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Fermentative potential of *Pseudomonas aeruginosa* strain for biosurfactant production

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ABSTRACT : Biosurfactants are amphiphilic compounds, which have the significant advantages over synthetic counterparts. The advantages of biosurfactants over their synthetic derivatives and wide range of applications have attracted the strong interest of scientific community. These have wide range of potential applications in areas of environmental protection and management, crude oil recovery, as antimicrobial agents in health care and food processing industries. The present paper reports the evaluation of *Pseudomonas aeruginosa* MTCC 2297 for the production of biosurfactants. The effect of different medium components and process parameters was monitored for the enhancement of emulsification activity. The maximum emulsification activity of 80% was obtained after 144 hrs of incubation period.

Keywords : Biosurfactants, *Pseudomonas aeruginosa*, emulsification activity

INTRODUCTION

Biosurfactants are amphiphilic compounds which are produced mainly by hydrocarbon degrading microorganisms. The molecular structures of these comprise a hydrophilic portion, which may consist of mono-, oligo- or polysaccharides, amino acids or peptides or carboxylate or phosphate groups, and a hydrophobic portion, which is composed of saturated or unsaturated (hydroxy) fatty acids or fatty alcohols (Dyke *et al*., 1991; Bodour and Miller-Maier, 2002). The advantages of biosurfactants over synthetic (chemically derived) counterparts include lower toxicity, biodegradability, selectivity, specific activity at extreme temperatures, pH, salinity, the possibility of their production through fermentation, their potential applications in environmental protection and management, crude oil recovery, as antimicrobial agents in health care and food processing industries. These advantages clearly put the biosurfactants ahead of the synthetic counterparts and have, as a result, elevated their commercial potential.

Biosurfactants have shown wide range of applications. These compounds are capable of reducing the surface tension of the culture broth and emulsification of insoluble carbon sources in the culture medium (Francy *et al*., 1991; Banat, 1995; Ron and Rosenberg, 2001). Such surface properties made them good candidates for enhanced oil recovery (EOR) (Banat *et al.*, 2002). Some biosurfactants are known to have therapeutic applications as antibiotics and antifungal or antiviral compounds. These can also be used in bioremediation of soil or sand or in the cleanup of hydrocarbon contamination in groundwater.

The compounds secreted by *Pseudomonas aeruginosa* constitute a heterogeneous mixture of mono- and di-rhamnolipids which has been used to obtain most of the published data. However, it is interesting to evaluate the individual contribution of each homologue to the biological properties of the mixture, and thus obtain a molecule with the desired properties for specific uses. The whole process of bacterial biosynthesis of rhamnolipids can be classified, therefore, as a green process. The nature of biosurfactant varies from strain to strain; therefore it is essential to evaluate different available strains for their biosurfactant potential and their characterization. In view of the above, the present work was carried out to evaluate the fermentative potential of *Pseudomonas aeruginosa* MTCC 2297 for biosurfactant production.

MATERIAL AND METHODS

Micro-organism. *Pseudomonas aeruginosa* MTCC 2297 was used in this work, and was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Maintenance of Culture. The bacterial culture were maintained on growth media containing: beef extract (1 g/L), yeast (2 g/L), peptone (5 g/L), sodium chloride (5 g/L). 250 mL Erlenmeyer flask containing 50 mL of a liquid medium were inoculated with the strain and incubated at 37°C for 24 hrs. The cultures were maintained by subculturing, aseptically at fortnight intervals and stored at 4°C, until further use.

Preparation of Starter Culture. The bacterial culture was grown in 50 ml of media in 250 ml capacity Erlenmeyer flask, having the same composition as described above. After sterilization, the flasks were inoculated with a loopful of culture from capsule and incubated at 37°C for 24 hrs.

Production of Biosurfactant. The composition of fermentation medium was (g/L): $NaNO_3$ (1.28), K_2HPO_4 (0.87),

$MgSO_4.7H_2O$ (0.1), NaCl (0.1), KCl (0.2), Tris (hydroxymethyl) aminomethane (6.5), glucose (20); mineral salt solution (5 mL). The mineral salt solution contained the following ingredients (g/L): $FeSO_4$ $(NH_4)_2SO_4.6H_2O$, H_3BO_3, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$, $MnSO_4.H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$, $ZnSO_4.7H_2O$. The pH of medium was initially adjusted to 6.7 by 1.0 M HCl. Different carbon and nitrogen sources were added to the fermentation media. The media was inoculated with the *Pseudomonas aeruginosa* MTCC 2297 strain and incubated at 37°C for 144 hrs.

