Cyclic siloxane biosurfactant-producing *Bacillus cereus* BS14 biocontrols charcoal rot pathogen *Macrophomina phaseolina* and induces growth promotion in *Vigna mungo* L.

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Abstract
Rhizobacteria are vital component of soil–plant interfaces which helps in plant growth responses and disease management. Precisely, the role of biosurfactant production by rhizobacteria in biocontrol mechanisms is underscored. The current study explores the destructive effect of a biosurfactant-producing bacterium *Bacillus cereus* BS14 on fungal growth under in vitro experiments and showed in vivo reduction of disease severity in pulse crop *Vigna mungo*. In this study, *B. cereus* BS14 was observed as plant growth-promoting rhizobacterium (PGPR) based on abilities of production of phytohormone and HCN, phosphate solubilization and biocontrol of *Macrophomina phaseolina*. The purified biosurfactant from BS14 inhibited the fungal growth by arresting radially growing mycelia. Scanning electron microscope (SEM) study revealed deformities at cellular level in the mycelia of *M. phaseolina*. The biosurfactant of *Bacillus* BS14 was identified as cyclic siloxane in GC–MS spectroscopy and FT-IR spectroscopy analyses. In the pot trial studies, *B. cereus* BS14 proved its efficiency for the growth promotion of *Vigna mungo* and significantly reduced disease severity index. The present study concludes that biosurfactant of rhizobacterial origin and rhizobacteria can serve for biological control, improvement in crop production and agricultural sustainability. In future, it can be developed as biological control and biofertilizer formulations for legume crops, and commercialized for routine farming practices.

Keywords *Bacillus* · Biosurfactant · Plant growth-promoting rhizobacteria · Biocontrol · Legume

Introduction
The bacterial metabolites, such as enzymes, antibiotics, vitamins, and other bioorganic acids have different applications in environment, industries, medicine, and agriculture (Lynch and Audus 1976; Singh et al. 2017). In agriculture, these are utilized to fulfil an aim of increasing crop productivity and soil-fertility management in an eco-safe way (Maheshwari et al. 2013; Maheshwari 2015). Such metabolites like biosurfactants or bio-emulsifiers are precious compounds as they are involved in soil-conditioning phenomenon to remediate soil, restrict the growth of phytopathogens and aid disease control in various field crops (Sachdev and Cameotra 2013). The rhizobacteria, the inhabitant of rhizosphere can enhance the plant health and growth via phytohormone production, secretion of iron-chelating siderophores, solubilization of insoluble phosphatic salts in the soil, induction of plant-immunity via systemic acquired resistance (SAR), and induced systemic resistance (ISR) (Sharma et al. 2018), water stress management by 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity, and biotic (density-dependent) stress regulation by biological control. Soil-borne fungal pathogens attacking on below-ground parts of plants are still need to be controlled by rhizobacteria and their novel metabolites like biosurfactant (Bee et al. 2019) irrespective to cell-wall degrading enzymes.
Recent science of biocontrol has emerged to describe the role of certain biosurfactant in destructions of fungal pathogens, enhancement of rhizobacterial colonization and other means of biological control (Sarwar et al. 2018a), bacilli are often considered for elite production of a comprehensive array of biologically active molecules, such as lipopeptides (LPs), chitinase, and glucanase which protect plant from fungal diseases (Agarwal et al. 2017). Earlier, Romano et al. (2013) have purified cyclic lipopeptides from *Bacillus amyloliquifaciens* strain BOSA and checked its antifungal activity against pathogenic fungi *Fusarium oxysporum*, *Aspergillus niger*, *Botrytis cinerea*, and *Penicillium italicum*. Moreover, production of a wide range of antimicrobial substances by aerobic endospore forming bacteria (AEFB), such as biosurfactants has also been reported (Adu and Hunter 2021). Biosurfactants demonstrate antibacterial and antifungal activities against a wide array of pathogenic bacteria and fungi (Kumar and Johri 2012). A biosurfactant-producing *Bacillus sonorensis* MBCU2 was isolated from vermin compost-amended soil which showed potential antagonistic activity against *M. phaseolina* (Pandya and Saraf 2015). After qualitative analysis, biosurfactant from *Bacillus* was studied for its antifungal properties (Sarwar et al. 2018b). Ample reports have been published to establish *Bacillus* as a biosurfactant-producing bacteria (Sarwar et al. 2018a, b; Hafeez et al. 2019). On the other hand, Al-Ali et al. (2018) have demonstrated the production of exopolysaccharide (EPS) and biosurfactant for biofilm formation and rhizosphere colonization. Naturally, exopolysaccharide (EPS) produces silicones, when reacting with siloxanes to form organic siloxane, which is also produced by *Bacillus mucilaginosus* var. *siliceous* (Avakyan et al. 1986). In the present study, biosurfactant produced by *Bacillus* BS14 is structurally similar to the cyclic siloxane. Cyclic siloxanes are particularly useful to produce silicone surfactants and foam polyurethane for foam applications (Hil 2002). Recently, a US patent (US20190059385A1) has appeared to increase agronomic yield using the functional approach of siloxanes for foam applications (Hil 2002). Micro-colonies were purified by streaking and designated with laboratory code prior to identification by cultural and biochemical tests, such as Gram-staining, spore staining, motility test, oxidase test, indole test, catalase test, citrate test, coagulase test, methyl red, and Voges–Proskauer test.

### Screening of biosurfactant production

All the bacterial isolates were initially screened for biosurfactant production via various screening procedures, such as mineral salt cetyl-trimethyl-ammonium-bromide (CTAB) — methylene blue agar plate assay (Siegmund and Wagner 1991), hemolytic activity (Youssef et al. 2004), bacterial adherence to hydrocarbons (BATH) assay (Rosenberg et al. 1980), drop collapse assay (Jain et al. 1991), oil spreading assay (Rodrigues et al. 2006), emulsification stability (E24) test (Das et al. 2008), and measurement of surface tension of cell-free culture broth according to Du Nouy’s ring method (Lunkenheimer and...
Wantke 1981), with slight modification as previously carried out in our study (Kumar et al. 2016).

