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"IDENTIFICATION AND CHARACTERIZATION OF GENETIC DETERMINANTS IN BACTERIA INVOLVED IN FROTH FORMATION AT NATURAL WATER BODIES"

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ABSTRACT

Industrialization, modernization, rapid urbanization, and unscientific disposal of inadequately treated sewage and effluent from the various source into surface water bodies has been a major concern leading to water pollution. Frothing of lakes and foam related issues is one such emerging environmental concern in South India. Although several researchers have dealt with the characterization of physical and chemical parameters to understand the mechanism of foam formation, the role of biotic factors has been largely ignored.

The main objective of this project is to identify the bacteria present in the froth forming lakes, to identify and characterize the biosurfactant of the bacteria and amplify the particular gene responsible for foaming. Surface water samples from three locations, namely, Bellandur lake in Bengaluru, Noyyal River in Coimbatore, Perungudi canal in Chennai, were collected to identify the basic biological elements involved in foam formation and seasonal studies were performed. Relevant physico chemical characteristics of seasonal water samples were determined through APHA standard methods. The bacteria is identified using 16 S rRNA gene sequencing method and with help of genbank. The characteristics of the biosurfactant, which is responsible for the formation of foam, was inspected. Basic characteristics of bio-surfactant such a Critical Micelle Concentration (CMC), Emulsifying index, decay constant Kd were also determined. The chemical compound in the biosurfactant is determined using Liquid Chromatography – Mass Spectrometer (LC-MS). After the identification of a particular gene, the primer is designed for the specific gene with the help of Genbank and FASTA tool. Then the gene is amplified with the help of gradient PCR.

After analyzing the characteristics of the water sample, phosphate is the important parameter to look for in the water sample because phosphate is the form of detergent, which exceeds the limit. However, phosphate foam does not last longer than 5 seconds. Despite the presence of highly diverse biological communities, a specific species of bacteria in water samples were to be found dominating and predominant. Interestingly, III laboratory-scale studies indicated that the bacteria alone could contribute to foam formation in growth media. If the bacteria were tested positive for the production of foam, the bacteria could be identified using 16 S rRNA gene sequences with the help of Genbank. The bacteria is identified as *bacillus sp.* The CMC value of biosurfactant (BS) is 10.02 mg/ml, which cannot be lowered further even with the addition of biosurfactant. The emulsifying index is analyzed for 30 days. BS with Diesel oil (DO) has a higher emulsifying index which lowers the surface tension of emulsion more. The

decay constant value for DO is -0.0163, Sunflower oil (SF) is -0.0042 and Kerosene (KO) is – 3 x 10-4. The decay constant for BS with KO is higher, so the stability of the emulsion is higher for KO. Using LC-MS, the biosurfactant produces rhamnolipid is identified. With the help of Genbank and FASTA tool, the primer is designed for rhamnolipid, and gradient PCR is used to amplify the particular gene. The sample DNA is amplified and the band is obtained at the temperature of 62°C and 64°5C.

Keywords: Froth, Foam, Lakes, Biosurfactant, Surface Tension, Genbank, 16 S rRNA, rhamnolipid