Production, characterization, and antioxidant activities of bacterial exopolysaccharides extracted from petroleum oil water

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Backgroundand objective
There are large quantities of water produced during the extraction of petroleum oil called ‘produced water’. The aim of this research is to isolate and identify bacteria produced exopolysaccharides (EPSs) present in this water and to evaluate their antioxidant activities as medicinal value.
Materials and methods
First isolation of Fe, Mn, and biofilm bacteria, next production of of EPSs, then morphological, physiological, and molecular characterization of isolates produced EPSs. Finally, study the characters of EPSs chemically and evaluate the antioxidant activity of EPSs by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging model, reducing power, and by superoxide anion-scavenging activity.
Results and conclusion
Three bacteria were isolated from water of petroleum oil (produced water).These isolates produced water-soluble EPSs called Fe, Mn, and BF-EPSs and the highest production were 4.5, 7.5, and 5 g/l, respectively. The isolates were identified as *Bacillus subtilis* SMM1, *Bacillus pumilus* SMM2, and *Bacillus tequilensis* SMM3. Results showed that the three EPSs were acidic with different compositions of monosaccharide and different molar ratio. Uronic acid and SO_3^- were estimated. EPSs scavenged superoxide radical (O^{2-}), DPPH radicals, and reducing power property. Fe-EPS was the most effective one in scavenging the superoxide radical and DPPH radicals while the highest reductant is BF-EPS. The obtained results demonstrated that all EPSs that have strong antioxidant activity can be used in medicinal and nutritional applications related to reduction of oxidative stress.

Keywords:
antioxidant activities, B. subtilis SMM1, B. pumilus SMM2, B. tequilensis SMM3, chemical composition, exoploysachharides

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Introduction

As the worldwide demand and production of petroleum product (oil and gas) keep on expanding, its production and investigation are associated with different ecological impacts. It is known to produce water in the production of oil, where it represents a source of waste generation of the oil because of the huge volume-generated toxicity and no treatment or appropriate transfer.

Microorganisms fit for surviving the high toxicity of water production have an incredible biotechnological interest [1,2]. These microorganisms have adopted extraordinary metabolic pathways; furthermore, they have defensive systems to survive and introduce a model to examine the roles and the stability of their biomolecules. Undoubtedly microorganisms from extreme habitats that produced exopolysaccharides (EPSs) were very promising in biotechnological applications [3] ranging from industrial to medical applications [4].

Sutherland [5] used the term EPS for the first time to describe high molecular weight carbohydrate polymers produced by bacteria. The advantages of microbial EPSs when compared with others sources like plants and marine macroalgal polymers are due to their physical and chemical properties besides their stability [6]. To the extent the microbial biodiversity is concerned, bacterial EPSs show an extensive variety of chemical structures: numerous EPSs demonstrate heteropolymeric arrangement in addition to a high molecular weight. EPSs produced by bacteria are found in two structures, as capsular or slime polysaccharides where EPSs are either bound covalently or remain attached (loosely bound) to the surface of the cell or occur in the extracellular medium [7].

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EPSs are used in the detoxification mechanisms of oil-polluted area furthermore as biosurfactants [8]. Recently, because of the advancement of new ways of life and work, there has been an expanding interest for useful nourishments, for example, chitins, polyphenols, and phospholipids. So, EPSs have been broadly examined and observed to be a standout among the most imperative biological macromolecules in nature due to their extensive variety of pharmacological activities, for example, antityrosinase, antioxidant, antitumor, antihypertensive [4,9,10]. The aim of this research is to isolate bacteria produced EPS from the remained polluted water (produced water) and test these polymers for their antioxidant activities by different assays.

Materials and methods

Water analysis

Physicochemical water analysis according to American public health association (APHA) [11] was received from the Qarun Petroleum Company and is recorded in Table 1.

Bacterial and culture conditions

Bacteria were isolated from samples of (produced water) obtained from Qarun Petroleum Company, Egypt, and they were grown at 37°C under aeration in flask batches of a Medium 1 used for the isolation of sulfate-reducing bacteria. This medium contained the following ingredients as g/l [12] of KH_2PO, 0.5; NH_4Cl 1.0; $CaSO_4$ 1.0; $MgSO_4.7H_2O$ 2.0; sodium lactate 3.5; yeast extract 1.0; ascorbic acid 0.1, thioglycolic 0.1, $FeSO_4.7H_2O$ 0.5; agar 20, and distilled water 1000 ml, pH 7.

Medium 2 which was used for iron-oxidizing bacteria and manganese-oxidizing bacteria contained the following ingredients as g/l [13]: (NH_4) SO4 0.5; $NaNO_3$ 0.5; K_2HPO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.5; citric acid 10; sucrose 2; tryptone 1; $MnSO_4 \cdot 5H_2O$ 4.7 (for manganese-oxidizing bacteria) or $FeSO_4 \cdot 7H_2O$ 5.9 (for iron-oxidizing bacteria); agar 20, and distilled water 1000 ml, pH 7.

Medium 3 (nutrient agar medium) was used for biofilm bacteria detection, isolation and counting, contained the following ingredients as g/l [12]: peptone, 5.0; meat extract, 1.0; yeast extract, 2.0; NaCl, 5; agar 20, and 1000 ml distilled water, pH 7.

