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Hidden inside desert rocks: Salinity triggers an increase in exopolysaccharides from endolithic cyanobacteria with anti-inflammatory potential

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ABSTRACT

Endolithic cyanobacteria thriving in rocks of deserts remain an unexplored source for the quest of novel bioproducts in extreme environments. In this work, 7 endolithic cyanobacteria from the polyextreme Atacama Desert, covering four genera and three lithic substrates, were investigated for the production of exopolysaccharides with anti-inflammatory potential. A moderate salinity (20 g NaCl L^{-1}) was tolerated by all strains, triggering a 3–9-fold increase in exopolysaccharides (EPS) yield in 4 of them that counteracted the growth decrease due to NaCl stress. EPS from all strains showed anti-elastase activity with inter-strain and inter-salinity variations. The moderate EPS productivity by *Gloeocapsa* sp. UAM572 (0.4 mg EPS L^{-1} day^{-1}), elevated anti-elastase capacity of *Chroococcidiopsis* sp. UAM579 EPS (IC_{50} = 78 μg mL^{-1}) and the first biotechnological data of genus *Pseudoacaryochloris*, provide a promising foundation for potential applications of EPS from endolithic cyanobacteria in cosmetics and biomedicine, whose opportunities and challenges are discussed herein.

1. Introduction

Extreme environments are gaining momentum regarding the quest for novel bioactive compounds synthesized by unparalleled microbial communities. Among those ecosystems, hot deserts host highly selective habitats requiring singular biochemical adaptations by their unique microbial inhabitants. The Atacama Desert (northern Chile) is such a polyextreme environment that is even considered an analog for Mars conditions due to its world records of highest ultraviolet (UV) irradiation and lowest mean annual precipitation (3–27 mm) [1], along with very broad daily temperature amplitudes (−4 °C to 49 °C) [2]. Such a hostile environment restricts most microbial life to endolithic habitats (inside rocks) with a photosynthetic community dominated by cyanobacteria thriving in cryptoendolithic (within pore spaces), chasmoendolithic (within cracks and fissures) and hypoendolithic (within microcave-like pores in the bottom layer) microhabitats [3].

Cyanobacteria produce countless bioactive compounds with diverse chemical natures (such as fatty acids, pigments, proteins -peptides, enzymes-, and polysaccharides) [4]. Many of them are not fully characterized for biotechnological purposes. Within those, exopolysaccharides (EPS) are among the most promising candidates for numerous applications in global industries like food (e.g. additives, nutraceuticals), wastewater treatments (e.g., heavy metal removal), biopolymers (gums, flocculants, sorbents, soil conditioners), biomedicine (wound healing; anti-inflammatory agents) and pharmaceuticals/cosmetics (e.g., anti-aging ingredients) [4]. Cyanobacterial EPS are very complex heteropolysaccharides of high molecular weight (composed of 6 or more different monosaccharides) that are located surrounding individual cells or cell groups. They have prominent roles in biofilm formation and substrate attachment, offering chemical and physical protection against biotic and abiotic stress factors [5]. Compared with non-photosynthetic bacteria like Lactobacilli [6], cyanobacterial EPS appear structurally richer while offering the advantage of potentially lower carbon footprints by CO_2 removal during photosynthetic growth. Some of the structural uniqueness of cyanobacterial EPS include broader monosaccharide diversity compared to other bacterial groups, the presence of particular non-sugar parts like uronic acids which are exclusive of cyanobacteria and a few microalgae, and sulphate groups

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that are only found in archaea and eukaryotes [4,5,7].

Experiments in lab cultures of cyanobacteria evidenced that both the structural features and the overall yield of EPSs are affected by abiotic factors like light irradiance, temperature, nutrients (N, P, Ca, Mg), and salinity (NaCl and $CaCl_2$) [4,7]. Most results concern aquatic and soil strains isolated from temperate habitats, with the very scarce data from desert cyanobacteria, specifically focused on soil biocrusts [8–10]. Nevertheless, there is a lack of information on endolithic cyanobacterial cultures from deserts, as far as we are aware.

During the last decade, cosmetic and biomedical industries have shown an increasing need for environmentally sustainable products, focused on natural sources and green processes like cyanobacterial-derived compounds. In the current rise of average life expectancy, it becomes essential for the cosmetic industry to find anti-aging bio-products that improve skin density and elasticity, avoid moisture loss, and reduce wrinkling [11]. One of the main causes of skin aging is chronic low-level inflammation resulting from environmental stressors (UV, among others) that turn into an inflammatory cascade process in the tissue. Part of this inflammatory cascade is the release of several metalloproteinases as collagenase, gelatinase, hyaluronidase, and elastase from circulating immune cells (e.g., neutrophils), and skin keratinocytes and fibroblasts. Chronic activation of human neutrophil elastase (a serine protease) is linked to increased skin aging, wrinkling, skin laxity and loss of resilience [12]. Neutrophil elastase (NE) over-expression is also involved in several chronic inflammatory diseases like cystic fibrosis, asthma, rheumatoid arthritis or chronic obstructive pulmonary disease [13]. As such, finding inhibitors of elastases is one of the hot topics within green cosmetics based on micro/macro algae and cyanobacteria. While several cyanobacterial peptides are known for inhibiting elastases [13], there is no information on whether EPS from endolithic cyanobacteria offer any anti-elastase, or anti-inflammatory/anti-aging effect in general that could be potentially relevant for downstream cosmetic or biomedical applications.

A previous study in endolithic cyanobacteria from Atacama found that moderate salinity (20 g NaCl L^{-1}) triggers a 50-fold increase in the content of scytonemin [14], a UV-screening pigment highly valued for its cosmetic potential. Interestingly, in this same study, some endolithic cyanobacteria from Atacama survived salinity exposure even when isolated from non-NaCl containing rocks like calcite or gypcrete. This could be hypothetically due to physiological strategies primarily addressed to tolerate desiccation which might include the synthesis of EPS to retain water [15] among others. However, the specific effect of NaCl on the production of EPS in terms of yield and chemical composition, by desert endolithic cyanobacteria remains so far unexplored. In this context finding halotolerant endolithic strains proves relevant not just for ecological purposes -i.e. to understand adaptation strategies of those extraordinary microorganisms- but also for biotechnology, since salinity can reduce cross-contamination by non-target microorganisms such as bacteria, viruses, fungi, zooplankton or protists [16] when cyanobacteria are grown in open cultures for commercial mass production [7].