Isolation of plant growth-promoting bacteria

The determination of IAA production by bacterial isolates was done by growing the cultures on LB broth and incubating at 28 °C for 24 h at 120 rpm. Exponentially grown culture (10⁸ cfu mL⁻¹) was centrifuged at 10,000 rpm for 20 min at 4 °C to collect the supernatant. 2 μL of orthophosphoric acid was added to 2 mL of supernatant with subsequent addition of Salkowski’s reagent. Appearance of pink colour confirms the IAA production. Further, HCN (cyanogen) production was determined following the modified method of Bakker and Schippers (1987). Siderophore production was evaluated on Chrome-azure S (CAS) medium by spot inoculating bacterial culture and incubated at 28 ± 1 °C for 48–72 h (Schwyn and Neilands 1987). The formation of orange to yellow halo around the bacterial colonies confirmed siderophore production. Phosphate solubilization ability of all isolates was detected by spotting them separately on Pikovskaya’s agar plates (De Freitas et al. 1997). These plates were then incubated at 28 ± 1 °C for 3 days and observed for the appearance of the clearing zone around the colonies. The qualitative assay for chitinase production was performed following the method of Dunne et al. (1997). Isolates were separately inoculated by spotting on the plates containing chitin minimal medium (CMM) as the sole source of carbon and incubated at 30 ± 2 °C for 7 days. These plates were examined for the development of clear zones around the bacterial colonies. For biofilm assay, sterile Muller Hinton broth (MHB) (5 mL) was poured in the pre-sterile test tubes inoculated separately with the test organisms along with proper control and incubated at 37 °C for 24 h. The broth was discarded, washed with 0.5 M phosphate buffer saline (PBS) and the internal surface of the tube was stained with 1% crystal violet solution to confirm biofilm formation (O’Toole 2011).

Biochemical and physiological characterization

The biochemical characterization of isolates was carried out followed by Bergey’s Manual of Determinative Bacteriology (Holt et al. 1994). Different phenotypic characters of these isolates were compared with the standard strains, such as Bacillus sp. (MTCC 297) and Bacillus subtilis (MTCC 441) which were procured from the Institute of Microbial Technology (IMTECH), Chandigarh (India). These eight isolates showed similarity with the species of Bacillus.

Molecular characterization

For molecular characterization of the isolate BS14, genomic DNA was extracted following Green and Sambrook (2012). The 16S rRNA gene of the isolate BS14 was amplified using polymerase chain reaction (PCR). A universal primer set consisting of 27F 5’AGAGTTTGATCMTGCTGAG3’ and 1492R 3’CGGTTACCTTGTTACGACTT5’ was used to amplify 16S rRNA gene using MJ Research PTC-100 Peltier Thermal Cycler (PCR). DNA amplicons were purified and directly sequenced from Institute of Microbial Technology (IMTECH), Chandigarh, India. All the sequences were compared with 16S rRNA gene sequences (type) available in the GenBank databases of NCBI using BLAST search. The 16S rDNA sequence was submitted to the GenBank Database to get an accession number. Phylogenetic analysis was performed using MEGA 6.0 version.

Production and purification of biosurfactant

The biosurfactant-producing potential isolate BS14 was transferred to 5 mL nutrient-rich broth (NRb) containing 1% yeast extract, 1.5% nutrient broth, and 1% ammonium sulfate and incubated at 37 °C for 12 h and 120 rpm as seed culture to get the optical density of 0.5 at 600 nm (equivalent to McFarland solution). Further, 5 mL suspension of BS14 was transferred to a 1000-mL Erlenmeyer flask containing 500 mL of LB medium and incubated on a rotary shaker incubator (150 rpm) at 37 °C. The bacterial cells were removed by centrifugation at 10,000 rpm at 4 °C for 20 min and the supernatant was acidified with 6 N hydrochloric acid to get the pH 2.0. The precipitate containing biosurfactant was allowed to settle at 4 °C for overnight and collected by centrifugation at 15,000 rpm for 20 min. The precipitate was dissolved in distilled water, pH 7.0 was maintained using 1 N NaOH and re-centrifuged at 10,000 rpm for 10 min to get the final precipitate of biosurfactant (Sanchez et al. 2007).

In vitro antagonistic activity

The fungal pathogen M. phaseolina was procured from the culture repository of Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar (India) (Singh et al. 2010). Antagonistic properties of bacterial isolates were tested against M. phaseolina on potato dextrose agar (PDA) plates following the dual culture technique of Skidmore and Dickinson (1976). 5-day-old mycelial discs of 5 mm diameter were placed in the center of solidified medium in plates containing modified PDA by adding 2% sucrose. Culture of the isolate BS14 (7 × 10⁸ cfu mL⁻¹) was spotted 2 cm apart from the fungal disc and incubated at 28 ± 1 °C for 5 days. Growth inhibition was calculated by measuring the distance between the bacterial and fungal...
colonies as compared to the control. The fungal growth inhibition (%) was calculated using the formula: $100 \times \frac{C - T}{C}$, where $C =$ radial growth of fungus in control and $T =$ radial growth of fungus in dual culture. Purified biosurfactant of isolate BS14 was used to test the fungal growth inhibition by disc-diffusion method (Kirby et al. 1957; Jorgensen and Turnidge 2015).

**Post-interaction events**

Fungal mycelia growing towards the zone of interaction were processed for scanning electron microscopic (SEM) studies. Agar discs of 1 mm thickness were cut and isolated from the zone of interaction and placed on a glass slide. These were treated with 2% glutaraldehyde solution at 20 °C for 24 h. The samples transferred to copper stubs over double adhesive tape were coated with gold in POLARON, AU/PD sputter coater, and scanned by microscope at 30 kV. The electron microscopic study was carried out at Wadia Institute of Himalayan Geology, Dehradun (India).

**Chemical characterization of biosurfactant**

Fourier transform-infrared spectroscopy (FT-IR) spectra of the dried biosurfactant

FT-IR spectra of the dried biosurfactants were recorded on a 8400S, FT-IR spectrometer (Shimadzu) (available at Patanjali Research Institute, Haridwar), and equipped with a mercury–cadmium–telluride (MCT) detector and cooled with liquid nitrogen. About 2 mg of dried biomaterial was milled with 200 mg of KBr to get a fine powder. The powder was compressed into a thin pellet to be analyzed by FT-IR spectra measurement in wavelength of 400–4000 cm$^{-1}$. The analysis of FT-IR spectra was carried out using OPUS 3.1 (Bruker Optics) software.

