



Comparative Study of Biosurfactant Producing Microorganisms Using Different Energy Sources

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ABSTRACT

Biosurfactants are amphiphilic compounds produced by microorganisms extracellularly, that reduces surface and interfacial tensions. The main objective of the present study was to know the influence of different energy sources on the biosurfactant production by two bacterial strains isolated from oil spilled area from VIT University campus. The biosurfactant producing potential of these two bacterial strains (Strain 1 and Strain 2) were compared using four different energy sources such as dextrose, peptone, yeast and beef extract at two different concentrations [0.5% and 1.5%]. Different qualitative tests such as oil displacement method, emulsification index, hemolytic activity, biosurfactant yield were carried out. Generally, dextrose showed the maximum activity in both the strains and beef extract showed the lowest activity. Strain 1 showed the maximum potential than Strain 2 to produce biosurfactant in the presence of different energy sources at different concentrations. This study showed that the production of biosurfactant could be influenced by the different energy sources and their concentrations.

Keywords: Bacterial strains, Biosurfactant, Energy sources, Potential.

INTRODUCTION

Biosurfactants are naturally synthesized amphiphilic surface active compounds.¹ These are natural surfactants synthesized by microorganisms including different yeast, fungal and bacterial species. Biosurfactants are produced either on the cell wall of living microorganisms or they can be excreted out of the cell in a surrounding environment.² Emulsification and transport of water insoluble substrates (hydrocarbons) are the important functions of biosurfactant in a living microbial cell.³ *Aspergillus spp.*, *Arthrobacter spp.*, *Bacillus subtilis*, *Bacillus stearothermophilus*, *Torulopsis bombicola*, *Pseudomonas aeruginosa* and *Pseudomonas putida* are some of the microorganisms that are known to produce biosurfactant.⁴

Production of biosurfactant may be influenced by the type of energy source used.² In the contaminated environment, the available energy sources are either labile or non-labile. The quantity and quality of organic matter are also expected to play a vital role in the production of biosurfactant by microorganisms. Concentration of yeast extract determined the synthesis of glycolipids by *Torulopsis spp.*⁴ It has been reported that ratio of nitrogen to carbon source determined the yield of biosurfactant (glycolipids) produced.⁴ Microorganisms either use water soluble substrate like carbohydrate, amino acids or only hydrocarbons for biosurfactant production.^{4,5}

Glycolipids (rhamnolipids, sophorolipids), phospholipids, fatty acids and natural lipids, peptides (gramacidine, polymyxins), polymeric (emulsan, liposan), particulae biosurfactants (lipoteichoic acid, M protein) are the major class of biosurfactants based on their chemical composition.⁸ Saponins, lecithins and humic acids are the

phytogenic biosurfactants that are released from decaying roots.⁶

Presence of hydrophobic and hydrophilic groups within the same molecule allows them to form a partition between fluid phases with different polarity.⁴ Surface tension reduction property along with heat and pH stability makes Biosurfactants applicable in many fields.⁴ Recently Biosurfactants are being used for degradation of organic pollutants in soil, bioremediation, oil recovery, ceramic processing etc. Biosurfactants are easily biodegradable, environmental friendly with low toxicity but because of the high cost effectiveness they are not used widely in industries.⁴ In this study four different sources were used to culture the bacteria and to know the extent of influence of these energy sources on biosurfactant production. One carbon source, dextrose and three different nitrogen sources, yeast, peptone and beef extract were selected for the purpose of this study.

MATERIALS AND METHODS

Sample Collection and Isolation of Microorganisms

Surface soil sample was collected from VIT University campus. 5g of soil sample was mixed in 50ml of nutrient broth and kept at 25°C for 3 days. Serial dilutions from 10⁻¹ to 10⁻⁶ were carried out in distilled water. 0.1ml of serially diluted sample from 10⁻⁵ and 10⁻⁶ was used for spread plating on sterilized nutrient agar plates. These plates were then incubated at 37°C for 24 hr. Morphology of grown colonies was studied. Selected bacterial strains were maintained on agar slants at 4°C for future study.^{1,7}



