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Exploring of Biosurfactant Producing Bacterial Isolates Combined with Plant Growth Promoting Activities

Arati[1*], Tamilvendan, K.[2], G. Selvakumar[3], P. S. Benherlal[4], N. Earanna[5] and Y. A. Nanja Reddy[6]
[1]Ph.D. Scholar, Department of Agricultural Microbiology, UAS, GKVK, Bangalore (Karnataka), India.
[2]Professor, Department of Agricultural Microbiology, UAS, GKVK, Bangalore (Karnataka), India.
[3]Principal Scientist (Microbiology), Division of Natural Resource IIHR, Hessaraghatta, Bengaluru (Karnataka), India.
[4]Associate Professor, Department of Biochemistry, UAS, GKVK, Bangalore (Karnataka), India.
[5]Professor, Department of Agricultural Microbiology, UAS, GKVK, Bangalore (Karnataka), India.
[6]Professor, Department of Crop Physiology, UAS, GKVK, Bangalore (Karnataka), India.

*(Corresponding author: Arati *)*
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ABSTRACT: The biosurfactants are the pool of secondary metabolites that are bounteously extravasated by multifarious microorganisms either extracellularly into the culture broth or anchored to the cell surface where from they are released. These biosurfactants are engrossed in crop growth and improvement as an appealing alternative to synthetic surfactants. There is paucity of information on biosurfactant having plant growth promoting activities. Hence, the present investigation was taken up to uncover the plant growth stimulating potential of efficient biosurfactant producers chosen after rigorous screening by qualitative and quantitative assays in particular oil spreading test, drop collapse assay, penetration assay, emulsification assay, bacterial adhesion to hydrocarbon (BATH) assay and surface tension reduction test. The seven bacterial isolates (BPB-17, BPB-34, BPB-48, BPB-49, BSB-18 and BSB-24) which were screened and selected from the previous study were evaluated for plant growth-promoting properties *viz.*, PO_4 solubilization, siderophore production, ammonia production, zinc and potassium solubilization, N_2- fixation and ACC deaminase activity. Significant increases in plant growth-promoting traits were registered by isolate BPB-17 mainly phosphate solubilization (2.4 mg/L), ammonia production (1.98 mg/L) and K-solubilization (2.46 mg/L). While the isolate BPB-48 recorded significant siderophore production (76.88 %) and Zn solubilization (2.71 mg/L).

Keywords: Microorganisms, Biosurfactant, plant growth promoting potential, solubilization.

INTRODUCTION

Increased agricultural production to fulfill the food demands of the ever-increasing human population is a major challenge. Using synthetic chemicals to meet food demand has a profusion of ravages on the environment. Accordingly, it has become mountingly crucial to seek novel alternatives, merely ecofriendly and bio-based polymeric surfactants that could embellish soil health (Foley *et al*., 2012). This can be wangled using green technology like microbes and their metabolite which ensures long-term viability in agriculture, one such microbe-produced compound is biosurfactant. Biosurfactants are amphiphilic compounds produced by distinct microorganisms on living surfaces or excreted extracellularly. Amphiphilic nature of biosurfactant comprehends hydrophilic moiety comprising an acid, peptide cations, or anions, mono-, di- or polysaccharides and a hydrophobic moiety of unsaturated or saturated hydrocarbon chains or fatty acids (Banat *et al*., 2010). These structures confer a jumble of properties, including its proficiency in lowering the surface and interfacial tension of liquids and to form micelles and micro emulsions between two disparate phases. They differ from conventional surfactants by their biological origin and have no chemical synthesis step added during its production. These unique properties have led to their increased applications in various fields, such as environment and agriculture (Markande *et al*., 2021). These biosurfactant producing microorganisms ameliorate plant growth by producing divergent plant growth promoting traits and embellishment of plant immunity against pests and diseases. Despite the lack of information on the role of biosurfactants that produce PGP bacteria in encouraging plant growth, the use of biosurfactants to produce PGP bacteria is still a viable option. The exploration of biosurfactants producing bacteria for greater plant growth promotion assumes significance in discovery of novel biosurfactants linked with their ecofriendly use in agriculture which has recently been targeted by the scientific community (Mishra *et al*., 2020). Exploring the plant growth promoting biosurfactants producing bacteria will overthrow the

precarious effects of chemical surfactant application, paving way for eco-friendly and prudent environment leading to sustainable agriculture. The present paper focuses on plant growth-promoting activities of biosurfactant-producing isolates isolated from hydrocarbon and fly ash contaminated areas.

MATERIAL AND METHOD

Source of bacteria. Bacterial isolates which recorded highest surface tension reduction and oil spreading activity namely, BPB-17, BPB-34, BPB-48, BSB-49, BSB-18 and BSB-24 documented by the earlier study (Arati and Tamilvendan 2022) were selected to screen for their plant growth promotion potential and bacterial strain *Bacillus* sp. (MCC4316) procured from NCMR, Pune was used as a reference strain for biosurfactant production.

Screening of biosurfactant producing bacteria for their plant growth promoting activities

***In vitro* solubilization of inorganic phosphate.** For the qualitative estimation of phosphate solubilization by biosurfactant-producing bacteria, the selected bacterial isolates were grown in tryptic soy broth and incubated at 30°C on constant shaking at 120 rpm for 24 h. Then, 5 µl of culture was spot inoculated into Pikovskaya's medium containing tricalcium phosphate as an insoluble phosphate source. The inoculated plates were incubated at 30°C for 3 days. After incubation, plates were observed for a clear halo zone around the colony.