Screening of Carbon Sources. The effect of different carbon substrates (glucose, kerosene, glycerol, soybean oil, mustard oil, jatropha oil) on biosurfactant production was investigated by supplementing individually in fermentation medium at the concentration of 2% (w/v).

Screening of Nitrogen Sources. Different nitrogen sources such as sodium nitrate, glycine, alanine, potassium nitrate, and ammonium nitrate were supplemented individually at the concentration equivalent to 0.12% N to test their effect on biosurfactant production.

Effect of Process Parameters. The effect of different process parameters such as pH, temperature, and incubation time was monitored by varying the respective parameters.

Estimation of Emulsification Activity. The procedure of Pruthi and Cameotra (1995) was employed to estimate the emulsification activity.

Estimation of Rhamnolipid Content. The estimation of rhamnolipid content was carried using phenol sulphuric acid test (Dubois et al., 1956).

RESULTS AND DISCUSSION

The experimentation undertaken to evaluate the *Pseudomonas aeruginosa* MTCC 2297 for production of biosurfactant has been presented and discussed below:

Screening of Carbon Sources. Screening experiments were conducted on five carbon sources, *i.e.*, glucose, glycerol, soybean oil, mustard oil, and jatropha oil for their effectiveness on biosurfactant production. The results of various carbon sources for emulsification activity under above mentioned conditions are as shown in Fig. 1.

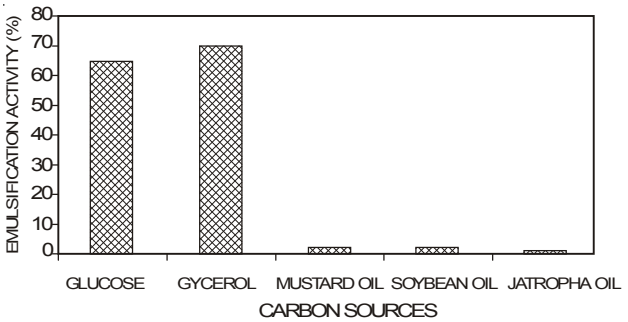

Fig.1. Effect of different carbon sources on emulsification activity of P. aeruginosa MTCC 2297.

As evident from the results that *Pseudomonas aeruginosa* MTCC 2297 was able to grow in a medium containing glycerol and glucose with maximum emulsification activity. Other carbon sources (soybean oil, mustard oil, and jatropha oil) used, showed very less emulsification activity. This suggested that the carbon source preference of the strain for biosurfactant production, which seems to be strain dependent (Wu *et al*., 2008). Most microbial surfactants were substrate specific, solubilizing or emulsifying different hydrocarbons at different rates (Ilori and Amund, 2001). Poor emulsification of other hydrocarbons might be due to the inability of the biosurfactant to stabilize the microscopic droplets. Also inefficient oxygen supply in the flask cultures may be responsible for poor growth in other carbon sources, as biodegradation of these oils are known as an oxygen-intensive metabolic event. On other hand, glycerol is the carbon source which is taken up very easily than compared to others. Our results are in agreement with those obtained using *Pseudomonas aeruginosa* CFTR-6, which produced glycolipids when glycerol (2%) was used as carbon source (Itoh *et al*., 1971). Among the five carbon sources tested, glycerol was the best carbon source with emulsification activity of 70%.

Screening of Nitrogen Sources. Nitrogen sources such as sodium nitrate, glycine, alanine, potassium nitrate, and ammonium nitrate were added to the fermentation media. Alanine and glycine were found most effective amongst these nitrogen sources (Fig. 2). Other nitrogen sources (potassium nitrate, sodium nitrate and ammonium nitrate) gave low emulsification activity, potassium nitrate being the lowest, as compared to glycerol. Among the nitrogen sources added glycine and alanine gave similar results, but considering the cost factor glycine was the most effective.

