.2	<.0001	<.0001	1.0000	<.0001
Bal20	*Bacillus altitudinis*	55.1	0.0353	0.0050	0.0003	<.0001
Bar15	*Bacillus aryabhattai*	90.5	<.0001	<.0001	1.0000	<.0001
Bar16	*Bacillus aryabhattai*	64.9	0.0180	0.0033	0.2079	<.0001
Bar21	*Bacillus aryabhattai*	57.5	0.0136	0.0016	0.0011	<.0001
Ble1	*Bacillus lentus*	74.2	<.0001	<.0001	0.4208	<.0001
Bmo3	*Bacillus mojavensis*	54.5	0.2720	0.0907	0.0117	0.0010
Bmt10	*Bacillus methylotrophicus*	51.4	0.4749	0.1896	0.0039	0.0033
Bmy19	*Bacillus mycoides*	66.9	0.0092	0.0015	0.3115	<.0001
Bmy32	*Bacillus mycoides*	77.7	0.0001	<.0001	0.9947	<.0001
Bpu6	*Bacillus pumilus*	78.4	<.0001	<.0001	0.9982	<.0001
Bsa25	*Bacillus safensis*	62.5	0.0378	0.0079	0.1200	<.0001
Bsa26	*Bacillus safensis*	74.1	0.0006	<.0001	0.8614	<.0001
Bsa27	*Bacillus safensis*	79.2	<.0001	<.0001	0.9997	<.0001
Bsp2	*Bacillus simplex*	60.2	0.0044	0.0004	0.0038	<.0001
Bsp3	*Bacillus simplex*	62.0	0.0437	0.0095	0.1061	<.0001
Bsp4	*Bacillus simplex*	93.9	<.0001	<.0001	1.0000	<.0001
Bsp8	*Bacillus simplex*	55.9	0.2035	0.0626	0.0186	0.0005
Bsp26	*Bacillus simplex*	64.5	0.0201	0.0038	0.1927	<.0001
Bsp53	*Bacillus simplex*	81.9	<.0001	<.0001	1.0000	<.0001
Bsp68	*Bacillus simplex*	87.1	<.0001	<.0001	1.0000	<.0001
Bsp90	*Bacillus simplex*	52.2	0.0340	0.0038	<.0001	<.0001
Bsp113	*Bacillus simplex*	63.3	0.0010	<.0001	0.0144	<.0001
Bsp123	*Bacillus simplex*	74.2	0.0005	<.0001	0.8715	<.0001
Bsp129	*Bacillus simplex*	99.9	<.0001	<.0001	1.0000	<.0001
Bsp130	*Bacillus simplex*	61.6	0.0490	0.0109	0.0960	<.0001
Bsp133	*Bacillus simplex*	73.7	0.0007	<.0001	0.8329	<.0001
Bsp139	*Bacillus simplex*	67.6	0.0072	0.0011	0.3548	<.0001
Bsp141	*Bacillus simplex*	99.9	<.0001	<.0001	1.0000	<.0001
Bsp146	*Bacillus simplex*	70.9	0.0021	0.0003	0.6075	<.0001
Bsp149	*Bacillus simplex*	64.7	0.0189	0.0035	0.2013	<.0001
Bsp153	*Bacillus simplex*	89.7	<.0001	<.0001	1.0000	<.0001
Bsp159	*Bacillus simplex*	56.8	0.1650	0.0480	0.0251	0.0004
Bsp165	*Bacillus simplex*	71.4	<.0001	<.0001	0.2188	<.0001
Bsp168	*Bacillus simplex*	69.1	0.0042	0.0006	0.4596	<.0001
Bsp171	*Bacillus simplex*	67.3	0.0079	0.0013	0.3390	<.0001
Bsp188	*Bacillus simplex*	73.0	0.0009	0.0001	0.7829	<.0001
Bsp196	*Bacillus simplex*	95.1	<.0001	<.0001	1.0000	<.0001
Bsssu2	*Bacillus subtilis* subsp. *subtilis*	74.8	0.0004	<.0001	0.9084	<.0001
Bsssu3	*Bacillus subtilis* subsp. *subtilis*	74.2	0.0005	<.0001	0.8715	<.0001
Bto10	*Bacillus toyonensis*	64.7	0.0005	<.0001	0.0250	<.0001
Bto11	*Bacillus toyonensis*	62.7	0.0013	0.0001	0.0114	<.0001
Bto22	*Bacillus toyonensis*	64.8	0.0004	<.0001	0.0265	<.0001
Bto23	*Bacillus toyonensis*	51.1	0.1304	0.0258	<.0001	<.0001
Bto51	*Bacillus toyonensis*	67.6	<.0001	<.0001	0.0718	<.0001
Bve2	*Bacillus velezensis*	54.7	0.2613	0.0861	0.0125	0.0009
Bwe6	*Bacillus weihenstephanensis*	93.3	<.0001	<.0001	1.0000	<.0001
Fso1	*Fictibacillus solisalsi*	59.6	0.0834	0.0206	0.0572	0.0001
Pata1	*Paenibacillus taichungensis*	64.4	0.0211	0.0040	0.1865	<.0001
Paxy1	*Paenibacillus xylanexedens*	74.8	<.0001	<.0001	0.4681	<.0001
Control	**Active ingredient**[c]					
Poncho/Votivo	Clothianidin and *B. firmus* I-1582	21.1	…	1.0000	<.0001	0.9885
Clariva	*Pasteuria nishizawae*	16.3	1.0000	…	<.0001	0.0000
Temik	Aldicarb	99.6	<.0001	<.0001	…	<.0001
Untreated control	Sterile distilled water	2.8	0.9885	1.0000	<.0001	…

[a]*In vitro* tests were performed in 96-well plates. Data collected were analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.05$. *P* value less than 0.05 indicate a significant effect. Adjusted *P* values were obtained according to Dunnett's method. The LS-means are presented in the tables with adjusted *P* values for statistical differences.

[b]Mortality was determined by calculating as the following equation:
[(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] × 100.

[c]Active ingredients for the nematicides Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Clariva is *Pasteuria nishizawae*, Temik is Aldicarb, and untreated control is sterile distilled water.

[d]Dunnett's option was used in the LSMEANS statement to assess the differences between bacterial strains and the Poncho/Votivo, Clariva, Temik, and the untreated control.

Table 2. Effect of ten PGPR strains on *Heterodera glycines* cyst numbers and total nematode population density in greenhouse trials at 60 DAP[a].

Treatment	Scientific Name	Cyst[b]	60 DAP Dunnett's *P* vs. (*P* ≤ 0.10)				Total *H. glycines*[d]	60 DAP Dunnett's *P* vs. (*P* ≤ 0.10)			
			Clothianidin + *B. firmus*[c]	*P. nishizawae*	Abamectin	Water		Clothianidin + *B. firmus*	*P. nishizawae*	Abamectin[d]	Water
Bal11	*B. altitudinis*	2458	0.9599	1.0000	0.0400	1.0000	2897	1.0000	1.0000	0.0876	1.0000
Bal13	*B. altitudinis*	2154	0.9860	1.0000	0.0556	1.0000	3817	0.9781	0.9939	0.0187	1.0000
Bmo3	*B. mojavensis*	1665	1.0000	0.9931	0.2698	0.9678	2319	1.0000	1.0000	0.2928	0.9900
Bsa26	*B. safensis*	2934	0.6536	0.9993	0.0092	1.0000	3781	0.9840	0.9960	0.0206	1.0000
Bsa27	*B. safensis*	2754	0.9449	1.0000	0.0353	1.0000	3132	1.0000	1.0000	0.0893	1.0000
Bsssu2	*B. subtilis* subsp. *subtilis*	2140	0.9940	1.0000	0.0759	1.0000	2474	1.0000	1.0000	0.1558	1.0000
Bsssu3	*B. subtilis* subsp. *subtilis*	2064	0.8248	1.0000	0.0184	1.0000	2780	0.9966	0.9995	0.0306	1.0000
Bve2	*B. velezensis*	1583	1.0000	0.9780	0.3331	0.9282	1822	0.9966	0.9859	0.5600	0.8386
Bve12	*B. velezensis*	3527	0.3012	0.9062	0.0018	0.9644	4197	0.7629	0.8500	0.0047	0.9865
Fso1	*Fictibacillus solisalsi*	1733	0.9991	1.0000	0.0944	1.0000	2326	1.0000	1.0000	0.1187	1.0000
Control	**Active ingredient**[c]										
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	1745	…	0.9832	0.3554	0.9424	2386	…	1.0000	0.1875	0.9999
Clariva	*Pasteuria nishizawae*	2245	0.9832	…	0.0594	1.0000	2562	1.0000	…	0.1446	1.0000
Avicta	Abamectin	1116	0.3715	0.0620	…	0.0352	1789	0.1963	0.1513	…	0.0562
Untreated control	Water	2304	0.9343	1.0000	0.0327	…	3274	0.9999	1.0000	0.0520	…

[a] Greenhouse trials were performed in plastic cone-tainers with mixed pasteurized soil and sand (60:40, v/v) for 45 days. Data collected were repeated twice and analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.10$. Adjusted *P* values less than 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. The LS-means and adjusted *P* values are presented in the tables.

[b]Cyst = cysts and white females at 60 DAP.

[c]Active ingredients for the nematicides Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Clariva is *Pasteuria nishizawae*, Avicta is Abamectin, and untreated control is water.

[d]Total *H. glycines* = total numbers of soybean cysts, white females, and juveniles at 60 DAP.

Table 3. Effect of ten PGPR strains on soybean plant height (PH) and plant biomass (Bio) in greenhouse trials at 60 DAP[a].

Treatment	Scientific Name	PH[b]	60 DAP Dunnett's *P* vs. ($P \leq 0.05$)				Bio[d]	60 DAP Dunnett's *P* vs. ($P \leq 0.05$)			
			Clothianidin + *B. firmus*[c]	***P. nishizawae***	**Abamectin**	**Water**		**Clothianidin + *B. firmus***	***P. nishizawae***	**Abamectin**	**Water**
Bal11	*B. altitudinis*	35.3	0.0164	1.0000	0.9971	1.0000	4.9	0.1865	0.9910	0.9971	0.9845
Bal13	*B. altitudinis*	35.1	0.0154	1.0000	0.9962	1.0000	5.4	0.0116	1.0000	1.0000	1.0000
Bmo3	*B. mojavensis*	40.8	0.0002	0.6444	0.3767	0.9827	4.6	0.1871	0.9909	0.9970	0.9842
Bsa26	*B. safensis*	38.9	0.0014	0.9352	0.6983	1.0000	4.8	0.0566	1.0000	1.0000	1.0000
Bsa27	*B. safensis*	35.4	0.0109	1.0000	0.9870	1.0000	4.8	0.0766	1.0000	1.0000	1.0000
Bsssu2	*B. subtilis* subsp. *subtilis*	41.4	0.0002	0.4976	0.2746	0.9227	5.4	0.0319	1.0000	1.0000	1.0000
Bsssu3	*B. subtilis* subsp. *subtilis*	34.9	0.0255	1.0000	0.9997	0.9999	5.2	0.0399	1.0000	1.0000	1.0000
Bve2	*B. velezensis*	34.7	0.0279	1.0000	0.9998	0.9998	5.3	0.0771	1.0000	1.0000	1.0000
Bve12	*B. velezensis*	37.9	0.0020	0.9654	0.7667	1.0000	6.1	0.0028	0.9972	0.9921	0.9986
Fso1	*Fictibacillus solisalsi*	35.0	0.0187	1.0000	0.9984	1.0000	4.3	0.1577	0.9963	0.9990	0.9930
Control	**Active ingredient[c]**										
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	27.1	…	0.0477	0.1414	0.0058	2.8	…	0.0319	0.0495	0.0227
Clariva	*Pasteuria nishizawae*	34.2	0.0477	…	1.0000	0.9990	5.0	0.0319	…	1.0000	1.0000
Avicta	Abamectin	33.7	0.1481	1.0000	…	0.9546	5.2	0.0517	1.0000	…	1.0000
Untreated control	Water	37.6	0.0056	0.9988	0.9385	…	5.3	0.022	1.0000	1.0000	…

[a] Greenhouse trials were performed in plastic cone-tainers with mixed pasteurized soil and sand (60:40, v/v) for 60 days. Data collected were repeated twice and analyzed in SAS 9.4 using Proc Glimmix procedure at significant level of 0.05. Adjusted *P* values less than 0.05 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. The LS-means are presented in the tables with adjusted *P* values for statistical differences.

[b] PH = plant height (cm) at 60 DAP.

[c] Active ingredients for the nematicides Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Clariva is *Pasteuria nishizawae*, Avicta is Abamectin, and untreated control is water.

[d] Bio = soybean plant biomass including shoot fresh weight (g) and root fresh weight (g) at 60 DAP.

Table 4. Effect of five PGPR strains and two mixtures of PGPR strains on *Heterodera glycines* population density on soybean in the microplot at 60 DAP [a].

Treatment	Scientific Name	Cyst[b]	60 DAP Dunnett's *P* vs. ($P \leq 0.10$)				Total *H. glycines*[d]	60 DAP Dunnett's *P* vs. ($P \leq 0.10$)			
			Clothianidin + *B. firmus*[c]	*P. nishizawae*	Abamectin	Water		Clothianidin + *B. firmus*	*P. nishizawae*	Abamectin	Water
Bal13	*B. altitudinis*	1123	0.0449	0.6546	0.0611	0.1065	1224	0.0791	0.8444	0.1114	0.2987
Bsa27	*B. safensis*	472	0.9998	0.3982	0.9986	0.9833	609	1.0000	0.7686	1.0000	0.9995
Bsssu2	*B. subtilis* subsp. *subtilis*	774	0.7977	1.0000	0.8814	0.9752	984	0.3261	1.0000	0.4340	0.8383
Bve12	*B. velezensis*	439	0.9899	0.1373	0.9678	0.8624	448	0.9960	0.1455	0.9793	0.7078
Bve2	*B. velezensis*	384	0.9042	0.0627	0.8277	0.6375	425	0.9875	0.1131	0.9543	0.6243
Mixture 1[e]		465	0.9996	0.3750	0.9977	0.9776	471	0.9998	0.3643	0.9980	0.9041
Mixture 2[e]		930	0.4621	0.9997	0.4589	0.6263	968	0.5817	1.0000	0.6898	0.9537
Control	**Active ingredient**[c]										
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	563	…	0.5400	1.0000	0.9999	584	…	0.4944	1.0000	0.9914
Clariva	*Pasteuria nishizawae*	832	0.5400	…	0.6467	0.7878	931	0.4944	…	0.6216	0.9537
Avicta	Abamectin	587	1.0000	0.6467	…	1.0000	620	1.0000	0.6216	…	0.9989
Untreated control	Water	632	0.9999	0.8361	1.0000	…	736	0.9914	0.9539	0.9989	…

[a] Microplot trials were performed in 26.5 liter pot. Data collected were repeated and analyzed in SAS 9.4 using Proc Glimmix procedure at significant level of $\alpha \leq 0.10$.
Adjusted *P* values less than 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method.
The LS-means are presented in the tables with adjusted *P* values for statistical differences.

[b] Cyst = cysts and white females from 100 cm^3 of soil at 60 DAP.

[c] Active ingredients for the nematicides Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Clariva is *Pasteuria nishizawae*, Avicta is Abamectin, and untreated control is water.

[d] Total *H. glycines* = total numbers of soybean cysts, white females, and vermiform stages per 100 cm^3 of soil at 60 DAP.

[e] Mixture 1 = strain Bve2 + strain Bal13; Mixture 2 = Abamectin + strain Bve2 + strain Bal13.

Table 5. Effect of five PGPR strains and two mixtures of PGPR strains on early plant growth at 60 DAP and yield on soybean at 160 DAP in the microplot[a].

Treatment	Scientific Name	PH[b]	60 DAP Dunnett's *P* vs. (*P* ≤ 0.10)				Bio[d]	60 DAP Dunnett's *P* vs. (*P* ≤ 0.10)				Yield[e]	160 DAP Dunnett's *P* vs. (*P* ≤ 0.10)			
			Clothianidin + *B. firmus*[c]	*P. nishizawae*	Abamectin	Water		Clothianidin + *B. firmus*	*P. nishizawae*	Abamectin	Water		Clothianidin + *B. firmus*	*P. nishizawae*	Abamectin	Water
Bal13	*B. altitudinis*	43.8	0.0476	0.0689	0.0938	0.0389	95.7	0.1523	0.1388	0.4995	0.0184	192.2	1.0000	0.9999	0.9748	0.9974
Bsa27	*B. safensis*	41.7	0.1396	0.1903	0.2450	0.1176	94.7	0.1308	0.1189	0.4508	0.0150	175.3	0.9998	1.0000	0.7707	1.0000
Bsssu2	*B. subtilis* subsp. *subtilis*	36.1	0.9462	0.9842	0.9965	0.9120	73.6	0.7390	0.7027	0.9986	0.1484	193.2	0.9996	0.9708	1.0000	0.8941
Bve12	*B. velezensis*	38.4	0.4498	0.5745	0.6872	0.3893	66.0	0.9773	0.9672	1.0000	0.3954	203.0	0.9997	0.9758	0.9999	0.9056
Bve2	*B. velezensis*	36.7	0.7664	0.8727	0.9388	0.7015	76.7	0.7185	0.6818	0.9979	0.1394	219.1	0.9782	0.8365	1.0000	0.6875
Mixture 1[f]		39.8	0.3309	0.4216	0.5098	0.2884	74.4	0.7053	0.6742	0.9898	0.1880	156.3	0.8853	0.9900	0.3279	0.9994
Mixture 2[f]		43.8	0.0478	0.0691	0.0940	0.0390	88.5	0.4328	0.4048	0.8835	0.0812	185.4	1.0000	0.9993	0.9925	0.9885
Control	**Active ingredient**[c]															
Poncho/Votivo	Clothianidin + *B. firmus* I-1582	33.3	…	1.0000	1.0000	1.0000	57.4	…	1.0000	0.9880	0.9435	181.6	…	1.0000	0.9718	0.9979
Clariva	*Pasteuria nishizawae*	33.6	1.0000	…	1.0000	1.0000	53.4	1.0000	…	0.9815	0.9585	178.9	1.0000	…	0.8163	1.0000
Avicta	Abamectin	34.2	1.0000	1.0000	…	0.9999	66.5	0.9880	0.9815	…	0.4460	204.6	0.9718	0.8163	…	0.6634
Untreated control	Water	32.9	1.0000	1.0000	0.9999	…	48.9	0.9435	0.9585	0.4460	…	160.8	0.9979	1.0000	0.6634	…

[a]Microplot trials were performed in 26.5 liter pot. Data collected were repeated and analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.10$. Adjusted *P* values less than 0.10 indicated a significant effect. Adjusted *P* values were analyzed according to Dunnett's method. The LS-means are presented in the tables with adjusted *P* values for statistical differences.

[b]PH = plant height (cm) at 60 DAP.

[c]Activie ingredients for the nematicides Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Clariva is *Pasteuria nishizawae*, Avicta is Abamectin, and untreated control is water.

[d]Bio = plant biomass including shoot fresh weight and root fresh weight (g) at 60 DAP.

