Potential of *Enterococcus faecium* LM5.2 for lipopeptide biosurfactant production and its effect on the growth of maize (*Zea mays* L.)

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**Abstract**

This study characterized the lipopeptide biosurfactants produced by lactic acid bacteria isolated from milk and milk products, in addition to their effect on maize plant growth. The oil displacement test was performed as a primary screening method to select the BS producing bacteria. The strain LM5.2 had the maximum emulsification index of 45.1 ± 3, reduced the surface tension to 32.98 ± 0.23% among all the isolates, and efficiently produced 945.26 ± 4.62 mg/l of biosurfactants within 48 h in MRS broth under the optimum conditions. The isolate LM5.2 was identified using physiochemical tests and 16S rRNA gene sequencing as *E. faecium*. The biosurfactant was purified by TLC and identified as lipopeptide-like iturins and surfactins based on *R*<sub>f</sub> values. Mass spectroscopy, NMR, and FTIR analysis also confirmed the biosurfactant’s identity as the derivatives of iturin and surfactin. In vitro biosurfactant application significantly enhanced seedling growth at an optimal concentration of 450 µg/ml. Plant treatment (pot experiment) with *E. faecium* LM5.2 significantly promoted *Zea mays* growth compared to the control. To the best of our knowledge, this is the first report of lipopeptide biosurfactant production by *E. faecium*. Moreover, the study also showed that the biosurfactant and biosurfactant-producing *E. faecium* LM5.2 could be an eco-friendly plant growth-promoting agent.

**Keywords** 16S rRNA gene sequencing · Biosurfacetant · Lactic acid bacteria · Mass spectroscopy · Plant growth · Proton nuclear magnetic resonance

**Abbreviations**  
BS: Biosurfactants  
EI<sub>24</sub>: Emulsification index  
TLC: Thin layer chromatography  
ESI and APCI MS: Electrospray ionization and atmospheric pressure chemical ionization mass spectrometry  
m/z: Mass/charge  
FTIR: Fourier transform infrared spectroscopy  
H<sup>1</sup>NMR: Proton nuclear magnetic resonance  
Ppm: Parts per million

**Introduction**

Sikkim is a small state situated in the North-East Himalayan region of India, which is widely known for its unique food culture and traditional practices of fermentation. One of the predominant bacteria responsible for the fermentation of these foods are lactic acid bacteria (LAB) (Tamang and Tamang, 2010). LAB isolated from traditional fermented food are a potential source of surface-active molecules such as lipopeptides, glycolipids, polysaccharide–protein complexes, lipopolysaccharides, phospholipids, fatty acids, and neutral lipids (Satpute et al. 2016). Biosurfactants (BS) are the active molecules of microbial origin that possess the ability to reduce the surface tension or interfacial tension between polar and non-polar phases or emulsify the two immiscible liquids (Rahman et al. 2002).

Many microorganisms produce different types of biosurfactants (BSs), which vary in structure and function. These BSs affect microbial activities such as microbial motility (Arutchelvi et al. 2008), biofilm formation (Hamme et al. 2006), and quorum sensing (Arutchelvi et al. 2008; Ron and Rosenberg 2001). Most of the BSs are very stable against the...
broad range of variation in pH, temperature, salt concentration and other environmental factors. Due to their surface activity and foaming property (Desai and Banat 1997; Jagtap et al. 2010), BSs are used in the formulations of many commercial products such as cosmetics, pharmaceutics and the agriculture sector (Salek and Euston 2019).