Isolation of exopolysaccharide-producing bacteria

The strategy of the present work aimed to isolate sulfate-reducing, iron-oxidizing manganese and biofilm bacteria. A small portion of the fresh polymeric material was inoculated by the streaking plate technique using a solid agar medium. There was a specific medium for each group. Each medium was autoclaved at 121°C for 20 min. Final pH was adjusted to 7.0. The incubation was applied at 37°C for 24 h. The bacterial growth was examined by naked eye. Bacterial isolates were applied for complete purification.

Bacterial count

The bacterial growth (intensity) was detected by the most probable number and colony count techniques (CFU) according to APHA [11].

Screening for exopolysaccharides production

Isolates were screened for the production of EPS using the medium composed of the following ingredients g/l [14] of glucose 20; $CaCO_3$ 0.1; NH_4NO_3 0.8; K_2HPO_4 0.6; KH_2PO_4 0.05; $MnSO_4.4H_2O$ 0.1; yeast extract 1.0; distilled water 1000 ml, pH 7. Fifty milliliters of the previous medium was prepared, sterilized, and inoculated with 5% of fresh pure enrichment culture (24 h) of each group in 250 ml flasks. The flasks were incubated for 3 days at 30°C and 150 rpm. After centrifugation at 5000 rpm for

Table 1 Physicochemical analysis of produced water

Salts		Dissolved solids		Physical properties	
Ions	Concentrations (ppm)	Salts	Concentrations (ppm)		
Sodium and potassium	1676.4101	Calcium carbonate	102.519	Specific gravity	1.0062
Calcium	675	Calcium sulfate	992.672	pH	7
Magnesium	675	Calcium chloride	946.311	Salinity as NaCl	6099.08 ppm
Chlorides	3700	Magnesium sulfate	0	Total alkalinity	102.459 ppm
Sulfate	125	Magnesium chloride	1233.62	Total hardness	2978.78 ppm
Bicarbonates	700	Sodium Sulfate	0	Temporary hardness	102.459 ppm
Carbonates	0	Sodium and potassium chlorides	3916.28	Permanent hardness	2876.32 ppm
Hydroxide	0	Total dissolved salts	7300		

30 min, EPS production at supernatant was determined according to Dubois *et al.* [15].

Isolation and purification of polymers

Bacteria were grown in liquid culture (g/l) [16] of sucrose 50; peptone 4 and yeast extract 2; dissolved in 1000 ml distilled water, pH 7 under shaking condition; 150 rpm for 3 days. Cells were initially precipitated by centrifugation (3000 rpm for 20 min at 4°C). The supernatant was deproteinized by adding 5% (w/v) trichloroacetic acid. The mixture was stirred for 4 h. After centrifugation (5000 rpm for 20 min at 4°C, 2K15; Sigma-Laborzentrifugen, Germany), the pH of the supernatant was adjusted to 7.0 with NaOH (0.1 M) solution and dialyzed three times (1000 ml×3). Cold ethanol one volume (1 : 1) ethanol: supernatant was added to the supernatant, then agitated vigorously, and kept at 4°C overnight. The precipitated polymer was collected by centrifugation at 5000 rpm and then the method was repeated using two and three volumes of the cold ethanol in order to obtain the main fraction of EPS. The precipitated EPS in each fraction was collected by centrifugation at 5000 rpm and then the main fraction of EPS was washed twice with acetone and dehydrated by diethyl ether and then was dried under vacuum. The yield of EPS was determined by the Dubois method [15].

Taxonomic identification of the isolates

The promising strains which produce the highest EPS levels were identified on the basis of morphological, physiological, and biochemical characteristics according to Berge's manual [17,18] and Sneath *et al.* [19] combined with 16s rRNA sequence analysis. The universal primers delineated by Weisburg *et al.* [20] were used to amplify the 16s rRNA gene sequence. Data were submitted to GenBank and the sequence compared with the GenBank database (http://www.ncbi.nlm.nih.gov) using BLAST [21].

Identification of polymeric materials

Analysis of monosaccharide composition

The polymer composition was determined after hydrolysis with formic acid 88% at 105°C in a sealed tube for 5 h. The excess acid was removed by flash evaporation on a water bath at 40 °C and co-distilled with water (1 ml×3). The monosaccharaide 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 (flow rate 0.5 ml/min) [22]. Uronic acid content was determined by the m-hydroxydiphenyl method using glucuronic acid as the standard [23]. The sulfate content of the EPSs was determined by using the turbidimetric method [24] together with sodium sulfate as the standard.

Fourier-transform infrared spectroscopy

Infrared spectrum samples were obtained by grinding a mixture of a sample with dry KBr and pressing in a mortar. An IR spectrum was recorded on a Fourier-transform infrared spectrophotometer (500-IR; Brucker Scientific) [25].

Antioxidant properties investigations

In all in-vitro assays, ascorbic acid and butylated hydroxytoluene were used as standards.

DPPH radical-scavenging activity

The antioxidant activity was determined using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging model [26]. Aliquots of 1 ml of each tested EPSs at concentrations of 20, 40, 80, and 120 μg/ml were prepared in methanol and 5 ml of freshly prepared 0.1 mmol/l DPPH was thoroughly mixed. The mixture was shaken vigorously (2500 rpm) for 1 min, then left to stand for 60 min in the dark. Scavenging capacity was measured spectrophotometrically at 517 nm. Scavenging activity (%) was plotted against the sample concentration in the reaction system. The percentage scavenging activity (inhibition of the DPPH radical) was calculated according to the following formula;

% Inhibition = $[(A_0\text{-}A_1)/A_0]\times100$ Where A_0 was the absorbance of the control, and A_1 was the absorbance of EPSs or standards.

