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Production and Characterization of Extracellular Polymeric Substances by marine *Halomonas* sp. NASH isolated from Wadi El-Natroun

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Abstract

Halophilic micro-organisms often synthesize and produce extracellular polysaccharides (EPS), whose physical, chemical properties and material properties vary greatly from each other. The extracellular polysaccharide (EPS) development of Halomonas sp. MN795630 strain type halophilic bacterium (NASH) was investigated and whether biotechnological applications were feasible. After 168 hours of incubation, 4 g/L of EPS was produced and all elements from the medium were completely used during the growth. Sucrose has been identified as the most favorable carbon source for production of EPS and maximum production (6 g/l). Beef extract level was shown to be the best for EPS production among different nitrogen sources. Optimum production of EPS (10 g/L) were achieved by supplementing the medium with 4M NaCl, pH adjusted at 9 and the medium was inoculated with 7% initial inoculum. The purified EPS were characterized chemically. Fourier transform infrared (FTIR) spectrophotometer was observed in several functional groups. EPS also demonstrated an significant inhibitor of *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 9027 (20.4 and 14.7 mm), respectively. EPS show satisfactory results when applied as anti-oxidant, anti-inflammatory and emulsifier.

Keywords: Exopolysaccharides, *Halomonas* sp. NASH, Optimum conditions, Antimicrobial activity, Antioxidant activity, Anti-inflammatory

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INTRODUCTION

Exopolysaccharides microbial are polysaccharides that are extracellularly formed as capsules or slimes. Such microbial EPS are typically categorized into 2 broader classes: homopolysaccharide consisting of a single monosaccharide unit and a heteropolysaccharide with two or more monosaccharide units. In nature, microbial EPS is non-toxic , biodegradable and renewable[1]. They play a major role in the defense of desiccation[2] and are also useful in the formation of biofilms and also useful in forming biofilms[3]. Use as gelling agents, biosurfactants, emulsifiers, viscosities[4,5], biosorbants[6,7] Antimicrobials, Anti-Cancer Agents and Antioxidants[8-10].

EPS is comparatively less reported from extremophilic microorganisms, especially halophilic ones. The generation of EPS from halophilic bacteria in intense marine ecosystems and its biological activities have been investigated[11]. Several workers have recorded the extracellular polysaccharides formed by halophilic archaea and bacteria, and the members of the *Halomonas* genus were classified as the most potential producers[12,13]. The literature survey has shown clearly that the knowledge on the extracellular polysaccharides development and characterization by various halophilic micro-organisms isolated from hypersalin environments is not adequate[12,14]. However, the demand for extracellular polysaccharides of halophiles with better properties than existing ones is increasing. The present study was carried out with the goal of producing EPS by *Halomonas* sp. NASH, optimizing some environmental parameters for the production of EPS by *Halomonas* sp. NASH, characterizing the EPS produced and finally evaluating its efficacy as an antimicrobial, antioxidant and as a biosurfactant.

MATERIALS AND METHODS

Microorganisms and cultural conditions

Halomonas sp. NASH was provided by Dr. Asmaa M. Youssif (Botany and Microbiology Department, Faculty of Science, Alexandria University) and isolated from sediment sample in Wadi El-Natroun lakes, and set in the Genbank as *Halomonas* sp. NASH with accession number MN795630. This halophilic isolate was maintained on IRAM media[15] with the following composition (g/L); Magnesium sulfate, 20; Potassium chloride, 5; Calcium chloride, 0.2; Yeast extract, 4; Peptone, 5; Sodium chloride 223.3 (4M). The pH of the media was changed to 8 before sodium bicarbonate sterilization. The medium was sterilized immediately after preparation at 121°C for 20 minutes. For solid media, 20g agar was added. Strain was incubated at 35-37 ℃ for 7 days. The isolate was stored in 20% (v/v) glycerol at -20°C for subsequent investigation.

EPS production

Inoculum was prepared for the production of EPS by growing the culture in IRAM medium. 250 ml Erlenmeyer flask containing 50ml of medium consisting of (g/l): Magnesium sulfate, 20; Potassium chloride, 5; Calcium chloride, 0.2; Yeast extract, 4; Peptone, 5; Sodium chloride 223.3 (4M). The pH of the media had been set at 8 before sodium bicarbonate sterilization. This flask was inoculated on an IRAM medium grown with old stock culture. The flask was incubated in a 35-37°C, 200 rpm rotator incubator shaker for 7 days and then was centrifuged to obtain filtrate and extract EPS from it.

