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Evaluation of elite biosurfactant producing isolates for their plant growth promotional activity in chilli under greenhouse conditions

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Abstract
Biosurfactants are the surfactants produced from microorganisms which contain both hydrophilic and hydrophobic moeity. Green compounds such as biosurfactants were evaluated for their plant growth promotion in a pot culture under greenhouse conditions. The efficient biosurfactant and plant growth promoting isolates BPB-17 and BPB-48 were evaluated in a pot culture under greenhouse condition and compared with the one reference strain (*Bacillus* sp.) and PGP bacteria (*Azotobacter chroococcum*) at different time interval (Upto 90 days after transplanting). The efficient isolates were evaluated in order to explore the effect of biosurfactants produced from these isolates in improving the plant growth of chilli (Arka Meghana). Among all the treatments, soil inoculated with BPB-17 showed the improvement in plant growth parameters like plant height, leaf area index, number of leaves, number of branches, shoot and root biomass and yield parameters such as number of leaves, number of fruits per plant and fruit yield which is followed by treatment inoculated with BPB-48.

Keywords: Biosurfactants, PGP bacteria, growth promoters, transplanting reference strain

Introduction

Biosurfactants are a group of secondary metabolites widely produced by bacteria, yeast, actinobacteria and fungi either extracellularly into the culture broth or adhered to the cell surface from which they are released. The hydrocarbon substrate is emulsified by the production of these chemicals for the facilitation of transportation into cells. Many rhizosphere and plant-associated microbes produce biosurfactant which plays a vital role in motility, signalling and biofilm formation, indicating that biosurfactant governs plant–microbe interaction [1]. The majority of known biosurfactants are synthesized by microorganisms grown on water-immiscible hydrocarbons, but some have been produced on such water-soluble substrates as glucose, glycerol and ethanol [2]. Bacteria belonging to genera *Pseudomonas*, *Bacillus*, *Corynebacterium*, *Klebsiella*, *Acinetobacter*, *Achromobacter* and *Flavobacterium* have been reported to produce biosurfactants [3]. Biosurfactant producing microorganisms promote plant growth through improvement in plant immunity against organic contaminants in the environment, are efficient in alleviating stress responses and promote plant growth and development [4]. Biosurfactants are generally classified based on molecular weight (low or high). Glycolipids, phospholipids and lipopeptides are the most commonly reported low molecular weight; however, high molecular weight biosurfactants are composed of polysaccharides, lipopolysaccharides and a complex mixture of biopolymers [5]. These biosurfactant producing microorganisms produce variety of biosurfactant which enhance plant growth through production of different plant growth promoting traits and improvement of plant immunity against pest and diseases [6]. Furthermore, they are reported to play possible roles in alleviating stress responses in plants along with strengthening plant growth and development [7]. In this perspective, two efficient BS producing isolates such as BPB-17 and BPB-48 which showed efficient BS production potential and PGP activity under *in vitro* along with reference strain (*Bacillus* sp. MCC 4316) were further evaluated under greenhouse conditions

Materials and Methods

The two bacterial isolates able to produce biosurfactant [8] and also having plant growth promoting activity [9] isolated from hydrocarbon contaminated sites

Preparation of the potting mixture

Ten kg capacity plastic pots were filled with a sterilized mixture of soil and Farm Yard Manure (FYM) in the ratio of 2:1 and sterilized using formalin (15 mL/m^2) under sealed conditions for 3 days. After three days, the sheets were removed; the potting mixture was mixed thoroughly and dried to vaporize all the formalin. The physiochemical properties of the sterilized soil were studied and the results are presented in table 1. Fifteen days old chilli (Arka Meghana) seedlings were transferred to pots containing soil and farm yard manure.

