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Extraction and partial characterization of exopolysaccharides and pigments from cyanobacterium *Oscillatoria pseudogeminata* G.Schmid

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Cyanobacterial pigments such as chlorophyll, carotenoids and phycobiliproteins are considered as ecofriendly natural colourants used in food and food additives as they have nutraceutical value, non-toxic and non-carcinogenic. The present study focused on extraction, partial characterization of extracellular polysaccharides (EPS), phycocyanin and mycosporin like amino acid (MAA) from a freshwater cyanobacterium *Oscillatoria pseudogeminata* G.Schmid.The purified isolate of *O. pseudogeminata*was deposited and maintained in the NRMC-F, Bharathidasan University. The EPS was extracted with the help of double volume of ice-cold acetone and confirmed by UV (260 and 280 nm), FTIR and XRD analyses. Phycocyanin and MAA were also extracted from *O. pseudogeminata* biomass using double distilled water. The UV-Vis Spectrophotometric results revealed that 520 and 230 nm peaks represent phycocyanin and MAA, respectively. Further, the process of scaling-up the biomass and increase the productivity of EPS, Phycocyanin and MAA from *Oscillatoria pseudogeminata* is under raceway pond system, already initialed in our laboratory.

Keywords: Biomass, Food colourant, Mycosporin like amino acid, Natural colourants, Phycocyanin

Cyanobacteria are Gram-negative photosynthetic prokaryotes which are widely distributed in many water bodies worldwide. They were originally considered as blue-green algae because of their microscopic morphology, pigmentation and oxygen evolving photosynthesis. They can grow well in all aquatic habitats such as rivers, ponds, lakes, water tanks, paddy fields, sea water, hypersaline salt pans, brackish waters, soda lakes, all types of soils, deserts, cave walls, hot springs, polar regions, tree barks, on leaf surfaces, rocks, in sewage, industrial effluents and other extreme environments. Cyanobacteria are considered as potential organisms useful to mankind in varied areas such as food, feed, fuels, fertilizers, fine chemicals, cosmetics and combating pollution[1,2]. They are capable of producing various metabolites, such as EPS, pigments (e.g. β-carotene, c-phycoerythrin, and phycobiliproteins), UV pigments, antioxidants etc. by presenting complex photosynthesis, adaptation and defense systems[2].

Many cyanobacterial species live in extreme environments, including high exposure to solar radiation and long periods of desiccation. In order to survive in such extreme conditions, cyanobacteria produce extracellular polysaccharides, commonly known as exopolysaccharides (EPS). It helps them to overcome stress from desiccation due to low water activity in the desert or other hypersaline areas. They are major composition of carbohydrates and proteins and widely used as viscosity, emulsifier, stabilizing and gelling agents in the food industry[3]. Park *et al.*[4] reported that EPS production by cyanobacteria can beinfluenced by environmental and nutritional conditions, including temperature. Branyikova *et al.*[5] and Mishra & Jha[6] reported that extracellular polymeric substances identified as a complex mixture of macromolecular polyelectrolytes containing primary amine-group, halide-group, aliphatic alkyl group, aromatic compound, alkyl amine and/or cyclic amine with polysaccharides in cyanobacteria and microalgae.

Pigments such as phycobiliproteins, carotenoids and chlorophyll as natural colourants in food are gaining importance over the synthetic ones as they are non-toxic and non-carcinogenic[7-9]. Phycobiliproteins (PBP) are naturally occurring water soluble fluorescent pigments produced by cyanobacteria and some eukaryotic algae[10-12]. PBPs are classified as

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phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) with reddish, blue and bluish colour, respectively[13-15]. Despite the huge variety of cyanobacteria containing PC, *Arthrospira* (*Spirulina*) *platensis*[16], *Synechococcus*sp.[17], *Anabaena* sp.[18] and *Oscillatoria*[19] are currently used for commercial production of PC. Particularly C-phycocyanin and related phycobiliproteins are used in the food, biotechnology and cosmetic industry because of their colour, florescent and antioxidant properties[20].