As a whole, the present work provides novel bioprospection insights into endolithic cyanobacteria from the Atacama Desert towards biotechnological applications. For this aim, seven cyanobacterial strains covering the main genera, lithic substrates and microhabitats found in Atacama, are screened for their halotolerance and for the effect of moderate salinity (20 g NaCl L^{-1}) on the EPS yield, characterization of functional groups by micro-FTIR spectroscopy, and anti-inflammatory activity approached by inhibition rates of the enzyme neutrophil elastase by EPS. Results will be discussed in the context of their biotechnological potential for industries like cosmetics or biomedicine, along with a critical view of the opportunities and challenges faced for future outdoor mass production of the most relevant strains.

2. Materials and methods

2.1. Strains characteristics

Experiments were performed on 7 endolithic cyanobacterial strains isolated from samples obtained from the Atacama Desert (Northern Chile) in previous studies [17,18] and maintained as uni-cyanobacterial cultures as part of the Universidad Autónoma de Madrid (UAM) culture collection. Strains belonged to 4 different genera (*Chroococcidiopsis*, *Gloeocapsa*, *Gloeocapsopsis*, and *Pseudoacaryochloris*), and were isolated from 3 lithic substrates (calcite, gypcrete and halite) and 3 different microhabitats (cryptoendolithic chasmoendolithic, hypoendolithic) (Table 1; see micrographs in Supplementary Material).

2.2. Effect of NaCl on growth

Cyanobacterial cultures were grown in batch for 14 days in flasks containing 300 mL BG11 culture medium [19] supplemented with 20 g L^{-1} NaCl and BG11 without NaCl as control. All cultures were grown in triplicate with orbital shaking (135 rpm), at a temperature of 25 °C and constant light of 30 μmol photons m^{-2} s^{-1}. An optical density at 750 nm (OD_{750nm}) of 0.1 was set as a starting point for all cultures. Growth was determined by measuring OD_{750nm} after 14 days with a UV–Vis spectrophotometer (Hitachi U-2000 Spectrophotometer) and expressed as growth rate (day^{-1}) following [20].

2.3. Exopolysaccharides (EPS) extraction and quantification

Exopolysaccharides were quantified in cultures exposed to 0 and 20 g NaCl L^{-1} for 14 days as detailed in the previous section. For this purpose, 10-mL culture aliquots of each condition and replicate were centrifuged (10.000 ×*g*; 30 min) and the biomass pellet was stored at −80 °C and freeze-dried (UniFreez™ FD). EPS were extracted from the freeze-dried pellet obtained from 300 mL of culture following the protocol proposed for cyanobacteria by [21], resuspended in 1 mL of Milli-Q water, and quantified by spectrophotometry using the phenol-sulfuric method [22] with glucose as a reference. The concentration of EPS was expressed as (mg L^{-1}).

2.4. EPS characterization by micro Fourier transform infrared spectroscopy (micro-FTIR)

To investigate structural characteristics and alternative functionalities of EPS extracted from cyanobacterial cultures Micro-Fourier transform infrared spectroscopy (micro-FTIR) was used using a Perkin-Elmer Spotlight 200 Spectrum Two apparatus equipped with an MCT detector. For this purpose, 0.5 mg of desiccated EPS were placed individually over potassium bromide (KBr) discs (25 × 4mm) by using a zircon microneedle. A total of 25 scans were performed on each sample with a spectral resolution of 8 cm^{-1} and a spectral range of 4000–550 cm^{-1}. Spectra obtained were compared with spectra available in the literature [23].

Table 1
Features of the 7 endolithic cyanobacterial strains from the Atacama Desert analyzed in this study.

Strain	Species	Lithic substrate	Microhabitat	Reference
UAM572	*Gloeocapsa* sp.	Gypcrete	Hypoendolithic	[18]
UAM574	*Chroococcidiopsis* sp.	Gypcrete	Chasmoendolithic	[18]
UAM575	*Gloeocapsopsis* sp.	Gypcrete	Chasmoendolithic	[18]
UAM577	*Chroococcidiopsis* sp.	Gypcrete	Cryptoendolithic	[18]
UAM579	*Chroococcidiopsis* sp.	Gypcrete	Hypoendolithic	[18]
UAM584	*Chroococcidiopsis* sp.	Calcite	Chasmoendolithic	[52]
UAM587	*Pseudoacaryochloris* sp.	Halite	Cryptoendolithic	[52]

2.5. Elastase inhibition assay

EPS extracts diluted in 1 mL Milli-Q water (Section 2.3) were analyzed for their elastase inhibition activity through the Neutrophil Elastase (NE) Activity Assay Kit MAK246 (Sigma-Aldrich, USA). The kit protocol was followed with slight modifications. A standard curve of NE enzyme (0–25 ng NE enzyme/well) was prepared in a 96-well plate by diluting different volumes of Enzyme working solution (5 ng NE μL^{-1}) with NE Assay buffer up to a total 50 μL volume per well. EPS extracts were also serially diluted (1:1 and 1:2) and exposed to 15 ng NE, as the central concentration used for the standard curve, to determine their inhibitory activity. Both standards and samples were run in duplicate as recommended by the kit manufacturer. After adding NE substrate MAK246C, fluorescence was measured for 20 min at 37 °C ($\lambda_{ex} = 380$ nm/$\lambda_{em} = 500$ nm) in kinetic mode (every 2 min) by using a Synergy HT Biotek multi-well plate reader. Then, the percentage of inhibition was calculated according to Eq. I:

$$\%Inhibition = \left(1 - \frac{\Delta RFU\ sample}{\Delta RFU\ enzyme}\right) \times 100 \quad \text{(I)}$$

where ΔRFU is the increase in relative fluorescence between two time points of the kinetic measurement (T2 and T1). Subsequently, the percentage inhibition of each sample was utilized to determine the inhibitory concentration 50 (IC_{50}), i.e., the concentration of EPS necessary to inhibit 50 % of the activity of the NE expressed as μg EPS mL^{-1}.

