Production and Characterization of biosurfactant from garage soil

[1]Nair Sreecha Chandran,[2]Prabha Kumari.C,
[1]Research Scholar,[2] Dept. Principal Scientist
Biotechnology department
CEPCI Laboratory and Research Institute, Kollam,Kerala.

Abstract : Biosurfactants are surface active compounds that reduce the interfacial tension between two liquids,or that between a liquid and a solid. Their unique property like non toxic easily biodegradable,eco-friendly and high stability and wide variety of industrial applications makes them highly useful group of compounds.Biosufactants are produced from variety of microorganisms.The objective of the study was to isolate and characterize the biosurfactant producing bacteria from oil contaminated soil and study their effect of biosurfactant production at different physiological parameters like ph, carbon source,nitrogen source,fermentation substrates and metal ions.The isolation and production study was carried out in Minimal salt medium.The isolated strain was identified as Bacillus velezensis using 16S rDNA procedure.The optimal biosurfactant production was analysed by OD at 660nm,which showed the optimal Ph as 9,carbon source as lactose,nitrogen source as peptone, fermentation substrate as kerosene and best metal ion as manganese sulphate.

Keywords **- Biosurfactants, eco-friendly ,microorganism,biodegaradable**

1.INTRODUCTION

Microorganisms can produce a wide range of extracellular products with many properties and applications.Biosurfactants are one such extracellular –surface active compound produced by various bacteria, yeast,fungi ,algae etc.They are amphiphilic compounds,which reduce surface and interfacial tension by accumulating at the interface of immiscible fluids and thus increase the solubility and biodegradation of hydrophobic or insoluble organic compounds(*Van Hamme et al.,2006*).Many bacterial genera have been reported to produce different types of biosurfactants.Based on the chemical nature there are different types of biosufactants ,such as glycolipids,lipopolysacharides,oligosaccharides and lipopeptides(*Benet I M .,2010,Franzetti A.,2010,Mulligan C N.,2005*).Nowadays,crude oil is one of the essential source of energy in our developing world.But the accidental or deliberate release of crude oil to the enviorment causes dramatic pollution problems.These problems may result in huge disturbance of both the biotic and abiotic components of the ecosystem(*Miller.,1995).*Biosurfactants are reported to be gained attention in the field of environmental remediation process such as dispersion of oil spills,treatment of wastes etc(I M *Banat.,1995, S.J Joshi et al.,2010*).They are efficient remediation agents,with environment friendly characteristics such as low toxicity and high biodegradability(*Mulligan CN.,2005; Das et al.,2009*) .They have better foaming properties and higher selectivity.They are active at extreme temperatures ,ph and salinity as well and can be produced from industrial waste and from byproducts.And thus,biosurfactant production is cheap and and allows utilizing waste substrates and reducing their polluting effect at the same time(Kosaric N.,2001,Rahman KSM et al.,2003).Biosurfactants are also a potent antimicrobial agent ,and this application can be exploited against plant,animal and human microbial pathogens(*Vann Hammeet al.,2006*).Thus they have many advantages over their chemically synthesized counter parts.Properties like biodegradability ,low toxicity, biocompatibility pave way to their applications in cosmetics ,pharmaceuticals and as functional food additives too. The objective of the present work is to isolate biosurfactant producing bacteria from oil contaminated soil , followed by optimization process to suggest the best medium for biosurfactant production.

2..Materials and methods

2.1.Microorganism and culture conditions

Samples were collected from oil exploration area of Chathanoor, Kollam.Five gram of collected sample was then suspended in 100ml sterile normal saline and mixed well.Samples(2ml) were taken from the upper phase of suspension to inoculate a 100ml minimal salt medium(MSM) containing (g/L): KH_2PO_4(1.0), $MgSO_4$(0.5), $FeSO_4$(0.01), $NaNO_3$(1.5),$CaCl_2$(0.002), NH_2SO_4(1.5),1% of kerosene supplemented as the sole carbon source in a conical flask.Prior to the addition of sample,the ph of

the medium was adjusted to 7.0 and sterilized by autoclaving for 20 min.Cultures were then incubated in rotary shaker incubator for 72 hr as an enriched method to isolate potent biosurfactant producing bacterium.Pure cultures were obtained through serially diluting the culture and spread plating 100 µl on MSM agar plates incubated at 37°C for 24 hr.This isolate has been identified based on 16S-rRNA.The pure bacterial culture was subsequently preserved at 4°C and subculturing was done every month.

2.2. Inoculum preparation for biosurfactant assay

Seed culture were prepared by transferring a loop-full of selected isolate to a 250ml Erlenmeyer flask containing 50 ml nutrient broth medium and incubation at 37°C for 24hr .Then 1% of bacterial inoculums was transferred to 250ml flask containing MSM supplemented by kerosene(1%) and incubated for 24h and samples were taken asceptically followed by centrifugation and supernatant used for biosurfactant assays.

