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RESEARCH ARTICLE

Anatomizing extracellular polymer of *Calothrix desertica* with its anti-oxidation and anti-nutrient profiling

Lavania R[1], Narayanasamy M[2] & Thajuddin N[1, 3*]

[1]Department of Microbiology, Bharathidasan University, Tiruchirappalli- 620 024, India
[2]Department of Microbiology, M.G.R college, Hosur, Affliated to Periyar University, Tamil Nadu-635 130, India
[3]National Repository for Microalgae and Cyanobacteria – Freshwater, Bharathidasan University, Tiruchirappalli- 620 024, India

***Email:** nthaju2002@yahoo.com

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Abstract

Calothrix desertica having a semilunar apical heterocyst is proficient at excreting 1.2 g/L of extracellular polymers (EPsC) in 45 days. The refined EPsC constitutes 430 mg/g of glycogen, 390 mg/g of protein, and 14.6 mg/g of glycoproteins (GPs). The glycoprotein estimation of EPsC was performed by two step hydrolysis methods with H_2SO_4. The peak between 10 mAU to 20 mAU in HPLC, 1400 cm^{-1} to 1700 cm^{-1} in FTIR, and 40kDa- 35kDa bands in SDS-PAGE authenticates the presence of glycoproteins in the EPsC. The EPsC agglomerate of 1000 nm to 3000 nm size with a Zeta potential of -20 mV to 5 mV was determined using DLS. Further EPsC of nanosizes of 30 nm to 150 nm in 50,000 X and 20 nm to 40 nm in 60,000 X was measured using FE- SEM. The DPPH assay and H_2O_2 scavenging assay showed 73.1% and 70.8% of anti-oxidant activity in EPsC, which is coequally efficient as standard gallic acid. EPsC biopolymer can also be used as a potential reducing agent, as per the anti-nutrient activity studies.

Keywords

Extracellular polymers; DLS; Zeta potential; glycoprotein; anti-oxidation

Introduction

Lithophilic cyanobacteria establish its thallus and anchor themselves firmly on their surface niche by defending other microbial communities. Our experimental organism, *Calothrix desertica* is attached to rocks and expels slimy discharge out of the cell for its sustainability. The quantity of extracellular polymers of *C. desertica* (EPsC) produced remains proportionate to the environmental stress imposed (1). The incorporation of extracellular polymers in the manufacturing of high value products and pharmaceuticals by manipulating their level of tension has become a relevant study (2). At the same time, the total production cost of extraction and downstream processing of EPsC still keeps it at the bottleneck (3).

EPsC is a complex mixture of polymers with extracellular polysaccharides and proteins, along with minerals. The enzyme protease breaks the biopolymer into tiny molecules that can be easily utilized as carbon and energy sources by other microbes (4). Recent studies reveal the presence of glycoproteins like glucosamine (GlcN) and *N*- acetyl glucosamine (GlcNAc) formed by the glycosylation of exoproteome that also share a major ratio of EPsC matrix.

The cyanobacteria EPsC extract could behave as a good substitution for extracellular polymers from plants, seaweed, and animals since they can easily grow under controlled ex-situ conditions (5). Still, due to its production and recovery costs, these extracellular polymers represent a very small fraction of the biopolymer market. The production method using cheaper substrates like waste materials and agricultural by-products is considered to be the best remedy to reduce biosynthesis expenses. The nanosized extracellular polymer forming capability of *C. desertica* is a noticeable physical feature that will encourage the incorporative capacity of this bio- product in different biotechnological applications.

Microbial extracellular polymers are becoming a research focus in the biomedical industry by delivering anti -oxidation and anti-microbial properties. Extracellular polymers synthesized by cyanobacteria also bear equal bio -functions. They are capable of acting on superoxide anions and free radicals based on the species from which EPS has been extracted.

This study aims at the extraction of EPsC using solvents and its physical and chemical anatomization using instrumentation techniques. This evaluation can lead to estimating its capability to act as an effective anti-oxidant (6) and also its capacity to leave the micronutrients free by avoiding the adsorption tendency of biopolymer on atomic nutrients.

Materials and Methods

Collection and preservation of the sample

The algal mats were peeled out from the wet rocky vicinity near Thommankuthu waterfalls at Idukki, Kerala, India lying at 9° 57' 33.0768'' N, 76° 49' 8.346'' E angular distance. The sample collection area is broadly categorised into three zones: bedrock surrounding the waterfall as outcropping (OC), running water touching rock edges as ephemeral region (ER), and the impacted base of the waterfall as plunge pool (PP).

Cyanobacterial biodiversity of washed and sieved (50µm mesh) natural samples was recorded with a Light microscope (MCX 500, Micros, Austria). Both the species richness and evenness of the sampling site were evaluated. Traditional water analysis protocols (7, 8) were followed to analyse the growth influencing nutrients like Dissolved Oxygen (DO), Nitrate (NO_3), Nitrite (NO_2), Calcium (Ca) and Magnesium (Mg) in the sampling site. The Atomic Absorption Spectroscopic technique (AA6200, Shimadzu, Japan) was adopted to determine the chemical content. Hand-ground assorted algal peels were spread plated in BG11 agar medium (0.80%) (9), incubated, and monitored for 7 days interval of time. The morphology of procured axenic *C. desertica* culture was profoundly defined via light microscopic (Nikon Eclipse TS100 Microscope, USA) and confocal laser scanning microscopic (CLSM) (LSM 60, Carl Zeiss, Germany) observation. Features of *C. desertica* were compared with existing digital (algae base) cyanobacterial records. The genetic similarity was confirmed by 16S rRNA amplification (PCR, Applied Biosystems™ Veriti) with distinct primers (CYA106F/CYA781) (10), nucleotide sequencing and aligning (BLAST) followed by evolutionary detailing with the MEGA6.06 program. The DNA sequence was submitted to the National Center for Biotechnology Information (NCBI) GenBank. Equally 1.0g of wet *C. desertica* cells were transferred in a 3L Haffkine's flask bearing nitrogen deficient modified BG 11 medium at 24˚C, pH 7.4 with photon a flux of 80±20 µmol m^{-2} S^{-1} in a 12 hrs dark and 12 hrs light regime (11, 12) under photo-protective shades to avoid bleaching and attain logarithmic growth (13).

