Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [1] 2023: 116-120

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Screening of Thermophiles for Bioemulsifier Production

S.A. Shaikh* and G.R. Pathade
Krishna Institute of Allied Sciences, KVVDU, Karad, Maharashtra

ABSTRACT

This study was carried out with the aim to isolate a thermophile producing bioemulsifier which is known to have far more value in conservation of environment than just a product for commercialization. Amongst the thermophilic isolates obtained from Aravali hot water sample (Maharashtra) only one isolate was able to produce bioemulsifier during screening study. The isolate was identified as Bacillus licheniformis SGRP 4. The bioemulsifier produced by B. licheniformis SGRP 4 was found to be stable at 50°C. Bioemulsifier produced was attributed with capability to emulsify engine oil and reduce the surface tension of water.

***Key words**: thermophile, bioemulsifier, Bacillus spp*

Received 28.04.2023 Revised 20.05.2023 Accepted 11.06. 2023

INTRODUCTION

"Bioemulsifier" as the name suggest is an emulsifying agent produced by living organisms. Emulsifiers are surface-active amphipathic polymers that can emulsify two immiscible liquids [1].Bioemulsifiers are produced by a large number of bacteria, yeasts and fungi [2].Bioemulsifiers are advantageous over chemically derived surface-active agents due lower toxicity, biodegradability and biocompatibility [1].Bioemulsifiers have higher molecular weight as compared to biosurfactants [3].Biochemically bioemulsifiers are polysaccharides, proteins, lipopolysaccharides lipoproteins, or a mixture of polysaccharides, proteins and lipids [2,4,5]. Bioemulsifier finds a variety of applications in various industries like food, detergents, pharmaceuticals, agricultural and cosmetic [3,6]. Despite a larger sphere of applicability, bioemulsifiers are less explored compared to biosurfactants [3]. Due to high production cost and lower yield bioemulsifier that to under mild environmental conditions, there is a need to search for new or promising strains of microorganisms producing bioemulsifier that could be produced and would be able to function under harsh environmental conditions like higher temperature [1,3]. Some of the organisms producing well studied emulsifier like emulsan, alasan, liposan include *Acinetobacter calcoaceticus RAG-I, A. radioresistens, C. lipolytica, C. tropicalis*, etc [3].

MATERIALS AND METHODS

Isolation of Thermophiles

For the isolation of thermophiles, sample from Aravali hot water spring (Maharashtra, India) was collected in a presterilized container. It was then serially diluted and spread inoculated on Thermus agar (ATCC medium 697) containing 0.5% NaCl, 0.5% peptone, 0.4% beef extract, 0.2% yeast extract and 2-3% agar, of pH 7 [7]. The plates were incubated for 24-48 h at 50°C.Well isolated representative colonies were selected, purified and maintained for further study.

Screening for Bioemulsifier Producing Organisms

Production of Bioemulsifier [3]

Suspension of purified culture having a density of approx. 10^8cell/mL was prepared and inoculated in 100 mL of production medium containing g/L of 5g/L of NaCl, 2g/L NH_4NO_3, 2g/L KH_2PO_4, 5g/L Na_2HPO_4 and 0.1g/L $MgSO_4$. $7H_2O$ with 2% edible oil as a carbon source. The medium was incubated at 50°C for 5-6 days at 120 rpm in a shaker incubator.

Detection of Bioemulsifier in the Culture Broth [3]

To detect bioemulsifier production, the culture broth was centrifuged at 8000 rpm for 15-20 mins. The cell-free supernatant obtained was used as a crude bioemulsifier sample for screening.

Oil Spread Test

In a clean petriplate,20 mL of distilled water was added. On the surface of it, 100 µL of oil containing Sudan black B stain was added. Sudan black B was added for clear visualization of the displacement of oil by bioemulsifier. On the oil then 50µL of supernatant was added. Immediate displacement of oil upon the addition of supernatant indicates the presence of bioemulsifier.

Drop Collapse Test

The supernatant was placed on a paraffin sheet and observed after 10s. Collapsing of drop indicates the presence of surface-active agent.

