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Exploration of Biosurfactant Producing Bacteria for the Management of Blast Pathogen *Pyricularia grisea* in Finger Millet (*Eleusine coracana*)

V. Mamtha and K. Tamilvendan
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : mamathavk98@gmail.com

Authors Contribution

V. Mamtha :
Conceptualization, carried out the experiment, data analysis and draft preparation

K. Tamilvendan :
Conceptualization, supervision and draft correction

Corresponding Author :
V. Mamtha

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Abstract

Finger millet (Eleusine coracana) is a staple food grain in over 25 countries across Asia and Africa. It's drought tolerance and high nutritional value are significant, but blast disease caused by Pyricularia grisea is a major challenge as it results in yield loss. Chemical fungicides, though effective in managing the blast disease but poses environmental risks. In view of the above, developing an alternative eco-friendly, cost-effective disease management strategy based on bioactive natural products assumes greater significance. Biosurfactants are one of the recently explored microbial synthesized biomolecules with antimicrobial properties. Exploiting the biosurfactants producing bacteria for the management of Pyricularia grisea in finger millet would be a viable option in the wake of health and environmental risks posed by chemical fungicides. In order to isolate biosurfactant producing bacteria, soil samples were collected from hydrocarbon contaminated areas of Bangalore (Peenya industrial area) and Raichur. Following the enrichment culture technique, a total of sixty-eight bacterial isolates were isolated. Upon rigorous in vitro screening, five bacterial isolates namely viz., BSB-2, BSB-4, BSB-11, BSB-41 and BSR-51 were selected as efficient biosurfactant producing bacteria. Bioassay of these efficient biosurfactant producing isolates against the pathogen revealed BSB-2 as an efficient biocontrol agent as it recorded highest (65.65 %) inhibition of the pathogen under in vitro condition. This biosurfactant producing bacterial isolate, was identified as Bacillus sp. GZT which would be an ideal biocontrol agent for the management of blast pathogen in finger millet which would reduce the input cost in agriculture and protect the environment.

Keywords : Biocontrol, Biosurfactant producing bacteria, *Eleusine coracana*, *Pyricularia grisea*

Finger millet [*Eleusine coracana* (L.) Gaertn.] is one of the India's oldest crops, referred as '*nrtta-kondaka*' in the ancient Indian Sanskrit literature, which means 'Dancing grain' and is also known as '*rajika*' or '*markataka*' (Achaya, 2009). Finger millet commonly known as ragi is important minor millet widely grown in Africa and Asia. Bellundagi (2016) reported that it is originally native to the Ethiopian highlands though it was introduced in India a long time ago. The earliest records of finger millet date back to around 2300 BC in Hallur, Karnataka, India (Singh, 2008).

It is the climate smart crop that are drought resistant, growing in areas with low rainfall and infertile soil. Because of this adaptability nature, finger millet has grabbed the attention of farmers. In India, finger millet is predominantly grown in Karnataka and Tamil Nadu, followed by Uttarakhand, Maharashtra, Orissa, Andhra Pradesh, Jharkhand, Gujarat, West Bengal and Bihar. Karnataka has the largest area 6.82 lakh ha (Department of Agriculture & Farmers Welfare, 2023-24) under finger millet and is the largest producer in India with 8.65 lakh Tonnes (Department of Agriculture & Farmers Welfare, 2023-24). In South

Karnataka, finger millet is a key staple food for the majority of the population. It is cultivated both as a rainfed and irrigated crop, principally by small and marginal farmers and is grown both as main and an intercrop. Within Karnataka, finger millet is primarily cultivated in the districts of Tumakuru, Ramanagara, Kolar, Mandya, Bengaluru Rural, Hassan, Chikkaballapura, Mysuru, Bengaluru Urban, Chitradurga, Chikkamagaluru, Chamarajanagar and Davanagere (Bellundagi, 2016).

Finger millet is nutritionally superior, because of its nutritional qualities and demand for consumption, 2023 is considered as International year of millets. It is challenged by wide range of fungal and bacterial diseases. The most devasting disease is blast caused by the fungal pathogen *Pyricularia grisea*. The disease is both economically significant and very destructive, the average yield loss due to finger millet blast has been reported to be around 28 per cent and has been reported as high as 80-90 per cent in endemic areas (Ramappa *et al.*, 2002 and Viswanath & Channamma, 1994). Environmental conditions of rainfall, temperature (25-30°C) and humidity (90 per cent) are the most important predisposing factors for blast severity.

In India, the blast disease was first reported from Tanjore delta of Tamil Nadu by McRae in 1920. Since then, the disease is known to occur almost every year during rainy season in all major finger millet growing areas and is perceived as one of the major disease causing recurring yield losses in all the states of India (Seetharam,1983).

Pyricularia grisea affects finger millet at all stages of plant development, from seedling to grain formation. The symptoms at the seedling stages appear as small brown spots that eventually coalesce into large elongated or spindle-shaped lesions, the centres of which develop a grayish over growth. The over growth consists of conidia and conidiospores of the fungus. Severe infections may lead to seedling death. The pathogen spreads primarily by airborne conidia and rain splash (Poonacha *et al*., 2023).

Control of blast disease is a serious and challenging issue relying heavily on chemical pesticides like organophosphorus fungicides which have been reported to be highly effective (Kumar & Kumar, 2011 and Magar *et al*., 2015). It is often unreliable and the non-judicious repeated application of fungicides also leads to the development of fungicide resistance due to high selection pressure and rapid mutation in the pathogen population (Chakraborty *et al*., 2021 and Dutta *et al*., 2018). However, extended use of chemical pesticides has resulted in the development of pesticide-resistant fungal pathogens with negative effects on the ecosystem, soil fertility and water quality, leading to serious health problems including birth defects (Hawkins *et al*., 2015 and Hollomon, 2017). Hence, globally there is a huge demand for pesticide-free food which is safe and nutritious. To mitigate these challenges, microbes and their products have been frequently utilized to enhance agricultural productivity and crop yield. Microorganisms as biocontrol agents have received increasing attention, as they are less toxic and more eco-friendly than chemical pesticides (Copping, 2004).

Novel strategies such as applying bioactive natural products against the blast fungus are required to control this economically important disease sustainably. Biosurfactants are one of the latest explored microbial synthesized biomolecules. Biosurfactants are surface-active compounds derived from various microbial sources, including bacteria, fungi, actinobacteria and algae (Naughton *et al*., 2019). Biosurfactants are microbial amphipathic compounds, which contains both hydrophilic and hydrophobic moieties. Due to the biosurfactant's ability to reduce the surface as well as interfacial tensions by interacting with boundary between two phases in a heterogeneous system by allowing it nature of surfactants allows it to absorb at the air-water or oil-water interface where, they are attached in such a way that, the hydrophobic part is in air or oil and hydrophilic part in water (Master and Markande, 2023). Biosurfactants are highly effective against a range of pathogens, making them promising for plant defense and exhibit notable antifungal properties

against various fungal phytopathogens. They disrupt membrane permeability by forming pores and ion channels in the lipid bilayer, which impacts both bacteria and fungi, leading to cell disorganization. They interact with phospholipids and sterols in fungal membranes. They are cytotoxic to fungal cells, alter cell surface charges, create ion channels, induce leakage of intracellular metabolites and compromise membrane permeability (Goswami and Deka, 2021). Consequently, they are considered as valuable alternatives in agriculture to reduce the need for chemical surfactants and fungicides. Therefore, the present study was undertaken to isolate and screen biosurfactant producing bacteria from hydrocarbon contaminated areas for the management of blast disease in finger millet.

