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Isolation and Characterization of Mucous Exopolysaccharide (EPS) Produced by *Vibrio furnissii* Strain VB0S3

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Abstract Marine bacterial strains were isolated from coastal regions of Goa and screened for the strains that produce the highest amount of mucous exopolysaccharide (EPS). Our screening resulted in the identification of the strain *Vibrio furnissii* VB0S3 (hereafter called VB0S3), as it produced the highest EPS in batch cultures during the late logarithmic growth phase. The isolate was identified as VB0S3 based on morphological and biochemical properties. Growth and EPS production were studied in mineral salts medium supplemented with NaCl (1.5%) and glucose (0.2%). The exopolymer was recovered from the culture supernatant by using three volumes of cold ethanol precipitation and dialysis procedure. Chemical analyses of EPS revealed that it is primarily composed of neutral sugars, uronic acids, and proteins. Fourier-transform infrared (FT-IR) spectroscopy revealed the presence of carboxyl, hydroxyl, and amide groups, which correspond to a typical heteropolymeric polysaccharide, and the EPS also possessed good emulsification activity. The gas chromatographic analysis of an alditol-acetate derivatized sample of EPS revealed that it was mainly composed of galactose and glucose. Minor components found were mannose, rhamnose, fucose, ribose, arabinose, and xylose. EPS was readily isolated from culture supernatants, which suggests that the EPS was a slime-like exopolysaccharide. This is the first report of exopolysaccharide characterization that describes the isolation and characterization of an EPS expressed by *Vibrio furnissii strain* VB0S3. The results of the study contribute significantly and go a long way towards an understanding of the correlation between growth and EPS production, chemical composition, and industrial applications of the exopolysaccharide in environmental biotechnology and bioremediation.

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Exopolysaccharides (EPSs) are high molecular weight carbohydrate polymers that make up a substantial component of the extracellular polymers surrounding most microbial cells in the marine environment. EPSs are organic macromolecules that are formed by polymerization of similar or identical building blocks, which may be arranged as repeated units within the polymer. Polysaccharides are the most abundant components, generally representing 40% to 95% of the extracellular polymeric substances [22]. Abundant microbial polysaccharides present in dissolved organic carbon, particulate material, or biofilms are of major significance in the marine environment. EPSs constitute a large fraction of the reduced carbon reservoir in the ocean and enhance the survival of marine bacteria by influencing the physicochemical environment around the bacterial cell [8].

Bacterial exopolymers are important in the interaction between bacteria and their environment and are chemically diverse. The major organic fractions of the EPS are carbohydrates, proteins, and humic substances [40]. A wide range of chemical structures of homopolymeric or heteropolymeric type, made up of sugar and nonsugar components, is possible and the range of monosaccharide combinations, together with noncarbohydrate constituents and varied linkage types, makes the exopolymer an excellent emulsifying agent and attributes diversity in bacteria [33]. The bacterial exopolymers are usually acidic heteropolysaccharides possessing the functional groups (*e.g.*, hydroxyl, carboxyl, and phosphoric acid) associated with EPS, which exhibit high affinity towards certain metal ions [36]. Many interesting physical and chemical

properties of microbial exopolysacharides, have found a wide range of applications in the field; *e.g.*, stabilizing, suspending, thickening, gelling, coagulating, film-forming, and water retention capabilities. They may be useful in detergents, textiles, adhesives, paper, paint, food and beverage industries [56], oil recovery, heavy metal polluted soils and water [12, 24, 52], mining industry and petroleum industries [53, 54], replacement of chlorinated solvents used in cleaning-up of oil-contaminated pipes, and the formation of stable oil-in-water emulsions for the food [51]. A wide range of bacteria from various environmental habitats produce complex and diverse EPSs occurring as capsular polysaccharides intensively associated with the cell surface, or as slime polysaccharides loosely associated with the cell [60]. Exopolysaccharides like xanthan, dextran, and gellan produced by bacteria are now widely accepted products of biotechnology [53, 54].

