Identification of UH-Biosurfactant Producing Bacteria Using the Molecular Method

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Abstract

In this study, UH-biosurfactant producing bacteria was isolated and identified as *Serratia* sp. using the molecular method. Using the Basic Local Alignment Search Tool (BLAST) available in the National Center for Biotechnology Information (NCBI) database, there was 100% matching with *Serratia* marcescens when the 16S ribosomal RNA (16S rRNA) gene was used, and highest matching of 91% with *Serratia* plymuthica when RNA polymerase sigma 70 (sigma D) factor (rpoD) gene was used.

1 Introduction

Biosurfactant is used widely in environmental remediation field for petroleum and heavy metal pollution removal, by reducing surface tension between surfaces. It is also non-toxic, and biodegradable without second pollution. UH biosurfactant has been proven to be quite effective for enhancing the solubility of perchloroethylene (PCE), tetrachloroethylene (TCE), toluene, naphthalene, and phenanthrene in water, and removal of lead (Ghurye 1993; Hariharan 1996; Ren 1998; Srinivas 2005; Harendra 2006). Identification of the bacteria using the molecular method is fast and accurate, and is commonly used for modern bacteria identification. The 16S rRNA is a 1542 nt long component of the small prokaryotic ribosomal subunit, and it is used for bacteria identification in phylogenetic studies as it is highly conserved and contains hypervariable regions, providing species-specific signature sequences. A sigma 70 factor encoded by rpoD gene is a factor controlling gene expression in bacteria, and is a protein essential for survival, this factor is also appropriate for bacteria identification in phylogenetic analysis, as it rarely spreads horizontally and has an appropriate rate of evolution.

2 Objective

The overall objective was to identify the biosurfactant producing bacteria by the molecular method using 16S rRNA gene and rpoD gene.

3 Materials and Methods

3.1 Bacteria Cultivation

After cultivation of autoclaved fermentation liquid with 5% inoculation for 7 days under 30 ˚C, 150 rpm, and initial pH 7, 0.1 mL of fermentation liquid was added to the 0.9 mL of Phosphate-Buffered Saline (PBS) liquid. Bacteria number in the liquid was determined by serial dilution and plate count method with 10 μL of liquid applied on each plate, and bacteria colonies were got after 24 h incubation in 30 ˚C. At least three samples were used under each condition.

3.2 16S rRNA and rpoD gene PCR Amplification

Deoxyribonucleic acid (DNA) of the bacteria was extracted using DNeasy Blood & Tissue Kit after sample enrichment from one single colony. The concentration of DNA extracted was quantified using Nano Drop Spectrophotometer as 126.8 ng/μL. Primers used for 16S rRNA amplification were: the forward 5'-GAGGCAG- CAGTGGGGAATA-3', the reverse 5'-CTAGCGATTCCGACTTCACG-3'. Primers used for rpoD gene amplification were: the forward 5'-ACGACTGACCCGGTA-CGCATGTA-3', the reverse 5'-ATAGAAATAAC- CAGACGTAAGTT-3'. Materials needed for Polymerase Chain Reaction (PCR) was forward primer 1 μL, reverse primer 1 μL, Go Taq Green 7.5 μL, DNA 0.5 μL, and Nuclease free H_2O 5 μL. PCR amplification was as follows: 58.8 ˚C 30', 72 ˚C 40', 94 ˚C 15', 30 circles for 16S rRNA; 51 ˚C 30', 72 ˚C 40', 94 ˚C 15', 35 circles for rpoD gene. Gel

electrophoresis of the products was performed after PCR. The PCR products were purified with QIA quick Spin Kit and used for sequencing.

4 Results and Discussion

The bacteria colonies obtained on agar plate from serial dilution of biosurfactant fermentation liquid showed the same physical characteristics (Fig. 1), and the concentration of bacteria was 10^{10}/L after calculation. Gel electrophoresis showed successful PCR amplification of 16S rRNA and rpoD gene (Fig. 2). BLAST in NCBI database with the sequencing results, 100% matching to *Serratia* marcescens of 16S rRNA gene was obtained, and the highest of 91% matching to *Serratia* plymuthica of rpoD gene was obtained. Thus the bacteria was identified as *Serratia* sp.

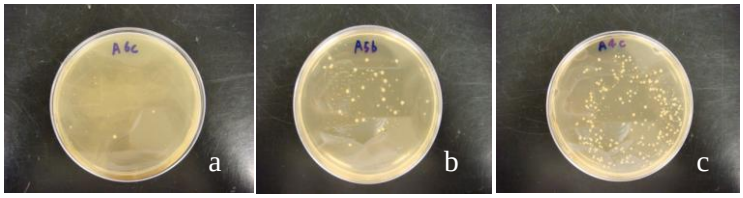

Fig. 1 Bacteria Colonies Obtained with Serial Dilution.

Colonies got after (a) 10^6, (b) 10^5 and (c) 10^4 dilution of 0.1 mL of fermentation liquid respectively, with 10 μL of liquid applied on each plate.

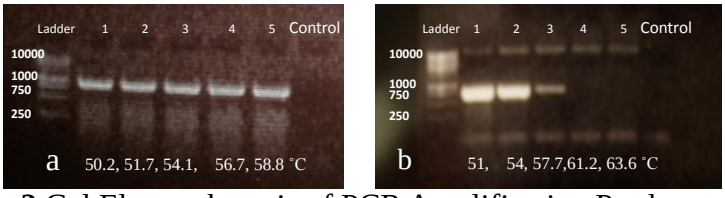

Fig. 2 Gel Electrophoresis of PCR Amplification Products of (a) 16S rRNA and (b) rpoD Gene

1-5 represent different annealing temperatures of (a) 50.2˚C, 51.7˚C, 54.1˚C, 56.7˚C, and 58.8 ˚C respectively, and (b) 51˚C, 54˚C, 57.7˚C, 61.2˚C, and 63.6 ˚C respectively.

5 Conclusions

Bacteria producing UH biosurfactant from used vegetable oil was identified to be *Serratia* sp. using the 16S rRNA and rpoD gene PCR amplification and BLAST in NCBI database after sequencing.

6 Acknowledgements

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