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Full Length Research Paper

Exopolysaccharide production by *Bacillus cereus* GU812900, a fouling marine bacterium

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The aim of the study was to isolate the exopolysaccharides (EPS) from the active biofilm bacterium *Bacillus cereus* GU 812900 which was isolated from the stainless steel test panel and deployed in the Vellar estuary for a period of about four weeks. The deployed stainless steel test panels when analyzed revealed a direct relationship between the rate of corrosion and the concentration of EPS that is, the rate of corrosion increased with an increase in the concentration of EPS and the period of immersion. Growth and EPS production by *B. cereus* strain GU812900 was studied in a basalt salt solution (BSS) containing (g/1); NaCl, 30.0; KCl 0.75; $MgSO_4.7H_2O$, 7.0; NH_4Cl, 1.0; K_2HPO_4, 0.7; KH_2PO_4. 0.3; glucose, 10.0 and 1 ml of trace metal solution. The rate of EPS production in batch cultures was highest during the late log phase of the growth then stationary phase. The EPS was recovered from the culture supernatant by using ethanol precipitation – dialysis procedure. Chemical composition of EPS revealed that it is primarily composed of natural sugars, proteins, phosphates and sulphates. The EPS production was influenced by carbon, nitrogen and phosphate concentrations in the growth medium. EPS produced by *B. cereus* GU812900 is a heteropolysaccharide which influence the corrosion. The FTIR analysis revealed the functional groups of the EPS. The results of the study was expressed the relationship between biocorrosion and biofilm formation of the stainless steel test panels.

Key words: Biofilm, exopolysaccharides (EPS), sequencing, secondary metabolite, characterization, FTIR analysis.

INTRODUCTION

Exopolysaccharides (EPS) are organic macromolecules that are formed by polymerization of similar or identical building blocks. Which may be arranged as repeated units within the polymer. Bacterial exopolysaccharides are important in the interaction between bacteria and their environment and are chemically diverse. These bacteria may produce new bioactive compounds including EPSs with novel structures and may have fascinating industrial applications (Querellou, 2003). There has been a growing interest in the isolation and identification of new marine microbial polysaccharides in recent years (Weiner 1997). Although many known marine bacteria can produce EPSs and some of the EPSs they produce have unique properties, the search for new EPS-producing microorganisms is still promising. Marine bacteria have become increasingly popular and novel sources of EPSs (Lee et al., 2003).

EPS are high molecular weight compounds and are sticky in nature (Passow et al., 1994). The production and chemical characteristics of EPS are controlled by nutrient dynamics, microbial physiology, phytoplankton species, age of phytoplankton bloom etc. Similarly, EPS produced by microorganisms may exist either as tight capsules or as loosely attached slimes (Decho, 1990) that generally differ in their physico-chemical characteristics (Hoagland et al., 1993). EPS in the natural environment is generally heteropolymeric (made of different monomeric units), Wingender et al. (1999). EPS contain non-sugar components like uronic acid, methyl esters, sulphates,

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Abbreviations: EPS, Exopolysaccharides; **BSS,** basalt salt solution; **PCR,** polymerase chain reaction; **rDNA,** ribosomal deoxyribonucleic acid.

pyruvates, proteins, nucleic acids and lipids (Sutherland, 1977). Marine microbes grown in laboratory cultures produce EPS when nutrients such as nitrogen, phosphorus, sulfur, and potassium are limited (Sutherland, 1982). Exo-polymer production may be enhanced in response to physical factors such osmotic stress and temperature (Krembs et al., 2002; Junge et al., 2004). The composition of polysaccharide is generally independent of the nature of the limiting nutrient.

In the present study has been revealed that the isolation of exopolysaccharide producing marine bacteria from the coastal regions of vellar estuary. Strain GU812900 was a gram negative, rod-formed bacterium, absolutely demanding 1-3% salt for growth. This strain was identified as *B. cereus* and the EPS production was isolated and characterised.

MATERIALS AND METHODS

Study area

The active bio fouling bacteria were isolated from the stainless steel panels that were deployed in the Vellar estuary for about one month. The Vellar estuary (lat. 11°29'N and long. 79°46'E) is always open with the Bay of Bengal.