Bacterial growth is often accompanied by production of EPSs, which have relevant ecological and physiological functions. The nutrient status and growth phase of surface-associated bacteria may influence the quality and composition of the EPS produced [14]. In recent years, there has been growing interest in the isolation and characterization of microbial EPSs owing to their importance in adhesion, nutrient sequestration, chelation of heavy metals, detoxification of toxic compounds, and protection against osmotic shock [14, 30]. Despite their importance, very few studies were carried out on the chemical characterization of EPSs from various marine bacteria such as *Vibrio*, *Pseudomonas*, *Alteromonas*, *Marinobacter*, etc. [7, 26, 35, 39, 46, 49, 50]. The biotechnological potential of these biopolymers from marine and estuarine environments remains largely untapped. Since EPSs are industrially important compounds, experiments were designed to isolate EPSs and characterize them chemically and physically from the bacterium *Vibrio furnissii* strain VB0S3.

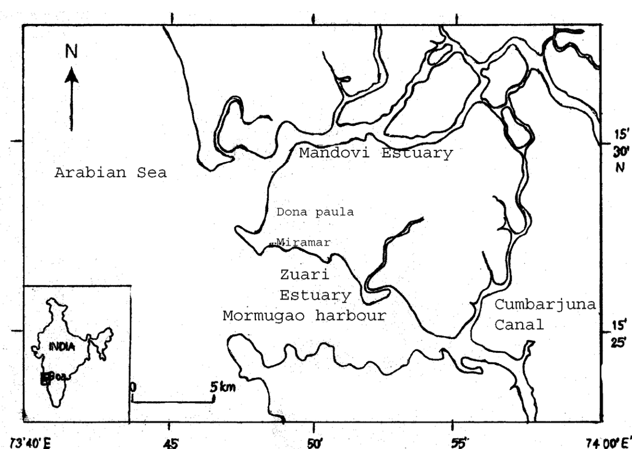

Fig. 1. Map showing sampling sites for collection of bacterial isolates from the west coast of India (Goa region).

MATERIALS AND METHODS

Sampling and Culture Conditions for EPS Production

Exopolymer-producing bacteria were isolated from water samples collected from different sampling sites of the river Mandovi and Zuari estuarine network connecting the coastal regions of Goa (Fig. 1). The bacterial isolates were obtained by serial dilution plating on TCBS agar plates incubated at 28±2°C for 24 h. The culture VB0S3 was grown and maintained in optimized mineral salts medium containing 0.2% glucose, 12.6% K_2HPO_4, 18.2% KH_2PO_4, 10% NH_4NO_3, 1% $MgSO_4 \cdot 7H_20$, 0.6% $MnSO_4$, 1% sodium molybdate, 1% $CaCl_2 \cdot 2H_20$, 0.06% $FeSO_4 \cdot 2H_20$, and 1.5% NaCl in 1 l of distilled water.

Strain Maintenance and Characterization

Strains were stored at –20°C in trypticase soya broth (TSB; Himedia Laboratories) containing 20% (v/v) glycerol and subcultured once in every 4 months. Before its use as inoculum, strains were subcultured on TSA, and plates were incubated at 28°C for 24 h. The bacteria were screened for their ability to produce exopolymer based on colony morphology (mucoid phenotypes). Among the screened isolates, strain VB0S3 was found to have the ability to form a viscous exopolymer after the ice-cold ethanol precipitation. The strain was tentatively identified as *Vibrio furnissii* based on Gram staining, cell morphology, and classical biochemical tests following *Bergey's Manual of Systematic Bacteriology*, Alsina and Blanch set of keys for biochemical identification of marine *Vibrio* sp. [3, 5]. For phylogenetic characterization, DNA preparations and 16S rDNA sequence analysis with primer consensus 16S rRNA gene forward primer 63f (59-CAG GCC TAA CAC ATG CAA GTC-39) and reverse primer 1387r (59-GGG CGG WGT GTA CAA GGC-39) (Integrated DNA technologies, U.S.A.) were used. In total, 1,324 bp were sequenced, and then 1,087 bp were compared with other sequences available in GenBank (www.ncbi.nlm.nih.gov). The isolate VB0S3 was selected for extraction, purification, and characterization of an exopolymer from *Vibrio furnissii.*