Material and Methods

Collection of Soil Samples from Hydrocarbon Contaminated Areas

The soil samples were collected from different hydrocarbon contaminated sites of Peenya industrial area, Bengaluru (Automobile oil and lubricators contaminated soil, Petroleum contaminated soil, Agro chemicals contaminated soil, Garage contaminated soil, paint and oil contaminated soil in Peenya Industrial area, Bengaluru) and Raichur (Fly ash contaminated soil (a), Shaktinagar, Fly ash contaminated soil (b), Shaktinagar, Automobile oil and lubricators contaminated soil, Tippu sultan Road, Petroleum contaminated soil, Station Road, Raichur). A total of fifteen soil samples were collected in clean and sterile plastic bags, transported to the laboratory and stored at 4°C for further use.

Isolation of Biosurfactant Producing Bacterial Isolates by Enrichment Culture Technique

The homogenized soil sample of five gram each was suspended in a 100 mL conical flask containing 50 mL sterile water and incubated on a rotary shaker at 30°C (180 rpm) for 2 hrs, followed by allowing it to stand for 30 min at room temperature. Subsequently, supernatant of 0.5 mL was transferred to 50 mL of Luria-Bertani (LB) broth and incubated in a rotary shaker at 37 °C with shaking (180 rpm) for 24 hrs to enrich microbial population. Then 1 mL sample of the enriched medium was added to 50 mL of minimal salt medium containing 2 per cent (v/v) crude oil as the sole carbon source and incubated at 37 °C with shaking (180 rpm) for 7 days. After incubation, the samples were serially diluted up to 10^{-7}. Then, an aliquot of 100 µL from 10^{-7} dilution was spread on tryptic soy agar plates and incubated at 30 °C for 48 hrs. The colonies with distinct morphology were selected and purified by streaking on the agar plates and maintained on nutrient agar slants.

Screening of Bacterial Isolates for Biosurfactant Production

Screening of bacterial isolates for biosurfactant activity was carried out by subjecting 24 hrs old bacterial culture grown in nutrient broth for 48 hours for centrifugation at 1200 rpm for 20 minutes at 4 °C (Cooper and Goldenberg, 1987) and then subjecting cell free supernatant to oil spreading test (Morikawa *et al.,* 2000) and drop collapse test (Bodour and Miller-Maier, 1998).

Further, screening of cultures was done by following emulsification index test (Bodour *et al.,* 2004) and surface tension test (Walter *et al.,* 2010).

Characterization of Efficient Biosurfactant Producing Bacteria

Morphological Characterization

All the selected isolates were streaked on agar plates and incubated at 30 °C for 24 hrs. There after colony morphological traits *viz.,* colour, form, elevation and margin were noted. Microscopic observations were performed to investigate the Gram reaction and motility characteristics as per the standard methods (Cappuccino and Sherman, 2002).

Molecular Characterization

Extraction of Genomic DNA

Molecular characterization was done by extracting DNA. The DNA isolated was used in polymerase chain reactions for the amplification of the 16S

rRNA gene, by using universal primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT. PCR mixture prepared contained 1x Taq buffer (2 µl), dNTP mix (2µl), forward and reverse primer (0.5 µl each) template DNA (1 µl) and Taq polymerase (0.3 µl) and final volume was adjusted to 20 µl of sterile water. The PCR amplified products were subjected for agarose gel electrophoresis and after 30 minutes the gel was visualized under UV light and documented using gel documentation unit. The amplified PCR product was sequenced by Barcode Bioscience Pvt. Ltd., Bengaluru.

In vitro Biocontrol Efficacy of Biosurfactant Producing Bacterial Isolates against Blast Pathogen *Pyricularia grisea*

This was done by dual culture method and then per cent inhibition over control was calculated by using the equation given by Vincent (1947)

$$I = \frac{C-T}{T} \times 100$$

Where, I = *Per cent* inhibition,

C = Growth of fungal plant pathogen in control (mm),

T = Growth of fungal plant pathogen in dual culture plate (mm)

Siderophore Production

Siderophore production was tested using chrome Azurol's (CAS) medium as described by Schwyn and Neiland (1987) and then it was quantified by CAS-Shuttle assay. The percentage of siderophore units were calculated by the using 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

Ammonia production was tested by following the method suggested by (Cappuccino and Sherman, 1992) Further, this was quantified by measuring the absorbance using UV-visible spectrophotometer (Thermo scientific, Biomate 3S, China) at 450nm.

Statistical Analysis

The data was statistically analysed using OPSTAT statistical tool and the means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Isolation of Biosurfactant Producing Bacterial Isolates by Enrichment Culture Technique

Contaminated soil is one of the major reservoirs of biosurfactant producers. Therefore, hydrocarbon contaminated sites and other polluted areas are considered as most promising locations for isolation of biosurfactant producing organisms (Bento *et al*., 2005). In the present study, a total of 15 soil samples were collected from Bengaluru (Peenya industrial area) and Raichur (Fig 1). The collected soil samples were used for isolation of biosurfactant producing bacteria by enrichment culture technique. A total of 68 bacterial isolates were extracted in minimal medium amended with 2 Per cent (v/v) crude oil as the sole carbon source (Plate 1). These bacterial isolates were purified continuously and the pure cultures were maintained on agar-slants.

The bacterial isolates obtained from Bangalore, Peenya industrial area soil were coded from 1 to 50 prefixed with three alphabets BSB indicating biosurfactant producing bacteria isolated from Bengaluru, the isolates isolated from Raichur soil were coded from 51 to 68 prefixed with three alphabets BSR indicating biosurfactant producing bacteria isolated Raichur (Table 1).

Similar procedure has been followed by El-Gebaly (2020) who have isolated 28 bacterial isolates from oil contaminated sites near gas stations in Egypt. Alyousif *et al*. (2020) isolated bacteria from soil samples contaminated with hydrocarbon which were collected from the area around the electricity generators in Al-dair district, Basrah governorate south of Iraq. Arati and Vendan (2022) carried out an