Biosurfactants have been reported to have antifungal or antimicrobial activity against many plant pathogens and thus can be used in place of chemical pesticides and insecticides to attain good health and environmental sustainability (Sachdev and Cameotra 2013). The most studied example is the rhizobacterial *Pseudomonas* sp. and *Bacillus* sp. which produces rhamnolipids and surfactin, respectively, that inhibits the growth of fungal pathogens (*Alternaria* sp., *Botrytis* sp., *Fusarium* sp., *Pythium* sp., *Phytophthora* sp., *Plasmopara* sp., *Rhizoctonia* sp.) and bacterial pathogens (*Pseudomonas syringae* and *Ralstonia solanacearum*) (Bais et al. 2004; Monnier et al. 2020; Penha et al. 2020; Rodrigues et al. 2017). Rhamnolipid of *Pseudomonas* also stimulates the plant’s immune system and protects them from pathogens (Vatsa et al. 2010). The BS plays a vital role in cell communication between rhizobacteria and plant root hairs (Dusane et al. 2010). They also increase the heavy soil’s wettability by hydrophilization which help the fertilizers to distribute evenly in the soil and in its bioabsorption. Growth enhancement by the biosurfactant-producing bacteria has been shown in the tea plants, *Brassica juncea* (L.), *Phaseolus vulgaris* (bean), *Raphanus* L. (radish), and *Beta vulgaris* L. (beetroot).

The objective of this study was to evaluate the potential of LAB isolated from the milk and milk products of Sikkim (India) to produce BS. The best BS producing bacterium was identified and its BSs were characterized. Furthermore, the ability of different concentrations of the purified BS was evaluated for in vitro seed germination and the potential of the selected bacterium was evaluated on plant growth promotion.

### Materials and methods

#### Sample collection, isolation and tentative identification of bacterial isolates

Five raw cow milk and five homemade dahi samples were collected from the local market of Gangtok, Sikkim (India), and processed for bacterial isolation on De Man, Rogosa and Sharpe (MRS) agar media with 1.0 % CaCO<sub>3</sub>. Bacteria were isolated by the serial dilution method at 10<sup>-4</sup> concentrations from the pure milk/dahi samples (Ghatani and Tamang 2017). Discrete colonies showing halo zone in the CaCO<sub>3</sub> supplemented MRS agar plate were selected, purified and maintained at − 20 °C. Tentative identification of BS producing bacteria was made by microscopic observations, Gram staining, growth at different conditions and sugar fermentation tests according to the “Berger’s Manual of Systematic Bacteriology” (Vos et al. 2009).

#### Screening for biosurfactant (BS) production

Pure isolates were cultured in MRS broth at 37 °C for 48 h. The culture broth was centrifuged at 8000 rpm for 20 min to obtain the cell free supernatant used for screening the BS.

#### Oil displacement test

A thin layer of 20 µl of engine oil (Servo2T Supreme, India) was made on 30 ml of distilled water in a 100 mm glass petri plate, and 10 µl of cell-free supernatant was added gently to the oil film. The displacement of oil showed the presence of BS in the media (Youssef et al. 2004).

#### Estimation of Emulsification Index (EI<sub>24</sub>)

5 ml of each sunflower oil and culture supernatant was taken in a test tube and vortexed for 5 min. The tubes were kept undisturbed overnight. After 24 hours, the total length of liquid in the media and the emulsified solution’s length were measured. EI<sub>24</sub> was then calculated (John et al. 2021).

#### Measurement of surface tension

DuNoüy platinum ring method was employed to measure the surface tension. The surface tension of the 48 hours culture supernatant was measured with the Kruss GmbH Hamburg tensiometer’s help at 25 °C (Gudiña et al. 2010).

#### Production and extraction of biosurfactant

Lactic acid bacteria were cultured in MRS broth for 48 hours at 25 °C in a shaker incubator at 120 rpm. The culture broth was centrifuged at 12,000 rpm at 4 °C, and the supernatant was acidified with 5M HCl by adjusting pH to 2. The supernatant was kept at 4 °C overnight to allow the biosurfactant to settle at the flask’s bottom. After harvesting the precipitate by centrifugation (12,000 rpm for 20 min and 4 °C), it was suspended in a CHCl<sub>3</sub>:CH<sub>3</sub>OH (2:1) solution and centrifuged again. Then, the surfactant was collected from the interphase of CHCl<sub>3</sub> and CH<sub>3</sub>OH. The collected biosurfactant was suspended in distilled water with an adjustment of pH to 7. Finally, the biosurfactant was lyophilized for further chemical analysis (Sharma et al. 2015).
Bacterial identification

