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## Full Length Article

[](http://crossmark.crossref.org/dialog/?doi=10.1016/j.ejbas.2018.05.009&domain=pdf)Characterization and applications of exopolysaccharide produced by marine *Bacillus altitudinis* MSH2014 from Ras Mohamed, Sinai, Egypt

Sahar S. Mohamed [a](#_bookmark0), Shaimaa K. Amer [b](#_bookmark1), Manal S. Selim [a](#_bookmark0),[⇑](#_bookmark3), Hala M. Rifaat [c](#_bookmark2)

a *Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Division, National Research Centre, Cairo, Egypt*

b *Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt*

c *Microbial Chemistry Department, Genetic Engineering and Biotechnology Research Division, National Research Centre, Cairo, Egypt*

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### a b s t r a c t

The present study aims to survey exopolysaccharides (EPSs) production in 29 bacterial strains isolated from the sediments around the mangrove trees in Ras Mohamed area, Red Sea Coast, Sinai Peninsula, Egypt. Two of the strains were able to produce EPSs. A higher yield of EPS was obtained from isolate No. 12. Strain identification resulted in a close similarity with *Bacillus altitudinis.* The produced EPS was characterised as a heteropolysaccharide containing mannouronic acid, glucose, and sulphate. A gel permeation chromatography was used to estimate the EPS molecular weight which found to be

4.23 × 105 Dalton. The typical pattern of polysaccharide absorbance was supported by the infrared

spectrum. The EPS appears significant in vitro antitumor activities against two cancers cells EACC and lung cancer A-549. Furthermore, a broad range of bacteria and fungi were inhibited with purified EPS.

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1. Introduction

Exopolysaccharides (EPSs) are long chains of high molecular mass polymers produced by different microorganisms, including bacteria, fungi and blue green algae [[1]](#_bookmark13). EPSs produced by bacteria exhibit significant structural diversity with novel biological prop- erties are considered as valuable sources of natural polymers with multiple biotechnological applications. Microbial EPSs are preva- lent in the extreme marine environment where they are essential for microbial existence. Most of the functions attributed to EPSs are of a protective nature and their accurate roles are dependent on the ecological functions in which the microorganisms live. They could support the microbial communities to suffer extremes of temperature, salinity and nutrient accessibility, construct a bound- ary between the bacterial cell and its included environment.

Bacteria in marine environments were forced with osmotic stress to produce EPSs with unique composition were studied for prospective application in various sectors [[2]](#_bookmark14) and for discovery of novel macromolecules [[3]](#_bookmark15). Recently, evidences for antioxidant, immune-modulation, antitumor and antimicrobial properties of EPSs producing microorganisms have increased [[4,5]](#_bookmark16).

Serious side effects of clinically used antitumor drugs direct the attention towards investigation of novel agents with a higher

\* Corresponding author.

*E-mail address:* [manalsleem@yahoo.com](mailto:manalsleem@yahoo.com) (M.S. Selim).

potency and fewer effects [[6]](#_bookmark24). Human pathogens, as well, have resistance formed from the abnormal use of commercial antimicro- bial drugs. This resistance with the undesirable side effect of cer- tain antibiotics motivated the scientists to look for new antimicrobial substituent from many sources [[7]](#_bookmark25).

The aim of this study is to deal with the isolation and character- isation of EPS producing bacteria from the marine sediments around the mangrove trees in Ras Mohamed area, Red Sea Coast. The in vitro activities of purified EPS as antitumor agent against Ehrlich Ascites carcinoma and lung cancer cell lines as well as the antimicrobial activities against Gram positive and Gram nega- tive bacteria, yeast and fungi were also studied.

1. Materials and methods
   1. *Isolation of bacteria and production of EPSs*

Marine sediment was collected from the rhizosphere of a man- grove trees (*Avicennia marina*) from Ras Mohamed, Sinai Peninsula, Egypt. The sample was serially diluted in 90 ml sterile water and plated on a medium containing (in g/l) [glucose (20); yeast extract (0.1); NH4NO3 (0.8); CaCO3 (1); K2HPO4 (0.6); KH2PO4 (0.5);

MgSO4·7H2O (0.05), MnSO4·4H2O (0.1) and agar (15)] which dis-

solved in 750 ml seawater and 250 ml distilled water [[8]](#_bookmark26). EPSs pro-

ducing bacterial isolates were selected based on colonies phenotype (smooth and mucoid).

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Pure strains were then inoculated in 250 ml conical flasks con- tain 50 ml of previous medium and incubated at 37 °C in a rotary shaker at 120 rpm for 48 h. After centrifugation at 5000 rpm for 15 min, supernatant was mixed with four volumes of chilled etha- nol, while the collected pellets were washed twice with acetone and diethyl ether [[5]](#_bookmark22) and then drying at 50 °C to obtain constant weight. EPSs formation were estimated by the phenol-sulphuric acid method [[9]](#_bookmark29).

* 1. *Identification of the strain*

The strain with the highest EPS production was identified according to their morphology, physiology and biochemistry fea- tures such as nitrate reduction, catalase, oxidase, Voges- Proskauer test, acid production from glucose [[10]](#_bookmark31) and 16S rRNA sequence. The 16S rRNA gene was amplified and sequenced using universal primers described by Weisburg et al. [[11]](#_bookmark32). The sequences were compared with the GenBank data base using BLAST [[12]](#_bookmark33).

