

# CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF EXOPOLYSACCHARIDE ISOLATED FROM MARINE BACTERIA

P. Maheswari<sup>1</sup>, S. Sankaralingam<sup>2</sup> and N. Sivakumar<sup>3</sup>

<sup>1</sup>Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamil Nadu,

<sup>2</sup>Department of Botany, Saraswathi Narayanan College, Madurai, Tamil Nadu, India.

<sup>3</sup>School of Biotechnology, Department of Molecular Microbiology, Madurai Kamaraj University, Madurai, Tamil Nadu, India.

## ABSTRACT

Exopolysaccharides producing bacteria was isolated from marine sediment soil. Optimization was done to get maximum EPS production, for those parameters such as pH 8, temperature 30C, galactose, yeast extract, sodium nitrate, Ferrous sulphate, Tween 80, NaCl 7% and Tris Hcl buffer. The physicochemical characterization was done for the EPS crude extract obtained from *Halomonas* sp. The bacterial EPS extract revealed characteristic absorption bands of EPS for *Halomonas* characterized by FT-IR, GC- MS and NMR analysis. Antimicrobial activity was analyzed for bacterial EPS extract which shows higher zone in *Klebsiella* sp and *Aspergillus ochraceus*.

Keywords: Exopolysaccharides, Optimization, DPPH, FT-IR, NMR and antimicrobial activity

## INTRODUCTION

Microbial exopolysaccharides are produced by various genera of bacteria and yeast. Many of these products have been shown to have a wide variety of applications in food, pharmaceutical and oil industries (Crescenzi, 1995). EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation, phagocytosis, phage attack, antibiotics or toxic compounds, predation by protozoans, osmotic stress and adhesion to solid surfaces and in cellular recognition.

In food industry, microbial exopolysaccharides are used as thickeners or viscosifiers, stabilizing or emulsifying agents and as gelling and water-binding agents or texturizers (Sutherland, 2001). Many researchers have additionally shown that the Exopolysaccharides obtained from different sources have shown repression of various microorganisms at different concentrations. It's been investigated that anti bacterial activities of crude saccharide of *Pleurotus tuber-regium* on some bacteria 10 pathogens showed to inhibit their growth through the agar cup diffusion and disc diffusion ways (Schuts *et al.*, 2016).

Liu *et al.*, (2011) worked on a levan-type exopolysaccharide (EPS) from *Paenibacillus polymyxa* EJS-3 was successfully acetylated, phosphorylated and benzylated, respectively, affording its derivatives of acetylated levan (AL), phosphorylated levan (PL) and benzylated levan (BL). Then, the antioxidant and antitumor activities in vitro of the natural polysaccharide and its derivatives were determined.

Many researchers have additionally shown that the Exopolysaccharides obtained from different sources have shown repression of various microorganisms at different concentrations. It's been investigated that anti bacterial activities of crude saccharide of *Pleurotus tuber-regium* on some bacteria 10 pathogens showed to inhibit their growth through the agar cup diffusion and disc diffusion ways (Schuts *et al.*, 2016).

## **MATERIALS AND METHODS**

### **Optimization of cultural conditions for EPS production**

The factors like pH, temperature, carbon, organic and inorganic nitrogen, incubation time, amino acids, surfactants, inoculum concentration, NaCl concentration and metal ions concentration which were expected to affect the production of EPS by the selected strain was optimized by selecting one parameter at a time. Growth of the organism was determined by optical density measured at 600 nm.

### **Effect of different pH on bacterial growth**

Different pH (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) was adjusted into the production medium to determine the effect of pH on bacterial growth for EPS production. Growth of the organism was determined by optical density measured at 600 nm.

### **Effect of different temperature on bacterial growth**

Different temperatures (20, 30, 40 and 50°C) were used to prepare production medium to determine the effect of temperature on bacterial growth and EPS production.

### **Effect of different incubation time on bacterial growth**

In the production medium bacterial culture was incubated at different incubation time (24, 48, 72, 96 and 120 hours) intervals to determine the effect of incubation time on bacterial growth and EPS production.

### **Effects of different carbon sources on bacterial growth**

Different carbon sources at 1% concentration (Dextrose, Glucose, Sucrose, Maltose, Lactose, Raffinose, Fructose, Xylose, Trahalose, Galactose, Mannitol and Maltose) were introduced to the production medium to determine the effect of carbon dose on EPS production.

### **Effects of nitrogen sources on bacterial growth**

Different nitrogen sources at 1% concentration (Urea, Glycine, Casein, Yeast extract, Peptone, Ammonium nitrate, Sodium nitrate, Potassium nitrate and Ammonium sulphate) were introduced into the production medium individually to determine the effect of nitrogen source on microbial growth and EPS production.

### **Effects of metal ions on bacterial growth**

Different metal ions at 1% concentration (Sodium carbonate, Zinc sulphate, Potassium chloride, Magnesium chloride, Ferrous sulphate, Sodium sulphate, Copper sulphate, Nickel sulphate and Lithium chloride) was introduced into the production medium individually to determine the effect of metal ions on microbial growth and EPS production.

### **Effects of amino acids on bacterial growth**

Different amino acids at 0.1% concentration (Glycine, Glutamine, Cysteine, Alanine, Methionine, Aspartic acid, Ornithine, Proline, Threonine, Phenyl alanine, Serine, Leucine, Hydroxy proline, 2-aminobutric acid, Norleucine, Arginine, Histidine, Tyrosine, Valine, Lysine, Isoleucine, Glutamic acid, Tryptophan and Dihydroxy phenylalanine) was introduced into the production medium.

### **Effects of surfactants on bacterial growth**

Different surfactants at 0.2% concentration (SDS, PEG, Tween 80, Tween 20, CTAB and Triton X 100) were introduced into the production medium individually to determine the effect of surfactants on microbial growth and EPS production.

### **Effects of NaCl concentration on bacterial growth**

Different concentrations of NaCl (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) were introduced into the production medium individually to determine the effect of NaCl on microbial growth and EPS production.

### **Effect of inoculum concentrations on bacterial growth**

Different inoculum concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5) were introduced into the production medium individually to determine the effect of inoculum concentrations on microbial growth and EPS production.

### **Mass scale production**

In order to increase the amount of EPS production in various pH, temperature, incubation time, carbon source, nitrogen source and agricultural residues; optimization processes was carried out using *Bacillus* sp. Fermentation was carried out in a 3L fermentor (Lark, Chennai) in optimized production media (Sankaralingam, 2012).

### **Statistical analysis**

The results obtained in the present investigation were subject to statistical analysis like Mean ( $\bar{x}$ ) and Standard deviation (SD) by Zar, (1984).

### **CCD and RSM-Statistical analysis**

From the optimized nutrient composition for *Bacillus* sp. growth rate, the effect of the Carbon sources (Lactose), Nitrogen sources (Ammonium nitrate), Metal ions (Zinc sulphate) and Surfactants (Tween 80) level were studied using Central Composite Design (CCD) (Song *et al.*, 2007).

### **Statistical analysis**

Experimental designs and the polynomial coefficients were calculated and analyzed using a trial version of Design-Expert software (version 8.0.4, Stat-Ease Inc., Minneapolis, USA). Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA).

### **Characterization of EPS**

#### **Fourier Transform Infrared Spectroscopy analysis of EPS**

The bacterial exopolysaccharide was characterized using Fourier transform infrared Spectrophotometer (Wang *et al.*, 2004).

#### **Gas chromatographic - Mass spectrophotometric analysis of the EPS**

EPS was examined by gas chromatography (GC) as their alditol acetates Albersheim *et al.* (1967).

#### **NMR spectroscopy**

$^1\text{H}$  nuclear magnetic resonance (NMR) and  $^{13}\text{C}$  NMR spectra will be recorded at 23°C using a Bruker, Switzerland 400 MHz spectrometer. 70 mg of polysaccharide will be dissolved in 1 ml of 99%  $\text{D}_2\text{O}$  followed by centrifugation and freeze dried. The process will be repeated twice and the final sample will be dissolved in 1.0 ml of 99.98%  $\text{D}_2\text{O}$  (Sivakumar *et al.*, 2012).