Preparation of Medium Using Different Energy Sources

Isolated bacterial strains were sub-cultured regularly and stored at 4°C. 120ml nutrient broth medium (1.5g nutrient broth in 120ml distilled water) was prepared in a sidearm flask and sterilized in an autoclave. 20ml of nutrient broth was taken out and stored it as a blank. Medium was then inoculated with a selected bacterial strain of either Strain 1 and Strain 2. Inoculated flask was kept at room temperature on a shaker (100rpm) for 24hr. After 24hr of incubation, O.D. of the culture was measured at 590nm. Sidearm flasks containing media with different concentrations (0.5% or 1% or 1.5%) of energy sources (dextrose, peptone, yeast, beef extract) were sterilized in an autoclave. 10ml of 24hr old culture was added into each sidearm flask. Inoculated flasks were kept on a shaker (100rpm) at room temperature. After 24hr, bacterial culture was centrifuged at 10,000rpm at 4°C for 10min. Supernatant was separated in a clean, dry centrifugation tube carefully. Collected cell free supernatant was used for different tests.

Qualitative Tests

Hemolytic Activity

Biosurfactants possess a property of blood cell lysis. This test was always used for preliminary screening of biosurfactant producing microorganisms. Wells were prepared by sterilized borer on blood agar plates. In a laminar airflow chamber, 40µl of supernatant (containing biosurfactant) was added in each well and incubated the plates in an incubator for 24hr at 37°C. After 24hr of incubation, the clearing zone around the well was measured.⁸

Oil Displacement

25 ml of distilled water was added in a petri plate. 200µl of oil was added drop by drop on water surface so that a thin film of oil layer was formed. Then 40µl of supernatant was added at the centre of oil layer. Displacement of oil will occur if the supernatant contains biosurfactant and the extent of oil displaced depends on the concentration of biosurfactant in the supernatant. Diameter of a displaced oil area was measured in cm. The diameter of clearing zone was expected to be proportional to the oil displacement activity of surfactant. Linear correlation was noticed between quantity of biosurfactant and diameter of clearing zone.⁸

Emulsification Index

Emulsification index (E_{24}) has been used to characterize the biosurfactant emulsifying activity. 1ml of oil was added in a clean, dry test tube. Then 0.5ml of supernatant was added to the test tube. Vortex the oil-supernatant mixture for 1min. Measure the height of emulsion layer, after 24hr.^{8,9} E_{24} was calculated using the following formula

$$E_{24} = \text{height of emulsion layer} / \text{height of total solution} \times 100$$

Correlation between biosurfactant concentration and E_{24} was reported.⁸

Biosurfactant Yield

Supernatant collected by centrifugation of bacterial cell broth and adjusted to a pH 2 by adding of 6N HCL and was stored overnight at 4°C for 24hr. Centrifuged at 10,000rpm at 4°C for 10min. The pellet was collected and the pH was adjusted to 7 by adding 2N NaOH. Equal volume of chloroform:methanol (2:1) mixture was added into a separating funnel and kept for separation. Organic layer (lower layer) containing biosurfactant was collected in a dry, clean eppendorf tube. The eppendorf tube was kept open at room temperature. After drying, the weight of the dried crude extract of biosurfactant in the eppendorf tube was measured. The weight of empty eppendorf and biosurfactant containing eppendorf were measured.⁸ Difference in weight of eppendorf tubes gave dry weight of crude extract of biosurfactant.

RESULTS

Isolation of Bacterial Strains

From collected soil sample, two bacterial strains were isolated successfully and they were named as Strain 1 and Strain 2.

Using four different energy sources (dextrose, peptone, yeast and beef extract) and three different concentrations (0.5%, 1% and 1.5%), biosurfactant production potential was estimated for these two bacterial strains. All these energy sources favored for biosurfactant production. However the yields of biosurfactant varied among the energy sources as well as between concentrations. It is clear from these results that change in energy sources would influence the growth and the productivity of biosurfactant. With increase in concentration (from 0.5% to 1.5%) of an energy source, yield of biosurfactant also increased from 60mg to 400mg for 100ml. Thus it was understood that these bacterial isolates produced surface active biosurfactant during their growth as a primary metabolite.

