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Isolation and characterization of thermophilic bacteria from different habitats and their assessment for antagonism against soil-borne fungal plant pathogens

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Three different biomaterials *viz*., boiled cow milk, compost manure and tomato rhizospheric soil were found as habitats of the thermophilic antagonistic bacteria. The isolated bacteria were able to grow satisfactorily at thermophilic temperature range (>55°C). Based on morphological, biochemical and physiological characters, the bacterial isolates were identified as *Bacillus licheniformis* (boiled cow milk), and *Bacillus stearothermophilus* (compost manure and tomato rhizospheric soil). All the three thermophilic bacterial isolates exhibited strong antagonism against tested soil-borne fungal plant pathogens in order of *B. licheniformis* (inhibition zone of 67.67 mm against *R. bataticola*) > *B. stearothermophilus* from compost manure (51.67 mm against *R. solani*) > *B. stearothermophilus* from tomato rhizospheric soil (38.33 mm against *P. aphanidermatum*). The ability to tolerate high temperature (>55°C), pH (6-8) and salt concentrations (up to 8%), and antibiotic resistance properties of the antagonistic thermophilic *Bacillus* isolates may hold them as potential biocontrol candidates, especially under stressed rhizosphere environments where other biocontrol agents fail. However, the results need further confirmation under field conditions where these bioagents will be applied in a formulated form.

Key words: Antagonism, antibiotic sensitivity, biocontrol activity, soil borne pathogens, thermophilic bacteria.

INTRODUCTION

Plant diseases caused by fungi, bacteria and viruses are the major factors limiting the agricultural productivity, reducing the crop yields to the tune of 16% globally (Oerke, 2006). The soil-borne disease complex consisting mainly of damping off, root rot, collar rot and wilts causes more than 50% losses in crop production (Biswas and Das, 1999). Globally, the chemical fungicides like metalaxyl, captan, benomyl, chlorothalonil, copper oxychloride and many more are being used desperately for the control of soil-borne crop diseases caused by pathogenic genera *viz*., *Pythium*, *Fusarium, Phytophthora, Rhizoctonia* and *Sclerotium* (Rao et al., 2007; Wightwick et al., 2010). However, risk of ground water pollution affecting the quality of life, destruction of non-target beneficial soil microflora, deleterious effects on mycorrhizal associations, depletion of soil nutrient

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dynamics, especially of nitrogen are some of the deleterious effects associated with excess chemical use for the control of soil-borne plant pathogens (Georgieva et al., 2002; Van-Zwieten, 2004; Rao et al., 2007; Wightwick et al., 2010). Under such circumstances, biological control assumes critical importance in crop disease management, especially for soil-borne fungal diseases.

Antagonistic bacteria are the ideal candidates for biocontrol of plant diseases of economically important crops; for example *Bacillus subtilis* GB-03 against *F. oxysporum f. sp. vasinfectum* and *R. solani* in cotton (Brannen and Backman, 1994), *Bacillus cereus* against *Phytopthora sojae* in soybean (Osburn et al., 1995). However, failure of bacterial antagonists to survive and accord long term disease control under environmental stress conditions is a major hindrance to the adoption of biocontrol strategies for management of soil borne plant diseases (Hoitink and Boehm, 1999). The antagonistic bacteria that could tolerate extreme environmental conditions and offer efficient control of fungal plant pathogens are supposed to be good alternative for plant disease management. Being endospore formers, thermophilic bacteria can tolerate heat and resist themselves from desiccation under high temperature stress, a feature makes them an attractive candidate for biocontrol of plant diseases (Rao et al., 2007; Wightwick et al., 2010). They exhibit excellent seed and root colonising ability, thereby suppressing the growth of fungal pathogens effectively (Kim et al., 1997). Besides, they are abundantly present in rhizospheric soils as saprophytes which make them as ideal biological control agents for soil-borne plant diseases.

The occurrence of thermophilic bacteria has been reported from various biomaterials like milk and milk products (Ruckert et al., 2004; Ronimus et al., 2006), compost manure (Miyatake and Iwabuchi, 2005), and crop rhizospheric soils (Sao Paulo, 2007; Santana et al. 2013). However, their use as potential bioagents for the control of soil-borne fungal plant pathogens has been less explored. The basic aim of the present study was to isolate and characterize the thermophilic bacteria from three different biomaterials *viz*., boiled cow milk, compost manure and tomato rhizospheric soil and to evaluate their antagonistic potential against important soil borne fungal pathogens for possible introduction as biocontrol agents in plant disease management. The study helped in identifying the promising candidate isolates of thermophiic bacteria which can further be up scaled for commercial bioformulation for control of destructive soil borne crop diseases.

