Uttar Pradesh Journal of Zoology

Volume 45, Issue 13, Page 275-281, 2024; Article no.UPJOZ.3639
ISSN: 0256-971X (P)

Isolation and Characterisation of Biosurfactant Producing Bacteria from Domestic Wastes

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i134154

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3639

Original Research Article

Received: 07/04/2024
Accepted: 11/06/2024
Published: 14/06/2024

ABSTRACT

Bio-surfactants are organic compounds that accumulate at the interface of immiscible fluids, effectively reducing interfacial tension and enhancing solubility. With their eco-friendly nature, characterized by low toxicity and high biodegradability, bio-surfactants have garnered attention across various industries including cosmetics, agriculture, and bioremediation. In this study, bacteria capable of producing bio-surfactants were isolated from domestic wastes. Soil samples from a cloth washing area were cultured in LB medium, and the isolated bacteria were screened and characterized using physical (gram staining) and chemical (catalase and indole tests) methods. The predominant bacterial strains isolated were identified as *Bacillus subtilis* and *Escherichia coli*, with B. subtilis being gram-positive and E. coli gram-negative. The bio-surfactant production capability of the isolated strains was confirmed through oil spreading assays. The findings suggest a potential for utilizing bio-surfactants in bioremediation applications, thereby contributing to

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Cite as: *Alappat, Meera Mary, and Remya V.K. 2024. "Isolation and Characterisation of Biosurfactant Producing Bacteria from Domestic Wastes". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (13):275-81. https://doi.org/10.56557/upjoz/2024/v45i134154.*

environmental pollution control efforts. This study underscores the importance of exploring bio-surfactant-producing bacteria from domestic waste sources for their future applications in sustainable environmental management.

Keywords: Biosurfactant; bioremediation; Bacillus subtilis; Luria bertani medium (LB); gram staining; catalase test; indole test.

ABBREVIATIONS

B. subtilis : Bacillus subtilis
LB : Luria Bertani

1. INTRODUCTION

"Surfactants are surface active agents that have a great role in to reducing the interfacial tension between two phases. It is a complex mixture of several phospholipids, lipoproteins and ions" [1]. Surfactants are organic compounds that contain hydrophobic and hydrophilic parts. Biosurfactants are the organic compounds that synthesized from bacteria, yeast and fungi. Biosurfactants also have properties like reduce the surface tension, used in cosmetics and also for pollution control. They are really biodegradable. Biosurfactant have little side effects than chemical surfactants [2].

Nowadays the biosurfactants are mainly concerned as a type of remediation technology for pollution control [3]. Biosurfactants are nontoxic and they act as emulsifiers. Biosurfactants are biologically produced from living organisms and they have advantageous characters like structural diversity, non-toxic, higher biodegradability [4]. So the Biosurfactant are mainly used in cosmetics, pharmaceuticals and pollution control. Biosurfactants are of different chemical composition such as glycolipids, lipopolysaccharides, oligosaccharides and lipopeptides [5] .The substrates for production of microbes are domestic wastes. Domestic wastes are wastes that generated in day-to-day life. The main house hold wastes are frying oils, vegetable wastes and waste water from drainage [6,6a-6b].

Microbial fermentation is the suitable method for biosurfactant productions. Major steps done in this work are sample collection, isolation of bacteria, screening and characterization of isolated strains and isolation is done in Mineral Salt Medium. Screening is done by oil spreading method, emulsification test, foaming activity. Biosurfactants can be efficiently used in handling industrial emulsion, control of oil spills, biodegradation and lowering the toxicity of industrial discharge and in bioremediation of polluted soil.

2. MATERIALS AND METHODS

2.1 Culture and Isolation of *Bacillus subtilis*

For the culturing of *Bacillus subtilis LB* medium, a culture medium was utilized. The significant fixings in the LB medium were Tryptone - 1g, Yeast – 0.5g, NaCl - 1g, and soil tests. To culture, the *Bacillus* bacteria 30g of soil test were gathered from the homegrown waste regions and warmed up to 90ºc. Then poured the warmed soil into a 50ml culture broth medium and shaken well in a shaker incubator and inoculated this culture in 48 hrs (Fig. 1). After the incubation time frame, the bacterial strain were separated. For the segregation nutrient agar plates were prepared. Agar plates were set up by utilizing nutrient agar that was completely blended in 100ml of refined water. Agar was boiled and autoclaved then poured into Petri dishes and allowed to solidify. After the agar sets the bacterial culture was streaked on agar plates by disinfected inoculation loop and incubated the agar plates at 30ºc for 48hrs.

