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ORIGINAL ARTICLE

Functional Groups Characterization of Exo- Polysachharides from Marine Cyanobacterium (*Anabaena* and *Nostoc* Species*)*, at Vellar Estuary, South East Coast of India

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ABSTRACT
The estuary is a semi-enclosed coastal water body and prominent in marine ecosystems. Marine cyanobacterium plays an important role considering high potential producing various beneficial materials. Many Cyanobacteria produce Exo-polysaccharides (EPS), which are high molecular weight biopolymers mainly composed sugar residues and significant in biotechnological importance due to their technological application in several industries. Fourier Transform Infrared (FTIR) spectroscopy is a rapid, nondestructive, time saving method that can detect a range of functional groups of individual organism cells. The Anabaena and Nostoc species are filamentous heterocyst cyanobacterium, the EPS of these organisms have been isolated, characterized through FTIR for various functional groups. The IR spectrum of EPS-A (Anabaena) showed the presence of OH stretching with O=C=O bond, C=N bond, N-O bond, S=O bond and C-O bond, indicating the presence of alcohols, carbonyl, aliphatic amines, nitro compound, sulfonyl and secondary alcohols groups. The IR spectrum of EPS-N (Nostoc) also revealed the most of the groups as same in EPS-A with an addition of C-H (Alkanes) and C = C (Alkenes). The cyanobacterial based EPS are naturally consisted of polysaccharides and soluble protein which may include the substantial amounts of neutral sugars and uronic acid. The presented research paper revealed to isolate, extract and partial characterization of EPS, exhibited the significant functional (Chemical) groups, from Anabaena and Nostoc species at Vellar estuary of South East Coast Zone of Tamil Nadu (India), , which may be the potentials for further commercial exploitation.
Keywords: *Biopolymers, Infra-Red spectrum, Functional groups and Cyanobacterium.*

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Introduction

A Cyanobacterium is oxygen evolving prokaryotic photosynthetic and gram negative microorganisms. Cyanobacteria are important contribution to primary productivity as globally and base of marine food web in World Ocean's [1]. Evolutionally it was formed before 3.5 billion years ago [2] and exhibit a variety of morphometric forms, either unicellular or filamentous in nature. The cyanobacteria are more abundant in nutrients rich surface water which can be distributed from tropical water to polar region. Cyanobacteria are prokaryotes and phylogenetically related to Eubacteria and Algae due to photosynthesis nutrition.

Many Cyanobacterial sp are surrounded by mucilaginous cells/filaments, this mucilaginous substance was called as Exopolysachharides (EPS), composed of carbohydrates which secrets from outside the cells [3].The organic polymer made up of polysaccharides with smaller quantities of proteins, lipids, glycoprotein's, etc [4]. The production of EPS depends on the producing microorganisms and its culturing conditions [5] a biotic factor such as temperature, lights and nitrogen concentration changes in the production of EPS [6-7].

Exopolysaccharides chemical compositions are different which produced by various cyanobacteria, mainly divided in to two groups, homo polysaccharides and hetero polysaccharides [8]. Cyanobacterial and other microbial EPS are involved in photosynthesis, stress resistance, symbiotic relationship and biofilm formation in microorganisms [9]. The scientific nature of EPS is basically to protect the microbial cells from environmental stresses for survival [10-12].

Several studies [13] have been reported regarding the EPS synthesis is the major end product of photosynthesis. Cyanobacterial EPS are basically complex in nature, because of, presence of, proteins, pyuruvic acid, uronic acids and sulfate groups [14]. Cyanobacterial EPS are high molecular weight and an ordered series of sugar units such as glucose, galactose, xylose and uronic acids. The cyanobacterial EPS could protect the Nitrogenase enzyme from harmful effects of oxygen, in filamentous heterocyst's N_2 fixing genus [15].

A cyanobacterium can be found ubiquitous, i.e. in Marine environments, fresh water ecosystem, dessert and some species in Antarctic rocks. Estuarine water is one of the most considerable and productive ecosystem in Marine environments [16]. The other name is so called "Transitional water" because its represents the transition between marine and freshwater ecosystem.