* 1. *Extraction and purification of EPS*

The selected strain was grown in a fermentation medium con- taining (g/l) [peptone (4.0), yeast extract (2.0), and sucrose (20.0)] which dissolved in 750 ml seawater and 250 ml distilled water (pH 7–7.5) at 37 °C for three days in an incubator shaker at 120 rpm to produce EPS [[13]](#_bookmark36). To precipitate proteins, 5% tri- chloroacetic acid was added to cell free culture supernatant and incubated at 4 °C for 24 h. After centrifugation at 5000 rpm for 15 min, supernatant pH was adjusted to 7 and dialyzed against dis- tilled water using dialysis tube (MWCO 2000). After dialysis, abso- lute ethanol were added to supernatant and incubated at 4 °C for 24 h. The precipitated EPS was dissolved, dialysed against deion- ized water and filtered using 0.45 mm filter, then applied to a

DEAE-cellulose column (1.5 cm × 70 cm). Elution was carried out

using continuous gradient NaCl solution (0.2–3.0 M). Subse-

quently, a further purification step was carried out for the collected fractions using a Sephadex G-200 column (2 cm × 80 cm) which was eluted with 0.1 M NaCl at a flow rate of 0.5 ml/min. The frac-

tions with EPS were collected in one fraction which dialyzed and lyophilized for further analysis.

* 1. *Chemical analyses of EPS*

Protein, uronic acid and monosaccharide sulphate were esti- mated for the produced EPS according to methods described by many authors [[10,14–16]](#_bookmark31) respectively. A high performance liquid chromatography (HPLC) was used to analyse the monosaccharide

content using a Shimadzu Shim-Pack SCR-101N column (7.9 mm ×

30 cm) and deionized water as mobile phase at flow rate

0.5 ml/min utilizing acid hydrolysis technique [[17]](#_bookmark40).

* 1. *Molecular weight estimation*

The molecular weight of EPS was estimated by gel permeation chromatography (GPC) on a Sephadex G-200 column (80 cm × 2 cm). Standard dextrans (40,000; 500,000 and 2,000,000) Daltons

were used for calibration and the molecular weight was deter- mined by plotting against standard graph [[18]](#_bookmark42).

* 1. *Fourier-transform infrared spectroscopy (FT-IR)*

The dried EPS was mixed to KBr powder, ground and pressed into a 1 mm pellets for FT-IR measurements in the range of

400–4000 cm—1 using Bruker scientific FT-500-IR spectrophotome-

ter [[19]](#_bookmark17).

* 1. *Periodate oxidation and Smith degradation*

Periodate oxidation and Smith degradation were determined by dissolving desulfated EPS (16 mg) in distilled water (6 ml) and then added to 0.1 M NaIO4 (50 ml) in round bottom flasks which kept at 4 °C in dark [[20]](#_bookmark17). Aliquots (0.1 ml) were taken every 24 h and diluted with distilled water (25 ml) which read in spectropho- tometer at 223 nm.

Periodate consumption was calculated based on absorbance change [[21]](#_bookmark17) and formic acid products were monitored by the phenol-sulphuric acid method [[9]](#_bookmark29). Ethylene glycol (2 ml) was added and the solutions were dialyzed against distilled water for 48 h. After that NaBH4 (100 mg) is added and the mixture was left for 24 h temperature and then ice cold acetic acid (4N) is added to stop the reaction. The solutions were again dialyzed as described above and lyophilized [[22]](#_bookmark17). The resulting polyalcohol was hydro- lyzed with HCOOH 90% for 5 h and the produced sugars and sugar alcohols were analyzed by HPLC.

* 1. *Biological activity of EPS*
     1. *Radical scavenging activity of EPS towards DPPH radical*

The free radical scavenging activity of EPS was estimated according to method describing by Yang et al. [[23]](#_bookmark17) using 1,1- diphenyl-2-picryl-hydrazyl (DPPH). Five ml of DPPH in ethanol

was added to 1 ml of purified EPS with concentrations (50–300 m g/ml). The mixture was incubated in dark for 30 min. and measured at 517 nm using spectrophotometer UV–Visible 2401PC (Shimadzu, Japan).

The DPPH absorbance goes down with a high free radical scav- enging ability. The free radicals scavenger capacity was calculated based on this equation: Scavenging ability (%) = [(A517 of control

— A517 of sample)/A517 of control] × 100. The EC50 value is con-

sidered as the effective concentration (mg) of EPS at which the

DPPH absorbance was reduced by 50%.

* + 1. *Antitumor activity against Ehrlich Ascites Carcinoma Cells (EACC)*

Tumor cells viability was measured with modified cytotoxic trypan blue exclusion technique as described by Yang et al. [[23]](#_bookmark17). Tumor cells proliferation inhibition was calculated based on this

equation: [(A—B/A] × 100%, where A and B are the average number

of viable tumor cells of the control and the samples, respectively.

* + 1. *Antitumor activity against lung cancer cell line A-549*

Cytotoxicity of purified EPS was measured against A-549 cell line using the MTT cell viability assay [[24]](#_bookmark17). Percentage of relative viability was calculated using the equation (Absorbance of treated

cells/Absorbance of control cells × 100). Then the half maximal

inhibitory concentration IC50 was calculated from the graph and

BJ-1 normal human cell line was used as control.

