Physical Characterization and Structure Elucidation of Exopolysaccharide from Marine Halotolerant Bacterium Enterobacter cloacae VHP-34

Article *in* Industrial Biotechnology · December 2023

DOI: 10.1089/ind.2023.0020

CITATIONS
0

READS
63

3 authors:

Vishal Patel
Ganpat University
8 PUBLICATIONS **72** CITATIONS
SEE PROFILE

Falguni Patel
Kadi Sarva Vishwavidyalaya
9 PUBLICATIONS **32** CITATIONS
SEE PROFILE

Kamlesh Shah
R G Shah Science College
70 PUBLICATIONS **394** CITATIONS
SEE PROFILE

Original Research

Open camera or QR reader and scan code to access this article and other resources online.

Physical Characterization and Structure Elucidation of Exopolysaccharide from Marine Halotolerant Bacterium *Enterobacter cloacae* VHP-34

Vishal H Patel[1], *Kamlesh Shah*,[2] *and Falguni R Patel*[3]

[1]*Department of Biotechnology, Mehsana Urban Institute of Sciences, Ganpat University, Kherva, Gujarat, India*
[2]*Department of Microbiology, RG Shah Science College, Vasna, Ahmedabad, Gujarat,*
[3]*Department of Biotechnology and Microbiology, Shree M.M Patel Institute of Science and Research, Gandhinagar, Gujarat India*

Abstract

An exopolysaccharide (EPS)-producing bacterium, Enterobacter cloacae *VHP-34, was isolated from a coastal area of Umargam, Gujarat. Water-soluble EPS produced by* Enterobacter cloacae *VHP-34 was recovered with the organic solvent acetone and then purified. Carbohydrate and protein content were measured by the phenol sulphuric method and the Folin-Lowry method, Carbohydrate and protein content were 40% and 0.002%, respectively. Acid hydrolysis was done to check the monosaccharide linkage within the EPS polymer. Strong acids like HCl and trifluoroacetic acid (TFA) were used to hydrolyze the EPS. Filtered hydrolysate was used to determine monosaccharide by thin layer, paper and HPLC techniques. It was found that the repeating unit of EPS consisted of glucose and galactose as main monosaccharide components. Purified EPS was checked for functional groups and for structural elucidation by FTIR and NMR. SEM was done to check porous structure of EPS of* Enterobacter cloacae *VHP-34. Based on the results of NMR and FTIR, the obtained EPS structure has monosaccharide fragments with backbones of α-D-Glcp (1 → 4)-α-D-Glcp-(1 → 6)-α-D-Galp, respectively.*

Keywords: *Enterobacter cloacae,* Thin Layer chromatography, Paper chromatography, Glucose, Galactose

Introduction

Exopolysaccharides (EPSs) are high molecular weight sugars that adhere to microbial cells in the marine system in the form of capsular polysaccharides. Currently, demand for biological, bioactive polymers has increased in many sectors.[1] Marine terrains are rich and largely unexploited sources of bacterial EPS that can be developed into different bioprospecting applications.[2]

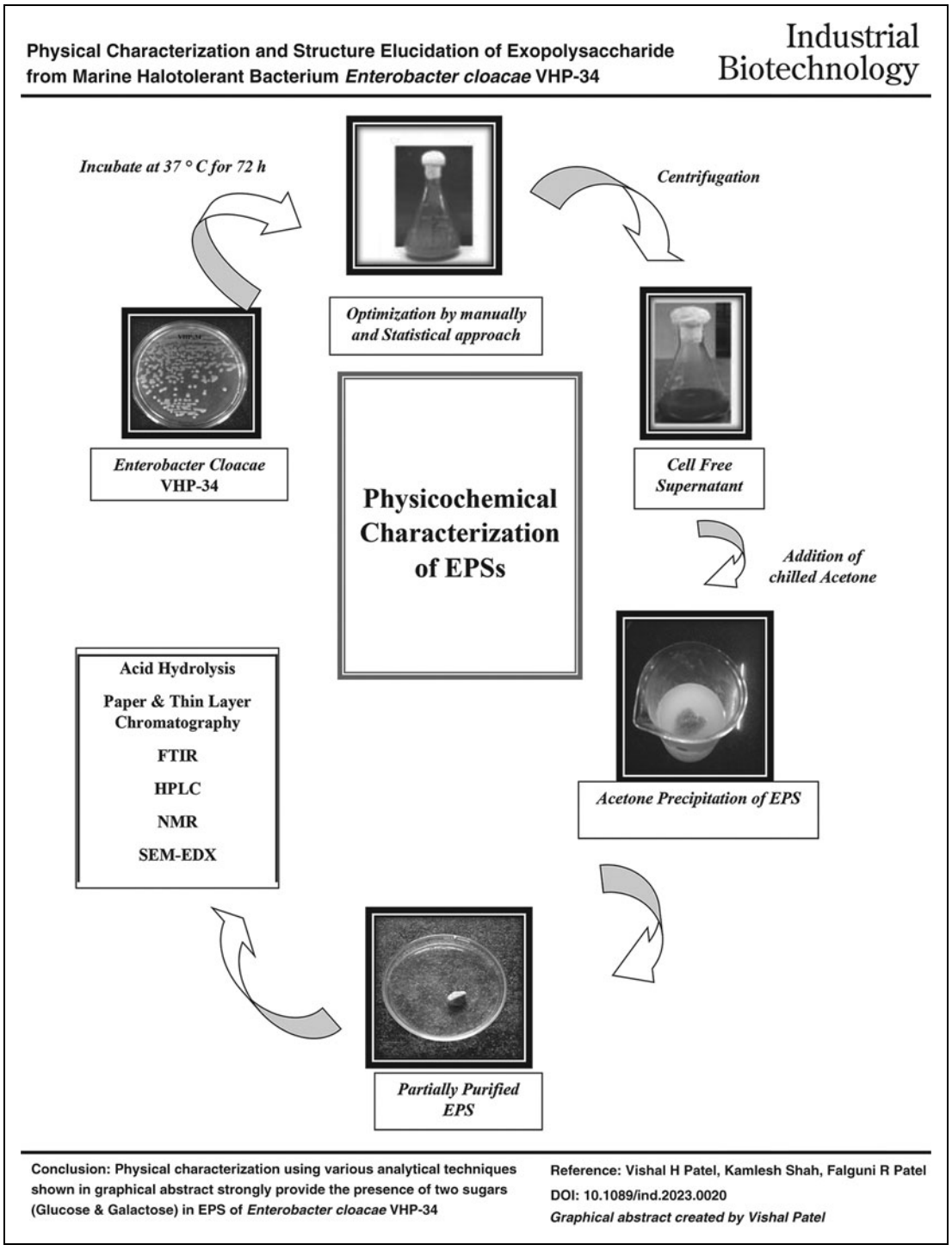
Considerable research attention is being placed on marine microorganisms due to the rich ecological and physiological diversity of marine environments.[3] Marine organisms have the ability to secrete distinctive bioactive compounds, including polysaccharides and enzymes, which can be used for societal needs.[4] Among these bioactive compounds, EPS is one of the most promising, since exopolysaccharides play important roles in both saline and hypersaline environments.[5]

EPS plays a pivotal role in many mechanisms held in marine ecosystem like nutrient uptake, aggregation, adhesion to shells, and biofilm formation.[6] Various Gamma-Proteobacteria were entrenched as EPS producers. Many halophilic EPS-producing bacteria are known for protecting membrane integrity of the cell. The most common halophilic EPS producers belong to the genus *Halomonas.*[7] The exopolysaccharides produced by bacteria exhibit vast structural diversity, and thus have found a broad range of implementation in different industrial sectors because of their physical and rheological properties.[8]

Marine strain *Enterobacter cloacae* Z0206 has glucose, galactose and mannose as part of its structural carbohydrates' monomers.[9] Another marine strain, *Enterobacter sakazakii,* has glucose, fucose and galactose as part of its monosaccharide backbone,[10] while *Enterobacter* A47 marine bacterium has glucose, fucose, galactose and glucouronic acid in structural composition.[11]

EPS secreted by marine bacteria have been used as constituents in food products, pharmacy, oil industry, hydrocarbons, vegetable, mineral oils and bioremediation for environment

 DOI: 10.1089/ind.2023.0020



protection.[12] EPS is used as a stabilizing and emulsifying agent in food industries. EPS is hydrophobic with high molecular mass and can encourage surface activity, making it useful in emulsification.[13] Gellan, one type of EPS produced from moderately halophilic bacterium *Sphingomonas paucimobilis* ATCC 31461, can be used as a food additive.[14] Glycosaminoglycans, a type of EPS, is principally used in glycobiology, which is used for therapeutic drugs.[15] EPS of *Azotobacter chroococcum* XU1 is used in metal bioremediation of Pb and Hg.[16] EPS of *Enterobacter sp.w*as able to do metal bioremediation of Pb.

