



Research article

Metabolite profiling and molecular characterization of NBAIR BSWG1: A potential strain of *Bacillus subtilis* against *Fusarium oxysporium* f. sp. *udum*

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ABSTRACT

To address the fungal wilt of pigeon pea caused by *Fusarium oxysporium* f. sp. *udum*, farmers currently rely on chemical fungicides, despite their harmful effects. However, there is a growing need for safer alternatives like green pesticides. Bacterial biocontrol agents and their derivatives serve as potential green pesticides in the management of plant pathogens. In the present study, we aimed to identify indigenous *Bacillus subtilis* strains effective against *F. oxysporium* f. sp. *udum*. We used PCR and MALDI-TOF analysis to identify the active components responsible for the efficiency of efficient strain. Biochemical studies of cell-free extracts extracted from *B. subtilis* strains demonstrated the highest biosurfactant activity in NBAIR BSWG1, with an oil displacement of 2 cm and an emulsification index of 60 %. Molecular characterization confirmed the presence of surfactin, fengycin, and iturin coding genes in the *B. subtilis* strains, among them, NBAIR BSWG1 showed the highest number of lipopeptide-producing genes. Meanwhile, NBAIR BSWG1 showed inhibition of 79.84 % against *F. oxysporium* f. sp. *udum* using cell-free extract. Further metabolite profiling of NBAIR BSWG1 using MALDI-TOF analysis further confirmed surfactin, fengycin, and iturin in the purified cell-free extract of NBAIR BSWG1. Two peaks with m/z of 923.77 and 1149.92 were identified as novel lipopeptide compounds which need further characterization. The present study identified NBAIR BSWG1 as an efficient bacterial strain for the inhibition of *F. oxysporium* f. sp. *udum* and its antifungal properties are mainly due to the production of cyclic lipopeptides.

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1. Introduction

Pigeon pea (*Cajanus cajan* L.) is one of the most important pulse crop grown worldwide. Fungal wilt of red gram caused by *Fusarium oxysporium* f. sp. *udum* is a very harmful disease of pigeon pea that causes significant yield loss globally. Farmers use chemical fungicides to manage the fungal wilt of red gram. Due to the extensive use of chemical fungicides target fungal strains became resistant to these chemicals, and negatively impacted the survival of many non-target beneficial microbial populations, causing an imbalance in the rhizospheric microbial diversity and a decline in the soil's quality [1]. Biocontrol agents are potential substitutes for controlling plant diseases and increasing crop yield without these negative impacts. Using biological control agents to manage plant pathogens is considered a sustainable strategy for safe and profitable agricultural productivity [2]. Using beneficial and antagonistic microorganisms for biological control offers a potential, environmentally friendly, and effective approach to control *F. oxysporium* f. sp. *udum* [3,4]. Among the biocontrol agents, *Bacillus* species stands highly advantageous over other bacteria for protection against root pathogens. Quick growth, easy manageability, and strong colonization capabilities make *Bacillus* species an ideal option for biocontrol [5]. *Bacillus subtilis* is one of the most successful bacterial biocontrol agents predominating the industry being a bacterial biocontrol agent, it can multiply very fast and dominate over pathogens and it can survive under adverse environmental conditions with benefits [6].

Bio-surfactant property bacterial strains can be characterized by simple biochemical tests like oil displacement assay and emulsification index [7,8]. Oil displacement assay is an indicator of the antimicrobial potential of lipopeptides due to bio-surfactant activity. Emulsification assay is also an indirect method used to screen biosurfactant production. The emulsification index denotes the emulsification activity of biosurfactants/lipopeptides produced by bacteria [9]. Hence in the present study, these two assays have been used for screening bio-surfactant properties of *B. subtilis* strains.

The polymerase chain reaction is considered one of the most important scientific advances in the field of molecular biology [10]. Polymeric chain reaction revolutionized molecular biology by simplifying the identification and validation of genes responsible for particular traits [11]. Bacterial biocontrol agents are known to produce antagonistic potential secondary metabolites such as lipopeptides [12]. There have been relatively few reports available about the molecular confirmation of genes involved in lipopeptides biosynthesis in indigenous *B. subtilis* strains. Hence, we employed PCR-based validation of lipopeptide genes in indigenous *B. subtilis* strains, isolated from the Western Ghats of India.