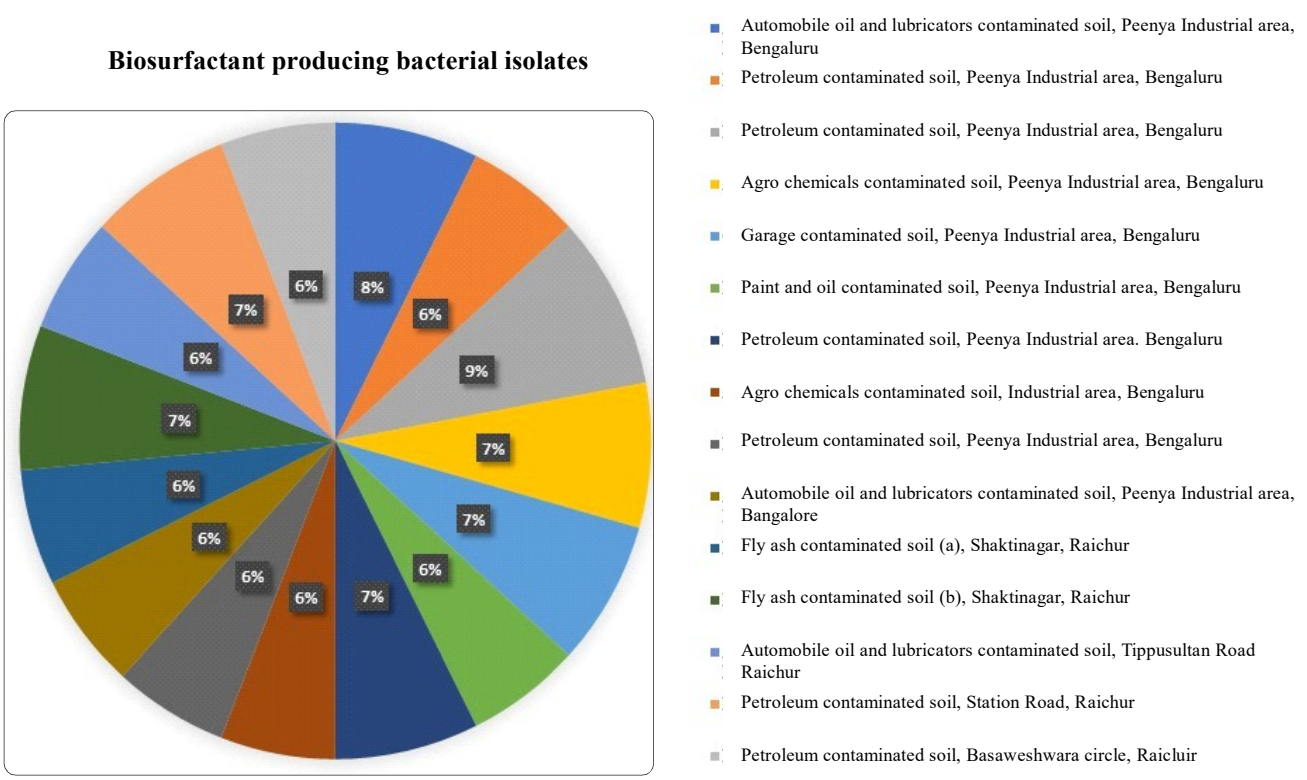

Fig. 1 : Biosurfactant producing bacterial isolates isolated from hydrocarbon contaminated soils collected from Bengaluru (Peenya industrial areas) and Raichur

Plate 1 : Biosurfactant producing bacterial isolates isolated from hydrocarbon contaminated soils on minimal media supplemented with 2% crude oil by enrichment culture technique

TABLE 1

Isolation and screening of bacteria from hydrocarbon contaminated soils for biosurfactant production potentials

Location	Isolates	Qualitative tests		Quantitative tests	
		Oil spreading test	Drop collapse test	Emulsification index test(%)	Surface tension (mN/m)
Automobile oil and lubricators contaminated soil, Peenya Industrial area, Bangalore	Control	-	-	0.00 (1.00^{p})	85.67 ^{a}
	BSB-1	+	+	43.26 (6.65ijk^{j})	67.04 ^{bcde}
	BSB-2	+	+	62.83 (7.99^{ab})	28.65 ^{z}
	BSB-3	+	+	51.66 (7.26^{defg})	55.56 ^{ghij}

Continued....

Table 1 Continued....

Location	Isolates	Qualitative tests		Quantitative tests	
		Oil spreading test	Drop collapse test	Emulsification index test(%)	Surface tension (mN/m)
	BSB-4	+	+	59.61 (7.79^{abc})	38.95 ^{wxy}
	BSB-5	+	+	50.33 (7.17^{defgh})	54.39 ^{hijk}
Petroleum contaminated soil, Peenya Industrial area, Bengaluru	BSB-6	+	+	40.00 (6.4^{kd})	62.81 ^{cdef}
	BSB-7	+	+	18.17 (4.08^{o})	61.29 ^{efg}
	BSB-8	+	+	50.04 (7.14^{defghij})	56.66 ^{ghi}
	BSB-9	+	+	43.29 (6.66^{ijk})	59.49 ^{fgh}
Petroleum contaminated soil, Peenya Industrial area, Bengaluru	BSB-10	+	+	33.39 (5.86^{m})	54.2 ^{hijkl}
	BSB-11	+	+	56.70 (7.6^{bcd})	37.18 ^{y}
	BSB-12	-	+	39.99 (6.4^{kl})	67.7 ^{bcd}
	BSB-13	-	+	43.48 (6.67^{hijk})	63.55 ^{bcdef}
	BSB-14	+	+	40.33 (6.43^{kl})	68.64 ^{bc}
	BSB-15	+	+	43.16 (6.65^{ijk})	67.16 ^{bcde}
Agro chemicals contaminated soil, Peenya Industrial area, Bengaluru	BSB-16	+	+	46.33 (6.88^{fghijk})	59.79 ^{fgh}
	BSB-17	+	+	45.33 (6.81^{ghijk})	52.89 ^{ijklm}
	BSB-18	+	+	46.51 (6.89^{ghijk})	62.69 ^{def}
	BSB-19	+	+	50.00 (7.14^{defghij})	63.82 ^{bcdef}
Garage contaminated soil, Peenya Industrial area, Bengaluru	BSB-20	+	+	53.30 (7.37^{cdef})	47.58 ^{mnopqrst}
	BSB-21	+	+	46.56 (6.9^{fghijk})	43.51 ^{rstuvwx}
	BSB-22	+	+	36.53 (6.13^{lm})	45.78 ^{opqrstuv}
	BSB-23	+	+	40.00 (6.4^{kl})	48.54 ^{klmnopqrs}
	BSB-24	+	+	40.10 (6.41^{kl})	43.3 ^{stuvwx}
Paint and oil contaminated soil, Peenya Industrial area, Bengaluru	BSB-25	+	+	40.10 (6.41^{kl})	45.4 ^{opqrstuv}
	BSB-26	-	+	43.18 (6.65^{ijk})	47.35 ^{mnopqrst}
	BSB-27	+	+	40.00 (6.40^{kl})	51.08 ^{ijklmno}
	BSB-28	+	+	39.96 (6.4^{kl})	48.89 ^{klmnopqrs}
Agro chemicals contaminated soil, Industrial area, Bengaluru	BSB-29	+	+	50.14 (7.15^{defghi})	46.98 ^{mnopqrstu}
	BSB-30	+	+	43.19 (6.65^{ijk})	52.24 ^{ijklmn}
	BSB-31	-	-	-	-
	BSB-32	+	+	26.00 (5.2n)	50.27 ^{jklmnop}
Petroleum contaminated soil, Peenya Industrial area, Bengaluru	BSB-33	-	+	39.99 (6.4^{kl})	44.59 ^{pqrstuvwx}
	BSB-34	-	+	46.56 (6.9^{fghijk})	51.05 ^{ijklmno}
	BSB-35	+	+	53.39 (7.38^{cdef})	46.76 ^{nopqrstu}
	BSB-36	+	+	640.0 (7.41^{kl})	49.44 ^{klmnopqr}
	BSB-37	-	-	-	-
Agro chemicals contaminated soil, Peenya Industrial area, Bengaluru	BSB-38	-	+	40.00 (6.4^{kl})	48.41 ^{lmnopqrs}
	BSB-39	-	-	-	-

Continued....