The identification of the isolate was done by 16S rRNA gene sequencing. Bacterial genomic DNA was isolated by a phenol–chloroform method (Sambrook et al. 2006). Full-length 16S rRNA gene region was amplified by 27F and 1492R primers with standard PCR protocol (Sachdev and Cameotra 2013). PCR product was subjected to Sanger sequencing, and the forward and reverse sequences were aligned by Codon Code Aligner 7.1.2 software. The identity of bacterial isolates was confirmed by the BLAST tool from the NCBI nr/nt database. Partial sequence data were deposited in the GenBank nucleotide sequencing data library. A phylogenetic tree was constructed using MEGA X software (Kumar et al. 2016). The evolutionary history was inferred using the Maximum Likelihood method and General Time Reversible model. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (16 categories (+G, parameter = 0.1735)).

Purification of biosurfactant

Lyophilized biosurfactant (100 µg) was dissolved in 1ml of methanol and applied on analytical Silica gel 60F254 plates (Merck, Germany). The chromatogram was developed in duplicate with mobile phase chloroform: methanol: ammonia water (60:35:5). One plate was treated with ninhydrin solution, and another plate was treated with hydrochloric acid. Biosurfactant was purified by preparative Thin Layer Chromatography (TLC) with the mobile phase chloroform: methanol: ammonia water: (65:35:5) on HF254 silica gel plate (Merck, Germany). The bands were observed under UV light at 254 nm (Antonious et al. 2015).

Characterization of biosurfactant

Chemical characteristics of isolated BS were determined by mass spectroscopy (MS), infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectrometry.

LC–MS

The various biosurfactant fractions were isolated from the purified BS by LC–MS (Thermo Finnigan LCQ Advantage Max Ion Trap Mass Spectrometer Hyphenated with the Thermo Finnigan Surveyor HPLC system). BS samples were dissolved in HPLC grade methanol (Merck, Germany) at a concentration of 2 mg/ml, and 2 µl was injected into the C18 column (5 µl, 4.6 × 250 mm) with a flow rate of 0.20 ml/min. The mobile phase was an acetonitrile/water (with 0.1% TFA) gradient (10–90%). ESI-MS was obtained in positive mode with a scanning range of 50–2000Th (Antonious et al. 2015).

FTIR

FTIR spectroscopy was used to elucidate the chemical bonds and functional groups present in the BS structure (Pornsunthorntawee et al. 2008). BS sample was analyzed in an Alpha FTIR spectrophotometer (Bruker Germany), equipped with an Opus graph plotter (Pornsunthorntawee et al. 2008). The lyophilized dry biosurfactant sample was mixed with potassium bromide (KBr) and compressed into a tablet form, scanned under 700–4000cm−1 with the resolution of 4 cm−1.

NMR

TLC purified BS sample was dissolved in deuterated water (D2O) in the concentration of 50 µg/ml, and 1H-NMR spectroscopy was done to determine the structural characteristics of the BS with the instrument ‘Bruker AFCEND-Germany spectrophotometer’. The scanning was done at the frequency of 400 mHz. The spectrograph was analyzed with ‘Mesterno’ software.

Effect of biosurfactant on seed germination

First, the seeds were washed with 3% sodium hypochlorite to make them free from undesired fungal spores. Five different sets of three petri plates were prepared with 10ml of 0, 150, 300, 450 and 600 µg/ml BS solutions soaked in filter papers and incubated in the dark. In each plate, five healthy maize seeds were placed, and the growth of the seedlings was observed for one week. The observation was taken at a regular interval of 24 h.