Processing culture exudate

The jellified culture medium with biomass of *C. desertica* was centrifuged at 10,000 rpm at 8°C for 10 min to segregate viscous EPsC (14). The viscid centrifugate was blended with an equal volume of acetone in a 2 L beaker, covered, and refrigerated (4°C) for 48 hrs. Sedimentary EPsC obtained by solvent extraction was resolved by centrifugation at 10,000 rpm at 10°C for 8 min. Dialysis of collected EPsC pellets with double distilled water across the 10 kDa molecular-weight-cut-off for 24 hrs with gently stirring water wash at 20°C for 2 times was done (15). The EPsC was further air dried at room temperature (30 °C) for 2 hrs then lyophilized at -40°C for 12 hrs (16).

Proteo: glyco content of EPsC

For polysaccharide precipitation, 0.1 mg of EPsC powder was dissolved in 40 mL of Na_2CO_3 (0.2 M) at 80°C for 2 hrs and centrifuged at 10,000 rpm for 10 min. The supernatant was mixed with 0.05M HCl and centrifuged at 10,000 rpm for 20 min and the pellet was washed with double distilled water. Finally, the pellet was centrifuged at 15,000 rpm for 20 min by adding 0.1M NaOH and ethanol (80%). Protein in EPsC was precipitated by adding 2.0M H_2SO_4 to the supernatant, regulating it to its isoelectric precipitation condition (pH 3.3), and centrifuged at 9000 rpm for 10 min (17, 18). The conventional colorimetric methods, in particular phenol sulphuric acid (19) and Lowry's protocol (20) for carbohydrates and proteins were followed to quantify and understand their rational distribution in EPsC.

Glycoprotein estimation in EPsC

Two-step hydrolysis method

The extracellular glycoprotein of EPsC was determined by two steps of hydrolysis: initially treating with concentrated H_2SO_4 at low temperature, then with diluted H_2SO_4 at high temperature, along with nitrous acid depolymerisation. Add 10 mg of EPsC to 0.3 mL of 72% (v/v) sulfuric acid in a 15 ml screw cap tube and keep shaking for 2 hrs. This partially diluted EPsC tube was autoclaved by adding 8.4 mL of water for complete dissolution. The obtained homogenous solution was transferred to a tube and added to 0.5 mL of 1 M $NaNO_2$. The tubes were kept closed and open for 6/6 hrs time regime to accomplish the depolymerization-deamination. To the anhydromannose bearing tubes, 0.5 mL of 0.5% MBTH (3-methyl-2-benzothiozolone-hydrazone-hydrochloride) and 0.5 mL of 0.5% $FeCl_3$ were added. As the blue colour developed (in 1 hr) absorbance was read with UV- Vis spectrophotometer

(Cary 60 UV-Vis, Agilent Technologies) at 650 nm and pure GlcNc hydrochloride in 2.48% (v/v) H_2SO_4 was taken as standard. The presence of glycoprotein was confirmed with High Performance Liquid Chromatography (HPLC) (Milford, MA, USA) at 60°C with 5 mM sulfuric acid as eluent and a UV-vis detector gauge of 254 nm (21, 22).

Electrophoretic method

The EPsC protein was separated using SDS-PAGE using the Bio-Rad Mini-PROTEAN Electrophoresis system. The test sample was diluted in 20% TCA and kept under refrigeration for 12 hrs to precipitate. The gel was casted with stacking gel and separating gel having concentration ratios of 5:12 and 1:4 partition area, respectively. 500 µg/mL of sample along with a protein ladder of 15–130 kDa size range were loaded in the gel and allowed to run under the applied voltage of 70 V for a period of 2 hrs 30 min (23).

Derivatization of EPsC

To improve the application capability of EPsC, a solubility test has been conducted for the biopolymer. The protic and aprotic solvents like 90% ethyl acetate, dimethyl sulfoxide (DMSO), diethyl ether, and ethanol with their combinations of DMSO to water, ethanol to water, diethyl ether to water, ethyl acetate to water, diethyl ether to ethanol, and ethanol to ethyl acetate in 1:9 ratio. The mixture containing vials was sonicated (V TECH Mild Steel Probe Sonicator) under a 10 kHz to 50 kHz frequency range, which was later kept in ultrasonic baths for 1 hr at 45°C to obtain homogenized EPsC solutions (24, 25).

Size determination of EPsC

Dynamic light scattering

Dynamic Light Scattering (DLS) is the precise technique used to determine the size of the EPsC particle in the suspension. The efficacy of biopolymers is significantly affected in their particle size and distribution. DLS determines the dimension of EPsC based on the Brownian motion of the particle and measures the hydrodynamic size in a range of 0.3 nm to 10000 nm. The rate of repulsion between the particles of the suspension was measured using the Zeta potential. This parameter measures the self-sedimentary characteristics of EPsC. As the value of Zeta potential increases, the rate of sedimentation decreases (26).

