Indian Journal of Experimental Biology
Vol. 60, December 2022, pp. 931-938
DOI: 10.56042/ijeb.v60i12.58955

Benchmarking of different microbes for their biosurfactants antifungal action against plant pathogens

Khem Raj Meena[1,4]*, Satyam[1], Ashutosh Singh[2], Aman Jaiswal[1] & Dinesh Rai[3]

[1]Department of Microbiology; [2]Center for Advanced Studies on Climate Change,[3]Department of Plant Pathology; Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar – 848 124, India

[4]Department of Biotechnology, School of Life Sciences, Central University of Rajasthan, Kishangarh, Rajasthan, India-305817

Received 06 January 2021; *revised 18 October 2022*

The biotic stress caused by phytopathogens (bacteria, fungus, yeast and insect pests) is a primary factor in yield loss of plants. Biocontrol agents and their active compounds are used to manage such plant pathogens. Here, in our study, we screened four bacterial isolates identified as *Bacillus cereus*, *B. anthracis*, *B. velezensis* and *Serratia marcescens* after morphological, biochemical and molecular characterization (16s rDNA sequencing) for production of biosurfactant by foam forming activity, oil spreading tests and emulsification activity. Highest foam stability (75 min) and maximum emulsification activity E24% (75%) was observed by *B. velezensis* strain. Among all the four isolates, *Bacillus velezensis* strain produced maximum biosurfactant (0.349±0.004 g/50 mL). Biosurfactant of all the four bacterial isolates were checked for fungal inhibiton on PDA plate(s). *Bacillus velezensis* showed comparatively the highest percent inhibition 58.82, 88.15, 78.45,72.68, 83.96, 75.47, 68.07 and 88.44% against *Colletotrichum falcatum*, *Fusarium oxysporum* f sp. *ciceri*, *Helminthosporium maydis*, *F. oxysporum* f. sp. *lycopersici*, *Aspergillus niger*, *Mucor* sp., *Helminthosporium oryzae* and *Rhizoctonia solani*, respectively. *Bacillus velezensis* biosurfactant among all the four bacterial isolates was found to be most effective against the tested phytopathogens.

Keywords: Antifungal activity, *Bacillus velezensis,* Biosurfactants, Biotic stress, Emulsification activity, Foam forming activity, Oil spreading test

Biosurfactants are important low molecular weight amphiphilic and microbial originated bioactive products produced by bacteria, yeast and fungi and have attracted a great attention in the recent years due to medical, industrial environmental, pharmaceuticals applications and use in the industrial processes such as foaming, emulsification, detergency, solubilization and wetting properties[1-3]. Amphipathic substances with hydrophilic tail and hydrophobic head ends are termed as biosurfactants[4]. These biosurfactants have certain unique properties that make different them from chemical surfactants and make them a preferable choice for utilization in various formulation developments and aggregation studies due to their more advantages *i.e.* minimum toxicity, high biodegradability, good environmental compatibility, lesser critical micelle concentration (CMC), synthesized from renewable resources, high selectivity,high foaming forming abilty, target specific activity at extent temperature, salinity and pH[1,4,5].

Biosurfactants can also be formed by the microbial fermentation processes utilizing cheaper agricultural based substrates and their crop residues. Chemically synthesized surfactant agents are generally non-biodegradable and toxic, and therefore biosurfactants increase more emphasis as they are ecofriendly and also biodegradable[6]. Continuously mounting biosurfactant production and reducing costs of production are the main factors which play a key role affecting the efficiency of biosurfactant production[7,8]. Biosurfactants seem to depend mainly on the use of huge and low cost substrates to optimize cultivation condition(s), which can increase the yield, largely[9-11]. Researchers have paid considerable attention towards isolation and characterization of biosurfactant(s) produced by extremophiles such as asthermophilic and halophilic bacteria[12-14]. Epiphytic and endophytic microbes are also involved in biosurfactant production that helps in seed germination[15].

Biosurfactants also inhibit the action of phytopathogens and help in bioremediation by acting as biocontrol agents. Since the use of pesticides on plants should be avoided to for obtaining the chemical

*Correspondence:
E-Mail: khemrajmeena88@gmail.com

free plant products; rhizospheric bacteria have been explored by researchers that secrete metabolites which increase the yield of plant products[16]. The bacterial biosurfactants which are rich source of antimicrobial peptides can be used for biological control of fungal phytopathogens. *Bacillus* is considered as a factory at large scale production of the secondary metabolite like molecules which are capble of inhbiting the growth of phytopathogens. The microbial biosurfactant molecules like lipopeptides (LPs) act as a potent multipurpose weapons to contract with a plenty of phytopathogens for their growth inhibition. Biosurfactants molecules are less toxic, biodegradable and effectiv at extreme or adverse temperatures, resistant to change at different pH values and salinities, make them an alternative to their chemical(s) used in different applications, including in agriculturefield, food as well as in bioremediation[17,18]. In this context, here, we investigated selected bacterial isolates in inhibiting the phytopathogens viz. *Colletotrichum falcatum*, *Fusarium oxysporum* f sp. *ciceri*, *Helminthosporium maydis*, *F. oxysporum* f. sp. *lycopersici*, *Aspergillus niger*, *Mucor* sp., *Helminthosporium oryzae* and *Rhizoctonia solani*, etc. to reduce the loss of the crops yield.