The 200 µl (10^9) 24 h old culture of seven bacterial isolates were inoculated into the 50 ml of National Botanical Research Institute's phosphate (NBRIP) broth amended with 5 g/L of tri-calcium phosphate (pH=7) and incubated at 30°C on a rotary shaker at 120 rpm/min for seven days. Uninoculated NBRIP broth served as control. After seven days of incubation broth was centrifuged at 10,000 rpm for 15 minutes. To the 0.5 ml supernatant, 1-2 drops of p-nitrophenol (0.25 %) was added as an indicator followed by the dropwise addition of 5 N HCl to neutralize the color. Further, the solution was diluted with 40 ml of double distilled water, and 8 ml of ammonium paramolybdate-ascorbic acid reagent was added, final volume was made up to 50 ml with distilled water and incubated at room temperature for 20 minutes. After incubation, the absorbance was read at 880 nm by using a UV-visible spectrophotometer (Thermo Scientific, Biomate 3S, China) (Murphy and Riley, 1962).

Siderophore production. Siderophore production was tested using chrome Azurol's (CAS) medium as described by Schwyn and Neiland (1987). Five µl of 24 h old seven bacterial inoculum was spot inoculated on to the tryptic soy medium with CAS dye and incubated at 30 °C for 3 days. The formation of a yellow to light orange halo surrounding the colony was considered positive for the production of siderophore.

Quantification of siderophore production. Biosurfactant-producing isolates that were positive for siderophore production were further used for quantitative estimation using CAS – Shuttle assay. Twenty four hours old culture of seven bacterial isolates were inoculated into TSB and incubated at 30°C on a rotatory shaker (Thermocon, Bangalore) with 200 rpm for 3 days. Following incubation, the broth was centrifuged at 10000 rpm (Eppendorf, India) for 15 min and 0.5 ml resultant supernatant was mixed with 0.5 mL of CAS dye, incubated at room temperature for 1 h, and absorbance was measured at 630 nm (Thermo scientific, Biomate 3S, China) to estimate the loss of blue color to orange against a reference consisting of 0.5 mL of uninoculated broth. The percentage of siderophore units was calculated by the formula

% Siderophore units = (Ar630 nm- As630 nm)/ Ar630 nm x 100

Where, Ar = Absorbance of reference at 630 nm

As = Absorbance of sample at 630 nm

Ammonia production. The seven bacterial isolates were examined for their ability to produce ammonia production in peptone water. Freshly grown bacterial cultures were inoculated into 10 ml peptone water and incubated at 30°C for 72h. After incubation 0.5 ml of Nessler´s reagent was added and observed for color development. The development of a brown to yellow color was an indication of ammonia production (Cappuccino and Sherman, 1992). Further, this was quantified by measuring the absorbance using UV-visible spectrophotometer (Thermo Scientific, Biomate 3S, China) at 450nm.

Hydrogen cyanide (HCN) production. Hydrogen cyanide (HCN) production was assessed by streaking the bacterial isolates on King's B agar plate amended with 4.4 g/l glycine. Whatman No.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. The plates were then sealed with parafilm and incubated at 30°C for 48 h. After 48 h, the color change in the filter paper from deep yellow to reddish-brown color was considered as an indication of HCN production.

Zinc solubilization. All bacterial strains were screened for their zinc solubilization using zinc oxide (ZnO) as an insoluble source. Five µl of overnight grown bacterial isolates were spot inoculated into zinc medium plates containing ZnO as an insoluble source. These plates were covered with aluminum foil and incubated in the dark at 28°C for 7 days. Isolates showing clear zones around colonies were considered positive for zinc solubilization. To know the amount of zinc solubilized in the broth quantitative assay was carried out. One mL of overnight grown culture was inoculated into 50 ml of minimal broth supplemented with 1% insoluble zinc source and incubated at 30 ºC for 15 days on a rotary shaker (Thermocon, Bangalore) at 150 rpm. Then the bacterial cultures were centrifuged at 15,000 rpm (Eppendorf, India) for 20 min and the supernatant was passed through a 0.2 mm membrane filter. Further, the sample was fed to an atomic absorption spectrometer (AAS) to find the concentration of available zinc present in the samples.

Potassium solubilization. Potassium solubilization was evaluated on Alexandrow agar medium supplemented with mica as an insoluble potassium source. Using a sterile cork borer 5mm holes were made on

Alexandrow agar plates, inoculated with a 5 µL of each bacterial culture, and incubated at 30°C for 3 days. The isolate exhibiting clear zones was considered positive for potassium solubilization. For quantification, one mL of overnight grown culture was inoculated into 50 ml of minimal broth supplemented with 2% insoluble potassium source and incubated at 30°C for 15 days on a rotary shaker (Thermocon, Bangalore) at 150 rpm. Then the bacterial cultures were centrifuged at 15,000 rpm for 20 min and the supernatant was passed through a 0.2 mm membrane filter. Further, the sample was fed to a flame photometer to find the concentration of available potassium present in the sample.

Free-living nitrogen fixation. Nitrogen fixation of seven bacterial isolates was qualitatively examined on Norris's glucose nitrogen-free medium. All the bacterial isolates were streaked on Norris's glucose nitrogen-free medium plates and these plates were incubated at 28°C for 7 days. The isolates growing on N free media were considered as positive free living nitrogen fixation.

ACC deaminase activity. The ACC deaminase activity of bacterial isolates was determined by using 1-aminocyclopropane-1-carboxylate (ACC) as the sole nitrogen source. Seven bacterial isolates were grown in 5 ml of TSB medium and incubated at 28°C at 120 rpm for 24 hrs. The cells were harvested by centrifugation at 5000 rpm for 5 minutes and washed twice with sterile 0.1 M Tris- HCL (pH 7.5). The harvested cells were resuspended in 1 ml of 0.1 M Tris-HCL (pH 7.5) and spot inoculated onto Petri plates containing DF (Dworkin and Foster 1958) salts minimal medium supplemented with 3mM ACC as the sole nitrogen source. Plates containing only DF salts and minimal medium without ACC served as a negative control. The plates were incubated at 28°C for 72 h. The bacterial isolates that grew on DF medium with ACC were considered positive for ACC deaminase activity.