MATERIALS AND METHODS

Sample collection

The samples of boiled cow milk and compost manure were obtained from the Cattle Improvement Scheme, Department of Animal Science and Dairy Science, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri. The rhizospheric soil of tomato crop was collected from experimental field of the Vegetable Improvement Scheme of Department of Horticulture, MPKV, Rahuri, Maharashtra, India. The temperatures of the sources at the time of the sampling were recorded as 81, 69 and 40°C for boiled cow milk, compost heap and tomato rhizospheric soil, respectively. The samples were collected in sterile polybags and immediately brought into the laboratory for isolation.

Isolation of thermophilic bacteria and determination of thermal death points

The bacteria from collected samples were isolated on Nutrient Agar (NA) medium (Himedia, Mumbai, India) following enrichment culture technique (Allen, 1953). The 250 ml conical flasks containing 10 g of sample and 90 ml sterile nutrient broth were homogenised in orbital shaker at 150 rpm and were heat shocked by placing in water bath at 80°C for 10 min. The suspensions thus obtained were serially diluted. The sterilie NA plates were inoculated with 1ml of solution from serial dilution of 10^7 using spread plate technique (), and were incubated at 55°C for 24 h. The distinct single colonies were subcultured onto freshly prepared manganese agar slants to facilitate further growth. The pure cultures of bacterial isolates were subjected to different temperatures ranging from 5 – 80□C for determination of thermal death points and survival at higher temperatures. For temperatures between 5 – 65°C, the bacterial isolates were tested using nutrient agar whereas for hyper thermophilic range (70 - 80°C) nutrient broth was used (Seeley and Vandemark, 1970). After incubation for 24 h, the NA plates were observed for growth pattern of bacterial isolates at respective temperatures while test tubes with NB were observed for turbidity.

Identification of thermophilic bacterial isolates

Overnight grown cultures of the isolated microorganisms were examined under microscope for colony morphology, cell shape, size, arrangement and motility. The gram staining and endospore staining were performed as per the standard procedures mentioned in Bergey's manual of determinative bacteriology (Snaeth, 1986). The isolates were subjected to biochemical tests *viz*., catalase test, oxidase test, acid and gas production, casein hydrolysis, gelatin liquefaction, starch hydrolysis, citrate utilization, nitrate reduction, hydrogen sulfide production, indole production, urease activity and methyl Red-Voges Proskeur (MR-VP) tests by following the standard procedures given by Seeley and Vandemark (1970) and Cappuccino and Sherman (1987).

Sensitivity to the concentrations of pH, NaCl and antibiotics in growth media

The effects of various concentrations of pH (2-10), NaCl (1-10%) and antibiotics on growth and sporulation of thermophilic bacterial isolates were determined by inoculating the NA slants with overnight cultures of thermophilic *Bacilli* and incubating at 50□C for 48 h (Gulati et al., 2007; Singh et al., 2010). Sensitivity to antibiotics *viz*., streptocyclin, streptomycin sulphate, bacterianashak and cyclohexamide with different concentrations (ppm) was evaluated (Imanaka et al., 1981). Growth pattern of bacterial isolates on different concentrations of pH, NaCl and antibiotics were characterised as no growth (-), slow growth (+), moderate growth (++), profused growth (+++) and very profused growth (++++).

In vitro screening for antagonism against soil borne fungal pathogens

The thermophilic bacterial isolates were screened for antagonistic

Table 1. Screening of bacterial isolates for thermotolerance.

Test temperature (°C)	*Growth pattern of bacterial isolates from		
	Boiled cow milk	Compost manure	Tomato rhizospehric soil
	Thermophilic range		
80	+	-	-
75	++	-	-
70	+++	++	++
65	++++	++	++
60	++++	++	+++
55	++++	+++	++++
50	++++	++++	++++
	Mesophilic range		
45	++++	++++	++++
40	++++	++++	++++
35	++++	+++	+++
30	++++	+++	+++
25	++++	++	+++
	Psychrophilic range		
20	++	+	+
15	+	+	+
10	-	-	-
5	-	-	-

*Growth patterns: -, no; +, slow; ++, moderate; +++, profuse; ++++, very profuse.