Mycosporine-like amino acids (MAAs) are water-soluble pigments that absorb UV radiation of 280-340 nm, and structurally distinct MAAs are known in taxonomically diverse organisms. In cyanobacteria, MAAs mainly protect the cells against solar radiation and they do not function as accessory pigments in photosynthesis[21]. In the present study, we attempted (i) Extraction of exopolysaccharides cyanobacteria cell free extracts; (ii) phycocyanin; and (iii) Mycosporine like amino acids extraction from cyanobacterial biomass (*Oscillatoria pseudogeminata*).

Materials and Methods

Isolation, identification, maintenance and cultivation of cyanobacteria

The sample *Oscillatoria pseudogeminata*G.Schmid was collected from the pond water from Karaikudi district, Tamil Nadu, India. Cyanobacteria were purified using standard method as described by Rippka *et al*.[22] and morphologically identified using the standard monograph of Desikachary[23]. The purified cyanobacteria were maintained in BG[11] with nitrogen rich medium in Erlenmeyer flask at 24°C±2°C under and illuminated with cool white Sylvania 40W T12 fluorescent lamps at an intensity of 14.4±1 Wm^2 for a 16/8 h light/dark cycle. The purity of isolate was checked under the microscope and scaled up to 20L culture vessel with above mentioned conditions for mass cultivation.

Extraction and Characterization

Cyanobacteria pigment Phycocyanin (*PC*)

The biomass of the purified cyanobacterium was harvested in their log phase and it was used for extraction of phycobilin by freeze thaw method. Extracted solution was characterized using UV–Vis Spectrophotometer (Cary 60, Agilent Technology, USA) at 200-800 nm.

Mycosporine like amino acids (*MAA*)

Extraction of mycosporine like amino acids (MAA) from the isolated cyanobacterium was done as described by Matsui *et al*.[24].

Exopolysaccharides (EPS)

EPS was extracted from cell free supernatant of cyanobacteria with double the volume of ice cold acetone Jain *et al.*[25] used and incubated at 20ºC for 2 days. EPS precipitate was collected by centrifugation and lyophilized for further analysis. For UV visible analysis, EPS was dissolved in distilled water and then detected with a UV-visible spectrophotometer at wavelengths ranging from 200-400 nm.

FTIR and GC- MS analysis

For detection of functional groups of Phycocyanin and EPS, the samples were mixed with KBr powder individually and then pressed into a pellet and it was used for FTIR analysis in the frequency range of 4000-400 cm^{-1} (Perkin Elmer, USA). For detection of FAME composition, the samples were analyedin GC–MS.

XRD analysis of EPS

The X-ray diffraction (XRD) technique is used to analyze the metallic nature of particle using ULTIMA (IV), SAMRT Lab, XRD, RIGAKU Corporation, Japan.

Bioflocculant of EPS

Bioflocculant capacity of the EPS extract was determined by Alcian Blue binding assay[26]. Alcian Blue was dissolved at a concentration of 1 mg/mL in acetic acid. About 0.5 mL of supernatant containing EPS was diluted in 4.25 mL of acetic acid and combined with 0.25 mL of the Alcian blue dye preparation. After 30 -min incubation at room temperature (30ºC), the solution was centrifuged at 3000 rpm for 10 min and the optical density of the supernatant was determined at 610 nm. Control assays contained Alcian Blue and acetic acid without any added EPS. Flocculating activity was calculated as:

Flocculating activity (%)= (B-A/B) 100 %

where A and B are the absorbance values of sample and control, respectively, at 610 nm.

Results and Discussion

Isolation, identification and mass production of cyanobacteria

Cyanobacteria were isolated and it was maintained in BG11 medium, the morphological characters were determined with the cell size and shape of comparison with standard monograph. From the microscopic observation the cyanobacteria were pale green thallus, not attenuated, 1.8 µm broad, calyptra absent, not granulated, and morphologically identified as *Oscillatoria pseudogeminata*by using light

microscopic followed by standard monograph Desikachary[23]. Identification was further supported with the confocal microscopic imaging (Carl Zeiss) (Fig. 1). The purified cyanobacteria were mass cultured and maintained in the National Repository for Microalgae and Cyanobacteria for Freshwater (NRMC-F) as mentioned above mentioned conditions.