Materials and Methods

Morphological and biochemical tests for isolated microbial isolates

Bacterial isolates (colony) were identified on the basis of morphological tests (Gram's staining), biochemical tests (methyl red, voges Proskauer's, indole, etc.).

Molecular characterization of bacterial isolate(s)

For identification of the bacterial isolates T1, T2, T3 and T4 which showed biosurfactant and antifungal activity, we used 16S rDNA analysis[19].

Screening of bacterial isolates for biosurfactant production

Foam forming activity

All 12 different combinations of 4 microbial isolates (PDBT1, NBT1, LBT1, PDBT2, NBT2, LBT2, PDBT3, NBT3, LBT3, PDBT4, NBT4 and LBT4) were grown separately in 100 mL of potato dextrose broth, nutrient broth and Luria bertani broth. The flasks were then incubated at 37°C on a shaker incubator at 150 rpm for 96 h. After that the foam activity was detected as duration of foam height, foam stability and foam shape[20].

Emulsification activity (E_{24})

Organic hydrocarbons (benzene, toluene, hexane and diesel oil) and cell-free broth of 4 different bacteria in different media (PDBT1, NBT1, LBT1, PDBT2, NBT2, LBT2, PDBT3, NBT3, LBT3, PDBT4, NBT4 and LBT4) each 2.5 mL were inoculated in test tubes and homogenized by vortexing for 2 min at a high speed. After 24 h, the emulsification activity (E24%) was measured using following formula[21]:

$$E_{24}\ (\%) = \frac{\text{Total height of emulsified layer (mm)}}{\text{Total height of liquid layer (mm)}} \times 100$$

Oil spreading test

Hundred microliter of diesel oil was added to 40 mL distilled water in a Petri dish to form a thin oil layer. Thirty µL each of 4 different bacterial culture(s) [T1, T2, T3 and T4] supernatant (PDBT1, NBT1, LBT1, PDBT2, NBT2, LBT2, PDBT3, NBT3, LBT3, PDBT4, NBT4 and LBT4) were then added on it. Results were observed and recorded accordingly[22].

Antifungal test against selected phytopathogens *Fusarium*, *Aspergillus* and *Mucor*sp.

The antifungal activity of the T1, T2, T3 and T4 bacteria was detected by performing dual culture plate's techniques[23]. Small loopful cultures of respective fungal isolates such as *Fusarium* sp., *Helminthosporium oryzae*, *Helminthosporium maydis*, *Colletotrichum falcatum*, *Aspergillus niger*, *Rhizoctonia solani* and *Mucor* sp. were placed at the centre of PDA Petri plate with the help of loop. The mycelial plug of fungal strains was placed at the centre of the Petri plate and the bacteria streaked at periphery of the plates. The plates were then incubated in BOD Incubator at temperature of 30°C being observed at an interval of 24 h and after 4 days, the percentage inhibition of mycelial growth was calculated using this formula:

$$I = 100\ (C - T) / C$$

where I = Percentage inhibition of mycelial growth, C = Growth of pathogen (fungus) in control plate (mm) and T = Growth of pathogen (mm) in dual cultures in tested condition.

Extraction of biosurfactant from selected bacterial isolates

Inoculum of 5% (v/v) and 1 O.D. cells of T1, T2, T3 and T4 bacteria were placed to freshly prepared Potato Dextrose Broth, Nutrient broth and Luria bertani broth and then kept in shaking incubator at 37°C for 72 h. The culture broth(s) were centrifuged at 10,000 rpm for 10 min at 4°C to obtain a cell-free supernatant[24]. The pH of the supernatant was adjusted to 5.0 using 6N HCl. The white precipitate pellet was separated by centrifugation at 10,000 rpm for 10 min at 4°C. The precipitate was then extracted with methanol (500 µL).

Antifungal activity of crude biosurfactant against selected fungal strains

The antifungal activity was detected by well diffusion method[25]. Small mycelium of fungal isolates of *Fusarium* sp, *Helminthosporium oryzae*, *Helminthosporium maydis*, *Colletotrichum fulcatum*, *Aspergillus niger* and *Mucor* sp. were placed at the centre of Petri plate with the help of loop and the biosurfactants crude preparation (50 µL) of four bacterium isolates like T1, T2, T3 and T4 were placed in the well made by cork borer away from the pathogen at four place(s) in a triangular fashion in the Petri plate. Plates incubated at 30°C and percentage inhibition was calculated by the above formula.

Results

Isolation of microbial isolates

Four microbial isolates (T1, T2, T3 and T4) were isolated from rhizosphere soil samples and all these strains were purified by repeating sub cultured in the petri plates containing Nutrient agar (pH 7.0; Fig. 1) and further preserved at 4°C for further identification

Morphological and biochemical characterization of bacterial isolates

Morphological and biochemical characteristics

Bacterial isolate(s) T1, T2 and T3 were found as Gram +ve, purple rod shaped bacterial cells while T4 was found as Gram –ve bacteria with small rod shaped pink color cells under the microscopes at 1000X (Fig. 2 and Table 1). The methyl red, oxidase and glucose utilization test were found +ve for T1 and in T2 one more test vogesproskauer test was positive while other tests were observed –ve. Indole, voges proskauers, rhamnose and sucrose tests were positive while other test found –ve for T3 isolate. Indole, methyl red, citrate utilization and adonitol test were +ve while others were found –ve for T4 bacterial isolate.

