Stress induced enhancement in exo-polysaccharide production in *Spirulina subsalsa* and its chemical characterization

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Abstract

Bound exocellular polysaccharide (EPS) from marine cyanobacterium Spirulina subsalsa was characterized using GLCMS and stress induced variation in EPS production was studied in batch culture mode. The neutral sugar composition of Spirulina biomass was identified by gas liquid chromatography showing monosaccharide composition as Fucose, Xylose, Mannose, Glucose and Galactose. It was evident from the study that the structural architecture of the extra cellular polysaccharide of Spirulina is highly complex in nature as common in algal system. The cyanobacterial cultures were subjected to different stress conditions like culture aging, phosphate and nitrate depleted condition, higher nitrate (10mM NO3-) and salinity (0.9M NaCl) levels. Growth was monitored by Chlorophyll content and total protein level in relation to polysaccharide production. Remarkable increase in EPS contents were noted in phosphate depleted condition, excess nitrate and high salinity level which were almost 3, 1.6 and 2.5 times more respectively. It was also observed that culture aging is an important factor for increasing EPS production(1.8 times). In different stress factors, the ratio and composition of monosaccharides were found to vary from that of control. Rhamnose and arabinose appeared in stress conditions which were not present in control condition. Rhamnose was recorded in nitrate and phosphate stress. Arabinose also appeared in phosphate and salinity stress. The altered carbohydrate polymer composition in nutrient-limited conditions, indicating that nutrient depletion used to influence the biosynthetic pathway; enhancing its bio-technological importance.

Keywords:-Extra cellular polysaccharide, Filamentous cyanobacteria, Nutritional stress, Spirulina subsalsa,Gas chromatography, Mass spectrometry.

Introduction

Cyanobacteria or blue green algae are photoautotrophic prokaryotes which consist of a large variety of species which are extensively present in different parts of the world including the extreme ones. They also posses diverse morphological, physiological and biochemical properties. Exocellular polysaccharide (EPS), mainly a product of photoassimilated carbon, is released in medium or form mucilaginous capsules in most of the cyanobacterial genera.This EPS with high biotechnological potential is much easier to exploit further, unlike the plant system.

In nature, cell wall polysaccharides fullfill a wide range of functions, eg. energy storage, contribution to structural integrity and mechanical strength, control of osmotic pressure, buffer layer that protects against drought and infective organisms such as viruses, bacteria, fungi (Arad 1988,1999,2004; Kloareg and Quatrano 1988; Lapsin R, Pricl 1995). Among the algal (including cyanobacteria) bio-chemicals of commercial importance, algal polysaccharides vary in structural and functional properties based on the type of organism and the growth conditions. Polysaccharides from algal sources have been found to possess a vareity of biological activities which may find application in cosmetic,food and pharmaceutical industries (Morris et al 2001; Chen et al. 1994; Tache et al. 2000; Berteau and Mulloy 2003).

Extra cellular polysaccharide extraction from various red and brown algae and their chemical characterization were done by a number of workers. Among the cyanobacterial genera, strains like, *Nostoc*, *Anabaena, Arthrospira, Mastigocladus* etc have been studied so far (Helm et al 2000; Otero and Vincenzini 2004; Xia et al 2001; Gloaguen et al 1999). Studies on algal EPS are performed on the chemical, structural and rheological attributes Gloaguen et al 1999; Belinger et al 2010; Li et al 2001; Richert and Golubic et al (2005). Changes in chemical nature or production rate were reported mainly due to nitrate and salinity stress. Influence of different growth factors, physical parameters, nutritional availability, aging etc. on the EPS production are some other key parameters, which have to be thoroughly studied for proper exploitation.

Spirulina is blue green algae of the Oscillateriaceae family which grows naturally in warm climate countries and has been considered as supplement in human and animal food. It has been utilized as a source of protein and vitamin supplements Its

safety for human consumption has also been established through numerous toxicological studies.They have been found to be a rich source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids. Though scattered repots of individual factors like salinity, nitrate stress etc. are present but the effect of different stress factors on EPS of *Spirulina subsalsa* are discussed here in a collective way.

In the present investigation, variation in total EPS production, related to other growth parameters of *Spirulina subsalsa* in long term controlled batch culture mode was studied in varied nutritional conditions such as nitrate avialability/depletion, phosphate depletion and excess salinity. Chemical characterization of the neutral sugars present in the EPS was also performed. It is worthwhile to mention here that, the EPS isolated from all stress conditions were observed to be both quantitatively and qualitatively diffrent from that of the control one. All stresses altered the monosaccharide composition of the EPS extracted,which is significant for bio-technological implementation.
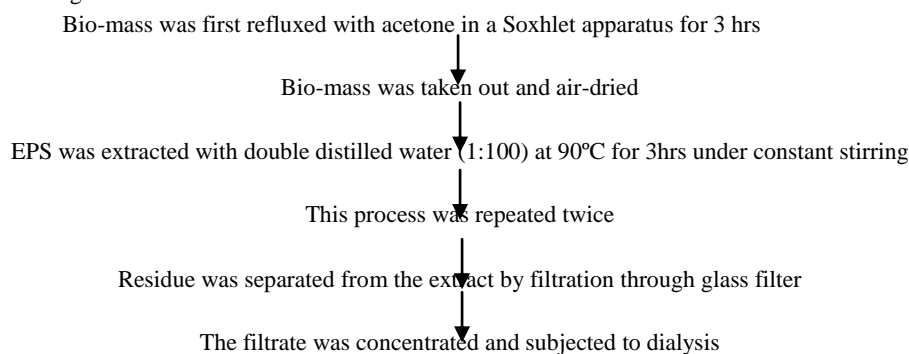
Materials and Methods

Organism and Culture Conditions:- The cyanobacterial strain *Spirilina subsalsa* was procured from National Facility for Marine Cyanobacteria (NFMC), Tiruchirapalli, Tamilnadu, India. It is a filamentous, multicellular organism showing stacks of coin like cells tightly coiled into a more or less regular spiral, broader than 3 μm. The strain is maintained in batch culture mode under sterile conditions in Artificial Sea Nutrient III liquid medium [salt composition- (g L^{-1}) : NaCl 25gm;MgCl$_2$ 6H$_2$O 2gm; KCl 0.5gm; NaNO$_3$ 0.75gm; K$_2$HPO$_4$,3H$_2$O 0.02gm; MgSO$_4$,7H$_2$O 3.5gm; CaCl$_2$ 0.5gm; Citric Acid 0.003 gm; Ferric Ammonium Citrate 0.003 gm; EDTA 0.0005 gm; Na$_2$CO$_3$ 0.02 gm; Trace Metal Mix A5 1 ml containing (mg mL^{-1}) H$_3$BO$_3$, 2.86; MnCl$_2$;4H$_2$0, 1.81; Na$_2$MoO$_4$;2H$_2$O, 0.390; ZnSO$_4$,7H$_2$O, 0.222; CuSO$_4$,5H$_2$O, 0.079 and Co(NO$_3$)$_2$;6H$_2$O, 0.0494]. The pH was maintained at 7.5 after sterilization.The culture sets are maintained by regular transfers into fresh liquid medium at 20˚C in 16/8 hour light/dark cycle under cool fluorescent light having light intensity 20-30 μmol photons m^{-2} s^{-1}.