2.3.Biosurfactant productivity tests

2.3.1.Oil spreading method

Oil spreading method was carried out by method described by *Morikawa et al.,2000*.In this,50ml of distilled water was added to petri plate followed by the addition of 100µl of diesel(crude oil) to the surface of the water.Then 10µl of cell free culture broth(as mentioned above) was dropped on the crude oil surface. Observe the flatness of mineral oil droplet in water after 1 minute compared to negative control(MSM without bacteria).

2.3.2.Emulsification index(EI%)

In order to demonstrate emulsification index,about 2ml of diesel added to equal volume of supernatant and vigourously mixed for 2 min in vortex and incubated for 24 hr to obtain the E24.Emulsification Index(EI%) was then calculated using equation:

EI(%) = Total height of emulsified layer/total height of liquid layer ×100(*Chandankere et al.,2013*)

2.4.Identification of bacterial isolate

The most efficient biosurfactant producing bacterial isolate was then identified using 16S rDNA analysis procedure ,performed in RGCB,Tvm.The obtained DNA sequence was then matched with deposited genes of Gene Bank using basic alignment search tool(BLAST).

2.5.Optimization for biosurfactant production

The bacterial culture was grown in nutrient broth(13g/l)for 20-24hr at 37°C and 2%(v/v)of inoculums was used for production of biosurfactant using MSM.All experiments were carried out in 250ml flask containing 100ml Minimal Salt medium.

2.5.1.Effect of different parameters on biosurfactant production

Different parameters used in the optimization process are: ph,carbon source, nitrogen source, fermentation substrates and metal ions.To investigate the effect of pH on biosurfactant production ,the intial ph was adjusted to 5.0,6.0,7.0,8.0,9.0 using 1N NaOH and 1N HCl.The 100ml production medium (MSM)with 2% of inoculums was grown at 37°C at 125 rpm for 5 days.Samples were collected for every 24h centrifuged and supernatant analyzed for biosurfactant production by checking OD at 660nm.To identify the best carbon source namely sucrose,glucose ,fructose ,lactose and maltose were supplied to each 100 ml production medium with the intial best ph,and 2% inoculums,analyzed the result after 24hr as intial method.To evaluate the appropriate nitrogen source for biosurfactant production the basic nitrogen source in msm, *sodium nitrate* was eliminated and added equimolar concentration of other nitrogen sources like urea,yeast,malt extract,peptone and beef extract ,along with the best carbon source analyzed the result after 24 hr.Then we investigate the effect of various fermentation substrates like olive oil,kerosene,glycerol petrol and diesel in the above similar manner.Finally, checking the effect of different metal ions:Zinc chloride,Potassium chloride,magnesium sulphate,Aluminium sulphate and manganese sulphate on biosurfactant production was analysed at 24 hr.

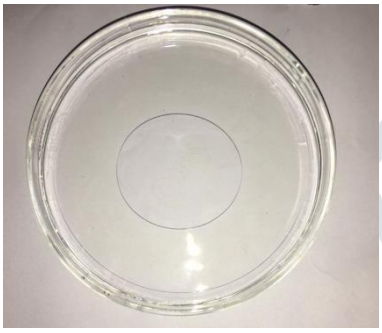
3.Result and discussion

Biosurfactants are attracting a pronounced interest owing to their potential advantages over their chemical counter parts(Eduardo et al.,2015).

3.1.Identification of efficient biosurfactant producer

Molecular identification showed that the isolate was closely related to *Bacillus velenzensis* with similarity of 100%.

3.2.Biosurfactant production tests



a)

b)

a) Biosurfactant positive *Bacillus velenzensis* showing oil displacement.

b)Emulsification test for *Bacillus velenzensis*

Oil displacement test the bacterial culture could ,displaced oil an area of 4.15cm^2.Maximum emulsification activity(E_{24}) test obtained 54.05% of emulsification.

3.3.Optimization of medium

3.3.1Effect of intial pH on biosurfactant production:

To study the effect of intial pH on biosurfactant production by *Bacillus velenzensis*, the intial pH of the medium was adjusted to 5.0,6.0,7.0,8.0 and 9.0 with the addition of 2% inoculum. To ensure the biosurfactant production , samples at 0,24,48 and 72 hours were centrifuged at 10000 rpm for 20minutes and supernatant were subjected to optical density reading., it was observed that biosurfactant production depends on pH and the bacterial strain showed maximum production at pH 9 at 24 hours(1.0550 OD units).After 48 hours the rate of production decreased.

