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# Structure and Characterization of Exopolysaccharide Producing Bacteria

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**ABSTRACT**
*Given the importance of microbial polysaccharide, the current study had two objectives: (1) isolate and extract exopolysaccharide from producing bacteria. (2) Characterization and analysis of structural elements.For this, a sample of water was taken from a nearby pond in Waghodia, Vadodara. This sample was used to grow exopolysaccharide-producing bacteria on BHM medium. The gram staining of these bacteria colony showed, rod shaped, motile colonies and were gram positive suggesting the strain belongs to genus Bacillus. Exopolysaccharide(extracellular polymeric material) is a type of polymeric substance found outside of cells. EPS was been isolated, and its structure and properties were determined. A solubility test in five different solvents revealed that EPS is only completely soluble in water. Thin Layer Chromatography is used to detect qualitative monomer units (TLC). The potential for using FT-IR as a pattern recognition and clustering tool with respect to EPS structures produced by Bacillus licheniformis strain extracted from water sample.*
***KEYWORDS :*** *exopolysaccharide , Bacillus licheniformis , thin-layer chromatography, isolation, solubility, FT-IR*

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## INTRODUCTION

Polysaccharides are the world's most abundant organic substance. They are everywhere because they may be retrieved from plants, animals, algae, and microbes in soil, water, and the environment. A polysaccharide's chemical structure is made up of monomers known as monosaccharides. They can be made up of a single kind of monosaccharide, as in homopolysaccharides, or a mixture of numerous types, generally up to 10, as in heteropolysaccharides.[1] The polysaccharide backbone, which can be linear or branched, can be decorated with a variety of inorganic and organic substituents (sulphates, phosphates, acetates, ethers, amino acids, lactates, and pyruvates). The sizes of polysaccharides range from 50 Da to several thousand KDa.[2]

Marine environments constitute a wide and distinct environment in which numerous species of bacteria serve critical functions for the sustainability of the planet's ecology. Microorganisms in the oceans account for around half of the primary creation of organic molecules on Earth, with exopolysaccharides playing a key role.[3] These polymers are found in dissolved organic carbon (DOC) in marine settings, where they contribute to a variety of processes including as particle production, sedimentation, and metal cycling. EPS are highly hydrated polymers, with water accounting for 99 percent of their wet weight . They range from the simple eL, 1-4 linked, unbranched glucose called dextrans to the complex and substituted heteropolysaccharides composed of oligosaccharide repeating subunits like xanthan and colanic acid. Exopolysaccharides are essential products of microbial biotechnologies that attempt to transform waste materials into bioenergy and biomaterials,[5] so contributing to a reduction in economic reliance on fossil fuels.

## MATERIAL AND METHODS

### SAMPLE COLLECTION

To collect water sample from water body pond/lake, a 150ml sterile sealed water bottle was rinsed with pond/lake water three times and filled 2-3 inches away from the top. If Refrigerated, sample should be taken into use within 30 hours.

### SCREENING AND IDENTIFICATION

Collected water sample was inoculated in the prepared nutrient broth,for which nutrient broth powder 2.6 grams was taken in 200 ml distilled water. It was mixed thoroughly and sterilized using autoclave at 121 degrees Celsius for 15 minutes.[6] It was allowed to cool at 45 degrees and was inoculated with collected water sample. Flask was closed with cotton plug and incubated for 48 hours. After 48 hours of growth of bacterial colonies, they were subjected to gram staining.

### EPS GROWTH IN CULTURE MEDIUM

The incubated sample was taken out after 48 hours. This sample was serially diluted, using distilled water or 0.9 % saline.[7] BHM broth was prepared aside by suspending 11.63 grams in 500 ml distilled water. Dextrose was added 15 grams in 500 ml distilled water. The heated and boiled culture medium is sterilized by autoclaving at 121 degrees Celsius for 15 minutes. BHM broth was transferred into 5 sugar tubes and each was serially diluted with the bacterial sample. These were kept in environmental shaker for 5 days for further growth.

### EXOPOLYSACCHARIDE EXTRACTION AND PURIFICATION

Bacterial cells grown overnight have been discarded by centrifugation at 10,000 rpm for 20 minutes. The supernatant in Eppendorf tubes is incubated for 1 hour. The supernatant is then taken out in beaker and treated with chilled acetone.[9] The precipitated EPS was then dried out in hot air oven and powdered.

### SOLUBILITY ASSAY

Solubility experiment was tested with five different solvents (dimethyl sulphoxide, methanol, methyl acetate, water, xylene and acetone). Each solvent was taken 3 ml with 1% (w/v) EPS. It was vortex for 1 minute and kept overnight to study the solubility of biopolymer.