#### **Antibacterial assay**

To study the antibacterial activity of the polysaccharides, human clinical pathogens and the plant pathogens (El-Masry *et al.*, 2000) were used.

### 3.13.3. Antifungal assay

To study the antifungal activity for the present study, the fungal were seeded on Sabouraud Dextrose Agar plates (Del Val *et al.*, 2001).

## RESULTS

### Optimization of Cultural Condition for EPS Production By Marine Bacteria

EPS produced by *Halomonas* sp. isolated from marine sample was adjusted to various cultural conditions. Maximum EPS production was recorded at pH 8 ( $11 \pm 0.5$ mg/ml), temperature  $30^{\circ}\text{C}$  ( $17 \pm 0.4$  mg/ml), carbon source is galactose ( $13 \pm 0.2$  mg/ml), organic nitrogen sources is yeast extract ( $9.6 \pm 0.5$ mg/ml), inorganic nitrogen sources is sodium nitrate ( $135 \pm 0.3$ mg/ml), metal source is ferrous sulphate ( $50 \pm 0.2$  mg/ml), aminoacid is DOPA ( $44.6 \pm 0.1$  mg/ml), surfactants is Tween 80 ( $27.8 \pm 0.5$  mg/ml), 7% NaCl ( $27.9 \pm 0.4$  mg/ml), 72 hours incubation period ( $34.6 \pm 0.6$  mg/ml), Tris Hcl buffer ( $20 \pm 0.6$  mg/ml) (Fig. 1 - 12).