[e]Yield = soybean yield (g) obtained at 160 DAP and adjusted to 13% moisture content per pot.

[f]Mixture 1 = strain Bve2 + strain Bal13; Mixture 2 = Abamectin + strain Bve2+ strain Bal13.

Table 6. Effects of five PGPR strains and two mixtures of PGPR strains on early soybean plant growth at 60 DAP and yield at 160 DAP in the field trials[a].

Treatment	Scientific Name	Bio[b]	60 DAP Dunnett's *P* vs. (*P* ≤ 0.10)				Cyst[d]	60 DAP Dunnett's *P* vs. (*P* ≤ 0.10)				Yield[e]	160 DAP Dunnett's *P* vs. (*P* ≤ 0.10)			
			Clothianidin +*B. firmus*[c]	*P. nishizawae*	Abamectin	Water		Clothianidin +*B. firmus*	*P. nishizawae*	Abamectin	Water		Clothianidin + *B. firmus*	*P. nishizawae*	Abamectin	Water
Bal13	*B. altitudinis*	70.1	1.0000	0.9993	1.0000	1.0000	136	0.9632	1.0000	0.9997	0.3704	4140.2	0.3705	0.9997	0.4113	0.9980
Bsa27	*B. safensis*	64.9	0.9970	1.0000	0.9572	1.0000	85	1.0000	0.9740	0.9972	0.0297	4273.3	0.9543	0.9998	0.9708	0.5994
Bsssu2	*B. subtilis* subsp. *subtilis*	66.8	0.9999	1.0000	0.9915	1.0000	163	0.3678	0.9160	0.7477	0.5509	4393.5	1.0000	0.7683	1.0000	0.1419
Bve12	*B. velezensis*	84.3	0.9783	0.6804	0.9992	0.8711	163	0.3678	0.9160	0.7477	0.5509	4373.8	1.0000	0.8552	1.0000	0.1886
Bve2	*B. velezensis*	78.2	0.9983	0.7980	1.0000	0.9553	118	0.9968	1.0000	1.0000	0.0448	4366.9	1.0000	0.8815	1.0000	0.2077
Mixture 1[f]		71.8	1.0000	1.0000	0.9979	1.0000	85	1.0000	0.9732	0.9971	0.0294	4296.1	0.9864	0.9975	0.9928	0.4842
Mixture 2[f]		77.5	1.0000	0.9700	1.0000	0.9987	169	0.5460	0.9500	0.8465	0.8607	4466.7	0.9999	0.4036	0.9996	0.0422
Control	**Active ingredient**[c]															
Poncho/Votivo	Clothianidin *B. firmus* I-1582	74.0	…	0.9966	1.0000	1.0000	95	…	0.9816	1.0000	0.0071	4405.6	…	0.7082	1.0000	0.1179
Clariva	*Pasteuria nishizawae*	64.3	0.9955	…	0.9373	1.0000	125	0.9816	…	1	0.0700	4208.9	0.7082	…	0.7547	0.8979
Avicta	Abamectin	75.1	1.0000	0.9477	…	0.9961	151	0.9993	1.0000	…	0.0330	4396.3	1.0000	0.7547	…	0.1360
Untreated control	Water	68.7	1.0000	1.0000	0.9961	…	222	0.0071	0.0700	0.033	…	4055.5	0.1179	0.8979	0.1360	…

[a]Field trials were performed in E.V Smith and Tennessee Valley Research and Extension Center in 2015. Data collected were repeated and analyzed in SAS 9.4 using Proc Glimmix procedure at significant level of 0.10. Adjusted *P* values less than 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. The LS-means are presented in the tables with adjusted *P* values to determine statistical differences.

[b]Bio = plant biomass including shoot fresh weight and root fresh weight (g) at 60 DAP.

[c]Activie ingredients for the nematicides Poncho/Votivo are Clothianidin plus B. firmus I-1582, Clariva is *Pasteuria nishizawae*, Avicta is Abamectin, and untreated control is water.

[d]Cyst = cysts and white females in 100 cm^3 of soil at 60 DAP.

[e]Yield = soybean yield (kg/ha) obtained at 160 DAP and adjusted to 13% moisture content.

[f]Mixture 1 = strain Bve2 + strain Bal13; Mixture 2 = Abamectin + strain Bve2 + strain Bal13.

Figure 1. Soybean plants treated with strain *B. altitudinis* Bal13 (Right) and untreated control (Left) at 60 DAP.

Figure 2. Soybean roots treated with strain *B. altitudinis* Bal13 (Right) and untreated control (Left) at 60 DAP.

Figure 3. Soybean treated with Mixture 2 (Right) and untreated control (Left) at 80 DAP.

Appendix 1. Effect of 669 PGPR strains on *Meloidogyne incognita* J2 mortality as compared to the commercial nematicides Clothianidin plus *B. firmus* I-1582 and Aldicarb as well as an untreated control[a].

Code	Scientific name	*Meloidogyne incognita* J2 mortality (%)[d]	Dunnett's *P* vs [c] ($P \leq 0.05$)		
			Clothianidin + *B. firmus*[b]	Aldicarb[b]	Water
Ad1	*Arthrobacter defluvii*	53.5	0.8886	0.0681	0.0150
Ae1	*Arthrobacter equi*	21.4	1.0000	<.0001	1.0000
Bal1	*Bacillus altitudinis*	10.3	1.0000	<.0001	1.0000
Bal2	*Bacillus altitudinis*	71.1	0.0535	0.9365	<.0001
Bal3	*Bacillus altitudinis*	96.4	<.0001	1.0000	<.0001
Bal4	*Bacillus altitudinis*	97.4	<.0001	1.0000	<.0001
Bal5	*Bacillus altitudinis*	100.0	<.0001	1.0000	<.0001
Bal6	*Bacillus altitudinis*	8.0	1.0000	<.0001	1.0000
Bal7	*Bacillus altitudinis*	33.3	1.0000	0.0001	0.7464
Bal8	*Bacillus altitudinis*	0.0	0.9987	<.0001	1.0000
Bal9	*Bacillus altitudinis*	2.2	1.0000	<.0001	1.0000
Bal10	*Bacillus altitudinis*	4.0	1.0000	<.0001	1.0000
Bal11	*Bacillus altitudinis*	59.7	0.9047	0.6812	0.0483
Bal12	*Bacillus altitudinis*	52.5	0.9375	0.0531	0.0201
Bal13	*Bacillus altitudinis*	75.9	0.1480	1.0000	0.0009
Bal14	*Bacillus altitudinis*	87.7	0.0003	1.0000	<.0001
Bal15	*Bacillus altitudinis*	94.5	<.0001	1.0000	<.0001
Bal16	*Bacillus altitudinis*	61.3	0.3699	0.3157	0.0013
Bal17	*Bacillus altitudinis*	84.4	0.0010	1.0000	<.0001
Bal18	*Bacillus altitudinis*	36.7	1.0000	0.0004	0.5056
Bal19	*Bacillus altitudinis*	48.2	0.9996	0.0174	0.0602
Bal20	*Bacillus altitudinis*	41.2	1.0000	0.0020	0.2547
Bal21	*Bacillus altitudinis*	16.8	1.0000	<.0001	1.0000
Bar1	*Bacillus aryabhattai*	6.5	1.0000	<.0001	1.0000
Bar2	*Bacillus aryabhattai*	15.9	1.0000	<.0001	1.0000
Bar3	*Bacillus aryabhattai*	56.3	1.0000	<.0001	1.0000
Bar4	*Bacillus aryabhattai*	25.9	1.0000	<.0001	0.9994
Bar5	*Bacillus aryabhattai*	14.1	1.0000	<.0001	1.0000
Bar6	*Bacillus aryabhattai*	78.2	0.0077	1.0000	<.0001
Bar7	*Bacillus aryabhattai*	75.1	0.0193	0.9992	<.0001
Bar8	*Bacillus aryabhattai*	88.1	0.0003	1.0000	<.0001
Bar9	*Bacillus aryabhattai*	79.4	0.0054	1.0000	<.0001
Bar10	*Bacillus aryabhattai*	45.6	1.0000	0.0082	0.1075
Bar11	*Bacillus aryabhattai*	33.3	1.0000	<.0001	0.5339
Bar12	*Bacillus aryabhattai*	15.9	1.0000	<.0001	1.0000
Bar13	*Bacillus aryabhattai*	37.0	1.0000	0.0005	0.4855
Bar14	*Bacillus aryabhattai*	63.3	0.2694	0.4263	0.0006
Bar15	*Bacillus aryabhattai*	64.6	0.2131	0.5124	0.0004
Bar16	*Bacillus aryabhattai*	67.8	0.4568	0.9930	0.0078
Bar17	*Bacillus aryabhattai*	60.3	0.4294	0.2671	0.0018
Bar18	*Bacillus aryabhattai*	40.1	1.0000	0.0014	0.3043
Bar19	*Bacillus aryabhattai*	87.6	0.0003	1.0000	<.0001
Bar20	*Bacillus aryabhattai*	86.8	0.0004	1.0000	<.0001
Bar21	*Bacillus aryabhattai*	90.8	<.0001	1.0000	<.0001
Bar22	*Bacillus aryabhattai*	88.3	0.0002	1.0000	<.0001
Bar23	*Bacillus aryabhattai*	33.3	1.0000	<.0001	0.5339
Bar24	*Bacillus aryabhattai*	55.8	0.7464	0.1116	0.0078
Bar25	*Bacillus aryabhattai*	54.1	0.8589	0.0768	0.0129
Bar26	*Bacillus aryabhattai*	12.9	1.0000	<.0001	1.0000
Bar27	*Bacillus aryabhattai*	100.0	<.0001	1.0000	<.0001
Bar28	*Bacillus aryabhattai*	95.4	<.0001	1.0000	<.0001
Bar29	*Bacillus aryabhattai*	96.7	<.0001	1.0000	<.0001
Bar30	*Bacillus aryabhattai*	45.6	1.0000	0.0082	0.1075
Bar31	*Bacillus aryabhattai*	68.5	0.0971	0.7917	<.0001
Bar32	*Bacillus aryabhattai*	62.8	0.2907	0.3990	0.0007
Bar33	*Bacillus aryabhattai*	83.5	0.0014	1.0000	<.0001
Bar34	*Bacillus aryabhattai*	28.9	1.0000	<.0001	0.9725
Bar35	*Bacillus aryabhattai*	27.5	1.0000	<.0001	0.9936
Bar36	*Bacillus aryabhattai*	17.8	1.0000	<.0001	1.0000
Bar37	*Bacillus aryabhattai*	5.3	1.0000	<.0001	1.0000
Bar38	*Bacillus aryabhattai*	11.1	1.0000	<.0001	1.0000
Bar39	*Bacillus aryabhattai*	7.2	1.0000	<.0001	1.0000
Bar40	*Bacillus aryabhattai*	4.1	1.0000	<.0001	1.0000
Bar41	*Bacillus aryabhattai*	97.6	0.0011	1.0000	<.0001
Bar42	*Bacillus aryabhattai*	1.7	1.0000	<.0001	1.0000
Bar43	*Bacillus aryabhattai*	4.8	1.0000	<.0001	1.0000
Bar44	*Bacillus aryabhattai*	3.5	1.0000	<.0001	1.0000
Bar45	*Bacillus aryabhattai*	34.5	1.0000	<.0001	0.4443
Bar46	*Bacillus aryabhattai*	84.2	0.0315	1.0000	<.0001
Bar47	*Bacillus aryabhattai*	57.8	0.5981	0.1692	0.0041
Bar48	*Bacillus aryabhattai*	20.6	1.0000	<.0001	1.0000
Bar49	*Bacillus aryabhattai*	66.7	0.1423	0.6615	0.0002
Bar50	*Bacillus aryabhattai*	1.1	0.9998	<.0001	1.0000
Bar51	*Bacillus aryabhattai*	54.4	0.9984	0.3906	0.1270
Bar52	*Bacillus aryabhattai*	24.8	1.0000	<.0001	1.0000

Bar53	*Bacillus aryabhattai*	7.1	1.0000	<.0001	1.0000
Bce1	*Bacillus cereus*	15.1	1.0000	<.0001	1.0000
Bce2	*Bacillus cereus*	14.1	1.0000	<.0001	1.0000
Bce3	*Bacillus cereus*	29.2	1.0000	<.0001	0.9670
Bce4	*Bacillus cereus*	71.1	0.0538	0.9355	<.0001
Bce5	*Bacillus cereus*	8.1	1.0000	<.0001	1.0000
Bce6	*Bacillus cereus*	73.7	0.0275	0.9940	<.0001
Bce7	*Bacillus cereus*	56.8	0.6688	0.1395	0.0056
Bce8	*Bacillus cereus*	94.3	<.0001	1.0000	<.0001
Bce9	*Bacillus cereus*	39.7	1.0000	0.0012	0.3235
Bce10	*Bacillus cereus*	13.4	1.0000	<.0001	1.0000
Bce11	*Bacillus cereus*	24.9	1.0000	<.0001	0.9999
Bce12	*Bacillus cereus*	13.9	1.0000	<.0001	1.0000
Bce13	*Bacillus cereus*	22.5	1.0000	<.0001	1.0000
Bce14	*Bacillus cereus*	79.3	0.0056	1.0000	<.0001
Bce15	*Bacillus cereus*	64.6	0.2121	0.5141	0.0004
Bce16	*Bacillus cereus*	31.3	1.0000	<.0001	0.8804
Bce17	*Bacillus cereus*	28.8	1.0000	<.0001	0.9753
Bce18	*Bacillus cereus*	16.7	1.0000	<.0001	1.0000
Bce19	*Bacillus cereus*	40.8	1.0000	0.0017	0.2729
Bce20	*Bacillus cereus*	40.7	1.0000	0.0017	0.2776
Bce21	*Bacillus cereus*	32.5	1.0000	<.0001	0.8051
Bce22	*Bacillus cereus*	27.4	1.0000	<.0001	0.9947
Bce23	*Bacillus cereus*	20.8	1.0000	<.0001	1.0000
Bce24	*Bacillus cereus*	33.3	1.0000	0.0001	0.7464
Bce25	*Bacillus cereus*	4.3	1.0000	<.0001	1.0000
Bce26	*Bacillus cereus*	22.4	1.0000	<.0001	1.0000
Bce27	*Bacillus cereus*	41.2	1.0000	0.0020	0.2547
Bce28	*Bacillus cereus*	16.1	1.0000	<.0001	1.0000
Bce29	*Bacillus cereus*	3.8	1.0000	<.0001	1.0000
Bce30	*Bacillus cereus*	18.2	1.0000	<.0001	1.0000
Bce31	*Bacillus cereus*	4.1	1.0000	<.0001	1.0000
Bce32	*Bacillus cereus*	5.1	1.0000	<.0001	1.0000
Bce33	*Bacillus cereus*	2.8	1.0000	<.0001	1.0000
Bce34	*Bacillus cereus*	4.9	1.0000	<.0001	1.0000
Bce35	*Bacillus cereus*	9.8	1.0000	<.0001	1.0000
Bce36	*Bacillus cereus*	47.8	0.9998	0.0157	0.0654
Bce37	*Bacillus cereus*	51.0	0.9798	0.0369	0.0298
Bce38	*Bacillus cereus*	61.7	0.3489	0.3354	0.0011
Bce39	*Bacillus cereus*	40.6	1.0000	0.0017	0.2788
Bce40	*Bacillus cereus*	14.7	1.0000	<.0001	1.0000
Bce41	*Bacillus cereus*	73.3	0.0312	0.9895	<.0001
Bce42	*Bacillus cereus*	94.2	<.0001	1.0000	<.0001
Bce43	*Bacillus cereus*	22.5	1.0000	<.0001	1.0000
Bce44	*Bacillus cereus*	79.1	0.0059	1.0000	<.0001
Bce45	*Bacillus cereus*	70.9	0.0564	0.9272	<.0001
Bce46	*Bacillus cereus*	94.3	<.0001	1.0000	<.0001
Bce47	*Bacillus cereus*	50.3	0.9898	0.0310	0.0355
Bce48	*Bacillus cereus*	9.7	1.0000	<.0001	1.0000
Bce49	*Bacillus cereus*	10.0	1.0000	<.0001	1.0000
Bce50	*Bacillus cereus*	29.1	1.0000	<.0001	0.9695
Bce51	*Bacillus cereus*	12.0	1.0000	<.0001	1.0000
Bfi1	*Bacillus firmus*	34.5	1.0000	0.0002	0.6633
Bga1	*Bacillus galliciensis*	11.4	1.0000	<.0001	1.0000
Ble1	*Bacillus lentus*	6.2	1.0000	<.0001	1.0000
Bmt1	*Bacillus methylotrophicus*	25.2	1.0000	<.0001	0.9999
Bmt2	*Bacillus methylotrophicus*	76.7	0.0120	1.0000	<.0001
Bmt3	*Bacillus methylotrophicus*	31.7	1.0000	<.0001	0.8574
Bmt4	*Bacillus methylotrophicus*	6.2	1.0000	<.0001	1.0000
Bmt5	*Bacillus methylotrophicus*	90.7	<.0001	1.0000	<.0001
Bmt6	*Bacillus methylotrophicus*	12.6	1.0000	<.0001	1.0000
Bmt7	*Bacillus methylotrophicus*	82.3	0.0021	1.0000	<.0001
Bmt8	*Bacillus methylotrophicus*	3.3	1.0000	<.0001	1.0000
Bmt9	*Bacillus methylotrophicus*	68.1	0.1058	0.7640	<.0001
Bmt10	*Bacillus methylotrophicus*	45.5	1.0000	0.1034	0.4502
Bmt11	*Bacillus methylotrophicus*	42.3	1.0000	0.0029	0.2092
Bmt12	*Bacillus methylotrophicus*	43.1	1.0000	0.0038	0.1775
Bmt13	*Bacillus methylotrophicus*	27.6	1.0000	<.0001	0.9930
Bmo1	*Bacillus mojavensis*	2.6	1.0000	<.0001	1.0000
Bmo2	*Bacillus mojavensis*	49.9	0.9940	0.0275	0.0398
Bmo3	*Bacillus mojavensis*	70.6	0.3255	0.9998	0.0039
Bmo4	*Bacillus mojavensis*	66.8	0.1402	0.6670	0.0002
Bmy1	*Bacillus mycoides*	75.9	0.0154	0.9998	<.0001
Bmy2	*Bacillus mycoides*	2.5	1.0000	<.0001	1.0000
Bmy3	*Bacillus mycoides*	1.0	0.9998	<.0001	1.0000
Bmy4	*Bacillus mycoides*	1.4	0.9999	<.0001	1.0000
Bmy5	*Bacillus mycoides*	1.8	1.0000	<.0001	1.0000
Bmy6	*Bacillus mycoides*	4.1	1.0000	<.0001	1.0000
Bmy7	*Bacillus mycoides*	39.8	1.0000	0.0341	0.7798
Bmy8	*Bacillus mycoides*	0.0	0.9987	<.0001	1.0000

