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Characterization and anti-inflammatory activity effect of exopolysaccharide from *Bacillus axarquiensison* paw rats carrageenan model

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Abstract

The exopolysaccharide (EPS) was produced by a halophilic bacterial strain isolated from marine sediments, Porto-Elsokhna Beach, Egypt. This isolate has been defined morphologically and physiologically and has been recognized by 16S rDNA as *Bacillus axarquiensis* NRC G6.This strain was deposited in the Gene Bank (http://www.ncbi.nlm.nih. gov) under the accession number of KU945823. The EPS produced was fractionated by precipitation and listed the primary fraction as EPSBa3. Through FT-IR and HPLC, further work attempted to elucidate the structural function of EPSBa3. The findings showed that EPSBa3 was an acidic EPS composed of glucuronic: mannose: galacturonic: glucose and xylose, respectively, in a molar ratio of 1:1:3:3:3. The proportion of uronic acid and sulfate was 13.9% and 7% respectively. The estimated median molecular weight (Mw) and molecular number (Mn) were 1.499 $X10^6$ and 1.29 X 10^6g / mol. In rats, the anti-inflammatory activity of exopolysaccharide (EPSBa3) was also explored using paw edema induced by carrageenan. EPSBa3 caused important anti-inflammatory effects on the carrageenan model by 50 mg/kg orally. In catalase (CAT) and superoxide dismutase (SOD) enzymes and GSH content, the EPSBa3 enhanced declines. In addition, the content of nitric oxide (NO), lipid peroxidation (LPO) and reactive oxygen species (ROS) arising from carrageenan-induced edema were considerably reduced following therapy with EPSBa3. In addition, the findings showed that interleukin-6 (IL-6) protein expression was decreased after EPSBa3 treatment leading to alleviate paw edema. These findings stated that to avoid inflammatory diseases, EPSBa3 could be utilized as a source of natural products.

Keywords:

Bacillus axarquiensis; exopolysaccharide, oxidative stress, carrageenan, and anti-inflammatory activity.

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1. Introduction

Marine environmental circumstances are generally distinct from terrestrial circumstances, which could confer distinctive features on marine microorganisms, enabling them to create various new biologically active compounds (1,2). The isolation of fresh microorganisms is a significant feature for taxonomy, production and function identification (3). The marine environment (Egypt's Porto-Elsokhna beach) could be viewed as a repository of many novel microorganisms that have the capacity to generate polysaccharide particular to secondary metabolites (4,5). The polymers excreted by some microorganisms are known as exopolysaccharides (EPS) as protective obstacles against damaging circumstances. *Bacillus axarquiensis* is one of the most widespread fleshy bacteria of the species bacilli and can be isolated from all nations. Many microbial EPS can be used in a broad spectrum of biotechnological applications, such as stabilizers, thickeners, and texturizers in the food industry, anti-aging molecules in the cosmetics sector, or flocculating agents in the sewage treatment sector (6-8). The EPS also contributes to multiple human physiological operations as an antioxidant, anti-tumor, anti-viral (9) and anti-inflammatory agents and can behave as inducers of interferon, platelet aggregation inhibition and colony-stimulating factor synthesis (10-13). Extremely variable salinity, temperature and pressure conditions are described in marine settings. Thus, the bacteria that are isolated from these settings are anticipated to vary in adaptation mechanisms compared to worldwide ones, such as the synthesis of EPS with individual components and different compositions that permit ongoing life there. This variety is one of the excellent concerns nowadays because it provides the chance of finding new molecules with exclusive characteristics, such as antibacterial, algaecide or anti-fouling operations (14-16). As a consequence of the study of several marine species producing EPS, novel macromolecules were found and isolated (17). Most marine-derived EPS are bacterial (i.e., mesophilic and heterotrophic classical bacteria; psychrophilic, thermophilic and halophilic extremophilic microorganisms); archaea have also been shown to generate EPS (17-19). Bacillus sp. a significant group of bacteria that has several benefits over other bacteria, such as simple cultivation and conservation, and is therefore conducive to industrial production (20). Bacillus sp. can generate more EPS than lactic acid bacteria, and more than one form of EPS may be produced by the same Bacillus strain (21). In addition, their immune-modulating and anti-cancer impacts are the most promising biological characteristics of these polysaccharides. A possible mechanism is that it is suggested that these polysaccharides improve in vivo and in vitro cell-mediated immune responses and act as modifiers of biological reaction. In addition, polysaccharide's anti-inflammatory activities were also researched in these years. Du *et al*., (2015) (22) assessed the fungal beta-glucan (a type of polysaccharide) anti-inflammatory effects. A beta-glucan from *Alcaligenes faecalis* was discovered to be able to induce CD4 (+) T cells producing IL-10 and to inhibit the growth of *Eosinophilic airway* inflammation (23). In addition, Du et al. (2016) (24) discovered S polysaccharide's anti-inflammatory activity. Ultrasonic therapy affected the municipality. It has been suggested that polysaccharide's powerful anti-inflammatory activity, potentially due to pro-inflammatory cytokine inhibition or enhanced anti-inflammatory cytokine manufacturing (25). In the present study was to purify and characterize the *Bacillus axarquiensis* exopolysaccharide by high performance liquid chromatography (HPLC) and Fourier transform infrared spectroscopy (FT-IR), Also its anti-inflammatory effects were evaluated after isolating and identifying the bacterial strain by 16S rDNA,. For exploring the application of the EPS as a functional ingredient in the food industry.

