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Isolation, Identification and Biocontrol Activity of Novel Chitinolytic Bacteria against *Meloidogyne incognita* Infecting *Capsicum annuum* L.

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ABSTRACT

In the era of climate change, environmental degradation caused by the unabated and indiscriminate usage of synthetic nematicides, there is urgent demand to develop safe alternatives for management of nematode diseases. In this respect, this study aimed to isolate, characterize, and evaluate novel chitinolytic rhizobacteria as biocontrol agents against *Meloidogyne incognita* infecting *Capsicum annuum* L. Among seventeen chitinolytic bacterial isolates, which were isolated from nematode suppressive soil, five isolates exhibited high chitinolytic activity. These isolates were identified based on 16S rRNA gene analysis as *Chryseobacterium daecheongense* (isolate AB1), *Bacillus toyonensis* (isolate AB2), *Pseudomonas lini* (isolate AB3), *Lactobacillus helveticus* (isolate AB4) and *Klebsiella oxytoca* (isolate AB5). *In vitro* studies revealed that maximum nematode mortality (100%) was recorded for isolates AB3 and AB5; while maximum egg hatching inhibition (90.86 and 86.27%) in AB3 and AB4, respectively. Under field conditions, maximum inhibition in gall formation, female numbers, egg-mass production, developmental stages and final population of juveniles in soil, was recorded for AB3, AB4 and AB5 isolates, respectively. The accumulation of phenolic compounds, peroxidase and polyphenol oxidase in pepper leaves were induced by different bacterial treatment which played a significant role in resistance of the plant to biotic stress. The histological study showed poor formation of regular giant cells in pepper roots treated with chitinolytic bacterial strains. Root transverse section of pepper plants infected with *M. incognita* and treated with isolates AB3 and AB4 showed maximum healthy pattern and less necrotic points compared to the control. In conclusion, this study demonstrated that among the tested bacterial stains, *P. lini* (AB3), *L. helveticus* (AB4) and *K. oxytoca* (AB5) showed high biocontrol prospects against *M. incognita* and could be introduced and formulated as a promising, affordable and safe bionematicides. Moreover, *C. daecheongense* (AB1) was recorded for the first time for its nematicidal activity.

Keywords: Rhizobacteria, Chitinase, Biological Control, *Meloidogyne incognita*, *Capsicum annuum* L.
Article type: Research Article.

INTRODUCTION

Meloidogyne species, root-knot nematode, is the most extensively studied nematode species attacking plants. Gall formation in roots infected with *Meloidogyne* species results in disruption of nutrient and water absorption causing above-ground symptoms such as stunting, wilting, chlorosis, and decreased crop yields (Collett *et al.* 2021). Plant-parasitic nematodes posed 15% of annual crop yield estimating losses of 100-157 billion USD all over the world (Phani *et al.* 2021). The negative impacts of agrochemicals have created urgent need to develop alternative safe and eco-friendly sustainable approaches. Biological control using rhizobacteria with characteristic metabolites is

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a promising approach that is required for its eco-friendly and sustainable management (Soliman *et al.* 2019; Mohammed Al-Shemmary & Salih Al-Taee 2021; El-Sayed *et al.* 2022; Hanash *et al.* 2022). Sweet pepper (*Capsicum annuum* L.) is a popular and economically-important vegetable crop in Egypt and worldwide due to its high quantities of ascorbic acid and important nutritional elements which provide beneficial impacts on human health (Abdel Aziz & Geeth 2018). It occupies the second rank among vegetable crop areas grown under plastic houses in Egypt (Alkharpotly 2018). Most common pepper varieties are susceptible to the southern root-knot nematode *M. incognita* causing excessive economic losses annually (Sorial *et al.* 2020). Chitin is an aminopolysaccharide that dispersed widely in nature. It is a rigid and resistant structural component that contributes to the mechanical strength of chitin-containing organisms. Chitin can be considered as the key structural constitute of nematode eggshell (Dukariya & Kumar 2020). The disturbance of chitin synthesis or its hydrolysis led to death of nematode embryos, laying defective eggs or moulting failure. Thus, the components involved in the chitin metabolic process are promising targets for development of biological nematicidal agents (Asaturova *et al.* 2022)**.** Enzyme technology is a promising to be used in many eco-friendly industrial sectors. Various microorganisms produce wide range of hydrolytic enzymes working effectively on different substrates, such as cellulose and chitin (Mohammed 2020). Chitinases have received increased attention due to their remarkable role in biotechnological applications, especially in agriculture for controlling phytopathogenic fungi, nematodes and harmful insects (Poria *et al.* 2021) However, the enhancement of microbial production of extracellular novel chitinases by modification of culture medium composition is critical to achieve optimal yield and productivity with a low cost (Doan *et al.* 2021; Subramani *et al.* 2022). Several bacterial species are important sources of bioactive natural compounds including chitinases and have advantages of being cultivated in a short time and could be produced effectively in a large-scale manner (Akeed *et al.* 2020). Several species of *Bacillus* such as *B. pumilus* (Agarwal *et al.* 2018) and *B. licheniformis* (Sasi *et al.* 2020) have shown promising chitinolytic activities. In addition, *Paenibacillus* sp. (Doan *et al.* 2021) *Lactobacillus* species (Horvath-Szanics *et al.* 2020) and rhizobacterial *Pseudomonas* species exhibited remarkable chitinolytic activities and mycolytic action, so that recommended as safe biological control agent (Dukare *et al.* 2020). The objective of the current study was to isolate, characterize and enhance the nematicidal activity of promising chitin-degrading rhizobacterial strains, under both *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Sample collection and isolation of chitinolytic bacteria

For isolation of bacteria, ten soil samples were randomly collected from nematode infected pepper field in Qalyubia Governorate in North-Eastern of Egypt. Plants showing healthy appearance were uprooted and shacked carefully to remove the excess soil around the roots. The adhering soil mixed to represent composite sample. The collected samples were transferred to the laboratory under controlled temperature conditions. The soil samples were mixed gently and transferred to one representative sample which then bi-fold serially diluted in saline (0.85%, NaCl w/v) followed by plating in triplicate on colloidal chitin agar (CCA) medium (Murthy & Bleakley 2012) The colonies surrounded by distinct clear zones on creamish background were considered as chitinase producer and were purified on Luria-Bertani (LB, HiMedia® Laboratories Ltd., Mumbai, India) medium.

Safety assessment of bacterial isolates

Hemolytic activity

The hemolytic activity test was performed by streaking the selected bacterial isolates on Columbia Blood Agar plates supplemented by 5% sheep blood and incubated at 30 °C for 48 h. The strains that sowed green-hued zones around the colonies (α-hemolysis) or did not produce any effect on the blood plates (γ-hemolysis) were considered non hemolytic, while strains produced blood lysis zones around the colonies were classified as hemolytic (β-hemolysis; Mangia *et al.* 2018).

Antibiotic sensitivity test

Seven antibiotics, listed in Table 1, were used for determination of the susceptibility/resistance of the selected bacterial strains to antibiotics. One hundred microliter of the active bacterial suspensions was spread separately on the surface of LB containing plates. The antibiotics discs were placed on surface of plates and incubated at 30°C for 24 h. The results were measured according to the instructions given by the manufacturer (Rzepkowska *et al.* 2017).

Table 1. Antibiotics and their concentrations used in antibiotic sensitivity test.

Number	Antibiotics	Final concentration (µg mL^{-1})
1	Fusidic Acid (FA)	10
2	Cefotaxime (CTX)	30
3	Vancomycin (VA)	30
4	Clindamycin (DA)	2
5	Ceftriaxone (CRO)	30
6	Amikacin (AK)	30
7	Erythromycine (E)	15

Molecular characterization of the selected rhizobacterial isolates

Molecular characterization of the five selected bacterial isolates was done according to the method of Rochelle *et al.* (1995) Genomic DNA was extracted from each single bacterial colony cultured on LB media at 30 °C for 24 h on a rotary shaker at 120 rpm. The culture was centrifuged at 13000 rpm for 5 min at 4°C and pellet was subjected to genomic DNA extraction using the DNeasy mini kit (Qiagen, CA, USA) according to the manufacturer's instructions. The extracted DNA was used as a template for PCR to amplify 16S rRNA gene. In order to analyse the 16S rRNA, forward primer 8F (5′AGT TGA TCC TGG CTC AG 3′) and reverse primer 1492R (5′TAC CTTGTT ACG ACT T3′) were used. Purified PCR products were sent for sequencing at Macrogen (South Korea). For obtaining accession numbers, the deduced sequences were submitted to the GeneBank database. Identification of phylogenetic neighbours and calculation of pairwise similarity of these sequences were performed using the National Centre for Biotechnology Information (NCBI) taxonomy tree building service and DNAMAN ver. 7 software.

Chitinase activity

Chitinase activity was assayed spectrophotometrically by estimating the amount of free reducing sugars formed after colloidal chitin hydrolysis according Somogyi method modified by Neish (Neish 1952). Single colony from each actively-growing bacterial strain were inoculated in colloidal chitin broth medium and incubated at 30°C for 5 days on rotary incubator at 180 rpm. Bacterial cultural filtrates were obtained by centrifugation at 8,000 rpm for 5 min at 4°C. A reaction mixture (1 mL supernatant + 1 mL of 1% colloidal chitin suspension in 0.2 M-phosphate buffer pH 6.5 was incubated on shaking water bath at 40°C and 70 rpm for 1 h. One mL of the previous mixture was added to 2 mL copper reagent then incubated at 100 °C for 20 min. Two mL of Arsenomolybdate reagent was added and finally the total volume was made up to 25 mL with distilled water. The released reducing sugars were measured at 520 nm. One unit of the chitinase activity was defined as the amount of enzyme which yields one µM of reducing sugar as N-acetyl-D-glucosamine (Glc NAC) equivalent per minute.

Biomass and culture filtrate of BCAs

Bacterial strains were cultured in conical flasks containing LB broth medium which modified by supplementation with 1% colloidal chitin as a sole carbon source for chitinase induction and incubated at 30 °C for 48 h on a shaker (180 rpm). For separation of culture filtrate, the bacterial culture (100 mL) was centrifuged at 6000 g for 20 min at 4 ºC and then the supernatant was passed through 0.22 µm in diameter nitrocellulose filter, sterile culture filtrates were then used in the following experiments.