Scanning electron microscopy

SEM micrographs of EPsC were taken using the field emission technique to understand the surface morphology. A scanning electron microscope (FE-SEM) (JEOL JSM-6701F, UK) incorporating a cold cathode field emission gun was used for imaging gold sputtered EPs particles mounted on the metal stub. An accelerating voltage of 3.0 keV and higher magnifications of 30,000X and 50,000X were employed to ensure a clear profile of EPs (27).

IR spectroscopy

Fourier transform infrared spectroscopy (FT-IR, Perkin Elmer with version 10.4.00, USA) with a transmittance range of 4000 cm^{-1}– 400 cm^{-1} was used to analyse the presence of different functional groups embedded in EPsC. The attenuated transmittance (ATR- FT-IR) principle incorporated into the protocol was followed. The spectrum obtained was interpreted and compared with library data (28).

Bio-processes

Anti-oxidation activity

In-vitro assessment of EPsC as an anti-oxidant was studied by understanding DPPH (2, 2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide scavenging capacity.

In the DPPH assay 1mg/mL of EPsC dissolved in DMSO was considered the sample solution. Make up the sample solution in 1 mL of 200 µg/mL to 1000 µg/mL concentration. 1 mL of 0.1 mM DPPH in ethanol was added to each concentration and incubated in the dark for 2 hrs. The absorbance was measured using a Cary 60 UV-Vis Agilent Technologies spectrophotometer at 517 nm with deionised water as a control. The capacity of EPsC to scavenge DPPH molecules determines its ability to act as an anti-oxidant.

DPPH scavenging activity or inhibition (%) = $[1 - A_s / A_c] \times 100$ where A_s is the absorbance of the sample, A_c is the absorbance of the control (29).

Hydrogen peroxide scavenging assay was performed by preparing 10 mM hydrogen peroxide solution in 0.1 M phosphate buffer saline (pH 7.4), 200–1000 µg of EPsC were mixed with 2 mL of hydrogen peroxide solution and incubated for 10 min. The absorbance was measured at 230 nm (Cary 60 UV-VIS, Agilent Technologies, USA).

$$\text{Percentage of scavenging (\%)} = \frac{(A_0 - A1)}{A_0} \times 100$$

Here, the absorbance of the control is (A_0) and the absorbance of the sample or standard is (A1).

Gallic acid is used as a standard anti-oxidant for both scavenging assays (30).

Anti- nutrient activity

Pre and post quantification of two essential elements, iron (Fe) and zinc (Zn), in the EPsC treated metal mixtures was performed with respective cathode lamps of flame atomic absorption spectroscopy (AAS) (AA6200, Shimadzu, Japan). To the 0.5 mg/mL and 1.0 mg/mL of Fe and Zn solutions, an equal volume of 1.0 mg/mL of EPsC mixture was added, shaken and kept stable for 20 min. The metal solution alone was considered the control, and a triplex of each experiment was set for confirmation (31).

Results

Biodiversity and isolate

Lithophilic green slimes bearing cyanobacterial samples showed an appreciable range of diversity (Fig.1). The microscope aided analysis of the collected samples showed evenness in the distribution of *Phormidium* sp. and *Oscillatoria* sp. throughout the three (OC, ER, and PP) areas with varying disturbance intensity. Extracellular polymer synthesizers such as *Nostoc* sp. and *Calothrix* sp.

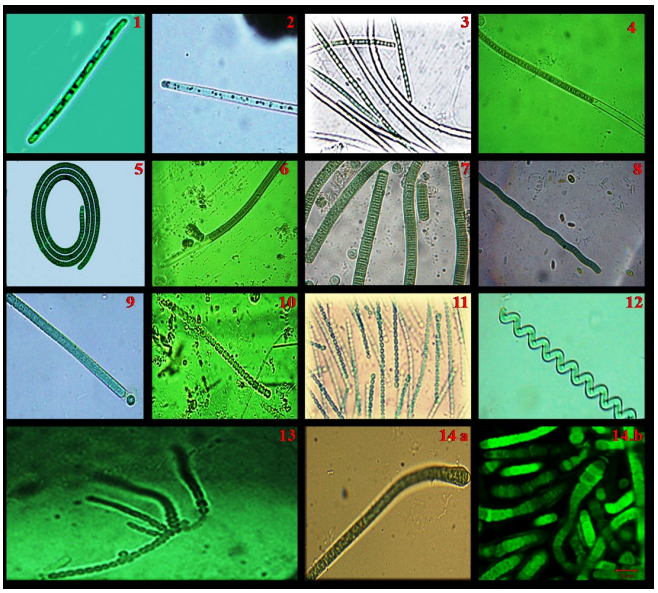

Fig. 1. Microscopic images of major cyanobacterial species distributed near Thommankuthu waterfalls: (1,2,3,4) *Phormidium* sp., (5,6,7,8,9), *Oscillatoria* sp., (10,11), *Nostoc* sp., (12) *Spirulina* sp., (13), *Stigonema* sp., *Calothrix desertica* (14 a, b)

among the collected samples were evenly present in the OC and ER regions. *Spirulina* sp. and *Stigonema* sp. are the two other major cyanobacterial groups distinguished in the moderately disturbed rocky outcrops of waterfalls. Planktons, green algae and diatoms were also found associated with these recorded predominant cyanobacterial groups (Table. 1). Plated natural samples developed green patches in the petri plates within 7- 10 days of incubation under controlled temperature and light (32). The ex situ acclimatization rate of *C. desertica* was comparatively high. By careful pricking and inoculating viable cells of *C. Desertica*, its growth initiated in 1mL BG 11 broth within 14- 20 days further established its biomass at a rate of 3.0 g/L in haffkine culture flask over 45 days.