2.6. Statistical analyses

Statistical comparisons of salinity cases (20 g NaCl L^{-1}) versus control (0 g NaCl L^{-1}) were performed using Student's *t*-tests. A significance level of $\alpha = 0.05$ was set in all cases. All statistical analyses were carried out using GraphPad Prism 9.1.0.221.

3. Results and discussion

3.1. Endolithic cyanobacteria tolerate a moderate NaCl concentration

All 7 strains belonging to 4 genera and 3 lithic substrates (Table 1) tolerated a moderate NaCl concentration (20 g NaCl L^{-1}) showing positive growth rates (Fig. 1).

NaCl exerted significant differences in growth (*t*-test $p < 0.05$; see *p*-values in Supplementary Material) for all strains except for *Gloeocapsopsis* sp. UAM575 and *Pseudoacaryochloris* sp. UAM587. There was a marked contrast between strain UAM587 isolated from a NaCl-containing rock (halite) for which NaCl induced a positive effect in growth rate (1.4-fold that of control), and the remaining 6 strains from other substrates (Table 1) for which NaCl induced a reduction in growth rates being 0.2 to 0.7-fold those without NaCl. Among them, the greatest halotolerance was observed in *Chroococcidiopsis* sp. UAM574 and *Gloeocapsopsis* sp. UAM575 (0.7-fold growth rate in salinity versus control) followed by UAM571, UAM572 and UAM575 (0.5 to 0.6-fold). Growth rates ranged from 0.1 to 0.3 day^{-1} at 0 g NaCl L^{-1} and lower 0.1–0.2 day^{-1} at 20 g NaCl L^{-1}. Both the moderate growth rates and the effect of NaCl were similar to previous findings in endolithic *Chroococcidiopsis* sp. strains (0.1–0.4 day^{-1}; growth rate at 20 g NaCl L^{-1} being 0.6-fold that of 0 g NaCl L^{-1}), yet from deserts other than Atacama (Negev Desert, Israel; Baja California Desert, USA) [24,25]. Interestingly, the present study provides the first data on the influence of NaCl on the ecophysiology (growth and EPS) of a *Pseudoacaryochloris* strain. Cultures of this genus, originally described from Sahara Desert gypsum rocks [26], have not been characterized for biotechnological purposes before the present work, as far as we know. Strain UAM587 is apparently not stressed within the NaCl range of the present experiment which seems reasonable considering its rock habitat in Atacama (halite composed of NaCl). It may hence be interesting to investigate whether *Pseudoacaryochloris* withstands salinities >20 g NaCl L^{-1} to evaluate the resilience of this genus with implications both for biotechnological

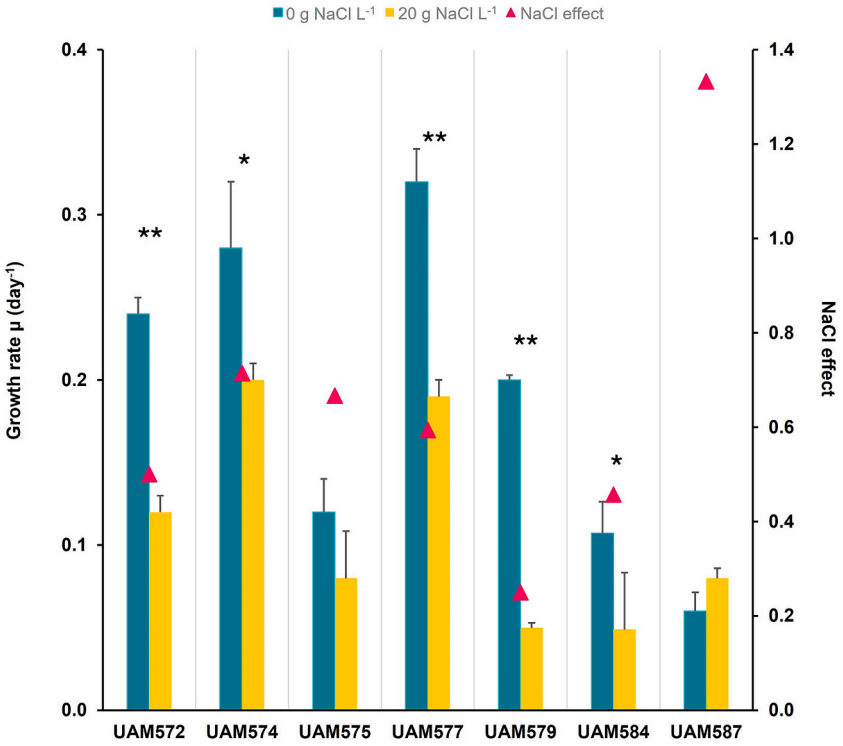

Fig. 1. Effect of NaCl on growth of 7 endolithic cyanobacteria. X axis stands for strain code. NaCl effect (right Y axis) is defined as the ratio of growth rate in BG11 culture medium supplemented with 20 g NaCl L^{-1} and growth rate in non-supplemented BG11 (0 g NaCl L^{-1}). Error bars represent standard deviation ($n = 3$). Statistical differences in growth rates between experimental conditions for each strain are indicated by * (*p*-value<0.05) and ** (*p*-value<0.01).

applications but also as a novel biological material for astrobiology-oriented experiments since the Atacama Desert is considered an analog for Mars habitability [27].

3.2. NaCl triggers EPS increase in 4 cyanobacteria from diverse genera and rock substrates

Results on EPS production were expressed both as concentrations per culture volume (mg EPS L^{-1}) (Fig. 2) and as average daily volumetric productivities (mg EPS L^{-1} day^{-1}) over the 14-day growth period (Table 2).

