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CHARACTERIZATION AND APPLICATIONS OF BIOSURFACTANT PRODUCED FROM BACILLUS LYCHENIFORMIS..

Dr R.S. Awasthi,
Principal, Shivaji Mahavidyalaya,
Renapur, Latur.

R.P. Shinde
Research Scholar,
Dr. Babasaheb Ambedkar Marathwada University, Aurangabad

ABSTRACT

Microorganisms are responsible for the production of biosurfactants, which are substances that have surface-active qualities. A number of industries, including bioremediation, medicine, and agriculture, have shown interest in these biosurfactants owing to the fact that they have the potential to be used in a variety of contexts and possess features that are beneficial to the environment. The primary objective of this research is to analyse the biosurfactants that are produced by bacteria in Lonar Lake, which is a unique alkaline crater lake located in India. The approach that will be used is qualitative. Many people from different parts of the globe are aware of the fact that Lonar Lake is home to a diverse range of bacteria. The objective of collecting water and sediment samples for the purpose of getting bacterial isolates was accomplished in a number of different sites, both within and outside of the actual water environment. For the purpose of bacterial extraction, a water sample was employed, and rRNA analysis was utilised in order to identify the bacterium Bacillus Lycheniformis. In order to define and identify the biosurfactant that we synthesised by adding 2% glucose to MSM medium, we used a battery of analytical procedures. These methods included thin-layer chromatography (TLC), infrared spectroscopy (IR), and high-performance liquid chromatography (HPLC). When it comes to biosurfactants, the recently created one has always been a lipopeptide. Examples of businesses that make use of biosurfactants include the cosmetics industry, the food and beverage industry, and the healthcare industry, to name just a few. The objective of this experiment was to produce nanoparticles by using a biosurfactant that was produced by microorganisms. The successful manufacturing of ***nanoparticles*** *was shown by the discovery of peaks at 310 and 440 nm, which were discovered by spectroscopic examination. Not only were the nanoparticles that were created able to effectively combat Pseudomonas and E. coli species, but they also demonstrated exceptional antibacterial and antifungal properties. The yeasts Candida and Aspergillus niger were also successfully eradicated by the nanoparticles notwithstanding their effectiveness. At this time, XRD research on nanoparticles was being carried out.*

Keywords: - *Bacillus lycheniformis, Nanoparticles, Biosurfactant, Lipopeptides, AgNo3 nanoparticles antimicrobial and antifungal activity.*

1.INTRODUCTION.

Lake Lonar, which is the third largest lake in the world, can be found in the area of India that is known as Buldhana. Approximately 50,000 years ago, a meteorite travelling at a high speed impacted the region, so producing it. The lakes are able to flourish in this secluded system because there are no influences from the outside world, and the influents are continuously maintained. It is estimated that the lake has a diameter of around 1.75 km and gets water via precipitation and seepage from the earth. However, the lake does not receive any additional discharges from industrial sources. It is the very high levels of salt and alkalinity that are responsible for the distinctive qualities that Lonar Lake has. Both the pH and salinity of lake water are measured at 10.5 and 6,290 milligrammes per litre, respectively. Early research on the variety of microorganisms concentrated mostly on isolating and characterising specific bacteria as their primary concerns. Amphiphilic substances, which include chemical and biosurfactants, have the ability to make hydrophobic compounds more mobile and soluble, while simultaneously reducing surface tension and interfacial forces at the interface of molecules that are incompatible with one another. Amphibolic compounds are released by degradable organisms during the process of hydrocarbon decomposition. These chemicals have an effect on the dynamics of the degradation process. This group of chemicals has been given the name biosurfactants by the scientific community. The low toxicity of biosurfactants makes them beneficial in a wide variety of industries, including medicine, food processing, medicine, oil reservoirs, and bioremediation, among others.

2.OBJECTIVE

1. To isolate Bacillus licheniformis from the unusual alkaline and saline environment of Lonar Lake.
2. To create biosurfactants in a lab utilising the isolated bacterial strain.
3. Formation of Nanoparticles by using extracted biosurfactant and check its Antimicrobial and Antifungal Activty.

3.MATERIALS AND METHODS

3.1 Microorganism and culture conditions

Strain LS-18 was determined to be Bacillus licheniformis by the use of phenotypic characterisation, numerical taxonomic and chemotaxonomic analysis. An uncontaminated soil sample was collected from Kroner lake.which and B-MSM (mineral salt medium) D H Tambekar; et al,2012.

3.2 Sample collection

Water and Soil samples were collected from different sites of lonar lake. Their pH, Alkalinity and Salinity were determined. And samples were stored at 4˚c until used.

3.3 Screening of Biosurfactant producing Microorganisms

Samples were Enriched in Nutrient broth for 48hrs, After Enrichment of samples loopful culture of enriched sample were inoculated on oil coated plates, and incubated at 37˚ c for 48-72hrs. (Morikawa M and Imanaka T (2000)), (DH. Tambekar and PV Gadakh(2012)). Oil degrading microorganisms were confirmed by Zone of clearance appeared on oil coated plates. Morphologically different colonies were selected for biosurfactant production. Production Medium Composition,

Kno3- 0.1gm

Kcl- 0.1gm

K2HPO4- 7.0gm

KH2PO4 – 3gm

CaCL2 – 0.01gm

MgSo4.7H2O - 0.5gm with 5ml of trace element solution

Content

Feso47H2O – 0.116g/l

H3BO3 -0.232g/l

CaCl2.6H20- 0.41g/l

CuSo4.5H2O - 0.008g/l

MnSo4.H2O – 0.008g/l

(NH4)6 Mo7024 – 0.022g/l

ZnSo4 – 0.174 g/l with 2% Glucose as a sole source of carbon for production medium. In Modified form was used. 100ml of sterile MSM medium was taken in 250ml Erlenmeyer flask and inoculated with Oil degrading microbial cultures.

Cultures were incubated at rotary shaker fermenter in the laboratory for 7 days at 37˚ c.

Primary Characterization of biosurfactant

Selection of high yield producing bacteria by the following tests Modified Drop Collapse Method, E-24 Index.

