Research Article

Indian Journal of Microbiology Research

ISSN 2394-546X(Print)
e-ISSN 2394-5478(Online)

IJMR

PRODUCTION OF BIOSURFACTANT OF MICROBIAL ORIGIN USING DIFFERENT RAW MATERIALS

P. W. Deshmukh[1,*], N.S .Kulkarni[1], M. G Bodhankar[2]

[1]Department of Microbiology, R.A.College, Washim.
[2]Department of Microbiology, Y.M.college, Bharati Vidyapeeth University, Pune

Correspondence Author:
E-mail: deshmukhp12687@gmail.com

Abstract: *Surfactantsare widely used for industrial, agricultural, food, cosmetic and pharmaceutical applications. Most of the compounds are chemically synthesized. However, it is only in the past few decades that surface active molecules of microbial origin, referred to as biosurfactants, have gained considerable interest. Biosurfactants have advantages over their chemicals counterparts because they are biodegradable, have low toxicity, effective at extreme temperatures or pH values and show better environmental compatibility. To overcome the production cost and to compete with synthetic surfactant inexpensive substrate and effective microorganism must be develop. The present study deals with the positive use of industrial and agricultural waste as the possible subatrate for biosurfactant production and oil has proved to be an attractive substrate for biosurfactant production.*

Keywords: - *Biosurfactants, Pseudomonas aeruginosa*

Introduction

Surfactants are amphiphilic compounds; they possess properties like surface and interface activity, emulsification, foaming, wetting, detergency and density reduction of heavy hydrophobic compounds. Surfactants produced by microorganism as secondary metabolites are called as biosurfactants.

Biosurfactants have advantages over their chemicals surfactant because they are biodegradable (Zajic et al., 1977), have low toxicity (Poremba et al., 1991), are effective at extreme temperatures or pH values (Cameotra and Makkarr 1998) and show better environmental compatibility (Georgiou et al., 1990).

Biosurfactants were first discovered as extracellular amphiphilic compounds of fermentation bacteria (Kitamoto et al., 2009). Initially they were seen interesting due to their ability to increase solubility of insoluble or poorly soluble hydrocarbons. However, the more and more popular trend of using renewable resources in industry (especially in food and pharmaceutical industries) have led to relentless interesting in gaining and application of natural surfactants, mainly biosurfactants (Nitschke and Costa, 2007). Nowadays, biosurfactants are produced using co- and by-products of different technologies as a carbon source for microorganisms (molasses, glycerol, whey, frying oil, animal fat, soapstock and starch-rich wastes e.g. potato wastes) (Maneerat 2005; Makkar and Cameotra, 2002).

Biosurfactants are classified mainly on the basis of their chemical structure and origin. The hydrophilic head is usually amino acid, peptide, mono-, di- or polysaccharide. The hydrophobic tail is usually saturated, unsaturated, linear,

branched or hydroxylated fatty acid. Great emphasis has recently been given to the environmental impacts caused by chemical surfactants due to their toxicity and difficulty in being degraded in the environment (Van Hamme et al, 2006). Increasing environmental concerns, the advance in biotechnology and the emergence of stringent laws have led to biosurfactants being a potential alternative to the chemical surfactants available on the market (Banat, I. M,et. al.,2000 and Henkel, M et.al.,2012).

Biosurfactants are potentially replacements for synthetic surfactants in several industrial processes, such as lubrication, wetting, softening, fixing dyes, making emulsions, stabilizing dispersions, foaming, preventing foaming, as well as in food, biomedical and pharmaceutical industry, and bioremediation of organic- or inorganic-contaminated sites. Glycolipids and lipopeptides are the most important biosurfactants (BS) for commercial purpose Shete et al. (2006)

Material and Methods

Isolation of Pseudomonas aeruginosa:

Isolation of Pseudomonas aeruginosa was done by inoculating composite soil sample collected from oil industry and petrol pump on plates containing selective medium of cetramide agar.1gm of soil sample was added to 10ml distilled water and vortexed thoroughly. The supernatant was serially diluted and spreaded on cetramide agar plates.All plates were then incubated at 30^0 c for 48 hrs. Pseudomonas species identify by cultural characteristics and biochemical test.

Screening of surfactant producing pseudomonas species:

All the isolates tentatively detected as pseudomonas species were inoculated in 10 ml nutrient broth and incubated at 37 c for 4 days. Followed by incubation all the tubes were subjected to centrifugation at 5000 rpm for 30 min. Screening of surfactant producing pseudomonas was done by adopting the phenol sulphuric acid method and Erythrocyte haemolysis method respectively. [Shete et al., 2006] The strain showing both the test positive were considered as biosurfactant producers.