Nematicidal activity assay (*In vitro* study)

The nematicidal activity of bacterial biomass and free cell culture filtrates was assayed in *in vitro* by the percentage of inhibition of egg hatch and second stage juveniles (J2s) mortality of *M. incognita* (Abdellatif *et al.* 2021)

Effect of bacterial strains on *M. incognita* (J2s) vitality

One hundred freshly hatched J2s *M. incognita* were added to plate (3 cm in diameter) containing 0.2 mL of each bacterial culture (10^9 CFU mL^{-1}), separately. Petri-dishes which containing nematode suspensions only were served as control. Each treatment was conducted in triplicates. After 24 h of exposure, J2s which did not move even after needle stimulation were considered dead (Cao *et al.* 2021). In order to determine the effects of extracellular metabolites of the antagonists on J2s mortality, 0.2 mL from each bacterial culture filtrate was used instead of bacterial culture and the same previous steps were followed. The nematode mortality rate (%) was calculated (Schneider & Orelli 1947).

Mortality (%) = number of dead nematodes/total number of nematodes × 100

Effect of bacterial strains on *M. incognita* egg hatching

One mL *M. incognita* egg suspension containing approximately 100 eggs was added to petri-dish containing 0.2 mL bacterial culture (10^9 CFU mL^{-1}) of each bacterial strain, separately. Water in petri dishes supplied with eggs suspension was served as control. To determine the effects of extracellular metabolites on egg hatching, 0.2 mL from each bacterial culture filtrate was used instead of bacterial culture and the same previous steps were followed. The incubation was performed in completely dark conditions at 30 ºC. After five days of exposure, the number of hatched eggs was counted under a stereomicroscope. Relative hatching and Hatching inhibition (%) were calculated as follows:

Relative hatching (%) = Number of hatched J2 in each treatment /number of hatched J2 in water × 100

Hatching inhibition (%) = 100 – Relative hatching (%)

Field experiment

Preparation of Natural Chitinous Amendment

The exoskeleton of shrimp was collected and cleaned carefully, then prepared as fine powder to be used as cost-effective chitinous source (Ledchumanakumar *et al.* 2021).

Preparation of bacterial treatments

The five bacterial strains were grown separately in 2 liter LB broth medium supplemented with 1% colloidal chitin as a sole carbon source and incubated on a rotary shaker at 180 rpm and 30 °C for 48 h.

Experimental design

The experimental area was naturally infested with *M. incognita* that was identified previously by Sequence Characterized Amplified Region (SCAR) marker system. Initial population densities of *M. incognita* were determined one week prior to planting according to Barker (1985). This field experiment was carried out during March 2021 at Wadi El Natrun, Beheira Governorate, Egypt. The environmental conditions during this season were temperature (25-35 °C) and humidity (40-60%). Two days after transplantation of pepper seedlings, the cultivated soil was irritated with liquid suspension of natural chitinous amendment (10 g L^{-1}) to act as food base for chitinolytic bacteria. The experiment was conducted in a completely randomized block design with five treatments representing the investigated bacterial strains. The positive control represented by Oxamyl active compound-based nematicide, while plants that did not received any treatment were acted as negative control (Liu *et al.* 2020) Each treatment was replicated three times and each replicate represented by 10 plants. Different bacterial treatments were applied monthly at the rate of 20 L /fed. Moreover, pepper plants in all treatments were fertilized based on the fertilization program that recommended by Centre Lab. of Organic Agriculture, Agriculture Research Centre, Egypt. Three months later, at harvesting time, three plants were chosen at random from each treatment, carefully uprooted and the following data were recorded:

a. Chitinolytic Bacterial Population in Soil

At the end of the experiment, different soil samples were collected from rhizosphere of each treatment separately. The population of chitinolytic bacteria was assessed by spreading 100 µL of each diluted soil suspension on colloidal chitin agar plate. Three replicates for each soil sample. The plates were incubated at 30 °C for 5 days. The numbers of bacteria with clear zones around the colonies were recorded (Kuddus & Ahmad 2013)

b. Plant Growth parameters

Length of shoot and root, fresh and dry weight of shoot and root, number and weight of fruits per plant were recorded. Also, the total chlorophyll was measured according to Askar and Treptow protocol (Askar & Treptow 1993)

c. Nematode parameters

Number of galls, egg-masses, and females per 5 g of root system, number of eggs per egg-mass as well as nematode population in 250 g soil were counted.

d. Biochemical studies

Estimation of peroxidase (PO) and polyphenol oxidase (PPO)

Enzymes such as PO and PPO were extracted by homogenizing leaves of pepper plants and estimated. In a precooled mortar and pestle, 3 mL of 50 mM ice-cold sodium phosphate buffer (pH 6.0) was used to homogenize the sample. The homogenate was centrifuged at 10,000 rpm and 4 °C for 10 min. Extracted crudes were used for the determination of ensuing enzyme activity (Wang *et al.* 2011; Tian *et al.* 2002)

Estimation of total phenolic compounds and proline

The Folin-Ciocalteau technique was used for colorimetric measurement of total phenols (Slinkard & Singleton 1977) and proline content was estimated according to Jinal and Amaresan methodology (Jinal & Amaresan 2020).

e. Fruit quality characteristics

Firmness measurements (g cm^{-2}) were determined using push/pull powers Dynamometer Model DT 101. Sugars were extracted from samples by 70% ethyl alcohol and clarified by lead acetate. The excess of lead acetate was precipitated by sodium oxalate. Total sugars were determined in the clarified solution as outlined in AOAC (2005) Ascorbic acid content was estimated in fresh fruits using 2,4- dichlorophenol indophenol according to the method described by AOAC (2005). The results were expressed as mg ascorbic acid per g of sample.

f. Histological studies

The selected portions of *M. incognita* infected pepper roots from all treatments were carefully washed from soil. Roots cut into 3-5 mm long sections, then fixed in FAA solution according to Southey (1986). The histological studies was performed according to the protocol described by Sorial *et al.* (2020).

Statistical analysis

Statistical analysis was conducted by SPSS 2008, version 17.0, applying the General Linear Model's procedure. Significant differences among treatments were evaluated using Duncan's multiple range test ($p \leq 0.05$; SPSS 2008).

RESULTS

Screening and isolation of chitinolytic bacteria

Seventeen rhizobacterial isolates were appeared on the chitinase producer selective medium containing colloidal chitin as a sole carbon source (Fig. 1). According to the diameter of clear zone (≥ 5 mm) around the bacterial colony, five chitinolytic bacterial colonies were selected and purified on LB medium and stored at 4ºC for further investigation. These isolates were named as AB1, AB2, AB3, AB4 and AB5 (Fig. 2).

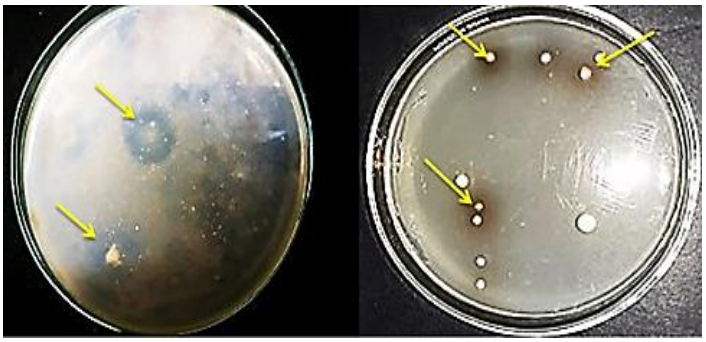

Fig. 1. Isolation of chitinolytic bacteria on colloidal chitin agar plates.

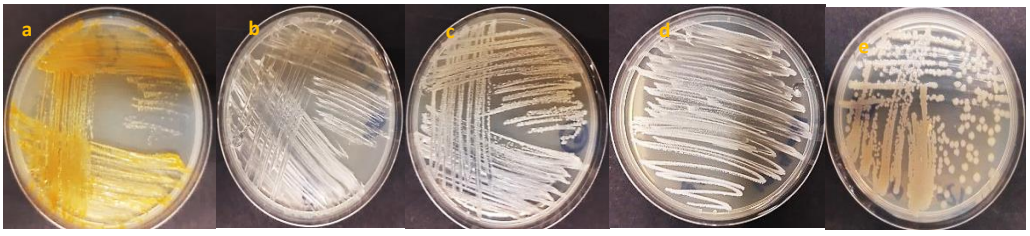

Fig. 2. Selected chitionolytic bacterial isolates on LB medium; a: AB1, b: AB2, c: AB3, d: AB4, e: AB5.

Safety assessment of bacterial isolates

On blood agar medium, no change in colour was observed around bacterial isolates AB1, AB2 and AB4 (γ-hemolysis). The other two isolates, i.e., AB3 and AB5 showed α-hemolysis. The absence of β-hemolysis in all isolates indicates their safe properties for agricultural applications. With respect to susceptibility to antibiotics, most isolates exhibited susceptibility to Cefotaxime and Amikacin, while most isolates were resistant to Clindamycin (Table 2).

Table 2. Antibiotic susceptibility profiles of the selected bacterial isolates.

Isolate code	FA (10 µg)	CTX (30 µg)	DA (2 µg)	VA (30 µg)	CRO (30 µg)	AK (30 µg)	E (15 µg)
AB1	S	S	R	R	I	I	S
AB2	R	S	R	R	S	S	R
AB3	R	S	R	R	I	S	R
AB4	R	S	R	R	I	S	R
AB5	S	S	S	R	S	S	S

Note: Resistant (R), intermediate (I), and susceptible (S); S: ≥ 21 mm, I: 16-20 mm, R: ≤ 15 mm. FusidicAcid (FA), Cefotaxime (CTX), Clindamycin (DA), Vancomycin (VA), Ceftriaxone (CRO), Amikacin (AK), Erythromycine (E).

Molecular characterization of the selected rhizobacterial isolates

The five bacterial isolates designated as AB1, AB2, AB3, AB4, and AB5 were identified by analysis of the sequence similarity of 16S rDNA gene sequence with those in Genbank database as *Chryseobacterium daecheongense, Bacillus Toyonensis, Pseudomonas lini, Lactobacillus helveticus* and *Klebsiella oxytoca,* respectively. The DNA sequence for the 16s rDNA of these strains has been deposited in the GenBank database with accession numbers shown in (Table 3). The sequence alignment and phylogenetic trees of the five bacterial strains were generated (Figs. 3, 4, 5, 6 and 7).