The water analysis results of the collection sites (OC, ER, and PP) (Fig. 2) revealed minimal human activity and a low pollution rate. The light microscopic and CLSM images under an Ar laser of 480- 540 nm of excitation wave length of *C. desertica* materialized a 200µm long filament with a 5µm broad and 3µm long trichome holding a thin sheath and an apical heterocyst (Fig.1. 14a). The CLSM

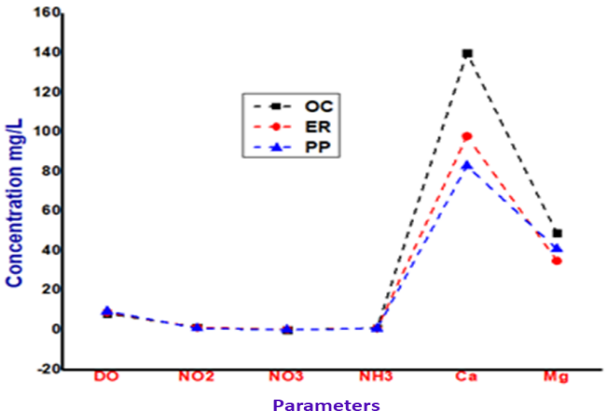

Fig. 2. Chemical composition of water in the three collection sites (Out Cropping (OC), Ephimeral Region (ER), Plunge Pool (PP))

images focused the slightly attenuated and constricted thallus with hemispherical and compressed heterocyst (Fig.1. 14b). The gene amplicons of the cyanobacterial isolate fall within ≈ 650 bp of the marker. Polymerised genetic material of *C. desertica* was sequenced and deposited in the GenBank (NCBI) with accession number MH144148. Alligning the genomic sequence of the amplified DNA figured out its genetic compatibility with *Calothrix* sp., *Nostoc* sp., and cyanobacteria. The phylogenetic tree (Fig. 3) was constructed by the maximum likelihood method to understand its descendants for better genus confirmation (33). The culture was preserved for public access in the germplasm of the National Repository for Microalgae and Cyanobacteria-Freshwater (NRMC-F), BDU, Tamil Nadu, India.

Extracted biopolymer

Jellification of the *C. desertica* culture medium began within 30 days (mid log phase) of its growth, with maximum EPsC synthesized in 45-50 days (stationary phase) (Fig. 4). The gelatinous *C. desertica* medium collected over a 60-day interval released 1.2 g/L of dry EPsC powder. Usually Nostocale enhances its biomass slowly, since it takes 60- 70 days to reach sufficient density of cells for polymer extraction (34); whereas *C. desertica* consumed less time for effective sustainable EPsC

Table 1. The chart depicting cyanobacterial species richness and evenness in the area divided (OC: Out Cropping; ER: Ephimeral Region; PP: Plunge Pool) around the water falls

	Cyanobacteria	OC	ER	PP
1.	*Phormidium* sp.	++	+	+
2.	*Phormidium* sp.	++	++	+
3.	*Phormidium* sp.	+++	++	++
4.	*Phormidium* sp.	+++	+	+
5.	*Oscillatoria* sp.	+	+	+
6.	*Oscillatoria* sp.	+	+++	+
7.	*Oscillatoria* sp.	+	++	-
8.	*Oscillatoria* sp.	++	+	-
9.	*Oscillatoria* sp.	+	++	-
10.	*Nostoc* sp.	+++	++	-
11.	*Nostoc* sp.	++	+	-
12.	*Spirulina* sp.	++	+	-
13.	*Stigonema* sp.	++	-	-
14.	*Calothrix desertica*	+++	-	-
(+++) Dominant, (++) abundant, (+) present, (-) absent				

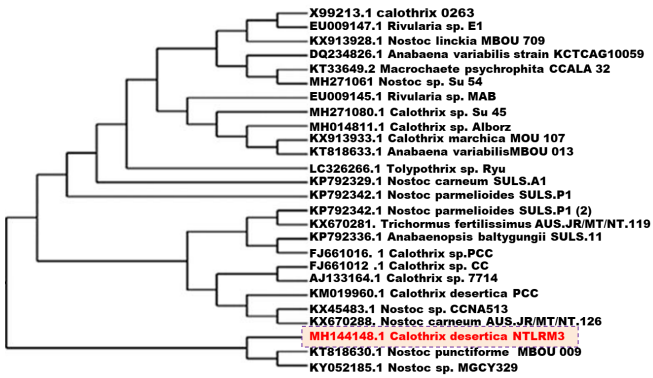

Fig. 3. Phylogenetic tree connecting the ancestry of *calothrix desertica* to other *Nostocales* proves its EPsC releasing efficacy

synthesis. A polar solvent like acetone can change the polarity of EPsC in water and decrease its relative solubility, which improves its precipitation. Thus, the quantity of sedimentary EPsC particles is elevated, and downstream processing of EPsC becomes more prolific at low temperatures (4°C). This hyporeactive biopolymer can be stored in a dark, cool place at room temperature.

Proteo- Glyco and glycoprotein ratio in EPsC

Extracellular polysaccharides and proteins were successfully extracted from *C. desertica* medium exudates. The UV-spectrum based chemical quantification detected 430 mg/g of glycogen and 390 mg/g of protein content. Glycoprotein content determined by two-step hydrolysis using H_2SO_4 and one-step nitrous acid depolymerization showed 14.6 mg/g of glycoprotein in EPsC. The peaks obtained between 15 to 20 retention times in the chromatogram of HPLC for acetic acid also indicate the chances for the occupancy of glycoprotein in EPsC (Fig. 5) (34).

Further investigation of the EPsC protein by performing SDS- PAGE revealed the existence of bonds between carbohydrates and proteins. The molecular weight of the EPsC protein was obtained in a range of 40kDa-35kDa, which confirms the formation of glycoprotein (Fig. 6).

