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Nutritional Factors Effecting Rhamnolipid Production by a Nosocomial *Pseudomonas aeruginosa*

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Abstract

Different *Pseudomonas* strains were isolated and grown on the basal medium and inspected for rhamnolipid biosurfactant production. They were incubated in a rotary shaker for 8 days at 30 ^{o}C and at 150 rev min^{-1}. In order to increase the rhamnolipid production, 7 different carbon sources, including glucose, glycerol, mannitol, hexadecane, fructose, maltose and lactose were used. Varying concentrations of carbon sources for maximum rhamnolipid concentration were also determined. Different nitrogen sources in the basal medium, such as $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4NO_3, KNO_3, $NaNO_3$ and peptone were tested. Resulting rhamnolipid concentration was 589.3 mg/L, when 2% mannitol was used as the carbon source and 2.0 g/L $NaNO_3$ as the nitrogen source.

Key Words: *Pseudomonas aeruginosa*, rhamnolipid, biosurfactant, nosocomial bacteria

INTRODUCTION

Biosurfactants are important surface active substances that are produced by microorganisms. These compounds are capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases. They are of great interest because of their indispensable properties and are widely used in many industries [1]. Biosurfactants have many advantages such as biological availability, ecological acceptability, variability, low toxicity, biodegradability, ability to be produced from renewable and cheaper substances. In addition, biosurfactants are ecologically safe and used for bioremediation, decontamination of oil contaminated areas, tank cleaning and microbial enhanced oil recovery [2]. Some other different industrial applications are in personal care, mining, paper industry, agricultural applications, textiles, food industry and therapeutic applications [2,3,4]. These compounds are receiving considerable attention because they are more effective than chemical surfactants in increasing the bioavailability of hydrophobic compounds [5]. In spite of these advantages, biosurfactants have to compete with chemical surfactants for their high production cost. Some bacteria, yeasts and fungi are able to produce biosurfactants. Although most of them are bacteria [6- 8] and yeasts [9-11] there are some strains of fungi [12] known to synthesize biosurfactants. According to Desai and Patel [13], there are five major classes of biosurfactants, i) glycolipids, ii) phospholipids and fatty acids, iii) lipopeptides / lipoproteins, iv) polymeric surfactants, v) particulate surfactants. Rhamnolipids which are glycolipids produced by *Pseudomonas aeruginosa*, are among the most effective surfactants known today [4]. Rhamnolipids are also used for the source of rhamnose [14]. The sugar rhamnose is a potential

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material as a fine chemical in scientific and industrial studies, as a component in chemical reactions and as a strating material in the synthesis of organic compounds. We were interested in isolating a bioemulsifier from *Pseudomonas aeruginosa*, which could potentially produce rhamnolipids. In this paper, we determined the conditions of rhamnolipid synthesis by isolated *P. aeruginosa* in detail.

MATERIAL AND METHODS

Microorganisms

The microorganisms used in this study were 12 different *Pseudomonas aeruginosa* strains isolated at our laboratory from hospital culture samples. They were maintained on nutrient agar slants and were kept at 4°C. Transfers were made at 1 month intervals. Inocula were prepared by growing cells at 30°C in nutrient broth in an incubator at 150 revmin^{-1} for 24 hours. The best rhamnolipid producer was selected by the method of drop-collapse [15] and studies were continued with this strain.

Media

The composition of the basal medium for *P. aeruginosa* used in this study consisted of a carbon source 20.0 gl^{-1}, KH_2PO_4 0.7 gl^{-1}, Na_2HPO_4 2.0 gl^{-1}, $MgSO_4.7H_2O$ 0.4 gl^{-1}, $CaCl_2.2H_2O$ 0.01 gl^{-1}, $FeSO_4.7H_2O$ 0.001 gl^{-1}. The medium was supplemented either with mannitol or glucose as carbon sources and transferred into a 250 mL Erlenmeyer flask containing 50 ml liquid basal medium. The final pH was adjusted to 6.7 with phosphate buffer and was autoclaved at 110°C for 20 minutes. Modifications in this basal medium were made in order to study the limitation effects of the microorganism as described in the text.