Gas chromatography–mass spectroscopy (GC–MS) analysis

GC–MS analysis of biosurfactant was done using a Varian 4000 Mass Spectrometer employing DB5 type capillary column and helium as a carrier gas at a flow rate of 0.5 mL/min. The sample volume was 1 μL and the temperature was gradually increased from 40 °C to 280 °C to identify the compound. The total run time was 45 min. The MS transfer line was maintained at a temperature of 280 °C. GC–MS analysis was done using electron impact ionization at 70 eV and data were evaluated using total ion count (TIC) for identification and quantification of the compound. A comparative study was done between the identified compound spectra and that of known compounds of the GC–MS library NIST.

**Pot trial experiments**

Pot trial experiments were carried out in triplicate and twenty seeds of V. mungo var. U-31 (procured from Plant Pathology Department, IARI, Delhi, India) were sown per pot randomly in the rabi season from November to April. A total of five treatments were used during pot trial. Treatment 1 was seed bacterization with antibiotic-resistant strain of Bacillus cereus BS14Cam+Ery+. The antibiotic-resistant mutant of B. cereus BS14 was developed using Chloramphenicol and Erythromycin by following the method of Dheeman et al. (2020). For seed bacterization, healthy pre-sterilized seeds soaked in sterile lukewarm water for overnight were used (Dubey et al. 2012). Treatment 2 was designed by infesting the seeds of V. mungo with sclerotia of M. phaseolina by mixing 0.5 mg sclerotia in 1% CMC or bio-priming. Treatment 3 was a mixture of treatment 1 and treatment 2 (include bacterization and infestation as a multistep protocol). In treatment 4, 0.5 mg/mL of purified biosurfactant in 1% CMC (in the ratio of 1:1 v/v) (for seed coating; at the rate of 10 mL semi-solid suspension per 100 g seed) was used. For treatment 5, treatment 2 was mixed with treatment 4 (include bacterization and biosurfactant treatment). Non-bacterized seed acted as a control. After all the treatments, seeds were sown in sterile pots in triplicates. The rhizosphere soil, sampled from treatments 1 and 2 were used to monitor root colonization and estimation of B. cereus BS14Cam+Ery+ at 30, 60, and 90 days after sowing (DAS). B. cereus BS14Cam+Ery+ strain colonizing V. mungo roots was screened on chloramphenicol and erythromycin amended medium. Colony numbers of indigenous bacteria were monitored on nutrient agar plates. Seed germination and plant growth parameters, such as root/shoot (length) weight (dry/fresh) were measured. As a sample ten fungus-infested plants were taken out after 60 days after plantation and symptoms of defoliation and wilting due to charcoal rot in plant roots were monitored. Disease index was calculated based on a scale 0 to 2, where $0 =$ no wilting/root rot, $1 =$ chlorosis and yellowing of leaves/wilting or root rot, $2 =$ dead plants. Disease severity was calculated as follows: disease severity = $\sum$ (number of diseased plants at each index value $\times$ disease index value)/(total number of plants investigated $\times$ 2) $\times$ 100% (Sotoyama et al. 2015; Agarwal et al. 2017).

**Statistical analysis**

The data were analysed statistically for the mean differences in values of control and treated plants using Microsoft excel and Graph pad prism 5.0 software. The data were subjected to two-way analysis of variance (ANOVA) to determine the effect of treatment conditions, period and its interaction on various parameters. The data were analysed employing the
Duncan’s Multiple Range Test (DMRT) by taking $p \leq 0.01$ as a significant level.

**Results**

**Isolation of putative Bacillus**

Based on morphological, physiological, and biochemical characteristics the isolates were found Gram-positive, rod-shaped endospore former, and producers of white, dry and folds, opaque and irregular edged colonies on NAM plates. Eight isolates showed positive reaction for catalase and oxidase test, utilized glucose, glucosamine, and sorbose. The isolates were negative for $\text{H}_2\text{S}$ production and methyl red test (Supplementary Table 1).

**Screening of biosurfactant**

Eight isolates were screened for biosurfactant production exhibited positive $\beta$-haemolytic activity, BATH assay, drop collapse test, oil spreading assay, emulsification assay, and surface tension assay. All the isolates except BS21 and BS24 displayed positive CTAB–methylene blue agar plate assay. Among all the isolates BS14 was found to show the best biosurfactant-producing properties (Table 1).

A dark blue halo zone with a sharply defined edge around the culture well was observed after 24 h in CTAB–methylene blue agar assay. In BATH assay, the bacterial cells indicated their affinity towards the hydrophobic substrate. BS14 showed $\beta$-haemolysis displaying the maximum haemolytic zone of ~2.94 cm. Cell adherence of BS14 to crude oil was 80.23%. Emulsification assay is an indirect method used to screen biosurfactant production. The cell-free culture broth of BS14, BS24, BS27, and BS41 showed more emulsification activity with petrol oil than the other isolates. BS14 showed significant emulsification activity with the emulsification index ($E_{24}$) of 70.58. In contrast, BS27 and BS41 displayed a better drop collapse test than the other isolates. Furthermore, the isolates BS12 and BS14 showed the maximum reduction in surface tension by 67.14 D/CM than the other isolates. BS14, BS27, and BS41 showed the maximum oil spreading activity forming the clearing zone (Table 1).

**Plant growth-promoting (PGP) activities of isolates**

Eight Bacillus isolates produced IAA and solubilized phosphate, whereas BS12, BS14, BS24, and BS27 produced HCN. The development of pink colour with and without tryptophan in cell-free supernatant indicated IAA production. Change in colour of filter paper from yellow to moderate and reddish-brown by adding $\text{FeCl}_3$ indicated HCN production by the isolates. Siderophore was produced only by BS12, BS14, BS28, BS40, and BS41. The formation of orange halos around the spots on CAS agar medium indicates siderophore production. However, all the isolates were solubilized phosphate. Formation of clear halos around bacterial spots in Pikovskaya’s medium after 48 h displayed phosphate solubilization. Moreover, none of the isolates produced chitinase except BS12 and BS14 (Table 2). All the isolates exhibited biofilm formation except BS12, BS40, and BS41. Bacterial cells adhered to the surface of test tubes which showed biofilm production.