Bmy9	*Bacillus mycoides*	16.8	1.0000	<.0001	1.0000
Bmy10	*Bacillus mycoides*	21.8	1.0000	<.0001	1.0000
Bmy11	*Bacillus mycoides*	18.8	1.0000	<.0001	1.0000
Bmy12	*Bacillus mycoides*	56.1	0.9899	0.4771	0.0945
Bmy13	*Bacillus mycoides*	13.4	1.0000	<.0001	1.0000
Bmy14	*Bacillus mycoides*	25.7	1.0000	<.0001	0.9996
Bmy15	*Bacillus mycoides*	46.0	1.0000	0.1118	0.4272
Bmy16	*Bacillus mycoides*	55.3	0.7762	0.1019	0.0089
Bmy17	*Bacillus mycoides*	71.1	0.0531	0.9375	<.0001
Bmy18	*Bacillus mycoides*	85.4	0.0007	1.0000	<.0001
Bmy19	*Bacillus mycoides*	37.1	1.0000	0.0190	0.9116
Bmy20	*Bacillus mycoides*	67.8	0.1122	0.7446	0.0001
Bmy21	*Bacillus mycoides*	48.7	0.9990	0.0198	0.0538
Bmy22	*Bacillus mycoides*	36.7	1.0000	0.0173	0.9264
Bmy23	*Bacillus mycoides*	32.0	1.0000	0.0056	0.9985
Bmy24	*Bacillus mycoides*	37.9	1.0000	0.0006	0.4263
Bmy25	*Bacillus mycoides*	54.4	0.8406	0.0822	0.0118
Bmy26	*Bacillus mycoides*	70.0	0.0693	0.8845	<.0001
Bmy27	*Bacillus mycoides*	3.3	1.0000	<.0001	1.0000
Bmy28	*Bacillus mycoides*	4.2	1.0000	<.0001	1.0000
Bmy29	*Bacillus mycoides*	4.5	1.0000	<.0001	1.0000
Bmy30	*Bacillus mycoides*	94.9	<.0001	1.0000	<.0001
Bmy31	*Bacillus mycoides*	50.7	1.0000	0.2363	0.2292
Bmy32	*Bacillus mycoides*	52.5	0.9999	0.3039	0.1744
Bmy33	*Bacillus mycoides*	0.0	0.9987	<.0001	1.0000
Bmy34	*Bacillus mycoides*	54.3	0.8453	0.0808	0.0121
Bmy35	*Bacillus mycoides*	25.6	1.0000	<.0001	0.9997
Bmy36	*Bacillus mycoides*	58.9	0.5226	0.2073	0.0029
Bmy37	*Bacillus mycoides*	47.1	1.0000	0.0129	0.0768
Bmy38	*Bacillus mycoides*	18.5	1.0000	<.0001	1.0000
Bmy39	*Bacillus mycoides*	33.1	1.0000	<.0001	0.7640
Bmy40	*Bacillus mycoides*	18.8	1.0000	<.0001	1.0000
Bmy41	*Bacillus mycoides*	8.3	1.0000	<.0001	1.0000
Bmy42	*Bacillus mycoides*	44.8	1.0000	0.0905	0.4901
Bmy43	*Bacillus mycoides*	32.9	1.0000	<.0001	0.7762
Bmy44	*Bacillus mycoides*	2.5	1.0000	<.0001	1.0000
Bps1	*Bacillus psychrosaccharolyticus*	26.8	1.0000	<.0001	0.9976
Bps2	*Bacillus psychrosaccharolyticus*	15.1	1.0000	<.0001	1.0000
Bps3	*Bacillus psychrosaccharolyticus*	12.1	1.0000	<.0001	1.0000
Bps4	*Bacillus psychrosaccharolyticus*	75.1	0.0193	0.9992	<.0001
Bps5	*Bacillus psychrosaccharolyticus*	39.2	1.0000	0.0010	0.3544
Bpu1	*Bacillus pumilus*	14.7	1.0000	<.0001	1.0000
Bpu2	*Bacillus pumilus*	20.4	1.0000	<.0001	1.0000
Bpu3	*Bacillus pumilus*	43.7	1.0000	0.0045	0.1603
Bpu4	*Bacillus pumilus*	25.3	1.0000	<.0001	0.9999
Bpu5	*Bacillus pumilus*	79.1	0.0059	1.0000	<.0001
Bpu6	*Bacillus pumilus*	60.0	0.8922	0.6990	0.0454
Bsa1	*Bacillus safensis*	96.8	<.0001	1.0000	<.0001
Bsa2	*Bacillus safensis*	34.5	1.0000	0.0002	0.6651
Bsa3	*Bacillus safensis*	45.5	1.0000	0.0079	0.1110
Bsa4	*Bacillus safensis*	66.0	0.1643	0.6089	0.0002
Bsa5	*Bacillus safensis*	40.0	1.0000	0.0013	0.3081
Bsa6	*Bacillus safensis*	92.9	<.0001	1.0000	<.0001
Bsa7	*Bacillus safensis*	87.9	0.0003	1.0000	<.0001
Bsa8	*Bacillus safensis*	100.0	<.0001	1.0000	<.0001
Bsa9	*Bacillus safensis*	53.7	0.8804	0.0705	0.0144
Bsa10	*Bacillus safensis*	3.5	1.0000	<.0001	1.0000
Bsa11	*Bacillus safensis*	12.9	1.0000	<.0001	1.0000
Bsa12	*Bacillus safensis*	90.2	0.0001	1.0000	<.0001
Bsa13	*Bacillus safensis*	8.1	1.0000	<.0001	1.0000
Bsa14	*Bacillus safensis*	2.8	1.0000	<.0001	1.0000
Bsa15	*Bacillus safensis*	5.4	1.0000	<.0001	1.0000
Bsa16	*Bacillus safensis*	2.6	1.0000	<.0001	1.0000
Bsa17	*Bacillus safensis*	21.1	1.0000	<.0001	1.0000
Bsa18	*Bacillus safensis*	5.1	1.0000	<.0001	1.0000
Bsa19	*Bacillus safensis*	14.8	1.0000	<.0001	1.0000
Bsa20	*Bacillus safensis*	11.7	1.0000	<.0001	1.0000
Bsa21	*Bacillus safensis*	9.9	1.0000	<.0001	1.0000
Bsa22	*Bacillus safensis*	19.2	1.0000	<.0001	1.0000
Bsa23	*Bacillus safensis*	43.7	1.0000	0.0046	0.1588
Bsa24	*Bacillus safensis*	40.1	1.0000	0.0014	0.3056
Bsa25	*Bacillus safensis*	52.6	0.9999	0.3080	0.1717
Bsa26	*Bacillus safensis*	64.6	0.6376	0.9319	0.0167
Bsa27	*Bacillus safensis*	46.7	1.0000	0.1271	0.3902
Bsa28	*Bacillus safensis*	56.9	0.6651	0.1409	0.0055
Bsa29	*Bacillus safensis*	24.1	1.0000	<.0001	1.0000
Bsa30	*Bacillus safensis*	30.2	1.0000	<.0001	0.9335
Bsa31	*Bacillus safensis*	64.9	0.2007	0.5346	0.0003
Bsa32	*Bacillus safensis*	48.7	0.9989	0.0202	0.0528
Bsa33	*Bacillus safensis*	26.1	1.0000	<.0001	0.9992

Bsa34	*Bacillus safensis*	64.4	0.2220	0.4972	0.0004
Bsa35	*Bacillus safensis*	96.5	<.0001	1.0000	<.0001
Bsi1	*Bacillus siamensis*	18.2	0.9880	0.4879	0.0912
Bsi2	*Bacillus siamensis*	40.0	1.0000	0.0013	0.3094
Bsi3	*Bacillus siamensis*	4.0	1.0000	<.0001	1.0000
Bsp1	*Bacillus simplex*	18.3	1.0000	<.0001	1.0000
Bsp2	*Bacillus simplex*	82.0	0.0023	1.0000	<.0001
Bsp3	*Bacillus simplex*	47.3	1.0000	0.1405	0.3621
Bsp4	*Bacillus simplex*	37.6	1.0000	0.0215	0.8889
Bsp5	*Bacillus simplex*	21.2	1.0000	<.0001	1.0000
Bsp6	*Bacillus simplex*	13.4	1.0000	<.0001	1.0000
Bsp7	*Bacillus simplex*	29.1	1.0000	<.0001	0.9695
Bsp8	*Bacillus simplex*	44.8	1.0000	0.0913	0.4874
Bsp9	*Bacillus simplex*	25.5	1.0000	<.0001	0.9997
Bsp10	*Bacillus simplex*	1.4	0.9999	<.0001	1.0000
Bsp11	*Bacillus simplex*	1.7	1.0000	<.0001	1.0000
Bsp12	*Bacillus simplex*	32.7	1.0000	0.0066	0.9966
Bsp13	*Bacillus simplex*	65.1	0.1942	0.5468	0.0003
Bsp14	*Bacillus simplex*	22.2	1.0000	<.0001	1.0000
Bsp15	*Bacillus simplex*	19.8	1.0000	<.0001	1.0000
Bsp16	*Bacillus simplex*	19.3	1.0000	<.0001	1.0000
Bsp17	*Bacillus simplex*	25.3	1.0000	<.0001	0.9998
Bsp18	*Bacillus simplex*	10.7	1.0000	<.0001	1.0000
Bsp19	*Bacillus simplex*	38.8	1.0000	0.0009	0.3770
Bsp20	*Bacillus simplex*	33.8	1.0000	0.0001	0.7142
Bsp21	*Bacillus simplex*	38.5	1.0000	0.0008	0.3930
Bsp22	*Bacillus simplex*	34.3	1.0000	0.0002	0.6797
Bsp23	*Bacillus simplex*	30.2	1.0000	<.0001	0.9335
Bsp24	*Bacillus simplex*	76.2	0.0139	0.9999	<.0001
Bsp25	*Bacillus simplex*	0.0	0.9961	0.4276	0.1117
Bsp26	*Bacillus simplex*	55.2	0.9987	<.0001	1.0000
Bsp27	*Bacillus simplex*	3.3	1.0000	<.0001	1.0000
Bsp28	*Bacillus simplex*	22.7	1.0000	<.0001	1.0000
Bsp29	*Bacillus simplex*	33.1	1.0000	0.0001	0.7605
Bsp30	*Bacillus simplex*	27.0	1.0000	<.0001	0.9966
Bsp31	*Bacillus simplex*	5.7	1.0000	<.0001	1.0000
Bsp32	*Bacillus simplex*	75.7	0.0161	0.9998	<.0001
Bsp33	*Bacillus simplex*	65.5	0.1801	0.5749	0.0003
Bsp34	*Bacillus simplex*	37.0	1.0000	0.0004	0.4872
Bsp35	*Bacillus simplex*	81.6	0.0027	1.0000	<.0001
Bsp36	*Bacillus simplex*	68.4	0.0986	0.7865	<.0001
Bsp37	*Bacillus simplex*	17.0	1.0000	<.0001	1.0000
Bsp38	*Bacillus simplex*	29.8	1.0000	<.0001	0.9460
Bsp39	*Bacillus simplex*	16.3	1.0000	<.0001	1.0000
Bsp40	*Bacillus simplex*	11.6	1.0000	<.0001	1.0000
Bsp41	*Bacillus simplex*	24.3	1.0000	<.0001	1.0000
Bsp42	*Bacillus simplex*	79.0	0.0061	1.0000	<.0001
Bsp43	*Bacillus simplex*	10.0	1.0000	<.0001	1.0000
Bsp44	*Bacillus simplex*	66.0	0.1643	0.6089	0.0002
Bsp45	*Bacillus simplex*	58.0	0.5856	0.1750	0.0039
Bsp46	*Bacillus simplex*	55.8	0.7429	0.1128	0.0077
Bsp47	*Bacillus simplex*	74.9	0.0199	0.9990	<.0001
Bsp48	*Bacillus simplex*	71.0	0.0172	0.7796	<.0001
Bsp49	*Bacillus simplex*	37.3	1.0000	0.0005	0.4675
Bsp50	*Bacillus simplex*	83.0	0.0017	1.0000	<.0001
Bsp51	*Bacillus simplex*	75.5	0.0170	0.9996	<.0001
Bsp52	*Bacillus simplex*	76.9	0.0116	1.0000	<.0001
Bsp53	*Bacillus simplex*	82.7	0.0426	1.0000	0.0001
Bsp54	*Bacillus simplex*	83.1	0.0016	1.0000	<.0001
Bsp55	*Bacillus simplex*	81.1	0.0031	1.0000	<.0001
Bsp56	*Bacillus simplex*	75.3	0.0179	0.9995	<.0001
Bsp57	*Bacillus simplex*	83.2	0.0016	1.0000	<.0001
Bsp58	*Bacillus simplex*	88.3	0.0002	1.0000	<.0001
Bsp59	*Bacillus simplex*	83.7	0.0013	1.0000	<.0001
Bsp60	*Bacillus simplex*	64.3	0.2240	0.4939	0.0004
Bsp61	*Bacillus simplex*	84.8	0.0009	1.0000	<.0001
Bsp62	*Bacillus simplex*	69.5	0.0773	0.8574	<.0001
Bsp63	*Bacillus simplex*	76.4	0.0132	1.0000	<.0001
Bsp64	*Bacillus simplex*	89.2	<.0001	1.0000	<.0001
Bsp65	*Bacillus simplex*	26.8	1.0000	<.0001	0.9974
Bsp66	*Bacillus simplex*	70.9	0.0568	0.9261	<.0001
Bsp67	*Bacillus simplex*	43.4	1.0000	0.0041	0.1692
Bsp68	*Bacillus simplex*	18.0	1.0000	<.0001	1.0000
Bsp69	*Bacillus simplex*	51.2	0.9764	0.0385	0.0285
Bsp70	*Bacillus simplex*	31.2	1.0000	<.0001	0.8832
Bsp71	*Bacillus simplex*	47.2	1.0000	0.0132	0.0755
Bsp72	*Bacillus simplex*	46.8	1.0000	0.0117	0.0827
Bsp73	*Bacillus simplex*	48.7	0.9989	0.0201	0.0531
Bsp74	*Bacillus simplex*	33.7	1.0000	0.0001	0.7232
Bsp75	*Bacillus simplex*	33.6	1.0000	0.0001	0.7268

Bsp76	*Bacillus simplex*	45.0	1.0000	0.0068	0.1227
Bsp77	*Bacillus simplex*	40.8	1.0000	0.0018	0.2706
Bsp78	*Bacillus simplex*	28.3	1.0000	<.0001	0.9841
Bsp79	*Bacillus simplex*	76.8	0.0117	1.0000	<.0001
Bsp80	*Bacillus simplex*	13.7	1.0000	<.0001	1.0000
Bsp81	*Bacillus simplex*	86.1	0.0006	1.0000	<.0001
Bsp82	*Bacillus simplex*	73.5	0.0292	0.9921	<.0001
Bsp83	*Bacillus simplex*	14.2	1.0000	<.0001	1.0000
Bsp84	*Bacillus simplex*	99.6	0.0006	1.0000	<.0001
Bsp85	*Bacillus simplex*	8.1	1.0000	<.0001	1.0000
Bsp86	*Bacillus simplex*	32.6	1.0000	<.0001	0.7984
Bsp87	*Bacillus simplex*	81.3	0.0568	1.0000	0.0002
Bsp88	*Bacillus simplex*	91.5	<.0001	1.0000	<.0001
Bsp89	*Bacillus simplex*	84.3	0.0010	1.0000	<.0001
Bsp90	*Bacillus simplex*	17.6	1.0000	<.0001	1.0000
Bsp91	*Bacillus simplex*	64.4	0.2220	0.4972	0.0004
Bsp92	*Bacillus simplex*	90.2	0.0001	1.0000	<.0001
Bsp93	*Bacillus simplex*	56.1	0.7196	0.1208	0.0070
Bsp94	*Bacillus simplex*	81.3	0.0029	1.0000	<.0001
Bsp95	*Bacillus simplex*	25.6	1.0000	<.0001	0.9997
Bsp96	*Bacillus simplex*	95.5	<.0001	1.0000	<.0001
Bsp97	*Bacillus simplex*	20.3	1.0000	<.0001	1.0000
Bsp98	*Bacillus simplex*	27.8	1.0000	<.0001	0.9914
Bsp99	*Bacillus simplex*	9.1	1.0000	<.0001	1.0000
Bsp100	*Bacillus simplex*	2.4	1.0000	<.0001	1.0000
Bsp101	*Bacillus simplex*	98.0	0.0010	1.0000	<.0001
Bsp102	*Bacillus simplex*	5.2	1.0000	<.0001	1.0000
Bsp103	*Bacillus simplex*	8.1	1.0000	<.0001	1.0000
Bsp104	*Bacillus simplex*	19.8	1.0000	<.0001	1.0000
Bsp105	*Bacillus simplex*	7.6	1.0000	<.0001	1.0000
Bsp106	*Bacillus simplex*	5.6	1.0000	<.0001	1.0000
Bsp107	*Bacillus simplex*	6.2	1.0000	<.0001	1.0000
Bsp108	*Bacillus simplex*	20.4	1.0000	<.0001	1.0000
Bsp109	*Bacillus simplex*	46.1	1.0000	0.0095	0.0971
Bsp110	*Bacillus simplex*	10.4	1.0000	<.0001	1.0000
Bsp111	*Bacillus simplex*	14.3	1.0000	<.0001	1.0000
Bsp112	*Bacillus simplex*	2.2	1.0000	<.0001	1.0000
Bsp113	*Bacillus simplex*	3.5	1.0000	<.0001	1.0000
Bsp114	*Bacillus simplex*	99.9	0.0006	1.0000	<.0001
Bsp115	*Bacillus simplex*	99.9	0.0006	1.0000	<.0001
Bsp116	*Bacillus simplex*	99.1	0.0007	1.0000	<.0001
Bsp117	*Bacillus simplex*	18.3	1.0000	<.0001	1.0000
Bsp118	*Bacillus simplex*	69.3	0.0818	0.8422	<.0001
Bsp119	*Bacillus simplex*	13.1	1.0000	<.0001	1.0000
Bsp120	*Bacillus simplex*	1.6	1.0000	<.0001	1.0000
Bsp121	*Bacillus simplex*	2.9	1.0000	<.0001	1.0000
Bsp122	*Bacillus simplex*	39.6	1.0000	0.0011	0.3327
Bsp123	*Bacillus simplex*	83.7	0.0350	1.0000	<.0001
Bsp124	*Bacillus simplex*	67.5	0.1195	0.7232	0.0001
Bsp125	*Bacillus simplex*	6.8	1.0000	<.0001	1.0000
Bsp126	*Bacillus simplex*	76.2	0.0142	0.9999	<.0001
Bsp127	*Bacillus simplex*	15.4	1.0000	<.0001	1.0000
Bsp128	*Bacillus simplex*	13.6	1.0000	<.0001	1.0000
Bsp129	*Bacillus simplex*	58.8	0.9358	0.6307	0.0571
Bsp130	*Bacillus simplex*	44.5	1.0000	0.0865	0.5038
Bsp131	*Bacillus simplex*	88.2	0.0003	1.0000	<.0001
Bsp132	*Bacillus simplex*	11.7	1.0000	<.0001	1.0000
Bsp133	*Bacillus simplex*	42.7	1.0000	0.0618	0.6066
Bsp134	*Bacillus simplex*	62.9	0.2847	0.4064	0.0007
Bsp135	*Bacillus simplex*	71.4	0.0497	0.9477	<.0001
Bsp136	*Bacillus simplex*	12.3	1.0000	<.0001	1.0000
Bsp137	*Bacillus simplex*	24.7	1.0000	<.0001	1.0000
Bsp138	*Bacillus simplex*	1.6	1.0000	<.0001	1.0000
Bsp139	*Bacillus simplex*	39.6	1.0000	0.0331	0.7882
Bsp140	*Bacillus simplex*	13.9	1.0000	<.0001	1.0000
Bsp141	*Bacillus simplex*	35.9	1.0000	0.0146	0.9501
Bsp142	*Bacillus simplex*	6.0	1.0000	<.0001	1.0000
Bsp143	*Bacillus simplex*	98.2	0.0009	1.0000	<.0001
Bsp144	*Bacillus simplex*	5.6	1.0000	<.0001	1.0000
Bsp145	*Bacillus simplex*	3.1	1.0000	<.0001	1.0000
Bsp146	*Bacillus simplex*	41.1	1.0000	0.0451	0.7014
Bsp147	*Bacillus simplex*	7.5	1.0000	<.0001	1.0000
Bsp148	*Bacillus simplex*	12.0	1.0000	<.0001	1.0000
Bsp149	*Bacillus simplex*	38.8	1.0000	0.0276	0.8339
Bsp150	*Bacillus simplex*	9.2	1.0000	<.0001	1.0000
Bsp151	*Bacillus simplex*	1.3	0.9999	<.0001	1.0000
Bsp152	*Bacillus simplex*	3.3	1.0000	<.0001	1.0000
Bsp153	*Bacillus simplex*	31.8	1.0000	0.0054	0.9988
Bsp154	*Bacillus simplex*	99.9	0.0006	1.0000	<.0001
Bsp155	*Bacillus simplex*	37.6	1.0000	0.0006	0.4466