3.3.1 Modified Drop Collapse Method

In this technique, microtiter plates are lightly coated with Pennzoil, and 5 µL of the culture broth is carefully placed at the center of each well. Observations are made within one minute to determine if the drop collapses on the oil-coated surface. A collapsing drop indicates the presence of biosurfactants (BS) in the sample (Jain et al., 1991; (Bodour and Miller-Maier, 1998). However, samples with minimal surfactant concentrations may produce false-negative results (Satpute et al., 2008).

3.3.2 Emulsification Index (EI):

The emulsification activity of biosurfactants is determined by calculating the Emulsification Index (EI), as described by Cooper and Goldenberg (1987). To measure EI, kerosene is mixed with culture broth in a 1:2 ratio (v/v), vortexed for 2 minutes, and left undisturbed for 24 hours. The height of the emulsion layer formed at the interface between the aqueous and kerosene layers is recorded.

3.3.3 Surface Tension measurement:

Surface Tension of the production medium was recorded During the fermentation period by capillary raise method. Reduction in surface tension Is the result of surface-active molecules interactions. (Kumar, S.et. al.1990.)

4.1 characterization of Biosurfactant

4.1.1 Biochemical characterization at primary level

Saponification, Foaming activity, Ninhydrin tests were performed to determine the Proteins Amino acids, in produced biosurfactant. Sawnhey et,at.(2000).

4.1.2 Fourier transformed infrared (FTIR) spectroscopic analysis of the Biosurfactant. The functional groups and the bond types present in the compound were determined bi FTIR Method. Formation of Were observed in a range 4000-400 $cm^{-}1$.

4.1.3 High performance Liquide Chromatography (HPLC) In this method components into the samples were separated on the basis of their non-covalent interactions with the stationary phase. Peaks were observe at various retention time.

5. Identification of the Isolate at molecular level. 16s rDNA sequence method were followed to identify the isolate.

BS recovery

Acetone Precipitation methos: By the process of acetone precipitation of substances, Following the combination of cell-free supernatant with ice-cold acetone, the resulting mixture is next suspended in phosphate buffer in order to precipitate emulsifiers. An incubation period of 15 to 20 hours at 4 degrees Celsius is required in order to get the emulsifier precipitate. The emulsifying activity fractions, protein fractions, and polysaccharide fractions are all investigated here. This approach has been employed by a number of workers in order to clean BS/BE containers. The process of extracting and purifying biosurfactants is referred to as production.

Biomass determination

The amount of time that passed between the collection of samples, centrifugation, and washing was variable. Drying the biomass in the air at a temperature of 105 degrees Celsius was performed until the biomass reached a weight that was constant throughout.

TLC analysis

Preliminary characterisation of biosurfactants by thin-layer chromatography (TLC) for biosurfactant identification. For the purpose of identification, additional methods were used. (Makkar and Cameotra, 1997; Haba et al., 2000). Even though IR and HPLC Through conducting research into the creation of AgNo3 nanoparticles, we were able to validate the utility of the biosurfactant that was ultimately retrieved (Lin et al., 1998a; 1998b.) X-ray diffraction (XRD) analysis and ultraviolet spectroscopy both independently confirmed the creation of nanoparticles. To determine whether or not the silver nanoparticles have antibacterial and antifungal capabilities, we conducted tests. (Moonjit Das et.at.2019)

Applications of Biosurfactant in nanotechnological studies

Synthesis of AgNo3 Nanoparticles.

AgNo3 nanoparticles were formed by using Extracted surfactant. Synthesis of Np's by using biosurfactants is a easy and less time consuming method. In this two step methos we used 0.1%(w/v) bacterial biosurfactant mixed with 100ml of 0.05mol aq solution of AgNo3 with constant stering for 10min. Then Autoclaved at 121 ˚c for 15min. The Formation Of colour from clear to muddy brown indicated the formation of Np's, which was later recovered by centrifugation at 10000 rmp for 10min at room temp followed by drying in oven at 60˚c.

RESULTS AND DISCUSSION

Biosurfactant Producing efficient Bacteria Primarily identified by grams staining

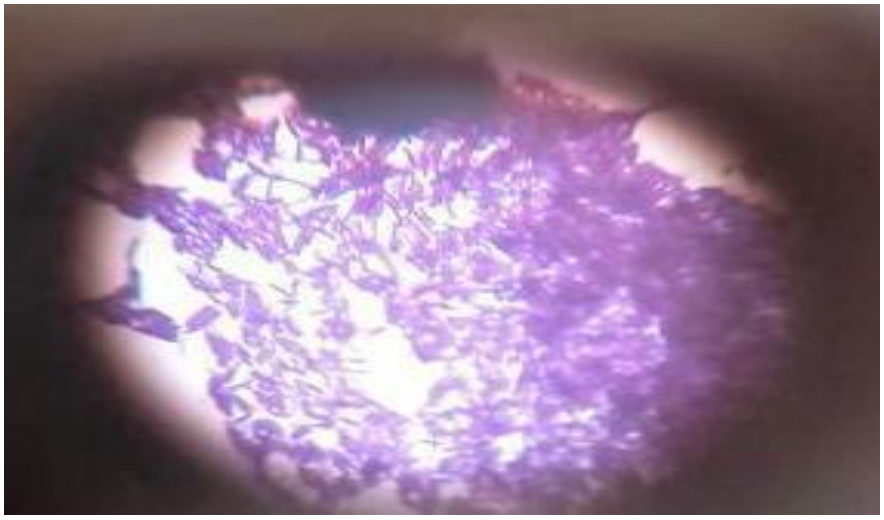
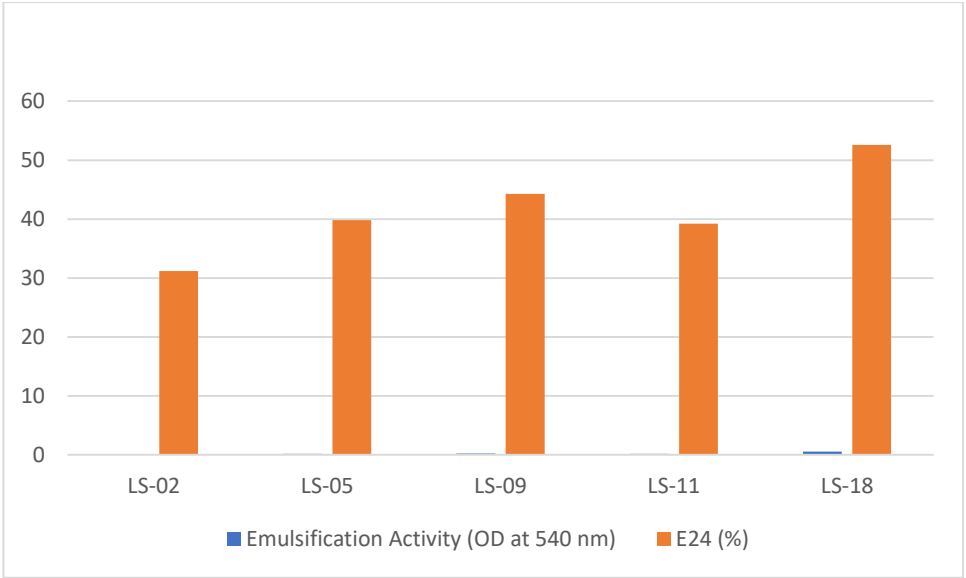