activity against major soil borne fungal pathogens *viz*., *Pythium aphanidermatum*, *Fusarium oxysporum* F. sp. *ciceri*, *Rhizoctonia bataticola, Sclerotium rolfsi, Fusarium oxysporum F. sp lycopersici* and *Rhizoctonia solani*. The test fungal plant pathogens used in present study were freshly isolated from the disease samples collected from experimental fields of Mahatma Phule Krishi Vidyapeeth, Rahuri. A dual culture technique (Morton and Stroube, 1955) was used for *P. aphanidermatum* and *S. rolfsi*, whereas seeding and disc assay method (Besson et al., 1978) was used of the rest of the pathogens. The thermophilic bacterial isolates exhibiting zone of inhibition against test pathogen were screened as potential antagonist. Morphological characters of approaching hyphae were observed every day under light microscope (400 IX70-S1F2, Olympus Optical Co. Ltd., Tokyo, Japan) and images were recorded with a digital camera (CAMEDIA C-3040 Zoom, Olympus Optical Co. Ltd.).

RESULTS AND DISCUSSION

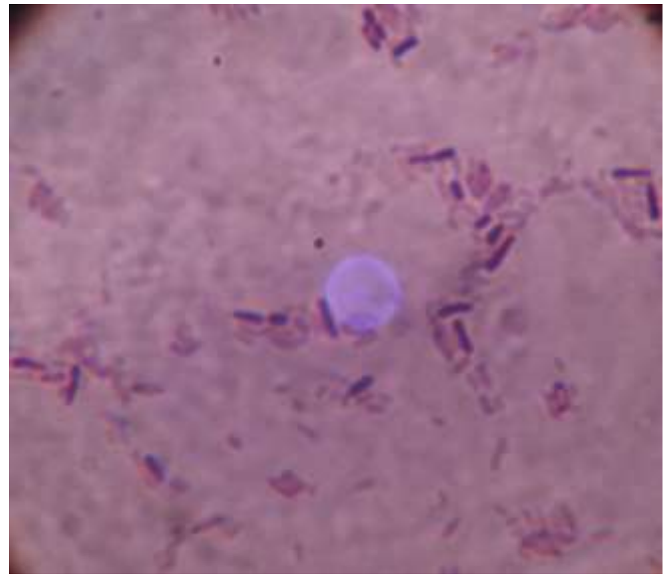
Occurrence of thermophilic bacteria in various biomaterials

The profuse growth of bacterial colonies after incubation at 55°C for 48 h confirmed the occurrence of the thermophilic bacteria in all the three biomaterials that is boiled cow milk, compost manure and tomato rhizospheric soil. All the three isolates were able to sustain highly thermophilic temperature range (70°C) thereby indicating their thermophilic nature. The thermal minima and maxima for all three thermophilic bacterial isolates were 10 and 80°C, respectively. The highest thermal death point (TDP) was observed in bacterial isolate from boiled cow milk (80°C), whereas the TDPs for bacterial isolates from compost manure and tomto rhizospheric soil were both at 75°C. The bacterial isolates could grow moderately at temperatures between 15-20°C. The favourable temperature range for bacterial isolate from boiled cow milk was broad (25-65°C) compared to bacterial isolate from compost manure and tomato rhizosperic soil (40-55°C) (Table 1). Our results are in line with the work of earlier researchers who also reported boiled cow milk, compost manure and crop rhizospheric soils as habitats for the thermophilic bacteria. Nakanishi (1963) isolated *B. licheniformis* from milk samples heat treated at 120°C. Janstova and Lukasova (2001) isolated the *Bacillus* strains from raw milk and farm environment for thermo resistance which grew at the temperature range of 95-135°C. Fujio and Kume (1991) found that thermophilic strains of bacteria *B. stearothermophilus* and *Thermus* sp. isolated from compost were able to grow at optimum temperature range 60-65°C. Miyatake and Iwabuchi ((2005) investigated the enzymatic activity and species diversity of thermophilic bacteria in cattle manure compost at 54, 60, 63, 66 and 70°C, which were dependent on composting temperature. They observed the highest level of thermophilic bacterial activity at 54°C. Xiao et al. (2011) observed an increased biomass of thermophilic bacteria due to continuous thermophilic composting (CTC) with incubation at 30, 40 and 50°C and concluded that CTC might have increased biomass of thermophilic bacteria, especially *Bacillus* spp. Nunes de Souza and Martin (2001) reported that the optimum growth temperature of a thermophilic bacterium isolated from a soil sample in Rio de Janeiro, Brazil was 55°C whereas upper temperature threshold for growth was around 70°C after which no growth was observed. Gulati et al. (2007) reported that thermophilic strain of *Bacillus laevolacticus* isolated from the rhizospheric soil of fenugreek plant (*Medicago falacata*) was optimally active at 70°C.