We observed three different trends in the effects of NaCl in EPS production (Fig. 2 and Table 2) where NaCl triggered (i) a significant increase in EPS, (ii) a lack of effect, or (iii) a significant EPS decrease, detailed as follows. NaCl induced a significant EPS rise in 4 strains of 3 different genera (*Gloeocapsa* sp. UAM572, *Chroococcidiopsis* sp. UAM579 and UAM584, and *Pseudoacaryochloris* sp. UAM587), with increases (EPS ratio of 20 g NaCl L^{-1} and 0 g NaCl L^{-1}) ranging from 3.3-fold (in *Pseudoacaryochloris* sp. UAM587) to 3.8–4.9-fold in *Chroococcidiopsis* sp. (UAM579, UAM584) and a remarkable 9-fold EPS increase in *Gloeocapsa* sp. UAM572. Maximum EPS concentrations measured at 20 g NaCl L^{-1} in these 4 strains varied from 0.9 mg EPS L^{-1} in UAM579 to 5.6 mg EPS L^{-1} in UAM572. In contrast, the single strain of genus *Gloeocapsopsis* analyzed in this study (UAM575) was the only one showing a significant decrease in EPS content when exposed to NaCl. EPS concentrations in UAM575 were low in general, being at 20 g NaCl L^{-1} 0.4-fold of those measured at 0 g NaCl L^{-1} (0.2 mg EPS L^{-1} and 0.5 mg EPS L^{-1}, respectively) (Fig. 2). Finally, NaCl did not produce a significant effect in two other *Chrococcidiopsis* sp. strains (UAM574 and UAM577) (*t*-test; $p > 0.05$) which again showed low EPS concentrations under all experimental conditions (0.2–1.2 mg EPS L^{-1}). The reasons behind these trends may be related to differential ecophysiological responses of cyanobacteria towards tolerating moderate salinities. Previous studies in cyanobacteria suggest that biological mechanisms behind salinity tolerance are similar to those for desiccation [9], a stress to which endolithic Atacama strains should be adapted. Along with the up-regulation of genes involved in osmoprotectant metabolisms, such as the K^+ transporting system; and down-regulation of genes related to photosynthesis, nitrogen-transport and ribosomal proteins [28], the three main strategies described for desiccation tolerance in photosynthetic organisms include the synthesis of EPS to retain and capture water; synthesis of proteins (dehydrins, hydrophilins) for membrane stabilization; and synthesis of intracellular compatible solutes to replace water (e.g., trehalose among others) [15]. In this context endolithic

Table 2
Maximal EPS productivities by 7 endolithic cyanobacteria.

Strain	EPS (mg L^{-1} day^{-1})	NaCl (g L^{-1}) in culture medium	NaCl effect on EPS production
Gloeocapsa sp. UAM572	0.39 ± 0.02	20	Positive
Chroococcidiopsis sp. UAM584	0.38 ± 0.04	20	Positive
Pseudoacaryochloris sp. UAM587	0.09 ± 0.01	20	Positive
Chroococcidiopsis sp. UAM577	0.08 ± 0.01	0	Not significant
Chroococcidiopsis sp. UAM579	0.06 ± 0.02	20	Positive
Chroococcidiopsis sp. UAM574	0.01 ± 0.006	0	Not significant
Gloeocapsopsis sp. UAM575	0.04 ± 0.003	0	Negative

The strains are arranged in decreasing order of productivity. NaCl (g L^{-1}) stands for the concentration for which the maximum productivity occurred in each strain. NaCl effect refers to whether 20 g NaCl affected EPS productivity by increasing it (positive) decreasing it (negative) or being not statistically significant (not significant), with a significance level of $\alpha = 0.05$.

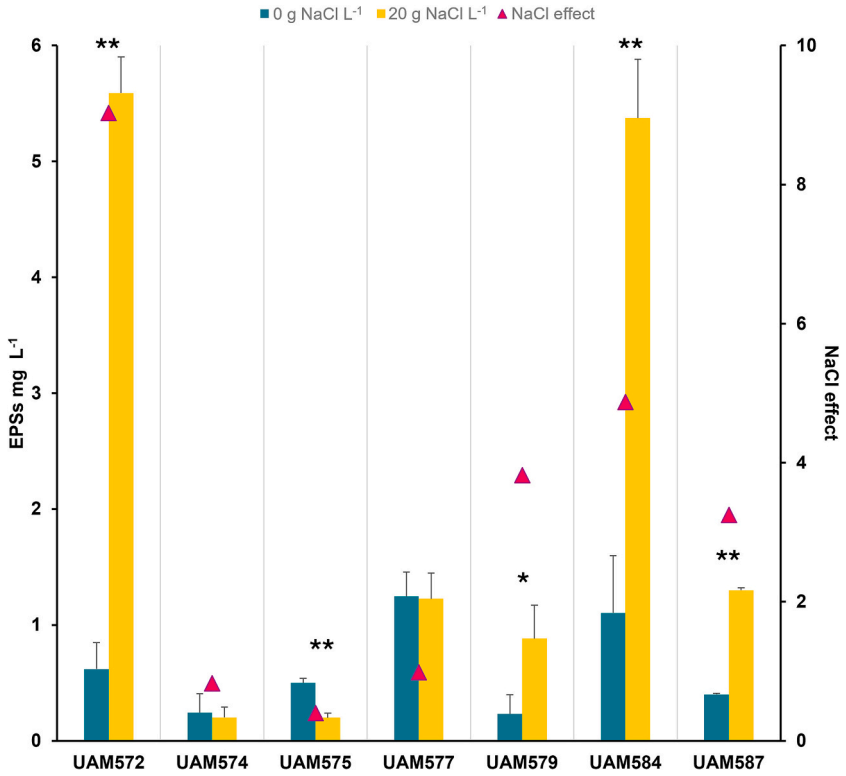

Fig. 2. Effect of NaCl on EPS yield by 7 endolithic cyanobacteria. X axis stands for strain code. NaCl effect (right Y axis) is defined as the ratio of EPS concentration in BG11 culture medium supplemented with 20 g NaCl L^{-1} divided by the same parameter in non-supplemented BG11 (0 g NaCl L^{-1}). Error bars represent standard deviation (n = 3). Statistical differences in EPS yield between experimental conditions for each strain are indicated by *(*p*-value<0.05) and ** (*p*-value<0.01).