Growth Curve and Exopolymer Production of Strain VB0S3

The bacterial cultures for EPS production were grown and maintained as batch cultures in 200 ml of MSM supplemented with NaCl to a final concentration of 1.5% (w/v), and 0.4% glucose in 500-ml Erlenmeyer flasks on a rotary shaker at 28±2°C for 2 days. The pH of the medium was adjusted to 7.0 with 1 N NaOH. Medium (250 ml) was dispensed in 500-ml Erlenmeyer flasks and inoculated with 2% (v/v) of an overnight grown culture in the same medium at room temperature (28±2°C) on a rotary shaker at 160 rpm. Subsamples of 5-ml aliquots were drawn at regular intervals for turbidity measurements of bacterial growth (A_{600} nm) and EPS production.

Extraction, Purification, and Quantification of Exopolymer VB0S3

The culture volume of 200 ml was centrifuged at 15,000 ×*g* for 20 min at 4°C. The cell pellets were freeze-dried and weighed. The supernatants were pressure-filtered through cellulose nitrate filters with the following pore sizes: 0.8, 0.45, and 0.25 μm (Millipore filters, Bangalore, India). EPSs were precipitated from the final filtrate after the addition of three volumes of cold ethanol and the solution was chilled to 4°C overnight. The resulting precipitate was recovered by vacuum filtration through a scintered glass apparatus. An additional 100 ml of cold ethanol was added to the filtrate and the solution was placed at −20°C overnight. The precipitate was recovered as above. The precipitates were washed with 70−100% ethanol-water mixtures. After washing with ethanol, the EPSs were combined and dried in a desiccator and stored at room temperature until needed. To remove excess salts, the EPS were redissolved in distilled water and dialyzed at 4°C for 24 h (molecular weight cutoff of 13 kDa; Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) against distilled water. Excess water was removed under vacuum before lyophilization. The EPS extracted was lyophilized using a Labonco lyophilizer (Kansas City, MO, U.S.A.) at 3,000 psi. The lyophilized exopolysaccharide was stored at room temperature until chemical and physical analyses were performed. Lyophilized exopolysaccharide was hydrolyzed with 2 N HCl for 2 h at 100°C in ampules flushed with N_2 before sealing. After hydrolysis, the solution was evaporated to dryness under reduced pressure at 40°C [49]. The lyophilized sample was used for chemical and physical characterization.

Emulsifying Activity of VB0S3 Exopolysaccharide

The emulsifying activity of EPS was assayed by modifying the method described by Nielsen and Jahn [38]. Lyophilized EPS (0.5 mg) was dissolved in 0.5 ml deionized water by heating at 100°C for about 15−20 min and allowed to cool to room temperature (28±2°C). The volume was then made up to 2 ml using phosphate-buffered saline (PBS). The sample mixures were vigorously vortexed for 1 min after the addition of the various hydrophobic substrates: diesel and crude oil supplied by the Indian Oil Corp. Ltd; and xylene, toluene, and Tween 80 by Qualigens Chemical Comp, Mumbai, India. Samples were incubated at 30°C with a reciprocal shaking speed of 160 rpm. The absorbance at 540 nm was read immediately before and after vortexing (A_0). The decrease in absorbance was recorded after incubation at room temperature for 30 and 60 min (A_t). A control was run simultaneously with 2 ml of PBS and 0.5 ml of hexadecane without EPS. The emulsification activity was expressed as the percentage retention of emulsion during incubation for time *t*: $A_t/A_0 \times 100$.