Fig: - Gram +ve Rods

Table 1

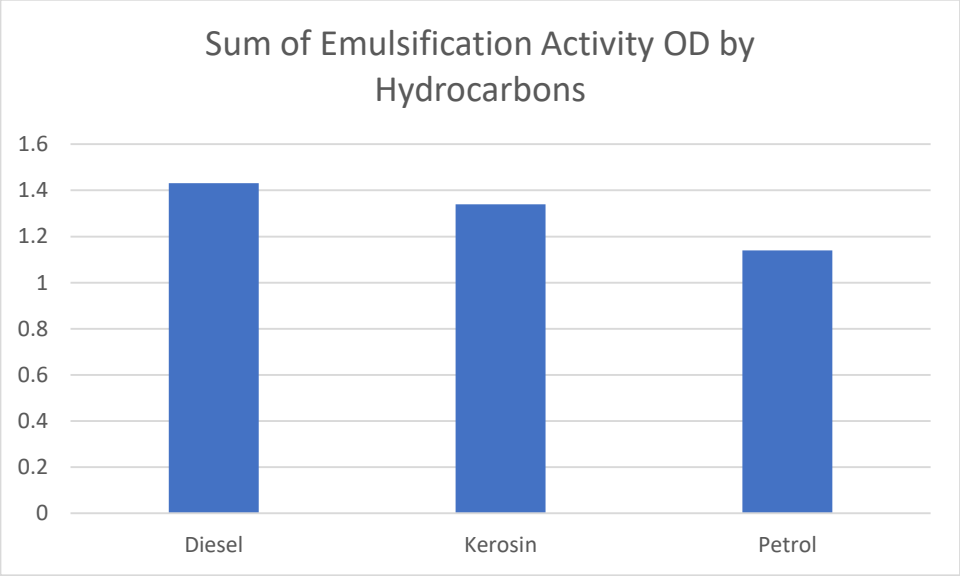
Isolates	Emulsification Activity (OD at 540 nm)	E24 (%)
LS-18	0.54	52.6



Graph showing emulsification activity of efficient bactria

Table 2

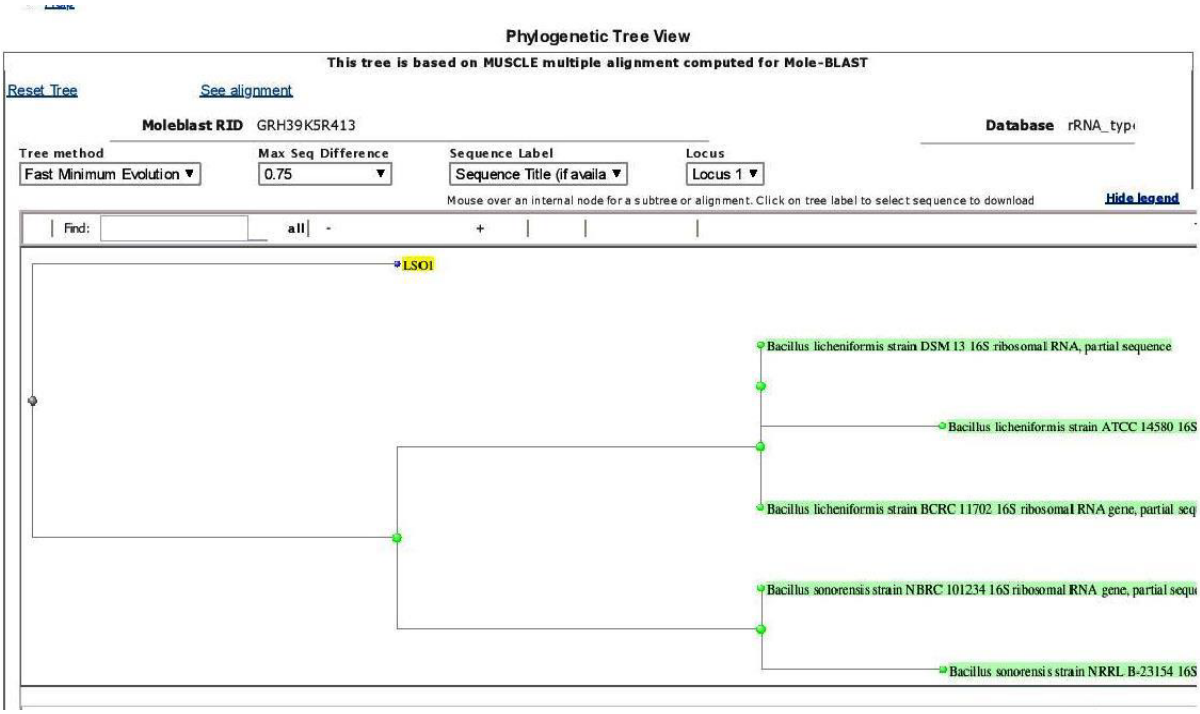
Isolates	Hydrocarbons	Emulsification Index %	Emulsification Activity OD
LS-18	Kerosin	52.6	0.51
	Petrol	42.81	0.24
	Diesel	39.73	0.19



Graph showing results for various hydrocarbons used for E-24 test

These results of the biosurfactant production method are shown in Tables 1 and 2, respectively. After an incubation period of seven days, the E-24 Index was evaluated using optical density (OD). In the case of an efficient organism, the E-24 Index is 52.6%, which is the maximum possible value. To validate the E-24 index, a number of different hydrocarbons derived from efficient organisms were used.