Effect of biosurfactant-producing bacteria on Zea mays’ growth

The soil samples were collected from different agriculture sites in Sumbuk village, situated in South Sikkim, India. The soil was sterilized by autoclaving at 121 °C for 20 min twice. Two sets of five pots were prepared and marked as lactic acid bacteria, and control treatment, respectively, and each pot was filled with 1.25 kg of soil. In parallel, a broth culture of E. faecium LM5.2 was prepared with the cell density of 108 cells/ml by adjusting the optical density to 0.8 at the wavelength of 600 nm with UV–Visible spectrophotometer (PerkinElmer Lambda 25) (Bhuvaneswari et al. 1980). A set of five pots was inoculated with 20 ml of culture broth.
with the initial population of LAB in each pot was about 8 × 10^5 cells per kg soil. An equal amount of sterilized MRS broth was added to the control pots. The pots were incubated for one week at room temperature in undisturbed conditions. Maize seeds were surface sterilized by washing with 3% sodium hypochlorite to eliminate fungal spores or other microbial contaminants, following that, the seeds were thoroughly washed with sterilized water to remove the hypochlorite; later, one seed was sown in each pot. For the first week, observation of the seedlings’ development was made every 24 hours. Later, the plant length, number of leaves, length of the leaves, and the plant’s width were measured every week for one month. After one month, the plants were removed from the soil carefully without damaging the roots, and fresh weight, dry weight and moisture content were recorded.

Effect of biosurfactant on rhizosphere microflora

After harvesting the plants, each potting soil’s microbial loads were analyzed using the serial dilution method. Mycorrhizal spores were extracted from each set of 100 g soil by the sieving method. Mycorrhiza spores were counted manually with the help of a zoom stereomicroscope.

Statistical analysis

All the data were replicated at least thrice to obtain the mean and the standard deviation. The initial screening of biosurfactant was analyzed by one-way ANOVA followed by Bonferroni post hoc analysis to determine the significant differences (p<0.05) among the means of different isolates. Student’s two-sample t test analyzed differences in the means of the effect of plant growth. All the analyses were performed in RStudio 3.6.3.

Results

Fifty discrete circular-shaped colonies, with a clear zone in calcium carbonate, supplemented MRS as lactic acid bacteria. The isolates were found to be a Gram-positive, rods or coccus, arranged in pairs or chains, catalase negative, KOH-test negative, and did not produce CO2 from glucose. Based on physiological and biochemical tests (Table 1), these bacteria were tentatively identified as *Enterococcus sp.* and *Lactobacillus sp.* and were screened for biosurfactant production.

Screening for biosurfactant production

Out of 50 isolates, only ten isolates showed an oil displacement zone of 1–3 cm in diameter (Table 2). Isolates that showed a diameter of less than 0.5 cm were not considered for further analysis. The isolate LM5.2 showed the maximum emulsification index with a value of 45.1± 0.89. Besides, isolates CLS6.1 and LM5.8 also showed a remarkable emulsification index compared to other isolates (Table 2). The isolate LM5.2 reduced the surface tension to 32.98 ± 0.23% as determined by the Du Noüy platinum ring method. The isolates CLS6.1, LM5.1 and LM5.3 also reduced the surface tension to 32.56 ± 0.42, 31.68 ± 0.76 and 31.68 ± 0.62, respectively. The maximum biosurfactant production was found in LM5.2 culture, which was 945.26 ± 4.62 mg/ml, which was statistically significant (p<0.05). Hence, based on the oil displacement test and the quantity of biosurfactant produced, isolate LM5.2 was selected for 16S rRNA sequencing and further analyses.

Identification of bacteria

The 16S rRNA sequence obtained was searched for the similarity with the NCBI data bank with the BLAST tool’s help and confirmed its species as *E. faecium*. The sequence was submitted to NCBI GeneBank with the accession number MH733938. The phylogenetic relationship based on 16S rRNA gene sequences also showed strain LK5.2 to be closely related to *E. faecium* (Fig 1).

TLC analysis

The thin layer chromatogram of the biosurfactant showed spots at the RF values of 0.26, 0.41, and 0.51 under UV254, which indicated that the BS contains conjugated double bonds in its structure. Upon ninhydrin treatment, pink color spots developed, which indicated the lipopeptide nature of the BS.