Chemical Analysis of VB0S3 Exopolysaccharide

Lyophilized exopolymer was dissolved in ultrapure milli-Q water (0.1 g/l) for chemical analyses. Exopolymers were assayed for total carbohydrate content using the phenol-sulfuric acid assay with glucose as standard [17]. Uronic acids were assayed using the method described by Dische [16] with glucuronic acid as standard. Methyl pentoses [15] and sulfated sugars were determined by measuring sulfates according to the method of Terho and Hartiala [55] after hydrolysis of the polymer, with K_2SO_4 was used as the standard. The protein content of the EPS was determined according to Keene and Lindberg [33], and BSA was taken as the standard.

Fourier Transformed Infrared Spectroscopy (FTIR)

The major structural groups of the purified EPS were detected using Fourier transformed-infrared spectroscopy [29]. Pellets for infrared analysis were obtained by grinding a mixture of 2 mg of exopolysaccharide with 200 mg of dry KBr, followed by pressing the mixture into a 16-mm diameter mold. The Fourier transform-infrared spectra were recorded on a Bruker Vector 22 instrument (Bruker SA, Wissembourg, France) in the region of 4,000−500 cm^{-1}, and the spectra were traced with a Hewlett Packard plotter (Palo Alto, CA, U.S.A.).

Analytical Gas Chromatography

Sugars released by hydrolysis from the lyophilized EPS were analyzed by capillary gas chromatography (Perkin - Elmer model 8310, Wellesley, MA, U.S.A.) equipped with a fused silica capillary column coated with CP Sil-88, (25 m, i.d. 0.32 mm, ds 0.12; Chrompack, Middleburg, Netherlands). The hydrolysates were reduced to their alditol acetate derivatives. A flame ionization detector (FID) was used to separate the alditol acetate mixture, and oxygen-free dry helium was used as carrier gas at a flow rate of 25−30 ml/min. The resulting methyl glycosides were converted to their trimethylsilyl derivatives. The gas chromatogram oven temperature was initially programmed at 70°C and was then rapidly raised to 150°C after the sample was injected. The final analysis temperature was set at 230°C for 40 min using inositol (1 mg/ml) as the internal standard.

RESULTS AND DISCUSSION

Bacterial isolates were identified by standard morphological, physiological, and biochemical tests and also by following the schemes of Alsina and Blanch set of keys [3] and Baumann and Schubert [5]. The bacterial strain VB0S3 was Gram-negative; a facultative anaerobe; a motile, curved rod; oxidase, catalase, and gelatinase positive; and produced green mucoid colonies on TCBS agar. In addition, the isolate failed to utilize indole, sorbitol, mannitol, and melibiose and was positive to arginine dehydrolase, ONPG test, nitrate reductase, and sensitive to the vibriostatic

agent 0/129. The sequence of the 16S rRNA-encoding gene of strain VB0S3 was determined and deposited in the EMBL sequence database under accession number AJ414399 [2]. Phylogenetic analyses using the BLAST program showed that strain VB0S3 belonged to the gamma subdivision of the phylum *Proteobacteria* and that it was closely related to *Vibrio furnissii.* The percentage sequence similarity between VB0S3 and *Vibrio furnissii* was 87%, clearly below the limit of intraspecies variability. The isolate was tentatively identified as *Vibrio furnissii*.