In light of this, the organism that had the highest E-24 index was selected for further investigation. The 16-segment rRNA sequencing technique was used to investigate the molecular level of the identified strain. A phylogenetic analysis was carried out using the sequences of individuals who were typical of the population. These are the sequences that are accessible for BLAST in the GenBank database (YouTube).

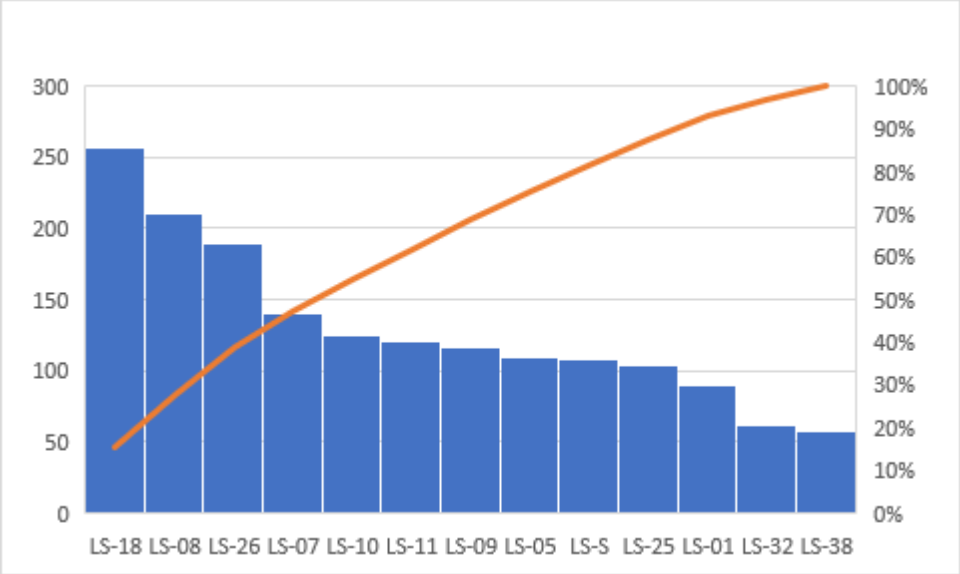


Through the use of Mol Blast NCBI, we were able to recover the phylogenetic sequence of a bacterial culture that demonstrated the most favourable outcomes when it came to the production of biosurfactant using 2% glucose as the only carbon feedstock. It may be concluded that Bacillus licheniformis has been positively identified.

Table 3

1. Isolates	Dry Weight Mg/100ml	Emulsification index
LS-18	257	56.16

E-24 index of Efficient bacteria(Bacillus Licheniformis)

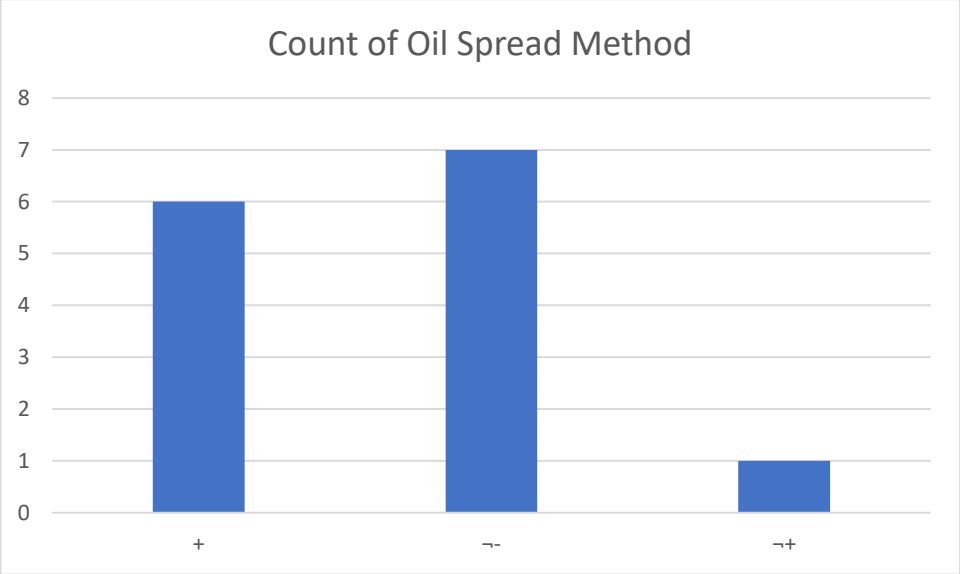


Graph showing Comparative study of E-24 Index

Optimum E-24 index recorded for LS-18 Isolate hence selected for biosurfactant production

Table no -4 Screening of Microorganisms for Biosurfactant production

Isolate	Oil Spread Method	Drop Collapse method	Saponofication test	Oil Emulsion Activity
LS-02	-	-	-	+
LS-05	-	-	+	+
LS-18	+	+	+	+
LS-09	-	+	-	+
LS-11	+	-	-	+