Correlation between growth & EPS production at different growth phases

A seed culture was prepared for the not each strain by inoculating 250ml conical flask containing a 50 ml IRAM medium with a loopful of the strain and rotary shaker (200 rpm) incubation, until the growth was OD_{600} = (0.8-1). Inoculate flasks each containing 50 ml of the sterilized optimized medium in regular 0,5 ml inoculum from the seed culture previously prepared. Flasks were incubated shacked at 200 rpm at 37°C for 7 days. Samples were taken at time intervals to measure growth at wave length 600nm. Flasks were centrifugal for 10 minutes at 10,000 rpm and EPS was removed with supernatant.

One-time variable (OVAT) method for optimizing EPS production

The effect of different parameters including carbon and nitrogen sources, concentrations of inoculum, pH and NaCl were the parameters investigated by *Halomonas* sp. NASH for optimum production of EPS. The NASH strain was grown in medium IRAM and incubated at 37°C, 120 rpm for 7 days. Both experiments were performed in 250 ml Erlenmeyer flask using a 50 ml IRAM medium. EPS was measured at the

end of each trial, and gravimetrically calculated. The effect of carbon sources and nitrogen on the development of EPS was assessed, organic and inorganic sources. Glucose, sucrose, lactose, maltose, fructose, galactose, glycerol and sodium citrate, respectively, which are organic and inorganic sources of carbon. Ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrate, yeast extract, tryptone, beef extract and casein hydrolysate, which were representative of inorganic and organic nitrogen sources. The effect of pH on the output of EPS was achieved by changing the medium pH at 6, 7, 8, 9 and 10. The results of different NaCl (0, 1, 2, 3, 4 and 5 M) concentrations have also been studied. *Halomonas* sp. NASH tested the effect of inoculum size on EPS production by inoculating the production medium with various inoculum sizes from 1 to 10 percent (v/v) and shaking at 37°C for 7 days[16].

EPS extraction and Purification

The fermented cells were harvested after seven days, and the cell suspension was heated to 100°C for 10 min to inactivate the enzymes. The suspension has been cooled to room temperature and centrifuged for 20 minutes to extract biomass at 4,000 rpm Sevage reagent (chloroform: n-butanol at 5:1 v/v) was further treatment of the crude solution three times for removal of protein. EPS was precipitated with cold ethanol (three times volume) and left at 4°C overnight. Centrifugation was used for the precipitation at 10,000 rpm for 15 min and was dissolved in Milli Q water. It was subsequently enveloped into a dialysis bag (12-14 KDa) and dialysed with Milli Q water at 4°C for 48 hours for partial purification[17].

Characterization of partial purified EPS

Carbohydrate and protein contents

According to the Dubois method the total carbon content was measured[18]. Adding 25 µl of 85 percent phenol to one ml of the sample solution, accompanied by adding 2.5 ml of concentrated sulphuric acid, shaking the mixture after each addition. The mixture was put in a boiling water path (100°C) for 10 min, then cooled to room temperature before reading spectrophotometrically at 488 nm against a blank of one ml distilled water The regular solution of glucose was used to produce a calibration curve which was used to measure the sample carbohydrate content. Using the method described by[19], the total soluble protein was determined quantitatively. The 100 µl sample was thoroughly mixed with 3 ml of the alkaline solution and allowed to stand in room temperature for at least ten minutes. An amount of 0.25 ml of the diluted Folin-Ciocalteau reagent (2:1, v/v) was added rapidly to the mixture and mixed immediately. The mixture was left to stand for 30 min. Thereafter, the extraction was measured at 760 nm against the blank. Using a calibration curve, constructed with Bovine Serum Albumin as a reference, the protein concentration of the unknown samples was estimated.

FT-IR analysis

In transmission mode, The FT- IR (BRUKER, Vertex 70) and OPUS pellets in the 4000 400 cm^{-1} range were used to obtain IR transmission spectroms using potassium bromide (KBr)[20].

