Chinese Journal of Polymer Science Vol. 33, No. 2, (2015), 236–244

Chinese Journal of Polymer Science

Prospecting Exopolysaccharides Produced by Selected Bacteria Associated with Marine Organisms for Biotechnological Applications*

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Abstract In this study, bacteria associated with marine organisms were screened for the production of exopolysaccharides (EPSs) on MY media containing sea salts (2.5%–10%). Three selected isolates were identified as *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 using 16S rRNA gene sequencing. Optimization of the growth and EPS production kinetics in relation to incubation time were assessed. The purified EPS yield was 590, 650 and 540 $mg \cdot L^{-1}$ culture media respectively in *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26. Biochemical and FTIR analyses revealed the presence of biologically important functional groups in the EPS produced by all the three isolates. The EPS produced by *Nitratireductor* sp. PRIM-24 and *Alteromonas* sp. PRIM-21 showed 2.0% sulfate content. These bacterial EPS also showed antioxidant and emulsifying activities and the EPS produced by *Enterobacter* sp.PRIM-26 showed significantly higher antioxidant activities in terms of superoxide (IC_{50} 0.33 $mg \cdot mL^{-1}$) and DPPH (IC_{50} 0.44 $mg \cdot mL^{-1}$) radical scavenging. It also showed higher emulsifying activities against selected hydrophobic substrates with EI_{24} values above 60%. From the results of the study, it can be concluded that the isolated bacteria produce EPS that can be investigated in detail for biotechnological applications.

Keywords: Marine bacterial exopolysaccharides; *Alteromonas; Nitratireductor*; *Enterobacter*; Antioxidant activity; Emulsification.

INTRODUCTION

Bacteria colonize ubiquitously, they are found associated with both plants and animals as symbionts. These host-microbe relationships are often mediated by some important metabolites produced by bacteria such as exopolysaccharides (EPSs)[1]. Exopolysaccharides have several key functions for the bacterial cell and/or its host as they create microenvironments for cell function, metabolism and reproduction[2]. Structurally, EPSs are high molecular weight carbohydrate polymers sometimes with substituted functional groups. Due to diverse functional and physico-chemical properties, in particular, antioxidant, immunological, prebiotic effects, water retention, emulsification and gelling abilities these carbohydrate polymers are used in a variety of industrial applications such as food, pharmaceuticals and cosmetics[3]. Several EPS molecules isolated from bacteria have shown potential antioxidant properties[4]. The growing demand for antioxidants and the emerging trend in replacing synthetic anti-oxidant food additives with natural alternatives stimulated the exploration of efficient biotechnological production approaches, where microbial EPS holds a promise.

Exopolysaccharide producing bacteria have been isolated from various environments, including bacteria associated with other organisms[5]. The EPS produced by associated symbiotic bacteria such as *Alteromonas*

* This work was financially supported by the India and author Priyanka P acknowledges the Yenepoya University Junior research fellowship. The authors thank BRNS (2013/34/19/BRNS/1210).
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Received April 3, 2014; Revised July 28, 2014; Accepted September 1, 2014
doi: 10.1007/s10118-015-1581-7

macleodii ssp. *fijiensis*[6] and *Vibrio diabolicus*[5] isolated from a marine polychaete annelid *Alvinella pompejana* and *Alteromonas* sp. isolated from marine shrimp[7] exhibited unique composition suitable for skin care formulations[3], bone healing material[8] and tissue regeneration[9]. A commercially important EPS with heterogenous composition was isolated from a seaweed associated bacteria, *Bacillus licheniformis*[10]. This offers promise for isolating EPS producing bacteria from marine environment. Hence, an investigation into EPSs produced by bacteria associated with marine multi-cellular organisms was undertaken.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals, DEAE-cellulose, sephacryl S-500 2,2-diphenyl-1-picryl hydrazyl (DPPH), and *m*-hydroxy biphenol were purchased from Sigma Aldrich (USA). Potassium ferricyanide, phenol, ethanol, hydrochloric acid, sulphuric acid, perchloric acid, Tween 80, xylene, toluene and hexane were purchased from Merck (India). Dialysis membranes 12 kDa cutoff, tris-base, crystal violet, MY media components, D-glucose, bovine serum albumin, glucose penta acetate, glucuronic acid and pyrogallol were purchased from HiMedia (India). All the reagents used were of analytical grade.

