BMC Microbiology

RESEARCH

Open Access

Characterization and identification of hot pepper-associated endospore-forming bacteria with potential applications as biofertilizers and in biocontrol of pepper wilt pathogens

Shiferaw Demissie Tola[1*], Diriba Muleta[2], Fassil Assefa[2], Farideh Ghadamgahi[3], Ramesh Raju Vetukuri[3*] and Beira Hailu Meressa[1*]

Abstract

Background Although hot pepper contributes significantly to Ethiopia's national economy, its production is hindered by devastating outbreaks of phytopathogens such as *Fusarium* wilt and *Meloidogyne incognita* disease complexes. It is known that bacteria in the pepper rhizosphere can promote plant growth by suppressing soil-borne pathogens and producing growth-promoting substances. Therefore, hot pepper-associated endospore-forming bacteria were evaluated for plant growth-promoting traits and in vitro antagonism to pepper wilt-causing pathogens, revealing some potentially valuable isolates.

Results One hundred and forty-seven heat-resistant endospore-forming rhizobacteria were recovered from 48 rhizosphere samples. Thirty-five of these isolates solubilized phosphate efficiently with solubilization index values of 2.8–10, and produced indole acetic acid (27. 31–59.16 µg/ml). Moreover, 20 isolates hydrolyzed chitin effectively, 21 of them reduced the radial growth of three pathogenic *Fusarium oxysporum* strains by between 26.7% and 79.2%, and cell-free supernatants of 12 isolates reduced the hatching of *M. incognita* eggs by 51–96.4% while also increasing juvenile mortality by 45–98.7%. After 16S rRNA gene sequence analysis, 31 of the isolates were identified as *Bacillus* spp. (*B. siamensis*, *B. velezensis*, and *B. cereus*; $n=26$) and *Paenibacillus polymyxa* ($n=5$).

Conclusions The bacterial strains JUBC7 (*B. cereus*) and JUBC12 (*B. siamensis*) have multiple phytobeneficial traits that make them promising microbial inoculants for protecting high value crops against phytopathogens.

Keywords Dual culture, Heat-tolerant, Plant growth-promoting, Phytopathogens, Supernatant, 16S rRNA

*Correspondence:
Shiferaw Demissie Tola
shiferaw.demissie@ju.edu.et
Ramesh Raju Vetukuri
Ramesh.Vetukuri@slu.se
Beira Hailu Meressa
beira.hailu@ju.edu.et
[1] College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box 307, Jimma, Ethiopia
[2] Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[3] Swedish University of Agricultural Sciences (SLU), Box 190, Lomma SE-234 22, Sweden

Background

Hot pepper (*Capsicum* spp.) cultivation in Ethiopia is hindered by the increasingly devastating impact of abiotic and biotic factors [1–4], causing pepper production to decline from 12.5 t/ha in 2010/11 to 6.5 t/ha in 2021/22 [5]. The primary factors limiting Ethiopian pepper production are diseases and pests such as *Fusarium* wilt and *Meloidogyne incognita*, which are particularly prevalent in southwest Ethiopia due to the local cropping system, intensive cultivation, and edaphic factors [6–8].

Such problems are not limited to Ethiopia; the emergence and wide distribution of virulent phytopathogens and formae speciales such as *Fusarium* wilt strains and root-knot nematodes (RKN) is a global problem. Of special concern is the formation of disease complexes in which one pathogen facilitates the invasion of another for example, infestation by RKN species such as *M. incognita* predisposes plants to *Fusarium* infection. This problem is especially severe in the tropics where diverse soil-borne diseases are found [9–11].

The synergy of *Fusarium* wilt and RKN during co-infection was first recognized in cotton [12, 13]. Previous studies showed that approximately 7.7 x 10^4*Fusarium* conidia (g soil)$^{-1}$ were required to cause visible foliar wilt symptoms on cotton but just 6.5 x 10^2 conidia (g soil)$^{-1}$ were sufficient to induce disease development in the presence of 50 *M. incognita* individuals [14]. Moreover, combined exposure to *M. incognita* and *Fusarium* spp. increased inka nut plant mortality by 25–30% when compared to *M. incognita* alone (5–10% mortality) [15], and several researchers have noted that pepper cultivation in Ethiopia is increasingly threatened by multiple pathogens, particularly those causing wilt complex diseases [1, 16–19].

Previous studies have demonstrated the high prevalence of *M. incognita* on pepper and other vegetables in Ethiopia as follows; this pest's reported prevalence is 58% in the country's central and southwestern regions [20], 90% in the eastern region [21], 55.3–57.7% in the central and western regions [22], and 38.5% in the Oromia, SNNP, Tigray, and Amhara regions [23]. In addition, a recent analysis of the *Nad5* gene revealed that all available *Meloidogyne spp.* samples from the pepper-growing region of the southwestern (Jimma Zone) could be assigned to the species *M. incognita*, whose prevalence in this region ranges from 73 to 89% [8]. *Fusarium* wilt is also a major pathogen of hot pepper in Ethiopia, with disease incidence rates of 50% in the Benishangul-Gumuz region (northwestern) [24], 18–37% in Wallaga (western) [18], 40–65% in Gojam (northwestern) [16, 25], and 58.3–93% in the central region [26]. Our recent analysis of the *ITS* region revealed *F. oxysporum* to be highly abundant in pepper fields within the Jimma Zone [7], which includes some of Ethiopia's main pepper-growing areas. Yield losses caused by *Fusarium* wilt are reportedly as high as 68–71% in Ethiopia's Central Rift Valley [27], demonstrating a clear need for reliable methods of managing soil-borne pathogens in order to sustainably increase Ethiopian pepper production.

Agrochemicals are widely used to control soil-borne pathogens but several studies have shown that these substances can be disappointingly ineffective in addition to causing significant ecological harm [28]. It is therefore notable that recent publications indicate that agricultural production can be sustainably increased by inoculation with plant growth-promoting rhizobacteria (PGPR), which help maintain biodiversity in ecosystems [29, 30]. In particular, endospore-forming rhizobacteria such as *Bacillus subtilis*, *B. velezensis*, *B. siamensis*, *B. amyloliquefaciens*, *B. cereus*, and *Paenibacillus* spp. have drawn attention because they can produce diverse plant growth-promoting substances and antimicrobial agents that combat a wide range of phytopathogens [29, 31–36].

The protective effects of rhizobacteria in the pepper rhizosphere have been demonstrated in several studies: rhizobacteria were found to inhibit bacterial wilt colony growth by 61.5% to 72%, and radial growth of *Fusarium* wilt by 18.6-43.7%, while also reducing *M. incognita* survival by 16.6-65.8% [29, 34, 37, 38]. Moreover, rhizobacteria have been shown to perform diverse plant growth-promoting functions including phosphate solubilization, nitrogen fixation, biosynthesis of antimicrobials and phytohormones such as indole-3-acetic acid, and induction of systematic resistance in pepper via overexpression of genes encoding enzymes involved in pathogen defense such as peroxidases, polyphenol oxidase, and superoxide dismutase [39–41].

Chitinolytic bacteria reduced the occurrence of visible *Fusarium* wilt symptoms in hot pepper by 29–61% while also promoting increases in shoot height and biomass of 6.5–14.4% and 17.4–87%, respectively [42]. Moreover, *P. polymyxa* inhibited the radial growth of *Fusarium* wilt in vitro and reduced the disease severity of *F. oxysporum* f.sp. *capsici* on pepper by 67.7% [43], and culture filtrates of the same species (25–100%) inhibited *M. incognita* egg hatching by 84–91% and increased J2 mortality by 100% in vitro [44]. In addition to producing phytohormones, *B. velezensis* induced systemic resistance in chili peppers and reduced *Fusarium* wilt disease severity by 60% [40]. Although the rhizosphere of hot pepper plants from Ethiopia could harbor diverse plant growth promoting rhizobacteria (PGPR), no bacterial species from this environment had previously been shown to exhibit the full range of advantageous traits, which include effective biofertilization and biopesticide activity.

This study aims to address two major challenges in Ethiopian pepper cultivation, namely nutrient depletion and the spread of phytopathogens, both of which severely affect the production of this high-value crop. The growing threat of soil-borne diseases such as *Fusarium* wilt and *M. incognita* has created an urgent need for sustainable solutions to protect pepper crops. Endospore-forming bacteria from the pepper rhizosphere with PGPR traits and antagonism towards pathogens could provide such a solution, acting as both biofertilizers and biocontrol agents with the potential to

enhance plant growth, improve nutrient uptake, and suppress soil-borne pathogens. By isolating, characterizing, and evaluating hot pepper rhizobacteria for beneficial traits and pathogen antagonism, this study lays a foundation for mass production of biofertilizers and the application of biocontrol agents in Ethiopian agriculture.

Results

Physiological assays and identification of pepper-associated endospore-forming bacteria

A total of 147 bacteria that can withstand heat treatment and dissolve phosphate were collected from 48 soil samples. Thirty-five of these isolates were highly effective at dissolving tricalcium phosphate, with phosphate solubilization index (PSI) values between 2.8 and 10; the isolates with the highest PSI values were JUBC11, JUBC24, and JUBC26 (Table 1 and Fig. 1A). In addition, 29 isolates hydrolyzed chitin, forming clear zones with diameters of 1 to 9 mm, corresponding to chitinolytic index (CI) values of 0.3 to 7.5 (Table 1). Twenty isolates had CI values above 1; those with the strongest chitinase activity were JUBC7 (*B. cereus*), JUBC12 (*B. siamensis*), and JUBC13 (*B. siamensis*) (Fig. 1B). Additionally, 27 isolates (77%) produced hydrogen cyanide (HCN) when grown on a glycine-supplemented tryptic soya agar medium, with JUBC7 (*B. cereus*) exhibiting the highest HCN production (Fig. 1C). All 35 isolates that could solubilize phosphate also produced indole-3-acetic acid (IAA) at concentrations between 27.31 and 59.16 (µg/ml). The strains JUBC5 and JUBC11 (*P. polymyxa*), JUBC8 (*B. velezensis*), and JUBC18 (*B. siamensis*) produced the largest quantities of IAA (Table 1 and Fig. 1D). The bacterial isolates tended to form white, rough, large, and irregular colonies on growth media. Microscopically, they appeared as Gram-positive endospore-forming rods (Fig. 1E).

