International Scholars Journals

Advanced Journal of Environmental Science and Technology ISSN 2756-3251, Vol. 15 (9), pp. 001-004, September, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Marine Bacteria as a Source of Biosurfactants: Isolation and Characterization

Seema Dhail[1]* and Nakuleshwar Dut Jasuja[2]

[1]Laboratory of Microbiology, Department of Biotechnology, International College for Girls, Rajasthan University, Jaipur-302004, India.

[2]Department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur- 303007, India.

Accepted 6 June, 2024

Biosurfactant-producing marine bacteria were isolated from oil-spilled seawater collected from harbors and docks in Mumbai, India. Haemolytic activity, emulsification activity toward n-hexadecane, emulsion of mixtures of oils, drop collapsing test as well as oil displacement test were used to determine biosurfactant producing activity of marine bacteria. Among 25 strains, 20 different strains showed biosurfactant activity in which only 6 strains had haemolytic activity and were able to emulsify mixtures of oils (kerosene + petrol + diesel) in Marine Broth 2216 during cultivation. Six strains named MW1, JN1, MS3, JN2, MS1, and MW2 were identified by various identification tests. MW2 (*Pseudomonas* sp.) Strain showed the highest emulsification activity against n-hexadecane. In addition, MW2 showed the highest activity for oil displacement test (3.14 ± 0.02) and emulsification test (70.5 ± 0.55) towards n-hexadecane.

Key words: Biosurfactant, emulsification, bacteria, haemolytic, extreme conditions, oil.

INTRODUCTION

Human activities have remained the major source of pollution of the environment especially soil and water. A spill of over 800 tonnes of oil being spilled into the Arabian Sea, on 7th of August 2010, due to the collision of Panamanian vessels MSC Chitra and MV Khalijia III has severely impacted around 1,273 hectares of mangroves. Further, according to the New Zealand government an oil spill from a grounded container ship in the Bay of Plenty has killed 1,250 seabirds on 10th October, 2011 with hundreds of others in rescue centers (Sukhada, 2011). The polluted or impacted soil and water would have to be restored to its natural or near natural state by remediation. Bioremediation is a viable option, that involves the use of microorganisms especially bacteria. It comprises the introduction of genetically engineered microorganisms or the augmentation of the activity of the native microorganisms to remediate the environment. Most of these microorganisms produce surface-active agents, which facilitate nutrient uptake and breakdown of interfacial tension that may exist in interacting with hydrophobic substance like hydrocarbons. However, it is the ability of biosurfactant producer to reduce interfacial surface tension, which has important tertiary oil recovery and bioremediation consequences (Bento et al., 2005). A drop collapsing test was advised for screening bacterial colonies that produce surfactants. Bento et al. (2005) used the reduction of surface tension and the emulsify capacity to screen biosurfactant producing micro-organisms. Hence, the study screened for the diversity and distribution of surface-active agent producing bacteria from contaminated soil and water that will be of potential use in the remediation of mixture of oils polluted areas.

*Corresponding author. E-mail: seemaresearch21@gmail.com, nakuljasuja@gmail.com. Tel: +91-9785235164.

MATERIALS AND METHODS

To carry out isolation of biosurfactant producing marine bacteria, oil-spilled seawater samples were collected from harbors and docks in Arabian Sea, Mumbai. The samples were spread on a nutrient agar (Difco, USA), dissolved in distilled water and incubated at room temperature for 1 to 2 days for water samples and for soil samples, 1 g of soil sample was taken and serially diluted in 0.85% sterile saline water. All dilutions were performed in triplicates and

then the samples were spread on nutrient agar plates and incubated at room temperature for 1 to 2 days. After incubation, plates were enumerated and morphologically different bacteria were selected for biosurfactant screening (approximately 5 to 6 isolates per plate) and purified by re-streaking twice. Isolated colonies were inoculated into 100 ml of Marine Broth 2216 (Difco) containing 2 to 3 drops of kerosene and kerosene with petrol oil, and incubated with continuous shaking (200 rpm) for 24 to 48 h at room temperature using a shaker. Colonies possessing biosurfactant-producing activity, as evidenced by emulsification of oil, were chosen for further experimentation. In addition, the cell suspensions of isolated strains were tested for presence of surfactant by using haemolytic activity, the qualitative drop-collapsing test, quantitative oil displacement test and emulsification activity.