2. Materials and methods

Chemicals

Reduced glutathione, 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA) and λ-carrageenan, type IV were obtained from Sigma

Chemical (St Louis, USA). All other chemicals and reagents used were of analytical grade.

Sample and culture medium

Sea sediment samples were collected from Porto-Elsokhna beach in Egypt. The sediments were collected at depths of 2–3 m. A medium composed of (g/l): 20, agar; 20, sucrose; 2, yeast extract and 4, peptone was used for isolation, purification, and short-time storage. The medium was prepared with natural seawater collected on-site and distilled water in the proportion of 75:25 (v/v).

Isolation of bacterium producing the EPS

The samples were processed using the following method: wet sediment (1 g) was diluted with 100 mL of sterile seawater (SSW). After mixing, the diluted sample was allowed to settle for a few minutes, and then serially diluted. One ml of each dilution was spread on the agar plate medium. Petri dishes were incubated at 37°C and observed occasionally for bacterial growth. The colonies on the plates were purified acceding to the streak plate method (26) and the purified isolates were inoculated on the agar medium and incubated at 37°C for 5 days.

Screening of EPS producing strain

Thirty colonies of strains exhibited shiny and slimy surfaces on the growth media. It indicates the production of EPS by bacteria (27). The strains were regularly subculture and sustained in nutrient agar slants as stock. The strains were cultivated in 25 mL shake flasks on liquid medium containing per liter (750 mL sea water and 250 mL distilled water): 20 g sucrose, 2 g yeast extract, and 4 g peptone. The cultures were incubated in a shaker, for 3 days, at 37°C and 120 rpm. The culture medium was centrifuged at 5000 rpm for 30 min to remove cells; the supernatant was further subjected to deproteinization by TCA 5% (28). The yield of EPS was determined by the phenol-H_2SO_4 method using glucose as standard (29). The EPS was precipitation by addition 5 volume absolute ethanol and collected by centrifuged at 5000 rpm for 20 min at 4 °C, the precipitate was washed by acetone and dried under vacuum.

Identification of screened strain

Morphological and Physiological Characterization

The most potent bacterial strain was identified by its morphological, physiological and biochemical experiments according to Bergey's Manual of Determinative Bacteriology (30). The identification was confirmed by comparing the results of a morphological shape, Gram's staining, growing tests at different temperatures and pH, catalase tests.

16S rDNA sequence identification

The extraction of DNA was carried out by a DNA extraction Kit and quality was evaluated on 1.2 % agarose gel, a single band of high Mw DNA has been observed. The forward primer was ITS1 (5′-TCCGTAGGTGAAC TTTGCGG-3′) and the reverse primer was ITS4 (5′-TCCTCCGCTTATTGAT ATGC-3′)(31). The PCR amplified was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplified was carried and then performs 35 amplification cycles at 94℃ or 45 s, 55°C for 60 s and 72°C for 60 s. DNA fragments are amplified about 988 bp in the case of bacteria. Include a positive control (*E. coli* genomic DNA) and negative control in the PCR. Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied Biosystems, USA). Sequencing products were resolved on an Applied Bio-systems model 3730XL automated DNA sequencing system. The forward and reverse 16S rDNA sequences obtained were checked for accurate base calling, assembled and analyzed using BLAST available on the NCBI website (http://www.ncbi.nlm.nih.gov). A neighbor-joining phylogenetic tree was constructed from 1630 bp using the Clustal X version 2 with 1000 bootstrap replicates (32).

Isolation and fractionation of EPS

EPS was separated from the culture medium by centrifuged at 5000 rpm for 30 min to remove cells;

the supernatant was subjected to deproteinization by TCA 5% (28). After that EPS was precipitated by adding 5 volumes of cold ethanol. The resulted precipitate was centrifuged, re-dissolved in the deionized water and dialyzed against water for 3 days at 4°C. The supernatant was centrifuged again to remove the insoluble materials and then fractionated using 1, 2, 3 and 4 volumes cold absolute ethanol. The white fractions powder was obtained for further work (33).

Chemical analysis

Total sugars were determined by the phenol-H_2SO_4 method (29) Dubois *et al.,* 1956 using glucose as standard. Uronic acid was measured by the m-hydroxybiphenyl method (34), using glucuronic acid as standard. Sulfate was determined after hydrolysis (formic acid 85% at 100°C, 5 h) using the turbidimetric method (35) with sodium sulfate as standard. A monosaccharides composition of the main fraction (EPS) was determined by high-performance liquid chromatography (HPLC). About 200 mg was hydrolyzed with 2 mL of formic acid 85% at 100°C in a sealed tube for 12 h. The plenty of acid was removed by flash evaporation on a water bath at a temperature of 50°C and co-distilled with water (1 mL× 4) and then evaporated to dryness (36). The monosaccharides contents were quantified by HPLC on a Shimadzu Shim-Pack SCR-101N column (7.9 mm × 30 cm), using deionized water as the mobile phase at flow rate 0.5 mL/min °C (37). The identification of sugar was done by comparison with authentic sugars.

Molecular weight analysis

The weight-average molecular weight (*Mw*) and number-average molecular weight (*Mn*) of EPS were determined on an Agilent 1100 HPLC system equipped with a RI Detector. The EPS was dissolved in 2 mL of solvent and then it filtrated through a 0.45 μm filter prior to injection. The polydispersity index (PI) calculated from the *Mw/Mn* ratio (38,39).