Results for Strain 1

Oil Displacement

It was clear that there was a relationship between the amount of oil displaced by amphophiles and the concentrations of energy sources used. With increase in carbon concentration, extent of oil displaced was also increased and vice versa. The maximum oil displacement activity (2.3cm) was observed for 1.5% of dextrose when compared to the other carbon sources. Lowest oil displacement activity (0.9cm) was noticed for 0.5% yeast.

Emulsification Index

The emulsification index for dextrose, yeast and peptone decreased with decreasing concentration (Figure 2). However for beef, emulsification index increased with decreasing concentration of beef extract. Amongst four



energy sources, dextrose showed high value of emulsification index (26.2%).

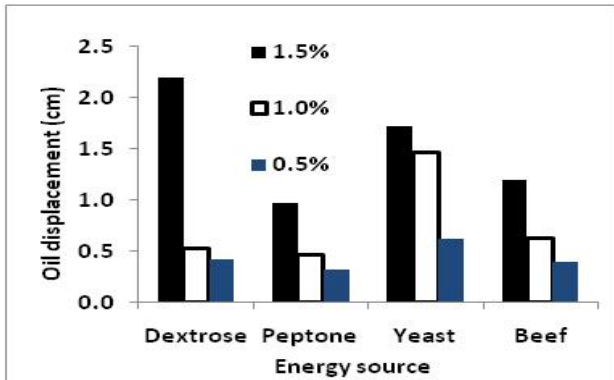


Figure 1: Comparison of oil displacement index for three different concentrations of various energy sources for Strain 1.

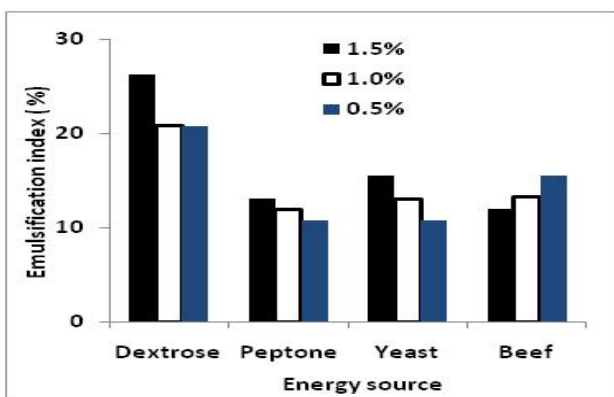


Figure 2: Comparison of emulsification index for three different concentrations of various energy sources for Strain 1.

Hemolytic Activity

From the results shown in Figure 3, it was clear that with increase in concentration of energy source, the area of clearing zone on blood-agar plate was also increased. For 1.5% of peptone, maximum clearing zone area was observed. For yeast and beef, the extent of haemolysis was less when compared to dextrose and peptone.

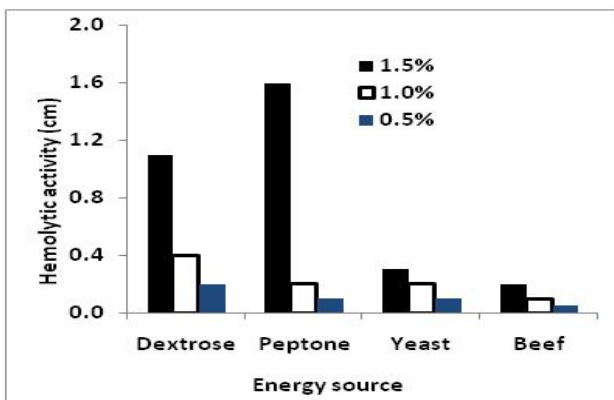


Figure 3: Comparison of hemolytic activity for three different concentrations of various energy sources for Strain 1.

Biosurfactant Yield

The influence of energy source concentration on biosurfactant yield (dry weight) was presented in Figure 4. With increase in concentration, yield of biosurfactant was also increased. For 1.5% of dextrose and yeast, the highest yield was obtained whereas, for 0.5% of peptone, the lowest yield was obtained for this bacterial strain.