Biosurfactant characterization

Mass spectroscopy

The biosurfactant of *E. faecium* LM5.2 showed the HPLC–MS peaks at m/z 1054, which corresponds to the surfactin linear chain of Glu–Leu^2–Val–Asp–Leu^2 with C14 (Ma et al. 2016). The peak at m/z 985 corresponds to the same amino acid chain with the 12 CH group in the hydrophobic moiety. The peak at m/z 906 indicated the presence of β-hydroxyl fatty acid chain associated with the Glu–Leu^2–Val–Asp–Leu amino acid chain of surfactin (Ma et al. 2016). The peak at m/z 832.52 corresponds to the sodium ion associated with C_7 fatty acid and the Glu–Leu^2–Val–Asp–Leu^2 amino acid chain (Ma et al. 2016). The next peak at 685 also showed the precursor of the surfactin amino acid chain [H]–Leu^2–Val–Asp–Leu^2–[OH] (Ma et al. 2016), which is the characteristic feature of surfactin. Peaks at m/z 758 showed the impurities of di-rhamnolipid.
### Table 1  Morphological and physiological characteristics of biosurfactant-producing LAB isolates

| Isolate code | Microscopy | NaCl conc | Growth in/at | Tentative identity |
|--------------|------------|-----------|--------------|--------------------|
|              |            | 6.5%      | 18%          |                    |
|              |            | 10°C      | 20°C to 45°C |                    |
|              |            | 3.6 to 9.6| pH           |                    |
|              | Cell Morphology | Arabinose | Cellubiose | Glactose | Lactose | Mellobiose | Rhamnose | Ribose | Sorbitol | Trihalose | Xylose |
| LM1.8        | C          | +         | –           | –        | –        | +         | +        | +      | –        | +        | –      | –      | –      | Enterococcus.sp |
| LM5.1        | R          | –         | –           | +        | –        | –        | +        | +      | +        | –        | –      | +      | –      | Lactobacillus.sp |
| LM5.2        | C          | +         | –           | –        | +        | –        | +        | +      | +        | –        | –      | +      | –      | Enterococcus.sp |
| LM6.1        | C          | +         | –           | –        | +        | –        | +        | +      | +        | –        | –      | +      | –      | Enterococcus.sp |
| LM5.3.4      | C          | +         | –           | –        | +        | –        | +        | +      | +        | –        | –      | +      | –      | Enterococcus.sp |
| LM5.8        | C          | +         | –           | –        | +        | –        | +        | +      | +        | –        | –      | +      | –      | Enterococcus.sp |
| LM5.9        | R          | –         | –           | +        | –        | +        | +        | +      | +        | –        | –      | +      | –      | Lactobacillus.sp |
| LM5.10       | C          | +         | –           | –        | +        | –        | +        | +      | –        | –        | +      | –      | –      | Enterococcus.sp |
| CL6.1        | C          | +         | –           | –        | +        | –        | +        | +      | +        | –        | –      | +      | –      | Enterococcus.sp |
| CL7          | C          | +         | –           | –        | +        | –        | +        | +      | +        | –        | –      | +      | –      | Enterococcus.sp |

All the isolates were gram positive, non-endospore producing and catalase negative and fermented sucrose, mannose, salicin and maltose

C coccus, and R rod shaped; All coccus cells were either paired or in chain
A strong peak of m/z at 610 could not be identified (Fig. 2).

FTIR

The biosurfactant’s spectroscopy showed the absorbance at 944 cm\(^{-1}\), representing CH=CH bonds in the structure (Silverstein et al. 2005). The sharp medium peak at 1026 cm\(^{-1}\) showed the C–O bond stretch, and minor peaks at 1046 cm\(^{-1}\) indicated the presence of alkanes of the fatty acid chain (Silverstein et al. 2005). Weak peaks at 1108 cm\(^{-1}\) show unsaturated alcohol or phenol group present in the structure (Silverstein et al. 2005). A weak peak at 1458 cm\(^{-1}\) could be because of lactones, which is the characteristic of surfactin. Weak absorbance at 1542 cm\(^{-1}\) shows the N–H bend of amide that can be present in the peptide chain of the biosurfactant. CO–N group of the peptide is associated with a medium height peak at 1646 cm\(^{-1}\) (Silverstein et al. 2005). A weak broad peak at 2512 and 2849 cm\(^{-1}\) corresponds to the lipopeptide biosurfactant’s carboxyl group, while small peaks at 2915 cm\(^{-1}\) indicate alkanes (C–H) stretch in the structure (Silverstein et al. 2005) (Fig. 3).