Anabaena sp and *Nostoc* sp are well known inhabitants in estuarine region [17]. Both are filamentous group of cyanobacteria. It's having the nitrogen fixing capabilities. The nitrogen fixing ability due its specialized cells called as "Heterocysts". As per the biotechnological interest it's a very potential organisms it can be replace the traditional polysaccharides in various industries, including food and cosmetology [18].

The productions of EPS from cyanobacteria for long time, meanwhile the importance of EPS get notified only from the increasing of industrializations [19]. In the recent years many researchers were concentrated the cyanobacterial exopolysachharides for various industrial applications, especially used as gelatinous and thickening agents. This Cyanobacterial Polysaccharides' widely used in Cosmetics and Pharma industries, competing with other natural Polysaccharides derived from microalgae and higher Plants [20].

For the past several years the Fourier transform infrared (FTIR) spectroscopy which would used as most prominent technique for absorption and emission of several materials, through measuring infrared (IR) region. The FTIR used to mapped cellular components, such as carbohydrates, proteins' and lipids to identify the abnormal cells [21-22]. The compounds properties which can be analyzed both qualitatively and quantitatively. The aim of this research paper is study the functional group characterization of exo polysaccharides through Fourier transform infra red spectroscopy, from Cyanobacterium (*Anabaena* and *Nostoc*), Vellar estuary of Tamilnadu (India).

MATERIAL AND METHODS

Sample Collection:

The cyanobacteria samples used in this study are isolated from the Vellar Estuary. The Vellar River, originating at Servarayan hills under Salem district merged with the Bay of Bengal Sea, near to Porto novo, Cuddalore district, Tamilnadu. (Parangipettai- Lat. 11 °30' N, long. 79°46' E) after flowing over a distance of 480 km. The estuary is about 600 m in width at its junction with sea. The samples were collected and they were kept in aspectical conditions.

Isolation:

The BG-11 media [23] was used to isolate the pure culture. The pH of media was buffered at 7.7 to 7.9. The culture grows on solid agar and liquid media of BG-11.The very minimal amount of samples was inoculated in to media. All the inoculated plates were sealed with paraffin taps and kept under culture room at 25^{o} C temp with continuous illumination (2500 lux) on 14:10 hours light and dark. The light provided by cool white fluorescent lamp for adequate growth of cultures. Repeated the process until get the pure cultures. The plates were observed regular interval and isolated colonies picked up and examined under microscope for morphological examinations. The pure cultures were transferred from the plate to slants for further maintenance. The isolated cultures from agar media / slants were inoculated in to 10 ml and 100 ml of broth containing 25 ml and 250 ml of Erlenmeyer flask, as respectively. The visible growth was observed during 7 to 14 days' interval. The mass multiplications were carried out using with increased quantity of liquid media at *in-vitro* conditions and the broth culture was regularly shaking to avoid clumping the cells.

Morphological characterization:

Morphological identification and cell dimensions of the strain from growing fresh cultures were identified through the taxonomical identification on in the 'classical' standard literature [24].

Extraction of Exo polysaccharide:

The extraction of exo polysaccharide was carried out by [25-26]. The cells were separated from the growth medium by centrifugations 14,000 *g*, 20 min at 10°C. The polysaccharide from this concentrated supernatant was precipitated by gradually adding an equal volume of cold ethanol to the supernatant and kept at 4°C overnight, dialyzed for 24 h against distilled water and then lyophilized.

FTIR Spectroscopy:

The partial characterization of EPS was done using FTIR spectroscopy. It was used to identify the structure and important functional groups in EPS.FTIR is wide popular techniques for monitoring EPS variation [27-28]. The range which was used 4000 to 400 cm^{-1} and spectra were recorded in transmittance mode on a Perkin Elmer Spectrum.

RESULTS AND DISCUSSION

As per the standard cyanobacterial identification manual, the morphometric characters were studied and viewed under the standard microscope. Results of the study on microscopic views confirmed as *Anabaena* species (Fig 1) and *Nostoc* species (Fig 2). In *Anabaena*, the thallus are blue green in colour with mucilaginous layer, cylindrical filaments, trichome single, and finally end cell conical akinetes. However, in *Nostoc* cells are spherical in shape; thallus is light blue green in colour with twisted filaments with heterocyst.