Reduction capability property

The reducing power of EPSs was determined according to the method of Oyaizu [27]. One milliliter of each tested EPSs at different concentrations and phosphate buffer (0.5 ml, 0.2 M, pH=6.6) with potassium ferricyanide $[K_3Fe\ (CN)_6]$ (2.5 ml, 1%) were placed in a test tube. The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a Shimadzu spectrophotometer. The higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide anion radical-scavenging property

Measurement of this property for different EPS concentrations are based on the method described by

Liu *et al.* [28], with slight modifications [29]. Superoxide radicals were generated in phenazine methosulfate (PMS)–NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 ml of Tris–HCl buffer (16 mmol/l, pH=8.0) containing 1 ml of NBT (50 μmol/l) solution, 1 ml NADH (78 μmol/l) solution, and 1 ml sample solution were mixed. The reaction was started by adding 1 ml of PMS solution (10 μmol/l) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm in a Shimadzu spectrophotometer was measured against blank samples. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Percent inhibition}=[(A_0-A_1)/A_0]\times 100.$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the extract or standards.

Results

Water analysis

The 'produced water' contains salts, metals, and radionuclides characteristic of the formation from which the water was extracted as shown in Table 1. It is well known that no produced water was alike; it depends on its region.

Isolation, count, and identification of bacteria

The microbial growth of all groups recorded a high activity as shown in Table 2. The bacterial total counts (CFU) and the MPN ranged from 1.135×10^5 to 1.175×10^6 and $>10^5>10^6$, respectively, which meant the presence of highly active microbial flora in this situation.

Table 2 Total bacterial count and most probable number isolated bacteria

Bacterial group	CFU/ml	MPN
BF-bacteria	1.175×10^6	$>10^6$
Fe-bacteria	4.165×10^5	$>10^5$
Mn-bacteria	1.135×10^5	$>10^5$

MPN, most probable number.

There are 10 isolates that have the ability to produce EPSs and three strains were chosen (highest EPS production) for the current study. The productivity of EPSs for Fe, Mn, and BF were 4.5, 7.5, and 5 g/l, respectively.

According to the physiological and biochemical characters as shown in Table 3, the bacterial isolates which produced a high yield of EPSs were identified as *Bacillus* spp. Comparison of 16 s rRNA gene sequence of strains with other bacteria from GenBank by BLAST analysis showed the closest strain (99% similarity) with accession numbers MF446913, MF446914, and MF446916 (Table 4 and Figs 1–3).

Production and characterization of exopolysaccharides

All the EPSs that produced from the three strains have various monosaccharide compositions with various molar ratios. As the result, the molar ratio of uronic ranged from 13.01 to 18.09% (Table 5).

The results in Table 5 show that EPSs (polymers) obtained from BF, Mn isolated bacterial groups contained galacturonic acid. Mn, BF produced polymer contained galacturonic acid by the same ratio. Iron bacteria produced polymer contained glucuronic acid by the same ratio as of galacturonic acid in the other bacteria. Furthermore each type of the isolated bacterial group produced polymers containing different monosaccharides represented as glucose, rhamnose and fucose. Results explained the plugging

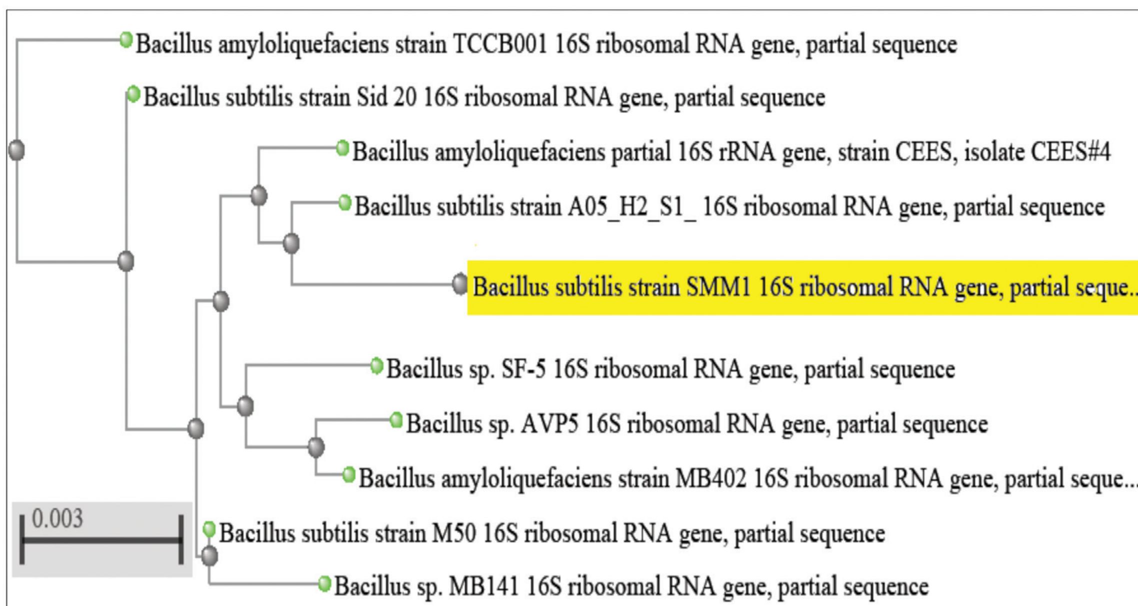
Table 3 Phenotypic characterizations of bacterial isolates according to Berge's manual