Biotechnological applications of EPS

Antimicrobial activity

For EPS antimicrobial potential determination, two tested Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, as well as two Gram negative bacteria; *Escherichia coli* ATCC 19404, *Pseudomonas aeruginosa* ATCC 9027 and one yeast strain *Candida albicans* ATCC 10231 were selected as indicator strains. These strains were cultivated in the medium Lauria Broth (LB) overnight at 30 °C. The culture was then diluted by LB media and incubated at an acceptable temperature at 5 hours under a 200-rpm agitation to an initial OD 600 of 10^6 CFU/mL indicator strain. The measurement inoculum for the indicator strain was based on the cell counts 'growth curve and optical diameter. By the agar diffusion test, the antimicrobial activity was detected[21]. 50 mm of agar nutrient are poured on all plates inoculated by a predictor microorganism. Upon solidification wells are punched and two drops of sterile water agar have been applied to each of their bodies with a 055 cm corkborer. 100 ul of filtered EPS were transferred to a well after sterilization by using 0.22 µl filters. All the plates were incubated for 24-48 h at the appropriate temperature. The clear zone radius around each well (Y) and the well radius (X) are measured linearly in mm after incubation, whereby Y2 is divided by X2 and determines an absolute unit (AU) for clear zone. According to the following equation: $AU= Y^2/X^2$,

The absolute unit of each EPS which implies a positive antibiotic action result[22].

Antioxidant activity

Free radical 1,1-diphenyl 2-picrylhydrazyl scavenging activity (DPPH) was assessed as a measure of antioxidant activity using the[23] approach of partially purified exopolysaccharide. Generic polysaccharides were used as a reference compound, such as ascorbic acid. Scavenging effect (%) = (Ac –As)/Ac× 100. Ac is control absorption, As is the sample absorption.

Anti-inflammatory assay

The Mizushima technique was tested in albumin denaturation inhibition[24],[25] to test the anti-inflammatory function.

Biosurfactant

Applying the same amount of paraffin oil to an EPS sample (v/v), then vortexing with high speed (2 min) and permitted to stand at 24 hours, the EPS emulation index (E24) was calculated. The percentage of E24 was calculated using the following equation: E24 = Height of emulsion formed (cm) ×100 / Total height of solution (cm)[26].

RESULTS AND DISCUSSION

Correlation between growth & EPS production at different growth phases

The correlation between growth and EPS production was planned to be investigated at different growth phases. In this experiment the growth of the bacterium was controlled in incubated at 37°C in batch cultures in IRAM medium shacked. Standard inoculum (1%) is taken from seed cultures previously prepared (OD600~ 0.8-1) and used to inoculate 50 ml of media in 100 ml flasks. Then, the flasks were shaken at 120 rpm at 37°C. Timely samples were used to measure spectro-metrical growth at wavelength 600 nm and output of EPS at regular intervals. Data in Fig. 1 reveal that cells of *Halomonas* sp. NASH entered the exponential phase of growth after 2 days of incubation and the stationary phase of growth after 9 days of incubation. EPS was growth phase dependent, low production was detected at the beginning of exponential growth, and increased exponentially with bacterial growth till it become constant at stationary phase. The maximum EPS production was recorded in the middle of exponential phase recording 4g/l.

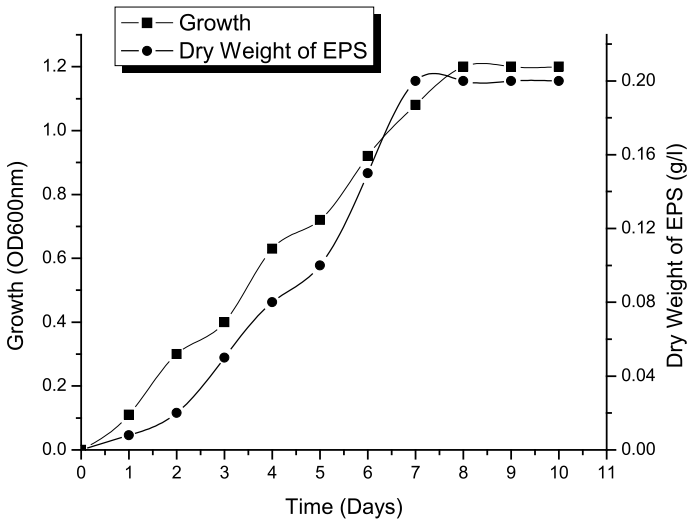

Fig. 1. Correlation between growth of *Halomonas* sp. NASH grown in IRAM and incubated at 37°C under shaken conditions for 10 days and EPS production