Table 1 Taxonomic identity and physiological traits of selected hot pepper rhizosphere-associated bacteria

Identity of rhizobacteria		PGPR traits			
Isolates	**Taxonomic status**	**PSI**	**CI**	**IAA(µg / ml)**	**HCN**
JUBC1	*Bacillus siamensis*	3.3	1.5	38.39	+
JUBC5	*Paenibacillus polymyxa*	2.8	1.8	49.13	+
JUBC6	*B. siamensis*	3.2	3.0	36.21	+
JUBC7	*B. cereus*	4.2	7.5	39.91	+ + +
JUBC8	*B. velezensis*	3.0	4.5	48.15	+
JUBC11	*P. polymyxa*	5.7	3.5	59.16	+
JUBC12	*B. siamensis*	3.4	6.3	36.26	+ +
JUBC13	*B. siamensis*	3.0	5.3	34.33	+
JUBC14	*B. siamensis*	3.1	2	27.31	-
JUBC16	*B. siamensis*	3.8	3.5	36.50	-
JUBC17	*B. velezensis*	4.0	1	34.56	+
JUBC18	*B. siamensis*	3.3	2.5	49.91	+
JUBC19	*B. cereus*	3.3	3.5	36.11	+ +
JUBC20	*B. siamensis*	4.0	1.0	32.60	-
JUBC24	*B. cereus*	10.0	0.7	36.86	+ +
JUBC25	*B. velezensis*	4.0	0.6	35.32	+
JUBC26	*B. velezensis*	5.0	0.8	36.08	+
JUBC27	*B. velezensis*	3.1	1.2	36.84	+ +
JUBC28	*B. siamensis*	2.8	1.5	37.47	+
JUBC32	*B. velezensis*	3.0	1.5	35.33	+
JUBC33	*P. polymyxa*	3.0	1.0	31.73	+ +

Data are only shown for the 21 bacterial isolates with the highest PSI values

The symbols -, +, + +, and + + + denote no, weak, moderate, and strong hydrogen cyanide production, respectively

JU Jimma University, *PSI* phosphate solubilization index, *CI* Chitinolytic index, *IAA* Indole acetic acid, *HCN* Hydrogen cyanide

Antagonistic effects of bacterial isolates against *Fusarium oxysporum*

Dual culture assays using three *Fusarium* wilt isolates were performed to evaluate the rhizobacterial isolates' ability to inhibit the pathogen's growth (Fig. 2). In total, 60% ($n=21$ isolates) of the rhizobacterial isolates effectively inhibited *Fusarium* growth.

The antagonistic bacteria demonstrated statistically significant ($F=7.9–19.7$, $P<0.0001$) levels of inhibition, with inhibition percentages between 26.7 and 79.2% (Table 2). Of the 21 tested bacterial isolates, over 95% markedly inhibited *Fusarium* radial growth, achieving significant (> 50%) inhibition percentages against FI4 and up to 52.4% inhibition of FI1 but only 28.6% inhibition of FI5 (Table 2). JUBC12 (*B. siamensis*) and JUBC7 (*B. cereus*) were the most efficient inhibitors, while JUBC22 (*B. velezensis*) was less effective against the tested *Fusarium* isolates (Table 2).

Effect of cell-free supernatant on *M. incognita*

Cell-free supernatants of 12 rhizobacterial isolates were tested for their ability to suppress egg hatching and the J2 activity of *M. incognita* (Fig. 3). All of the isolates significantly ($P<0.0001$, $LSD=1.3–4.6$, $CV=0.9–4.5$) suppressed the hatchability of the eggs (> 50%) and increased J2 mortality by over 45% ($P<0.0001$, $LSD=1.7–3.3$, $CV=1.2–4.1$) for all supernatant concentrations) compared to the control group (Fig. 3). The best-performing isolate was JUBC7 (*B. cereus*), which had the highest egg-hatching inhibition percentage and J2 mortality rate. Increasing the concentration of JUBC7 supernatant from 25 to 100% caused a corresponding increase in egg hatchability inhibition (76–96%) and J2 mortality (73–99%). Similarly, increasing the applied concentration of JUBC12 (*B. siamensis*) increased inhibition of egg hatching (75–96%) and J2 mortality (64–97%).

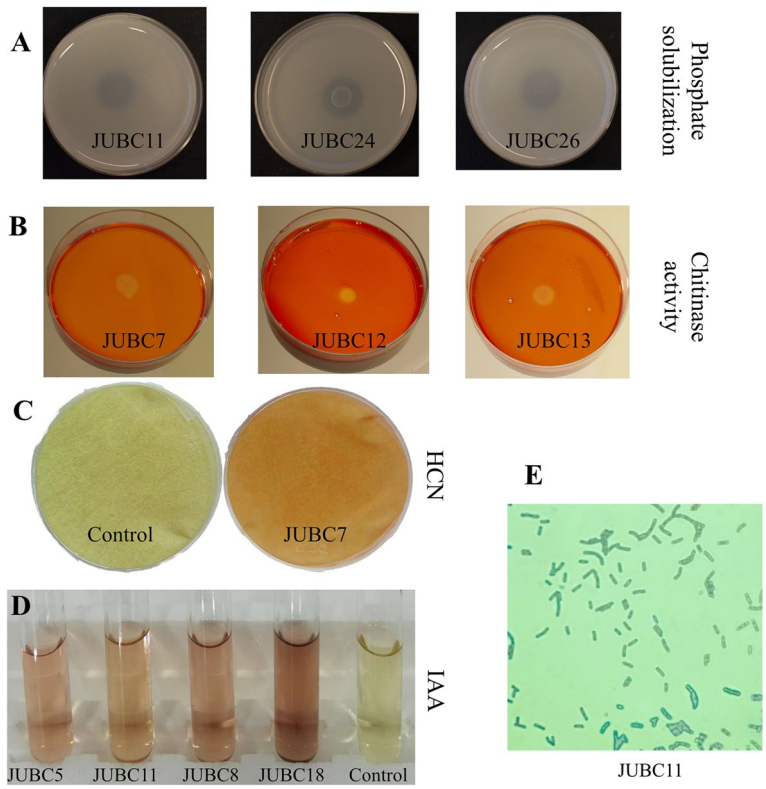

Fig. 1 Morphological and physiological traits of hot pepper-associated endospore-forming rhizobacterial isolates. **A** Phosphate solubilization, **B** Chitinase activity **C** HCN production, **D** Indole-3-acetic acid (IAA) production, and **E** Micrograph showing the rod-like shape of the most active isolates

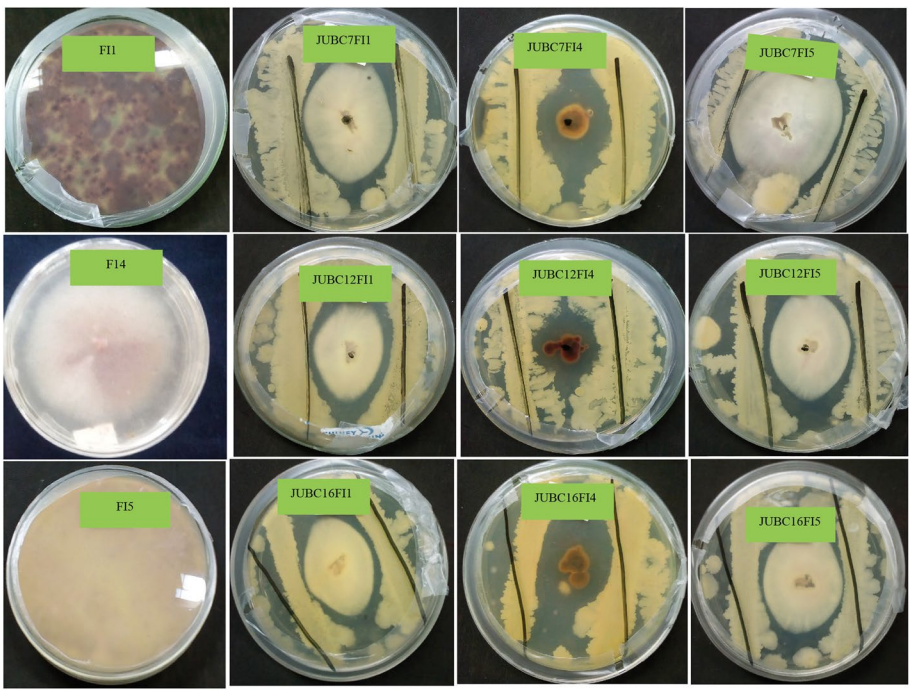

Fig. 2 Antagonistic effects of endospore-forming rhizobacteria against *Fusarium* wilt. Control plates (left-most column) and treatment plates using three antagonistic rhizobacterial isolates. JUBC7; *Bacillus cereus*, JUBC12 and JUBC16; *B. siamensis*. **FI1, FI4, FI5; *Fusarium oxysporum* isolates 1, 4, and 5

Table 2 Antagonistic effects of pepper-associated rhizobacteria against mycelial growth of *Fusarium* pepper wilt isolates. Data are presented as means ± SD