Characteristic	Isolate no. 1	Isolate no. 2	Isolate no. 3
Gram stain	Positive	Positive	Positive
Motility	Motile	Motile	Motile
Catalase	Positive	Positive	Positive
Citrate	Positive	Negative	Positive
Indole production	Negative	Negative	Positive
Urease production test	Negative	Negative	Negative
Oxidase	Positive	Positive	Positive
Nitrate reduction test	Positive	Negative	Positive
H_2S production test	Positive	Negative	Negative
VP (Voges–Proskauer)	Positive	Positive	Positive

Table 4 Names of bacteria according to 16 s rRNA

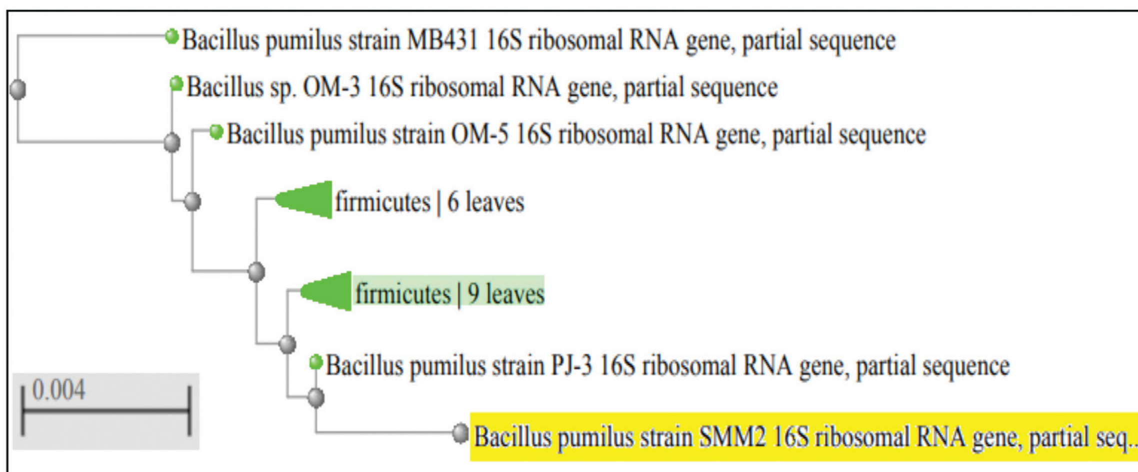
Isolates number	Name of organisms	Type of bacteria	Isolation source	Accession number
1	*Bacillus subtilis* SMM1	Fe-bacteria	Produced water	MF446913
2	*Bacillus pumilus* SMM2	Mn-bacteria	Produced water	MF446914
3	*Bacillus tequilensis* SMM3	BF-bacteria	Produced water	MF446916

Figure 1



Phylogenetic tree of the partial sequence of 16 s rRNA of the local isolate (*Bacillus subtilis* SMM1) with respect to closely related sequences available in GenBank databases.

Figure 2



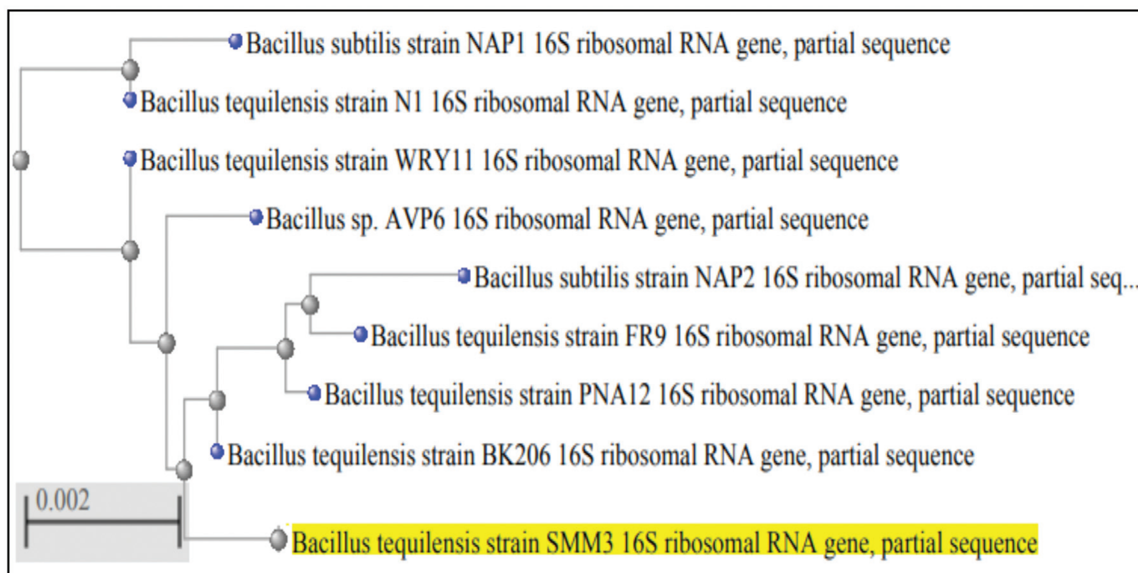
Phylogenetic tree of the partial sequence of 16 s rRNA of the local isolate (*Bacillus pumilus* SMM2) with respect to closely related sequences available in GenBank databases.

of the filter within few days of working operation due to the bacteria in produced water and their exopolymeric secretions. In addition, sulfate content was obtained from the three strains but with different percentage as shown in Table 5 and these results refer to their antioxidant activity.