To manage crop diseases by biological means, we need to identify locally adaptable, biologically active biocontrol agents. However, very few Indigenous *B. subtilis* isolates and their bio-efficacy on *F. oxysporium* f. sp. *udum* are available. In our previous studies, we have isolated, and characterized *B. subtilis* strain NBAIR BSWG1 from the Western Ghats of India, tested their antagonistic potential, and found it very effective against *Sclerotium sclerotiorum* [13], *S. rolfsii* [14], *Alternaria alternata* [15]. However, the bio-efficacy of the same strain against *F. oxysporium* f. sp. *udum* is not been tested, hence in the present study, we have tested the bio-efficacy NBAIR BSWG1 by poison food technique using different concentrations of cell-free extract.

Bacillus subtilis produces a variety of secondary metabolites in their growth processes, which have many functions, such as inhibiting the growth of pathogens and promoting plant growth. *Bacillus subtilis* is known to produce some lipopeptides such as surfactin, fengycin, and iturin, which induce systemic resistance in plants [16]. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) has efficiently identified and characterized microbial secondary metabolites [17]. Although the cell-free extract of NBAIR BSWG1 successfully controlled many phytopathogens [13–15], the constituent antimicrobial compounds present in the cell-free extract of NBAIR BSWG1 has not been studied, hence the current investigation being conducted to characterize cell-free extract from NBAIR BSWG1 isolated from Western Ghats of India using MALDI-TOF.

MALDI-TOF with PCR offers easy platforms to characterize the antifungal lipopeptides. The identification of antifungal lipopeptides helps to formulate prospective biocontrol by-products, which have the wide-scale utility biological management pigeon pea wilt. Thus, the study aimed to test the bio-control activity of NBAIR BSWG1 against *F. oxysporium* f. sp. *udum* and decipher molecular mechanisms involved by DNA fingerprinting and metabolite profiling.

2. Materials and methods

2.1. Culture collection and maintenance of *B. subtilis* and *Fusarium oxysporium* f. sp. *udum*

Bacillus subtilis strains used in the present study were isolated and characterized in our previous study [13]. The pure culture of *Fusarium oxysporium* f. sp. *udum* was collected from microbial culture collection, Insect Bacteriology Laboratory, ICAR-NBAIR, Bengaluru. All isolates of *B. subtilis* were stored on slants of Luria Bertani at 4 °C in a refrigerator (BPL frost-free, India) and also in glycerol stocks (50 % v/v) at –80 °C in a bio-freezer (Vestfrost, Denmark). Pure culture *F. oxysporium* f. sp. *udum* maintained on potato dextrose agar slants at 4 °C in a refrigerator.

2.2. Biochemical characterization of *Bacillus subtilis* strains

Bacillus subtilis strains were screened biochemically using an oil spread assay and emulsification index (E24) assay to test the biosurfactant properties, which are the indirect measure of the antimicrobial properties of bacterial strains.

2.2.1. Oil displacement assay

The oil displacement assay is an indicator of the production of antimicrobial compounds that have surfactant activity. For this assay, 10 μ L of crude oil was added to the surface of 40 mL of distilled water in a Petri dish to form a thin oil layer. Then, 10 μ L of the cell-free extract was gently placed on the center of the oil layer, which will displace the oil and a clear zone if the bio-surfactant activity is present in the cell-free extract. The diameter of the clearing zone on the oil surface was visualized under visible light and measured after 30 s, which correlates to the surfactant activity, also known as oil displacement activity [18].

2.2.2. Emulsification index (E24) assay

The emulsification activity of *B. subtilis* strains was analyzed using tubes containing 3 mL of the purified lipopeptides solution at a concentration of 1.0 g/L and 2 mL of hydrocarbon substrate. The test was performed using vegetable oil. The tubes containing vegetable oil and cell-free extract were mixed by vortex for 2 min and maintained under static conditions at room temperature for 24 h. The emulsion stability was determined after 24 h and the emulsification index (E24) was calculated by dividing the measured height of the emulsification layer by the mixture's total height and multiplying by 100 [19].