Molecular characterization of bacteria isolates by 16s rDNA sequencing

On the basis of sequence homology with the help of RDP (Ribosomal Database Project) and phylogenetic analysis, the bacterial isolate T1, T2, T3 and T4 identified as *Bacillus cereus*,

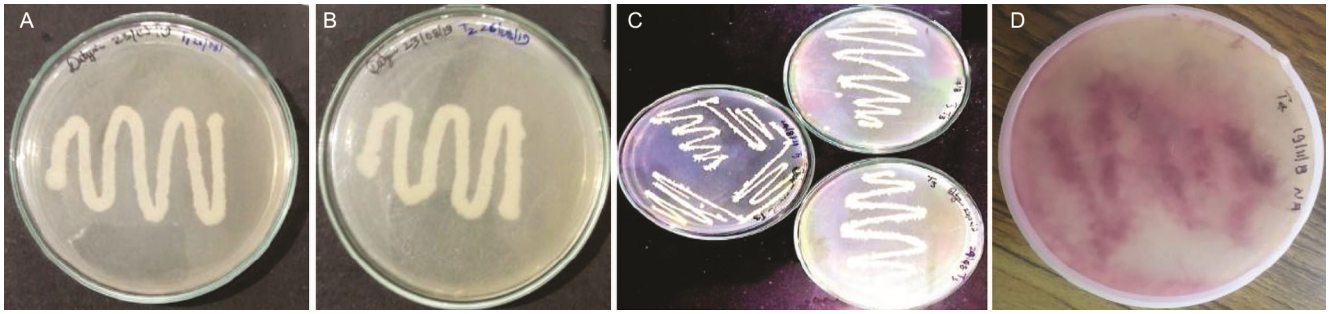

Fig. 1 — Bacterial cultures on Petri plates. (A-D) Bacterial isolates T1, T2, T3 and T4, respectively on nutrient agar plate.

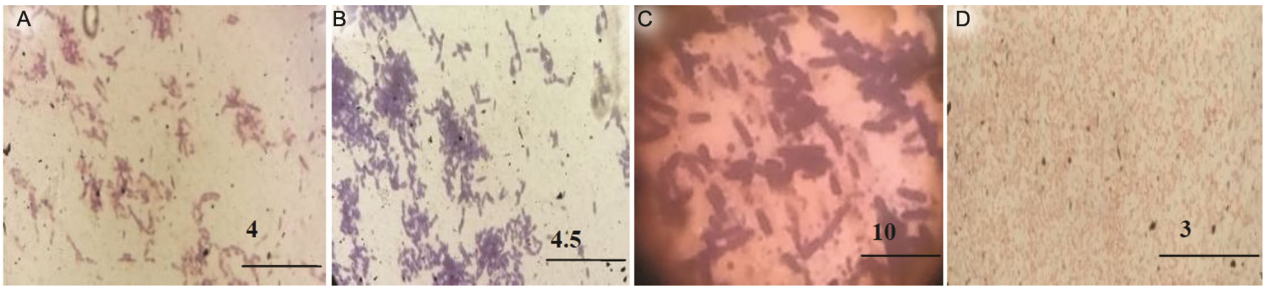

Fig. 2 — Gram's staining images of four [T1, T2, T3 and T4 bacterial isolates (A-D, respectively)]. (A & C) purple coloured rod shaped cells depict Gram +ve nature of bacterium; (B) purple blue coloured rod shaped cells depicted Gram +ve nature of bacterium; and (D) pink coloured short rod shaped cells depict Gram –ve nature of bacterium.

Table 1 — Morphological characteristic of bacterial isolates

Bacterial Isolate	Colony colour	Cell shape	Colony shpae	Gram's staining
T1	purple	Rod shaped	Streptobacilli	+ve
T2	Purple blue	Rod shaped	Streptobacilli	+ve
T3	purple	Rod shaped	Streptobacilli	+ve
T4	Pink	Short rod shaped	Staphylobacillus	–ve

Bacillus anthracis, *Bacillus velezensis* and *Serratia marcescens*, respectively (Fig. 3).

Foam forming activity

Highest foam stability (75 min) was observed for *B. velezensis* in potato dextrose broth (Table 2).

Emusifification activity of bacteria in with hydrocarbons

The maximum emulsification activity E24% (75%) was obserbved in the case of *Bacillus velezensis* with PDB (Table 3).

Oil spreading test

The highest zone of clearance (28.73 mm) against diesel oil was observed by *Bacillus velezensis* cell-free broth with LB broth and lest zone of clearance (11.27 mm) was observed by *B. cereus* in LB broth.

