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Screening, production, optimization, characterization, pharmacological analysis and applications of Exopolysaccharide from Marine Bacteria: A Review

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ABSTRACT

Exopolysaccharides (EPS) is a group of high molecular weight biopolymers produced during the metabolic process of some microorganisms. A broad class of exopolysaccharides (EPSs) are produced by microbes in response to environmental stressors such salt, pH, temperature, and light intensity. Microbial marine exopolysaccharides (EPS) are one of the essential and functional commercial-grade products and have several biotechnological applications. Exopolysaccharides (EPSs) from microorganisms are essential harmless natural biopolymers used in applications including medications, nutraceuticals and functional foods, cosmetics, and insecticides. Isolating new EPSs with unique functionalities and unique chemical and physical characteristics is gaining attention. In this sense, a vast number of unidentified and uncultivated bacteria that are capable of producing and excreting EPSs can be found in the marine environment. The structure of EPS is complex and difficult to analyze, resulting in difficulties in investigating their structure–activity relationship, and its specific mechanism of action has not yet been revealed. . The characterization studies, structural and pharmaceutical analysis are crucially important parameters that need to be studied and those headings are discussed in this review.

Key Words: Exopolysaccharide, Marine organisms, Characterization technique, pharmaceutical analysis.

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Introduction

The extreme environments have made the microorganisms capable of surviving extreme conditions which has given them their unique capabilities. Extracellular polysaccharide or Exopolysaccharides are produced by microorganisms on their cell surface as a result to protect themselves from the harsh environment thus producing tolerant enzymes and other bio substances that can be used for various industrial purposes (Subramani and Sipkema, 2019). In recent years the production of exopolysaccharides from the marine microorganism is growing interest among researchers, there is various isolation and identification of microorganisms is being researched. These polymers are mainly involved in the maintenance of the environments of marine by contributing to various processes such as particle formation sedimentation dissolved metal cycling and organic carbon dissolution**.** (Verdugo, P., 2012). The characterization of the exopolysaccharides is a hard process for researchers because of their structural complexity even the main component of exopolysaccharides which is carbohydrate is very difficult to obtain their monomer linkage and its biochemical properties. So there are various methods such as acid hydrolysis, high-performance liquid chromatography, gas chromatography with mass spectroscopy, acid hydrolysis, fourier transform infrared spectroscopy (FTIR), monosaccharide analysis, HPLC, NMR and glucose assay, and various other characterization analyses. (Liang, T et al., 2015). In this review the isolation, screening, optimization of microorganisms that produce exopolysaccharides and characterisation and various applications of the exopolysaccharide produced from the microorganisms of marine source are discussed

Screening, optimization and Characterisation of EPS

Screening is a selective process where a sample taken from the environment in a large population is isolated and detected according to our interest. Zobell is the most common medium used for isolation of marine microorganisms as they have glycerol and glucose (Dave, S et al ., 2020). *V. harveyi* strain VB23 was isolated with samples taken from river Mandovi and Zuari estuarine coastal area of Goa. They are grown in normal glycerol-based agar and TCBS agar. Further purification is done by mineral media with NaCl in compositions (Bramhachari, P et al ., 2022).

Exopolysaccharides production will be at the maximum level at two different phases in the growth of microorganisms. Some studies have shown that one of the phases for maximum

production is the exponential phase and the other is the stationary phase where the nitrogen be limited by the maximum production. The yield of exopolysaccharides and their really logical characteristics mainly depend upon some of the factors that are related to fermentation conditions such as the utilization of carbon and nitrogen sources, optimal temperature, requirements of minerals, optimal PH, along with the education rate and consumption of oxygen. From various studies, we can say that the production of exopolysaccharides can be increased by using the physiological control (Suresh Kumar, A et al., 2007) as well as the nutrition limitations in marine bacteria such as phosphorus, nitrogen, potassium, and sulfur can lead to the increase in the exopolysaccharide production (Sutherland, I. 1982). The carbon sources that are used as constant can be replaced by different other carbon sources for the production of exopolysaccharides and check the effect of its production. The medium with different carbon sources are inoculated with the organism and can be incubated. After incubation, the exopolysaccharide production will be measured using phenol sulphuric acid method (Shankar, T et al., 2013). Nitrogen sources also replaced with other nitrogen sources and exopolysaccharide production is measured in comparison to the standard exopolysaccharide production medium. After the inoculation of the microorganism and incubation the production of exopolysaccharides are compared with standard medium (Hwang, H et al., 2005). The production medium that is considered as standard is inoculated with the microorganism at different pH levels and incubated after the incubation the production of exopolysaccharide is measured and the pH range that gives the effective production is used for the optimisation process (Patil, S et al., 2010).

Response surface methodology is the method that is used for optimisation. The chemically defined medium that is considered as standard or optimised by one variable at a time approach and the data obtained will be used for the optimisation process by using surface methodology method of central composite design (Mustafa, M et al., 2020). The structural surface is important to know the rigidity and hardness for many aspects in cosmetics, cancer and biofilm. It can be determined using NMR, IR, and FTIR and so on. Microorganisms like *Pseudomonas* sp. ID1 taken from the antarctic sea sediments is subjected to FTIR analysis which showed the presence of O-H and other bonds on a broad range of 3400nm. The proton spectrometer agreed with the presence of anomeric protons with glycan compounds (Carrión, O et al., 2015). The monosaccharide composition, sulfate and uronic acid was determined using Thin layer chromatography the composition was determined by HPLC, Thin layer chromatography, and colorimetric methods are widely used in monosaccharide analysis.

Pharmacological applications of Exopolysaccharide:

Exopolysaccharide can also be used as the pharmaceutical excipients such as binders, diluents, wetting agents, filling agents, disintegrating agents, dissolution enhancers, and so on.

As binder

The exopolysaccharide that is produced from marine bacteria used for filling the binders can be used for the creation of tablets by using the wet granulation method for fabrication (Abdelhamid, S et al., 2020)

As diluents

The diluted can also be called as filler that are used in that form of tablets which can be used for the centralization of therapeutic agents helping in the procedure by increasing the solid reproducibility (De Jesus Raposo et al., 2015).

As disintegrants

The materials that are used for the dose shapes that improve the separation of tablets in two little particles by breaking them quickly without any external particle are called disintegrants. The usage of disintegrants is necessary for the tablet dissolving inside the body and the marine exopolysaccharide can be used as the disintegrant because of its rate of determining advanced RDS in a quicker medication releasing process (Abdelhamid, S et al., 2020). The marine bacterial exopolysaccharide can act as a decent operator by expanding the porosity, slender activity, wettability and swelling contact of water in liquids helps in the disintegration of granules when reaching the stomach (Thomas, N., & Kim, S. 2013).

Dissolution enhancers

Exopolysaccharides produced from Marine bacteria can fore disintegration of poor dissolvable medications these can help in decreasing the firmness of the tablet and helps in separation disintegration into smaller granules expanding the surface area for disintegration (Hoare, T., & Kohane, D. 2008).

As a drug delivery carrier

Exopolysaccharides produced from Marine microorganisms can also be used for the synthesis of drug delivery carriers. The properties such as bio perfect dangerous biodegradable and so on are the most proper quality needed as a drug delivery carrier (Dhanaraju, M et al., 2010).

In-vitro antioxidant activity

The antioxidant activity also called as free radical scavenging activity of the marine microorganism-produced exopolysaccharide can be measured against the (1, 1-diphenyl-2 picrylhydrazyl) DPPH Radicals (Asker, M., & Shawky, B. 2010). Some other types of antioxidant activity can be analysed in vitro with the help of methods such as hydrogen peroxide radical scavenging assay, hydroxyl scavenging assay, superoxide anion radical scavenging assay, and DPPH radical scavenging assay

Anti-bacterial and antiviral activity

Exopolysaccharides obtained from the marine microorganisms of certain concentrations can be used for the antibacterial and antiviral test. Here the bacteria are incubated overnight and the freshly prepared culture will be seeded in the exopolysaccharide then incubated for over 24 hours and the turbidity is measured for the quantification process. in the same way viruses are infected to the cells that are treated with exopolysaccharides and the lactate dehydrogenase activity in the culture medium will be checked (Yan, Y et al., 2011; Allen, M et al., 1994).

Anticoagulant activity

Some of the carbohydrates have anticoagulant activities by showing the thrombin activities or initiating the expansion of anticoagulant time or by acting against the thrombin 3. Similarly, these molecules can have antithrombotic effects by blocking the movement of the thrombin by interfering with heparin cofactor 2 (De Jesus Raposo, M et al., 2015; Li, W et al., 2008). Some researchers have proved that the sulfated EPS can be used for the anticoagulation activity

Acute toxicity study

In this analysis exopolysaccharides are dissolved in distilled water and rats are used as models. After the observation, the changes in the morphology such as skin and fur along with the internal organ system such as respiratory, circulatory, central nervous system, autonomic systems, and somato motor activities and behaviour patterns are all checked and noted (Yamasaki Y et al., 2016).

CONCLUSION:

The review explains the detailed phase from the isolation of microorganisms from marine ecosystem, the use and composition of media in which they are grown and isolated, screening techniques like string and other methods to detect the presence of EPS. The optimization of media components, fermentation, solvents and incubation methods gives a detailed view on how to optimize and what are the parameters that are needed to be in the check list before and after isolating a new microorganism. Pharmaceutical analysis and structural analysis gives an insight to the structural composition, techniques like HPLC, NMR and others that can be used to determine what type of monosaccharide's are used hence their application depends on the structural analysis. Pharmaceutical analysis like toxicity, skin irritation, biopolymers, anti-microbial and anti-viral describes not only the future perspectives of the EPS but also the caution signs and the necessities.

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