The FT-IR spectrum of EPS

The FTIR spectrum of EPS was determined using a Bucker scientific 500-IR FTIR spectrophotometer (Bucker Co., Ettlingen, Germany) at a range of 4000–400 cm^{-1}. The purified EPS was ground with spectroscopic grade KBr powder and then pressed into pellets for FTIR measurement (40).

Experimental Animals

Male albino rats (160±20 g) from the laboratory stock colony of National Organization for Drug Control and Research (NODCAR) were used in the present study. The animals were kept under normal environmental conditions for one week before the initiation of the experiment. The animals were allowed free access to water and were fed on a basal diet. The animals were kept individually in stainless steel cages at air condition 20-22 °C and relative humidity of about 55%.

Anti-inflammatory activity of EPS

Thirty-six male albino rats were divided into six groups each comprised of six rats. Before any treatment, the thickness of the back paw of each animal was determined using an electronic digital caliper, Germany. EPS samples were prepared by dissolving them in d water. Rats were administered orally with EPS at dose 50 mg/kg, One hour after these administrations, each rat received in its left back paw a sub planter injection of 1% carrageenan suspension (0.1 ml per animal) (41). The thickness of the back paw of each rat was measured at 1, 2, 3 4 and 5 hours after the injection of the carrageenan.

Biochemical investigation

Two grams of paw tissues were taken, rinsed in ice-cold distilled water and immediately placed in three times their volume of cold 1.15% KCl containing 0.2% Triton X-100 and homogenized. The homogenate was centrifuged at 8000 g for 10 min to obtain the supernatant stored at -20°C (42).

Determination of oxidative stress parameters

Measurement of malondialdehyde (MDA)

MDA content was assayed using the thiobarbituric acid (TBA) test as described by

Ohkawa *et al*., 1979 (43). MDA reacts with TBA to form a colored complex. Absorbance was measured at 532 nm to determine the MDA content. The specific activity is defined as nm/g tissue.

Measurement of reactive oxygen species (ROS) content

ROS was estimated according to Vrablic *et al.*,2001 (44). A modified version of a previously described assay for the intracellular conversion of nitro blue tetrazolium (NBT) to formazan by superoxide anion was used to measure the generation of reactive oxygen species

Measurement of Nitric Oxide

NO was estimated according to Wang *et al.*, (2002) (45) using Griess reaction. Briefly, to an aliquot of supernatants, Griess reagent was added and the colored product formed was read at 540 nm. NO was quantified using a standard curve.

Determination of reduced glutathione (GSH)

Levels of GSH were assayed by the method of Beutler et al. (1963) (46). Briefly, the deproteinization of homogenate was made by 10% trichloroacetic acid and centrifuged at 3500 rpm for 10 min. 50µL supernatant was mixed with 0.32 mol/L disodium hydrogen phosphate and 0.04% 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) solution. The yellow-colored substance formed by the reaction of GSH and DTNB was measured at 412 nm. The results were expressed as GSH mmol/g tissue.

Determination of Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity in kidney homogenate was determined according to the method of Minami and Yoshikawa (1979) (47). This method is based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan color development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development). SOD activity is expressed as U/g tissue.

Measurement of catalase (CAT)

The CAT activity was measured according to the method described by Aebi (1984) (48) by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25°C. CAT activity is expressed as mmol/g tissue.

Protein expression of IL-6

IL-6 contents were analyzed using a commercially available ELISA kit (BioSource International, Inc., Camarillo, CA, USA) according to the instructions of the manufacturer in the supernatant.

Statistical analysis

Statistical analyses were performed with SPSS software and were calculated using one-way ANOVA followed by Post Hoc Duncan. $P<0.05$ was considered to indicate a statistically significant result.

3. Results and Discussion

Isolation, screening, and identification of the bacteria-producing EPS Thirty strains from a total of seventy single colonies were chosen from separate marine specimens based on their morphological variations in agar plates. Finally, G6 strain was selected as one of the best EPS' producers according to the yield of polysaccharide per liter (2.6 g / L). The most powerful bacterial isolate G6 was recognized with morphological and physiological features on a molecular basis (16s rDNA). With 99 percent resemblance to **Bacillus axarquiensis** NRC G6, G6 was discovered to belong to the genus Bacillus. The nucleotide sequence was placed under the KU945823 accession number in the GeneBank (http:/www.ncbi.nlm.nih. gov). Table (1) and Fig. (1), respectively, show morphological and physiological features along with the phylogenetic tree of *Bacillus axarquiensis*.

