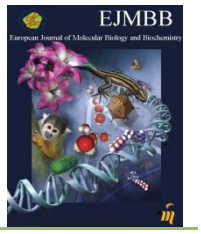
e - ISSN - 2348 - 2206
Print ISSN - 2348 - 2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



PRODUCTION AND OPTIMIZATION OF BIOSURFACTANT FROM MICROORGANISMS ISOLATED FROM WINDMILL OIL CONTAMINATED SOIL SAMPLES

K.Mohan[1*] and Dorisa Jenifer[2]

[1]Professor, Department of Bio-technology, Udaya School Engineering, Vellamodi, K.K Dist, Tamil Nadu, India.
[2]P.G. student, Department of Bio-technology, Udaya School Engineering, Vellamodi, K.K Dist, Tamil Nadu, India.

Article Info

Received 23/05/2014
Revised 16/06/2014
Accepted 19/06/2014

Key words:-
optimization, low toxicity, specificity, biodegradability, chemical surfactants, detergents.

ABSTRACT

Isolation and screening of extracellular biosurfactant producing bacteria from windmill oil contaminated soil samples and selection of positive strain so that optimization parameters can be carried out for better production. Biosurfactant synthesized by microorganisms are capable of reducing surfaces and interfacial tension with low toxicity, high specificity and biodegradability, leads an increasing interest on these products alternative to chemical surfactants. Its production has exceeded 2.5 million tons per year, as they are the key ingredients in detergents, shampoos, toothpaste, oil additives other consumer and industrial products.

INTRODUCTION

Surfactants are amphiphilic compounds that reduce the free energy of the system by replacing the bulk molecules of higher energy at an interface [1]. Because of their amphiphilic nature, surfactants tend to accumulate at interfaces and surfaces. As a result, surfactants reduce the forces of repulsion between unlike phases at interfaces or surfaces and allow the two phases to mix more easily [2].

Biosurfactants can be divided into low molecular mass molecules, which efficiently lowers surface and interfacial tension and high molecular mass polymers which are more effective as emulsion stabilizing agents. Some low mass surfactants include glycolipids, lipopeptides and phospholipids whereas high molecular biosurfactants include polymeric and particulate surfactants [3].

Corresponding Author

K.Mohan
Email:-kumaresanmhn@yahoo.co.in

A special property of a biosurfactant is their ability to reduce the surface tension of water from 72 mNm^{-1} to below 40 mNm^{-1} [4].

Effective physiochemical properties which are low interfacial tension and critical micelle concentration (CMC) and temperature stability are the characteristics of these compounds [5].

Biosurfactants have become an important product of biotechnology for industrial and medical applications. They can be used as emulsifiers, de-emulsifiers, wetting agents, spreading agents, foaming agents, functional food ingredients and as detergents in various industrial sectors as Petroleum, Petrochemicals, Organic chemicals, Food beverages, Cosmetics, Pharmaceuticals, Mining, Metallurgy, Agrochemicals, Fertilizers, Environmental Control Management and many others.

MATERIALS AND METHODS

Glasswares and media to be used were cleaned and sterilized at 121°C with 15 lb pressure for 20 minutes. Bacteria involved in this study were isolated from different soil samples present in windmill.

Screening for Biosurfactant Production

Heamolytic Assay

It was performed using blood agar. Fresh cultures of bacterial isolates were prepared and results were based on clear zone observed that indicates heamolysis [6].

Drop Collapsing Test

Crude oil was taken in a 96 well microtitre plate and was left to equilibrate. A flattened drop was observed with the aid of magnifying glass after one minute [7].

Emulsification Test

Several colonies of pure culture were suspended in 2ml of mineral salt medium. Hydrocarbons were added to this mixture and vortexed. The emulsion index (E_{24}) is the height of the emulsion layer (cm) divided by total height (cm) multiplied by 100 [8].

$$\text{Emulsification Index} = \frac{\text{Height of the emulsion layer}}{\text{Total Height}} \times 100$$

Biosurfactants Production

The primary innoculum was prepared in Luria Bertani medium and inoculated with stock culture. The positive isolates of the biosurfactant producing organisms were selected and grown in medium containing mineral salt and agitated. Trace elements like Zn $SO_4.7H_2O$-0.29g, $CaCl_2.4H_2O$-0.24g, $CuSO_4.5\ H_2O$-0.25g, $MnSO_4.H_2O$-0.17g and distilled water 1000ml.

DETERMINATION OF BIOSURFACTANT

Orcinol Method

Orcinol assay was used for the assessment of glycolipids in the sample. Extracellular glycolipids concentration was evaluated in triplicate by measuring the concentration of rhamnose. To the sample Orcinol was added and heated at 80° C. Standard curves are prepared and rhamnose equivalents (RE) were determined.

OPTIMISATION PARAMETERS

Effect of Incubation Time

500ml of inoculated medium was incubated at 37° C by shaking around at 100 RPM. 10ml of this culture was drawn aseptically at 12 hours intervals up to 72 hours followed by Orcinol assay.

Effect of pH

100ml of medium was prepared and was adjusted to different pH such as 5ph, 6pH, 7pH, 8pH using 0.1N NaOH and 0.1N HcL. Then these mixtures were innoculated followed by Orcinol assay.

Effect of Carbon Sources

100ml of production medium at pH 7.5 was prepared in different flasks. Carbon sources (1%) such as fructose, glucose, dextrose, maltose and starch were added into each and then culture was innoculated followed by Orcinol assay.

Effect of Nitrogen Sources

100ml of production medium was prepared in different conical flasks. In each of them different nitrogen sources (0.5%) such as Peptone, Yeast Extract, Ammonium Sulphate, Sodium Acetate and Urea. The flasks were innoculated and biosurfactants production was determined using Orcinol assay.