Determination and examination of the chemical compositions and structural elucidations of EPS are necessary to understand their structure–function relationship.[17] The chemical characterization of EPS has been performed by many advanced techniques, including FTIR, NMR, acid hydrolysis, high-performance liquid chromatography (HPLC), methylation analysis, gas chromatography with mass spectroscopy (GC-MS) and many more.[18]

To determine the primary structure of EPS, it is crucial to examine and identify the repeating unit of monosaccharide. The monosaccharide composition analysis of EPS usually involves different methods such as acid hydrolysis or detection by HPLC, HPTLC and GC.[19] There are several available methods for hydrolysis of EPS, which includes acid hydrolysis, enzymatic hydrolysis and combined acid and enzymatic hydrolysis. Acid hydrolysis is common and makes use of some common chemical catalysts like hydrochloric acid (HCl), and trifluoroacetic acid (TFA). FTIR provides information of functional groups present at polysaccharide whereas NMR provides the structural perception of carbohydrate polymer by giving information regarding possible linkages between monosaccharide and their orientations to each other.[20]

Various analytical methods exist to explore EPS structure and determine the relative amount of monosaccharides present in polymer.[21] Different spectroscopic methods like such as FTIR and NMR are well-known methods that can provide significant information regarding polysaccharide structure.[22]

The present study focuses on (A) partial purification of EPS from bacterium *Enterobacter cloacae* VHP-34 with acid hydrolysis, paper and thin layer chromatography and (B) characterization of EPS by NMR, FT-IR, HPLC, and Scanning Electron Microscope-Energy Dispersive X-ray crystallography.

Materials and Methods

ISOLATION AND IDENTIFICATION OF EPS PRODUCING BACTERIA

Potent EPS producer VHP-34 was isolated from Umargam coast area of Gujarat and grown on modified GYE agar. The 16s rRNA analysis was compared with other 16 nucleotide sequences. Evolutionary analyses were conducted by MEGA7. From the results of 16s rRNA sequencing, the isolate VHP-34 was found to be *Enterobacter cloacae* (GenBank, Accession No: MW492544).[23]

PRODUCTION OF EPS IN MODIFIED GYE MEDIUM

Enterobacter cloacae VHP-34 was inoculated in modified glucose yeast extract medium and incubated at 37°C for 72 h on shaker at 150 rpm. At intervals of 24 h, 10 mL broth was withdrawn and centrifuged at 10,000 rpm for 10 min. The sticky supernatant was collected in a centrifuge tube and mixed with double volume of chilled acetone (RANKEM, 99% pure). Sticky precipitates were filtered on a pre-weighted Whatman filter paper. The dried Whatman filter paper was weighed after overnight drying. The increase in the weight of filter paper was calculated, with the weight indicating dry mass of EPS produced by bacterium.[23]

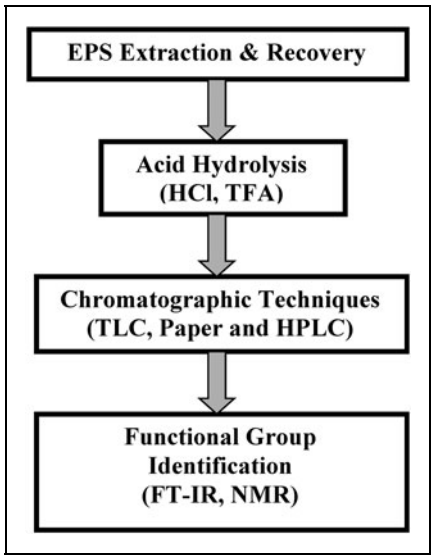


Fig. 1. Process outline for purification and characterization of EPS

PURIFICATION AND RECOVERY OF EPS

Organic solvent was used to precipitate EPS content in liquid broth. The efficacy of various solvents—acetone, methanol, ethanol and isopropanol—to extract the EPS from fermented broth was evaluated.[24] EPS was dried in a hot air oven for 24 to 48 h and then ground in a mortar and pestle (*Fig. 1*).

DETERMINATION OF TOTAL CARBOHYDRATE AND PROTEIN CONTENT OF EPS

The phenol sulfuric acid method is commonly used for the fast determination of total carbohydrate content of bacterial EPS. Total carbohydrate content of EPS was measured by phenol sulfuric method with glucose as a standard[25] and total protein content of EPS was measured by Folin-Lowry method with bovine serum albumin as a standard.[26]

ACID HYDROLYSIS OF EPS FOR ANALYSIS OF MONOSACCHARIDE

EPS was hydrolyzed with two strong acids. 20 mg of dry EPS powder hydrolyzed with 6N Hydrochloric acid and 2M

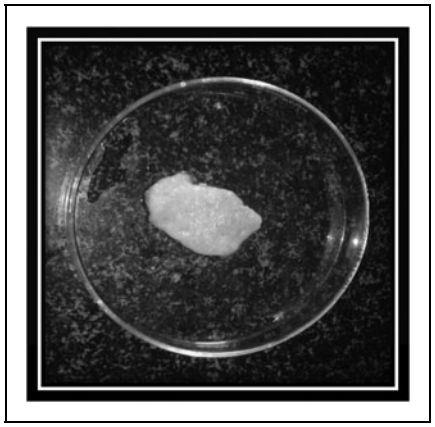


Fig. 2. Extracted EPS in organic solvent (acetone)

Trifluoroacetic acid was incubated at 100°C for 20 min to 1 h. Hydrolyzed sample was neutralized with sodium carbonate until pH reached 7.0. Aliquot of hydrolysate was filtered through Whatman filter paper before using it for paper chromatography, thin layer chromatography (TLC) and HPLC.

PAPER AND THIN LAYER CHROMATOGRAPHY OF HYDROLYSED EPS

Monosaccharide composition of the EPS was analyzed by paper chromatography and TLC. Neutralized hydrolysate was filtered using Whatman filter paper no.1 for chromagraphy analysis. Standard chromatographic sheet of Whatman filter was used for paper chromatography and silica gel 60G (Merck) plates were used for TLC. For both chromatographic procedures, the same solvent system was used. Standardized solvent system was butanol: isopropanol: pyridine: water in proportions of 103:56:28:8. Hydrolyzed sample of EPS was spotted on paper as well as TLC sheet along with standards like glucose, galactose, sucrose, maltose, fructose, and lactose. After each run, sheets of paper and TLC were sprayed with developer aniline diphenyl amine (orthophosphoric acid, acetone) reagent and incubated at 105°C for 10 min to develop the spots.

$$\text{Rf value} = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$$

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

For glycosyl compositional analysis of monosaccharides present in EPS, HPLC analysis was done using Model Waters Inc, S5 amino column, with RI detector (R 410, Waters) and H_2O as mobile phase. Flow rate was 0.6 mL/min and runtime was 25 min. EPS-hydrolyzed sample along with standards were injected in column. The retention time obtained was compared to those determined using D-Glucose and D-Galactose as standards.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) OF EXTRACTED EPS

FTIR of EPS was performed using Bruker IR spectrophotometer. Sample was scanned from 500 to 4000 cm^{-1}.

