International Journal of Education and Science Research Review
E-ISSN 2348-6457
Volume-6, Issue-3 May- June 2019
P-ISSN 2349-1817
www.ijesrr.org
Email- editor@ijesrr.org

STUDIES ON BIOSURFACTANT PRODUCING BACTERIA ISOLATED FROM LONAR LAKE WATER.

Dr R.S.Awasthi,
Principal, shivaji mahavidyalaya,
Renapur,Latur.

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

Abstract- studies on Biosurfactant producing Bacteria isolated from Lonar Lake.

Lonar lake is situated in Buldhana District is a Big source of biological Diversity . It is world's third Largest ,Hyper Velocity Meterotic impact crater.It is unique in world for its alkalinity& salinity. Alkanity (pH 9.5) & salinity (6382mg/l).

Some Micro organisms produces Biosurfactant as their by product. Biosurfactants are Amphiphlic compounds they can reduce surface tension n can increase solubility of two immiscible fluids.

In the present study microorganisms isolated from lonar lake water were used to study biosurfactant production. water sample is enriched in MSM (Mineral salt medium). Oil coated plates were used to isolate efficient Bacteria, Hydrocarbon degraders were selected for further production..

Production medium ie MSM medium was used with 2% Glucose as a soul source of carbon. 7days incubation were followed on at 37®c , after every 24 hrs E-24 index were checked. At the end of 7thday comparative study was carried on. E-24 Index test , oil spread method tests were done. The efficient Bacterial culture was identified at its molecular level by 16s rRNAananalysis. The identified Organism Bacilluslycheniformis is under study, for further Qualitative and Quantitative tests of Biosurfactant.

Key words:-Biosurfactants, Hydrocarbon Degraders, Lonar Lake, Emulsification Index.

Introduction-

Lonar Lake situated In District Buldhana, India. is the World's 3rd Largest & the only Hyper Velocity Meteoritic Impact Crater formed about 50,000yrs ago.it is closed system,not having outlates and regular influents are responsible for lakes existence .TheDaimeter of Lake isaroud, 1.75km and a water enters in lake through rain , ground water seepage and there is no other industrial discharges received by lake (1).

Lonar lake having very high Alkalinity and Salinity, wich makes this lake Unique in the World.pH of lakes water is (PH- 9.5) and Salinityis(6388 mg/ltr).The Diversity of microbes were studied preliminarily by isolation and characterization of Microorganisms.(2.3.4.5)

Chemical and Biosurfactant's are Amphiphilic compounds which reduces interfacial and surface tention by accumulating at the interface of immiscible fluids and increase the solubility and mobility of Hydrophobic compound.(6.7.8) During the degradation of Hydrocarbons the degradative organisms produces an Amphibolic compounds that influenc the degradation rules . This compounds are known as Biosurfactants.

Because of there Low Toxicity ,Biosurfactants having Various Applications in Medical Industries, Oil reservoirs, Chemical Industries, food processing, Pharmaceuticals , And Envioronment Bioremediation(9).

Hence the objective of research is to Examine the Biosurfactant Production Potential of Optimise Bacterial isolates from Lonar Lake and Biosurfactant Producing Strange were further Characterise By Genetic methods using the 16s rRNA sequencing technique to find out the species level.

Fig 1-Lonar Lake Soil & Water Samples were collected.

Materials & Methods-

Sampling site and Sample Collection:-

Different samples were collected from different sites of Lonar Lake, Water samples were labelled as LS-01, LS-02, LS-03, LS-04, LS-05. Sediment sample were labelled as LSD-1 ,LSD-2,

LSD-3. The weter sample were collected in sterile Bottles while sediment samples were collected in sterile polythene Bags.

Chemical Analysis of the Sediments And Water samples:-

pH :- pH of all samples was recorded using digital ph meter Model No- ELICO L-1120

Alkalinity:- Alkalinity was determined by potentiometric titration method,using sodium carbonate as a Std and phenoplthalein as an indicator, Against 0.1 N sulphuric acid as a described in Stdmethods.Alkalinity was deteremined in terms of CaCo3(10, 11).

Salinity:- Salinity was determined in terms of both Cl^- and Nacl by usingArgentometric method. In which std AgNo3 used as a titrant as described in stdmethods(Grenberg et al.1992),(11).