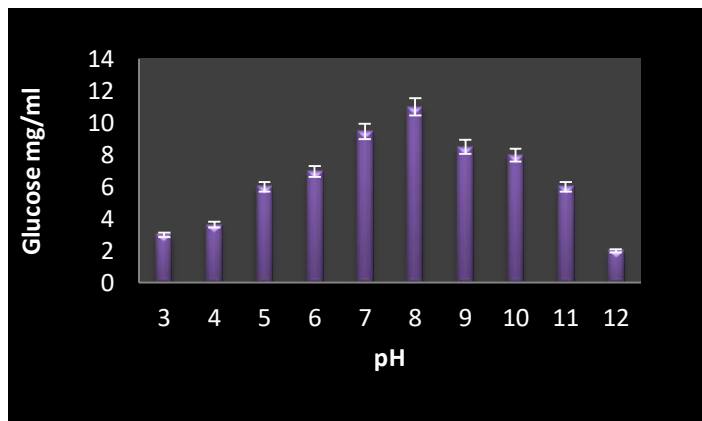


Fig. 1 Effect pH on EPS production

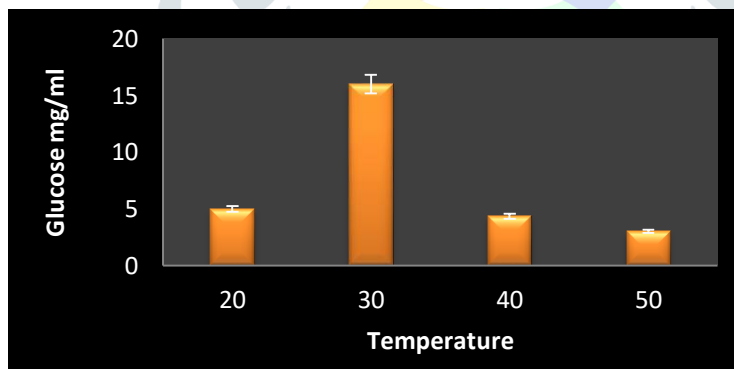


Fig. 2 Effect Temperature on EPS production

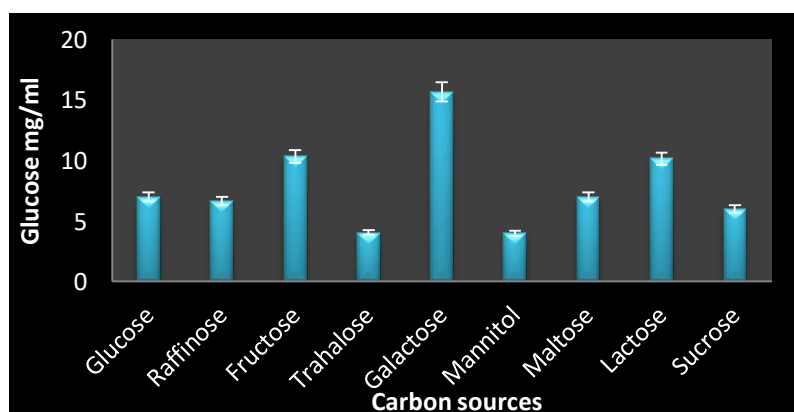


Fig. 3 Effect of carbon sources on EPS production

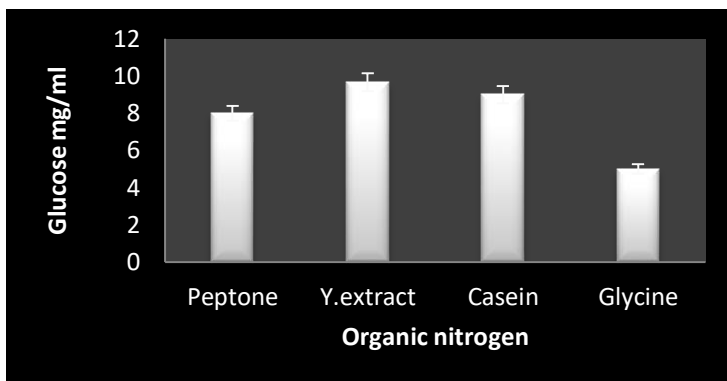


Fig. 4 Effect of Organic nitrogen sources on EPS production

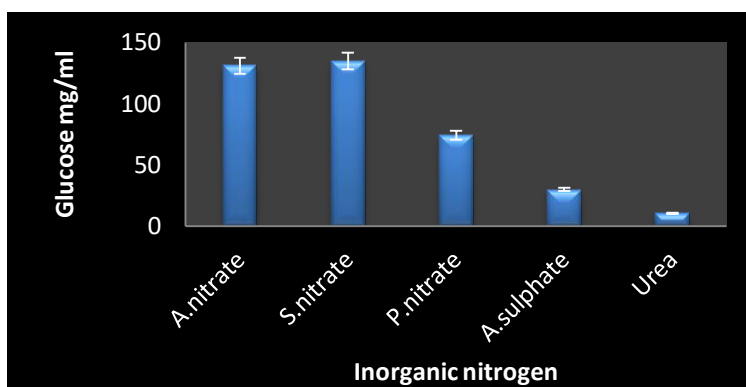


Fig. 5 Effect of inorganic nitrogen sources on EPS production

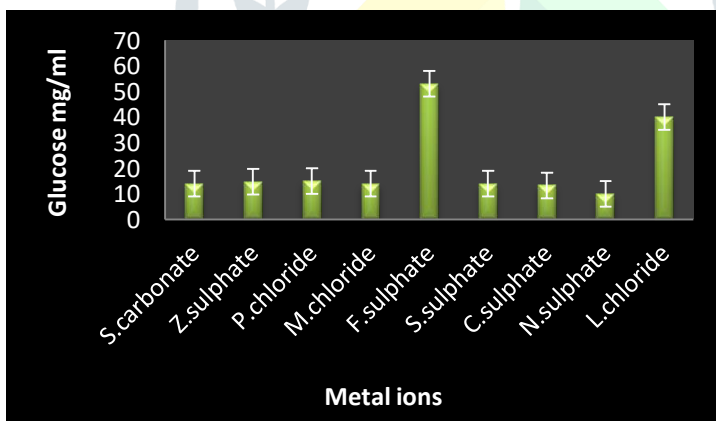
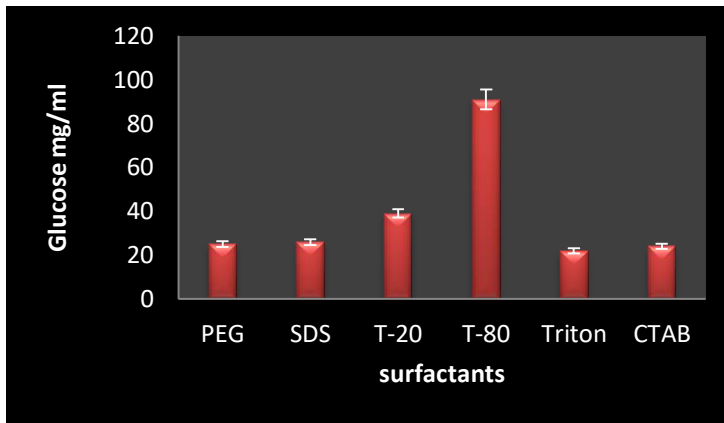
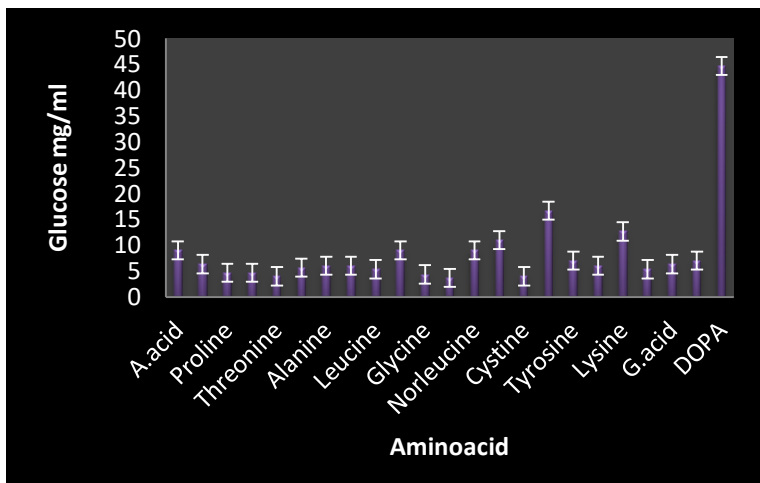


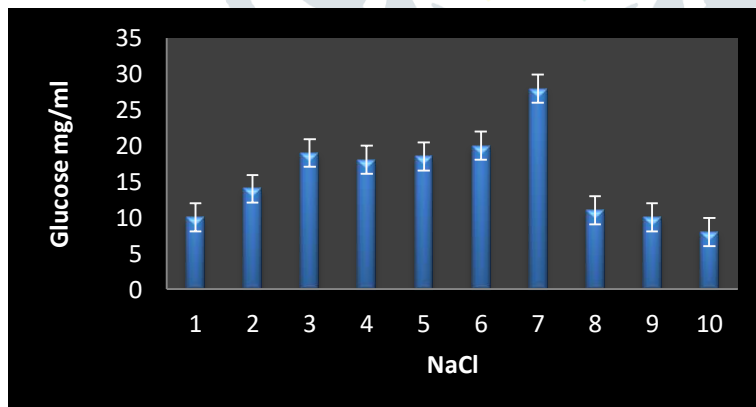
Fig. 6 Effect of metal ions on EPS production



**Fig. 7 Effect of Surfactants on EPS production**



**Fig. 8 Effect of amino acids on EPS production**



**Fig. 9 Effect of NaCl on EPS production**

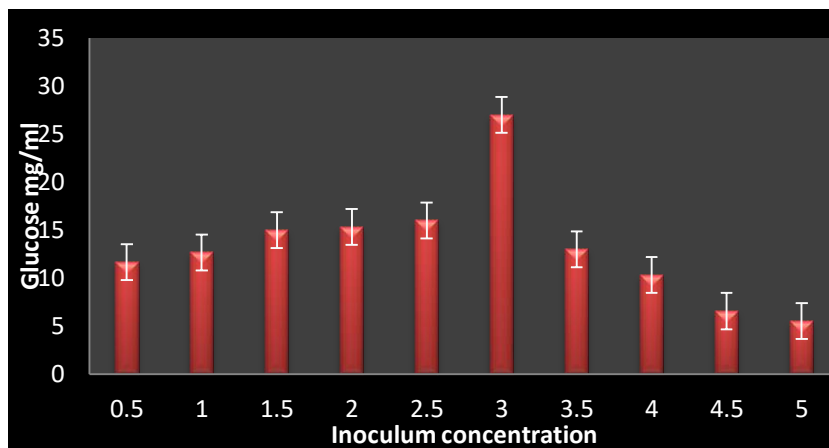


Fig. 10 Effect of Inoculum concentration on EPS production

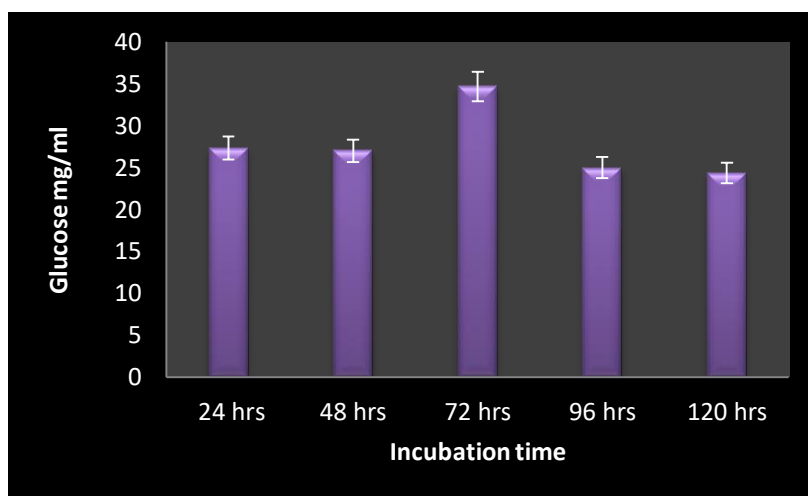


Fig. 11 Effect of Incubation time on EPS production

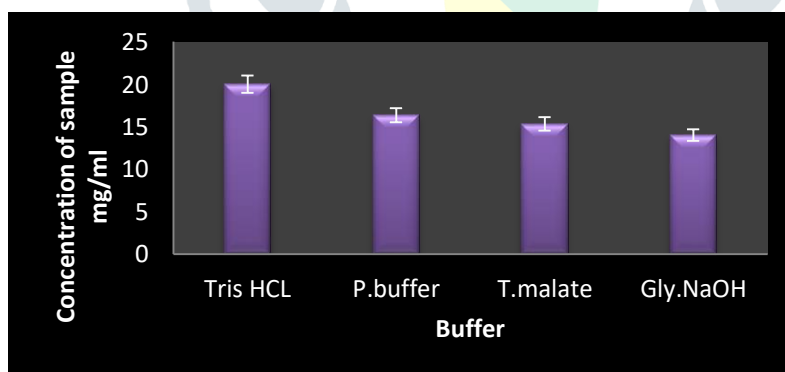


Fig. 12 Effect of buffer on EPS production

Central Composite design

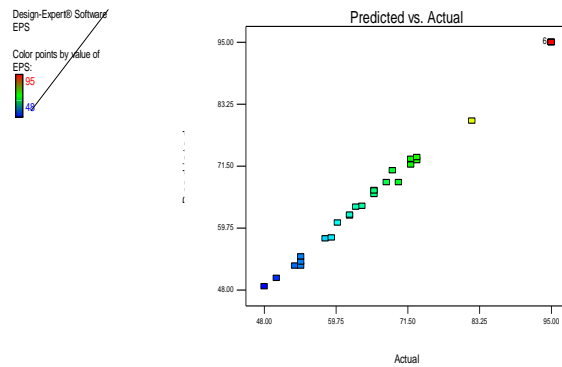


Fig.13. Predicted response versus actual value

The interaction effects of variables on EPS production were studied by plotting 3D surface curves against any two independent variables, while keeping another variables at its central (0) level. The 3D curves of the calculated response (EPS production) and contour plots from the interactions between the variables are shown in the figure 14a-14f.

The conventional method (i.e., change-one-factor-at-a-time) traditionally used for optimization of multiactor experimental design had limitations because (i) it generates large quantities of data which are often difficult to interpret (ii) it is time consuming and expensive (iii) ignores the effect of interactions among factors which have a great bearing on the response. To overcome these problems, a central composite design (CCD) and RSM were applied to determine the optimal levels of process variables on EPS production. Only 30 experiments were necessary and the obtained model was adequate ( $P < 0.001$ ). By solving the regression equation, the optimum process conditions were determined. A maximum EPS yield of 95mg/ml was obtained at the optimized process conditions (Fig.15a-15f).