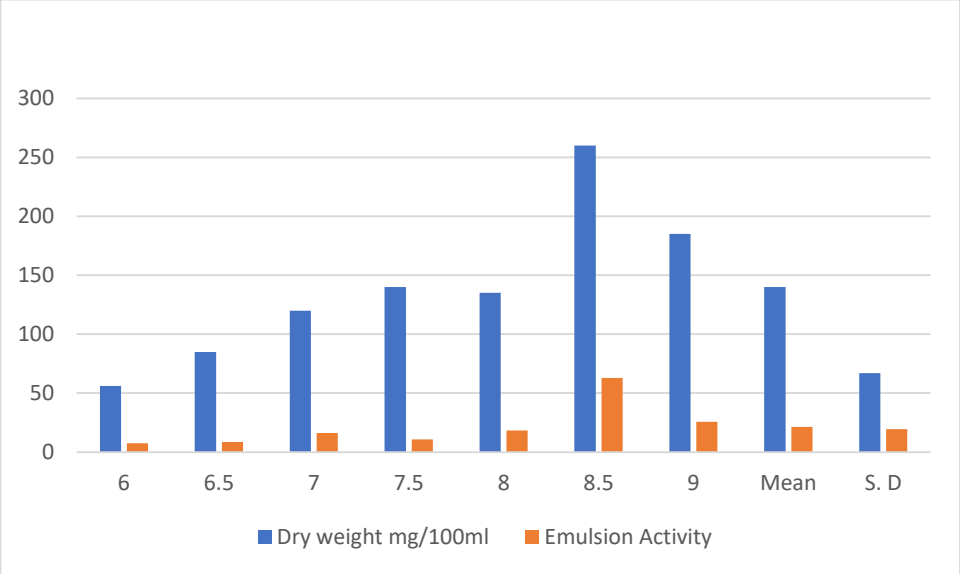


Graph showing results for screening methods for biosurfactant identification

Studies on isolates for Growth Optimization and Biosurfactant Production.

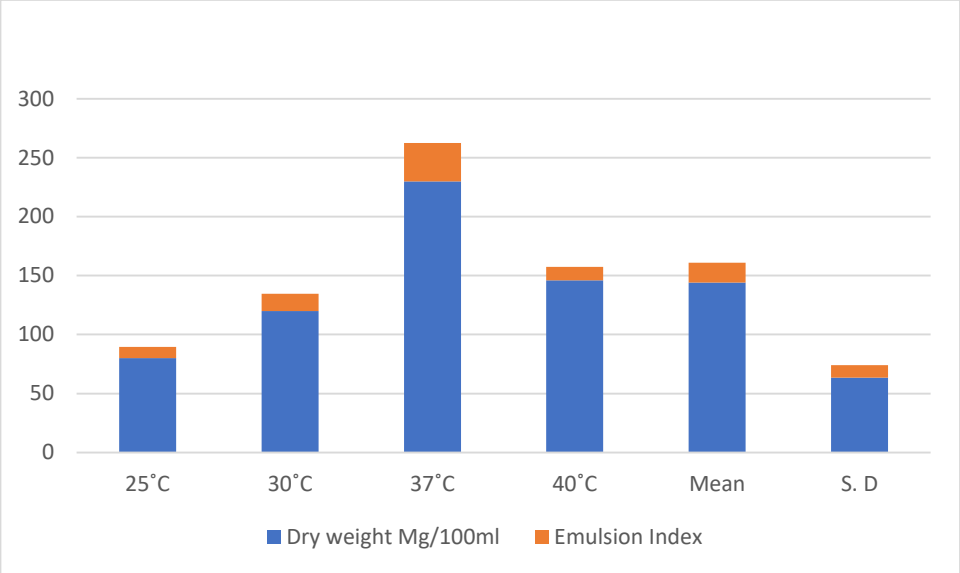
Effect of pH

Bacillus licheniformis was cultured in a controlled environment with a pH range of 5 to 8 for a period of seven days on MSM medium with 2% glucose. The purpose of this experiment was to investigate the impact of pH on the development and generation of biosurfactant.The Optimum growth was observed at pH 8.5



Effect of pH on growth and production of biosurfactant

Effect of Temperature:



Effect of Temperature on growth and biosurfactant production

Optimum growth was observed at 37˚ c.

.

In this study the factors affecting growth and biosurfactant production, their optimum values were concluded as follows:

pH-8.5

Temperature- 37˚C.

Incubation Period- 7 days

Substrate Concentration (Glucose)- 2%(w/v)

KNo3 concentration- 0.1%(w/v)

Chemical Composition of MSM Medium:

Kcl- 0.1gm

K2HPO4- 7.0gm

KH2PO4 – 3gm

CaCL2 – 0.01gm

MgSo4.7H2O - 0.5gm with 5ml of trace element solution

Content

Feso47H2O – 0.116g/l

H3BO3 -0.232g/l

CaCl2.6H20- 0.41g/l

CuSo4.5H2O - 0.008g/l

MnSo4.H2O – 0.008g/l

(NH4)6 Mo7024 – 0.022g/l

ZnSo4 – 0.174 g/l with 2% Glucose as a sole source of carbon for production medium.

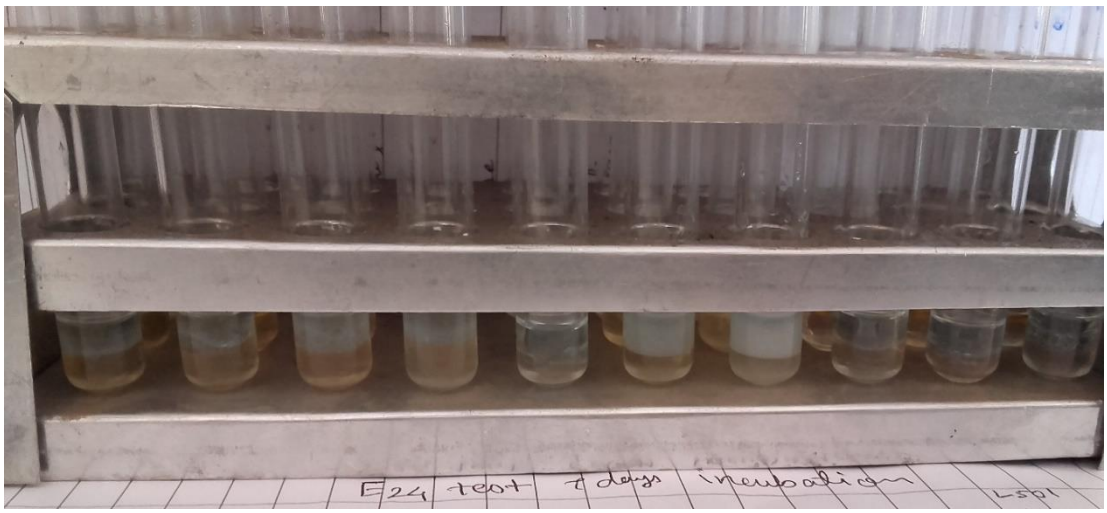


Fig: - E-1 index Activity.