Antifungal screening of bacterial strains against *Fusarium*, *Aspergillus* and *Mucor* sp

Bacillus velezensis was found to be the most effective and it showed 70-80% inhibition against the *Fusarium oxysporum* spp., *Helminthosporium oryzae*,

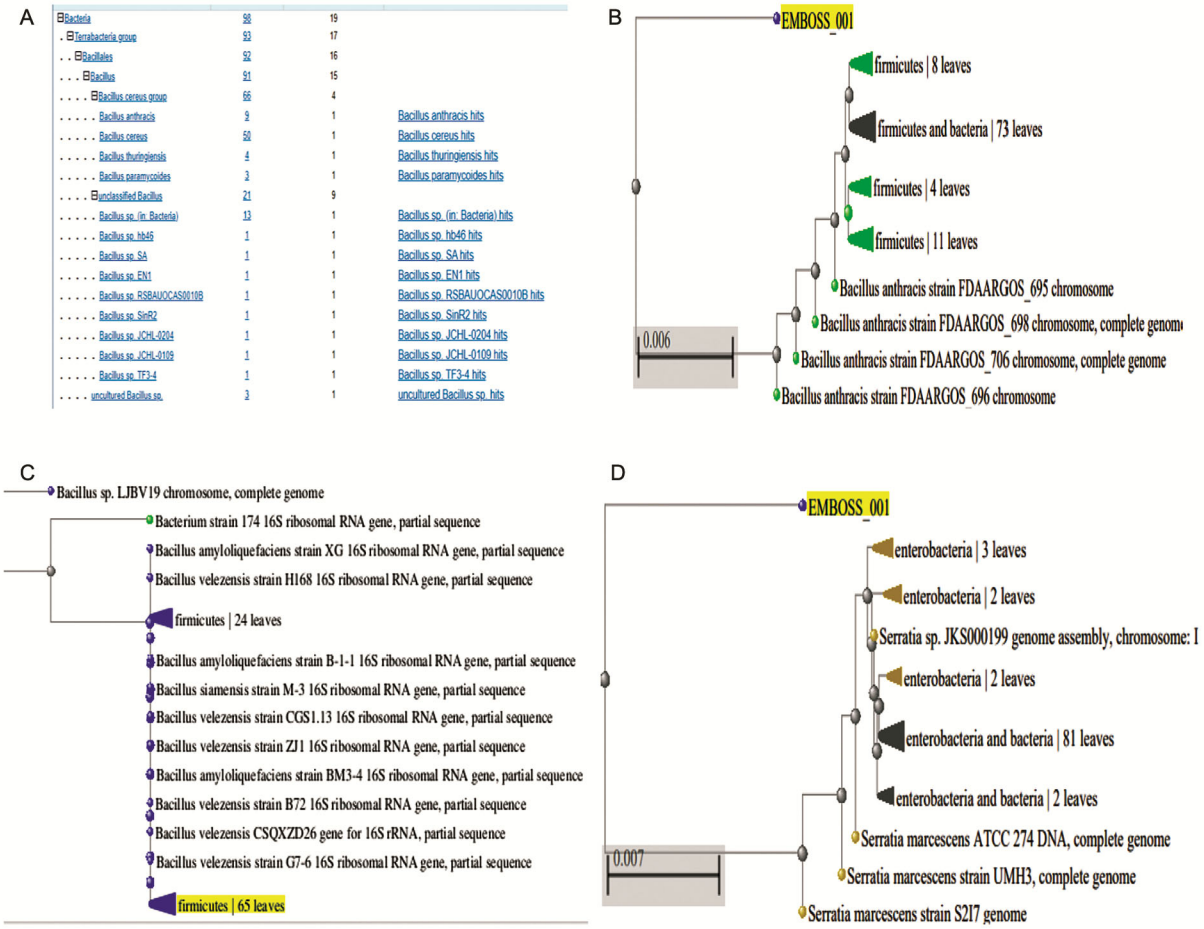

Fig. 3 — (A) Ribosomal Database project (RDP) of islate T1 confirms *Bacillus cereus* strain; (B) Nucleotide BLAST data of bacterial isolate T2 confirms *B. anthracis*; and (C) distrance tree result of bacterial isolate T3 confirms *Bacillus velezensis* and distrance phylogenetic tree of isolate T4 confirms *Serratia marcescens*.

Table 2 — Foam forming activity of four bacterial isolates

Bacteria	Nutrient Broth			Potato Dextrose Broth			Luria Bertani Broth		
	Foam height (mm)	Foam stability (min)	Foam properties	Foam height (mm)	Foam stability (min)	Foam properties	Foam height (mm)	Foam stability (min)	Foam properties
Bacillus cereus	1.017±0.009	50	+	2.077±0.038	60	++	2.047±0.026	66	++
B. anthracis	2.023±0.019	61	++	5.030±0.017	72	+++	3.018±0.009	69	++
B. velezensis	5.020±0.012	70	++	5.090±0.059	72	+++	6.177±0.034	75	+++
Serratia marcescens	6.133± 0.067	73	+++	3.160± 0.021	64	++	3.107± 0.007	59	++

Table 3 — Emulsification index (E_{24}) of cell-free broth of bacteriadifferent hydrocarbons