Experimental design:-. Each experimental set was inoculated in 150 ml of culture media in 250ml culture flask with known amount of live bio-mass of exponential growth phase (from 14 days old regularly sub-cultured stock).One set was maintained as control.The sets were subjected to different stresses, like a) culture aging; nutritional stresses like b) PO$_4$$^-$ defficiency c) NO$_3$$^-$ defficiency d) 10mM NO$_3$$^-$ conc. e) 0.9M NaCl. Bio-mass were harvested at regular intervals of 7 days from 14 days after inoculation (acclimatization period) up to 56days of culture.Samples were taken in triplicate from one culture for extra-cellular polysaccharide,protein and chlorophyll estimation.Axenity of the cultures was checked by plating on agar medium and by microscopic observation.

Extraction of EPS:- Most of the extraction procedure so far reported, dealt with soluble released polysaccharides (RPS) which were released in the culture medium by mainly unicellular cyanobacteria.The main obstacle faced in the present investigation is that the filamentous algal starin, *Spirulina* do not release EPS in this way.The extraction procedure was modified from the standard protocols for better extraction [23],[18], (Helm et al 2000; Li et al 2001; Chattopadhyay et al 2007).

The modified procedure is given in flowchart as follows-

Bio-mass was first refluxed with acetone in a Soxhlet apparatus for 3 hrs

↓

Bio-mass was taken out and air-dried

↓

EPS was extracted with double distilled water (1:100) at 90ºC for 3hrs under constant stirring

↓

This process was repeated twice

↓

Residue was separated from the extract by filtration through glass filter

↓

The filtrate was concentrated and subjected to dialysis

Purification: - The recovered polymer was re-dissolved in water and further purified by repeated precipitation with ethyl alcohol (4vol).The final precipitate was dissolved in water and lyophilized.

Estimation of EPS:- For chemical characterization and biochemical assay, biomass of known weight were taken and EPS were extracted first with dH_2O followed by 4M NaOH solution. Different procedures such as 1M NaCl, EDTA salt,0.1-0.2 H_2SO_4 were also tested. However, best result was obtained when extracted with dH_2O followed by 4M NaOH solution. Bio-mass was washed with ethanol and loosely bound polysaccharide fraction was extracted by dH_2O. The cell-free supernatant was separeted by centrifugation and 90% ethanol (3times) was added and kept overnight in 4°C. The precipitated polysaccharide was collected by centrifugation. The residual biomass was treated with 4M NaOH at 90°C for 1hour. The cell-free supernatant was collected by centrifugation and to it, 90% ethanol (3times) was added and kept overnight in 4°C. The precipitated polysaccharide was collected by centrifugation and washed with alcohol till it was free from residual alkali. The residue after 4M NaOH extrction which contained the intact cells was again washed with dH_2O to remove alkali, grinded in presence of 10% TCA and kept overnight in 4°C. The amount of extracellular polysaccharide present in the precipitate from dH_2O and 4M NaOH fractions, and the intracellular polysaccharide in the supernatant from the TCA fraction were quantified by the Standard phenol-sulfuric acid method (Dubois et al 1956).

Chlorophyll (Arnon 1949) and protein (Lowry et al 1951) were estimated following standard protocols.

For microscopic observation of mucopolysaccharides layer, algal bio-mass was used afresh from cuture without any stain. Slides were observed by **Differential interference contrast microscopy** (Plate 1a and b).
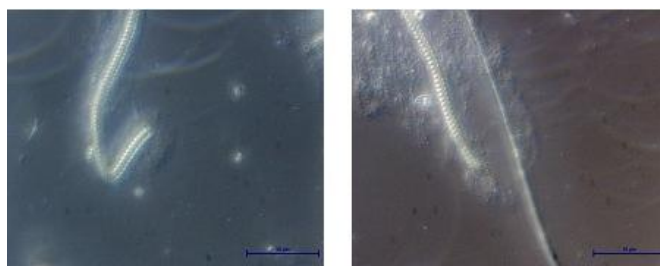

Plate 1a   **Plate 1b**

Plate 1a and 1b:- DIC or NIC image clearly showing the peptidogycan layer around the algal sample.

Fourier transform infrared spectroscopy:- Fourier transform– infrared spectroscopy (FT-IR) were performed on KBr plate. FT-IR spectra were recorded on a Jasco 410 instrument. with a resolution of 4 cm^{-1}. Spectra were obtained in the 4000-400 cm. region. (Fig 1)
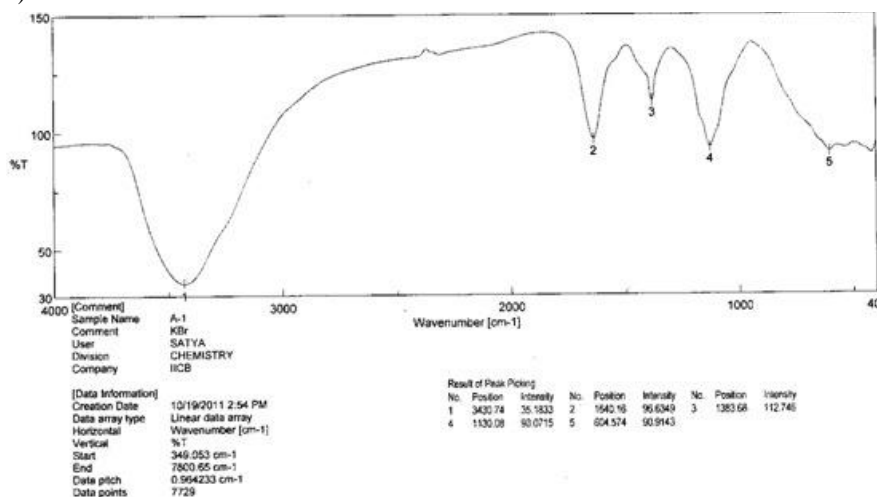

Fig 1:- Fourier transform infrared spectrum of the purified EPS.

