



Functional, rheological, and antioxidant properties of extracellular polymeric substances produced by a thermophilic cyanobacterium *Leptolyngbya* sp.

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Abstract

Extracellular polymeric substances (EPSs) produced by the filamentous cyanobacterium identified as *Leptolyngbya* sp. IkmLPT16 were isolated and characterized chemically, and their antioxidant, functional, and rheological properties were studied. The strain produces a significant amount of EPSs (2.15 g L^{-1}) conjointly with a biomass production achieved at a maximum of 1.35 g L^{-1} after nine production days. Chemical analysis of EPSs revealed the presence of mannose (35%), arabinose (24%), glucose (15%), rhamnose (2%), and one uronic acid (8%). Fourier transformed infrared spectrum of EPSs further revealed the presence of $\nu\text{C-N}$ groups indicating the presence of peptide moieties. Elemental analysis of EPSs showed the presence of sulfate groups ($S=0.59\%$) as inorganic substituents. Functional properties of *Leptolyngbya* EPSs were determined based on water holding capacity, oil holding capacity, foaming ability, and metal sorption ability. Experimental results showed high water holding capacity (119%), water solubility index (97.43%), and oil holding ability (87.52%), with a strong metal sorption ability and consequent foam stability (22%). The rheological properties of EPSs were comparable with commercial xanthan gum with higher resistance to Temperature. *Leptolyngbya* sp. EPSs displayed an effective antioxidant activity via directly scavenging free radicals particularly DDPH• ($\text{IC}_{50}=4 \text{ mg. mL}^{-1}$ against 10 mg. mL^{-1} for L-ascorbic acid) and •OH ($\text{IC}_{50}=10 \text{ mg. mL}^{-1}$ against 20 mg. mL^{-1} for L-ascorbic acid) and as an iron-chelating agent ($\text{IC}_{50}=40 \text{ mg. mL}^{-1}$ against 60 mg. mL^{-1} for EDTA). The outcomes of this study demonstrate the potential use of *Leptolyngbya* sp. EPSs in several food and pharmaceutical applications.

Keywords Extracellular polymeric substances · *Leptolyngbya* · Cyanobacteria · Composition · Functional property · Rheology · Antioxidant property

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Introduction

Despite its high potential, in the context of the transition from a fossil fuel-based economy and the global mass-market industry to a “circular and territorialized bioeconomy” (Vigani et al. 2015), the microalgae market is still limited to only a few exploited microalgae strains. Thus, according to many experts, a census of novel natural resources must be made and ambitious plans for sustainable development established.

Among these resources, thermophilic microalgae and cyanobacteria strains have an exceptional potential (Barbarino & Lourenço 2005), in particular strains whose versatility of applications (food, feed, and bioactive) makes it possible to envisage original contributions. These renewable resources and their valorization have received increased attention since they contain higher levels of

proteins (Lourenço et al. 2004; Becker 2007) and high-value thermostable bioactive compounds (Haki & Rakshit 2003) compared to terrestrial crops, such as soybean and pulse legumes. Furthermore, as a consequence of growth at high temperatures, thermophiles can possess wide possibilities for physiological adaptation and genetic modifications that make them potential producers of extracellular polymeric substances (EPSs) with high production yields.

Recently, the development of new lifestyles and work pressure increased demand for the use of several functional foods, such as polyphenols, phospholipids, chitins, and microbial EPSs (Barrow & Shahidi 2007). Microbial extracellular polymeric substances (EPSs) are organic biodegradable polymers made up mainly of polysaccharides. EPSs are synthesized by a multitude of diverse microorganisms, including bacteria, fungi, microalgae, and cyanobacteria (Wingender et al. 1999; Aires et al. 2011; Xiao & Zheng 2016). EPSs such as xanthan from *Xanthomonas campestris*, gellan gum from *Sphingomonas paucimobilis* (Sutherland 1998), or pullulan from *Aureobasidium fungus* (Morris & Harding 2009) have captured the commercial market and are being used as food additives, stabilizers, and suspending agents.

In searching for new sources of novel exopolysaccharides, attention toward cyanobacterial EPSs has increased due to their massive/hyperproduction, rheological features, and variable possible applications in the different fields of biotechnology (De Philippis & Vincenzini 1998; Khattar et al. 2010; Ozturk & Aslim 2010). Cyanobacterial EPSs have a potential advantage among other commercial EPSs mainly due to their renewability, stable cost, constant, and reproducible physico-chemical properties (Selbmann et al. 2002; Parikh & Madamwar 2006; Pereira et al. 2009). The production of EPSs by cyanobacteria depends on species and growth conditions with a maximum EPSs yield observed during the exponential or the stationary growth phases (Delattre et al. 2016; Xiao & Zheng 2016).

Cyanobacterial EPSs are biopolymers that can remain associated with the cell surface (cell-bound polymers) and/or be liberated into the surrounding environment as released polysaccharides (De Philippis & Vincenzini 1998; Arad & Levy-Ontman 2010). They have numerous industrial and health applications, such as antioxidants, anti-tumor, antiviral, anti-inflammatory, drug carriers, bioabsorbant, encapsulation materials, flocculants, emulsifying agents, and ion exchange resins, because of their distinctive physical and chemical properties (Yim et al. 2004; Abed et al. 2009; De Jesus Raposo et al. 2013; Kumar et al. 2018). Bioactive properties of EPSs are related to their molecular weight, monosaccharide composition, glycosidic linkage, conformation, and non-carbohydrate substituents such as protein, uronic acid, nucleic acids, lipids and O-methyl, O-acetyl, and sulfate groups (Pereira

et al. 2009; Ozturk & Aslim 2010; Delattre et al. 2016; Rani et al. 2017).

In this context, the present study aims to identify new sources of novel cyanobacterial EPSs released into the surrounding environment by a newly identified filamentous cyanobacterium, *Leptolyngbya* sp. IkmLPT16, isolated from a Tunisian hot water spring and cultivated in laboratory conditions. Biomass and EPS production were evaluated and further chemical and physical proprieties and antioxidant activity of EPSs were studied.