**Molecular identification**

The 16S rRNA gene sequence of the BS14 incorporated 1425 bp (NCBI GenBank Accession No. KU991962). It showed 99.44% sequence similarity with Bacillus cereus ATCC 14579. Therefore, the isolate BS14 has further been referred to as *B. cereus* BS14.

### Table 1 Biosurfactant-producing assays of rhizosphere bacilli from *Vigna mungo*

| Isolates | CTAB methylene blue agar plate assay | $\beta$-hemolytic activity | BATH assay | Emulsification assay | Drop collapse test | Surface tension measurement (D/CM) | Oil spreading assay |
|----------|-------------------------------------|---------------------------|------------|---------------------|-------------------|-------------------------------|-------------------|
| BS12     | ++                                  | ++                        | ++         | +                   | +                 | 52.12                         | +                 |
| BS14     | +++                                 | +++                       | ++         | ++                  | +                 | 67.14                         | ++                |
| BS21     | −                                   | +                         | +          | +                   | +                 | 31.23                         | +                 |
| BS24     | −                                   | ++                        | ++         | +                   | +                 | 28.32                         | +                 |
| BS27     | +                                   | +                         | ++         | ++                  | +                 | 32.31                         | ++                |
| BS28     | +                                   | +                         | +          | +                   | +                 | 21.12                         | +                 |
| BS40     | +                                   | +                         | +          | +                   | +                 | 40.13                         | +                 |
| BS41     | ++                                  | +                         | +          | ++                  | +                 | 24.12                         | ++                |

− absence of halo formation or no activity, + small halo < 0.5 cm, ++ medium halo formation > 0.5 cm wide surrounding to colonies, +++ large halo > 1.0 cm wide surrounding to colonies.
In vitro antagonistic activity of *Bacillus cereus* BS14 and pure biosurfactant

The pure culture of *B. cereus* BS14 and its biosurfactant inhibited the radial growth of *M. phaseolina* by 70.10% and 53.6%, respectively, after 7 days of incubation at 28 ± 1°C (Fig. 1A, B). However, fungal inhibition was more pronounced in dual culture as compared to that of pure biosurfactant. Further, fungal growth inhibition corresponded with the incubation period.

Post-interaction events in mycelia of *M. phaseolina*

*B. cereus* BS14 in the zone of interaction resulted in halo cell formation and caused mycelial deformities and hyphal degradation of *M. phaseolina*. Formation and development of *M. phaseolina* sclerotia were arrested towards the zone of interaction; consequently, such mycelia and sclerotia lost their vigour. The SEM study shows the dissolution of fungal septa, hyphal fragmentation, and perforation in the cell wall of *M. phaseolina* (Fig. 2A–C).

Chemical analysis of biosurfactant

The chemical structure of the purified biosurfactant from *B. cereus* BS14 was preliminarily investigated using FT-IR spectroscopy. The peak at 1568.02 cm⁻¹ indicates the chemical structure identical to that of a cyclic compound consisting of a hexane ring. The peak at 2329.85 cm⁻¹ indicates the Si–H group. The peak between 3394.48 and 3558.42 cm⁻¹ shows the relatedness of the Si–NH₂ group. The FT-IR analysis shows the similarity with the cyclic compound produced by *B. cereus* BS14 (Fig. 3). FT-IR responsiveness within different absorption regions, absorbance peak heights corresponding to the Si–O bond and Si–CH₃ bond were evidenced and indicated a strong correlation with the expected concentration in the siloxane. For external verification of the calibration, FT-IR results were compared to those produced by GC–MS. The GC–MS analysis of the concentrated methanol extract resulted in many compounds. The peaks in the chromatogram were integrated and were compared with the database of the spectrum of known components stored in the GC–MS library of NIST to confirm the FT-IR structure analysis. Based on GC–MS analysis, the compound showed the relatedness with cyclic compound ‘cyclic siloxane’ (Fig. 4).

Pot trial experiments

Seed germination was enhanced maximally with the treatment of *B. cereus* BS14, while the germination of the fungal-infested seeds significantly decreased followed by mixed treatment of *M. phaseolina* and *B. cereus* BS14. Following the pattern of robustness, treatment BS14 evidenced approximate 25% enhancement in shoot length over control but significantly lowered in the fungal treatment after 90 days of sowing. It is also evidenced that the disease severity index reached at a peak in the fungal infestation. The development of roots can never be ignored as the attained maximum length in the bacterial treatment is similar to the treatment

Table 2  Plant growth-promoting attributes, chitinase production and biofilm assay of rhizosphere bacilli from *V. mungo* in vitro

| Isolates | Siderophore | HCN | IAA | Chitinase production | Phosphate solubilization | Biofilm assay |
|----------|-------------|-----|-----|----------------------|-------------------------|--------------|
| BS12     |            |     | +++ | ++                   | ++                      | −            |
| BS14     | +++         | +++ | +++ | ++                   | +++                     | +            |
| BS21     | −           | −   | +   | −                    | +                       | +            |
| BS24     | −           | +   | +   | −                    | +                       | +            |
| BS27     | −           | −   | −   | −                    | −                       | −            |
| BS28     | ++          | −   | −   | −                    | +                       | +            |
| BS40     | +           | −   | +   | −                    | −                       | −            |
| BS41     | ++          | −   | −   | −                    | ++                      | −            |

IAA indole-3-acetic acid, HCN hydrogen cyanide, − absence of halo formation or no biofilm former, + small halo < 0.5 cm or biofilm forming, ++ medium halo formation > 0.5 cm wide surrounding to colonies, +++ large halo > 1.0 cm wide surrounding to colonies

Fig. 1 Antagonistic effect of (A) *Bacillus cereus* BS14 against *M. phaseolina* in dual culture media (B) purified biosurfactant of *Bacillus cereus* BS14 against *M. phaseolina* in fungal culture media
of biosurfactant. Enhancement in the shoot weight was observed high in the bacterial treatment but the biosurfactant treatment was equally effective in improving the plant biomass. B. cereus BS14Cam+Ery+ strain successfully colonized V. mungo roots both in control and M. phaseolina-untreated pots and significantly ($p < 0.01$) enhanced seed germination, plant growth, and plant biomass over control (Table 3). B. cereus BS14Cam+Ery+ plus M. phaseolina inoculation displayed disease reduction at 30, 60, and 90 days after sowing (DAS). The effect of biosurfactant exclusively did not
improve the growth parameters of *V. Mungo*, however, reduced the disease severity index as compared to bacterial inoculation (Table 3). *B. cereus* BS14Cam+Ery+ displayed an effective root colonization of *V. mungo* as evidenced by the recovery of significant bacteria population from *V. mungo* rhizosphere at 90 DAS (Table 4).