2.3. PCR validation of antimicrobial lipopeptides genes in *Bacillus subtilis* strains

All five *B. subtilis* strains were validated for the presence of antimicrobial lipopeptides genes. Based on the literature surfactin (*urfA*), iturin (*ituC*), fengycin (*fenD*), bacillomycin (*bmyB*), and bacilycin (*bacA*) specific PCR oligonucleotides were selected, and synthesized, details of primers used in this study listed in Table 1 [20]. The PCR conditions for the *bacA*, *urfA*, and *bmyB* genes are as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles comprised of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min 30 s and one cycle of final extension at 72 °C for 10 min. Conversely, for the *fenD*, the PCR commenced with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, followed by annealing at 57 °C for 1 min, and extension at 72 °C for 1 min 45 s, and one cycle of final extension at 72 °C for 10 min [20]. PCR-amplified products were confirmed through gel electrophoresis and subjected to Sanger sequencing. The sequences obtained were searched for homology matches using the BLAST algorithm in the nr NCBI database. Further phylogenetic tree was constructed using surfactin gene sequences of our strains along with the sequences available in the database.

2.4. The antimicrobial activity of *Bacillus subtilis* strains

Based on lead obtained from biochemical characterization, and PCR validation of lipopeptides coding genes, antimicrobial activity of efficient *B. subtilis* strain was tested against *Fusarium oxysporium* f. sp. *udum* using poison food technique [21]. The syringe-filtered cell-free extract from efficient *B. subtilis* strain was mixed with molten PDA medium @ 12.5 μ L/mL, 25 μ L/mL, 37.5 μ L/mL, 50 μ L/mL, 62.5 μ L/mL, 75 μ L/mL, 87.5 μ L/mL and 100 μ L/mL of media. The medium was poured into the Petri plates and allowed to solidify, after solidification, agar plugs (5 mm) of *F. oxysporium* f. sp. *udum* maintained in pure culture was placed at the center of the plate and incubated at room temperature [22]. Petri plates without lipopeptides are maintained as control and the assay was conducted in three replications. Observation on radial mycelial growth of *F. oxysporium* f. sp. *udum* was measured and antagonistic efficacy was calculated using the following formula

$$E (\%) = (RC - RI) \times 100 / RC$$

Where, E (%) = antagonistic efficacy; RC = radius of the mycelia control colony (mm), RI = radius of the mycelia growth in treated plates.

2.5. Metabolite profiling of *Bacillus subtilis* strain using MALDI-TOF analysis

Based on lead obtained from bioassay, biochemical tests, and molecular characterization of five *B. subtilis* strains, we have chosen to characterize a cell-free extract of the most efficient strain selected for identification of biocontrol potential secondary metabolites through MALDI-TOF analysis. The syringe-filtered cell-free extract of efficient strain was subjected to MALDI-TOF analysis by

Table 1
Lipopeptide-specific PCR primers used for the characterization of *Bacillus subtilis* strains.

Sl. No.	Name of genes	Primer sequences	Tm	Amplicon size (bp)	Reference
1.	Surfactin (<i>urfA</i>)	F-AACGGGGAGCCTGTTC AATA R-ACAAGTTCAGGCACCGATTC	57 °C	420	Mardanov et al., 2017
2	Fengycin (<i>fenD</i>)	F-AAAGGTGTGTGGAATTGATG R-GCTGTCTCCTCTATCAAAAA	48 °C	670	Mardanov et al., 2017
3	Iturin (<i>ituC</i>)	F-TGCCATTATTGTCTACGGAG R-ATAAATCATACAGCCGAC	50 °C	270	Mardanov et al., 2017
4	Bacilycin (<i>bacA</i>)	F-CATTTCCAATTTTACTCTTC R-TACTTTTGGCCGTGCAAGCTC	48 °C	410	Mardanov et al., 2017
5	Bacillomycin (<i>bmyB</i>)	F-ACGGCAGGTTTTGATTTTT R-CGTTCCCTATCTCCGGA	47 °C	290	Mardanov et al., 2017

functioning in the positive mode using Rapiflex (Bruker-Daltonics Bremen, Germany) in the m/z range of (500–2000 Da). The sample was mixed with matrix (Dihydroxy benzoic acid) in a 1:1 ratio and spotted on the target plate. Data was acquired using the software Flex control and analyzed using Flex analysis software.

2.6. Statistical analysis

Bioassay data were analyzed by ANOVA, with bacterial strains as the independent variable and Student's t-test for simple pair-wise comparisons using R software version 4.2.2 and variables were arc sin transformed [2].