Materials and methods

Organism and culture conditions

The strain was isolated from microbial mats anchored to submerged stones in hot water (Ain Echfa, 60 °C) located in the northern part of Tunisia (36° 49' N, 10° 34' E). Isolation and purification were performed by dilution and plating of water samples. The amplification and sequencing of the 16S rRNA gene fragment showed a similarity of 94.3% with *Leptolyngbya* sp., (Bravakos et al. 2016) and 93.6% with *Leptolyngbya frigida* ANT.L52.2 (Taton et al. 2006).

The strain was identified as a new species of the genus *Leptolyngbya* and named *Leptolyngbya* sp. The nucleotide sequence determined has been deposited in the GenBank database (accession number SUB3434644 clone-38-pGEMT-EUB28f-1492r-1454pb-MG753795GU552680). The strain was referenced IkmLPT16 and conserved in the Tunisian National Institute of Sciences and Technology collection.

Growth condition

The strain *Leptolyngbya* sp. IkmLPT16 was placed under conditions that optimize the algal growth: BG11 medium (Bischoff & Bold 1963; Stanier et al. 1971); 40 ± 1 °C; $85 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and performed in a 20 L capacity closed cylindrical reactor for 9 days. Experimental cultures were exposed to a series of LED tubes (type C-LED tubes, Cefla, Italy) with a light/dark photoperiod of 16:8 h. The strain was inoculated with an initial optical density (680 nm) of 0.07. Growth was monitored by measuring daily the dry biomass to determine the biomass density (g L^{-1}). Samples (100 mL) in triplicate were filtered through prewashed $0.2 \mu\text{m}$ microfiber filters (GF/F filter, Whatman Plc., UK) and desiccated overnight at 80 °C. The filters were cooled to room temperature in a desiccator before the weighing.

The instantaneous growth rate (GR, $\text{g L}^{-1} \text{ day}^{-1}$) was determined using a fitting program applied to the growth curve using the following formula: $GR = (dx/dt)$, where (x) is the biomass density and (t) is the time.

Isolation, purification, and quantification of EPSs

The EPSs were extracted from the culture by stirring the algal culture for 30 min, followed by filtration using a filter with a 20 μm pore size to separate the algal cells from the culture medium containing the released EPSs. The total recovered supernatant (culture medium and soluble EPSs) was concentrated (tenfold) via tangential ultrafiltration (Vivaflow 50, Sartorius) using Millipore membranes with a cut-off of 8 kDa. The removal of low molecular weight substances and inorganics was ensured by a cycle of consecutive concentrations and dilutions with ultrapure water until constant conductivity was reached (0.08 mS^{-1}). The recovered filtrate, rich in EPSs, was freeze-dried and weighed for gravimetric EPSs determination. The EPSs productivity $\text{g EPS L}^{-1} \text{ day}^{-1}$ was evaluated.

Chemical composition of *Leptolyngbya* sp. EPSs

The biochemical composition of *Leptolyngbya* sp. EPSs were determined using colorimetric and gravimetric methods. Total carbohydrates content was determined by the phenol sulfuric acid method according to (Dubois et al. 1965) using D-glucose (Sigma, 50–99-7) as standard. Proteins content was determined according to (Lowry et al. 1951) using bovine serum albumin (Sigma, 10,711,454,001) as standard. The total lipid content of EPSs was determined gravimetrically using the method of Folch et al. (1957). The EPSs elemental analysis (C, N, H, S, and P) was performed using a Flash Elemental Analyzer 1112 (ThermoQuest, Italy).

The *Leptolyngbya* EPSs monosaccharide profile was separated by gas chromatograph type GC 5890AGC 5890A (Hewlett Packard, USA) at 240 °C and detection was performed via FID. The separation column RTX2330 (Restek, Germany) was used with a 30 m length and 25 μm diameter. Nitrogen and air-hydrogen mixture was used as the carrier gas and as fuel, respectively. EPSs (5 mg) were hydrolyzed in 2 mL trifluoroacetic acid (2 M) for 2 h at 100 °C and transferred into target vials and dried under nitrogen gas (Streeter & Strimbu 1998). The extracts were subjected to derivatization before GC–MS analysis as described by Streeter & Strimbu (1998), with the following modifications: 310 μL of pyridine and 250 μL of STOX solution were used to resuspend dried samples, and 400 μL of HMDS and 40 μL of TFA were used to derivatize extracts. The separated monosaccharides were quantified using external calibration with an equimolar mixture of nine monosaccharide standards (analytical standard, Sigma Aldrich): hexoses (mannose, glucose, and galactose), pentoses (xylose and arabinose), deoxyhexoses (rhamnose and fucose), and acidic hexoses (galacturonic and glucuronic acids). Derivative sugars were identified by comparison of retention times and mass spectra

to those of authentic standards and quantified using standard curves generated from each authentic standard.

UV–visible spectrum and Fourier transform infrared spectroscopy (FT-IR) of EPSs

Leptolyngbya EPSs were dissolved in ultrapure water (10 mg mL^{-1}) and the UV spectrum of the EPSs solution was obtained using a UV–visible spectrophotometer (U-3900H, HITACHI, Japan) scanning in a wavelength range of 190–800 nm. The infrared analysis (FT-IR) was prepared by grinding 2 mg dry EPS with 200 mg dry KBr and pressing it in a mold (Gongi et al. 2021). The FT-IR spectra were recorded in transmittance mode in the region of 4,000 to 500 cm^{-1} with a resolution of 4 cm^{-1} on a Perkin Elmer spectrum GX FT-IR system (Perkin Elmer, USA).

Functional proprieties of EPSs

The functional properties of *Leptolyngbya* sp. EPSs were determined by the water solubility index (WSI, %), water holding capacity (WHC, %), oil holding capacity (OHC, %), foam capacity (FC, %) and foam stability (FS, %), and metal sorption capacity (%).