Isolation of EPS Producing Bacteria Associated with Marine Organisms

Several marine animals and sea weed samples were collected fresh in sterile containers from the coastal regions between Someshwara (12.7862N, 74.8534E) and Malpe (13.3524N, 74.7015E) located along the west coast of India. After transported to lab, the samples were washed and surface sterilized with 70% (*V*/*V*) ethanol dissected aseptically and contents were homogenised. Serial dilutions of the collected samples were done and plated on a complex agar media (MY)[11], supplemented with sea salt solution to obtain a final salt concentration of 7.5% (*W*/*V*)[12]. Based on the mucoid phenotype of the colonies, three EPS producing bacteria were selected, pure cultured and preserved in 30% (*V*/*V*) glycerol at −80 °C. The three isolates were designated as PRIM-21, PRIM-24, PRIM-26.

Quantitative Biofilm Assay

The biofilm formation is often mediated by extracellular polymeric substances, and hence, biofilm formation by the three EPS producing isolates was determined according to the method described earlier[13]. The overnight broth cultures of the isolates were adjusted to 0.8 $O.D._{600}$ and inoculated to 2 mL MY media taken in polystyrene cuvettes (Volex, India). The cultures were incubated at 32 °C for 48 h under static conditions following which liquid culture was decanted, and the biofilm was gently washed with normal saline and stained with 0.1% (*W*/*V*) crystal violet. It was solubilized in acetic acid (33%) and $O.D._{590}$ was read spectrophotometrically for the quantification of the biofilm. Four repeats were carried out to obtain mean biofilm values.

Taxonomic Identification of the Selected Isolates

Taxonomic identification of the strains was carried out by 16S rRNA gene sequencing as described elsewhere[14]. Briefly, the genomic DNA was extracted from the 48 h grown bacterial culture using genomic DNA extraction kit (Mobio Inc). Then PCR amplification of the 16S rRNA gene was carried out using 3F/9R universal primer pair. BigDye terminator cycle sequencing kit was used for sequencing (ABI PRISM 310, Applied Biosystem, USA). Sequenced data was aligned and compared with available standard sequences of culturable and non-culturable bacterial lineage in the web based EzTaxon[15].

Culture Conditions and EPS Production

Incubation time required for the maximum EPS production by the isolates, *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter cloacae* PRIM-26 was studied by culturing them in 250 mL MY media supplemented with 7.5% sea salts for seven days in quadruples. Quantification of the EPS yield at specified time intervals (24, 48, 72, 120 and 168 h) was carried out by the previously described method[16]. Salinity tolerance and EPS production in response to salinity were observed by plating the isolates on MY media supplemented with sea salts to reach the required final concentrations (1, 2.5, 5, 7.5, 10 and 15% *W*/*V*).

For EPS extraction, the bacteria were grown in MY media under optimum salinity conditions for 72 h at 32 °C under agitation (100 rev·min^{-1}). EPS was extracted by cold ethanol precipitation of the culture supernatant, and precipitated EPS was recovered by centrifugation[16]. The EPS was dissolved in deionised water and dialysed against Milli Q water using a 12 kDa cut off dialysis membrane for 24 h, lyophilized and dry weight was recorded. The extracted EPS was purified by gel permeation chromatography (GPC) on a Sephacryl S500 column (Sigma) and anion exchange chromatography (AEC) on a DEAE-cellulose column (Sigma). The purified sample was lyophilized and stored in vacuum desiccator till analysis.

Characterization of EPS

Biochemical characterization of the EPS was carried out by standard spectrophotometric analysis (Shimadzu UV-1800, Japan). Total carbohydrate content was estimated by phenol-sulphuric acid method[17], total proteins by Lowry's method[18], uronic acids by *m*-hydroxybiphenyl method[19], acetyl residues by ferric perchlorate method[20], sulfate content by rhodizonate method[21] and phosphate content by micro method[22].

FTIR spectroscopy

Structural characterization of the EPS was carried out by FTIR spectroscopy. For this, purified EPS (10 mg) was pelleted with KBr and FTIR spectrum was recorded in the frequency range of 4000–500 cm^{-1} using an IR spectrometer (Prestige 21, Shimatzu, Japan).