Phenol sulphuric acid method:

Phenol sulphuric acid method was used to detect the presence of biosurfactant. 1ml of 5% phenol was added to the supernatant to this 5ml of concentrated sulphuric acid was added drop by drop, colour changes from yellow to orange shows the presenceof biosurfactant.

Erythrocyte haemolysis method:

10 ml of culture supernatant [pH 6.5] were concentrated by the addition of ZnCl2. The precipitated material was dissolved in 10 ml sodium phosphate buffer [pH6.5] and extracted twice with equal volume of diethyl ether. The pooled organic phases were evaporated to dryness and the pellets dissolved in 100 ml of methanol. 10 µl of the concentration culture supernatant were spotted on filter paper disc and then put onto agar plates containing 5% sheep blood .The blood agar plates were incubated at room temperature for 2 days.

Biosurfactant fermentation:

Surfactant production process was carried out utilizing five different medium Whey medium, Oil medium,Nutrient broth [2% glucose], Inorganic salt medium [2% glucose], Triptic soy broth medium.Allthe medium were sterilized in autoclave at 121^0 c for 15 min at 15 lbs. The surfactant producing strain of pseudomonas species was aseptically inoculated in each medium @15 per cent v/v as inoculum volume.Uninoculated set for each medium was maintained as control. The inoculated mediums werefurther incubated on rotary shaker for 8 days at room temperature.

Extraction of biosurfactant:

The extraction of biosurfactant was done by subjecting the enriched broth to centrifugation at 5000 rpm for 30 min. The rhamnose was estimated spectrophotometrically by adopting the method suggested by Chandrasekaran et al., [1980]. Rhamnolipid concentrationwas determined by the orcinol assay.

Evaluation of surfactant activity was done by measuring surface tension of each media before inoculation and after inoculation and incubation. Density of the media was determined using formula as Density of medium= weight of medium/ volume of medium. Surface tension (ST) was calculated by formula.

$$\text{Surface Tension} = \frac{h.r.p.g}{2\cos\theta}$$

Where, h= height of medium in capillary tube, p= density of medium, r= radius of bore of capillary, θ=angle of contact, Cos θ=1.

Result and Discussion

The composite soil samples collected from different oil mills were screened for the presence of *pseudomonas* species. The isolated strains were subjected for determination of morphological,cultural and biochemical characteristic. Total three strains were isolated and confirmed as pseudomonas species [Bergeys 2001].The confirmed isolates were further examine for the presence of surfactant production ability adopting phenol sulphuric acid method and erythrocyte haemolysis method from the result[table 1] it was observed that out of all the three pseudomonas isolates strain no.1 [PS1] showed positive results for both procedures. Whereas [PS2] was positive for phenol sulphuric acid method and [PS3] was positive for erythrocyte haemolysis method. Hence, strain [PS1] was confirmed as biosurfactant producer and further used for biosurfactant production at laboratory level.

The experimentation on biosurfactant production was carried out using five different medium of which whey and oil was of environmental interest whereas Triptic soy broth , Nutrient broth, Inorganic salt medium are frequently reported for production of microbial surfactant . The result of surfactant produce using pseudomonas species was express in terms of Rhamnose [table1] and the surfactant activity was analysed by evaluating the percent reduction in surface tension. [table2]

Table - 1:
Concentration of Rhamnose

Sr.no	Media	Optical density [540 nm]	Rhamnose
1	**Nutrient broth**	0.19	21
2	**Inorganic salt medium**	O.33	37
3	**Oil medium**	0.87	97
4	**Whey medium**	0.33	37
5	**Triptic soy broth**	0.22	24
6	**Control**	o.18	20

Table – 2:
Percent reduction in surface tension

Media	Surface tension before incubation[dyne/cm]	Surface tension after incubation [dyne/cm]	Per cent reduction in surface tension
Nutrient broth	0.283	0.270	4.59
Inorganic salt medium	0.281	0.268	4.62
Oil medium	1.194	0.245	79.73
Whey medium	1.044	0.30	71.26
Triptic soy broth	1.02	0.288	28.23
Control	1.104	1.104	0

It was observed that, the maximum surfactant was produced [97mg/ml] when the pseudomonas was cultivated using oil as a substrate, followed Whey [37mg/ml],

Triptic soy broth [24mg/ml] and Nutrient broth [21 mg/ml]. Incase of inorganic salt medium as an offered substrate it was at part with Whey medium in producing the biosurfactant during eight days of incubation. Oil as an attractive substrate for the production of biosurfactant and proved to be significant for enhancebiosurfactant production by *pseudomonas* over other substrate analysed. It may be due to the hydrocarbon status of substrate, which has ultimately accelerated the surfactant producing metabolism and growth of the *pseudomonas* species.