* + 1. *Antimicrobial activity*

The antimicrobial potential of the purified EPS was tested against a wide set of microorganisms including: (i) Gram positive bacteria *(Bacillus subtilis* NRRL B-941 and *Staphylococcus aureus* NRRL B-767), (ii) Gram negative bacteria (*Pseudomonas aeruginosa* NRRL B-23 and *Escherichia coli* NRR-B 210)*,* (iii) yeast (*Saccha- romyces cerevisiae* Y-2034 and *Candida albicans* NRRL Y-477) and

(iv) fungi *(Aspergillus niger* NRRL-3 and *Fusarium oxysporum* NRRL 26406). The inhibition zones produced by different concentration of EPS (75, 100, 150, and 200 lg/disc) were determined. Rimactane

as antibacterial (200 lg/disc) and flucoral as antifungal (200 lg/

disc) were used as control [[25]](#_bookmark17).

1. Results and discussion
   1. *Isolation, screening, and identification of the EPS producing bacteria*

Many marine bacteria produce EPSs for their growth to adhere to solid surfaces and to overcome extreme conditions. In the pre- sent study, 29 bacterial strains were isolated from the rhizosphere sediment of mangrove trees in Ras Mohamed area, Red Sea; Sinai, Egypt. Two of them had the ability to produce EPSs. The highest production of EPS (10.33 g/l) was detected at strain No. 12. Liu et al. [[26]](#_bookmark17) reported in a study that 19 strains of bacteria produced EPSs in 2.28–9.02 g/l, where they selected a strain because of its highest EPS production and identified it as *Bacillus licheniformis*.

Colonies of strain No. 12 showed mucoid appearance on solid medium. The cells were Gram positive, rod shaped, motile and spore forming when observed under phase contrast microscope. In nitrate reduction, Voges-Proskauer tests and acid production from glucose, catalase and oxidase testes, the strain was positive, while negative for gas production from glucose. Hence, strain No. 12 was temporary identified as *Bacillus* sp. particularly, the par- tially sequenced 16S rRNA genes showed 99% similarity with *Bacil- lus altitudinis*, so strain No. 12 was identified as *Bacillus altitudinis* MSH2014 with accession No. (KY550404) ([Fig. 1](#_bookmark4)).

* 1. *Characterisation of EPS*
     1. *Purification of EPS*

The crude EPS produced from *Bacillus altitudinis* MSH2014 were purified using DEAE-cellulose anion exchange chromatography column ([Fig. 2](#_bookmark5)). Elution profile showed one major peak (fraction 100–120). Such fraction was collected, dialyzed and lyophilized to get EPS which applied for further analysis.

* + 1. *Molecular weight determination of EPS*

The molecular weight of the EPS produced by *Bacillus altitudinis* MSH2014 determined by a gel filtration technique was 4.23 × 105 Da. EPS appeared as a single symmetrical narrow peak ([Fig. 3](#_bookmark6)),

which represent the homogeneity of EPS. Previous reports of the molecular weight of the EPS produced by *Bacillus licheniformis*

was 2.826 × 104 Da [[26]](#_bookmark17).

* + 1. *Chemical composition of EPS*

The purified EPS produced a negative response by Bradford test indicating the absence of protein. Monosaccharide analysis by HPLC revealed that EPS composed of mannuronic acid and glucose with molar ratio 1:2.2. The chemical composition of EPS indicated the presence of uronic acid (14.26%) and sulphate (15.47%).

