

Production of exopolysaccharide by *Vagococcus carniphilus* MCM B-1018 isolated from alkaline Lonar Lake, India

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Abstract A Gram positive bacterium obtained from a sediment sample from the alkaline Lonar Lake, India, was identified as *Vagococcus carniphilus* by morphological, physiological and biochemical characterisation and 16S rDNA sequencing. The bacterium grew in sodium chloride (NaCl) up to 2.5 M and at pH 7–11, 10 being optimum. Mucoid, glistening colonies were produced on exopolysaccharide (EPS) production medium (pH 10); the organism produced 1,126 mg l⁻¹ EPS under optimized conditions. The gross chemical composition of *Vagococcus* EPS indicated 20% protein and 75% neutral sugars, while the monosaccharide composition revealed the presence of galactose and mannose with an additional three spots corresponding to unidentified compounds. FTIR analysis confirmed the presence of an alkyl group in the polysaccharide and revealed 75% similarity with standard dextran. *Vagococcus carniphilus* isolated from Lonar Lake was different from a previously reported strain with respect to its biotechnological potential for EPS production. In the present study, the newly isolated *V. carniphilus* from Lonar Lake was characterized and studied for production of EPS.

Keywords Microbial exopolysaccharide · *Vagococcus* · Lonar Lake · Alkaliphile

Introduction

Microbes form functional consortia via biosynthesis of extracellular polymeric substances or exopolysaccharide (EPS), which becomes attached to solid surfaces or released in the form of slime. EPS plays a structural role in helping bacterial attachment to surfaces, improving nutrient acquisition or providing protection from environmental stresses and host defences (Iqbal et al. 2002). EPS production by microbes has been studied for decades because of its many interesting physicochemical and rheological properties with novel functionality. Microbial polysaccharides have found a wide range of applications in the food, pharmaceutical, petroleum and other industries (Sutherland 1990, 1998; Desai and Banat 1997; Tombs and Harding 1998). They are also used in the removal of heavy metals from industrial effluents and potable water (Norberg and Perrsson 1984).

Microbial polysaccharides possess properties not found in traditional polymers of plant or algal origin. Extreme environments are a valuable source of microbes that secrete interesting new molecules. It has been hypothesised that the synthesis of exo-cellular polysaccharides in microbes plays a major role in protecting these cells from stress in extreme habitats (Nicholaus et al. 1999a, b). There are some reports on polysaccharide production and development of polysaccharides with superior properties compared to known polymers. The diverse microbes found in extreme environments hold great potential for the discovery of new polysaccharides. There is therefore great interest in the search for high polysaccharide producing microbes.

Lonar Lake—an Indian soda lake situated in Lonar village, District Buldhana, Maharashtra, India (latitude 19° 58', longitude 76° 36'), in the formerly volcanic Deccan-trap geological region. It is a circular depression with its longest and shortest diameters being 1,875 m and 1,787 m, respec-

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tively, a raised rim (30 m) and a depth of 135 m (La Touche and Christie 1912). Based on geological studies, the lake (in crater form) originated due to a meteorite impact about 50,000–60,000 years ago (Fredriksson et al. 1973). Salinity and high alkalinity (pH 9.8–10) are the notable features of its water. According to Jhingram and Rao (1954), the high alkalinity is due to a high concentration of sodium carbonate. During a previous study carried out from 2002 to 2006, 86 alkaliphilic bacterial cultures were isolated from Lonar Lake (Joshi et al. 2008). These were screened for production of EPS. One of these bacteria, *Vagococcus carniphilus*, has the ability to produce EPS at alkaline pH. The present paper describes the characterisation of *Vagococcus carniphilus* and its EPS production.