*Determination of molecular weight:-*Name of HPLC: Shimadzu; Column used: PWXLG3000; Flow rate:Water, 0.5 ml/min; Detector used: RI (refractive index).

In standard (Fig 2), peaks appear at retention time 12.541 min and 29.598 min correspond to 40,000 and 10,000 MW. So by comparing with the standard data, it may be concluded that the Molecular Weight of the sample (Fig 3) is approx. 10,000-11,000 (peak appears at 28.916 min).
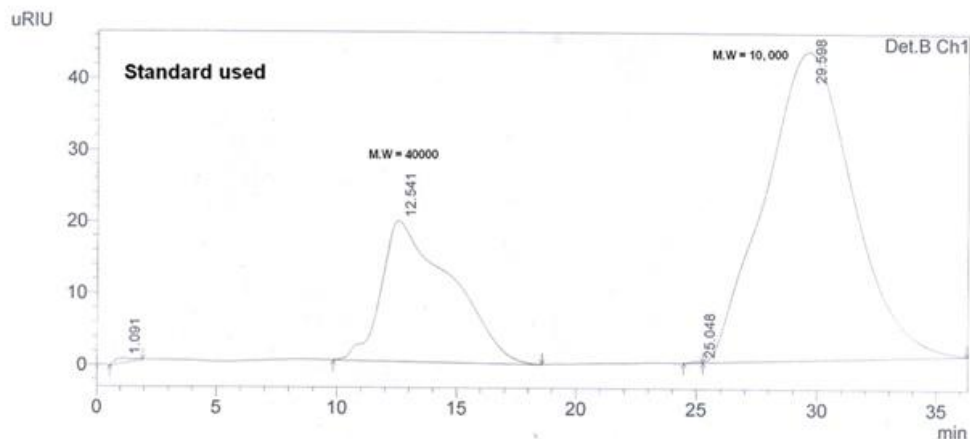

Fig 2:- HPLC profile showing MW of standard polysachharide (10,000 and 40, 000).
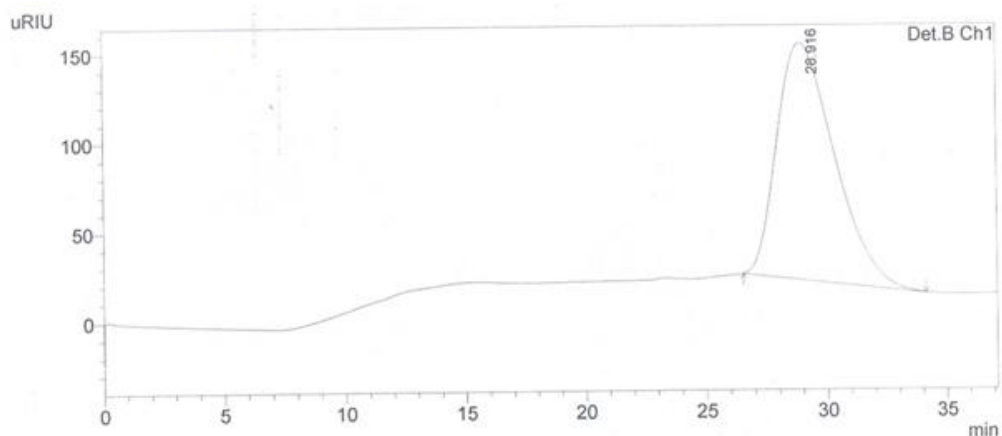
Fig 3:- HPLC profile showing MW of purified algal EPS.

Monosachharide analysis:- Purified samples (1-2 mg) were hydrolyzed with 2N TFA at 120°C for 2 h in sealed glass tube to produce monosaccharides. For the detection and estimation of sugar by GLC as their alditol acetates, the liberated monosaccharides were reduced with sodium borohydride followed by neutralization with aquous acetic acid to adjust its pH to 4. The resulting alditol was acetylated and traces of the reagents were removed by repeated co-evaporation with dry toluene. The neutral sugars were analyzed as alditol acetates by GLC-MS analysis. A Hewlett Packard 5890 plus GC tandemly linked to a JEOL mass spectrophotometer (JEOLAX-500) with electron impact ionisation (EI) at 70 ev and ion source temparature at 200°C was used. For resolution, DB-5MS capillary column (0.25 mm x 0.25 µ x 30 m) was used using temperature programming (150°C-2min-5°C/min-200°C-10min). Analysis were carried by using a HP-5 column equipped with Agilent Chemstation software.

*Paper chromatography:-*The hydrolyzed sugars were examined by descending paper chromatography technique for the separation and identification of monosaccharides and lower oligosaccharides on Whatman No.1 MM chromatography paper.

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Isolation and purification on preparative scale were done on Whatman No.3 MM chromatography paper using solvent system (v/v) Ethyl acetate - Pyridine - Acetic acid - Water (5:5:1:3).

For the visualization of spots, alkaline silver nitrate staining (Trevelyan staining) procedures were performed:

 Uronic acid estimation:- Galacturonic acid was detected by paper chromatography and GLC. The sample (5 mg) was hydrolyzed by 2N trifluoroacetic acid (2 ml) in a sealed tube at 120°C **.** The acid was removed under reduced pressure in a rotavapour and traces of acid was removed by co-distillation with water. The sample was then analyzed by paper chromatography using solvent [acetic acid-water-pyridine-ethyl acetate, 1:3:5:5 (v/v)]. The spots were visualized by using alkaline-sliver nitrate reagent. In a separate experiment, the hyrolyzed sample was heated with anhydrous methanolic HCl in a sealed tube at 100^0C for 12 hours. The HCl was removed in a ratovapour and traces of acid was removed by repeated co-distillation with anhydrous methanol. The resulting methy glycoside methyester of uronic acid was acetylated as describe above. The resulting compund was analyzed as mentioned earlier. In both cases, strandard samples of glucuronic acid and glacturoinc acid was used for comparision. The galacuronic acid was estimated by using colourimentic method (REF) using m-hydroxy diphenyl (Blumenkratz and Asbae-Hansen G 1973).

Results

The DIC image confirmed the presence of copiously produced extrapolysachharide material outside the thallus. The EPS of *Spirulina subsalsa* (Plate. 1a, b) showed prominent slime layer which was seen as amorphous mucilaginous material dispersed around the organism. Differential interference contrast microscopy or DIC, also known as Nomarski Interference Contrast (NIC) or Nomarski microscopy has been used to identify the peptidogycan layer around the algal sample.