The Fourier transforms infrared spectroscopy (FTIR) analysis of exo-polymeric substances from *Anabaena* sp revealed that the distinct stretching frequencies of chemical groups (Fig.3). The dominant peak observed 3344.55 cm^{-1} which representing the O-H stretching (Alcohol) vibration peak. The peak 2358.94 cm^{-1} indicating O=C=O stretching of carbonyl group-general. The 1639.49 cm^{-1} and 1550.77cm^{-1} groups mainly correlate to C=N, N-O as respectively. The functional groups of above peaks as indicate of aliphatic amines and nitro compound. The peaks noted in 1406.11 cm^{-1} and 1111.00 cm-1 which represents S=O (sulfonyl chloride), C-O stretching (secondary alcohol).

The FTIR analysis of exo polymeric substances from *Nostoc* sp revealed that the distinct stretching frequencies of chemical groups (Fig.4).The dominant peaks observed at 3739.13 cm^{-1} and 3254.18 cm^{-1} which represents O-H stretching (Alcohols / phenols). The peak of 2889.37 cm^{-1} as C-H stretching (Alkanes) groups. The frequencies were observed at 2360.87 cm^{-1} and 1645.28 cm^{-1} as represents O=C=O (carbonyl groups), C=C (Alkenes) as respectively. The peaks of 1548.84, 1400.32 and 1100.02 cm^{-1} which indicate as N-O stretching (Nitro compounds), C-C stretching (Aromatics) and C-N bond (Aliphatic amines) respectively.

Cyanobacterial exo-polysaccharide's produce diverse properties in terms of physical and chemical which used wide industrial applications [29].The [13] has reported regarding the EPS synthesis is the major end product of photosynthesis. The results of the study on Exo-polysaccharides revealed that *Nostoc* and *Anabaena* strongly could have been widely used in the food and pharma industries. Especially, countries like China and Beru, the EPS derived from *Nostoc* used as a dietary supplement [30]. The nature of EPS components was identified through FTIR spectroscopy which is non destructive technique for monitoring EPS variation [28]. During the samples investigation this IR spectrum provides specific vibrational fingerprints. There are several evidence reported the similar works done in the aspects of cyanobacterial EPS production with partial characterization [32-33]. In the study, we clearly recorded that various functional groups of extracted EPS from *Anabaena* and *Nostoc* species at VellarEstuary.

The study conducted [35] on *Anabaena* sp. PCC 7120, extraction and partial characterization of EPS (0 m M $CaCl_2$) in these, the dominant peaks as 3388.30 cm^{-1} as OH groups, 2926.32 cm^{-1} as CH_2 groups and 1650.97 cm^{-1} as C=O groups were observed. As compared with our works, the dominant peaks were observed 3344.55 cm^{-1} as OH (Alcohol) group, 2358.94 cm^{-1} as O=C=O stretching (Carbonyl Group - General), 1639.49 cm^{-1} as C=N,1550.77 cm^{-1} N-O and 1111.00 cm^{-1} as C-O stretching. These findings demonstrate the existence of protein function and peptide bonds in the EPS composition. The absorption at 1,111 cm−1 could be attributed to the presence of sulphate groups as S = O and C−O−S [9]. In addition, some peaks that were less than 1,000 cm^{-1} were also present due to several visible bands [36] and/or to the occurrence of possible linkages between two monosaccharide molecules [9].

There are some important studies on *Nostoc* sp especially on exo polysaccharides [37-39].According to The studies based on the EPS production the highest level is *Nostoc* sp. BTA97 and followed by *Anabaena* sp. BTA990 [40]. According to [41], *Anabaena* sp. BTA 992 and *Nostoc* specie BTA12 was the best EPS extraction among the other eight isolates originally isolated from Indo-Burma biodiversity hotspots of north-eastern India. In according with [42] conducted the study on isolated strain of *Nostoc* commune. The peaks at ~3291 cm^{-1} corresponding to O-H groups, ~1023 cm^{-1}represents to stretching vibration of the C-O-C in glucose units, and ~1596 cm^{-1}, indicated to absorbance of aldehyde.