Materials and methods

Collection and isolation

Six sediment and two water samples were collected from alkaline Lonar Lake for isolation of alkaliphilic bacteria (Joshi et al. 2008). Isolation was carried out by enrichment of sediment and water samples in Horikoshi I, Horikoshi II, nutrient agar (pH 10), nutrient agar (pH 10) with 30 g l⁻¹ sodium chloride, Davis Mingioli's synthetic medium (pH 10) with 5 g l⁻¹ peptone, and Tindall's medium, using a previously described method (Joshi et al. 2008). Well-isolated and differentiated colonies were transferred to corresponding agar slants.

Screening for EPS production

All bacterial isolates were screened for EPS producing ability by inoculation on respective enrichment medium with 5% glucose as carbon source. The plates were incubated at 30°C for 48 h and observed for mucoid, glistening colonies. Of all the isolates tested, only two, designated as ARI 341 and ARI 349, showed this type of morphology. The isolate ARI 341 was selected for further characterisation and detailed studies.

Identification and 16S rDNA sequencing

Isolate identification was carried out according to *Bergey's Manual of Systematic Bacteriology* (Krieg and Holt 1984) and by comparing with standard culture described earlier (Shewmaker et al. 2004) on the basis of morphological, biochemical and physiological tests, and further confirmed by 16S rDNA sequencing. Morphological studies were carried out by microscopic observations of Gram stained cells under an oil immersion objective on a light microscope and scanning electron microscopy (SEM) using a previously described procedure (Joshi et al. 2007). For 16S rDNA sequencing, DNA of the isolate was extracted and purified

using standard phenol-chloroform extraction procedures (Sambrook et al. 1989). 16S rRNA gene amplification, sequencing and sequence analysis was carried out as described earlier by Savant et al. (2002) and Dighe et al. (2004). The sequence has been submitted to the GenBank database (accession number DQ 166854).

Salt and pH tolerance

The salt tolerance of isolate ARI 341 was tested in nutrient broth medium (pH 10) using various NaCl concentrations (0, 1, 2, 3, 4, 5 M), while the pH range for the growth was tested at pH values 7–11. The inoculated tubes were incubated at 30°C for 48 h and optical density was measured at 600 nm using a μ -Quant instrument (μ -quant; Bio-Tek Instruments, Winooski, VT).

Production of EPS

Inoculum preparation

The organism was grown in nutrient broth medium (pH 10) and incubated overnight at 120 rpm at 28±2°C. Inoculum was prepared by collecting the cell pellet obtained after centrifugation (10,000 rpm for 15 min); the cells were washed with sterile saline and suspended in distilled water. Suspensions were added to 100 ml of same medium supplemented with 5% glucose in 500 ml Erlenmeyer flasks and incubated at 28±2°C under shake culture conditions for 8 days. After incubation, culture broth was removed for determination of EPS produced.

Isolation and purification of EPS

Culture broth was centrifuged (12,000 rpm at 4°C for 30 min) and 8 g v/v trichloroacetic acid was added to the supernatant and shaken for 45 min; the suspension was then centrifuged to remove proteins. The EPS was then precipitated from the supernatant with two volumes of ice cold isopropanol followed by storage at 4°C. The precipitate was recovered by centrifugation (8,500 rpm for 20 min at 4°C) and washed with 70–100% ethanol-water mixtures. EPS was dried in a desiccator and the yield determined by weight (in terms of milligrams per litre). Excess salts were removed by dissolving the EPS in distilled water and dialysing against the same for 2 days at room temperature (28±2°C) before lyophilising (Nichols et al. 2004). EPS was stored at room temperature (28±2°C) until analysis.

Optimization of different environmental conditions for EPS production

Physical (medium composition, incubation period, culture condition) and biological (inoculum density) parameters

have a significant effect on EPS production. Therefore, to optimize production process parameters, EPS was isolated and purified using the procedure described by Nichols et al. (2004). The experiments were performed in triplicate and results were analysed by Minitab 14 statistical software (<http://www.gtpcc.org/gtpcc/minitab14.htm>).

Production of EPS in different media

Five different media were used. The media contained ingredients given in the list below as g l^{-1} of distilled water.