Table 1: Initial physicochemical and chemical properties of the soil used for pot culture experiment

Parameters	Value
Physiochemical	
pH	5.92
EC(dSm^{-1})	0.52
Primary nutrients	
Available Nitrogen (kg ha^{-1})	145.61
Available Phosphorus (kg ha^{-1})	8.47
Available Potassium (kg ha^{-1})	100.66

Preparation of biosurfactant producing bacterial inoculum: Two efficient bacterial isolates were selected based on the performance of biosurfactants production and plant growth promotional activity under *in vitro* experiments and they were compared with reference strain and non-biosurfactant producing PGPR in the pot culture studies. Selected isolates were used for the development of inoculum. A loopful of bacterial cultures were inoculated into tryptic soy broth and incubated for 48 h at 30 °C. Each culture was monitored for the attainment of a population of 10^9 cells/ml of the broth. Once a sufficient population was attained, the cultures were inoculated into the pots following different application methods. For soil inoculation, the inoculum was applied directly to the soil at 0, 15 and 30 DAT

Treatment details for soil application

T_1	Control
T_2	Tryptic soy broth (TSB)
T_3	*Azotobacter chroococcum*
T_4	BPB-17
T_5	BPB-48
T_6	*Bacillus* sp. (MCC4316)

Observations recorded

Plant height: The plant height was recorded on the 30, 60 and 90 days after transplanting. The plant height was measured from the ground level to the tip of the top most leaf of the plant.

Number of leaves per plant: The total number of fully grown green leaves produced per plant was counted on the 30, 60 and 90 days after transplanting.

Number of branches: The number of branches was recorded on the 30, 60 and 90 days after transplanting.

Number of flowers: Numbers of flowers were recorded on the 60 and 90 days after transplanting.

Plant shoot and root biomass: The root and shoot dry weight of the plant was recorded 90 days after planting. The uprooted plant root and shoot portions were separated and dried separately in a hot air oven at 60 °C for 3 days to attain constant weights and then shoot and root dry weights were recorded and expressed in grams per plant.

Fruit yield: The number of fruits per plant was recorded at harvest. Values are the average of three pickings.

Results and Discussion: Green technology like microbes and metabolites produced by microbes in agriculture ensures long-term viability. One such microbe derived products is biosurfactant. These biosurfactant producing microorganisms produce variety of biosurfactant which enhance plant growth through production of different plant growth promoting traits and improvement of plant immunity. In the present study, chilli (Arka Meghana) was used as a test crop to study the effect of efficient biosurfactant producing isolates on plant growth and yield.

Influence of efficient biosurfactant producing bacteria on plant height and leaf area index (LAI) of chilli

The effect of the BS producing isolates on plant height and leaf area index was studied at 30, 60 and 90 days of transplanting (Table 2). Soil inoculation of BS producing isolates showed significantly higher plant height and leaf area index as compared to the reference strain, (*Bacillus* sp.) and *Azotobacter*. The maximum plant height of 74.26 cm was recorded by the isolate BPB-17 cm at 90th day which is significantly higher compared to all other treatments followed by isolate BPB-48 which recorded 69.46 cm. The reference and PGPB has shown 67.15 cm and 57.05 cm of plant height and LAI, respectively. The highest leaf area index (89.55 cm^2/plant was observed in treatment which was inoculated with BPB-17 at 90th day which was followed by BPB-48 which recorded 85.40 cm^2/plant at 90th day. To support this point, biosurfactants have been reported to serve a major role in plant development as these biosurfactants increases the bioavailability of nutrients for microbes which are associated with plants [10].

Table 2: Influence of soil application of efficient biosurfactant producing bacteria on plant height and leaf area index of chilli