RESULT AND DISCUSSION

The study of bacterial isolates establishing positive interaction with plants is increasing day by day because of their potential utilization for enhancing crop yield and soil fertility for sustainable agriculture and environments. Beneficial microbes improve plant growth by enhancing the availability of nutrients, the regulation of phytohormones, and increasing plant tolerance against biotic and abiotic stresses. The BS-producing isolates which additionally contain plant growth-promoting potential would tag them as potential candidates for crop production in agriculture. With this background, all the biosurfactant-producing bacterial isolates were subjected to PGP screening.

A. Phosphate solubilization

Phosphorus (P) is the second most vital macronutrient that plays a significant role in the overall growth and development of the plant. Inorganic phosphorus readily gets transformed into less available forms by forming a complex with Al and Fe in acid soils or with Ca in calcareous soils (Banerjee *et al*., 2010). It is documented that some of the soil bacteria are capable of solubilizing fixed soil phosphorous and applied phosphates and make it available to the plants. These phosphate solubilizing bacteria have been reported to play a considerable role in increasing the P efficiency of both native and applied P and improving the growth and yield of crops (Thakur *et al*., 2014). In our study, all seven BS producing bacteria solubilized the inorganic phosphate, as evidenced by the formation of a clear halo around the colony on the Pikovskaya's agar medium. Further, the quantitative estimation of the phosphate solubilizing abilities of the isolates was examined (Table 2; Fig 1 and 6). Among the seven isolates, BPB-17 exhibited significantly the highest phosphate solubilization (2.40 mg/L) being the most efficient phosphate solubilizer as compared to all other isolates followed by BPB-48 (1.90 mg/L). The results are in agreement with Wang *et al*. (2020) who used tricalcium phosphate as an insoluble phosphate from Bacillus subtilis had shown a strong capability to solubilize tricalcium phosphate with soluble phosphorus content of 276.3 µg/mL and Gupta *et al*. (2002) showed the varied phosphate solubilization capability of the bacterial isolates and reported that maximum P solubilization was recorded by PS4 at 7th day (10.22 mg/ml) followed by PS3 (9.42 mg/ml). Phosphate solubilization of organisms could be due to the combined effect of pH decrease and organic acid production (Yu *et al*., 2011; Ravinder *et al*., 2022).

Siderophore production. Iron (Fe) is an essential element needed by all living organisms from unicellular to multicellular as a vital element for their numerous cellular processes (Sarwar *et al*., 2020). In the iron-deficient condition, these microorganisms produce the siderophore, low molecular weight chelators that trap iron molecules from the atmosphere and host for their survival (Ahmed and Holmstrom, 2014). Siderophores are high affinity iron chelating compounds that help to chelate a few elements essential for plants. It is one of the vital mechanisms for disease suppression and plant growth promotion. All the seven efficient BS-producing isolates were screened for their in vitro siderophore production on CAS agar plates, to evaluate the siderophore production capacity of BS producing isolates, taking into account the formation of orange-colored halo zone around the bacterial colonies as positive for siderophore production, all the seven isolates were identified to be siderophore producers (Table 2; Fig. 2 and 6).

The quantification results of siderophore revealed that, among these seven isolates BPB- 48 produced a significantly higher amount (76.88%) of siderophore followed by BPB-17 46.14% as compared to other isolates. Different organisms produced different percentages of siderophores in their culture as reported by many authors (Breidbach *et al*., 2002; Hussein and Joo, 2012; Sayyed *et al*., 2005) which might be attributed to the genetic variability among the isolates. The present study is in accordance with Ghosh *et al*. (2015) who evaluated siderophore production from three isolates both qualitatively and quantitatively and recorded that Pseudomonas aeroginosa yielded the

highest siderophore (80.50%). Wu *et al*. (2018) reported that BS producing *P. aeruginosa* L10 produced a siderophore by forming a halo zone around the colony measuring 4.1 to 4.5 cm. Gururani *et al*. (2012) evaluated the capacity of siderophore producing bacteria influencing the uptake of various metals including Fe, Zn, and Cu by plants.

Ammonia production. Ammonia production is one of the important attributes of PGP bacteria that influence plant growth indirectly by enhancing root and shoot elongation, and increasing plant biomass by supplying nitrogen to host plants (Wani *et al*., 2007). The ammonia production potential of BS-producing isolates was evaluated on peptone broth (Table 2; Fig 3).