Isolates	Identity(16SrRNA)	*Fusarium* hyphal growth inhibition (%)		
		FI1*	FI4*	FI5*
JUBC1	*Bacillus siamensis*	45.5 ± 3^{def}	75 ± 4.2^{ab}	33.3 ± 2.7^{fgh}
JUBC3	*B. siamensis*	42.4 ± 6.1^{ef}	72.2 ± 6.4^{b}	26.7 ± 1.8^{h}
JUBC4	*B. siamensis*	48.5 ± 3^{cde}	66.7 ± 4.2^{cd}	36.7 ± 3.3^{fgh}
JUBC5	*Paenibacillus polymyxa*	53.5 ± 1.7^{abc}	58.3 ± 4.2^{e}	33.3 ± 3.3^{fgh}
JUBC6	*B. siamensis*	39.4 ± 3^{f}	61.4 ± 2.7^{de}	55.6 ± 2^{bc}
JUBC7	*B. cereus*	56.8 ± 1.8^{ab}	78.7 ± 2^{a}	61.4 ± 1.7^{ab}
JUBC8	*B. velezensis*	57.2 ± 2.9^{a}	66.7 ± 4.2^{cd}	33.3 ± 3.3^{fgh}
JUBC9	*P. polymyxa*	51.5 ± 3^{abcd}	58.3 ± 4.2^{e}	36.7 ± 3.3^{fg}
JUBC10	*B. siamensis*	39.4 ± 3^{f}	79.1 ± 4.2^{a}	36.7 ± 3.3^{fg}
JUBC11	*P. polymyxa*	57.4 ± 3^{a}	70.8 ± 4.2^{bc}	30 ± 3.3^{gh}
JUBC12	*B. siamensis*	57.6 ± 1.7^{a}	79.2 ± 4.2^{a}	65.6 ± 5.1^{a}
JUBC13	*B. siamensis*	39.4 ± 3^{f}	61.1 ± 2.5^{de}	43.3 ± 6.7^{de}
JUBC14	*B. siamensis*	51.53 ± 6.1^{abcd}	58.3 ± 4.2^{e}	33.3 ± 10.7^{ef}
JUBC15	*B. siamensis*	45.5 ± 3^{def}	79.1 ± 4.2^{a}	33.3 ± 3.3^{fgh}
JUBC16	*B. siamensis*	55.1 ± 2.8^{abc}	61.1 ± 4.2^{de}	51.4 ± 1.7^{cd}
JUBC17	*B. velezensis*	42.4 ± 3^{ef}	58.3 ± 4.2^{e}	30 ± 0.3^{gh}
JUBC18	*B. siamensis*	51.5 ± 1.3^{abcd}	61.4 ± 2.8^{de}	54.4 ± 5.1^{bcd}
JUBC19	*B. cereus*	39.4 ± 3^{f}	63.7 ± 2.6^{de}	53.7 ± 9^{cd}
JUBC20	*B. siamensis*	52.3 ± 1.3^{abcd}	58.3 ± 4.2^{e}	26.7 ± 1.9^{h}
JUBC21	*P. polymyxa*	52.1 ± 2.2^{abcd}	50.6 ± 2.4^{f}	33.3 ± 3.3^{fgh}
JUBC22	*B. velezensis*	45.5 ± 3^{def}	41.2 ± 3.4^{g}	30 ± 3.3^{gh}

Means labeled with different letter(s) across the columns differ significantly according to Duncan's Multiple Range Test ($p < 0.0001$). SD: standard deviation. *FI1, FI4, FI5; *Fusarium oxysporum* isolates 1, 4, and 5

Two of the tested bacterial strains, JUBC7 and JUBC12, significantly outperformed the other bacterial isolates in terms of their effects against *M. incognita*, even at the lowest tested supernatant concentration (25%). For example, JUBC7 and JUBC12 induced 76% and 75% inhibition of egg hatching, respectively, whereas the corresponding values for JUBC11 and JUBC16 were 51% and 55%, respectively. Similar outcomes were observed for J2 mortality (73% for JUBC7 and 64.2% for JUBC12, compared to 45.1% and 45.3% for JUBC13 and JUBC18, respectively). JUBC19 (*B. cereus*) and JUBC11 (*P. polymyxa*) also effectively reduced egg hatchability and caused substantial J2 mortality at concentrations of 75% and 100%, respectively (Fig. 3).

Molecular analysis of endospore-forming bacterial isolates

The 16S rRNA gene sequences of 31 bacterial isolates were analyzed, revealing that they belonged to the genera *Bacillus* and *Paenibacillus* (Table 3). Each isolate was assigned a unique GenBank accession number starting with OM766257-OM766283, although four were excluded from subsequent analysis due to poor sequence quality. We compared the newly identified isolates to the NCBI database and found that they were all highly similar to known strains, with similarities of 99% to 100% (Table 3).

The new isolates were closely related to bacteria from diverse sources, as shown by the topology of a Maximum Likelihood tree with bootstrapping values > 50 (Fig. 4). The majority of the isolates (26, corresponding to 83.9% of the total) were assigned to the genus *Bacillus*, while the remaining 5 (16.1% of the total) belonged to *Paenibacillus*. Within the *Bacillus* genus, three different species were identified: *B. siamensis* (50%), *B. velezensis* (34.6%), and *B. cereus* (15.4%). All *Paenibacillus* isolates belonged to the species *P. polymixa* (Fig. 4).

Discussion

Heat treatment-resistance rhizobacteria were successfully isolated from the rhizosphere of hot peppers (Table 1). Thirty-five of these isolates displayed multiple plant growth-promoting traits including phosphate solubilization, chitin hydrolysis, and production of IAA and HCN, all of which are (along with production of ammonia, siderophores, and antimicrobials) valuable for promoting plant growth [34]. Additionally, 21 isolates effectively suppressed the mycelial growth of pepper wilt fusarium, while 12 strongly inhibited egg hatchability and J2 survival in the disease-causing nematode parasite *M. incognita*. These findings suggest that the pepper rhizosphere is a rich source of heat-tolerant bacterial strains with remarkable plant growth-promoting properties and strongly antagonistic activity against phytopathogens. This is consistent with previous reports showing that pepper-associated rhizobacteria exhibit biopesticidal and biofertilizing activity in vitro resulting from production of plant growth substances and antagonistic activity against bacterial wilt (*R. solanacearum*) [29, 34]. In particular, the endospore-forming pepper rhizobacteria *B. subtilis, B. amyloliquefaciens*, and *B. cereus* reportedly inhibited bacterial wilt colony growth by 66–68.4%, 64.5%, and 61.5%, respectively [29]. In another study, the inhibition of fungal mycelial growth of Sorghum anthracnose (*Colletotrichum graminicola*) due to *Trichoderma* spp. in the range of 60.4- 76.5% was observed on a dual culture plate [45]. The use of beneficial rhizobacteria as biofertilizers and biocontrol agents could thus offer substantial environmental and economic benefits, providing sustainable and eco-friendly alternatives to chemical treatments and reducing reliance on agrochemicals that harm both ecosystems and human health [41, 45, 46]. By suppressing pathogens such as *Fusarium* wilt and parasitic nematodes, these microbial inoculants enhance the