Table 1. Morphological, cultural and physiological characteristics of G6 isolate

Character	**Result**
Gram's stain	Positive
Spore formation	Endospore forming
Cell shape	round-ended rods -shaped
Motility	Motile
Colony shape	the colonies are cream-coloured, slightly irregular in shape and bulge upward
Oxygen requirement	Aerobic
pH and salinity	Haloalkaliphilic
Catalase production	Positive

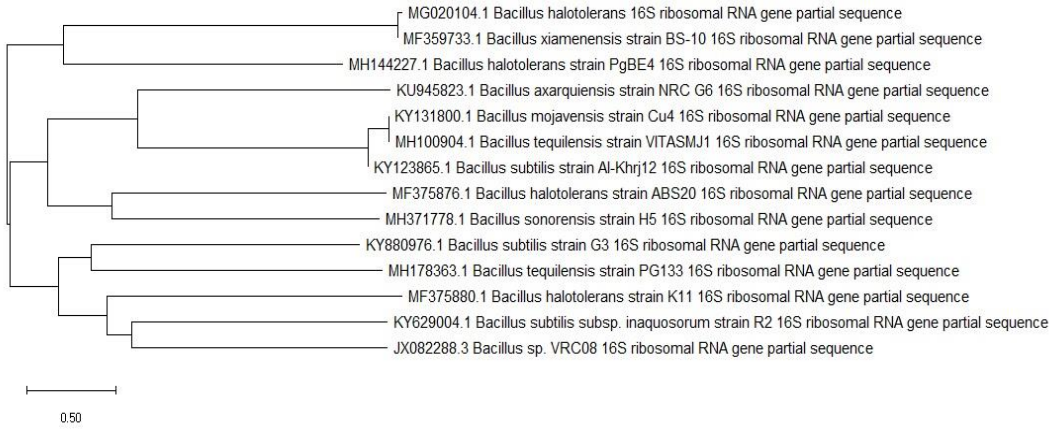

Figure 1. Phylogenetic tree based on 16S rDNA gene sequencing, showing the phylogenetic relationship of ***Bacillus axarquiensis*** within representative species of the genus *Bacillus*

EPSBa3 isolation and chemical structure

Many marine bacteria, such as *Edwardsiella tarda*, *Paenibacillus polymyxa* and *Alteromonas*, could generate EPSs (49). Bacillus axarquiensis NRC G6's EPS output was 2.6 g / l of medium development after 5 days. After fractionation with ethanol precipitation from crude EPS, the primary fraction EPSBa3 was achieved. The EPSBa3 has been gathered for further structural assessment. It emerged as a white-yellow powder, with the Bradford test having an adverse response. The data that the UV spectra did not detect absorption at 260 and 280 nm stated that RNA, DNA and/or protein were not present. EPSBa3 contained 13.9% uronic acid as assessed by colorimetric m-hydroxydiphenyl and 7% sulfate. HPLC determined the structure of

monosaccharides of EPSBa3 hydrolysate, in which glucuronic: mannose: galacturonic: glucose and xylose were recognized in the hydrolysate and their molar proportions were 1:1:3:3:3, respectively. Smiderle, *et al.* (2008) and Silveira*et al.* (2015) (50,51) were found to have a marked antinociceptive impact when tested in mice, showing that mushroom heteropolysaccharides may also have therapeutic characteristics such as anti-inflammatory activity. The EPSBa3's Mw, Mn and polydispersity index (PI) was evaluated by chromatography of gel permeation. The EPSBa3 was widely dispersed (PI) 1.16 in the GPC chromatogram and had a total Mw of 1.499 $X10^6$ g / moL and Mn of 1.29 X 10^6 g / moL (Fig.2). Bacillus sp.1-450's molecular weight of EPS was 2.2103 KDa and the functional groups in the EPS chains are key determinants for biological operations (52). Sulfate groups are generally not found in microorganism EPSs, whereas they are found in all halophilic bacterial EPSs and in several marine bacterial EPSs and brown and red algae PSs in cell walls (53-56).

Sulfated EPSs are of large potential interest in medicine because they have a number of bioactive properties (57,58). Band intensity at 3855.97 and 3432.67 cm−1 bands showed OH axial deformation that matched the intermolecular and intermolecular hydrogen bond (59,60). The soft band for the secondary and primary (CH2) bands at 2934.16 cm−1 were ascribed to the axial deformation of the CH (61-60). New bands emerged in the area of 1644.98 cm−1 correspondings to the stretching vibration of C-O and COO groups. CH2 and OH bonding (Fig. 3) were depicted by the absorptions around 1433.82 cm−1. Strong absorption at 1261.22cm−1 was subjugated by vibration-stretching glycosidic linkage (C–O–C) (62). In addition, the glucosyl residue B-pyranose shape was stated by the band at 928.56 cm−1. The FT-IR assessment, therefore, proposed that the EPSBa3 could very well belong to the B-anomeric setup (63).