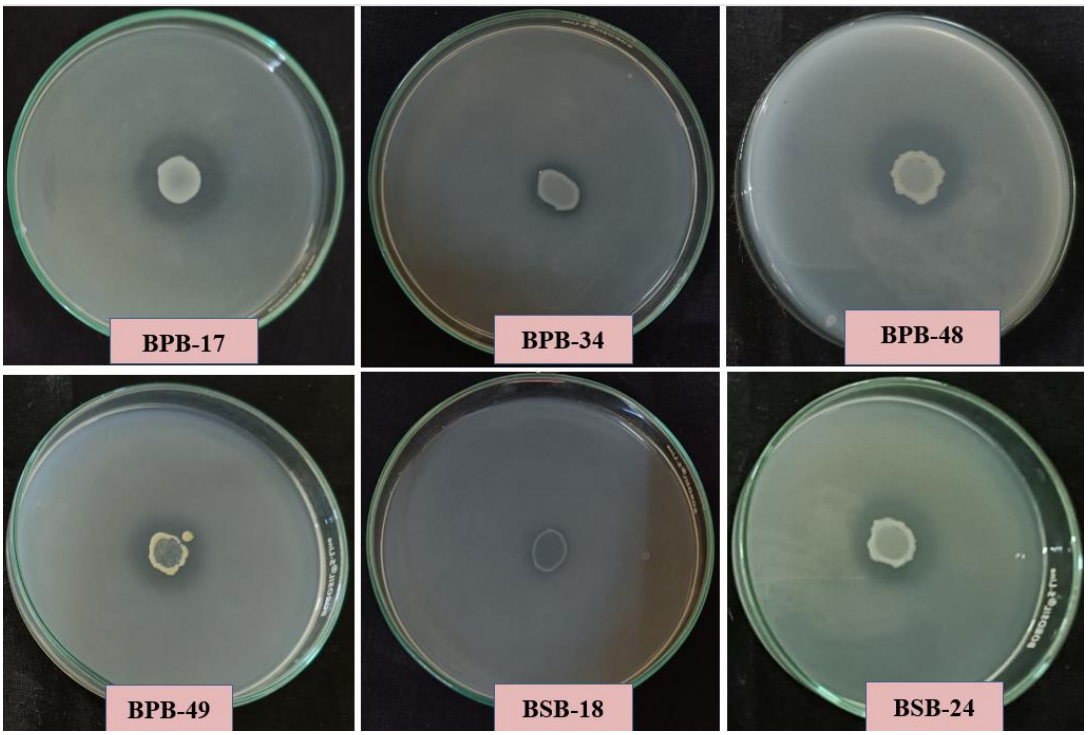


Fig. 1. Phosphate solubilization zone produced by efficient biosurfactant producing isolates on Pikovskaya's agar.

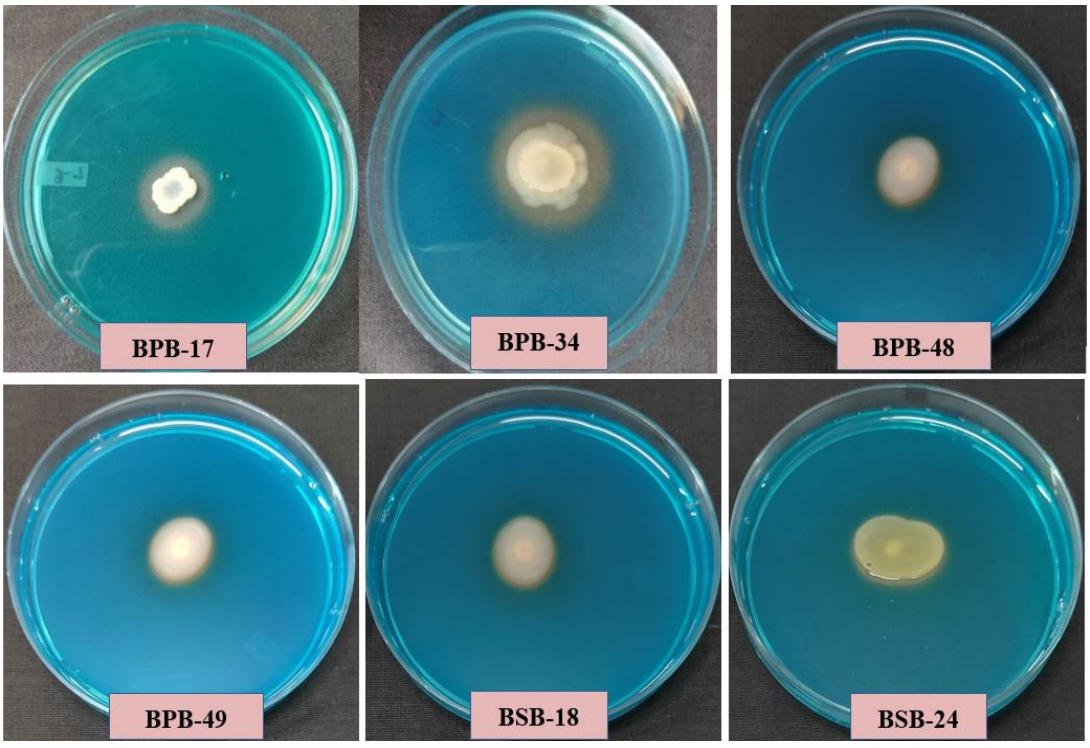


Fig. 2. Orange halo zone of siderophore produced by efficient biosurfactant producing isolates on CAS agar plates.

Table 1: Plant growth promoting activities of biosurfactant producing bacteria.

Biosurfactant Producing Bacterial Isolates	HCN Production	Free living N_2 Fixation	ACC Deaminase activity
BPB-17	+	+	+
BPB-34	+	+	+
BPB-48	+	+	+
BPB-49	-	-	+
BSB-18	-	-	+
BSB-24	+	+	+
Bacillus sp. (MCC4316)	-	-	+

Note: (+) - Positive, (-) – Negative

The quantitative estimation of ammonia production revealed that a significant increase in ammonia production was observed in BPB-17 (1.98 mg/L) compared to all other isolates. The results of the current investigation are in accordance with the findings of Chrouqi *et al.* (2017) which reported that among eight bacterial isolates strains S48 and S54 recorded higher ammonia production (2.07 µg/ml). Similar results were obtained for ammonia production in 95% of isolates from rice rhizosphere (Samuel and Muthukkaruppan, 2011) and 85% of strains from wheat rhizosphere (Cherif-Silini *et al*., 2016). The ammonia production by the plant growth-promoting bacterium in the soil increase the pH (9-9.5) creating an alkaline condition that suppresses the growth of certain fungi which has a potent inhibition effect and also inhibits germination of spores of many pathogenic fungi (Swamy *et al*., 2016). Microorganisms hydrolyze urea into ammonia and carbon dioxide to produce ammonia which aids in meeting the nitrogen needs of the host plant, lessens pathogen colonization of plants (Rodrigues *et al*., 2016), and influences plant growth indirectly (Geetha *et al*., 2014). Pahari *et al.* (20016) tested five bacterial isolates for the production of ammonia in peptone water and reported that all the isolates exhibited strong production of ammonia from peptone water except SBBA-2.