This study reports for the first time the isolation and biochemical characterization of EPSs produced by a strain of *Vibrio furnissii* species. Most of the EPS-producing marine bacteria, isolated from various sites, are Gram-negative rods belonging to the genus *Vibrio*, *Flavobacterium*, *Pseudomonas*, *Alcaligenes* sp., *Alteromonas* sp., *Alteromonas atlantica*, and *Alteromonas colwelliana* and are known to produce acidic polysaccharides [24]. Strain VB0S3 synthesizes and excretes exopolysaccharides and shows a mucoid phenotype on TCBS agar plates. The total amount of EPSs (sum of released EPS fractions) increased rapidly with the number of cells and reached a maximum of 136.8 mg/l during the late log phase of growth to stationary growth. The exopolymer yields of the batch culture were 6.4 μg/ml at 6 h to 31.2 μg/ml after 24 h of incubation and did not vary much, followed by the decrease of EPS production after 36 h (Fig 2). When the cultures were centrifuged at 12,000 ×*g* for 30 min at 4°C, the supernatants from the isolates were highly viscous and formed stringy precipitates with 95% cold ethanol, and to enhance the precipitation, the samples were stored at 4°C for 24 h. The addition of three volumes of ice-cold ethanol showed a better precipitation and recovery of the biopolymer. The precipitates recovered by centrifugation at 12,000 rpm for 20 min were vacuum dried at reduced pressure to obtain a crude biopolymer

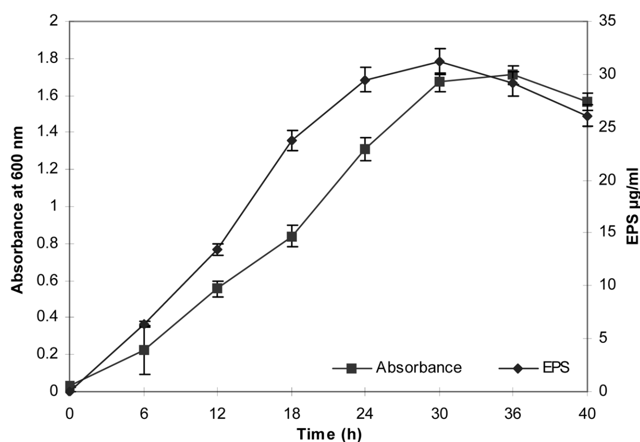

Fig. 2. Growth and EPS production of *V. furnissii* strain VB0S3. Culture conditions: MSM broth+1.5% NaCl+0.2% glucose, temperature 28°C, pH 7.0, and agitator speed 160 rpm. ■, Absorbance at 600 nm; ◆, EPS μg/ml.

or were directly dissolved in distilled water. This VB0S3 exopolysaccharide exhibited good solubility in distilled water. Most exopolysaccharides are produced both in the exponential and stationary phases [58], but an exception has been noted by Williams and Wimpenny [59] for another nonmarine pseudomonad, which produces exopolysaccharide only in the stationary phase. Various methods including high-speed cold centrifugation [14], mild alkali, EDTA, amd NaCl [9] were reported for extraction of EPS from microbial cultures. However, fairly effective extraction of exopolysacchardies was found in the present study by cold ethanol precipitation and incubation at 4°C for 24 h. This biopolymer exhibited a high solubility in distilled water, following the solubility principle "like dissolves like." The hydroxyl groups present within the polysaccharide had the possibility of hydrogen bonding with one or more water molecules. Thus, the polymer could imbibe water and even dissolve partially or completely [6].

Chemical Analysis

Chemical analysis of the EPS expressed by strain VB0S3 showed gross differences in composition of the exopolysaccharide. The analysis showed that the exopolymer produced by *Vibrio furnissii* was acidic in nature, and the contents of the neutral sugars (total carbohydrates), proteins, uronic acids, and methyl pentoses were 166.17, 28.6, 16.48, and 5.8, respectively. In general, carbohydrate content of the polymer was higher than uronic acids and proteins, a feature that was also been observed in other bacterial EPSs (Table 1). The sulfate contents were below detection limits. During the course of screening for EPS production, *Vibrio furnissii* strain VB0S3 showed the highest exopolymer production with reference to yield (μg/ml) and chemical contents, *i.e.*, total carbohydrates, uronic acids, proteins, and methyl pentoses. The binding of divalent cations to biopolymer occurs through electrostatic interactions with negatively charged functional groups such as (i) uronic acids, (ii) phosphoryl groups associated with membrane components, and (iii) carboxyl groups of amino acids [27]. Uronic acids confer anionic characteristics upon the EPSs and are responsible for their biotechnological applications: their use in biodetoxification of heavy metals and waste waters [54]. Proteins also play an important role in emulsification activity. Fazio *et al.* [19] have previously shown that exopolymer from a marine bacterium contains high quantities of galacturonic acid.