strains of *Gloeocapsa*, *Chroococcidiopsis* and *Pseudoacaryochloris* of our study seem to respond to NaCl mostly by increasing EPS production as observed in non-endolithic cyanobacteria from genera *Arthrospira*, *Anabaena*, *Cyanothece*, *Microcoleus*, *Synechococcus*, *Synechocystis* and *Spirulina* among others [4] (and references therein). In contrast, the genus *Gloeocapsopsis* (strain UAM575) showed an apparently different tolerance response which may involve (among others) trehalose synthesis as already demonstrated in another endolithic *Gloeocapsopsis* strain from Atacama exposed to desiccation [15]. Other strategies like the synthesis of proteins like dehydrins cannot be discarded either for UAM575 nor for the remaining strains, deserving further investigations preferably with transcriptomics to understand expression patterns of gene clusters potentially involved in salinity tolerance.

This study provides novel results on the effect of NaCl in EPS from desert endolithic cyanobacteria. Previous research in cyanobacterial cultures from other habitats (soils, freshwaters, marine and hypersaline habitats) showed various trends (reviewed in [4,7]), with most studies finding a positive effect of NaCl on EPS yield [29] but also some without a clear effect [30], and even in some specific strains a decrease of EPS upon NaCl exposure [31]. Beyond this physiological complexity inherent to the study of biologically diverse cultures, the use of moderate salinity proved an efficient strategy from a biotechnological perspective in the present study. This idea is supported by the volumetric EPS productivity data (Table 2) since the 3 overall maximum productivities of this study occurred at 20 g NaCl L^{-1} in 3 different cyanobacterial genera from 3 different rock substrates. Over the 14-day growth period, *Gloeocapsa* sp. UAM572 isolated from gypcrete showed the highest productivity (0.39 mg EPS L^{-1} day^{-1}) closely followed by *Chroococcidiopsis* sp. UAM584 from calcite (0.38 mg EPS L^{-1} day^{-1}) and by *Pseudoacaryochloris* sp. UAM587 from NaCl-based halite with a lower 0.09 mg EPS L^{-1} day^{-1} productivity. To our knowledge, these are the first results on daily EPS productivities by endolithic cyanobacterial cultures from deserts. Those are nearly 7-fold higher than productivities measured in endolithic Atacama strains for another industrially relevant bioproduct (scytonemin) [14]. Maximum EPS productivities of *Gloeocapsa* sp. UAM572 (0.4 mg EPS L^{-1} day^{-1}; equivalent to 0.2 mg g^{-1} DW day^{-1} based on DW-standardized EPS content shown in Supplementary Material) are within ranges measured for desert cyanobacteria from non-endolithic habitats. Namely, experiments in cultures of filamentous genera (*Microcoleus*, *Nostoc*, *Phormidium*, *Scytonema*) from soil crusts in the Chinese Tengger Desert showed maximum productivities of 0.4–0.8 mg EPS L^{-1} day^{-1} [9] and 0.09–0.20 mg EPS g DW^{-1} day^{-1} [10]. These EPS yields in desert isolates are undoubtedly lower than those reported for aquatic cyanobacterial cultures ranging from 0.7 to >300 mg EPS L^{-1} day^{-1} (reviewed in [4]). However, there are still a number of potential advantages of using desert isolates that are worth being explored especially in a global context of climate warming and increased desertification. Those include their growth in arid land not suitable for any agricultural use, and/or with reduced freshwater consumption, especially for halotolerant strains that could be grown on partially diluted seawater; or for those strains hypothetically suited for low-water consuming biofilm bioreactors where EPS from endolithic or soil crust strains may be helpful for substrate attachment and biofilm growth [5].

3.3. NaCl influences EPS chemical properties as evidenced by micro-FTIR spectroscopy

Micro-FTIR spectroscopy as a low-cost and fast technique, is commonly used to explore the structure and functionality of EPS produced by cyanobacteria and other microorganisms [21,29,32–34]. In