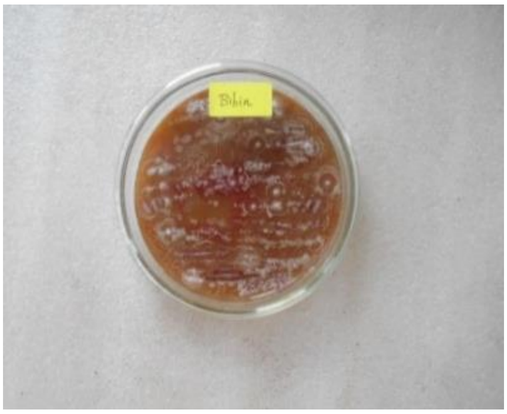

Fig. 1. Culture plate showing the colonies of *Bacillus subtillis*. This shows bacteria culture streaked on solidified agar. Each white spots denotes bacterial colonies

Table 1. Identification of bacterial strain

Sl. No.	Test	Samples		
		S1-Nattika	S2-Thrithaloor	S3- Kodungaloor
1	Gram staining	G-ve	G+ve	G+ve
2	Catalase Test	Positive	Positive	Positive
3	Indole Test	Positive	Negative	Negative
4	Bacterial strains	*E.coli*	*B.subtillis*	*B.subtillis*

3. IDENTIFICATION METHODS

3.1 Biochemical Characterization

For the biochemical identification of the isolates here, two simple methods were used. The chemical methods are called Catalase Test and Indole Test.

Fig. 1. Indole Test; Test tubes showing dark color (cherry red) indicates indole positive and strain is *E. coli* and other tubes are negative for indole test

3.1.1 Catalase test

The catalase test is a common method used in microbiology to determine the presence of the enzyme catalase in bacteria. Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen gas. When hydrogen peroxide is added to a bacterial culture that produces catalase, bubbles of oxygen gas will be liberated, indicating a positive catalase test. This test is particularly useful in differentiating between certain bacterial species, as catalase production can vary among them.

3.1.2 Indole test

"The indole test is another common biochemical test used in microbiology to determine the ability of bacteria to produce indole from the amino acid tryptophan" [7-10]. Bacteria that possess the enzyme tryptophanase can break down tryptophan into indole, pyruvic acid, and ammonia. As you described, after incubating the bacteria in tryptone broth, a small portion of the culture is transferred to a separate test tube and Kovac's reagent is added. Kovac's reagent contains p-dimethylaminobenzaldehyde, which reacts with indole to produce a red color. The appearance of a cherry red color in the test tube indicates a positive result for the indole test.

4. RESULTS AND DISCUSSION

4.1 *Bacillus subtilis*

Bacillus subtilis is a gram positive bacteria. *Bacillus* strains that produced biosurfactant are confirmed by using oil spread assay [11]. "This oil spreading method is rapid and easy to carry out. The oil spreading technique is a reliable method to detect biosurfactant production by different microorganisms" [12]. Surfactin is the major biosurfactant screened from *Bacillus subtilis* suggests that in a specific study [13], researchers identified surfactin as the primary biosurfactant produced by *Bacillus subtilis*. Surfactin have the capability of oil biodegradation was also confirmed by testing the petroleum oil contaminated soil from North East India. Surfactin is a well-known biosurfactant with various industrial and environmental applications due to its surface-active properties. It is a cyclic lipopeptide produced by *Bacillus subtilis* and other closely related species. Its ability to reduce surface tension and form stable emulsions makes it valuable in applications such as bioremediation, agriculture, cosmetics, and pharmaceuticals. The researchers investigated methods to enhance the production of surfactin from *Bacillus subtilis* through two specific techniques: continuous product removal and the addition of metal cations. Surfactin is a crystalline peptide lipid surfactant produced by *Bacillus subtillis*. Lipopeptide is a another biosurfactant derived mainly from a marine *bacillus* and also described another anti-fungal biosurfactant Iturin from *Bacillus* is also reported [14] .The culture of *Bacillus subtilis* were used for the introduction of antibiotics. Lipopeptide is used for insecticidal activity against fruit fly. *Bacillus subtilis* utilize crude oil and hydrocarbons as sole carbon sources used for oil spill cleans up [15].