NUCLEAR MAGNETIC RESONANCE ANALYSIS OF EPS

NMR spectrum of EPS was analyzed using JEOL, ECZR 600 MHz spectrometer. EPS was dissolved in deuterium oxide (D_2O), at a concentration of 10 mg/mL for 1H NMR. The spectrum was recorded at 600 MHz.

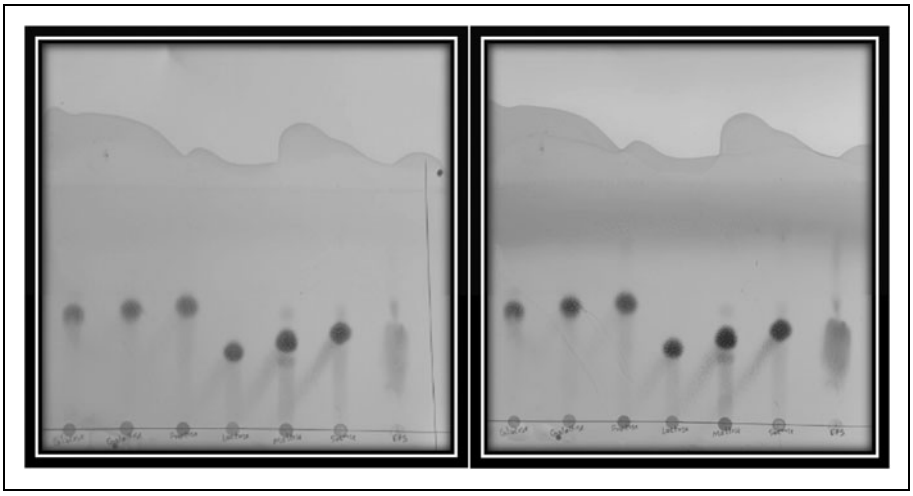


Fig. 3. Paper and TLC chromatogram of EPS hydrolyzed with HCl along with standards

SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSIVE X RAY SPECTROSCOPY (EDX) OF EPS

SEM was done to study the surface morphology of EPS. EPS solution (1 mg/mL) was air dried. The sample was analyzed by scanning electron microscopy (JEOL, JSM-7100F). EDX analysis was done for elemental analysis of EPS.

Results

PRODUCTION OF EPS IN MODIFIED GYE MEDIUM

Optimization was done manually using statistical tools like Central Composite Design and Response surface method to optimize EPS production by *Enterobacter cloacae.* The optimum condition was glucose-10.0%, yeast extract-2.0%, peptone-0.2%, NaCl- 5.0%, inoculum concentration 2%, 72 h incubation, pH 7, and 37°C temperature.[24]

PURIFICATION AND RECOVERY OF EPS

Fermented broth was centrifuged at 10,000 rpm for 10 min. After centrifugation supernatant was taken. For the precipitation and recovery of EPS, organic solvent was used. Double the

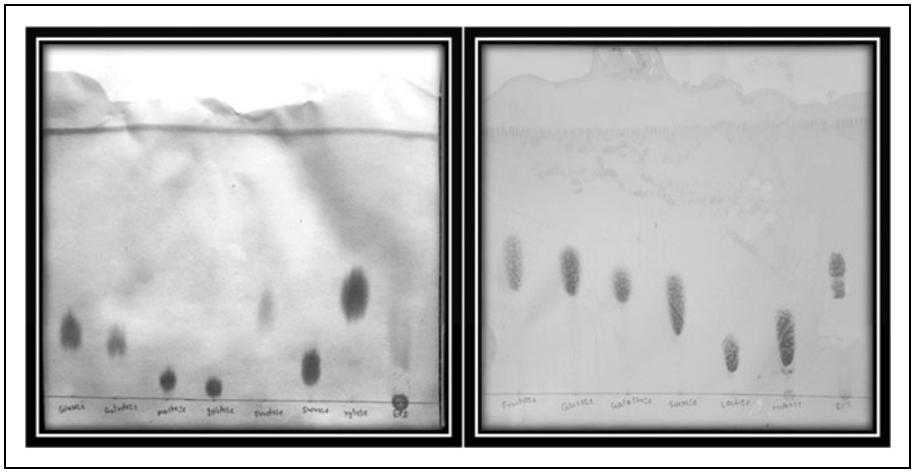


Fig. 4. Paper and TLC chromatogram of EPS hydrolyzed with TFA along with standards

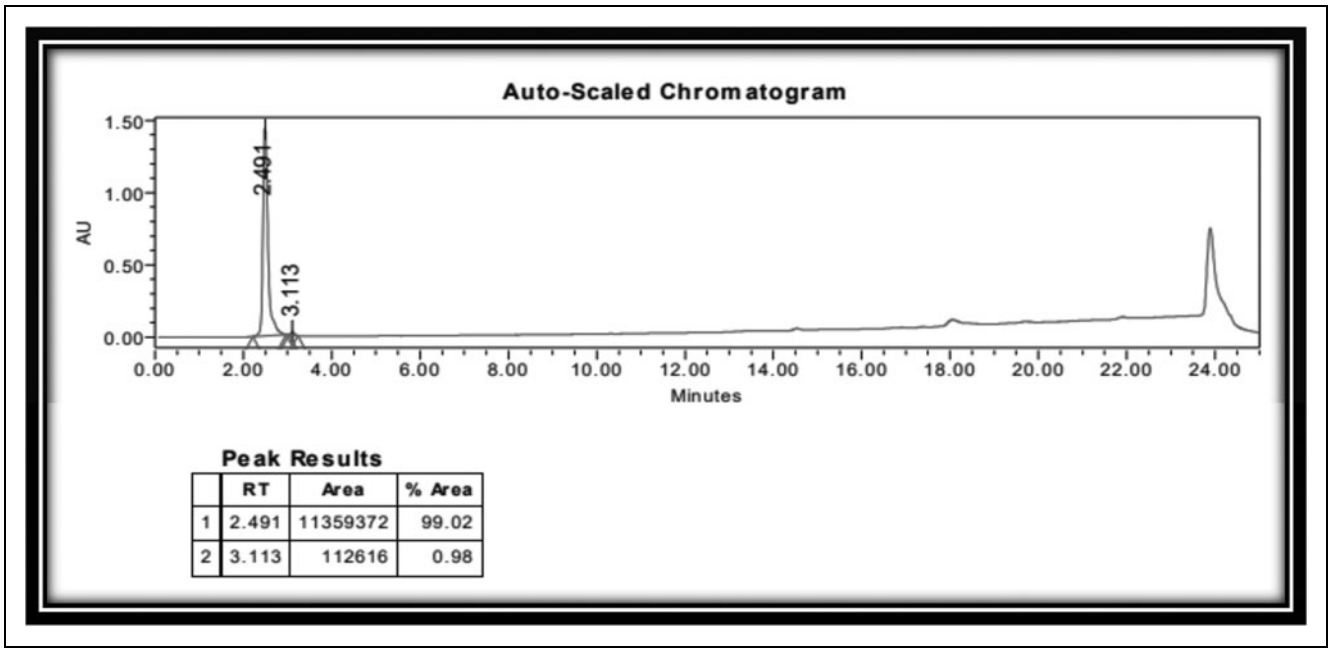


Fig. 5. HPLC chromatogram showing analysis of EPS of *Enterobacter cloacae* VHP-34

volume of acetone was used for the recovery of EPS, which resulted in good quantity of EPS with 32.0 g/L for *Enterobacter cloacae* VHP-34. Comparison of our data with other solvents, like ethanol, gave 16 g/L; methanol yielded 0.9 g/L, and isopropyl alcohol gave 26 g/L of EPS.[24] During the study, it was observed that acetone not only decreased the solubility of polymer but also washed out impurities such as color components, salts, and cells. Crystalline powder again dissolved in double distilled water and was precipitated again using 1:2 proportions of acetone. This procedure was done three times to remove maximum protein contamination from EPS. Purified EPS powder can be stored at 60°C for long periods of time. *Figure 2* shows recovery of EPS in acetone and pure EPS after three to four repeated washes with acetone.