Enrichment & isolation of Bacteria:-

- Enrichment of sample - sediment (1gm) and water (10ml) samples from Lonar Lake inoculated separately in 250 ml conical flasks, containing 100ml mineral salt medium .

- **Composition of Mineral Salt Medium (MSM) g/l -**

NaNo3- 2.5gm

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% Fresh soyabenes

Oil as a sole source of carbon (ll)

All the samples were insulated at 37'C for 7days at 150 rpm on Rotary shaker

Isolation and Biochemical characterization.:-

After the enrichment of samples (72hrs) the samples were inoculated on solid nutrient agar plate. Well isolated & morphologically different colonies were selected for further study.

Isolated colonies were characterised by standard Biochemical test according to Bergeys Manual of systematic bacteriology (Systematic Bacteriology By Bergeys section 13, Vol-2 BAMU Library.)

Testing of Biosurfactant Production from Bacteria:-

1) **Oil Coating Plates Technics**- Oil coated MSM medium plates were prepared without using any other carbon source (12) used for the test.
   Cultures grown on oil coated plats were selected for BS production. MSM medium was prepared by using 2% glucose as a carbon source.
   Selected colonies were inoculated in production medium for 7 days on 150 rpm on rotary shaker. BS activity was checked every day various confirmative testes were followed for BS production like oil spreading method, Emulsification index (E24).

Analysis of Biosurfactants:-

1) Solubility Test- Small amount of isolated biosurfactant was taken in three test tubes and water, alcohol & chloroform was added to each tube their solubility was tested.
2) Saponification Test- 2ml of 2% NaOH solution was added to the small amount of Biosurfact& shaken well the formation of soup was observed (14)
3) Oil Spreading Method:- 50mililiter of distlled water added to the petridish 20µl of oil was added in it, thin layer was allowed to form on surface. 10µl of cell free supernatant was dropped on surface of oil.The diameter of zone of clearance of oilsurface was measured.(15)
4) Emulsification Index(E_{24}):- Confirmation of biosurfactant production as cheked by E24 test by adding 2ml Oil and 3ml of culure supernatant centrifuge this mixture for 2min with high speed and leave it to stand for 24hrs.(16,17)

$$E_{24} = \frac{\text{Height of emulsion layer}}{\text{Height of total solution}} \times 100\%$$

5) Surface Tention Measurement:- Surface Tension was measured by Tentiometer Distilled water was used to compaire results(15)

16 S rRNA sequencing &phylogenetic analysis along with BS producingbacterial isolated biochemical tests & 16S rRNA sequencing.

Results and Discussions:-

For the study of Biosurfactant producing bacteria from Lonar Lake total 8 samples were examined from sediment & water samples. Samples was collected in Winter season Nov 2013 and enrichment of samples was followed on MSM medium containing oil after enrichment sub culturing was done for 4-5 times.
Isolated bacteria was used for production of Biosurfactantby using MSM medium with 2% glucose without Oil, Bacteria were analysed for standard biochemical test and further identified by 16S rRNA sequencing as Bacillus Lychemfarmus.
Biosurcant producing bacteria were preliminary isolated by oil coated plate method.

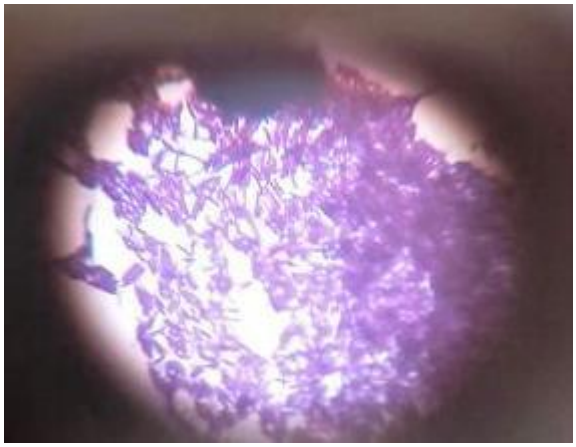


Fig-2 Gram Positive rods
Fig-2 showing Gram Positive Rods Efficient Bacteria ForBiosurfactant Production