Surface tension and topology

Solubility and surface tension are inversely proportionate characteristics. To obtain a homogenous solution of EPsC temperature was elevated, which reduced the surface tension among water molecules. The maximum solubility of EPsC was obtained in 90 % of DMSO (a polar aprotic

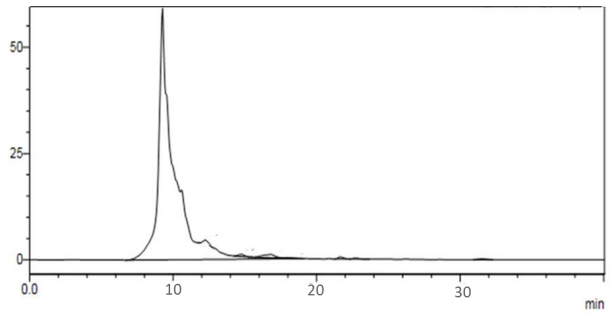

Fig. 5. The HPLC chromatogram tapering peak at R_t 10 min. indicating *N*- acetyl glucosamine composition in EPsC

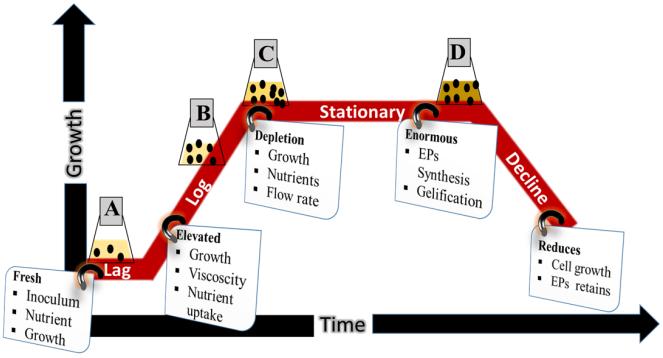

Fig. 4. Graphical plot representing stages of nutrient requirement and EPsC synthesis during growth of *Calothrix desertica*

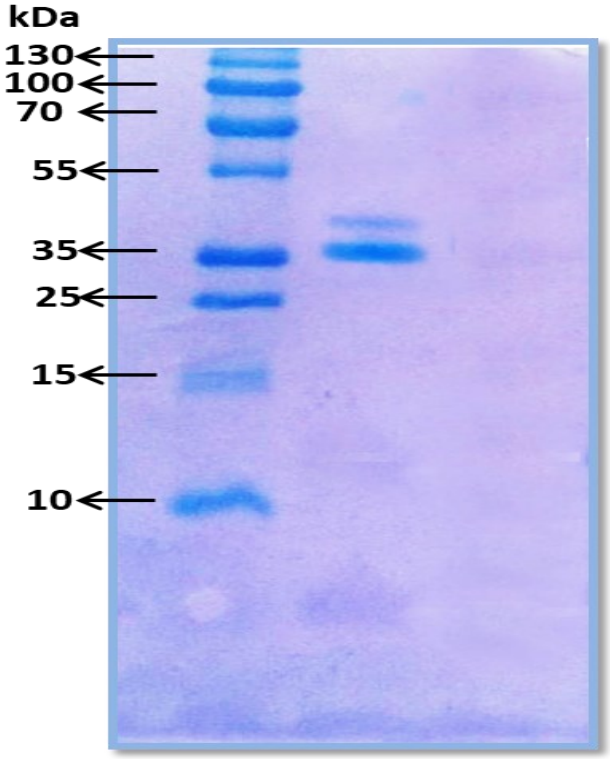

Fig. 6. SDS- PAGE depicting glycoprotein bands of EPsC.

solvent). DLS determined the size of the EPsC particle between 1000 nm to 3000 nm by recording the light scattered by the particles with an incident monochromatic coherent light source (Fig. 7a). The size of the EPsC powder can be brought down to nanometres by working on minimizing the agglomeration tendency among molecules (35). The Zeta potential of EPsC ranges from -20 mV to 5 mV, which is closer to zero (Fig. 7b). As Zeta potential value increases and remains closer to zero, the rate of self-sedimentation efficacy decreases. The intermolecular Van der Waals forces will be high if the Zeta potential is closer to zero. Since the value of the Zeta potential of EPsC indicates the stability of its suspension at high levels (36).

Field emission scanning electron microscopy (FE-SEM) images of EPsC focused on irregularly clumped globular granules (Fig. 8). The size of the particles fell in a range of 30 nm to 150 nm at 50,000 X and 20 nm to 40 nm at 60,000 X magnifications. The particle size obtained indicates the habit of nanoparticle biosynthesis in *C. desertica* (27). Having a homogenous solution with smaller particle sizes is a necessity for enhancing the biotechnological properties of any natural extract.

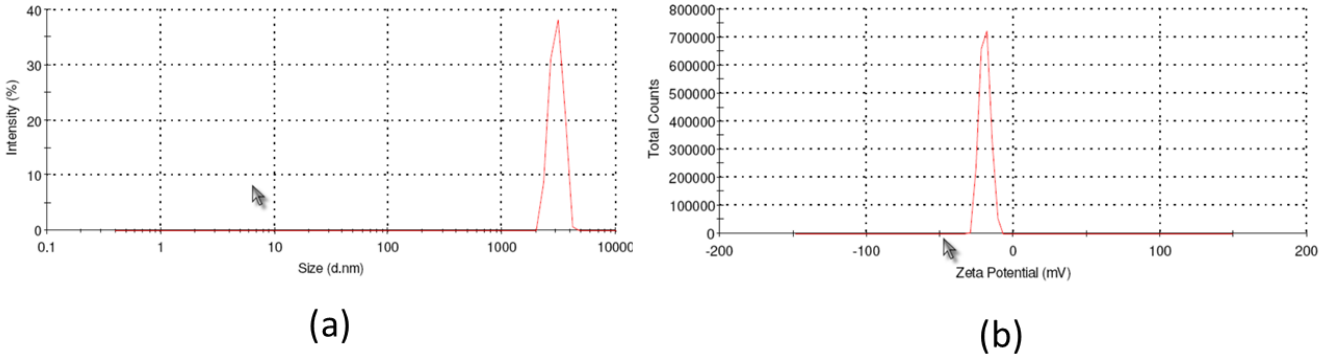

Fig. 7. Graphs showing the particle size (a) and Zeta potential (b) of EPsC.