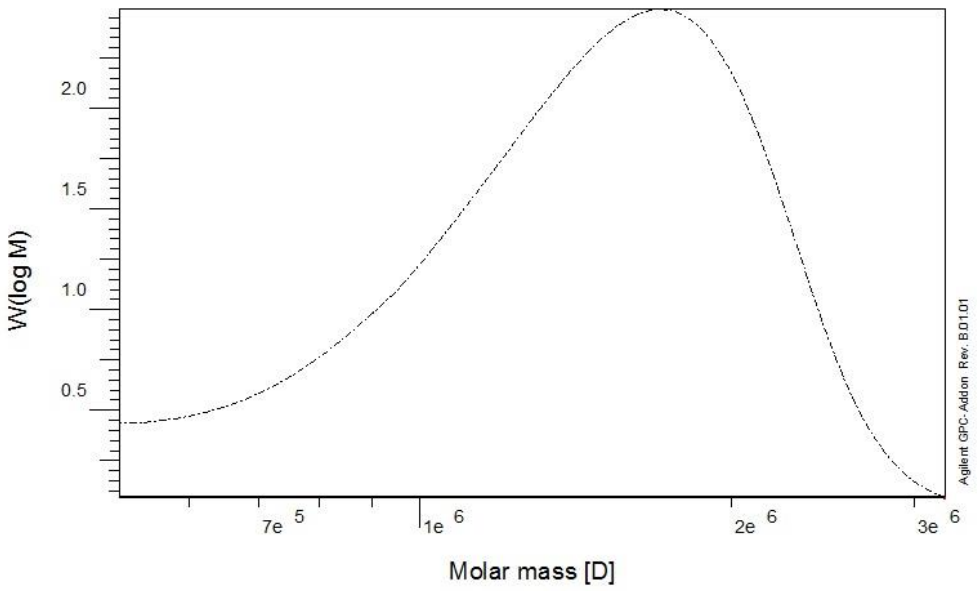

Figure 2. Molecular weight distribution of EPS Ba3 from *Bacillus axarquiensis* NRCG6

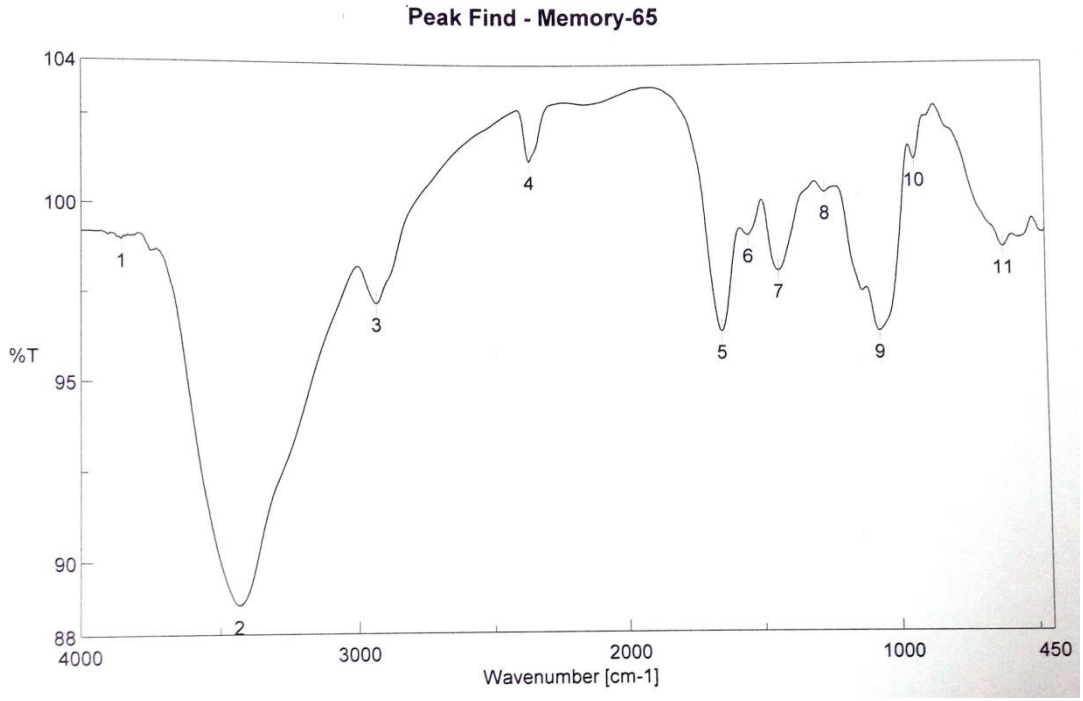

Figure 3. FT-IR spectrum of EPSBa3 from *Bacillus axarquiensis* NRC G6

Antioxidant activity

EPSBa3's antioxidant activity at distinct levels (200, 400, 600, 800 and 1000 µg / ml) was assessed as free radical DPPH scavenging and its outcomes are shown in Figure (4). It is evident that the largest scavenging activity for EPSBa3 was discovered at 1000 µg / ml for 60 min. followed by 1000 µg / ml (91.3%), 45 min. (90.9%), 800 µg / ml for 60 min. (85.8%) for 45 min. and 1000 µg / ml (84.5%) for 30 min. In diminishing order. The EPSBa3 was discovered to have DPPH radical-scavenging activity with an IC50 value of 400µg / ml in the in vitro antioxidant assay. The findings are comparable to those of Zhang *et al.,* (64) who discovered that the sulfated galactan fraction F1 (isolated from red seaweed, Porphyrahaitanensis) had substantial in vitro antioxidant activity, Seng *et al.,* (65) discovered that polysaccharide extracts from Ganodermatsugae had excellent antioxidant characteristics except for their capacity to scavenge against hydroxyl radicals and could be good candidates. As a fresh dietary supplement and functional food, Asker *et al.,* (66) assessed the radical scavenging assay (RSA) antioxidant activity of CPS, CPSI, and CPSII in vitro. CPSI fraction showed the greatest antioxidant activity among the three fractions, EPS analysis showed elevated antioxidant activity and this could explain the pharmacological foundation for ill avoidance, inflammation, and atherosclerosis of polysaccharides (67).

Anti-inflammatory activity

Inflammation is the body's complicated biological reaction to diseases, irritations or other wounds, and harm to cells. Inflammation plays a significant role in multiple illnesses such as rheumatoid arthritis, asthma, inflammatory intestinal disease, neurodegenerative illnesses, and cancer (68). Several pro-inflammatory mediators, including IL-6, IL-12, TNF, COX-2 and inducible nitric oxide synthase (iNOS) are published during an inflammatory reaction (69). We examined the in vitro anti-inflammatory impact of EPSBa3 in rats using the carrageen-induced paw edema model in this research.