**Discussion**

Rhizobacteria as soil ecological population is a heterogeneous group of bacteria and beneficial for the improvement of legume crop production (Baliyan et al. 2018). Biosurfactant-producing *Bacillus* with plant growth-promoting traits as a rationale was studied to prove a better alternative for biocontrol agents of phytopathogens, bioinoculants for plant growth promotion and overall improvement in legume crop yield via eco-balanced ways. In this study, *B. cereus* BS14 was found the most feasible biosurfactant-producing plant growth-promoting rhizobacteria among the other eight isolates. *B. cereus* BS14 established a new functional niche in the

![Fig. 4 GC–MS spectra of purified biosurfactant produced by *Bacillus cereus* BS14](image-url)
rhizosphere or soil habitat, as able to produce biosurfactants and involve in soil-conditioning of indigenous farms. We have observed that, *B. cereus* BS14 has plant growth-promoting characters, such as IAA, HCN and siderophore production. Earlier, *Bacillus* is reported to bear a PGPR’s characteristics as evidenced in our study (Kumar et al. 2012). In this trend, plant hormones like indole-3-acetic acid (IAA), gibberellins, cytokinin, and certain volatiles have also been reported in PGPRs (Mehta et al. 2010). *Bacillus* has significant effects on the biocontrol of phytopathogenic fungi (Dheeman et al. 2020). Similarly, *B. cereus* BS14 was found effective to restrict the growth of phytopathogenic fungi, and caused cytotoxic effects in the fungi. In vitro inhibition of fungal pathogen by the purified biosurfactant of *B. cereus* BS14 is interesting to note the biocontrol behaviour of biosurfactants. The biocontrol of phytopathogens by such metabolites has been evidenced from the study conducted on antifungal activity and characterization of *Bacillus* against *M. phaseolina* (Hussain and Khan 2020). Under our investigation, we have observed phosphate solubilization efficiency of biosurfactant-producing bacteria. Previously, impact of biosurfactant production has been correlated to the phosphorus solubilization activity in certain rhizobacteria (Mishra et al. 2020). Phosphorus (P) is a very crucial plant growth-limiting nutrient and available in insoluble form in a substantial amount in the soils for plant growth nutrient. The abilities of *B. cereus* BS14 in phosphate solubilization was observed as an important trait of PGPR, which most probably improved the available P in the soil and directly affected the plant growth. However, in the biosurfactant as responsible mechanisms of P-solubilization has not been understood well and still in the nutshell. Still, the role of biosurfactants in phosphate and other mineral solubilization as the bioremediation approach is underscored. Besides, Prakash and Arora (2019) evidenced the abilities of *Bacillus* strains for solubilization of phosphate and other mineral nutrients. Further, this study opens a scope to utilize bacterial origin surfactant for soil remediation and enhancement of soil nutrition improvement.

Furthermore, iron is a crucial nutrient for all forms of life and all microorganisms require iron for their growth and metabolism too (Neilands 1995). *B. cereus* BS14 was found a prominent siderophore-producing bacterium, in vitro. Many rhizobacteria have been reported to produce antifungal metabolites like HCN (Bhattacharyya and Jha 2012). In the present study, *B. cereus* BS14 has been shown to produce HCN; similar ability of *Bacillus* has been reported by Dheeman et al. (2020). *B. cereus* BS14 produced a clear zone in the chitin-containing growth medium and showed chitinase producing abilities, as the desired mechanisms of the biocontrol agent (Kumar et al. 2012). Chitinase production by rhizosphere bacilli from rice (Chen et al. 2010) and *Phaseolus vulgaris* (Kumar et al. 2012) has been reported earlier. *B. cereus* BS14 possesses all necessary characters of PGPR along with biosurfactant producing abilities. Beneficial rhizobacteria can promote plant growth not only by facilitating mineral nutrient uptake and phytohormone production but also more indirectly by protecting against the infection of fungal pathogens. They can antagonize pathogens by producing low molecular-weight toxin or extracellular lytic enzymes (Haas and Keel 2003) and more indirectly by triggering the defensive capacities in the host plant (Cameotra and Makkar 2004). We found that *B. cereus* BS14 inhibited the growth of *M. phaseolina* in vitro and resulted in several types of abnormalities in mycelia. It may be due to the secretion of many inhibitory compounds leading to multifarious abnormalities in fungal hyphae as observed by scanning electron microscopy (SEM). Similar work on biocontrol of *M. phaseolina* has been carried out by Kumar et al. (2012). Furthermore, fungal inhibition was more pronounced in dual culture as compared to pure biosurfactant. Earlier, *Bacillus* species have been reported for the biological control of fungi including *Fusarium* species and *M. phaseolina* (Kumar et al. 2012). The broad-spectrum antagonistic activity of bacilli has been executed by the secretion of several metabolites including antibiotics (Haas and Keel 2003), volatile HCN (Chen et al. 2010) and siderophores (Gupta et al. 2002).