DETERMINATION OF TOTAL CARBOHYDRATE AND PROTEIN CONTENT OF EPS

Total carbohydrate content in EPS of *Enterobacter cloacae* was 40%, as measured by phenol sulfuric method. Total protein content was measured by Folin Lawry method, and it was found that EPS did not contain any protein residues. Furthermore, qualitative test determination for carbohydrates via the Molish test, Benedict test and Seliwanoff's tests were positive for EPS,

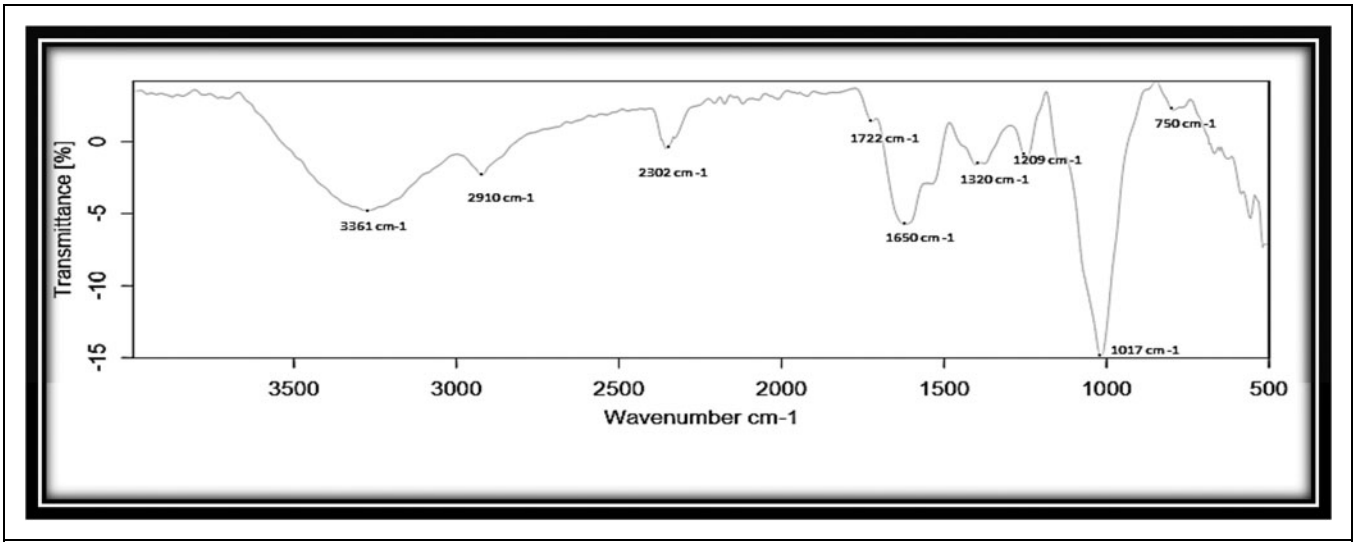


Fig. 6. IR Spectrogram of EPS of *Enterobacter cloacae* VHP-34

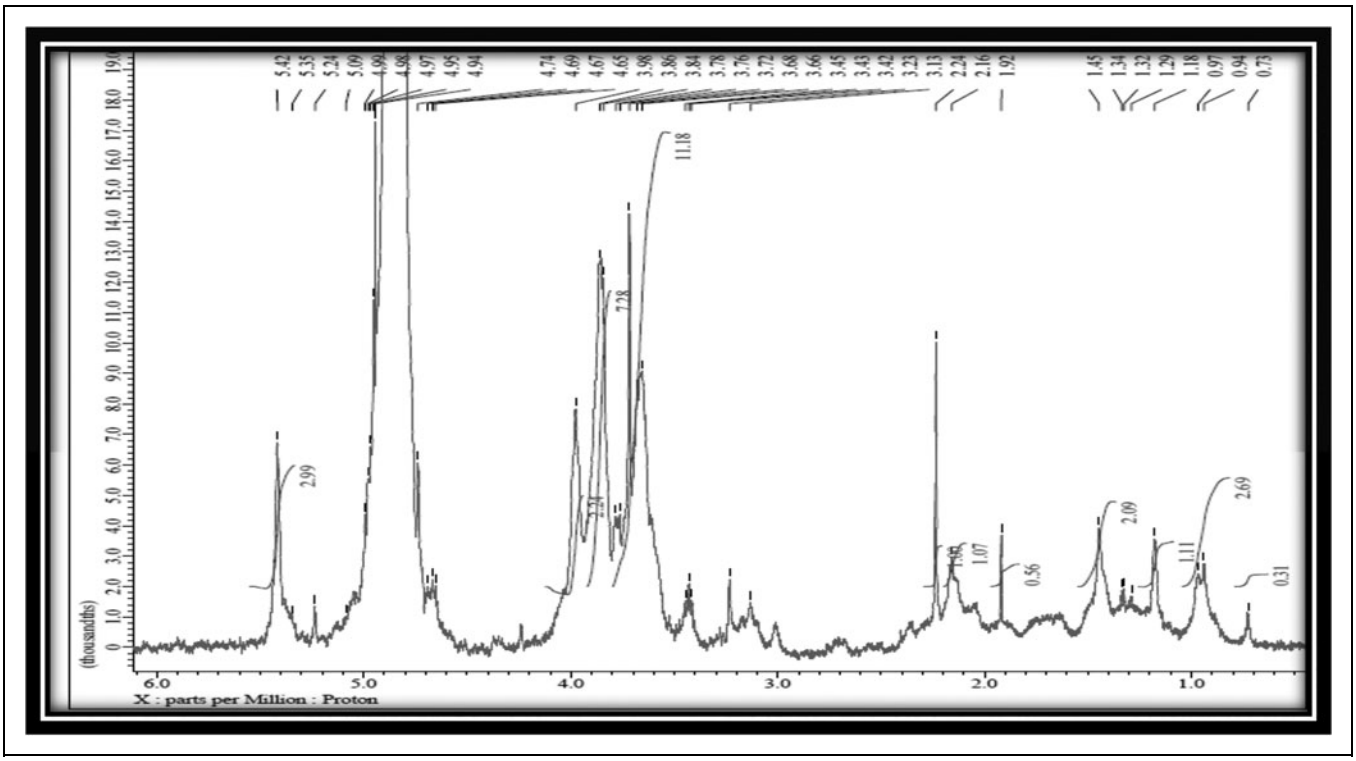


Fig. 7. 1H NMR of EPS of *Enterobacter cloacae* VHP-34

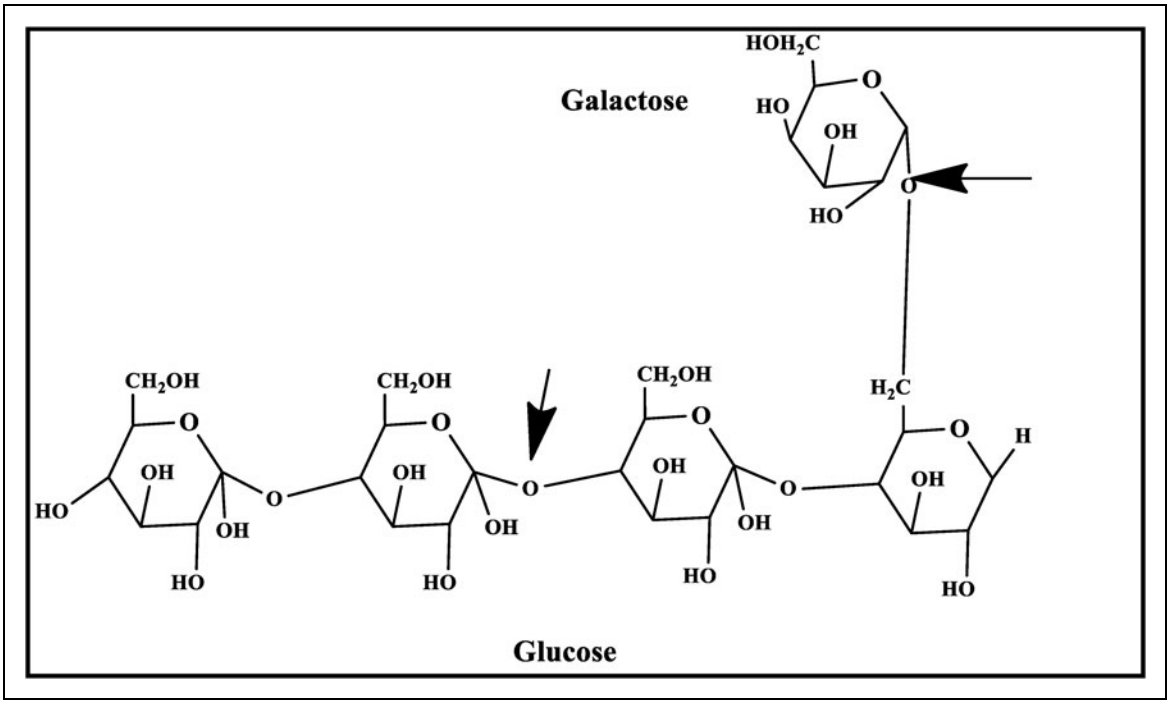


Fig. 8. Proposed structure of EPS of *Enterobacter cloacae* VHP-34

Table 1. Elemental Analysis of EPS of *Enterobacter cloacae* VHP-34

ELEMENT	ATOMIC%
C	52.04
N	7.61
O	39.13
Na	0.98
P	0.08
S	0.09
Cl	0.07
Total	100

indicating the presence of carbohydrates by detecting reducing sugars as hexo and aldo forms.

ACID HYDROLYSIS OF EPS FOR ANALYSIS OF MONOSACCHARIDE

Acid hydrolysis was done using hydrochloric acid and trifluoroacetic acid. Comparison between the two strong acids showed that TFA gave good results for hydrolysis of polysaccharide. Research suggested that TFA has become the first choice for most sugar derivative analysis due to its effectiveness with glycosidic bonds without destroying the monosaccharide components. Moreover, TFA is itself volatile, so there are other methods for neutralization, like evaporation, which minimizes the interference in between the whole process.[27]

PAPER AND THIN LAYER CHROMATOGRAPHY OF HYDROLYSED EPS

The depolymerization of EPS by acid hydrolysis is the first step in composition study and for further chromatographic determination. The number of spots on paper as well as thin layer chromatogram, and their Rf value, were used as basic criteria for the selection of optimal hydrolysis conditions. HCl is often considered too harsh for hydrolysis and degrades the liberated monosaccharides. Paper and TLC chromatogram of EPS, which was hydrolyzed with HCl, are shown in *Fig. 3*. Spots of hydrolyzed EPS on paper as well as TLC were wide, but not distinct and trailing with variable Rf value from 0.20 to 0.44 in the same solvent system.