Table 3. Genetic identification of the selected bacterial isolates

Genus (PCR)	Species (16S rDNA)	Strain symbol	No. of base pairs amplified	NCBI accession number	Similarity with
Chryseobacterium	*daecheongense*	AB1	700	MZ148124	*Chryseobacterium daecheongense* N15101- 93.35%
Bacillus	*toyonensis*	AB2	550	MZ148324	*Bacillus toyonensis* strain 1/4R2A-Z-4 - 99.2%
Pseudomonas	*lini*	AB3	539	MZ148421	*P. lini* JZY5-59- 99.81%
Lactobacillus	*helveticus*	AB4	550	MZ148422	*Lactobacillus helveticus* strain IMAU94192 - 89.10%
Klebsiella	*oxytoca*	AB5	813	MZ148423	*Klebsiella oxytoca* strain CdS - 99.7%

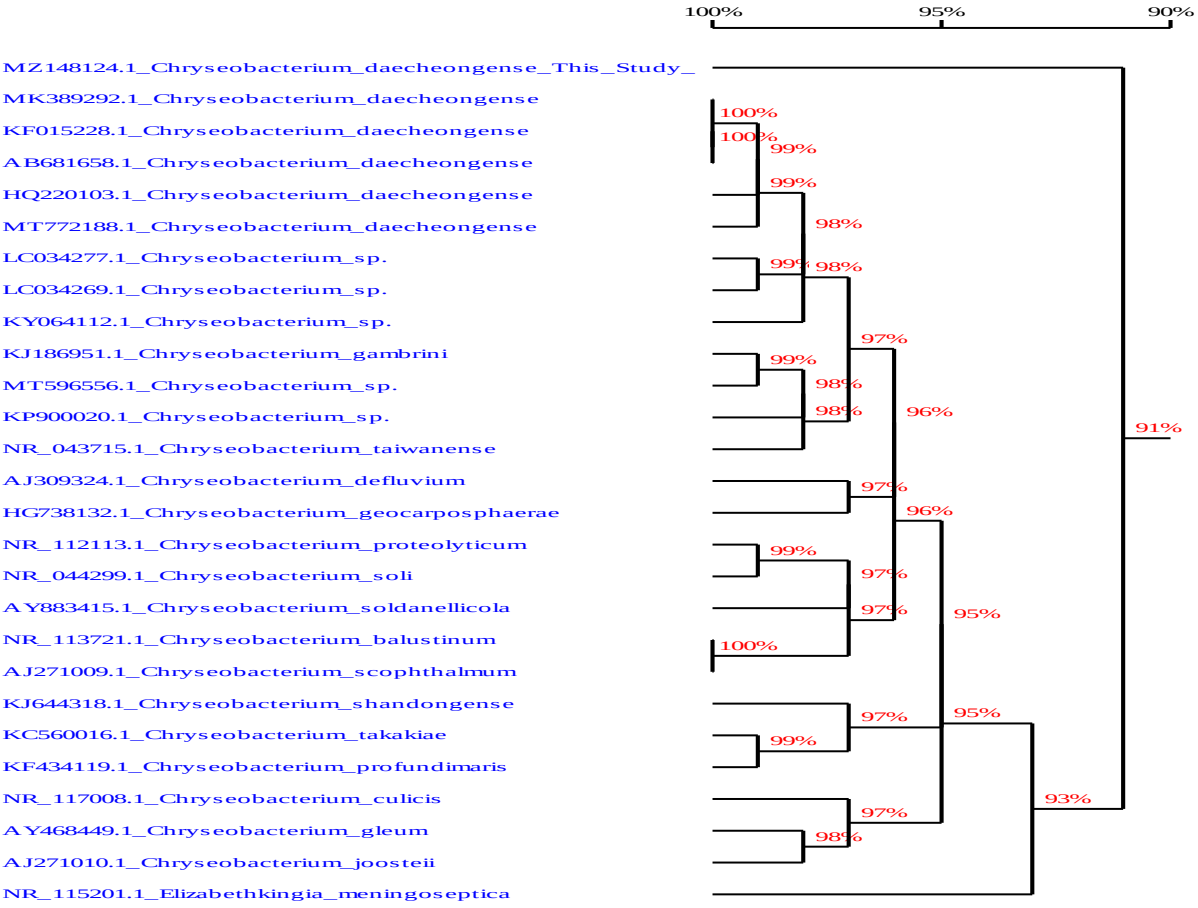

Fig. 3. Phylogenetic relationship based on 16S rDNA gene sequence showing relationships of *Chryseobacterium daecheongense* strain AB1 with other close homologous strains.

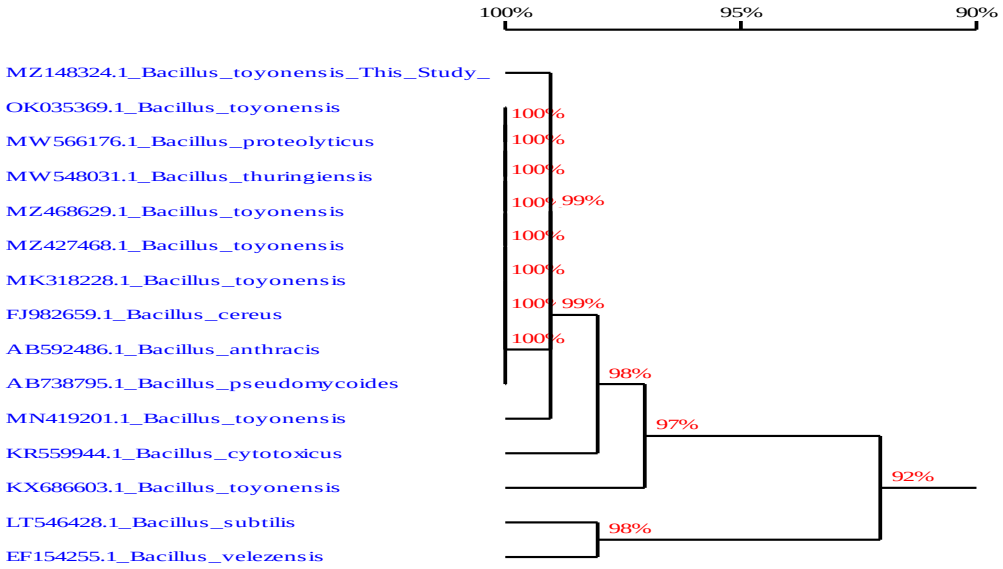

Fig. 4. Phylogenetic relationship based on 16S rDNA gene sequence showing relationships of *Bacillus toyonensis* strain AB2 with other close homologous strains.

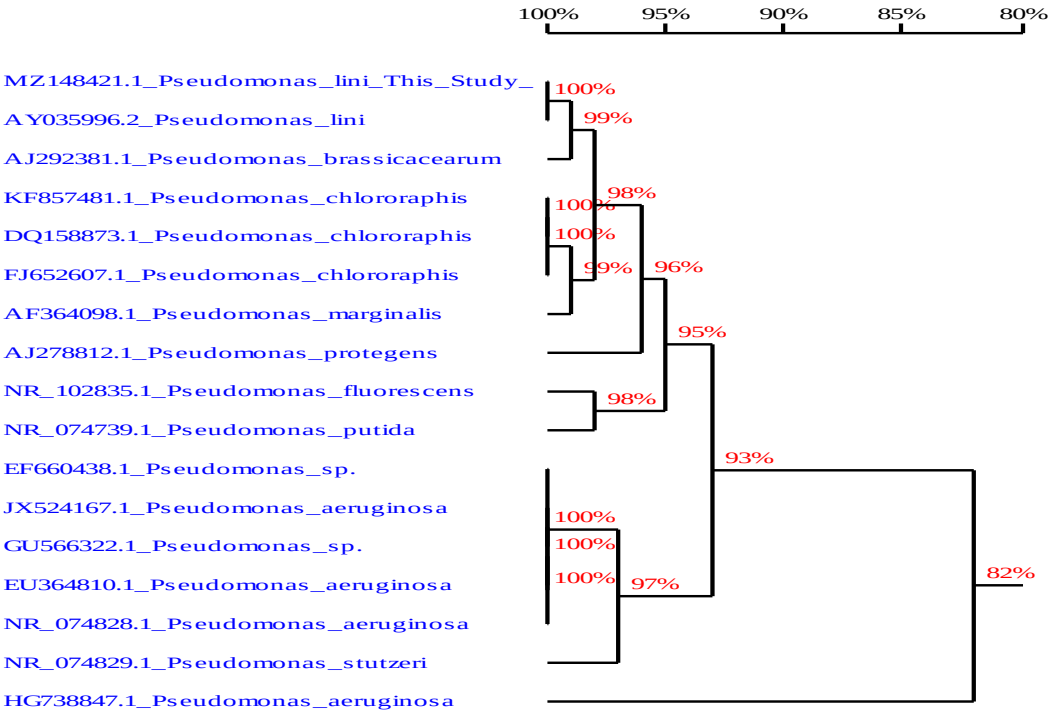

Fig. 5. Phylogenetic relationship based on 16S rDNA gene sequence showing relationships of *Pseudomonas lini* strain AB3 with other close homologous strains.

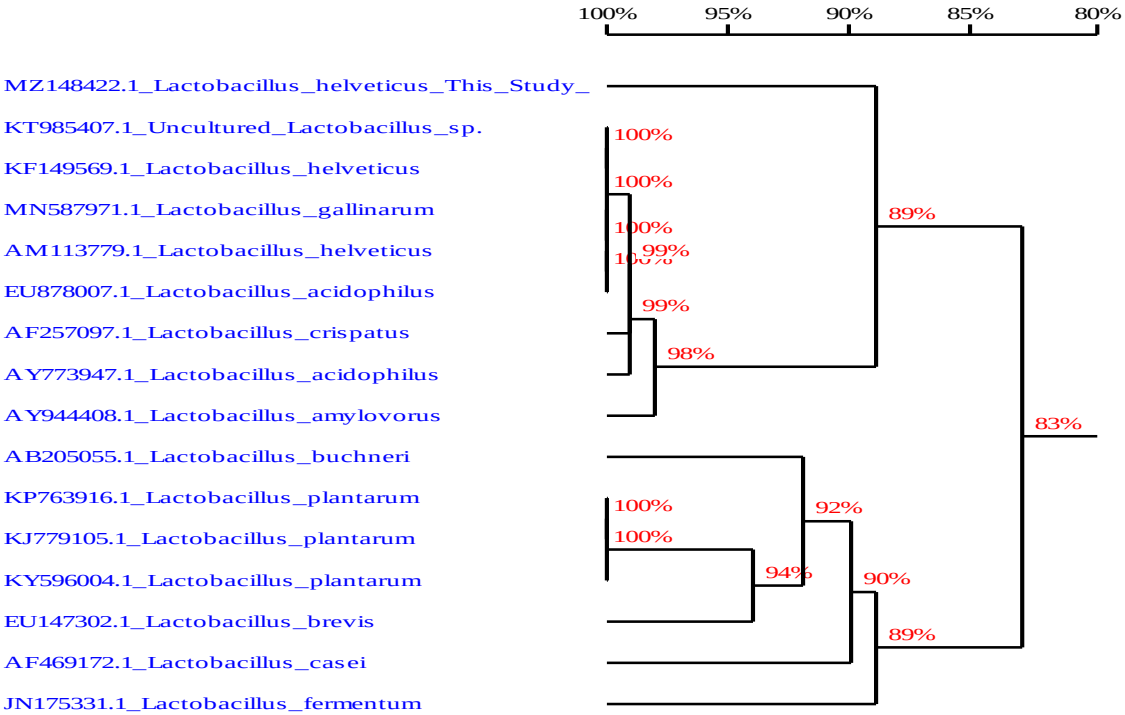

Fig. 6. Phylogenetic relationship based on 16S rDNA gene sequence showing relationships of *Lactobacillus helveticus* strain AB4 with other close homologous strains.