Bsp156	*Bacillus simplex*	9.0	1.0000	<.0001	1.0000
Bsp157	*Bacillus simplex*	7.0	1.0000	<.0001	1.0000
Bsp158	*Bacillus simplex*	33.9	1.0000	0.0175	0.9247
Bsp159	*Bacillus simplex*	36.7	1.0000	0.0001	0.7033
Bsp160	*Bacillus simplex*	0.8	0.9997	<.0001	1.0000
Bsp161	*Bacillus simplex*	13.9	1.0000	<.0001	1.0000
Bsp162	*Bacillus simplex*	31.1	1.0000	<.0001	0.8912
Bsp163	*Bacillus simplex*	26.6	1.0000	<.0001	0.9983
Bsp164	*Bacillus simplex*	23.9	1.0000	<.0001	1.0000
Bsp165	*Bacillus simplex*	1.9	1.0000	<.0001	1.0000
Bsp166	*Bacillus simplex*	17.3	1.0000	<.0001	1.0000
Bsp167	*Bacillus simplex*	5.9	1.0000	<.0001	1.0000
Bsp168	*Bacillus simplex*	45.8	1.0000	0.1090	0.4348
Bsp169	*Bacillus simplex*	17.1	1.0000	<.0001	1.0000
Bsp170	*Bacillus simplex*	41.1	1.0000	0.0019	0.2581
Bsp171	*Bacillus simplex*	40.2	1.0000	0.0375	0.7541
Bsp172	*Bacillus simplex*	19.7	1.0000	<.0001	1.0000
Bsp173	*Bacillus simplex*	31.0	1.0000	<.0001	0.8938
Bsp174	*Bacillus simplex*	24.3	1.0000	<.0001	1.0000
Bsp175	*Bacillus simplex*	19.4	1.0000	<.0001	1.0000
Bsp176	*Bacillus simplex*	4.7	1.0000	<.0001	1.0000
Bsp177	*Bacillus simplex*	28.6	1.0000	<.0001	0.9789
Bsp178	*Bacillus simplex*	18.2	1.0000	<.0001	1.0000
Bsp179	*Bacillus simplex*	25.3	1.0000	<.0001	0.9998
Bsp180	*Bacillus simplex*	24.7	1.0000	<.0001	1.0000
Bsp181	*Bacillus simplex*	30.4	1.0000	<.0001	0.9217
Bsp182	*Bacillus simplex*	12.1	1.0000	<.0001	1.0000
Bsp183	*Bacillus simplex*	26.0	1.0000	<.0001	0.9993
Bsp184	*Bacillus simplex*	25.9	1.0000	<.0001	0.9994
Bsp185	*Bacillus simplex*	31.0	1.0000	<.0001	0.8938
Bsp186	*Bacillus simplex*	35.2	1.0000	0.0002	0.6143
Bsp187	*Bacillus simplex*	67.7	0.1146	0.7375	0.0001
Bsp188	*Bacillus simplex*	41.7	1.0000	0.0503	0.6688
Bsp189	*Bacillus simplex*	10.7	1.0000	<.0001	1.0000
Bsp190	*Bacillus simplex*	22.5	1.0000	<.0001	1.0000
Bsp191	*Bacillus simplex*	8.3	1.0000	<.0001	1.0000
Bsp192	*Bacillus simplex*	16.9	1.0000	<.0001	1.0000
Bsp193	*Bacillus simplex*	17.0	1.0000	<.0001	1.0000
Bsp194	*Bacillus simplex*	15.7	1.0000	<.0001	1.0000
Bsp195	*Bacillus simplex*	90.0	0.0001	1.0000	<.0001
Bsp196	*Bacillus simplex*	40.8	1.0000	0.0420	0.7221
Bsp197	*Bacillus simplex*	64.3	0.2240	0.4939	0.0004
Bsp198	*Bacillus simplex*	80.6	0.0037	1.0000	<.0001
Bsp199	*Bacillus simplex*	52.8	0.9251	0.0571	0.0185
Bsp200	*Bacillus simplex*	71.9	0.0437	0.9643	<.0001
Bsp201	*Bacillus simplex*	7.4	1.0000	<.0001	1.0000
Bsp202	*Bacillus simplex*	42.8	1.0000	0.0005	0.0843
Bsp203	*Bacillus simplex*	10.0	1.0000	<.0001	1.0000
Bsp204	*Bacillus simplex*	20.9	1.0000	<.0001	1.0000
Bsp205	*Bacillus simplex*	39.8	1.0000	0.0012	0.3222
Bsp206	*Bacillus simplex*	23.0	1.0000	<.0001	1.0000
Bsp207	*Bacillus simplex*	36.5	1.0000	0.0004	0.5192
Bsp208	*Bacillus simplex*	29.1	1.0000	<.0001	0.9676
Bssin1	*Bacillus subtilis* subsp. *inaquosorum*	0.0	0.9987	<.0001	1.0000
Bssin2	*Bacillus subtilis* subsp. *inaquosorum*	3.3	1.0000	<.0001	1.0000
Bssin3	*Bacillus subtilis* subsp. *inaquosorum*	3.2	1.0000	<.0001	1.0000
Bssin4	*Bacillus subtilis* subsp. *inaquosorum*	12.9	1.0000	<.0001	1.0000
Bssin5	*Bacillus subtilis* subsp. *inaquosorum*	24.9	1.0000	<.0001	0.9999
Bssin6	*Bacillus subtilis* subsp. *inaquosorum*	4.4	1.0000	<.0001	1.0000
Bssin7	*Bacillus subtilis* subsp. *inaquosorum*	24.3	1.0000	<.0001	1.0000
Bssin8	*Bacillus subtilis* subsp. *inaquosorum*	88.4	0.0002	1.0000	<.0001
Bssin9	*Bacillus subtilis* subsp. *inaquosorum*	94.6	<.0001	1.0000	<.0001
Bssin10	*Bacillus subtilis* subsp. *inaquosorum*	94.6	<.0001	1.0000	<.0001
Bssin11	*Bacillus subtilis* subsp. *inaquosorum*	54.1	0.8544	0.0781	0.0126
Bssin12	*Bacillus subtilis* subsp. *inaquosorum*	94.3	<.0001	1.0000	<.0001
Bssin13	*Bacillus subtilis* subsp. *inaquosorum*	29.4	1.0000	<.0001	0.9599
Bssin14	*Bacillus subtilis* subsp. *inaquosorum*	94.5	<.0001	1.0000	<.0001
Bssin15	*Bacillus subtilis* subsp. *inaquosorum*	90.6	<.0001	1.0000	<.0001
Bsssu1	*Bacillus subtilis* subsp. *subtilis*	23.6	1.0000	<.0001	1.0000
Bsssu2	*Bacillus subtilis* subsp. *subtilis*	84.4	0.0302	1.0000	<.0001
Bsssu3	*Bacillus subtilis* subsp. *subtilis*	82.4	0.0457	1.0000	0.0001
Bsssu4	*Bacillus subtilis* subsp. *subtilis*	1.2	0.9999	<.0001	1.0000
Bte1	*Bacillus tequilensis*	36.6	1.0000	0.0004	0.5158
Bte2	*Bacillus tequilensis*	93.5	<.0001	1.0000	<.0001
Bte3	*Bacillus tequilensis*	1.4	0.9999	<.0001	1.0000
Bth1	*Bacillus thuringiensis*	0.0	0.9987	<.0001	1.0000
Bth2	*Bacillus thuringiensis*	58.8	0.5312	0.2026	0.0030
Bth3	*Bacillus thuringiensis*	0.0	0.9987	<.0001	1.0000
Bth4	*Bacillus thuringiensis*	9.2	1.0000	<.0001	1.0000
Bto1	*Bacillus toyonensis*	2.1	1.0000	<.0001	1.0000

Bto2	*Bacillus toyonensis*	6.1	1.0000	<.0001	1.0000
Bto3	*Bacillus toyonensis*	7.1	1.0000	<.0001	1.0000
Bto4	*Bacillus toyonensis*	6.6	1.0000	<.0001	1.0000
Bto5	*Bacillus toyonensis*	11.5	1.0000	<.0001	1.0000
Bto6	*Bacillus toyonensis*	6.8	1.0000	<.0001	1.0000
Bto7	*Bacillus toyonensis*	9.4	1.0000	<.0001	1.0000
Bto8	*Bacillus toyonensis*	12.1	1.0000	<.0001	1.0000
Bto9	*Bacillus toyonensis*	16.8	1.0000	<.0001	1.0000
Bto10	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto11	*Bacillus toyonensis*	20.2	1.0000	<.0001	1.0000
Bto12	*Bacillus toyonensis*	13.1	1.0000	<.0001	1.0000
Bto13	*Bacillus toyonensis*	23.9	1.0000	<.0001	1.0000
Bto14	*Bacillus toyonensis*	8.9	1.0000	<.0001	1.0000
Bto15	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto16	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto17	*Bacillus toyonensis*	1.6	0.9999	<.0001	1.0000
Bto18	*Bacillus toyonensis*	87.5	0.0003	1.0000	<.0001
Bto19	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto20	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto21	*Bacillus toyonensis*	63.5	0.2603	0.4387	0.0006
Bto22	*Bacillus toyonensis*	82.9	0.0017	1.0000	<.0001
Bto23	*Bacillus toyonensis*	73.3	0.0310	0.9898	<.0001
Bto24	*Bacillus toyonensis*	76.8	0.0118	1.0000	<.0001
Bto25	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto26	*Bacillus toyonensis*	6.3	1.0000	<.0001	1.0000
Bto27	*Bacillus toyonensis*	31.1	1.0000	<.0001	0.8912
Bto28	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto29	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto30	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto31	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bto32	*Bacillus toyonensis*	0.0	1.0000	<.0001	1.0000
Bto33	*Bacillus toyonensis*	2.6	1.0000	<.0001	1.0000
Bto34	*Bacillus toyonensis*	74.0	0.0258	0.9957	<.0001
Bto35	*Bacillus toyonensis*	12.4	1.0000	<.0001	1.0000
Bto36	*Bacillus toyonensis*	93.1	<.0001	1.0000	<.0001
Bto37	*Bacillus toyonensis*	34.1	1.0000	0.0001	0.6924
Bto38	*Bacillus toyonensis*	26.2	1.0000	<.0001	0.9991
Bto39	*Bacillus toyonensis*	42.3	1.0000	0.0029	0.2083
Bto40	*Bacillus toyonensis*	98.5	<.0001	1.0000	<.0001
Bto41	*Bacillus toyonensis*	1.1	0.9999	<.0001	1.0000
Bto42	*Bacillus toyonensis*	4.3	1.0000	<.0001	1.0000
Bto43	*Bacillus toyonensis*	28.1	1.0000	<.0001	0.9870
Bto44	*Bacillus toyonensis*	9.0	1.0000	<.0001	1.0000
Bto45	*Bacillus toyonensis*	82.1	0.0022	1.0000	<.0001
Bto46	*Bacillus toyonensis*	64.2	0.2271	0.4889	0.0004
Bto47	*Bacillus toyonensis*	2.0	1.0000	<.0001	1.0000
Bto48	*Bacillus toyonensis*	1.0	0.9998	<.0001	1.0000
Bto49	*Bacillus toyonensis*	66.2	0.1572	0.6252	0.0002
Bto50	*Bacillus toyonensis*	37.1	1.0000	0.0005	0.4773
Bto51	*Bacillus toyonensis*	87.2	0.0004	1.0000	<.0001
Bto52	*Bacillus toyonensis*	89.4	0.0002	1.0000	<.0001
Bto53	*Bacillus toyonensis*	75.6	0.0167	0.9997	<.0001
Bto54	*Bacillus toyonensis*	81.3	0.0029	1.0000	<.0001
Bto55	*Bacillus toyonensis*	91.3	<.0001	1.0000	<.0001
Bto56	*Bacillus toyonensis*	5.9	1.0000	<.0001	1.0000
Bto57	*Bacillus toyonensis*	87.0	0.0004	1.0000	<.0001
Bto58	*Bacillus toyonensis*	81.0	0.0032	1.0000	<.0001
Bto59	*Bacillus toyonensis*	68.3	0.1019	0.7762	<.0001
Bto60	*Bacillus toyonensis*	19.9	1.0000	<.0001	1.0000
Bto61	*Bacillus toyonensis*	91.3	<.0001	1.0000	<.0001
Bto62	*Bacillus toyonensis*	4.8	1.0000	<.0001	1.0000
Bto63	*Bacillus toyonensis*	84.5	0.0010	1.0000	<.0001
Bto64	*Bacillus toyonensis*	66.6	0.1445	0.6560	0.0002
Bto65	*Bacillus toyonensis*	86.6	0.0004	1.0000	<.0001
Bto66	*Bacillus toyonensis*	72.3	0.0398	0.9737	<.0001
Bto67	*Bacillus toyonensis*	24.8	1.0000	<.0001	0.9999
Bto68	*Bacillus toyonensis*	17.8	1.0000	<.0001	1.0000
Bto69	*Bacillus toyonensis*	16.6	1.0000	<.0001	1.0000
Bto70	*Bacillus toyonensis*	0.0	0.9987	<.0001	1.0000
Bve1	*Bacillus velezensis*	8.5	1.0000	<.0001	1.0000
Bve2	*Bacillus velezensis*	72.8	0.2386	1.0000	0.0021
Bve3	*Bacillus velezensis*	11.6	1.0000	<.0001	1.0000
Bve4	*Bacillus velezensis*	54.1	0.8559	0.0777	0.0127
Bve5	*Bacillus velezensis*	54.7	0.8216	0.0879	0.0108
Bve6	*Bacillus velezensis*	42.8	1.0000	0.0034	0.1888
Bve7	*Bacillus velezensis*	23.3	1.0000	<.0001	1.0000
Bve8	*Bacillus velezensis*	6.6	1.0000	<.0001	1.0000
Bve9	*Bacillus velezensis*	0.0	0.9987	<.0001	1.0000
Bve10	*Bacillus velezensis*	9.8	1.0000	<.0001	1.0000
Bve11	*Bacillus velezensis*	1.1	0.9999	<.0001	1.0000