Biosurfactant produced by *B. cereus* BS14 showed antifungal activity against *M. phaseolina*. Earlier, Mnif et al. (2015) isolated *Bacillus* sp. SPB1 that produced a lipopeptide biosurfactant and exhibited antifungal activity against *Rhizoctonia bataticola* (perfect state *M. phaseolina*) and *Rhizoctonia solani*. Already, the concept of microbial biosurfactants and their antimicrobial activity has been prolonged (Goswami and Deka 2021). We have observed that *B. cereus* BS14 produced cyclic siloxanes type biosurfactant, which are especially important and valuable compounds from the industrial application point of view. The cyclic siloxanes are used in the manufacture of silicones, carriers, lubricants, and solvents in a variety of commercial applications (Si 2006). The importance of the cyclic siloxane type biosurfactant has rarely been covered in the literature. This study is the first report on *Bacillus* capable of producing siloxane biosurfactants. The FT-IR and CG–MS analyses evidenced that biosurfactant produced by *B. cereus* BS14 has similarity with cyclic siloxanes. Extracellular polysaccharide plays a crucial role in silica release, especially in the case of quartz. Such polysaccharides can react with siloxanes to form organic siloxanes. It can be of bacterial provenance for example *B. mucilaginosus* var. *Siliceous* (Avakyan et al. 1986). Strains of *Bacillus thuringiensis* (Bt) produce crystalline proteins (δ-endotoxins) during their stationary phase of growth. Many authors used surfactants (1,2-benzo-thiazolizin-3-one) of the inert ingredients in Foray 48 B; the siloxane (organosilicones) Triton-X-100, Tween 20, and Latron CS-7 as surfactants for *Bt* formulations (Helassa
et al. 2009). Biosurfactants are also known to play multifarious roles in biofilm formation. In the present study, B. cereus BS14 produced biosurfactants and formed biofilm. The best biofilm-forming activity was found in other isolates also those produce biosurfactants too. The role of bacterial biofilms and surface components in plant-bacterial associations has been evidence by Bogino et al. (2013).

Pot trial experiments illustrated the potential of B. cereus BS14 to be developed as an effective commercial biological control agent. Bacterial broth culture of B. cereus BS14Cam+Ery+ effectively enhanced plant growth and decreased charcoal rot disease. Effect of cyclic siloxane biosurfactant was observed in disease reduction irrespective of a direct impact on plant growth promotion. This may be due to living actions and the involvement of other traits of bacteria in the rhizosphere. Thus, B. cereus BS14 played a distinguished role in declining charcoal rot disease along with plant growth promotion of V. mungo. Increase in bacterial dynamic and assemblage of B. cereus BS14 in V. mungo rhizosphere with a high number of colonies forming unit, evidenced by lower fungal infestation, which may be due to rhizosporic effect leading to higher bacterial load in the rhizosphere for natural competition in the rhizospheric niche. Also, the synergistic effect of rhizobia and Bacillus (Menéndez and Paço 2020) cannot be ruled out but the possibility of this factor cannot be ignored under this investigation. Further, the effect of fungal load in soil influences bacterial community or how bacteria adopt r-strategy for high reproduction and colonization under fungal stress is a future research. As per evidence from the results, the isolate was putative to produce novel cyclic siloxane type of biosurfactants. This study reflects beneficial gears of biosurfactant production as an indirect approach of PGPR, like enzyme production, antibiotic production responsible for biocontrol. Furthermore, the study concreted with pot trial assay in which exclusive growth and health improvement were acquainted by plants, and disease severity index was reduced significantly in the biosurfactant treatment. Therefore, we postulate the effects of siloxane biosurfactant in biocontrol of phytopathogens and growth promotion of leguminous crop.

Conclusions

It may be concluded that biosurfactant-producing bacteria like Bacillus cereus BS14 are available in the rhizosphere of legume crops and exhibit strong plant growth-promoting properties and biocontrol potential against M. phaselina causing charcoal rot in V. mungo. Further use of biosurfactants(s) or biosurfactant-producing bacteria for biocontrol of charcoal rot disease is important. Future of this study has insights of biosurfactant-producing PGPR-bioinoculants in various carrier materials for cultural and environmental sustainability.