Deployment of test panels

A study on the corrosion and biofilm formation of stainless steel was carried out at Vellar estuary during October/ November, 2009. Before use the stainless steel panels (10 x 15 x 0.3 cm) were treated with 10% HC1, washed with water and dried in an oven, cooled in a desiccator, weighed on a balance and kept in a desiccator until used. Panels were immersed in surface waters (~l m) of the Vellar Estuary at about 1meter depth at low tide mark. The samples were scraped at weekly intervals by the following (Bhosle et al., 1990; Bragadeeswaran and Kanagarajan, 2007).

Corrosion rate

The rate of corrosion was calculated (Majumdar et al., 1999). After retrieval, the corrosion products were removed using a stainless-steel knife. They were dried in an oven, powdered in a pestle and mortar, transferred to acid-cleaned vials and stored at 4°C until analysis. After the removal of corrosion, product panels were cleaned and reweighed as above to estimate weight loss. The corrosion rates (mg. dm^{-2} d^{-1}) were then calculated using the formula:

$C = W1- W2/At$

Where, *C* is the corrosion rate (mg. dm^{-2} d^{-1}), *W1* and *W2* are weights in gram of mild stainless steel and copper panels before and after immersion, *A* is the area of the panels (cm^2) and t the duration of immersion in days. Precision of the method based on six replicates was ± 7.67 %.

Isolation of bacteria

In order to isolate bacterial cultures, corrosion products were removed using a brush and sterile sea water. Samples were serially diluted. A known aliquot was plated onto Zobell marine agar plates. The plates were incubated at room temperature (28 ± 2°C) for 24 hours. Colonies were randomly selected, purified and stored on Zobell marine agar slants. Ten cultures from these were randomly selected to assess their exopolysaccharide production. These strains were tentatively identified using standard taxonomic methods (Buchanan et al., 1975). The preliminary characterization of bacterial strains was achieved according to standards for microbial identification in Bergy's manual of systematic bacteriology, on the basis of colony morphology, biochemical test and microbial examination.

Characterisation of bacterial isolates

DNA isolation and 16S rDNA gene amplification

The selected strain was cultured in LB broth (Hi Media, Mumbai) for 2 days and total genomic DNA was the 16S rDNA sequences were amplified by polymerase chain reaction (PCR) using extracted universal primers 8f (30GAGTTTGATCCTGTGCTCAG50) and 1490r (50GACTTACCAGGGTATCTAATCC-30). The PCR mixture consisted of 5 ll of 109 buffer (mg 2? free), 5 ll of 2.5 lM MgCl2, 8 ll of dNTP mixtures (2.5 lM each), 1 ll of each primer ll of template DNA and 0.5 ll of Taq DNA polymerase (5 U/ll) (Genei, Bangalore), in a final volume of 50 ll. PCR was performed in a thermal cycler (Genei, Bangalore) using an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 90 s and final extension at 72°C for 7 min by modified method of Guomin et al. (2007).

Nucleotide sequences accession number

The partially complete (*1498 bp) 16S rDNA sequences of *B. cereus* isolate have been deposited in the GenBank database under accession number JBS10 GU812900.

Mass culture conditions for *B. Cereus* JBS10 GU812900

Bacterial culture for EPS production was grown in a BSS containing (g/1); NaCl, 30.0; KC1 0.75; MgSO4.7H2O, 7.0; NH4Cl, 1.0; K2HPO4, 0.7; KH_2PO_4. 0.3; glucose, 10.0 and 1 ml of trace metal solution (Bhosle, 1985). Carbon and nitrogen sources and concentrations, and the concentrations of phosphate were varied as required. pH of the medium was adjusted to 7.5 with 1N NaOH. The medium was sterilised by autoclaving for 20 min at 121°C and was inoculated with 2% (V/V_0) of an 18-h-old culture grown in the same medium at room temperature on a rotary shaker at 150 rpm.

