

Understanding biofilm formation among different marine bacteria especially *Bacillus* in salt stress environment

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In the Western part of India, world's biggest manmade freshwater reservoir is proposed by constructing a dam on the Gulf of Khambat (GoK) and gradually converting seawater in to fresh water through inflow of incoming river waters. In order to understand the possible impact of this project on marine microbial community, a detailed study was initiated to evaluate the impact of salinity reduction on physiology of bacteria inhabiting in this environment.

A total of 12 different bacterial species isolated from different geographical location of GoK were screened for salinity tolerance in the laboratory. Two strains, identified as *Bacillus alcalophilus* and *Grimontia hollisae*- developed bio film when they cultured at lower salinity, might be due to some salt related stress. Further mechanism of biofilm formation was studied for *B. alcalophilus*. A detailed chemical analysis viz. total protein, carbohydrate, C, H, N, S analysis and Fourier Transform-Infrared Spectroscopy (FT-IR), different microscopic images of biofilms were obtained after 6, 12, 18 and 24 h of incubation. Scanning Electron Microscopy (SEM), and Atom force Microscopy (AFM) were also carried out with the same time scale to understand mode of biofilm formation and possible changes in surface structure during bio film development.

SEM revealed multilayer bacterial deposition and increase in exopolymer content with the growth of biofilm. AFM study also revealed the similar findings. Considerable changes were observed in FT-IR spectra and chemical composition with the formation of biofilm. C, H, N, S analysis revealed that concentration of elemental C, H, and N was low at the initial stage (6 h) as well as at maturation stage (24 h) of membrane formation while maximum after 18 h of incubation time. Elemental sulphur was absent in membrane at any stage of development. In the present study, sincere attempts were made to understand the chemical changes in biofilm of *B. alcalophilus* during its formation under salt stress condition.

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