ABSTRACT

Ecophysiological group of bioemulsifier producing isolates obtained from twelve intertidal zone sampling sites spanning the entire western and one eastern coastal states of India yielded 227 isolates which were screened to acquire twelve isolates belonging to ecophysiological group of sporulating, mesophilic, heterotrophic bioemulsifier producing bacteria capable of quorum quenching with ability to form biofilm and reduce the surface tension. This is the first report of bioemulsifier production by *Solibacillus*, *Sporosarcina*, *Lysinibacillus*, *B. thuringiensis* and *B. flexus*. In this group *Solibacillus silvestris* AM1 was found to possess maximum emulsification activity of 62.5% with broad spectrum of solvent specificity and was therefore selected for further studies. This strain even though showed higher similarity with the type strain HR3-23 in FAME analysis and DNA-DNA hybridization studies, significant differences were observed in its carbon substrate utilization and transition/transversion ratio analyses suggesting evolutionary adaptations towards its niche. *S. silvestris* AM1 produced cell-bound bioemulsifier after 6h and was released into the environment after 16h. The statistical experimental design demonstrated that the bioemulsifier production was influenced by presence of peptone and yeast extract significantly in the Zobell marine medium while non-protein media cited in literature for bioemulsifier production gave negligible results. In natural environment, the organism must be producing the bioemulsifier selectively in proteinaceous but oligotrophic conditions prevailing in its niche. *S. silvestris* AM1 produced an extracellular, homo-multimeric glycoprotein bioemulsifier with a MW of more than 200 kDa and containing 30 kDa monomeric subunits comprising of minor carbohydrate components galactose and ribose/xylose which was also found to have homology with a bacterial flagellin according to Mascot analysis of LC/MS-MS data. It exhibited stability in broad pH and salinity range and also possessed resistance to moderate levels of surfactants and sensitivity to proteinase K. The bioemulsifier also exhibited an interesting feature typical of bacterial functional amyloids i.e., presence of fibrous structure with antiparallel β strand characteristics noted in TEM, CD spectrum and FTIR analysis. The emulsions formed by bioemulsifier AM1 in presence of trichloobenzene and paraffin oil exhibited pseudoplastic non-Newtonian rheological property, as observed by particle size and shear stress analysis. From the
ecophysiological studies undertaken in present work, the natural role of bioemulsifier in *S. silvestris* AM1 is envisaged as follows: It changes the cell surface hydrophobicity and acts as a protectant against the hydrocarbon toxicity. It aids in cell aggregation and adhesion to substratum and consequently helps in biofilm formation by decreasing the interfacial interaction energy. The bioemulsifier possessed the ability to influence the biofilm formation of other bacteria like *Staphylococcus aureus* and *Paracoccus* sp. In addition to emulsification activity, the bioemulsifier AM1 also exhibited biodispersant and hydrocarbon solubilization properties and therefore it has ability to facilitate other compatible hydrocarbon degrading bacteria. In microcosm studies of interaction of *S. silvestris* AM1 and its bioemulsifier with hydrocarbon degrading bacterium *Rheinheimera* sp.C06 representing another ecophysiological group of bacteria isolated from shared niche of *S. silvestris* AM1 revealed their bioremediation potential.