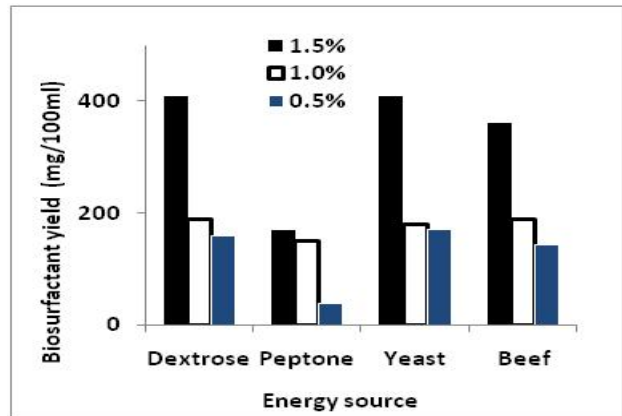


Figure 4: Comparison of biosurfactant yield for three different concentrations of various energy sources for Strain 1.

Results for Strain 2

Oil Displacement

The results of Strain 2 were shown in Figure 5 and they revealed that the oil displacement index was directly proportional to the concentration of energy source as observed for Strain 1. With increase in concentration of energy source, oil displacement activity was also increased and vice versa. Amongst four different energy sources, the maximum oil displacement activity (0.73cm) was noticed for 1.5% yeast.

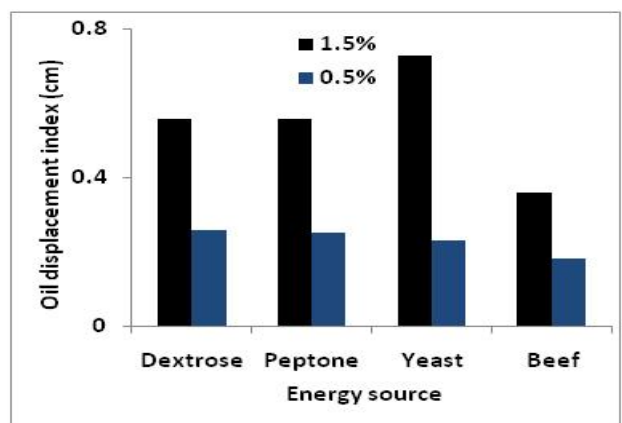


Figure 5: Comparison of oil displacement activity for two different concentrations of various energy sources for Strain 2.

Emulsification Index

Results of emulsification index for the bacterial Strain 2 was presented in Figure 6. From these results, it was clear that with increase in concentration of energy sources used, emulsification index value was also increased.

However in case of beef extract, emulsification index was high for 0.5% as compared to 1.5%. At 1.5% dextrose, highest emulsification index (30.9%) was noticed when compared to other energy sources.

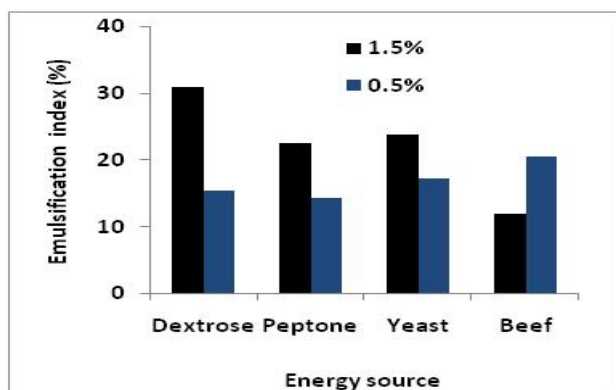


Figure 6: Comparison of emulsification index for two different concentrations of various energy sources for Strain 2.

Hemolytic Activity

It was clearly understood that with increase in concentration of carbon source, the area of clearing zone on blood-agar plate also increased (Figure 7). For 1.5% of dextrose, maximum clearing zone was observed. For beef, the extent of haemolysis was less as compared to dextrose, peptone and yeast.