One-time variable (OVAT) method for optimizing EPS production

Effect of different carbon sources

Halomonas sp. NASH has shown that the addition of sucrose, glucose, and sodium citrate at the average level of 1% has resulted in different bacterium rates of growth and EPS production. Sucrose was most effective in EPS production (6g/l) followed by glucose (5g/l) and sodium citrate (4.4g/1). However, compared to sucrose, glucose

and sodium citrate exerted maximum influence on the growth of the isolate. Carbon sources like maltose, glycerol, fructose, lactose along with galactose produced poor to moderate EPS (Fig. 2). Similar results were obtained by Biswas and paul[27] who mentioned that glucose was one of the carbon sources which affected on the growth and production of EPS by *Halomonas xianhensis* SUR308. Also, Llamas[13] proved that EPS production and maximum growth by *Halomonas almeriensis* was obtained by using 1% glucose as a carbon source. Glucose has been reported to influence EPS production in a number of bacterial species including *Halomonas* spp, glucose at >1.0% level retarded the EPS production[28-30].

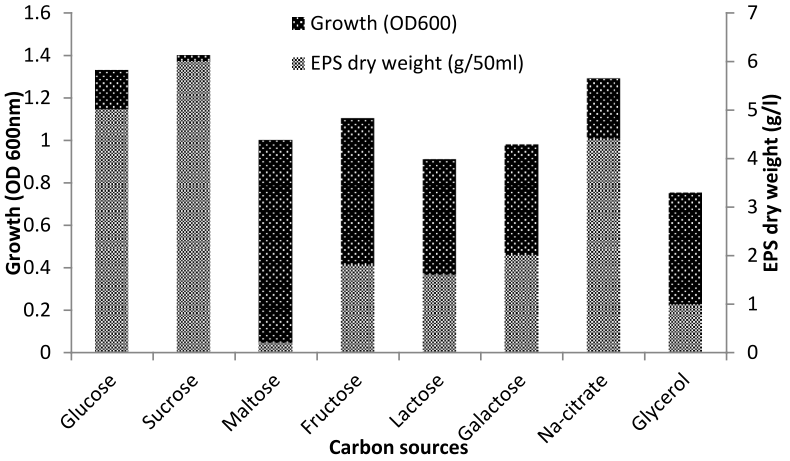

Fig. 2. Effect of various sources of carbon on growth and EPS production by *Halomonas* sp. NASH. Fermentations were carried out in IRAM medium supplemented with 1% carbon source under continuous shaking (200 rpm) at 37°C and pH 8 with 1% (v/v) initial inoculum for 7 days.

Effect of different nitrogen sources

Ammonium sulfate, peptone, sodium nitrate, urea and yeast extract, however, are known to encourage both a rate of growth and the EPS production due to the presence of organic nitrogen sources[31]. From the results as illustrated in Fig. 3, it was evident that all the organic nitrogen sources like peptone, yeast extract, casein hydrolysate, beef extract, tryptone have positive influence on the EPS synthesis. In presence of organic nitrogen sources, the isolate was capable of accumulating remarkable amounts of EPS (5–7g/L). However, amongst the different organic nitrogen source, beef extract was most preferred one which led to the production of 7 g/L of EPS. This may be due to vitamins and cofactors present in organic nitrogen sources which could have played the key role in inducing growth and EPS production[32]. Biswas and paul[27], proved that organic nitrogen sources promote both growth rate of *Halomonas xianhensis* SUR308 and the EPS production than inorganic nitrogen sources especially casein hydrolysate gave maximum EPS production reached 6.5g/l. Gu and his team also said that the production of EPS from *halophilic Kocuriarosea* ZJUQH was influenced by organic nitrogen such as peptone, yeast extract and casein hydrolysate[33].

Effect of pH

The figure 4 showed that both growth and output of EPS were significantly increased by growing the medium pH until 9. The maximum output of EPS at the pH 9 was reached, reaching 7.5g/L and gradually decreased by a pH rise of over 9. This is means that EPS production by *Halomonas* sp. NASH preferred alkaline condition. On the other hand, Biswas and paul[27] mentioned that maximum EPS production by *Halomonas xianhensis* SUR308 was 2.99g/l at pH 7.5. Also, Gu et al.[33] stated that maximum EPS production by halophilic *Kocuria rosea* was obtained at neutral pH. While[16], proved that pH 6 was the suitable pH for the maximum production of EPS (23g/l) by *Halomonas smyrnensis* SVD III.