Characterization and identification of thermophilic bacteria

The microscopic observations of thermophilic bacteria revealed that, all the three isolates were gram +ve, motile rods and capsule formers with endospores produced terminally (Figure 1). On nutrient agar (NA) medium, bacterial isolate from boiled cow milk produced creamy yellow, circular colonies with smooth glistening surface, raised elevation and smooth edges. Similarly, the colonies of bacterial isolates from compost manure and tomato rhizospheric soil were creamy white, flat with no elevation, rough and dry surface and with wavy edges. The characters observed were typical of the family *Bacillaceae* and were similar to those reported in literature (Nunes de Souza and Martin, 2001; Esikova et al., 2002; Pathak and Rekadwad, 2013).

The biochemical tests indicated that all the three thermophilic bacterial isolates were strict aerobes, catalase positive, oxidase positive, and were able to produce the acid from glucose, fructose, lactose and sucrose, except bacterial isolate from tomato rhizospheric soil which did not produce acid from lactose (Figure 2). None of the isolates could produce gas from glucose. The tests of casein hydrolysis and starch hydrolysis (Figure 3), and gelatin liquefaction were positive for all three isolates. The test of citrate utilization was positive only for the bacterial isolate from boiled cow milk (Figure 4). The other biochemical tests like H_2S production, indol formation and urease activity were negative for all three thermophilic isolates. The test of methyl red was positive for all the three bacterial isolates whereas the Voges-Proskaer test was positive only when pH of the growth medium was less than 6.0. The biochemical characterization revealed that all the three thermophilic isolates are distinctly different from each other (Table 2). Our results are in larger agreement with literature reports. Nunes de Souza and Martin (2001) reported that thermophilic bacterium isolated from a soil sample in Rio de Janeiro, Brazil was strictly aerobic and catalase +ve. Fujio and Kume (1991), Akanbi et al. (2010) and Panda et al. (2013) also identified the thermophilic strains of bacteria as *Bacillus* sp. based on conventional gram straining technique, physiological and biochemical characterizations. Singh et al. (2010) reported that thermophilic *Bacillus cereus* SIU1 strain isolated from slightly alkaline soils of Uttar Pradesh was strict aerobe with positive catalase and oxidase activity and was able to hydrolyze casein and gelatin. All these reports are closely in agreement with present findings indicating that our thermophilic antagonistic bacterial isolates were of *Bacillus* sp.



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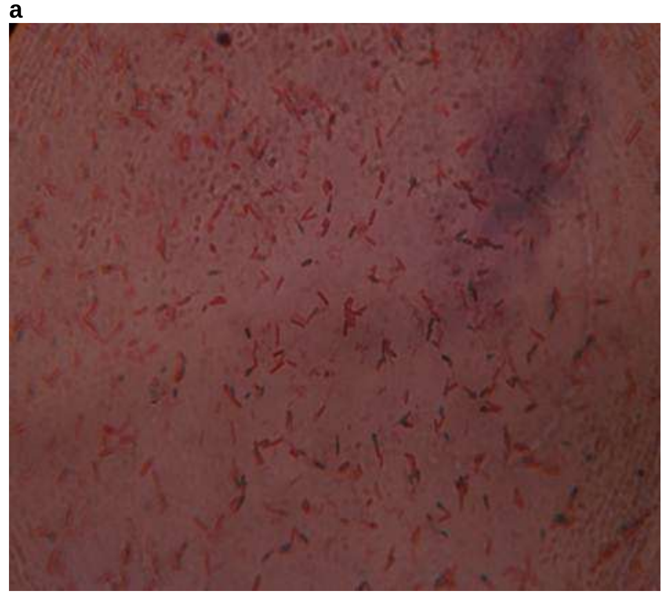

b

Figure 1. Gram +ve rods of thermophilic bacteria isolated from tomato rhizospheric soil (a) and rods showing endospores terminally (b).

All the three thermophilic bacterial isolates preferred a pH range between 6.0-8.0, at which profuse growth was observed, even though they could grow at a wider pH range between 4 and 10. None of the isolates preferred highly acidic (2.0) and highly alkaline (12.0) pH range