Bve12	*Bacillus velezensis*	81.1	0.0591	1.0000	0.0002
Bve13	*Bacillus velezensis*	61.9	0.7951	0.8106	0.0303
Bve14	*Bacillus velezensis*	89.3	0.0099	1.0000	<.0001
Bve15	*Bacillus velezensis*	28.7	1.0000	0.0024	1.0000
Bve16	*Bacillus velezensis*	1.0	0.9998	<.0001	1.0000
Bve17	*Bacillus velezensis*	7.6	1.0000	<.0001	1.0000
Bve18	*Bacillus velezensis*	10.5	1.0000	<.0001	1.0000
Bve19	*Bacillus velezensis*	11.6	1.0000	<.0001	1.0000
Bve20	*Bacillus velezensis*	2.3	1.0000	<.0001	1.0000
Bve21	*Bacillus velezensis*	52.4	0.9423	0.0516	0.0208
Bve22	*Bacillus velezensis*	5.3	1.0000	<.0001	1.0000
Bve23	*Bacillus velezensis*	40.0	1.0000	0.0013	0.3081
Bve24	*Bacillus velezensis*	0.0	0.9987	<.0001	1.0000
Bve25	*Bacillus velezensis*	13.8	1.0000	<.0001	1.0000
Bve26	*Bacillus velezensis*	12.3	1.0000	<.0001	1.0000
Bve27	*Bacillus velezensis*	11.0	1.0000	<.0001	1.0000
Bve28	*Bacillus velezensis*	60.9	0.3960	0.2932	0.0015
Bve29	*Bacillus velezensis*	21.8	1.0000	<.0001	1.0000
Bve30	*Bacillus velezensis*	9.1	1.0000	<.0001	1.0000
Bve31	*Bacillus velezensis*	6.0	1.0000	<.0001	1.0000
Bve32	*Bacillus velezensis*	21.6	1.0000	<.0001	1.0000
Bve33	*Bacillus velezensis*	39.8	1.0000	0.0012	0.3209
Bve34	*Bacillus velezensis*	58.9	0.7050	0.3464	0.0108
Bve35	*Bacillus velezensis*	44.6	1.0000	0.0061	0.1319
Bve36	*Bacillus velezensis*	36.7	1.0000	0.0004	0.5090
Bve37	*Bacillus velezensis*	76.5	0.1352	1.0000	0.0007
Bve38	*Bacillus velezensis*	22.9	1.0000	<.0001	1.0000
Bve39	*Bacillus velezensis*	39.2	1.0000	0.0010	0.3544
Bve40	*Bacillus velezensis*	76.5	0.1341	1.0000	0.0007
Bve41	*Bacillus velezensis*	45.9	1.0000	0.0091	0.1003
Bwe1	*Bacillus weihenstephanensis*	23.4	1.0000	<.0001	1.0000
Bwe2	*Bacillus weihenstephanensis*	83.6	0.0013	1.0000	<.0001
Bwe3	*Bacillus weihenstephanensis*	31.1	1.0000	<.0001	0.8886
Bwe4	*Bacillus weihenstephanensis*	15.2	1.0000	<.0001	1.0000
Bwe5	*Bacillus weihenstephanensis*	57.8	0.5999	0.1684	0.0042
Bwe6	*Bacillus weihenstephanensis*	8.2	1.0000	<.0001	1.0000
Bwe7	*Bacillus weihenstephanensis*	0.0	0.9987	<.0001	1.0000
Bwe8	*Bacillus weihenstephanensis*	7.9	1.0000	<.0001	1.0000
Bwe9	*Bacillus weihenstephanensis*	13.0	1.0000	<.0001	1.0000
Bwe10	*Bacillus weihenstephanensis*	94.3	<.0001	1.0000	<.0001
Bwe11	*Bacillus weihenstephanensis*	0.8	0.9997	<.0001	1.0000
Bwe12	*Bacillus weihenstephanensis*	39.1	1.0000	0.0010	0.3600
Bwe13	*Bacillus weihenstephanensis*	43.3	1.0000	0.0040	0.1725
Bwe14	*Bacillus weihenstephanensis*	31.8	1.0000	<.0001	0.8468
Bwe15	*Bacillus weihenstephanensis*	75.4	0.0174	0.9996	<.0001
Bwe16	*Bacillus weihenstephanensis*	81.8	0.0025	1.0000	<.0001
Bwe17	*Bacillus weihenstephanensis*	46.1	1.0000	0.0096	0.0965
Bwe18	*Bacillus weihenstephanensis*	27.3	1.0000	<.0001	0.9950
Bwe19	*Bacillus weihenstephanensis*	41.2	1.0000	0.0020	0.2514
Bwe20	*Bacillus weihenstephanensis*	3.5	1.0000	<.0001	1.0000
Bwe21	*Bacillus weihenstephanensis*	18.3	1.0000	<.0001	1.0000
Brep1	*Brevibacterium epidermidis*	83.8	0.0013	1.0000	<.0001
Brep2	*Brevibacterium epidermidis*	42.3	1.0000	0.0029	0.2092
Brep3	*Brevibacterium epidermidis*	44.2	1.0000	0.0054	0.1430
Brep4	*Brevibacterium epidermidis*	29.2	1.0000	<.0001	0.9663
Brep5	*Brevibacterium epidermidis*	54.9	0.8084	0.0919	0.0102
Brep6	*Brevibacterium epidermidis*	67.8	0.1122	0.7446	0.0001
Brep7	*Brevibacterium epidermidis*	52.2	0.9486	0.0494	0.0218
Brio1	*Brevibacterium iodinum*	87.8	0.0003	1.0000	<.0001
Enx1	*Enterobacter xiangfangensis*	36.9	1.0000	0.0004	0.4955
Exs1	*Exiguobacterium sibiricum*	0.0	0.9987	<.0001	1.0000
Fso1	*Fictibacillus solisalsi*	70.3	0.3385	0.9997	0.0042
Lma1	*Lysinibacillus macroides*	64.8	0.6287	0.9368	0.0161
Lpa1	*Lysinibacillus parviboronicapiens*	0.7	0.9997	<.0001	1.0000
Paam1	*Paenibacillus amylolyticus*	17.7	1.0000	<.0001	1.0000
Paam2	*Paenibacillus amylolyticus*	58.0	0.5838	0.1758	0.0039
Paam3	*Paenibacillus amylolyticus*	58.3	0.5608	0.1871	0.0035
Paam4	*Paenibacillus amylolyticus*	25.4	1.0000	<.0001	0.9998
Paam5	*Paenibacillus amylolyticus*	47.6	0.9999	0.0148	0.0689
Paam6	*Paenibacillus amylolyticus*	70.8	0.0574	0.9240	<.0001
Paam7	*Paenibacillus amylolyticus*	82.8	0.0018	1.0000	<.0001
Paam8	*Paenibacillus amylolyticus*	95.2	<.0001	1.0000	<.0001
Paam9	*Paenibacillus amylolyticus*	29.4	1.0000	<.0001	0.9599
Paba1	*Paenibacillus barcinonensis*	22.4	1.0000	<.0001	1.0000
Pagl1	*Paenibacillus glycanilyticus*	7.7	1.0000	<.0001	1.0000
Pail1	*Paenibacillus illinoisensis*	32.9	1.0000	<.0001	0.7745
Pala1	*Paenibacillus lautus*	0.0	0.9987	<.0001	1.0000
Pala2	*Paenibacillus lautus*	7.6	1.0000	<.0001	1.0000
Pala3	*Paenibacillus lautus*	13.8	1.0000	<.0001	1.0000
Pala4	*Paenibacillus lautus*	70.4	0.0628	0.9065	<.0001

Pamd1	*Paenibacillus macquariensis* subsp. *defensor*	3.3	1.0000	<.0001	1.0000
Paod1	*Paenibacillus odorifer*	45.6	1.0000	0.0082	0.1081
Pata1	*Paenibacillus taichungensis*	32.8	1.0000	0.0069	0.9958
Path1	*Paenibacillus thiaminolyticus*	4.0	1.0000	<.0001	1.0000
Patu1	*Paenibacillus tundrae*	79.6	0.0050	1.0000	<.0001
Pava1	*Paenibacillus validus*	11.4	1.0000	<.0001	1.0000
Paxy1	*Paenibacillus xylanexedens*	45.2	1.0000	0.0073	0.1170
Paxy2	*Paenibacillus xylanexedens*	90.4	0.0001	1.0000	<.0001
Panag1	*Pantoea agglomerans*	3.4	1.0000	<.0001	1.0000
Rhil1	*Rhizobium larrymoorei*	31.9	1.0000	<.0001	0.8453
Rhoq1	*Rhodococcus qingshengii*	2.8	1.0000	<.0001	1.0000
Soi1	*Solibacillus isronensis*	53.5	0.9996	0.3466	0.1486
Spg1	*Sporosarcina globispora*	27.6	1.0000	<.0001	0.9932
Spg2	*Sporosarcina globispora*	0.0	0.9987	<.0001	1.0000
Spg3	*Sporosarcina globispora*	39.6	1.0000	0.0012	0.3288
Spg4	*Sporosarcina globispora*	44.6	1.0000	0.0061	0.1325
Spg5	*Sporosarcina globispora*	29.3	1.0000	<.0001	0.9629
Spg6	*Sporosarcina globispora*	4.7	1.0000	<.0001	1.0000
Spg7	*Sporosarcina globispora*	1.9	1.0000	<.0001	1.0000
Spg8	*Sporosarcina globispora*	84.4	0.0010	1.0000	<.0001
Spg9	*Sporosarcina globispora*	27.4	1.0000	<.0001	0.9947
Spg10	*Sporosarcina globispora*	6.2	1.0000	<.0001	1.0000
Uid1	*Bacillus aerophilus/stratosphaericus**	43.0	1.0000	0.0654	0.5890
Uid2	*Bacillus aerophilus/stratosphericus**	28.7	1.0000	<.0001	0.9774
Uid3	*Bacillus aerophilus/stratosphericus**	7.7	1.0000	<.0001	1.0000
Uid4	*Bacillus aerophilus/stratosphericus**	89.1	0.0002	1.0000	<.0001
Uid5	*Bacillus aerophilus/stratosphericus**	2.7	1.0000	<.0001	1.0000
Uid6	*Bacillus aerophilus/stratosphericus**	88.2	0.0002	1.0000	<.0001
Uid7	*Bacillus aerophilus/stratosphericus**	54.8	0.8134	0.0904	0.0104
Uid8	*Bacillus altitudinis/stratosphericus/aerophilus**	98.9	<.0001	1.0000	<.0001
Uid9	*Bacillus altitudinis/stratosphericus/aerophilus**	96.2	<.0001	1.0000	<.0001
Uid10	*Bacillus altitudinis/stratosphericus/aerophilus**	96.8	<.0001	1.0000	<.0001
Uid11	Unidentified species*	0.0	0.9987	<.0001	1.0000
Uid12	Unidentified species*	5.3	1.0000	<.0001	1.0000
Control	**Active ingredient** [b]				
Poncho/Votivo	Clothianidin and *B. firmus* I-1582	24.4	…	<.0001	1.0000
Temik	Aldicarb	99.2	<.0001	…	<.0001
Untreated control	Sterile distilled water	2.0	1.0000	<.0001	…

[a]*In vitro* tests were performed in 96-well plates. All the PGPR strains had 4 replications and controls were based on 17 repeats. Data collected were analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.05$. *P* value less than 0.05 indicate a significant effect. Adjusted *P* values were obtained according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

[b]Active ingredients for Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Temik is Aldicarb, and untreated control is sterile distilled water.

[c] Dunnett's option was used in the LS-means statement to assess the differences between bacterial isolates and the Poncho/Votivo, Temik, and the untreated control.

[d]Mortality was determined by the following equation: [(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] ×100.

*Indistinguishable species and unidentified strains.

Appendix 2. 670 PGPR isolates effect on *Heterodera glycines*J2 mortality as compared to the industry standard biologicals Poncho/Votivo, Clariva, and chemical Temik as well as an untreated control[a].

Code	Scientific name	*Heterodera glycines* J2 mortality (%)[b]	Dunnett's *P* vs[d] ($P \leq 0.05$)			
			Clothianidin + *B. firmus*[c]	*P. nishizawae*	Aldicarb	Water
Ad1	*Arthrobacter defluvii*	7.9	1.0000	1.0000	<.0001	1.0000
Ae1	*Arthrobacter equi*	6.3	1.0000	1.0000	<.0001	1.0000
Bal1	*Bacillus altitudinis*	24.0	1.0000	1.0000	<.0001	0.9982
Bal2	*Bacillus altitudinis*	5.7	1.0000	1.0000	<.0001	1.0000
Bal3	*Bacillus altitudinis*	33.5	1.0000	0.9985	<.0001	0.1058
Bal4	*Bacillus altitudinis*	5.3	1.0000	1.0000	<.0001	1.0000
Bal5	*Bacillus altitudinis*	2.9	0.9897	1.0000	<.0001	1.0000
Bal6	*Bacillus altitudinis*	14.4	1.0000	1.0000	<.0001	1.0000
Bal7	*Bacillus altitudinis*	8.4	1.0000	1.0000	<.0001	1.0000
Bal8	*Bacillus altitudinis*	11.1	1.0000	1.0000	<.0001	1.0000
Bal9	*Bacillus altitudinis*	51.7	0.1099	0.0206	<.0001	<.0001
Bal10	*Bacillus altitudinis*	24.1	1.0000	1.0000	<.0001	0.8203
Bal11	*Bacillus altitudinis*	64.0	0.0236	0.0045	0.1725	<.0001
Bal12	*Bacillus altitudinis*	54.7	0.0408	0.0059	0.0002	<.0001
Bal13	*Bacillus altitudinis*	81.2	<.0001	<.0001	1.0000	<.0001
Bal14	*Bacillus altitudinis*	3.8	0.9982	1.0000	<.0001	1.0000
Bal15	*Bacillus altitudinis*	3.4	0.9959	1.0000	<.0001	1.0000
Bal16	*Bacillus altitudinis*	2.8	0.9885	1.0000	<.0001	1.0000
Bal17	*Bacillus altitudinis*	1.3	0.9322	1.0000	<.0001	1.0000
Bal18	*Bacillus altitudinis*	15.7	1.0000	1.0000	<.0001	1.0000
Bal19	*Bacillus altitudinis*	13.4	1.0000	1.0000	<.0001	1.0000
Bal20	*Bacillus altitudinis*	55.1	0.0353	0.0050	0.0003	<.0001
Bal21	*Bacillus altitudinis*	41.2	0.9150	0.4566	<.0001	0.0059
Bar1	*Bacillus aryabhattai*	0.0	0.8384	0.9999	<.0001	1.0000
Bar2	*Bacillus aryabhattai*	17.8	1.0000	1.0000	<.0001	1.0000
Bar3	*Bacillus aryabhattai*	24.5	1.0000	1.0000	<.0001	1.0000
Bar4	*Bacillus aryabhattai*	6.4	1.0000	1.0000	<.0001	1.0000
Bar5	*Bacillus aryabhattai*	8.8	1.0000	1.0000	<.0001	1.0000
Bar6	*Bacillus aryabhattai*	5.3	1.0000	1.0000	<.0001	1.0000
Bar7	*Bacillus aryabhattai*	8.8	1.0000	1.0000	<.0001	1.0000
Bar8	*Bacillus aryabhattai*	8.3	1.0000	1.0000	<.0001	1.0000
Bar9	*Bacillus aryabhattai*	38.1	0.9991	0.7674	<.0001	0.0215
Bar10	*Bacillus aryabhattai*	3.3	0.9950	1.0000	<.0001	1.0000
Bar11	*Bacillus aryabhattai*	22.2	1.0000	1.0000	<.0001	0.8195
Bar12	*Bacillus aryabhattai*	9.3	1.0000	1.0000	<.0001	1.0000
Bar13	*Bacillus aryabhattai*	19.4	1.0000	1.0000	<.0001	0.9996
Bar14	*Bacillus aryabhattai*	20.3	1.0000	1.0000	<.0001	0.9969
Bar15	*Bacillus aryabhattai*	90.5	<.0001	<.0001	1.0000	<.0001
Bar16	*Bacillus aryabhattai*	64.9	0.0180	0.0033	0.2079	<.0001
Bar17	*Bacillus aryabhattai*	14.8	1.0000	1.0000	<.0001	1.0000
Bar18	*Bacillus aryabhattai*	27.5	1.0000	1.0000	<.0001	0.4728
Bar19	*Bacillus aryabhattai*	8.1	1.0000	1.0000	<.0001	1.0000
Bar20	*Bacillus aryabhattai*	27.0	1.0000	1.0000	<.0001	0.5278
Bar21	*Bacillus aryabhattai*	57.5	0.0136	0.0016	0.0011	<.0001
Bar22	*Bacillus aryabhattai*	28.9	1.0000	1.0000	<.0001	0.3562
Bar23	*Bacillus aryabhattai*	25.9	1.0000	1.0000	<.0001	0.4167
Bar24	*Bacillus aryabhattai*	17.6	1.0000	1.0000	<.0001	1.0000
Bar25	*Bacillus aryabhattai*	34.1	1.0000	0.9943	<.0001	0.0864
Bar26	*Bacillus aryabhattai*	23.0	1.0000	1.0000	<.0001	0.9065
Bar27	*Bacillus aryabhattai*	49.4	0.2090	0.0478	<.0001	<.0001
Bar28	*Bacillus aryabhattai*	46.7	0.3950	0.1141	<.0001	0.0004
Bar29	*Bacillus aryabhattai*	4.7	0.9998	1.0000	<.0001	1.0000
Bar30	*Bacillus aryabhattai*	7.3	1.0000	1.0000	<.0001	1.0000
Bar31	*Bacillus aryabhattai*	43.6	0.6961	0.2660	<.0001	0.0019
Bar32	*Bacillus aryabhattai*	4.2	0.9994	1.0000	<.0001	1.0000
Bar33	*Bacillus aryabhattai*	2.6	0.9845	1.0000	<.0001	1.0000
Bar34	*Bacillus aryabhattai*	0.0	0.8384	0.9999	<.0001	1.0000
Bar35	*Bacillus aryabhattai*	15.8	1.0000	1.0000	<.0001	1.0000
Bar36	*Bacillus aryabhattai*	19.3	1.0000	1.0000	<.0001	0.9998
Bar37	*Bacillus aryabhattai*	11.8	1.0000	1.0000	<.0001	1.0000
Bar38	*Bacillus aryabhattai*	5.5	1.0000	1.0000	<.0001	1.0000
Bar39	*Bacillus aryabhattai*	10.0	1.0000	1.0000	<.0001	1.0000
Bar40	*Bacillus aryabhattai*	1.5	0.9446	1.0000	<.0001	1.0000
Bar41	*Bacillus aryabhattai*	5.3	1.0000	1.0000	<.0001	1.0000
Bar42	*Bacillus aryabhattai*	7.5	1.0000	1.0000	<.0001	1.0000
Bar43	*Bacillus aryabhattai*	2.1	0.9705	1.0000	<.0001	1.0000
Bar44	*Bacillus aryabhattai*	1.1	0.9246	1.0000	<.0001	1.0000
Bar45	*Bacillus aryabhattai*	10.3	1.0000	1.0000	<.0001	1.0000
Bar46	*Bacillus aryabhattai*	33.1	1.0000	1.0000	<.0001	0.4749
Bar47	*Bacillus aryabhattai*	0.7	0.8939	1.0000	<.0001	1.0000
Bar48	*Bacillus aryabhattai*	21.7	1.0000	1.0000	<.0001	0.9746
Bar49	*Bacillus aryabhattai*	10.8	1.0000	1.0000	<.0001	1.0000
Bar50	*Bacillus aryabhattai*	2.7	0.9871	1.0000	<.0001	1.0000
Bar51	*Bacillus aryabhattai*	47.0	0.8294	0.4394	0.0007	0.0155
Bar52	*Bacillus aryabhattai*	9.5	1.0000	1.0000	<.0001	1.0000