Bacteria	Media	E_{24}(%)±SD of cell-free broth of different bacteria with hydrocarbons				Stability (days)
		Benzene	Toluene	Hexane	Diesel oil	
Bacillus cereus	NB	60.0±0.58	52.15±1.14	No emulsification activity	5±0.14	1
	LB	65.166±0.44	62.5±1.20		17.75±0.14	1
	PDB	52.58±0.65	40.90±0.66		7.5±0.14	1
B. anthracis	NB	62.67±0.44	54.23±2.34		5±0.14	1
	LB	63.75±0.43	63.88±1.38		16.58±0.16	1
	PDB	52.58±0.50	52.08±1.80		5.33±0.22	1
B. velezensis	NB	57.5±0.28	41.66±2.40		12.5±0.14	2
	LB	72.41±0.30	54.16±0.23		12.75±0.14	2
	PDB	75.0±0.29	8.40±0.30		2.91±0.41	2
Serratia marcescens	NB	73.17±1.90	63.88±1.83		20.5±0.14	1
	LB	72.5±2.88	64.58±1.20		20.25±0.14	1
	PDB	70.17±0.44	66.66±1.20		2.58±0.08	1

Table 4 — Antifungal test against the selected phytopathogens like *Fusarium*, *Aspergillus* and *Mucor* in Petri plate by co-culture method

Name of diseases	Name of phytopathogen	Control	*Bacillus cereus*	*Bacillus anthracis*	*Bacillus velezensis*	*Serratia marcescens*
Tomato vascular wilt	*Fusarium oxysporum* f. sp. *lycopersici*	-	+++	++++	++++	+
Brown spot disease in rice	*Helminthosporium oryzae*	-	-	-	+++	+
Fusarium wilt of chickpea	*Fusarium oxysporum* f sp. *ciceri*	-	-	-	++++	+
Mucor rot	*Mucor* sp.	-	-	-	++++	+
Rice sheath blight	*Rhizoctonia solani*	-	_	+	++++	_
Black mould of onion	*Aspergillus niger*	-	-	+	++++	-
Red Rot of Sugarcane	*Colletotrichumfalcatum*	-	-	-	++	+
Maydis Leaf Blight Disease of Maize	*Helminthossporium maydis*	-	++	++	++++	+

[++++ represents 70-80 % inhibition of phytopathogens by bacteria. +++ represents 60-70% inhibition, ++ represents 50-60% inhibition, + represents 1-50% inhibition and – indicates no inhibition]

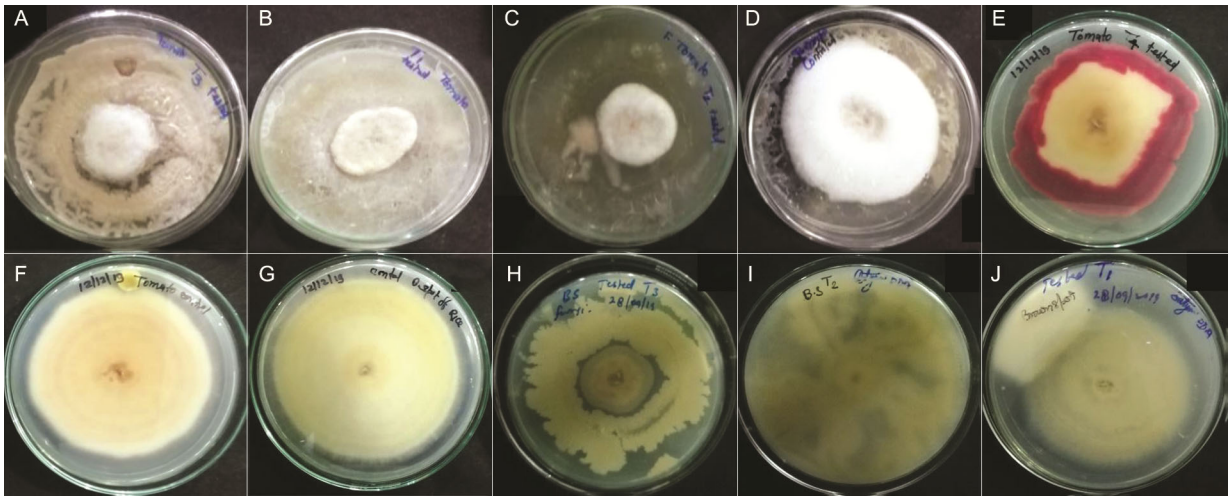

Fig. 4 — Inhibiton of *Fusarim oxysporum f.* sp. *lycopersici*. with *B. velezensis*, *B. cereus*, *B. anthracis*, respectively (A-C) while (D) is control or placebo of *Fusarim oxysporum f.* sp. *lycopersici*; (E) tested; and (F) control pictures show inhibiton of *Fusarim oxysporum f.* sp. *lycopersici* by *Serratia*; (G) Control represents only fungus *Helminthosporium oryzae*; and (H-J) Petri plates depict inhibitory action against *Helminthosporium oryzae* inoculated with *Bacillus velezensis*, *B. cereus* and *B. anthracis*, respectively.