Table 1 Continued....

Location	Isolates	Qualitative tests		Quantitative tests	
		Oil spreading test	Drop collapse test	Emulsification index test(%)	Surface tension (mN/m)
	BSB-40	+	+	43.23 (6.65^{ijk})	44.69 ^{nopqrs}
	BSB-41	+	+	56.50 (7.58^{bcde})	38.65 ^{xy}
Petroleum contaminated soil, Peenya Industrial area, Bengaluru	BSB-42	+	-	36.43 (6.12^{lm})	46.55 ^{nopqrstu}
	BSB-43	-	-	-	-
	BSB-44	-	-	66.35 (8.21^{a})	43.87 ^{rstuvwx}
	BSB-45	+	-	46.59 (6.9^{fghijk})	44.72 ^{pqrstuvw}
	BSB-46	+	+	33.29 (5.86m)	46.81 ^{nopqrstu}
Automobile oil and lubricators contaminated soil, Peenya Industrial area, Bengaluru	BSB-47	-	-	-	-
	BSB-48	-	+	43.22 (6.65^{ijk})	50.76 ^{ijklmno}
	BSB-49	-	+	-	-
	BSB-50	+	-	33.26 (5.85^{m})	30.25 ^{z}
Fly ash contaminated soil (a), Shaktinagar, Raichur	BSR-51	+	+	53.27 (7.37^{cdef})	45.3 ^{opqrstuv}
	BSR-52	+	+	36.47 (6.12^{lm})	40.53 ^{vwxy}
	BSR-53	+	+	43.37 (6.66^{efghij})	54.25 ^{hijkl}
	BSR-54	-	-	-	-
Fly ash contaminated soil (b), Shaktinagar,	BSR-55	+	+	53.38 (7.37^{cdef})	49.91 ^{jklmnopq}
	BSR-56	+	-	50.04 (7.14^{defghij})	47.83 ^{mnopqrs}
	BSR-57	-	-	- -	
	BSR-58	+	-	46.45 (6.89^{fghijk})	41.65 ^{tuvwxy}
	BSR-59	+	+	36.40 (6.12^{lml})	43.43 ^{stuvwx}
Automobile oil and lubricators contaminated soil, Tippu sultan Road Raichur	BSR-60	+	+	43.13 (6.64^{ij})	39.9 ^{vwxy}
	BSR-61	+	+	40.20 (6.42^{kl})	59.94 ^{fgh}
	BSR-62	+	+	52.44 (5.21^{cdefg})	41.36 ^{uvwxy}
	BSR-63	+	+	49.73 (6.13^{degij})	45.29 ^{opqrstuv}
Petroleum contaminated soil, Station Road, Raichur	BSR-64	+	+	53.19 (7.36^{cdef})	64.07 ^{bcdef}
	BSR-65	+	+	50.75 (6.41$^{defg)}$	68.98 ^{b}
	BSR-66	+	+	50.07 (7.15^{defghi})	49.43 ^{klmnopqr}
	BSR-67	+	+	26.27 (5.22^{n})	41.2 ^{uvwxy}
	BSR-68	+	+	46.33 (6.88^{fghijk})	44.07 ^{qrstuvwx}

Note : BSB- Biosurfactant producing bacteria isolated from Bengaluru; BSR – Biosurfactantproducing bacteria isolated from Raichur, (+) - positive and (-) – negative; Isolates which showed negative for qualitative screening were not subjected for quantitative screening

experiment to isolate and screen potential biosurfactant producing bacteria from contaminated soils and phyllosphere of different plants grown in oil and heavy metal contaminated soils, where they have isolated 95 bacterial isolates by leaf imprint method and soil enrichment culture technique from phyllosphere and soil.

Screening of Bacterial Isolates for Biosurfactant Production

Several methods were adopted to identify potential biosurfactant producers among the isolated bacteria from hydrocarbon contaminated sites. These included qualitative and quantitative tests, which are as follows: oil spreading test, drop collapse test, emulsification index (E_{24}) and surface tension (Pandey *et al*., 2021).

The 68 bacterial isolates were screened for biosurfactant production by following oil spreading assay is a very reliable and rapid test as it needs only a small amount of sample and doesn't require any specific equipment. It was observed that 52 bacterial isolates were turned out to be efficient by oil displacement assay. The clearing zone on the crude oil surface diameter indicates the surfactant activity, which is also known as the oil displacement activity (Plate 2, Table 1). Alyousif *et al*. (2020) isolated wide variety of bacteria from soil samples contaminated with hydrocarbon which were collected from the area around the electricity generators in Al-dair district, Basrah governorate south of Iraq, these isolated bacteria were biosurfactant producers as it showned the oil displacement on oil - water surface when subjected to oil displacement test.

The drop collapse assay established earlier confide on the property of destabilizing the liquid droplets which is due to the activity of surfactants. The drop collapse assay is easy to perform as well as very sensitive. It also has several advantages like requirement of very less amount of sample, not requiring specialized equipment, being rapid and simple to perform, 55 bacterial isolates were positive for this test (Table 1, Plate 3).

Habib *et al*. (2020) screened *Rhodococcus* sp. ADL36 which produced lipopeptide, a diesel-degrading Antarctic bacterium for the capacity to produce surface-active molecules. The strain showed a positive result for drop collapse test, oil displacement activity, microplate assay, maximal emulsification index at 45 per cent and ability to reduce water surface tension.

Plate 2 : Oil spreading test confirming biosurfactant production by the bacterial isolates