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Declarations

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References

Adu FA, Hunter CH (2021) Screening and identification of lipopeptide biosurfactants produced by two aerobic endospore-forming bacteria isolated from Mfabeni Peatland, South Africa. Curr Microbiol 14:1–8
Agarwal M, Dheeman S, Dubey RC, Kumar P, Maheshwari DK, Bajpai VK (2017) Differential antagonistic responses of Bacillus pumilus MSUA3 against Rhizoctonia solani and Fusarium oxysporum causing fungal diseases in Fagopyrum esculentum Moench. Microbiol Res 205:40–47
Al-Ali A, Deravel J, Krier F, Béchet M, Ongena M, Jacques P (2018) Biofilm formation is determinant in tomato rhizosphere colonization by Bacillus velezensis. Environ Sci Pollut Res 25(30):29910–29920
Arora NK, Kang SC, Maheshwari DK (2001) Isolation of siderophore-producing strains of Rhizobium meliloti and their biocontrol potential against Macrophomina phaseolina that causes charcoal rot of groundnut. Curr Sci 81:673–677
Avakyan ZA, Pivovarova TA, Karavaiko GI (1986) Properties of new species Bacillus mucilaginosus. Mikrobiologiya 55:477–482
Ayed HB, Azabou MC, Hmidet N, Triki MA, Nasri M (2019) Economic production and biocontrol efficiency of lipopeptide biosurfactants from Bacillus mojavensis A21. Biodegradation 30(4):273–286
Bais HP, Fall R, Vivanco JM (2004) Biocontrol of Bacillus subtilis against infection of Arabidopsis roots by Pseudomonas syringae is facilitated by biofilm formation and surfactin production. Plant Physiol 134(1):307–319
Bakker AW, Schipper B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated plant growth-stimulation. Soil Biol Biochem 19:451–457
Baliyan N, Dheeman S, Maheshwari DK, Dubey RC, Vishnoi VK (2018) Rhizobacteria isolated under field first strategy improved chickpea growth and productivity. Environ Sustain 4:461–469
Bee H, Khan MY, Sayyed RZ (2019) Microbial surfactants and their significance in agriculture. Plant growth promoting rhizobacteria (PGPR): prospects for sustainable agriculture. Springer, Singapore, pp 205–215
Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350
Bogino PC, Oliva MDLM, Sorroche FG, Giordano W (2013) The role of bacterial biofilms and surface components in plant-bacterial associations. Int J Mol Sci 14:15838–15859
Comeotra SS, Makkar RS (2004) Recent applications of biosurfactants as biological and immunological molecules. Curr Opin Microbiol 7:262–266
Castaldi S, Pettrillo C, Donadio G, Piazza FD, Cimmino A, Masi M, Evidente A, Istitaico R (2021) Plant growth promotion function of Bacillus sp. strains isolated from Salt-Pan rhizosphere and their biocontrol potential against Macrophomina phaseolina. Int J Mol Sci 22(7):3324
Chen F, Wang M, Zheng Y, Luo J, Yang X, Wang X (2010) Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber Fusarium wilt by Bacillus subtilis B579. World J Microbiol Biotechnol 26(4):675–684
Das P, Mukherjee S, Sen R (2008) Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. Chemosphere 72(9):1229–1234
De Freitas JR, Banderjee MR, Germida J (1997) Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). Biol Fertil Soil 24(4):358–364
Dheeman S, Baliyan N, Dubey RC, Maheshwari DK, Kumar S, Chen L (2020) Combined effects of rhizo-competitive rhizosphere and non-rhizosphere Bacillus in plant growth promotion and yield improvement of Eleusine coracana (Ragi). Can J Microbiol 66(2):111–124
Dubey RC, Maheshwari DK, Kumar V, Pandey RR (2012) Growth enhancement of Sesamum indicum L. by rhizosphere-competent Azotobacter chroococcum AZ02 and its antagonistic activity against Macrophomina phaseolina. Arch Phytopathol Plant Prot 45:437–454
Dunne C, Crowley JJ, Moënne-Loccoz Y, Dowling DN, O’Gara F (2019) Microbial metabolites in sustainable agroecosystems. Springer Nature, Co, Baltimore, p 787
Hussain T, Khan AA (2020) Determining the antifungal activity and characterization of Bacillus siamensis AMU03 against Macrophomina phaseolina (Tassi). Goid. Indian Phytopathol 73:507–516
Hänsel R, Kruse D, Sieverding E, Riedl C, Ludwig JS (2019) Use of polyether modified short-chain siloxanes in agriculture in order to increase harvest yield. US Patent Application No. 16/073,091
Helassa N, Quinquampoix H, Noinville S, Szponarski W, Staunton S (2009) Adsorption and desorption of monomer-Mte (Bacillus thuringiensis) Cry1Aa toxin on mont-morillonite and kaolinite. Soil Biol Biochem 41:498–504
Hil RM (2002) Silicone surfactants-new developments. Curr Opin Colloid Interface Sci 7(5–6):255–261
Holt JC, Krieg NR, Sneath PHA, Staley JT (1994) Bergey’s manual of determinative bacteriology, 9th edn. The Williams and Wilkins Co, Baltimore, p 787
Hussain T, Khan AA (2020) Determining the antifungal activity and characterization of Bacillus siamensis AMU03 against Macrophomina phaseolina. Appl Biochem Biotechnol 171(8):2176–2185
Jain DK, Collins-Thompson DL, Lee H, Trevors TA (1991) Drop collapsing test for screening surfactant-producing microorganisms. J Microbiol Method 13:271–279
Jørgensen JH, Turnidge JD (2015) Susceptibility test methods: dilution and disk diffusion methods. Man Clin Microbiol. https://doi.org/10.1128/9781555873817.ch71
Kirby WMM, Yoshihara GM, Sundsted KS, Warren JH (1957) Clinical use of a single disc method for antibiotic sensitivity testing. Antimicrob Agents Chemother 15:534–536
Kumar A, Johri BN (2012) Antimicrobial lipopeptides of Bacillus: natural weapons for biocontrol of plant pathogens. In: Satyanarayana T et al (eds) Microorganisms in sustainable agriculture and biotechnology. Springer Netherlands, pp 91–111
Kumar P, Dubey RC, Maheshwari DK (2012) Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 167:493–499
Kumar S, Dubey RC, Maheshwari DK (2016) Biosurfactant-mediated biocontrol of Macrophomina phaseolina causing charcoal rot in Vigna mungo by a plant growth promoting Enterococcus sp. BS13. J Plant Pathol Microbiol 7(385):2
Lunkenheimer K, Wamke KD (1981) Determination of the surface tension of surfactant solutions applying the method of Lecomte du Noüy (ring tensiometer). Colloid Polym Sci 259:354–366
Lynch JM, Audus LJ (1976) Products of soil microorganisms in relation to plant growth. Crit Rev Microbiol 5(1):67–107
Machion F, Sambrook J (2012) Molecular cloning: a laboratory manual, 4th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
Gupta CP, Dubey RC, Maheshwari DK (2002) Plant growth enhancement and suppression of Macrophomina phaseolina causing charcoal rot of peanut by fluorescent Pseudomonas. Biol Fertil Soil 35:399–405
Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease. Ann Rev Phytopathol 41:117–153
Hafeez FY, Naureen Z, Sarwar A (2019) Surfactin: an emerging biocontrol tool for agriculture sustainability. Plant growth promoting rhizobacteria for agricultural sustainability. Springer, Singapore, pp 203–213
