JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2014. Vol. **8**(6), p. 5045-5050

Biosurfactant Production by Bacterial Isolates from Marine Sediments

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(Received: 04 October 2014; accepted: 25 November 2014)

The application of detergents in the fabrics leads to pollution to the environment and causes harmful effect to aquatic organism. This study aims at using surfactants produced by bacteria to curb the hurdles posed by use of synthetic detergents. In the present study bacterial isolates were obtained from marine mangrove sediments collected from Rameshwaram coast, which harbours diverse population of microorganisms. The isolates were found to be gram negative. Two isolates were assessed for the ability to produce biosurfactants. After fermentation in MSM media supplemented with glycerol fabric assay was performed and the isolates VITDAMJ 1 and 2 was found to be capable of producing biosurfactants. The effective isolates were characterized by 16S rRNA sequencing.

Key words: Enterobacteriaceae and "Fabric assay.

The major component of the earth'sbiosphere is represented by the marine environment which covers 70% of the earth surface. The marine ecosystem represents a vast biodiversity and a rich source of novel products. It is facilitated with diverse groups of microorganisms which are capable of producing unique metabolites (Fenical, 1993). Possessing a healthy and rich bioresource,the marine ecosystem provides a wide area for the efficient production of novel products like antibiotics, enzymes, vitamins, drugs, biosurfactants (BS), bioemulsifier (BE) and other commercially important products (Jensen and Fenical, 1994). BS or BE are considered to have major importance among the various marine bioactive compound because of their structural and functional diversity as well as their wide industrial applications (Banat*et al*., 1991; Banat, 1995a; Banat, 1995b; Rodrigues*et al*., 2006).

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Surfactants are the surface active organic compounds which can be broadly classified into two distinct groups: synthetic surfactants and biosurfactants (Pornsunthorntawee*et al*., 2008). Synthetic surfactants are the products obtained from organic chemical reactions whereas biosurfactants are produced by biological means(Banat *et al*., 2000). Pollution by various chemical products in the environment has been a major drawback in recent days and one of them is due to wide usage of the synthetic surfactants.

Biosurfactants are regarded as surface active compounds generated by the living cells like microorganisms (Janek*et al*., 2013). They are found to be ampiphillic in nature which are composed of both hydrophilic as well as hydrophobic moiety. Therefore they show variety of surface activities and involve in solubilisation of hydrophobic substrates (Desaiand Banat, 1997). Biosurfactants are the natural compounds which play a major role over the synthetic surfactants due to their high biodegradability, non-toxicity, low irritancy and compatibility with human skin. They have the large scale industrial as well as practical

applications such as detergents, fabric softeners, cosmetics, laxatives, pharma products, emulsions, pesticides, bioremediation, oil recovery etc. (Makkaret al., 2011). Four common types of BS produced by microorganisms are glycolipid, lipopeptides and lipoproteins, phospholipids and polymeric substances (Healy et al., 1996). Among all the four BS, glycolipids are most challenging biosurfactants which comprises of carbohydrates and long chain aliphatic and hydroxyaliphatic acid. Glycolipids are again classifiedintorhamnolipids, sophorolipids and trihalose lipids. rhamnolipids are the dominant class of glycolipids mainly produced by bacteria. Its structure comprises of a glycosyl head group i.e. a rhamnose moiety and a 3-(hydroxyalkanoyloxy) alkanoic acid i.e. HAA (Soberon-Chavez *et al*., 2005).

The present study was focused in the isolation andidentification of biosurfactants producing bacterial strain VITDAMJ1 obtained from marine sediment of Mandapam, Rameshwaram. And the effective production of biosurfactant was examined by fabric assay analysis on the basis of its stain removal ability.

MATERIALS AND METHODS

Sampling

Marine sediment samples were collected in sterile screw capped bottle from the east coast of Mandapam, Rameshwaram. Random sampling was performed at different locations at a depth of 20-40 meters (Fig.1). All the samples obtained were brought to the laboratory and was processed for isolation of bacteria.

Isolation of Biosurfactant producing bacteria

Bacteria capable of producing biosufactants were isolated after serially diluting the marine sediments. The samples were plated onto Zobell's marine agar by spread plate technique and were incubated at 28 ± 2ºC temperature (Pornsunthorntawee*et al*., 2008).

Morphological and biochemical characterization

Morphological andbiochemical characterizations were performed for the isolates VITDAMJ1 which includes Gram's staining, IMViC, TSI and the test results were observed after 24h of incubation except for VP test which was incubated for 48h.