Characterization of EPS and Phcyocyanin and MAA

UV-visible analysis

UV-visible spectroscopy is widely used for the analysis of chromophore groups of atoms characterized by strongly absorbing electronic transitions. The results indicated that there was a significant amount of absorption in the spectrum for the tested compounds. UV–vis absorption spectra of the EPS from *Oscillaotoriapseudogeminata*showed absorption at 280 and 260 nm, implying the presence of both nucleic acid and protein present in the EPS sample (Fig. 2A). Moreover, a shoulder peak was observed around 260~280 nm clearly indicates the presence of EPS without any other material, such as protein. These results indicate that EPS of *Oscillatoria pseudogeminata* are very large and complicated substances that contain multiple UV-absorbing groups.

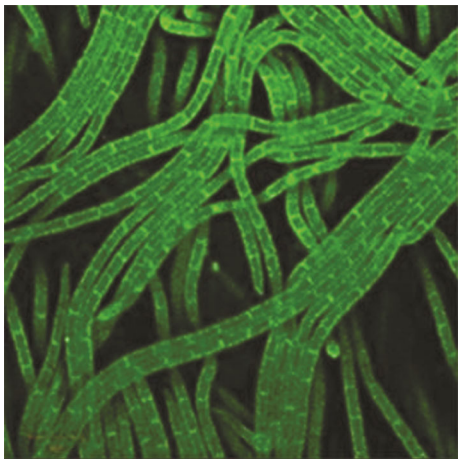

Fig. 1 — Confocal microscopic image of *Oscillatoria pseudogerminata*

Moreover, the UV spectrum showed an absorption zone between 300~400 nm, which can be attributed to the absorbance of cyanobacterial water-soluble compounds such as mycosporine like amino acids (MAA) and scytonemin[27]. Despite enormous diversity in cyanobacteria, only *Spirulina* sp. are cultivated for the production of phycoyanin, which makes it to be considered as the best source of natural blue colour for food[28]. Spectral scanning of the phycocyanin displayed a sharp absorbance peak at 612nm (Fig. 3A). Notably, no absorbace peak was detected at either 650 or 540 nm. This is attributed to the absence of phycoerythrin and allophycocyanin. Antelo *et al.*[29] reported that phycocyanin of *A. platensis* was more stable when incubated at temperature 50–55°C at acidic pH 6. The addition of preservatives may further enhance PBP stability[30].

Fourier transforms infrared spectroscopy (FTIR) and GC MS analysis

In the present study, FTIR results reveal the presence of different functional groups of extracted EPS of *O. pseudogeminata*. (Table 1 and Fig. 2A). The band at 2932 cm^{-1} is characteristic for the C-H stretching and the vibration of CH_2 (3273 cm^{-1}) stretch, the literature supported the presence of biopolymers[31]. The amine and amide groups support that the biopolymer is not only composed of polysaccharides but also some peptides and/or proteins as documented by Pagnanelli *et al.*[32]. The Absorption at 1633 cm^{-1} is assigned to the stretching vibrations of the carboxylate group. The absorption at 1391 cm^{-1} is possibly due to the CH_2 bending. The peak at 1033 cm^{-1} might be due to the contribution of C-O bond of polysaccharide. The bands lie in the range of 1000-1125 cm^{-1} is characteristic of uronic acids and O-acetyl ester linkage bond. Trabelsi *et al.*[33] and Khattar *et al.*[34] reported that the occurrence of