Antioxidant Properties of the EPS

Antioxidant activity of the EPS in terms of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging was assayed at concentrations of 0.25, 0.50 and 1.0 mg·mL^{-1} according to a previously described method[23]. Superoxide radical scavenging activity was measured according to Li[24] at concentrations between 40 and 320 µg/mL. The IC_{50} (inhibitory concentration for 50% reduction of the free radicals) was calculated by the regression analysis of the graph plotted with the % inhibition data against the concentration. Ferric (Fe^{3+}) reducing power (FRP) of EPS was determined according to the method of Oyaizu[25] and total antioxidant capacity (TAC) was measured by assaying the ability of EPS to covert Mo (VI) to Mo (V)[26] at concentrations between 0.25 and 1.0 mg·mL^{-1}. For all these experiments, the mean values were calculated from four independent repeats.

Emulsification Activity of the EPS

Emulsification activity of the EPS against different hydrophobic oils was carried according to the method of Cooper and Goldenberg[27]. For this, 5 mL EPS solution (0.5% *W*/*V*) in distilled water was mixed with an equal volume of different hydrophobic substrates namely olive oil, sunflower oil, petrol, kerosene, hexane, xylene and toluene. The mixture was vortexed vigorously for 5 min and kept under static condition for 24 h at 4 °C. Tween 80 at a concentration of 0.5% *V*/*V* was used as a positive control. The experiment was repeated (n = 4) to obtain a mean emulsification index (EI_{24}). The turbid emulsified layer was measured and EI_{24} was calculated using the following formula.

$$EI_{24} = \frac{\text{Volume of the emulsified layer}}{\text{Total volume}}$$

Statistical Analysis

All values represent the mean of quadruples (n = 4) that were analysed by one way analysis of variance (ANOVA) using the STATISTICA (Stat Soft, Inc.). Unless specified, the results were considered significantly different if, $p < 0.01$.

RESULTS

Taxonomic Identification of the Bacteria

All the three selected isolates were gram negative rods and produced prominent mucoid colonies with entire margin on MY media supplemented with sea salts (2.5%–7.5%, *W*/*V*) (Fig. 1). Taxonomic identification of the

isolates by 16S rRNA gene sequencing identified strain PRIM-21 isolated from a sea weed as *Alteromonas* sp. with 98.78% similarity to *Alteromonas hispanica* F-32^T. Strain PRIM-24 isolated from the gut content of marine crab was identified as *Nitratireductor* sp. PRIM-24 with 99.77% similarity to *Nitratireductor kimnyeongensis* sp. KY 101^T and PRIM-26 isolated from the gastro-vascular contents of a sea anemone as *Enterobacter* sp. with 99.46% similarity to *Enterobacter cloacae* ssp. *dissolvens* LMG 2683^T. The 16S rRNA gene sequences of the isolates *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 were submitted to GenBank under the accession number KJ210054, KJ210055 and KJ210057 respectively.

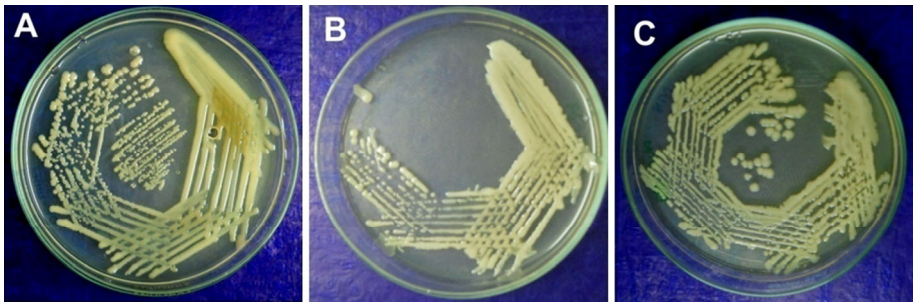

Fig. 1 Growth rate of the isolates (A) *Alteromonas* sp. PRIM-21, (B) *Nitratireductor* sp. PRIM-24 and (C) *Enterobacter* sp. PRIM-26 on MY agar plates following 72 h incubation

Biofilm Formation

It was observed that all the three isolates could form biofilms on the polystyrene surface with a corresponding OD_{590} of the stained biofilms being (1.093 ± 0.264), (0.728 ± 0.194) and 0.362 ± 0.122 for *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 respectively.