- Medium I: Sucrose 40 g, KNO_3 1 g, MgSO_4 0.2 g, CaSO_4 0.5 g, NaMoO_4 0.05 g (Ashtaputre and Shah 1995).
- Medium II: Yeast extract 1 g, peptone 5 g, NaCl 32 g, glucose 30 g (Nichols et al. 2004).
- Medium III: Glucose 20 g, yeast extract 1 g, casamino acids 1 g, Na_2HPO_4 10 g, KH_2PO_4 3 g, K_2SO_4 1 g, NaCl 1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, CaCl_2 0.01 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001 g (Seo et al. 2004).
- Medium IV: Glucose 20 g, peptone 30 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25 g, KH_2PO_4 0.1 g, NaCl 0.02 g (Yun and Park 2003).
- Medium V: EPS production medium (GYMK): Glucose 30 g, yeast extract 5 g, K_2HPO_4 2 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g.

All media were adjusted to pH 10 with 1 N NaOH; 100 ml of each medium in a 500 ml flask was inoculated with ARI 341 culture with a density of 2×10^7 cells ml^{-1} and incubated at room temperature ($28 \pm 2^\circ\text{C}$) under shake culture conditions along with an uninoculated control of each medium for 1 week. After incubation, the culture broth was harvested and tested for EPS produced.

Production of EPS with different incubation periods and different culture conditions

Incubation time and culture conditions are the two major factors influencing the quantity of EPS produced in culture medium (Arias et al. 2003). GYMK medium (100 ml; pH 10) in a 500 ml flask was inoculated with ARI 341 culture to attain an initial inoculum density of 2.4×10^7 cells ml^{-1} and incubated under both shaking (120 rpm) and stationary culture conditions (30°C for 10 days). The medium incubated under the same conditions without culture served as a control. Culture broth was harvested after every 2 days, centrifuged and tested for EPS produced.

Effect of initial inoculum density on EPS production

Initial cell density has a profound effect on final density attained, rate, and extent of EPS production. Hence it is

important to determine the optimum initial culture density. To study this, organism was grown in 100 ml GYMK medium (pH 10) in a 250 ml Erlenmeyer flask for 24 h under shake culture conditions. Bacterial cells were harvested by centrifugation (12,000 rpm for 10 min). The cell pellet was resuspended in sterile saline and the cell density of the suspension was determined on double beam UV-VIS spectrophotometer (Spectra scan, Chemito 2600, India) with saline as a blank. Culture suspension was added to 100 ml GYMK medium (pH 10) in 500 ml Erlenmeyer flasks to attain initial cell densities at 600 nm to 4.9×10^6 , 2.4×10^7 , 4×10^8 and 2.4×10^8 cells ml^{-1} . An equal amount of saline instead of culture suspension in GYMK medium served as a control. All flasks were incubated at 30°C on a rotary shaker for 6 days. After incubation, culture broth was harvested and tested for EPS produced.

Effect of glucose concentration on EPS production

GYMK medium (100 ml; pH 10) with varying concentrations of glucose (1, 3, 5, 7 and 10 g%) in 500 ml flasks was inoculated with ARI 341 culture to attain an initial inoculum density of 2.4×10^7 cells ml^{-1} and incubated at 30°C under shake (120 rpm) culture conditions for 6 days. Medium incubated under the same conditions without culture served as the control. The culture broth was harvested after 6 days, centrifuged and tested for EPS produced.

Characterisation of EPS

Colorimetric analysis

After dialysis, purified EPS was subjected to determination of total carbohydrate and protein content. The total carbohydrate content of EPS was determined by the Anthrone method (Jayaraman 2003), and the protein content by the Folin-Ciocalteu method (Lowry et al. 1951).