Assessment of EPS production in *B. cereus* JBS10 GU812900

Bacterial culture was grown in the BSS medium wherein glucose was replaced by trisodium citrate (5.0 g/1) for 48 hours. Cells were removed by centrifugation at 6,000 rpm at 4°C for 15 min and discarded. 1 ml supernatant in replicate was used to assess the production of EPS. The EPS was estimated by the phenol-sulphuric acid method as above. Of the cultures examined, the culture SSJS which was tentatively identified as *B. cereus* JBS10 GU812900 produced the highest amount of EPS. This culture was used for further work as described below.

Effect of carbon source and concentration on EPS production by *B. cereus* JBS10 GU812900

The effect of carbon source on the production of EPS was studied

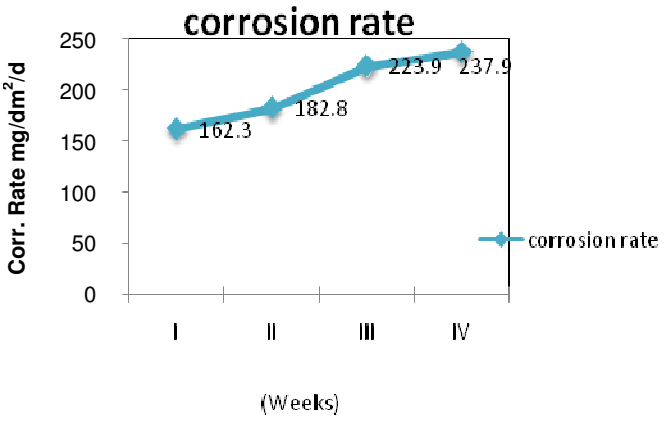


Figure 1. Corrosion rate of stainless steel during study period.

using BSS medium supplemented with 1% of fructose, galactose, glucose, maltose, sucrose and xylose and 0.5% of citrate as the carbon and energy source. Sucrose concentration in the growth medium was varied from 0.25 to 3% in order to assess the effect of carbon concentrations on EPS production by *B. cereus* JBS 10 GU812900. After 8 days, cells were removed by centrifugation at 6,000 rpm for 15 min at 4°C. The supernatant was passed through 0.2-µm-pore-size filter. Filtrate was dialyzed against distilled water at 4°C to eliminate any low molecular weight sugars and salts using dialysis bags (MW cutoff of 8,000). The dialyzed supernatant was again concentrated using rotary vacuum evaporator at 40°C and adjusted to a known volume. A known aliquot was used to estimate EPS by the phenol sulphuric acid method.

Effect of nitrogen source and concentration on EPS production by *Bacillus cereus* JBS10 GU812900

The BSS growth medium containing 1% sucrose and 0.013% of nitrogen either as ammonium chloride, ammonium sulphate, sodium nitrate or urea was used to assess the effect of nitrogen source on EPS production. The nitrogen (as ammonium sulphate) concentration in the growth medium was varied from 0.001 to 0.1% to assess the effect of nitrogen concentration on the exopolysaccharide production. The growth medium was inoculated with the 18h-old *B. cereus* JBS 10 GU812900 culture grown in the same medium. The culture was grown at room temperature for 8 days on rotary shaker at 150 rpm. Exopolysaccharide was estimated following the method described as above.

Effect of phosphate concentration on EPS production by *B. cereus* JBS10 GU812900

The BSS medium containing 1% sucrose and 0.013% ammonium sulphate was supplemented with various concentrations of dipotassium hydrogen phosphate and potassium dihydrogen phosphate (0.25 to 2.0 µg/ml) to assess the effect of phosphate concentration on the EPS production. The culture was grown and the EPS was estimated as above.

Growth and EPS production by *B. cereus* JBS10 GU812900

BSS containing 1% sucrose, 0.05% ammonium sulphate (0.006% N) and 1 µg/ml of phosphate was used as a growth medium to monitor the growth of EPS production by *Bacillus cereus* JBS 10 GU812900. Growth was monitored at room temperature at 150 rpm. At regular intervals, 5-ml aliquots were removed for turbidity measurement (A 540 nm). Samples were centrifuged (6,000 rpm for 15 min) and 1-ml supernatant was removed and dialyzed. A suitable aliquot was used to estimate EPS concentration using phenol sulphuric acid method. The experiment was carried out until the culture reached the stationary growth phase.