Characterization of Extracted Biosurfactant

TLC Of Extracted Biosurfactant:

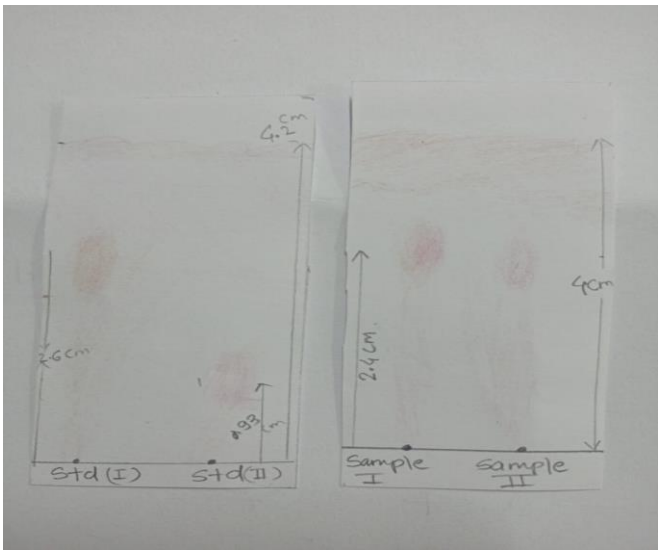
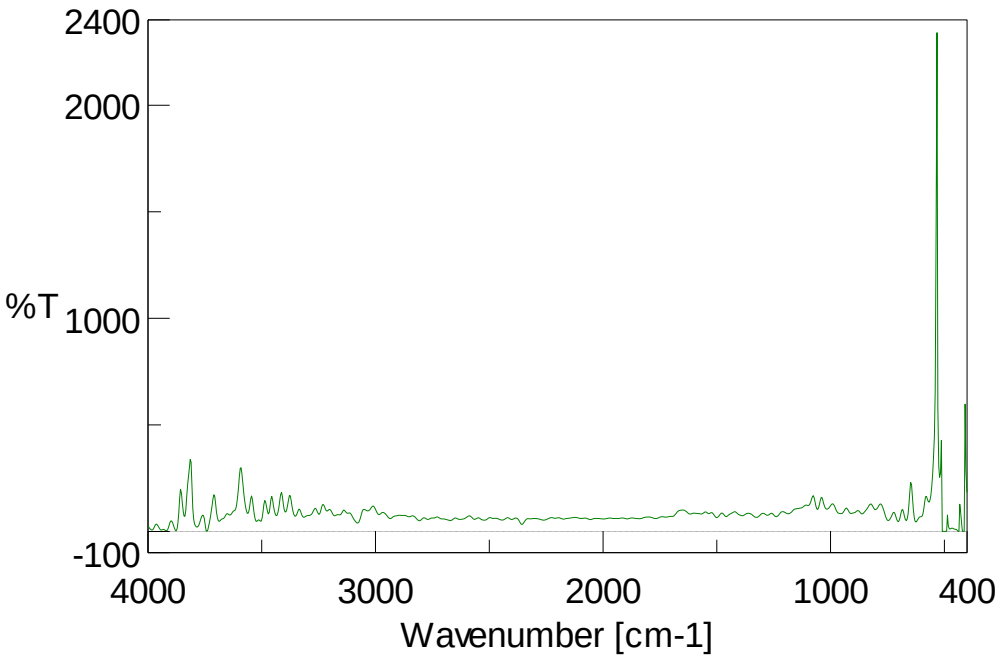


Fig 2:- TLC Result Std(1) LippopeptideStdStd (2)Glycolipids std. Sample (1,2) Cruide Supernatant Of Bio surfactant

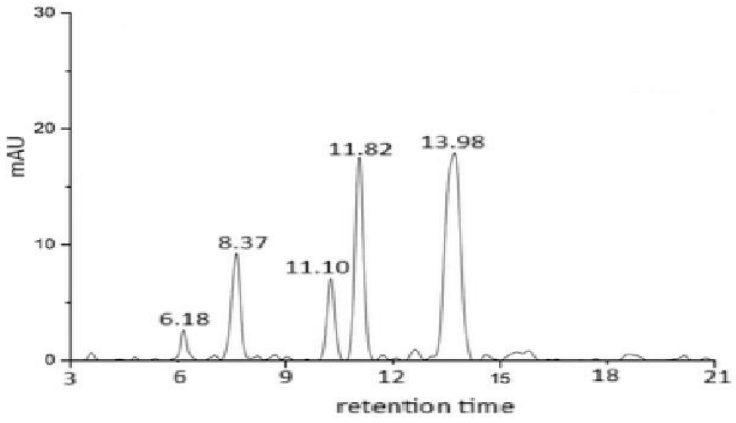
Obtaining Rf values for the identification of pure biosurfactant was accomplished by the use of the TLC approach.. The Rf value of 60 that was obtained for the biosurfactant that was extracted via the use of the TLC technique is in accordance with the usual Rf value of 67 that is associated with surfactin.