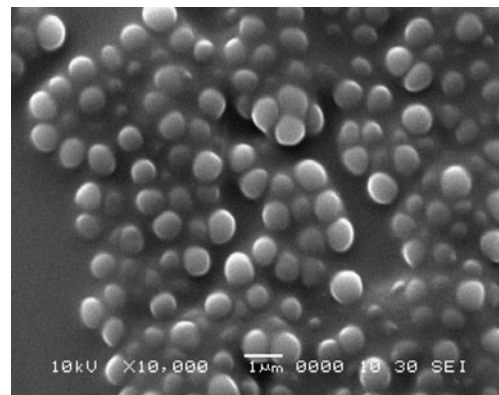


Fig. 1 Scanning electron micrograph (SEM) of *Vagococcus carniphilus*; cells occur singly. Cells were grown in nutrient broth (pH 10) containing 5 g% w/v glucose at 30°C for 24 h. Magnification $\times 10,000$

Table 1 Physiological and biochemical characteristics of isolate ARI 341 and type strain of *Vagococcus carniphilus*

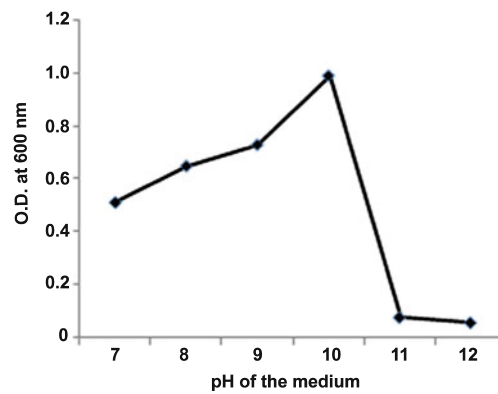
Biochemical tests	Isolate ARI 341	Type strain 1843-02 ^T (=ATCC BAA-40 ^T =CCUG 6823 ^T) (Shewmaker et al. 2004)
Catalase	+ ^a	-
Oxidase	+	ND ^b
Carbohydrate utilisation		
Glucose	+	ND
Glycerol	-	+
Fructose	+	ND
Ribose	-	+
Galactose	-	ND
Mannitol	-	-
Mannose	-	ND
Sorbitol	-	-
Lactose	-	-
Trehalose	-	+
Cellobiose	-	ND
Inositol	-	ND
Xylose	+	ND
Citrate utilisation	-	ND
Nitrate reduction	-	ND
Growth at 37°C	+	+
Growth at 45°C	-	Variable
Growth on NaCl		
6.5% NaCl	+	+
7.5% NaCl	+	ND
10% NaCl	+	ND
17% NaCl	-	ND
Hydrolysis of		
Starch	+	ND
Gelatin	-	ND
Casein	-	ND
Urea	-	-
Arginine	+	-
Tween 80	-	ND
Esculin	-	+

^a + positive reaction, – negative reaction

^b Not determined

Monosaccharide analyses

Thin layer chromatography (TLC) using hydrolysed samples of purified EPS was used to determine monosaccharide composition. Hydrolysis was done using 2 M trifluoroacetic acid at 100°C for 4 h. Samples were dried under a stream of nitrogen gas and reconstituted in deionised water. TLC analysis was performed using silica gel 60F 254 HPTLC plates prewashed by running a blank chromatogram in a mixture of methanol-water (9:1 v/v) and activated at 120°C for 20 min (Dogsa et al. 2005). A standard

**Fig. 2** Tolerance of ARI 341 to various pH values

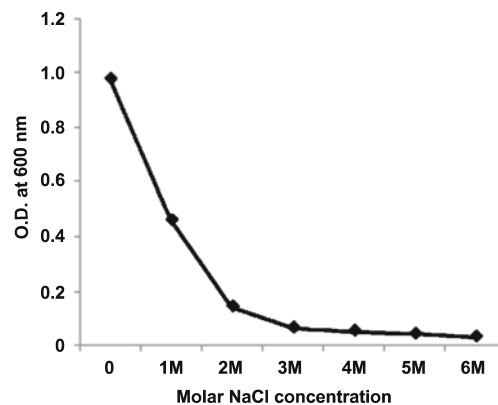
mixture containing galactose, mannose, rhamnose, arabinose and fructose was used for comparison. Separation was carried out in a normal, unsaturated chamber at room temperature (28±2°C) using acetonitrile:water (85:15 v/v) as the solvent system. The dried plates were dipped in aniline-diphenylamine reagent and heated at 120°C for 10 min to observe coloured spots of sugars. The spots of hydrolysed EPS were compared with the spots of standard sugars.