Table 2

| Isolate | Source | Oil displacement zone (cm) | Emulsification index (%) | Surface tension reduced (%) | Surfactant produced (mg/L) |
|---------|--------|---------------------------|--------------------------|-----------------------------|---------------------------|
| LM1.8   | Milk 1 | 37.89 ± 0.75\(^{ab}\)     | 30.44 ± 0.5\(^{bc}\)     | 694.82 ± 9.83\(^{a}\)    |                            |
| LM5.1   | Milk 1.5| 38.51 ± 1.47\(^{ab}\)    | 31.68 ± 0.76\(^{abc}\)  | 736.00 ± 8.01\(^{b}\)    |                            |
| LM5.2   | Milk 3 | 45.1 ± 0.98\(^{a}\)      | 32.98 ± 0.23\(^{a}\)    | 1050.60 ± 4.62\(^{e}\)   |                            |
| LM5.3   | Milk 3 | 40.2 ± 0.98\(^{ab}\)     | 31.68 ± 0.62\(^{abc}\)  | 453.43 ± 3.54\(^{d}\)    |                            |
| LM5.3.4 | Milk 3 | 37.41 ± 1.30\(^{ab}\)    | 29.70 ± 0.39\(^{c}\)    | 384.80 ± 2.11\(^{f}\)    |                            |
| LM5.8   | Milk 1 | 41.7 ± 1.79\(^{ab}\)     | 31.46 ± 0.75\(^{abc}\)  | 258.47 ± 4.28\(^{f}\)    |                            |
| LM5.9   | Milk 3 | 38.09 ± 0.95\(^{ab}\)    | 30.05 ± 0.41\(^{c}\)    | 166.00 ± 8.86\(^{g}\)    |                            |
| LM5.10  | Milk 2 | 41.18 ± 0.00\(^{ab}\)    | 30.11 ± 0.28\(^{bc}\)   | 635.78 ± 4.27\(^{h}\)    |                            |
| CL6.1   | Curd 1 | 44.65 ± 0.53\(^{a}\)     | 32.56 ± 0.42\(^{ab}\)   | 766.60 ± 5.12\(^{i}\)    |                            |
| CL7     | Curd 2 | 36.19 ± 0.95\(^{ab}\)    | 30.60 ± 0.60\(^{abc}\)  | 563.58 ± 2.73\(^{j}\)    |                            |

Data represent the mean ± SD, Standard deviation of three independent replication. Means with different superscripts along the column are statistically significant at \(p < 0.05\).

(2017). A strong peak of m/z at 610 could not be identified (Fig. 2).

Fig. 1 Maximum likelihood phylogenetic tree based on 16S rRNA gene sequence of the biosurfactant-producing bacteria Enterococcus faecium LM5.2. The tree with the highest log likelihood (− 3876.16) is displayed. The percentage of trees with the associated taxa clustered together is shown alongside the branches.

Fig. 2
NMR

The BS isolated from *E. faecium* LM5.2 showed a duplet peak at 0.8 ppm, indicating the presence of a terminal primary alkyl group of the fatty acid (Fig 4). A strong triplet peaks at 1.20, 1.25, and 1.26 ppm, associated with peaks at 1.47 and 1.66 ppm, showed a secondary alkyl group of the hydrocarbon chain. A small duplet peak at 1.84 and 1.85 ppm indicated the non-saturated hydrocarbon chain of fatty acid. The peaks at 2.10 and 2.32 ppm showed –CH2–COO−, duplet at 3.2 ppm corresponds to the –O–CH– group present in the structure, and a sharp, strong peak at 4.35 ppm showed the terminal amino group of the amino acid (Balan et al. 2017). The peak at 8.1 and 8.3 ppm indicated terminal amide that is indicative of the presence of amino acid in the BS (Tiwary and Dubey 2018).