Mucor sp., *Rhizoctonia solani*, *Aspergilus niger*, *Helminthosporium maydis* (Table 4 and Fig. 4).

Antifungal activity of crude biosurfactant against phytopathogens

Bacillus cereus, *B. anthracis*, *B. velezensis* and *Serratia marcescens* were tested for their antifungal activity using crude biosurfactant. Here also, the *Bacillus velezensis* bacteria was most effective inhibiting the growth of the *Fusarium oxysporum* f sp. *ciceri* (88.15%) and *Aspergillus niger* (83.96 %) and *Mucor* sp. (75.47 %) (Table 5).

Table 5 — Antifungal activity of crude biosurfactant against the selected fungal strains

Treatment	% inhibition											
	Colletotrichum falcatum		*Fusarium oxysporum* f sp. *ciceri*		*Helminthossporium maydis*		*Aspergillus niger*		*Mucor* sp.		*Helminthosporium oryzae*	
	Diameter ± S.E (cm)	% Inhibitn.	Diameter ± S.E (cm)	% Inhibitn.	Diameter ± S.E (cm)	% Inhibitn.	Diameter ± S.E (cm)	% Inhibitn.	Diameter ± S.E (cm)	% Inhibitn.	Diameter ± S.E (cm)	% Inhibitn.
Control	5.1±0.06	0	8.967±0.03	0	7.1±0.06	0	8.833±0.167	0	8.967±0.033	0	7.1±0.06	0
Bacillus cereus	5.133 ±0.09	-0.65	8.963 ±0.037	0.05	3.5 ±0.06	50.70	8.833 ±0.167	0	8.933 ±0.067	0.38	7.1 ±0.06	0
Bacillus anthracis	5.2 ±0.06	-1.96	8.967 ±0.03	0	3.43 ±0.07	51.69	5.867 ±0.186	33.58	8.9 ±0.058	0.75	7.1 ±0.06	0
Bacillus velezensis	2.1 ±0.06	58.82	1.063 ±0.03	88.15	1.53 ±0.01	78.45	1.417 ±0.044	83.96	2.2 ±0.058	75.47	2.27 ±0.09	68.07
Serratiamarcescens	3.2 ±0.12	37.25	5.063 ±0.03	43.54	3.97 ±0.03	44.08	8.833 ±0.133	-0.38	8.3 ±0.153	7.44	4.2 ±0.15	40.84
C.D.	0.252		0.107		0.158		0.473		0.269		0.29	
SE(m)	0.079		0.033		0.05		0.148		0.084		0.091	
SE(d)	0.112		0.047		0.07		0.209		0.119		0.128	
C.V.	3.295		0.877		2.199		3.794		1.958		2.829	

Discussion

Biosurfactants are important bioactive secondary metabolite molecules produced by yeast, bacteria and some filamentous fungi in exponential growth phase or on starting of stationary phase and have versatile applications, and therefore, these compound have extended extensive interest in the latest previous years that have develop an important product of the biotechnology for industrial, medical and agricultural purposes[1,2]. On the basis of the Gram staining and 16s rDNA sequencing followed by RDP (Ribosomal Database Project) the bacterial isolates T1, T2, T3 and T4 were identified as *Bacillus cereus*, *B. anthracis*, *B. velezensis* and *Serratia marcescens*, respectively. *Bacillus velezensis* bacteria showed the highest foam height (6.177±0.034 mm) and the minimum foam height (2.047±0.026 mm) was observed *Bacillus cereus* in Luria Bertani broth. Out of 160 strains, only 11 strains had foaming activity with foam stability 30-135 min which was lesser than our research results. The bacterium *Serratia marcescens* showed maximum foam forming activity in nutrient broth (6.133±0.067 mm) and minimum in Luria Bertani (3.107±0.007 mm) and medium in potato dextrose broth (3.160±0.021 mm). In our study, *Bacillus cereus* showed maximum emulsification activity (E24%; 65.166±0.44%) in Luria Bertani broth with benzene while lowest E24% in potato dextrose broth was 52.58±0.65% in benzene. In the case of hexane, no emulsification activity (E24%) was observed in any broth media by bacterial isolates *Bacillus cereus*, *B. anthracis*, *B. velezensis* and *Serratia marcescens.* This may be due to the complex structure of hexane compared to other hydrocarbon tested. Previous study reports that out of 13 strain only 4 strain showed E24% activity (CQ1-45.7±1.17, CQ2- 61.5±1.07, CQ4 -56.8±0.53, CQ13 -52.4±2.16) and these strain were only biosurfactant producers[24]. E_{24} of CQ2 was the highest among the four strains, which could reach up to 61.5±1.07%. He used E24% for secondary screening for biosurfactants producer.