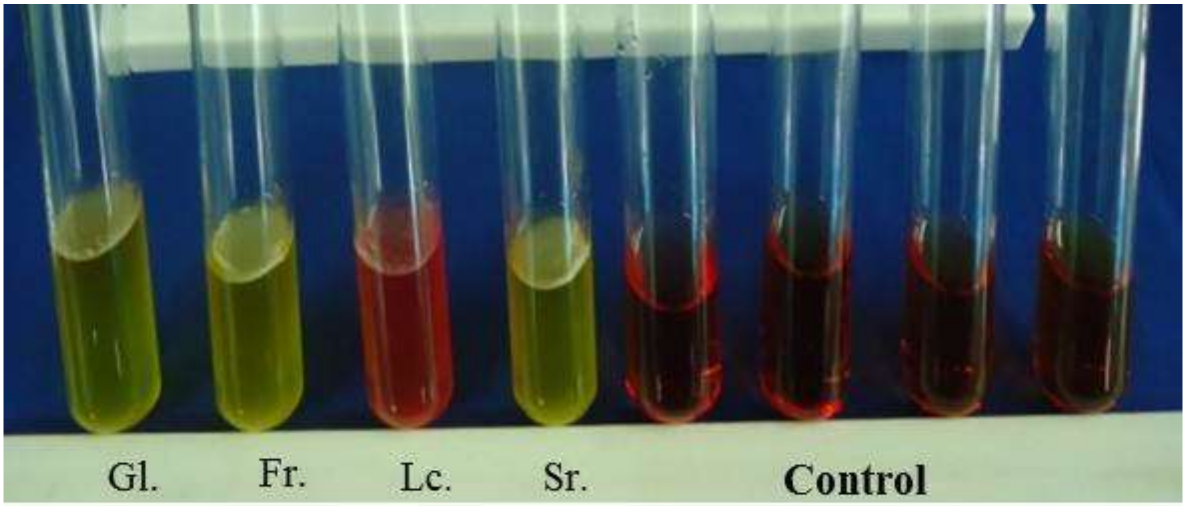

Figure 2. Acid production test in phenol red carbohydrate broth for thermophilic bacterial isolate from tomato rhizospheric soil. Gl = Glucose; Fr = Fructose; Lc = Lactose; Sr = Sucrose.

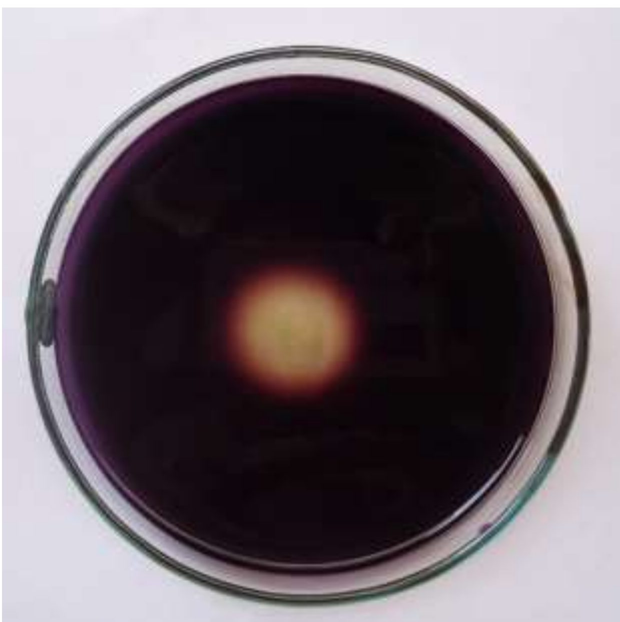

Figure 3. Starch hydrolysis test for isolate from compost manure. Formation of clear hollow zone around bacteria upon smearing with Gram's Iodine on starch agar plates indicates that bacteria are starch hydrolysis +ve.

Figure 4. Citrate utilization test for thermophilic bacterial isolate from boiled cow milk. T = treatment; C = control. Change in colour from green to purple in Simmons's citrate slant indicates citrate +ve reaction.

(Table 3). Our results are closely in agreement with earlier reports on pH tolerance of thermophilic antagonistic bacteria. Nunes de Souza and Martin (2001) observed the optimum growth of a thermophilic bacterium from a soil sample in Rio de Janeiro, Brazil at pH 7.0. Gulati et al. (2007) reported that thermophilic strain of *Bacillus laevolacticus* isolated from the rhizospheric soil of fenugreek plant (*Medicago falacata*) was optimally active at pH between 7.0 8.0. Singh et al. (2010) reported that thermophilic *Bacillus cereus* SIU1 strain isolated from slightly alkaline soils of Uttar Pradesh grew over a wide range of pH between 5.0 -12.0.

All the three thermophilic bacterial isolates from

Table 2. Identification of antagonistic thermophilic bacterial isolates based on morphological and biochemical characterization.