*Quantification of EPS in control and stress exposed condition***:-** In the present investigation, variations in EPS production were determined from *Spirulina subsalsa* at different growth phase from '0' to 56 days of culture which denotes the effect of culture aging on EPS production. Growth of the samples in relation to EPS production is another important parameter to be checked. Protein constitutes one of the main primary metabolites of cyanobacterial cell. So,along with EPS, chlorophyll and protein content were also measured under different stresses including culture aging. Correlation matrix between EPs content, day intervals, protein content and chlorophyll content are presented in Table 1, showing the level of significance.

From the result it was observed that *Spirulina* produced higher amount of EPS in respect to culture age (upto 56 days). Extra cellular polysaccharide production increased 1.8 times in *S.subsalsa* (Table 2) due to aging stress. Statistical analysis showed that the increase in amount of EPS were significant.

A comparative account of time bound EPS production in different stress conditions are represented in Table-3. From the results it revealed that Phosphate depletion produced maximum EPS in comparison with all the nutritional stress given to *Spirulina subsalsa*. Fig.4a showed that after 14 days exposure, 121.35mg/gm EPS had been produced which is highest among other stress given to *Spirulina subsalsa.* Growth was measured by estimating chlorophyll content, increased upto 21 days of culture in control set, whereas the experimental biomass showed the similar trend as in EPS production. Protein content gradually increased upto 21st days of culture in control condition, and a gradual decrease in total protein content under phosphate depleted condition (Fig.4b).

EPS production under nitrate depletion increased initially (up to 14days) compared to control and was found to be highest (56.31mg/gm dry biomass) at the onset of log phase, then gradually decreased (Fig 5a). Chlorophyll content was less than control in nitrate depleted condition at different time intervals. Protein production was found to drop off under nitrate depletion as expected (Fig.5b).
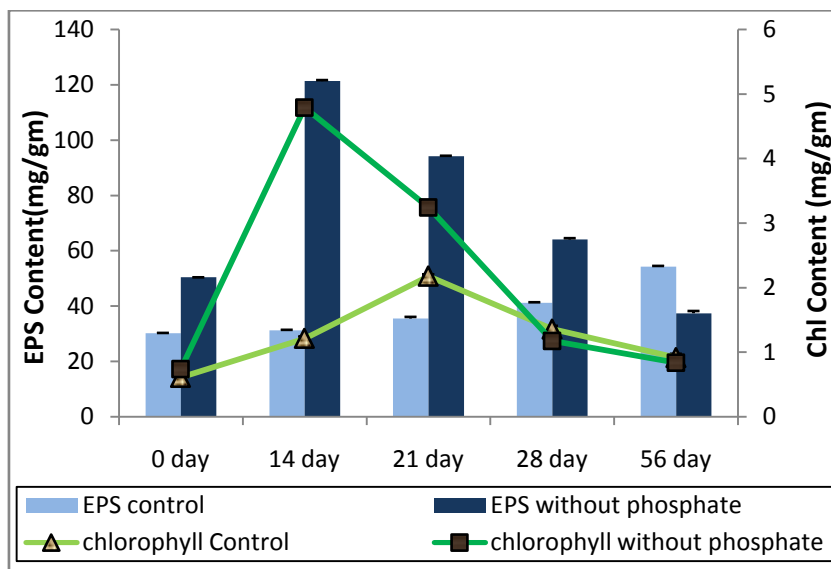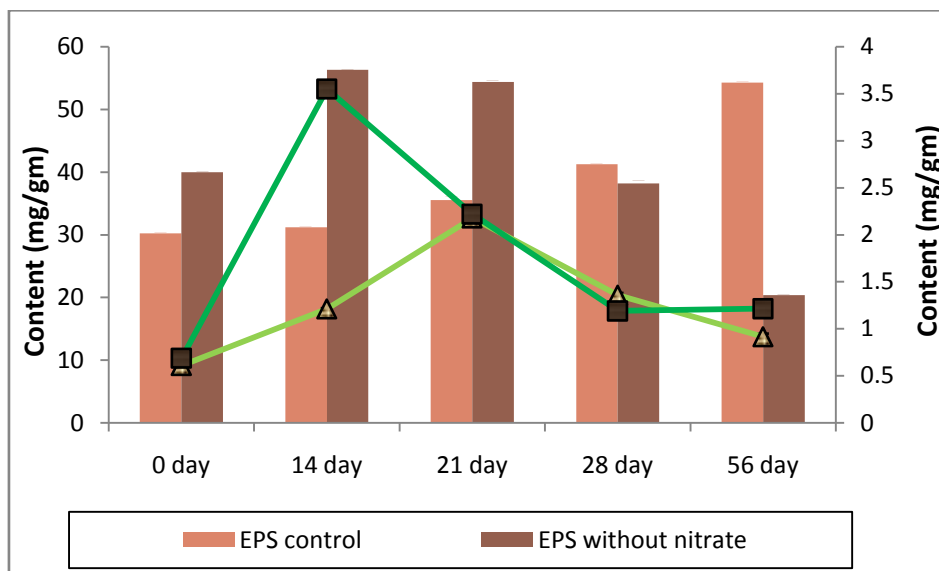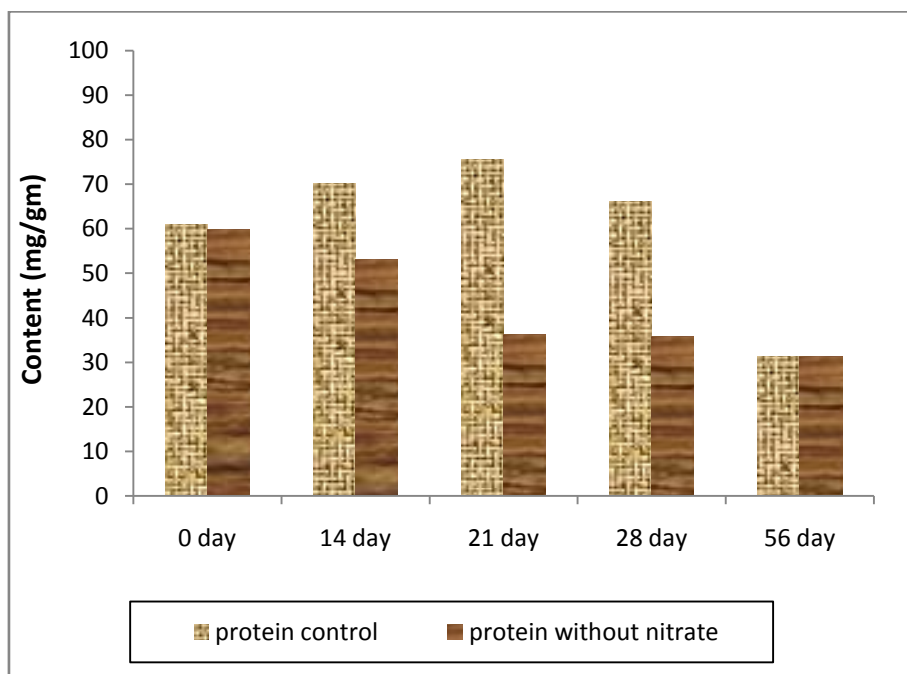
4A
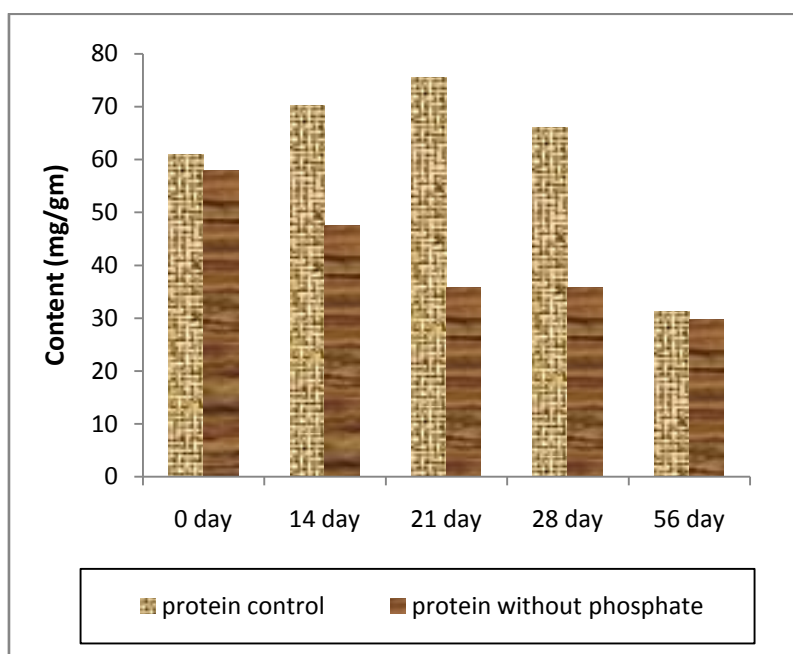
4B