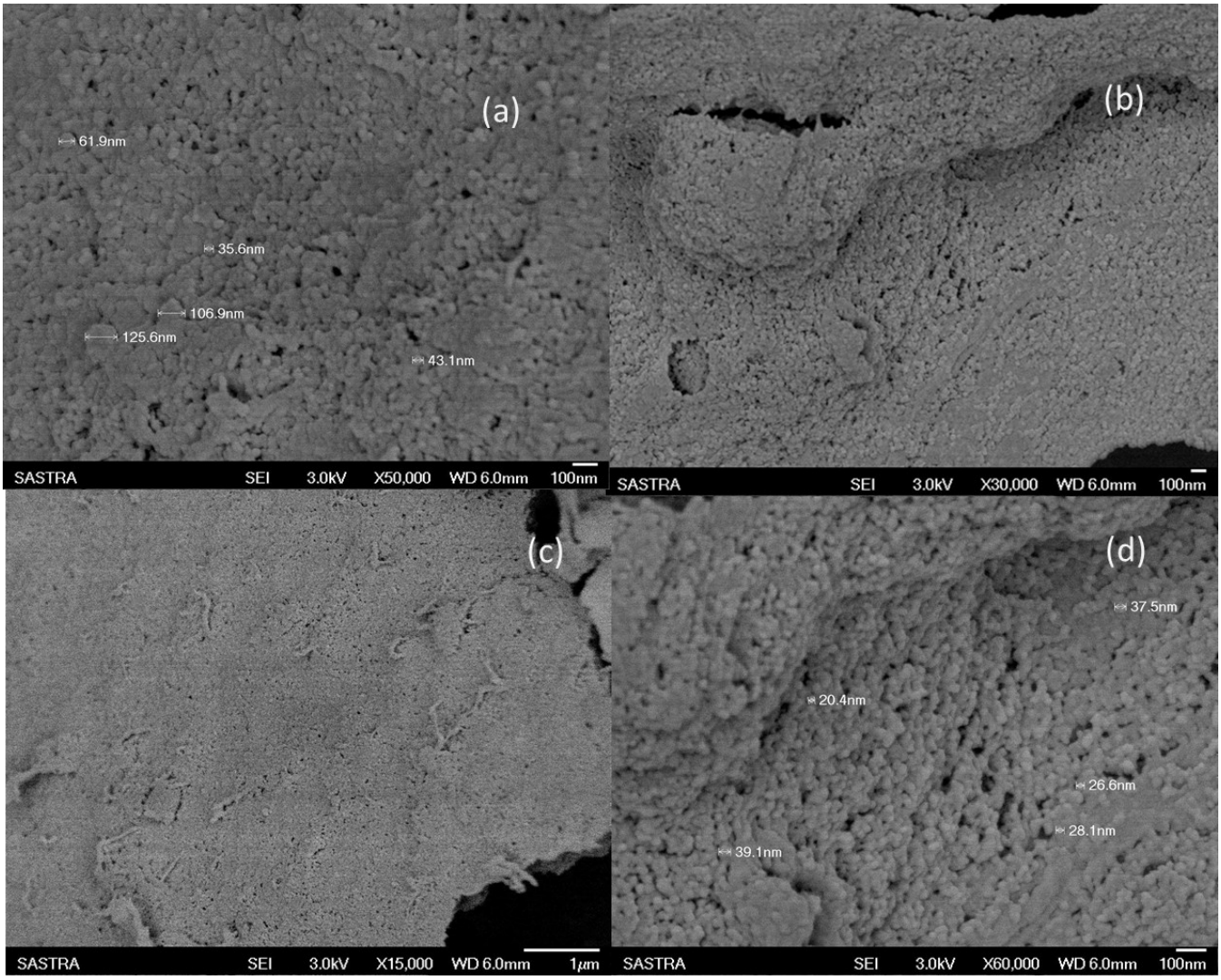

Fig. 8. FE- SEM images of EPsC focusing at different magnifications

Molecular Vibrations

IR spectrum of EPsC with 14 peaks was obtained, which covers the wavelength of 300 cm^{-1} to 3000 cm^{-1} indicating the presence of proteins, glycoproteins, polysaccharides, and hydrocarbons. Narrowly differentiated peaks were obtained by the stretching forces and intermolecular bonds developed by the EPsC molecules if a particular quantum of energy fell on them. The peak at 3366.8 cm^{-1} indicates asymmetric O-H stretching, 1637.7 cm^{-1} represents the Amide I band, and 1408.08 cm^{-1} represents C-N stretching, N-H deformation, and C-H deformation of Amide II amino acids (37). The various peaks between 800 and 600 cm−1 indicate the presence of a mineral matrix (Fig. 9).