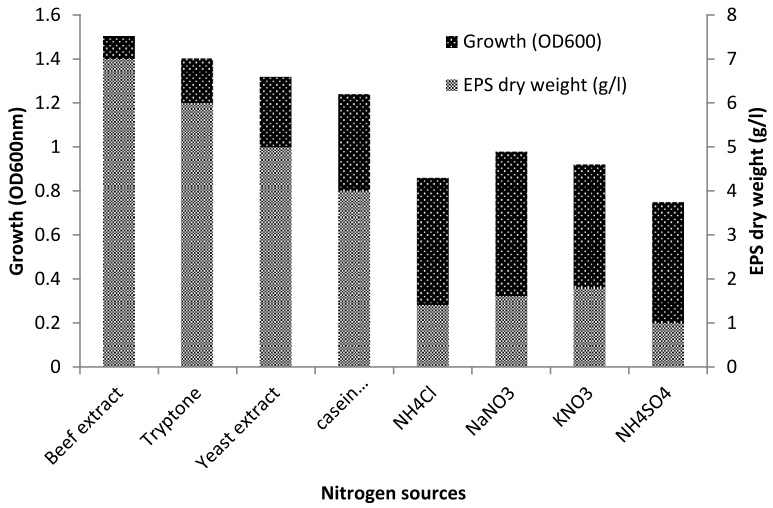

Fig. 3. Effect of different sources of nitrogen on growth and EPS production by *Halomonas* sp. NASH. Fermentations were carried out in IRAM medium supplemented with 9g/l nitrogen source under continuous shaking (200 rpm) at 37°C and pH 8 with 1% (v/v) initial inoculum for 7 days.

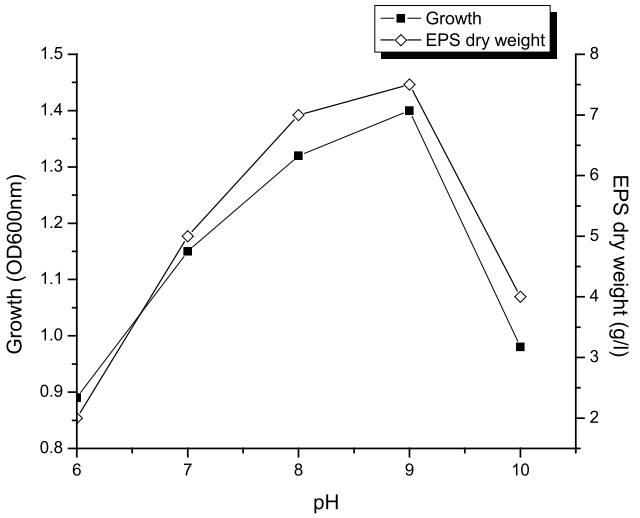

Fig. 4. Effect of pH on growth and EPS production by *Halomonas* sp. NASH. Fermentations were carried out in IRAM medium under continuous shaking (200 rpm) at different initial pH ranging from 6-10 and temperature at 37°C with 1% (v/v) initial inoculum.

Effect of NaCl concentrations

As a halophilic organism, the isolate NASH showed a wide degree of tolerance to NaCl for growth and EPS production was more or less constant in the range of 2 to 4M NaCl in the medium, where by EPS production varied from 6.5 to 8 g/L. It was evident from the results (Fig. 5) that the production of EPS was maximum (8 g/L) at 4M NaCl. Similarly[16], mentioned that maximum production of EPS (23g/l) by *Halomonas smyrnensis* SVD III obtained by adding 20% NaCl in the medium. Arias[28] reported that optimum salt concentrations for EPS production by *Halomonas maura* was 2.5%, While the best for maximum production of EPS was 7.5 percent salt concentration by *Halomonas eurihalina*, *Halomonas ventosae* and *Halomonas anticariensis*.