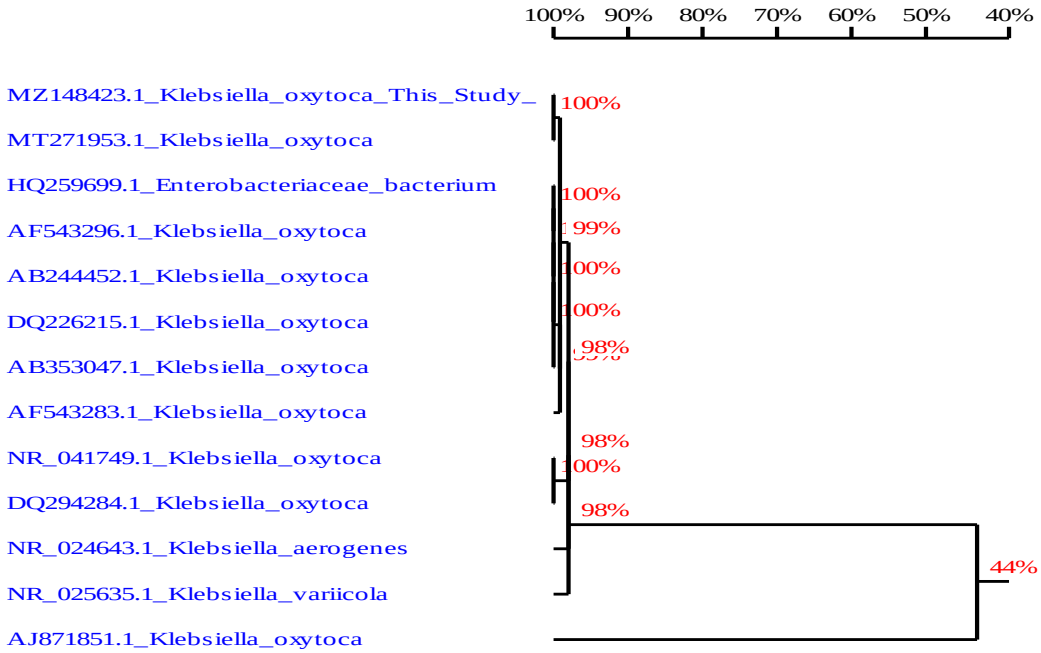

Fig. 7. Phylogenetic relationship based on 16S rDNA gene sequence showing relationships of *Klebsiella oxytoca* strain AB5 with other close homologous strains.

Assay of chitinase activity

All selected bacterial isolates recorded significant chitinase activity. *P. lini* showed the highest chitinase activity (8.00 U mL^{-1}) followed by *K. oxytoca* (7.32 U mL^{-1}). Other bacterial isolates recorded moderate chitinolytic activity (Fig. 8).

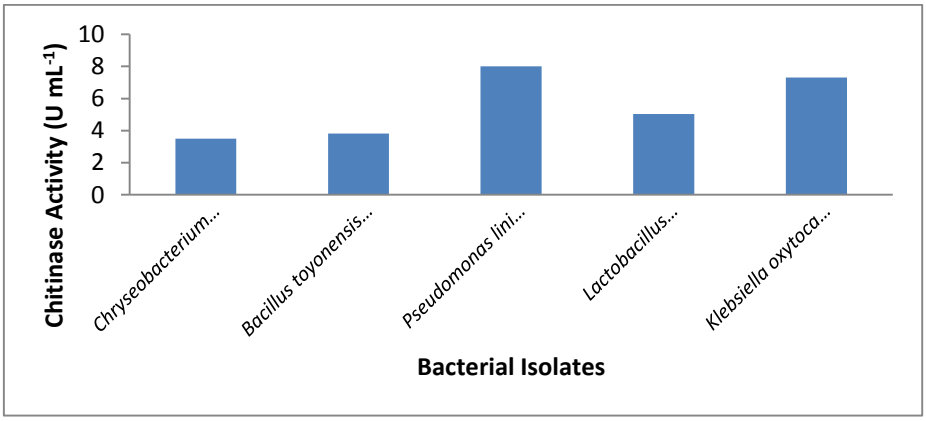

Fig. 8. Chitinolytic activity of the isolated bacteria.

Nematicidal Activity of the Chitinolytic Bacteria

Biomasses and culture filtrates of the tested bacteria showed significant mortality in *M. incognita* J2 after 24 h of exposure, compared to control. *P. lini* and *K. oxytoca* exhibited the highest percentage of mortality (100%). *L. helveticus* displayed also remarkable mortality rate (97.28 and 93.21%) when applied as bacterial biomass and culture filtrates, respectively (Table 4). Moreover, the microscopic study revealed that distinct morphological distortions have been observed in J2s treated with different bacterial strains compared to untreated control (Fig. 9). Remarkable lysis followed by collapse in the inner content of J2s was the most distinguished morphological malformation for all bacterial treatments.

Table 4. Nematicidal activity of biomasses and culture filtrates of isolated bacteria.

Treatments	Juveniles Mortality (%)			
	Biomass		Culture Filtrate	
	No. of immobile juveniles	Mortality (%)	No. of immobile juveniles	Mortality (%)
Chryseobacterium daecheongense	88.66^{c}	88.46	75.00^{e}	74.57
Bacillus toyonensis	95.33^{b}	95.25	81.33^{d}	81.01
Pseudomonas lini	100.00^{a}	100.00	100.00^{a}	100.00
Lactobacillus helveticus	97.33^{ab}	97.28	93.33^{b}	93.21
Klebsiella oxytoca	100.00^{a}	100.00	86.00^{c}	85.76
Oxamyl active compound-based nematicide	100.00^{a}	100.00	100.00^{a}	100.00
Control (Nematode Alone)	1.66^{d}	1.66	1.66^{f}	1.66

Note: Values with the same letter are not significantly different.

Ovicidal Activity of Chitinolytic Bacteria

Both biomasses and culture filtrates of the tested bacteria exhibited significant reduction in *M. incognita* eggs hatching as compared to control. The inhibitory impact varied depending on bacteria species. The highest ovicidal activity was detected in *P. lini* and *L. helveticus* biomasses after 5 days of exposure. With respect to culture filtrate, *P. lini* and *K. oxytoca* recorded the highest inhibition (Table 5). These results revealed that, the high rate (%) of nematicidal and ovicidal activities of the *P. lini* and *K. oxytoca* culture filtrates are in accordance with their chitinase activity.

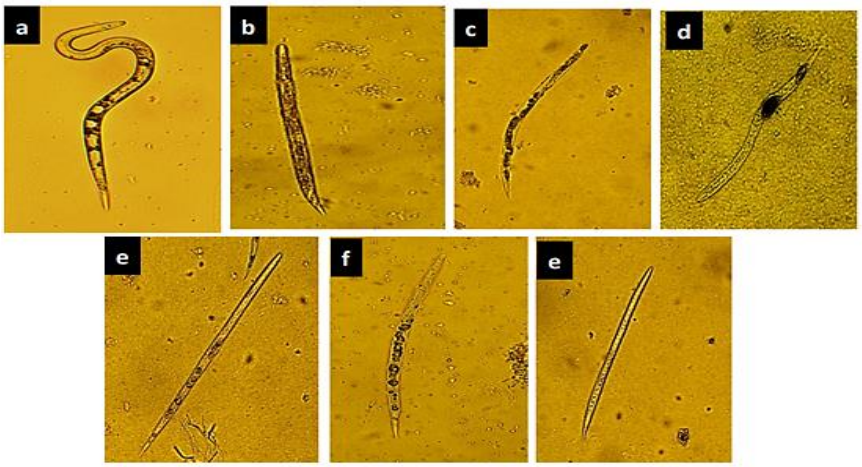

Fig. 9. Malformation effect of the tested bacteria strains on M. incognita J2s (a) Untreated control, (b) *Chryseobacterium daecheongense*, (c) *Bacillus toyonensis*, (d) *Pseudomonas lini*, (e) *Lactobacillus helveticus*, (f) *Klebsiella oxytoca*, (e) Oxamyl active compound-based nematicide.

Field Experiment

Effect of bacterial treatments on the total count of chitinolytic bacteria in rhizosphere of pepper plant

The treatment of soil with different chitinolytic bacteria in combination with natural chitin increased remarkably the total population of chitinolytic bacteria. The maximum total count was attained in *K. oxytoca* followed by *P. lini* and *L. helveticus*. Treatment with oxamyl active compound-based nematicide, negatively affected the population of chitinolytic bacteria even when compared to untreated control (Table 6).

Table 5. Ovicidal activity of biomasses and culture filtrates of the isolated bacteria.

Treatments	Ovicidal Activity (%)					
	Biomass			Culture Filtrate		
	No. of hatched eggs	Relative hatching (%)	Inhibition (%)	No. of hatched eggs	Relative hatching (%)	Inhibition (%)
Chryseobacterium daecheongense	17.66^{c}	18.65	81.35	45.00^{b}	47.53	52.47
Bacillus toyonensis	24.66^{b}	26.05	73.95	54.66^{b}	57.74	42.26
Pseudomonas lini	8.66^{e}	9.14	90.86	22.00^{c}	23.24	76.76
Lactobacillus helveticus	13.00^{de}	13.73	86.27	38.00^{b}	40.14	59.86
Klebsiella oxytoca	14.33^{cd}	15.13	84.87	26.00^{c}	27.46	72.46
Oxamyl active compound-based nematicide	23.00^{b}	24.29	75.71	23.00^{c}	24.29	75.71
Control (Nematode Alone)	94.66^{a}	-	-	94.66^{a}	-	-

Note: Values with the same letter are not significantly different.