The results indicated that RSM not only help us to locate the optimum conditions of the process variables in order to enhance the maximum EPS production, but also proves to be well suited to evaluating the main and interaction effects of the process variables on EPS production.

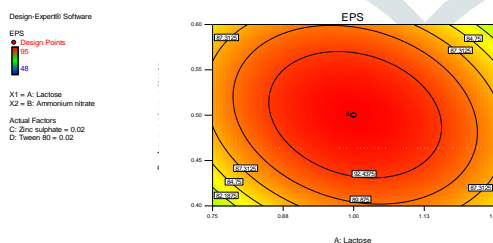


Fig.14a. Contour plot showing the effect of Lactose and Ammonium nitrate on EPS production.

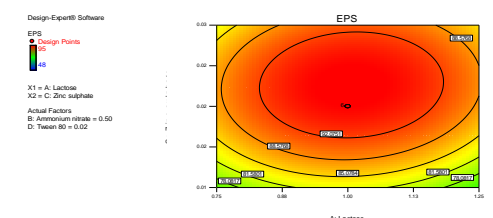


Fig.14b. Contour plot showing the effect of Lactose and Zinc sulphate on EPS production

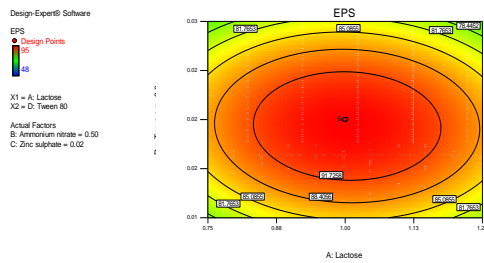


Fig.14c. Contour plot showing the effect of Lactose and Tween 80 on EPS production

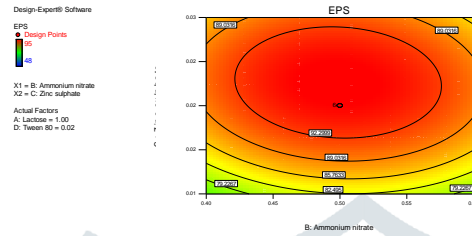


Fig.14d. Contour plot showing the effect of Ammonium nitrate and Zinc sulphate on EPS production

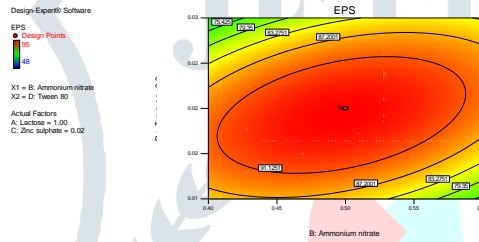


Fig.14e. Contour plot showing the effect of Ammonium nitrate and Tween 80 on EPS production

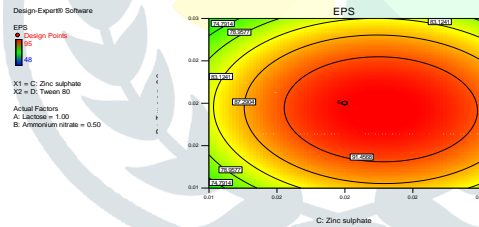


Fig.14f. Contour plot showing the effect of Zinc sulphate and Tween 80 on EPS production

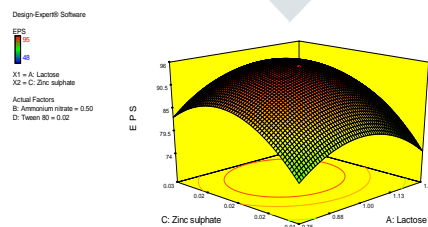


Fig.15a. 3D plot showing the effect of Lactose and Zinc sulphate on EPS production

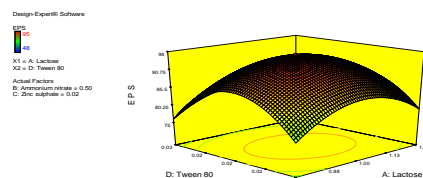


Fig.15b. 3D plot showing the effect of Lactose and Tween 80 on EPS production

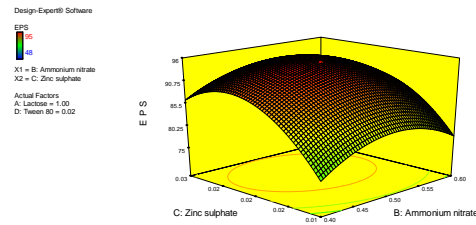


Fig.15c.3D plot showing the effect of Ammonium nitrate and Zinc sulphate on EPS production

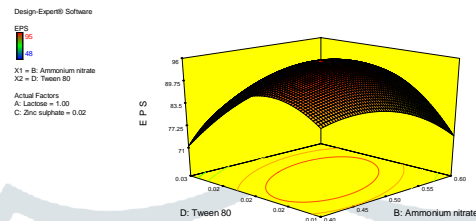


Fig.15d. 3D plot showing the effect of Ammonium nitrate and Tween80 on EPS production

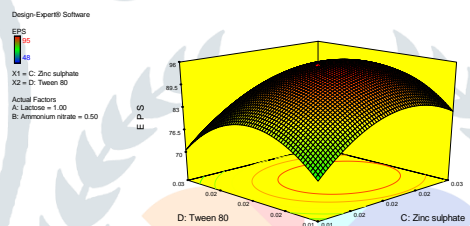


Fig.15e.3D plot showing the effect of Zinc sulphate and Tween 80 on EPS production

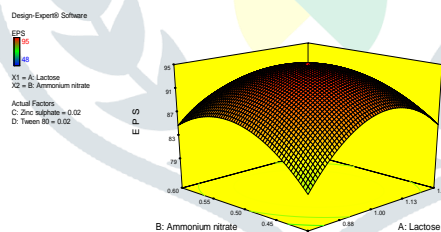


Fig.15f.3D plot showing the effect of Lactose and Ammonium nitrate On EPS production

### FT-IR spectrophotometer analysis

FT-IR spectrum of purified EPS of *Halomonas* sp. was studied. The strong peak at  $3442.70\text{cm}^{-1}$  explained O-H stretching vibration (alkyl group) and it shows even the signal at  $338.95\text{ cm}^{-1}$  revealed N-H stretching vibration (amide). The signal at  $2360.71\text{ cm}^{-1}$  contributed H-C=O: C-H medium stretching vibration (aldehyde). The signal at  $1653.85\text{ cm}^{-1}$  explained C=C- medium stretching vibration (alkenes). The signal at  $1283.54\text{ cm}^{-1}$  concluded C-H wag ( $-\text{CH}_2\text{X}$ ) (alkyl halides). The signal at  $1119.60$  and  $1067.53\text{ cm}^{-1}$  cleared C-N stretch vibration (aliphatic amines). The signal at  $990.63\text{ cm}^{-1}$  concerns =C-H strong stretching vibration (alkenes). The signal at  $947.95\text{ cm}^{-1}$  it shows O-H stretching vibration (Carboxylic acids). In weak signal at  $865.01$ ,  $620.07$ ,  $536.17\text{cm}^{-1}$  designed ring formation of C-C<sub>1</sub> stretching it showed alkyl halides (Fig. 16).

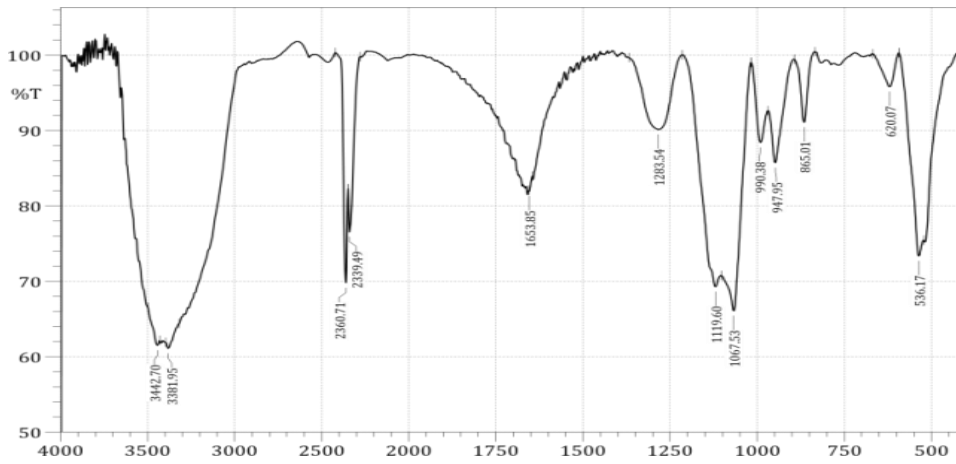


Fig.16. FT- IR spectrum of exopolysaccharides from *Halomonas* sp.