The water solubility index (WSI) was measured as reported by Vinothini et al. (2019).

$$\text{WSI}(\%) = \frac{\text{Weight of dry solids in supernatant}}{\text{Initial sample weight}} \times 100 \quad (1)$$

The water holding capacity (WHC) and the oil holding capacity (OHC) were determined following the procedure described by Wang & Kinsella (1976). Briefly, samples (50 mg) were dispersed in 5 mL of distilled water or 5 mL of peanut oil. The mixture was centrifuged at $4800 \times g$ for 10 min and the supernatant was removed. The WHC and OHC (%) represent the ratio between the weight of the tube content after draining and the weight of the EPS samples. They are reported as grams of water or oil bound per gram of the polysaccharides on a dry basis.

The foaming capacity (FC) and foam stability (FS) were determined according to Shen et al. (2019). EPS aqueous solution, 0.5% (w/v) was prepared and FC and FS were estimated using Eqs. 3 and 4.

$$\text{FC}\% = \frac{V_T - V_0}{V_0} \times 100 \quad (2)$$

$$\text{FS}\% = \frac{V_t - V_0}{V_0} \times 100 \quad (3)$$

where V_T and V_0 are the total volumes after and before whipping. V_t is the total volume after the solution is left at room temperature for 30 min.

The metal sorption capacity of *Leptolyngbya* EPSs was measured based on the method described by Abid et al. (2019). Volumes of 50 mL of Cu^{2+} , Fe^{2+} , Zn^{2+} , or Pb^{2+} metal solution (10 mg L^{-1}) were mixed separately with 2 mL (10 mg L^{-1}) EPSs solutions. Mixtures were then incubated in dark at $25 \text{ }^\circ\text{C}$ for 5 h and separated by centrifugation at $10,000 \times g$ for 10 min at $4 \text{ }^\circ\text{C}$. An atomic absorption spectrometer (iCE 3500 Thermo Scientific, USA) was used to determine the residual metal concentrations. For each metal, the adsorption capacity was expressed as the proportion (%) of residual to initial metal concentrations.

Rheological properties of EPSs

The flow behavior of EPSs was studied according to the method described by Moreno and al. (2000) using a Brookfield R/S Rheometer equipped with a double gap cylinder. The inner and outer radii of the measuring bob (R2 and R3) of the instrument were 22.75 and 23.50 mm, whereas the inner and outer radii of the measuring cup (R1 and R4) were 22.25 and 24 mm, respectively. The shear program applied consisted of an increasing shear-rate ramp from 0.1 to 900 s^{-1} for 15 min.

Tested samples consisted of aqueous dispersions of EPS produced by *Leptolyngbya* prepared at 0.2% and 0.4% (w/w). Aqueous dispersions of xanthan gum (XG; Sigma) were also characterized for comparison purposes.

Furthermore, the viscosities of EPS and xanthan were compared according to an increase of temperature from 15 to $60 \text{ }^\circ\text{C}$. To control the temperature a Julabo circulator thermostat was utilized.

Antioxidant activity evaluation of EPSs

The scavenging ability of 1,1-diphenyl-2-picrylhydrazyl ($\bullet\text{DPPH}$) radical and superoxide hydroxyl ($\bullet\text{OH}$) radicals was measured in the concentration range of 2 – 100 mg mL^{-1} of *Leptolyngbya* EPSs water solution, based on the methods described by (Bersuder et al. 1998) and (Halliwell et al. 1987) respectively. Ascorbic acid was used as the positive control.

The metal chelating activity and the iron-reducing power of *Leptolyngbya* EPSs water solution in the concentration range 2 – 100 mg mL^{-1} were performed with EDTA as the positive control, based on the methods described by Wettasinghe and Shahidi (2002) and Adjimani and Asare (2015) respectively.

Statistical analysis

The results were expressed as average \pm standard deviation (SD) over experimental replicates. Statistical analysis of the data was carried out using the software SPSS Statistics 20.

Differences between treatments were assessed with a one-sided Student's *t* test, and *p* values < 0.05 were considered to be statistically significant.

Results

Growth and EPSs production

The kinetics of biomass growth and EPSs production of *Leptolyngbya* sp. were evaluated under optimal growth conditions (BG11 medium, $40 \text{ }^\circ\text{C}$, $85 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and over 9 days of batch culture (Fig. 1). The maximum biomass density and the maximum EPSs production obtained after 9 days of culture (Fig. 1a) were $1.35 \pm 0.04 \text{ g L}^{-1}$ and $2.15 \pm 0.03 \text{ g L}^{-1}$, respectively.

The daily productivity time course of (Fig. 1b) illustrates that cell biomass and EPS production were correlated. The maximum daily production ($0.32 \text{ g L}^{-1} \text{ day}^{-1}$) of biomass was attained after 5 days of cultivation, and the maximum daily production of EPS ($0.55 \text{ g L}^{-1} \text{ day}^{-1}$) was obtained after 6 days of cultivation. These findings demonstrate that the kinetics of EPS production and the kinetics of cellular growth are practically associated.

EPSs biochemical characterization

Leptolyngbya sp. EPSs were analyzed for their carbohydrate, protein, and lipid contents expressed as EPSs ash-free dry weight (AFDW) (Table 1). Results show high content of carbohydrates reaching $66.44\% \pm 2.14$ of the dry weight and a relatively high amount of proteins ($24.20\% \pm 1.54$) when compared to the lipids content ($9.36\% \pm 1.21$). Element analysis of the crude EPSs shows that the mass ratio of hydrogen-carbon was near $1/6$ ($10/60$) and the presence of nitrogen (23.52%) and sulfate element (0.59%).

FT-IR and UV spectrum analysis

The FTIR spectrum of the *Leptolyngbya* sp. EPSs (Fig. 2A) displayed broadband between 3200 cm^{-1} and 2940 cm^{-1} indicating the O–H and C–H stretching vibrations related to polysaccharides (Santhiya et al. 2002). The peak at 1640 cm^{-1} was related to the asymmetrical stretching vibration of the carboxylate group and may correspond to the ring stretching of mannose (Mishra et al. 2011; Rani et al. 2017). The absorption observed at 1300 cm^{-1} is attributed to the bending vibration (δ) of $\nu\text{C-N}$ of amino acids from peptide/proteins in the EPSs (Simonova & Karamancheva 2013). The presence of pyranose units, C–O–C group and C–O–S bindings were consistent with absorption band at 1020 cm^{-1} , 840 cm^{-1} , and 697 cm^{-1} , respectively, and further evidenced sulfate groups (Lloyd et al. 1961; Santhiya et al. 2002).