Carrageen-induced paw edema is a model commonly used to screen anti-inflammatory drug impacts (70).

Effect of EPSBa3 on carrageenan-induced paw edema in rats Paw thickness was improved in a time-dependent way following carrageenan injection in rats (Table 2). Carrageenan administration improved paw thickness at 1 hour and was maximum after 5 hours. However, after therapy with carrageenan, paw edema ($P<0.05$) was considerably decreased after therapy with EPSBa3 at 3, 4 and 5 h (Table 2).

The current research shows that EPS plays an important role as a protective agent against the growth of acute inflammation induced by carrageenan. The formation of edema in the paw is the consequence of a synergy between multiple inflammatory mediators that increases lung permeability and/or blood flow mediators (71). Inflammatory processes are the organism's physiological reaction to various stimuli such as trauma, diseases or mechanisms of immunology. During inflammation, the arachidonic acid cascade is extremely activated, leading in eicosanoid formation, and it is mediated by cyclooxygenase and5-lipoxygenase enzymes (72). It is thought that carrageenan-induced rat paw edema is biphasic. The first stage (1 h) includes the release of histamine and serotonin and the second stage (more than 1 hour) is due to the release of drugs similar to prostaglandin. Based on this, a cyclooxygenase inhibition or the exercise of anti-oxidative characteristics may explain the second stage (73).

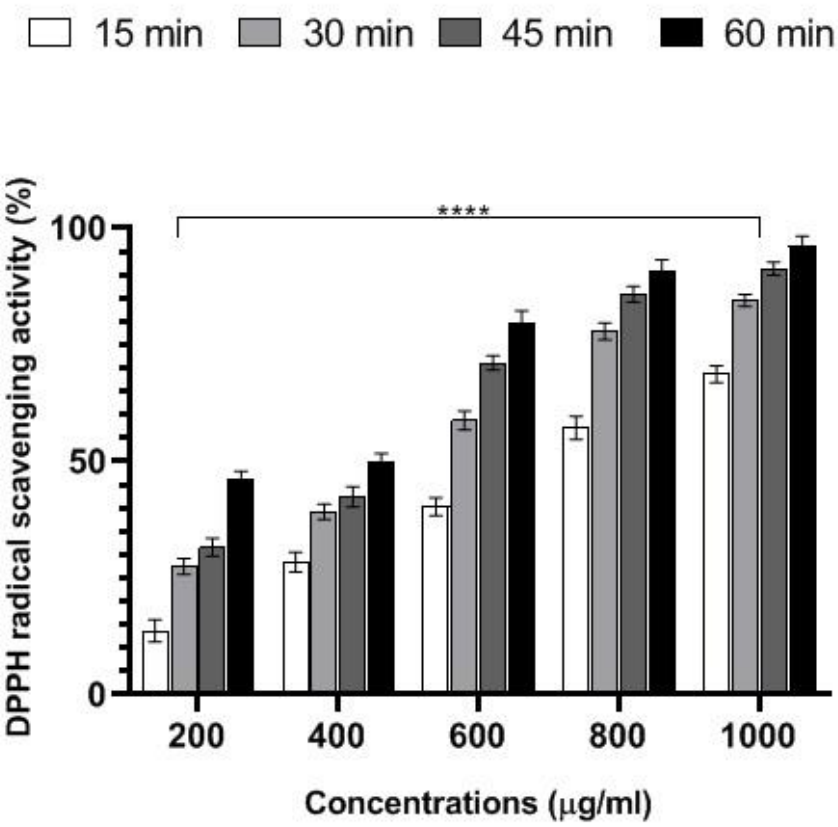

Figure 4. The DPPH radical-scavenging activities of EPS Ba3

Table 2. Anti-inflammatory effects of EPSBa3 on carrageenan-induced hind paw edema in rats

Groups	Swelling (thickness) (mm)				
	1 hour	2 hours	3 hours	4 hours	5 hours
Carrageenan	11.16±1.06[a]	15.41±1.33[a]	18.21±1.12[a]	18.11±1.7[a]	16.77±1.17[a]
IM (10 mg/kg)	11.19±1.03[a]	14.71±1.01[a]	7.60±0.54[b]	8.81±0.76[b]	7.14±0.56[b]
EPSBa3 (50 mg/kg)	11.08±1.05[a]	15.14±1.15[a]	12.54±1.10[c]	12.24±1.07[c]	10.32±0.30[c]

Values are expressed as means±SEM of six rats per group. Values on the same column not sharing the same superscript letters were significantly different (P<0.05), IM: indomethacin.

Pow tissues were analyzed for biochemical parameters such as ROS, NO, GSH and LPO (Fig. 5) and SOD and CAT operations after 5 h of carrageenan paw injection (Fig. 6). NO, after carrageenan therapy, the concentrations of ROS and LPO (P<0.05) were considerably increased in the paw tissues. However, in rats treated with carrageenan, GSH content and SOD and CAT operations were reduced. Significantly improved pretreatment of EPS (P<0.05) NO, ROS and LPO, GSH and SOD and CAT (Fig. 5and 6). As shown in Fig. 7 after the injection of carrageenan, the protein expression of IL-6 was considerably improved (P<0.05) in the paw tissues, whereas EPSBa3 improved the expression of IL-6.