The basal medium was inoculated with 2.0 ml of seed culture of *P. aeruginosa* [16] and was incubated in a rotary shaker for 8 days at 30°C and 150 rev min^{-1}.

Analytical Methods

To determine the cell concentration, cultures were centrifuged at 6000 x *g* for 10 minutes. The cell pellet was washed with distilled water and centrifuged again. Cell growth was monitored by measuring the absorbance at 550 nm.

Rhamnose concentration was estimated by an indirect method based on the determination of rhamnose described by Chandrasekaran and Bemiller [17]. Rhamnolipid concentrations were calculated from standart curves prepared with L-rhamnose corresponding approximately to 2.5 mg of rhamnolipids [16].

RESULTS AND DISCUSSION

Rhamnolipid production was observed when the strain was grown in basal medium. *P. aeruginosa* is well known for its production of rhamnolipid. In this study, to improve the rhamnolipid production, 7 different carbon sources including glucose, glycerol, mannitol, hexadecane, fructose, maltose and lactose were tested. The production was relatively high when glucose was the carbon source. However, a better yield was noted in the medium containing glycerol. In addition, rhamnolipid concentration reached at its maximum level when mannitol was used as the carbon source. It seems *P. aeruginosa* can produce rhamnolipids without any induction with hydrophobic carbon sources, such as hydrocarbons. Figure 1 and Figure 2 compare the effects of different carbon and nitrogen sources on the rhamnolipid production of the *P. aeruginosa* when it was grown on the basal medium.

The effect of varying concentrations of mannitol as a sole carbon source on rhamnolipid production was also determined and it was shown on Figure 3. Maximum production was observed when the mannitol concentration remained at 2%.

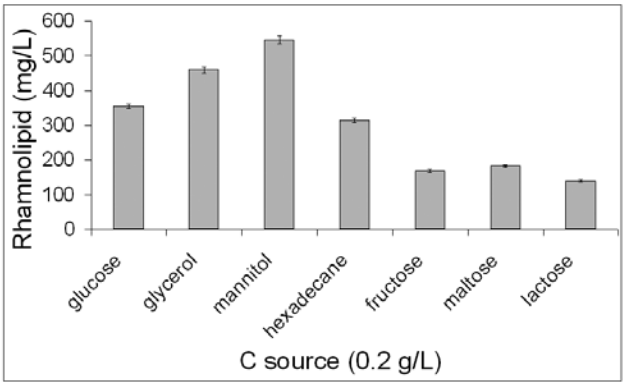

Figure 1. Rhamnolipid production by *P. aeruginosa* on various carbon sources. Rhamnolipid production was expressed as rhamnose. Values represent the averages from three cultures.

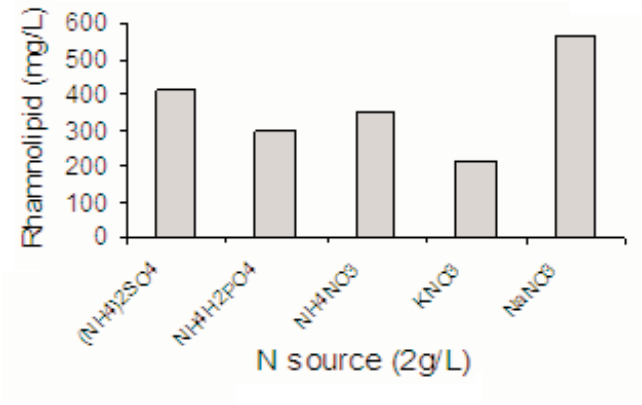

Figure 2. Rhamnolipid production by *P. aeruginosa* on various nitrogen sources. Rhamnolipid production was expressed as rhamnose. Values represent the averages from three cultures.