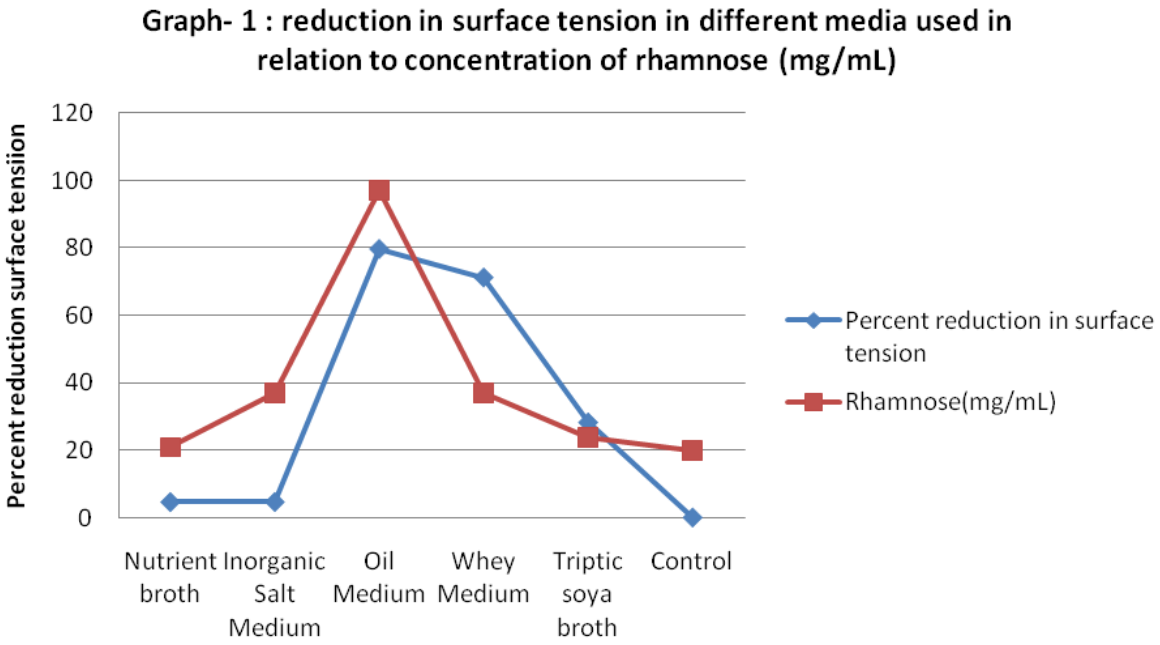
Our results are in accordance with the finding of Nitschke et.al. [2005] however, their studies are on vegetable oils. In the present study the maximum biosurfactant produce by pseudomonas species especially on oil as subtracts may be due to the availability of optimum waste oil which might have resulted in the high yield of rhamnose as compared to whey medium.triptic soy broth, inorganic salt medium & Nutrient broth.

The nature of carbon source present in the oil substrate might be favoured by the *pseudomonas* species & hence, resulted in enhanced productivity. The quality & quantity if biosurfactant production are affected & influenced by the nature of the carbon in the substrate have been reported by Singer [1985].

On the other hand, several studies with the plant derived oils have shown that they can act as an effective & cheap raw materials for biosurfactant production. Sunflower, Soybean, Rabassu & Corn oil respectively , have been frequently focused as an excellent substrate for biosurfactant production [Trummler et.al ,2003 Vance-Harrop et.al ,2003 & Pekin et.al ,2005] It was also observed that , the biosurfactant produced when *pseudomonas* species was cultivated on whey as substrate was significant over Nutrient broth , Triptic soy broth & control which indicates the whey as the second in priority after oil could be used as substrate for biosurfactant production in whey may be due to the accelerated growth of *pseudomonas* species . Dubey et al. 2004 reported that, the effluent from the dairy industry supports good microbial growth & can be used as cheap raw material for biosurfactant.