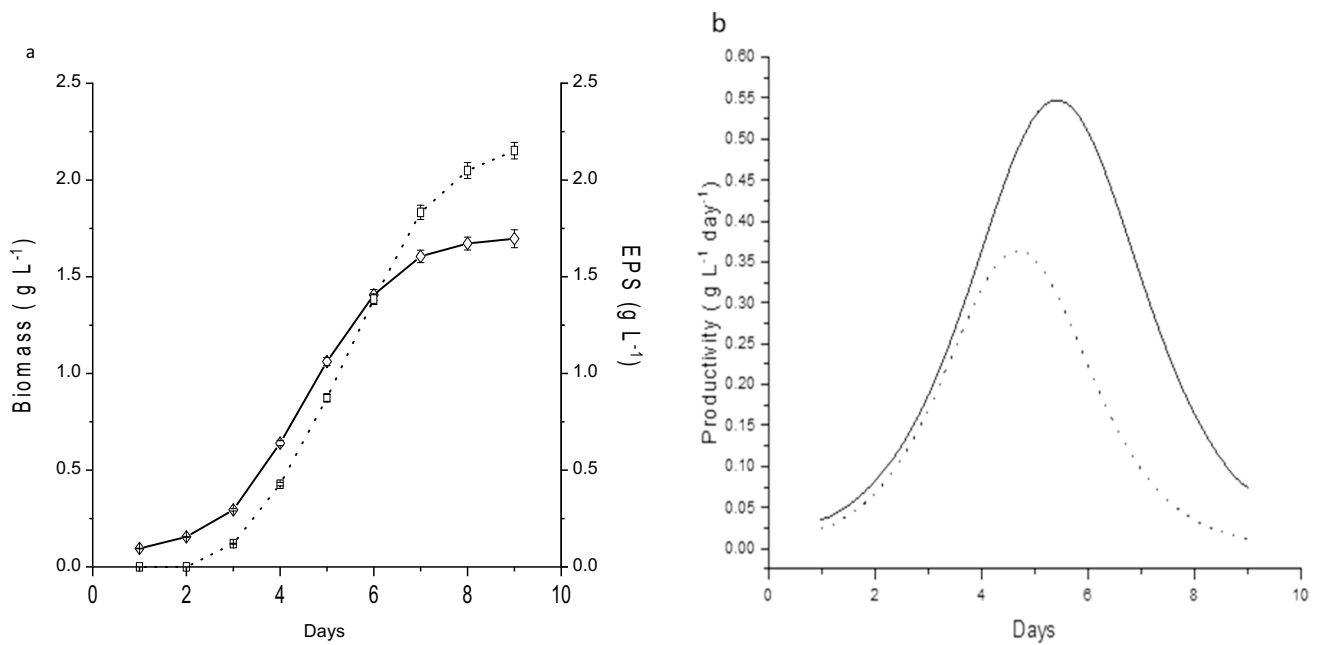
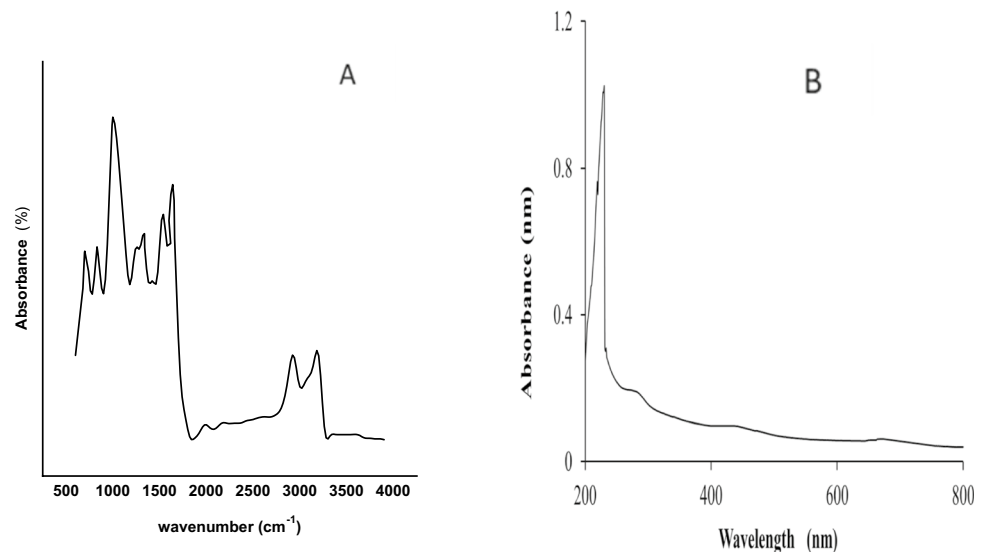


Fig. 1 Kinetics of growth (full line) and EPS production (dotted line) by *Leptolyngbya* sp. **a** Daily course of biomass density. **b** Daily biomass and EPS productivity. Data are expressed as mean \pm SD, $n = 3$. Error bars indicate SD

Table 1 Protein, carbohydrates, lipids, and elemental analysis composition of EPS produced by *Leptolyngbya* sp. (Expressed in % EPS AFDW)

	Proximal composition			Element composition			
	Proteins	Carbohydrates	Lipids	C	H	N	S
EPS (%)	24.20 \pm 1.54	66.44 \pm 2.14	9.36 \pm 1.21	60.00	10.00	23.52	0.59

Fig. 2 FTIR spectrum (A) and UV-vis (B) of the *Leptolyngbya* EPSs



On the other hand, the UV spectra of the EPSs (Fig. 2B) showed a single peak at 210 nm, which is a common characteristic of the electron transition of carbohydrates (Kwak et al. 2016). Moreover, a shoulder observed around

260~280 nm is commonly attributed to electron transitions in aromatic and polyaromatic compounds found in most conjugated molecules, including proteins (Jia et al. 2007), and the absorption zone between 300 and 400 nm, can be

attributed to the extracellular UV-protectant pigments such as mycosporine-like amino acids (MAAs) and scytonemin (Sinha and Häder 2008).