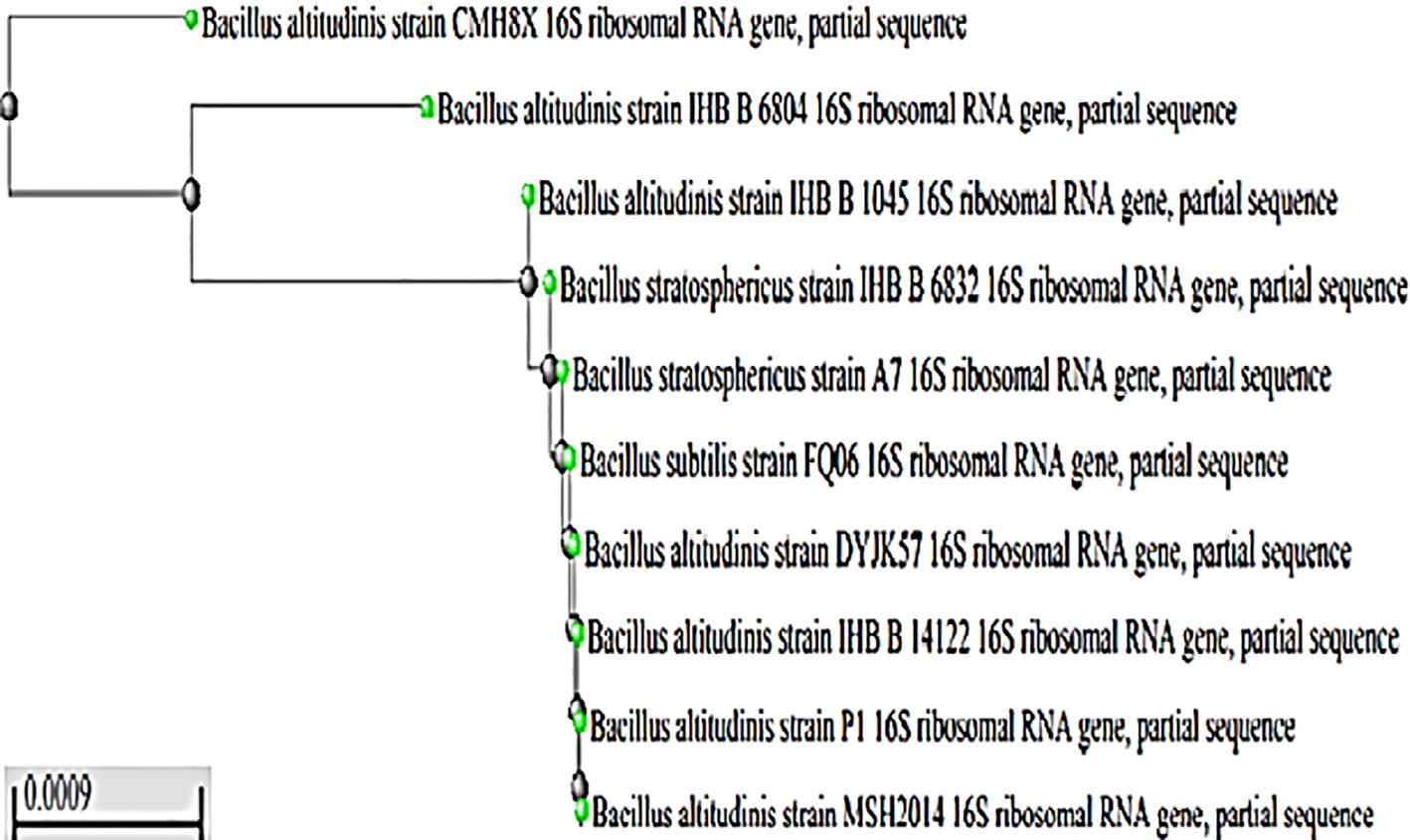


Fig. 1. Phylogenetic tree of the partial sequence of 16S rRNA with closely related sequences available in GenBank databases.

1.4

1.2

0.4

1.0

O.D at 490 nm

0.8

0.6

0.3

0.2

NaCL (M)

0.4

0.2

0.1

0.0 0.0

0 20 40 60 80 100 120 140 160 180

### No. of fractions

Fig. 2. DEAE cellulose column elution profile for EPS from *Bacillus altitudinis* MSH2014.

1.4

1.2

O.D at 490 nm

1.0

0.8

0.6

0.4

0.2

0.0

0 20 40 60 80 100 120 140 160 180

Tube number

Fig. 3. Gel filtration chromatography of EPS from *Bacillus altitudinis* MSH2014*.*

It was found that bacteria isolated from deep sea which pro- duced EPS was typically acidic due to containing 10–40% uronic acid [[27]](#_bookmark17) and polyanionic due to uronic acids, ketal-linked pyru-

vate or inorganic residues such as PO— or SO— existence [[28]](#_bookmark17).

process of periodate oxidation. But desulfated exopolysaccharide consumed periodate (0.942 mol) to produce formic acid (0.000801 mol) per one mole of anhydrosugar. HPLC analysis of

EPS from *Bacillus altitudinis* MSH2014 shows erythritol, glycerol,

4 3

According to the literatures, the presence of SO— group enhanced

3

the medical potential of EPS [[29]](#_bookmark17). The sugar composition had an

important role in the biological activities [[30]](#_bookmark18).

The FT-IR spectrum of EPS from *Bacillus altitudinis* MSH2014 showed a typical pattern for polysaccharide absorbance ([Fig. 4](#_bookmark8)).

The broad band at 3435.6 cm—1 is due to AOH groups of carbo- hydrate residues stretching vibration [[31]](#_bookmark19). The 2360.0 cm—1

band is due to CAH stretching vibration. The weak absorbance peak at 1630 cm—1 was due to the stretching vibration of C@O that may be associated with the mannuronic acid and internal hydrogen bonds [[32]](#_bookmark20). Band at 1340 cm—1 is due to the presence

of S@O and/or CAOAS bonds [[33]](#_bookmark21) and the band at 863 cm—1

indicates the a-configuration which is simultaneously present

in EPS.

* 1. *Periodate oxidation and Smith degradation*

Periodate oxidation was done to both sulphated and desul- phated EPS where in case of sulphated EPS did not produce for- mic acid and consumed periodate due to the presence of sulphate groups on the carbon atom 2 or 3 which impede the

and erytheric acid presence ([Table 1](#_bookmark7)). The presence of a small amount of glycerol and free erythritol in large amount partially demonstrate existence of (1 ? 4) linkages between monosaccha- rides units and sulphate groups on the C2 and/or C3 in EPS. Erythritol was produced from C3, C4, C5 and C6 of the (1 ? 4) glycosidic linkages of glucose after hydrolysis of the backbone, while erytheric acid was liberated from C3, C4, C5 and C6 of the (1 ? 4) glycosidic linkage of mannouronic acid. The presence of relatively small quantities of glycerol from the glucose units and erytheric acid from the mannouronic unit in the hydrolysis of polysaccharides, gave the information that glucose may be found at the non-reducing end. These outcomes are in good agreement with those mentioned by other workers [[34,35]](#_bookmark23).