The infrared spectrum of BF-EPS and Fe-EPS Figs 4 and 5 revealed typical major broad stretching peaks around 3423.99 cm^{-1} for the OH, and a weak band at 2924.52 cm^{-1} demonstrates the C-H stretching vibration. The expansive band at 1628.59 cm^{-1} indicates bound water, the absorption at 1457.92 cm^{-1} was possible due to nonsymmetric CH_3 bending. The absorbance at 1792.5 cm^{-1} indicated the presence of uronic acid.

The infrared spectrum of Mn-EPS Fig. 6 recorded typical major broad stretching peaks at around 3429.78 cm^{-1} for the OH; a weak band at 2925.48 cm^{-1} revealed the C-H stretching vibration. The expansive band at 1624.73 cm^{-1} was because of the bound water; the absorption at 1457.92 cm^{-1} was possible due to nonsymmetric CH_3 bending. The absorbance at 1792.5 cm^{-1} demonstrates the existence of uronic acid and 1113.69 cm^{-1} due to

Figure 3

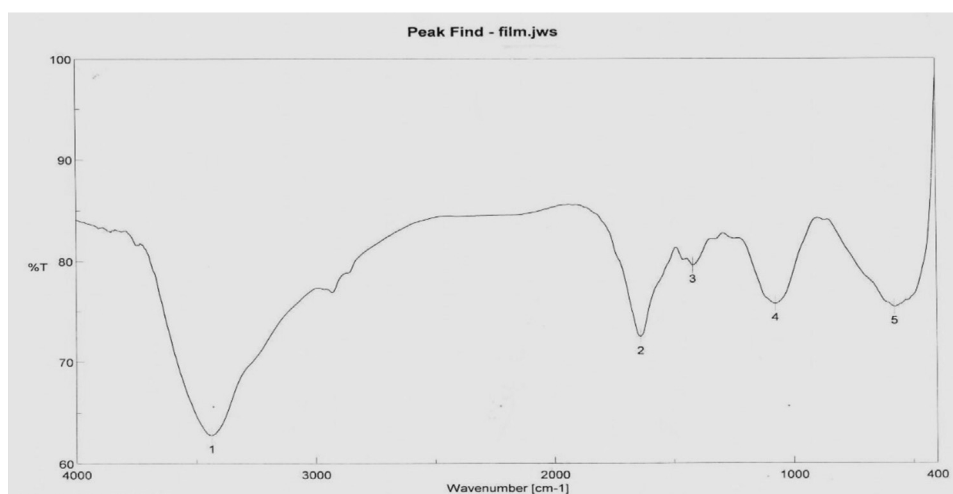


Phylogenetic tree of the partial sequence of 16 s rRNA of the local isolate (*Bacillus tequilensis* SMM3) with respect to closely related sequences available in GenBank databases.

Table 5 Sulfate content, uronic, and molar ratios of the isolated exopolysaccharides

Source of polymer	Molar ratio of monosugars	Uronic acid (%)	Sulfate content (%)
BF-bacteria	Galacturonic acid : glucose 1.0 : 2.7	16.54	11.09
Fe-bacteria	Glucuronic acid : rhamnose 1.0 : 4.1	13.01	14.36
Mn-bacteria	Galacturonic acid : fucose 1.0 : 1.6	18.09	18.18

Figure 4



Fourier-transform infrared spectroscopy chart for exopolysaccharide obtained from biofilm bacteria.

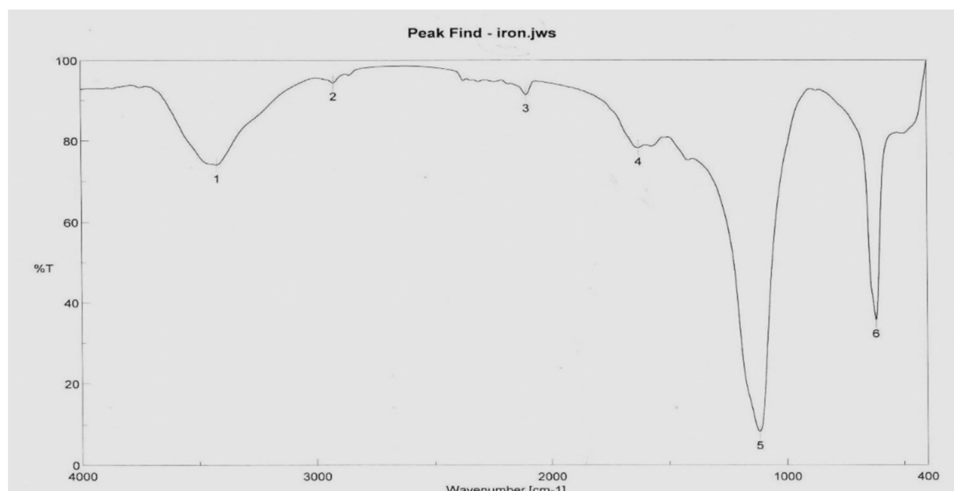
the sulfate group. These charts indicated that all polymeric materials were of microbial origin. These polymers are similar in some groups and different in another one.

EPS activities can be influenced by numerous factors such as chemical composition, molecular weight, and conformation; also they can be influenced by the ways of their isolation and extraction, where these factors could play an important role in the antioxidant characters [30].