Bar53	*Bacillus aryabhattai*	27.1	1.0000	1.0000	<.0001	0.5132
Bce1	*Bacillus cereus*	4.8	1.0000	1.0000	<.0001	1.0000
Bce2	*Bacillus cereus*	7.5	1.0000	1.0000	<.0001	1.0000
Bce3	*Bacillus cereus*	3.1	0.9926	1.0000	<.0001	1.0000
Bce4	*Bacillus cereus*	1.2	0.9307	1.0000	<.0001	1.0000
Bce5	*Bacillus cereus*	22.8	1.0000	1.0000	<.0001	0.9231
Bce6	*Bacillus cereus*	5.1	1.0000	1.0000	<.0001	1.0000
Bce7	*Bacillus cereus*	23.1	1.0000	1.0000	<.0001	0.8994
Bce8	*Bacillus cereus*	16.2	1.0000	1.0000	<.0001	1.0000
Bce9	*Bacillus cereus*	4.7	0.9998	1.0000	<.0001	1.0000
Bce10	*Bacillus cereus*	15.3	1.0000	1.0000	<.0001	1.0000
Bce11	*Bacillus cereus*	15.1	1.0000	1.0000	<.0001	1.0000
Bce12	*Bacillus cereus*	32.7	1.0000	0.9998	<.0001	0.1333
Bce13	*Bacillus cereus*	15.7	1.0000	1.0000	<.0001	1.0000
Bce14	*Bacillus cereus*	1.5	0.9459	1.0000	<.0001	1.0000
Bce15	*Bacillus cereus*	0.0	0.8384	0.9999	<.0001	1.0000
Bce16	*Bacillus cereus*	0.0	0.8384	0.9999	<.0001	1.0000
Bce17	*Bacillus cereus*	0.0	0.8384	0.9999	<.0001	1.0000
Bce18	*Bacillus cereus*	1.7	0.9544	1.0000	<.0001	1.0000
Bce19	*Bacillus cereus*	2.2	0.9738	1.0000	<.0001	1.0000
Bce20	*Bacillus cereus*	23.9	1.0000	1.0000	<.0001	0.8384
Bce21	*Bacillus cereus*	10.4	1.0000	1.0000	<.0001	1.0000
Bce22	*Bacillus cereus*	5.1	1.0000	1.0000	<.0001	1.0000
Bce23	*Bacillus cereus*	13.1	1.0000	1.0000	<.0001	1.0000
Bce24	*Bacillus cereus*	9.4	1.0000	1.0000	<.0001	1.0000
Bce25	*Bacillus cereus*	3.7	0.9979	1.0000	<.0001	1.0000
Bce26	*Bacillus cereus*	3.9	0.9985	1.0000	<.0001	1.0000
Bce27	*Bacillus cereus*	0.7	0.8957	1.0000	<.0001	1.0000
Bce28	*Bacillus cereus*	6.1	1.0000	1.0000	<.0001	1.0000
Bce29	*Bacillus cereus*	2.9	0.9897	1.0000	<.0001	1.0000
Bce30	*Bacillus cereus*	1.4	0.9393	1.0000	<.0001	1.0000
Bce31	*Bacillus cereus*	0.6	0.8882	1.0000	<.0001	1.0000
Bce32	*Bacillus cereus*	22.5	1.0000	1.0000	<.0001	0.9393
Bce33	*Bacillus cereus*	12.2	1.0000	1.0000	<.0001	1.0000
Bce34	*Bacillus cereus*	5.5	1.0000	1.0000	<.0001	1.0000
Bce35	*Bacillus cereus*	12.7	1.0000	1.0000	<.0001	1.0000
Bce36	*Bacillus cereus*	9.6	1.0000	1.0000	<.0001	1.0000
Bce37	*Bacillus cereus*	0.4	0.8725	1.0000	<.0001	1.0000
Bce38	*Bacillus cereus*	10.4	1.0000	1.0000	<.0001	1.0000
Bce39	*Bacillus cereus*	2.3	0.9762	1.0000	<.0001	1.0000
Bce40	*Bacillus cereus*	4.5	0.9997	1.0000	<.0001	1.0000
Bce41	*Bacillus cereus*	38.4	0.9981	0.7347	<.0001	0.0189
Bce42	*Bacillus cereus*	48.3	0.2775	0.0701	<.0001	0.0002
Bce43	*Bacillus cereus*	22.5	1.0000	1.0000	<.0001	0.9406
Bce44	*Bacillus cereus*	13.9	1.0000	1.0000	<.0001	1.0000
Bce45	*Bacillus cereus*	37.6	0.9998	0.8133	<.0001	0.0258
Bce46	*Bacillus cereus*	26.1	1.0000	1.0000	<.0001	0.6134
Bce47	*Bacillus cereus*	24.4	1.0000	1.0000	<.0001	0.7919
Bce48	*Bacillus cereus*	0.6	0.8882	1.0000	<.0001	1.0000
Bce49	*Bacillus cereus*	6.9	1.0000	1.0000	<.0001	1.0000
Bce50	*Bacillus cereus*	8.2	1.0000	1.0000	<.0001	1.0000
Bce51	*Bacillus cereus*	10.6	1.0000	1.0000	<.0001	1.0000
Bfi1	*Bacillus firmus*	12.7	1.0000	1.0000	<.0001	1.0000
Bga1	*Bacillus galliciensis*	7.8	1.0000	1.0000	<.0001	1.0000
Ble1	*Bacillus lentus*	74.2	<.0001	<.0001	0.4208	<.0001
Bmo1	*Bacillus mojavensis*	0.6	0.8863	1.0000	<.0001	1.0000
Bmo2	*Bacillus mojavensis*	6.5	1.0000	1.0000	<.0001	1.0000
Bmo3	*Bacillus mojavensis*	54.5	0.2720	0.0907	0.0117	0.0010
Bmo4	*Bacillus mojavensis*	1.0	0.9183	1.0000	<.0001	1.0000
Bmt1	*Bacillus methylotrophicus*	1.6	0.9497	1.0000	<.0001	1.0000
Bmt2	*Bacillus methylotrophicus*	37.3	0.9999	0.8384	<.0001	0.0286
Bmt3	*Bacillus methylotrophicus*	3.3	0.9955	1.0000	<.0001	1.0000
Bmt4	*Bacillus methylotrophicus*	1.3	0.9322	1.0000	<.0001	1.0000
Bmt5	*Bacillus methylotrophicus*	0.0	0.8384	0.9999	<.0001	1.0000
Bmt6	*Bacillus methylotrophicus*	9.8	1.0000	1.0000	<.0001	1.0000
Bmt7	*Bacillus methylotrophicus*	38.3	0.9986	0.7499	<.0001	0.0201
Bmt8	*Bacillus methylotrophicus*	1.2	0.9292	1.0000	<.0001	1.0000
Bmt9	*Bacillus methylotrophicus*	2.7	0.9866	1.0000	<.0001	1.0000
Bmt10	*Bacillus methylotrophicus*	51.4	0.4749	0.1896	0.0039	0.0033
Bmt11	*Bacillus methylotrophicus*	12.5	1.0000	1.0000	<.0001	1.0000
Bmt12	*Bacillus methylotrophicus*	11.8	1.0000	1.0000	<.0001	1.0000
Bmt13	*Bacillus methylotrophicus*	9.0	1.0000	1.0000	<.0001	1.0000
Bmy1	*Bacillus mycoides*	35.2	1.0000	0.9730	<.0001	0.0613
Bmy2	*Bacillus mycoides*	5.1	1.0000	1.0000	<.0001	1.0000
Bmy3	*Bacillus mycoides*	7.8	1.0000	1.0000	<.0001	1.0000
Bmy4	*Bacillus mycoides*	0.0	0.8384	0.9999	<.0001	1.0000
Bmy5	*Bacillus mycoides*	2.0	0.9669	1.0000	<.0001	1.0000
Bmy6	*Bacillus mycoides*	1.8	0.9589	1.0000	<.0001	1.0000
Bmy7	*Bacillus mycoides*	47.9	0.7589	0.3763	0.0010	0.0115
Bmy8	*Bacillus mycoides*	18.7	1.0000	1.0000	<.0001	1.0000

Bmy9	*Bacillus mycoides*	19.8	1.0000	1.0000	<.0001	1.0000
Bmy10	*Bacillus mycoides*	23.1	1.0000	1.0000	<.0001	0.8994
Bmy11	*Bacillus mycoides*	9.7	1.0000	1.0000	<.0001	1.0000
Bmy12	*Bacillus mycoides*	10.4	1.0000	1.0000	<.0001	1.0000
Bmy13	*Bacillus mycoides*	19.4	1.0000	1.0000	<.0001	0.9997
Bmy14	*Bacillus mycoides*	34.2	1.0000	0.9940	<.0001	0.0857
Bmy15	*Bacillus mycoides*	31.3	1.0000	1.0000	<.0001	0.6203
Bmy16	*Bacillus mycoides*	5.2	1.0000	1.0000	<.0001	1.0000
Bmy17	*Bacillus mycoides*	6.5	1.0000	1.0000	<.0001	1.0000
Bmy18	*Bacillus mycoides*	5.4	1.0000	1.0000	<.0001	1.0000
Bmy19	*Bacillus mycoides*	66.9	0.0092	0.0015	0.3115	<.0001
Bmy20	*Bacillus mycoides*	10.3	1.0000	1.0000	<.0001	1.0000
Bmy21	*Bacillus mycoides*	32.4	1.0000	0.9999	<.0001	0.1464
Bmy22	*Bacillus mycoides*	21.0	1.0000	1.0000	<.0001	1.0000
Bmy23	*Bacillus mycoides*	28.0	1.0000	1.0000	0.0002	1.0000
Bmy24	*Bacillus mycoides*	5.8	1.0000	1.0000	<.0001	1.0000
Bmy25	*Bacillus mycoides*	14.2	1.0000	1.0000	<.0001	1.0000
Bmy26	*Bacillus mycoides*	38.7	0.9963	0.7013	<.0001	0.0166
Bmy27	*Bacillus mycoides*	1.1	0.9246	1.0000	<.0001	1.0000
Bmy28	*Bacillus mycoides*	1.0	0.9166	1.0000	<.0001	1.0000
Bmy29	*Bacillus mycoides*	0.6	0.8863	1.0000	<.0001	1.0000
Bmy30	*Bacillus mycoides*	14.9	1.0000	1.0000	<.0001	1.0000
Bmy31	*Bacillus mycoides*	5.3	1.0000	1.0000	<.0001	1.0000
Bmy32	*Bacillus mycoides*	77.7	0.0001	<.0001	0.9947	<.0001
Bmy33	*Bacillus mycoides*	0.0	0.8384	0.9999	<.0001	1.0000
Bmy34	*Bacillus mycoides*	0.0	0.8384	0.9999	<.0001	1.0000
Bmy35	*Bacillus mycoides*	1.2	0.9277	1.0000	<.0001	1.0000
Bmy36	*Bacillus mycoides*	0.0	0.8384	0.9999	<.0001	1.0000
Bmy37	*Bacillus mycoides*	16.4	1.0000	1.0000	<.0001	1.0000
Bmy38	*Bacillus mycoides*	13.9	1.0000	1.0000	<.0001	1.0000
Bmy39	*Bacillus mycoides*	26.5	1.0000	1.0000	<.0001	0.5751
Bmy40	*Bacillus mycoides*	1.0	0.9166	1.0000	<.0001	1.0000
Bmy41	*Bacillus mycoides*	1.9	0.9650	1.0000	<.0001	1.0000
Bmy42	*Bacillus mycoides*	46.2	1.0000	0.9717	0.0389	0.2572
Bmy43	*Bacillus mycoides*	1.8	0.9599	1.0000	<.0001	1.0000
Bmy44	*Bacillus mycoides*	5.8	1.0000	1.0000	<.0001	1.0000
Bps1	*Bacillus psychrosaccharolyticus*	12.6	1.0000	1.0000	<.0001	1.0000
Bps2	*Bacillus psychrosaccharolyticus*	21.6	1.0000	1.0000	<.0001	1.0000
Bps3	*Bacillus psychrosaccharolyticus*	9.7	1.0000	1.0000	<.0001	1.0000
Bps4	*Bacillus psychrosaccharolyticus*	0.0	0.8384	0.9999	<.0001	1.0000
Bps5	*Bacillus psychrosaccharolyticus*	2.9	0.9905	1.0000	<.0001	1.0000
Bpu1	*Bacillus pumilus*	0.0	0.8384	0.9999	<.0001	1.0000
Bpu2	*Bacillus pumilus*	7.0	1.0000	1.0000	<.0001	1.0000
Bpu3	*Bacillus pumilus*	19.1	1.0000	1.0000	<.0001	0.9999
Bpu4	*Bacillus pumilus*	17.7	1.0000	1.0000	<.0001	1.0000
Bpu5	*Bacillus pumilus*	0.0	0.8384	0.9999	<.0001	1.0000
Bpu6	*Bacillus pumilus*	78.4	<.0001	<.0001	0.9982	<.0001
Brep1	*Brevibacterium epidermidis*	32.1	1.0000	1.0000	<.0001	0.1605
Brep2	*Brevibacterium epidermidis*	20.4	1.0000	1.0000	<.0001	0.9968
Brep3	*Brevibacterium epidermidis*	1.2	0.9292	1.0000	<.0001	1.0000
Brep4	*Brevibacterium epidermidis*	1.7	0.9556	1.0000	<.0001	1.0000
Brep5	*Brevibacterium epidermidis*	5.0	0.9999	1.0000	<.0001	1.0000
Brep6	*Brevibacterium epidermidis*	3.8	0.9982	1.0000	<.0001	1.0000
Brep7	*Brevibacterium epidermidis*	1.1	0.9215	1.0000	<.0001	1.0000
Brio1	*Brevibacterium iodinum*	2.6	0.9856	1.0000	<.0001	1.0000
Bsa1	*Bacillus safensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bsa2	*Bacillus safensis*	3.6	0.9974	1.0000	<.0001	1.0000
Bsa3	*Bacillus safensis*	1.6	0.9521	1.0000	<.0001	1.0000
Bsa4	*Bacillus safensis*	2.0	0.9688	1.0000	<.0001	1.0000
Bsa5	*Bacillus safensis*	0.6	0.8882	1.0000	<.0001	1.0000
Bsa6	*Bacillus safensis*	4.7	0.9998	1.0000	<.0001	1.0000
Bsa7	*Bacillus safensis*	0.5	0.8805	1.0000	<.0001	1.0000
Bsa8	*Bacillus safensis*	2.2	0.9746	1.0000	<.0001	1.0000
Bsa9	*Bacillus safensis*	1.1	0.9199	1.0000	<.0001	1.0000
Bsa10	*Bacillus safensis*	13.1	1.0000	1.0000	<.0001	1.0000
Bsa11	*Bacillus safensis*	46.1	0.4453	0.1353	<.0001	0.0005
Bsa12	*Bacillus safensis*	6.4	1.0000	1.0000	<.0001	1.0000
Bsa13	*Bacillus safensis*	14.3	1.0000	1.0000	<.0001	1.0000
Bsa14	*Bacillus safensis*	21.5	1.0000	1.0000	<.0001	0.9790
Bsa15	*Bacillus safensis*	0.6	0.8882	1.0000	<.0001	1.0000
Bsa16	*Bacillus safensis*	33.1	1.0000	0.9994	<.0001	0.1194
Bsa17	*Bacillus safensis*	5.6	1.0000	1.0000	<.0001	1.0000
Bsa18	*Bacillus safensis*	0.6	0.8844	1.0000	<.0001	1.0000
Bsa19	*Bacillus safensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bsa20	*Bacillus safensis*	1.1	0.9231	1.0000	<.0001	1.0000
Bsa21	*Bacillus safensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bsa22	*Bacillus safensis*	5.6	1.0000	1.0000	<.0001	1.0000
Bsa23	*Bacillus safensis*	18.2	1.0000	1.0000	<.0001	1.0000
Bsa24	*Bacillus safensis*	1.5	0.9446	1.0000	<.0001	1.0000
Bsa25	*Bacillus safensis*	62.5	0.0378	0.0079	0.1200	<.0001

Bsa26	*Bacillus safensis*	74.1	0.0006	<.0001	0.8614	<.0001
Bsa27	*Bacillus safensis*	79.2	<.0001	<.0001	0.9997	<.0001
Bsa28	*Bacillus safensis*	9.7	1.0000	1.0000	<.0001	1.0000
Bsa29	*Bacillus safensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bsa30	*Bacillus safensis*	2.2	0.9738	1.0000	<.0001	1.0000
Bsa31	*Bacillus safensis*	25.4	1.0000	1.0000	<.0001	0.6884
Bsa32	*Bacillus safensis*	9.3	1.0000	1.0000	<.0001	1.0000
Bsa33	*Bacillus safensis*	39.4	0.9889	0.6366	<.0001	0.0129
Bsa34	*Bacillus safensis*	3.4	0.9963	1.0000	<.0001	1.0000
Bsa35	*Bacillus safensis*	2.1	0.9697	1.0000	<.0001	1.0000
Bsi1	*Bacillus siamensis*	8.9	1.0000	1.0000	<.0001	0.9955
Bsi2	*Bacillus siamensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bsi3	*Bacillus siamensis*	2.8	0.9885	1.0000	<.0001	1.0000
Bsp1	*Bacillus simplex*	0.8	0.9047	1.0000	<.0001	1.0000
Bsp2	*Bacillus simplex*	60.2	0.0044	0.0004	0.0038	<.0001
Bsp3	*Bacillus simplex*	62.0	0.0437	0.0095	0.1061	<.0001
Bsp4	*Bacillus simplex*	93.9	<.0001	<.0001	1.0000	<.0001
Bsp5	*Bacillus simplex*	22.1	1.0000	1.0000	<.0001	0.9589
Bsp6	*Bacillus simplex*	40.1	0.9688	0.5575	<.0001	0.0093
Bsp7	*Bacillus simplex*	29.3	1.0000	1.0000	<.0001	0.3273
Bsp8	*Bacillus simplex*	55.9	0.2035	0.0626	0.0186	0.0005
Bsp9	*Bacillus simplex*	12.8	1.0000	1.0000	<.0001	1.0000
Bsp10	*Bacillus simplex*	15.7	1.0000	1.0000	<.0001	1.0000
Bsp11	*Bacillus simplex*	8.6	1.0000	1.0000	<.0001	1.0000
Bsp12	*Bacillus simplex*	21.6	1.0000	1.0000	<.0001	0.9762
Bsp13	*Bacillus simplex*	35.2	1.0000	0.9722	<.0001	0.0608
Bsp14	*Bacillus simplex*	20.9	1.0000	1.0000	<.0001	0.9920
Bsp15	*Bacillus simplex*	29.6	1.0000	1.0000	<.0001	0.3035
Bsp16	*Bacillus simplex*	9.8	1.0000	1.0000	<.0001	1.0000
Bsp17	*Bacillus simplex*	2.2	0.9746	1.0000	<.0001	1.0000
Bsp18	*Bacillus simplex*	1.7	0.9533	1.0000	<.0001	1.0000
Bsp19	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp20	*Bacillus simplex*	22.7	1.0000	1.0000	<.0001	0.9262
Bsp21	*Bacillus simplex*	17.8	1.0000	1.0000	<.0001	1.0000
Bsp22	*Bacillus simplex*	0.9	0.9099	1.0000	<.0001	1.0000
Bsp23	*Bacillus simplex*	11.1	1.0000	1.0000	<.0001	1.0000
Bsp24	*Bacillus simplex*	44.8	0.5776	0.1983	<.0001	0.0011
Bsp25	*Bacillus simplex*	3.2	0.9938	1.0000	<.0001	1.0000
Bsp26	*Bacillus simplex*	64.5	0.0201	0.0038	0.1927	<.0001
Bsp27	*Bacillus simplex*	2.9	0.9897	1.0000	<.0001	1.0000
Bsp28	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp29	*Bacillus simplex*	3.9	0.9985	1.0000	<.0001	1.0000
Bsp30	*Bacillus simplex*	5.4	1.0000	1.0000	<.0001	1.0000
Bsp31	*Bacillus simplex*	3.9	0.9985	1.0000	<.0001	1.0000
Bsp32	*Bacillus simplex*	14.4	1.0000	1.0000	<.0001	1.0000
Bsp33	*Bacillus simplex*	12.0	1.0000	1.0000	<.0001	1.0000
Bsp34	*Bacillus simplex*	5.9	1.0000	1.0000	<.0001	1.0000
Bsp35	*Bacillus simplex*	31.4	1.0000	1.0000	<.0001	0.1918
Bsp36	*Bacillus simplex*	11.4	1.0000	1.0000	<.0001	1.0000
Bsp37	*Bacillus simplex*	12.2	1.0000	1.0000	<.0001	1.0000
Bsp38	*Bacillus simplex*	5.2	1.0000	1.0000	<.0001	1.0000
Bsp39	*Bacillus simplex*	3.3	0.9955	1.0000	<.0001	1.0000
Bsp40	*Bacillus simplex*	4.7	0.9999	1.0000	<.0001	1.0000
Bsp41	*Bacillus simplex*	2.0	0.9679	1.0000	<.0001	1.0000
Bsp42	*Bacillus simplex*	21.1	1.0000	1.0000	<.0001	0.9885
Bsp43	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp44	*Bacillus simplex*	7.3	1.0000	1.0000	<.0001	1.0000
Bsp45	*Bacillus simplex*	4.3	0.9995	1.0000	<.0001	1.0000
Bsp46	*Bacillus simplex*	34.4	1.0000	0.9916	<.0001	0.0804
Bsp47	*Bacillus simplex*	15.8	1.0000	1.0000	<.0001	1.0000
Bsp48	*Bacillus simplex*	3.6	0.9648	1.0000	<.0001	1.0000
Bsp49	*Bacillus simplex*	5.8	1.0000	1.0000	<.0001	1.0000
Bsp50	*Bacillus simplex*	8.2	1.0000	1.0000	<.0001	1.0000
Bsp51	*Bacillus simplex*	10.0	1.0000	1.0000	<.0001	1.0000
Bsp52	*Bacillus simplex*	1.8	0.9578	1.0000	<.0001	1.0000
Bsp53	*Bacillus simplex*	81.9	<.0001	<.0001	1.0000	<.0001
Bsp54	*Bacillus simplex*	3.0	0.9923	1.0000	<.0001	1.0000
Bsp55	*Bacillus simplex*	2.9	0.9909	1.0000	<.0001	1.0000
Bsp56	*Bacillus simplex*	6.2	1.0000	1.0000	<.0001	1.0000
Bsp57	*Bacillus simplex*	28.1	1.0000	1.0000	<.0001	0.4186
Bsp58	*Bacillus simplex*	6.4	1.0000	1.0000	<.0001	1.0000
Bsp59	*Bacillus simplex*	10.9	1.0000	1.0000	<.0001	1.0000
Bsp60	*Bacillus simplex*	7.1	1.0000	1.0000	<.0001	1.0000
Bsp61	*Bacillus simplex*	8.5	1.0000	1.0000	<.0001	1.0000
Bsp62	*Bacillus simplex*	7.3	1.0000	1.0000	<.0001	1.0000
Bsp63	*Bacillus simplex*	4.6	0.9998	1.0000	<.0001	1.0000
Bsp64	*Bacillus simplex*	16.5	1.0000	1.0000	<.0001	1.0000
Bsp65	*Bacillus simplex*	8.3	1.0000	1.0000	<.0001	1.0000
Bsp66	*Bacillus simplex*	25.8	1.0000	1.0000	<.0001	0.6495
Bsp67	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000