Table 6. Effect of different treatments on chitinolytic bacterial count.

Treatments	Chitinolytic Bacterial count/g soil
Chryseobacterium daecheongense	1.3×10^{5cd}
Bacillus toyonensis	2.3×10^{5bcd}
Pseudomonas lini	4.8×10^{5ab}
Lactobacillus helveticus	3.3×10^{5abc}
Klebsiella oxytoca	5.6×10^{5a}
Oxamyl active compound-based nematicide	2×10^{3d}
Untreated Control	1×10^{4d}

Note: Values with the same letter are not significantly different.

Effects of bacterial treatments on pepper growth parameters, yield and fruit quality

Treatments with different bacterial strains significantly induced bell pepper growth parameters including fresh and dry shoot and root weights (Table 7). *P. lini* and *L. helveticus* showed the best improvement in pepper growth; followed by *K. oxytoca*, *C. daecheongense* and *B. toyonensis* (Fig. 10). The control treatment recorded the lowest number of fruits, weight of fruit and fruit yield per plant. Plants treated with *P. lini* recorded the highest number of fruits, weight of fruit and fruit yield per plant (Table 8). With respect to fruit quality, the highest fruit firmness was recorded in Oxamyl active compound-based nematicide (3.26 g cm^{-2}), followed by *K. oxytoca* (3.19 g cm^{-2}), then *P. lini* (3.05 g cm^{-2}), while the lowest in untreated control (2.08 g cm^{-2}). The total sugar content of pepper fruits was increased significantly by different bacterial treatments. The highest amount of total sugar was recorded in *L. helveticus* and *P. lini*. Total sugar contents of fruits treated with oxamyl active compound-based nematicide remained unaffected.

Ascorbic acid content of bell pepper was also increased significantly in treated plant. *L. helveticus* was found to be the best bacterial treatments in elevating vitamin C contents of bell pepper fruits, while the lowest vitamin C in untreated control (Table 9).

Table 7. Effect of the isolated bacteria on pepper growth parameters.

Treatments	Growth parameters				
	Shoot			Root	
	length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)
Chryseobacterium daecheongense	51.33^{b}	121.66^{cd}	17.33^{b}	21.66^{c}	55.00^{b}
Bacillus toyonensis	51.33^{b}	111.66^{cd}	17.00^{b}	19.66^{cd}	53.33^{b}
Pseudomonas lini	70.33^{a}	248.33^{a}	33.33^{a}	32.66^{a}	73.33^{a}
Lactobacillus helveticus	70.66^{a}	218.33^{a}	28.33^{a}	32.00^{a}	55.00^{b}
Klebsiella oxytoca	67.33^{a}	165.00^{ab}	20.00^{b}	25.00^{b}	49.33^{bc}
Oxamyl active compound-based nematicide	68.00^{a}	173.00^{ab}	20.33^{b}	26.00^{b}	63.33^{ab}
Untreated control	41.66^{c}	73.00^{c}	14.00^{b}	16.00^{d}	34.00^{c}

Note: Values with the same letter are not significantly different.

Fig. 10. Effect of different treatments on Bell pepper growth **(**a) *Chryseobacterium daecheongense*, (b) *Bacillus toyonensi*, (c) *Pseudomonas lini*, (d) *Lactobacillus helveticus*, (e) *Klebsiella oxytoca*, (f) Oxamyl active compound-based nematicide, (g) Untreated control.

Table 8. Effect of bacteria on Yield components of pepper.

Treatments	No. of fruits/plant	Weight of one fruit (g)	Fruit yield/plant (g)
Chryseobacterium daecheongense	5.66^{de}	24.00^{b}	150.00^{c}
Bacillus toyonensis	7.00^{cd}	24.00^{b}	173.33^{b}
Pseudomonas lini	9.33^{a}	25.00^{ab}	215.00^{a}
Lactobacillus helveticus	9.00^{ab}	26.00^{b}	235.00^{a}
Klebsiella oxytoca	7.66^{bc}	24.00^{b}	183.33^{b}
Oxamyl active compound-based nematicide	8.00^{abc}	25.00^{ab}	182.66^{b}
Untreated control	5.33^{e}	20.00^{c}	126.66^{d}

Note: Values with the same letter are not significantly different.

Table 9. Effect of bacterial treatments on fruit quality.

Treatments	Firmness (g cm$^{-2)}$	Total sugars (%)	Ascorbic acid (mg /100g FW)
Chryseobacterium daecheongense	2.34^{cd}	3.65^{b}	42.66^{c}
Bacillus toyonensis	2.64^{bc}	3.23^{c}	41.86^{c}
Pseudomonas lini	3.05^{ab}	4.26^{a}	53.86^{a}
Lactobacillus helveticus	2.98^{ab}	4.31^{a}	55.73^{a}
Klebsiella oxytoca	3.19^{a}	3.05^{c}	54.66^{a}
Oxamyl active compound-based nematicide	3.26^{a}	2.08^{d}	47.86^{b}
Untreated control	2.08^{d}	2.17^{d}	40.93^{c}

Note: Values with the same letter are not significantly different.

Effect of bacterial treatments on different nematode parameters

Population densities of *M. incognita* in soil and root were significantly suppressed with all bacterial treatments. *K. oxytoca* and *L. helveticus* exerted the maximum suppression of nematode population (81.94 and 82.45%) with

the least reproduction factor (Rf = 0.38 and 0.37) respectively (Table 10). *P. lini* also exhibited remarkable inhibition in nematode population (76.77 %). Root galling was reduced significantly in *P. lini* (82.59%), *L. helveticus* (77.27%) and *K. oxytoca* (70.45%; Table 11). Similar trend was found in the number of egg masses with the reduction rate of 82.66, 72.44 and 59.27% in *P. lini*, *L. helveticus* and *K. oxytoca*, respectively. Fecundity expressed by number of eggs /single egg mass was negatively affected by the five bacterial treatments. Noteworthy, Oxamyl active compound-based nematicide exceeded all bacterial treatments and significantly recorded the highest reduction in total nematode population (88.64%), the least of Rf (0.24), RGI (1.00) as well as the least EI (1.00).

Table 10. Effect bacterial treatments on the population density of *M. incognita*.

Treatments	No. of juveniles /250 g soil	No. of females/ 5 g of root	No. of developmental stages/ 5 g of root	Final population	Red. %	RF	Efficiency
Chryseobacterium daecheongense	195.00^{c}	19.66^{c}	10.33^{b}	224.99^{c}	30.34	1.40	34.88
Bacillus toyonensis	230.00^{b}	24.66^{b}	8.66^{b}	263.32^{b}	18.47	1.75	18.60
Pseudomonas lini	65.00^{d}	6.00^{d}	4.66^{c}	75.00^{d}	76.77	0.50	76.74
Lactobacillus helveticus	50.00^{d}	5.66^{d}	2.66^{c}	58.32^{de}	81.94	0.38	82.32
Klebsiella oxytoca	45.00^{d}	8.66^{d}	3.00^{c}	56.66^{de}	82.45	0.37	82.79
Oxamyl active compound-based nematicide	33.33^{d}	1.33^{e}	2.00^{c}	36.66^{e}	88.64	0.24	88.83
Control (Nematode Alone)	270.00^{a}	38.66^{a}	14.33^{a}	322.99^{a}	-	2.15	-

Note: Values with the same letter are not significantly different; Final population is calculated as the sum number of juveniles, females and developmental stages; Red. (%) (Reduction percentage) = (F.C-F. T)/F.C × 100 where, F.C: final population in untreated control and F.T: final population in treated plant; Reproduction factor (RF) = Nematode final population/Nematode initial population.

Table 11. Effect of bacterial treatments on development and reproduction of *M. incognita.*

Treatments	No. of galls / 5 g of root	Red. %	RGI	No. of egg masses/ 5 g of root	Red. %	EI	No. of eggs/ egg mass	Red. %
Chryseobacterium daecheongense	21.66^{b}	50.77	3	14.33^{b}	56.12	3	231.66^{bc}	24.04
Bacillus toyonensis	23.33^{b}	46.97	3	16.33^{b}	50.00	3	228.33^{bc}	25.13
Pseudomonas lini	7.66^{c}	82.59	2	5.66^{c}	82.66	2	158.33^{d}	48.08
Lactobacillus helveticus	10.00^{c}	77.27	2	9.00^{c}	72.44	2	245.00^{b}	19.67
Klebsiella oxytoca	13.00^{c}	70.45	3	13.30^{b}	59.27	3	220.00^{bc}	27.86
Oxamyl active compound-based nematicide	1.00^{d}	97.72	1	1.00^{d}	96.93	1	216.66^{c}	28.96
Control (Nematode Alone)	44.00^{a}	-	4	32.66^{a}	-	4	305.00^{a}	-

Note: Values with the same letter are not significantly different; RGI: Root gall index and EI: egg masses index was as follows:
0 = no galls or egg masses, 1= 1-2; 2= 3-10; 3= 11-30; 4= 31-100 and 5= more than 100 galls or egg masses.

Effect of bacterial treatments on accumulation of resistance compounds in leaves of pepper infected with M. incognita

It was found that nematode infection increased the accumulation of resistance compounds in leaves of pepper. The highest amounts of proline were accumulated in leaves of untreated control. The lowest proline values were found in plants which treated with *L. helveticus*, *P. lini* and *K. oxytoca*. Accumulation of total phenolic compounds increased in *L. helveticus*, *K. oxytoca* and *P. lini* treatments. There is no significant difference in other treatments. Peroxidase (PO) and polyphenol oxidase (PPO) activities were obviously raised in pepper leaves of plants treated with *P. lini*, *L. helveticus* and *K. oxytoca* (Table 12).

Histological studies

Giant cells are smaller than those of untreated infected roots in case of *C. daecheongense* and *B. toyonensis* treatments (Figs. 11-12). The transverse sections of roots treated with *P. lini* (Fig. 13) and *L. helveticus* (Fig. 14) exhibited healthier pattern and less necrotic points compared to untreated control. Giant cells contained less or free of cytoplasm in the case of *K. oxytoca* (Fig. 15); Pepper roots infected with *M. incognita* and treated with Oxamyl active compound-based nematicide, was poorly formed giant, while the xylem cells exhibited small abnormalities in the structure when compared to control (Fig. 16). Generally, all treatments did not prevent *M. incognita* from penetrating the roots but different time was required to reach the vascular cylinder to develop giant cells and to complete life cycle. *M. incognita* induced alterations in cells of cortical region in pepper roots. In untreated control, giant cells were found in vascular parenchyma cells with different shapes from circular to irregular shape (Fig. 17).