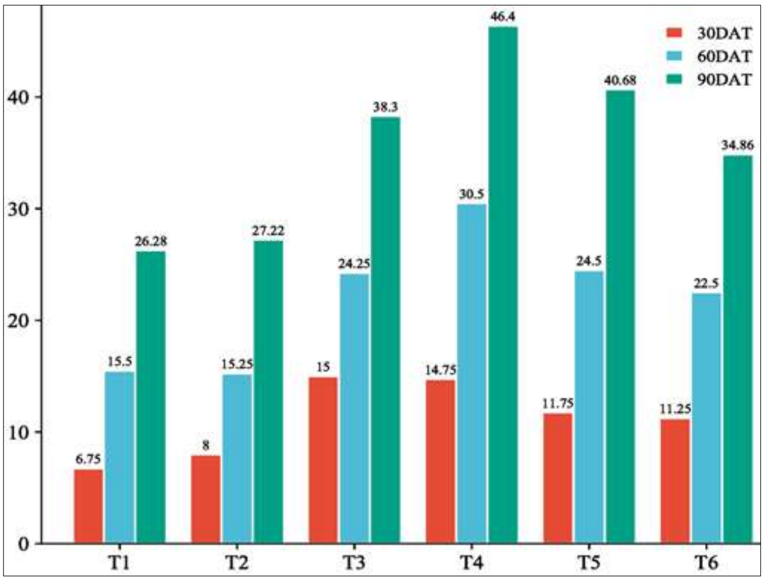
Treatments	Plant Height (cm)			Leaf Area Index (cm^2/plant)		
	30DAT	60DAT	90DAT	30DAT	60DAT	90DAT
T_1: Control	9.2^e	34.76^e	43.97^d	10.14^d	31.76^e	57.99^d
T_2: TSB	9.77^e	36.79^e	46.24^d	10.37^d	32.81^e	58.83^d
T_3: A. *chroococcum*	26.25^c	45.21^d	67.15^b	25.34^c	46.85^d	73.25^c
T_4: BPB-17	30.92^a	57.74^a	74.26^a	36.66^a	61.03^a	89.55^a
T_5: BPB-48	28.83^b	55.28^b	69.46^b	33.30^b	56.44^b	85.40^b
T_6: *Bacillus* sp. (MCC4316)	22.21^d	49.81^c	57.05^c	26.61^c	50.02^c	74.32^c

Note: Values are mean (±SE) (n=4) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p>0.05)

Influence of efficient biosurfactant producing bacteria on number of leaves and number of branches

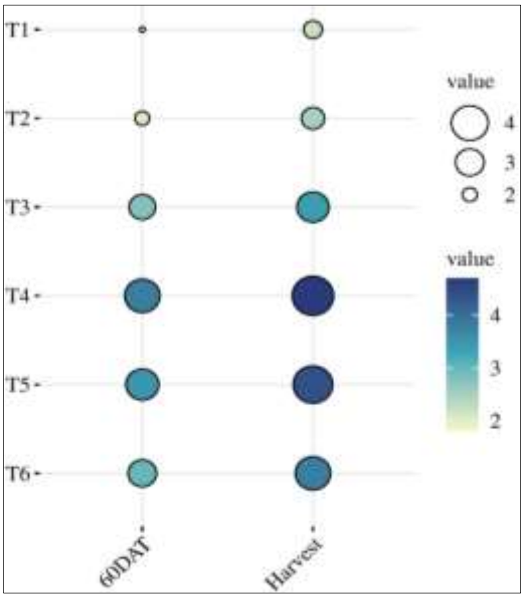
The total leaves and branches were counted to understand the influence of the isolates at 30, 60 and 90 days. Leaf number and branches gradually increased from 30 days to 90 days and results are represented in (Fig 1 and 2). In soil inoculation of BPB-17 (T_4) showed significantlly higher number of leaves and number of branches at 90th day (46.85 and 4.40, respectively). The reference strain and PGPB were inferior as compared to the BS producing isolates.

The treatment that received only TS broth showed slightly increased values as compared to control in soil application, compared to chemical treatment in foliar and soil and foliar application methods. The PGP substances and biosurfactants produced by the isolates might have had a positive influence on leaf numbers and branches as these compounds have been implicated in plant growth promotion and the organism were nitrogen fixers which might have possible supplied nitrogen plays a role in leaf growth. The findings of the present study is in accordance with [11] which reported that among the isolate examined for biosurfactant production potential *Enterobacter* sp. (A5C) was selected for *in vitro* plant microbe interaction assay based its biosurfactant production potential and this treatment showed significantlly higher number of leaves as compared to the control plant



T_1- Control; T_2-TSB; T_3- A. *chroococcum*; T_4 – BPB-17; T_5 – BPB-48; T_6- *Bacillus* sp. (MCC4316).