Growth Kinetics and EPS Production

Among the three isolates, the maximum EPS was produced by *Alteromonas* sp. PRIM-21 followed by *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 (Fig. 2). In the isolate, *Alteromonas* sp. PRIM-21, a significant increase in the EPS production was observed until 72 h. *Nitratireductor* sp. PRIM-24 produced highest EPS at 120 h growth however it was not significantly higher than the 72 h yield. *Enterobacter* sp. PRIM-26 showed relatively lower EPS yield till 48 h, followed by a significant increase till 120 h. A significant decrease in EPS content was observed after 168 h of growth in both *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26.

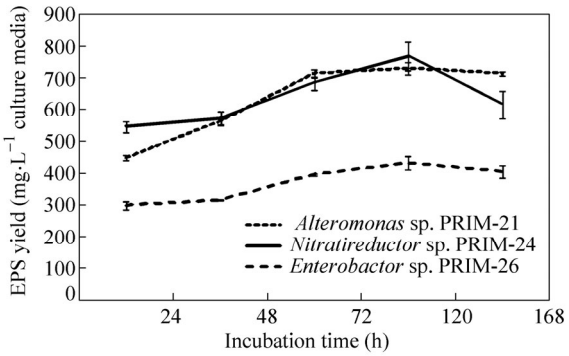

Fig. 2 Kinetics of EPS production in *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 (Values are mean ± SD, n = 4)

The salinity tolerance studies indicated that *Alteromonas* sp. PRIM-21 was tolerant to higher salinity compared to *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp.PRIM-26. *Alteromonas* sp.PRIM-21 and

Enterobacter sp. PRIM 26 showed highest growth with significantly high EPS production at 7.5% salinity, however the optimum salinity for EPS production by *Nitratireductor* sp. PRIM-24 was at 2.5%. *Nitratireductor* sp. PRIM-24 produced maximum EPS (650 mg·L^{-1}) under optimum salinity (2.5%) conditions. Whereas, EPS yield under the optimum salinity (7.5%) by *Alteromonas* sp. PRIM-21 and *Enterobacter* sp.PRIM-26 were 590 and 540 mg·L^{-1} respectively.

Biochemical Characterization of the EPS

Biochemical characteristics of the EPS produced by the three strains are presented in Table 1. Among the isolates, total carbohydrate content was highest (62%) in the EPS produced by *Enterobacter* sp. PRIM-26 followed by *Alteromonas* sp. PRIM-21 (51%) and *Nitratireductor* sp. PRIM-24 (39%). The uronic acid content was 92%, 56% and 40% of the estimated total sugar content in EPS produced by the isolates *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24, *Enterobacter* sp.PRIM-26 respectively. Functional groups namely phosphate and acetyl were detected in all the EPS studied. Interestingly, the EPS produced by *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 contained sulfate groups (2%).

Table 1. Biochemical composition of the EPSs produced by *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 (Data are mean ± SD; $n = 4$)

Biochemical Composition (mg·g^{-1} EPS)	Isolates		
	Alteromonas sp. PRIM-21	*Nitratireductor* sp. PRIM-24	*Enterobacter* sp. PRIM-26
Carbohydrate	507.5 ± 11.9	390.7 ± 17.7	625.2 ± 6.7
Protein	63.4 ± 0.9	119.9 ± 1.5	31.7 ± 0.3
Uronic acid	466.0 ± 11.1	218.7 ± 5.0	253.3 ± 6.1
Acetyl	18.6 ± 0.3	7.4 ± 0.2	11.7 ± 0.9
Phosphate	2.2 ± 0.1	7.5 ± 0.2	1.1 ± 0.1
Sulfate	19.5 ± 0.4	22.0 ± 0.2	ND