3. Results

3.1. Biochemical characterization of *Bacillus subtilis* strains

Biochemical characterization of cell-free extract extracted from *B. subtilis* strains was carried out to check biosurfactant activity through oil displacement activity. Among all the strains NBAIR BSWG1 showed high oil displacement value of 2.0 cm clearing zone followed by NBAIR BSWG4 (1.8 cm), NBAIR BSWG3 (1.7 cm), NBAIR BSWG2 (1.5 cm), and NBAIR BSWG5 (1.6 cm) (Fig. 1a). The emulsification index of *B. subtilis* strains ranged from 46 to 60 %. The emulsification index shows the ability of the cell-free extract to disperse two or more immiscible liquids together to form a semi-stable mixture. Among all the strains NBAIR BSWG1 showed the highest emulsification index of 60 %, followed by NBAIR BSWG4 (57 %), NBAIR BSWG3 (54 %), NBAIR BSWG2 (48 %), and NBAIR BSWG5 (46 %) (Fig. 1b).

3.2. Validation of antimicrobial lipopeptides genes using PCR

The genes responsible for lipopeptide production have been molecularly validated using lipopeptide-specific PCR primers in all five *B. subtilis* strains. Amplification of surfactin gene (*urfA*) was found in all five strains of *B. subtilis* (Fig. 2a) with 420 bp amplicon size at 57 °C annealing temperature, meanwhile, BLAST analysis against NCBI database showed an exact match with the surfactin gene. Fengycin gene got amplified only in NBAIR BSWG1 and NBAIR BSWG4 at an annealing temperature of 48 °C with 430 bp amplicon size using *fenD* primer sets (Fig. 2b), similarly, BLAST analysis confirmed the matching amplified gene sequence with fengycin gene. Parallely, Bacilysin gene (*bacA*) amplification was found only in NBAIR BSWG1 (Fig. 2c) with 410 bp amplicon size at an annealing temperature of 48 °C, BLAST analysis also confirmed the similarity of the sanger sequence with *bacA* in NCBI database. However, there was no amplification seen for the bacillomycin gene (*bmyB*) in any of the strains. The phylogenetic tree constructed using surfactin gene sequences is depicted in Fig. 3.

3.3. Antimicrobial activity of *Bacillus subtilis* against *Fusarium oxysporum* f. sp. *udum*

The antimicrobial activity of NBAIR BSWG1 against *F. oxysporum* f. sp. *udum* by poison food technique using different concentrations of cell-free extract, results of the same been tabulated in Table 2 and depicted in Fig. 4. The antagonistic activity of the cell-free extract showed inhibition ranging from 15.31 to 79.84 %. The cell-free extract concentration of 100 $\mu\text{L}/\text{mL}$ showed the highest percent inhibition of 79.84 % against *F. oxysporum* f. sp. *udum* and the lowest percent inhibition of 15.31 % was observed in a concentration of 12.5 $\mu\text{L}/\text{mL}$.

3.4. Identification of antimicrobial lipopeptides using MALDI-TOF analysis

Mass-spectrophotometric analysis of the cell-free extract extracted from NBAIR BSWG1 using MALDI-TOF shown most of the peaks

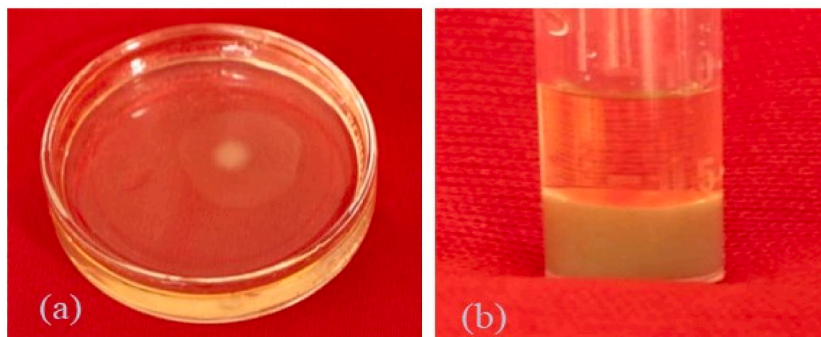


Fig. 1. Biochemical characterization of *Bacillus subtilis* isolates for confirmation of biosurfactant activity. (a) Oil displacement assay of NBAIR BSWG1, (b) Emulsification index assay of NBAIR BSWG1.