Emulsification Index (E-24)

6 mL of oil was mixed with 4mL of culture supernatant and vortexed for 2min at high speed to facilitate proper mixing. The mixture was allowed to stand still for 24h and then the % emulsification was calculated as

$$\text{E-24 Index, \%} = (\text{height of emulsion/total height}) \times 100$$

Generally, bioemulsifier showing 50 % E-24 index is considered to be a good emulsifier.

For all the above tests Sodium lauryl sulphate was used as the positive control and Phosphate buffered saline was used as the negative control.

On the basis of the above screening test, a promising isolate was selected for further study.

Determination of Surface Tension [8,9]

The surface tension of the culture supernatant was determined by drop count method using a stalagmometer with the help of formula.

$\gamma_1 = (d1/d2) \times (n1/n2) \times \gamma_2$

where γ_1 is the surface tension of the test sample, d is density, n signifies no of drops and γ_2 is the surface tension of water. Water was used here as a reference liquid.

Characterization of Bioemulsifier [3]

Effect of Temperature

The stability of the emulsifier at different temperatures was checked by determining the E-24 index as mentioned above. The supernatant of culture broth of promising isolate was mixed with oil and incubated at different temperatures like 10°C, 25°C,37°C and 50°C.

Capability to Emulsify Different Hydrocarbons

The ability of culture supernatant to emulsify different hydrocarbons like engine oil, toluene, petrol and diesel was determined by the E-24 index.

Identification of Isolate

The isolate capable of producing a bioemulsifier was identified using 16S rRNA gene sequencing. The gene sequence was then subjected to the BLAST algorithm to determine homology for the identification of the isolate. The gene sequence of the identified isolate was submitted to the NCBI gene bank and the accession number was obtained.

RESULTS AND DISCUSSION

Isolation and Screening of Thermophiles for Bioemulsifier Production:

Five thermophilic isolates were obtained from Aravali hot water spring. Isolates obtained were designated as A1.1, A1.2 and so on till A1.5 until identified.(Table-1)

On the basis of the oil spread test, drop collapse test, and E-24 index results, only one isolate A1.2 was found to produce bioemulsifier. The cell-free supernatant also reduced the surface tension to 49.21dynes/cm. Although the surface tension was found to be reduced, to categorize this surface active agent as a potential biosurfactant and not bioemulsifier, the surface tension has to be reduced to 35dynes/cm [10].

Characterization of Bioemulsifier

The stable emulsion was seen to be produced at 25, 37, 50°C but not at 10°C. Thus, among the temperatures used 50°C is the highest temperature at which the bioemulsifier was able to form a stable emulsion (Table 2). Panjiar *et al* reported that the crude bioemulsifier produced by *Lysinibacillus sp*. SP1025 and *Bacillus cereus* SP1035 could produce stable emulsion in a temperature range of 10-80°C.

The bioemulsifier produced by the isolate was found to emulsify edible oil and engine oil but it did not emulsify toluene, petrol and diesel. Amongst engine oil and edible oil, maximum emulsification was seen in the case of engine oil. In one of study, the bioemulsifier produced by *B. cereus* SP1035 was found to be more compatible with aromatic and aliphatic hydrocarbons but not with ester-based oils [3]. In one of the report by Noudeh et al., (2010) maximum bioemulsifier production by *B. licheniformis* PTCC 1595 was obtained with olive oil.

(Table 3 and Photoplate 1)

Identification of Isolate

Isolate was found to have maximum similarity with *Bacillus licheniformis* in phylogenetic analysis of the 16Sr-RNA gene sequence. The 16Sr RNA gene sequence has been submitted to the NCBI gene bank (Accession no: OQ346293). There are many reports of the isolation of organisms belonging to the genus *Bacillus* which have the ability to produce surface active agents [3,10,11]. (Fig 1, Photoplate 2)

Table 1: Screening of Isolates

Sr. No	Isolate	Oil displacement Test	Drop collapse Test	E-24 index (%)
1	A1.1	-	+	-
2	A1.2	+++	+	39%
3	A1.3	-	-	-
4	A1.4	-	-	-
5	A1.5	-	-	-