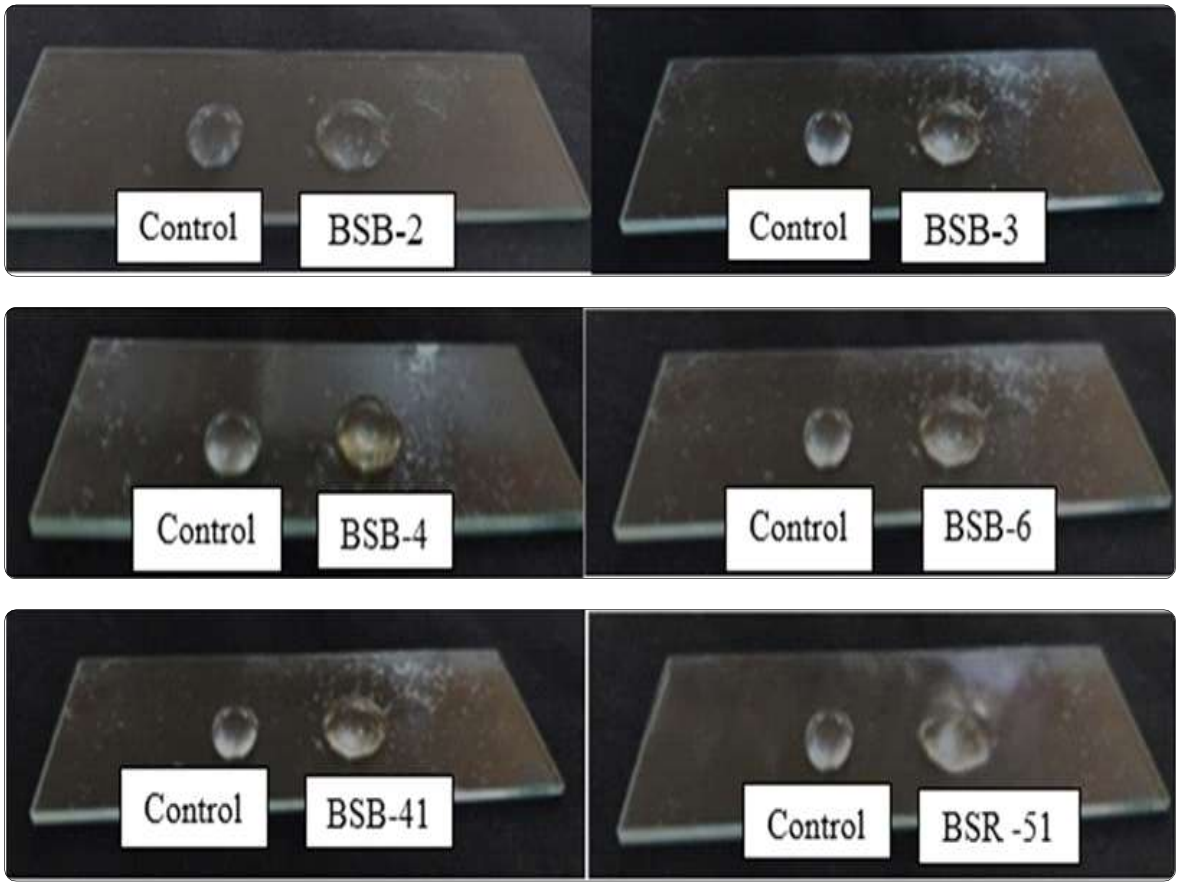

Plate 3 : Drop collapse test confirming biosurfactant production of bacterial isolates BSB and BSR

Among the 68 bacterial isolates, BSB-2, BSB-3, BSB-4, BSB-5, BSB-8, BSB-11, BSB-19, BSB-20, BSB-29, BSB-35, BSB-41, BSR-53, BSR-60, BSR-61, BSR-62, BSR-63, BSR-64 and BSR-65 showed highest emulsification index (Table 1). Variety of methods are established for measuring surface and interfacial tension like stalagmometric method, pendant drop shape technique, du-nouy-ring method and axisymmetric drop shape analysis (Walter *et al*., 2010). In case of stalagmometric method the surface tension can be determined on the basis of the number of drops which fall per volume, the density of the sample and the surface tension of a reference liquid. The isolates BSB-2, BSB-4, BSB-11, BSB-41, BSR-52, BSR-59, BSR-60, BSR-62, BSR-63 and BSR-67 showed higher surface tension (Table 1). Rani *et al*. (2020) screened bacterial isolates retrieved from stagnant water and hydrocarbon polluted soil using enrichment culture technique were screened for biosurfactant production and reported that this Bacterial isolates exhibited highest oil displacement, highest emulsification index and reduction in surface tension. The glycolipid produced by *Achromobacter kerstersii* LMG3441 from contaminated sites in an oil refinery plant showed high surface activation capacity with reduction of surface tension (Kazemzadeh *et al.,* 2020). Shaimerdenova *et al*. (2024) isolated 26 isolates from wells in the Akingen oilfield in West Kazakhstan. Stalagmometric method (Traubeg's Stalagmometer) was used to measure surface tension of isolates. Based on the morphological features, biochemical activities and the 16S rRNA gene, the isolates were classified to the *Bacillus subtilis* group. *Geobacillus stearothermophilus* DSM2313 is a bioemulsifier which was identified by surface tension and emulsification activity, surface tension was measured using stalagmometric method. (Czinkoczky and Nemeth, 2023).

Based on qualitative and quantitative screening methods for biosurfactant production isolates BSB-2, BSB-4, BSB-11, BSB-41 and BSR-51 were selected as efficient biosurfactant producers for characterization.

Characterization of Selected Biosurfactant Producing Bacteria

Bacterial isolates carry their own unique morphological characteristics. Hence, the preliminary observation of the morphological characters is the initial step for identification of bacteria.

Morphological Characterization

Five efficient biosurfactant producing bacterial isolates were selected based on their qualitative and quantitative screening methods were subjected to morphological characterization. Colonies of these five isolates were observed on nutrient agar plates for their morphological characters (Table 2). The colony colour of biosurfactant producing bacterial isolates were pale yellow and white with circular and irregular form, raised and flat elevation, surface of all the bacterial colonies were smooth except BSB-41. Further, Gram staining revealed that BSB-2, BSB-4, BSB-11, BSR-51 were gram positive and BSB-41 was Gram negative. Microscopic observation revealed that BSB-2, BSB-4, BSB-41, BSR-51 were rod shaped, whereas BSB-11 was cocci. Ja'afaru *et al*. (2022) identified the Biosurfactant producing isolates based on morphology as *Bacillus* sp. while *Staphylococcus* sp., *Micrococcus* sp, were also obtained. The best biosurfactant producer - isolate B3, appeared on nutrient agar as cream colored, small circular colonies with entire margin. It is a Gram-positive rod and an endospore former and was preliminarily identified as *Bacillus* sp.

Molecular Characterization of Selected Biosurfactant Producing Isolates

DNA of the five efficient biosurfactant producing bacterial isolates were isolated and amplified using PCR with 16S rRNA universal primers. The amplified DNA products were viewed by gel electrophoresis using 1.5 per cent agarose gel. The PCR product of 16S rRNA gene was bi-directionally sequenced using forward and reverse primer through Sanger Sequencing (Barcode biosciences, Bengaluru -77) method. To identify the five unknown isolates, the obtained sequences were checked for their nearest similarity through NCBI BLAST (Basic Local Alignment Search Tool) (Table 3). Clustal W1.6 was used to align the 16S rRNA gene sequences with the BLAST sequences John *et al*. (2020) isolated biosurfactant producing organisms from different contaminated soil, among the isolates, the potential biosurfactant- producing isolates were considered as *Bacillus* sp. by 16S rRNA characterization.

Table 2

Morphological characteristics of biosurfactant producing bacteria

Isolate code	Colony Characteristics					Gram's Reaction	Cell Shape
	Colour	Form	Elevation	Margin	Surface		
BSB-2	White	Circular	Raised	Entire	Smooth	Gram +ve	Rod
BSB-4	Yellow	Irregular	Raised	Entire	Smooth	Gram +ve	Rod
BSB-11	White	circular	Raised	Entire	Smooth	Gram ve	Cocci
BSB-41	White	Irregular	Flat	Entire	Smooth	Gram -ve	Rod
BSR-51	Pale yellow	Irregular	Raised	Entire	Rough	Gram +ve	Rod

Note : BSB-Biosurfactant producing bacteria isolated from Bengaluru; BSR-Biosurfactant producing bacteria isolated from Raichur

TABLE 3

Molecular characteristics of biosurfactant producing bacterial isolates

Isolate	Closest homology	% Similarity
BSB-2	*Bacillus* sp. GZT	99.57
BSB-4	*Bacillus* sp. A55	98.00
BSB-11	*Bacillus* sp. AG-453-K03	99.93
BSB-41	*Pseudomonas fluorescens* FC6846	98.75
BSR-51	*Bacillus paramycoides*V 37	98.00