IR result of Extracted Biosurfactant:



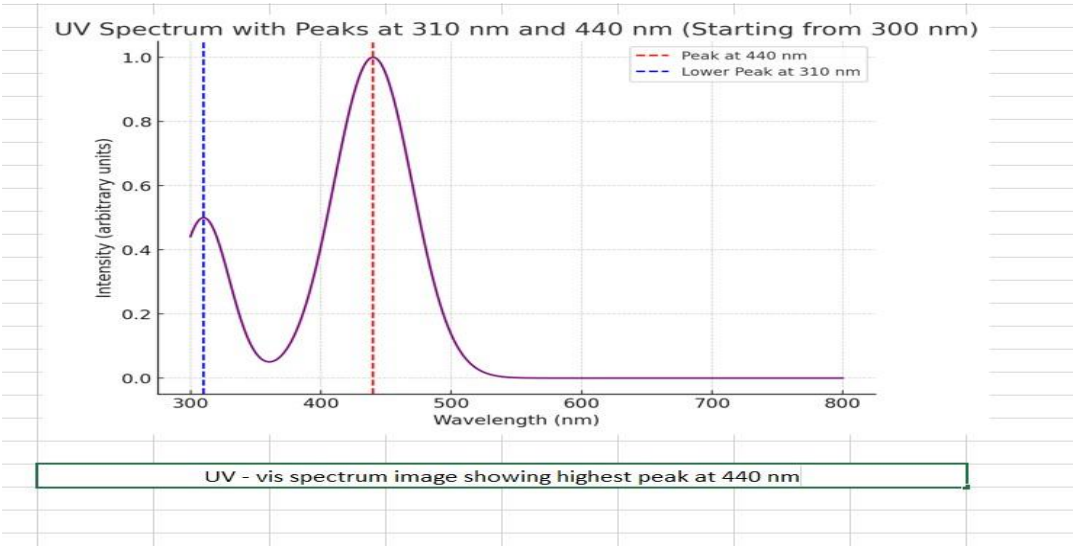
In accordance with the protocols of IR and HPLC, a qualitative analysis of biosurfactant was performed. Bands in the region of 4000-400 cm-1 were identified in the Fourier transform infrared spectra.

HPLC Result of Extracted Biosurfactant:



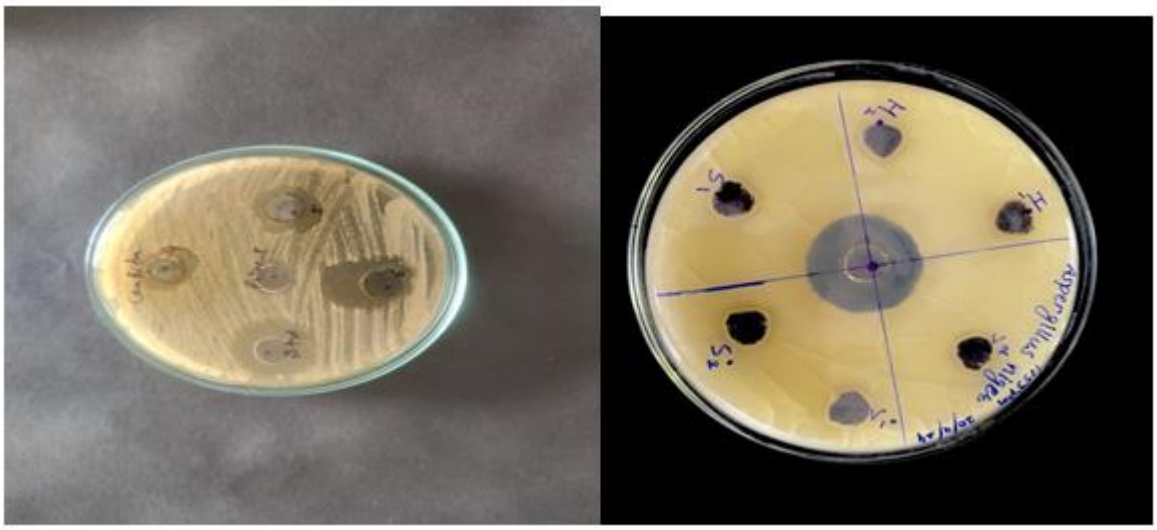
In HPLC peaks were observed at various Retention time (6.18,8.37,11.10,11.82,13.98).

Synthesis of AgNo3 Nanoparticles



UV Spectroscopic Analysis of Silver nanoparticles UV Spectroscopic Peaks observed at 310 and 440nm.

- Antibacterial properties of nanoparticles composed of AgNo3 For the preparation of it, the crude biosurfactant was used. In the centre, the zone of inhibition for E. coli is 30mm±1mm, whereas in the other zones, it varies from 30mm±1mm to 36mm±1mm.It has been shown that S. aureus does not possess a zone of inhibition against nanoparticles. When it comes to Pseudomonas species, the zone of inhibition measures 2(BS)-14mm ±1mm. 3 nanoparticles 16 mm ± 1 mm



- AgNo3 Nanoparticles' antifungal effects Made with the use of Rusty Biosurfactant Inhibition zone for Candida albicans is 19mm ±1mm at its maximum standard deviation.±1mm, 4-14mm.±1 millimetre for 5 to 16 millimetres.6–17 mm with a tolerance of ±1 mm. The zone of inhibition for the standard flucomazole against Aspergilus niger is 31-32mm ±1mm. Zones of inhibition are not shown by others.

- XRD Results for Nanoparticles Are Still Awaiting

CONCLUSION

Bacillus licheniformis, a bacterium that was isolated from the uniquely acidic and saline Lonar Lake, was responsible for the development of a biosurfactant that, according to a qualitative evaluation, had extraordinary surface-active features. According to the findings of the study, the very effective emulsification, foaming, and surface tension reducing properties of the biosurfactant demonstrated that it has the potential to be used in both industrial and environmental settings. Because of its low impact on the environment and its origin in microorganisms, biosurfactant is an excellent and environmentally responsible alternative to synthetic surfactants. Our findings emphasize the value of researching extremophilic bacteria from uncommon habitats such as Lonar Lake in order to advance biotechnology and propose creative solutions to environmental challenges. They also highlight the importance of environmental research. Synthesis of AgNo3 was easily carried out by using Extracted Biosurfactant where as, The formation of Np's was confirmed by UV Spectroscopic analysis.

Np's of AgNo3 synthesized by biosurfactant produced from bacillus licheniformis shows strong Antimicrobial as well as Antifungal activity against various Bacteria and Fungi.

REFERENCE

1. Lin S, Carswell KS, Sharma MM, Georgiou G. 1998b. Continuous production of

2. Lin SC, Lin KG, Lo CC, Lin YM. 1998a. Enhanced biosurfactant production by a

4. Moonjit Das,Debajit Borah, Kaustuvmani Patowary, Mayuri Borah, Ankita

   Khatanair,8741; 2019.