Media preparation and culture condition

Seed culture was prepared using LB broth and was incubated at 37ºC for 48 h. To assessthe ability of the isolates to degrade hydrocarbons 2ml seed culture was inoculated into 250ml Erlenmeyer flask containing 100ml mineral salt medium (MSM) with the following composition: 1 g/L $(NH_4)_2PO_4$, 3 g/L KH_2PO_4, 0.2 g/L $MgSO_4.7H_2O$ (pH 7.0) by taking 1% v/v glycerol as carbon source. It was then kept for incubation at 37º C in rotary shaker at 200 rpm for 3 days (Venkata and Karanth., 1989).

Fermentation for biosurfactant production

For the production of biosurfactant, 3.5ml of fermented sample was inoculated in 500ml Erlenmeyer flask containing 350ml MSM (1 g/L $(NH_4)_2PO_4$, 3 g/L KH_2PO_4, 0.2 g/L $MgSO_4.7H_2O$ (pH 7.0) by taking 1% v/v glycerol as carbon source). It was then kept for incubation at 200 rpm for 4 days (Janek*et al*., 2008).

Fabric assay analysis

Clean white cotton clothes (2 cm^2) were stained with blood, ketchup, crystal violet and chocolate sauce: ten microliter of blood, ketchup and chocolate sauce was applied onto cloth and was kept for drying overnight. The stained fabrics were subjected to wash analysis with commercial detergent (Surf excel), fermented sample, MSM medium and distilled water. The stained fabrics weretransferred into centrifuge tubes; tube containing distilled water, commercial detergentand fermented sample, uninoculatedMSM mediaserved as control. The tubes were kept in agitation at 1500 rpm for 5 minutes at room temperature. After incubation, fabric pieces were taken out, rinsed with water and dried (KuttuvanValappilSajna*et al*., 2013).

Molecular characterization by 16S rRNA sequencing

Genetic characterization of bacterial strain was carried out by using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Extraction of DNA fromthecells was performed and the 16S rRNA sequence was determined by fluorescent dye terminator method using the sequencing kit (ABI Prism Big dye terminator cycle sequencing ready reaction kit v.3.1). Products were allowed to run on an ABI13730XL capillary DNA sequencer (ABI Prism 310 genetic analyzer, Tokyo, Japan). The

aligned sequences were computed using ClustalW software and sequence homologies were determined using BLASTn search to create an evolutionary distance matrix (Poonguzhali*et al*., 2009).

RESULTS

Isolation of BS producing bacteria

A total of two isolates were obtained using Zobell's marine agar (SeghalKiran*et al*., 2010). The two isolates were named as VITDAMJ1 and VITDAMJ2).Among the two isolates VITDAMJ1 showed positive results for BS production.

Morphological and biochemical characterization

The bacterial strain was found to be gram-negative rod shaped. Bacterial strain was found to produce mixed acid by showing positive result in MR test which was confirmed by occurrence of pink colour where as it showed negative result for VP test. The organism was found to give positive result for citrate utilization test by utilizing sodium citrate as its carbon source and inorganic ammonium salt as its nitrogen source, development of Prussian blue colour confirmed the positive result. In TSI test theformation of bubbles and upliftmentof media in the tube was obtained which indicated the production of CO_2 and change in the colour of media from red to yellow indicated the production of acidwhereas it showed negative result for Indole test.

Fabric Assay

Among the two isolates VITDAMJ 1 was capable of removing strains of blood, ketchup, crystal violet, chocolate sauce. Percentage of stain removal was analysed for all the fabric pieces stained with blood, ketchup, chocolate and crystal violet and were washed with fermented sample, surf excel, uninoculated MSM medium and distilled water. In this analysis surf excel showed complete stain removal for ketchup stain and about 90-95% stain removal for blood and chocolate stain. Whereas the sample showed an effective stain removing ability up to 95% for ketchup stain and

Fig.1. Map of Mandapam, Rameshwaram

a) b) c) d)

Fig. 2. C: Control i.e. MSM medium, S.A.: Fermented sample, D.W.: Dis. H_2O, D: Detergent,A: Blood stain, B: Ketchup, C: Chocolate, D: Crystal violet