Bacterial isolates from	Morphological characters			Biochemical characters																			Species
						Carbohydrate fermentation						Hydrolysis									Voges - Proskaer		
	Rod shaped	Endospore produced	Stain Gram +ve in young cultures	Catalase	Oxidase	Glucose	Fructose	Lactose	Sucrose	Gas from glucose	Strict aerobes	Casein	Gelatin	Starch	Citrate utilization	Nitrate reduction	H2S production	Indol production	Urease activity	Methyl red	pH < 6.0	pH > 7.0	
Boiled cow milk	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	*Bacillus licheniformis*
Compost manure	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	-	-	+	+	-	*Bacillus stearothermophilus*
Tomato rhizospehric soil	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-	-	-	+	+	-	*Bacillus stearothermophilus*

Table 3. Effect of pH of nutrient medium on growth of thermophilic bacterial isolates.

pH	*Growth pattern of bacterial isolates from		
	Boiled cow milk	Compost manure	Tomato rhizospehric soil
2	-	-	-
4	+++	+++	+++
6	++++	++++	++++
8	+++	++++	++++
10	++	++	++
12	-	-	-

*Growth patterns: -, No; +, slow; ++, moderate; +++, profuse; ++++, very profuse.

present study were able to tolerate salt concentration as high as 8.0%; however the sensitivity to NaCl concentrations differ significantly among the three isolates (Table 4). The bacterial isolate from boiled cow milk was comparatively more sensitive to NaCl concentration which grew profusely only up to 3.0 % salt concentration, whereas the other two isolates grew very profusely up to 5% salt concentration. Very slow growth was observed in the bacterial isolates from compost and tomato rhizospwhric soil at 9.0% NaCl concentration, whereas the isolate from boiled cow milk failed to grow at this concentration. None of the three thermophilic bacterial isolates were able

Table 4. Effect of different NaCl concentrations on growth of thermophilic bacterial isolates.

NaCl concentration (%)	Growth pattern of bacterial isolates*		
	Boiled cow milk	Compost manure	Tomato rhizospehric soil
1	++++	++++	++++
2	++++	++++	++++
3	++++	++++	++++
4	+++	++++	++++
5	+++	++++	++++
6	++	+++	+++
7	++	+++	++
8	++	++	++
9	-	+	+
10	-	-	-

*Growth patterns: -, No; +, slow; ++, moderate; +++, profuse; ++++, very profuse.

to grow at 10.0% NaCl concentration. Our results on high salt tolerance in antagonistic thermophiles suggest possible utilization of these bacteria as effective biocontrol agents for soil borne fungal pathogens, especially in saline and alkaline conditions. The literature reports exist on NaCl tolerance of thermophilic antagonistic bacteria. Elnasser et al. (2007) reported that thermophilic bacterial strain *Bacillus justea* I isolated from hot springs of Jordan grew optimally at NaCl concentration of 0.5%. The range of NaCl tolerance observed in our study is comparatively much higher than reported by Elnasser et al. (2007), which may be due to dissimilar strains. Singh et al. (2010) reported that thermophilic *B. cereus* SIU1 strain isolated from slightly alkaline soils of Uttar Pradesh was highly halotolerant, which was able to grow in the presence of 0.0-10% NaCl. These reports are in line with present findings with respect to salt tolerance of thermophilic *Bacillus* sp.

The antibiotic sensitivity of three thermophilic isolates varied for each of the antibiotic group tested. The isolate from boiled cow milk could tolerate concentrations of streptocyclin and streptomycin sulphate as high as 500 ppm, whereas the other two isolates could tolerate only up to 50 ppm. The isolate from tomato rhizospheric soil was more resistant to *Bacterianashak* (a bactericide) and could tolerate up to 1000 ppm, whereas the remaining two were able to tolerate only up to 500 and 250 ppm, respectively. The isolate from boiled cow milk (*B. licheniformis*) was less sensitive to cyclohexamide and could grow at concentration as high as 7000 ppm. On the other hand, rest of the two bacterial antagonists could grow only up to 4000 ppm concentration cyclohexamide (Table 5). Very few literature reports exist on antibiotic sensitivity of thermophilic bacteria for comparison with our results. Imanaka et al. (1981) tested the thermophilic *Bacillus subtilis* strains isolated from hot springs and compost for resistance to antibiotics. They reported that bacteria were resistant to antibiotics like tetracycline (250 ppm) and streptomycin sulfate (1000 ppm). The antibiotic resistance of the thermophilic bacterial antagonists from present study indicates them as potential biocontrol candidates for safe integration with bactericides (antibiotics) commonly used in disease management.