ND: Not detectable

FTIR Analysis

The FTIR spectra of the EPS produced by the bacterial isolates showed characteristic absorption bands for polysaccharide at 3600–3200 and 1075–1010 cm^{-1} corresponding to the hydroxyl groups and peaks at 2926, 2850 and 1458 cm^{-1} assigned to alkyl groups (Fig. 3). The absorption peak corresponding to uronic acids in the region 1740–1210 cm^{-1} was observed in all the three EPS, however intensity of the peaks was maximum for the EPS produced by *Alteromonas* sp. PRIM-21. The IR band at 1650–1500 cm^{-1} representing amino group corresponding to proteins was also detected in all the three selected EPS[28]; intense peaks at this region were observed in the EPS produced by *Nitratireductor* sp. PRIM-24. Peaks characteristic to sulfate groups at 1250 cm^{-1}[29] were observed in the EPSs of *Alteromonas* sp. PRIM-21 and *Nitratireductor* sp. PRIM-24. The peaks indicative of the substituent groups of the EPS correlated with the results obtained in the biochemical characterization data.

Antioxidant Activities of the EPSs

Antioxidant activities of the EPSs were evaluated based on the ability to inhibit a stable non-biological radical DPPH and a biologically harmful radical superoxide. The EPS produced by *Enterobacter* sp. PRIM-26 was more potent (IC_{50} 0.44 mg·mL^{-1}) against DPPH radical followed by *Nitratireductor* sp. PRIM-24 (IC_{50} 0.49 mg·mL^{-1}) and *Alteromonas* sp. PRIM-21 (IC_{50} 0.61 mg·mL^{-1}). The superoxide radical scavenging activities (IC_{50}) of the EPS produced by *Alteromonas* sp. PRIM-21 and *Enterobacter* sp. PRIM-26 were 0.65 mg·mL^{-1} and 0.33 mg·mL^{-1} respectively. The EPS produced by *Nitratireductor* sp. PRIM-24 did not show superoxide radical scavenging activity. The EPS produced by *Enterobacter* sp. PRIM-26 showed significantly higher antioxidant activities in terms of both superoxide radical and DPPH radical scavenging. However, the TAC and FRP activities of this EPS were lower than the other two EPSs (Fig. 4).

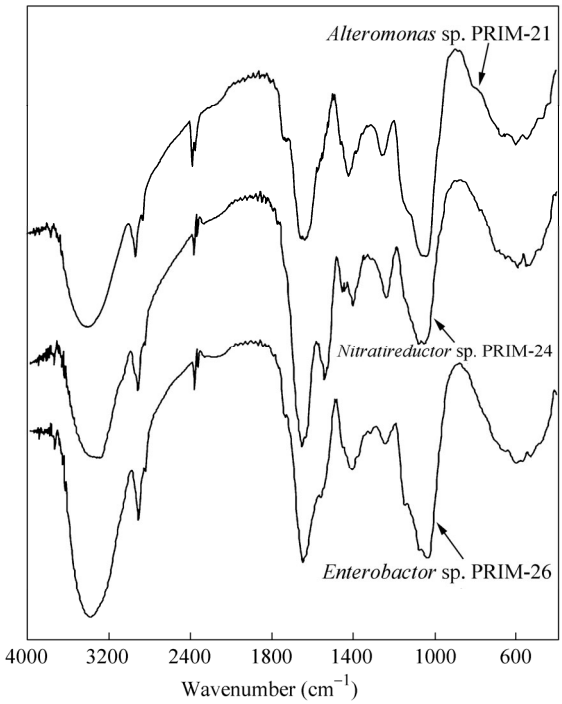

Fig. 3 The FTIR overlay spectra of the EPSs produced by *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26