4.2 *Escherichia coli*

E. coli is the gram negative bacteria. In this study the E.coli was detected by indole test. They conducted study about the methods for E.coli identification in food and water. Rhamnolipids were the major biosurfactant isolated from *E.coli* [16].Production of rhamnolipids from *pseudomonas* and *E.coli* was also confirmed by another studies [17]. Nowadays E.coli have great role in biotechnology were used to produce heterologous proteins, recombinant proteins [18]. The modified *E.coli* cells are used for the vaccine development, bioremediation and biofuels [19]. “One of the major application of biosirfactants is to control the environment pollution control and medical science. Bioremediation in general aims at providing cost effective contaminant specific treatments to reduce the concentration of individual or mixed environmental contaminants” [20]

“Microorganisms capable of hydrocarbon degradation have often been isolated from aquatic environments tested a biosurfactant from *Pseudomonas aeruginosa* for its ability to remove oil from contaminated Alaskan gravel samples under various conditions” [21]. “Bio detox (Germany) described a process to decontaminated soils, industrial sludge and waste water and investigated the effects of the effects of rhamnolipid biosurfactants on in situ biodegradation of hydrocarbon entrapped in a porous matrix and reported a mobilization of hydrocarbon entrapped with in the soil matrix at biosurfactant concentrations higher than critical micelle concentration (CMC)”[22]. “It is well known that microbial cells may chelate metals from solution”[23]. “There is also another study conducted to investigate the potential of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* in the removal of metals from soil contaminated with cadmium reported”[24]. “Another application of biosurfactants is oil storage tank cleaning. Surfactants have been studied for use in reducing the viscosity of heavy oils thereby facilitating recovery, transportation and pipelining” [25]. An area of considerable potential for biosurfactant applications is in the field of microbial enhanced oil recovery (MEOR). Biosurfactants can also aid oil emulsification and assist in the detachment of oil films rocks. Biosurfactants have some therapeutic applications.

Biosurfactants may be used for the dispersion of inorganic minerals in mining and manufacturing processes. Some other applications of biosurfactants are in the coal oil mixture coal water slurry as dispersants, emulsifiers in cosmetics, paints, additives for rolling oil. They also used as foaming agents in toiletries, cosmetics, ore floatation, as metal sequestering agents in mining, as emulsifiers in waste treatment. Biosurfactants are beginning to acquire a status as potential performance effective molecules in various fields.

5. CONCLUSION

This study investigated the surfactant activity of bacterial strains isolated from industrial waste-contaminated soils in cloth washing areas, confirming the influence of environmental factors on the metabolism of these microbes. The research highlights that *Bacillus subtilis* and *E. coli* isolated from contaminated soil exhibit biosurfactant-producing abilities. Furthermore, these bacterial strains demonstrated oil recovery properties, as confirmed by the oil spreading [26]. In this assay, 10 µl of crude oil added to 40 ml of distilled water in a petri dish forms a thin oil layer. The presence of biosurfactant in the supernatant displaces the oil, forming a clearing zone whose diameter correlates with surfactant activity, also known as oil displacement activity.

Biosurfactants have therapeutic applications. Lipopeptides produced by *Bacillus subtilis* exhibit antimicrobial and antifungal activities, with surfactin and iturin being the primary antifungal agents. Biosurfactants are also useful in personal and health care fields, and as therapeutic agents. Concerns about pesticide pollution have driven global efforts to develop alternative biological control technologies. Stanghellini and Miller evaluated the biological control potential of rhamnolipid-producing strains, concluding that biosurfactants have significant potential for controlling zoospores plant pathogens.