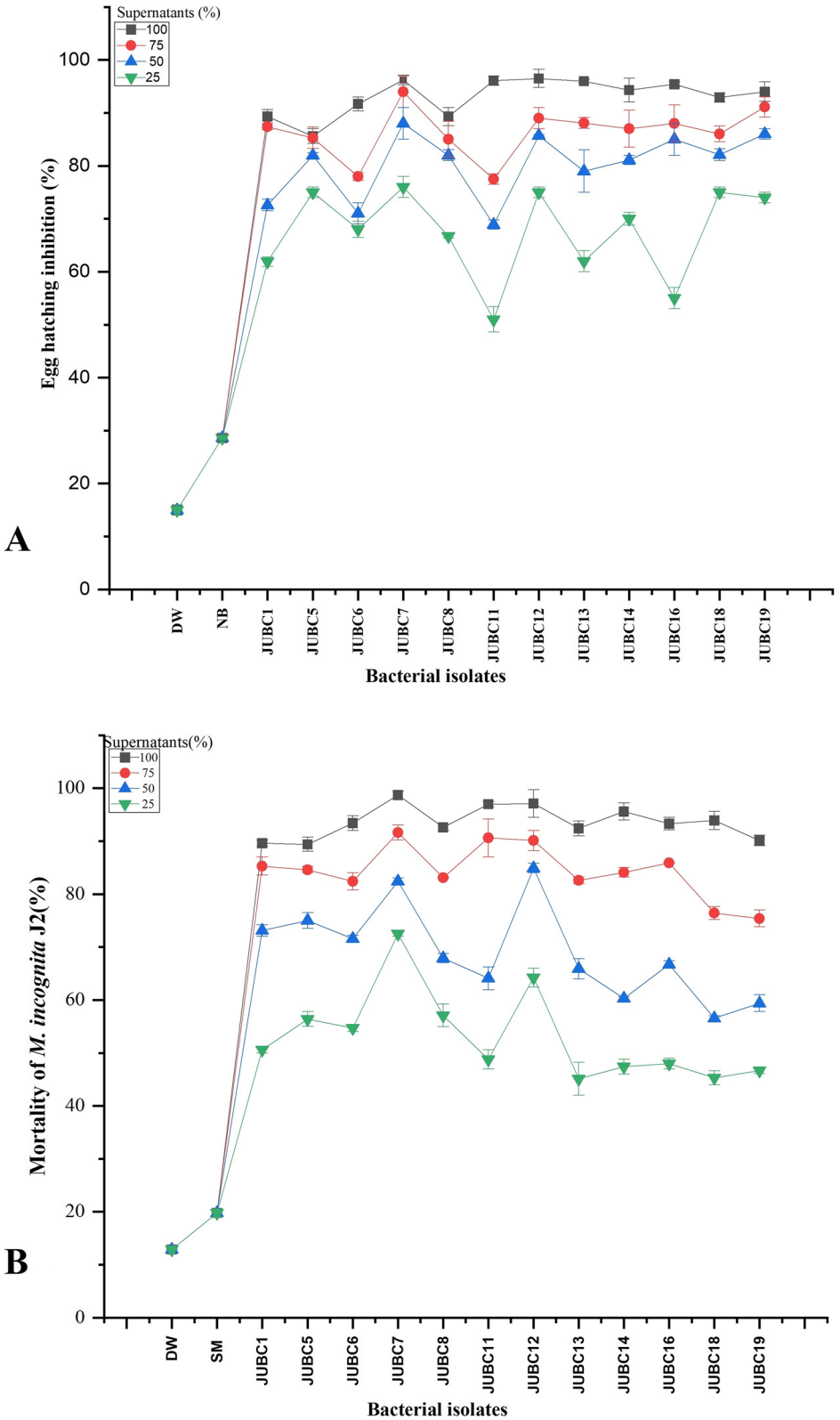

Fig. 3 Effects of cell-free supernatants of 12 pepper-associated endospore-forming rhizobacterial isolates on egg hatching (**A**) and J2 mortality of *Meloidogyne incognita* (**B**) in vitro. DW; distilled sterile water. NB; nutrient broth. SM; solid medium. JUBC1, JUBC6, JUBC12, JUBC13, JUBC14, JUBC16, JUBC18; *Bacillus siamensis*. JUBC5 and JUBC11; *Paenibacillus polymyxa*. JUBC7 and JUBC19; *B. cereus*. JUBC8; *B. velezensis*. Line graphs with error bars generated using Origin Pro 2024 version 10.10.178

Table 3 Similarity of the identified isolates to strains retrieved from databases

Blast match						
Isolate Code[a]	Closely related strain[b]	Accession Nº.[c]	Similarity (%)	% Query coverage	Accession Nº[d]	Nº of isolates[e]
JUBC1	*B. siamensis* HoB-1	OM074300	100	100	OM766257,	1
JUBC3	*B. siamensis* LMR2	OM471772	100	100	OM766258,	8
JUBC4	*B. siamensis* JA1	OM455487	100	100	OM766259,	1
JUBC5	*P. polymyxa* ZF197	MK961276	99.9	100	OM766283,	5
JUBC7	*B. cereus* P20-08	OM061692	100	100	OM766261,	1
JUBC8	*B. velezensis* CR-502	OM074020	100	100	OM766262,	8
JUBC13	*B. siamensis* WB6	OL636035	99.9	99	OM766265,	1
JUBC15	*B. siamensis* XB12	OM536024	99.2	100	OM766267,	1
JUBC19	*B. cereus* DS-2	ON005108	100	100	OM766271,	3
JUBC26	*B. velezensis* XC1	MT649755	100	100	OM766276,	1
JUBC28	*B. siamensis* SDI-28	KT021508	99.3	100	OM766278	1

[a] Codes assigned to currently identified bacterial isolates

[b] Related bacterial strains retrieved from the NCBI database

[c] Accession number of bacterial strains obtained from the NCBI

[d] Accession number of currently identified bacterial isolates

[e] Number of isolates assigned to a given related strain from the database. *B. siamensis*; *Bacillus siamensis*, *P. polymyxa*; *Paenibacillus polymyxa*

health of soil and crops, which in turn fosters biodiversity and minimizes long-term environmental damage caused by synthetic pesticides and fertilizers. Another important advantage of adopting strategies based on inoculation with beneficial rhizobacteria is that it could significantly reduce input costs for farmers. This is particularly important in tropical agricultural regions such as Ethiopia, where chemical pesticides and fertilizers are expensive and difficult to apply, and farming is predominantly done on smallholdings with high turnover of pathogens/pests. The strains identified in this work could potentially be incorporated into integrated pest management (IPM) systems to improve vegetable production and increase yields [47, 48]. For instance, combined use of biocontrol agents and fungicides increased pepper yields by over 22% in one study [49]. The bacterial strains identified here could further enhance crop production after validation under varied field conditions, offering a cost-effective way to improve both productivity and profitability for pepper growers. Such a shift towards biological control would increase agricultural sustainability and support long-term food security while reducing environmental risks.

Phosphate solubilization activity was assayed using Pikovskaya's medium. Several isolates produced clear zones indicating varying levels of organic acid production and phosphate mobilization [34, 50], with PSI values ranging from 2.8–10. Three stood out as being especially suitable for use in the acidic soils of Ethiopia's Jimma areas, where complexation by Fe^{2+} and Al^{3+} ions greatly limits phosphate availability to crops [51]: JUBC11 (*P. polymyxa*), JUBC24 (*B. cereus*), and JUBC26 (*B. velezensis*). The genera *Bacillus* and *Pseudomonas* thus appear to be the best phosphate solubilizers and suppliers of available phosphate to plants [52]. This is notable because both this work and previous studies have shown that *Bacillus* spp. are also effective producers of plant growth-promoting substances and antagonists of soil-borne phytopathogens [41].

All 35 of the bacterial isolates produced the phytohormone indole-3-acetic acid (IAA), in concentrations ranging from 27.3 to 59.16 (µg/ml). The highest concentrations were generated by the strains JUBC5 (*P. polymyxa*), JUBC8 (*B. velezensis*), JUBC11 (*P. polymyxa*), and JUBC18 (*B. siamensis*). Other studies have similarly demonstrated effective IAA production by pepper-associated rhizobacteria [34, 53]. Exogenous IAA can enhance root growth and improve nutrient absorption by plants, especially in nutrient-deficient soils, making IAA production a valuable plant growth-promoting trait. Additionally, 27 of the bacterial isolates produced HCN, with JUBC7 (*B. cereus*) producing the most. HCN is a rhizobacterial secondary metabolite that can suppress pathogen activity by inhibiting energy metabolism while also promoting plant growth by sequestering metal ions and thus increasing phosphate availability [54].

Twenty-nine of the rhizobacterial isolates demonstrated at least some chitinase activity, forming distinct clear zones on the assay plates. This is desirable for pest control because microbial chitinases can degrade fungal

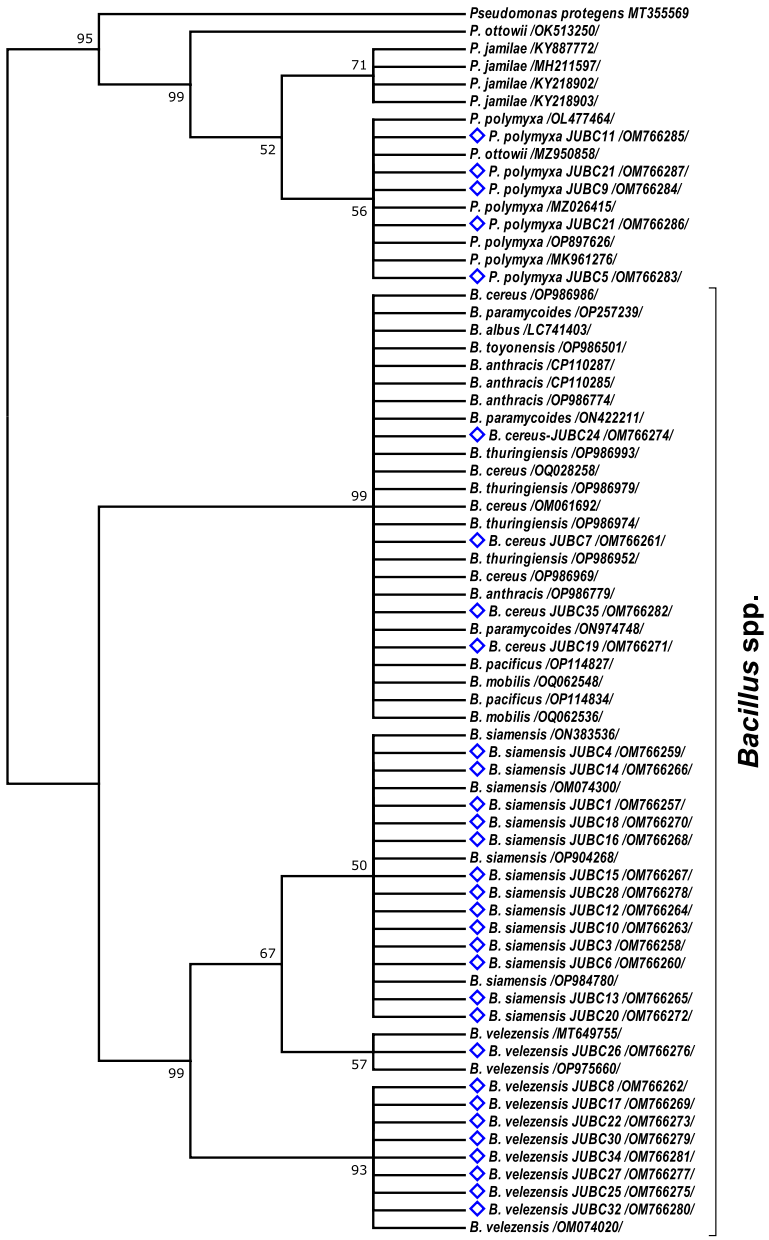

Fig. 4 Maximum Likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the potential PGPR isolates from the hot pepper rhizosphere and reference strains from the GenBank database including *Pseudomonas protegens* (MT355569), which was used as an outgroup taxon. Newly identified species are enclosed in diamond-shaped blue boxes. The numbers on the tree indicate the bootstrap percentages after 1000 iterations

cell walls as well as nematode eggshells and cuticles [37]. Formerly, chitinase-producing bacteria isolated from rhizosphere soil were shown to effectively inhibit the growth of phytopathogenic fungi [55], and treatment with the metabolite of pepper rhizobacteria was recently shown to induce disintegration of the bacterial wilt cell wall and deformation of bacterial wilt cells [29]. The isolates with the highest chitinolytic activity were *Bacillus* species, namely JUBC7 (*B. cereus*), JUBC12 (*B. siamensis*), and JUBC13 (*B. siamensis*). The CI values of these isolates were all above 1, indicating that they surpass the useful activity threshold for potential biocontrol agents [56]. *B. cereus* was previously confirmed to produce chitinase [57].