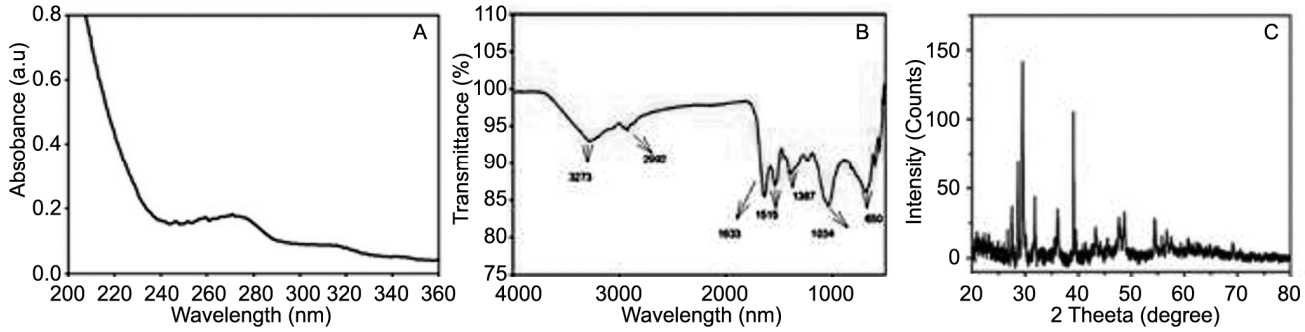

Fig. 2 — (A) UV spectrum; (B) FTIR sepcturm; and (C) XRD pattern of extracted EPS from *Oscillatoria pseudogerminata*

numerous bands fewer than 1,000 cm^{-1} possibly due to several visible bands and/or to the presence of probable linkages between monosaccharides. These results suggest that EPS from *Oscillatoria* sp. contain uronic acids, sulfate groups and peptides in their composition which were further confirmed by FTIR analysis of EPS.The FAME analysis of *Oscillaotoriapseudogeminata* was shown in (Table 2 and Fig. 4).

XRD of EPS

The composition, purity and crystallinity (i.e., either crystalline or amorphous) of the EPS from *Oscillatoria* sp. were analyzed by the XRD technique, and the obtained result is depicted in (Fig. 2C). The sharp thin characteristic diffraction peaks centered at 30.17, 35.72, 39.10, 48.75, 56.72 and 59.02° correspond to the crystalline parts of EPS. Here, the *Oscillatoria* sp. has been used and it corrolates with the finding of Angelaalincy*et al.*[35]. In addition to the aforementioned diffraction peaks, a broad peak exhibited at 28.637° and 29.500° was ascribed to the amorphous component of EPS. The results also synchronous the finding of Li *et al.*[36] and Kiviake *et al.*[37], where characterization of (xanthan gum) produced by kitchen waste as the sole substrate and galactan EPS produced by *Weissella confuse* KR780676 was shown two sharp narrow diffraction peaks that indicate the crystalline nature whereas broad peak indicates amorphous component,

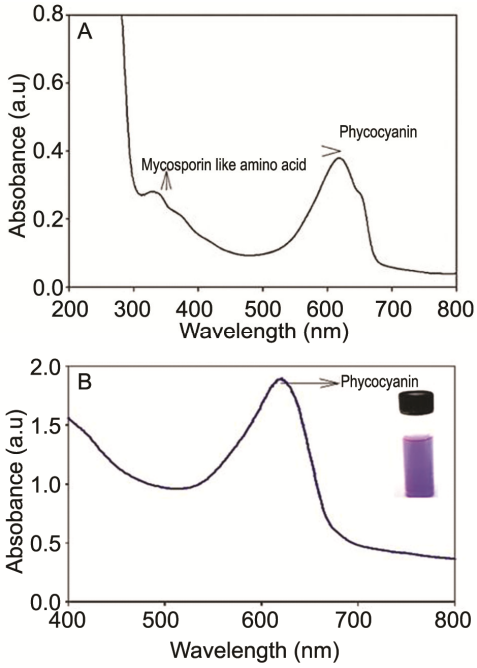

Fig. 3 — UV spectrum of extracted (A) phycocyanin pigment; and (B) MAA from *Oscillatoria pseudogerminata*

Table 1 — FTIR functional groups EPS extracted from *Oscillatoria pseudogerminata*