### QUALITATIVE IDENTIFICATION OF MONOSACCHARIDLAL UNITS BY TLC

Thin layer chromatography (TLC) was used to identify the monosaccharide units of EPS. TLC was performed using a modified approach developed by Khattar et al. (2010)[12]. EPS (50mg) was hydrolyzed in 5 ml 4M trifluoroacetic acid (TFA) sealed screw cap bottles with Teflon tape in an autoclave at 121 degrees Celsius for two hours.[10] TFA was evaporated in a vacuum evaporator, and the dried hydrolyzed extract was diluted in 1ml of sterile distilled water.[11] TLC was performed on this sample. The flowing solvent for TLC was ethyl acetate/methanol/glacial acetic acid/water (60:15:15:10), and the separated sugars on the plate were spotted by spraying reagent anisaldehyde/sulfuric acid/ethanol (1:1:20). Plates were cooked in a hot air oven at 100 degrees Celsius until colour spots appeared.[12]

### FT-IR ANALYSIS

The NICOLET 6700 FT-IR (Thermo Scientific) was used to perform Fourier transform infrared spectroscopy to determine the presence of key structural groups as well as EPS replacements in the 400-4000 per cm range with a scan speed of 32. According to Maio et al., the polysaccharide sample was crushed into KBr pellets at a sample: KBr ratio of 2:100.[13][14]

## RESULTS AND DISCUSSION

### IDENTIFICATION OF BACTERIAL STRAIN

In gram staining of the bacterial sample, rod shaped, gram positive colonies were observed of *Bacillus licheniformis.*

### SOLUBILITY ASSAY

The extracted CAS EPS from the sample was added in five different solvents at a concentration of 1% (w/v). The findings of the table and the visual depiction indicates that EPS was insoluble in all solvents but soluble in water as a solvent. These findings are consistent with those of Maliehe et al (2016).[15] Solubility assay showed that EPS sample is only soluble in water and insoluble in other four tested solvents.

### QUANTIFICATION IDENTIFICATION OF MONOSACCHARIDAL UNITS BY TLC

On the chromatogram of hydrolyzed biopolymer, four spots with Rf values comparable to glucose, galactose, rhamnose and arabinose were identified, indicating that CAS EPS was made of arabinose, rhamnose, glucose, and galactose monomer sugar units. The glucose band was black and dense, in contrast to the galactose spot, which seemed faint, and rhamnose and arabinose, which were weaker than the rest. CAS EPS included a higher concentration of glucose.These findings, which were in line with previous research, corroborated the work of Moosavi-Nasab et al.; Bakhtiyari et al (2015).[16] TLC

results depict that CAS EPS was composed of glucose, galactose, arabinose and rhamnose monomers - xylose, maltose, rhamnose, galacturonic acid, galacturonic and glucuronic acid.

**FT-IR SPECTROSCOPY**

An FTIR spectrometer obtains high-resolution spectral data over a large spectral range at the same time. Because of the comparable absorption frequencies of functional groups in various molecules, infrared is a great technique for identifying functional groups.[17] The substituent distribution and functional groups of the polysaccharide isolated from the sample were investigated using FT-IR spectroscopy. The band from hydroxyl group stretching (O-H) measured at 3418.59 cm-1 was unambiguously connected to 3000-3700 cm-1. The band measured at 2920.30 cm-1 and 2851.46 cm-1 is caused by the asymmetric vibration of CH. The role of the CH2 and CH3 groups (Fusconi et al., 2010).[18] In addition, the highest point, the absorption band at 1615.78 cm-1 was ascribed to asymmetrical carboxylate groups stretch (-COO) from acid residues such as succinate groups, and the broadband at 1557.75 cm-1 related more particularly to the stretching vibration of the C=O function of the succinate and acetate functions. The signal at 1471.30 cm-1 might be attributed to the methyl group's specific vibration (-CH3). Finally, the presence of C-O vibrations of the CH3-C=O function and carbohydrate C-O functions resulted in peak at 1075.06 cm-1 (Delattre et al., 2015).[19] That shows OH stretching peak at 3399 cm-1 ; -CH stretching at 2918 cm-1 whereas CAS EPS shows it very close at 2920.30 cm-1 , similarly -COO asymmetrical stretch of standard X shows at 1628 cm-1 and CAS EPS sample reading shows it at 1615 cm-1 .Also sugar backbone C-C or C-O stretching of CAS EPS shows 1075.60 cm-1 reading and standard sample X also shows sugar backbone C-C or C-O stretch at 1069.30 cm-1.[20] The FT-IR gives the presence of Carbon containing groups.

**Table-1 Solubility assay of EPS in various solvents**

| SAMPLE | SOLVENTS | RESULTS |
|---|---|---|
| **A** | Dimethyl sulphoxide | - |
| **B** | Methanol | - |
| **C** | Methyl ace | - |
| **D** | Water | + |
| **E** | Xylene | - |

+ refers to positive for solubility assay - refers to negative for solubility assay

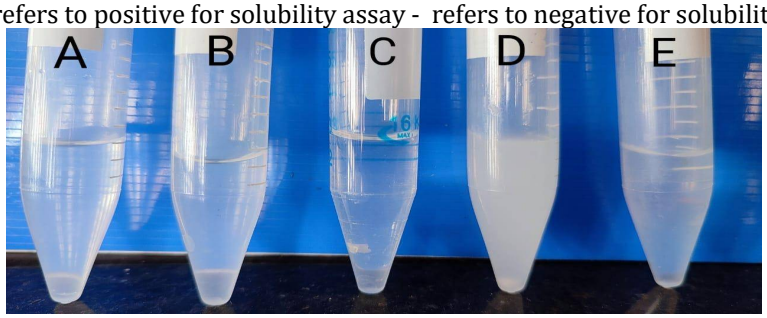


Fig.1 Solubility assay showed that EPS sample is only soluble in water and insoluble in other four tested solvents.