Previous studies have identified the production of diverse secondary metabolites and tolerance of harsh

environments as characteristic traits of endospore-forming bacteria [31, 58]. The isolates identified in this work displayed both traits. For example, *B. velezensis* can combat diverse fungal pathogens including *F. oxysporum*, *F. moniliforme*, and *C. falcatum* due to its production of metabolites that effectively suppress pathogen growth, including antibiotics, lipopeptides, hydrolytic enzymes, and HCN [29, 46, 59].

Supernatants from twelve of the isolates identified in this work also significantly reduced the hatchability of nematode eggs and caused severe J2 mortality in *M. incognita*. This is consistent with an earlier report noting that a cell-free supernatant from a *B. cereus* strain isolated from the rhizosphere of healthy tomato plants grown on Chinese farmland infested with *M. incognita* increased J2 mortality by 92.8% and reduced egg hatching by 33.8% [60]. In another study, supernatants from *P. polymyxa* reduced *M. incognita* egg hatchability by 91% and caused 100% J2 mortality in vitro [44]. These nematicidal effects may be due to the production of metabolites such as HCN, chitinase, and other toxic compounds that would both increase J2 mortality and reduce egg hatchability by degrading the cuticle and changing the behavior of nematodes [61–63]. The high efficacy of *Bacillus* strains in this context is supported by the literature: multiple studies have shown that *Bacillus* spp. increase *M. incognita* mortality more than other bacterial genera [41, 64]. This may be due to their ability to produce diverse bioactive substances and to form endospores while also competing effectively with phytopathogens.

Several of the isolated bacterial strains thus exhibited both potential PGPR features and strong antagonism towards the phytopathogens *Fusarium* and *M. incognita*. Strains exhibiting particularly good activity in both senses included JUBC7 (*B. cereus*), JUBC12 (*B. siamensis*), JUBC11 (*P. polymyxa*), JUBC13 (*B. siamensis*), JUBC16 (*B. siamensis*), and JUBC8 (*B. velezensis*). The results of greenhouse experiments also indicated that JUBC7 (*B. cereus*) and JUBC12 (*B. siamensis*) effectively suppressed *Fusarium* wilt infection and *M. incognita* infestation while increasing the growth of pepper plants (data to be presented in a forthcoming publication). Previous studies have shown that several of these bacterial species possess diverse phytobeneficial properties that can increase crop production and productivity [29, 40, 43, 44, 65].

The 16S *rRNA* gene sequence was used to identify 35 phytobeneficial bacterial isolates (four isolates excluded from analysis due to their bad sequences), which were assigned to different species of *Bacillus* such as *Bacillus cereus*, *B. siamensis*, *B. velezensis*, and *Paenibacillus polymyxa*. Similarly, earlier workers indicated the presence of those bacteria in the plant rhizosphere, but in this study considerable number and effective strains of *P. polymyxa* were obtained [29, 31, 34, 38, 66, 67]. The earlier studies also found that endospore-forming bacteria from the pepper rhizosphere produce multiple plant growth-promoting substances and are antagonistic towards various soil-borne pathogens [37, 38] as well as suppressing bacterial wilt [34].

The plant growth promoting activity and phytopathogen antagonism observed among the rhizobacterial isolates identified here is consistent with previous reports. For example, *B. velezensis* was shown to contain genes promoting plant growth and biocontrol activity, making it a preferred species for developing inoculants [56], and another study showed that *B. cereus* has typical PGPR characteristics and can combat pathogenic fungi due to its abundance of gene clusters for producing plant growth promoting and antifungal compounds [66]. Additionally, *Paenibacillus* spp. from plant roots can have multiple growth-promoting traits and a wide range of antimicrobial activities [68], and a *B. siamensis* strain was found to be antagonistic towards *F. oxysporum* both in vitro and in vivo [69]. Our results confirm these reported activities and demonstrate the high diversity of endospore-forming bacteria in the pepper rhizosphere.

Conclusions

The results presented here show that the hot pepper rhizosphere harbors diverse microbial inoculants with beneficial plant growth promoting activity and antagonism towards important phytopathogens. It was particularly rich in *Bacillus* species, with *B. siamensis* being dominant, and many of the identified strains could potentially be used to manage pepper wilt caused by *Fusarium* and *M. incognita* in the major pepper growing areas of Ethiopia's Jimma Zone. Our analyses suggest that the isolates JUBC12 (*B. siamensis*) and JUBC7 (*B. cereus*) have particularly high potential for development as biofertilizers and biocontrol agents against *Fusarium* wilt and nematode infection. Thus, to exploit these microbial inoculums efficiently, in future, their genes and or metabolites that are responsible for phytopathogen antagonism and producing of plant growth-promoting substances should be determined.

Materials and methods

Origin of pathogens and biocontrol agents

Endospore-forming rhizobacteria were isolated from healthy pepper rhizospheres, while *Fusarium* isolates were collected from infected pepper plants and *M. incognita* was collected randomly from nematode-infested pepper fields in the Jimma Zone, where the crop is widely cultivated [7, 8]. Characterization of the *Fusarium* wilt and rhizobacterial isolates as well as in vitro antagonism tests were performed at Jimma University.

DNA extraction and sequencing of *Fusarium* wilt and rhizobacterial isolates were performed at the Swedish University of Agricultural Sciences (Sweden). Molecular identification of *Meloidogyne* spp. was conducted at Ghent University (Belgium).

Sample collection and isolation of endospore-forming bacteria from pepper rhizosphere

During the 2018 pepper growing season, 48 pepper plant samples were collected from major pepper cultivating districts in the Jimma Zone. These samples included 24 from the Omo Nada district and 12 each from Sekoru and Kersa districts. In all cases, the sampling sites were similar to those from which *M. incognita* and *Fusarium* wilt samples were collected. Healthy plant samples were uprooted along with non-rhizosphere soil from different sites within the study areas. Intact roots and the adhered soil were transferred to a sterile polythene bag with a proper label and transported to laboratories at the Biology Department of Jimma University. After removing loosely adhered soil by gently shaking, the roots were cut into 2 cm pieces along with firmly adhered soil using sterile scissors.

From each rhizosphere sample, 10 g of soil was suspended in a flask containing 90 ml standard saline solution (0.85% NaCl) and kept on a rotary shaker at 125 rpm for 30 min. Upon preparing appropriate dilutions (10^{-1}–10^{-6} ml^{-1}), the serially diluted samples were heat treated at 80 °C for 10 min to eliminate non-endospore-forming rhizobacteria.

Screening of phosphate solubilizing bacteria

Aliquots (0.1 ml) of each heat-treated diluted sample were spread onto pre-solidified Pikovskaya's agar plates (10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $NH_4(SO_4)_2$, 0.5 g yeast extract, 0.1 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.2 g KCl, 0.002 g $MnSO_4$, 0.002 g $FeSO_4$, and 15 g Agar in 1L distilled water) and incubated at 32 °C for 2–5 days [70]. Pure cultures were then obtained by separately transferring 10–15 bacterial colonies that formed clear zones on each Pikovskaya's agar plate into screw cap test tubes containing nutrient broth (i.e., one colony per test tube). One loopful of each of the resulting overnight cultures was re-streaked onto nutrient agar medium (Oxoid, 1.5 g beef extract, 5 g peptone, 1.5 g yeast extract, 5 g NaCl, 15 g agar in 1L distilled water) for verification of purity and further study.

Evaluation of isolates for phosphate solubilization

The phosphate solubilization activity of the rhizobacterial cultures was evaluated by spotting them on a Pikovskaya agar plate that was then incubated for 2 to 5 days at 32 °C. The sizes of the resulting colonies and halo zones were measured with a transparent ruler and the phosphate solubilization index (PSI) was calculated using the following equation [71]:

$$PSI = \frac{Colony\ diameter + Halo\ zone\ diameter}{Colony\ diameter}$$

Assays of plant growth-promoting traits

HCN production assay

A 24 h old culture was spread on pre-solidified Tryptic soya agar medium (5 g peptic digest of soybean meal, 5 g sodium chloride, 15 g agar in 1L distilled water) supplemented with glycine (4.4 g/l). A sterilized Whatman No.1 filter paper was soaked with 2 ml of picric acid solution (2.5 g picric acid and 12.5 g Na_2CO_3 in 1L distilled water) and placed on the plate's lid, which was then sealed with parafilm, after which the plate was incubated at 32 °C for seven days. HCN production was evaluated based on the development of orange to red coloration as described previously [54].