**GC –MS analysis**

The purified EPS extract was subjected to GC-MS analysis. GC-MS of bacterial EPS peaks are corresponding to the presence of D-mannitol (0.91%) and D-galactose (4.06%) (Fig.17a, 17b and 17c).

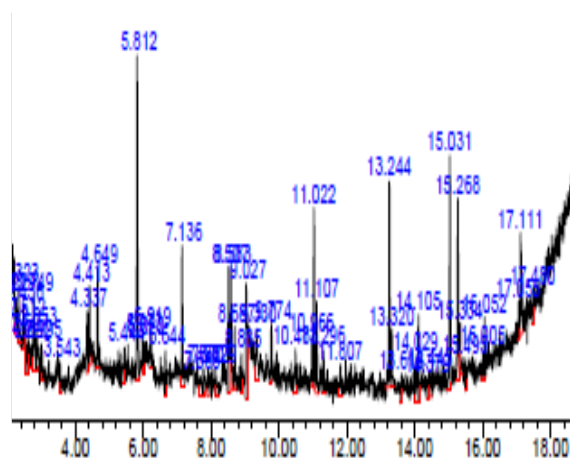


Fig.17a. GC –MS analysis of *Halomonas* sp EPS

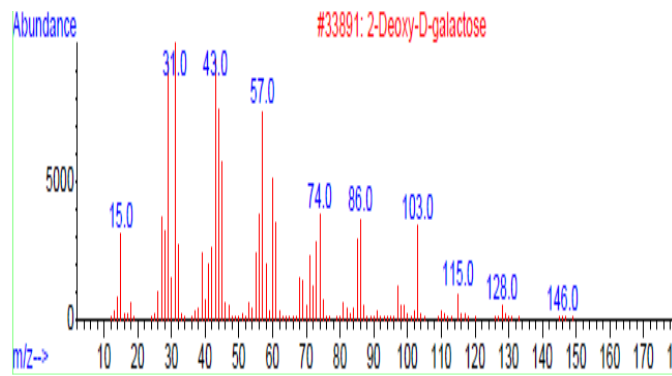


Fig.17b. GC –MS analysis of *Halomonas* sp EPS-D-galactose

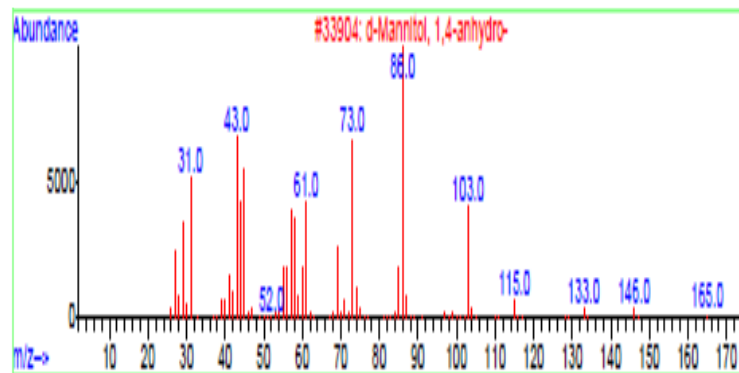


Fig.17c. GC –MS analysis of *Halomonas* sp EPS-D-mannitol

### NMR analysis

$^1\text{H}$  NMR spectrum of *Halomonas* sp EPS was analyzed. The signal appearing at substituted peaks are corresponding to the presence of D-mannitol (0.91%) and D-galactose (4.06%) residues (Fig.18a and 18b).

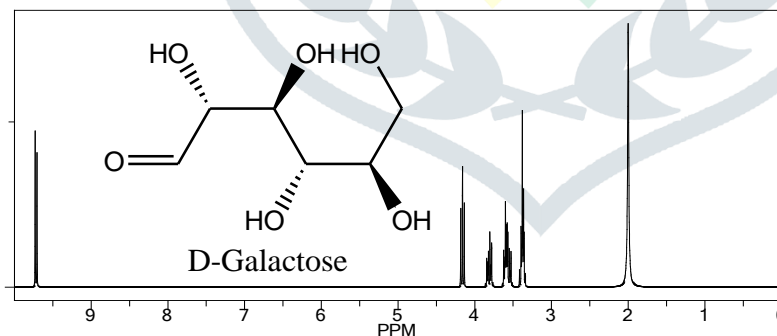


Fig.18a.  $^1\text{H}$  NMR Spectrum of D-Galactose

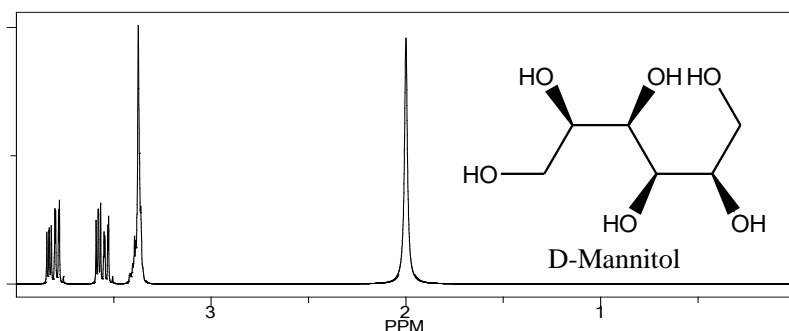


Fig.18b.  $^1\text{H}$  NMR Spectrum of D-Mannitol

### Antibacterial activity of polysaccharides against pathogens

The exopolysaccharide from *Halomonas* sp was assessed against nine different human pathogens such as *Klebsiella* sp, *Escherichia coli*, *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Serratia* sp, *Listeria monocytogenes*, *Streptococcus* sp and *Vibrio cholera*. The two bacterial plant pathogens *Erwinia amylovora* and *Erwinia caratovora* were also tested. The exopolysaccharides showed maximum zone of inhibition against *Streptococcus* sp (40mm), *Serratia* sp (32mm), *Staphylococcus* sp (25mm), *Erwinia caratovora* (25mm), *Erwinia amylovora* (20mm), *Bacillus* sp (20mm), *Klebsiella* sp (20mm), *Listeria* sp (20mm) and minimum zone of inhibition(17mm) was observed against *E. coli*, *Pseudomonas* sp, and *Vibrio cholera* is shown in table 1.

**Table. 1. Antibacterial activity of exopolysaccharide against pathogens**

S. No	Test organisms	<i>Halomonas</i> sp exopolysaccharide (mm)
<b>Human pathogens</b>		
1	<i>Klebsiella</i> sp	20mm
2	<i>E. coli</i>	17mm
3	<i>Bacillus</i> sp	20mm
4	<i>Staphylococcus</i> sp	25mm
5	<i>Pseudomonas</i> sp	17mm
6	<i>Streptococcus</i> sp	40mm
7	<i>Serratia</i> sp	32mm
8	<i>Vibrio cholera</i>	17mm
9	<i>Listeria monocytogenes</i>	20mm
<b>Plantpathogens</b>		
1	<i>Erwinia caratovora</i>	25mm
2	<i>Erwinia amylovora</i>	20mm

### Antifungal activity of EPS against pathogens

The exopolysaccharides was assessed against different fungal pathogens such as *Rhizopus stolonifer*, *Aspergillus japonicus*, *Aspergillus* sp, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus tamarii*, *Rhizopus microspores*, *Aspergillus aculentensis*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus fumigatus*, *Rhizopus sporotrichoides* and *Fusarium oxysporum*. The *Halomonas campaniensis* showed maximum of 41 mm of inhibition zone against *Aspergillus ochraceus* and maximum of 20 mm of inhibiting zone against *Aspergillus tamarii* is shown in Table 2.