Emulsification Activity

The emulsification activity of the exopolymer is determined by its strength in retaining the emulsion breaks rapidly within an initial incubation of 30 min. The absorbance reading after 30 and 60 min gives a fairly good indication of the stability of the exopolymer emulsion. The emulsifying activity of strain VB0S3 exopolysaccharide was tested on

Table 1. Chemical characterization of exopolymer components (%) isolated from *Vibrio furnissii* strain VB0S3 in comparison with other bacterial isolates.

Strain	Total carbohydrates	Proteins	Uronic acid	Sulfates	Methyl pentoses	References
Vibrio furnissii (VB0S3)	166.2	28.6	16.5	2.3	5.8	Present study
Vibrio sp.	51.9	1.8	14.4	ND	ND	[33]
Alteromonas infernus	57.0	4.0	42.0	8.8	ND	[26]
Alteromonas macleodi	42.0	4.0	38.0	5.0	ND	[47]
Marinobacter	16.9	43.9	17.6	2.7	ND	[7]

ND, not detected.

each of the hydrophobic substances (Fig. 3). Crude oil was the substrate most effectively emulsified, followed by xylene, diesel, and toluene. The dialyzed fraction of the exopolymer produced by *Vibrio furnissii* strain VB0S3 retained 41.68% and 10.41% in crude oil, 38.09% and 4.7% in diesel, 40.42% and 12.76% in xylene, and 31.25% and 10% in toluene of the emulsification activity after 30 min and 60 min, respectively (Fig. 3). This EPS was capable of emulsifying hydrocarbons (crude oil, xylene, diesel, and toluene) at a percentage higher than the controls. The functional groups in the molecular chains of the polymer are considered as important determinants for emulsification activity. The emulsification activity of the exopolymer from *Vibrio furnissii* VB0S3 was comparable to other EPS-producing bacterial isolates reported earlier [47].

Fourier Transformed-Infrared Spectroscopy (FTIR)

The FTIR spectrum of the purified EPS of *Vibrio furnissii* strain VB0S3 revealed characteristic functional groups such as a broad-stretching hydroxyl group at 3,416 cm^{-1} and a weak C-H stretching peak of methyl group at 2,925 cm^{-1} (Fig. 4). Furthermore, an asymmetrical stretching peak was noticed at 1,656 cm^{-1}, which corresponds to amide I>C=O str and C-N bending of protein and peptide amines, and a peak at 1,404 cm^{-1} could be assigned to >C=O str (sym) of the COO^{-} and C-O bond from COO^{-} [29]. A broad stretch of C-O-C, C-O at 1,000–1,200 cm^{-1} corresponds to the presence of carbohydrates [10]. Specifically, the peaks at 871 cm^{-1} ascertain the presence of glycosidic linkage bonds. The FTIR spectra of the polymer evidenced the presence of carboxyl groups, which may serve as binding sites for divalent cations. A comparison of functional groups revealed that EPS having a higher number of variable functional groups was more complex than the other exopolysaccharides previously reported by Abu *et al.* [1]. The presence of acidic sugars in the EPS may be important, considering the heavy-metal-binding properties of this polymer. The EPS excreted by *Vibrio furnissii* was highly surface active, which is probably due to an uronic-acid containing polymer. Grobe *et al.* [25] have analyzed the chemical composition of EPS excreted by *Pseudomonas aeruginosa.*