Table 12. Effect of bacterial treatments on concentration of resistance compounds in leaves of pepper infected with *M. incognita*

Treatments	Proline (mg g^{-1}) fresh leaves	Total phenol (mg g^{-1}) fresh leaves	PO	PPO
			Δ Absorbance units/mg protein	
Chryseobacterium daecheongense	0.420^{b}	14.56^{b}	1.050^{d}	0.171^{ab}
Bacillus toyonensis	0.343^{c}	14.79^{b}	1.206^{c}	0.139^{bc}
Pseudomonas lini	0.240^{de}	15.50^{a}	1.580^{a}	0.186^{a}
Lactobacillus helveticus	0.206^{e}	16.07^{a}	1.480^{b}	0.201^{a}
Klebsiella oxytoca	0.253^{d}	15.59^{a}	1.523^{ab}	0.197^{a}
Oxamyl active compound-based nematicide	0.420^{b}	14.65^{b}	0.990^{de}	0.148^{bc}
Control (Nematode Alone)	0.606^{a}	14.43^{b}	0.970^{e}	0.128^{c}

Note: Values with the same letter are not significantly different; PO: peroxidase, PPO: polyphenol oxidase.

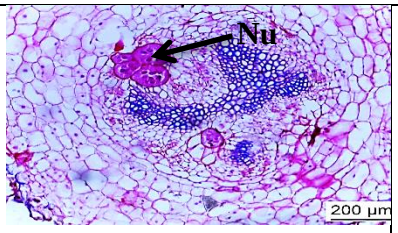

Fig. 11. Root transverse section of pepper plants infected with M. incognita and treated with *Chryseobacterium daecheongense* strain AB1.

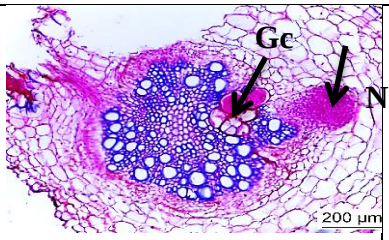

Fig. 12. Root transverse section of pepper plants infected with M. incognita and treated with *Bacillus toyonensis* strain AB2.

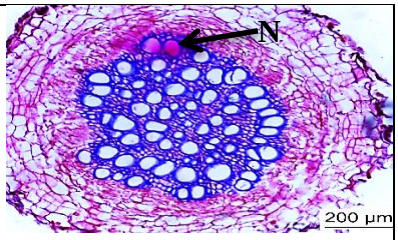

Fig. 13. Root transverse section of pepper plants infected with *M. incognita* and treated with *Pseudomonas lini* strain AB3.

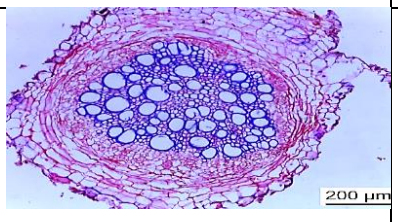

Fig. 14. Root transverse section of pepper plants infected with M. incognita and treated with *Lactobacillus helveticus* strain AB4.

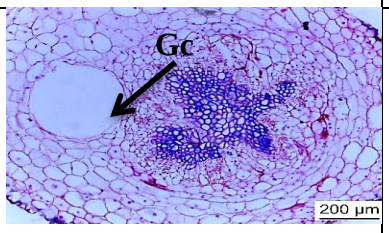

Fig. 15. Root transverse section of pepper plants infected with M. incognita and treated with *Klebsiella oxytoca* strain AB5.

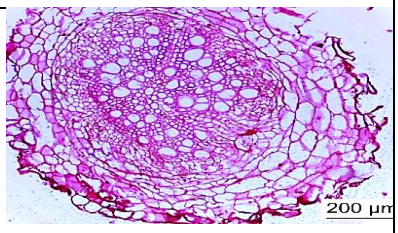

Fig. 16. Root transverse section of pepper plants infected with *M. incognita* and treated with *Oxamyl active compound-based nematicide.*

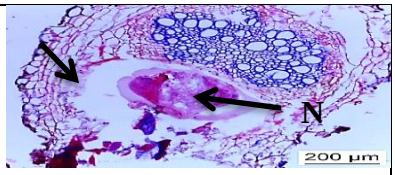

Fig. 17. Root transverse section of pepper plants infected with *M. incognita.*

Note: Gc: Giant cells; N: Necrosis; Nu: Nuclei

DISCUSSION

Soil microbial communities play a crucial role in soil fertility, sustainability, and plant health. However, intensive agriculture with increasing chemical inputs and recent changes in environmental conditions had influenced native soil microbial communities. Recently, research on the application of microorganisms, such as bacteria, fungi and actinobacteria for management of phytonematodes has drawn attention (Migunova & Sasanelli 2021). During the present study five promising chitinolytic bacterial isolates were isolated and selected for investigation of their nematicidal activity. They have been identified molecularly as *C. daecheongense*, *B. toyonensis*, *P. lini*, *L. helveticus* and *K. oxytoca*. Chitinolytic bacteria can decompose chitin under both aerobic and anaerobic conditions, and have been isolated from soil, compost, and shellfish waste. Bacterial members belonging to *Alteromonas*, *Aeromonas*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Chromobacterium* have been popularly reported for their chitinase activity (Poria *et al.* 2021; Abdellatif *et al.* 2021). Furthermore, the chitinolytic activity of *Bacillus toyonensis* PNTB1 (Tallur *et al.* 2016). *Lactobacillus* spp. (Horvath-Szanics *et al.* 2020) and *Klebsiella oxytoca* (Tamrela *et al.* 2021) was proved. The critical role of microbial chitinase production to cause disruption and malformation in eggs and larvae of root-knot nematodes was stated by Jha & Modi (2018) and Sayed *et al.* (2019). These results were confirmed by the recent study as the five isolated novel chitinolytic bacterial strains were successful in suppressing the egg hatching and vitality of larvae of *M. incognita*. In addition, application of the five chitinolytic bacterial isolates to soil amended with natural chitin was effective against *M. incognita* and enhances the soil population of chitinolytic bacteria especially in the rhizosphere zone. Most of these chitinolytic strains suppressed galling and reduced population densities of *M. incognita*. *P. lini* was the most effective strain as biocontrol agent. In addition, *C. daecheongense* was found to be biocontrol agent against *M. incognita* for the first time. Moreover, the growth and quality parameters of nematode infected pepper plants were promoted by different bacterial treatments. In unison with the present results, numerous studies have reported suppression in nematode populations with the application of bacterial treatment under field conditions. The prominent efficacy of five bacterial strains (*Bacillus cereus, B. subtilis, Pseudomonas putida, P. fluorescens*, and *Serratia proteamaculans*) against *M. javanica* was stated (Zhao *et al.* 2018). In addition to chitinase production, a cyclic dipeptide Cyclo (L-Pro-L-Leu), was identified as promising nematicidal compound that secreted by *P. simiae* MB751 (Sun *et al.* 2021). The highest reduction in *M. incognita* population and galling were noticed in tomato plants treated with *P. fuorescens*. Such reduction was also suggested to be related to the production of phytohormones that indirectly enhance plant health (Noureldeen *et al.* 2021). Genome analysis of *B. toyonensis* as a novel agent against phytopathogens proved its production to vital antimicrobial compounds such as chitinases, novel bacteriocins, non-ribosomal peptides and N-acyl homoserine lactonase at high frequency (Lopes *et al.* 2017). Moreover, both genetic and experimental evidence demonstrating that *B. toyonensisis* can be considered as plant growth-promoting bacterium (Contreras Pérez *et al.* 2019). Recently, *Lactobacillus brevis* have been recorded as novel promising natural nematicidal agents for their production of wide variety of key organic acids such as lactic acid, succinic acid and malic acid which have lethal effect on nematode J2s (Seo *et al.* 2019). The nematicidal activity of natural organic acids produced by lactic acid bacteria was confirmed by (Ibrahim *et al.* 2022) The current study suggested that the chitinase production by *L. helveticus* may has synergetic effect with organic acids and promote the nematicidal potentiality of this bacterial strain. It was found that, coating of soybean seeds with *Klebsiella pneumoniae* SnebYK culture not only decrease the infection rate of *Heterodera glycines*, but also reduce the proportion of adult female nematodes in soybean roots under field conditions (Liu *et al.* 2018). The field study conducted by El-Ashry *et al.* (2022) showed the effectiveness of rhizobacteria in decreasing *M. incognita* reproduction in pepper plants. Moreover, the combination between rhizobacteria and other natural materials such as composted cattle manure, promoted tomato growth effectively

and enhanced the plant tolerance to root-knot nematodes infection (El Ashry *et al.* 2020) In another study, the application of waste shrimp water as organic amendment increased pepper yield relative to untreated control (Zheljazkov *et al.* 2011). The present study noticed a significant decrement in fruit quality of nematode infected plants. Application of different chitinolytic bacterial strains treatments improved the fruit quality remarkably, compared to untreated control. This is consistent with previous findings that application of three plant growth-promoting rhizobacterial strains i.e. *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21 improved sweet pepper fruit quality in terms of soluble sugar, soluble solid and vitamin C (Zhang *et al.* 2019). Proline can accumulates in many plant species in response to both biotic and abiotic stresses and plays a role in protection of plant cells against oxidative damage (Janmohammadi *et al.* 2013). During the current study proline content of pepper leaves was accumulated significantly due to nematode infection. The highest values were recorded for uninoculated control, while pepper inoculated with bacterial treatments showed significant decrease in proline quantity. It was found that proline accumulation was negatively correlated with disease incidence. The present study also revealed an increase in the levels of total phenol of plants treated with *Pseudomonas lini* strain AB3, *Lactobacillus helveticus* strain AB4 and *Klebsiella oxytoca* strain AB5, compared to un-inoculated control. These are in accordance with Abd-el-Khair *et al.* (2019) who noticed remarkable stimulation total phenols in levels of cowpea infected with *M. incognita* in the presence of *B. subtilis, B. pumilus* and *P. fluorescens* treatments. It was suggested that the control of phenolic compounds activity by microorganisms mainly occurred due to the induction of activities of the polyphenol oxidase and chalcone synthase activities that mediated the accumulation of phenolic compounds at infection sites to protect the cell wall from nematode attack (Khanna *et al.* 2019). Moreover, Polyphenol oxidase (PPO) and peroxidase (PO) are modulated by biotic and abiotic stresses and responsible for induced systemic resistance. They are also related to the interaction between plants and microorganisms (Sahebani *et al.* 2020). PO and PPO are thought to reinforce cell walls lignification and suberization at the border of infection and so these limit spread of pathogens (Passardi *et al.* 2004). In the present studies the activity of both PO and PPO raised significantly in pepper plants treated with *P. lini* strain AB3, *L. helveticus* strain AB4 and *K. oxytoca* strain AB5. In harmony with Abdelrahman *et al.* **(**2021) the treatment of *Rhizoctonia solani* infected onion with *B. subtilis, P. fluorescens, or B. megaterium* increase the induction of the defence enzymes, i.e., PO and PPO and reduce the disease severity, compared to un-inoculated infected control. Rhizosphere inhabiting bacteria, such as *P. fluorescens* and *B. subtilis* activate the peroxidase activity in tomato plants which can be effective against results obtained by Sahebani & Hadavi (2009). In addition, the present investigation of histological alterations in pepper roots infected with *M. incognita* indicated that roots treated with *P. lini* and *L. helveticus* showed a few galls but no formation of giant cells while *K. oxytoca* showed less malformation and disruption in xylem layer, compared to un-inoculated control.