In the study, *Nostoc* sp displayed IR spectrum a broad peak at 3,254 cm^{-1} that corresponded to the stretching vibration of the OH group, 2889.37 cm^{-1} to C-H stretch groups, 2360.87 cm^{-1} to C-C stretch groups, 1645.28 cm^{-1} to C=C groups, 1548.84 cm^{-1} to N-O stretching groups and 1400.32 cm^{-1} to C-H bending groups. According to [43] studied exo cellular polysaccharides on *Nostoc carneum*, the peak at 3419 cm^{-1}, can be assigned to the stretching –OH or –NH (Hydroxyl or amines) groups, the bands 2364 and 2147 cm^{-1} can be attributed to C ≡ C and C ≡ N, along with they mentioned carboxylic and asymmetrical vibration groups also. The bands observed less than 1,000 cm^{-1} which may indicate various visible bonds or presence of probable linkages between monosaccharides [44-45]. The studies showed [46] was comparing to other cyanobacterial EPS the *Anabaena* sp having some advantageous properties, such as mitigation capabilities to temperature, pH and salt concentration. In Taiwan, isolated the *Anabaena* sp from water reservoir, then extracted the EPS along with identified the monosaccharide's [47]. The analysis of FTIR clearly showed a shift in major and minor band which would indicate changes in EPS. The basically mid range IR spectra useful to identifying phosphoric and carboxyl groups. According to [49] so many main constituents of EPS produced by filamentous heterocyst cyanobacteria, especially, including *Anabaena* sp and *Nostoc* sp. As a conclusion, in this current study the functional groups of EPS were moderate according to literature values [48]. The present studies revealed that the isolated *Anabaena* and *Nostoc* spp.,exo-polysaccharides exhibited the significant functional (Chemical) groups, which may be the potentials for further commercial exploitation.

Fig.1. A Microscopic view of *Anabaena* sp. (10 X)

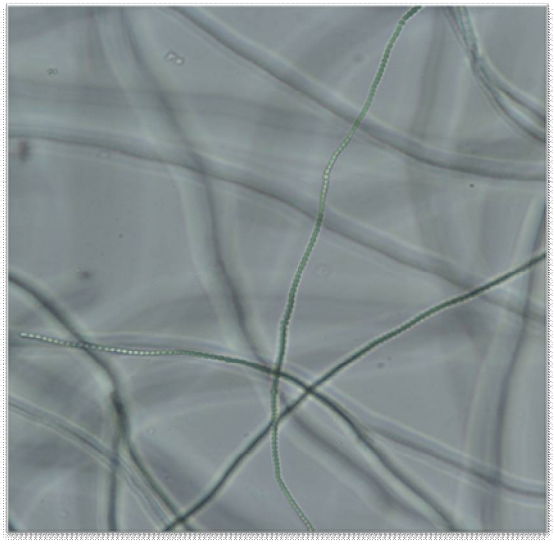

Fig: 2. A Microscopic view of *Nostoc* sp (10 X)