The usefulness of biosurfactants in bioremediation is expected to gain more importance in coming year and it is the most versatile process chemicals for use in the near future. The usefulness of biosurfactant in other fields is emerging, especially in personal health care and as therapeutic agents. With increased efforts on developing improved application technologies strain improvement and production process biosurfactant are expected to be among the most versatile process, biosurfactants are expected to be among the most versatile process chemicals for use in the near future.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENT

The creator expresses gratitude toward Dr.Remya V.K. for the confinement and support of microbes and prathibha for her important assistance to gather the data. The creator expresses gratitude toward Dr.Anitha Sankar C. principle, Sree Narayana College, Nattika, for sympathetically allowing me to utilize the library and lab offices of the College.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sambanthamoorthy K, Feng X, Patel R, Patel S, Paranavitana C. Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. BMC Microbiol. 2014;14.
Available:https://doi.org/10.1186/1471-2180-14-197
2. Ławniczak Ł, Woźniak-Karczewska M, Loibner AP, Heipieper HJ, Chrzanowski Ł. microbial degradation of hydrocarbons-basic principles for bioremediation: A review. Molecules. 2020;25.
Available:https://doi.org/10.3390/MOLECULES25040856.
3. Ron EZ, Rosenberg E. Biosurfactants and oil bioremediation. Curr Opin Biotechnol. 2002;13:249–52.
Available:https://doi.org/10.1016/S0958-1669(02)00316-6.
4. Santos DKF, Rufino RD, Luna JM, Santos VA, Sarubbo LA. Biosurfactants: Multifunctional biomolecules of the 21st century. Int J Mol Sci. 2016;17.
Available:https://doi.org/10.3390/ijms17030401.
5. Eraqi WA, Yassin AS, Ali AE, Amin MA. Utilization of Crude Glycerol as a Substrate for the Production of Rhamnolipid by *Pseudomonas aeruginosa*. Biotechnol Res Int 2016;2016: 3464509.
Available:https://doi.org/10.1155/2016/3464509
6. Han Z, Liu Y, Zhong M, Shi G, Li Q, Zeng D, et al. Influencing factors of domestic waste characteristics in rural areas of developing countries. Waste Management.2018;72:45–54.
Available:https://doi.org/10.1016/j.wasman.2017.11.039.
6a. Chizike, Ezekwu, Williams Janet Olufunmilayo, Ugboma CJ. Degradation of polyethylene using bacteria from waste dump sites in Obio/Akpor local government area in Rivers State, Nigeria. South Asian Journal of Research in Microbiology. 2022;14(1-2):9-16.
Available:https://doi.org/10.9734/sajrm/2022/v14i1259.
6b. Swapna TH, Narendra Kumar P, Yahya Khan, Gopal Reddy, and Hameeda Bee. Extracellular synthesis of Fe-NPs by bacteria/biosurfactant, kinetic parameter and reduction of Cr (VI) from Pollutant Samples. Journal of Advances in Microbiology. 2023;23(6):38-51.
Available:https://doi.org/10.9734/jamb/2023/v23i6730.
6c. Walter V, Syldatk C, Hausmann R. Screening concepts for the isolation of biosurfactant producing microorganisms. Biosurfactants. 2010:1-3.
7. Hassanshahian M. Isolation and characterization of biosurfactant producing bacteria from Persian Gulf (Bushehr provenance). Mar Pollut Bull. 2014;86:361–6.
Available:https://doi.org/10.1016/J.MARPOLBUL.2014.06.043.
8. Sanders ER. Aseptic laboratory techniques: plating methods. J Vis Exp 2012:1–18.
Available:https://doi.org/10.3791/3064.
9. Iwase T, Tajima A, Sugimoto S, Okuda KI, Hironaka I, Kamata Y, et al. A simple assay for measuring catalase activity: A visual approach. Sci Rep. 2013;3.

Available:https://doi.org/10.1038/srep03081.