CONCLUSION

In conclusion, five chitinolytic bacterial isolates, *C. daecheongense*, *B. toyonensis*, *P. lini*, *L. helveticus* and *K. oxytoca*, isolated from nematode suppressive soil were identified as eco-friendly alternatives for controlling root-knot nematodes. Among them, *P. lini* exhibited the highest chitinolytic and nematicidal activities in the laboratory and could be stably used under field conditions to control root-knot nematodes and promote pepper growth significantly. Proline, phenolic compounds, peroxidase and polyphenol oxidase accumulation was induced by different bacterial treatment and play a role in the resistance of pepper against nematodes. However, the commercial applications of these isolates require further investigation to develop effective bio-formulations for a sustainable agricultural approach.

Abbreviations

PCR: Polymerase chain reaction; CCA: Colloidal chitin agar; rDNA: Ribosomal DNA; J2s: Juveniles

REFERENCES

Abdel Aziz, MA & Geeth, RH 2018, Effect of foliar spray with some silicon sources and paclobutrazol on growth, yield and fruit quality of sweet pepper (*Capsicum annuum* L.) plants under high-temperature conditions. *Egyptian Journal of Agricultural Research*, 96: 577-593, https://doi.org/10.21608/ejar.2018.135762.

AbdEl Khair, H, El Nagdi, WM, Youssef, MM, Abd Elgawad, MM & Dawood, MG 2019, Protective effect of *Bacillus subtilis*, *B. pumilus*, and *Pseudomonas fluorescens* isolates against root knot nematode

Meloidogyne incognita on cowpea. *Bulletin of the National Research Centre*, 43, https://doi.org/10.1186/s42269-019-0108-8.

Abdellatif, AA, Tahany, MA, Sayed, AM, Ragab, AA, Dina, SSI & Elmaghraby, MK 2021, Activity of *Serratia* spp. and *Bacillus* spp. as biocontrol agents against *Meloidogyne incognita* infecting tomato. *Pakistan Journal of Biotechnology*, 18: 37-47. https://doi.org/10.34016/pjbt.2021.18.2/3.37.

Abdelrahman, H, Zaghloul, R, Abou Aly, H, Ragab, AA & Elmaghraby, MMK 2021, Application of some organic farming methods to enhancement the growth and production of green onion. *Journal of Agricultural Chemistry and Biotechnology*, 12: 79-89, https://doi.org/10.21608/jacb.2021.162616.

Agarwal, M, Dheeman, S, Dubey, RC, Kumar, P, Maheshwari, DK & Bajpai, VK 2017, Differential antagonistic responses of *Bacillus pumilus* MSUA3 against *Rhizoctonia solani* and *Fusarium oxysporum* causing fungal diseases in *Fagopyrum esculentum* Moench. *Microbiological Research*, 205: 40-47. https://doi.org/10.1016/j.micres.2017.08.012.

Akeed, Y, Atrash, F & Naffaa, W 2020 Partial purification and characterization of chitinase produced by bacillus licheniformis B307. *Heliyon*, 6: e03858. https://doi.org/10.1016/j.heliyon.2020.e03858

Alkharpotly, A 2018, Growth and yield responses of sweet pepper (*Capsicum annum* L.) to organic and NPK mineral fertilization under plastic houses conditions at arid regions. *Journal of Plant Production*, 9: 299-305. https://doi.org/10.21608/jpp.2018.35501.

AOAC 2005, Official Methods of Analysis. Association of Official Analytical Chemists. Published by the AOAC International, 18th ed., Washington DC, USA.

Asaturova. AM, Bugaeva. LN, Homyak. AI, Slobodyanyuk. GA, Kashutina. EV, Yasyuk. LV, Sidorov, NM, Nadykta, VD & Garkovenko, AV 2022, *Bacillus velezensis* strains for protecting cucumber plants from root-knot nematode *Meloidogyne incognita* in a greenhouse. *Plants*, 11: 275. https://doi.org/10.3390/plants11030275.

Askar, A & Treptow, H 1993, Analytical methods. In: Askar A, Treptow H, editors. Quality assurance in tropical fruit processing. Springer-Verlag, New York, pp. 27-28.

Barker, KR 1985, Nematode extraction and bioassay. In: Kr, Barker CC, Carter & JN, Sasser (Eds.), Advanced treatise on *Meloidogyne*, Vol. 11- Nematology. North Carolina State University, Graphics, Raleigh, pp. 19-35.

Cao, X, Zhang, R, Meng, S, Ren, Q, Mo, M & Liu, Y 2021, Biocontrol potential of *Agromyces allii* 130935 and its metabolites against root-knot nematode *Meloidogyne incognita*. *Rhizosphere*, 19: 100378. https://doi.org/10.1016/j.rhisph.2021.100378.

Collett, RL, Marais, M, Daneel, M, Rashidifard, M & Fourie, H 2021, *Meloidogyne enterolobii*, a threat to crop production with particular reference to sub-Saharan Africa: An extensive, critical and updated review. *Nematology*, 23: 247-285. https://doi.org/10.1163/15685411-bja10076.

Contreras Pérez, M, Hernández Salmerón, J, Rojas Solís, D, Rocha Granados, C, Orozco Mosqueda, MD, Parra Cota, FI, *et al*. 2019, Draft genome analysis of the endophyte, *Bacillus toyonensis* COPE52, a blueberry (*Vaccinium* spp. var. *biloxi*) growth-promoting bacterium. *3 Biotech*, 9. https://doi.org/10.1007/s13205-019-1911-5.

Doan, CT, Tran, TN & = Wang, S 2021, Production of thermophilic Chitinase by *Paenibacillus* sp. TKU052 by Bioprocessing of chitinous fishery wastes and its application in N-acetyl-D-glucosamine production. *Polymers*, 13: 3048. https://doi.org/10.3390/polym13183048.

Dukare, A, Paul, S & Arambam, A 2020, Isolation and efficacy of native chitinolytic rhizobacteria for biocontrol activities against *Fusarium* wilt and plant growth promotion in pigeon pea (*Cajanus cajan* L.). *Egyptian Journal of Biological Pest Control*, 30. https://doi.org/10.1186/s41938-020-00256-7

Dukariya, G & Kumar, A 2020, Distribution and biotechnological applications of Chitinase: A review. *International Journal of Biochemistry and Biophysics*, 8: 17-29.

El Ashry, R, Ali, A & Awad, S 2020, Enhancing application efficiency of *Pseudomonas* spp. and *Serratia marcescens* isolates against *Meloidogyne incognita* in tomato plants. *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control*, 12: 127-145.

El Ashry, RM, Hillal, AE, Qattan, SY, Mostafa, EM, Elshaer, N, Farag, A et al. 2022, Effects of rhizobacteria and seed oils as eco-friendly agents against *Meloidogyne incognita* infested pepper plants under greenhouse and repeated applications field conditions. *Saudi Journal of Biological Sciences*.

El-Sayed, SA, El-Shennawy, RZ, Ahmed Mohammed, T & Attia, AMF 2022, Induction of systemic resistance against damping-off and root-rot of white lupine (*Lupinus albus* L.) using some bioagents, chemical inducers and a mycorrhizal fungus. *Caspian Journal of Environmental Sciences*, 20: 571-583.

Hanash, AAR, Abed, RJ & AL-Magsoosi, AAR 2022, Nymphicidal and adulticidal action of *Beauveria bassiana* isolates in whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in vitro. *Caspian Journal of Environmental Sciences*, 20: 967-975.

Horvath Szanics, E, Perjéssy, J, Klupács, A, Takács, K, Nagy, A, Koppány Szabó, E, Hegyi, F, Németh Szerdahelyi E, DuM, Wang, Z, Kan, J & Zalán, Z 2020, Study of chitinase and chitinolytic activity of *Lactobacillus* strains. *Acta Alimentaria*, 49: 214-224. https://doi.org/10.1556/066.2020.49.2.11.

Ibrahim, DS, Amer, MN, El Shishtawy, HM, Elmaghraby, MMK, Abdellatif, AAM & El Deriny, MM 2022, Nematicidal activity of lactic acid bacteria against root-knot nematodes. *Open Access Journal of Microbiology & Biotechnology*, 7: 000216. https://doi.org/10.23880/oajmb-16000216.

Janmohammadi, M, Bihamta, M & Ghasemzadeh F 2013, Influence of Rhizobacteria inoculation and lead stress on the physiological and biochemical attributes of wheat genotypes. *Cercetari agronomice in Moldova*. 46: 49-67, https://doi.org/10.2478/v10298-012-0074-x.

Jha, S & Modi, HA 2018, Statistical optimization of chitinase production by Streptomyces rubiginosus SP24 and efficacy of purified chitinase to control root-knot nematode infection in *Vigna radiata* under controlled conditions. *Chemical and Biological Technologies in Agriculture*, 5. https://doi.org/10.1186/s40538-018-0133-0.

Jinal, NH & Amaresan, N 2020, Evaluation of biocontrol bacillus species on plant growth promotion and systemic-induced resistant potential against bacterial and fungal wilt-causing pathogens. *Archives of Microbiology*, 202: 1785-1794. https://doi.org/10.1007/s00203-020-01891-2.