Table No-1

Colony Characteristic	I	II	III	IV	V	VI	VII	VIII	IX	X
Size (cm)	0.5-0.7	0.1-0.2	0.7-0.8	0.3-0.6	0.5-0.8	0.3-0.6	O.8-1.0	0.2-0.3	0.7-0.8	1.0-1.3
Shape	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
Colour	White	White	pale yellow	White	pale yellow	White	pale yellow	White	pale yellow	White
Consistency	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid
Margin	Irregular	Regular	Regular	Entire	Irregular	Regular	Regular	Regular	Regular	Regular
Elevation	Flat	Raised	Flat	Flat	Flat	Raised	Raised	Raised	Raised	Raised
Gm Nature	Gm +ve	Gm +ve	Gm +ve	Gm +ve	Gm +ve	Gm-ve	Gm-ve	Gm +ve	Gm +ve	Gm-ve
Motility	Motile	Non Motile	Motile	Motile	Motile	Non Motile	Motile	Motile	Non Motile	Motile

Colony Characteristic	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
Size (cm)	0.3-0.7	0.1-0.4	0.7-0.9	0.3-0.6	0.5-0.8	0.3-0.7	0.4-0.9	1.0-1.2	0.2-0.4	0.7-1.0
Shape	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
Colour	White	pale yellow	pale yellow	White	White	White	White	White	pale yellow	pale yellow
Consistency	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid
Margin	Irregular	Entire	Irregular	Regular	Entire	Regular	Regular	Irregular	Regular	Entire
Elevation	Flat	Flat	Flat	Raised	Raised	Raised	Flat	Raised	Flat	Flat
Gm Nature	Gm-ve	Gm +ve	Gm +ve	Gm-ve	Gm +ve	Gm +ve	Gm +ve	Gm +ve	Gm-ve	Gm-ve
Motility	Non Motile	Motile	Non Motile	Motile	Non Motile	Motile	Motile	Motile	Motile	Motile

Table No-1 showing Biochemical Characterization of Isolates of Lonar Lake. Selected Isolates were followed by Oil Coated Plates Method for Hydrocarbon Degradation.

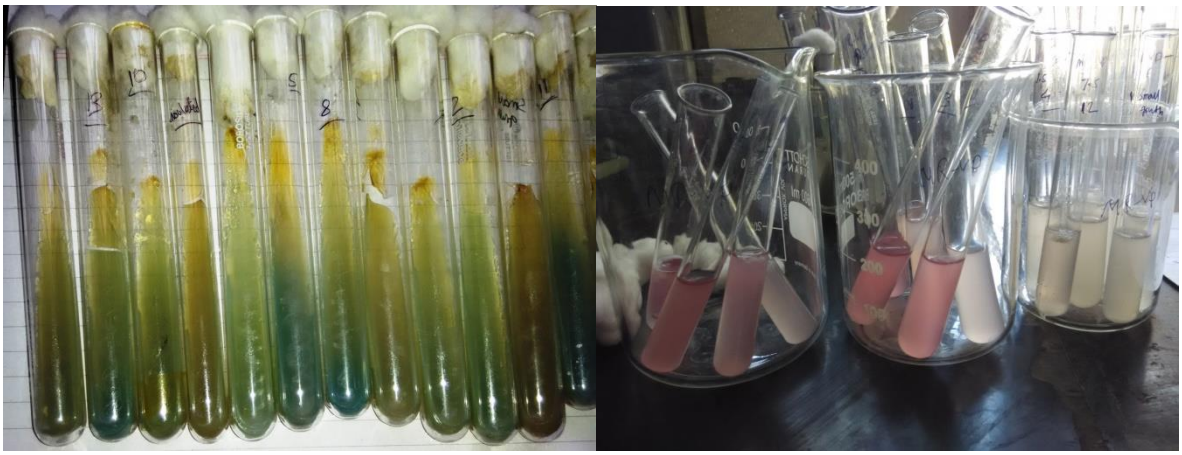


Fig-3 Images Of Biochemical Characterization of samples

Table No-2

Isolates	Hallow Zone Appeared on Oil Coated Plates
1	Negative
2	Positive
3	Negative
4	Negative
5	Positive
6	Negative
7	Negative
8	Negative
9	Positive
10	Negative
11	Positive
12	Negative
13	Negative
14	Negative
15	Negative
16	Negative
17	Negative
18	Positive

Table No-2 Showing results of Hydrocarbon degrading organisms. Total 5 organisms showing Hallow Zone which indicates oil degradation.

