Selection and Characterization of *Pseudomonas aeruginosa* EMS1 Mutant strain Showing Enhanced Biosurfactant Production

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Abstract

A new bacterial strain, was isolated from activated sludge, identified and named *P. aeruginosa* EMS1. The new strain produced surface-active rhamnolipids by batch cultivation in mineral salts medium with waste frying oils. The mutant strain KH7, designated *P. aeruginosa* EMS1, derived by random mutagenesis with N-methyl-N-nitro-N-nitrosogoanidine treatment producing high levels of the biosurfactants was selected by an ion-pair plate assay. The mutant strain KH7 showed 4-5 times more hydrocarbon emulsification as compared to the parent when grown on waste frying oils and various hydrocarbons. Furthermore, *P. aeruginosa* EMS1 and mutant strain KH7 was also able to use whey as a co-substrate for growth and biosurfactant production. As results of this study, mutant strain KH7 is a very efficient biosurfactant producer, and its culture conditions are relatively inexpensive and economical.

Rhamnolipid is synthesized by the *rhlAB*-encoded rhamnosyltransferase. To be convinced of these genes, we performed PCR based on *P. aeruginosa* PAO1 whole-genome database. *rhl* gene cluster nucleotide and amino acid sequences were compared for both parent and mutant. Comparison of nucleotide sequence of *rhlAB,* there were usually terminal's codons exchange.

Introduction

Biosurfactants have several advantages over the chemical surfactants, such as lower toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperatures, pH, and salinity and the ability to be synthesized from renewable feed-stocks (Banat, 2000; Maier, and Soberon,

2000; Muligan, 2001; Matthew, 2000; Lin, 1996).

There are many potentially useful biosurfactants, including both ionic and non-ionic surfactants, which range from short fatty acids to large polymers. This wide range results in a broad spectrum of potential industrial applications. The present study pursues the possibility of increasing the production of biosurfactant through various mutagenesis of *P. aeruginosa* EMS1. And *rhl* gene cluster nucleotide and amino acid sequences were compared for both parent and mutant.

Materials and Methods

1. Random mutagenesis

P. aeruginosa EMS1 isolated and characterized previously was used in these studies. Conditions for growth and metabolite production from this strain have also been optimized. The present study comprise of enhancing emulsifying activity and hyperproduction of biosurfactant through random mutagenesis with *N*-Methyl-*N*-nitro-*N*-nitrosoguanidine of *P. aeruginosa* EMS1.

2. Emulsifying activity

Emulsifying activity was measured by vortexing (Scientific Industries, Vortex-2 gene, U.S.A) for 2 min in 7.5 ㎖ test tube added soybean oil 200 ㎕ and determining the percentage of volume occupied by the emulsion. Turbidity was the determined at 540 nm (Spectronic Genesys 5, Milton Roy Co.).

3. Surface tension and Fcmc

The surface tensions of the culture supernatant were determined using a Tensiometor (Fisher Scientific surface tensiomat 21, U.S.A) according to the Du Nouy ring method. For the determination of Fcmc value, the supernatant was serially diluted and surface tension was measured for each dilution.

4. DNA sequencing analysis

DNA sequencing was performed on a DNA auto sequencer (model ABI PRISM 377, Perkin Elmer) at the Pusan branch of the Basic Science Institute. Computer analyses of the DNA sequence data and the deduced amino acid sequence were done

with programs available on the Expasy Molecular Biology server (http://www.expasy.ch/tools) on the worldwide web. The GenBank and Swiss-Prot databases were searched for protein sequence homology by using BLAST search engines.

Results and Discussion

1. Isolation of the enhanced biosurfactant producing mutant strain

Among the three hundred of mutant strains screened on the basis of plate assay, four mutants were selected for their capacity to produce biosurfactant. Mutant strain KH7, MNNG-induced mutant, showed the highest emulsifying activity.

2. Effect of acidified soybean oil concentration

The yield of biomass obtained in case of mutant strain KH7 was comparatively higher than the wild strain, which indicate that the mutant strain has improved growth kinetics. Mutant strain KH7 was found to emulsifying activity about 2 times greater than wild strain. Mutant strain KH7 gave surface tension reduction of 28 dyne/cm at 2% acidified soybean oil concentration and remained below 30 dyne/cm until 6%, while the wild strain gave surface tension reduction of 29.3 dyne/cm at 2%. Dilution factor (yield a concentration of biosurfactant in culture broth, Fcmc) was highest i. e. 24 in case of mutant strain at 3%, while in case of wild strain Fcmc value of 6 was obtained at 1%. Therefore, biosurfactant production was increased 2-3 times by mutant strain KH7.

3. Comparison of nucleotide and amino acid sequence

In the pairwise alignment comparison of *rhl* gene cluster nucleotide sequences of wild strain with those of mutant strain, *rhlA and rhlB,* genes showed 96%, 98% identities, respectively. Pairwise alignment comparisons of rhl locus of wild strain with that of mutant stain KH7 were performed. There were usually C-terminals amino acids were exchanged.

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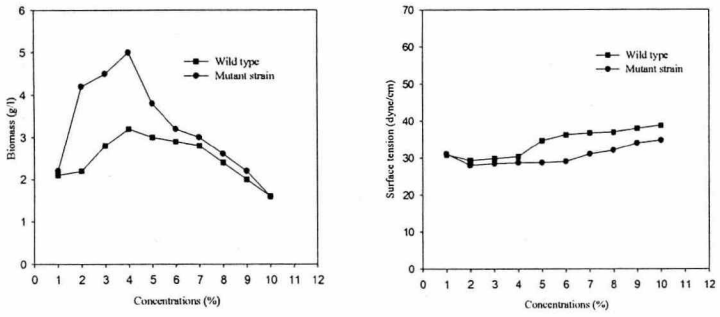

Fig. 1. Comparison of the *P. aeruginosa* EMS1 and mutant strain KH7 using acidified soybean oil as the sole carbon source.