Monosaccharide's composition

Monosaccharide analysis was carried out by GC-FID after acid hydrolysis by comparing their retention time with that of the monosaccharide standards. A standard curve was drawn using the area normalization method (data not shown) to calculate the content of each monosaccharide component in *Leptolyngbya* EPSs. The result revealed a heterogeneous composition (Table 2) of four monosaccharides, D-mannose (35.2%) and D-arabinose (24.3%) as pentoses and D-glucose (15.6%) and D-rhamnose (2.6%) as hexoses and, one uronic acid D-galacturonic acid (8.7%). The non-identified (13.6%) molar proportion could be attributed to amino sugars identified in some cyanobacterial and bacterial EPSs and the analytical methods used in this work, were not suitable for detecting these components (Nicolaus et al. 1999; De Philip-pis et al. 2001).

Functional properties of EPSs

The functional properties of *Leptolyngbya* sp. EPSs (Table 3) were determined by the water solubility index (WSI; %), water holding capacity (WHC, %), oil holding capacity (OHC, %), foam capacity (FC, %) and foam stability (FS, %), and metal sorption ability (%).

The water solubility (WSI) of *Leptolyngbya* sp. EPSs reached $85.5 \pm 3.8\%$ of the initial sample weight. Moreover, *Leptolyngbya* sp. EPSs showed great water and oil retaining ability, with a water holding capacity (WHC)

Table 2 Monosaccharide composition of *Leptolyngbya* sp. EPSs

Monosaccharide	Composition (mol %)
D-mannose	35.2
D-arabinose	24.3
D-glucose	15.6
D-rhamnose	2.6
D-galacturonic acid	8.7
n.d	13.6

n.d not determined

Table 3 Water solubility, water and oil holding, and foaming properties of *Leptolyngbya* sp. EPSs

	Water solubility Index (WSI %)	Water holding capacity (WHC %)	Oil holding capacity (OHC %)	Foaming capacity (FC %)	Foaming stability (FS %)
EPS (0.5% w/v)	85.5 ± 3.8	119.5 ± 2.7	87.5 ± 7.3	22.4 ± 0.5	10.3 ± 0.6

of $119.5 \pm 2.7\%$ and an oil holding capacity (OHC) of $87.5 \pm 7.3\%$, relative to the EPS dry weight.

On the other hand, *Leptolyngbya* sp. EPSs bound significant amounts of divalent heavy metal ions. For 10 mg L^{-1} of each experimented metal as initial concentration, results show (Fig. 3) high affinity toward Cu^{2+} ($93.25 \pm 1.25\%$) and Fe^{2+} ($90.57 \pm 1.03\%$). The efficiency on Zn^{2+} and Pb^{2+} sorption was significantly lower, with a metal adsorption capacities value of $87.33 \pm 1.23\%$ and $84.29 \pm 1.71\%$ (Fig. 3).

Rheological proprieties of EPSs

The flow curves of apparent viscosity variation (mPas), as a function of the shear rate (s^{-1}), of *Leptolyngbya* sp. EPSs were compared (Fig. 4) to that of xanthan gum (0.4% w/v). *Leptolyngbya* sp. EPSs presented a lower viscosity (max 14 mPa) than xanthan gum (20 mPa). However, both the aqueous dispersions of EPS (0.2%, and 0.4%) exhibited a rheological behavior similar to that of xanthan gum. Within the shear-rate range 100 to 450 s^{-1} , the apparent viscosity (mPa s) decreased, in both cases, with increasing the shear rate (s^{-1}), indicating a non-Newtonian shear-thinning behavior. Beyond the critical shear rate 450 s^{-1} , the apparent viscosity was stabilized and was associated with a viscosity at the infinite shear rate corresponding to a Newtonian region.

In another hand, the viscosity of EPSs increased with the increase in the EPS concentration from 0.2% (w/v) to 0.4% (w/v), and flow curves of both concentrations were parallel demonstrating that no conformational changes occurred. Moreover, the apparent viscosity increased

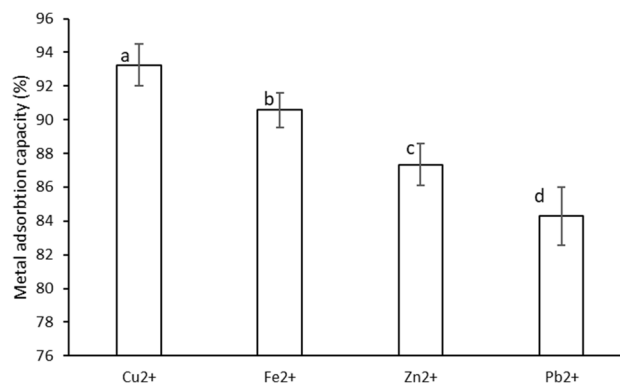


Fig. 3 Metal adsorption capacities (expressed in %, g residual metal/g initial metal) of *Leptolyngbya* sp. EPSs

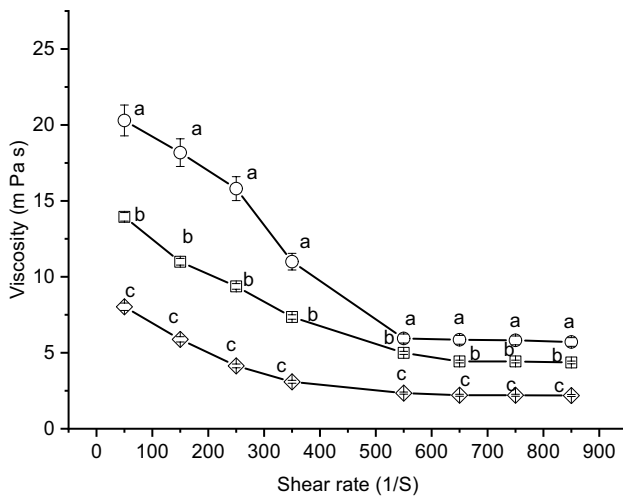


Fig. 4 Effect of shear rate on viscosity of *Leptolyngbya* sp. EPSs; 0.4% (w/v; empty square) and 0.2% (w/v; empty diamond) and xanthan gum, 0.4% (w/v; empty circle) at 25 °C