Identification of bacteria

The isolated colonies identified through microbiological and biochemical tests such as Gram staining, carbohydrate fer-mentation test, H_2S production test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test, catalase test, oxidase test, litmus milk reaction, starch hydrolysis test, gelatin hydrolysis test, lipid hydrolysis test.

Extraction of biosurfactant

Each culture was centrifuged at 8000 rpm, 4°C for 10 min to harvest the cells. The culture supernatant was taken and the pH of the culture supernatant was lowered to 2 with 5 M HCl and incubating at 4°C for 24 h. The precipitate was separated by centrifugation at 8000 rpm for 20 min. White precipitate formed culture was selected for further experimentation.

Biosurfactant activity tests

Haemolytic activity

Biosurfactant producing capacity was found to be associated with haemolytic activity. Haemolytic activity therefore appears to be a good screening criterion for surfactant-producing strains. Isolated strains were screened on blood agar plates containing 5% (v/v) goat blood and incubated at room temperature for 24 h. Haemolytic activity was detected as the occurrence of a define clear zone around a colony (Carrillo et al., 1996).

Drop collapsing test

Two micro-liter of mineral oil was added to each well of a 96-well micro-liter plate lid. The lid was equilibrated for 1 h at room temperature, and then 5 ∝l of the cultural supernatant was added to the surface of oil. The shape of the drop on the oil surface was inspected after 1 min. Biosurfactant-producing cultures giving flat drops were scored as positive '+'. Those cultures that gave rounded drops were scored as negative '-', indicative of the lack of biosurfactant production (Youssef et al., 2004).

Emulsification measurement

Emulsification activity was measured according to the method of Cooper and Goldenberg (1987) with a slight modification. To 4 ml of culture supernatant or biosurfactant crude extract (0.5%, w/v), 4 ml of n-hexadecane were added and vortexed at high speed for 2 min. The mixture was allowed to stand for 10 min prior to measurement. The emulsification activity is defined as the height of the emulsion layer divided by the total height and expressed as percentage.

Emulsification Activity = Height of emulsion layer/Total height

Oil displacement test

15 ∝l of weathered crude oil was placed on the surface of distilled water (300 ∝l) in a Petri dish (150 mm in diameter). Then, 20 ∝l of the culture supernatant were gently put on the center of the oil film. The diameter and area of clear halo visualized under visible light were measured and calculated after 30 s.

RESULTS

This study revealed that six strains MW1 (*Acinetobacter* sp.), JN1 (*Bacillus* sp.), JN2 (*Pseudomonas* sp.), MS3 (*Gluconobacter* sp.), MS1 (*Arthrobacter* sp.), MW2 (*Pseudomonas* sp.) out of 20 isolated strains had shown the higher biosurfactant activity, in which MW2 (*Pseudomonas* sp.) strain showed the highest emulsification activity, that is, 70.5 ± 0.55 (Figure 1), and oil displacement test was found 3.14 ± 0.02 (Figure 2). It was observed that MW2 was more effective in degrading the mixtures of oils as compare to other strains. Emulsification activity and results of drop collapsing test summarized in Table 1. It was noted that the strain with higher emulsifying activity toward oil showed the greater oil displacement activity and emulsification activity.

DISCUSSION

Present study revealed that biological tools can reduce pollution of soil and water that occurred due to increased transport and industrialization, etc. The chemical treatment to this kind of pollution has several disadvan-tages. Thus, biological treatments may be preferred because they have many advantages over chemical methods. In the present study, six biosurfactant-producing marine bacteria were isolated from oil-spilled sea water and soil samples, which have a capacity to emulsify mixtures of oils. Biosurfactant crude extract produced from strain MW2 showed the highest emul-sification and oil-displacement activity. The usefulness of biosurfactant producing strains in bioremediation of sites highly contaminated with crude petroleum–oil hydro-carbons was confirmed by Das and Mukherjee (2007), which also confers the present study. They detected the ability of three biosurfactant producing strains: *Bacillus subtilis* DM-04, *Pseudomonas aeruginosa* M and *Pseudomonas aeruginosa* NM to remediate petroleum crude oil contaminated soil. They inoculated the tested soil with mineral salts media containing a specified amount of all the three isolated strains. They also analysed the soil–phase total petroleum hydrocarbons