Fig 4a:- Variation in EPS and chlorophyll content in control and phosphate depleted condition with function of time. 4b:- Variation in protein content in control and phosphate depleted condition with function of time
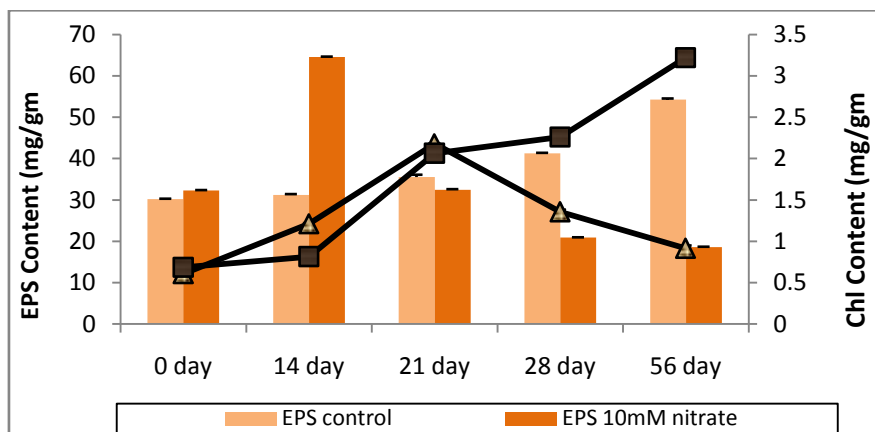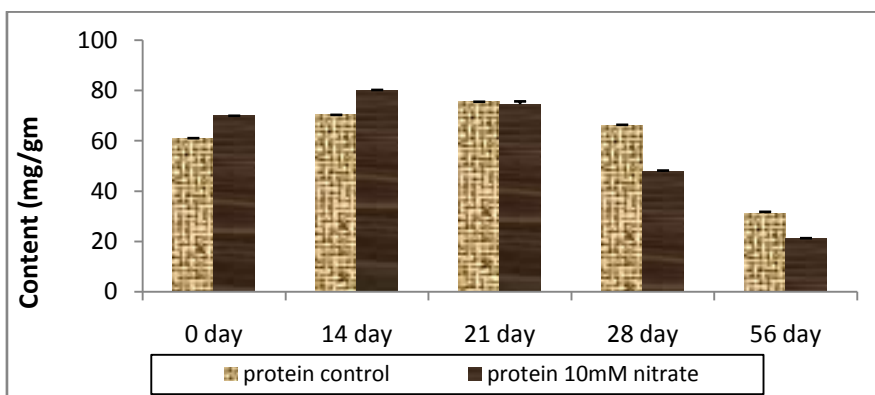
5a

5b

Fig 5a:- Variation in EPS and chlorophyll content in control and nitrate depleted condition with function of time. 5b:- Variation in protein content in control and nitrate depleted condition with function of time