Bsp68	*Bacillus simplex*	87.1	<.0001	<.0001	1.0000	<.0001
Bsp69	*Bacillus simplex*	24.3	1.0000	1.0000	<.0001	0.7967
Bsp70	*Bacillus simplex*	19.8	1.0000	1.0000	<.0001	0.9992
Bsp71	*Bacillus simplex*	31.7	1.0000	1.0000	<.0001	0.1792
Bsp72	*Bacillus simplex*	23.3	1.0000	1.0000	<.0001	0.8863
Bsp73	*Bacillus simplex*	17.1	1.0000	1.0000	<.0001	1.0000
Bsp74	*Bacillus simplex*	10.5	1.0000	1.0000	<.0001	1.0000
Bsp75	*Bacillus simplex*	5.6	1.0000	1.0000	<.0001	1.0000
Bsp76	*Bacillus simplex*	14.5	1.0000	1.0000	<.0001	1.0000
Bsp77	*Bacillus simplex*	23.1	1.0000	1.0000	<.0001	0.8975
Bsp78	*Bacillus simplex*	0.8	0.9047	1.0000	<.0001	1.0000
Bsp79	*Bacillus simplex*	49.4	0.2104	0.0483	<.0001	<.0001
Bsp80	*Bacillus simplex*	12.0	1.0000	1.0000	<.0001	1.0000
Bsp81	*Bacillus simplex*	9.6	1.0000	1.0000	<.0001	1.0000
Bsp82	*Bacillus simplex*	4.3	0.9995	1.0000	<.0001	1.0000
Bsp83	*Bacillus simplex*	2.7	0.9871	1.0000	<.0001	1.0000
Bsp84	*Bacillus simplex*	39.0	1.0000	0.9826	<.0001	0.1470
Bsp85	*Bacillus simplex*	0.9	0.9065	1.0000	<.0001	1.0000
Bsp86	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp87	*Bacillus simplex*	4.9	1.0000	1.0000	<.0001	1.0000
Bsp88	*Bacillus simplex*	22.0	1.0000	1.0000	<.0001	0.9630
Bsp89	*Bacillus simplex*	38.1	0.9991	0.7674	<.0001	0.0215
Bsp90	*Bacillus simplex*	52.2	0.0340	0.0038	<.0001	<.0001
Bsp91	*Bacillus simplex*	10.6	1.0000	1.0000	<.0001	1.0000
Bsp92	*Bacillus simplex*	7.9	1.0000	1.0000	<.0001	1.0000
Bsp93	*Bacillus simplex*	26.4	1.0000	1.0000	<.0001	0.5802
Bsp94	*Bacillus simplex*	44.1	0.6469	0.2363	<.0001	0.0015
Bsp95	*Bacillus simplex*	0.4	0.8725	1.0000	<.0001	1.0000
Bsp96	*Bacillus simplex*	1.3	0.9365	1.0000	<.0001	1.0000
Bsp97	*Bacillus simplex*	1.7	0.9544	1.0000	<.0001	1.0000
Bsp98	*Bacillus simplex*	0.5	0.8785	1.0000	<.0001	1.0000
Bsp99	*Bacillus simplex*	1.5	0.9472	1.0000	<.0001	1.0000
Bsp100	*Bacillus simplex*	7.9	1.0000	1.0000	<.0001	1.0000
Bsp101	*Bacillus simplex*	49.9	0.5910	0.2574	0.0022	0.0057
Bsp102	*Bacillus simplex*	2.1	0.9714	1.0000	<.0001	1.0000
Bsp103	*Bacillus simplex*	49.3	0.2146	0.0495	<.0001	<.0001
Bsp104	*Bacillus simplex*	4.3	0.9995	1.0000	<.0001	1.0000
Bsp105	*Bacillus simplex*	12.0	1.0000	1.0000	<.0001	1.0000
Bsp106	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp107	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp108	*Bacillus simplex*	4.3	0.9995	1.0000	<.0001	1.0000
Bsp109	*Bacillus simplex*	3.1	0.9935	1.0000	<.0001	1.0000
Bsp110	*Bacillus simplex*	4.6	0.9998	1.0000	<.0001	1.0000
Bsp111	*Bacillus simplex*	2.8	0.9885	1.0000	<.0001	1.0000
Bsp112	*Bacillus simplex*	8.0	1.0000	1.0000	<.0001	1.0000
Bsp113	*Bacillus simplex*	63.3	0.0010	<.0001	0.0144	<.0001
Bsp114	*Bacillus simplex*	26.8	1.0000	1.0000	<.0001	0.9455
Bsp115	*Bacillus simplex*	48.2	0.7383	0.3598	0.0011	0.0106
Bsp116	*Bacillus simplex*	9.8	1.0000	1.0000	<.0001	1.0000
Bsp117	*Bacillus simplex*	16.1	1.0000	1.0000	<.0001	1.0000
Bsp118	*Bacillus simplex*	25.8	1.0000	1.0000	<.0001	0.6469
Bsp119	*Bacillus simplex*	38.0	0.9993	0.7773	<.0001	0.0223
Bsp120	*Bacillus simplex*	5.4	1.0000	1.0000	<.0001	1.0000
Bsp121	*Bacillus simplex*	18.7	1.0000	1.0000	<.0001	1.0000
Bsp122	*Bacillus simplex*	2.7	0.9871	1.0000	<.0001	1.0000
Bsp123	*Bacillus simplex*	74.2	0.0005	<.0001	0.8715	<.0001
Bsp124	*Bacillus simplex*	14.3	1.0000	1.0000	<.0001	1.0000
Bsp125	*Bacillus simplex*	6.7	1.0000	1.0000	<.0001	1.0000
Bsp126	*Bacillus simplex*	3.6	0.9972	1.0000	<.0001	1.0000
Bsp127	*Bacillus simplex*	0.8	0.8994	1.0000	<.0001	1.0000
Bsp128	*Bacillus simplex*	18.3	1.0000	1.0000	<.0001	1.0000
Bsp129	*Bacillus simplex*	99.9	<.0001	<.0001	1.0000	<.0001
Bsp130	*Bacillus simplex*	61.6	0.0490	0.0109	0.0960	<.0001
Bsp131	*Bacillus simplex*	1.1	0.9199	1.0000	<.0001	1.0000
Bsp132	*Bacillus simplex*	9.0	1.0000	1.0000	<.0001	1.0000
Bsp133	*Bacillus simplex*	73.7	0.0007	<.0001	0.8329	<.0001
Bsp134	*Bacillus simplex*	13.6	1.0000	1.0000	<.0001	1.0000
Bsp135	*Bacillus simplex*	10.1	1.0000	1.0000	<.0001	1.0000
Bsp136	*Bacillus simplex*	10.9	1.0000	1.0000	<.0001	1.0000
Bsp137	*Bacillus simplex*	7.1	1.0000	1.0000	<.0001	1.0000
Bsp138	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp139	*Bacillus simplex*	67.6	0.0072	0.0011	0.3548	<.0001
Bsp140	*Bacillus simplex*	4.6	0.9998	1.0000	<.0001	1.0000
Bsp141	*Bacillus simplex*	99.9	<.0001	<.0001	1.0000	<.0001
Bsp142	*Bacillus simplex*	6.0	1.0000	1.0000	<.0001	1.0000
Bsp143	*Bacillus simplex*	3.8	1.0000	1.0000	<.0001	1.0000
Bsp144	*Bacillus simplex*	10.8	1.0000	1.0000	<.0001	1.0000
Bsp145	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp146	*Bacillus simplex*	70.9	0.0021	0.0003	0.6075	<.0001
Bsp147	*Bacillus simplex*	6.1	1.0000	1.0000	<.0001	1.0000

Bsp148	*Bacillus simplex*	17.6	1.0000	1.0000	<.0001	1.0000
Bsp149	*Bacillus simplex*	64.7	0.0189	0.0035	0.2013	<.0001
Bsp150	*Bacillus simplex*	2.1	0.9705	1.0000	<.0001	1.0000
Bsp151	*Bacillus simplex*	2.8	0.9889	1.0000	<.0001	1.0000
Bsp152	*Bacillus simplex*	14.7	1.0000	1.0000	<.0001	1.0000
Bsp153	*Bacillus simplex*	89.7	<.0001	<.0001	1.0000	<.0001
Bsp154	*Bacillus simplex*	48.2	0.7383	0.3598	0.0011	0.0106
Bsp155	*Bacillus simplex*	4.8	0.9999	1.0000	<.0001	1.0000
Bsp156	*Bacillus simplex*	3.4	0.9961	1.0000	<.0001	1.0000
Bsp157	*Bacillus simplex*	16.6	1.0000	1.0000	<.0001	1.0000
Bsp158	*Bacillus simplex*	30.8	1.0000	1.0000	<.0001	0.2245
Bsp159	*Bacillus simplex*	56.8	0.1650	0.0480	0.0251	0.0004
Bsp160	*Bacillus simplex*	9.6	1.0000	1.0000	<.0001	1.0000
Bsp161	*Bacillus simplex*	4.0	0.9989	1.0000	<.0001	1.0000
Bsp162	*Bacillus simplex*	7.1	1.0000	1.0000	<.0001	1.0000
Bsp163	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp164	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp165	*Bacillus simplex*	71.4	<.0001	<.0001	0.2188	<.0001
Bsp166	*Bacillus simplex*	1.8	0.9589	1.0000	<.0001	1.0000
Bsp167	*Bacillus simplex*	1.3	0.9365	1.0000	<.0001	1.0000
Bsp168	*Bacillus simplex*	69.1	0.0042	0.0006	0.4596	<.0001
Bsp169	*Bacillus simplex*	2.6	0.9851	1.0000	<.0001	1.0000
Bsp170	*Bacillus simplex*	3.1	0.9926	1.0000	<.0001	1.0000
Bsp171	*Bacillus simplex*	67.3	0.0079	0.0013	0.3390	<.0001
Bsp172	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp173	*Bacillus simplex*	4.9	0.9999	1.0000	<.0001	1.0000
Bsp174	*Bacillus simplex*	5.3	1.0000	1.0000	<.0001	1.0000
Bsp175	*Bacillus simplex*	6.1	1.0000	1.0000	<.0001	1.0000
Bsp176	*Bacillus simplex*	34.7	1.0000	0.9866	<.0001	0.0730
Bsp177	*Bacillus simplex*	1.4	0.9379	1.0000	<.0001	1.0000
Bsp178	*Bacillus simplex*	1.7	0.9567	1.0000	<.0001	1.0000
Bsp179	*Bacillus simplex*	3.6	0.9975	1.0000	<.0001	1.0000
Bsp180	*Bacillus simplex*	7.3	1.0000	1.0000	<.0001	1.0000
Bsp181	*Bacillus simplex*	5.9	1.0000	1.0000	<.0001	1.0000
Bsp182	*Bacillus simplex*	1.6	0.9521	1.0000	<.0001	1.0000
Bsp183	*Bacillus simplex*	3.3	0.9948	1.0000	<.0001	1.0000
Bsp184	*Bacillus simplex*	4.8	0.9999	1.0000	<.0001	1.0000
Bsp185	*Bacillus simplex*	9.5	1.0000	1.0000	<.0001	1.0000
Bsp186	*Bacillus simplex*	8.3	1.0000	1.0000	<.0001	1.0000
Bsp187	*Bacillus simplex*	35.6	1.0000	0.9589	<.0001	0.0540
Bsp188	*Bacillus simplex*	73.0	0.0009	0.0001	0.7829	<.0001
Bsp189	*Bacillus simplex*	7.3	1.0000	1.0000	<.0001	1.0000
Bsp190	*Bacillus simplex*	2.2	0.9754	1.0000	<.0001	1.0000
Bsp191	*Bacillus simplex*	2.5	0.9822	1.0000	<.0001	1.0000
Bsp192	*Bacillus simplex*	15.1	1.0000	1.0000	<.0001	1.0000
Bsp193	*Bacillus simplex*	4.6	0.9998	1.0000	<.0001	1.0000
Bsp194	*Bacillus simplex*	7.8	1.0000	1.0000	<.0001	1.0000
Bsp195	*Bacillus simplex*	32.1	1.0000	1.0000	<.0001	0.1594
Bsp196	*Bacillus simplex*	95.1	<.0001	<.0001	1.0000	<.0001
Bsp197	*Bacillus simplex*	38.2	0.9987	0.7524	<.0001	0.0203
Bsp198	*Bacillus simplex*	48.4	0.2692	0.0672	<.0001	0.0002
Bsp199	*Bacillus simplex*	41.0	0.9246	0.4705	<.0001	0.0063
Bsp200	*Bacillus simplex*	30.9	1.0000	1.0000	<.0001	0.2174
Bsp201	*Bacillus simplex*	1.7	0.9567	1.0000	<.0001	1.0000
Bsp202	*Bacillus simplex*	14.7	1.0000	1.0000	<.0001	1.0000
Bsp203	*Bacillus simplex*	13.2	1.0000	1.0000	<.0001	1.0000
Bsp204	*Bacillus simplex*	21.1	1.0000	1.0000	<.0001	0.9164
Bsp205	*Bacillus simplex*	5.5	1.0000	1.0000	<.0001	1.0000
Bsp206	*Bacillus simplex*	0.0	0.8384	0.9999	<.0001	1.0000
Bsp207	*Bacillus simplex*	2.2	0.9738	1.0000	<.0001	1.0000
Bsp208	*Bacillus simplex*	27.5	1.0000	1.0000	<.0001	0.4728
Bssin1	*Bacillus subtilis* subsp. *inaquosorum*	0.0	0.8384	0.9999	<.0001	1.0000
Bssin2	*Bacillus subtilis* subsp. *inaquosorum*	1.0	0.9166	1.0000	<.0001	1.0000
Bssin3	*Bacillus subtilis* subsp. *inaquosorum*	7.5	1.0000	1.0000	<.0001	1.0000
Bssin4	*Bacillus subtilis* subsp. *inaquosorum*	6.8	1.0000	1.0000	<.0001	1.0000
Bssin5	*Bacillus subtilis* subsp. *inaquosorum*	14.4	1.0000	1.0000	<.0001	1.0000
Bssin6	*Bacillus subtilis* subsp. *inaquosorum*	25.6	1.0000	1.0000	<.0001	0.6625
Bssin7	*Bacillus subtilis* subsp. *inaquosorum*	2.4	0.9810	1.0000	<.0001	1.0000
Bssin8	*Bacillus subtilis* subsp. *inaquosorum*	4.6	0.9998	1.0000	<.0001	1.0000
Bssin9	*Bacillus subtilis* subsp. *inaquosorum*	4.8	0.9999	1.0000	<.0001	1.0000
Bssin10	*Bacillus subtilis* subsp. *inaquosorum*	6.1	1.0000	1.0000	<.0001	1.0000
Bssin11	*Bacillus subtilis* subsp. *inaquosorum*	0.0	0.8384	0.9999	<.0001	1.0000
Bssin12	*Bacillus subtilis* subsp. *inaquosorum*	0.0	0.8384	0.9999	<.0001	1.0000
Bssin13	*Bacillus subtilis* subsp. *inaquosorum*	1.4	0.9379	1.0000	<.0001	1.0000
Bssin14	*Bacillus subtilis* subsp. *inaquosorum*	1.7	0.9533	1.0000	<.0001	1.0000
Bssin15	*Bacillus subtilis* subsp. *inaquosorum*	0.5	0.8805	1.0000	<.0001	1.0000
Bsssu1	*Bacillus subtilis* subsp. *subtilis*	0.0	0.8384	0.9999	<.0001	1.0000
Bsssu2	*Bacillus subtilis* subsp. *subtilis*	74.8	0.0004	<.0001	0.9084	<.0001
Bsssu3	*Bacillus subtilis* subsp. *subtilis*	74.2	0.0005	<.0001	0.8715	<.0001
Bsssu4	*Bacillus subtilis* subsp. *subtilis*	0.7	0.8920	1.0000	<.0001	1.0000