Table 1

HPLC results of Smith’s degradation of EPS produced by *Bacillus altitudinis* MSH2014.

Sugar alcohol

Erythritol Glycerol Erytheric acid

3.3 0.4 0.9

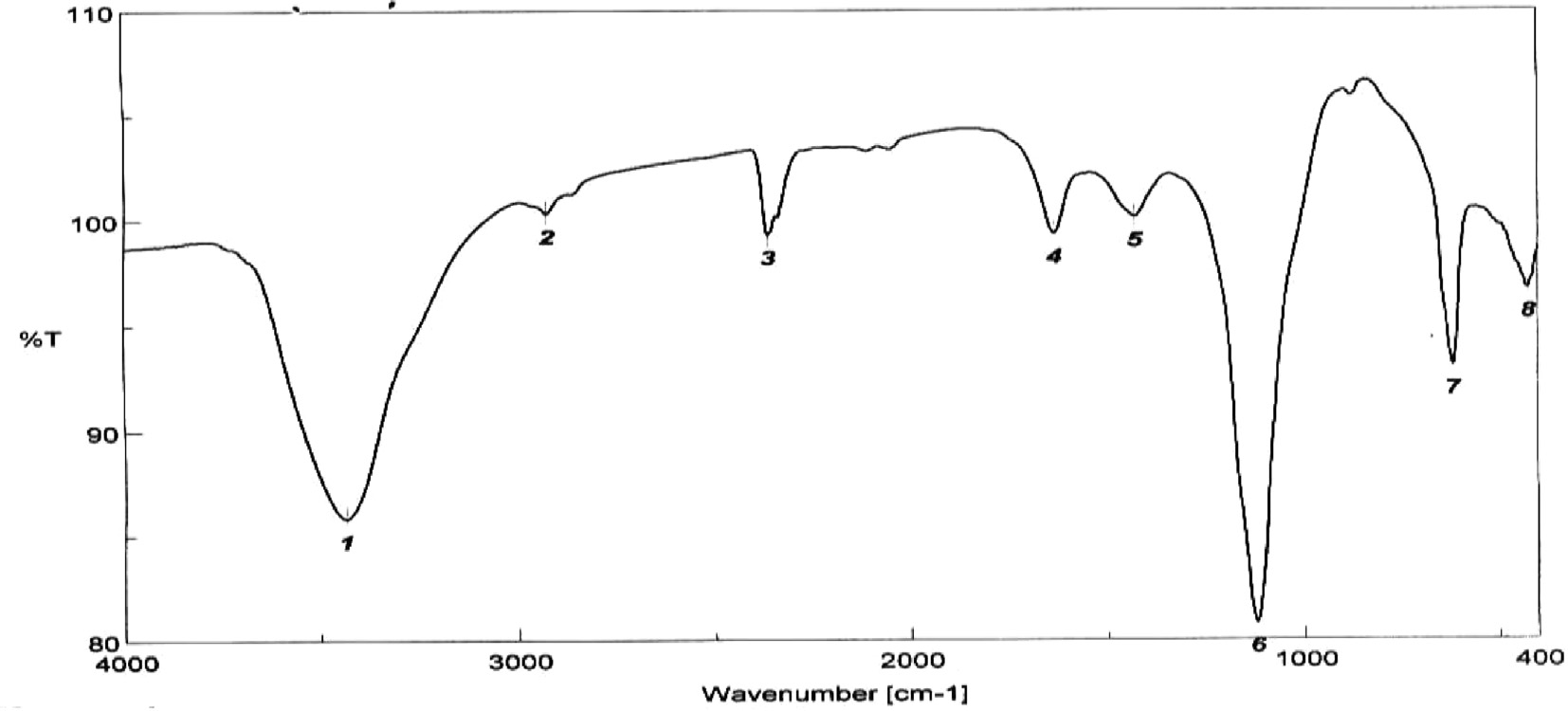


Fig. 4. FT-IR spectrum of the EPS from *Bacillus altitudinis* MSH2014*.*

100.00

Scavening activity (%)

80.00

60.00

40.00

20.00

0.00

0.00 50.00 100.00 150.00 200.00 250.00 300.00

Concentrations of EPS (μg/ ml)

* 1. *Biological activity of EPS produced by Bacillus altitudinis MSH2014*
     1. *Free radical scavenging activities of EPS*

The free radical scavenging activity was estimated with DPPH [[36]](#_bookmark27). The scavenging activity of EPS illustrates in [Fig. 5](#_bookmark9) showed a concentration-dependent manner, with an EC50 value of 150 (mg/ml). It was reported that EPS was isolated from *Bacillus coagu-*

*lans* RK-02 and purified by size exclusion chromatography which

showed in vivo antioxidant activities [[37]](#_bookmark28). Agili and Mohamed [[38]](#_bookmark30), also, mentioned that EPSs from *Padina pavonica* had antioxi- dant activities. The antioxidant properties of polysaccharides are mainly correlated to their confirmation, structure, molecular mass and their monosaccharides components [[39]](#_bookmark34).

Fig. 5. Free radical scavenging effects of EPS from *Bacillus altitudinis* MSH2014.