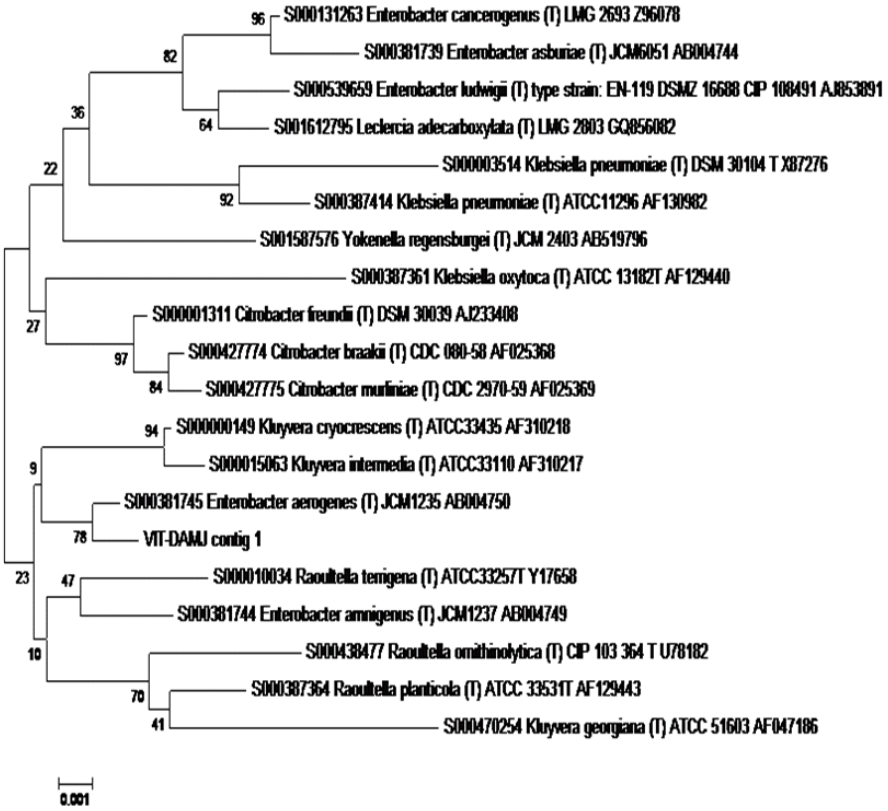

Fig. 3. Phylogenetic tree of the isolate VIT DAMJ1(*) showing evolutionary relationship with other isolates

about 90% of stain removal for both blood and chocolate stain. It was also noticed that both sample and surf excel showed least removal of stain for crystal violet that is up to 50% and 60% respectively (Fig. 2).

Molecular characterization by 16S rRNA sequencing

The isolate obtained from the marine sediment VITDAMJ 1 was found to be the closest neighbour of *Enterobacteriaceae sp*. *w*ith a percentage similarity of 99%. After performing molecular characterization using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') 1492R (5'-GGTTACCTTGTTACGACTT-3') the phylogenetic tree was constructed and the bacteria was categorized under *Enterobacteriaceae sp.*

DISCUSSION

Bacteria are known to synthesize biosurfactants, some of them includes *Pseudomonas fluorescens* BD5, *Micrococcus luteus* BN56, *Pseudomonas putida* BD2, *Pseudomonas aeruginosa* SP4, *Ochrobactrum* sp. 1C and *Brevibacterium* sp. 7G. Though several surfactants have been previously reported there is a need for an effective biosufactant, hence in the present study bacteria was isolated from the marine sediment of Rameshwaram coast due to the increased report on the bacterial communites capable of producing wide range of bioactive compounds (SeghalKiran*et al*., 2010; Abhijit*et al*., 2012). Hence in our study we have focused on the isolation of bacteria using zobell's marine agar media (Karthikeyan*et al*., 2013;Hahnke and Harder, 2013; Rajeev Kumar and Chandrasekharan, 2003).

Two isolates obtained namely VITDAMJ1 and VITDAMJ2 were screened for their potential for BS production. Among the two isolates VITDAMJ1 showed effective results in removal of stains in fabric wash analysis which was similar to that of the study reported Sajna*et al*., 2013.Previous

studies showed that several compounds of bacterial source were capable of removing stain in fabric assay (ValappilSajna*et al.*, 2013; Sagheer*et al*., 2009). Some of the BS includes Rhamnolipid, lipopeptide,Sophorolipids are capable of removing stains (Hirata *et al*., 2009).

The effective strain VITDAMJ1 hence obtained was characterized by various phenotypic and genotypic methods and it was identified by 16S rRNA sequence that the BS producing bacteria is a close neighbour of *Enterobacter* sp.

CONCLUSION

Bacterial strain capable of producing BS was isolated from marine sediment of Rameshwaram coast and biosurfactant was successfully extracted from the bacterial culture. The fabric assay confirmed the effective removal of stain by the strain VITDAMJ 1.

ACKNOWLEDGEMENTS

The authors are thankful to Vellore Institute of Technology and its management for providing facilities for conducting the research successfully.

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