In our study, *Bacillus cereus* showed zone of clearance of 15.96±0.013 mm, 16.16±0.003 mm and 11.27±0.009 mm in nutrient broth, potato dextrose broth and in Luria Bertani broth, respectively. *Bacillus velezensis* showed 28.73±0.015 mm zone of clearance in Luria Bertani, 27.4±0.031 mm in nutrient broth and 27.78±0.048 mm zone in potato dextrose broth. Zone of clearance size was possibly due to biosurfactants concentration present in supernatant of respective bacterial isolates. Concentration of biosurfactants in the broth may decide the size of zone of clearance which might be due to its exceptionally high hydrophile-lipophileintractions.Similar results for *Bacillus subtilis* PL2015 produceed optimal lipopeptide yield of 547 mg/L at 7 pH and maximum production by *B. subtilis* KLP2015 observed at 30ºC (545 mg/L)[27]. It was found that production of biosurfactants 2.0 g/L for *Brevibacterium* 7G and 2.5 g/L for *Ochrobactrum* 1C[28]. Production of biosurfactants for bacterial strains *Rhodococcus* sp. NJ2 and *Pseudomonas* sp. BP10 were 0.01 g/L and 0.05 g/L, respectively[29].

Seven bacterial isolates, *Alcaligenes faecalis* S18 and *B. cereus* S42 were most effective in reducing yellowing and wilt symptoms by 94 and 88% and the vascular browning by 95 to 97.5%, respectively as compared to untreated control and FOL-inoculated[30].

In greenhouse experiments, egg plants treated with *Bacillus* isolates (EC4, EC13), *Pseudomonas* isolates (EB67, EB9) and *Enterobacter* isolates (EB44, EB89) decreased the incidence of wilt by more than 70%. In our study, Inhibition of different phytopathogens by biosurfactants of *Bacillus velezensis* was *Fusarium solani* was 68.07%, *Colletotrichum falcatum* (58.82%), *Fusarium oxysporum* f sp. *ciceri* (88.15%), *Helminthosporium maydis* (78.45%), *Fusarium oxysporum* f. sp. *lycopersici* (72.68%), *Aspergillus niger* (83.96%), *Mucor piriformis* (75.47%), *Helminthosporium oryzae* (68.07%) and *Rhizoctonia solani* (88.44%). This activity of percentage inhibition of phytopathogens by bacterial biosurfactants might be due to amphiphilic nature of biosurfactant produced by *Bacillus cereus*, *B. anthracis*, *B. velezensis* and *Serratia marcescens* which binds with the fungal cell membrane by hydrophobic intractions and damage the cell membrane. Similar research of % inhibition against *Mucor* sp. and *Aspergillus niger* by application of lipopeptides produce by *Bacillus subtilis* PL2015 and found (75.1%) and (41.9%) % inhibition for *Mucor*spp and *A. niger*, respectively[27].

Conclusion

Isolated bacteria were identified to be *Bacillus cereus*, *B. anthracis*, *B. velezensis* and *Serratia marcescens* after morphological, biochemical and 16s rDNA sequencing. All four bacterial isolates were screened for biosurfactant production by foam forming activity, oil spreading tests and emulsification activity. *Bacillus velezensis* was found to produce maximum biosurfactant (0.349±0.004 g/50 mL). Biosurfactant of all four bacterial isolates checked for fungal inhibiton on PDA plate. *Bacillus velezensis* showed percent inhibition of 68.07, 58.82, 88.15, 78.45,72.68, 83.96, 75.47, 68.07 and 88.44% against *Fusarium solani*, *Colletotrichum falcatum*, *Fusarium oxysporum f.* sp. *ciceri*, *Helminthosporium maydis*, *F. oxysporum f.* sp. *lycopersici*, *Aspergillus niger*, *Mucor piriformis*, *Helminthosporium oryzae* and *Rhizoctonia solani*, respectively. Our results suggest *Bacillus velezensis* biosurfactant to be most effective against tested phytopathogens.

Acknowledgement

All authors are very thankful to Department of Microbiology and College of Basic Sciences, RPCAU, Pusa for provdindg facilities and space to conduct experiments.

Conflicts of interest

Authors declare no competing interests.