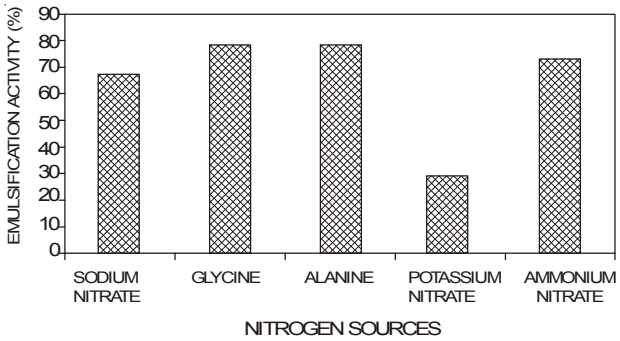

Fig.2. Effect of different nitrogen sources on emulsification activity of P. aeruginosa MTCC 2297

The studies showed that glycine is more effective in the production of biosurfactants than nitrates, which is in agreement with other studies reported in the literature in view of nitrogen limitation (Arino *et al*., 1996; Shrive *et al*., 1995).

Effect of Concentration of Glycerol

The effect of concentration of glycerol on emulsification activity was investigated by using different glycerol

concentrations (1-5%) in the fermentation media. General trend of biosurfactant production initially increases with increasing carbon substrate concentration Fig.3, until it reached a maximum value and then leveled off (Wei *et. al.*, 2005). Maximum activity was seen with glycerol concentration with 3%. It is evident from the results that emulsification activity was highest with glycerol concentration of 3% after 144 hrs of incubation.

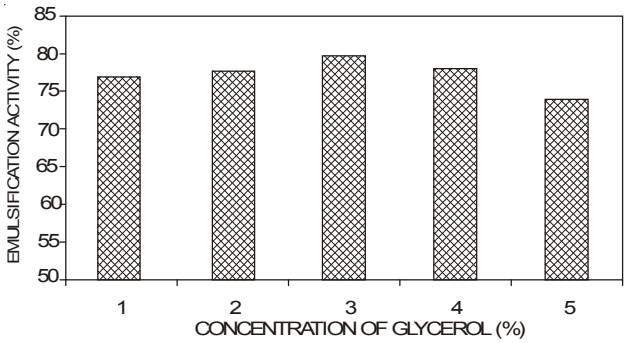

Fig.3. Effect of glycerol concentration on emulsification activity of P. aeruginosa MTCC 2297.

Effect of Concentration of Glycine. Glycine was added to the fermentation media at different concentration of 0.15-0.55%. It is evident from Fig. 4 that the increase in the concentration of glycine hinders the increase in emulsification activity. At very high concentration of glycine, very low emulsification activity was observed, which could be due to the fact that an optimum C/N ratio is required by *P. aeruginosa* for maximum biosurfactant production and an increase in the nitrogen concentration results in lower rhamnose concentration as well as higher interfacial tension values (Guerra-Santos *et al.*, 1984). Some reports mentioned that biosurfactant production is more efficient under nitrogen-limiting conditions (Benincasa *et al.*, 2002). The results show that a possible inhibitory effect on the bacterial metabolism may occur due to a likely nutrient transport deficiency.

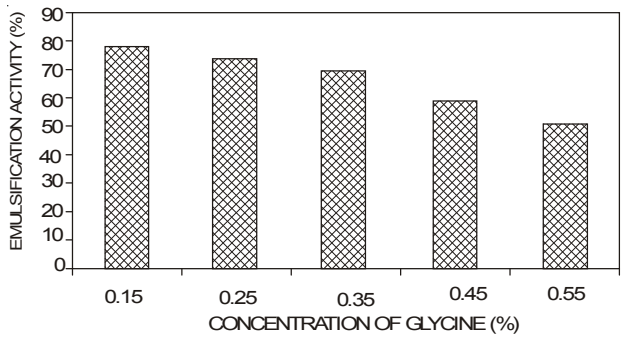

Fig.4. Effect of glycine concentration on emulsification activity of P. aeruginosa MTCC 2297.

Effect of pH. The pH of the fermentation was varied from 6.0 to 8.0 to study its effect on emulsification activity. At very low and very high pH *i.e.* at 6.0 and 8.0, very low emulsification activity was observed Fig.5. This may be due to the reason that the *P. aeruginosa* MTCC 2297 strain grows only near neutral pH. Any change in media alkalinity or acidity hinders the biosurfactant production. Biosurfactant production was maximum between pH 6.7 and 7. These results suggested that the optimal pH for biosurfactant production with the MTCC 2297 strain was in the range of 6.7-7.