10. Alves MS, Da Silva Dias RC, De Castro ACD, Riley LW, Moreira BM. Identification of clinical isolates of indole-positive and indole-negative Klebsiella spp. J Clin Microbiol 2006;44:3640–6. Available:https://doi.org/10.1128/JCM.00940-06.
11. Nayarisseri A, Singh P, Singh SK. Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain ANSKLAB03. Bioinformation. 2018;14:304–14. https://doi.org/10.6026/97320630014304.
12. Biniarz P, Łukaszewicz M, Janek T. Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: A review. Crit Rev Biotechnol. 2017;37:393–410. Available:https://doi.org/10.3109/07388551.2016.1163324.
13. Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM, McInerney MJ. Comparison of methods to detect biosurfactant production by diverse microorganisms. J Microbiol Methods. 2004;56:339–47. Available:https://doi.org/10.1016/j.mimet.2003.11.001.
14. Yaraguppi DA, Bagewadi ZK, Patil NR, Mantri N. Iturin: A Promising Cyclic Lipopeptide with Diverse Applications. Biomolecules. 2023;13. Available:https://doi.org/10.3390/biom13101515.
15. Meena KR, Kanwar SS. Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics. Biomed Res Int..2015;2015. Available:https://doi.org/10.1155/2015/473050.
16. Kathiravan N, Rajesh A, Kim JW, Davoodbasha MA. Isolation and Characterization of Biosurfactant-Producing Soil Fungus Penicillium sp. Appl Biochem Biotechnol; 2023. Available:https://doi.org/10.1007/S12010-023-04704-6.
17. Production of rhamnolipids-producing enzymes of Pseudomonas in E. coli and structural characterization - Search Results - PubMed n.d. Available:https://pubmed.ncbi.nlm.nih.gov/?term=Production+of+rhamnolipids-producing+enzymes+of+Pseudomonas+in+E.+coli+and+structural+characterization&filter=datesearch.y_10 (accessed May 30, 2024).
18. Jia B, Jeon CO. High-throughput recombinant protein expression in Escherichia coli: current status and future perspectives. Open Biol 2016;6. Available:https://doi.org/10.1098/RSOB.160196.
19. Reed B, Chen R. Biotechnological applications of bacterial protein secretion: From therapeutics to biofuel production. Res Microbiol. 2013;164:675–82. Available:https://doi.org/10.1016/j.resmic.2013.03.006.
20. Azubuike CC, Chikere CB, Okpokwasili GC. Bioremediation techniques-classification based on site of application: Principles, advantages, limitations and prospects. World J Microbiol Biotechnol. 2016;32. Available:https://doi.org/10.1007/S11274-016-2137-X.
21. Pacwa-Płociniczak M, Płaza GA, Poliwoda A, Piotrowska-Seget Z. Characterization of hydrocarbon-degrading and biosurfactant-producing Pseudomonas sp. P-1 strain as a potential tool for bioremediation of petroleum-contaminated soil. Environ Sci Pollut Res Int. 2014;21:9385–95. Available:https://doi.org/10.1007/S11356-014-2872-1.
22. Makkar RS, Rockne KJ. Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. Environ Toxicol Chem. 2003;22:2280–92. Available:https://doi.org/10.1897/02-472.
23. White C, Sayer JA, Gadd GM. Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. FEMS Microbiol Rev 1997;20:503–16. Available:https://doi.org/10.1111/J.1574-6976.1997.TB00333.X.
24. Sandrin TR, Chech AM, Maier RM. A rhamnolipid biosurfactant reduces cadmium toxicity during naphthalene biodegradation. Appl Environ Microbiol. 2000;66:4585–8. =Available:https://doi.org/10.1128/AEM.66.10.4585-4588.2000.
25. Dobler L, Ferraz HC, Araujo de Castilho LV, Sangenito LS, Pasqualino IP, Souza dos Santos AL, et al. Environmentally friendly rhamnolipid production for

petroleum remediation. Chemosphere. 2020;252. Available:https://doi.org/10.1016/j.chemosphere.2020.126349.

26. Evaluation of Screening Methods for the Isolation of Biosurfactant Producing Marine Bacteria Rengathavasi Thavasi1 *, Shilpy Sharma1 and Singaram Jayalakshmi2 - Search Results - PubMed n.d. Available:https://pubmed.ncbi.nlm.nih.gov/?term=Evaluation+of+Screening+Methods+for+the+Isolation+of+Biosurfactant+Producing+Marine+Bacteria+Rengathavasi+Thavasi1+*%2C+Shilpy+Sharma1++and+Singaram+Jayalakshmi2 (accessed May 31, 2024).

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3639