Hydrogen cyanide (HCN) production. Many PGPBs can produce HCN, which is a very common volatile toxic antimicrobial secondary metabolite produced by several microorganisms (Devi *et al*., 2007). Bacteria that generate hydrogen cyanide modulate plant growth by combating fungal infections by acting as biocontrol agents. In the present study it is observed that among seven isolates tested for HCN production, four isolates BPB-17 BPB-34, BPB-48, and BSB-24 were positive for HCN production (Table 1; Fig 4 and 6). The results are in accordance with the findings of Jayaprakashvel *et al*. (2010) which reported the color change of sodium picrate impregnated filter paper strips, clearly indicating HCN production by 24 bacterial isolates. Apart from the bio control studies, HCN produced by PGPR plays a significant role in the formation of complexes with transitional metals present in minerals (Faramarazi and Brand, 2006; Fairbrother *et al*., 2009) and also with irons (Keel *et al*., 1997) and thus reduce the available iron levels for phytopathogens to contribute or additional dimension of biocontrol and metabolism of nutrient elements from natural rocky environments (Wongfun *et al*., 2014; Lapanje *et al*., 2014; Frey *et al*., 2010).

B. Zinc solubilization

Zinc (Zn) is the most vital micronutrient for optimum plant growth. In plants, zinc plays a key role as a structural constituent or as a regulatory co-factor of a wide range of different enzymes and proteins involved in many important biochemical pathways, mainly concerned with photosynthesis, in the conversion of sugars to starch, protein metabolism, pollen formation and the maintenance of the integrity of biological membranes (Singh *et al*., 2005). Zn is present in the soil as an unavailable form for plant uptake (Mumtaz *et al*., 2017). All the isolates were able to solubilize zinc by forming a solubilization zone around the colony on a minimal medium supplemented with ZnO agar plates. Further, in quantitative estimation (Table 2) the significant increase in zinc solubilization was recorded by isolate BPB-48 (2.71 mg/L) followed by isolate BPB-17 (2.33 mg/L). These findings are consistent with the findings of Othman *et al*. (2022) who reported that among the 88 bacterial isolates screened, 9 isolates were able to solubilize Zn on mineral salts media, showing a clear halo zone and TM56 had the highest solubilization ability (20.3%).

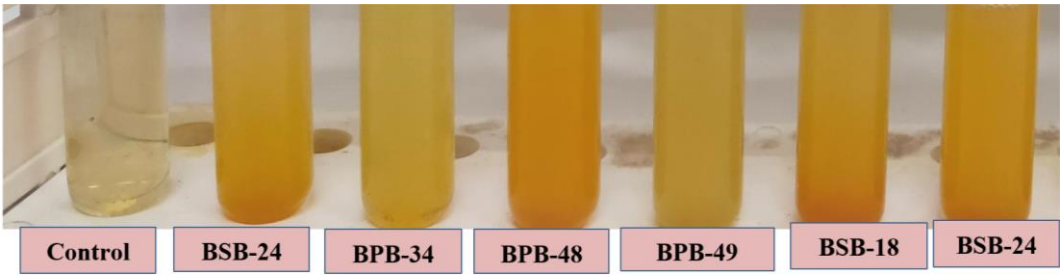


Fig. 3. Ammonia produced by efficient biosurfactant producing bacterial isolates indicated.

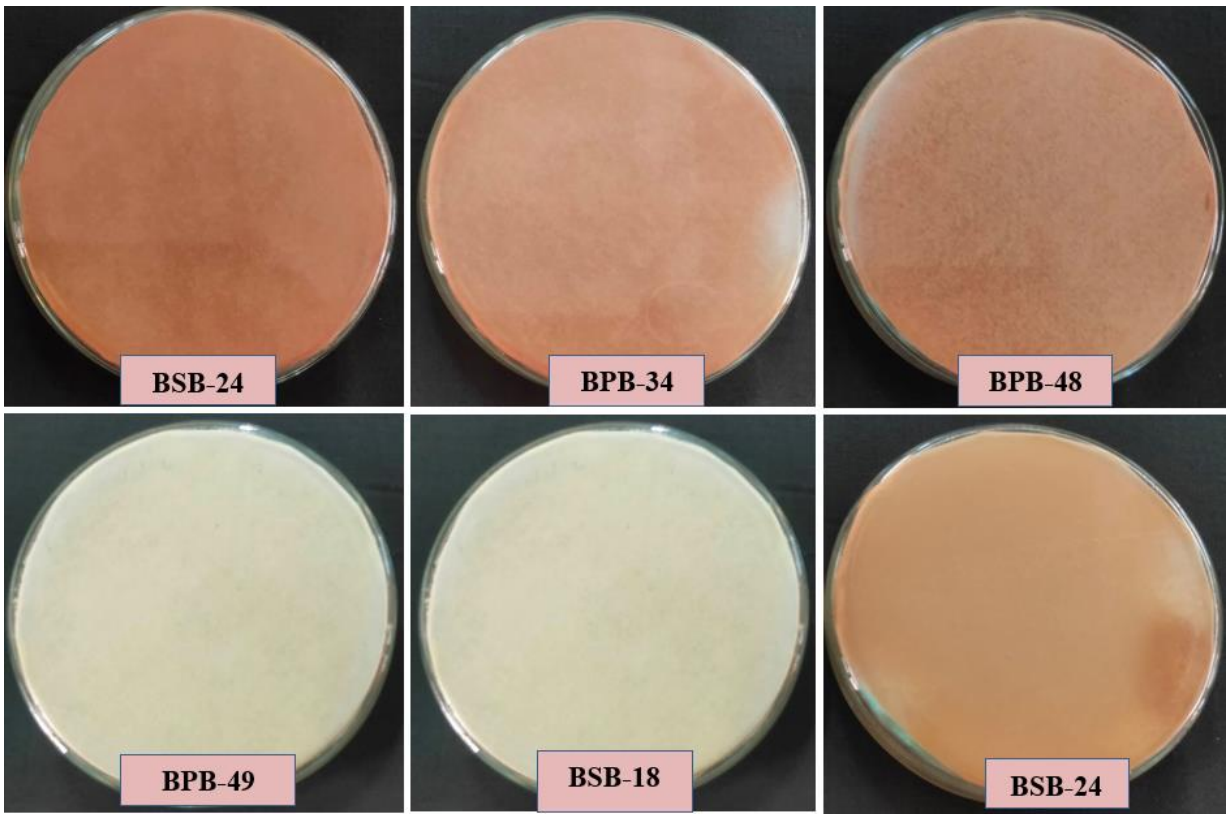


Fig. 4. HCN production by changing colour of filter paper indicating by efficient biosurfactant producing isolates.