Characterization of EPS

Total carbohydrate was estimated by the phenol sulphuric acid method (Dubois et al., 1956) Protein was analysed using the method of Smith et al. (1985). Pyruvate was determined by the method of (Slonecker and Orbntas, 1962) Uronic acids were estimated by the method of (Filisetti-Cozzi and Carpita, 1991).

Fourier transform infrared spectroscopy (FT-IR)

The major structural groups of the purified EPS were detected using Fourier transform infrared spectroscopy (FT-IR; Abu et al., 1991).

RESULTS

Corrosion rate on stainless steel

The experiments were conducted at Vellar estuary. Corrosion rate of stainless steel generally increased with increasing immersion period (Figure 1). The initial corrosion was occurring in first week of immersion (162.3 mg/dm^2/d) and gradually increased at IVth week (237.9 mg/dm^2/d) due to macro fouling.

Isolation of biofilm (microfouling) bacteria

The biofilm samples were collected from stainless steel test panels. The samples were placed on Zobell agar medium 2216 (Himedia, Mumbai) for bacterial isolation. The bacterial strains were isolated based on colony morphology, colour, shape and size of each strain. The different strains were isolated for pure culture by streak plate method. Finally the pure cultures were maintained by slants. The Biochemical Characterisation of Bacterial Isolate showed in Table 1.

Cultivation of isolate SSJS

Bacterial strain SSJS was isolated from stainless steel test panels. The strain showed enhanced growth on MA + Glu and after 14 days of growth at 20°C on this medium, produced rod shaped, facultative aerobes which were milky white and produce endospores 1- 2 mm in diameter with a mucoid texture and an entire margin. *B. species* was Gram positive and curved rods.

Table 1. Phenotypic characteristics of the strain SSJS isolated from biofilm stainless steel test panel.

Phenotypic characters	*Bacillus cereus* GU812900
Gram stain	'+' rod
Motility	+ or -
Indole	-
Aerobic growth	+
Anaerobic growth	+
Endospores	+
Catalase activity	+
Benzidine reaction	+
Oxidase reaction	+ or -
Glucose fermentation	-
Glucose o/f medium	-

Table 2. Top 10 sequencing producing significant alignments of *Bacillus cereus* GU812900.

Accession	Description	Max score	Total score	Query coverage (%)	E value	Max ident (%)
GQ383905.1	*Bacillus* sp. 4CCS8 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
CP001407.1	*Bacillus cereus* 03BB102, complete genome	2702	3.765e+04	100	0.0	100
FJ685763.1	*Bacillus cereus* strain PEBC08010810 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
CP001176.1	*Bacillus cereus* B4264, complete genome	2702	3.781e+04	100	0.0	100
EU855219.1	*Bacillus cereus* strain CTSP45 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
EU558976.1	*Bacillus sp.* cp-h43 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
EU373359.1	*Bacillus cereus* strain HNR10 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
EU350369.1	*Bacillus cereus* strain ST307 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
EF100616.1	*Bacillus cereus* strain HS-MP13 16S ribosomal RNA gene, partial sequence	2702	2702	100	0.0	100
DQ207729.2	*Bacillus cereus* strain CCM 2010 16S ribosomal RNA gene, complete sequence	2702	2702	100	0.0	100

16S rDNA sequences

Analysis of partial 16S ribosomal deoxyribonucleic acid (rDNA) sequences from SSJS (1498 bp) also indicated that the isolate belonged to the genus *Bacillus* species with the closest species *B. cereus* haloplanktis sequence similarity SSJS at 100% (Table 2). *B. cereus* genomic DNA was isolated and PCR amplified with universal