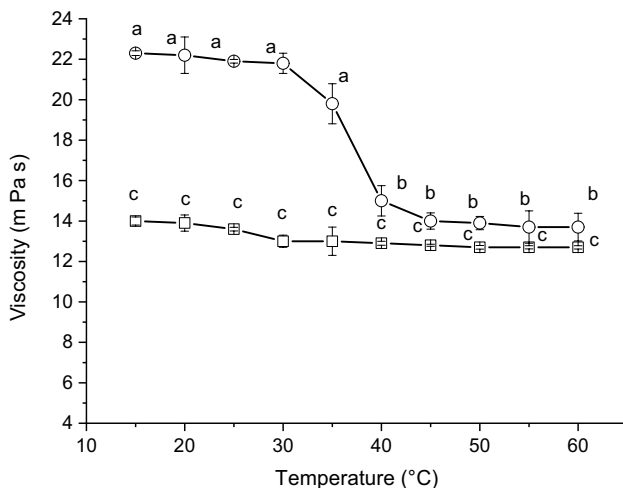


Fig. 5 Effect of temperature on viscosity of EPS of *Leptolyngbya* sp., and xanthan 0.4% (w/v) EPS (empty square) and 0.4% (w/v) xanthan (empty circle)

with the concentration of the EPSs solutions. Increasing the EPSs concentration makes the molecular bonds more cohesive and thus requires more intense shears to be destructed and thus suggesting viscoelastic gel-like behavior.

Leptolyngbya EPSs and xanthan's apparent viscosities were compared according to an increase of temperature from 15 to 60 °C (Fig. 5). As can be seen, the EPSs aqueous solutions showed more stability against temperature increase. The viscosity of EPSs remains constant (14 m Pa s) with the increase of temperature while the viscosity of the xanthan aqueous solution was highly affected since the temperature exceeded 40 °C.

Antioxidant activity of EPSs

The antioxidant activity of *Leptolyngbya* sp. EPSs was evaluated by different assays, namely, the scavenging activity, the iron reduction activity, and the metal chelating capacity (Fig. 6).

Total EPSs scavenging activity was evaluated on •DPPH (Fig. 6A) and •OH radicals (Fig. 6B). The present findings showed that the higher concentration of EPSs, the higher level of scavenging ability. As shown in Fig. 6A, *Leptolyngbya* sp. EPSs had a perceptible •DPPH scavenging activity significantly higher than ascorbic acid. *Leptolyngbya* sp. EPSs scavenging activity varied from $35.2 \pm 1.7\%$ at the concentration of 2 mg mL^{-1} and reached $90.4 \pm 1.5\%$ at the concentration of 100 mg mL^{-1} . The IC_{50} of *Leptolyngbya* sp. EPSs on •DPPH was 4 mg mL^{-1} , significantly lower than ascorbic acid (10 mg mL^{-1}).

EPSs showed also high scavenging abilities on hydroxyl radical •OH of $90.21 \pm 0.4\%$ at 100 mg mL^{-1} , whereas the scavenging activity of the ascorbic acid was significantly lower with the highest value of $70.5 \pm 0.1\%$ (Fig. 6B). The IC_{50} of *Leptolyngbya* sp. EPSs on •OH were 10 mg mL^{-1} , twofold lower than of ascorbic acid (20 mg mL^{-1}).

The reducing activity of *Leptolyngbya* sp. EPSs was evaluated by measuring their ability to reduce ferric ion (Fe^{3+}) to its ferrous form (Fe^{2+}). Data (Fig. 6C) showed that EPSs exhibited significantly lower electron-donating ability ($\text{OD}_{700} = 0.3$ at 0.5 nm) than EDTA ($\text{OD}_{700 \text{ nm}} = 2.9$ at 3 nm). Nevertheless, the EPSs from *Leptolyngbya* sp. presented a higher metal chelating activity with a maximum of 91% at 100 mg mL^{-1} which was higher than that obtained with EDTA (77%) (Fig. 6D). The IC_{50} of the metal chelating ability of EPSs and EDTA were 40 and 60 mg mL^{-1} respectively. In both cases, the metal chelating capacity was dose-dependent.

Discussion

The strain *Leptolyngbya* sp. IkmLPT16 is the first filamentous cyanobacterium isolated from Tunisian hot springs and cultivated in the laboratory. The genus *Leptolyngbya* (sensu lato) is one of the commonest cyanoprokaryotic organisms in several world's biotopes. Several *Leptolyngbya* strains are common in thermal and mineral springs from 40 to 60 °C (Sompong et al. 2005). However, based on the comparison of the nucleotide sequences, the species isolated in the present work could be considered a new strain.

Leptolyngbya sp. IkmLPT16 showed high biomass productivity exceeding that reported for common cyanobacteria cultivated in the same conditions (Zili et al. 2015; Chentir et al. 2017). Furthermore, it revealed a high ability to produce EPSs compared to that recorded by several