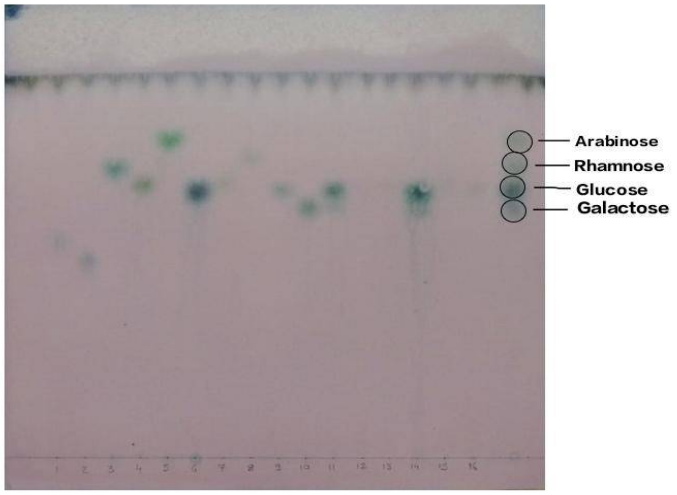


Fig-2 TLC results depict that CAS EPS was composed of glucose galactose arabinose and rhamnose monomers 1) xylose 2) maltose 3) rhamnose 4) galacturonic acid 5) arabinose 6) glucose7) galacturonic acid 8) rhamnose 9) glucuronic acid 10) galactose 11) glucose 12)-16) glucose

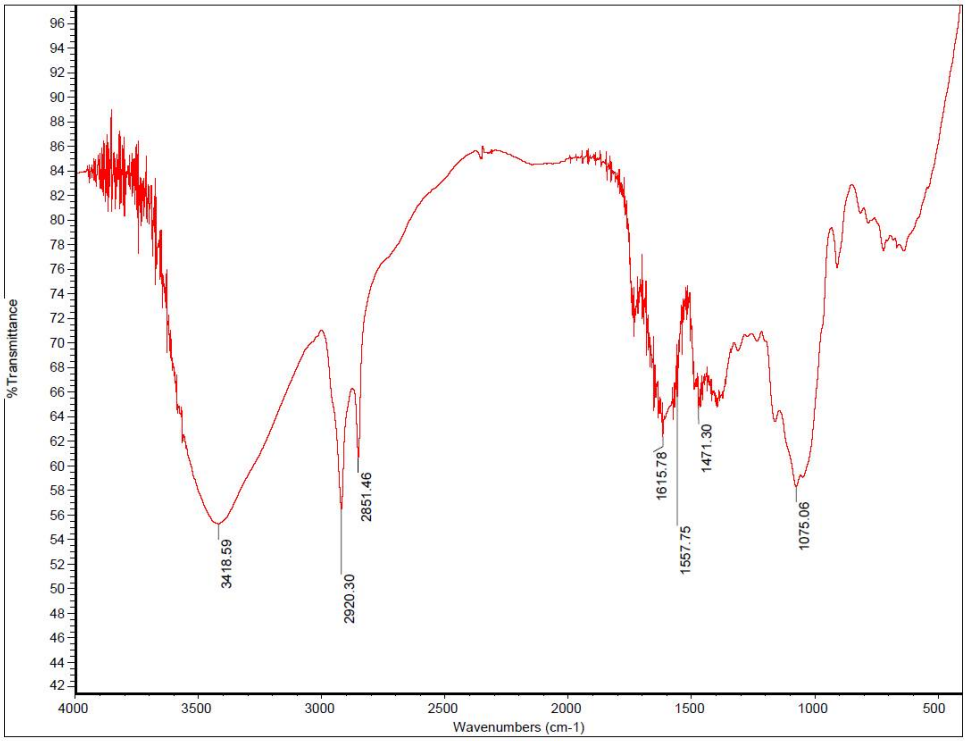


**Fig-3 Complete FT-IR spectra of EPS extracted from sample showing characteristic peaks of microbial polysaccharide**

## CONCLUSION

In accordance to the monosaccharide units found in EPS sample of *Bacillus licheniformis*, glucose, galactose were the most important moieties found via thin layer chromatography. The temperature of the incubator showed an inverse effect on EPS growth. EPS produced by the Bacillus strain was a water-soluble polysaccharide. FT-IR spectra summarized that extracted sample of EPS is a carbon containing biopolymer.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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