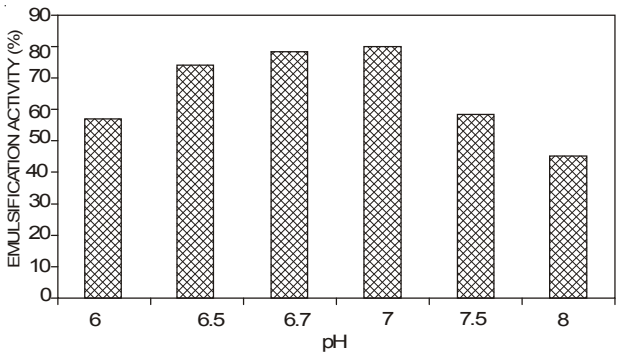

Fig.5. Effect of pH on emulsification activity of P. aeruginosa MTCC 2297

Effect of temperature. The effect of temperature on emulsification activity was studied by cultivating the bacterial strain in media at temperature range of 30-45°C. Biosurfactant production increased with temperature until 37°C and then decreased sharply above 37°C Fig.6. At very high temperature like 40°C and above there was very low emulsification activity. Optimal growth for biosurfactants from *Pseudomonas* has been reported at 37°C (Wei *et. al.*, 2005; Gunther *et. al.*, 2005). The results suggested the maximum emulsification activity in batch culture of *P. aeruginosa* MTCC 2297 at 35-37°C.

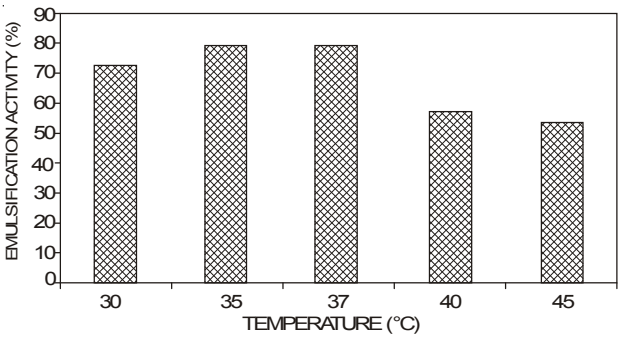

Fig.6. Effect of temperature on emulsification activity of P. aeruginosa MTCC 2297

Effect of incubation time. The fermentation media was prepared from the above set of parameters i.e. glycerol concentration of 3 (%, v/v), glycine concentration 0.12 (%, w/v), having pH 7.0 was incubated with stationary conditions at 35°C and samples were taken at regular intervals. From the results it can be observed that there was continuous increase in the emulsification activity of biosurfactant as a function of incubation period (Fig.7). Emulsification activity increased up to 144 hrs and beyond this incubation period, it decreased, which may be due to exhaustion of nutrients, and metabolic changes in the medium. Rhamnolipids production with the *P. aeruginosa* MTCC 2297 strain was confirmed with phenol sulphuric acid

test and the rhamnolipid content of 354 mg/L with emulsification activity of 80% was observed at 144 hrs.

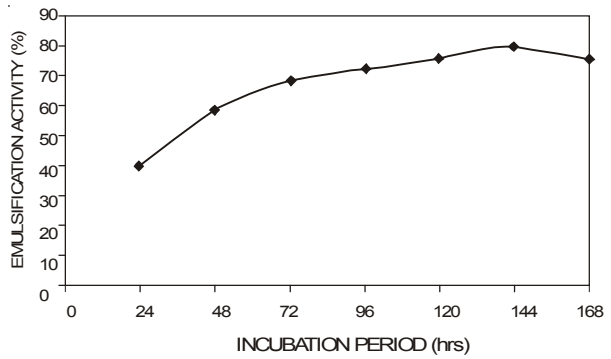

Fig.7. Effect of incubation period on emulsification activity of P. aeruginosa MTCC 2297

From the above studies, it can be concluded that *P. aeruginosa* MTCC 2297 has good potential for biosurfactant production. Among the screened media components, the best carbon source and nitrogen source for the production for this strain was found to be glycerol and glycine, respectively. The fermentation media having glycerol (3%) and glycine (0.12%) at pH 6.7-7.0 incubated at 35-37°C gave maximum emulsification activity (80%) with the rhamnolipid content of 354 mg/L after 144 hrs of incubation period.

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