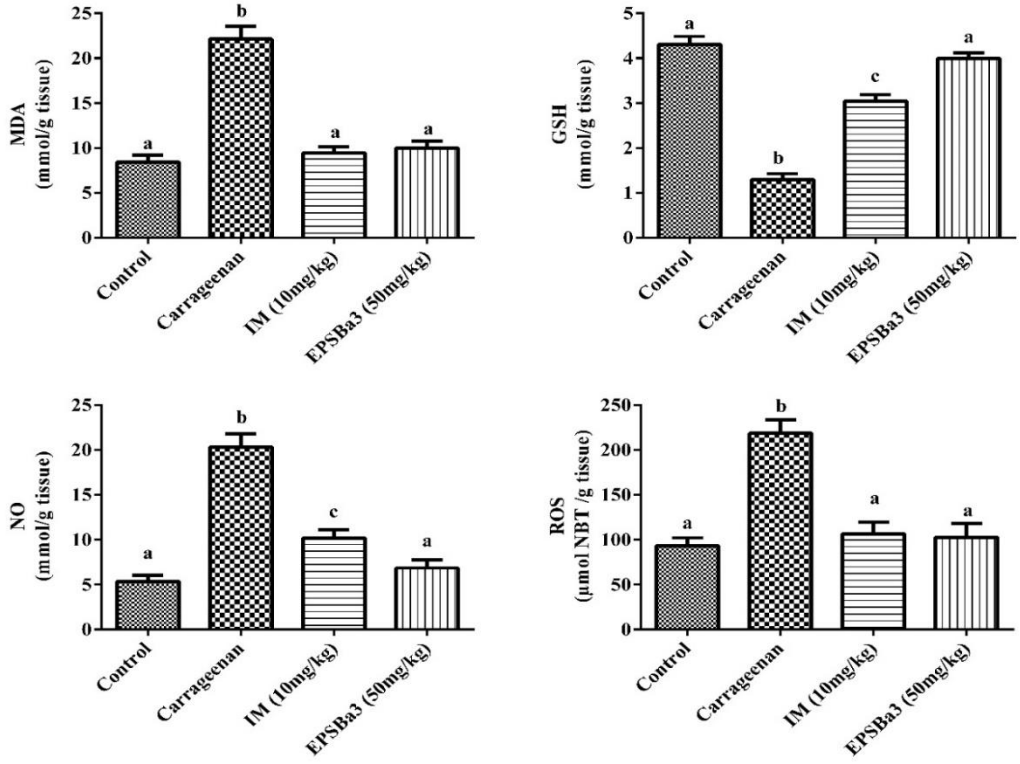

Figure 5. Effect of EPSBa3 on changes in MDA, GSH, NO and ROS levels in carrageenan-induced paw edema inrats. Values are expressed as means±SE of six rats per group. Values on the same column not sharing the same superscript letters were significantly different (P<0.05), IM: indomethacin.

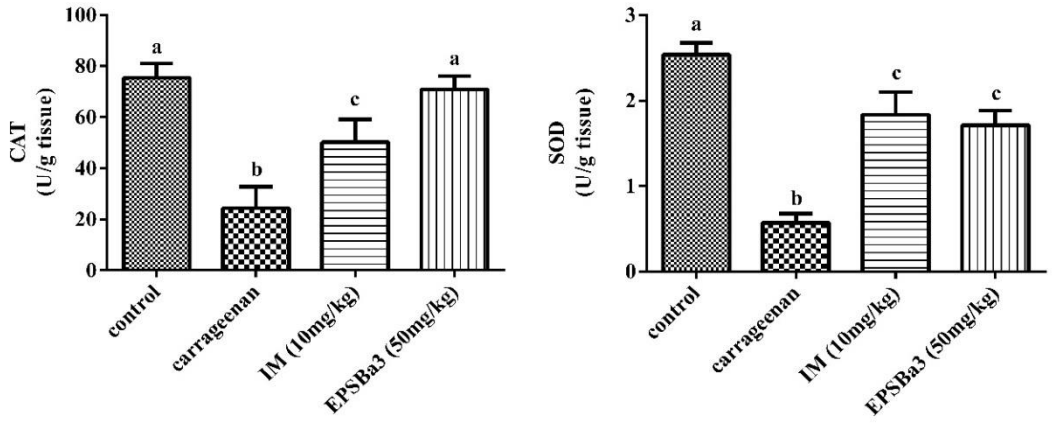

Figure 6. Effect of EPSBa3 on changes in CAT and SOD activities in carrageenan-induced paw edema inrats. Values are expressed as means±SE of six rats per group. Values on the same column not sharing the same superscript letters were significantly different (P<0.05), IM: indomethacin