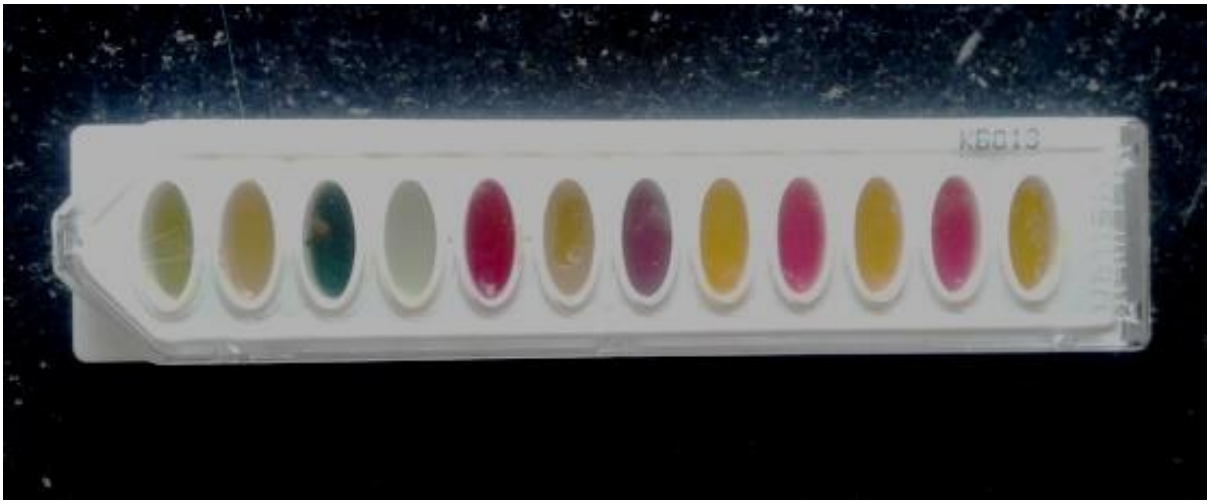


Fig 4- Bacillus Confirmative Test Kit (Hi-media)