IAA production assay

The IAA production of the bacterial isolates was determined using an established method [72]. Briefly, a loopful of a young bacterial culture was inoculated into nutrient broth amended with 5 mg/ml L-tryptophan and incubated at 32 °C for 48 h. After incubation, the culture was centrifuged at 5000 rpm for 20 min, then 2 ml of the supernatant was mixed with two drops of orthophosphoric acid and 4 ml of Solawaski's reagent (50 ml, 35% perchloric acid, 1 ml of 0.5 M $FeCl_3$) in a test tube that was then kept at room temperature for 20 min. The development of pink coloration indicated the production of IAA, which was quantified by measuring absorbance at 530 nm using a V-600 UV spectrophotometer (Japan Spectroscopic Company, JASCO) and the level of IAA production was determined using a standard IAA curve.

Chitinase activity assay

Chitinolytic activity was evaluated using a modification of a published method [56]. A loopful of a young bacterial culture was spotted on pre-solidified minimal salt medium (Na_2HPO_4 0.65 g, 12.25 g NaCl, 1.5 g KH_2PO_4, 0.12 g $MgSO_4$, 0.5 g NH_4Cl, 0.005 g $CaCl_2$ and 5 g colloidal chitin dissolved in 1L distilled water) in two locations and incubated at 32 °C for seven days, then soaked with Congo red dye 0.1% (w/v) for ten min and washed with distilled water. The diameters of the bacterial colonies and the clear zones formed around them were measured using a transparent ruler, then the chitinolytic index was calculated as follows:

$$Chitinolytic\ index = \frac{Clear\ zone\ diameter - Colony\ diameter}{Colony\ diameter}$$

Characterization of the endospore-forming bacterial isolates

Cultural and microscopic characteristics of isolates

Bacterial isolates considered effective were characterized based on their colonial morphology (i.e., colony color, shape, size, and texture). Additionally, gram and endospore staining was performed for microscopic observation after smear preparation [73].

In vitro assay for antagonism of isolates towards *Fusarium* and nematodes

Source of Fusarium and nematode pests

Fusarium oxysporum (FI1, FI4, and FI5) isolates were selected based on a preliminary pathogenicity test using the Mareko Fana pepper variety [7]. *M. incognita* is known to be a major parasite of hot peppers and was collected from infested fields in the Jimma Zone, where its activity is a significant problem [8].

Antagonism towards *Fusarium* isolates

Thirty-five rhizobacterial isolates (JUBC1-35) displaying PGPR traits were identified using the assays described in the preceding section. Their activity against *Fusarium* wilt fungi was evaluated using a dual culture technique in which the positions of the pathogen and antagonistic microbes are interchanged on the same medium. For primary screening, a fungal suspension (10^7 spores/ml) was seeded on a pre-solidified 1:1 mix of PDA (potato dextrose agar) and NA (nutrient agar). Then, 48 h-old bacterial agar blocks (1×1.5 cm^2) were placed at two positions on the pathogen-inoculated medium and incubated at 28 °C for 5–7 days [74].

Under similar conditions to the preliminary screening test, the degree of fungal radial growth inhibition caused by the bacterial isolates was quantified by placing a 5-day-old *Fusarium* mycelial disc (1×2 cm^2) at the center of a 90 mm Petri dish containing a pre-solidified 1:1 mixture of PDA and NA. A loopful of overnight bacterial culture (10^9 CFU/ml) was then streaked in a broadband fashion about 3 cm away from the mycelial block on two opposite sides of the plate and incubated at 28 °C for 5–7 days until complete plate coverage was achieved by the mycelium of a *Fusarium* disc on a control plate without antagonistic microbes. These experiments were performed in triplicate. The antagonistic effect of the bacterial strains against the fungal pathogen was quantified by computing the degree of fungal radial growth inhibition relative to the control using the equation shown below [45], where *P* is the growth inhibition percentage (%), *R1* is the diameter of the *Fusarium* mycelium in the absence of antagonistic bacteria (mm), and *R2* is the diameter of the *Fusarium* mycelium in the presence of antagonistic bacteria.

$$P = \frac{R_1 - R_2}{R_1} \times 100$$

Assay of activity against *Meloidogyne incognita*

Twelve of the rhizobacterial isolates that inhibited *Fusarium* wilt growth were tested for effects on nematode egg hatching and second-stage juvenile (J2) mortality.

Preparation of cell-free extract

A single colony from each antagonistic bacterium was inoculated into a screw-cap test tube containing 10 ml sterilized nutrient broth and incubated at 32 °C for 48 h. An aliquot of the bacterial culture was then placed in a 2 ml Eppendorf tube and centrifuged at 12,000 rpm for 10 min. The supernatant was passed through a 0.22 µ Millipore filter and used directly in the intended assay while the cell pellet was discarded [75].

Meloidogyne incognita egg hatching and J2 mortality assays

Effects on egg-hatching and J2 mortality were evaluated separately, as described previously [75]. For the egg-hatching assay, the cell-free rhizobacterial supernatants were used as stock solutions (100%) and also diluted with distilled sterile water to obtain dilutions at 75, 50, and 25% of the initial supernatant concentration. For each concentration, 1 ml of supernatant from each of the 12 bacterial isolates was separately placed in one well of a 12-well plate. *M. incognita* eggs harvested from a susceptible tomato rootstock using 0.5% NaOCl solution with a mean density of 50 eggs per 100 µl) were then placed in the wells and incubated at room temperature. The number of hatched eggs in each well was counted under a stereomicroscope at 24 h intervals for 120 h. The trial was repeated twice and each treatment was tested in triplicate in both cases. Distilled sterile water and autoclaved nutrient broth were used as controls. The egg hatching inhibition rate was calculated using a previously reported equation [75]: $I\ (\%) = (C\text{-}T)/C \times 100$, where *I* represents the egg hatching inhibition percentage, *T* is the hatchability of the eggs in the treatment groups and *C* is the hatchability of the eggs in the control group.

To evaluate effects on J2 mortality, a freshly hatched 30/100 µl J2 suspension of *M. incognita* was placed in each well of a 12-well plate containing 1 ml of cell-free extract (separate multi-well plate were prepared for extract concentrations of 25, 50, 75, and 100%) and in a control plate containing sterile nutrient agar and distilled sterile water

in place of the bacterial extract. The plates were then covered with parafilm and incubated at room temperature for 120 h. J2 survival was determined by counting under a stereomicroscope at 24 h intervals for 120 h. Nematodes were considered dead if they did not move when probed with a fine needle. The experiment was repeated twice, with three replicates of each treatment (including controls) in both runs. J2 mortality was calculated using the equation $JM\ (\%) = (T/C) \times 100$, where *JM* is the J2 mortality, *T* is the number of dead individuals, and *C* is the total number of J2 individuals used in the test [75].

Molecular identification of endospore-forming bacterial isolates

Thirty-five (35) endospore-forming bacterial isolates that showed multiple plant growth traits and antagonism towards phytopathogens were selected for molecular identification. Pure cultures of the bacterial colonies were inoculated into Luria–Bertani (LB) broth (Duchefa Biochemie, Netherlands) and incubated at 28 °C for 12 h. Genomic DNA was then extracted from each isolate using a Quick-DNA Fungal/Bacterial Microprep Kit (Zymo Research, Irvine, CA, USA). DNA yield was measured using a NanoDrop microphotometer (NanoDrop Technologies, South San Francisco, CA, USA) and its integrity was checked by electrophoresis on a 1% agarose gel.

The 16S rRNA gene of each isolate was amplified with the following primer pair: 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 907R (5′-CCG TCA ATT CMT TTR AGT TT-3′) [76]. PCR was performed using 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 10 pmol of each primer, 1 U HotStar *Taq* polymerase (Qiagen Valencia, CA), and 10 ng of genomic DNA as the template set, with the following thermal profile: heating at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 50 °C for 30 s, and 72 °C for 30 s with a final incubation for 5 min at 72 °C.

The PCR products were purified using the Qiagen PCR purification kit (Qiagen, UK) and sequenced at the Eurofins sequencing facility (Konstanz, Germany). BioEdit version 7.0.5 was used to visualize raw sequences and edit low-quality sequences. The newly obtained sequences were used to perform BLAST similarity searches against the GenBank NCBI database [77]. Search hits for sequences from records in the database were analyzed for sequence coverage and identity, and the best-matched NCBI accession was recorded.

After aligning and trimming the unaligned sequences using Seaview version 5, the newly obtained 16S rRNA gene sequences together with the sequences retrieved from GenBank and a sequence from the outgroup taxon *Pseudomonas protegens* (MT355569) were used to reconstruct phylogenetic trees using the maximum likelihood method. The support for each branch was estimated using the bootstrap method with a heuristics search and 1000 replicates.

Data analysis

Data on bacterial physiology, antagonistic activity against *Fusarium* wilt, and inhibition of egg hatching and J2 mortality in *M. incognita* were analyzed using SAS statistical software version 9.3 (SAS Institute, 211). Duncan's multiple range test (DMRT) was used to compare the means for each treatment at $P < 0.05$. Graphs showing the results of the nematode antagonism assays were generated using Origin Pro 2024 version 10.10.178.

Abbreviations

CI	Chitinolytic index
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
IPM	Integrated pest management
JM	J2 mortality
J2	Second-stage juvenile
LB	Luria-Bertani broth
NA	Nutrient agar
PDA	Potato dextrose agar
PGPR	Plant growth-promoting rhizobacteria
PSI	Phosphate solubilization index

Acknowledgements

The authors would like to express their gratitude to Jimma University for providing financial support and access to facilities. RRV acknowledges support from FORMAS (2019–01316), the Carl Tryggers Foundation for Scientific Research (CTS 20:464), the Crafoord Foundation (20200818), the Novo Nordisk Foundation (0074727), and the SLU Centre for Biological Control.