Fourier transform infrared spectroscopy

Pellets for infrared analysis were obtained by grinding a mixture of 2 mg EPS with 200 mg dry KBr. The Fourier transform infrared (FTIR) spectra were recorded on Perkin Elmer instrument (Perkin Elmer, Spectrum one FTIR spectrometer) with a resolution of 4 cm⁻¹ in 4,000–400 cm⁻¹ regions along with a standard sample of dextran.

Flocculating activity of EPS

A flocculating test was carried out in a test tube containing a mixture of 10 ml 0.5% activated carbon and 100 µl 1% CaCl₂ using the method described by Yun and Park (2003). To the test tube, 100 µl of 0.02% polysaccharide solution

**Fig. 3** Tolerance of ARI 341 to various NaCl concentrations

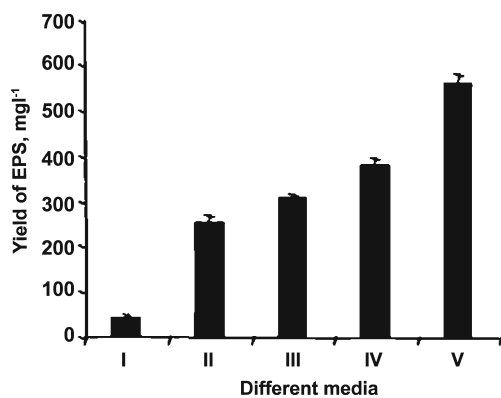


Fig. 4 Exopolysaccharide (EPS) production by *V. carniphilus* using different nutrient media

was added and mixed well. The resulting suspension was observed for the flocculation of activated carbon for 60 min during incubation at room temperature. A similar experiment was carried out using water and xanthan gum (Sigma, UK) as a control.

Results and discussion

Morphology

Bacterial strain ARI 341 isolated from a sediment sample collected from Lonar Lake was found to be a Gram positive coccus using nutrient agar medium (pH 10). A SEM photograph of the isolate indicated that it occurred singly (Fig. 1). On EPS production medium it produced round, large, entire, orange-coloured, glistening mucoid colonies.

Identification of the isolate

Biochemical and physiological tests, growth and metabolic properties (Table 1) and 16S rDNA sequencing indicated

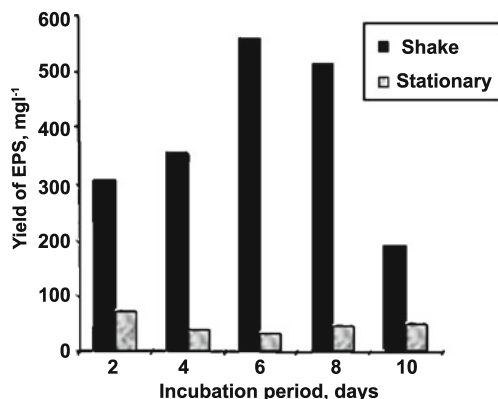


Fig. 5 EPS production by *V. carniphilus* under different culture conditions for different incubation periods

that the bacterial isolate obtained from the Lonar Lake sediment sample is *Vagococcus carniphilus*. The isolate is deposited with the MACS Collection of Microorganisms (MCM), which is affiliated to World Federation of Culture Collection (WFCC), code 561 as MCM B-1018.

Salt and pH tolerance

The isolate was found to grow in a wide pH range (7–11) with 10 as the optimum (Fig. 2). The organism could grow without added NaCl in the growth medium with tolerance up to 2.5 M, i.e. 12.5% (Fig. 3). *Vagococcus carniphilus* from ground beef was previously isolated and described by Shewmaker et al. (2004). It was observed that the organism could grow in the presence of 6.5% NaCl. However, there was no report of its pH tolerance.