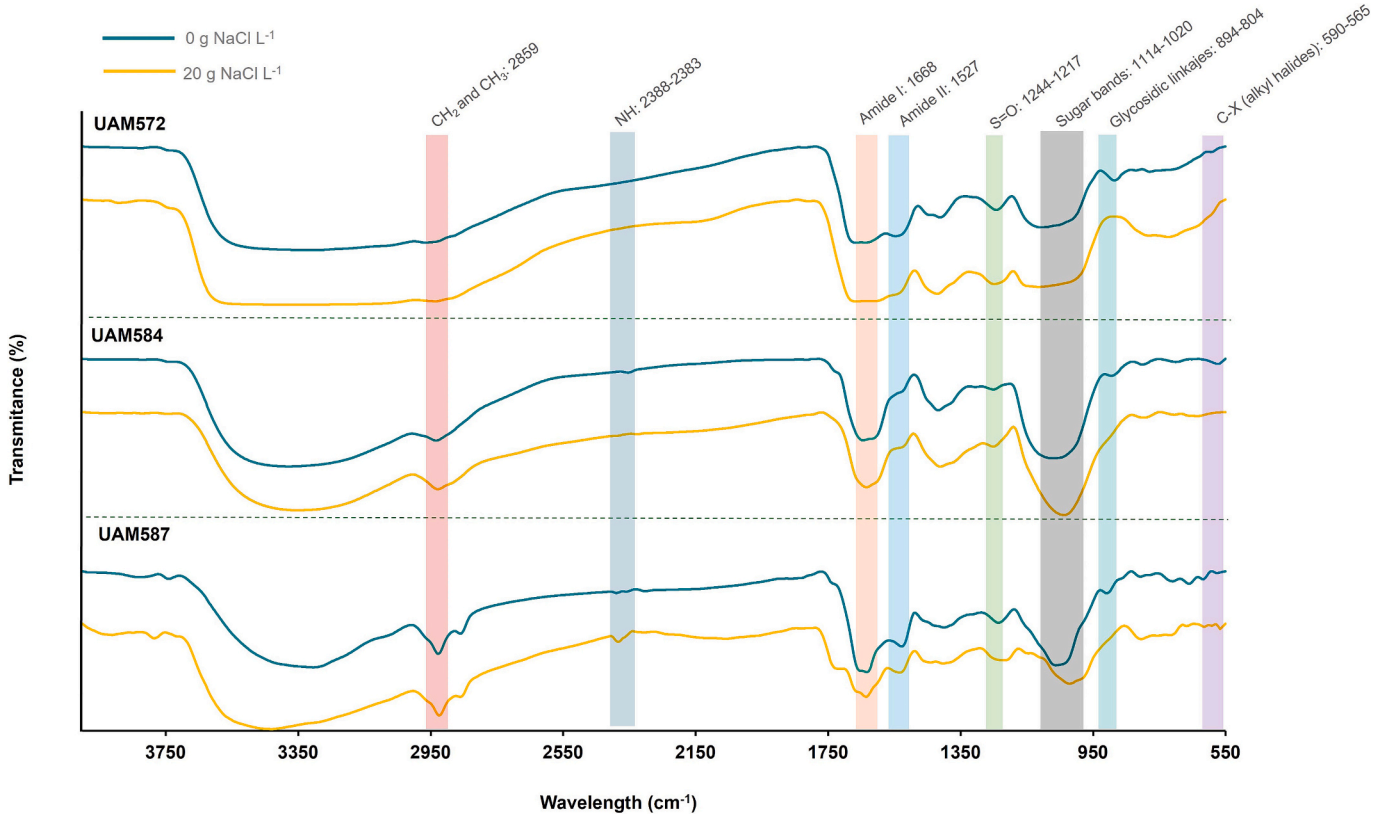

Fig. 3. FTIR spectra of EPS produced by the 3 strains that showed the highest productivities. Green line stands for EPS spectra under control conditions (0 g NaCl L^{-1}) and yellow line for EPS spectra under NaCl exposure (20 g NaCl L^{-1}). CH_2 and CH_3 absorb in 2859 cm^{-1} (red area), NH absorbs at 2383–2388 cm^{-1} (dark blue), amides I–II absorb at 1527–1668 cm^{-1} (orange and yellow areas), sulfate groups at 1217–1244 cm^{-1} (green area) and polysaccharides at 1020–1114 cm^{-1} (grey area). The -glycosidic linkages are visible as a shoulder at 804–894 cm^{-1} (blue area) and C-X of alkyl halides at 565–590 cm^{-1} (purple area). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this study, it allowed us to determine the main functional groups present in EPS extracts and to characterize them based on differences in those functional groups (Fig. 3; and detailed results of peaks identified in Supplementary Material). As a whole, IR spectra confirmed the presence of polysaccharides and polysaccharide-like structures in all EPS samples such as C—O bond in C-OH, stretching vibration of C-O-C, or the O—H and C—H groups. Other groups found in all strains and conditions, often observed in cyanobacterial EPS [21,29,32,35], included Amide *I* and S═O asymmetric stretching vibration of the sulfate group [36]. Interestingly, EPS containing sulfated groups among bacteria seems to be exclusive to cyanobacteria, conferring them a negatively charged surface with ecological advantages over bacterial competitors [32] as well as a number of biotechnological properties against viruses (e.g., Herpes virus among others), and the inhibition of enzymes involved in skin inflammation and hyperglycemia [7,37] and bioremediation capacity attracting cationic metal ions [38].

Beyond functional groups found in all strains, certain bands were only observed in EPS from some strains such as those corresponding to CH_2 and CH_3 deformations (bends) of proteins (only found in UAM575 and UAM587) or C-X stretch of alkyl halides (found in all strains except UAM575). Apparently, EPS from *Pseudoacaryochloris* sp. UAM587 seemed to be the most diverse showing bands in all peaks observed in the present study, some of them such as the N—H bond only found in this strain. Whether this apparent singularity of UAM587 is related to its saline habitat (NaCl-containing halite rock) remains to be solved. Concerning the general effect of NaCl on all strains, the main changes in FTIR bands after exposure to 20 g NaCl L^{-1} include the disappearance of some peaks and/or shifts of others (Supplementary Material), which has already been reported when characterizing cyanobacterial EPS produced under different growth conditions [21] or phases [32]. Namely, in this study, exposure to 20 g NaCl L^{-1} triggered the disappearance of the C-X stretch of alkyl halides (in 5 out of 7 strains) and Amide II (in 2 out of the 4 strains where it has been detected). On the other hand, the bands corresponding to Amide *I* and C-O-C stretching vibration showed a shift with strain-specific behaviors. Particularly, Amide I peak shifted in UAM574 while this effect was not clearly seen in any other strain. Regarding C-O-C, it seemed to shift towards a decrease in cm^{-1} in strains UAM584 and UAM587 while an increase in cm^{-1} seemed to occur in strains UAM572 and UAM574.

Altogether this points out to the structural complexity of EPS in desert endolithic strains already observed in cyanobacteria from other habitats, most likely with species (or even strain)-specific compounds, whose chemical and biological properties can vary in response to NaCl. Previous structural studies in cyanobacteria exposed to NaCl have not specifically focused on changes in functional groups but mostly on substitutions of sugar monomers constituent of EPS. For instance, 3 aquatic *Synechocystis* strains exposed to 12 g NaCl L^{-1} showed changes in monomer ratios with variations in sugars like glucose, xylose, arabinose and rhamnose [39]. Similarly, the halophilic cyanobacterium *Aphanothece halophytica* showed shifts in the relative amounts of two (rhamnose and galactose) out of the seven monosaccharides in EPS when grown in different NaCl concentrations [40]. The possible sugar monomers shift in endolithic cyanobacteria as well as their implications for EPS biological properties, remain to be disentangled by further detailed analyses. Such future research might focus mostly on strains with greater EPS productivity (e.g., UAM572, 584 and 587 in our study) and/or greater biological activity to inhibit industrially relevant targets (see results Section 3.4).