DPPH radical-scavenging activity

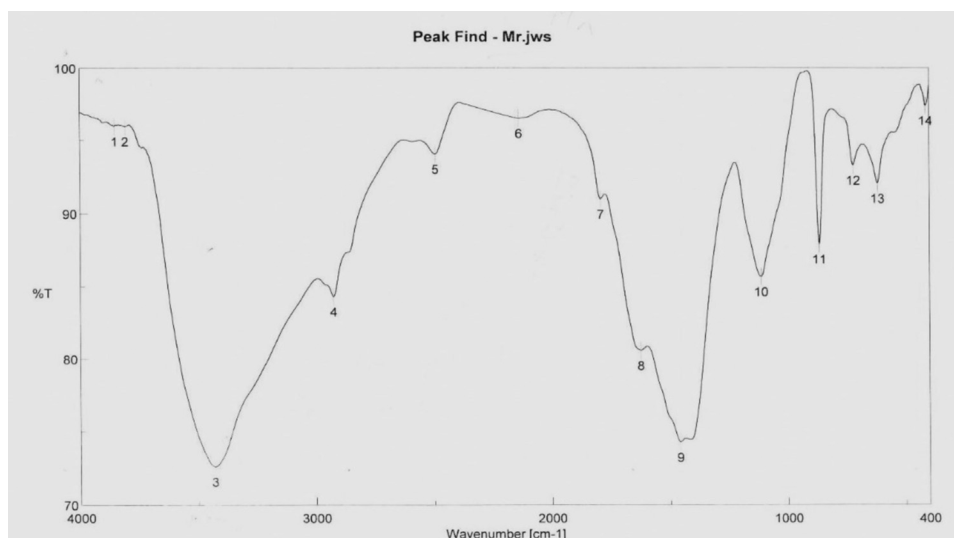
To study the antioxidant activities of three EPSs, antioxidant properties were based on SO_3^-, hydroxyl, and uronic groups. The outcomes of DPPH radical-

Figure 5



Fourier-transform infrared spectroscopy chart for exopolysaccharide obtained from iron-oxidizing bacteria.

Figure 6



Fourier-transform infrared spectroscopy chart for exopolysaccharide obtained from manganese-oxidizing bacteria.

scavenging assay showed that EPSs have the ability to scavenge free radicals Fig. 7. The activity was conditionally increased by increasing the concentration of EPSs. This observation was true with all tested polysaccharides and standards to reach the highest impact at 120 mg/l. The best outcomes were obtained with Fe-EPS (40.84, 48.2, 53.93, and 58.28% for 20, 40, 80, and 120 mg/l) while the lowest effect was found with BF-EPS (30.8, 37, 46.7, and 49% for 20, 40, 80, and 120 mg/l).

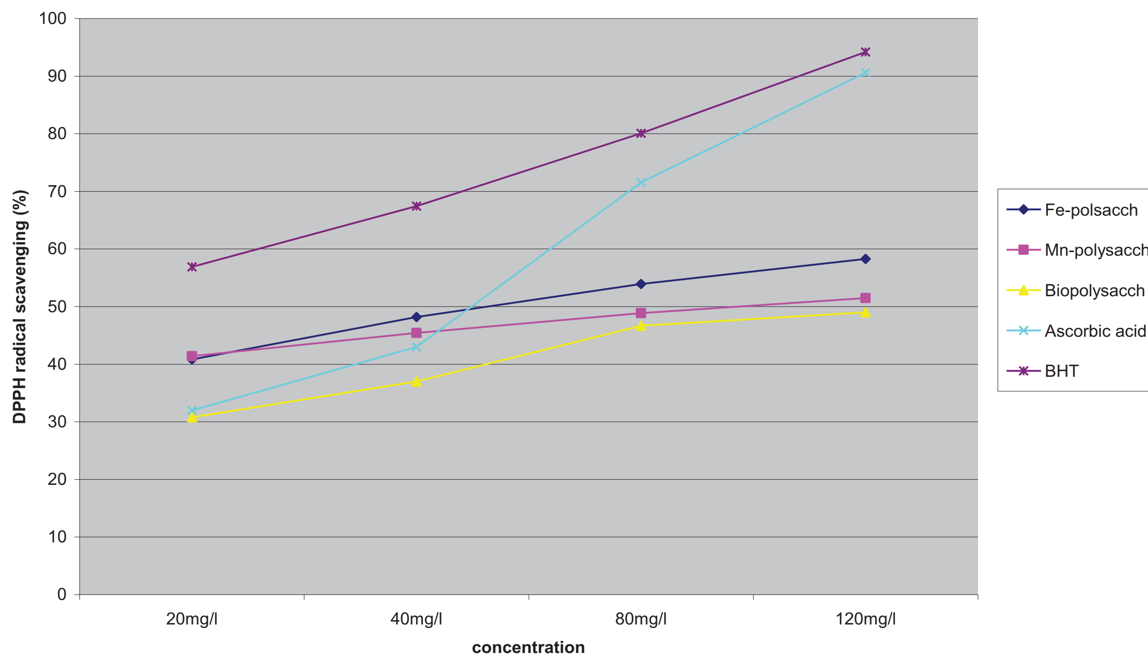
Reducing power

All EPSs at all concentrations revealed the reducing capacity and the outcomes were shown in Fig. 8. In all samples, the reducing capacity increased by increasing the concentration. In addition, the reducing capacity of BF-EPS was higher than the other samples at 80 and 120 mg/l. It has the same level of the other two EPSs as a reductant at 20 and 40 mg/l, so that it was the most active one at the highest concentration, while the weaker one was Fe-EPS. The reducing power was conditionally increased by increasing the concentration; all EPSs demonstrated a similar pattern of information.