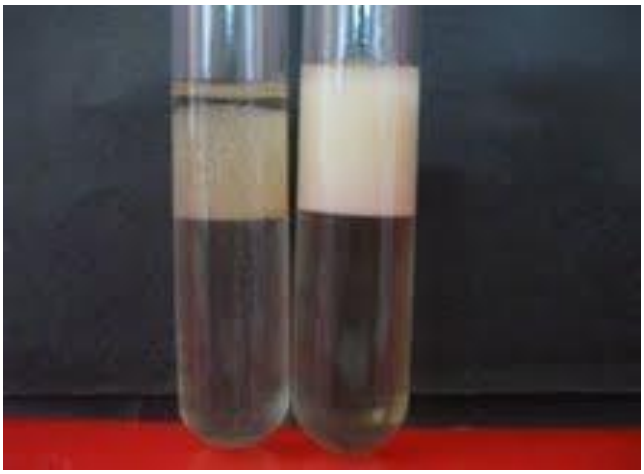

Figure 1. Emulsification activity assay test (a) *Pseudomonas sp* (MW2).

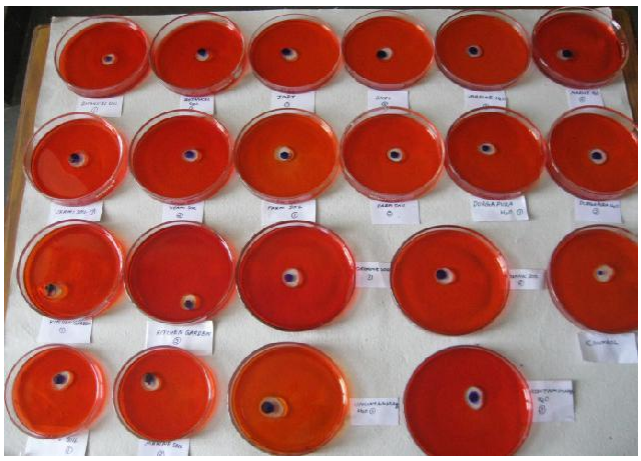

Figure 2. Oil displacement test.

(TPH) concentrations after 120 days and compared to a control where the soil was treated with un-inoculated medium. Bioaugmentation of studied soil with *P. aeruginosa* M and NM consortium and *B. subtilis* strain showed that TPH levels were reduced from 84 to 21 and 39 g kg^{-1} of soil, respectively. In contrast, the TPH level was decreased to 83 g kg^{-1} in control soil (Das and Mukherjee, 2007). Various microorganisms, which are already surviving in different conditions of nature, can produce biosurfactant used in biodegradation of various harmful chemicals present in oils. Kiran et al. (2010) suggested that the single screening method is unsuitable for identifying all types of biosurfactants, and recommended that more than one screening method should be included during primary screening to identify potential biosurfactant producers. In the present study, hemolytic activity assay, oil displacement activity, and emulsification activity measurement were used to screen the biosurfactant producer. Strain *Pseudomonas* sp. showed positive results in all of the four screening methods used. Thus, we confirm that this bacterium can produce biosurfactants with positive responses. The main advantages of microbiological method of bioremediation of hydrocarbon polluted sites are use of biosurfactant producing bacteria without necessarily characterization of the chemical structure of the surface active compounds. The cell free culture broth containing the biosurfactants can be applied directly or by diluting it appropriately to the contaminated site. The other benefit of this approach is that the biosurfactants are very stable and effective in the culture medium that was used for their synthesis (Płociniczak et al., 2011).

Conclusion

Application of biosurfactant and biosurfactant-producing bacteria in environmental cleaning (bioremediation) is a

Table 1. Oil displacement activity, emulsification activity and drop collapsing test of cultural supernatant from different-different strains.