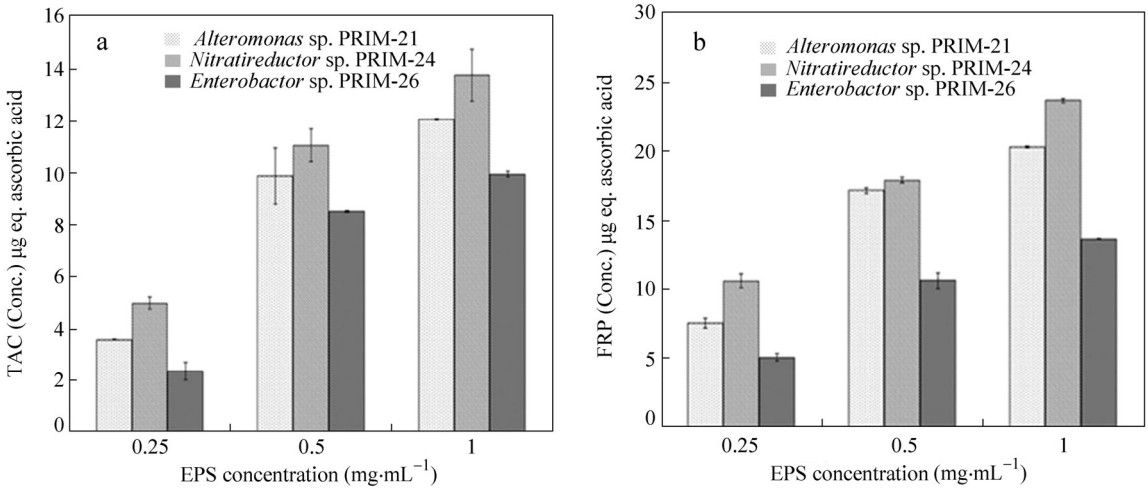

Fig. 4 The antioxidant potential assessed based on (a) the total antioxidant capacity and (b) ferric reducing power of the EPSs produced by *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 (Values are mean ± SD, n = 4)

Emulsification Index

All the three EPSs at a concentration of 0.5% *W*/*V* could emulsify most of the hydrophobic substrates tested such as hydrocarbons (petrol, kerosene, hexane, xylene, toluene) and vegetable oils (sunflower, olive oil) (Table 2). The EPS produced by *Enterobacter* sp. PRIM-26 could emulsify all the tested hydrophobic substrates and *Alteromonas* sp. PRIM-21 could emulsify all except sunflower oil, olive oil and petrol.

Table 2. Emulsification index of the EPSs produced by *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 against the hydrophobic substrates after 24 h (Data are mean ± SD, $n = 4$, Tween 80 at a concentration of 0.5% (*V*/*V*) was used as positive control.)

Hydrophobic substrate	Isolates (EI_{24})			
	Alteromonas sp. PRIM-21	*Nitratireductor* sp. PRIM-24	*Enterobacter* sp. PRIM-26	Tween 80
Olive oil	NE	53 ± 2	65 ± 4	62 ± 2
Sunflower oil	NE	NE	63 ± 2	60 ± 1
Petrol	NE	49 ± 4	58 ± 2	56 ± 1
Kerosene	61 ± 2	70 ± 3	61 ± 9	23 ± 1
Hexane	52 ± 1	64 ± 2	61 ± 3	10 ± 2
Xylene	57 ± 5	69 ± 2	68 ± 2	52 ± 2
Toluene	60 ± 2	71 ± 5	70 ± 2	38 ± 3

NE: No emulsion

DISCUSSION

In the present study, EPS producing bacteria associated with marine organisms have been isolated. Three bacterial isolates, *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp.PRIM-26 were selected based on the mucoid appearance of the colonies. Earlier reports have revealed that members of the genus *Alteromonas* are mainly isolated from sea water and a few members are also reported to be associated with marine animals[6]. *Nitratireductor* sp. PRIM-24 closely resembles *Nitratireductor kimnyeongensis* KY 101^T isolated from a dried seaweed sample in Korea[30]. Members of *Enterobacteriacea* occur as normal gut flora in many terrestrial animals, and also reported to be associated with marine organisms such as sea urchin[31]. *Alteromonas* and *Enterobacter* species generally produce EPS with important biological activities[6, 31], however; limited information is available on the EPS production by *Nitratireductor* species.

The three selected isolates were capable of producing strong biofilms on polystyrene surface. In the marine habitats the biofilm formed mediate host-bacteria associations or surface adhesions. Formation of biofilms by secretion of EPSs along with proteins, nucleic acids and some organic compounds have important physiological role in bacteria as it positively influences the survival of marine organisms by increasing the availability of nutrients and carbon substrates[2].