Production of EPS

EPS is an important class of biological ligand (Perry et al. 2005) that biofilm bacteria produce in large amounts. It has been hypothesised that synthesis of EPS in microorganisms plays a major part in protecting cells from stress in extreme habitats (Nicholaus et al. 1999a, b). Studies in the Arctic have shown that high concentrations of EPS in brine channels may provide buffering against harsh winter conditions and high salinity, and cryoprotect microbes against ice crystal formation (Nichols et al. 2004). Krulwich et al. (2001) reviewed the role of the Na⁺ cycle in pH homeostasis of aerobic, alkaliphilic *Bacillus* species. According to him, one of its mechanisms is that of Na⁺/H⁺ antiporters, which achieve net H⁺ accumulation that is coupled with Na⁺ efflux. The process of pH homeostasis is also enhanced, perhaps during transitions to high pH, by different arrays of secondary cell wall polymers, e.g. non-peptidoglycan acidic polymers like s-layer, polyglutamate capsule, etc. (Krulwich et al. 2001). The negative charge on acidic non-peptidoglycan polymer gives the cell surface its ability to adsorb sodium and hydronium ions and

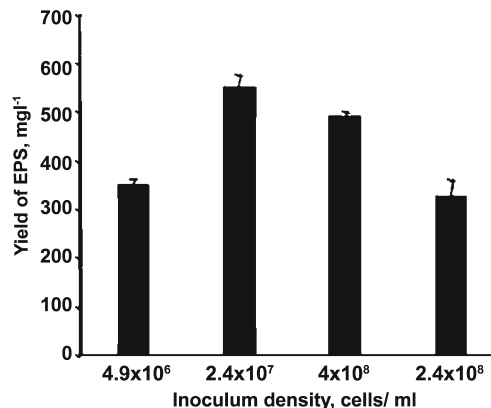


Fig. 6 EPS production using different inoculum densities

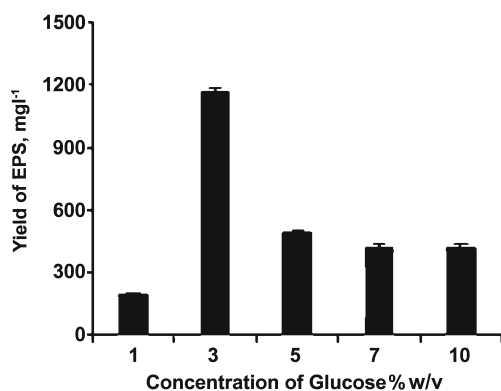


Fig. 7 EPS production at different concentrations of glucose

repulse hydroxide ions and thus enables cells to grow in alkaline environments. This may be true for bacteria growing under extreme conditions at Lonar Lake. Moreover, it could be hypothesized that, as a secondary cell wall polymer, EPS acts as binding site for Na⁺ ions and helps bacterial Na⁺/H⁺ antiporter systems. It thus generates a sodium motive force that drives substrate accompanied by Na⁺ into cells thus playing an important role in adaptation (or growth) at high pH.

With this baseline idea, Lonar Lake bacterial isolates were screened for production of EPS. After preliminary screening, *V. carniphilus* was found to exhibit appreciable potential for EPS production and hence was used for further optimisation studies. Moreover, there are no reports on EPS production from *V. carniphilus* and this is the first report on the isolation of an EPS producing organism from the alkaline Lonar Lake.