Hydrolysis of EPS with 2M TFA at 100°C for 2 h (*Fig. 4*) showed significant hydrolysis, with thin spots and good separation observed. The results of hydrolysis showed that 2 h of treatment with 2M TFA at 100°C satisfactorily hydrolyzed the EPS. As per comparison with standard sugars, it was revealed that hydrolyzed EPS had glucose and galactose as a main sugar backbone. Two spots in TLC sheet had similar Rf values of 0.36 mm and 0.45 mm for glucose and galactose, respectively. Developer aniline diphenyl amine, with strong ortho-phsophoric acid, could affect results on paper and TLC sheet. Paper showed poor results compared to TLC sheet, as strong acid affected cellulose fibers of paper. Research has been done with EPS produced by *Enterobacter sp.* ACD_2 and the Paper Chromatography of the EPS acid hydrolysate identified the monosaccharides galactose, glucose, fucose, and uronic acid, with traces of fructose. [28]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatogram obtained by HPLC revealed that EPS of *Enterobacter cloacae* VHP-34 was a hetero-polysaccharide of glucose and galactose (*Fig. 5*). Glucose is the predominant sugar composition showing the peak at retention time of 2.491 min followed by galactose at 3.113 min. As per covered area in particular Retention time the weightage of glucose was higher than galactose. This HPLC system was connected only with RI detector and not with LC-MS system, so exact amount of sugar contents were unknown. Similar results were published by Xiao et al.,[10] where EPS of *Enterobacter sakazakii* was mainly composed of L-Fucose, D-Glucose, and D-Galactose on the basis of HPLC analysis.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) OF EXTRACTED EPS

FTIR (*Fig. 6*) results revealed that this EPS has broad stretch at 3,361 cm^{-1}, which showed presence of hydroxyl group (OH). Weak stretch at 2,910 cm^{-1} was caused by the asymmetric stretching vibration of the C–H bond in the alkyl group of the polysaccharide chain structure. EPS of *Enterobacter faecalis* MSI12 showed peaks at 2,926 and 2,962 cm^{-1}, which represent C-H stretch.[29] Broad stretch at 1,650 cm^{-1} confirmed the presence of C = O group, indicating conjugated ketone. The strong and sharp absorption peak at 1,017 cm^{-1} came from the

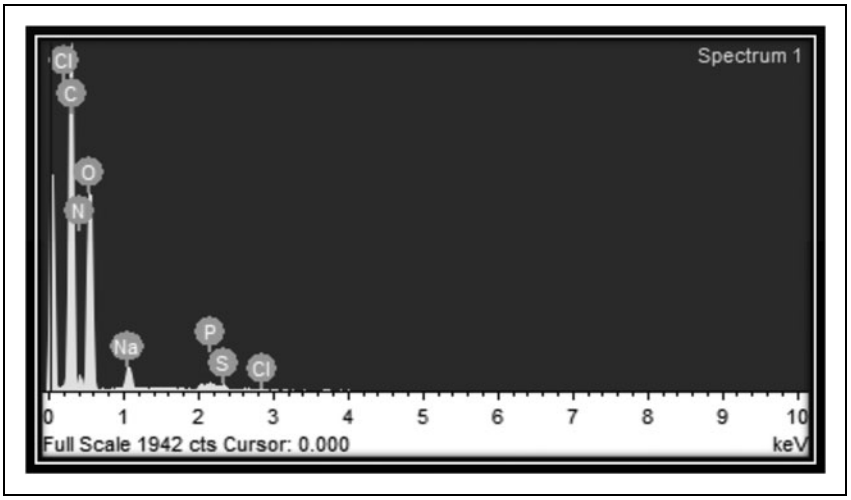


Fig. 9. Full scale spectrum of elemental analysis of EPS of *Enterobacter cloacae* VHP-34

stretching vibration of C–O, which was also a characteristic absorption peak of polysaccharides.