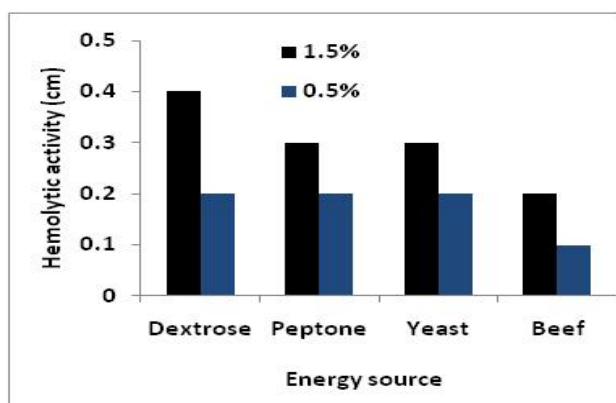


Figure 7: Comparison of hemolytic activity for two different concentrations of various energy sources for Strain 2.

Biosurfactant Yield

As observed for Strain 1, it was distinct that with increase in concentration of energy source, biosurfactant yield was also increased for bacterial Strain 2. Yeast showed the lowest biosurfactant yield (102mg/100ml) when compared to other energy sources. For 1.5% of peptone, the highest biosurfactant yield (177mg/100ml) was obtained.

DISCUSSION

Strains were able to use substrates such as glucose (dextrose), starch and glycerol to produce Biosurfactants but the results of glucose (dextrose) as carbon source seems to be more interesting when compared to other

energy sources. The result of Ghridi and Chaabouni, (2011) suggested that the carbon sources had been taken up more easily when compared to the other sources which are rich in protein or nitrogen.¹⁰

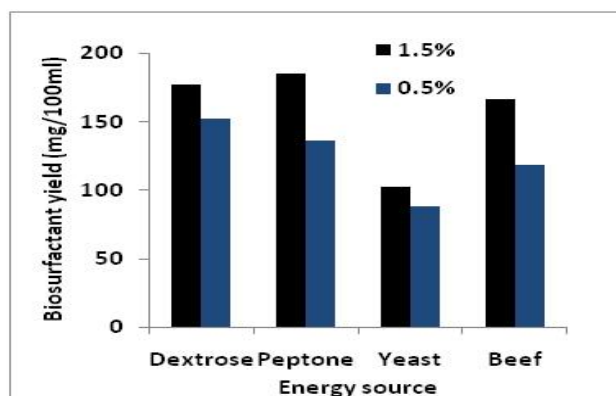


Figure 8: Comparison of biosurfactant yield for two different concentrations of various energy sources for Strain 2.

As the concentration of energy sources increased, the production of biosurfactant also increased indicating that increase in concentration of energy source (from 0.5% to 1.5%) might have favored the production of biosurfactant. Dextrose showed maximum biosurfactant yield, suggesting that bacteria grow more effectively in the presence of dextrose and thus biosurfactant production was also high when compared to other energy sources. For Strain 1, high biosurfactant yield was observed at 1.5% dextrose concentration revealing that increase in carbon source concentration might have speed up the production of biosurfactant. However with other glucose concentration, Biosurfactants production in the media was significantly decreased. This could be explained by the fact that the produced cells at these glucose concentrations were not physiologically adequate to synthesize Biosurfactants as noticed by Ghridi and Chaabouni, (2011). Yeast is reported to be more useful nitrogen source as compared to beef and peptone.¹⁰ However, it has been reported that the use of peptone along with meat extract may yield a higher production as compared to standard medium.¹¹

For Strain 2, both dextrose and peptone induced high yield of biosurfactant (Figure 8). This result may be due to the preference of dextrose and peptone by Strain 2. Bacterial Strain 1 showed good results of various tests such as oil displacement index, emulsification index, hemolytic activity and biosurfactant yield when compared to Strain 2. These results suggested that Strain 1 may have better potential to produce biosurfactant than Strain 2. Further Strain 1 produced the maximum biosurfactant in presence of dextrose at a concentration of 1.5% indicating that dextrose as a carbon source was most probably supported the production of biosurfactant.

Nitrogen rich sources also exhibited influence on the production of biosurfactant of these two strains, but they were not as pronounced as dextrose. Difference in

various test results of these two strains indicated that the responses of these bacterial strains were not most probably the same. It could be suggested that a combination of carbon and nitrogen sources might have synergistic effect on the biosurfactant producing potential of the bacteria. Since the natural environment may obtain energy sources from various sources, a combined use of few carbon and nitrogen sources may have additional advantage of accelerating the functional processes.

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