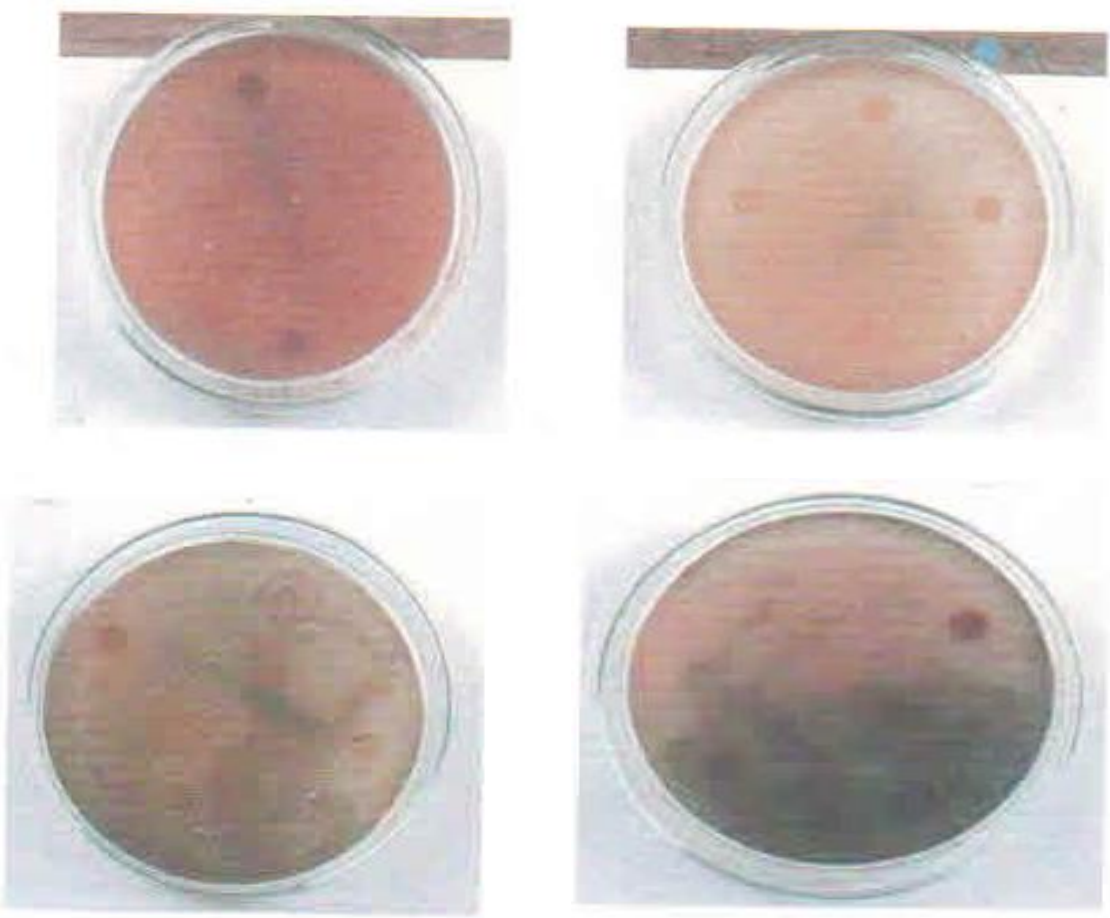
The study on evaluation of surfactants activity was done express in the terms of per cent reduction in surface tension with & without biosurfactant treatment. The result are presented in table [2] & graphically illustratedin [graph 1]. From the result , it was observed that maximum reduction in surface tension [79.73] per cent was achieved by the surfactant extracted from the fermentation set up , In which oil was taken as a substrate , followed by 71.26 in case of whey medium , 28.23 in triptic soy broth . Whereas, in case of Nutrient broth & inorganic salt medium the reduction in surface tension recorded was very low viz 4.59 & 4.62 per cent respectively.

The correlative studies on the quality of biosurfactant produced , growth performance of *pseudomonas*& Rhamnose produced indicates the positive correlation between all three parameters .The result on the present studies are in contradiction with the finding of Shete et.al.[2006]. They reported 33.14 % reduction in surface tension using inorganic salt medium + glucose as substrate , whereas , the present studies have enlightened only 4.62 %reduction in surface tension . It may be due to the difference in the strains utilised for the biosurfactant production .However, our result are in accordance with the experimental findings of ferraz et.al. , [2002], they reported oil as a best substrate for the goods quality of biosurfactant Secondly the probable reason for the quality of biosurfactant produced on oil may be due to pseudomonas strain which may have significant lipase activity & hence it has facilitated assimilation of fat contained in oil . They also reported that the growth of strain on oily substrate decreased the surface tension .The result on the present studies enlight the oil as most suitable substrate for biosurfactant production.



Phenol Sulphuric acid method

Erythrocyte Haemolysis Method



Conclusion:

Biosurfactant producer are present in hydrocarbon rich soil. Oil has proved to be an attractive substrate for biosurfactant production. Burnt oil can be use as substrate for biosurfactant production and may extend the solution on its disposable problem.