EPS production in the isolates began early, during the late exponential growth phase (24–48 h) and continued till stationary phase. The EPS production kinetics was similar to some other reported bacteria such as *Halomonas maura*[32]. After the optimum incubation time there was a decrease in the quantity of EPS in the culture, which may be due to enzymatic degradation[33]. Increased EPS production was obtained at higher salinity (> 1% *W*/*V*) in all the three isolates. It is known that EPS plays a protective role against osmotic stress and overproduction under increased salinity is also reported elsewhere[34]. To provide buffering action against increased salinity, increased secretion of EPS is observed in some sea ice bacteria[35]. Further, biochemical characterization of the selected EPS revealed the presence of uronic acid containing sugars and other groups such as sulfate, phosphate and acetyl associated with it. These functional groups and carboxylated sugars in the EPS have important physiological role in marine bacteria and are ionisable at the sea water pH (8) thereby give a negative charge to the polymer. This negative charge helps buffering the cells against fluctuating salinity experienced in the marine environments[36].

Sulfated EPSs isolated from bacteria have shown to possess anticoagulant, antioxidant and bone healing properties[8, 37], similarly acetyl and phosphate groups in the EPS also render biologically important properties[38, 39]. Ability of the EPS to scavenge free radicals is another property of biological importance. The accumulation of free radicals is a key player in the pathogenesis of many degenerative diseases like atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degeneration of eyes[40]. Hence, natural antioxidants are used as prophylactic agents in such conditions. It is interesting to note that the antioxidant activity shown by the three EPSs is higher compared to mannans isolated from *Edwardsiella tarda*[4]. The protein content and functional groups often contribute towards higher antioxidant activity[41]. The

superoxide radical scavenging activity obtained for EPSs produced by *Alteromonas* sp. PRIM-21 and *Enterobacter* sp. PRIM-26 was higher compared to that in earlier reports on some non-sulfated EPSs produced by *Bacillus licheniformis*[42], however it was lesser compared to some highly sulfated EPSs[39]. Though the isolate *Enterobacter* sp. PRIM-26 showed potent radical scavenging activities, the TAC and FRP were lower compared to those of the EPSs of *Alteromonas* sp. PRIM-21 and *Nitratireductor* sp. PRIM-24. The TAC and FRP activities measure the ability of the EPSs to render the transition metals less active to fenton's reaction by chelation/change the oxidation state, and thereby inhibit the generation of hydroxyl radical. It is found that functional groups are important components that take part in chelation reactions and the relative higher content of functional groups such as sulfate in the EPSs produced by *Alteromonas* sp. PRIM-21 and *Nitratireductor* sp. PRIM-24 could be the cause for the significantly higher TAC and FRP activities. Antioxidant activities of the EPS have various biotechnological applications. The EPS isolated from a probiotic bacteria, *Lactobacillus plantarum* shows potent antioxidant activities conferring additional health benefits[43].

The EPS due its surface active property enables emulsification of hydrophobic substrates. Emulsifiers are used extensively in food, cosmetic and environment remediation sectors. All the three EPSs studied showed emulsification activity against at least four hydrophobic substrates studied. The formation of emulsions is associated with the high molecular weight of the EPS which is known to prevent the coalescence of oil droplets by forming an efficient coating around them[44]. The emulsification activity can be further enhanced by the presence of proteins and anionic functional groups. In some protein containing EPSs, the protein moiety binds initially to the hydrophobic substrate irreversibly following which the emulsion is stabilized by the polysaccharide component[45]. The emulsification activity obtained for the EPS produced by *Enterobacter* sp. PRIM-26 was comparable to that of the EPS produced by *Salipiger mucosus* $A3^T$ and Tween 80 at similar concentrations[46]. Moreover, light microscopic observations of the emulsions revealed small uniform droplets with smooth consistency. Emulsifying agents that can form uniform and stable emulsions have application as emulsifiers or surfactants.

CONCLUSIONS

It can be concluded that bacteria living in association with marine organisms can be looked upon as ideal source for isolating important carbohydrate polymers. The EPSs produced by the isolates *Alteromonas* sp. PRIM-21, *Nitratireductor* sp. PRIM-24 and *Enterobacter* sp. PRIM-26 have shown important structural and functional properties. Further, optimisation of the media components can enhance the yield and economical feasibility of production. The presence of biologically important functional groups, antioxidant and emulsifying activities obtained for the EPS holds a promising scope for further exploration into potential biotechnological applications.

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