**Effect of biosurfactant on seedlings germination**

The seeds germinated in petri plates with the treatment of different concentrations of BS showed maximum growth
at 450 µg/ml with a mean length of 4.3 cm on the sixth day, while the mean length of the control set of seedlings was 2.4 cm under the same conditions (Fig 5). The two-way ANOVA analyses followed by multiple pairwise comparisons with Bonferroni adjustment showed that the effect of BS at 450 µg/ml had a significant effect ($p<0.05$) on the germination and seedlings growth.

**Fig. 4** $^1$H NMR spectroscopic pattern of purified biosurfactant form *E. faecium* LM5.2 dissolved in deuterated methanol-$d_4$ solvent. The peaks were developed in the range of 1.2–1.8 ppm indicates hydrocarbon chains of fatty acids; however, from 2.1 to 8.3 showed the different bonds of peptidal chain

**Fig. 5** Effect of different concentrations of biosurfactant produced by *E. faecium* LM5.2 on the germination and growth of corn seeds under in vitro condition
Effect of biosurfactant-producing *E. faecium* LM5.2 on the potted plants

*E. faecium* LM5.2 imparts a significant effect on different aspects of maize growth, including seed germination, plant height, leaf development, leaf growth, weight, and moisture content of the plant. Plant growth was estimated in terms of the number of days for seed germination, plant height, number of leaves developed and the length of leaves for one month (Fig 6, supplementary table 1). The germination of seedlings in the *E. faecium* LM5.2 took 5 ± 0.4 days, while it took 8 ± 0.8 days in the control pots, which was statistically significant (*p* < 0.05). The mean plant height in treated soil was observed to be about 28.82 ± 1.6 cm, while the control plants showed a mean height of 15.48 ± 2.2 cm (significant at *p* < 0.05). The average length of leaves in one month in a treated set of plants was 15.91 ± 1.99 cm; on the other hand, the average leaf length of control plants was 8.64 ± 1.10 cm. The fresh weight and moisture content of the treated plant set was 0.989 ± 1.79 g and 61.69 ± 0.06%, respectively, while the fresh weight and moisture content of control plants was 0.667 ± 3.65 g and 52.82 ± 0.07%. No significant difference was observed in the number of leaves.

Effect of *E. faecium* LM5.2 on soil microbial count: the total mycorrhizal spores per 100 g soil in the treated and control pots were 1424.44 ± 551.26 and 275.56 ± 38.23, respectively, which may be directly responsible for the enhanced plant growth in the treated soil. The total microbial count in the treated soil was 3.32 × 10^8 cfu/g, while the control set of soil samples had 8.9 × 10^6 cfu/g.

![Fig. 6 Boxplot showing effect of *E. faecium* LM5.2 on the plant growth with the respective *p* values.](image_url)

|   |   |
|---|---|
| a | Effect on the germination time, |
| b | Effect on the height of the plant, |
| c | Effect on the leaf length, |
| d | Effect on the fresh weight of the plant, |
| e | Effect on the dry weight of the plant, |
| f | Effect on the moisture content. Boxplot with red color represents the control set while the boxplot in peacock green represents the treatment group (LAB). |

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Discussion

Milk and milk products are rich sources of lipids/fats and lactose, which provide ideal conditions for the growth of BS producing lactic acid bacteria (Yilmaz et al. 2009). Many reports are available on the BS produced by Bacillus and Pseudomonas species. However, LAB has also been increasingly studied as a potential source of BS (Gudiña et al. 2010). These reports have mentioned LAB as an efficient source of rhamnolipids and other glycolipid biosurfactants (Sharma et al. 2015; Velraeds et al. 1996). To our knowledge, this is the first report that mentions E. faecium as a potential source of lipopeptide surfactant. For primary screening, the oil displacement method was used, as it is easy, fast, and cheap. EI24 is used as a quantitative screening method for evaluating the emulsification efficiency of biosurfactants (Ghasemi et al. 2019), which depends on the biosurfactant-producing strain and the type of oil. The emulsification of soybean oil by the lipopeptide BS from E. faecium LM5.2 was similar to that reported by Ghasemi et al (Ghasemi et al. 2019) though higher emulsification capacities of BSs have also been reported, especially from non-lactic isolates (Dalili et al. 2015; Janek et al. 2013). The reduction of surface tension by the isolate LM5.2 was similar to that of E. faecium MRTL9 (Sharma et al. 2015) and slightly higher as shown by Lactobacillus sp.(Morais et al. 2017).