50

45

40

Viability cells (%)

35

30

25

20

15

10

5

0 200 400 600 800 1000 1200 1400 1600

## Concentrations of EPS (μg/ml)

Fig. 6. Viability of EACC cells for the EPS from *Bacillus altitudinis* MSH2014*.*

100

viability ( % of control)

80

60

40

20

0

0 20 40 60 80 100

# Concentration of EPS μg/ml

Fig. 7. Antitumor activity against lung cancer of the purified EPS from *Bacillus altitudinis* MSH2014*.*

* + 1. *Antitumor activity*
       1. *Antitumor activity against Ehrlich Ascites Carcinoma Cells (EACC).* As shown in [Fig. 6](#_bookmark10), the EPS exerted inhibitory activity on EACC in concentration dependent manner. The viability of tumor cells after incubation with purified EPS drastically decreased from 47 to 20% and then gradually decreased further to 10% by increas- ing the concentration of EPS from 100 to 500 till 1500 mg/ml

respectively, which is in agreement with the results obtained by

Ahmed and Ahmed [[40]](#_bookmark35).

* + - 1. *In vitro antitumor activity against lung cancer cell.* Lung cancer cell line A-549 was treated with the purified EPS at different concentrations (12.5, 25, 50 and 100 mg/ml) and showed in [Fig. 7](#_bookmark11). The data shows a gradual decrease in tumor cells viability with

high concentrations of EPS. The highest cells viability (71%) was found at 12.5 mg/ml which decreases to 63%, 51% and 39% at 25 mg/ml, 50 mg/ml, 100 mg/ml of EPS, respectively, and IC50 was

51.94 mg/ml. While in case of normal cell line the viability of cells

reached 95%, 90%, 88% and 77% when treated with the different

concentrations of EPS 12.5, 25, 50 and 100 mg/ml respectively.

The differences in the antitumor activities of EPS may be due to their different physicochemical properties such as chain shape, molecular weight and monosaccharide composition [[41]](#_bookmark37). EPS molecular weight may affect its bioactivities, as the high molecular weight showed more antitumor activity [[42]](#_bookmark38).

* + 1. *Antimicrobial activities*

The antimicrobial activities ([Table 2](#_bookmark12)) demonstrate that the puri- fied EPS possessed antimicrobial activities against all of the tested microorganisms and by increasing the concentration of EPS the inhibition zone increased which ranged from 6.3 to 24.9 mm. Orsod et al. [[43]](#_bookmark39) isolated two EPSs producers marine bacteria and screened their antimicrobial activities against both Gram positive bacteria (*Lysinibacillus* and *Paenibacillus* sp.) and Gram negative bacteria (*Pseudomonas* sp*., Escherichia coli*) had shown presence of inhibition zones.

The mechanism of the antimicrobial activity of the purified EPSs were studied by various investigators and they mentioned that the

Table 2

Inhibition zones of microbial growth by different concentrations of the EPS from *Bacillus altitudinis* MSH2014*.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Concentrations of EPS (mg/disk) Gram+ve bacteria Gram—ve bacteria Yeast Fungi | | | | | | | | | | | | | |
|  | | *B. subtilis* | *St. aureus* |  | *E. coli.* | *P. aeruginosa* |  | *S. cerevisiae* | *C. albicans* |  | *A. niger* | *F. oxysporum* |  |
| Inhibition zone (mm) | | | | | | | | | | | | | |
| 75 | 11.2 | | 12.2 | 12.9 | | 7.7 | 10.2 | | 7.7 | 15.2 | | 6.3 |  |
| 100 | 13.1 | | 15.1 | 17.7 | | 10.6 | 12.2 | | 9.5 | 16.7 | | 7.1 |  |
| 150 | 15.7 | | 17.3 | 19.8 | | 13.4 | 14.7 | | 13.7 | 18.7 | | 8.3 |  |
| 200 | 17.8 | | 18.8 | 24.9 | | 15.6 | 17.6 | | 17.3 | 20.0 | | 10.5 |  |
| Rimactane | 16.7 | | 16.8 | 21.9 | | 14.1 | 0.0 | | 0.0 | 0.0 | | 0.0 |  |
| Flucoral | 0.0 | | 0.0 | 0.0 | | 0.0 | 25.9 | | 24.1 | 24.5 | | 26.4 |  |

antimicrobial activity of anionic polysaccharides such as sulphated polysaccharide occurs by several mechanisms through its chelation activities and the deprivation of metal, trace elements or essential nutrients which limit the growth of microorganisms [[44]](#_bookmark41).

1. Conclusion

Although EPSs are one of the abundant bioactive components, their study still remains an interesting scope for their high molec- ular diversity, complexity and high capability for drugs design and preparation. In addition, microorganisms producing EPSs are promising sources that meet actual industrial demand, especially, marine microorganisms which offer great opportunities for new bioactive compound discovery.

Conflict of interest statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inap- propriately influence this work.