Anti- oxidant and anti- nutrient

The antioxidant character of EPsC is clearly disclosed by performing two scavenging assays. In the DPPH assay, the percentage of DPPH inhibition is calculated as an anti-oxidation parameter, which showed a logarithmic increase with EPsC concentration. The maximum of 73.1% of anti-oxidation activity was indicated by EPsC at 1000 µg/mL but in the hydrogen peroxide scavenging assay, the percentage reduced a little to 72.1% (Fig. 10). The standard

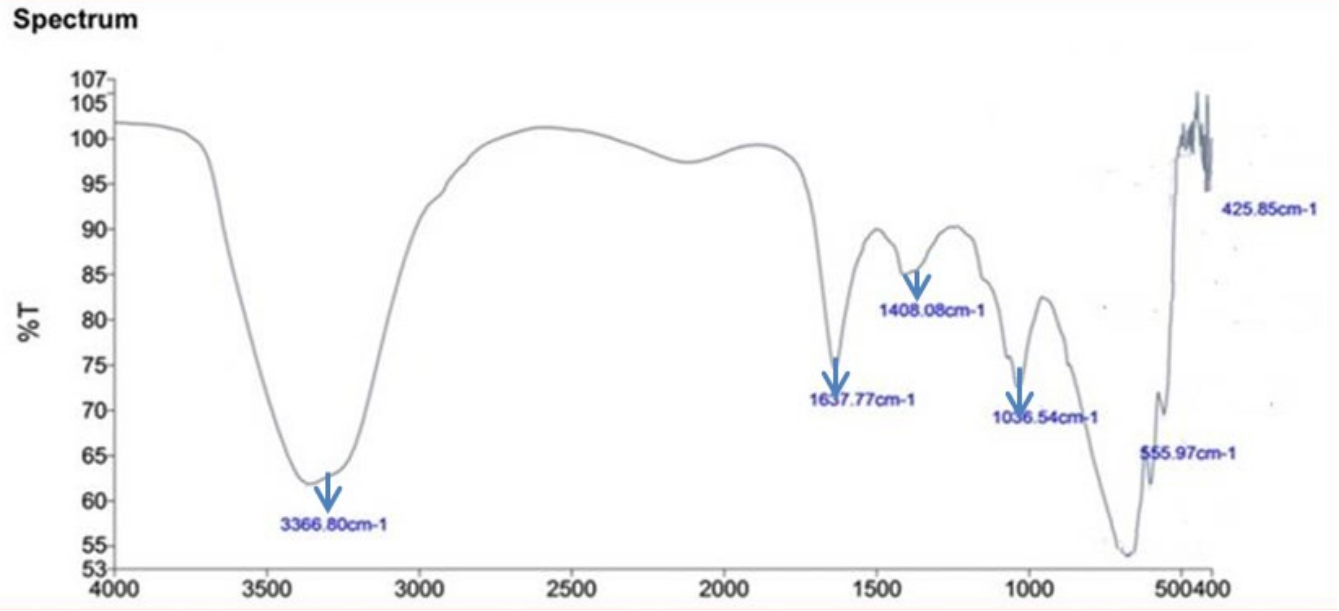

Fig. 9. FTIR peaks of EPsC showing amide bands and hydrocarbons

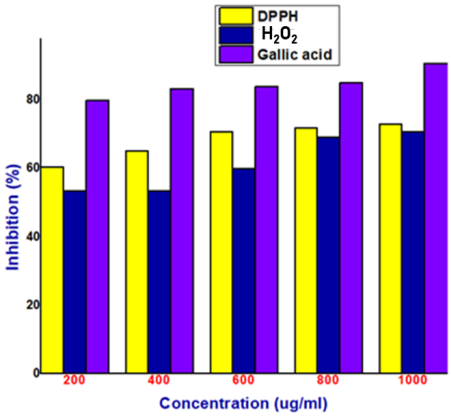

Fig. 10. Graph representing the percentage of anti-oxidation via DPPH and hydrogen peroxide scavenging assays

reducing agent, gallic acid, showed 90% electron removal capacity, which in effect should increase the anti-oxidation activity with the increasing concentration of the reducing agent.

The EPsC has been dissolved directly without following any intermediary elution steps, and this itself is showing an appreciable amount of antioxidant activity. This depicts the easy releasing tendency of electron acceptors by this natural polymer (38). Anti-nutrient analysis with AAS denoted no change in the quantity of the atoms (Fe and Zn) and zero precipitation tendency in the 'EPsC treated and untreated' metal solutions.

Discussion

Extracellular polymer synthesis

Calothrix desertica is an ecologically important genus with thick walled apical heterocysts that help nitrogen fixing enzymes. While comparing the morphology of *C. Desertica*, it resembles the most ubiquitous and ample taxa like *Calothrix fusca* and *Rivularia minutula* to some extent. As the apical heterocyst is a common character among the three species, *C. desertica* differs by having a perfect semilunar heterocyst compared with *C. fusca* and the absence of apical or terminal hair, which is present in *R. minutula* (39). Genetically, this cyanobacterium is determined by its ability to synthesize hairs, while the expression of this feature is totally based on its ecology. The reproduction by cracking hormogonia bearing transparent necridic cells can also count as one of the characteristic features of *C. desertica.* The cladistic data for understanding the evolution of *C. desertica* reveals evidence of polyphyly, which has some evolving elements missing in its ancestry (40). Hence, some taxonomical researchers with similar data suggested modifying the family Calotrichaceae by putting *Dulcicalothrix, Calothrix*, and *Macrochaete* under it. Together, they strongly recommend the reclassification of *C. desertica* to *Dulcicalothrix desertica* (41).