Note : BSB-Biosurfactant producing bacteria isolated from Bengaluru; BSR-Biosurfactant producing bacteria isolated from Raichur

TABLE 4

Biocontrol efficacy of biosurfactant producing bacterial isolates

Isolate	Per cent inhibition (%)	Siderophore production (%)	Ammonia production (mg/L)
Control	0 ^{e}	13.03 ^{f}	0.00 (0.71) ^{d}
BSB-2	65.68 ^{a}	69.92 ^{a}	3.35 (1.96) ^{a}
BSB-4	65.39 ^{ab}	35.56 ^{e}	1.06 (1.25) ^{c}
BSB-11	64.53 ^{c}	40.58 ^{c}	1.34 (1.36) ^{b}
BSB-41	65.31 ^{b}	39.47 ^{d}	1.23 (1.31) ^{bc}
BSR-51	63.87 ^{d}	41.45 ^{b}	1.07 (1.25) ^{c}

Note : BSB-Biosurfactant producing bacteria isolated from Bengaluru; BSR-Biosurfactant producing bacteriaisolated from Raichur

In vitro Biocontrol Efficacy of Biosurfactant Producing Bacterial Isolates against Blast Pathogen *Pyricularia grisea*

Dual Culture Method

Biological control agents are the most preferred natural choice to manage plant diseases that can enhances crop yields by growth promoting attributes of environment friendly microorganisms. Now a days, microbial biosurfactants are gaining very much importance in plant protection. These biosurfactants are surface active compounds produced by a variety of microorganisms and their mode of action, in biological control involves the formation of channels in the cell wall and perturbations of the cell surface of the pathogen (Raaijmakers *et al*., 2006). Primary screening of biocontrol agents is usually done by dual culture method where in per cent inhibition is judged by zone of inhibition. However, these methods are good only for those biocontrol agents, which produces antifungal antibiotics or hydrolytic enzymes. In the recent years, there are several reports on the production of volatile organic compounds like HCN, ammonia and others (Bahroun *et al.,* 2018) which also need to be considered for screening.

Among five isolates BSB-2, showed highest inhibition against blast pathogen *P. grisea* followed by BSB-4, BSB-41, BSR-51 and BSB-11 (Table 4). Kumar *et al*. (2021) found that biosurfactant producing bacterium *Bacillus cereus* BS14 inhibited the fungal growth under *in vitro* condition by arresting radially growing mycelia against *Macrophomina phaseolina* in *Vigna mungo* Reddy and Shivaprakash (2018) was carried out *in vitro* experiment to study the efficiency of endophytic bacteria isolated from millets for biocontrol of *Rhizoctonia solani*. *Kuhn*, a causal organism of sheath blight in millets. Results obtained showed the isolates obtained from small millets, inhibited mycelial growth of *Rhizoctonia solani* compared to control which received only pathogen. Kumar and Naik (2015) evaluated biocontrol activity of bioagents *Trichoderma viride, Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* against wilt pathogen *Fusarium oxysporum* f. sp. *ricini* under *in vitro* condition. *Trichodermaviride* fungal bioagent recorded the maximum inhibition of 92.35 per cent, followed by bacterial bioagent *Bacillus subtilis* (88.75%). *Trichoderma viride* (92.35%) and *Bacillus subtilis* (88.75%) recorded the maximum inhibition of *Fusarium* wilt pathogen, compared to all the bioagents.

Siderophore Production

Siderophores are high affinity iron chelating compounds which help to chelate few elements essential for plants. It is one of the vital mechanisms

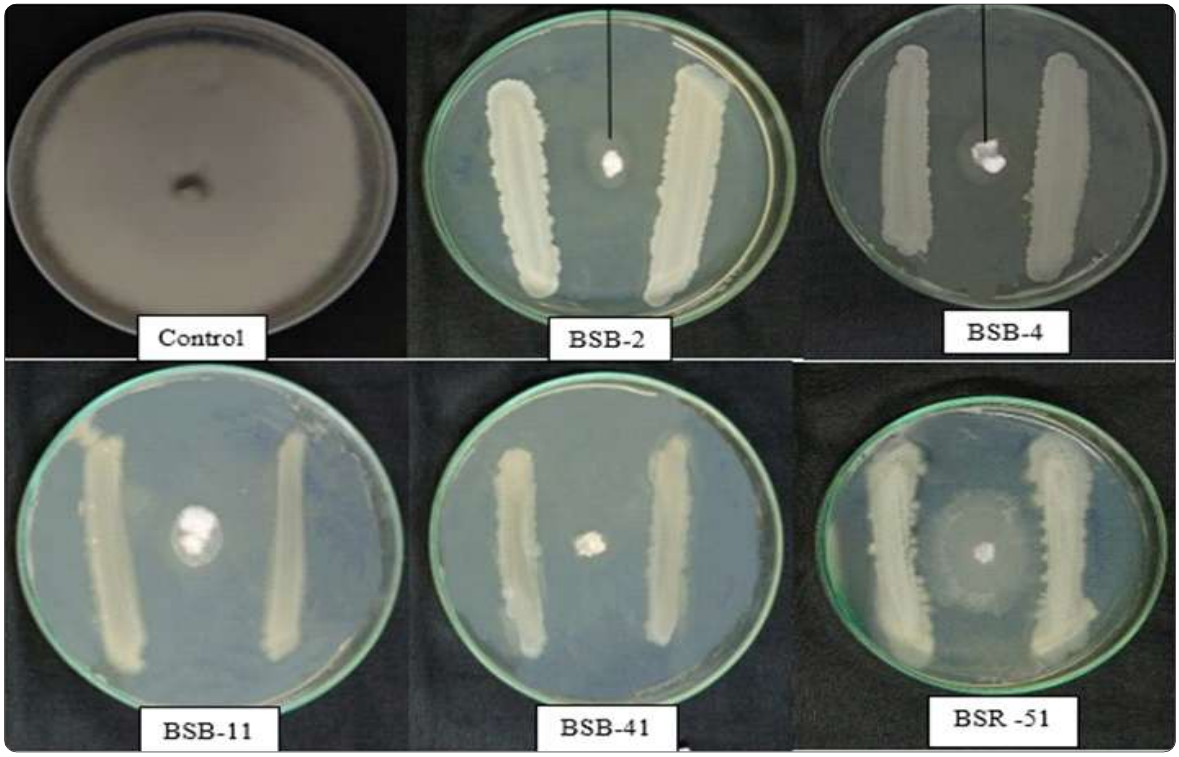

Plate 4 : Inhibition of blast pathogen *(P. grisea)* by biosurfactant producing bacterial isolates in dual culture method

for disease suppression and plant growth promotion (Sarwar *et al*., 2020). In order to evaluate siderophore production capacity of biosurfactant producing isolates, all the five efficient biosurfactant producing isolates were screened for their *in vitro* siderophore production on CAS agar plates Taking into account the formation of orange-colored halo zone around the bacterial colonies as positive for siderophore production, all the five isolates were identified to be siderophore producers (Table 4). After preliminary confirmation of siderophore producing ability of the isolates, siderophore production potentials of the isolates were estimated quantitatively using nutrient broth supplemented with CAS reagent. The quantification results of siderophore revealed that, percentage of siderophore produced by the biosurfactant producing bacterial isolates ranged from 35.56 to 69.92 per cent.