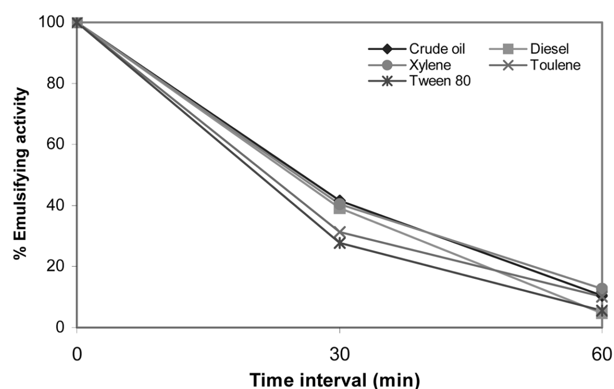

Fig. 3. Percent Emulsification activity of various hydrocarbons, after 0.5 ml was added to 0.5 ml EPS (1 mg/ml) diluted to 2 ml with phosphate-buffered saline (PBS), vortexed for 1 min, and the absorbance monitored at 540 nm.
A control was run with 2-ml PBS without EPS.

Composition of Exopolysaccharides

Different strains of *Vibrio* sp. were reported to produce different EPSs [38, 46]. The EPS from the present study contained differential amounts of galactose and glucose. No structural information on this EPS was given earlier as this is the first description of its chemical characterization in *Vibrio furnissii* strain VB0S3. A variety of EPSs are synthesized by Pseudomonads [11, 50]. It is well known that in microorganisms, the carbon source used for growth

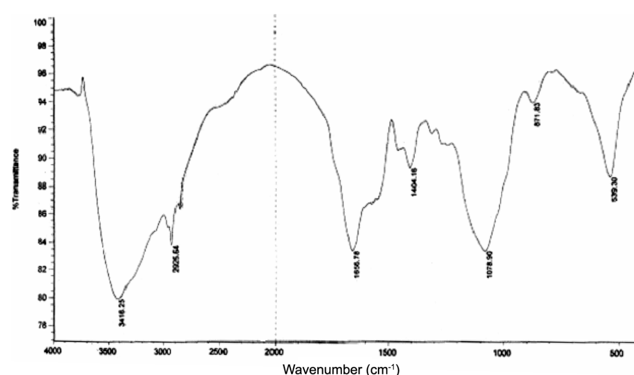

Fig. 4. FTIR spectrum of the exopolysaccharide produced by *Vibrio furnissii* VB0S3.

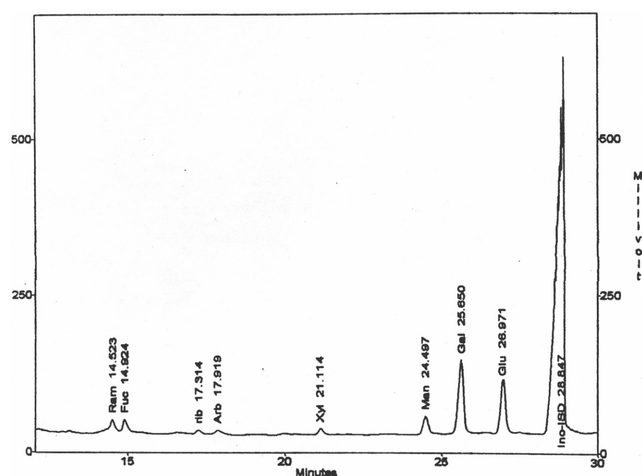

Fig. 5. Gas chromatogram of the alditol acetate derivative of hydrolyzed exopolysaccharide from *Vibrio furnissii* VB0S3.

determines both the quality and quantity of polysaccharide formation.