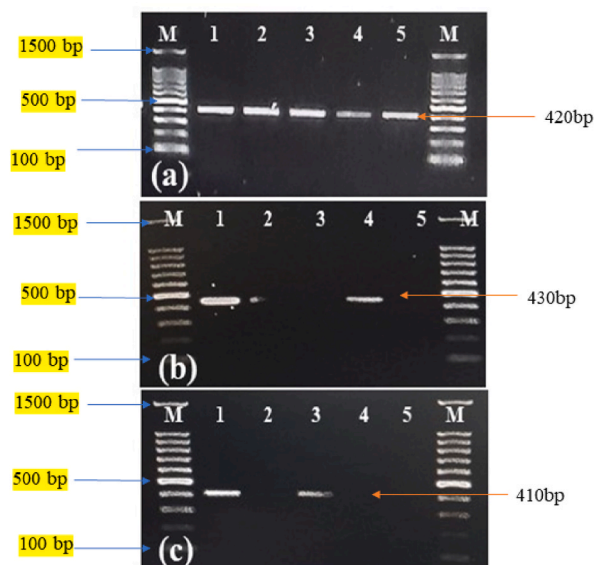


Fig. 2. Gel electrophoresis pictures showing amplification of surfactin (a) *srfA*, (b) *fenD*, (c) *bacA* in *Bacillus subtilis* isolates, M = 100 bp ladder, 1. NBAIR BSWG1, 2. NBAIR BSWG12, 3. NBAIR BSWG13, 4. NBAIR BSWG14, 5. NBAIR BSWG15.

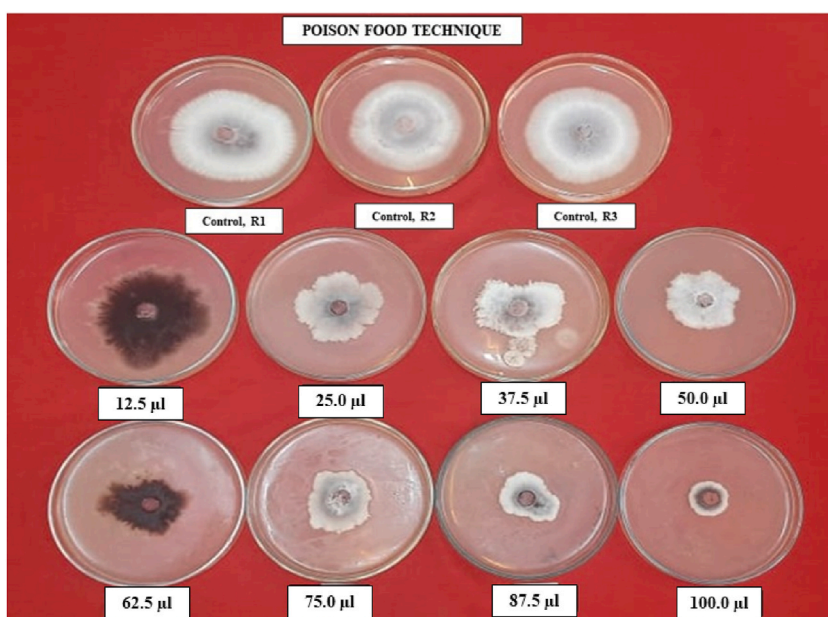


Fig. 3. Comparative phylogenetic analysis of surfactin gene present in *Bacillus subtilis* strains.

in the range of 600–2400 m/z (Fig. 5A). The mass to charge ratio was narrowed from 1350 to 1650 m/z to identify antimicrobial 189 lipopeptides (Fig. 5B). Based on the Mass spectrometry Contaminant Database (MaConDa) [23], the peaks observed at m/z 679, 701, and 905 were identified as contaminants as they appeared sporadically during the replications, indicating contamination. The presence of fengycin was confirmed at m/z of 1477, 1478, 1492, 1505, and 1528 m/z as per mass-to-charge ratio data available in the literature [24–26]. The presence of surfactin was confirmed at m/z of 1058, 1030 and 1131 and iturin at m/z of 1080.78 by comparing the m/z value with the literature m/z value [24–27]. (Table 3).