NUCLEAR MAGNETIC RESONANCE ANALYSIS OF EPS

Figure 7 shows an NMR spectrogram of EPS. 1H NMR spectra of EPS indicated that the presence of anomeric protons of α hexoses residue at 4.9 ppm and between 3.5 to 4 ppm. 4.09 has high field H while 3.88, 3.76, 3.60 and 3.52 have low field H. The possible linkages of polysaccharide of *Enterobacter cloacae* VHP-34 can be α-1, 6 linkage between glucose-galactose (gluconopyranosyl and galctopyaronosyl) and α-1, 4 linkage between glucose-glucose. EPS of *Enterobacter ludwigii* showed anomeric protons of hexoses in region of 5.5–4.4.[30] On the basis of 1 H NMR chemical shift, *Fig. 8* shows proposed structure of polymer of EPS have repeat units of glucose and galactose. The chemical shift of 3.2 to 4.5 ppm corresponds to the chemical movement of protons, indicating presence of saccharide cycle.[31]

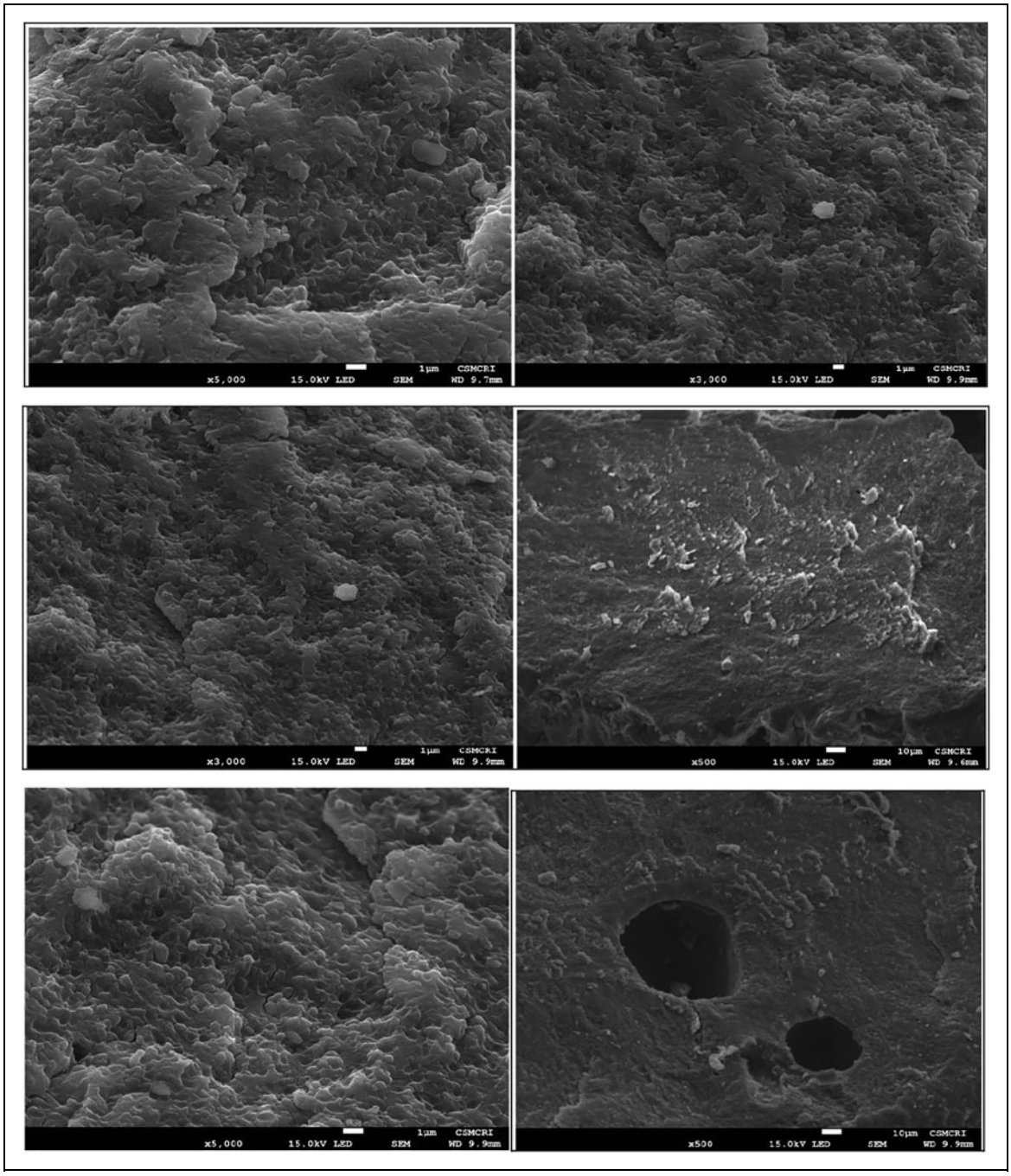


Fig. 10. Scanning Electron Microscope images of EPS of *Enterobacter cloacae* VHP-34

SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSIVE X RAY SPECTROSCOPY (EDX) OF EPS

Figure 10 illustrated the structure of EPS produced by *Enterobacter cloacae* VHP-34 by SEM. SEM analysis of the EPS showed compact, porous, and irregular web-like structure.[32] Researchers have also reported the porous nature of EPS. EDX was also performed to study the elemental composition of EPS, which showed presence of different elements, such as C, H, O, Na, Cl, P and S in EPS. Carbon and Hydrogen are building blocks of EPS polymer. Nitrogen also has an important role as protein source in EPS structure. *Table 1* represents elemental analysis of EPS by Energy dispersive X-ray spectroscopy. *Figure 9* represents graphical spectrums of elemental analysis. EDX results showed the presence of highest amount of Carbon (52.04%), which might serve as the major component of EPS.

Table 2. EPS-Producing Marine Bacteria with Carbohydrate Monomers

BACTERIA	MONOMERS OF EPS	REFERENCE
Alteromonas sp. JL2810	Galacturonic acid, mannose, rhamnose and galactose	36
Bacillus licheniformis	Glucose, galactose , mannose and arabinose	8
Streptomyces carpaticus No. 3	Galacturonic acid, glucose, xylose, galactose, mannose, and fructose	37
Geobacillus thermodenitrificans ArzA-6	Galactose, arabinose, Fructose and glucose	7
Shewanella livingstonensis	Arabinose and xylose	38
Bacillus altitudinis MSH2014	Mannuronic acid and glucose	12
Idiomarina fontislapidosi F32	Glucose, mannose and galactose	39
Alteromonas hispanica F23	Glucose, mannose and xylose	39
Salipiger mucosus A3	Fucose, galactose, glucose and mannose	40
Pseudoalteromonas sp. Strain SM20310	Xylose, mannose, glucose, galactose and rhamnose	21
Paenibacillus Spp.	Glucose, mannose, galactose and glucuronic acid	17
Kosakonia sp. CCTCC M2018092	L-fucose, d-glucose, d-galactose, d-glucuronic acid	41
Halomonas elongata S6	Glucose, rhamnose, mannose and glucosamine	42
Halorubrum sp. TBZ112	Mannose, glucosamine, galacturonic acid, arabinose, and Glucuronic acid	43
Klebsiella sp	Glucose, fructose, galactose, fucose and uronic acid	44
Vibrio harveyi strain VB23	Galactose, glucose, rhamnose, fucose, ribose, arabinose, xylose and Mannose	45
Pseudoalteromonas sp. AM	Glucose	3

Discussion

Table 2 represents various marine bacteria with their sugar monomers in structure of EPS with comparison results of EPS of *Enterobacter cloacae* VHP-34 . Nagraj et.al described that EPS of *Enterobacter* sp. YG4 EPS was composed of 61.89% sugars and 9.32% proteins.[33] Another study on *Enterobacter sp.* suggested that it was contained total carbohydrates and protein content of 91.7% and 1.8% respectively.[34] An EPS of *Enterobacter sakazakii* was dissolved in 3 mL of 2M TFA and hydrolyzed at 110°C for 6 h.[10] For the analysis of the sugar composition from EPS of *Enterobacter* A47, 5 mg of purified EPS was hydrolyzed with 0.1 mL TFA 99% at 120°C for 2h.[35] Research on EPS from *Enterobacter* sp. suggested use of TFA for hydrolysis. Certain amounts of polysaccharide was hydrolyzed at 100°C for different time interval at 1.5h, 5h and 10h with 1 mL of 2 M TFA in a sealed test tube.[28]

