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Effects of Light Intensity on Components and Topographical Structures of Extracellular Polysaccharides from the Cyanobacteria *Nostoc* sp.

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A study on the effects of light intensity (40 and 80 μE/m^2/sec) on the components and topographical structures of extracellular polysaccharides (EPS) was carried out in cyanobacteria *Nostoc* sp.. EPS yield increased with light intensity. However, light intensity did not significantly affect the EPS fractions and monosaccharide composition. Higher light intensity generally resulted in higher protein content of EPS in similar fractions. The topographical structure of EPS, investigated by atomic force microscopy, appeared as spherical lumps, chains and networks. The long chains were observed at higher light intensity. Thus, light intensity affected the yield and nature of EPS.

Keywords: cyanobacteria, extracellular polysaccharides, light intensity, *Nostoc* sp., atomic force microscopy

Many cyanobacterial species can synthesize and secrete extracellular polysaccharides (EPS), which contained amino acids, polypeptides, amides, proteins, vitamins, especially polysaccharides (Neu and Marshall, 1990). Cyanobacterial EPS can be divided in two main groups: the polysaccharides released into the surrounding environment (RPS), and the capsular polysaccharides associated with the cell surface (CPS), which can be referred to as sheaths, capsules, and slimes (De Philippis and Vincenzini, 1998, 2003). EPS is increasingly gaining particular research attention because of its potentially useful applications (De Philippis and Vincenzini, 1998, 2003; De Philippis *et al*., 2001, 2011; Suresh Kumar *et al*., 2007).

Various culture parameters affect the synthesis of EPS (De Philippis and Vincenzini, 1998; Pereira *et al*., 2009). In particular, light is one of the most important factors that influence the synthesis and release of EPS. Several recent studies on the influence of light intensity (Friedman *et al*., 1991; Moreno *et al*., 1998; Otero and Vincenzini, 2003; Trabelsi *et al*., 2009; Yu *et al*., 2010; