

Exopolysaccharides from *Enterobacter* sp. ACD2 isolated from the Tabuk area of Saudi Arabia have biological and microbiological activity

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ABSTRACT

Exopolysaccharides (EPSs) from bacteria have been shown to have industrial and medicinal applications. By improving the culture conditions of *Enterobacter* sp. isolated from the marine environment of Haqel Beach in the Tabuk region of Saudi Arabia, our group has previously obtained a high yield of EPS production (8.6 gm/L). The chosen strain was then identified using traditional techniques and molecular characterization utilizing 16S Ribosomal RNA (16S rRNA) gene sequencing. The composition of this EPS was established in this study, and its anticoagulant, fibrinolytic, antibacterial, and prebiotic actions were assessed. We used acid hydrolysis and qualitative and quantitative paper chromatography to identify the monosaccharide

moieties of this EPS (PC). This EPS had almost 25% glucose, 25% galactose, 40% fucose, 10% uronic acid, and traces of fructose in its monosaccharide composition. Sulfation was done to the EPS that was created. Both the native and sulfated EPS were tested for biological and microbiological (anticoagulation, fibrinolytic, antimicrobial, and prebiotic) activities, and both showed higher antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, but neither form showed antifungal activity against *Candida albicans*. The native and sulfated EPS's minimum inhibitory concentration (MIC) that maintained the maximum bacterial inhibition zone was 15 mg/dl. There were no prebiotic activities seen in either EPS. Finally, the generated biopolymer possesses considerable biological activity that will require additional adaption for practical and industrial uses.

Key Words: *Exopolysaccharide; Enterobacter sp.; Marine Bacteria; Prebiotic; Antimicrobial*

INTRODUCTION

An exopolymer, or biopolymer released into the environment by an organism, is an Exopolysaccharide (EPS) (i.e., external to the organism). These exopolymers include bacteria's biofilms, which serve to anchor them and protect them from the elements. Polysaccharides and proteins make up the majority of exopolymers, although other molecules including DNA and lipids can also be found. Diverse bacterial species may be found in the ocean, and they can be used to make important chemicals such as EPSs, which have biotechnological uses. A bacterial population in a marine habitat has enormous promise as a source of novel natural products. Furthermore, EPSs appear to have a broad variety of uses, including emulsification, thickness, absorption, film formation, gel organization, and anticancer therapies. Natural products, such as EPSs, are thought to be valuable sources of potential chemotherapeutic drugs. Investigations into aquatic ecosystems have been expanded in the search for new bioactive chemicals. EPSs have important biological functions, such as regulating cell division, differentiation, immunological modulation, anticancer, antioxidant, and antiviral activity. EPSs can help prevent illnesses, inflammation, and atherosclerosis by acting as antioxidants. EPSs have been found in a variety of medical applications, including arthritis therapy, wound dressings, surgery, and pharmaceutical tablets. The health benefits of these substances, particularly those from *Lactobacillus* species used as probiotics, have been widely documented. In addition, antiviral, antioxidative, immunostimulatory, tumor-resistant, and antibacterial characteristics have been discovered in EPSs. Rare monosaccharide sugars like L-fucose and L-rhamnose, as well as uronic acids, are not commonly found in nature, but both have a variety of interesting properties that make them appealing in a variety of fields of application as anti-inflammatories, antioxidants, and building blocks for synthesizing the nucleoside analogues that are used as antiviral agents, justifying the efforts to synthesize them.

Marine EPSs have a lot of promise for usage as natural bioactive medicines in medicinal applications. The increase of bacterial resistance to currently available antibiotics has piqued interest in marine microorganisms as a potential new source of antibacterial chemicals. The prebiotic potential

of several lactic acid bacteria-produced EPSs has been investigated. Chitin or its sulfated derivative (chitosan) is one of the most prevalent marine polysaccharides, and following chemical sulfation, it may be employed in biological applications such as blood anticoagulant. Carrageenan's polysaccharide also has strong anticoagulant and fibrinolytic properties. Previously, we identified and selected a marine *Enterobacter* sp. and optimized multiple culture conditions to increase its EPS production. We were able to produce a high yield of EPS (8.6 g/l) using these adjusted settings. The goal of this work was to examine the biological and microbiological activities of marine *Enterobacter* sp. native and sulfated EPS, including anticoagulant, fibrinolytic, antibacterial, and prebiotic properties.