Highest siderophore production was observed in BSB-2 which recorded 69.92 per cent followed by BSR-51 with 41.45 per cent, BSB-11 with 40.58 per cent, BSB-41 with 39.47 per cent BSB-4 with 35.56 per cent and control recorded 13.03 per cent siderophore production. Bacteria produce a wide array of siderophores, such as bacillibactin, pyochelin, pyoverdine and petrobactin (Miljakovie *et al*., 2020). Siderophores play an important role in biological control, making Fe unavailable to soil-borne pathogens. Siderophores produced by *Bacillus* spp. and other biocontrol agents have a much higher affinity for iron than the siderophores produced by plant pathogens (Saha *et al*., 2013). The present study is in accordance with Ghosh *et al*. (2015) who evaluated siderophore production both qualitatively and quantitatively for three bacterial isolate *Bacillus subtilis*, *B. megatericus* and *Pseudomonas aeruginosa.* All the bacterial isolates were positive for qualitative assay. However, quantitative assay of *Pseudomonas aeruginosa* yielded highest siderophore (80.50 %) in broth containing CAS dye.

Ammonia Production

The ammonia production potential of biosurfactant producing isolates was evaluated on peptone broth

(Table 4). The quantitative estimation of ammonia production revealed that the ammonia production ranged from 1.06 to 3.35 mg/L, BSB-2 recorded highest ammonia production of 3.35 mg/L followed by BSB-11 with 1.34 mg/L, BSB-41 with 1.23 mg/L ammonia production, BSR -51 with 1.07 mg/L ammonia production and BSB-4 with 1.06 mg/L ammonia production and in control no colour development was observed upon addition of Nessler's reagent in peptone broth. The results of current investigation are in accordance with Chrouqi *et al*. (2017) in which all the eight bacterial isolates subjected to ammonia production test were able to produce ammonia and highest value of 2.07µg/ml was measured for strains S48 and S54.

The present study documented that the biosurfactant producing bacterial isolate BSB-2 performed better than other isolates in inhibiting the blast pathogen *P. grisea*. This isolate could be recommended as a bioagent for commercial purpose.

References

Achaya, K. T., 2009, *The illustrated foods of India: A-Z*. Oxford University Press.

Alyousif, N. A., Al-tamimi, W. H. and Al-luaibi, Y. Y., 2020, Screening, enhance production and characterization of biosurfactant produced by *Pseudomonasaeru ginosa* isolated from hydrocarbon contaminated soil. *Eurasia. J. Biosci.,* **14** (2) : 4377 - 4391.

Arati and Vendan, T., 2022, Isolation and screening of potential biosurfactant producing bacteria from phyllosphere and soils of different contaminated areas. *Mysore J. Agric. Sci*., **56** (4) : 31 - 43.

Bahroun, A., Jousset, A., Mhamdi, R., Mrabet, M. and Mhadhbi, H., 2018, Anti-fungal activity of bacterial endophytes associated with legumes against *Fusariumsolani*: Assessment of fungi soil suppressiveness and plant protection induction. *Appl. Soil Ecol.*, **124** : 131 - 140.

Bellundagi, V., Umesh, K. B. and Ravi, S. C., 2016, Growth dynamics and forecasting of finger millet (Ragi) production in Karnataka. *Econ. Aff.*, **61** (2) : 195 - 201.

Bento, F. M., Camargo, F. A. O., Okeke, B. C. and Frankenberger, W. T., 2005, Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. *Microbiol. Res*., **160** : 249 - 255.

Bodour, A. A., Guerrero-Barajas, C., Jiorle, B. V., Malcomson, M. E., Paull, A. K., Somogyi, A., Trinh, L. N., Bates, R. B. and Maier, R. M., 2004, Structure and characterization of flavolipids, a novel class of biosurfactants produced by *Flavobacterium* sp. Strain MTN11. *Appl. Environ. Microbiol.,* **70** : 114 - 120.

Bodour, A. A. and Miller-Maier, R. M., 1998, Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant producing microorganisms. *J. Microbiol. Methods,* **32** : 273 - 280.

Cappuccino, J. G. and Sherman, N., 1992, Microbiology: A laboratory manual. Pearson Education.

Cappuccino, J. G. and Sherman, N., 2002, Microbiology Laboratory Manual, 4th Edition.

Chakrabortya, M., Mahmuda, N. U., Ullahb, C., Rahman, M. and Islam, T., 2021, Biological and biorational management of blast diseases in cereals caused by *Magnaporthe oryzae*. *Crit. Rev. Biotechnol*., **41** (7) : 994 - 1022.

Chrouqi, L., Lahcen, O., Jadrane, I., Koussa, T. and Alfeddy, M. N., 2017, Screening of soil rhizobacteria isolated from wheat plants grown in the Marrakech region (Morocco, North Africa) for plant growth promoting activities. *JMES*, **8** : 3382 - 3390.

Cooper, D. G. and Goldenberg, B. G., 1987, Surface-active agents from two *Bacillus* species. *Appl. Environ. Microbiol.*, **53** (2) : 224 - 229.

Copping, L. G., 2004, The manual of biocontrol agents. *British Crop Protection Council*, pp. : 702.

Czinkoczky, R. and Nemeth, A., 2023, Modeling the biosurfactant fermentation by *Geobacillus stearothermophilus* DSM2313. *Period. Polytech. Chem. Eng.*, **67** (1) : 104 - 115.

DUTTA, S., ZAHAN, S., GUPTA, D. R., MAHMUDA, N., CHANCLUD, E., WIN, J., KAMOUN, S. AND ISLAM, T., 2018, Genomic analyses reveal that biocontrol of wheat blast by *Bacillus* spp. may be linked with production of antimicrobial compounds and induced systemic resistance in host plants. Fig share, pp. : 17 - 18.

EL-GEBALY, E., 2020, Screening of biosurfactant production by bacterial strains isolated from oil contaminated sites near gas stations in Egypt. *Al-Azhar J. Pharm. Sci.,* **61** (1) : 134 - 146.

GHOSH, S. K., PAL, S. U. J. O. Y. AND CHAKRABORTY, N. I. L. O.Y., 2015, The qualitative and quantitative assay of siderophore production by some microorganisms and effect of different media on its production. *Int. J. Chem. Sci.*, **13** (4) : 1621 - 1629.

GOSWAMI, M. AND DEKA, S., 2021, Biosurfactant mediated biocontrol of pathogenic microbes of crop plants. *Biosurfactants for a sustainable future: production and applications in the environment and biomedicine*. Wiley Online Library, **5** : 491 - 509.

HABIB, S., AHMAD, S. A., WAN JOHARI, W. L., ABD SHUKOR, M. Y., ALIAS, S. A., SMYKLA, J., SARUNI, N. H., ABDUL RAZAK, N. S. AND YASID, N. A., 2020, Production of lipopeptide biosurfactant by a hydrocarbon-degrading Antarctic *Rhodococcus*. *Int. J. Mol. Sci*., **21** (17) : 6138.

HAWKINS, L. K., MYLROIE, J. E., OLIVEIRA, D. A., SMITH, J. S., OZKAN, S., WINDHAM, G. L., WILLIAMS, W. P. AND WARBURTON, M. L., 2015, Characterization of the maize chitinase genes and their effect on *Aspergillus flavus* and aflatoxin accumulation resistance. *PloSOne*, **10** (6) : 0126185.

