Production of Biosurfactant by Indigenous Isolated Bacteria in Fermentation System

Tayebeh Fooladi\textsuperscript{a}, Aidil Bin Abd Hamid\textsuperscript{a}, Wan Mohtar Wan Yusoff\textsuperscript{a}, Nasrin Moazami\textsuperscript{b} and Zahra Shafiee\textsuperscript{a}

\textsuperscript{a} School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Bangi 43600, Selangor, Malaysia
\textsuperscript{b} Biotechnology center, Iranian Research Organization for Science & Technology (IROST), Tehran, Iran

\textbf{Abstract.} \textit{Bacillus pumilus} 2IR is a soil isolate bacterium from an Iranian oil field that produces promising yield of biosurfactant in medium E. The production of biosurfactant by strain 2IR has been investigated using different carbon and nitrogen sources. The strain was able to grow and to produce surfactant, reducing the surface tension of the medium from 60 mN/m to 31 mN/m on glucose after 72 h of cultivation. The strain was able to produce the maximum amount of biosurfactant (0.72 g/l) when potassium nitrate and glucose used as a nitrogen and carbon sources respectively. Production of biosurfactant reaches to highest amount at a C/N ratio of 12.

\textbf{Keywords:} Biosurfactant, \textit{Bacillus pumilus}, production.
\textbf{PACS:} 82.35.Pq

\textbf{INTRODUCTION}

Biosurfactant or microbial surface active compounds are amphiphiles with polar and non polar moieties that tend to preferentially partition at interfaces and thus reduce the interfacial surface tension [1]. With increasing environmental awareness and emphasis on a sustainable society in harmony with the global environment, during the recent years, natural surfactants of microbial origin, commonly referred to as biosurfactants are getting much more attention compared to chemical surfactants owing to mild production condition, lower toxicity, higher biodegradability and environmental compatibility [2-3]. All the stated qualities of biosurfactants have prompted their tremendous applications in environmental protection as well as in food, cosmetic, biopesticide and pharmaceutical industries [4-5]. A survey of literature shows that biosurfactants are produced by a wide variety of microorganisms; however the chemical nature of biosurfactant is dependent on the producing species [5]. Among the biosurfactant producing potential microbes, \textit{Bacillus subtilis} are known to produce cyclic lipopeptides (CLPs) including surfactins, iturins, fengycins, and lichenysins, as the major classes of biosurfactants. Depending upon the nature of the biosurfactant and the producing microorganisms, the following patterns of biosurfactant production by fermentation are possible: (a) growth-associated production, (b) production under growth limiting conditions, and (c) production associated with the precursor augmentation. In the case of growth-associated biosurfactant production, there exists a parallel relationship between the substrate utilization, growth and biosurfactant production [4,5]. Cell growth and the accumulation of metabolic products are strongly influenced by medium compositions such as carbon sources, nitrogen sources, growth factors, and inorganic salts, because they are essential components for growth of microbes and production of biosurfactant in fermentation process. In the present investigation we report the effect of carbon and nitrogen sources and C/N ratio on growth and biosurfactant production by strain \textit{Bacillus pumilus} 2IR.

\textbf{MATERIALS AND METHODS}

\textbf{Microorganism and fermentative production of surfactin}
The microorganism used in this study, *Bacillus pumilus* 2IR was isolated from Iranian oil field. The pre culture was prepared by transferring a loopful from a fresh culture grown on to nutrient agar in to 50 ml medium E in 250 ml Erlenmeyer flask .The flask was incubated at 150 rpm and 30°C for 14-18h. The composition of medium E was consisted of (g/l): 10 glucose, 50 NaCl, 1 (NH4)2SO4, 0.25 MgSO4, 13.9 K2HPO4, 2.7 KH2PO4, 1 NaNO3, 0.5 yeast extract and 10ml trace salt solution. Trace salt solution had the following composition (g/l): 1 EDTA, 3 MnSO4·H2O, 0.1 FeSO4·7H2O, 0.1 CaCl2·2H2O, 0.1 CoCl2·6H2O, 0.1 ZnSO4·7H2O, 0.01 CuSO4·5H2O, 0.01 AlK (SO4)2·12H2O, 0.01 H3BO3 and 0.01 Na2MoO4·2H2O [6].