Influence of initial inoculum dose

Since the initial inoculum added to this medium is known to affect the development of

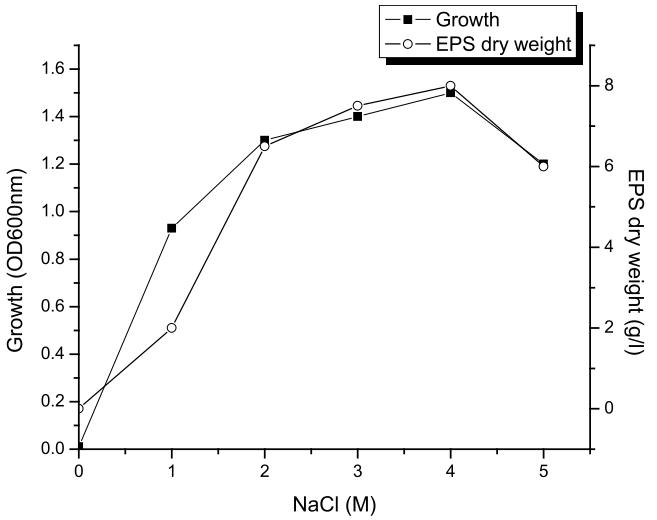

Fig. 5. Influence of NaCl concentration on growth and EPS production by *Halomonas* sp. NASH. Fermentations were carried out in IRAM medium under continuous shaking (200 rpm) at NaCl concentrations ranging from 0–5M, pH and temperature of 9 and 37°C with 1% (v/v) initial inoculum

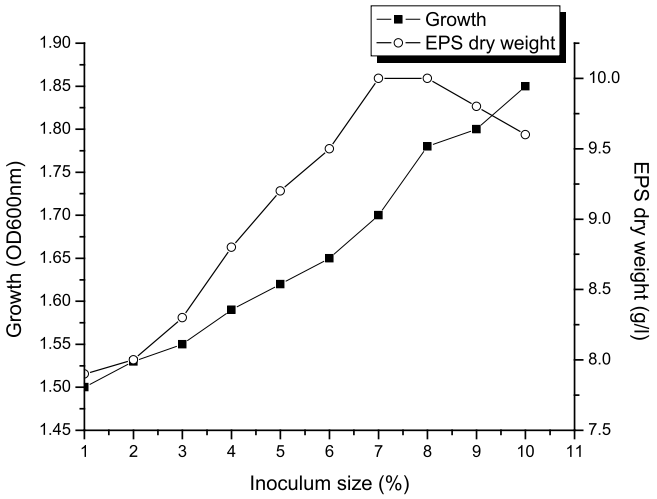

Fig. 6. Influence of initial inoculum dose on growth and EPS production by *Halomonas* sp. NASH. Fermentations were carried out in IRAM medium 4M NaCl at different inoculum dose of 1–10% (v/v) under continuous shaking (200 rpm) at pH and temperature of 9 and 37°C.

the EPS, the IRAM medium added to 4M NaCl, 1% sucrose and 9g of beef at pH 9 were inoculated in a newly cultivated isolate level at 1–10 percent (v/v) and incubated in a continuous shaking (200rpm), at 37°C. As shown in Fig. 6, 10 g/L of EPS was produced by inoculating the medium with an initial inoculum of 7%. At this stage, the culture density (OD) turned so thick and prevented normal shaking of the medium. To extract the EPS, the culture medium was initially diluted to separate the cell mass by centrifugation. Our results are similar to Biswas[27], who mentioned that 7% of *Halomonas xianhensis* SUR308's inoculum size was optimal for 7.87 g/L EPS. While[16], mentioned that maximum production of EPS (23g/l) by using 10% inoculum size of *Halomonas smyrnensis* SVD III.

Characterization of EPS produced by *Halomonas* sp. NASH

Carbohydrate and protein contents

The study showed that the EPS from *Halomonas* sp. NASH are acidic in nature, with a total content of 80,5±4 mg/g of carbohydrates

and a total protein content of 9±2 mg/g. In similar results, the *Bacillus subtilis* basal medium and the malt medium respectively, had total carbohydrate of 0.91 mg/100 ml and 0. 43 mg/100 ml, which indicates his presence significantly in the extract[34]. In all samples examined, a low protein content (around 1%) makes the methods used to differentiate proteins against polysaccharides to be more effective[35]. Maalej 's tests for EPS extracted from *Pseudomonas stutzeri* AS22 (1 percent of protein) were identical[36]**.**

The FTIR Spectrum

The exopolysaccharide spectrum FTIR (Fig. 7) displays different bands concentrations. The FTIR of exopolysaccharide spectrum (Fig. 7) shows different rates of bands. The spectrum shows characteristic absorption peaks at 3448.72, 2970.38, 2252.86, 1643.35, 1381.03, 1134.14, 640.96 and 617.22 cm^{-1}; at 3448.72 cm^{-1} the large vibration of the O-H stretch suggested that carbohydrates were in free hydroxy classes. The band at 2970.38 cm-1 approves the stretching vibration of C–H stretching of alkane group.

