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Effects of Fe - Mg Limitations and Physiological Factors on *P. aeruginosa* Rhamnolipid

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Abstract

A selected *Pseudomonas aeruginosa* strain was grown on the basal medium and inspected for rhamnolipid biosurfactant production. In order to increase the rhamnolipid production, limitations of both magnesium and iron elements in the basal medium were tested. The Fe concentrations of 0.05 g/L and above inhibited the increase in both cell growth and rhamnolipid production. Mg concentration had a negligible effect on rhamnolipid levels than Fe. While Carbon/Mg ratios were between 400-2000, biosurfactant concentration increased and when Carbon/Fe ratio was above 5000, biosurfactant production increased until the ratio reached about to 10^5. In addition to the nutrients, effects of pH and temperature were also investigated. Culture broths were incubated at between 20-40°C. Maximum rhamnolipid production and cell growth were observed at 34.5°C. Optimum pH values for rhamnolipid production were found between 6.5-7.0.

Keywords: Biosurfactant; *Pseudomonas aeruginosa*; rhamnolipid; Fe limitation; Mg limitation.

INTRODUCTION

Biosurfactants are surface active substances synthesized by living cells. There is a great interest on microbial biosurfactants in recent years because of their indispensible properties such as, ecological acceptability, structural diversity, low toxicity and biodegradability. Biosurfactants are ecologically safe and used for bioremediation, decontamination of oil contaminated areas, tank cleaning, removing heavy metals from sediments and microbial enhanced oil recovery [1, 2]. In spite of these advantages, biosurfactants have to compete with chemical surfactants for their high production cost. Commercial biosurfactants are generally more expensive than the chemical surfactants [3]. Some bacteria, yeasts and fungi are able to produce biosurfactants. Although most of them are bacteria [4-6] and yeasts [7-9], there are some strains of fungi [10] known to synthesize biosurfactants. According to Desai and Patel [11] , 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, are among the most effective surfactants known today [12]. Rhamnolipids are also used for the source of rhamnose [13]. The sugar rhamnose is a potential material as a fine chemical in scientific and industrial studies, as a component in chemical reactions and as a starting material in the synthesis of organic compounds. This paper describes the relationship between the rhamnolipid production of an isolated *Pseudomonas aeruginosa* strain and effects of some various environmental factors and limitations of medium components.

MATERIALS AND METHODS

Microorganisms and media

Pseudomonas aeruginosa strain used in this study was selected among 12 different *Pseudomonas aeruginosa* strains from the hospital culture samples in a previous study [14]. The selected strain was maintained on Nutrient agar and slants were kept at 4°C. It was transferred at 1 month intervals. Inocula were prepared by growing cells at 30°C for 24 hours in Nutrient broth in an incubator at 150 rev min^{-1}.

The basal medium for *P. aeruginosa* used in this study contained 20.0 g l^{-1} mannitol, 0.7 g l^{-1} KH_2PO_4, 2.0 g l^{-1} Na_2HPO_4, 0.4 g l^{-1} $MgSO_4.7H_2O$, 0.01 g l^{-1} $CaCl_2.2H_2O$, 0.001 g l^{-1} $FeSO_4.7H_2O$. The final pH was adjusted to 6.7 with phosphate buffer and was autoclaved at 110°C for 20 minutes. The medium was inoculated with 2.0 ml of seed culture of *P. aeruginosa* [15] and was incubated in a rotary shaker for 8 days at 30°C and 150 rev min^{-1}. Modifications of the basal medium and culture conditions were made as described in the text.

Analytical methods

To determine the cell biomass, 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 a method based on the determination of rhamnose described by Chandrasekaran and Bemiller [16]. Rhamnolipid concentrations were calculated from standard curves prepared with L-rhamnose [15].

RESULTS AND DISCUSSION

Effects of Magnesiumx and Iron

Limitations of both Fe and Mg in the basal medium were tested. The results indicated that, similar to the nitrogen limitations stated before [17], the stimulation of rhamnolipid synthesis occurs under Mg and Fe limited conditions. However, Mg concentration had less influence on the production of rhamnolipid than Fe concentration. Comparison of these two nutrients was shown in Figure 1. To understand the effect of Mg concentrations, basal medium (original composition is given in Materials and methods) with 0.0005 g l^{-1}, 0.001 g l^{-1}, 0.05 g l^{-1} and 0.1 g l^{-1} of Mg ($MgSO_4$)

were prepared. The N and Fe concentrations were kept constant. The maximum production of rhamnolipid (549 mg l^{-1}) was observed at the concentration of 0.001 g l^{-1} Mg.

Although not being an effective growth-limiting factor, Fe had a great influence on rhamnolipid production. The rising Fe concentrations (0.05 g l^{-1} and above) inhibited the increase in both cell growth and rhamnolipid production. However, the limiting concentrations of Fe (0.001 g l^{-1} and below) stimulated the increase in rhamnolipid production. Maximum production of rhamnolipid (614 g l^{-1}) was observed when Fe concentration was 0.0001 g l^{-1}. Some researchers have found higher values than this concentration [14, 18]. It was observed that not only Fe, but also Mg effected the rhamnolipid production of *P. aeruginosa* when it was grown on basal medium at pH 7.0.

Literature revealed that the Carbon/Nitrogen ratio is an important factor that affects the production yield of rhamnolipid [19]. Correlatively, additional experiments were carried out to investigate the effects of the carbon to iron (C/Fe) ratio and the carbon to magnesium (C/Mg) ratio on performance of rhamnolipid production. As shown in Figure 2, when C/Fe ratios were above 5000, performance of rhamnolipid production increased until the ratio was about 10^5. Therefore, C/Fe ratio was found to play a crucial role in rhamnolipid production of this strain. C/Mg ratios were also investigated with the basal medium. When C/Mg ratio was between 400-2000, biosurfactant production increased (Figure 3).