The production process was carried out in Erlenmeyer (1000 ml) containing 300 ml aliquots of the fermentation medium under test. The flasks were inoculated with the pre culture at 2% (v/v) and incubated in a shaking incubator (150 rpm) at 30°C for 3 days. The culture broth was centrifuged at 10000 rpm for 10 min to prepare the cell free supernatant. An aliquot of supernatant was acidified to pH 2 using 6N HCL, left overnight at 4°C and then centrifuged at 10000 rpm for 20 min. The pellet was dried and weight as the crude precipitate biosurfactant [6].

**Surface Tension Activity**

The surface tension was measured using ring tensiometer (K 6, Kruss, Hamburg, Germany). All measurement was made on cell free broth obtained by centrifuging the culture at 10000 for 10 min [7-8].

**Biomass Determination**

Samples were centrifuged (10000 rpm) for 10 min at 4°C. The Biomass dry weight was determined by drying at 105°C for 24 h [7-8].

**Nutritional Factors Affecting Surfactin Production**

In order to optimize nutritional parameters, different carbon and nitrogen sources in medium E was studied to determine the ability of the strain *Bacillus pumilus* 2IR on biosurfactant production. In all cases; the monitored fermentation parameters included biomass, biosurfactant concentration and surface tension activity. The optimum C/N ratio was also selected from these studies.

**Effect of Different Carbon Sources**

For the selection of carbon sources different carbohydrates and hydrocarbons; glucose, sucrose, starch, cheese whey, lactose, molasses and hexadecane, crude oil glycerol and paraffin oil were tested for the biosurfactant activities by the isolated strain 2IR. The concentration of all compound used in this study were 3% (w/v) [9-11].

**Effect of Different Nitrogen Sources**

For the selection of nitrogen sources in the fermentation medium different simple Urea, potassium nitrate, ammonium chloride, ammonium sulfate and complex nitrogen sources; yeast extract and peptone were studied. The concentration of all compounds used in this study was 0.3% (w/v). In the present investigation the surface activity of the biosurfactant, cell mass concentration and biosurfactant production were measured, then the appropriate carbon and nitrogen sources were selected by optimization of the medium. The optimum C/N ratio was also selected from these studies [10, 12-13].

**RESULTS AND DISCUSSION**

**Growth Characteristics and Biosurfactant Production on Different Carbon Sources**

FIGURE 1 shows the reduction in surface tension of cell free broth and growth concentration of *Bacillus pumilus* 2IR using different carbohydrates (starch, sucrose, cheese whey, molasses, lactose and glucose) and hydrocarbons (hexadecane, glycerol, paraffin oil and crude oil) as carbon sources. All these carbon sources are good substrate for growth and production of biosurfactant by *Bacillus pumilus* 2IR. Results revealed that surface tension reduction was greater with glucose, starch, cheese whey and sucrose as carbon sources in comparison with other carbon sources as
carbohydrates such as crude oil and hexadecane. From this experimental results glucose showed significant reduction (31mN/m) as compared to the other carbon sources therefore the use of glucose as carbon sources for producing biosurfactant seems to be more interesting and low cost alternative. After selection of the appropriate carbon source the concentration was optimized, for this purpose the different concentration of glucose were used for the production of biosurfactant and cellular growth. The results were shown in TABLE (1) (a) revealed that glucose at a concentration level of 3% indicated the maximum biosurfactant activity (31mN/m) as compared with either lower or higher concentrations. So, optimum concentration of glucose was considered as 3% and selected for further work.

**Effect of Nitrogen Sources on Biosurfactant Production**

Biosurfactant production was affected depending on the nitrogen sources used in the fermentation. Simple nitrogen sources like urea, potassium nitrate, ammonium chloride and ammonium sulfate, were used for biosurfactant production. FIGURE 2 shows potassium nitrate is the best simple nitrogen source of the six tested sources. It causes the highest reduction of surface tension followed by ammonium sulfate. According to the results as shown in FIGURE 2, Yeast extract as a complex nitrogen source significantly affected the concentration of biomass rather than other complex nitrogen sources but it cannot reduce the surface tension as well as other compounds. The basic aspect for the improvement of biosurfactant productivity is the ratio of C/N. The results were obtained using glucose and potassium nitrate as a carbon and nitrogen sources respectively. The best result for optimum C/N ratio was found to be 12:1 (FIGURE 3).