Superoxide anion-scavenging activity

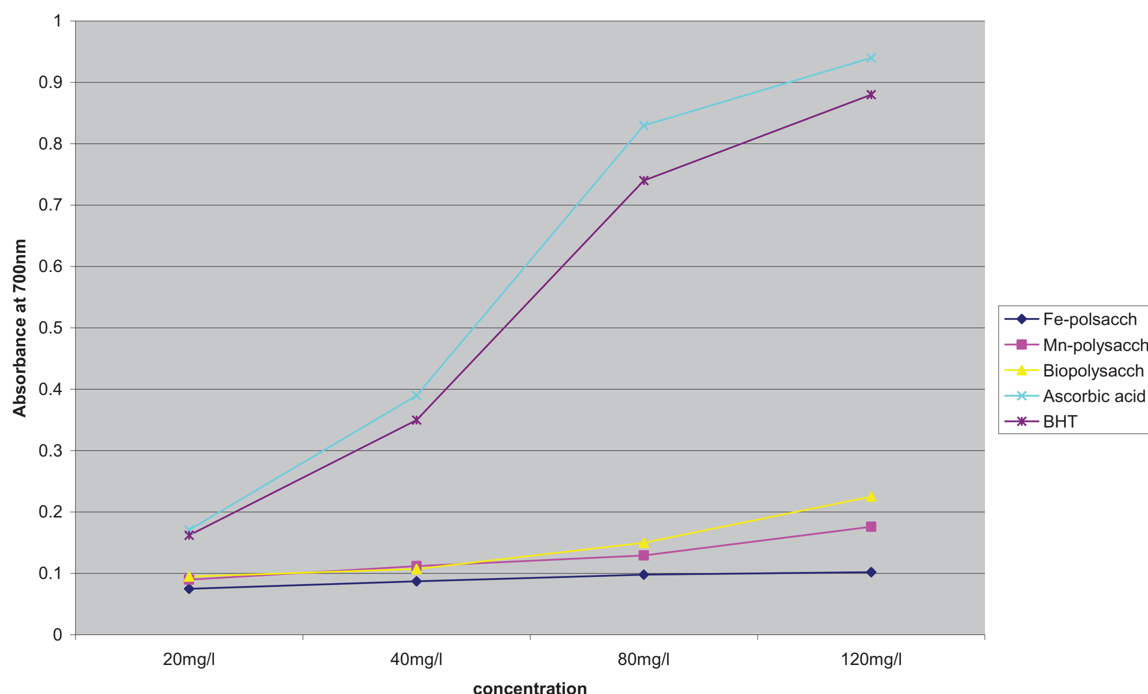
Superoxide anion-scavenging impact of the three EPSs was examined using the PMS-NADH-NBT method and data appeared in Fig. 9. Recorded outcomes demonstrated the elevated activity of every tested EPS at various concentrations. Both Fe-EPS and Mn-EPS displayed high recorded values when

Figure 7



DPPH free radical-scavenging effect of three exopolysaccharides and standards. Values are mean of triplicates.

Figure 8



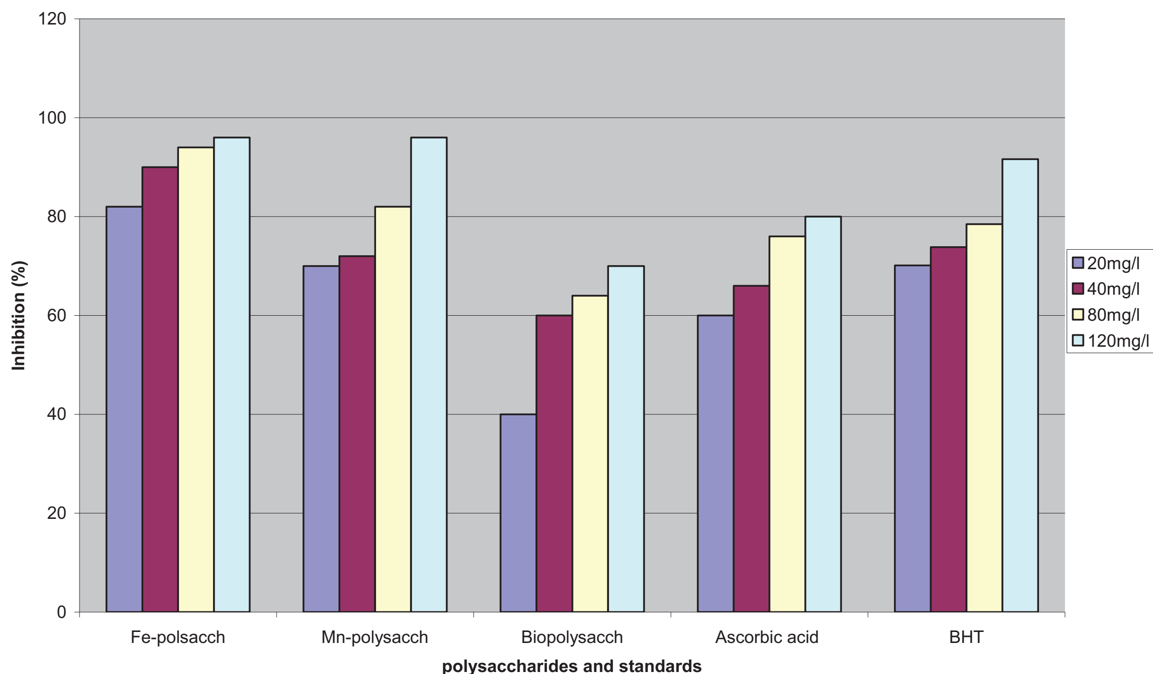
Reducing power of three exopolysaccharides and standards. Values are mean of triplicates.