Uronic acid was one of the main residues present in species of *Enterobacter* with aldose, hexose and pentose sugar moieties. *Enterobacter cloacae* VHP-34 have very small traces of uronic acid. Another research on same bacterium, *Enterobacter cloacae* Z0206, produced acidic EPS with sugar monomers of fucose, glucose, galactose, glucuronic acid and pyruvic acid.[41] EPS of *Enterobacter sakazakii* mainly has a glucose, galactose and fucose backbone.[10] A study of EPS of *Enterobacter cloacae* Z0206 was made up of glucose, galactose, mannose and fucose.[9] EPS of *Enterobacter ludwigii* was made up of repeating units composed of galactose, glucose, fucose, glucuronic acid.[30]

An EPS of strain *Enterobacter cloacae* P2B was made up of arabinose, mannose, glucose, galactose and xylose.[46] EPS of *Enterobacter* strain A47 DSM 23139 was made up of a fucose sugar backbone,[35] while EPS of marine Gram-positive bacterium *Bacillus licheniformis* was made up of glucose, galactose, mannose and arabinose.[8] EPS of *Bacillus licheniformis* 14580 was made up of galactose, fructose and glucose as the predominating sugar monomers. [47]

Conclusion

Many analytical techniques were employed to detect physical appearance of numerous functional groups as well as chemical composition of EPS of *Enterobacter cloacae* VHP-34. Chemical analysis revealed that EPS has acidic nature with negative charge. Structural analysis revealed that it has repeating unit of glucose and galactose as main sugar monomers, while SEM microscopy proved that EPS of *Enterobacter cloacae* VHP-34 has a porous structure. EDX study suggested the presence of higher amounts of carbon in the EPS structure.

EPS of *Enterobacter cloacae* VHP-34 can be used in heavy metal bioremediation, particularly with biosorption mechanism due to its negative charge. It leads to adsorption of positively

charged heavy metal on the surface of EPS. Many therapeutic applications could also be possible, including the use of EPS as anti-inflammatory and anticoagulanting agents. It can also be used as emulsifying agents in food industries. Finally, this EPS is the product of gram-negative bacteria, so the antimicrobial effects of EPS could be used against many pathogens.

Acknowledgments

The authors are thankful to the Management of Kadi Sarva Vishwavidyalaya (KSV), Gandhinagar, Gujarat for providing a laboratory facility.

Author Contributions

Conceptualization, Kamlesh Shah; experiments execution, Vishal Patel; writing and original draft preparation, Vishal Patel; data analysis, Falguni Patel and Kamlesh Shah; review, editing, visualization, Falguni Patel, Kamlesh Shah. All authors have read and agreed to the published version of the manuscript.

Author Disclosure Statement

No competing financial interests exist.

Funding Statement

This research did not receive funding.

REFERENCES

1. Poli A, Anzelmo G, Nicolaus B. Bacterial exopolysaccharides from extreme marine habitats: Production, characterization and biological activities. *Mar Drugs* 2010;8(6):1779-802. doi:10.3390/md8061779
2. Gutierrez T, Morris G, Green DH. Yield and physicochemical properties of EPS from Halomonas sp. strain TG39 identifies a role for protein and anionic residues (sulfate and phosphate) in emulsification of n-hexadecane. *Biotechnol Bioeng* 2009;103(1):207-16. doi:10.1002/bit.22218
3. Al N. Characterization of an exopolysaccharide-producing marine bacterium, isolate Pseudoalteromonas sp. AM. *Afr J Microbiol Res* 2011;5(22).
4. Marine bacterial extracellular polysaccharides: A review. *J Coastal Life Medicine* 2017;5(1):29-35. doi:10.12980/jclm.5.2017J6-207
5. Dave SR, Upadhyay KH, Vaishnav AM, et al. Exopolysaccharides from marine bacteria: Production, recovery and applications. *Environ Sustain* 2020;3(2):139-154. doi:10.1007/s42398-020-00101-5
6. Nicolaus B, Kambourova M, Oner ET. Exopolysaccharides from extremophiles: From fundamentals to biotechnology. *Environ Technol* 2010;31(10):1145-58. doi:10.1080/09593330903552094
7. Panosyan H, Di Donato P, Poli A, et al. Production and characterization of exopolysaccharides by *Geobacillus thermodenitrificans* ArzA-6 and *Geobacillus toebii* ArzA-8 strains isolated from an Armenian geothermal spring. *Extremophiles: Life Under Extreme Conditions* 2018;22(5):725-737. doi:10.1007/s00792-018-1032-9
8. Singh RP, Shukla MK, Mishra A, et al. Isolation and characterization of exopolysaccharides from seaweed associated bacteria *Bacillus licheniformis*. *Carbohydr Polym* 2011;84(3):1019-1026. doi:10.1016/j.carbpol.2010.12.061
9. Xu CL, Wang YZ, Jin ML, et al. Preparation, characterization and immunomodulatory activity of selenium-enriched exopolysaccharide produced by bacterium *Enterobacter cloacae* Z0206. *Bioresour Technol* 2009;100(6):2095-2097. doi:10.1016/j.biortech.2008.10.037
10. Xiao M, Fu X, Wei X, et al. Structural characterization of fucose-containing disaccharides prepared from exopolysaccharides of *Enterobacter sakazakii*. *Carbohydr Polym* 2021;252(117139. doi:10.1016/j.carbpol.2020.117139
11. Torres CA, Antunes S, Ricardo AR, et al. Study of the interactive effect of temperature and pH on exopolysaccharide production by Enterobacter A47 using multivariate statistical analysis. *Bioresour Technol* 2012;119:148-56. doi:10.1016/j.biortech.2012.05.106
12. Mohamed SS, Amer SK, Selim MS, et al. Characterization and applications of exopolysaccharide produced by marine *Bacillus altitudinis* MSH2014 from Ras Mohamed, Sinai, Egypt. *Egyptian J Basic Appl Sci* 2019;5(3):204-209. doi:10.1016/j.ejbas.2018.05.009
13. López-Ortega MA, Chavarría-Hernández N, López-Cuellar MdR, et al. A review of extracellular polysaccharides from extreme niches: An emerging natural source for the biotechnology. From the adverse to diverse! *Int J Biological Macromol* 2021;177:559-577. doi:10.1016/j.ijbiomac.2021.02.101
14. Prajapati VD, Jani GK, Zala BS, et al. An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydr Polym* 2013;93(2):670-678. doi:10.1016/j.carbpol.2013.01.030
15. Delbarre-Ladrat C, Sinquin C, Lebellenger L, et al. Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecules. *Front Chem* 2014;2:85. doi:10.3389/fchem.2014.00085
16. Rasulov BA, Yili A, Aisa HA. Biosorption of metal ions by exopolysaccharide produced by *Azotobacter chroococcum* XU1. *J Environmen Protect* 2013;04(09):989-993. doi:10.4236/jep.2013.49114
17. Liang TW, Wang SL. Recent advances in exopolysaccharides from Paenibacillus spp.: Production, isolation, structure, and bioactivities. *Marine Drugs* 2015;13(4):1847-1863. doi:10.3390/md13041847
18. Emaga TH, Rabetafika N, Blecker CS, Paquot M. Kinetics of the hydrolysis of polysaccharide galacturonic acid and neutral sugars chains from flaxseed mucilage. *Biotechnol Agron Soc Environ* 2012;16(2):139.
19. Sajna KV, Sukumaran RK, Gottumukkala LD, et al. Studies on structural and physical characteristics of a novel exopolysaccharide from Pseudozyma sp. NII 08165. Int J Biol Macromol 2013;59:84-89. doi:10.1016/j.ijbiomac.2013.04.025
20. Rice KM, Walker EM, Wu M, et al. Environmental mercury and its toxic effects. *J Prevent Med Public Health* 2014;47(2):74-83. doi:10.3961/jpmph.2014.47.2.74
21. Liu SB, Chen XL, He HL, et al. Structure and ecological roles of a novel exopolysaccharide from the arctic sea ice bacterium Pseudoalteromonas sp. Strain SM20310. *Appl Environ Microbiol* 2013;79(1):224-30.doi:10.1128/AEM.01801-12
22. Casillo A, Lanzetta R, Parrilli M, et al. Exopolysaccharides from marine and marine extremophilic bacteria: Structures, properties, ecological roles and applications. *Marine Drugs* 2018;16(2):69. doi:10.3390/md16020069
23. H Patel V, P Soni H, R Patel F. Isolation, screening and molecular characterization of exopolysaccharide producing moderately halotolerant bacteria from various coastal sites of Gujarat. *Biosci Biotechnol Res Asia* 2022;19(1):231-242. doi:10.13005/bbra/2981
24. Patel V, Soni HP, Patel F. Statistical optimization of medium for enhanced production of exopolysaccharide by marine bacteria *Enterobacter cloacae* VHP-34. *Ind Biotechnol* 2021;17(6):326-334. doi:10.1089/ind.2021.0014
25. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith FA. Colorimetric method for the determination of sugars. *Nature* 1951;168(4265):167-167.
26. Lowry O, Rosebrough N, Farr AL, et al. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193(1):265-275. doi:10.1016/s0021-9258(19)52451-6
27. Boual Z, Kemassi A, Khelil AOEHK, Michaud P, Hadj MDE. Partial characterization and hydrolysis procedure of water soluble polysaccharides extracted from onesaharian medicinal plant: Malvaaegyptiaca L. *Int J Biosci Biochem Bioinform* 2012;2(2).
28. Almutairi MH, Helal MMI. Biological and microbiological activities of isolated Enterobacter sp. ACD2 exopolysaccharides from Tabuk region of Saudi Arabia. *J King Saudi University Sci* 2021;33(2):101328. doi:10.1016/j.jksus.2020.101328
29. Kiran GS, Priyadharshini S, Anitha K, et al. Characterization of an exopolysaccharide from probiont *Enterobacter faecalis* MSI12 and its effect on the disruption of *Candida albicans* biofilm. *RSC Adv* 2015;5(88):71573-71585. doi:10.1039/c5ra10302a