**Table. 2. Antifungal activity of exopolysaccharides against pathogens**

S. No	Test organisms	<i>Halomonas</i> sp exopolysaccharide (mm)
1	<i>Aspergillus oculentensis</i>	38mm
2	<i>Aspergillus ochraceus</i>	41mm
3	<i>Rhizopus stolonifer</i>	33mm
4	<i>Fusarium oxysporum</i>	40mm
5	<i>Aspergillus oryzae</i>	18mm
6	<i>Aspergillus flavus</i>	22mm
7	<i>Fusarium sporotrichodes</i>	41mm
8	<i>Aspergillus niger</i>	35mm
9	<i>Aspergillus nidulens</i>	39mm
10	<i>Rhizopus microspores</i>	40mm
11	<i>Aspergillus fumigatus</i>	23mm
12	<i>Aspergillus tamarii</i>	20mm
13	<i>Aspergillus</i> sp	21mm
14	<i>Aspergillus japonicus</i>	29mm

## Discussion

In the present study maximum production of EPS was observed from *Bacillus* sp. in Zobell marine agar. The optimal cultural condition for EPS production are as follows: After 48hours incubation at pH 8.0 ( $11 \pm 0.5$ mg/ml), temperature  $30^{\circ}\text{C}$  ( $17 \pm 0.4$  mg/ml), carbon source is galactose ( $13 \pm 0.2$  mg/ml), organic nitrogen sources is yeast extract ( $9.6 \pm 0.5$ mg/ml), inorganic nitrogen sources is sodium nitrate ( $135 \pm 0.3$ mg/ml), metal source is ferrous sulphate ( $50 \pm 0.2$  mg/ml), aminoacid is DOPA ( $44.6 \pm 0.1$  mg/ml), surfactants is Tween 80 ( $27.8 \pm 0.5$  mg/ml), 7% NaCl ( $27.9 \pm 0.4$  mg/ml), 72 hours incubation period ( $34.6 \pm 0.6$  mg/ml), Tris Hcl buffer ( $20 \pm 0.6$  mg/ml). *Bacillus* sp. EPS content under the optimized conditions was better than that under the basic culture medium and initial conditions.

Rao *et al.* (2013) described on the carbon source for the EPS production. He reports maltose as the carbon source gave maximum EPS 10.45 g/L (12). An EPS yield of  $0.065 \pm 0.013$  and of  $0.297 \pm 0.054$  g/L substrate after 72 hours was obtained for glucose and jute, respectively. *Pseudomonas* sp shows production of 7–18 g/L of EPS using glycerol as the sole carbon source. The 5% sucrose concentration gave 19.3 g/L EPS and 15.0 g/L biomass.

Kanmani *et al.* (2011) investigated on the optimal conditions for EPS production by *S. phocae* PI80, different temperature ( $25$ – $50^{\circ}\text{C}$ ), pH (5.0–7.5) and NaCl concentrations (0–4%) were analyzed in MRS broth. The optimal temperature, pH and NaCl for cell growth and EPS production were  $35^{\circ}\text{C}$ , 6.5 and 2–3%, respectively with the corresponding cell growth ( $\text{OD}-1.333 \pm 0.02$ ,  $1.335 \pm 0.05$  and  $1.358 \pm 0.02$ ) and EPS (g/L) production ( $7.8 \pm 0.29$ ,  $7.9 \pm 0.34$  and  $8.1 \pm 0.27$ ). Effect of carbon sources on cell growth and EPS production by *S.phocae* was investigated in MRS broth. Among the carbons sources lactose ( $15 \text{ g L}^{-1}$ ) was found to be best for EPS production.

Cerning *et al.* (1994) stated that the three growth conditions (temperature, pH, and bactocasitone concentration) likely to affect EPS production. The influence of the carbon source on EPS production was not examined because other studies have shown in general, that glucose (10 to 20 g/l) provides the highest yield of EPS. Carbon source is one of the most important factors affecting EPS production. A wide variety of carbon sources, including sucrose, glucose, lactose, maltose, mannitol, sorbitol, whey, starch and even non sugar sources like methanol and C9 to C16 n-alkanes, can be used to produce microbial EPS.

Muralidhar *et al.* (2001) reported that a circular contour plot of response surfaces indicates that the interaction between the corresponding variables can be ignored, while an elliptical or saddle nature of the contour plot suggests that the interaction between the corresponding variables is significant. Meng *et al.* (2010) determined the optimal levels of the test variables for the amount of EPS extracted. The 3D response surface was described by the regression model.

Kemavongse and Teanpaisan (2007) reported on the primary components of fermentation medium, namely, Peptone, Lab lemco powder, dextrose, yeast extract,  $\text{KH}_2\text{PO}_4$ ,  $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ ,  $((\text{NH}_4)_3\text{C}_6\text{H}_5\text{O}_7)$ ,  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ ,  $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$  and Tween 80 for EPS production were identified by PBD. The effects of these components on the response indicated that there was a wide variation of biomass from 0.21- 1.73 g/l and EPS production from 0.89 to 1.83 g/L, respectively in the 12 trials. This variation reflected the importance of medium optimization to attain higher yields.

Ismail and Nampoothiri (2010) employed the model of response surface methodology (RSM) in the optimization of major encapsulation conditions such as concentration of sodium alginate, calcium chloride and curing time for the EPS producing microorganism *Lactobacillus plantarum*. The second-order quadratic model with the optimum conditions (sodium alginate 2 % (by mass per volume), calcium chloride 0.5 M and curing time 3 h) resulted in a maximum titre of  $(0.9 \pm 0.1)$  g/L of exopolysaccharides (EPS) at 72 h. The nearness of the coefficient of determination ( $R^2=0.97$ ) to 1 ensures the satisfactory adjustment of the quadratic model to the experimental data.

Sivakumar *et al.* (2013) reported that the RSM model was employed in the optimization of major EPS producing conditions such as jaggery, glutamine, ferric chloride. The second-order quadratic model with the optimum conditions (Jaggery-1%, Ferric chloride-0.02% and glutamine-0.2%). The nearness of the coefficient of determination ( $R^2=0.8716$ ) to 1 ensures the satisfactory adjustment of the quadratic model to the experimental data.

In the present study FT-IR spectrum of purified EPS of *Bacillus* sp. was studied. The strong peak at  $3442.70\text{cm}^{-1}$  explained O-H stretching vibration (alkyl group) and it shows even the signal at  $338.95\text{cm}^{-1}$  revealed N-H stretching vibration (amide). The signal at  $2360.71\text{cm}^{-1}$  contributed H-C=O: C-H medium stretching vibration (aldehyde). The signal at  $1653.85\text{cm}^{-1}$  explained  $\text{C}\equiv\text{C}$ - medium stretching vibration (alkenes). The signal at  $1283.54\text{cm}^{-1}$  concluded C-H wag ( $-\text{CH}_2\text{X}$ ) (alkyl halides). The signal at  $1119.60$  and  $1067.53\text{cm}^{-1}$  cleared C-N stretch vibration (aliphatic amines). The signal at  $990.63\text{cm}^{-1}$  concerns =C-H strong stretching vibration (alkenes). The signal at  $947.95\text{cm}^{-1}$  it shows O-H stretching vibration (Carboxylic acids). In week signal at  $865.01$ ,  $620.07$ ,  $536.17\text{cm}^{-1}$  designed ring formation of C-C<sub>1</sub> stretching it showed alkyl halides.

Nisha and Thangavel (2014) investigated the FTIR spectra of the crude EPS. The value  $3366.07\text{ cm}^{-1}$  indicates cell proteins delivered several amide related bands  $1^\circ$ ,  $2^\circ$  amines,  $2260\text{--}2100\text{ cm}^{-1}$  –C–C– stretch alkynes.  $1631.7\text{ cm}^{-1}$  indicate  $1^\circ$  amines/amide,  $1001.36\text{ cm}^{-1}$  indicates C–O and  $900\text{--}675\text{ cm}^{-1}$  indicates C–H aromatics.