Refrences

1. Banat, I. M, Makkar, R. S, & Cameotra, S. S. (2000) Potential commercial applications of microbial surfactants. ApplMicrobialBiotechnol., 53:495-508
2. Cameotra, S.S. and Makkar, R.S. (1998).Synthesis of biosurfactants in extreme conditions. Appl. Microbiol. Biotechnol. 50: 520-529
3. Cameotra S.S and Makkar R.S (1997) recent application of biosurfactant as biological and immunological molecules. Curr. Opin. Microbial .7 262-266.
4. Chandrasekaran E.V., Bemiller J. N. (1980). Constituent Analysis of Glycosaminoglycans in; Whistler, R.I Wolfrom. M. L (Eds).Methods in carbohydrate chemistry. Academic Press, New York. P89-96
5. Dubey K. and Juwarkar A. (2004). Distillery and curd whey wastes as viable alternative sources for biosurfactant production. World J. Microbial. Biotechnol. 17:61-69.
6. Ferraz C, De Arujo A.A and Pastore G.M. [2002]. The influence of vegetable oils on biosurfactant production by *Serratia marcescens*, Appl. Biochem. Biotechnol. 100(1-3) 841-848
7. Georgiou, G., Lin, S.C. and Sharma, M. M. (1990).Surface active compounds from microorganisms. Bio/Technology, 10: 60-65.
8. Henkel, M, Müller, M. M, Kügler, J. H, Lovaglio, R. B, Contiero, J, Syldatk, C. (2012) Rhamnolipids as biosurfactants from renewable resources: Concepts for next-generation rhamnolipid production. Process Biochemistry, 47(8): 1207-19.
9. Kitamoto D., Morita T., and Fukuoka T., Konishi M-A., Imura T. (2009): Self-assembling properties of glycolipidbiosurfactants and their potential applications. Curr. Op. Colloid Interface Sci., 14(5): 315-328,

10. Makkar R.S and Cameotra S.S (2002).An update on the use of unconventional substrates for biosurfactants production and their new application.Appl. Microbial.Biotechnol. 58:428-434
11. Maneerat S. (2005). : Production of biosurfactants using substrates from renewable resources. Songklanakarin. J. Sci. Technol., 27(3):675-683, 25. Nitschke M., Costa S.G.V.A.O. (2007): Biosurfactants in food industry.Trends Food Sci. Technol., 18:252-259.
12. Nitschke M., Costa S.G.V.A.O. (2005): Biosurfactants in food industry.Trends Food Sci. Technol., 18:252-259.
13. Poremba, K., Gunkel, W.,Lang, S. and Wagner, F. (1991).Marine biosurfactants, III. Toxicity testing with marine microorganisms and comparison with synthetic surfactants. Z. Naturforsch, 46c: 210-216.
14. Pekin G. et al. [2005] Production of sophorolipids from candida bombicola ATCC 22214 using Turkish corn oil and honey .Eng. Life Sci.5, 357-362.
15. Shete H.G., Chitanand M.P., Joshi P. S (2006). Production of biosurfactant by pseudomonas aeruginosa.J. Microb. World, 8(1):136-139
16. Singer M.E., in Microbes and Oil Recovery ed. Zajic, J.E. and Donaldson,E. C (1985). BioresourcePublications, E1 Paso, Texas. Pp. 19-38.
17. Trummler K. et al.,(2003) An integrated microbial / enzymatic process for production of rhamnolipids and 1-(+) rhamnose from rapeseed oil with Pseudomonas sp. DSM 2874. Eur. J .lipid. Sci. Technol., 105:563-571.
18. Van Hamme, J. D, Singh, A, & Ward, O. P. (2006) Physiological aspects. Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnology Advances.., 24(6), 604-20.
19. Vance-Harrop M.H.et al., [2003] .New bioemulsifiers produced by candida lipolytica using D-glucose and Babassu oil as carbon sources. Braz .J.Microbial .34, 120-123.
20. Zajic, J.E., Gignard, H. and Gerson, D.F. (1977). Properties and biodegradation of a bioemulsifier from Corynebacteriumhydrocarboclastus. Biotechnol.Bioeng. 19: 1303-1320
21. Zajic J.E., Cooper D.G., Jack T.R. and Kosaric N. (1984) (ed), Microbial enhanced oil recovery. Pennwell Books, Tulsa, Okla.

How to cite this article: Deshmukh PW, Kulkarni NS, Bodhankar MG, Production of biosurfactant of microbial origin using different raw materials. Indian J Microbiol Res 2014;1(1):84-90.