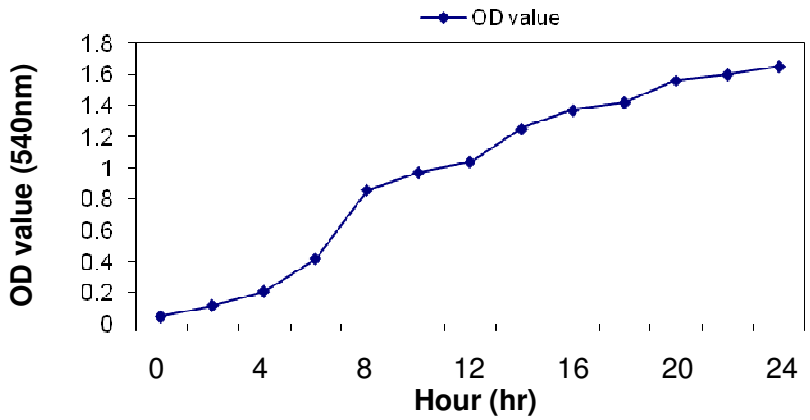


Figure 2. Growth curve of *Bacillus cereus* JBS 10 GU812900.

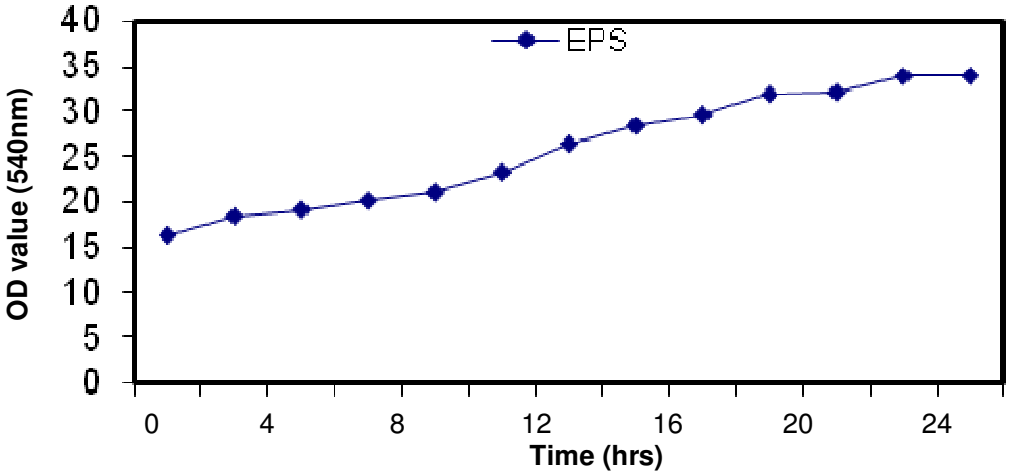


Figure 3. Exopolysaccharide (EPS) production of *Bacillus cereus* GU812900.

primer. The amplified product was sequenced and a 1498 base pairs DNA fragment was compared to the sequences available in GenBank, NCBI (GU812900). Partial sequencing of 16S rRNA was performed by Bioserve Pvt. Ltd, Hyderabad. BLAST analysis of the sequence data revealed most identity with *B. cereus* (GenBank accession number GU812900), when the sequences were compared with *B. thuringiensis* and *B. cereus* available in the databases (Figure 3). Thus, the bacteria isolated from the stainless steel test panels was confirmed up to species level and had a maximum of 100% similarity with top ten existing *B. cereus* strains. Analysis of partial 16S rDNA sequences from SSJS (1498 bp) also indicated that the isolates belonged to the genus *Bacillus* species with the closest species *B. cereus* haloplanktis (sequence similarity SSJS, 100%) (Table 1). *Bacillus cereus* genomic DNA was isolated and PCR amplified with universal primer. The amplified product was sequenced and a 1498 bp base pair DNA fragment was compared to the sequences available in GenBank, NCBI (GU812900).

Characterisation of exopolysaccharide

Factors influencing the EPS production

Various factors influencing EPS production by the culture of *B. cereus* JBS10 GU812900 was assessed. Of the various carbon sources and sucrose produced the highest amount of EPS (Table 4). The production of EPS increased with increase in sucrose concentration (0.25% of 60.2 mg/l to 3.00% of 426.06 mg/l). Of the nitrogen sources used ammonium sulphate, produced the highest (100.88 mg/l) quantities of EPS. When the growth medium was supplemented with 1µg/ml of phosphate, the EPS production was the highest. Phosphate concentration was increased (2.00 µg/ml of 498.04 mg/l).