3.4. EPS from endolithic cyanobacteria show a relevant anti-elastase activity

The inhibition of the enzyme elastase has been linked to a number of anti-inflammatory properties potentially relevant to avoid skin aging and diseases like cystic fibrosis, asthma or rheumatoid arthritis [13]. In the present study, the Neutrophil elastase activity assay evidenced the inhibitory capacity of all 7 strains (Fig. 4).

IC_{50} values showed marked inter-strain variations, ranging from a remarkably low IC_{50} (i.e., high inhibitory capacity) of 78 μg EPS mL^{-1} in *Chroococcidiopsis* sp. UAM579 to a high IC_{50} (low inhibitory capacity) of 830 μg EPS mL^{-1} in *Chroococcidiopsis* sp. UAM577. These IC_{50} are between 6 (UAM577) and 64-fold (UAM579) lower than the 5410 μg EPS mL^{-1} reported for elastase inhibition by *Porphyridium cruentum* [41]. EPS of the red microalgal genus *Porphyridium* has been extensively studied [42] with a number of patents registered by companies like Solazyme and L'Oréal on their anti-inflammatory effects (anti-aging and anti-arthritis) and many other biotechnological applications such as antivirals and nutraceuticals (see [7], and references therein).

Compared to EPS from non-photosynthetic bacteria, endolithic cyanobacteria from Atacama showed an anti-elastase capacity similar to those reported for 60 Lactobacilli strains [6]. Furthermore, EPS from 4 of the present strains covering 4 different cyanobacterial genera (UAM575, UAM579, UAM584, UAM587) are within the IC_{50} range for strains tagged as "highly effective" (85–142 μg EPS mL^{-1}) by these authors [6], with a fifth very close to it (IC_{50} = 160 μg EPS mL^{-1} for UAM572). Those IC_{50} are also in the range or, in many cases, more powerful than anti-elastase activity reported for plant extracts in poly-herbal formulations (anti-elastase IC_{50}: 127.3–172.1 μg mL^{-1}) [43].

Regarding the effect of salinity, NaCl exposure was necessary to detect anti-elastase activity in EPS from 3 strains (UAM572, UAM577 and UAM579) where no measurable IC_{50} was found in the control (0 g NaCl L^{-1}). In the remaining 4 strains (UAM574, UAM584, UAM587) NaCl induced a decrease in anti-elastase activity (1.7–2.6-fold increased IC_{50} at 20 g NaCl L^{-1} compared to control). Looking in detail at strains with the highest EPS productivity (UAM572, UAM584, UAM587), a positive NaCl effect on anti-elastase activity was observed for one of them (UAM572) whereas the negative effect of NaCl on anti-elastase activity in UAM584–587 seemed less pronounced than its positive effect on EPS productivity (3.3–4.9-fold increase in NaCl). These diverse behaviors suggest that EPS polymers may show important structural differences both between strains and between salinities, potentially affecting their biochemical interactions with the enzyme. Yet prior research has evidenced the difficulties in establishing a clear scheme of structure-function relationship for EPS [7], microalgae/cyanobacterial data suggest that EPS biological activity relies mostly on molecular weight, sulfate, uronic acids, or charged group content of the polysaccharides [29]. Particularly for elastases, the level of inhibition of human leukocyte elastase (HLE) by the potent anti-inflammatory drug heparin (a sulfated glycosaminoglycan naturally produced in human/animal body) varied depending on the molecular weight and degree of sulfation of its polysaccharides [29]. Given that salinity can influence the proportion of uronic acids and/or the chain length in EPS from cyanobacteria [39,44] and the sulfation pattern in EPS from red algae [45], similar (or additional) structural shifts may be hypothetically occurring in EPS from endolithic cyanobacteria and thus affecting their inhibitory effects on human neutrophil elastase. These intriguing structural aspects of EPS and their possible inhibitory kinetics deserve further research on Atacama cyanobacteria, especially in the strain showing the greatest anti-elastase activity (*Chrooccoccidiopsis* sp. UAM579) and those with high yield of EPS (especially *Gloeocapsa* sp. UAM572).

3.5. Biotechnological potential of endolithic cyanobacterial EPS: Considerations for mass production

The novel and promising small-scale results from the present study invite further development of mass production of endolithic cyanobacteria towards obtaining EPS yields sufficient for potential downstream applications in industries like cosmetics, pharmaceuticals, biopolymers or even food (nutraceuticals). Future steps may include large-scale tests in different types of bioreactors with the best performers, i.e., *Gloeocapsa* sp. UAM572, *Chroococcidiopsis* sp. UAM584 and *Pseudoacaryochloris* sp.