Authors' contributions

SDT and BHM designed and executed the experiments and carried out the study. SDT wrote the draft manuscript. RRV and FG carried the molecular part. SDT analyzed the data including the bioinformatics aspect. SDT, BHM, DM, FA, and RRV read and approved the final manuscript.

Funding

This work was supported by Jimma University.

Data availability

The nucleotide sequences of the 16S rDNA gene and accession numbers of bacterial isolates deposited in the GenBank database under accession numbers OM766257- OM766283(https://www.ncbi.nlm.nih.gov/Genbank/update.html) and also ITS region sequences and accession numbers of the *Fusarium* isolates; OQ771938- OQ771941 (https://submit.ncbi.nlm.nih.gov/subs/?search=SUB13039407). For the rest of the experiments, their analyzed data and material are available in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 19 September 2023 Accepted: 14 March 2025
Published online: 08 April 2025

References

1. Assefa M, Dawit W, Lencho A, Hunduma T. Assessment of wilt intensity and identification of causal fungal and bacterial pathogens on hot pepper (Capsicum annuum) in Bako Tibbe and Nonno Districts of West Shewa Zone, Ethiopia. Int J Phytopathol. 2015;4(1):21–8.
2. Zeleke A, Derso E. Production and management of major vegetable crops in Ethiopia. Addis Ababa: Eth-Cana Printing Press; 2015.
3. Nigussie G, Zewdia I. Achievements of hot pepper and it agro-ecological requirements in Ethiopia: a review paper. Adv Agron Plant Breed Hortic. 2021;9(4):01–6.
4. Sirba HY, Begna T, Gojam M. Adaptability evaluation of recently released hot pepper (capsicum anum) varieties at highland areas of west Hararghe, Ethiopia. Int J Res Agron. 2021;4(2):87–93.
5. CSA. Report on area and production of major crops, agricultural sample survey, the Federal Democratic Republic of Ethiopia, Volume I, Addis Ababa, Ethiopia, 2010–2022.
6. Mekonen S, Chala A. Assessment of hot pepper (*Capsicum* species) diseases in southern Ethiopia. Int J Sci Res. 2014;3(3):91–5.
7. Demissie S, Megersa G, Meressa BH, Muleta D. Resistance levels of Ethiopian hot pepper (Capsicum spp.) varieties to a pathogenic Fusarium sp. and in vitro antagonistic effect of Trichoderma spp. Arch Phytopathol Plant Prot. 2021;54(11–12):647–63.
8. Tola SD, Meressa BH, Muleta D, Assefa F. Biodiversity of parasitic nematodes associated with hot pepper (Capsicum spp.) in Jimma area, Ethiopia. Russ J Nematol. 2022;30(2):161–73.
9. Ploetz RC. Tropical fruit crops and the diseases that affect their production. Trop Biol Conserv Manag Agri. 2009;3:71.
10. Bucki P, Paran I, Ozalvo R, Iberkleid I, Ganot L, Braun MS. Pathogenic variability of *Meloidogyne incognita* populations occurring in pepper-production greenhouses in Israel toward Me1, Me3 and N pepper resistance genes. Plant Dis. 2017;101(8):1391–401.
11. Shaheen N, Khan UM, Azhar MT, Tan DK, Atif RM, Israr M, Yang SH, Chung G, Rana IA. Genetics and genomics of fusarium wilt of chilies. Rev Agron. 2021;11(11):2162–81.
12. Atkinson GF. Some diseases of cotton. Bull Ala Agr Exp Sta. 1892;41:51.
13. Martin WJ, Newson LD, Jones JE. Relationship of nematodes to the development of *Fusarium* wilt in cotton. Phytopathol. 1956;46:285–9.
14. Garber RH, Jorgenson EC, Smith S, Hyer AH. Interaction of population levels of Fusarium oxysporum f. sp. vasinfectum and Meloidogyne incognita on cotton. J Nematol. 1979;11(2):133–7.
15. Guerrero-Abad JC, Padilla-Domínguez A, Torres-Flores E, López-Rodríguez C, Guerrero-Abad RA, Coyne D, Oehl F, Corazon-Guivin MA. A pathogen complex between the root knot nematode Meloidogyne incognita and Fusarium verticillioides results in extreme mortality of the inka nut (Plukenetia volubilis). J Appl Botany Food Qual. 2021;94:162–8.
16. Abebe A, Abera M. Pepper disease assessment and identification in major growing districts of west Gojam zone in Northwestern Ethiopia. Int J Sustain Agric Res. 2019;6(1):8–20.
17. FDRE, JICA. Pepper Production, Ministry of Agriculture, Horticulture Development Technology Transfer Directorate (HDTTD) of FDRE and Japan International Cooperation Agency. 2019. https://www.jica.go.jp/Resource/project/english/ethiopia/010/materials/pnjn3r000000ob5f-att/02_08_01_03.pdf. Accessed 21 May 2022.
18. Biri B, Gomathinayagam P. Response of hot pepper (Capsicum annuum L.) to major fungal diseases under field and greenhouse conditions in Horo Guduru Wollega, Oromia, Ethiopia. Afr J Agric Res. 2021;17(6):923–32.
19. Adina G, Desalegn Y, Muluadam B. Survey on major diseases of horticultural crops in South Gondar Zone, Ethiopia. Int J Novel Res Eng Sci. 2022;8(2):1–4.
20. Mandefro W, Dagne K. Morphological variation of root-knot nematode populations from Ethiopia. PMJOE. 2000;4(2):19–28.
21. Tefera T, Huluka M. Distribution of Meloidogyne incognita (root-knot nematode) in some vegetable fields in Eastern Ethiopia. PMJOE. 2000;4(1, 2):77–84.
22. Mandefro W, Mekete T. Root-knot nematodes on vegetable crops in the central and western Ethiopia. PMJOE. 2002;2002(6):37–44.
23. Feyisa B, Yimer F, Kebede G. Survey and identification of plant parasitic nematodes associated with pepper in Ethiopia. JBAH. 2023;13(6):12–8.
24. Kebede M, Gidesa A. Survey and identification of diseases on major crops of Assosa and Kamashi zones, Ethiopia. GIJASH. 2017;1(1):21–5.
25. Guta A, Abera G. Assessments of distribution and status of major disease of hot pepper (Capsicum annuum L.) in West Shoa and East Wollega Zones, Ethiopia. J Plant Sci. 2022;10(2):57–63.
26. Oljira T, Berta S. Isolation and characterization of wilt-causing pathogens of local growing pepper (C. annuum L.) in Gurage Zone, Ethiopia. Int J Agron. 2020;6638683:1–8.
27. Gabrekiristos E, Demiyo T. Hot pepper fusarium wilt (Fusarium oxysporum f. sp. capsici): epidemics, characteristic features and management options. J Agric Sci. 2020;12(10):347–60.
28. Noumavo PA, Agbodjato NA, Baba-Moussa F, Adjanohoun A, Baba-Moussa L. Plant growth promoting rhizobacteria: beneficial effects for healthy and sustainable agriculture. Afr J Biotechnol. 2016;15(27):1452–65.
29. Kashyap AS, Manzar N, Nebapure SM, Rajawat MVS, Deo MM, Singh JP, Kesharwani AK, Singh RP, Dubey SC, Singh D. Unraveling microbial volatile elicitors using a transparent methodology for induction of systemic resistance and regulation of antioxidant genes at expression levels in chili against bacterial wilt disease. Antioxid. 2022;11(2):404.
30. Santoyoa G, Orozco-Mosquedaa MC, Govindappa M. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. Biocontrol Sci Technol. 2012;22(8):855–72.
31. McSpadden Gardener BB. Ecology of Bacillus and Paenibacillus spp. in agricultural systems. Phytopathol. 2004;94(11):1252–8.
32. Lyngwi NA, Joshi SR. Economically important *Bacillus* and related genera: a mini-review. Biol Useful Plants Microb. 2014;3:33–43.
33. Yadav DK, Devappa V, Kashyap AS, Kumar N, Rana VS, Sunita K, Singh D. Boosting the biocontrol efficacy of *Bacillus amyloliquefaciens* DSBA-11 through physical and chemical mutagens to control bacterial wilt disease of tomato caused by Ralstonia solanacearum. Microorganisms. 2023;11(7): 1790.
34. Kashyap AS, Manzar N, Rajawat MVS, Kesharwani AK, Singh RP, Dubey SC, Pattanayak D, Dhar S, Lal SK, Singh D. Screening and biocontrol potential of rhizobacteria native to Gangetic plains and hilly regions to induce systemic resistance and promote plant growth in chili against bacterial wilt disease. Plants. 2021;10(10):2125.
35. Ghadamgahi F, Tarighi S, Taheri P, Saripella GV, Anzalone A, Kalyandurg PB, Catara V, Ortiz R, Vetukuri RR. Plant growth promoting activity of *Pseudomonas aeruginosa* FG106 and its ability to act as a biocontrol agent against potato, tomato, and taro pathogens. Biol. 2022;11(1):140–67.
36. Djian-Caporalino C, Molinari S, Palloix A, Ciancio A, Fazari A, Marteu N, Ris N, Castagnone-Sereno P. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. Eur J Plant Pathol. 2011;131:431–40.
37. Wiratno WI, Syakir M, Sucipto I, Pradana AP. Isolation and characterization of endophytic bacteria from roots of *Piper nigrum* and their activities against *Fusarium oxysporum* and *Meloidogyne incognita*. Biodivers J of Biological Diversit. 2019;20(3):682–7.
38. Le KD, Kim J, Yu NH, Kim B, Lee CW, Kim JC. Interactional mechanisms of Paenibacillus polymyxa SC2 and pepper (Capsicum annuum L.) suggested by transcriptomics. BMC Microbiol. 2021;21:70–86.
39. Shin JH, Park BS, Kim HY, Lee KH, Kim KS. Antagonistic and plant growth-promoting effects of *Bacillus velezensis* BS1 isolated from rhizosphere soil in a pepper field. Plant Pathol J. 2021;37(3):307–14.
40. Martínez-Raudales I, Cruz-Rodríguez Y, Alvarado-Gutiérrez A, Vega-Arreguín J, Fraire-Mayorga A, Alvarado-Rodríguez M, Balderas-Hernández V, Fraire-Velázquez S. Draft genome sequence of Bacillus velezensis 2A–2B strain: a rhizospheric inhabitant of Sporobolus airoides (Torr.) Torr., with antifungal activity against root rot causing phytopathogens. Stand Genom Sci. 2017;12:60–73.
41. Kashyap AS, Manzar N, Meshram S, Sharma PK. Screening microbial inoculants and their interventions for cross-kingdom management of wilt disease of solanaceous crops-a step toward sustainable agriculture. Front Microbiol. 2023;14: 1174532.
42. Suryanto D, Patonah S, Munir E. Control of fusarium wilt of chili with chitinolytic bacteria. HAYATI J Biosci. 2010;17(1):5–8.
43. El-Kazzaz MK, Ghoneim KE, Agha MK, Helmy A, Behiry SI, Abdelkhalek A, Saleem MH, Al-Askar AA, Arishi AA, Elsharkawy MM. Suppression of pepper root rot and wilt diseases caused by *Rhizoctonia solani* and *Fusarium oxysporum*. Life. 2022;12(4):587.