3.3.2Effect of different carbon sources on biosurfactant production:

The effect of different carbon sources such as maltose,glucose,fructose lactose and sucrose on biosurfactant production by *Bacillus velenzensis* was studied.The pH of the medium were adjusted at 9,at which the maximum production obtained earlier.About 2% culture inoculum were added and the samples were analyzed for 24 hr.The supernatant of fermented sample was subjected to optical density reading. Among the tested carbon source ,lactose produced maximum of 0.699 OD units .This confirmed that maximum biosurfactant production can be obtained using lactose as the carbon source.Hence, lactose of 2%(w/v) was used as carbon source for further studies.

3.3.3.Effect of different nitrogen sources on biosurfactant production:

Effect of different nitrogen sources such as urea,yeast,malt,peptone and beef extract on biosurfactant production were studied by replacing the intial nitrogen source,Amonium Sulphate from MSM.The medium was supplemented with lactose as carbon source and concentration of nitrogen source as 1.5%(w/v) ,Ph 9 and 2% inoculum.After 24 hr ,the supernatant subjected to optical density reading. Peptone produced 0.3584 OD units ,which was the best among all nitrogen source tested.Hence peptone used as the nitrogen source for further studies.

3.3.4Effect of fermentation substrates on biosurfactant production

Effect of different fermentation substrates such as olive oil,kerosene ,diesel,petrol,and glycerol on biosurfactant production were studied.About 2% of fermentation substrates were used,with medium ph as 9 and 2% inoculum.Samples were collected at 24 hr,centrifuged and supernatant subjected to optical density reading. Among the substrates used,kerosene produced maximum of 0.3616 OD units.Hence kerosene used as the best fermentation substrate for further studies.

3.3.5.Effect of different metal ions on biosurfactant production

Effect of different fermentation substrates such as Zinc chloride,Potassium chloride,magnesium chloride,Aluminium sulphate and Manganese sulphate on biosurfactant production were studied.0.2% of metal ions,and 2% inoculums were added and analysed at 24 hr.The sample was centrifuged and supernatant subjected to optical density reading. The highest production obtained for Manganese Sulphate ie,0.053 OD units.Thus ,the best metal ion could used was concluded as Manganese sulphate.

CONCLUSION

Biosurfactant have a potential in bioremediation and in treatment of oilspills .In this study the isolated Bacillus strain were tested for its biosurfactant activity using oil displacement and emulsification activity.Both assays showed positive results ,followed by identifying the best medium conditions for biosurfactant production.The study revealed that among the used parameters,the medium with ph 9 showed maximum production at 24 hours,the best carbon source as lactose ,nitrogen source as peptone,fermentation substrate as kerosene and metal ion as manganese sulphate.

ACKNOWLEDGMENT

We are thankful to CEPCI Laboratory for allowing us to carry out this work.

Reference

- Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J., Marchant, R., 2010. Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol. 87, 427-444.
- Chandankere, R., Yao, J., Choi, M.M., Masakorala, K., Chan, Y., 2013. An efficient biosurfactant-producing and crude-oil emulsifying bacterium Bacillus methylotrophicus USTBa isolated from petroleum reservoir. Biochem. Eng. J. 74, 46-53.
- Eduardo J. Gudiña, Elisabete Rodrigues, A. Teixeira, L.R. Rodrigues"Biosurfactant production by *Bacillus subtilis* using corn steep liquor as culture medium Fornt. Microbiol. (2015), 10.3389/fmicb.2015.0005.
- I M. Banat, "Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review," Bioresour. Technol. 51, pp. 1-12, 1995.
- Miller, R.M. 1995. **Biosurfactant** – facilitated remediation of metal– contaminated soils. Environ. Health Persp.103 (1): 59-62.
- Morikawa, M., Hirata, Y., Imanaka, T., 2000. A study on the structure–function relationship of lipopeptide biosurfactants. Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids. 1488, 211-218.
- Mulligan CN (2005) Environmental applications for biosurfactants. Environ Pollut 133: 183-198.

- N. Kosaric**Biosurfactants in industry.**Pure Appl. Chem., 64 (11) (1992), pp. 1731-1737.
- P. Das S,Mukherjee, and R. Sen, "Rapid quantification of a microbial surfactant by a simple turbidometric method," J. Microbiol. Methods, vol. 76, pp. 38–42, 2009.
- Rahman, K.S.M., Rahman, T.J. McClean, S. Marchant, R. andBanat, I.M. 2002. Rhamnolipd **biosurfactant** production bystrains of Pseudomonas aeruginosa using low cost rawmaterials Biotechnol. Prog.18(6):1277-1281.
- S J. Joshi and A. J. Desai, "Biosurfactants: Role in bioremediation of NAPL & fermentative production," In: Biosurfactants, (Ed. R. Sen), 2010, Springer and Landes Bioscience publications, USA, pp. 222-235.
- Van Hamme JD, Singh A, Ward OP (2006) Physiological aspects: Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv 24: 604-620.