Production of Biosurfactants from Agro products

Natural agro products like rice bran, wheat bran, groundnut oil, coconut oilcake and jackfruit powder were used as substrates (2%concentration). They were innoculated and biosurfactants production was estimated.

Extraction of Biosurfactant

The cells were removed by centrifugation at 1000rpm, 10° C for 40 minutes. The Biosurfactant in the supernatant will be precipitated out by acidification with HcL at pH 2. This proteins and lipids were recovered by centrifugation at 12000rpm, 4° C for 20 minutes. A volume of 200µl of acidified culture supernatant was extracted three times with 1ml of diethyl ether and then the fractions were pooled, dried and resuspended in 1ml of 0.05M sodium bicarbonate, which was utilized for further studies.

RESULTS AND DISCUSSION

In this study 14 bacterial strains were isolated from different soil samples in windmills and from that *Pseudomonas sp* was selected for biosurfactant production.

Effect of Incubation Time

The Pseudomonas isolate was analyzed between 12th hour and 72th hour in mineral salt medium.

It was determined that biosurfactant production gradually increased between 36th hour and 72nd hour. Maximum production was seen in 4

It was determined that biosurfactant production gradually increased between 36th hour and 72nd hour. Maximum production was seen in 4th hour.

Effect of Temperature

The influence of temperature on bacterial growth and biosurfactant production is presented as:

It is claimed that increased concentration of biosurfactant during the stationary growth phase has some relation to the consumption of organic acid produced during active catabolism of glucose.

In our study maximum production was observed at 37° C. Temperature was observed to play its role in changing the pattern of biosurfactant production from primary to secondary metabolites.

Effect of pH

In order to improve biosurfactant production of the selected isolate, the influence of pH was studied.

Table 1. Effect of Time

Time of Culture Withdrawal	Biosurfactant (mg/ml)
12th hour	0.14
24th hour	0.22
36th hour	0.48
48th hour	0.7
60th hour	0.64
72nd hour	0.52

Table 2. Effect of Temperature

Temperature(°C)	Biosurfactant (mg/ml)
27	0.74
30	0.82
35	0.82
37	0.85

Table 3. Effect of pH

pH	Biosurfactant (mg/ml)
5	0.74
6	0.78
7	0.85
8	0.67

Table 4. Effect of Carbon Source

Carbon Source	Biosurfactant (mg/ml)
Glucose	0.95
Dextrose	1.15
Maltose	0.53
Starch	0.63

Table 5. Effect of Nitrogen Sources

Nitrogen Sources	Biosurfactant (mg/ml)
Peptone	0.63
Yeast extract	0.85
Urea	0.55
Ammonium Sulphate	0.53
Sodium Acetate	0.36

Table 6. Production of Biosurfactant from Agro products

Cheap Sources	Biosurfactant (mg/ml)
Rice bran	0.74
Wheat bran	1.39
Groundnut oil cake	1.15
Coconut oil cake	0.74
Jack fruit powder	1.15

In our study maximum biosurfactant production was observed in pH 7. This pH played an important role in affecting biosurfactant production through their effects on cellular growth and metabolic activity.

Effect of Different Carbon Sources

Dextrose as a source of carbon could be an important key to regulate biosurfactant synthesis. There were many evidences on importance of carbon and its concentration in the production of surface active compounds by microbes.

Effect of Different Nitrogen Sources

Various nitrogen sources were screened for maximum production of biosurfactant for the selected isolates. In our study yeast extract showed higher productivity rate. Certain reports have shown that NH_4NO_3 could be the best nitrogen source for the production of biosurfactant by facultative aerobes. Urea led to be a satisfactory production.

Major restriction in the commercialization of biosurfactant is their high production cost. The use of readily available cheap agro-industrial residues as the carbon source may reduce the higher cost. Among the cheap sources, wheat bran was the best substrate for biosurfactant production.

REFERENCES

1. Mulligan CN and Gibbs BF. (1993). Factors influencing the Economics of Biosurfactants, In, Kosaric, N(ed), Biosurfactants production, Properties and Applications, New York, Marcel Dekker, inc, 329-371.
2. Bodour AA and Miller-Maier RM. (2002). Biosurfactants, Types, Screening, Methods and Applications, *Encyclopedia of environmental Microbiology*, Wiley, NY, 750-770.
3. Kosaric N. (1992). Biosurfactants in industry. *Pure Applied Chemistry*, 11, 1731-1737.
4. Cooper DG and Paddock DA. (1984). Production of Biosurfactants from *Torulopsis bombicola*. *Applied Environmental Microbiology*, 47, 173-176.
5. Banat IM. (1995). Biosurfactants, Production and possible uses in Microbial enhanced oil recovery and oil pollution Remediation, A review, *Bioresource Technology*, 511-512.
6. Carrillo PG, Mardana C, Pitta Alvarez SJ and Giulietti AM. (1996). Isolation and Selection of Biosurfactant producing Bacteria. *World J Microbiol Biotechnol*, 12, 82-84.
7. Jain DK, Thompson DLC, Lee H and Trevors JT. (1991). A drop collapsing test for Screening Surfactant producing microorganism. *Journal of Microbiology methods*, 13, 271-279.
8. Francy DS, Thomas JM, Raymond RL and ward CM. (1991). Emulsification of hydrocarbons by subsurface bacteria. *Journal of Indian Microbiology*, 8, 237-246.

9. Desai JD and Banat IM. (1997). Microbial production of Surfactant and their commercial Potential. *Microbiology and Molecular Biology reviews*, 61, 47-64.
10. Cameotra SS and Makkar RS. (1998). Synthesis of Biosurfactants in extreme condition. *Applied Microbiology*, 50, 520-529.