Table 3. Effect of sucrose, nitrogen, phosphate concentration on EPS production by *Bacillus cereus* GU812900.

Sucrose (%)	OD (540 nm)	EPS(mg/l)
0.25	1.06	60.2
0.50	1.212	146.57
1.00	0.783	215.19
2.00	0.547	316.84
3.00	1.325	426.06
Nitrogen sources		
NH_4Cl	0.872	76.06
$(Na_4)_2SO_4$	1.546	100.88
Phosphate (µg/ml)		
0.25	1.256	227.08
0.50	1.46	464.2
1.00	0.897	521.14
1.50	1.362	460.36
2.00	1.692	498.04

Table 4. Chemical Composition of EPS produced by *Bacillus cereus* GU812900.

Component	µg/ml	%
Sugar	540.124	54.01
Protein	18.521	1.85

Chemical composition of EPS from *B. cereus* JBS10 GU812900

The gross chemical composition of bacterial EPS in presented in Table 3. The sugar and Protein component of EPS showed 540.124 and 18.521 µg/ml and the percentage composition of sugar and protein was 54.01 and 1.85%, respectively.

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE)

Upon SDS - PAGE on 12 % gel, crude protein of *Bacillus cereus* GU812900 yielded six bands ranging from 14 to 97.4 kDa with well defined bands at 14, 31, 43, 66.2 and 97.4 kDa From the above results, it's clearly indicating that the sample have more or less same type of molecular weight proteins (Figure 4).

Growth and EPS production

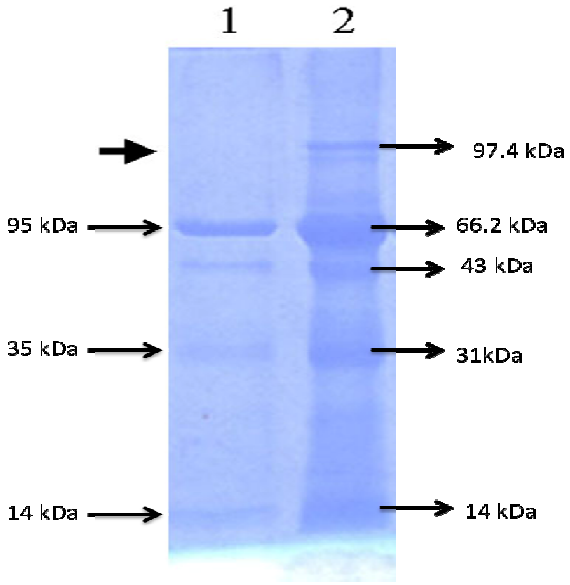
The production of EPS and the growth curve experiments were performed, wherein EPS synthesis was measured during the growth in batch cultures (Figures 1 and 2). The culture showed a characteristic sigmoidai growth curve with a lag phase of about 4 h and reached the stationary phase after 20 h. EPS production was observed at all stages of culture growth. It was higher during the stationary phase of growth and its concentration did not show any decline during 24 h of growth. The highest amount of EPS was produced during log and stationary phase.

Fourier transform infrared spectroscopy

The FTIR spectra of the EPS exhibited bands at various levels are obtained. A dominant absorption that is often attributed to C-H stretching in aliphatic of cell walls (fatty acids, carbohydrates) were recorded from 2853.98 to 2923.88 cm^{-1} (Figure 5). The IR absorption of cell proteins delivered several amide related bands. Quite a spectral peak was obtained at 1645.79 cm^{-1} indicated amide II band. The value obtained at 1560.53 cm^{-1} indicate NH_2 bending, C=O, C=N stretching (amide I band) and the values recorded at 618 cm^{-1} indicate C-O-C, P-O-C bonding (Phospholipids, RNA, aromatics).

DISCUSSION

Biofilm formation and exopolysaccharide production were measured from stainless steel test panels in the surface waters of the Vellar estuary. With increasing biofilm biomass, the concentration of EPS also increased over



Lane 1. Standard protein marker
Lane 2. Crude EPS profile of *Bacillus cereus* GU812900

Figure 4. PAGE (Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis).