The current work examined the anticoagulant, fibronogenic, antibacterial, and prebiotic effects of an EPS isolate after analyzing and modifying its structure by sulfation. Acid hydrolysis of such EPS can be used to separate the monomers, which can then be separated using paper chromatographic techniques to give pure monosaccharides. The sugar content can then be utilized as a starting point for creating compounds for high-value applications. The sugar content of the EPSs produced by the chosen bacteria was determined using full acid hydrolysis. The findings showed that there was 25% galactose, 25% glucose, 40% fucose, and 10% uronic acid, with traces of fructose. This finding is consistent with that of Iyer et al. (2005), who discovered that *Enterobacter cloacae* is composed of glucose, galactose, fucose, and glucuronic acid in a 2:1:1:1 molar ratio. The EPS product also contains two unusual monosaccharides: fucose and uronic acid, in addition to these sugar moieties. Both native and sulfated EPSs had robust anticoagulant (prolonged anticoagulation period) and fibrinolytic (100 percent clot destruction) effects in the current investigation. These findings support assertion that sulfated polysaccharides, such as mannan sulphate, chondroitin sulphate, heparin, and sulphoevernan, have well-known anticoagulant properties.

However, it was found that specific carbs also interfere with the prothrombin route, indicating that they are not yet ready to impact the outer coagulation pathway. On *E. coli* and *S. aureus*, the antibacterial activity of both the native and sulfated derivative EPSs was almost identical to that of

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the controls. Neither the native nor the sulfated EPS, on the other hand, demonstrated any anti-*Candida albicans* action. Onbasli and Aslim (2008) tested *Pseudomonas aeruginosa* B1 and B2 strains for the ability to generate inhibitory compounds, including EPSs, against the growth of *B. subtilis*, *E. coli*, *S. aureus*, and *C. albicans*, but only found action against *E. coli* and *S. subtilis*. Other researchers have reported antibacterial activity of EPSs isolated from the *Aeromonas hydrophila* strain An4 from marine catfish as well as four biofilm bacteria (*Galionella* sp, *Alteromonas* sp, *S. aureus*, and *Klebsiella* sp) from marine waters.

The Minimum Inhibitory Concentration (MIC) of a material necessary to inhibit or kill a bacterium is known as the MIC. Both the native and sulfated EPSs with the greatest inhibitory zone had MICs of 15 mg/dl. This finding suggests that this isolated EPS might be useful as an antibacterial agent for both Gram-positive and Gram-negative bacteria. The word "probiotic" refers to a wide range of microorganisms, the majority of which are Gram-positive bacilli such as *Lactobacilli* or *Bifidobacteria*. According to several recent research, a large and rising percentage of hospitalized patients are receiving probiotics as part of their treatment. Given the paucity of evidence for the effectiveness and safety of probiotic usage in hospitalized patients, these data suggest that further study is required to guide the use of

these medicines in this environment. However, research into the prebiotic activity of our EPS product revealed that neither its native nor sulfated forms exhibited any probiotic activity, since the probiotic index for all of the EPSs tested was less than 1.0. An *L. delbrueckii* bulgaricus EPS, on the other hand, exhibited the highest prebiotic indices (I), ranging from 7.9 to 10.1, according to a recent research. EPSs from *L. helveticus* and *L. casei*, on the other hand, had the lowest prebiotic indices (I), ranging from 1.4 to 2.4. The marine *Enterobacter* sp. ACD2 EPS offered a renewable source of components for industrial and medicinal uses. As a result, this EPS may be employed in a number of industrial biomedical applications, such as antibacterial antibiotics, thrombolytic anticoagulants, and clot solvents.

CONCLUSION

Both the native and sulfated versions of our biopolymer have considerable and promising biological properties as antibacterial, anticoagulant, and fibrinolytic compounds. Furthermore, the EPS composition contains rare monosaccharide moieties such as fucose and uronic acid, which are of great interest in medical applications and will almost certainly require further research and optimization for industrial applications such as antitumor, antiviral, immunomodulatory, and antioxidant activities.