From Fig.6a it became evident that excess nitrate increased EPS production almost 2fold after 14 days in *S.subsalsa,* but further the production rate gradually declined compared to control. Growth was found to be almost similar as compared to control up to 21 days, after that it maintained almost a steady state unlike control. Protein production was almost similar as control, but after 21days it decreased (Fig.6b).
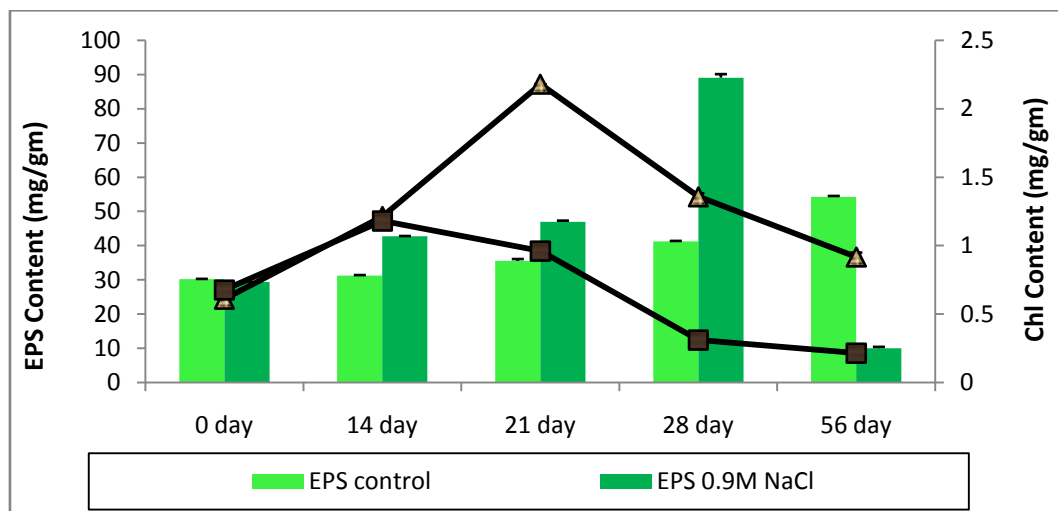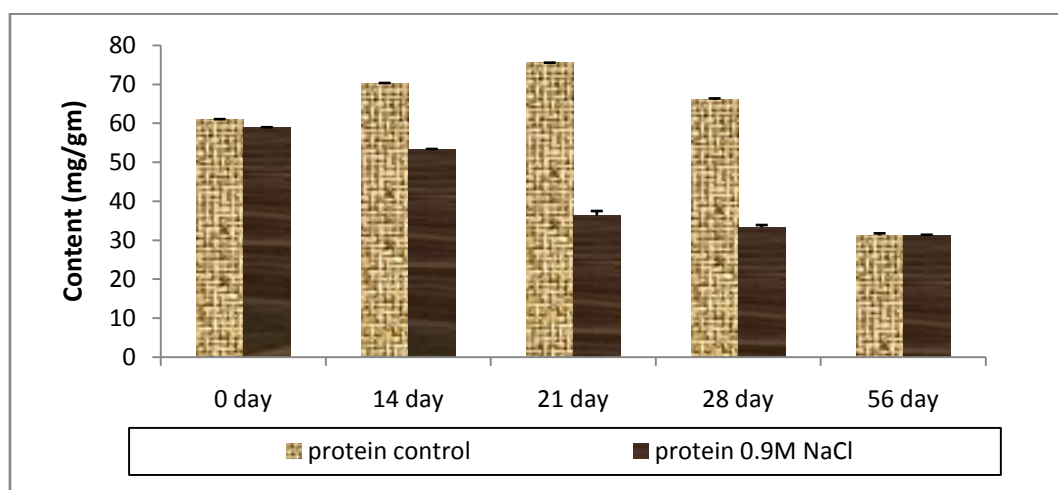
6a

6b

Fig 6a:- Variation in EPS and chlorophyll content in control and excess nitrate (10 mM) condition with function of time. 6b:- Variation in protein content in control and excess nitrate (10 mM) condition with function of time

The result (Fig.7a) shows that double salinity (0.9 M) stimulated the EPS production to a considerable amount also (maximum 89.1 mg/gm dry biomass after 28 days) until the aging stress became as an important factor. After 56 days EPS production declined. Growth pattern was similar to control but amount of chlorophyll was less than that of control. Protein production though gradually decreased throughout the experimental tenure. (Fig.7b).

7a

7b

Fig 7a:- Variation in EPS and chlorophyll content in control and salinity stress (0.9M NaCl) with function of time. 7b:- Variation in protein content in control and salinity stress (0.9M NaCl) with function of time.

Peaks in FT-IR spectra appeared in 2800-3200 cm-1 region corresponds to hydroxyl groups present in the polysaccharide. Peaks appeared in 1300-1450 cm^{-1} was due to C-H bending vibration. The C=O absorption of uronic acids occured at 1650 cm^{-1}(Fig 1).

The GLC-Mass spectrum of all the monosaccharides are shown in Fig 8. Analysis of the neutral sugars were carried by by GLC-MS analysis using a HP-5 column equipped with Agilent Chemstation software. In control condition, the EPS of *Spirulina subsalsa* was found to be composed of five different monosaccharides namely fucose, xyloose, mannose, glucose and galactose (in 1:1:2:4:3 ratio) and one was unidentified. The analysis showed that number and molar ratios notably changed due to the exposer to different stresses (Table-4, Fig 9-12). Rhamnose and arabinose occurred in stress conditions which were not present in control condition. Glucose, galactose were the predominant sugars present in almost all cases. Phosphorous depletion resulted in maximum EPS production (as stated earlier) which also showed marked changes in sugar compostion. A relative decrease in all sugars but fucose and xylose content was found to be similar, compared to the control. Arabinose was uniquely present in the EPS of P-depleted biomass. The glacuturonic acid (30%) was estimated by

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colourimetric assay. The C=O absorption of uronic acid occurred at 1650 cm^{-1}.Peaks in FT-IR spectra appeared in the range of 3300-3400 cm-^{1} corresponds to hydroxyl groups present in the polysaccharides of the EPS sample. Peaks appeared in 1380 or 2125 cm^{-1} was may be due to C-H bending vibration, the absence of azide was confirmed by chemical analysis. The C=O groups of uronic acids occurred at 1608-1650 cm^{-1} in the sample (Fig 13).
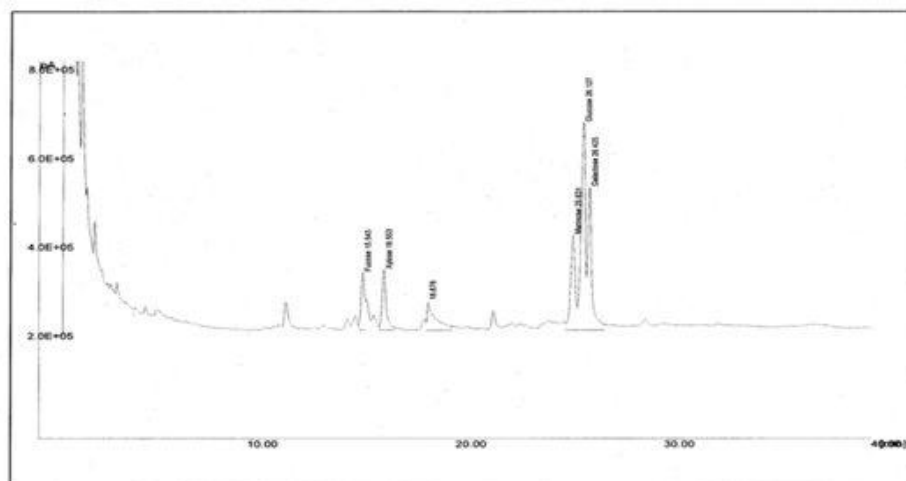