Strain	Emulsification activity (%)	Oil displacement test (cm^2)	Drop collapsing test
MW1	62.0 ± 0.40^{c}	2.45 ± 0.02^{c}	+
MW2	70.5 ± 0.55^{a}	3.14 ± 0.02^{a}	+
MW3	45.0 ± 0.15^{e}	1.13 ± 0.05^{g}	+
MW4	50.0 ± 0.30^{d}	1.14 ± 0.03^{f}	+
MW5	45.0 ± 0.20^{e}	0.13 ± 0.04^{l}	+
MW6	30.0 ± 0.19^{g}	1.10 ± 0.01^{i}	+
MS1	65.0 ± 0.36^{b}	2.20 ± 0.03^{e}	+
MS2	40.0 ± 0.18^{f}	1.11 ± 0.02^{h}	+
MS3	60.0 ± 0.30^{d}	2.30 ± 0.03^{d}	+
MS4	50.0 ± 0.30^{d}	1.14 ± 0.02^{f}	+
MS5	25.0 ± 0.13^{i}	1.05 ± 0.01^{k}	+
MS6	50.0 ± 0.27^{d}	1.13 ± 0.03^{g}	+
MS7	28.0 ± 0.15^{h}	1.09 ± 0.02^{j}	+
JN1	65.0 ± 0.50^{b}	2.55 ± 0.03^{b}	+
JN2	65.0 ± 0.45^{b}	2.55 ± 0.02^{b}	+
JN3	50.0 ± 0.31^{d}	1.13 ± 0.01^{g}	+
JN4	45.0 ± 0.21^{e}	0.13 ± 0.02^{l}	+
JN5	50.0 ± 0.25^{d}	0.13 ± 0.02^{l}	+
JN6	45.0 ± 0.19^{e}	0.13 ± 0.03^{l}	+
JN7	45.0 ± 0.21^{e}	0.04 ± 0.02^{m}	+

Different letters in the same column indicates significant differences ($p < 0.05$). MW (marine water sample from Mumbai), MS (marine soil sample from Mumbai).

potential area of more research as revealed from the present study. Both organic and inorganic contaminants can be removed through different processes (physico-chemical and biological) in which biosurfactants are involved. Due to their biodegrading potential and low hazard to the environment and human health, these microbes are very promising for use in environmental biotechnologies. The commercial success of such technologies is still limited by their high production cost. Optimized growth conditions using cheap renewable substrates (agro-industrial wastes) and efficient methods for cultivation of microbes could make these technologies more economically feasible. Although, they are mostly eco-friendly, still their environmental impact needs to be assessed thoroughly. Nevertheless, careful and controlled use of these interesting surface active molecules will surely help in the enhanced clean up of the toxic environmental pollutants, and provide us with a clean environment.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Panckaj Garg, Founder and Advisor of Jayoti Vidyapeeth Women's University, Jaipur, India for providing the facilities for this work.

REFERENCES

Bento FM, de Oliveria Camargo FA, Okeke BC, Frenkenberger Jr. WT (2005). Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. Microbiol. Res., 160: 249-255.

Carrillo PG, Mardaraz C, Pitta–Alvarez SI, Giulietti AM (1996). Isolation and selection of biosurfactant–producing bacteria. World J. Microbiol. Biotechnol., 12: 82-84.

Cooper DG, Goldenberg BG (1987). Surface active agents from two *Bacillus* species. Appl. Environ. Microbiol., 53: 224-229.

Das K, Mukherjee AK (2007). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresour. Technol., 98: 1339-1345.

Kiran GS, Thomas TA, Selvin J, Sabarathnam B, Lipton AP (2010). Optimization and characterization of a new lipopeptide biosurfactant produced by marine *Brevibacterium aureum* MSA13 in solid state culture. Bioresour. Technol., 101: 2389-2396.

Płociniczak MP, Płaza GA, Seget ZP, Cameotra SS (2011). Environmental Applications of Biosurfactants: Recent Advances. Int. J. Mol. Sci., 12: 633-654.

Sukhada T (2011). "Oil spill damage to soil irreversible: Report". The Times of India (Bennett, Coleman & Co. Ltd.). Retrieved 24 January 2011.

Youssef NH, Dunacn KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ (2004). Comparison of methods to detect biosurfactant production by diverse microorganism. J. Microbiol. Meth., 56: 339-347.