30. Pau-Roblot C, Lequart-Pillon M, Apanga L, et al. Structural features and bioremediation activity of an exopolysaccharide produced by a strain of *Enterobacter ludwigii* isolated in the Chernobyl exclusion zone. *Carbohydr Polym* 2013;93(1):154-162. doi:10.1016/j.carbpol.2012.09.025

31. Jivkova D, Sathiyanarayanan G, Harir M, et al. Production and characterization of a novel exopolysaccharide from *Ramlibacter tataouinensis*. *Molecules* 2022; 27(21):7172. doi:10.3390/molecules27217172

32. Wang J, Zhao X, Tian Z, et al. Characterization of an exopolysaccharide produced by *Lactobacillus plantarum* YW11 isolated from Tibet Kefir. *Carbohydr Polym* 2015;125:16-25. doi:10.1016/j.carbpol.2015.03.003

33. Devasya RP, Bhagwath AA. Exopolysaccharide produced by Enterobacter sp. YG4 reduces uranium induced nephrotoxicity. *Int J Biol Macromol* 2016;82: 557- 561. doi:10.1016/j.ijbiomac.2015.11.020

34. Lei B, Tang W, Song L, et al. Production, characterization, and flocculation mechanism of cation independent, ph tolerant, and thermally stable bioflocculant from Enterobacter sp. ETH-2. *PloS One* 2014;9(12):e114591, doi: 10.1371/journal.pone.0114591

35. Freitas F, Alves VD, Torres CAV, et al. Fucose-containing exopolysaccharide produced by the newly isolated Enterobacter strain A47 DSM 23139. *Carbohydr Polym* 2011;83(1):159-165. doi:10.1016/j.carbpol.2010.07.034

36. Zhang Z, Chen Y, Wang R, et al. The fate of marine bacterial exopolysaccharide in natural marine microbial communities. *PloS one* 2015;10(11):e0142690. doi: 10.1371/journal.pone.0142690

37. Selim MS, Amer SK, Mohamed SS, et al. Production and characterisation of exopolysaccharide from *Streptomyces carpaticus* isolated from marine sediments in Egypt and its effect on breast and colon cell lines. J Gene Eng Biotechnol 2018;16(1):23-28. doi:10.1016/j.jgeb.2017.10.014

38. Nichols CM, Lardiere SG, Bowman JP, et al. Chemical characterization of exopolysaccharides from Antarctic marine bacteria. *Microbial Ecol* 2005;49(4): 578-89. doi:10.1007/s00248-004-0093-8

39. Mata JA, Bejar V, Bressollier P, et al. Characterization of exopolysaccharides produced by three moderately halophilic bacteria belonging to the family Alteromonadaceae. *J Appl Microbiol* 2008;105(2):521-8. doi:10.1111/j.1365-2672.2008.03789.x

40. Llamas I, Mata JA, Tallon R, et al. Characterization of the exopolysaccharide produced by *Salipiger mucosus* A3, a halophilic species belonging to the Alphaproteobacteria, isolated on the Spanish Mediterranean seaboard. *Mar Drugs* 2010;8(8):2240-2451. doi:10.3390/md8082240

41. Li S, Xia H, Xie A, et al. Structure of a fucose-rich polysaccharide derived from EPS produced by Kosakonia sp. CCTCC M2018092 and its application in antibacterial film. Int J Biol Macromol 2020;159:295-303. doi:10.1016/j .ijbiomac.2020.05.029

42. Joulak I, Azabou S, Finore I, et al. Structural characterization and functional properties of novel exopolysaccharide from the extremely halotolerant *Halomonas elongata* S6. *Int Journal Biol Macromol* 2020;164:95-104. doi:10 .1016/j.ijbiomac.2020.07.088

43. Hamidi M, Mirzaei R, Delattre C, et al. Characterization of a new exopolysaccharide produced by Halorubrum sp. TBZ112 and evaluation of its anti-proliferative effect on gastric cancer cells. *3 Biotech* 2018;9(1). doi:10 .1007/s13205-018-1515-5

44. Essawy A, Shady H, Kher A, et al. Antimicrobial, anticoagulation, fibrinolytic and prebiotic activities of exopolysaccharide produced by Marine Klebsiella Sp. *Egypt J Exper Biol (Botany)* 2017;1. doi:10.5455/egyjebb.20161115114843

45. Bramhachari PV, Dubey SK. Isolation and characterization of exopolysaccharide produced by *Vibrio harveyi* strain VB23. *Lett Appl Microbiol* 2006;43(5):571-577. doi:10.1111/j.1472-765X.2006.01967.x

46. Naik MM, Pandey A, Dubey SK. Biological characterization of lead-enhanced exopolysaccharide produced by a lead resistant *Enterobacter cloacae* strain P2B. *Biodegradation* 2012;23(5):775-783. doi:10.1007/s10532-012-9552-y

47. Malick A, Khodaei N, Benkerroum N, et al. Production of exopolysaccharides by selected Bacillus strains: Optimization of media composition to maximize the yield and structural characterization. *Int Jo Biol Macromol* 2017;102:539-549. doi:10.1016/j.ijbiomac.2017.03.151

Address correspondence to:
Kamlesh Shah
Department of Microbiology
RG Shah Science College
Vasna, Ahmedabad, Gujarat,

E-mail: krshah2030@gmail.com