TABLE (1) (b) shows that the optimum concentration of potassium nitrate for maximum biosurfactant production was attained at 0.3% (w/v) due to maximum reduction in surface tension.

![FIGURE 1. Growth and biosurfactant production on different carbon sources by Bacillus pumilus 2IR in medium E after 72 h cultivation.](image)
FIGURE 2. Growth and Biosurfactant production on different nitrogen sources by *Bacillus pumilus* 2IR in medium E after 72 h cultivation.

TABLE (1). The effect of glucose concentration (a) and potassium nitrate concentration (b) on biosurfactant production and growth of biomass by strain of *Bacillus pumilus* 2 IR.

| Glucose Concentration (%w/v) | Surface Tension (mN/m) | Weight of Cell (g/l) | Potassium Nitrate Concentration (%w/v) | Surface Tension (mN/m) | Weight of Cell (g/l) |
|-------------------------------|------------------------|----------------------|----------------------------------------|------------------------|----------------------|
| 1%                            | 48                     | 1.33                 | 0.1%                                   | 45                     | 2.01                 |
| 2%                            | 42                     | 1.78                 | 0.2%                                   | 39                     | 2.89                 |
| 3%                            | 31                     | 2.75                 | 0.3%                                   | 31                     | 3.46                 |
| 4%                            | 38                     | 2.57                 | 0.4%                                   | 35                     | 3.11                 |
| 5%                            | 41                     | 1.76                 | 0.5%                                   | 43                     | 2.64                 |
FIGURE 3. The effect of C/N ratio on biomass, surface tension and biosurfactant production by *Bacillus pumilus* 2IR in medium E after 72 h cultivation.

CONCLUSION

In the present study *B. pumilus* 2IR was isolated from petroleum hydrocarbon contaminated soil sample. Optimum levels of biosurfactant were produced by *B. pumilus* 2IR when grown in medium E containing 30g/l glucose and 3g/l potassium nitrate with C/N ratio of 12:1. The biosurfactant produced reduced surface tension to 31mN/m.

ACKNOWLEDGMENT

The author would like to thank School of bioscience and biotechnology, Department of Science and Technology, University Kebangsaan Malaysia (UKM), Malaysia and Department of biotechnology Iranian Research Organization Science and Technology (IROST), Iran.

REFERENCES

1. P. Singh and S.S. Cameotra, *Trends Biotechnol.* **22**, 142-146 (2004).
2. L.G. Rodrigues, J. Teixeira, R.Oliveira and H. C. van der Mei, *Process Biochemistry* **41**, 1-10 (2006).
3. R.S. Makkar and S.S. Cameotra, *Appl. Microbiol. Biotechnol.* **58**, 428-434 (2002).
4. S.S. Cameotra and R.S. Makkar, *Curr. Opin. Microbiol.* **7**, 262-266 (2004).
5. K. Das and A. K. Mukherjee, *Process Biochemistry* **42**, 1191-1199 (2007).
6. H. Ghojavand, F. Vahabzadeh and A.K. Shahraki, *Journal of Petroleum Science and Engineering* **81**, 24-30 (2012).
7. E. Rismani, J. Fooladi and G.H. Ebrahimi, *Pakistan Journal of Biological Science*. 9, 2498-2502 (2006).
8. H. Youssef, K. E. Duncan, D. P. Nagle, K. N. Savage, R. M. Knapp and M. J. McInerney, *J. Microbiol. Methods* **5**, 339-347 (2004).
9. R.S. Makkar and S.S. Cameotra, *Journal of Surfactants and Detergents* **5**, 11-17 (2002).
10. A. M. A. Mawgoud, M. M. Aboulwafa and N.A.H Hassouna, *Appl. Biochem. Biotechnol.* **150**, 305-325 (2008).
11. H.Sik Kim, B.D.Yoon, C.H. Lee, H.H. Suh, H. Mock T. Katsuragi and Y. Tani, *Journal of Fermentation and Bioengineering* **1**, 41-46 (1997).
12. C. Li, K. Bai, Z. Cai and F. Ouyang, *J. Biotechnol.* **93**, 27-34 (2002).
13. T. Jiraporn, R. Niran, K. Takauki, H. Mitsuru, I. Tadayuki, M. Manasaki, and K. Shigenori, *Biosci. Biotechnol. Biochem.* **67**, 1239-1244 (2003).