44. Khan Z, Kim SG, Jeon YH, Khan HU, Son SH, Kim YH. A plant growth-promoting rhizobacterium, Paenibacillus polymyxa strain GBR-1, suppresses root-knot nematode. Bioresour Technol. 2008;99(8):3016–23.
45. Manzar N, Singh Y, Kashyap AS, Sahu PK, Rajawat MVS, Bhowmik A, Sharma PK, Saxena AK. Biocontrol potential of native Trichoderma spp. against anthracnose of great millet (Sorghum bicolour L.) from Tarai and hill regions of India. Biol Control. 2021;152: 104474.
46. Manzar N, Kashyap AS, Goutam RS, Rajawat MVS, Sharma PK, Sharma SK, Singh HV. Trichoderma: Advent of versatile biocontrol agent, its secrets and insights into the mechanism of biocontrol potential. Sustainability. 2022;14:12786.
47. Le KD, Kim J, Yu NH, Kim B, Lee CW, Kim JC. Biological control of tomato bacterial wilt, kimchi cabbage soft rot, and red pepper bacterial leaf spot using *Paenibacillus elgii* jck-5075. Front Plant Sci. 2020;11:775.
48. Saad AM, Salem HM, El-Tahan AM, El-Saadony MT, Alotaibi SS, El-Shehawi AM, Abd El-Mageed TA, Taha AE, Alkahtani MA, Ahmed AE, Swelum AA. Biological control: an effective approach against nematodes using black pepper plants (Piper nigrum L.). Saudi. J Biol Sci. 2022;29:2047–55.
49. Abada KA, Attia AM, Zyton MA. Management of pepper verticillium wilt by combinations of inducer chemicals for plant resistance, bacterial bioagents and compost. J Appl Biotechnol Bioeng. 2018;5(2):117–27.
50. Huang Y, Wu Z, He Y, Ye BC, Li C. Rhizospheric *Bacillus subtilis* exhibits biocontrol effect against *Rhizoctonia solani* in pepper *(Capsicum annuum)*. Biomed Res Int. 2017;2017:1–10.
51. Demissie S, Muleta D, Barecha G. Effect of phosphate solubilizing bacteria on seed germination and seedling growth of Faba Bean (Vicia faba L.). Int J Agric Res. 2013;8(3):123–36.
52. Wahyudi AT, Astuti RP, Widyawati A, Meryandini A, Nawangsih AA. Characterization of Bacillus sp. strains isolated from the rhizosphere of Soybean plants for their use as potential plant growth for promoting rhizobacteria. J Microbiol Antimicrob. 2011;3(2):34–40.
53. Admassie M, Wold-Hawariat Y, Alemu T. Characterization and diversity of plant growth promoting endophytic and rhizosphere bacteria isolated from the rhizosphere and tissues of pepper and their effect on plant growth promotion and disease suppression of Phytophthora capsici. Res Sq. 2021. https://doi.org/10.21203/rs.3.rs-422729/v1.
54. Bakker AW, Schippers B. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp.-mediated plant growth stimulation. Soil Biol Biochem. 1987;19(4):451–7.
55. Parani K, Shetty GP, Saha BK. Isolation of *Serratia marcescens* SR1 as a source of chitinase having potentiality of using as a biocontrol agent. Indian J Microbiol. 2011;51:247–50.
56. Gupta M, Aziz N, Choudhary DK, Shrivastava N, Varma A, Paul B. Identification of chitin degrading bacterial strains isolated from bulk and rhizospheric soil. J Pure Appl Microbial. 2018;12(1):133–41.
57. Ajayi AA, Onibokun EA, George FO, Atolagbe OM. Isolation and characterization of chitinolytic bacteria for chitinase production from the African catfish, *Clarias gariepinus* (Burchell, 1822). Res J Microbiol. 2016;11(4):119–25.
58. Goswami SK, Kashyap AS, Kumar R, Gujjar RS, Singh A, Manzar N. Harnessing Rhizospheric microbes for eco-friendly and sustainable crop production in saline environments. Curr Microbiol. 2024;81(1):14.
59. Shahid I, Han J, Hanooq S, Malik KA, Borchers CH, Mehnaz S. Profiling of metabolites of Bacillus spp. and their application in sustainable plant growth promotion and biocontrol. Front Sustain Food Syst. 2021;5: 605195.
60. Xiao TJ, Tan SY, Shen QR, Ran W. *Bacillus cereus* X5 suppresses root-knot nematode of tomato by colonizing in roots and soil. Afr J Microbio Res. 2012;6(10):2321–7.
61. Xie Z, Li M, Wang D, Wang F, Shen H, Sun G, Feng C, Wang X, Chen D, Sun X. Biocontrol efficacy of *Bacillus siamensis* LZ88 against brown spot disease of tobacco caused by *Alternaria alternate*. Biol Control. 2021;154: 104508.
62. El-Sayed WS, Akhkha A, El-Naggar MY, Elbadry M. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Front Microbiol. 2014;5: 118165.
63. Rao MS, Kamalnath M, Umamaheswari R, Rajinikanth R, Prabu P, Priti K, Grace GN, Chaya MK. *Bacillus subtilis* IIHR BS-2 enriched vermin compost controls root knot nematode and soft rot disease complex in carrot. Sci Hortic. 2017;218:56–62.
64. Xiang N, Lawrence KS, Kloepper JW, Donald PA, John A. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton. Plant Dis. 2017;101:774–84.
65. Niu DD, Wang CJ, Guo YH, Jiang CH, Zhang WZ, Wang YP, Guo JH. The plant growth-promoting rhizobacterium Bacillus cereus AR156 induces resistance in tomatoes with induction and priming of defense response. Biocontrol Sci Technol. 2012;22(9):991–1004.
66. Zhou H, Ren ZH, Zu X, Yu XY, Zhu HJ, Li XJ, Zhong J, Liu E-M. Efficacy of plant growth-promoting bacteria *Bacillus cereus* YN917 for biocontrol of rice blast. Front Microbiol. 2021;12: 684888.
67. Lyngwi NA, Nongkhlaw M, Kalita D, Joshi SR. Bioprospecting of plant growth promoting bacilli and related genera prevalent in soils of pristine sacred groves: biochemical and molecular approach. PLoS ONE. 2016;11(4): e0152951.
68. Ali MA, Lou Y, Hafeez R, Li X, Hossain A, Xie T, Lin L, Li B, Yin Y, Yan J, An Q. Functional analysis and genome mining reveal high potential of biocontrol and plant growth promotion in nodule-inhabiting bacteria within Paenibacillus polymyxa complex. Front Microbiol. 2021;11:618601.
69. Shen N, Li S, Li SY, Zhang H, Jiang M. The siderophore-producing bacterium, *Bacillus siamensis* Gxun-6, has an antifungal activity against *Fusarium oxysporum* and promotes the growth of bananas. Egypt J Biol Pest Control. 2022;32:34–43.
70. Pikovskaya RI. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiol. 1948;17:362–70.
71. Karpagam T, Nagalakshmi PK. Isolation and characterization of phosphate solubilizing microbes from agricultural soil. Int J Curr Microbiol Appl Sci. 2014;3(3):601–14.
72. Bric JM, Bostock RM, Silversone SE. Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol. 1991;57(2):535–8.
73. Gram HC. Uber die isolierte färbung der schizomyceten in schnittund trockenpräparaten. Fortschr Med. 1884;2:185–9.
74. Muleta D, Assefa F, Granhall U. In vitro antagonism of rhizobacteria isolated from Coffea arabica L. against emerging fungal coffee pathogens. Eng Life Sci. 2007;7(6):577–86.
75. Wei L, Shao Y, Wan J, Feng H, Zhu H, Huang H, Zhou Y. Isolation and characterization of a rhizobacterial antagonist of root-knot nematodes. PLoS ONE. 2014;9(1): e85988.
76. Morales SE, Holben WE. Empirical testing of 16S rRNA gene PCR primer pairs reveals variance in target specificity and efficacy not suggested by in silico analysis. Appl Environ Microbiol. 2009;75(9):2677–83.
77. Altschul SB. Local alignment search tool. J Mol Biol. 1990;215:403–10.

Publisher's Note