Table No-3

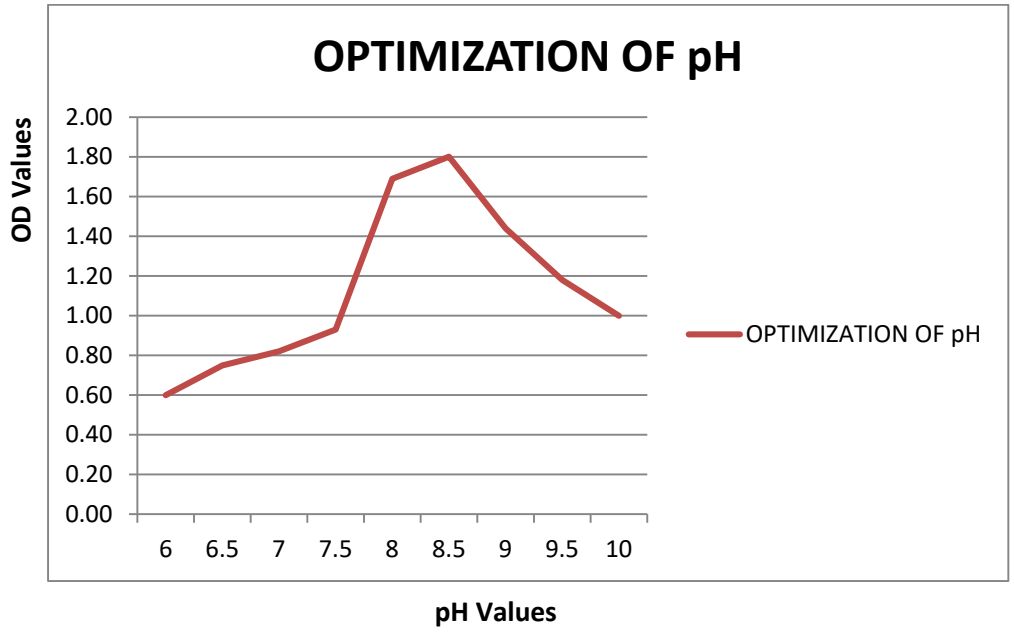


Table No-4

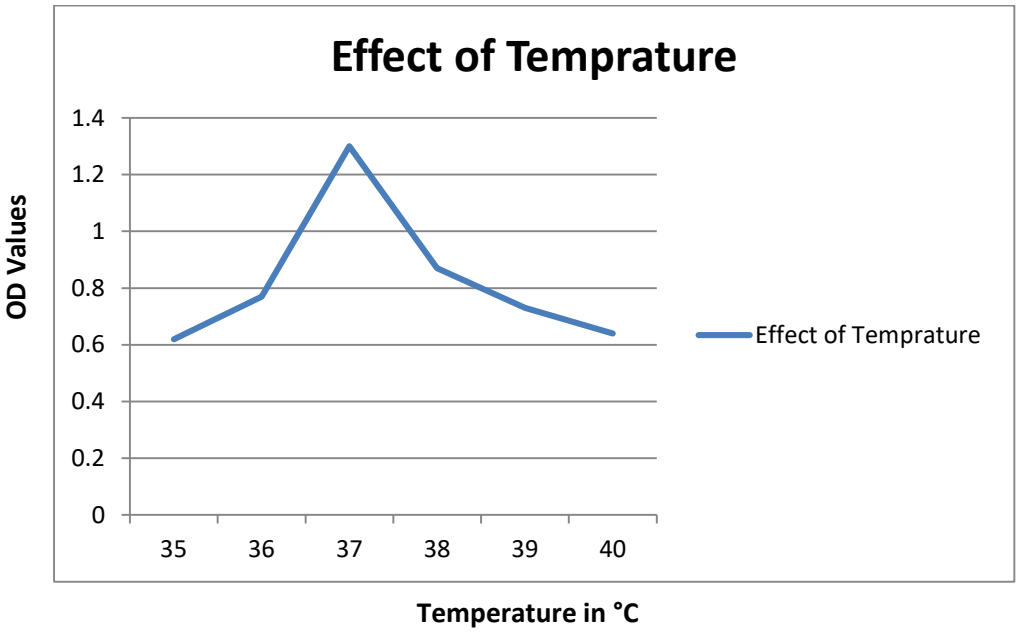


Table no 3- Showing results of effect of pH on growth of Bacteria Hence it is concluded that Bacteria Shows Highest growth at pH 8.5.

Table no 4- Showing results of effect of Temperature on growth of Bacteria Hence it is concluded that Bacteria showing Highest growth at 37'C.

Table No-5Table No-6

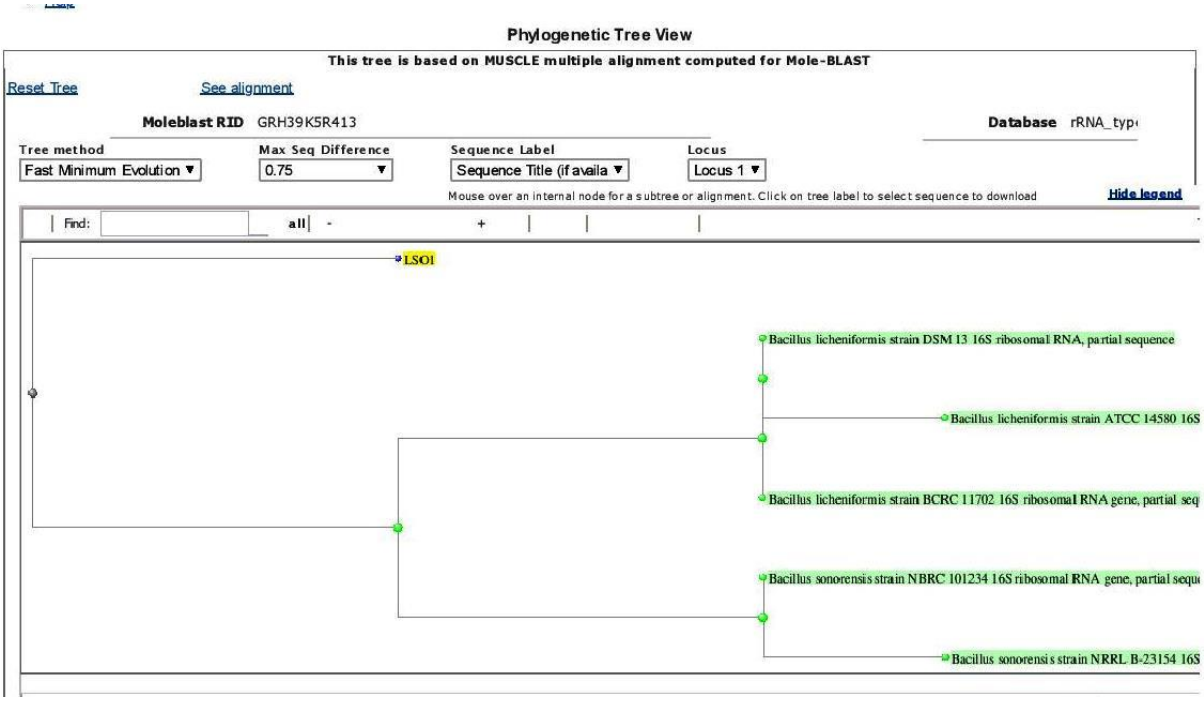
Isolates	Emulsification Activity (OD at 540 nm)	E24 (%)
1	0.11	31.2
2	0.17	39.8
3	0.54	52.6
4	0.18	39.2
5	0.23	44.3