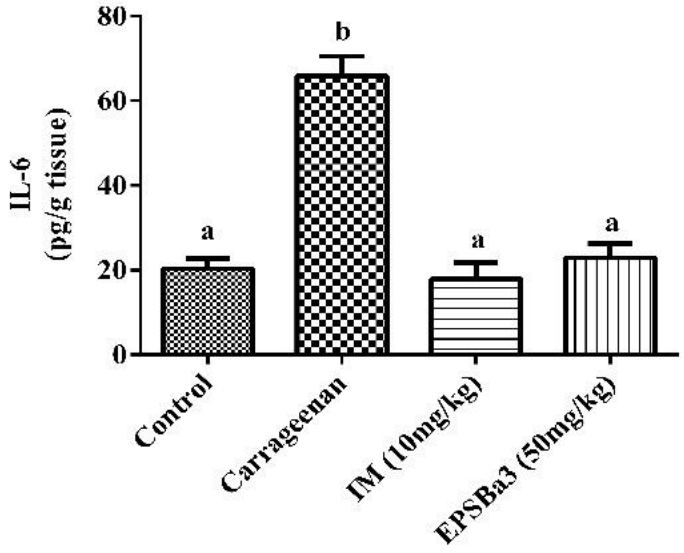

Figure 7. Effects of EPSBa3 on IL-6 protein expressions of edema paw in rats Values are expressed as means±SE of six rats per group. Values on the same column not sharing the same superscript letters were significantly different (P<0.05), IM: indomethacin.

The antioxidant activities (SOD and CAT activities) were assessed in all-paw tissues to investigate the impacts of antioxidant defenses on the acute inflammation system. In this research, it was discovered that SOD in carrageenan-induced paw edema was enhanced by EPS in which the SOD concentration decreased considerably. Catalase is a highly reactive enzyme that reacts to water and molecular oxygen with H_2O_2 and, by donating hydrogen, can form methanol, ethanol, formic acid or phenols (74).

We found that all doses of EPS and indomethacin improved catalase activity in the current research. Our findings are in agreement with (75,76) Kataoka *et al.* (2012) and Golechha *et al.*, *(*2014) who discovered a significant reduction in catalase activity in carrageenan ($P<0.001$). The pathogenesis of inflammatory processes involves lipid peroxidation (77). In our research, lipid peroxidation (LPO) was increased in carrageenan-treated rats in accordance with Ahmed and Bastawy (2014), Ahmed and Eid (2015) (78,79). By reducing the content of LPO, EPS enhanced this impact. Antioxidant and anticancer impacts of EPS have been discovered (80). NO is a powerful vasodilator made from inducible-nitric oxide synthase that has been engaged in maintaining inflammatory reactions, including increased vascular permeability and edema through alterations in local blood flow (81) (Moncada and Higgs, 1993). Furthermore, NO can respond to peroxynitrite with superoxide anion, a powerful oxidized molecule that can cause lipid peroxidation and cell harm (82) (Rubbo *et al.*, 1994). It is well known that the release of IL-1, IL-6, and IL-8 was stimulated by TNF-α. IL-6 improved the COX in turn. In the current research, after carrageenan injection, therapy with EPS considerably inhibited the paw edema (3-5 h). In addition, EPS considerably reduced the expressions of IL-6 proteins; this stated that EPS had anti-inflammatory impacts by inhibiting IL-6 and growing antioxidant enzymes (CAT and SOD) and GSH together with reducing the content of NO, ROS and LPO. Exopolysaccharide's anti-inflammatory activity was evaluated by inhibiting macrophage production of nitric oxide (NO), inducible synthase of nitric oxide (iNOS), and5-lipoxygenase (5-LOX). This exopolysaccharide significantly ($p<0.05$) inhibited dose-dependent levels of iNOS-induced lipopolysaccharides expression in the neurons. The protective impact of EPSBa3 on oxidative stress caused by carrageenan may be due to its antioxidant activity by reducing GSH and increasing LPO and ROS. EPS separated from *Micrococcus luteus* (83) and *Bacillus subtilis* SH1 (84) has concentration-dependent DPPH radical scavenging activity.

Intense edema was produced in the carrageenan group, characterized by infiltrates of inflammatory cells as compared to the carrageenan untreated group (normal control) (Table 3). EPSBa3 (50 mg/kg) or indomethacin (10 mg/kg) groups treated with carrageenan there were edema decreases as well as decreases in inflammatory cell infiltration.

Table 3. Histological analyses of rats paw in the carrageenan-induced edema model

Groups	**Histological score**		
	Pannus formation	**Inflammatory cells infiltration**	**Oedema**
Control (untreated)	-	-	-
Carrageenan	++	+++	+++
IM (10 mg/kg)	-	+/+	+/-
EPSBa3 (50 mg/kg)	-	++	++

(-) no (+) mild (++) moderate (+++) severe

Conclusion

In conclusion, the findings showed that the *Bacillus axarquiensis*-isolated EPSBa3 was purified and described using several distinct analytical methods, and the EPSBa3's in vitro antioxidant property was also assessed. The EPS consisted mainly of glucuronic: mannose: galacturonic: glucose and xylose, respectively, in a molar ratio of 1:1:3:3:3 The proportion of uronic acid and sulfate was 13.9% and 7% respectively. The average molecular weight (Mw) and molecular amount (Mn) of FT-IR were estimated at 1.499 $X10^6$ and 1.29 X 10^6g / mol respectively. The EPSBa3 had an anti-inflammatory impact on paw edema induced by π-carrageenan. EPSBa3's anti-inflammatory impact may be due to a reduction in IL-6 concentration and a reduction in NO material to avoid edema. It could also be through growing antioxidant enzyme activity (CAT and SOD), where free radicals (ROS) were scavenged and MDA levels decreased. Therefore, to avoid inflammatory diseases, EPSBa3 could be used as a source of dietary supplements.

Conflict of interest

The authors declare no conflict of interest.

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