C. desertica secretes polymers bearing valuable elements out of the cell (EPsC) under starvation and during reproduction, like all other nostocales. The earlier study reports show that this exocytosis activity starts after two weeks in cyanobacteria. This mechanism is eccentric among each cyanobacterium, since it depends on physical, chemical, and biological factors. Related results on the quantity of EPsC extract fall in a range of 0.5 g/L to 1.0 g/L of EPS in 21 to 40 days, which is approximately equal to the ability of *C. Desertica*, synthesizing 1.2 g/L in 45 days. Extraction is preferably done with solvents like acetone or ethanol in a single step; two-step extraction using lukewarm water and 0.1 M EDTA was also followed, which didn't make any noticeable difference in the quantity of product (42).

Embedded glycoprotein

Anatomization of cyanobacterial extracellular polymers traced the presence of polysaccharides and proteins, which were always 70% of total moieties. Earlier, the major studies were based on focusing on extracellular

polysaccharides alone. The micro algal groups like Zygnematophyceae and Chlorophyceae excrete more carbohydrates than proteins (43). Under optimized culture conditions and involving different bioreactors the productivity of EPS can be made promising (34). In Nostocales like *C. desertica*, estimated ratio for carbohydrate and protein lies approximately on the linear level, which is a unique feature. The species-specific exoproteome and exopolysaccharides, which remain stable outside the cell, can be associated with cosmic biotechnological applications.

The protein-polysaccharide linkage in the exocytotic polymers adds to the relevance of the bio-product. The composition of glycoprotein was confirmed by the primary treatment of EPsC with concentrated sulfuric acid at room temperature, which dissolved the molecule to a large extent. Final autoclaving helps to dissolve any remaining residues and enhances deacetylation. The precipitation rate of EPsC after bringing it to room temperature was found to be comparatively low. To avoid the sedimentation risk, the solution can be kept at a temperature higher than room temperature. Generally, the results of different groups show that the quantity of the glycoprotein exists in the 15 to 20 mg/g range (42).

Interpreting the patterns of molecular vibrations, bonds such as (–CO–NH–, C=O,N–C, N–H) represent the exoproteomic content. A similar FTIR analysis of another cyanobacterial exocytotic polymer reported the same atomic excitement peaks except for the common aliphatic (C-H) stretching for carbohydrates. They observed the peak of amine (–NH2 or –NH) along with carboxylic acid (–C=O) at 3233 cm^{-1} and 1622 cm^{-1} respectively. Merging our findings with those based on the IR spectrum proves the plentifulness of proteins over polysaccharides in EPsC (36). The presence of the Amide I band predicts the glycoprotein secondary structure having an absorbance value around 1700 cm^{-1} to 1600 cm^{-1} (44). Particularly in amino acids, β-sheets create a number of transmittance bands around 1600 cm^{-1} to 1400 cm^{-1}.This indicates the development of covalent linkage among amide and monosaccharide groups that mould glycoproteins in EPsC (45).

The massive band with highly retarded electrophoretic movement in SDS-PAGE indicates glycoprotein. The presence of limited bands indicates the absence of a large quantity of multiple protein distributions, which occurred due to the extraction procedure rather than the strain involved. The EPsC delays precipitating protein due to its unique topology and atomic planes (46). Besides comparing to the previous studies, amount of glycoprotein in EPsC is less than that in fungal extracellular matrix while being much higher than that in bacterial biofilms.

Anti-oxidation potential of EPsC

DPPH assay that can scavenge the free radicals is the most preferably used anti-oxidation property determining test by the researchers. We observed a gradual increase in the inhibition of free radicals in the EPsC treated assay, which correlates with the comparative anti-oxidation study conducted among *Calothrix* sp. and *Phormidium* sp. They reported very specific data holding up the inhibition of DPPH radicals by an ethnolic extract of *Calothrix* with an inhibitory concentration of (IC50)=30.72±3.31 µg/mL (47). Other suggested assays to test the reducing properties of EPsC are the ferric reducing capacity of plasma assays and phosphomolybdate complex forming assays (37).

A rapid and effortless colorimetric assay from the natural extracts was developed, which involves H2O2, phenol and 4-aminoantipyrine, along with horseradish peroxidase (HRP). Superoxide, the precursor of active radicals found free in the organisms forms reactive oxygen compounds like hydrogen peroxide promoting tissue damage, DNA damage and protein damage. The presence of this reactive oxygen species (ROS) breaks anti-oxidation activity. The existence of organisms is highly influenced by ROS scavengers, and here EPsC reveals its potential to act as an electron scavenger based on the concentration parameter. Besides the in vitro activities performed by various research groups and species, it is disclosed that antiradical activity is a culture specific property (48).

Anti-oxidants are crucial factors for the body because they act as anti-nutrients in some cases based on their chemical composition. Studies on some higher plants provide information that antioxidant compounds like tannin act as efficient anti-nutrients, which directly act on the metabolic system. Hence, the EPsC is devoid of such a specific compound that assimilates or deposits the essential nutrients (49).

Conclusion

The chemical profiling of extracellular polymers from *Calothrix desertica* has extended its application spectrum to more biological aspects. The value of EPsC exudate along with its thalloid biomass was able to be understood by precipitating, refining, solubilising, and bioprospecting. The glycoprotein composition detected in EPsC made the biopolymer worthier than other bacterial and fungal extracellular matrixes. The polar-aprotic nature of EPsC enhanced the surface tension of the homogenous solution. The probability of biosynthesis of nanoparticles was also observed using DLS and FE-SEM. These base-line characteristics support EPsC's ability to act as an efficient anti-oxidant and anti-nutrient.

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Authors contributions

Lavania. R performed the investigation, data interpretation and optimisation of methodology. Narayanasamy. M involved in the data analysis, draft preparation and adding scientific suggestions. Thajuddin. N done Formal analysis and Revision of manuscript. All the authors looked through the manuscript for accession .

Compliance with ethical standards

Conflict of interest : The authors not have any competing interests to declare.

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