Hydrocarbons	Emulsification Index	Emulsification Activity
Kerosene	52.6	0.38
Petrol	42.81	0.24
Diesel	39.73	0.19

Table No-5 &6 showing results of Bio-surfactant production. After 7days Incubation period E-24 Index were checked with OD. Efficient Organism shows highest E-24 Index i.e 52.6%. E-24 Index were checked by using different Hydrocarbons of efficient organism.

Hence organism which gave highest E-24 index was selected for further study. Selected Isolate was studied at molecular level as 16s rRNAsequencing .The sequences of representative isolates were used for phylogenetic analysis. The sequences available in the GenBank database by BLAST (Youtub).

Supernatant of Purified Biosurfactant was studied using different methods of identification



After 16SrNA sequencing of a Bacterial Culture which shows Best Result for the production of Bio surfactant Using 2% Glucose As A sole source of carbon Phylogenetic Sequence was obtained with the help of Mol Blast NCBI. Hence the organism is confirmed that Bacillus Licheniformis.

Table No 7 :- Properties of selected biosurfactant Producing Bacteria

Isolate	Oil Spreading Method	Emulsification Index test	Saponification test	ST of Supernatant (mN/m)	ST of Media With Oil (mN/m)

LS-O3	+ve	52.06	+ve	54.34	65.70

Table no-7 showing results for the properties of selected bio surfactant producing Bacteria, It gives positive results for Oil Spreading method, saponification test,And Emulsification Index 52.06mN/m . Reduces the surface tention of supernatant 54.34% after 3-4 days incubation which was initially 65.70mN/m of Media with oil.

TLC:-

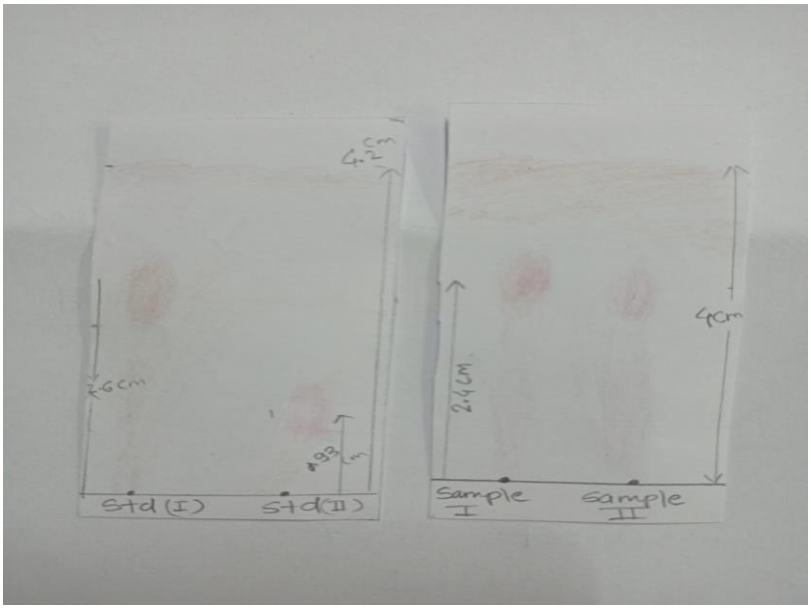


Fig 5 :- TLC Result Std(1) LippopeptideStdStd (2)Glycolipids std. Sample (1,2) Cruide Supernatant Of Bio surfactant