4. Discussion

The use of biocontrol agents to manage phytopathogens has been explored as an alternative to synthetic fungicides [28,29]. Among

Table 2Antagonistic activity of *Bacillus subtilis* NBAIR BSWG1 against *Fusarium oxysporum* f. sp. *udum* using poison food technique.

Concentration $\mu\text{L/mL}$	**Average radial growth of mycelial \pm SD (cm)	Percent inhibition (%)
12.5	3.50 ± 0.08^b	15.31
25.0	3.06 ± 0.05^c	25.79
37.5	2.73 ± 0.05^d	33.87
50	2.43 ± 0.05^e	41.13
67.5	2.13 ± 0.05^f	48.39
75.0	1.63 ± 0.08^g	60.48
87.5	1.26 ± 0.05^h	69.34
100	0.83 ± 0.05^i	79.84
Control	4.13 ± 0.05^a	0.00
C.D.	0.11	–

*DAI - Days after inoculation. **Average radial growth of three replications. Values in the same column followed by same letter do not differ significantly and the different letter indicates significant differences according to Duncan's Multiple Range Test ($P \leq 0.05$).



Fig. 4. Antagonistic activity of *Bacillus subtilis* NBAIR BSWG1 against *Fusarium oxysporum* f. sp. *udum* through Poison food technique using cell-free extract of NBAIR BSWG1 at 12.5 $\mu\text{L/mL}$, 25 $\mu\text{L/mL}$, 37.5 $\mu\text{L/mL}$, 50 $\mu\text{L/mL}$, 62.5 $\mu\text{L/mL}$, 75 $\mu\text{L/mL}$, 87.5 $\mu\text{L/mL}$ and 100 $\mu\text{L/mL}$ of media.

biocontrol agents *B. subtilis* has been extensively used for the management of phytopathogenic fungi because of its capacity to produce cyclic lipopeptides [30]. Lipopeptides are viewed as green alternatives for chemical surfactants/pesticides in agriculture. The production of lipopeptide depends on various parameters including pH, temperature, and oxygen. The optimum pH is neutral to slightly alkaline pH (7–7.5), with the temperature around 37 °C, sufficient oxygen along with a maximum incubation duration increases the secondary metabolite production, and meanwhile regulated agitation improves oxygen for the growth of bacterium for enhanced metabolite production.

Previously, we isolated, *B. subtilis* strains from the Western ghats of India and characterized them morphologically and molecularly by the 16S rRNA gene. However, they were not been characterized for biosurfactant activities. Hence, in the present study, the biosurfactant activities of strains have been evaluated by oil dispersion assay and emulsification index. Among five strains of *B. subtilis*, NBAIR BSWG1 showed the highest oil dispersion coefficient (2 cm) and highest emulsification index (60 %), these results indicated that NBAIR BSWG1 had the highest biosurfactant activity. The biocontrol agents that have maximum biosurfactant activity are more efficient in inhibiting the phytopathogens, we also found the highest inhibition of *Fusarium oxysporum* f. sp. *udum* by NBAIR BSWG1. Oil displacement assay outcomes were compared with those of positive findings. When the oil surface was repelled by a drop of surface-active metabolites found in the supernatants and formed clear halos, demonstrating the ability of lipopeptide to alter the nature of the oil surface and penetrate inside [31]. Similarly, Leelasupaksh 2008 [18] also showed that lipopeptides produced by *B. subtilis* strains influence the emulsification properties of cell-free extract which is a crucial indicator of the biocontrol potential of a bacterial strain.

Molecular characterization using lipopeptide-specific PCR primers DNA fingerprinting of *B. subtilis* strains. In the present study, surfactin was amplified at an amplicon size of 420 bp, fengycin at an amplicon size of 430 bp, and bacilysin at an amplicon size of 410 bp which confirms the presence of genes responsible for efficacy of *B. subtilis* strains. The inhibition of plant pathogens using cell-free