Vijayabasker *et al.* (2011) reported on the IR spectrum of the crude polysaccharide sample. It showed the band at  $1000\text{--}1500\text{ cm}^{-1}$  which is characteristic to glucan. The list of the bands at  $400\text{--}950\text{ cm}^{-1}$  interval is present. In addition, the spectra showed bands around  $1000$ ,  $1200$ ,  $1400$ ,  $1500$  and  $1600\text{ cm}^{-1}$  revealed the (1,3) glucan linkages in addition to the bands in the region of  $2900$  and  $3400\text{ cm}^{-1}$  chemical bands were presented.

Mahendran *et al.* (2013) reported on the IR spectroscopy of intact exopolysaccharides (EPS). It showed the presence of hydrogen bonded compound, possible acid or amine salt. The *G. lucidum* EPS extract revealed characteristic absorption bands of EPS as observed in the reference compound dextran sulphate. The IR spectrum of the crude polysaccharide sample showed the band at  $693.43$ ,  $799.52$ ,  $1099.46$ ,  $1383.97$ ,  $1632.80$ ,  $2927.08$  and  $3439.19\text{ cm}^{-1}$  in IR spectrum of *G. lucidum* malt EPS extract. The spectrum of polysaccharide sample showed the band at  $606.63$ ,  $929.72$ ,  $1036.77$ ,  $1077.28$ ,  $1404.22$  and  $1456.30\text{ cm}^{-1}$ .

In present study purified EPS extract was subjected to GC-MS analysis. GC-MS of bacterial EPS peaks are corresponding to the presence of D-mannitol (0.91%) and D-galactose (4.06%). Similarly Vijayabasker *et al.* (2011) reported that the fully methylated products were hydrolyzed with as it, converted into the alditol acetate and analyzed by GC-MS. Methylation analysis of the polysaccharides glucan such as 2,3,4, tetra-Me. Glu. and 2,3,6- tri. Me Glu. When the oligosaccharide alditol contain hexoses (Gal) or deoxyhexoses (Rha), the sequence of this saccharide may be determined on the basis of MS. The electron impact fragmentation patterns of the mass spectra of derived alditol acetates were prepared from the hydrolysed EPS.

Mahendran *et al.* (2013) investigated the electron impact fragmentation patterns of the mass spectra of derived alditol acetates prepared from the hydrolyzed exopolysaccharides. GC-MS analysis of fungal exopolysaccharide exhibited peaks corresponding to 1-methyl-2-formylimidazole (53.81%).

Zhang *et al.* (2010) obtained polysaccharides from *Amphora* sp. was subjected to GC-MS analysis. The peaks showed the presence of rhamnose, fucose, xylose, mannose, galactose and mannitol. Dawson *et al.* (1978) investigated on two major peaks present corresponds to 1,1, Ethanediol, diacetates, 1-Tri decenaldichlorobenzene, hexoses, deoxyhexoses for bacterial EPS. A number of small peaks are also present, but these do not necessary correspond to alditol acetate.

Sivakumar *et al.* (2012) studied the electron impact fragmentation patterns of the mass spectra of derived alditol acetates prepared from the hydrolysed exopolysaccharides. GC-MS of bacterial exopolysaccharide peaks are corresponding to 2- furancarboxyaldehyde, 5-(hydroxymethyl) (33.10%), Levoglucosone (21.41%), n-Hexadecanoic acid (16.24%), Phthalic anhydride (8.34%) and stigmast-5-en-3-ol, oleate (5.28%).

In present study  $^1\text{H}$  NMR spectrum of *Bacillus* EPS was analyzed. The signal appearing at substituted peaks are corresponding to the presence of D-mannitol (0.91%) and D-galactose (4.06%) residues. Similarly Sivakumaret al. (2012) reported  $^1\text{H}$  NMR spectrum of *Frateria aurentia* EPS. The anomeric proton signal at  $\delta 5.28\text{ppm}$  was attributed to  $\alpha$  configuration pyranose units. The chemical shifts from  $\delta 3.46$  to  $4.6\text{ppm}$  were assigned to protons of C-2-C-6 of hexosylglycosidic ring. In the anomeric region of the  $^{13}\text{C}$  NMR spectrum four anomeric carbon signals occurred at  $\delta 99.59\text{ppm}$ . The signal appearing at  $\delta 76.59\text{ppm}$  was due to the C-2 substituted mannose residues.

Chowdhury et al. (2011) reported that EPS is composed to glucose, mannose, xylose, arabinose and N- acetyl glucosamine monomer units. In terms of peak area, glucose and mannose were present as major constituents, whereas, xylose, arabinose, and N- acetyl glucosamine were as the minors.

In present study the antibacterial activity was carried out for EPS extracted from *Halomonas*. The EPS was tested against nine different human pathogens such as *Klebsiella* sp, *Escherichia coli*, *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Serratia* sp, *Listeria monocytogenes*, *Streptococcus* sp and *Vibrio cholera*. Two plant pathogen *Erwinia amylovora* and *Erwinia caratovora* was also tested.

Mahendran et al. (2013) reported on the antibacterial activity of EPS extracted from *G. Lucidum* and *L. fusiformis*. The EPS was tested against seven bacterial strains such as *E.coli* MTCC741, *S. aureus* MTCC96, *Proteus* sp, *Bacillus subtilis* MTCC121, *Pseudomonas aeruginosa* MTCC741, *Klebsiella* sp and *Bacillus cereus*. Both the fungal and bacterial EPS possess potent antibacterial activity against the tested pathogens. *G. lucidum* EPS from both malt and basal medium shows highest activity against the growth of *Bacillus cereus* ( $23 \pm 0.61\text{mm}$  and  $18 \pm 0.38\text{mm}$  respectively). *L. fusiformis* EPS from both malt and basal medium shows highest activity against the growth of *Klebsiella* sp ( $18 \pm 0.49\text{mm}$ ) and *E. coli* MTCC741 ( $10 \pm 0.28\text{mm}$ ) respectively.

Nisha and Thangavel (2014) reported on the antibacterial activity of crude EPS and was checked against *Escherichia coli*, *Klebsiella* sp, *Salmonella typhi* and *Staphylococcus* sp. Crude EPS produced zone of inhibition against all test organisms.

Veera Jothi et al. (2012) reported that the two *Lactobacillus* sp. namely LAB VJ 15 and LAB VJ 32 were found to show antibacterial activity in the well diffusion assay. The antibacterial substance produced by *Lactobacillus* inhibited pathogen such as *Staphylococcus aureus*, *Klebsiella* sp, *Pseudomonas* sp, *E. coli*, *Proteus* sp, *Bacillus* sp and *Salmonella* sp. The inhibitory effect was significant against Gram positive strain of *Bacillus*, the inhibitory effect was significant against of Gram Negative bacteria, *Salmonella* sp (12mm).

Rao (2015) studied the antibacterial activity for five extracts of microalgae against five pathogenic bacteria. The antibacterial activity exhibited maximum zone of inhibition (15.50 mm) was found in acetone culture crude extract of *Anabaena oryzae* against strain of *Salmonella typhi* followed by (12.60 mm) was noticed in petroleum ether extract against *Bacillus cereus*, 10.33 mm inhibition zone was found in methanol extract against *Klebsiella aerogenes* 9.66 mm of inhibition zone was observed in chloroform extract against *Micrococcus luteus*.

Similarly, Shankar *et al.* (2015) reported on the extracellular polymeric substance isolated from the three bacterial strains and was tested for their antimicrobial activity against the four bacterial strains (*Galionella* sp., *Alteromonas* sp., *S. aureus* and *Klebsiella* sp.). The EPS isolated from the strain A showed inhibitory activity against all the four target bacteria and showed a maximum inhibition zone against *Galionella* sp. 10 mm and minimum of 8 mm against *S. aureus*. The zone of inhibition against *Alteromonas* sp. was 9 mm and the zone of inhibition against *Klebsiella* sp. was 9 mm. The EPS isolated from the strain B showed inhibitory activity against *Alteromonas* sp. and *Klebsiella* sp. It showed a maximum inhibition zone against *Alteromonas* sp. 15 mm and minimum of 10mm against *Klebsiella* sp. The EPS of strain B did not show inhibitory activity against *Galionella* sp and *S. aureus*. The EPS isolated from the strain C showed inhibitory activity against all the four target bacteria and showed a maximum inhibition zone against *Alteromonas* sp. and the diameter of zone was 13 mm. The minimum inhibition zone of 9 mm was observed against *Galionella* sp. and *S. aureus*. The zone of inhibition against *Klebsiella* sp. was 10 mm.

