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Exopolysaccharide producing bacteria associated with brown seaweed- *Sargassum wightii*

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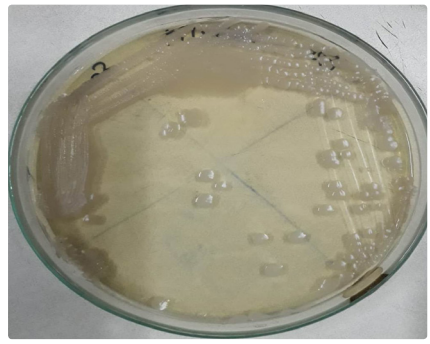
Biopolymers are the polymers obtained from various biological organisms containing covalently bonded monomers and are classified into polysaccharides, polypeptides and polynucleotides. The main source of biopolymers from marine environment includes macro algae, micro algae, bacteria, and fungi. Among the microorganisms, bacteria are widely accepted as the source of exopolysaccharide with different functional properties and can be exploited for novel industrial and biotechnological applications. Exopolysacchrides (EPS) are high molecular weight polymers secreted by bacteria, consisting of different functional groups such as acetyl, succinyl or pyruvyl, sulfate etc. Biodegradation ability of EPS from bacterial origin can replace the traditional polysaccharide sources from various fields such as biomedicals, food and textile industries in larger extent. However, the high cost of production and low yield from bacterial sources may limit the use in industry scale.

In the present study, an attempt was made to screen brown seaweeds viz, *Sargassum wightii*, *Padina gymnocephalus*, and *Turbinaria connoides* for EPS producing bacteria. Bacterial isolation was carried out by homogenizing the dried seaweed samples (25 g) in 225 ml 1X phosphate buffer saline and plated on trypticase soya agar (TSA) with 2% NaCl. A total of five distinct morphological isolates were selected based on their slimy mucoid appearance on the TSA plate. EPS was extracted according to the method by Berekaa and Ezzeldin (2018). Initial screening for EPS production by the bacterial isolates was carried out on the basis of EPS yield after 10 days of incubation in a shaker incubator with 180 rpm at 37 ^{o}C in trypticase soya broth (TSB). The EPS production in these isolates varied from nil to 0.62 mg ml^{-1} .Out of five isolates, one isolate from *Sargassum wightii* showed maximum production and was further inoculated into Luria bertani (LB), and Brain Heart Infusion (BHI) broth and the dry weight was measured. The dry weight of EPS was recorded maximum in BHI (1.27 mg ml^{-1}) followed by LB broth (1.12 mg ml $^{-1}$). The EPS production is often accompanied with the aging of the culture and exhaustion of available nutrients in the media. During chemical analysis, it was found that EPS from BHI broth contained 59.9% carbohydrate, 8.1% protein, 3.2% total uronic content and 1.5% sulphate content. The isolate was identified as *Bacillus cereus* by 16S rRNA sequencing.

Structural analysis of EPS by FT-IR analysis (Fig. 2) which showed a characteristic N-H and OH stretch at around 3292.93 cm^{-1} and a C-H stretching vibration at around 2925 cm^{-1} (Deepika et al., 2016). The absorption peaks within 1650-1540 cm^{-1} attributed to vibrations of a C O, NH and CN bending of protein and peptides. The absorption peaks within 1200-1000 cm^{-1} attributed to vibrations of a broad stretch of C O and C O C

glycosidic bands, which revealed the presence of carbohydrates (Zhang et al., 2013) that, would be sugar monomers in the EPS. The absorption peak at 600 cm^{-1} and 492 cm^{-1} could be attributed to the S-S stretch. The absorption observed at 1500-1600 cm^{-1} could be attributed to the stretching vibration of C=C and C–N groups. Peaks at 884 cm^{-1} ascertain the presence of glycosidic linkage bonds. The composition and components of exopolymeric substance of bacteria have large implications in their bioactive properties. Further research may be carried out to exploit the unique properties of exopolysaccharide from *Bacillus cereus* to find the practical applications in various fields such as textiles, pharmaceuticals and food industry.