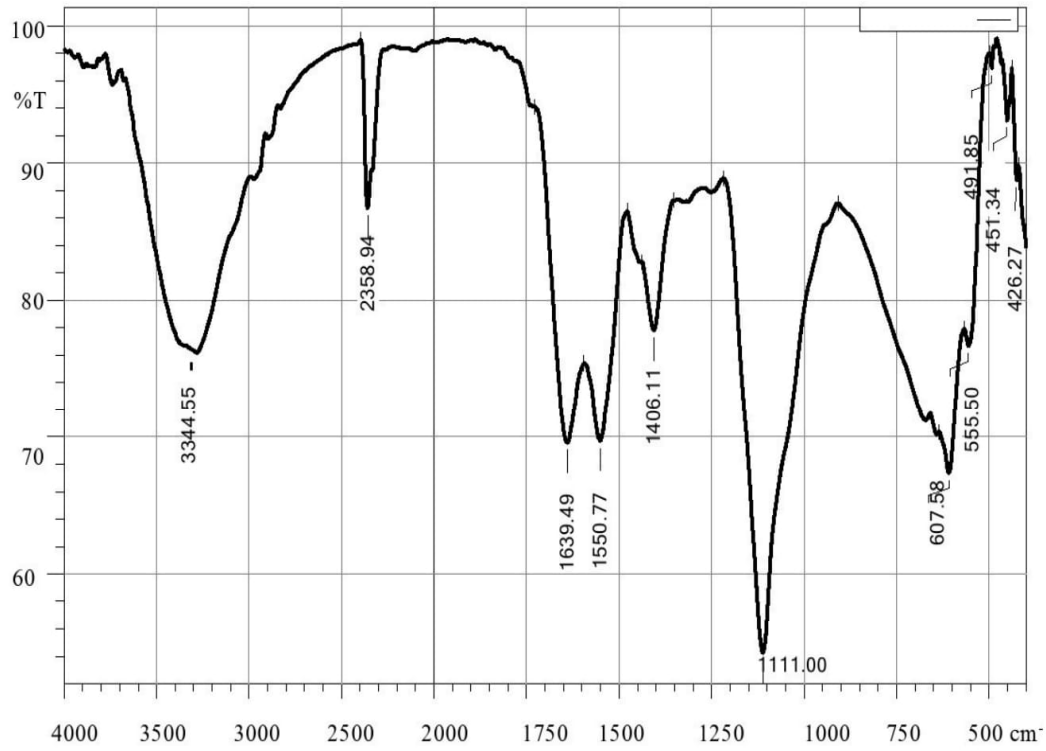

Fig.3:FTIR analysis of *Anabaena* sp-EPS.

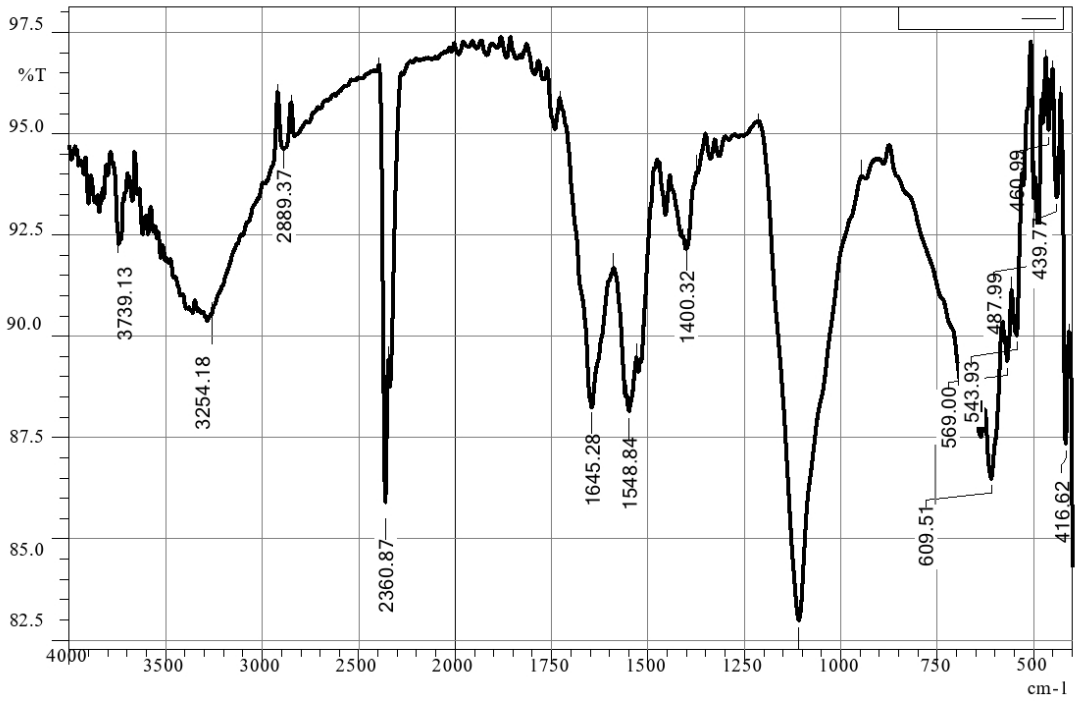

Fig. 4: FTIR analysis of Nostoc sp-EPS.

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