Fig 8:- Total ion chromatogram of the alditol acetates showing the neutral sugar composition in control.
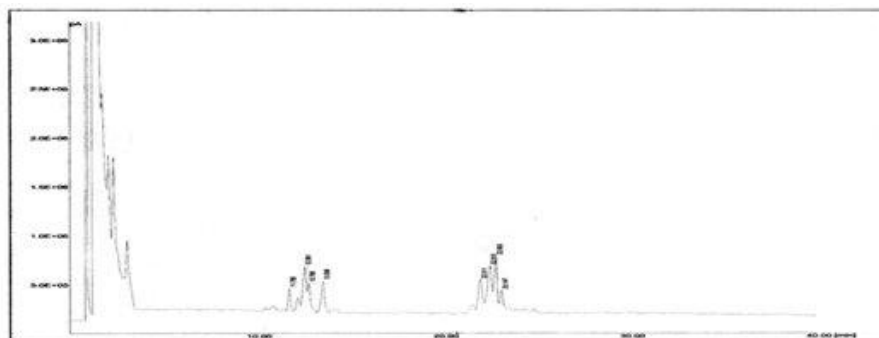

Fig 9:- Total ion chromatogram of the alditol acetates showing the neutral sugar composition in phosphate depleted conition.
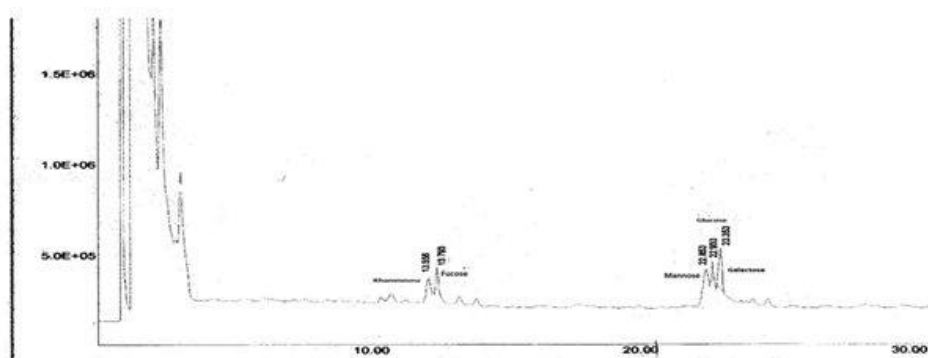

Fig 10.:- Total ion chromatogram of the alditol acetates showing the neutral sugar composition in nitrate depleted condition
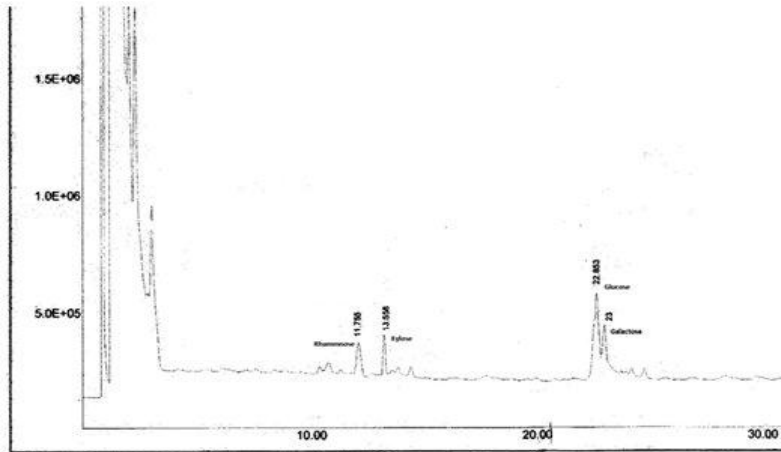
33

Fig11:- Total ion chromatogram of the alditol acetates showing the neutral sugar composition in excess nitrate stress.
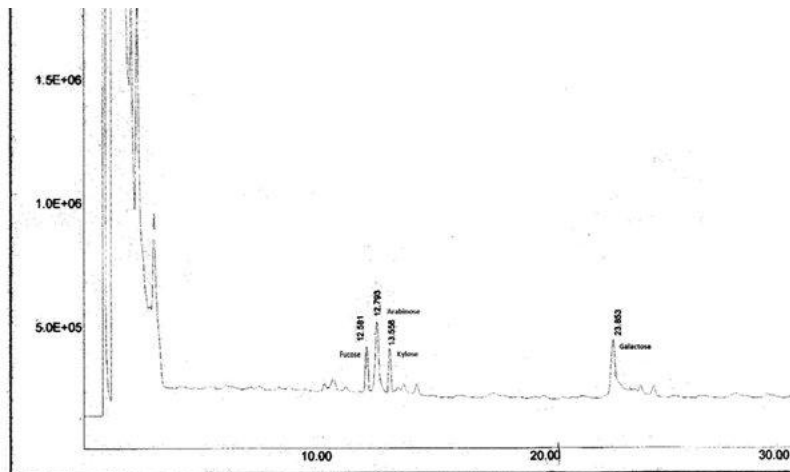

Fig12:- Total ion chromatogram of the alditol acetates showing the neutral sugar composition in double salinity stress
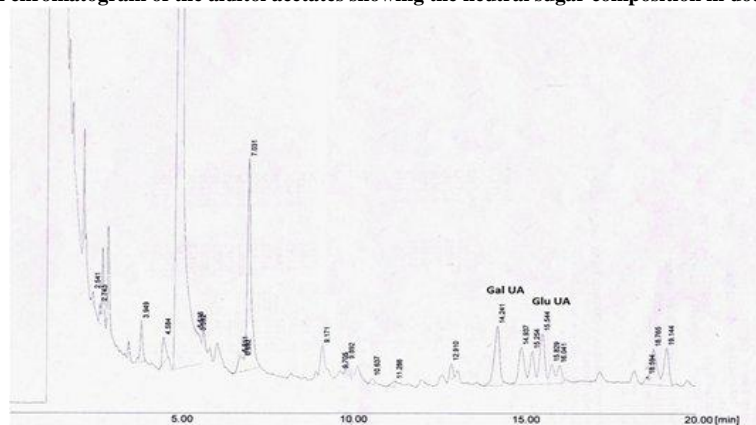

Fig 13:-. Total ion Chromatogram of the EPS in control condition for the determination of uronic acids.

