

Title of the Thesis

Molecular characterization of a biosurfactant from *Franconibacter* sp. and its application in oil recovery

Abstract

Surfactants of biological-origin are called biosurfactants. In comparison to synthetic surfactants, biosurfactants are biodegradable, less-toxic to the environment and are functional under extreme environmental conditions (temperatures, pH and salt concentrations). They are structurally more diverse as compared to the synthetic ones. All these properties make them a potential alternative to synthetic surfactants for various applications in petroleum, food, pharmaceuticals, cosmetic, agriculture and bioremediation etc. Despite having better physico-chemical properties and structural diversity, biosurfactant production suffers from limitations such as low productivity, high downstream production cost and lack of knowledge about the metabolic pathways for their production at larger scale for commercialization.

Although, several microorganisms have been reported for biosurfactant production, the latter has been characterized from only a few of them. Furthermore, very few biosurfactant synthesizing microbes have been genetically characterized. This thesis aimed at the production of biosurfactant from a potent bacterium obtained from the oil contaminated soil, its molecular characterization and its utilization in secondary oil recovery.

In this study, a Gram-negative, rod-shaped bacterium was isolated from the oil contaminated soil and its biosurfactant production ability was determined. The isolated bacterium was identified as *Franconibacter* sp. IITDAS19 through 16S rRNA sequencing.

The produced biosurfactant was isolated, purified and characterized by TLC, FTIR, GC-MS and LC-MS. It was identified as a glycolipid. It was found to be very stable at wide range of temperatures, pH and salt concentrations. It could reduce the surface tension of water from 71 mN/m to 31 mN/m. It also showed very high efficacy towards both aliphatic and aromatic hydrocarbons. The concentration of the crude biosurfactant was found to be 4.1 ± 0.5 g/l. The emulsion produced from the biosurfactant was found to be stable for more than 2 months. All these properties make the isolated biosurfactant useful for industrial scale applications. Whole genome sequencing of *Franconibacter* sp. IITDAS19 was also conducted to find out the genes responsible for the biosurfactant production. The genes were overexpressed in a heterologous host and in the native host for enhanced production of the biosurfactant. The produced biosurfactant showed about 63% recovery of residual oil in a sand pack column, which establishes its potential value for microbial enhanced oil recovery.

Thus, this study has led to the production and characterization of a potent biosurfactant from the oil contaminated soil isolate, named as *Franconibacter* sp. IITDAS19. Biosurfactant potential for microbial enhanced oil recovery was also confirmed. This study also provides understanding of the genetic basis of biosurfactant biosynthesis in *Franconibacter* sp. IITDAS19. This can be utilized for metabolic engineering for enhanced biosurfactant production in future.