Bte1	*Bacillus tequilensis*	29.6	1.0000	1.0000	<.0001	0.3035
Bte2	*Bacillus tequilensis*	23.7	1.0000	1.0000	<.0001	0.8537
Bte3	*Bacillus tequilensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bth1	*Bacillus thuringiensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bth2	*Bacillus thuringiensis*	2.6	0.9845	1.0000	<.0001	1.0000
Bth3	*Bacillus thuringiensis*	2.8	0.9889	1.0000	<.0001	1.0000
Bth4	*Bacillus thuringiensis*	1.6	0.9497	1.0000	<.0001	1.0000
Bto1	*Bacillus toyonensis*	3.2	0.9943	1.0000	<.0001	1.0000
Bto2	*Bacillus toyonensis*	14.2	1.0000	1.0000	<.0001	1.0000
Bto3	*Bacillus toyonensis*	3.1	0.9926	1.0000	<.0001	1.0000
Bto4	*Bacillus toyonensis*	11.0	1.0000	1.0000	<.0001	1.0000
Bto5	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto6	*Bacillus toyonensis*	5.2	1.0000	1.0000	<.0001	1.0000
Bto7	*Bacillus toyonensis*	13.2	1.0000	1.0000	<.0001	1.0000
Bto8	*Bacillus toyonensis*	7.8	1.0000	1.0000	<.0001	1.0000
Bto9	*Bacillus toyonensis*	5.7	1.0000	1.0000	<.0001	1.0000
Bto10	*Bacillus toyonensis*	64.7	0.0005	<.0001	0.0250	<.0001
Bto11	*Bacillus toyonensis*	62.7	0.0013	0.0001	0.0114	<.0001
Bto12	*Bacillus toyonensis*	1.9	0.9650	1.0000	<.0001	1.0000
Bto13	*Bacillus toyonensis*	21.0	1.0000	1.0000	<.0001	0.9905
Bto14	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto15	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto16	*Bacillus toyonensis*	13.5	1.0000	1.0000	<.0001	1.0000
Bto17	*Bacillus toyonensis*	0.7	0.8939	1.0000	<.0001	1.0000
Bto18	*Bacillus toyonensis*	5.5	1.0000	1.0000	<.0001	1.0000
Bto19	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto20	*Bacillus toyonensis*	5.9	1.0000	1.0000	<.0001	1.0000
Bto21	*Bacillus toyonensis*	11.5	1.0000	1.0000	<.0001	1.0000
Bto22	*Bacillus toyonensis*	64.8	0.0004	<.0001	0.0265	<.0001
Bto23	*Bacillus toyonensis*	51.1	0.1304	0.0258	<.0001	<.0001
Bto24	*Bacillus toyonensis*	8.7	1.0000	1.0000	<.0001	1.0000
Bto25	*Bacillus toyonensis*	18.2	1.0000	1.0000	<.0001	1.0000
Bto26	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto27	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto28	*Bacillus toyonensis*	6.0	1.0000	1.0000	<.0001	1.0000
Bto29	*Bacillus toyonensis*	17.3	1.0000	1.0000	<.0001	1.0000
Bto30	*Bacillus toyonensis*	36.9	1.0000	0.8765	<.0001	0.0338
Bto31	*Bacillus toyonensis*	3.8	0.9982	1.0000	<.0001	1.0000
Bto32	*Bacillus toyonensis*	10.3	1.0000	1.0000	<.0001	1.0000
Bto33	*Bacillus toyonensis*	0.3	0.8664	1.0000	<.0001	1.0000
Bto34	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto35	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto36	*Bacillus toyonensis*	1.1	0.9246	1.0000	<.0001	1.0000
Bto37	*Bacillus toyonensis*	0.5	0.8785	1.0000	<.0001	1.0000
Bto38	*Bacillus toyonensis*	31.7	1.0000	1.0000	<.0001	0.1792
Bto39	*Bacillus toyonensis*	0.8	0.9047	1.0000	<.0001	1.0000
Bto40	*Bacillus toyonensis*	1.0	0.9133	1.0000	<.0001	1.0000
Bto41	*Bacillus toyonensis*	1.6	0.9484	1.0000	<.0001	1.0000
Bto42	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto43	*Bacillus toyonensis*	1.6	0.9509	1.0000	<.0001	1.0000
Bto44	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto45	*Bacillus toyonensis*	11.9	1.0000	1.0000	<.0001	1.0000
Bto46	*Bacillus toyonensis*	0.6	0.8882	1.0000	<.0001	1.0000
Bto47	*Bacillus toyonensis*	0.9	0.9099	1.0000	<.0001	1.0000
Bto48	*Bacillus toyonensis*	0.6	0.8901	1.0000	<.0001	1.0000
Bto49	*Bacillus toyonensis*	0.4	0.8725	1.0000	<.0001	1.0000
Bto50	*Bacillus toyonensis*	1.8	0.9599	1.0000	<.0001	1.0000
Bto51	*Bacillus toyonensis*	67.6	<.0001	<.0001	0.0718	<.0001
Bto52	*Bacillus toyonensis*	1.9	0.9620	1.0000	<.0001	1.0000
Bto53	*Bacillus toyonensis*	20.3	1.0000	1.0000	<.0001	0.9971
Bto54	*Bacillus toyonensis*	33.1	1.0000	0.9994	<.0001	0.1185
Bto55	*Bacillus toyonensis*	42.2	0.8384	0.3722	<.0001	0.0038
Bto56	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto57	*Bacillus toyonensis*	34.2	1.0000	0.9932	<.0001	0.0837
Bto58	*Bacillus toyonensis*	1.1	0.9215	1.0000	<.0001	1.0000
Bto59	*Bacillus toyonensis*	1.7	0.9567	1.0000	<.0001	1.0000
Bto60	*Bacillus toyonensis*	0.9	0.9116	1.0000	<.0001	1.0000
Bto61	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto62	*Bacillus toyonensis*	1.7	0.9544	1.0000	<.0001	1.0000
Bto63	*Bacillus toyonensis*	0.4	0.8745	1.0000	<.0001	1.0000
Bto64	*Bacillus toyonensis*	5.1	1.0000	1.0000	<.0001	1.0000
Bto65	*Bacillus toyonensis*	1.0	0.9133	1.0000	<.0001	1.0000
Bto66	*Bacillus toyonensis*	0.8	0.9012	1.0000	<.0001	1.0000
Bto67	*Bacillus toyonensis*	3.5	0.9969	1.0000	<.0001	1.0000
Bto68	*Bacillus toyonensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bto69	*Bacillus toyonensis*	19.3	1.0000	1.0000	<.0001	0.9998
Bto70	*Bacillus toyonensis*	0.9	0.9116	1.0000	<.0001	1.0000
Bve1	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve2	*Bacillus velezensis*	54.7	0.2613	0.0861	0.0125	0.0009
Bve3	*Bacillus velezensis*	11.4	1.0000	1.0000	<.0001	1.0000

Bve4	*Bacillus velezensis*	32.1	1.0000	1.0000	<.0001	0.1572
Bve5	*Bacillus velezensis*	10.2	1.0000	1.0000	<.0001	1.0000
Bve6	*Bacillus velezensis*	7.1	1.0000	1.0000	<.0001	1.0000
Bve7	*Bacillus velezensis*	7.9	1.0000	1.0000	<.0001	1.0000
Bve8	*Bacillus velezensis*	17.7	1.0000	1.0000	<.0001	1.0000
Bve9	*Bacillus velezensis*	15.0	1.0000	1.0000	<.0001	1.0000
Bve10	*Bacillus velezensis*	11.8	1.0000	1.0000	<.0001	1.0000
Bve11	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve12	*Bacillus velezensis*	46.4	0.8749	0.4883	0.0005	0.0192
Bve13	*Bacillus velezensis*	39.8	1.0000	0.9616	<.0001	0.1216
Bve14	*Bacillus velezensis*	15.3	1.0000	1.0000	<.0001	1.0000
Bve15	*Bacillus velezensis*	20.7	1.0000	1.0000	<.0001	1.0000
Bve16	*Bacillus velezensis*	23.8	1.0000	1.0000	<.0001	0.8472
Bve17	*Bacillus velezensis*	17.0	1.0000	1.0000	<.0001	1.0000
Bve18	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve19	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve20	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve21	*Bacillus velezensis*	9.4	1.0000	1.0000	<.0001	1.0000
Bve22	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve23	*Bacillus velezensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bve24	*Bacillus velezensis*	1.9	0.9650	1.0000	<.0001	1.0000
Bve25	*Bacillus velezensis*	1.1	0.9231	1.0000	<.0001	1.0000
Bve26	*Bacillus velezensis*	4.9	0.9999	1.0000	<.0001	1.0000
Bve27	*Bacillus velezensis*	2.3	0.9769	1.0000	<.0001	1.0000
Bve28	*Bacillus velezensis*	2.7	0.9866	1.0000	<.0001	1.0000
Bve29	*Bacillus velezensis*	3.8	0.9982	1.0000	<.0001	1.0000
Bve30	*Bacillus velezensis*	0.9	0.9082	1.0000	<.0001	1.0000
Bve31	*Bacillus velezensis*	33.9	1.0000	0.9961	<.0001	0.0920
Bve32	*Bacillus velezensis*	1.1	0.9215	1.0000	<.0001	1.0000
Bve33	*Bacillus velezensis*	15.1	1.0000	1.0000	<.0001	1.0000
Bve34	*Bacillus velezensis*	6.7	1.0000	1.0000	<.0001	1.0000
Bve35	*Bacillus velezensis*	4.8	0.9999	1.0000	<.0001	1.0000
Bve36	*Bacillus velezensis*	13.6	1.0000	1.0000	<.0001	1.0000
Bve37	*Bacillus velezensis*	39.2	1.0000	0.9795	<.0001	0.1420
Bve38	*Bacillus velezensis*	2.7	0.9876	1.0000	<.0001	1.0000
Bve39	*Bacillus velezensis*	3.3	0.9955	1.0000	<.0001	1.0000
Bve40	*Bacillus velezensis*	12.5	1.0000	1.0000	<.0001	1.0000
Bve41	*Bacillus velezensis*	3.8	0.9982	1.0000	<.0001	1.0000
Bwe1	*Bacillus weihenstephanensis*	8.0	1.0000	1.0000	<.0001	1.0000
Bwe2	*Bacillus weihenstephanensis*	21.1	1.0000	1.0000	<.0001	0.9889
Bwe3	*Bacillus weihenstephanensis*	31.5	1.0000	1.0000	<.0001	0.1892
Bwe4	*Bacillus weihenstephanensis*	47.0	0.3722	0.1050	<.0001	0.0003
Bwe5	*Bacillus weihenstephanensis*	1.2	0.9277	1.0000	<.0001	1.0000
Bwe6	*Bacillus weihenstephanensis*	93.3	<.0001	<.0001	1.0000	<.0001
Bwe7	*Bacillus weihenstephanensis*	0.7	0.8975	1.0000	<.0001	1.0000
Bwe8	*Bacillus weihenstephanensis*	3.4	0.9957	1.0000	<.0001	1.0000
Bwe9	*Bacillus weihenstephanensis*	6.7	1.0000	1.0000	<.0001	1.0000
Bwe10	*Bacillus weihenstephanensis*	1.1	0.9199	1.0000	<.0001	1.0000
Bwe11	*Bacillus weihenstephanensis*	0.6	0.8901	1.0000	<.0001	1.0000
Bwe12	*Bacillus weihenstephanensis*	11.1	1.0000	1.0000	<.0001	1.0000
Bwe13	*Bacillus weihenstephanensis*	1.1	0.9231	1.0000	<.0001	1.0000
Bwe14	*Bacillus weihenstephanensis*	0.0	0.8384	0.9999	<.0001	1.0000
Bwe15	*Bacillus weihenstephanensis*	14.3	1.0000	1.0000	<.0001	1.0000
Bwe16	*Bacillus weihenstephanensis*	17.9	1.0000	1.0000	<.0001	1.0000
Bwe17	*Bacillus weihenstephanensis*	29.9	1.0000	1.0000	<.0001	0.2792
Bwe18	*Bacillus weihenstephanensis*	39.1	0.9932	0.6676	<.0001	0.0146
Bwe19	*Bacillus weihenstephanensis*	1.3	0.9336	1.0000	<.0001	1.0000
Bwe20	*Bacillus weihenstephanensis*	1.1	0.9246	1.0000	<.0001	1.0000
Bwe21	*Bacillus weihenstephanensis*	0.6	0.8901	1.0000	<.0001	1.0000
Enx1	*Enterobacter xiangfangensis*	0.0	0.8384	0.9999	<.0001	1.0000
Exs1	*Exiguobacterium sibiricum*	28.7	1.0000	1.0000	<.0001	0.3702
Fso1	*Fictibacillus solisalsi*	59.6	0.0834	0.0206	0.0572	0.0001
Lma1	*Lysinibacillus macroides*	13.1	1.0000	1.0000	<.0001	1.0000
Lpa1	*Lysinibacillus parviboronicapiens*	20.6	1.0000	1.0000	<.0001	0.9946
Paam1	*Paenibacillus amylolyticus*	16.5	1.0000	1.0000	<.0001	1.0000
Paam2	*Paenibacillus amylolyticus*	26.5	1.0000	1.0000	<.0001	0.5751
Paam3	*Paenibacillus amylolyticus*	22.1	1.0000	1.0000	<.0001	0.9589
Paam4	*Paenibacillus amylolyticus*	11.0	1.0000	1.0000	<.0001	1.0000
Paam5	*Paenibacillus amylolyticus*	11.6	1.0000	1.0000	<.0001	1.0000
Paam6	*Paenibacillus amylolyticus*	4.1	0.9992	1.0000	<.0001	1.0000
Paam7	*Paenibacillus amylolyticus*	4.4	0.9996	1.0000	<.0001	1.0000
Paam8	*Paenibacillus amylolyticus*	3.4	0.9961	1.0000	<.0001	1.0000
Paam9	*Paenibacillus amylolyticus*	9.6	1.0000	1.0000	<.0001	1.0000
Paba1	*Paenibacillus barcinonensis*	17.4	1.0000	1.0000	<.0001	1.0000
Pagl1	*Paenibacillus glycanilyticus*	7.5	1.0000	1.0000	<.0001	1.0000
Pail1	*Paenibacillus illinoisensis*	16.8	1.0000	1.0000	<.0001	1.0000
Pala1	*Paenibacillus lautus*	0.0	0.8384	0.9999	<.0001	1.0000
Pala2	*Paenibacillus lautus*	15.3	1.0000	1.0000	<.0001	1.0000
Pala3	*Paenibacillus lautus*	7.3	1.0000	1.0000	<.0001	1.0000
Pala4	*Paenibacillus lautus*	10.1	1.0000	1.0000	<.0001	1.0000

Pamd1	*Paenibacillus macquariensis* subsp. *defensor*	12.2	1.0000	1.0000	<.0001	1.0000
Panag1	*Pantoea agglomerans*	0.0	0.8384	0.9999	<.0001	1.0000
Paod1	*Paenibacillus odorifer*	25.7	1.0000	1.0000	<.0001	0.6573
Pata1	*Paenibacillus taichungensis*	64.4	0.0211	0.0040	0.1865	<.0001
Path1	*Paenibacillus thiaminolyticus*	1.7	0.9544	1.0000	<.0001	1.0000
Patu1	*Paenibacillus tundrae*	11.7	1.0000	1.0000	<.0001	1.0000
Pava1	*Paenibacillus validus*	13.7	1.0000	1.0000	<.0001	1.0000
Paxy1	*Paenibacillus xylanexedens*	74.8	<.0001	<.0001	0.4681	<.0001
Paxy2	*Paenibacillus xylanexedens*	22.5	1.0000	1.0000	<.0001	0.9379
Rhil1	*Rhizobium larrymoorei*	0.7	0.8975	1.0000	<.0001	1.0000
Rhoq1	*Rhodococcus qingshengii*	0.0	0.8384	0.9999	<.0001	1.0000
Soi1	*Solibacillus isronensis*	8.9	1.0000	1.0000	<.0001	1.0000
Spg1	*Sporosarcina globispora*	22.2	1.0000	1.0000	<.0001	0.9533
Spg2	*Sporosarcina globispora*	1.1	0.9246	1.0000	<.0001	1.0000
Spg3	*Sporosarcina globispora*	6.1	1.0000	1.0000	<.0001	1.0000
Spg4	*Sporosarcina globispora*	20.2	1.0000	1.0000	<.0001	0.9978
Spg5	*Sporosarcina globispora*	45.5	0.5083	0.1639	<.0001	0.0008
Spg6	*Sporosarcina globispora*	18.5	1.0000	1.0000	<.0001	1.0000
Spg7	*Sporosarcina globispora*	2.6	0.9845	1.0000	<.0001	1.0000
Spg8	*Sporosarcina globispora*	6.0	1.0000	1.0000	<.0001	1.0000
Spg9	*Sporosarcina globispora*	26.0	1.0000	1.0000	<.0001	0.6211
Spg10	*Sporosarcina globispora*	0.0	0.8384	0.9999	<.0001	1.0000
Uid1	*Bacillus aerophilus/stratosphaericus**	31.7	1.0000	1.0000	<.0001	0.5868
Uid2	*Bacillus stratosphericus/aerophilus**	11.4	1.0000	1.0000	<.0001	1.0000
Uid3	*Bacillus stratosphericus/aerophilus**	0.0	0.8384	0.9999	<.0001	1.0000
Uid4	*Bacillus stratosphericus/aerophilus**	6.6	1.0000	1.0000	<.0001	1.0000
Uid5	*Bacillus stratosphericus/aerophilus**	10.2	1.0000	1.0000	<.0001	1.0000
Uid6	*Bacillus stratosphericus/aerophilus**	24.4	1.0000	1.0000	<.0001	0.7846
Uid7	*Bacillus stratosphericus/aerophilus**	47.4	0.3386	0.0920	<.0001	0.0003
Uid8	*Bacillus altitudinis/stratosphericus/aerophilus**	0.0	0.8384	0.9999	<.0001	1.0000
Uid9	*Bacillus altitudinis/stratosphericus/aerophilus**	5.1	1.0000	1.0000	<.0001	1.0000
Uid10	*Bacillus altitudinis/stratosphericus/aerophilus**	1.3	0.9336	1.0000	<.0001	1.0000
Uid11	Unidentified species*	8.2	1.0000	1.0000	<.0001	1.0000
Uid12	Unidentified species*	21.8	1.0000	1.0000	<.0001	0.9697
Uid13	Unidentified species*	1.9	0.9650	1.0000	<.0001	1.0000
Control	**Active ingredient**[c]					
Poncho/Votivo	Clothianidin and *B. firmus* I-1582	21.1	…	1.0000	<.0001	0.9885
Clariva	*Pasteuria nishizawae*	16.3	1.0000	…	<.0001	0.0000
Temik	Aldicarb	99.6	<.0001	<.0001	…	<.0001
Untreated control	Sterile distilled water	2.8	0.9885	1.0000	<.0001	…

[a]*In vitro* tests were performed in 96-well plates. Data collected were analyzed in SAS 9.4 using PROC GLIMMIX procedure at significant level of $\alpha \leq 0.05$. *P* value less than 0.05 indicate a significant effect. Adjusted *P* values were obtained according to Dunnett's method. The LS-means are presented in the tables with adjusted *P* values to determine statistical differences.

[b]Mortality was determined by calculating as the following equation: [(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] × 100.

[c]Active ingredients for the nematicides Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Clariva is *Pasteuria nishizawae*, Temik is Aldicarb, and untreated control is sterile distilled water.

[d]Dunnett's option was used in the LSMEANS statement to assess the differences between bacterial isolates and the Poncho/Votivo, Clariva, Temik, and the untreted control.

*Indistinguishable species and unidentified strains.

Appendix 3. PGPR strains were not viable on TSA plate.

Code	Scientific name
Bce52	*Bacillus cereus*
Bpu7	*Bacillus pumilus*
Bsp209	*Bacillus simplex*
Bsp210	*Bacillus simplex*
Bsp211	*Bacillus simplex*
Bsp212	*Bacillus simplex*
Bsp213	*Bacillus simplex*
Bsp214	*Bacillus simplex*
Bsp215	*Bacillus simplex*
Bsp216	*Bacillus simplex*
Bsp217	*Bacillus simplex*
Bsp218	*Bacillus simplex*
Bsp219	*Bacillus simplex*
Bto71	*Bacillus toyonensis*
Pape1	*Paenibacillus peoriae*
Spg11	*Sporosarcina globispora*
Spg12	*Sporosarcina globispora*
Sps1	*Sporosarcina psychrophila*
Uid13	*Bacillus stratosphericus/aerophilus**
Uid14	Unidentified species*
Uid15	Unidentified species*

*Indistinguishable species and unidentified strains.