5. A simple thin layer chromatography based method for the quantitative analysis of biosurfactantsurfactinvis-a-vis the presence of lipid and protein impurities in the processing liquid BagcineleDlamini , VivekRangarajan*, Kim G. Clarke 1 Department of Process Engineering, Stellenbosch University, Private Bag X1, Stellenbosch, 7602, South Africa B. Dlamini et al.

6. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

7. Arima K, Kakinuma A, Tamura G (1968) Surfactin, a crystalline peptidelipid surfactant produced byBacillus subtilis: isolation, characterization and its inhibition of fibrin clot formation. Biochem BiophysRes Commun 31:488–494

8. *Bacillus licheniformis* mutant. Enzyme Microbiol Technol 23: 267–27

   Bibhuti bhusan Kakoti."Antimicrobial Activity of silver nanoparticles synthesized by

   a Biotechnol 41: 281–285.

9. Biria D, Maghsoudi E, Roostaazad R, Dadafarin H, Sahebghadam L, Amoozegar MA (2010) Purification and characterization of a novel biosurfactant produced by Bacillus licheniformis MS3. World J Microbiol Biotechnol 26:871–878

10. Burgos-Díaz C, Pons R, Teruel JA, Aranda FJ, Ortiz A, Manresa A, Marqués AM (2013) The production and physicochemical properties of a biosurfactant mixture obtained from Sphingobacterium detergens. J Colloid Interf Sci 394:368–379

11. Chooklin CS, Petmeaun S, Maneerat S, Saimmai A (2014) Isolation and characterization of a biosurfactant from Deinococcus caeni PO5 using jackfruit seed poder as a substrate. Ann Microbiol 64:1007–1020

12. Cohen SA, Michaud DP (1993) Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. Anal Biochem 211:279–287

Determining Pore Geometrical Characteristics in Soils of

13. Euzéby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol 47:590–592. Accessed 30 September 2014

14. Ghribi D, Ellouze-Chaabouni S (2011) Enhancement of Bacillus subtilis lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration. Biotechnol Res Int 2011:653654.

15. Gogotov IN, Miroshnikov AI (2009) The influence of growth medium composition and physicochemical factors on biosurfactant production by the bacterium Bacillus licheniformis VKM B-511. Appl Biochem Microbiol 45:588–592

16. Gudiña EJ, Rangarajan V, Sen R, Rodriguez LP (2013) Potential therapeutic applications of biosurfactants. Trends Pharmacol Sci 34:667–675

17. Jacques P (2011) Surfactin and other lipopeptides from Bacillus spp. In: Soberón-Chávez G (ed) Biosurfactants, from genes to applications. Springer, Berlin, pp 57–92

18. Jenny K, Kappeli O, Fiechter A (1991) Biosurfactants from Bacillus licheniformis: structural analysis and characterization. Appl Microbiol Biotechnol36:1669–1671

19. Jin-Feng Liu, Serge Maurice Mbadinga, Shi-Zhong Yang, Ji-Dong Gu and Bo-Zhong Mu "Chemical Structure, Property and Potential Applications of Biosurfactants Produced by Bacillus subtilis in Petroleum Recovery and Spill Mitigation" Int. J. Mol. Sci. 2015, 16, 4814-4837.

20. Joshi S, Yadav S, Nerurkar A, Desai AJ (2007) Statistical optimization of medium components for the production of biosurfactants by Bacillus licheniformis K51. J Microbiol Biotechnol 17:313–319

21. Kanlayavattanakul M, Lourith N (2010) Lipopetides in cosmetics. Int J Cosmetic Sci 32:1–8

22. Kumar, S.; Malik, R.S. Verification of Quick Capillary Rise Approach for

23. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A et al (2007) CLUSTAL W and CLUSTAL_X version 2.0. Bioinformatics 23:2947–2948

24. Li Y, Yang S, Mu B (2010) The surfactin and lichenysin isoforms produced by Bacillus licheniformis HSN 221. Analytical lett 43:929–940

25. Li Y-M, Haddad NI, Yang S-Z, Mu B (2008) Variants of lipopeptides produced by Bacillus licheniformis HSN221 in different medium components evaluated by a rapid method ESI-MS. Int Pept Res Ther 14:229–235

26. Llarch A, Logan NA, Castellví J, Prieto MJ, Guinea J (1997) Isolation and characterization of thermophilic Bacillus spp. from geothermal environments on Deception island, South Shetland Archipelago. Microb Ecol 34:58–65

27. Madslien EH, Rønning HT, Lindbäck T, Hassel B, Andersson MA, Granum PE (2013) Lichenysin is produced by most Bacillus licheniformis strains. J Appl Microbiol 115:1068–1080

28. Makkar RS, Cameotra SS. 2002. An update on the use of unconventional substrates for biosurfactant production and their new applications. Appl Microbiol Biotechnol 58: 428–434

29. Mittal and Sing. Isolation of hydrocarbon degrading bacteria from the soil contaminated with crude oil spills. Ind Expt Biol. 2009;47:760-765.

newly isolated Bacillus vallismortis MDU6 strains " IET Nanotechnology, ISSN 1751-

30. Patil JR, Chopade BA. 2001a. Distribution and in vitro antimicrobial susceptibility of Acinetobacter species on the skin of healthy humans. Natl Med J India 14: 204–208.

31. Patil JR, Chopade BA. 2001b. Studies on bioemulsifier production by Acinetobacter strains isolated from healthy human skin. J Appl Microbiol 91: 290–298.

32. Patil JR, Chopade BA. 2003. Bioemulsifier production by Acinetobacter strains isolated from healthy human skin. US patent No. US 2004/0138429 A1.

33. Rosenberg E, Zuckerberg A, Rubinovitz C, Gutnick DL. 1979. Emulsifier of Arthrobacter RAG-1: isolation and emulsifying properties. Appl Environ Microbiol 37: 402–408.

34. Tambekar DH, Pawar AL and Dudhane MN, Lonar lake water; Past and Present Nature Environ And Poll Technol.2010;9(2);217-221

the lipopeptide biosurfactant ob *Bacillus licheniformis* JF-2. Appl Microbiol

Varying Texture. Soil Sci. **1990**, 150, 883–888.