The quantity and quality of EPS produced by a microbe depends on the culture conditions (Giavasis et al. 2000; Laws et al. 2001). Understanding the characteristics of the organism, and optimisation of operating variables for production of any compound are useful to maintain environmental parameters, which may influence production in other situations. EPS production was therefore

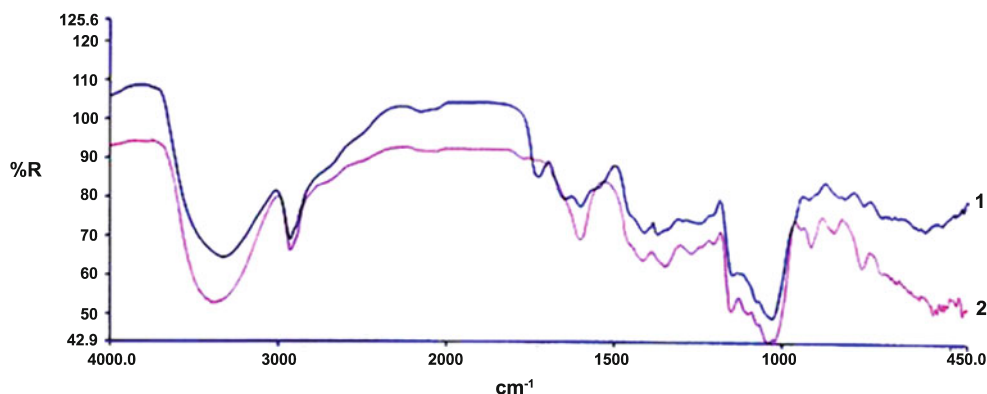
studied, concentrating on parameters such as medium composition, incubation period, inoculum density, shake/static conditions and glucose concentration, etc. *Vagococcus carniphilus* produced a maximum amount of EPS (560 mg l⁻¹) after 1 week using medium V (GYMK) at pH 10 (Fig. 4) and thus these conditions were used for further experiments.

The organism produced 560 mg l⁻¹ EPS on the 6th day under shake culture condition (Fig. 5). In the present study, EPS production increased with incubation period; a 6 day incubation was found to be optimum for EPS production as yield lowered to 511 and 188 mg l⁻¹ on days 8 and 10 of incubation, respectively. This may be due to enzymatic degradation as reported in other studies on EPS (Pham et al. 2000). Our results are in accordance with results found by Arias et al. (2003) in the case of the EPS producing halophilic bacterium, *Halomonas maura*. Stationary culture conditions gave lower EPS yield from the 2nd to 10th day of incubation and thus was not suitable for EPS production.

An initial inoculum density of 2.4 × 10⁷ cells ml⁻¹ gave maximum EPS production (550 mg l⁻¹) after 6 days incubation at 30°C (Fig. 6). Maximum EPS production, 1,126 mg l⁻¹ was achieved at 3% glucose concentration (Fig. 7), and this concentration was thus selected for further experiments. One noteworthy result was that the bacterium could grow and produce EPS with all carbon sources assayed. The yield of EPS obtained from glucose-containing medium was found to be maximum and hence glucose was selected as carbon source for the present study. Production of EPS was found to be maximum in medium V, i.e. GYMK, as compared to other four media tested. Under optimised conditions (GYMK medium at pH 10, 3% glucose, initial inoculum density 2.4 × 10⁷ cells ml⁻¹, shake culture conditions, 30°C, 6 days), *V. carniphilus* produced 1,126 mg l⁻¹ EPS.

There are few reports on production of EPS by extremophilic bacteria, and none from Lonar Lake, Maharashtra, India. Studies of haloalkaliphilic *Bacillus* sp. I-450 obtained from soil samples of the heavily polluted tidal

Fig. 8 Fourier transform infrared (FTIR) spectra of 1 *V. carniphilus* EPS, 2 dextran