Khanna, K, Jamwal, VL, Sharma, A, Gandhi, SG, Ohri, P, Bhardwaj, R, Al Huqail, AA, Siddiqui, MH, Marraiki, N & Ahmad, P 2019, Evaluation of the role of rhizobacteria in controlling root knot nematode (RKN) infection in *Lycopersicon esculentum* plants by modulation in the secondary metabolite profiles. *AoB PLANTS*, 11: plz069. https://doi.org/10.1093/aobpla/plz069.

Kuddus, SM & Ahmad, I 2013, Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. *Journal of Genetic Engineering and Biotechnology*, 11: 39-46.

Ledchumanakumar, S, Aruchchunan, N, Kandiah, P & Gunasingham, M 2021, Root-knot nematode management using chitin-rich fish industry by-product in organic brinjal cultivation. *Biology and Life Sciences Forum*, 3: 57, https://doi.org/10.3390/IECAG2021-09693.

Liu, D, Chen, L, Zhu, X, Wang, Y, Xuan, Y, Liu, X, Chen, L & Duan, Y 2018, Klebsiella pneumoniae SnebYK mediates resistance against *Heterodera glycines* and promotes soybean growth. *Frontiers in Microbiology*,9, https://doi.org/10.3389/fmicb.2018.01134.

Liu, G, Lin, X, Xu, S, Liu, G, Liu, F & Mu, W 2020, Screening, identification and application of soil bacteria with nematicidal activity against root-knot nematode (*Meloidogyne incognita*) on tomato. *Pest Management Science*, 76: 2217-2224. https://doi.org/10.1002/ps.5759.

Lopes, R, Cerdeira, L, Tavares, GS, Ruiz, JC, Blom, J, Horácio, EC, Mantovani, HC & Queiroz, MV 2017, Genome analysis reveals insights of the endophytic *Bacillus toyonensis* BAC3151 as a potentially novel agent for biocontrol of plant pathogens. *World Journal of Microbiology and Biotechnology*, 33, https://doi.org/10.1007/s11274-017-2347-x.

Mangia, NP, Saliba, L & Deiana, P 2018, Functional and safety characterization of autochthonous lactobacillus paracasei FS103 isolated from sheep cheese and its survival in sheep and cow fermented milks during cold storage. *Annals of Microbiology*, 69: 161-170, https://doi.org/10.1007/s13213-018-1416-1

Migunova, VD & Sasanelli, N 2021, Bacteria as biocontrol tool against Phytoparasitic nematodes. *Plants*, 10: 389. https://doi.org/10.3390/plants10020389.

Mohammed, AF 2020, Optimization of cellulase and chitinase enzymes production by plant growth promoting rhizobacteria. *Novel Research in Microbiology Journal*. 4: 641-652. https://doi.org/ 10.21608/nrmj.2020.73437.

Mohammed Al-Shemmary, A & Salih Al-Taee, M 2021, Response of sorghum to effect of two azo dye bacteria, *Caspian Journal of Environmental Sciences*, 19: 251-260.

Murthy, N & Bleakley, B 2012, Simplified method of preparing colloidal chitin used for screening of chitinase-producing microorganisms. *International Journal of Microbiology*, 10: 1-5. https://doi.org/10.5580/2bc3

Neish, AC 1952, Analytical methods for bacterial fermentations. Report 46-8-3, 2nd Revision. National Research Council of Canada, Saskatoon, Canada.

Noureldeen, A, Asif, M, Ansari, T, Khan, F, Shariq, M, Ahmad, F et al 2021, Effect of individual, simultaneous and sequential inoculation of *Pseudomonas fluorescens* and *Meloidogyne incognita* on growth, biochemical, enzymatic and nonenzymatic antioxidants of tomato (*Solanum lycopersicum* L.). *Plants*, 10:1145. https://doi.org/10.3390/plants10061145.

Passardi, F, Penel, C & Dunand, C 2004, Performing the paradoxical: How plant peroxidases modify the cell wall. *Trends in Plant Science*, 9: 534-540. https://doi.org/10.1016/j.tplants.2004.09.002

Phani, V, Khan, MR & Dutta, TK 2021, Plant-parasitic nematodes as a potential threat to protected agriculture: Current status and management options. *Crop Protection*, 144: 105573. https://doi.org/10.1016/j.cropro.2021.105573.

Poria, V, Rana, A, Kumari, A, Grewal, J, Pranaw & K, Singh S 2021, Current perspectives on Chitinolytic enzymes and their agro-industrial applications. *Biology*, 10: 1319. https://doi.org/10.3390/biology10121319.

Rochelle, PA, Will, JAK, Fry, JC, Jenkins, GJS, Parkes, RJ, Turley, CM & Weightman, AJ 1995, Extraction and amplification of 16S rRNA genes from deep marine sediments and seawater to assess bacterial community diversity. In: JT, Trevors *et al*. (Eds.). Nucleic Acids in the Environment. Springer-Verlag Berlin Heidelberg, pp. 219-239.

Rzepkowska, A, Zielińska, D, Ołdak, A & Kołożyn Krajewska, D 2017, Safety assessment and antimicrobial properties of the lactic acid bacteria strains isolated from Polish raw fermented meat products. *International Journal of Food Properties*, 20: 2736-2747.

Sahebani, N & Hadavi, NS 2009, Induction of H_2O_2 and related enzymes in tomato roots infected with root-knot nematode (*M. javanica*) by several chemical and microbial elicitors. *Biocontrol Science and Technology*, 19: 301-313. https://doi.org/10.1080/09583150902752012

Sahebani, N & Omranzade, F 2020, Assessment of plant defence induction and biocontrol potential of Bacillus megaterium wr101 against *Meloidogyne javanica*. *Nematology*, 22: 1091-1099.

Sasi, A, Duraipandiyan, N, Marikani, K, Dhanasekaran, S, Al Dayan, N & Venugopal D 2020, Identification and characterization of a newly isolated chitinase-producing strain *Bacillus licheniformis* SSCL-10 for chitin degradation. *Archaea*, 1-9. https://doi.org/10.1155/2020/8844811

Sayed, M, Abdel Rahman, T, Ragab, A & Abdellatif, A 2019, Biocontrol of root-knot nematode *Meloidogyne incognita* by Chitinolytic Trichoderma spp. *Egyptian Journal of Agronematology*, 18: 30-47. https://doi.org/10.21608/ejaj.2019.52842

Schneider, P & Orelli O 1947, *Entomologisches praktikum* [Entomological internship]. Verlag, HR Sauerlander Co., Aarau, Switzerland, 237 p.

Seo, HJ, Park, AR, Kim, S, Yeon, J, Yu, NH, Ha, S *et al.* 2019, Biological control of root-knot nematodes by organic acid-producing *Lactobacillus* brevis WiKim0069 isolated from kimchi. *The Plant Pathology Journal*, 35: 662-673, https://doi.org/10.5423/ppj.oa.08.2019.0225

Slinkard, K & Singleton, VL 1977, Total phenol analysis: Automation and comparison with manual methods. *The American Journal of Enology and Viticulture*, 28: 49-55.

Soliman, GM, Ameen, HH, Abdel Aziz, SM & El Sayed, GM 2019, In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. *Bulletin of the National Research Centre*, 43. https://doi.org/10.1186/s42269-019-0200-0.

Sorial, M, Mahdy, M, Zawam, H, Aboayana, A & Mousa, E 2020, Induced resistance in pepper plants against root knot nematode by some inducers in relation to the histological changes. *Egyptian Journal of Crop Protection*, 15: 1-21, https://doi.org/10.21608/ejcp.2020.205590

Southey, JF 1986, Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture. Fisheries and Food, London, 202 p.

Spss 2008, Statistical Package for Social Science. Version 17.0.0 Spss Corporation.

Subramani, AK, Raval, R, Sundareshan, S, Sivasengh, R & Raval K 2022, A marine chitinase from *Bacillus aryabhattai* with antifungal activity and broad specificity toward crystalline chitin degradation. *Preparative Biochemistry & Biotechnology*, 52: 1160-1172.

Sun, X, Zhang, R, Ding, M, Liu, Y & Li, L 2021, Biocontrol of the root-knot nematode *Meloidogyne incognita* by a nematicidal bacterium, *Pseudomonas simiae* MB751 with cyclic dipeptide. *Pest Management Science*, 77: 4365-4374, https://doi.org/10.1002/ps.6470.

Tallur, PN, Sajjan, DB, Mulla, SI, Talwar, MP, Pragasam, A, Nayak, VM, Ninnekar, HZ & Bhat, SS 2016, Characterization of antibiotic resistant and enzyme producing bacterial strains isolated from the Arabian Sea. *3 Biotech*, 6. https://doi.org/10.1007/s13205-015-0332-3.

Tamrela, HO, Sugiyanto, A, Santoso, I & Fadhilah, QG 2021, The qualitative screening of cellulolytic, chitinolytic, IAA-producing, and phosphate solubilizing bacteria from Black soldier fly larvae (*Hermetia illucens* L.). IOP Conference Series: Earth and Environmental Science, 948: 012065. https://doi.org/10.1088/1755-1315/948/1/012065.

Tian, S, Xu, Y, Jiang, A & Gong Q 2002, Physiological and quality responses of longan fruit to high O2 or high CO2 atmospheres in storage. *Postharvest Biology and Technology*, 24: 335-340. https://doi.org/10. 1016/s0925-5214(01)00153-3.

Wang, C, Lu, J, Zhang, S, Wang, P, Hou, J & Qian, J 2011, Effects of PB stress on nutrient uptake and secondary metabolism in submerged macrophyte, *Vallisneria natans*. *Ecotoxicology and Environmental Safety*, 74: 1297-1303. https://doi.org/10.1016/j.ecoenv.2011.03.005.

Zhang, L, Wang, D, Hu, Q, Dai, X, Xie, Y, Li, Q, Liu, H & Guo J 2019, Consortium of plant growth-promoting Rhizobacteria strains suppresses sweet pepper disease by altering the rhizosphere microbiota. *Frontiers in Microbiology*, 10. https://doi.org/10.3389/fmicb.2019.01668.

Zhao, D, Zhao, H, Zhao, D, Zhu, X, Wang, Y, Duan, Y, Xuan, Y & Chen, L 2018, Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Biological Control*, 119: 12-19. https://doi.org/10.1016/j.biocontrol.2018.01.004.

Zheljazkov, VD, Horgan, TE, Astatkie, T, Fratesi, D & Mischke, CC 2011, Study on shrimp waste water and Vermicompost as a nutrient source for Bell peppers. *HortScience*. 46: 1493-1496. https://doi.org/10.21273/hortsci.46.11.1493.

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