## REFERENCE

- Albersheim, P. Nevins, D.J. English, P.D. and Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. *Carbohydr Res.*, 5: 340-345.
- Cerning, J. Renard, C.M. Thibault, G.C. Bouillanne, J.F. Landon, C, Desmazeaud, M. and Topisirovic, L. (1994). Carbon source requirements for exopolysaccharide production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. *Appl. Env. Microbiol.*, 60: 3914-3919.
- Crescenzi, V. (1995). Microbial polysaccharides of applied interest on going research activities in Europe. *Biotechnol. Prog.* 11: 251-259.
- Chowdhury, S.R. Basaka, R.K. Sen, R. and Adhikari, B. (2011). Characterization and emulsifying property of a carbohydrate polymer produced by *Bacillus pumilus* UM -02 isolated from waste water irrigated agricultural soil. *Int. Biol. Macromolecules.*, 48: 705-712.
- Del Val, A.G. Platas, G. Basilio, A. Cabello, A. and Gorrochategui, J. (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.*, 4: 35-40.
- El-Masry, H.A. Fahmy, H.H. and Abdelwahed, A.S.H. (2000). Synthesis and antimicrobial activity of some new benzimidazole derivatives. *Molecules.*, 5: 1429-1438.
- Ismail, B. and Nampoothiri, K.M. (2010). Exopolysaccharide production and prevention of synthesis in starch using encapsulated probiotic *Lactobacillus plantarum*. *Food Technol. Biotechnol.*, 48(4): 484-489.
- Kanmani, R. Satishkumar, N. Yuvaraj, K.A. Paari, V. Pattukumar, M. and Venkatesan A. (2011). Production and purification of a novel exopolysaccharide from lactic acid bacterium *Streptococcus phocae* pi80 and its functional characteristics activity *in vitro*. *Bioresourcetchnology.* 102: 4827-4833.
- Kemavongse, K. and Teanpaisan, R. (2007). Psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913. *Eur. J. Lipid Sci. Technol.* 109(8): 629-642.

- Kim, H.O. Lim, J.M. Joo, J.H. Kim, S.W. Hwang, H.J. Chio, J.W. and Yun, J.W. (2000). Optimization of submerged culture condition for the production of mycelial biomass and exopolysaccharides by *Agrocybocylinracea*. *Bioresour. Technol.* 96: 1175-1182.
- Liu, C. Lu, J. Lu, L. Liu, Y. Wang, F. and Xiao, M. (2010). Isolation, structural characterization and immunological activity of an exopolysaccharide produced by *Bacillus licheniformis*. *Bioresour. Technol.* 8(37): 5528-5533.
- Liu, S.B. Qiao, L.P. He, H.L. Zhang, Q. and Chen, X.L. (2011). Optimization of fermentation conditions and rheological properties of exopolysaccharide produced by Deep-Sea bacterium *Zunongwangia profunda* SM-A87. *PLoS ONE*. 6(11): 268-273.
- Mahendran, S. Saravanan, S. Vijayabaskar, P. Anandapandian, K.T.K. and Shankar, T. (2013). Antibacterial potential of microbial exopolysaccharide from *Ganoderma lucidum* and *Lysinibacillus fusiformis*. *International Journal of Recent Scientific Research*. 4: 501-505.
- Mahendran, S. Vijayabaskar, P. Saravanan, S. Anandapandian, K.T.K. and Shankar, T. (2013). Structural characterization and biological activity of exopolysaccharide from *Lysinibacillus fusiformis*. *Afr. J. Microbiol. Res.* 7(38): 4666-4676.
- Meng, F. Zhou, B. Lin, R. Jia, L. Liu, X. Deng, P. Fan, K. Wang, G. Wang, L. and Zhang, J. (2010). Extraction optimization and *in vivo* antioxidant activities of exopolysaccharide by *Morchella sculeta* SO-01. *Bioresour. Technol.* 101: 4564 -4569.
- Muralidhar, R.V. Chirumanila, R.R. Marchant, R. and Nigam, P.A. (2001). Response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochem. Eng.* 9: 17-23.
- Nisha, P. and Thangavel, M. (2014). Isolation and characterization of biofilm producing bacteria from Arabian Sea. *Res. J. Recent. Sci.* 3: 132-136.
- Rao, B. (2015). Antibacterial activity of fresh water cyanobacteria. *J. Algal Biomass Utiln.* 6(3): 60- 64.
- Rao, B. Sudharsan, K. Reshma, C.H. Sekaran, G. and Mandal, A.B. (2013). Characterization of exopolysaccharide from *Bacillus amyloliquefaciens* BPRGS for its Biofloculant activity. *International Journal of Scientific and Engineering Research*. 4(10): 38-46.
- Sankaralingam, S. Shankar, T. Sendeshkannan, K. Ramasubburayan, R. and Prakash, S. (2012). Production of Protease from *Pseudomonas* sp. by immobilization approach on different matrices. *Eur. J Appl Sci.* 4(4):146-156.

- Schuts, E.C. Hulscher, M.E. Mouton, J.W. Verduin, C.M. Stuart, J.W. Overdiek, H.W. van der Linden, P.D. Natsch, S. Hertogh, C.M. Wolfs, T.F. et al. (2016). Current evidence on hospital antimicrobial stewardship objectives: A systematic review and meta-analysis. *Lancet Infect. Dis.* 16: 847–856.
- Shankar, M. Satheesh, S. and Viju, N. (2015). Antibacterial and biofilm inhibitory activities of bacteria associated with polychaetes. *Journal of Coastal Life Medicine.* 3(6): 495-502.
- Sivakumar, T. Sivasankaranarayani, S. Shankar, T. and Vijayabaskar, P. (2012). Optimization of cultural conditions for exopolysaccharides production by *Frateuria aurentia*. *International Journal of Applied Biology and Pharmaceutical Technology.* 3(3): 132-149.
- Sivakumar, T. Sivasankaranarayani, S. Shankar, T. and Issacdhinakaran, D. (2013). Characterization of exopolysaccharide producing bacterium *Frateuriaaurentia*. *World Applied Sciences Journal.* 27 (9): 1151-1157.
- Song, X.Y. Xie, S.T. Chen, X.L. Sun, C.Y. and Zhang, Y.Z. (2007). Solid-state fermentation for Trichokonins production from *Trichoder makoningii* SMF2 and preparative purification of Trichokonin VI by a simple protocol. *Journal of Biotechnology.* 131: 209-215.
- Sutherland, I.W. (1982). Biosynthesis of microbial exopolysaccharides. *Adv. Microbial Phys.* 23: 79-150.
- Sutherland, I.W. (2001). Microbial polysaccharides from Gram-negative bacteria. *International Dairy Journal.* 11: 663–674.
- VeeraJothi, V. Anandapandian, K.T.K. and Shankar, T. (2012). Bacteriocin production by probiotic bacteria from curd and its field application to poultry. *Archives of Applied Science Research.* 4(1): 336-347.
- Vijayabaskar, P. Babinastarlin, S. Shankar, T. Sivakumar, T. and Anandapandian, K.T.K. (2011). Quantification and characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121). *Advances in Biological Research.* 5(2): 71-76.
- Wang, Y. Zhang, M. Ruan, D. Shashkov, A.S. Kilcoyne, M. and Savage, A.V. (2004). Chemical components and molecular mass of six polysaccharides isolated from the sclerotium of *Poriacocos*. *Carbohydrate Research.* 339(2): 327-334.
- Zar, J.H. (1984). *Biostatistical analysis*. Englewood Cliffs, NJ: Prentice-Hall, 718 P. (Cited 790 times.).
- Zhang, X.Y. Lu, Y.N. Zhang, L.G, Qin, J.B. and Zhang, L. (2010). Exopolysaccharide in biotechnological application. *Biol. Technol.* 51(3): 581-585.