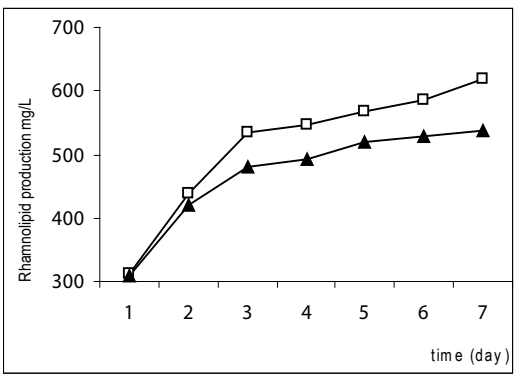

Fig. 1. Comparison of Fe and Mg limitations on rhamnolipid production.
Symbols: (□) Fe limitation, (▲) Mg limitation.
Values represent the averages from three cultures.

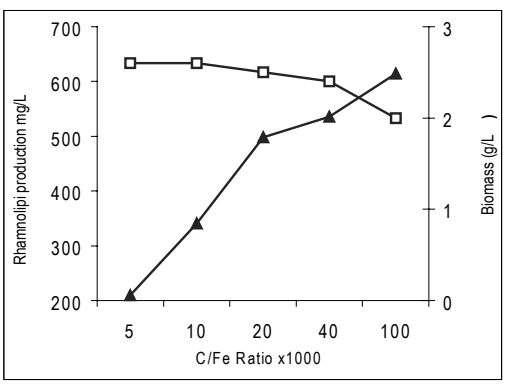

Fig. 2. Effect of C/Fe ratios on rhamnolipid production and cell growth.
Symbols: (▲) Rhamnolipid production expressed as rhamnose,
(□) Biomass (OD_{550}). Values represent the averages from three cultures.

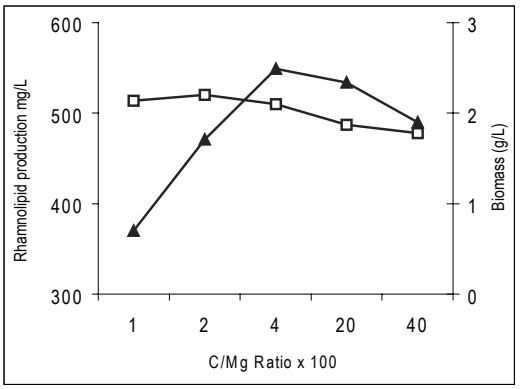

Fig. 3. Effect of C/Mg ratios on rhamnolipid production and cell growth.
Symbols: (▲) Rhamnolipid production expressed as rhamnose,
(□) Biomass (OD_{550}). Values represent the averages from three cultures.

Effects of Temperature and pH

In order to investigate the effect of temperature on rhamnolipid production, culture broths were incubated at between 20-40°C. It has been reported that the ranges between 32-34°C resulted in high biosurfactant production [20]. However, as shown in Figure 4, we observed the maximum rhamnolipid production and cell growth at 34.5°C with our strain. There was a sharp decrease above 36°C. Rhamnolipid production was increased within the range of 32-36°C. Higher and lower values than these temperatures, which were not suitable for our microorganisms, effected microbial metabolism. This condition was resulted low biosurfactant production. As the microbial metabolism slowed, rhamnose concentration also followed.

One of the parameters investigated in this study, was the effect of pH on rhamnolipid production. Studies on pH dependent rhamnolipid production by *P. aeruginosa* showed that values between 6.5-7.0 were the most suitable points for maximum rhamnolipid production (Figure 5). Lower or higher pH values caused rapid decrease. Temperature and pH are the factors that effect rhamnolipid production through their effects on cellular activity. It was shown that, temperature makes the biosurfactant composition different in *Pseudomonas* sp. Strain DSM-2874 [21].

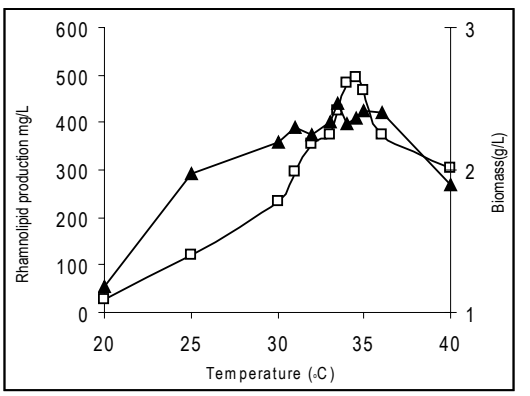

Fig. 4. Effect of temperature on rhamnolipid production and cell growth.
Symbols: (▲) Rhamnolipid production expressed as rhamnose,
(□) Biomass (OD_{550}). Values represent the averages from three cultures.

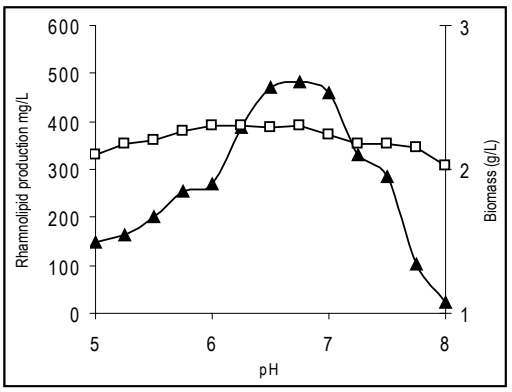

Fig. 5. Effect of pH on rhamnolipid production and cell growth.
Symbols: (▲) Rhamnolipid production expressed as rhamnose,
(□) Biomass (OD_{550}). Values represent the averages from three cultures.

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