Based on the morphological, biochemical and physiological characters, the bacterial isolates were identified upto species level as *B. licheniformis* (boiled cow milk), *B. stearothermophilus* (compost manure) and *B. stearothermophilus* (tomato rhizospheric soil). However, isolates from compost manure and tomato rhizospheric soil were different from each other in respect of their antagonistic potential (zone of inhibition produced) against test soil-borne fungal pathogens and therefore seems to be two different strains of same species *B. stearothermophilus*. The isolated bacterial strains were given names as BLbcm for *B. licheniformis* strain from boiled cow milk, BScm for *B. stearothermophilus* strain from compost manure and BStrs for *B. stearothermophilus* strain from tomato rhizosperhic soil.

In vitro **screening for antagonism against soil borne fungal plant pathogens**

All the three thermophilic isolates produced strong inhibition zones against test pathogens in dual culture assay (Table 6, Figure 5). *B. licheniformis* has shown highest antagonism against four test soil borne fungal pathogens *viz*., *P. aphanidermatum, F. oxysporum* F. sp. *ciceri, R. bataticola* and *F. lycopersici* compared to *B. stearothermophilus* (compost manure) and *B. stearothermophilus* (tomato rhizospheric soil). However, the isolates of *B. stearothermophilus* were comparatively more effective against *R. solani* and *S. rolfsii* respectively than *B. licheniformis*. In *S. rolfsii* colour of mycelia/ hyphae approaching to antagonistic bacteria was

Table 5. Antibiotic sensitivity of thermophilic *Bacilli.*

Antibiotic concentration (ppm)	Bacterial isolates from		
	Boiled cow milk	Compost manure	Tomato rhizospehric soil
Streptocyclin			
50	++++	+	+
100	+++	-	-
250	++	-	-
500	+	-	-
1000	-	-	-
Streptomycin sulphate			
50	++++	+	+
100	++++	-	-
250	++++	-	-
500	+++	-	-
1000	++	-	-
Bacterianashak			
50	++	++++	++++
100	+	+	+
250	+	+	+
500	-	+	+
1000	-	-	+
Cyclohexamide			
100	++++	++++	++++
500	++++	++++	++++
1000	+++	++++	++++
3000	+++	+++	+++
4000	+++	++	++
6000	++	-	-
7000	++	-	-

Table 6. *In vitro* efficacy of three thermophilic antagonistic *Bacilli* against six major soil borne fungal pathogens.

Test pathogen	Host plant	Inhibition zone (mm)			Inhibition (%)		
		B. licheniformis	**B. *stearothermophilus* (compost manure)**	***B. Stearothermophilus* (tomato rhizospheric soil)**	***B. licheniformis***	***B. stearothermophilus* (compost manure)**	***B. stearothermophilus* (tomato rhizospheric soil)**
P. aphanidermatum	Brinjal	63.00 ± 1.80^{b}	41.33 ± 1.80^{b}	38.33 ± 1.80^{a}	70.00	45.93	42.59

Table 6. Contd.

F. ciceri	Chick pea	34.17±0.29^{d}	26.33±0.29^{c}	28.67±0.29^{c}	37.97	29.26	31.86
R. bataticola	Chick pea	67.67±2.25^{a}	43.00±2.25^{b}	34.83±2.25^{b}	75.19	47.78	38.7
S. rolfsi	Chick pea	18.50± 0.25^{f}	19.25±2.25^{d}	23.00±0.25^{d}	20.56	46.67	25.56
F. lycopersici	Tomato	30.83±1.15^{e}	19.67±1.15^{d}	23.33±1.15^{d}	34.26	21.86	25.93
R. solani	Tomato	40.50±0.50^{c}	51.67±0.50^{a}	36.33±0.50^{ab}	45.00	57.42	40.37
SE (mean)		0.75	0.48	0.96			
CD (@0.05%)		2.30	1.48	2.94			

The different superscript letters a-f within the column indicates the treatment is significantly different from each other.