C. Potassium solubilization

Potassium is a mobile nutrient fixed in silicate minerals such as feldspars and micas, which are unavailable for plant uptake (Zhang and Kong, 2014). Plant- microbe interaction plays a very important role in the cycling of minerals. Microbes produce different types of acid which helps in the release of the nutrient from the insoluble substrate (Singh, 2018). In the present study, the potassium solubilization ability of the bacterial isolates was evaluated. All the isolates were able to solubilize K by forming a solubilization zone around the colony on the Aleksandrow agar plates (Fig. 5). The positive isolates selected and their pure cultures were inoculated to broth amended with mica for the quantitative determination of K solubilization. The results of K solubilization are presented in (Table 2). In the present study, the amount of K solubilized by all the isolates ranged between 0.3 to 2.4 mg/L. The isolate BPB-17 recorded 2.46 mg/L of K solubilization which is significantly higher compared to all other isolates followed BPB-48 solubilized 2.23 mg/L. These results are in agreement with Verma *et al*. (2016) who reported that among the 14 bacterial isolates isolated from the different rhizospheric regions of different zones of India, seven strains showed a zone of clearance on modified Aleksandrow medium plates supplemented with mica powder. Similarly, bacteria showing a zone of clearance on mica plates have been isolated from the roots of cereal crops (Mikhailouskaya, 2005).

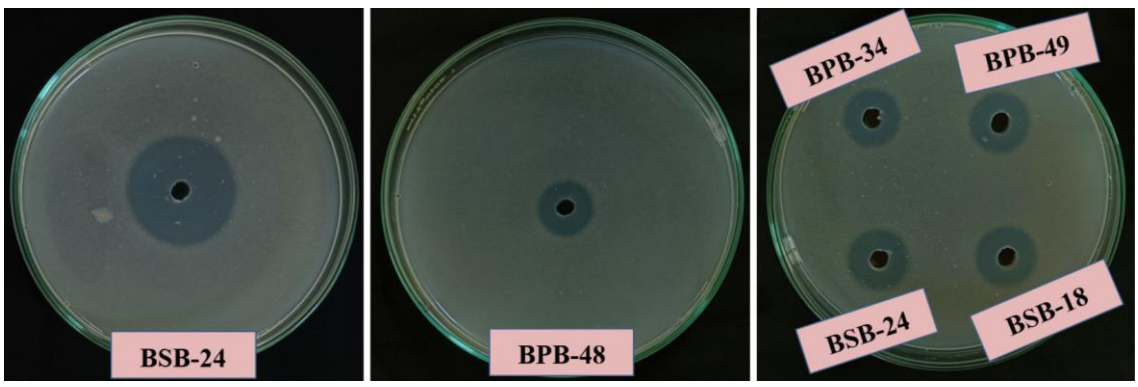


Fig. 5. Potash solubilization zones produced by efficient biosurfactant producing bacterial isolates on Aleksandrow agar plates.

The potassium solubilization process of KSB is completed by various mechanisms, including acidolysis, chelation, dissolution, active absorption, *etc*., which promote each other and eventually lead to the gradual precipitation of K+ in minerals (Du *et al*., 2008; Zhou *et al*., 2010; Lian *et al*., 2020).

Free-living Nitrogen fixation. Nitrogen is one of the most abundant and important elements because it is part of nucleic acids and proteins. Atmospheric nitrogen, which is the diatomic molecule or dinitrogen cannot be taken up by plants because they do not have the necessary enzymes to convert it into biologically useful forms. Nitrogen-fixing microorganisms play significant roles in converting atmospheric N_2 gas to ammonia. The quantity of N_2 fixed varies with each bacterium. In the present study, only four isolates BPB-17, BPB-34, BPB-48 and BSB-24 were able to grow on Norris's glucose nitrogen-free medium (Table 1; Fig 1) indicating that they are nitrogen fixers. Nihorimbere *et al*. (2011) showed that nitrogen fixing microbes produced many biosurfactants and they gained importance in agriculture (Gopalakrishnan *et al*., 2017). It is encouraging to note that the isolates used in the present study produced lipopeptide type of biosurfactants and also fixed nitrogen under *in vitro* conditions (unpublished) offering a viable option for crop cultivation contaminated soil ecosystems.

Table 2: Quantitative estimation of plant growth promoting traits of biosurfactant producing bacteria.

Biosurfactant Producing Bacterial Isolates	Phosphate solubilization (mg/L)	Siderophore production (%)	Ammonia production (mg/L)	Zn Solubilization (mg/L)	K Solubilization (mg/L)
Control	0.40^{g}	11.00^{f}	0.40^{f}	0.30^{g}	0.10^{f}
BPB-17	2.40^{a}	46.14^{b}	1.98^{a}	2.33^{b}	2.46^{a}
BPB-34	1.27^{f}	40.21^{de}	0.75^{e}	2.32^{b}	0.50^{d}
BPB-48	1.90^{b}	76.88^{a}	1.54^{b}	2.71^{a}	2.23^{b}
BPB-49	1.41^{e}	44.60^{bc}	1.25^{c}	0.84^{f}	1.76^{c}
BSB-18	1.72^{c}	42.27^{cd}	1.04^{d}	2.13^{c}	1.85^{c}
BSB-24	1.55^{d}	37.50^{e}	1.52^{b}	1.06^{e}	1.84^{c}
Bacillus sp. (MCC4316)	1.28^{f}	38.10^{e}	1.46^{b}	1.34^{d}	0.31^{e}

Note: Values are mean (±SE) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p>0.05).