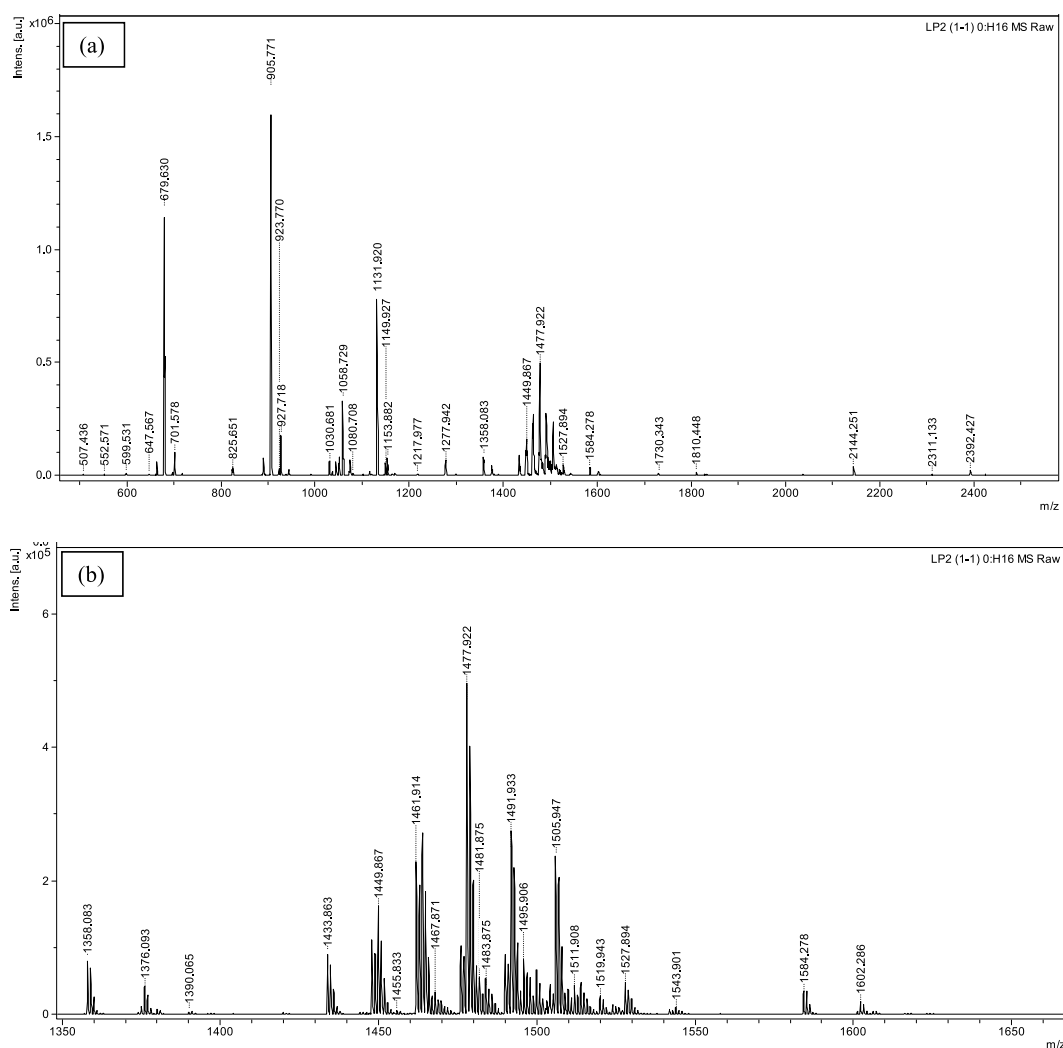


Fig. 5. MALDI-TOF mass spectra of NBAIR BSWG1 cell-surface extracts (a) range of 600–2400 (m/z ratio) (b) range of 1350–1650 (m/z ratio), analysis confirms the presence of the antibiotic lipopeptides *viz.*, surfactin, fengycin and iturin.

Table 3

Cyclic lipopeptide produced by *Bacillus subtilis* NBAIR BSWG1 identified by MALDI- TOF analysis.