References

1. [Guo S, Mao W, Li Y, Tian J, Xu J. Structural elucidation of the](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0005) [exopolysaccharides produced by fungus *Fusarium oxysporum* Y24. Carbohydr](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0005) [Res 2013;365:9–13](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0005).
2. [Nichols MCA, Guezennec J, Bowman JP. Bacterial exopolysaccharides from](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0010) [extreme marine environments with special consideration of the Southern](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0010) [Ocean, Sea Ice, and Deep-Sea hydrothermal vents. Marine Biotechnol](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0010) [2005;7:253–71](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0010).
3. [Finore I, Di Donato P, Mastascusa V, Nicolaus B, Poli A. Fermentation](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0015) [technologies for the optimization of marine microbial exopolysaccharides](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0015) [production. Mar Drugs 2014;12:3005–24](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0015).
4. [Zhang Y, Kong H, Fang Y, Nishinari K, Philips GO. Schizophyllan: a review of its](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0020) [structure, properties, bioactivity and recent developments. Bioact Carbohydr](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0020) [Dietary Fibre 2013;1:53–71](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0020).
5. [Li W, Ji J, Rui X, Yu J, Tang W, Chen X, et al. Production of exopolysaccharides by](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0025) [*Lactobacillus helveticus* MB2-1 and its functional characteristics in vitro. LWT-](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0025) [Food Sci Technol 2014;59:732–9](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0025).
6. [Zhang ZH, Tang JH, Zhan ZL, Zhang XL, Wu HH. Cellular toxicity of isoniazid](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0030) [together with rifampicin and the metabolites of isoniazid on QSG-7701](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0030) [hepatocytes. Asian Pac J Trop Med 2012;5:306–9](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0030).
7. [Ramachandran M, Thirumalai T, Vinothkumar P. Antibacterial activity of](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0035) [various leaf extracts of *Merremiae marginata* EK Elumalai. Asian Pac J Tropical](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0035) [Biomed 2011;1:406–8](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0035).
8. [Kim YO, Kim HK, Bae KS, Yu JH, Oh TK. Purification and properties of a](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0040) [thermostable phytase from *Bacillus* sp. DS11. Enzyme Microb Technol](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0040) [1998;22:2–7](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0040).
9. [Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0045) [determination of sugars and related substances. Anal Chem 1965;28:350–6](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0045).
10. [Holt JG, Sharpe ME, Williams ST. Bergey’s manual of systematic](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0050) [bacteriology. Baltimore, London: Williams & Williams; 1989](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0050).
11. [Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0055) [amplification for phylogenetic study. J Bacteriol 1991;173:697–703](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0055).
12. [Tamura K, Dudley J, Nei M, Kumar S. Molecular evolutionary genetic analysis](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0060) [(MEGA) software version 4.0. Mol Biol Evol 2007;24:1596–9](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0060).
13. [Jiang ZD, Jensen PR, Fenical W, Lobophorins A. New anti-inflammatory](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0065) [macrolides produced by a tropical marine bacterium. Bioorg Med Chem Lett](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0065) [1999;9:2003–6](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0065).
14. [Bradford MM. A rapid and sensitive method for the quantitation of microgram](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0070) [quantities of protein utilizing the principle of protein–dye binding. Anal](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0070) [Biochem 1976;72:248–54](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0070).
15. [Filisetti-Cozzi TMC, Carpita C. Measurement of uronic acids without](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0075) [interference from neutral sugars. Anal Biochem 1991;197:157–62](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0075).
16. [Dodgson KS, Price RG. A note on the determination of the ester sulfate content](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0080) [of sulfated polysaccharides. Biochem J 1962:106–10](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0080).
17. [Kwon HJ, Kim J. Determination of monosaccharides in glycoproteins by](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0085) [reverse-phase high- performance liquid chromatography. Anal Biochem](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0085) [1993;215:243–52](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0085).
18. [Luo D. Identification of structure and antioxidant activity of a fraction of](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0090) [polysaccharide purified from *Dioscorea nipponica* Makino. Carbohydr Polym](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0090) [2008;74:544–9](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0090).
19. [Ray B. Polysaccharides from *Enteromorpha campressa*: isolation, purification](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0095) [and structural features. Carbohydr Polym 2006;66:408–16](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0095).
20. [Yalin W, Yuanjing P, Cuirong S. Isolation, purification and structural](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0100) [investigation of a water-soluble polysaccharide from *Solanumlyratum*](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0100)[*lyratum* Thunb. Inter J Biolog Macromol 2005;36:241–5](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0100).
21. [Jeans A, Wilhan A. Periodic oxidation of Dextran. J Amer Chem Sci](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0105) [1950;72:2655–7](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0105).
22. [Mondal S, Chakraborty I, Pramanik M, Rout D, Islam S. Structural studies of](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0110) [water soluble polysaccharides of an edible mushroom, *Termitomyces eurhizus*](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0110)[(Areinvestigation). Carbohydr Res 2004;339:1135–40](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0110).
23. [Yang ZF, Zheng YH, Cao SF. Effect of high oxygen atmosphere storage on](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0115) [quality, antioxidant enzymes, and DPPH-radical scavenging activity of Chinese](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0115) [bayberry fruit. J Agric Food Chemist 2009;57:176–81](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0115).
24. [Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0120) [precise and rapid dye method for measuring cell growth/cell kill. J Immunol](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0120) [Methods 1989;119:203–10](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0120).