A very spectral peak of 2252,86 cm^{-1} was reached, which suggested –C≡C– stretched alkyne vibration. At 1643.35 cm^{-1}, the sharp band indicated C=C deepening vibration, indicating the ring of phenyl or the presence of conjugated carbonyl groups. The peak at 1381.03 cm^{-1} identifies the vibration bending of O-H alcohol group. The band is 1134.14 cm^{-1} in which the vibration (CO, alcohol, ester, ether and phenol) groups are extended. Alkyne in the exopolysaccharide can occur at bands 640,96 and 617,22 cm^{-1}. As a Fourier infrared spectroscopy transform, a fast and sensitive analysis technique was used to qualitative microbial and cell components such as EPS[37]. The broad FTIR peaks (Fig. 7), 3448,72, 2970,38, 2252,86, and 1381,03 cm^{-1} of EPS were obtained from *Halomonas* NASH have confirmed the carbohydrate presence. Orsod and his team stated that marine bacteria had extracted EPS from which alkenes, ketones and isocyanate, alcohols and ethers, carboxylic acid ester and phenol groups were indicated for absorption of the EPS[38].

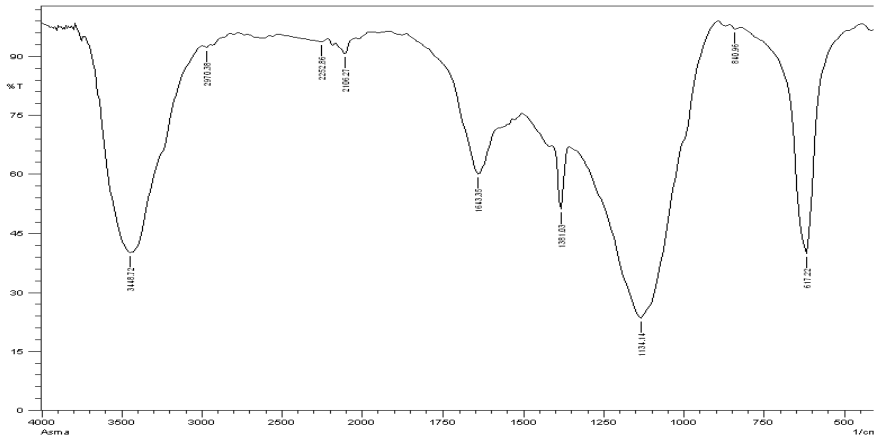

Fig. 7. FTIR spectra of the EPS produced by *Halomonas* sp. NASH

Biotechnological applications of EPS

Antimicrobial activity

EPS revealed a wide spectrum of antimicrobials against measured Gram positives *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, Gram negative bacteria; *Escherichia coli* ATCC 19404, *Pseudomonas aeruginosa* ATCC 9027 and yeast strain *Candida albicans* ATCC 10231. Table 1 data shows that EPS has specific antibacterial activity levels [Table 1].

Table 1. Antimicrobial activity of the EPS produced by *Halomonas* sp. NASH expressed as absolute unit (AU)

Pathogens	AU
Bacillus subtilis ATCC 6633	10.5
Staphylococcus aureus ATCC 6538	12.6
Escherichia coli ATCC 19404	8.5
Pseudomonas aeruginosa ATCC 9027	14.7
Candida albicans ATCC 10231	20.4

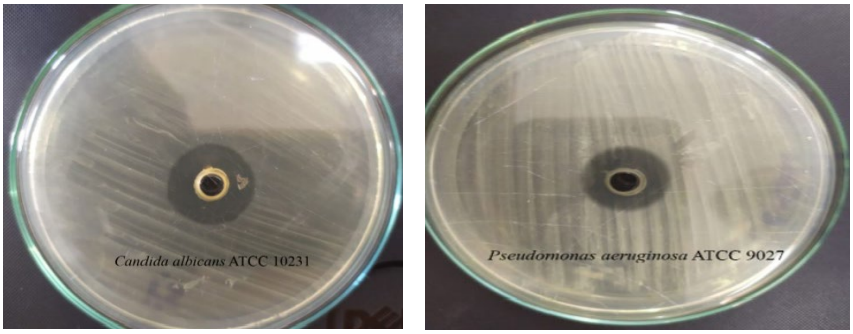

Fig. 8. Illustration of the antimicrobial activity of the EPS production by *Halomonas* sp. NASH against *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 9027.