References

1 Nitschke M & Costa SGVAO, Biosurfactants in food industry. *Trends Food Sci Technol*, 18 (2007) 252.
2 Makkar RS, Cameotra SS & Banat IM, Advances in utilization of renewable substrates for bio-surfactant production. *AMB Exp*, 1 (2011) 5.
3 Rodrigues L, Banat IM, Teixeira J & Oliveira R, Biosurfactants: potential applications in medicine. *J Antimicrob Chemother*, 57 (2006) 609.
4 Abbot V, Paliwal D, Sharma A & Sharma P, A review on the physicochemical and biological applications of biosurfactants in biotechnology and pharmaceuticals. *Heliyon*, 8 (2022) e10149.
5 Chen SY, Wei YH & Chang JS, Repeated pH-stat fed-batch fermentation for rhamnolipid production with indigenous *Pseudomonas aeruginosa* S2. *Appl Microbiol Biotechnol*, 76 (2007) 67.
6 Kumar V & Saravanan V, Bio-surfactants-types, sources and applications. *Res J Microbiol*, 10 (2015) 181.
7 Kosaric N, Biosurfactants in industry. *Pure Appl Chem*, 64 (1992) 1731.
8 Bognolo G, Biosurfactants as emulsifying agents for hydrocarbons. *Colloids Surf A*: *Physicochem and Eng Asp*, 152 (1999) 41.
9 Mukherjee S, Das P & Sen R, Towards commercial production of microbial surfactants. *Trends Biotechnol*, 24 (2006) 509.
10 Mukherjee S, Das P, Sivapathasekaran C & Sen R, Enhanced production of biosurfactant by a marine bacterium on statistical screening of nutritional parameters. *Biochem Eng J*, 42 (2008) 254.
11 Mutalik SR, Vaidya B, Joshi RM, Desai M & Nene SN, Use of response surface optimization for the production of biosurfactant from *Rhodococcus*spp MTCC 2574. *Bioresour Technol*, 99 (2008)7875.
12 Kumar AS, Mody K & Jha B, Evaluation of biosurfactant/bioemulsifier production by a marine bacterium. *Bull Environ Contam Toxicol*, 79 (2007) 617.
13 Joshi S, Bharucha C, Jha S, Yadav S, Nerurkar A & Desai AJ, Biosurfactant production using molasses and whey under thermophilic conditions. *Bioresour Technol*, 99 (2008) 195.
14 Mnif S, Chamkha M & Sayadi S, Isolation and characterization of *Halomonas* sp. strain C2SS100, a hydrocarbon-degrading bacterium under hyper saline conditions. *J Appl Microbiol*, 107 (2009) 785.
15 Saleh D, Jarry J, Rani M, Aliferis KA, Seguin P & Jabaji SH, Diversity, distribution and multi-functional attributes of bacterial communities associated with the rhizosphere and endosphere of timothy (*Phleumpratense* L.). *J Appl Microbiol*, 127 (2019) 794.
16 Taiwo LB, Oyedele AO, Ailenokhuoria BV & Okareh OT, Plant–microbes relationships in soil ecological system and benefits accruable to food health. In: *Field Crops: Sustainable management by PGPR*. (Eds. Maheshwari DK & Dheeman S; Springer International Publishing, Cham.), 2019, pp. 177.
17 Marchant R & Banat IM, Biosurfactants: a sustainable replacement for chemical surfactants. *Biotechnol Lett*, 34 (2012) 1597.

18 Mishra N, Agsar D, Deepthi SS, Kattegouda UM, Shetty PR & Pandey V, Novel application of Nerium leaf and Image J software in drop collapse assay for rapid screening of biosurfactant producing microorganisms. *Indian J Exp Biol*, 59 (2021) 484.

19 Tamura K, Dudley J, Nei M & Kumar S, MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol*, 24 (2007) 1596.

20 Chayabutra C, Wu J & Ju LK, Rhamnolipid production by *Pseudomonas aeruginosa*under denitrification: Effects of limiting nutrients and carbon substrates. *Biotechnol Bioeng*, 72 (2001) 25.

21 Lai CC, Huang YC, Wei YH & Chang JS, Biosurfactant enhanced removal of total petroleum hydrocarbons from contaminated soil. *J Hazard Mater*, 167 (2009) 609.

22 Plaza GA, Zjawiony I & Banat IM, Use of different methods for detection of thermophilic biosurfactants producing bacteria from hydrocarbon contaminated bio remediated soils. *J Pet Sci Eng*, 50 (2006) 71.

23 Jasim B, Sreelakshmi S, Mathew J & Radhakrishnan E, Surfactin, iturin, & fengycin biosynthesis by endophytic *Bacillus* sp. from *Bacopamonnieri*. *Microb Ecol*, 7 (2016) 106.

24 Gover M, Nain L, Singh SB & AK, Molecular and biochemical approaches for characterization of antifungal trait of potent biocontrol agent *Bacillus subtilis* RP24. *Curr Microbiol*, 60 (2010) 99.

25 Balouiri M, Sadiki M & Ibnsouda SK, Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal*, 6 (2016) 71.

26 Sun W, Cao W, Jiang M, Saren G, Liu J, Cao J, Ali I, Yu X, Peng C & Naz I, Isolation and characterization of biosurfactant-producing and diesel oil degrading *Pseudomonas* sp. CQ2 from Changqing oil field, China. *RSC Adv*, 8 (2018) 39710.

27 Meena KR, Sharma A, Kumar R & Kanwar SS, Two factors at a time approach by response surface methodology to aggrandize the *Bacillus subtilis* KLP2015 Surfactin to use as antifungal agent. *J King Saud Univ Sci*, 32 (2018) 337.

28 Ferhat S, Mnif S, Badis A, Eddouaouda K, Alouaoui R, Boucherit A, Mhiri N, Moulai-Mostefa, N & Sayadi S, Screening and preliminary characterization of biosurfactants produced by *Ochrobactrum* sp. 1C and *Brevibacterium* sp. 7G isolated from hydrocarbon-contaminated soils. *Int Biodeterior Biodegradation*, 65 (2011) 1182.

29 Kumari B, Singh SN & Singh DP, Characterization of two bio-surfactant producing strains in crude oil degradation. *Process Biochem*, 47 (2012) 2463.

30 Abdallah, RAB, Mokni-Tlili S, Nefzi A, Jabnoun-Khiareddine H & Daami-Remadi M, Biocontrol of *Fusarium* wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Nicotianaglauca* organs. *Biol Control*, 97 (2016) 80.