Sugars play an important role in the EPS synthesis as activated precursors [20]. The inorganic composition of the microbial EPS varies with the chemical composition of its surrounding medium and affects the physical and biological functioning and structure of the microbial biofilms [13]. The sugar composition of the EPS, analyzed using gas chromatography (Fig. 5), showed the percentage relative contribution of hexoses (galactose, 5.21%; glucose, 4.68%), deoxyhexoses (rhamnose, 1.0%; fucose, 0.79%), and pentoses (ribose, 0.16%; arabinose, 0.19%; xylose, 0.3%; mannose, 1.43%). Whereas in *Vibrio furnissii* strain VB0S3, galactose and glucose were the most abundant neutral sugars in differential amounts, *Vibrio parahaemolyticus* was shown to produce abundant levels of polysaccharide, consisting of four major sugars, *viz*. glucose, galactose, fucose, and *N*-acetylglucosamine [18]. Similar biochemical compositions were observed in previous studies of the EPS from *Alteromonas* species isolated from hydrothermal vent communities [44, 48] and adhesive *Pseudomonas* sp. [45]. Apparently, the monosaccharide composition appears to be variably composed from that of the EPS produced by a number of marine bacteria like *Flavobacterium* and *Pseudoalteromonas* sp. [35]. The presence of different sugar moieties suggests that the exopolymer is a heteropolysaccharide. The occurrence of nonsugars (uronic acids, methyl pentoses, sulfate, and proteins) indicates the acidic nature of the exopolymer. The heteropolysaccharide containing multiple sugars was earlier reported in different Gram-negative bacteria, such as *Pseudomonas fluorescens* strain III, which was found to contain glucose, glucosamine, rhamnose, fucose, arabinose, and acetate. The plant pathogen *Pseudomonas andropogonis* produces an acidic exopolymer containing glucose, glucouronic acid, mannose, rhamnose, and galactose [21], whereas the exopolymer of *Pseudomonas mendocina* P_2d contains rhamnose, fucose, glucose, ribose, arabinose, and mannose [48].

Upon biochemical examination of the EPS produced by *Vibrio furnissii* strain VB0S3, several prominent functional groups typical for heteropolysaccharides (such as hydroxyl, carboxylic, and amides) were found. The EPS also possessed good emulsification activity. High level of EPS production in *Vibrio furnissii* VB0S3 may possibly be involved in biofilm formation, which is regulated by a quorum-sensing signal molecule. The observations of Hammer and Bassler [28], and Parsek and Greenberg [43] in *Pseudomonas aeruginosa* and *Vibrio cholerae* lend support of such view, where quorum-sensing signals are involved in the development of biofilms [28, 43]. Implications for the role of these bacterial polysaccharides in the marine environment requires further characterization using thermogravimetric analysis, and NMR and mass spectroscopies, to elucidate the complete molecular structure of the EPS produced by *Vibrio furnissii* strain VB0S3.

Over the past few years, several bacteria originating from marine sources belonging to *Vibrio* or *Alteromonas* genus were shown to produce exopolysaccharides under laboratory conditions. The chemical composition of the polysaccharide secreted by VB0S3 differed slightly from those produced by other bacteria from marine origin. In addition, the presence of both hydroxyl and carboxyl groups could be responsible for the high mucous polysaccharide. This criterion makes this EPS a good alternative to other viscous polymers used in many industrial and biotechnological applications. On the basis of preliminary structural studies with gas chromatography and FTIR of sugar derivatives, the repeating unit of this polysaccharide was deduced to be a heteropolysaccharide. Recent advances in the knowledge of the biosynthetic pathways and genetics of EPS production can perhaps be applied to improve the quality and yield of these polymers [52]. Though the biotechnological potential of the biopolymer production by *Vibrio* sp. from the marine environment of coastal regions of India remains virtually unexplored, it is thought that this report on the new EPS produced by *Vibrio furnissii* strain VB0S3 will help in understanding the biochemical strategies of biopolymer formation by this proteobacterium. Moreover, it is envisaged that this new bioemulsifier may have many industrial and biotechnological applications. Additional studies are in progress to achieve the structural analysis of this polymer.

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