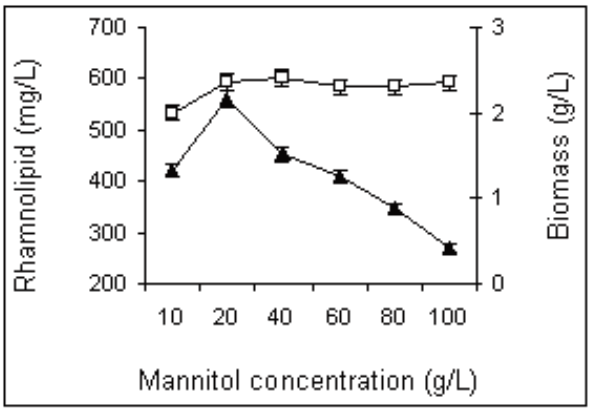

Figure 3. Effect of varying concentrations of mannitol as a sole carbon source on rhamnolipid production. Symbols: (▲) Rhamnolipid production expressed as rhamnose, (□) Biomass (OD550). Values represent the averages from three cultures.

Although the literature shows the hydrocarbons as suitable substrates for rhamnolipid production [18], the use of mannitol with a selected strain of *P. aeruginosa* resulted in better performance.

In order to understand the effect of nitrogen sources, rhamnolipid production was measured using different nitrogen sources such as $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4NO_3, KNO_3, $NaNO_3$ and peptone, with different concentrations in the basal medium.

Maximum productions were observed at the same concentrations as for the nitrogen sources tested. $NaNO_3$ at a concentration of 2.0 g/L was found to give the highest production of rhamnolipid (Figure 4). Nitrogen limitation had been reported to increase

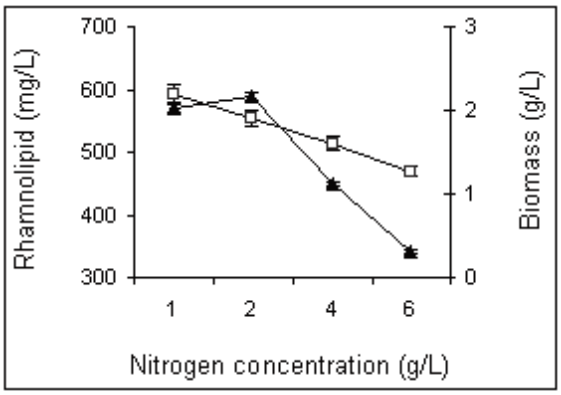

Figure 4. Effect of the nitrogen concentration on rhamnolipid production by *P. aeruginosa*. Symbols: (▲) Rhamnolipid production expressed as rhamnose, (□) Biomass (OD550). Values represent the averages from three cultures.

the rhamnolipid production by Kappeli and Guerra-Santos [19]. However, the results of our study showed that *P. aeruginosa* cell mass and rhamnolipid production decreased after 5 days of limitation. The highest concentration of rhamnolipids was obtained when mannitol was used as carbon source, and $NaNO_3$ as nitrogen source (589.3 mg/L), while $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4NO_3, KNO_3 and peptone, resulted in lower concentrations. The results of this study showed that

the rhamnolipid production of the *P. aeruginosa* is dependent not only on carbon source but also on limiting portion of nitrogen sources. Although some of the researchers [14, 20, 21] have studied nutrient effects on rhamnolipid production, microorganism strains, culture conditions and media are different in our study. Therefore, the production results are varied and higher. All experiments were repeated 3 times. In all instances, similar trends were observed. Because of their interesting properties, biosurfactants, especially rhamnolipids seem to have potential applications in future. Although *Pseudomonas aeruginosa* have a disadvantage of being an opportunistic human pathogen, these bacteria represent a valuable source for new compounds with surface active properties and it is likely to regenerate new nonpathogenic strains by genetic engineering.

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