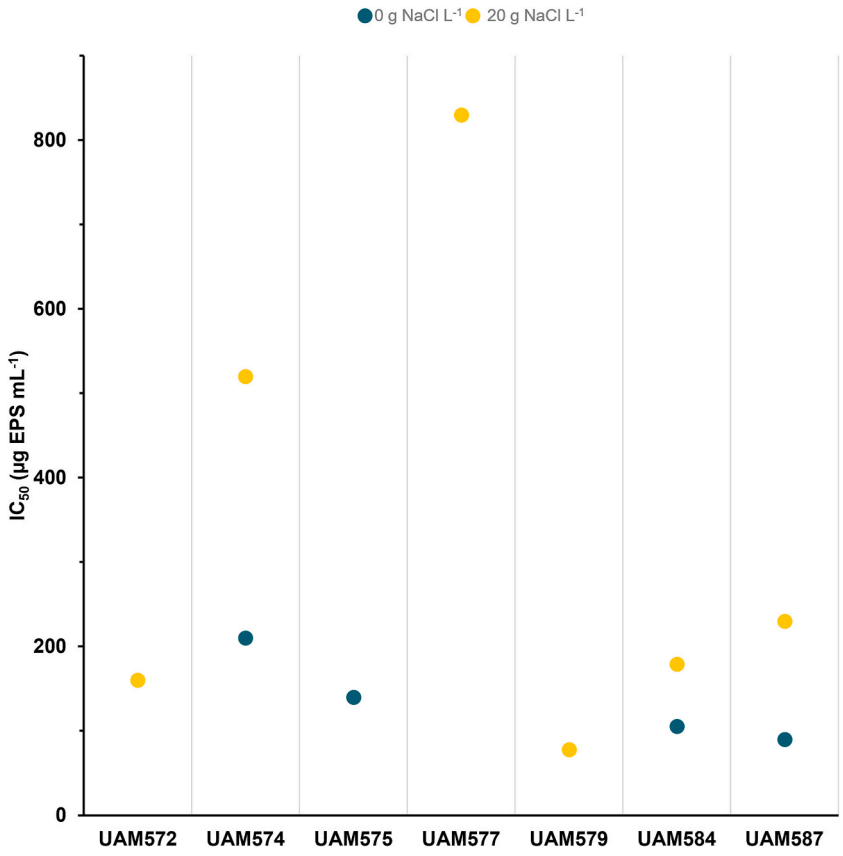

Fig. 4. Anti-elastase activity of EPS from 7 endolithic cyanobacteria. Y axis shows inhibitory concentration 50 (IC_{50}), i.e., the concentration of EPS necessary to inhibit 50 % of the activity of the NE expressed as µg EPS mL^{-1} per each strain and salinity (0 g NaCl L^{-1}; 20 g NaCl L^{-1}). Lower IC_{50} values indicate greater inhibitory capacity. No inhibitory activity (or $IC_{50} > 1$ mg mL^{-1}) was detected for EPS from strains UAM572, UAM577 and UAM579 grown with 0 g NaCl L^{-1}; and for UAM575 grown with 20 g NaCl L^{-1}, and thus they are not represented in the graph.

UAM587, grown under 20 g NaCl L^{-1} in order to evaluate whether productivities observed hereby remain upon upscaling. Open outdoor cultures are the most usual option for a cost-benefit viable mass production, for which the halotolerance found in endolithic cyanobacteria poses advantage in reducing potential contamination from air-suspended microbiota [16].

Looking at small-scale data within a broader context of potential mass production, [46] in their work with cyanobacterial cultures proposed a simplified simulation towards deriving potential areal productivity (per m^2) of biomass/bioproducts if grown in outdoors vertical bioreactors arranged in different multi-systems configurations [47,48]. These simulations have also been applied recently in [14] for a rough estimate of potential areal production of scytonemin by endolithic cyanobacteria derived from lab data. Following this same approach, strains from the present study may be hypothetically able to produce from 20.6 to 128.0 mg EPS per square meter per day (Supplementary Material) depending on the strain, salinity and bioreactor arrangements (wall-to-wall, inter-row) affecting sunlight exposure due to shading [47,48].

This indicates a potentially moderate EPS production with a relatively low land use, moreover, considering that these strains could be grown in desert or arid land not competing with agricultural food production while contributing to CO_2 fixation during growth. However, for sustainable production, it would still be necessary to reduce production costs to the minimum. Given that dewatering itself can take nearly 20–30 % of total biomass production costs in microalgal/cyanobacterial cultures, it would be desirable to optimize a low-cost method for biomass concentration, i.e., by growing cultures in biofilm, and/or investigating the natural self-settling capacity expected for EPS-rich cyanobacteria [49]. This self-settling could be further modulated by low-frequency ultrasonication pulses as already tested for endolithic *Chroococcidiopsis* sp. from Atacama before [50]. Together with optimized harvesting methods, it would also be reasonable to squeeze the full biorefinery potential of desert endolithic strains. One of the many possible pathways would be combining EPS extraction with other commercially relevant bioproducts like pigments. This alternative was successfully tested in two desert cyanobacteria, proposing EPS extraction followed by biomass freeze-drying and subsequent phycobiliproteins, chlorophyll *a* and carotenoids extraction [51].

Beyond these strategies with native strains, metabolic engineering would be an interesting field to explore towards achieving an increased EPS yield. In this sense, Billi indicated that desert strains of *Chroococcidiopsis* are suitable for genetic manipulation i.e. to include inducible promoters from *E. coli* that drive gene expression [27]. For this purpose, it remains of great interest to obtain whole genome sequences of endolithic cyanobacteria allowing description of biosynthetic clusters relevant for biotechnology, such as gene-clusters EPS (*exo* genes) or scytonemin (*scy* genes), among others. Having these biosynthetic machineries fully disentangled in Atacama endolithic cyanobacteria might open gates for biotechnology -e.g., facilitating research on directed manipulation of cyanobacteria and/or heterologous over-expression in *E. coli* - and ultimately improve our understanding of microbial strategies to survive at the limits of life on Earth.

4. Conclusion

This study provides novel biotechnological insight into endolithic cyanobacteria from the Atacama Desert. Moderate salinity (20 g NaCl L^{-1}) was tolerated by all strains, proving a biotechnologically efficient stress that triggered a 3–9-fold EPS increase in 4 strains of three genera

and lithic substrates. The anti-elastase activity was demonstrated for all strains, with inhibition capacity (IC_{50}) showing inter-strain and inter-salinity variations. Micro-FTIR evidenced shifts induced by NaCl in functional groups of EPS opening gates for future structural determinations. Relevant strains showing the highest EPS productivity (UAM575) and anti-elastase potential (UAM579) are proposed as candidates for further investigation on outdoor upscaling in arid land.

CRediT authorship contribution statement

María Cristina Casero: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David Velázquez:** Writing – review & editing, Funding acquisition, Conceptualization. **Adrián Pereira:** Writing – review & editing, Methodology, Investigation. **María del Mar Tejedor:** Writing – review & editing, Methodology, Investigation. **Luis García:** Writing – review & editing, Methodology, Investigation. **Antonio Quesada:** Writing – review & editing, Validation. **Samuel Cirés:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103817.

Data availability

Data will be made available on request.

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