+ indicates positive - indicates negative

Table 2: Stability of Bioemulsifier at Different Temperatures

Sr. No	Temperature (°C)	E-24 (%)
1	10	-
2	25	40
3	37	43
4	50	44

Table 3: Capability to Emulsify Different Hydrocarbons

Sr.No	Hydrocarbons used	E-24 (%)
1	Edible oil	38
2	Engine oil	49
3	Toluene	-
4	Petrol	-
5	Diesel	-

Photoplate 1: Emulsification of Engine Oil

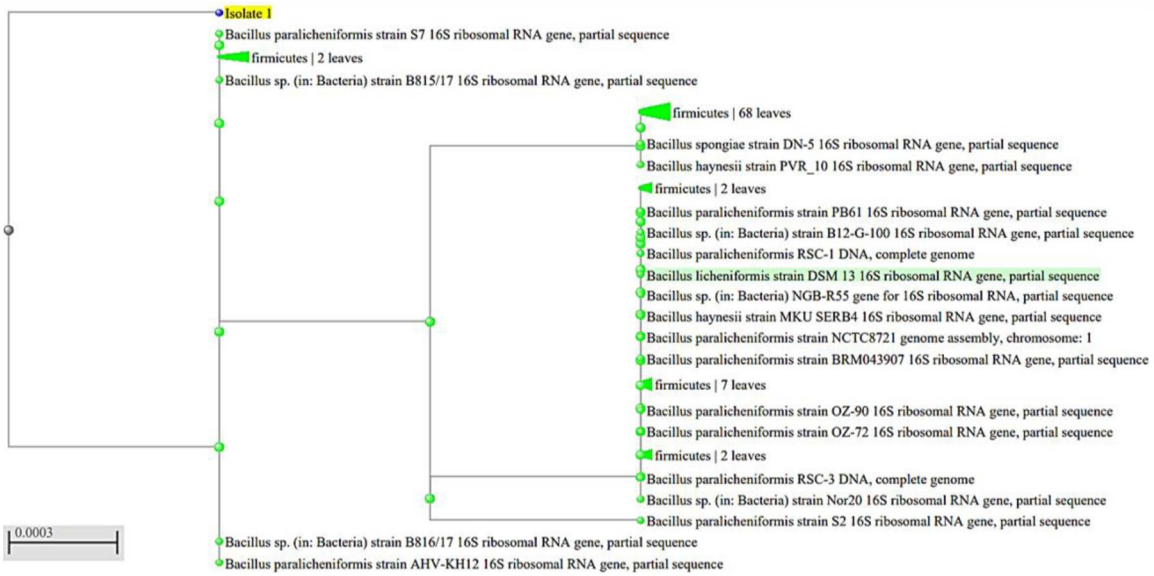
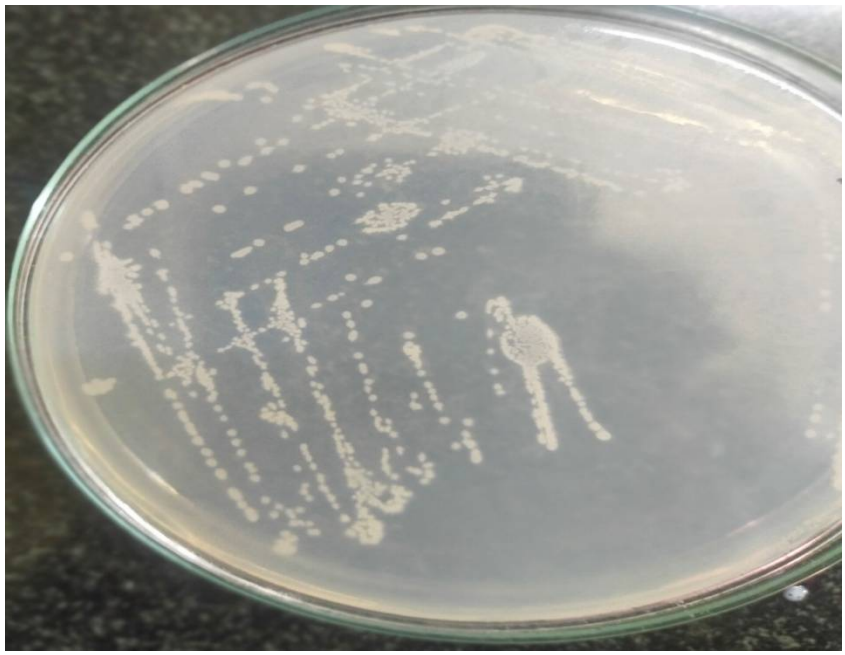