Sl. No.	Mass-to-charge ratio	Compound	References
1	1030.6	Surfactin	[23–25]
2	1058.7	Surfactin	[23,25]
3	1131.92	Surfactin	[27]
3	1449.8	Fengycin	[23,24]
4	1477.8	Fengycin	[23,25]
5	1527.9	Fengycin	[23,24]
6	1477.92	Fengycin	[23,24]
7	1491.7	Fengycin	[23,24]
8	1505.8	Fengycin	[23–25]
9	1527.9	Fengycin	[23]
10	1080.6	Iturin	[23,24]

extract of *B. subtilis* is mainly due to cyclic lipopeptides, these compounds having an antagonistic property by interfering with the target pathogen's cell membrane, resulting in alterations to its shape and permeability through rupture, solubilization, or by the creation of ion-conducting pores [6]. Some of the most well-known cyclic lipopeptides produced by the NBAIR BSWG1 strain are surfactins, fengycins, and bacilysin. They vary in kind of biosurfactant based on chemical composition, biological and physiochemical properties [32].

Based on biochemical analysis and PCR studies we tested the efficacy of NBAIR BSWG1 against *Fusarium oxysporium* f. sp. *udum* using the poison food technique and obtained an inhibition of 79.84 %. Similarly, in our previous studies the bioefficacy of NBAIR BSWG1 against *S. rolfisii*, *A. alternata*, and *S. sclerotiorum*, was 88.89 %, 82.73 %, and 85.82 %, respectively [13–15]. The highest inhibition of *F. oxysporium* f. sp. *udum* using cell-free extract of NBAIR BSWG1 is mainly due to cyclic lipopeptides, which includes surfactins, fengycins, and bacilycin as per molecular characterization of NBAIR BSWG1 strain.

Based on oil displacement assay, emulsification index, molecular characterization, and bioassay results, NBAIR BSWG1 was chosen for metabolite profiling. Cell-free extract of NBAIR BSWG1 used for bioassay studies has been analyzed using MALDI-TOF for identification of constituent compounds responsible for bio-efficacy of the strain. Mass-to-charge ratios of 1042, 1056, 1056, 1070, and 1084 Da, confirm the production of surfactin by NBAIR BSWG1 strain [24–27]. Surfactin has been reported for suppression of various phytopathogenic fungi including oomycete *Plasmopara viticola* which causes grapevine downy mildew [33], *Collectotrichum trifolii*, *Ascochyta medicaginicola* and *Phytophthora medicago* in *Medicago sativa* [34]. Members of fengycin and surfactin elicit induced systemic resistance in plants and create pores in membranes of plant pathogens, resulting in an osmotic imbalance leading to the death of plant pathogens [35]. The fact that the surfactin effect is more marked than that of iturin A, is not surprising since surfactin can cover more space than iturin A because of its larger molecular area. The lipopeptide effect on the cell surface hydrophobicity arises from the amphiphilic structure of such compounds by considering the peptide cycle and hydrocarbon chain as polar head and non-polar tail, respectively [36]. Mukherjee and Das (2005) [37] also reported surfactin and fengycin peaks at m/z between 1030 and 1060, and 1449 to 1530 respectively. Additionally, m/z peaks viz., 923.77 and 1477.92 are proposed as novel lipopeptide compounds which needs further characterization for confirmation of these compounds.

5. Conclusion

In the present study, we explored the biocontrol potential of NBAIR BSWG1 against *F. oxysporium* f. sp. *udum*, studied constituent secondary metabolite present in cell-free extract, and validated the presence of these antimicrobial compounds by MALDI-TOF analysis as well as PCR amplification. The current research elucidates the intricate molecular mechanisms underlying the biocontrol efficacy of NBAIR BSWG1. The present study identified NBAIR BSWG1 as an efficient bacterial strain for the inhibition of *F. udam* and its anti-fungal properties are mainly due to the production of cyclic lipopeptides.

Ethical approval

This article does not contain any studies with human participants or animals.

Data availability statement

All data accessed and analyzed in this study are available in the article.

Additional information

No additional information is available for this paper.

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CRediT authorship contribution statement

Ruqiya S: Investigation. **Shivakumara KT:** Writing – review & editing, Data curation. **Aditya K:** Data curation. **Kandan A:** Writing – review & editing. **Sivakumar G:** Writing – review & editing. **Prasannakumar MK:** Writing – review & editing. **Pramesh D:** Writing – review & editing. **Manjunatha C:** Supervision, Investigation, Conceptualization. **Hosam O. Elansary:** Writing – review & editing. **Mohamed A. El-Sheikh:** Writing – review & editing. **Ihab Mohamed Moussa:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hosam O. Elansary reports article publishing charges was provided by Plant Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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