Discussion

Some strains of cyanobacteria are well-known for their capability to excrete mucilaginous material. Indeed, several cyanobacterial strains possess, outside their outer membrane, additional surface structures, mainly of a polysaccharidic nature, that comprise a wide variety of outermost investments differing in thickness, consistency and appearance.

These structures, in spite of the rather arbitrary terminology sometimes used, can be referred to as three distinct types, namely sheaths, capsules and slimes. (Pereira et al, 2009).The EPS of *Spirulina subsalsa* is a good example of the slime as seen in the DIC image.

Since the early 1950s, more than 100 cyanobacterial strains belonging to almost 30 different genera have been investigated with regard to production of exopolysaccharide (EPS) and it was observed that the production of EPS varied greatly among different genera, even within different species of same genus. EPS production also changed significantly in different growth phases (De Philippis & Vincenzini, 1998).

It is necessary to identify different parameters that influence the productivity of the exopolymer of cyanobacterial polysaccharides for bio-technological exploitation .Several factors like, energy availability and the C: N ratio, controlling the production of the cyanobacterial EPS have been identified (De Philippis et al 1998; Li et al 2002) but very few detailed reports are available in the literature regarding the role of nutritional factors influencing the production of cyanobacterial EPS.

In the present study, age of culture play an important role in increasing EPS content of *Spirulina* biomass. Probably nutritional stress condition is the main controlling factor of producing EPS in batch culture condition of cyanobacteria. Jones and Yopp (1979) also found that the extracellular carbohydrates increased with the age of cultures of *A. halophytica.*It was also corroboraed from the previous study that many algae produce polysaccharides, mainly when they enter stationary growth phase (Hellebust1974)

 Phosphate limitation resulted in maximum EPS production by almost 3 fold.Similar results were also obtained in *Synechococcus* (Roux 1996) and in *Cyanothece* (De Philippis et al 1993). Interestingly, growth was also much higher than control after 14 days of exposure in phosphate defficiency. As previously reported, (Healey 1982) no certain relationship has been found between growth rates and phosphate concentrations in the present study. The relationship between the available amounts of phosphate and the production of EPS is also not clearly understood, as the overall effect might be dependent on a set of interlinked variables such as the amount of phosphate, nitrate and sulphate (Grillo and Gibson 1979; Pereira et al, 2009).

  In *S. subsalsa*, nitrogen defficiency also resulted in increase of EPS production. Nitrogen starvation has also been described as a condition that enhances EPS synthesis in *Cyanothece* (De Philippis et al 1993); *Nostoc* (Otero and Vincenzini 2003) probably because this contributes to the increase in the C: N ratio, which plays a critical role in the production of exopolysaccharide (Cho,2001). Elevated C:N ratio results in ample availability of carbon for the incorporation into the exopolymers, thus producing more EPS (Otero and Vincenzini 2003;Kumar et al 2007; Pereira et al, 2009). Growth was elevated at initial stage but afterwards it deceased as a result of nutrient limitation. Excess nitrogen stress affects the EPS production possibly in the opposite way. The result obtained in this study, also supports the same except that the EPS production amplified initially (within 21 days of exposure). Excess nitrate do not affect EPS significantly probably because it is more metabolisable source of nitrogen compared to ammonium or urea which significantly induces EPS production (Roux 1996).Growth was almost linear in the treated set unlike control where there was a decline. Therefore, from the observed data and earlier reports (Roux 1996), it can be stated that an increase in nutrient availability would not affect the EPS production; however, an increase in biomass would be expected.

The data obtained from the salinity (0.9M) stress in *S. subsalsa* showed great increase in EPS production at stationary phase of growth (2.5 fold). It is well-known that extra cellular polysaccharide functions as an osmotic solute protecting membranes from desiccation (Chen 2006). Under salt stress, cyanobacteria exports large amounts of EPS which improves salt tolerance and carbohydrate metabolism (Chen et al 2003). EPS production increased only in the stationary phase, possibly because a nutrient limitation is necessary for the activation of EPS production (Roux 1996). During the experimental tenure, salinity stress became lethal in long term exposure resulting in the death of bio-mass.

In the present experiment, protein content got declined or remained unchanged in all stress conditions, compared to that of control, indicating that the biosynthetic activities of cyanobacterial cells are directed preferentially toward carbohydrate synthesis, being hindered in protein formation.

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From the composition of the neutral sugars, it was evident that the structural architechture of the extra cellular polysaccharide is highly complex in nature similar to algal systems (Pereira et al, 2009). It contained 5 different sugars like Fucose, Xylose, Mannose, Glucose and Galactose. The ratio and composition of consisting sugars were found to change as a result of stress exposure. Rhamnose was present in both nitrate stress and in phosphate depletion. On the other hand, arabinose appeared in phosphate, salinity stress. Galactose was found to be predominant in all cases. Fucose was absent from excess nitrate and phosphate depleted condition. Xylose on the other hand, disappeared from nitrate depleted condition only. Mannose was observed to be absent from EPSs, subjected to excess nitrate, phosphate depletion and double salinity stress. Glucose was only absent in double salinity condition. The composition ratios of Mannose, Glucose and Galactose slightly decreased under almost all stresses, suggesting that polysaccharide(s) that contained large amounts of mannose, glucose and galactose are not easily released from the EPS under this stress. These results suggested that *Spirulina* cells tend to resist the effect of stresses by changing the composition and ratio of sugars in the EPS. Efrat et al (2004) reported earlier that arabinose content of EPS stimulates cell aggregation which takes place during reduced metabolic cell activity and could constitute a protected model that allows survival in stress environment. The presence of arabinose in some stresses in present work goes well with that finding.

Conclusion

Apart from being a rich source of vitamins, proteins and being a very useful food supplement, several studies show that EPS of *Spirulina* species exhibit various biological activities such as reducing body weight, antitumour, antimicrobial, strengthening immune system, radio protective, metalloprotective and anti-inflammatory effects. Present study showed that this useful biopolymer can be effectively produced and also could be enhanced by applying specific stress factor. Phosphate depletion or excess salinity was proved to have such an enhancing effect on the EPS of *Spirulina subsalsa.* Presence of uronic acid in the form of galacturonic acid in this study material, is a crucial factor for sequestering metal impurities. The carbohydrate polymer composition was altered in nutrient-limited conditions, indicating that nutrient depletion had influenced the biosynthetic pathways. These results indicate that polysaccharide production, triggered by diverse conditions may be due to different mechanisms of polysaccharide synthesis. Therefore with detailed knowledge in hand, one can think to alter the monosaccharide ratio at desired for further biotechnological exploitation.

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