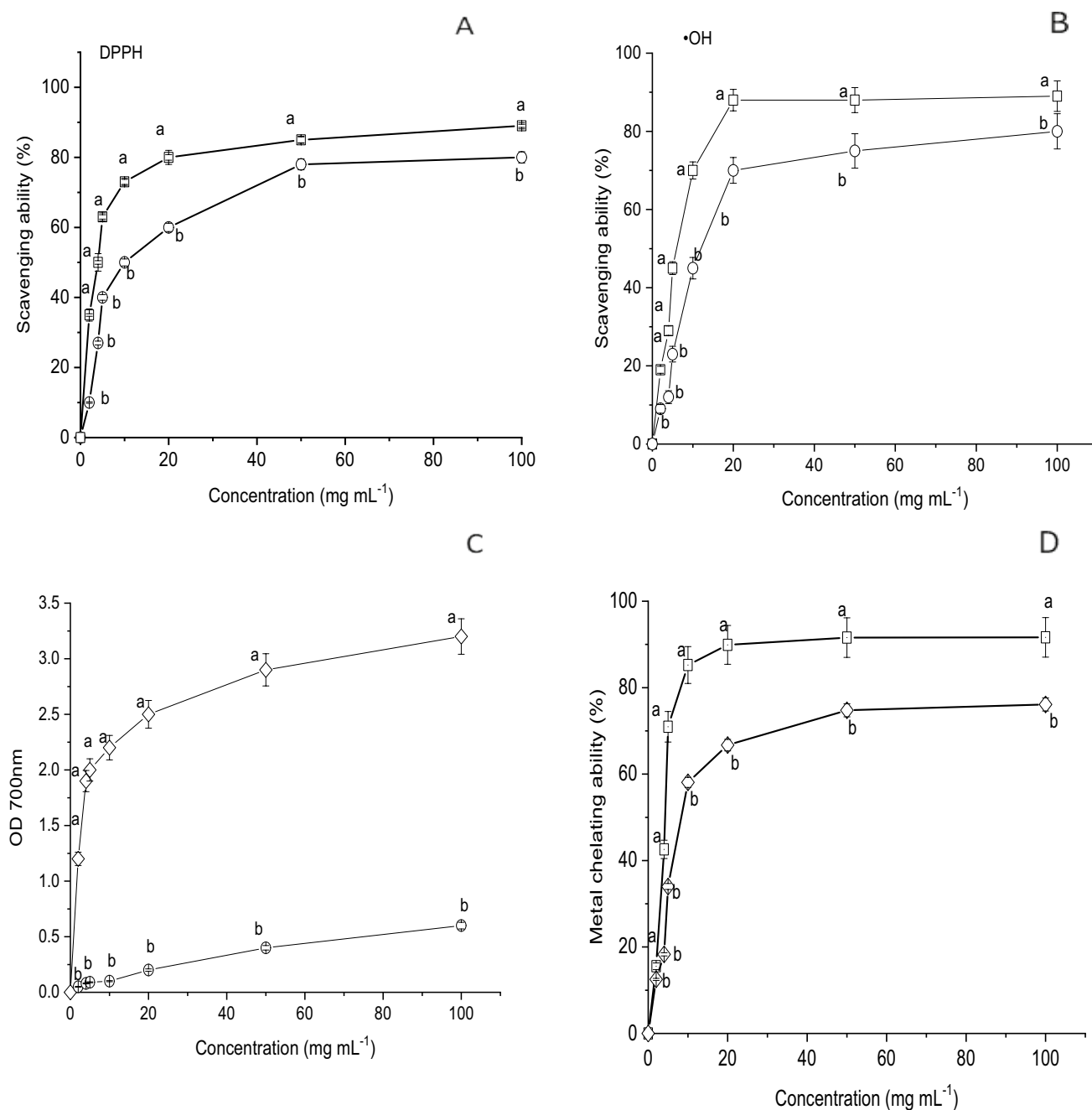


Fig. 6 The antioxidant property of *Leptolyngbya* sp. function of the concentration of EPSs (empty square) compared to ascorbic acid (empty circle) and EDTA (empty diamond). **A** •DPPH scaveng-

ing activity, **B** •OH scavenging activity, **C** iron reduction activity, **D** metal chelating activity

cyanobacteria strains including *Arthrospira platensis*, *Cyanotheca* sp. CCY 0110, and *Nostoc flagelliforme* (Mota et al. 2015; Chentir et al. 2017; Shen et al. 2019) and to that of representative EPS-producing lactic acid bacteria (Ruas-Madiedo & De Los Reyes-Gavilán 2005).

Commonly, EPSs production by microorganisms is often associated with growth depletion. EPSs are considered secondary metabolites linked to the defense strategy

against stress (De Philippis et al. 2001). In this work, the production of EPSs was observed during the whole culture phase, and the maxima of biomass and EPS production are practically correlated. This tendency has been observed in some cyanobacteria strains as *Anabaena flos-aquae* A37 (Moore & Tischer 1964), cylindrical *Anabaena* (Lama et al. 1996), or the green alga *Botryococcus braunii* (Fernandes et al. 1989). It can be attributed to the ability of

EPSs to retain nutrients thus increasing their availability for the growth metabolism (Comte et al. 2006).

Cyanobacterial EPSs are regarded as a very abundant source of structurally diverse polysaccharides, which may possess exclusive properties for special applications in varied fields involved in food processes like mixing, pouring, or pumping (Velasco et al. 2009; Ozturk & Aslim 2010).

The composition of *Leptolyngbya* EPSs IkmLPT16 revealed a heterogeneous nature including in addition to carbohydrates, several organic substituents as protein, lipids, and mineral compounds as sulfates groups. This trend is common to several microalgae and cyanobacteria strains, although the respective concentrations of these components could differ significantly from the species and the culture conditions (Mishra et al. 2011; Mota et al. 2015; Delattre et al. 2016; Xiao & Zheng 2016). Compositional analysis of *Leptolyngbya* sp. EPSs were within limits found in most cyanobacteria strain with carbohydrates (48 to 80%), protein (1 to 42%), and lipids (2 to 10%) (Mishra et al. 2011; Mota et al. 2015; Delattre et al. 2016; Xiao & Zheng 2016). Moreover, the presence of uronic acids was previously reported in many cyanobacterium EPSs (Micheletti et al. 2008; Mota et al. 2015).

The monosaccharide composition of *Leptolyngbya* EPSs was limited in this work to four monosaccharides and one uronic acid. Most cyanobacterial EPSs can enclose up to 12 different monosaccharide residues (De Philippis et al. 2001). However, EPSs with only four monosaccharides (ribose, xylose, glucose, and mannose) were also detected in *Oscillatoria*, *Nostoc*, and *Cyanothece* EPSs (Parikh & Madamwar 2006).