Wave number (cm^{-1})	Assignment	Intensity
3273	dimer OH stretch	Strong
2992	C-H stretch	Medium
1633	C=O stretch	Strong
1515	C-C stretch	Medium
1034	C-N stretch	Strong
650	C-Br stretch	Medium

Table 2 — Fatty acid methyl ester of *Oscillatoria pseudogeminata*

Compound Name	Area (%)	Retention time	Carbon No.	Nature
Hexadecanoic acid, methyl ester	18.58	19.560	C16:0	SFA
Octadecanoic acid, 9,10-dichloro-, methyl ester	14.35	32.384	C18:0	SFA
9-Octadecenoic acid (Z)-, methyl ester	4.39	23.759	C18:1	MUFA

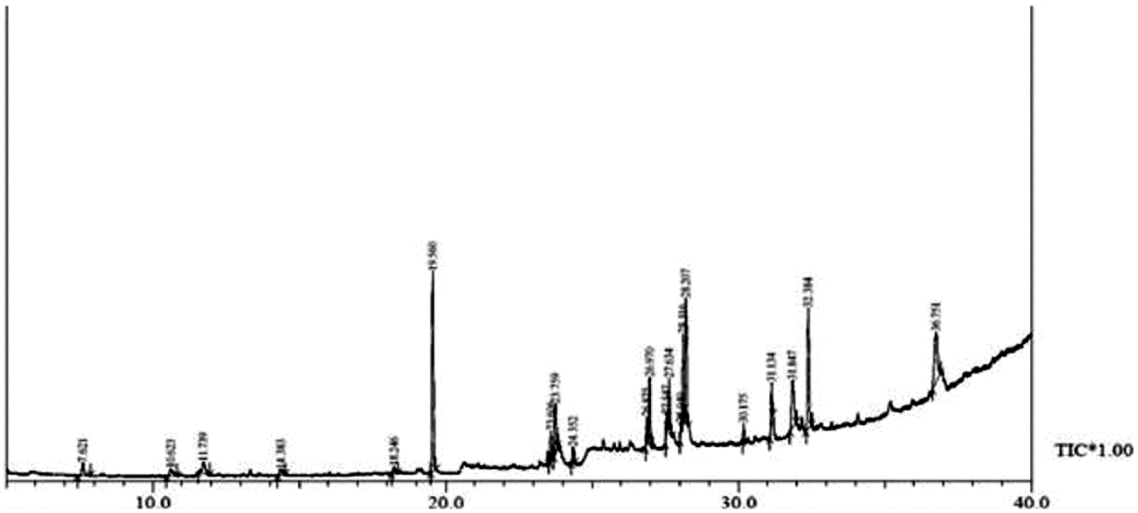

Fig. 4 — GC chromatogram of FAME analysis of *Oscillatoria pseudogeminata*

Bioflocculant activity

Borah *et al*[38] stated that flocculation is the opposite of emulsification and is an important property that is industrially useful and in clarifying muddy water and solutions. In the present study, bioflocculant activity was achieved maximum 84% at concentration of 20 mg/mL.Yim*et al*.[39] stated that EPS from marine dinoflagellate *Gyrodiniumimpudicum*had above 90% flocculating activity. The results presented here suggest that higher productivities can be expected from *Oscillatoria pseudogerminata*cultured under optimum conditions.

Conclusion

The study identifies a multifold approach of cyanobacteria in view of high value commodities. The selected strain *Oscillatoria pseudogeminata* isolated from the fresh water body exhibited its potential to produce phycocyanin, exopolysaccharides (EPS) and Mycosporine like amino acids (MAA). Spectroscopic analysis confirms the presence of phycocyanin and MAA with its signature peaks at specific wavelengths respectively. Also the in milieu exration of EPS was characterized with its noted functional groups. Stability of EPS was also identified implies its application in biotechnological applications. EPS produced from the selected freshwater *O. pseudogeminata* exhibited bioflocullant activity of about 80%. Thus, the study portrays the multifarious approach of the freshwater cyanobacterium in biotechnological applications.

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Conflicts of interest

Authors declare no competing interests.

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