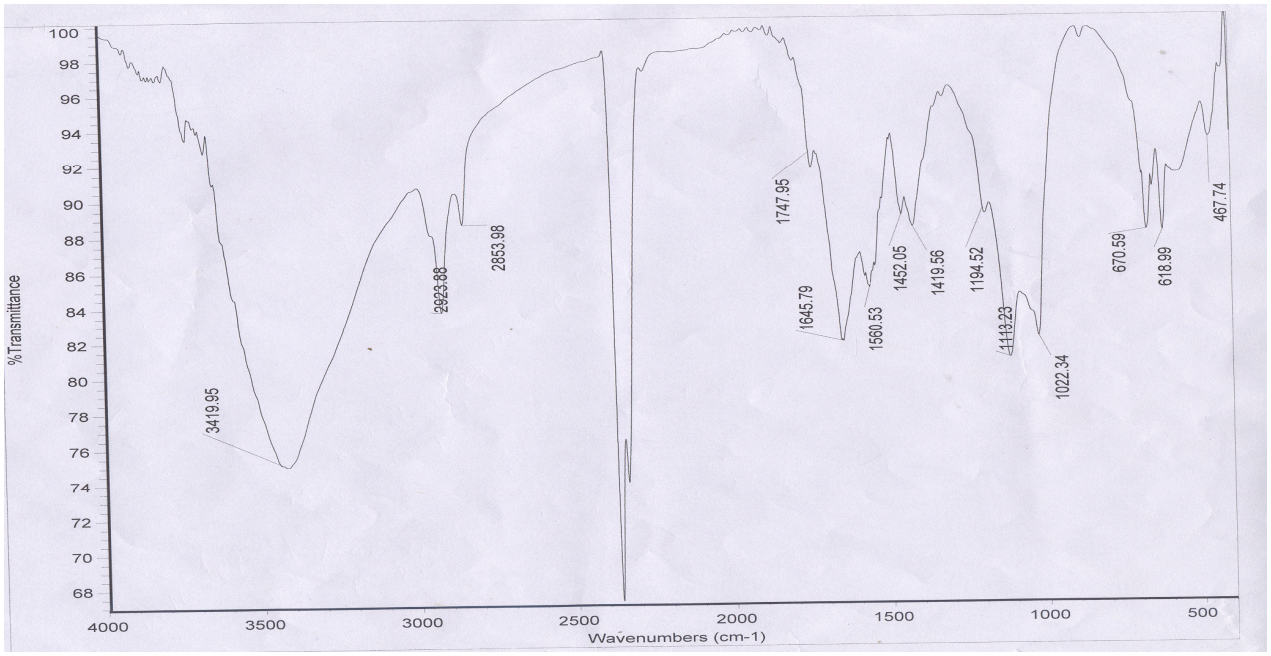


Figure 5. FTIR analysis of exopolysaccharide form *Bacillus cereus* GU812900.

the period of immersion. Biofilm biomass and EPS concentration showed significant and direct relationship with corrosion rates of stainless steel. While colonising the surface, microorganisms produce exopolysaccharides which may have direct influence on the corrosion behavior of metals (White et al., 1986; Ford et al., 1988 and Majumdar et al., 1999). Biofilm and its exopolysaccharides may provide sites for aerations cells (Little et al., 1986) ion concentration cells (Geesey et al., 1986) and sites for metal bindings. The present findings have also reported that with the increase in biofilm biomass the corrosion has also increased. The production of EPS

while growing seems to be an advantage for a bacterium fouling a surface as it may help to increase the strength of the adhesive bond and also to build a protective glycocalyx (Read and Costerton, 1987).

Previous data suggest that biofilm EPS influences the corrosion of mild steel in natural marine waters. All the cultures produced EPS although at different levels. Of these cultures, *Vibrio* sp. (isolate SS-21 D/8) produced the highest amount of EPS and therefore was used for the study on production, isolation and chemical characterization of EPS (Majumdar et al., 1999). It is that such studies will help in assessing the potential of exopolysaccharides as anticorrosive agents. The present study also suggested that biofilm EPS also has some influence on the corrosion of stainless steel in estuarine waters.