The highest antibacterial activity (14.7 and 12.6 AU) was recorded against *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538 respectively (Fig. 8). On another hand, antifungal activity of EPS produced by *Halomonas* sp. NASH against *Candida albicans* ATCC 10231 was (20.4 AU). Nwodo states that surface active EPSs contain molecules with amphiphilic behaviour, have various chemical and surface structures and can include bio-film formation and/or have antibacterial oranti-fungal activity at times[39]. Several studies have shown that EPS from microbial organisms have high antibiotic activity and several antibacterial mechanisms for EPS have been suggested, including cell division, cell wall destruction, cytoplasmic membrane decomposition and DNA decomposition[40,41]. While the antagonistic function of polysaccharides had not been identified in these studies, protocols of these studies may lead to new areas where antibacterial activity of polysaccharide's can be studied[42].

Antioxidant activity

The obtained data indicate that EPS from *Halomonas* sp. NASH has good antioxidant activity. Exopolysaccharides demonstrated 61.38±0,22% antioxidant activity at a 2 mg/ml concentration, while ascorbic acid had 83.08±0,30% antioxidant activity at a 2 mg/ml concentration. The findings show that EPS can be used as a natural antioxidant alternative to synthetic antioxidants. EPS also exhibited free radical scavenging activity for DPPH from various micro-organisms[43-46]. Free radicals, which lead to chronic conditions, such as atherosclerosis, diabetes, rheumatoid arthritis, post-infarction, heart disease and cancer, stroke and septic shocks, aging, and other human degenerative diseases can damage bio-molecules such as lipids, protein and DNA[47]. The antioxidant and free radical scaving behavior of exopolysaccharide isolated from the *Pseudomonas* AB1 was observed by Abdrabo and his team[22]. Challouf and his team was found to have moderate antioxidant activity through the use of the Trolox Equivalent Antioxidants activity check for exopolysaccharide extract from Cyanobacterium *Arthrospira platensis*[48].

Anti-inflammatory Assay

Anti-inflammatory agent EPS from *Halomonas* sp. NASH was tested with an albumin denaturation assay inhibition. Tests showed that EPS displayed anti-inflammatory activity (66.04%) compared with ascorbic acid as control. Exopolysaccharides which produced by *Cordyceps sinensis* Cs-HK1 have significant anti-inflammatory activities[49]. Several EPSs have been identified as being anti-inflammatory[50]. Normal EPS can be isolated from *Bacillus circulants* with anti-inflammatory activity[51]. In addition, the marine bacterium *Bacillus amyloliquefaciens* 3MS2017 can generate an acidic EPS with anti-inflammatory, antioxidant and antitumor activity[52]. An EPS provided by *Lactobacillus paraplantarum* BGCG11 also showed anti-inflammatory action in rats, by decreasing regulations for the IL-1β and iNOS mRNA and increasing levels of IL-6 and IL-10 anti-inflammatory cytokines[53].

Biosurfactant activity

Biosurfactant activity of EPS which production from *Halomonas* sp. NASH was tested by emulsifying capacity and calculation of emulsification percentage. The findings suggest

high levels of biosurfactant activity for *Halomonas* sp. NASH with 74.74 percent emulsification index, 67.47 and 54.96 percent respectively for paraffin oil, xylene and benzene. The biotechnological potential of microbial polysaccharides is demonstrated as immunomodulators and healers in Pharmaceutical and Gelling and Thickening Industries in food processing industries. In detoxifying areas contaminated with petrochemical oil, some EPS are used as biosurfactants[54]. EPSs are also used as surfactants and emulsifiers noted for the biodegradability of these agents[55].

CONCLUSION

Halomonas sp. NASH synthesized large quantities of exopolysaccharide when grown under the best growth conditions, and this EPS is suitable as an antimicrobial, antioxidant , anti-inflammatory and emulsifier.

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None.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

LSA carried out the statistical experiment analysis. NMF wrote the manuscript. All authors read and approved the manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated during and/or analysed the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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