HOLLOMON, J. M., 2017, *The biology of morphology: Insight into regulation of Candida albicans hyphal growth by pH and Cdk8*. Dartmouth College.

JA'AFARU, M. I., ABBAS, T., AJUNW, O. M. AND OLAIFA, K., 2022, Characterization and statistical optimization of biosurfactant production using *Bacillus subtilis* isolated from automotive oil-contaminated soil in Yola, Nigeria. *Sci. Afr.,* **17** : e01357.

JOHN, W. C., OGBONNA, I. O., GBERIKON, G. M. AND IHEUKWUMERE, C. C., 2020, Screening and characterization of biosurfactant-producing *Bacillus* species isolated from contaminated soils in Makurdi Metropolis. *Niger J. Biotechnol.*, **37** (2) : 165 - 176.

KAZEMZADEH, S., NAGHAVI, N. S., EMAMI-KARVANI, Z., EMTIAZI, G. AND FOULADGAR, M., 2020, Production of glycolipid biosurfactant during crude oil degradation by the novel indigenous isolated *Achromobacter kerstersii* LMG3441. *Water Sci. Technol.,* **82** (10) : 2134 - 2147.

KUMAR, B. AND KUMAR, J., 2011, Management of blast disease of finger millet (*Eleusine coracana*) through fungicides, bioagents and varietal mixture. *Indian Phytopathol.*, **64** (3) : 272.

KUMAR, J. A. AND NAIK, L. K., 2015, *In vitro* evaluation of biocontrol agents against isolated wilt pathogen of castor (*Ricinus communis* L.). *Mysore J. Agric. Sci.,* **49** (2) : 221 - 223.

KUMAR, S., DHEEMAN, S., DUBEY, R. C., MAHESHWARI, D. K. AND BALIYAN, N., 2021, Cyclic siloxane biosurfactant-producing *Bacillus cereus* BS14 biocontrols charcoal rot pathogen *Macrophomina phaseolina* and induces growth promotion in *Vigna mungo* L. *Arch. Microbiol.*, **203** (8) : 5043 - 5054.

MAGAR, P. B., ACHARYA, B. AND PANDEY, B., 2015, Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at Jyotinagar, Chitwan, Nepal. *Int. J. Appl. Sci. Biotechnol.*, **3** (3) : 474 - 478.

MASTER, N. G. AND MARKANDE, A. R., 2023, Importance of microbial amphiphiles: interaction potential of biosurfactants, amyloids and other exo-polymeric-substances. *World J. Microbiol. Biotechnol.*, **39** (11) : 320.

MCRAE, W., 1920, Detailed administration report of the government mycologist for the year. Imperial Institute of Agriculture Research, Pusa.

MILJAKOVIĘ, D., MARINKOVIĘ, J. AND BALE EVIĘ-TUBIĘ, S., 2020, The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*, **8** (7) : 1037.

MORIKAWA, M., HIRATA, Y. AND IMANAKA, T., 2000, A study on the structure–function relationship of lipopeptide biosurfactants. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids,* **1488** : 211 - 218.

NAUGHTON, P. J., MARCHANT, R., NAUGHTON, V. AND BANAT, I. M., 2019, Microbial biosurfactants: Current trends and applications in agricultural and biomedical industries. *J. Appl. Microbiol.*, **127** (1) : 12 - 28.

PANDEY, R., SHARMA, P., RATHEE, S., SINGH, H. P., BATISH, D. R., KRISHNAMURTHY, B. AND KOHLI, R. K., 2021, Isolation and characterization of a novel hydrocarbonoclastic and biosurfactant producing bacterial strain: *Fictibacillus phosphorivorans* RP3. *Biotech.*, **11** (2) : 105.

POONACHA, T. T., BHAVANA, C. S., RAMESH, G. V., GAVAYI, N., KOTI, P. S., PALANNA, K. B., RAJASHEKARA, H., RAJESH, G. AND DAS, I. K., 2023, Blast disease of millets: Present status and future perspectives. *Millets-Rediscover Ancient Grains*.6: https://doi.org/10.5772/intechopen.111392

RAAIJMAKERS, J. M., BRUIJN AND KOCK, 2006, Cyclic lipopeptide production by plant-associated *Pseudomonas* species: diversity, activity, biosynthesis and regulation. *Mol. Plant Microbe Interact.*, **19** : 699 - 710.

RAMAPPA, H. K., RAVISHANKAR, C. R. AND PRAKASH, P., 2002, Estimation of yield loss and management blast disease in finger millet (ragi). *Proc. Asian Cong. Mycol. Pl. Pathol.*, **1** (4) : 195.

RANI, K., SANGWAN, S., SINGH, S., SHARMA, P., KAUR, H. AND SINDHU, M., 2020, Biosurfactant production by bacteria retrieved from hydrocarbon polluted environment. *Int. J. Chem. Stud.*, **8** (4) : 1059 - 1065.

REDDY, M. R. AND SHIVAPRAKASH, M. K., 2018, Antagonistic activity of bacterial endophytes isolated from millets against *Rhizoctonia solani* in foxtail millet. *Mysore J. Agric. Sci.,* **52** (2) : 241 - 247.

SAHA, R., SAHA, N., DONOFRIO, R. S. AND BESTERVELT, L. L., 2013, Microbial siderophores: A mini review. *J. Basic Microbiol.*, **53** (4) : 303 - 317.

SAMBROOK, J., MANIATIS, T. AND FRITSCH, E., 1989, Molecular cloning. NY: Cold Spring Harbor Laboratory. New York, USA.

SARWAR, S., KHALIQ, A., YOUSRA, M., SULTAN, T., AHMAD, N. AND KHAN, M. Z., 2020, Screening of siderophore-producing PGPRs isolated from groundnut (*Arachis hypogaea* L.) rhizosphere and their influence on iron release in soil. *Commun. Soil Sci. Plant Anal.*, **51** (12) : 1680 - 1692.

SCHWYN, B. AND NEILANDS, J. B., 1987, Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem*., **160** : 47 - 56.

SEETHARAM, A., 1983, Identification of sources of resistance to ragi blast and its utilization in breeding. Plants for resistance to pests and diseases. National seminar on breeding crop plants for resistance to pests and diseases, Coimbatore, Tamil Nadu, India, pp. : 30 - 31.

SHAIMERDENOVA, U., KAIYRMANOVA, G., LEWANDOWSKA, W., BARTOSZEWICZ, M., SWIECICKA, I. AND YERNAZAROVA, A., 2024, Biosurfactant and biopolymer producing microorganisms from West Kazakhstan oilfield. *Sci. Rep.*, **14** (1) : 2294 - 2299.

SINGH, P., 2008, History of millet cultivation in India. History of science, philosophy and culture in Indian civilisation, **5** (1) : 107 - 119.

VINCENT, J. M., 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nat.,* **159** (4051) : 850.

VISWANATH, S. AND CHANNAMMA, L, K. A., 1994, Production technology for ragi and other small millets. *National level specialised orientation Training Programme*, pp. : 5 - 7.

WALTER, V., SYLDATK, C. AND HAUSMANN, R., 2010, Screening concepts for the isolation of biosurfactant producing microorganisms. *Biosurfactants*, **12** : 1 - 13.