For a detection of purified Biosurfactant TLC Technique was used to obtain Rf values. On 1st TLC plate Standard 1 Lippopeptide and Standard 2nd Glycolipids were used as standards. On 2nd TLC plate Purified Biosurfactant (Using Acid Precipitaion Method) were used as a solute. Using Solvent system Chloroform,Methanol, and Acetic Acid respectively in a ratio of 65:15:2. With Developing Agent Ninhydrine and Iodine(18)

Rfvalue :

Name of sample	Standard 1	Standard 2	Purified Biosurfactant
Rf Value	0.67	0.22	0.60

Rf Values indicate that standard 1 (Surfactine) And Rf value of Purified Biosurfactant shows similar Rf values so It can be concluded that the purified Biosurafactant resembles with Surfactine. The purified Biosurafctant is under Study for Applications.

References:-

1. Thakkar CD and Ranade Dr. AlkalophilicMethanosarcina isolated from Lonar lake. Currsci 2002;82;455-458.
2. Jones BE, Grant WD, Duckworth AW and Owenson GG. Microbial diversity of soda lakes, Extremophiles 1998;2;191-20.

3. HorikoshiK , alkalophiles:some applications o their products for biotechnology. MicrobiolMol Bio Rev.1999;63:735-750.
4. Joshi AA, KanekarPP,Sarnaik S, and Kelkar A. Bacterial Diversity of Lonar lake ecosystem. In :Banker SK, Mishra VR (Eds)Biodiversity of Lonar Crater Anamaya , New Delhi,2005;71-75.
5. TambekarDH ,Pawar AL and Dudhane MN, Lonar lake water; Past and Present Nature Environ And Poll Technol.2010;9(2);217-221.
6. Prince R. C . 1997. Bioremediation of Marine Oil Spills: Trends Biotech; 15;158-60.
7. Ron E.Z and E Rosenberg.2001, Natural roles of Biosurfactants , Environ Microbiol; 3:229-236.pubmed doi:10. 1046/j 1462-2920. 2001.
8. Mulligan, C.N 2005. Environmental applications of biosurfactants. Environ pollut 2005;133:183-98.
9. Muthusamy,K.S. Gopalkrishnan, T. K. Ravi AndP.Sivachidambaran, 2008, Biosurfactants; properties, Commercial production and application, curr. Sci, 94;736-74.
10. Greenberg A. E, Clesceri L.S, Eaton A.D, (1992). Standard Methods for the examination of water and waste water , 18thEdn. Published Jointly by APHA, AWWA, and WPCF, Washington DC.
11. American public Health Association(APHA): Standard Methods for the examination Water and Waste water 23rdEdn. 2320;2-36-37. 2520; 2-59-62.
12. TH Tambekar, PN Dose,SR. Gunjankar And PV. Gadakh; studies on Biosurfactant production from Lonar lakes Achromobacterxylosoxidons Bacterium; IJAPBC-vol(3), Jul-Sept,2012; ISSN:2277-4688:416.
13. Morikawa M, Hirata Y Imanaka.T.2000. A study on the structure- functution relationship of lippopeptidebiosurfactants. BiochemBiophysic ACTA 1488:211-218.
14. Bhavani T.M.B and Hemashenpagam .N.2013.production of Biosurfactant and Characterization by 16s rRNA sequencing technique of Bacteria Degrading Hydrocarbon isolated from petroleum contaminated soil ; Vol-1;300-305
15. SudiMR,Nasr S, Mehrnia MR and Sarrafzadeh MH. Characterization of novel biosurfactant producing strains of Bacillus spp. Isolated from petroleum containing soil. Iran J Microbiol.2009;1(2):54-61.
16. Lu Yi-tong, Chen Xiao-bin, Zhou Pei and Li Zhen-hong . Screening on oil decomposing microorganisms and application in organic waste treatmentmachine. J Env Sci. 2005; 17(3):440-444.
17. Mittal and Sing. Isolation of hydrocarbon degrading bacteria from the soil contaminated with crude oil spills. IndExpt Biol. 2009;47:760-765.
18. 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.*