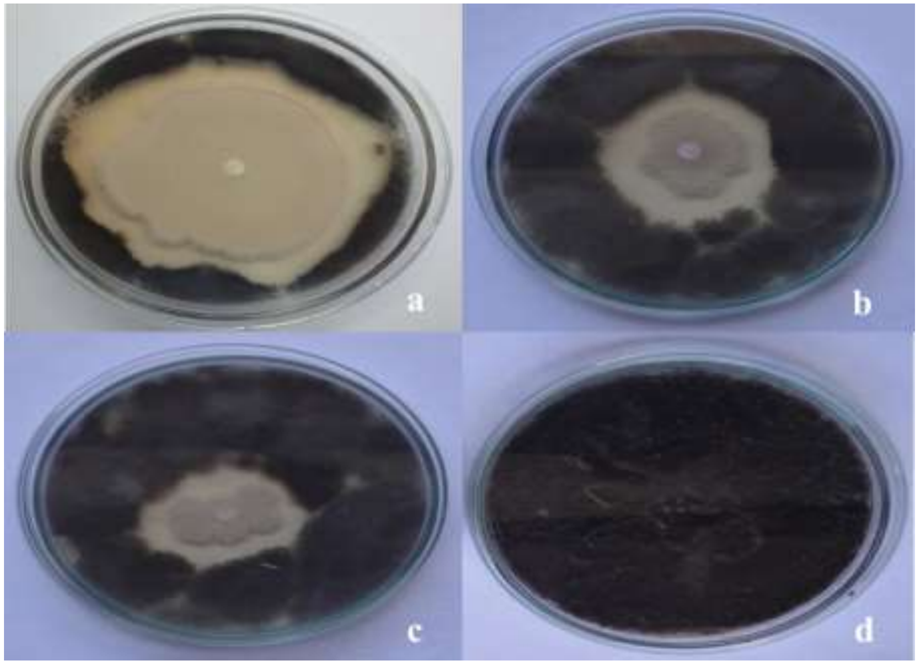

Figure 5. *In vitro* antagonism of thermophilic bacterial isolates against *Rhizoctonia solani* by inhibition zone technique. **a.** *B. licheniformis*; b. *B. stearothermophilus* from compost manure; c, *B. stearothermophilus* from tomato rhizospheric soil; **d,** control.

changed from white to brown which may due to antifungal compounds secreted by the bacterial antagonists. Similar results have been reported by earlier researchers. Landa et al. (2004) reported that approximately 32% of 74 bacterial isolates from the chickpea rhizosphere inhibited growth of

F. oxysporum f. sp. *ciceri* in dual cultures under *in vitro* studies. Foldes et al. (2000) reported that *B. subtilis,* strain IFS-01 isolated from rhizosphere of cereals produced clear inhibition zone against major phytopathogens *viz*., *F. oxysporum, Alternaria alternata, Botrytis cineria and Aspergillus niger*. Montealegre et al. (2003) reported that *B. subtilis* strain 639 and *B. lentimorbus* strain 640 isolated from rhizoplane and rhizosphere of healthy and diseased tomato plants produced halo zone against *R. solani* in dual culture assay. Garima et al., (2005) reported that the rhizobacteria, *Bacillus* sp. (GF23 and A555) significantly inhibited radial growth of soil borne fungal pathogens viz, *R. solani, S rolfsi, A. niger, A. flavus, Fusarium semitectum, F. udum, F. oxysporum f.* sp. *ciceri., F. moniliforme* and *F. oxysporum f.* sp. *lycopersici* by dual culture plate assay, thus showing a broad spectrum antagonism. Nakkeeran et al. (2006) reported that rhizobacteria *B. subtilis* strain BSCBE4 isolated from vegetable crops (tomato, brinjal and hot paper) showed the highest inhibitory effect on mycelial growth of *P. aphanidermatum* on PDA medium. Mehetre and Kale (2011) reported *in vitro* antagonism of thermophilic bacterium *B. licheniformis* against *P. aphanidermatum* causing chilli damping off. Sabet et al. (2013) reported that *Bacillus* sp. isolated from five commercial composts had antagonistic effects on soil borne fungal pathogens of cucumber *in vitro*. The treatment of antagonistic *Bacillus* strains B3, B5, B7, B9, and B11 suppressed the radial mycelial growth (24.4 to 57.8%) of *F. solani, P. ultimum*, *R. solani*, and *S. rolfsi* causing root rot in cucumber. Sharma et al. (2013) reported that *B. amyloliquefaciens* strain sks_bnj_1 isolated from diseased roots of soybean showed antagonism against *S. rolfsi, Sclerotinia* sp and *F. nivale*.

Conclusion

The present study revealed strong antagonistic potential of three thermophilic bacteria against major soil-borne fungal plant pathogens. The biochemical and physiological characterisation of thermophilic bacterial isolates indicated their ability to tolerate high temperature, pH, salt and antibiotic concentrations. We propose the thermophilic bacterial isolates as the potential biocontrol candidates for management of soil-borne fungal plant pathogens, especially under stressed environments where other biocontrol agents fail. Further studies in relation to efficacy of thermophilic bacterial antagonists for control of soil borne plant pathogens under field conditions and development of suitable bioformulations for their efficient filed delivery are required before recommending them for biocontrol.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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