Fig 1: Effect of soil application of biosurfactant producing bacterial isolates on number of leaves in chilli under greenhouse conditions at diffrent days of transplanting.



T_1- Control; T_2-TSB; T_3- *A. chroococcum*; T_4 – BPB-17; T_5 – BPB-48; T_6- *Bacillus* sp. (MCC4316)

Fig 2: Ballon plot representing effect of soil application of biosurfactant producing bacterial isolates on number of branches in chilli under greenhouse conditions at diffrent days of transplanting.

Dinfluence of efficient biosurfactant producing bacteria on yield parameters of chilli

The yield parameters such as number of flowers, number of fruits and fruit yield per plant were recorded. The flowers were counted at an interval of 60 and 90 days. The number of fruits was counted after harvest and measured the weight. Soil inoculation of BPB-17 (T_4) showed significantly higher number of flowers and increased drastically from 60 days to 90 days (Table 3). The number of flowers in plant has a direct impact on number of fruits and finally the fruit yield per plant. The present study is in accordance with findings of [12] which reported the development of bioformulation using biosurfactant produced by isolate LF3 and showed that LE3 cells amended with biosurfactant had best results of plant growth promotion and yield enhancement of sunflower as compared to other treatments.

Table 3: Influence of soil application of efficient biosurfactant producing bacteria on yield parameters of chilli

Treatments	Number of Flowers		Number of Fruits per Plant	Fruit Yield (g/ Plant)
	60DAT	Harvest		
T_1: Control	0.99^e	11.30^d	12.83^f	49.23^e
T_2: TSB	1.51^d	12.12^d	17.32^e	77.73^d
T_3: *A. chroococcum*	5.30^b	16.05^c	28.22^c	98.48^c
T_4: BPB-17	9.57^a	16.05^a	47.65^a	152.87^a
T_5: BPB-48	5.30^b	17.30^b	39.08^b	142.97^b
T_6: *Bacillus* sp. (MCC4316)	4.04^c	15.59^c	20.06^d	95.45^c

Note: Values are mean (±SE) (n=4) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p>0.05).

Influence of efficient biosurfactant producing bacteria on shoot and root biomass of chilli

The fresh weight and dry weight of the shoot and root from all the treatments were calculated and results are presented in (Table 4). The results obtained were complementary to the previously observed plant physiological parameters. Soil inoculation of BPB-17 (T_4) recorded significantly higher shoot biomass (46.87 and 9.00 fresh and dry weight respectively) and root biomass (3.76 and 2.39 fresh weight and dry weight respectively) compared to all other treatment. Present study is in accordance with findings of [13] which showed effect of surfactin in plant improvement and revealed that surfactin have a positive impact on seedling germination which also indirectly helps to increase biomass.

Table 4: Influence of soil application of efficient biosurfactant producing bacteria on shoot and root biomass of chilli

Treatments	Shoot (g/plant)		Root (g/plant)	
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
T_1: Control	25.51^d	4.44^e	1.50^e	0.77^e
T_2: TSB	27.08 ^{d}	4.90^d	1.63^e	0.88^d
T_3: A. *chroococcum*	42.58^b	8.03^b	2.19^d	1.00^c
T_4: BPB-17	46.87^a	9.00^a	3.76^a	2.39^a
T_5: BPB-48	44.05^b	8.91^a	3.58^b	2.00^b
T_6: *Bacillus* sp. (MCC4316)	36.48^c	7.39^c	2.79^c	2.00^b

Note: Values are mean (±SE) (n=4) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p>0.05).

Conclusion

Soil application of biosurfactant producing bacterial isolates had a positive influence on plant growth and development. Plants inoculated with BPB-17 recorded significantly higher plant height, number of leaves, number of branches, number of flowers, plant biomass, Number of fruits per plant and fruit yield. PGPB with the ability to solubilize various nutrients in the soil might serve as an alternative to chemical fertilizers and supplements commonly used to improve nutrient content in crops.

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