Hil RM (2002) Silicone surfactants-new developments. Curr Opin Colloid Interface Sci 7(5–6):255–261
Holt JC, Krieg NR, Sneath PHA, Staley JT (1994) Bergey’s manual of determinative bacteriology, 9th edn. The Williams and Wilkins Co, Baltimore, p 787
Hussain T, Khan AA (2020) Determining the antifungal activity and characterization of Bacillus siamensis AMU03 against Macrophomina phaseolina. Appl Biochem Biotechnol 171(8):2176–2185
Jain DK, Collins-Thompson DL, Lee H, Trevors TA (1991) Drop collapsing test for screening surfactant-producing microorganisms. J Microbiol Method 13:271–279
Jørgensen JH, Turnidge JD (2015) Susceptibility test methods: dilution and disk diffusion methods. Man Clin Microbiol. https://doi.org/10.1128/9781555873817.ch71
Kirby WMM, Yoshihara GM, Sundsted KS, Warren JH (1957) Clinical use of a single disc method for antibiotic sensitivity testing. Antimicrob Agents Chemother 15:534–536
Kumar A, Johri BN (2012) Antimicrobial lipopeptides of Bacillus: natural weapons for biocontrol of plant pathogens. In: Satyanarayana T et al (eds) Microorganisms in sustainable agriculture and biotechnology. Springer Netherlands, pp 91–111
Kumar P, Dubey RC, Maheshwari DK (2012) Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 167:493–499
Kumar S, Dubey RC, Maheshwari DK (2016) Biosurfactant-mediated biocontrol of Macrophomina phaseolina causing charcoal rot in Vigna mungo by a plant growth promoting Enterococcus sp. BS13. J Plant Pathol Microbiol 7(385):2
Lunkenheimer K, Wamke KD (1981) Determination of the surface tension of surfactant solutions applying the method of Lecomte du Noüy (ring tensiometer). Colloid Polym Sci 259:354–366
Lynch JM, Audus LJ (1976) Products of soil microorganisms in relation to plant growth. Crit Rev Microbiol 5(1):67–107
Maheshwari DK (2015) Bacterial metabolites in sustainable agroecosystem. Springer Science & Business Media
Maheshwari DK, Saraf M, Aaron A (2013) Bacteria in agrobiology: crop productivity. Springer Science & Business Media
Mehta P, Chauhan A, Mahajan R, Mahajan PK, Shirkot CK (2010) Strain of Bacillus circulans isolated from apple rhizosphere showing plant growth promoting potential. Curr Sci 98:538–542
Menéndez E, Paço A (2020) Is the application of plant probiotic bacterial consortia always beneficial for plants? Exploring synergies between rhizobial and non-rhizobial bacteria and their effects on agro-economically valuable crops. Life 10(3):24
Mishra I, Fatima T, Egamberdieva D, Arora NK (2020) Novel bioformulations developed from Pseudomonas putida B3P9 and its biosurfactant for growth promotion of Brassica juncea (L.). Plants 9(10):1349
Mnif I, Mnif S, Sahnoun R, Makout S, Ayedi Y, Ellouze-Chaabouni S, Ghribi D (2015) Biodegradation of diesel oil by a novel microbial consortium: comparison between co-inoculation with biosurfactant-producing strain and exogenously added biosurfactants. Environ Sci Pollut Res 22:14852–14861
Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726
O’Toole GA (2011) Microtiter dish biofilm formation assay. JoVE 47:e2437
Pandey AK, Burlakoti RR, Rathore A, Nair RM (2020) Morphological and molecular characterization of Macrophomina phaseolina isolated from three legume crops and evaluation of mungbean genotypes for resistance to dry root rot. Crop Prot 127:104962
Pandya U, Saraf M (2015) Isolation and identification of allelochemicals produced by B. sonorensis for suppression of charcoal rot of Arachis hypogaea L. J Basic Microbiol 55:635–644
Prakash J, Arora NK (2019) Phosphate-solubilizing Bacillus sp. enhances growth, phosphorus uptake and oil yield of Mentha arvensis L. 3 Biotech 9(4):126
Rodrigues LR, Teixeira JA, van der Mei HC, Oliveira R (2006) Physicochemical and functional characterization of a biosurfactant produced by Lactococcus lactis 53. Colloids Surf b: Biointerfaces 49:79–86
Rodrigues AI, Gudiña EJ, Teixeira JA, Rodrigues LR (2021) Biosurfactants as biocontrol agents against mycotoxigenic fungi. Biosurfactants for a sustainable future: production and applications in the environment and biomedicine, vol 5. Wiley, pp 465–490
Romano A, Vitullo D, Senatore M, Lima G, Lanzotti V (2013) Antifungal cyclic lipopeptides from Bacillus amyloliquefaciens strain BO5A. J Nat Prod 76:2019–2025
Rosenberg M, Gutnick D, Rosenberg E (1980) Adherence of bacteria to hydrocarbons a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol Lett 9:29–33
Sachdev DP, Cameotra SS (2013) Biosurfactants in agriculture. Appl Microbiol Biotechnol 97(3):1005–1016
Sanchez M, Aranda FJ, Espuny MJ, Marqués A, Teruel JA, Manresa A, Ortiz A (2007) Aggregation behavior of a dirhamnolipid biosurfactant secreted by Pseudomonas aeruginosa in aqueous media. J Colloid Interface Sci 307:246–253
Sarwar A, Brader G, Corretto E, Aleti G, Abaidullah M, Sessitsch A, Hafeez FY (2018a) Qualitative analysis of biosurfactants from Bacillus species exhibiting antifungal activity. PLoS ONE 13(6):e0198107
Sarwar A, Hassan MN, Imran M, Iqbal M, Majeed S, Brader G, Sessitsch A, Hafeez FY (2018b) Biocontrol activity of surfactin A purified from Bacillus NH-100 and NH-217 against rice bakanae disease. Microbiol Res 209:1–13
Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56
Shahid S, Khan MR (2019) Evaluation of biocontrol agents for the management of root-rot of mung bean caused by Macrophomina phaseolina. Indian Phytopathol 72(1):89–98
Sharma CK, Vishnoi VK, Dubey RC, Maheshwari DK (2018) A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen Macrophomina phaseolina in mung bean. Rhizosphere 5:71–75
Si O (2006) Need to assess efficacy of public health actions. In: Meeting of the California environmental contaminant biomonitoring program (CECBP) scientific guidance panel (SGP) December 4–5.
Sieg mund I, Wagner F (1991) New method for detecting rhamnolipids excreted by Pseudomonas species during growth on mineral agar. Biotechnol Tech 5:265–268
Singh N, Kumar S, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Biological control of Macrophomina phaseolina by chemotactic fluorescent Pseudomonas aeruginosa PN1 and its plant growth promotory activity in chir-pine. Crop Prot 29(10):1142–1147
Singh R, Kumar M, Mittal A, Mehta PK (2017) Microbial metabolites in nutrition, healthcare and agriculture. 3Biotech 7(1):15
Skidmore AM, Dickinson CH (1976) Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Trans Br Mycol Soc 66:57–64
Sotoyama K, Akutsu K, Nakajima N (2015) Biological control of Fusarium wilt by Bacillus amyloliquefaciens IUMC7 isolated from mushroom compost. J Gen Plant Pathol 82(2):105–109
Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ (2004) Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Methods 56:339–347

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