mudflats of Yellow Sea, Korea indicated a pseudo plastic behaviour of the EPS produced, with yields of $4,000 \text{ mg l}^{-1}$ (Ganesh Kumar et al. 2004). Hence, EPS produced by bacteria from extreme environments show significant potential. An alkaliphilic *Bacillus* spp. isolated from Natron, a soda lake in the Kenyan Tanzanian Rift Valley, have also yielded large amounts of EPS in alkaline medium (Carsaro et al. 1999). The moderately halophilic *Halomonas* S-30 isolated from hyper-saline habitats in Morocco produced $2,800 \text{ mg l}^{-1}$ EPS and exhibited pseudo plastic behaviour (Bouchotroch et al. 2001). Another isolate of *Halomonas eurihalina* H-28 from soil samples of solar slattern at Alcinat, Spain, produced EPS with yields of $1,200 \text{ mg l}^{-1}$, was capable of emulsifying crude oil, and thus could be applied in bioremediation processes (Martinez-Checa et al. 2002). The EPS known as *Mauran* was produced by *Halomonas maura* with a yield of $3,800 \text{ mg l}^{-1}$ (Arias et al. 2003). *Mauran* is highly viscous, displays pseudo plastic behaviour and has the capacity to bind lead and other cations. Antarctic marine isolates of *Pseudoalteromonas* produced 100 mg/gdw (gram dry weight) EPS (Nichols et al. 2004) and had ‘sticky’ behaviour with respect to metal ions. It is clear from previous reports that yields of EPS from our isolate ($1,126 \text{ mg l}^{-1}$) are comparable to the amount of EPS produced by other extremophilic bacteria.

Characterisation of EPS

Determining the chemical composition of EPS provides valuable information about the relationship between the composition of polysaccharides and their physical properties. The colorimetric analysis of purified EPS revealed the gross chemical composition of *V. carniphilus* EPS as 20% protein content and 75% neutral sugars, indicating that the polymer was mainly a polysaccharide.

Many researchers have used TLC to analyse various monosaccharides present in their polymers. This technique was used here and revealed that our EPS is composed of two neutral sugars, viz. galactose and mannose, with three additional spots representing unidentified compounds.

The FTIR spectrum of EPS (Fig. 8) displayed absorbances above $3,000 \text{ cm}^{-1}$ indicative of O–H stretch, and absorbance at $1,727 \text{ cm}^{-1}$ indicating the presence of carboxyl groups. Absorbances between $1,650 \text{ cm}^{-1}$ and $1,050 \text{ cm}^{-1}$ were characteristic of polysaccharides. It is noticeable that the FTIR spectrum of EPS produced by our isolate showed 75% similarity with standard dextran. FTIR spectra of *Vagococcus* EPS revealed characteristics typical of polysaccharides with O–H and carboxyl groups. Our results are similar to FTIR analysis of Antarctic *Pseudoalteromonas* with minor differences (Nichols et al. 2004).

Flocculating activity of EPS

There are many reports on the flocculating effect of microbial polysaccharides; the latter can replace synthetic flocculants and are used industrially (Toeda and Kurane 1991). Therefore, in the present study, we examined the flocculating effect of the purified polysaccharide against a suspension of activated carbon in water. Xanthan gum was used as a control to compare its flocculating activity. The *V. carniphilus* polysaccharide showed flocculating activity after 10 min, while flocculation occurred after 3 min of incubation with xanthan gum. The flocculating activity of the polysaccharide of *V. carniphilus* was found to be comparable to that of xanthan gum, suggesting that the polysaccharide has potential as a flocculating agent. Gao et al. (2006) isolated and characterised EPS-producing *Vagococcus* sp. W31 from a sewage sample collected from Little Moon River in Beijing. The organism exhibited good flocculating activity for Kaolin clay and thus has a potential application in wastewater treatment.

In conclusion, *Vagococcus carniphilus* isolated from Lonar Lake was found to differ from the *V. carniphilus* type strain 1843-02^T (=ATCC BAA-640^T=CCUG 46823^T) previously reported by Shewmaker et al. (2004), with respect to some morphological and biochemical characteristics but mainly in production of the biotechnologically important EPS. It was interesting to note that the organism produced $1,126 \text{ mg l}^{-1}$ EPS under an optimised set of conditions, had good flocculating activity, and thus can be used as a flocculating agent. Implications for the role of this bacterial polysaccharide in the alkaline Lonar Lake environment require further investigation, which will also provide insights into the possible commercial uses of this polymer.

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