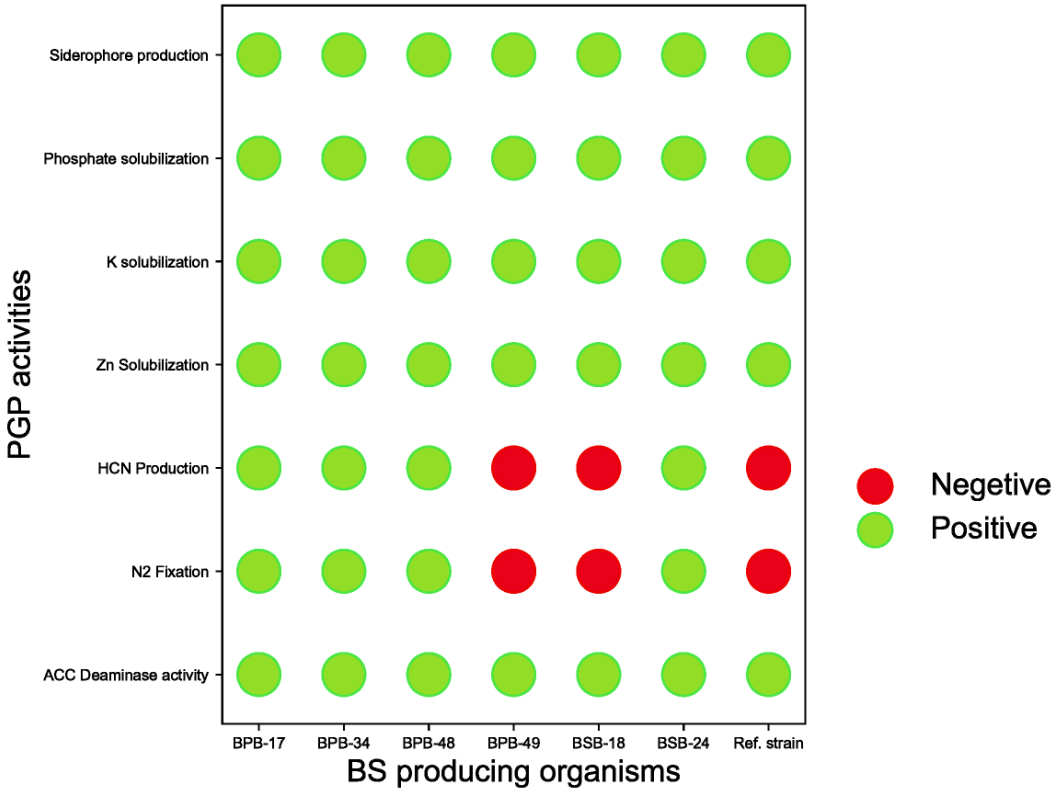
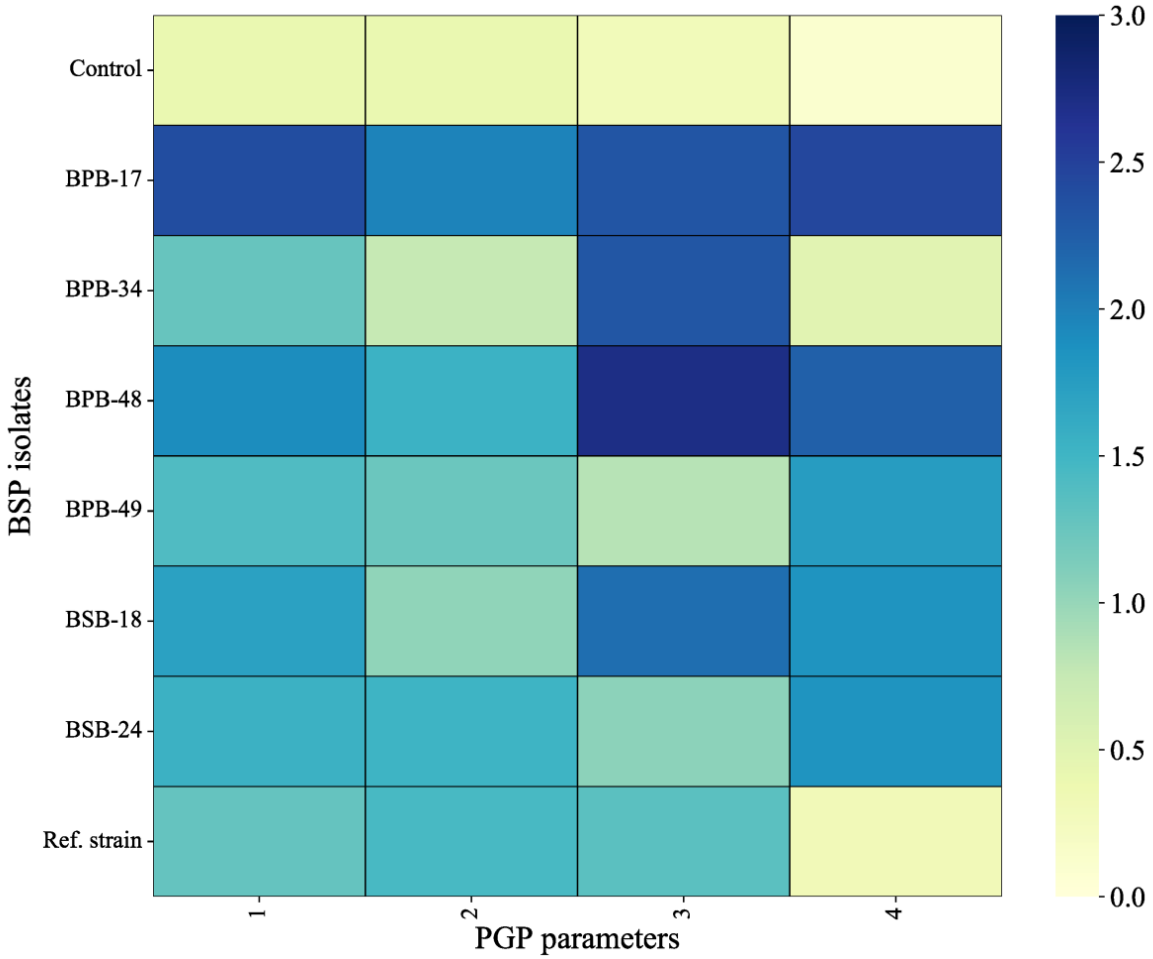


Fig. 6. Binary category plot representing qualitative test results of plant growth promoting activities of biosurfactant producing bacterial isolates.