Fig 1: Phylogenetic Tree



Photoplate 2: Pure Culture of the Isolate

CONCLUSIONS

Total five thermophilic isolates were obtained from Aravali hot water spring sample. Only one isolate that produced bioemulsifier was identified as a strain of *B licheniformis* SGRP 4 through the 16sr RNA gene sequencing and phylogenetic analysis. Hence, thermophiles producing surface-active compounds are widely distributed in nature and can be isolated from hot water springs. When the stability of bioemulsifier was checked at different temperatures, it was found to produce emulsion at 50°C maximally. Amongst different ester-based oils and hydrocarbons used, engine oil was emulsified to more extent. The bioemulsifier produced could emulsify ester-based oils but not aliphatic and aromatic hydrocarbons. This study has paved a way for newer research regarding the optimization of parameters to achieve maximum production of bioemulsifier economically.

REFERENCES

1. Uzoigwe, C., Burgess, J.G., Ennis, C.J. and Rahman, P.K., (2015). Bioemulsifiers are not biosurfactants and require different screening approaches. *Frontiers in microbiology*, *6*, p.245.
2. Atre, C., Bhandari, T., Kothawade, M. and Mujumdar, S., (2015). Isolation, characterization and screening of bioemulsifier producing micro-organisms from soil sample (Kaas Pathar, Satara, India) and its application. *International Journal of current microbiology and Applied Science. Special*, pp.65-81.
3. Panjiar, N., Sachan, S.G. and Sachan, A., (2015). Screening of bioemulsifier-producing micro-organisms isolated from oil-contaminated sites. *Annals of Microbiology*, *65*(2), pp.753-764.
4. Barkay, T., Navon-Venezia, S., Ron, E.Z. and Rosenberg, E., (1999). Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. *Applied and environmental microbiology*, *65*(6), pp.2697-2702.
5. Banat, I.M., Makkar, R.S. and Cameotra, S.S., (2000). Potential commercial applications of microbial surfactants. *Applied microbiology and biotechnology*, *53*, pp.495-508.
6. Saikia, R.R., Deka, S., Deka, M. and Banat, I.M., (2012). Isolation of biosurfactant-producing Pseudomonas aeruginosa RS29 from oil-contaminated soil and evaluation of different nitrogen sources in biosurfactant production. *Annals of microbiology*, *62*, pp.753-763.
7. Munster, M.J., Munster, A.P., Woodrow, J.R. and Sharp, R.J., (1986). Isolation and preliminary taxonomic studies of Thermus strains isolated from Yellowstone National Park, USA. *Microbiology*, *132*(6), pp.1677-1683.
8. Morikawa, M., Daido, H., Takao, T., Murata, S., Shimonishi, Y. and Imanaka, T., (1993). A new lipopeptide biosurfactant produced by Arthrobacter sp. strain MIS38. *Journal of bacteriology*, *175*(20), pp.6459-6466.
9. Sudip D., Shubrajit M., Ravi S., (2018). A practical book of physical pharmaceutics, Nirali prakashan.
10. Joshi, S.J., Suthar, H., Yadav, A.K., Hingurao, K. and Nerurkar, A., (2013). Occurrence of biosurfactant producing Bacillus spp. in diverse habitats. *International Scholarly Research Notices*, *2013*.
11. Noudeh, G.D., Moshafi, M.H., Kazaeli, P. and Akef, F., (2010). Studies on bioemulsifier production by Bacillus licheniformis PTCC 1595. *African Journal of Biotechnology*, *9*(3).
12. Satpute, S.K., Banpurkar, A.G., Dhakephalkar, P.K., Banat, I.M. and Chopade, B.A., (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Critical Reviews in Biotechnology*, *30*(2), pp.127-144.

CITATION OF THIS ARTICLE

S.A. Shaikh and G.R. Pathade. Isolation and Screening of Thermophiles for Bioemulsifier Production. Bull. Env. Pharmacol. Life Sci., Spl Issue [2]: 2023: 116-120.