25. [Bauer A, Kirby W, Sherris J, Turck M. Antibiotic susceptibility testing by](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0125) [standardising single disc method. Am J Clin Pathol 1966;36:493–6](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0125).
26. [Liu C, Lu J, Lu L, Liu Y, Wang F, Xiao M. Isolation, structural characterization](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0130) [and immunological activity of an exopolysaccharide produced by *Bacillus*](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0130)[*licheniformis* 8–37-0-1. Bioresour Technol 2010;101:5528–33](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0130).
27. [Priyanka P, Arun A, Rekha P. Sulfated exopolysaccharide produced by *Labrenzia*](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0135)[sp. PRIM-30, characterization and prospective applications. Inter J Biolog](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0135) [Macromol 2014;69:290–5](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0135).
28. [Poli A, Anzelmo G, Nicolaus B. Bacterial exopolysaccharides from extreme](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0140) [marine habitats: production, characterization and biological activities. Mar](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0140) [Drugs 2010;8:1779–802](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0140).
29. [Senni K, Pereira J, Gueniche F, Delbarre-Ladrat C, Sinquin C, Ratiskol J. Marine](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0145) [polysaccharides: a source of bioactive molecules for cell therapy and tissue](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0145) [engineering. Mar Drugs 2011;9:1664–81](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0145).
30. [Jong-Min P, Ki-Baik H, Sang-Oh K, Eun-Hee K. The anti-inflammatory effects of](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0150) [acidic polysaccharide from *Artemisia capillaris* on *Helicobacter pylori* infection. J](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0150) [Cancer Preven 2013;18:161–8](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0150).
31. [Brock-Neely W. Infrared spectra of carbohydrates. Advances in carbohydrates](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0155) [chemistry, 12. New York: Academic Press Inc; 1957. p. 13–33](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0155).
32. [Synytsya A, Mickova K, Synytsya A, Jablonsky I, Spevacek J, Erban V, et al.](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0160) [Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0160) [*Pleurotus eryngii*: Structure and potential prebiotic activity. Carbohydr Polym](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0160) [2003;76:548–56](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0160).
33. [Hasui M, Matsuda M, Okutani K, Shigeta S. Structural analysis of the lactate](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0165) [associated galactan sulfate produced by *Gymnodinium* sp.A3. In: Yasumoto T,](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0165) [Oshima Y, Fukuyo Y, editors. Armful and toxic algal booms. Inc., Inter](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0165) [Governmental Oceanographic Commission of UNESCO; 1996](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0165).
34. [Abdel-Akher M, Hamilton JK, Montgomery R, Smith F. A new procedure for the](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0170) [determination of the fine structure of polysaccharides. J Am Chem Soc](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0170) [1952;74:4970–1](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0170).
35. [Danishefk J, Whistler LR, Bettelheim FA. Introduction to polysaccharide](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0175) [chemistry. In: Waid P, Dprek H, Anthory H, editors. The carbohydrates](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0175) [chemistry, Vol. IIA. New York and London: Academic Press Inc; 1970. p.](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0175) [375–411](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0175).
36. [Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0180) [extracts for antioxidant activity. Life Sci 2003;73:167–79](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0180).
37. [Kodali VP, Perali RS, Se R. Purification and partial elucidation of the structure of](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0185) [an antioxidant carbohydrate biopolymer from the probiotic bacterium *Bacillus*](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0185)[*coagulans* RK-02. J Nat Prod 2011;74:692–7](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0185).
38. [Agili FA, Mohamed SF. Polysaccharides from *Padina pavonica*:](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0190) [chemical structural and antioxidant activity. Aust J Basic Appl Sci 2012;6:](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0190) [277–83](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0190).
39. [Bamigboye CO, Oloke JK, Dames JF. Biological activity of extracellular and](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0195) [intracellular polysaccharides from pleurotus tuber-regium hybrid and mutant](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0195) [strains. J Food Nutrit Res 2016;4:422–8](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0195).
40. [Ahmed OM, Ahmed RR. Anti-Proliferative and apoptotic efficacies of Ulvan](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0200) [polysaccharides against different types of carcinoma cells in vitro and in vivo. J](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0200) [Cancer Sci Ther 2014;6:202–8](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0200).
41. [Wu Q, Tun HM, Leung FCC, Shah NP. Genomic insights into high](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0205) [exopolysaccharides producing dairy starter bacterium *Streptococcus*](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0205)[*thermophilus* ASCC 1275. Sci Rep 2014;4:1–8](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0205).
42. [Peng Y, Zhang L, Zeng F, Kennedy FJ. Structure and antitumor activities of the](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0210) [water soluble polysaccharide *Ganoderma tsugae* mycelium. Carbohydr Polymer](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0210) [2005;59:385–92](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0210).
43. [Orsod M, Joseph M, Huyop F. Characterization of exopolysaccharides s](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0215) [produced by *Bacillus cereus* and *Brachybacterium* sp. isolated from Asian Sea](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0215) [Bass (Latescalcarifer). Malaysian. J Microbiol 2012;8:170–4](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0215).
44. [Skalicka-Woz´niak K, Szypowski J, Los R, Siwulski M, Sobieralski K, Glowniak K,](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0220) [Malm A. Evaluation of polysaccharides content in fruit bodies and their](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0220) [antimicrobial activity of four *Ganoderma lucidum* (W Curt.: Fr.) P. Karst.](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0220) [Strains cultivated on different wood type substrates. Acta Soci Botani Poloniae](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0220) [2012;81:17–21](http://refhub.elsevier.com/S2314-808X(18)30117-9/h0220).