ACC deaminase activity. The enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase is a pyridoxal phosphate dependent and inducible enzyme that cleaves the ACC, an intermediate precursor for ethylene production in plants into ammonia and α-ketoglutarate by opening the cyclopropane ring (Honma and Shimomura, 1978). ACC deaminase is an efficient marker for bacteria to improve plant growth by diminishing ethylene concentration. For many plants, an ethylene burst is necessary to break the dormancy of the seed, but once the seed has germinated, a persistent high dose of ethylene may prevent root extension. When bound to a plant root or the seed coat of a sprouting seedling, PGPB that produces the ACC deaminase enzyme may serve as a mechanism to prevent the ethylene level in the plant's tissues from rising to the point where root (or shoot) growth is compromised. The BS-producing isolates identified in the study, if found to possess this enzyme would be a welcoming trait to promote plant growth under contaminated and drought stress conditions. Therefore, all the seven isolates BPB-17, BPB-34, BPB-48, BPB-49, BSB-18 BSB-24, and *Bacillus* sp. were screened qualitatively for ACC deaminase activity on DF minimal medium with 3Mm ACC as the sole source of nitrogen. Interestingly, all the isolates could grow on ACC amended medium (Table 2; Fig 1) indicating that all the isolates used ACC sole nitrogen source. Safari *et al*. (2018) evaluated *Pseudomonas fluorescens* strains for their ability to utilize ACC, an immediate precursor of stress ethylene, using DF minimal medium containing 3 mM ACC and revealed that they were all able to use ACC as the sole nitrogen source in vitro conditions. Garcia *et al*. (2017) reported that 37 bacterial isolates obtained from 25 different rhizospheric soils were able to grow in DF minimal media amended with ACC as the sole nitrogen source.

The plant growth promoting activity (PO_4, K, Zn solubilization and ammonia production) of efficient biosurfactant producing bacterial isolates along with reference strain can be better visualised by drawing matrix heat map (Fig. 7). The values can be correlated with the colour scale *i.e* lower the value lighters the colour and higher the value darker colour the colour. From the heat map it was clear that the isolate BPB-17 and BPB-48 had higher value to all the PGP activities as compared to other isolates and siderophore production was represented by Donut map (Fig. 8).



Note: 1. Phospate solubilization, 2. Ammonia production 3. K- solubilization 4. Zn solubilization

Fig. 7. Plant growth promoting parameters influenced by inoculation of efficiant biosurfactant bacterial isolates as represented by heat map.

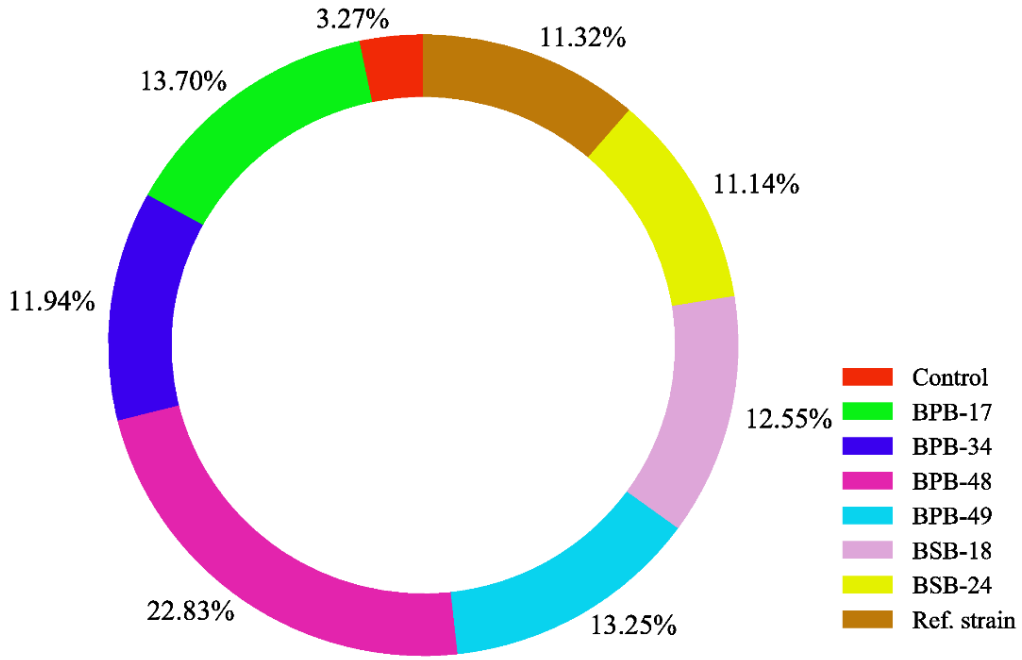


Fig. 8. Siderophore production influenced by inoculation of biosurfactant producing bacterial isolates represented by donut map.

CONCLUSION

Biosurfactants are amphiphilic compound that possesses the ability to reduce surface tension between solids, liquids, and gases interface in addition these are found to induce the production of plant growth-promoting substances. The present study illustrates the significance of biosurfactant producers screening for plant growth-promoting traits under *in vitro* conditions. These biosurfactant producers having a plant growth-promoting activity is an additional advantage for enhanced crop productivity in contaminated areas. The isolates BPB-17 and BPB-48 showed significant PGP activities and these isolates can be used for the inoculant development for enhancing crop growth and yield.

FUTURE SCOPE

The biosurfactant producing bacterial isolates having plant growth promoting activity will help in plant improvement.

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Conflict of interest. None.

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