contrasted with standards or BF-EPS. Fe-EPS indicated higher superoxide-scavenging activity than Mn-EPS at 20, 40, and 80 mg/l whereas they had a similar activity at 120 mg/l. BF-EPS produced reduced scavenging impact at all tested EPSs or standards. The most extreme impact was at 120 mg/l which is equivalent with the least impact of both Mn-EPS and butylated hydroxytoluene at 20 mg/l.

Discussion

It is known that free radical-mediated oxidation of biological molecules, for example, DNA, proteins, and lipids are associated with a variety of disorders and illness [31]. Free radicals incite an assortment of biochemical and physiological lesions and upgrade degenerative diseases like Alzheimer's, aging, and

Figure 9



Superoxide anion radical-scavenging effect of three exopolysaccharides, ascorbic acid, and butylated hydroxytoluene using the PMS-NADA-NBT method.

tumors [32]. Most importantly, lipids are attacked by free radical and lipid peroxidation initiates changes in the integrity and makes disturbances in fine structure furthermore causing loss of biomembrane functions [33]. Also, lipid peroxidation continues by a chain mechanism, increasing the harming impact of free radicals [34]. Changes of proteins and DNA were done due to lipid peroxidation; besides it was related with the pathogenesis of various sicknesses [31,34,35]. Furthermore, the effect of free radical-scavenging antioxidants has received much consideration. This study was carried out to evaluate the antioxidant activity of three EPSs, Fe-EPS, Mn-EPS, and BF-EPS by various assays.

The DPPH method includes a single-electron transfer mechanism, besides a hydrogen atom move characteristically in other antioxidant methods like the oxygen radical absorbance capacity assay [36]. In spite of the fact that the DPPH technique is not discriminative of the radical species, and it was demonstrated that the total antioxidant capacity value does not precisely indicate the antioxidant capacity; it introduces thought of the radical quenching ability of the samples [37].

DPPH-scavenging impact of all tested EPSs increased concentration dependently and the highest effect was recorded with Fe-EPS while the lowest effect was recorded with BF-EPS. Both Fe-EPS and Mn-EPS exhibited higher effect more than ascorbic acid at the lowest concentrations (20 and 40 mg/l).

In the reducing power assay, the shade of test solution was changed from yellow to various colors of green and blue colors. The reducing capacity of a compound was indicated as a huge marker of its antioxidant activity. The existence of reductants like antioxidant substances in the samples leads to the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) which can be observed by estimating the formation of Perl's Prussian blue at 700 nm [38]. The antioxidant activity has been accounted to have an immediate, positive relationship with this assay [39,40].

Our outcomes on the reducing power of all samples demonstrate the antioxidant effect. All tested EPSs were weaker reductants compared with standards. As compared in between them, BF-EPS was the best reductant while Fe-EPS was the weaker one. Superoxide anions are the widely recognized free radicals in vivo and are created in different biological systems besides the concentration of superoxide anion increments under conditions of oxidative stress [41]. Thus, an examination was done to test three disengaged EPSs at various concentrations as a scavenger for superoxide anion radicals. Polysaccharide-scavenging effect conditionally increased with increasing concentration. Fe-EPS and Mn-EPS exhibited the maximum scavenging effect than standards at all

concentrations, in inversely manner of DPPH scavenging effect or reducing power effect. Fe-EPS had an intense scavenging impact compared with the standards or other polysaccharides at all concentrations and it is similar to Mn-EPS at 120 mg/l only. In view of the distributed writings, the antioxidant activity of EPSs may be related with different factors [42,43]. Antioxidant activities were correlated with uronic acids, where it was revealed that the more the uronic acids found in EPSs the higher the antioxidant activities detected [44]. It was reported that the polysaccharide PUP60S2 (has a backbone composed of B (1-6)-D from *Polyporus umbellatus* sclerotia) that have more uronic glucopyranosyl acid residues and larger amounts of branching indicates better antioxidant activity. Besides that, the structure of EPS assumes a critical part of their biological and physiological functions [45,46].

Few specialists have announced that EPSs with more mannose and rhamnose exhibit higher antioxidant activities [47].

Conclusion

The main objective of this study was to isolate and define bacteria as well as investigation of these exopolysaccharides bacteria can potentially act as antioxidant where the tested bacteria are known to be produce polysaccharides. This study demonstrated the antioxidant effect of Fe-EPS, Mn-EPS, and BF-EPS. The tested polysaccharides significantly scavenged superoxide radical (O^{2-}) and (DPPH) radicals in a dose-dependent manner also exhibited reducing power activity. Fe-EPS was the most effective one in scavenging superoxide radical and DPPH radicals while the highest reductant is BF-EPS. Thus, these EPSs could apply a gainful activity in the nourishment industries, cosmetic and medicine as an antioxidant agent.

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Conflicts of interest

There are no conflicts of interest.

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