A

B

Figure 1: A) *Bacillus cereus* producing exopolysaccharide on trypticase soya agar; B) crude extract of exopolysaccharide produced by *Bacillus cereus* in Luria Bertani (LB) and Brain Heart Infusion (BHI) broth

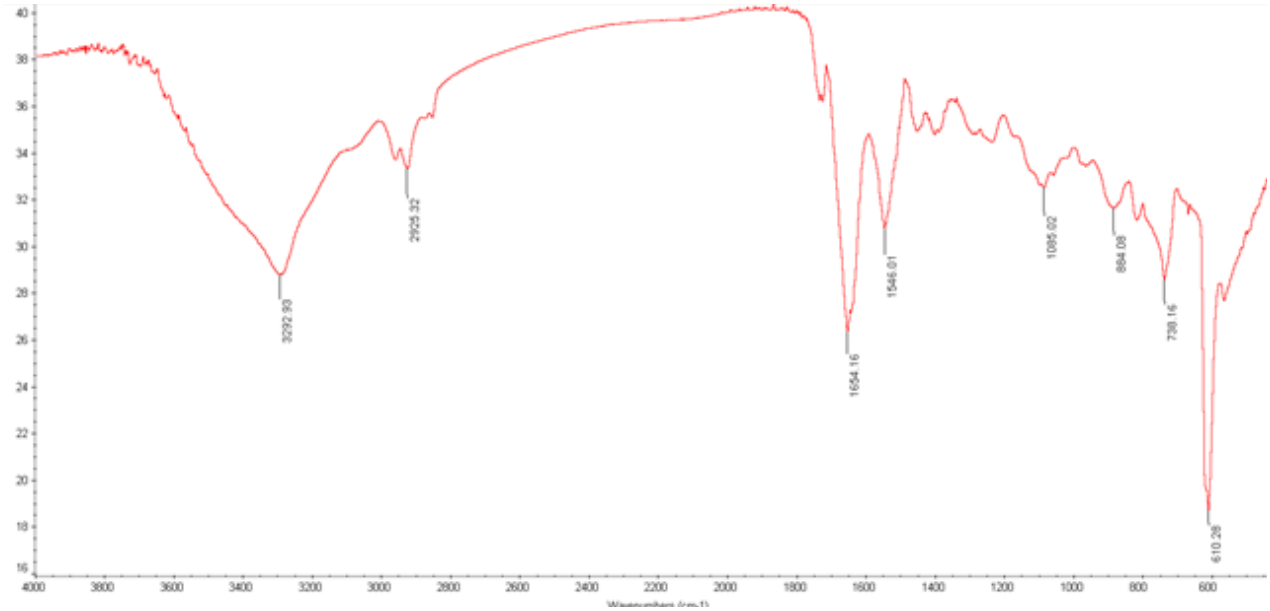


Figure 2: FT-IR Spectrum of crude EPS from Bacillus cereus

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Microbiological changes of Pangasius hypophthalmus fillets with Moringa oleifera (Lam) leaves in chilled storage condition

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Worldwide fishery products consumption is on the rise as it is a rich source of high-quality protein, essential vitamins, polyunsaturated fatty acids *etc* (Verbeke et al., 2005). But the unique biological composition along with high water content of fishery products make them susceptible to rapid enzymatic and microbial spoilage. Therefore, their shelf life is limited and several synthetic chemical additives are being used indiscriminately as preservatives. But continuous usage of such chemicals including antibiotics results in cancer, other foodborne illness, and development of multidrug resistance (MDR) in bacterial strains (Thomas et al., 2015).

To address such issues, bio-preservation has emerged as a novel technology which extends the shelf-life and safety of food products by the use of natural products like essential oils, phytoextracts, animal enzymes, microbial bacteriocins, organic acids, naturally occurring polymers *etc (*Pilar et al., 2010). *Moringa Oleifera (Lam)* belongs to family *Moringaceae* is often called as 'Miracle tree' or 'Tree of life' because of its wide range of medicinal uses with high nutritive value. *Moringa* leaf is well known for its potential antibacterial as well as antioxidant activity and is being used as a natural preservative for shelf life extension of various food products. There are limited reports on the use of *Moringa* leaf juice as a natural source of antimicrobial substance in fish processing and this study evaluates the antibacterial and antioxidant potential of *Moringa* leaf juice for extension of shelf life in vacuum packed pangasius fillets during chilled storage at 4 ± 1 °C.

About 5.5 kg of *Pangasius hypophthalmus* were procured in fresh condition from the local market, Mysore, Karnataka and immediately brought to the laboratory in the chilled condition. The fish were cut into pieces of equal size approximately weighing 30 gm and used for analysis and storage studies. Fresh *Moringa* leaves were collected and juice was extracted based on Rahman *et al*. (2009). The yield of juice is 29% and this