Depending on their structure, EPSs vary in their physicochemical properties (Nielsen & Jahn, 1999; Sheng et al. 2010; Xiao & Zheng 2016). The water solubility of the EPSs was attributed to their absorptive structure which can hold water through hydrogen bonds (Yang et al. 2019). The WSI range of bacterial EPSs extends from 14.2 to 92.15% (Saravanan et al. 2019; Vinothini et al. 2019), so with a WSI exceeding 85% *Leptolyngbya* sp., EPSs are considered among the most water-soluble ones.

The water and oil holding ability of cyanobacterial EPSs are rarely studied. These properties are highly considered in the food industry particularly as fat adsorber and flavor retention (Insulkar et al. 2018). The WHC of *Leptolyngbya* EPSs is in the range of those reported for bacterial ones; 117 up to 134% (Yang et al. 2019) while the OHC is much higher than that reported for EPS produced by bacteria (Devi et al. 2016; Trabelsi et al. 2018). The EPSs ability to hold and entrap water and oil molecules were related to the high molecular weight porous structure of polymer chains (Insulkar et al. 2018; Trabelsi et al. 2018; Gan et al. 2020). The peptidic moieties and deoxysugar rhamnose contribute

hydrophobic behavior to otherwise hydrophilic macromolecules (Khattar et al. 2010).

On the other hand, EPSs can create a network that stabilizes the dispersed air–water phase (Trabelsi et al. 2018) which explains their foaming properties. The FC and FS of *Leptolyngbya* EPSs are in the range of other bacterial EPSs with values from 16 to 44%. However, the foam stability evaluated to $10.32 \pm 0.56\%$ was lower than that of bacterial ones (20 to 30%) (Trabelsi et al. 2018); Shen et al. 2019; Gomaa & Yousef 2020. The high ability of *Leptolyngbya* EPSs to entrap both hydrophilic and hydrophobic groups makes them suitable for the stabilization of foams and also to trap metal ions which can be exploited by the cosmetic industry for product formulation.

Leptolyngbya sp. EPSs have a metal adsorption capacity similar to that recorded by several cyanobacteria (Pratap et al. 2021) and bacteria (Qin et al. 2007) strains. The sorption properties of the EPSs have been attributed to the presence of uronic acid, which confers a high affinity for positively charged molecules (Mota et al. 2015). Obtained results suggest that the *Leptolyngbya* EPS has potential use in industrial applications as a novel bio-resource for the removal of heavy metals from polluted environments.

The most common industrial use of microbial polysaccharides is related to the capability of these biopolymers to alter the rheological behavior of water, acting as thickening agents (Sutherland 1998; Deep et al. 2012) and to stabilize the flow properties of their aqueous solutions under drastic changes of temperature, ionic strength, and pH (Xiao & Zheng 2016; Trabelsi et al. 2018). *Leptolyngbya* EPSs, like most cyanobacterial exopolymers (Lapasin et al. 1992; Filali Mouhim et al. 1993; Parikh & Madamwar 2006), display a non-Newtonian behavior and an extremely strong pseudoplastic characteristic that both appear at concentrations as low as $0.02 \text{ g EPS L}^{-1}$. These properties may result from the polyelectrolytic character of the molecule, which originates in the electronegative charges brought by uronic acid residues and sulfate groups, and that would modify the conformation of the molecule through electrostatic interactions (Filali Mouhim et al. 1993; De Philippis & Vincenzini 1998). Furthermore, *Leptolyngbya* EPS aqueous solutions showed appreciable stability of the viscosity over a wide range of temperatures. The same behavior was also observed in the case of EPSs produced by *Nostoc PCC 7423* (De Philippis et al. 2001). As suggested for other cyanobacterial EPSs (Kaur & Sandhu 2010; Hu et al. 2020), *Leptolyngbya* sp. EPSs could be included as stabilizing as a bio-thickener, an additive, a stabilizer, or as a viscosifier and thickening agent in the food colloid systems.

Compared to ascorbic acid, *Leptolyngbya* EPSs showed high scavenging ability against •DPPH and the hydroxy radicals. The weak dissociation energy of the polysaccharide O–H bond gives to EPSs a high potential to donate H•

implied in the stabilization of free radicals (Yin et al. 2010; Minbo 2012). The hydrogen-donating ability was reckoned to be the dominant antioxidant property of EPSs obtained from bacterial strains (Andrew & Jayaraman 2020).

Furthermore, *Leptolyngbya* EPSs exhibit antioxidant activity via capturing iron ions and thus leading to the decrease of oxidation state and the suppression of the metal oxidant effects. The high iron-chelating activity was also identified for several cyanobacteria EPSs (Parwani et al. 2014). Several reports illustrated the high metal chelating ability of cyanobacterial and bacterial EPSs due to their capacity to bind several metal ions (Wang et al. 2014; Raj et al. 2018). In this context, Chang et al. (2010) reported that the presence of galacturonic acid is essential for the ability of chelating ferrous ions.

Such findings demonstrated that EPS is not just an arrangement of randomly associated biopolymers, but it constitutes an active matrix that protects the cell against free radicals mainly produced at high temperatures.

Conclusion

The filamentous cyanobacteria strain *Leptolyngbya* sp. IkmLPT16, isolated from the Tunisian hot water spring, represents a high potential for intensive industrial production allowing combining high yields in both biomass and EPSs. In addition, its filamentous structure gives greater ease of separation of the biomass and the EPS by simple filtration.

The EPSs obtained from this strain were sulfated heteropolysaccharides with protein moieties and contain four neutral sugars and one uronic acid. EPSs exhibited good functional properties, particularly as water and oil holders, and metal absorber. They showed non-Newtonian, pseudoplastic shear thinning properties which were conserved with the temperature increase. Moreover, the EPSs have potent radical scavenging activity and exert high iron-chelating ability.

Obtained results suggest that *Leptolyngbya* sp. EPSs may be valuable for use in the pharmaceutical and food industries. However, the valorization of these results acquired in the laboratory, requires validation in pilot-scale experiments integrating socio-economic aspects for a spin-off to industry. A step of normalization of the resulting products (biomass or EPSs) involving bacteriological, biochemical and toxic, and heavy metals tests is also needed.

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