The measurement of surface tension and emulsification index helps in the identification of the best quality biosurfactant produced among our isolates. In our screening, out of 50 isolates, ten were found to be efficient biosurfactant producers, which showed that LAB could provide some novel biosurfactants with different applications in the food and pharma industry. TLC showed the spot at an Rf value of 4.1, which corresponds to iturin. The next spot was at an Rf value of 5.1, which may be the surfactin's derivative (Fernandes et al. 2007). The Rf value at 2.6 can be fengycin (Dlamini 2017). MS spectrograph showed the series of m/z values of 1054, 980, 906, 832, 758, 684 and 610, which is actually obtained by repeated subtraction of 74 from 1054. The values m/z 1054, 906, 832 and 685 belonged to different sodium-associated surfactin A derivatives (Ma et al. 2016). The values in the range of m/z 1050–1058 has also been reported for sodium-associated surfactin A (Chaurasia et al. 2020; Ma et al. 2016). FTIR spectra showed CH=CH, CO–N and N–H, which indicated peptidyl group in the BS (Habib et al. 2020). Peaks of alkanes and alkenes were also found, which suggested the lipid hydrocarbon chain present in the structure (Sriram et al. 2011). NMR analyses also support the presence of similar structural properties (Silverstein et al. 2005). All these pieces of evidence prove that the E. faecium LM5.2 is producing lipopeptide biosurfactant. To the best of our knowledge, this is the first report of a lipopeptide biosurfactant from E. faecium.

Corn plant variety ‘Vivek Maize Hybrid 53’ was selected for our experiment as this variety is suitable for cultivation in the hilly regions like Sikkim. The BS from E. faecium LM5.2 showed a remarkable effect on the germination of the seedlings under in vitro conditions (Sachdev and Cameotra, 2013). Similarly, significant (p<0.05) growth enhancement by the BS producing bacterium was also observed in the potted plant experiment. LAB, including E. faecium, produces organic acids in the soil to help solubilize phosphorus and other essential minerals for the plant (de Lacerda et al. 2016). Simultaneously, the biosurfactant reduces the surface tension, interfacial tension, and forms micelles that help in the bioavailability of the nutrients to the plant. Sachdev and Cameotra have also reported that BS increases the bioadsorption of the water and nutrients from the soil to the roots, which results in the plants’ fast growth (Sachdev and Cameotra 2013). Moreover, BS reduces the stress inflicted by pathogenic bacteria and fungi due to their antimicrobial and antifungal activities (Monnier et al. 2020; Penha et al 2020; Rodrigues et al 2017; Bais et al. 2004). Both surfactin and fengycin have been found to induce systemic resistance in the plant (Cawoy et al. 2014; Li et al. 2019). Iturin A, another lipopeptide BS, has also been reported to induce plant defence genes in strawberry leaves (Yamamoto et al. 2015) and in cotton plants against Verticillium dahliae attack (Han et al. 2015). E. faecium LM5.2 also supported mycorrhizal growth in the rhizosphere, which may be another reason for relatively better plant growth than the control ones (Frey-Klett et al. 2007; Gamalero et al. 2009).

Conclusion

This study showed that the E. faecium LM5.2 isolated from raw cow milk is a potent source of lipopeptide BS, especially surfactin. Though purified BS fastened the seed germination, BS producing E. faecium LM5.2 also showed good seed germination and growth of maize plants in in vivo conditions. This study indicated that the BS producing E. faecium LM5.2 has the potential as a biofertilizer for crop improvement to boost organic agriculture. Sikkim is an organic state where chemical fertilizers and pesticides have been completely banned since 2016. The GRAS bacterial isolate, as a potent producer of BS, can be used as a biofertilizer for crop improvement to boost organic agriculture in the region.

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Declarations

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