Growth and exopolysaccharide production may be more prolific under attached conditions for some bacteria and attachment to solid surfaces may stimulate polysaccharide synthesis as suggested by Vandevivere and Kirchman (1993). Also, Allison and Sutherland (1987) demonstrated that two strains of freshwater bacteria only synthesized significant amounts of EPS after attachment, indicating that the polymers were not needed for initial adhesion to inert surfaces. The present study also reported that the EPS production was maximum during prolific stages of bacterial cell (log Phase).

The 16S rDNA sequence of strain 13737 showed 100% homology to that of *B. cereus.* Previous studies reports that, Lee et al. (2003) have isolated the 16S rDNA sequences which indicated that the 17 strains belonged to a-Proteobacteria, g-Proteobacteria, CFB group bacteria, high GC, Gram-positive bacteria and low GC, gram-positive bacteria. Kumaran et al. (2010) isolated from sea bass liver and kidney of all artificially infected moribund fish and confirmed as *Pseudomonas* sp. KUMS3. In the present investigation isolation of biofilm bacteria from stainless steel which was gram positive and contained 53% GC content was carried out. In the present investigation the bacterium isolated from biofilm test panels was indentified up to species level by using 16sRNA analysis. Strain SSJS showed 100% homology to the bacterium, *B. thuringiensis* and *B. cereus.*

The physiological factors controlling EPS production have been investigated primarily in marine planktonic bacteria that secrete polysaccharide in the laboratory cultures. The present study used various physiological conditions to regulate the EPS production. When grown in batch culture (2.0 1), using 2% sucrose, 0.006% nitrogen and 1 µg/ml of phosphate, the yield of EPS produced by the culture *Vibrio* sp. was 700 mg. EPS produced by *Vibrio* sp. resembles in many aspects the most common exopolymers of other bacteria. For example, the synthesis of EPS was first detected in early exponential phase and continued at stationary phase as observed in several other bacteria (Sar and Rosenberg, 1989; Read and Costerton, 1987). Similar study was conducted in B. cereus batch culture using 25% sucrose, 0.006% nitrogen and Phosphates, EPS production of B. cereus was 426.06 mg/l, 100.88 mg/ml and 498.04 mg/l respectively. The production of EPS while growing seems to be an advantage for a bacterium fouling a surface as it may help to increase the strength of adhesive bond and also to build a protective glycocalyx (Read and Costerton 1987). Most bacteria release the largest quantity EPS during stationary growth phase in laboratory culture (Decho, 1990 and Manca et al., 1996).

The release of EPS by bacteria is generally low during exponential growth and it accumulates during the stationnary phase. For example, secretion of exopolysaccharide by *Pseudomonas putida* and *Pseudomonas fluorescence* increased in stationary cells compared to exponential cells (Read and Costerton, 1987). This agrees well with the findings on the marine fouling bacterium *Vibrio* sp. Environmental conditions, especially availability of nitrogen, affected the carbohydrate and protein content of bacteria. Furthermore, nitrogen starvation may enhance production of carbohydrate. Moreover, the nutrient-deficient cultures may increase their carbohydrate and/or lipid production at the expense of protein. Therefore, higher production of EPS by *Vibrio* sp. during the stationary phase was perhaps associated with the deficiency of nutrients in the growth medium. In the present study the physiological factors such as sucrose, nitrogen and Phosphate concentrations which influence the production of EPS were altered and the results were observed in the laboratory conditions. As the sucrose concentration increased the EPS production also increased. Similarly the largest amount of EPS production was observed during stationary growth phase of the batch culture of the *B. cereus.*

Conclusion

Corrosion rate of stainless steel generally increased with increasing immersion period. The maximum corrosion was occurring an increasing immersion period and macro fouling settlement of the surface. The production of EPS was increased with influence of nutritional value of the media. The highest amount of EPS was produced during the stationary phase. The biofilm formed microorganisms produce EPS, which may serve as corrosion inhibitor for stainless steel. A number of bacteria isolated from the corrosion products showed potential for EPS production.

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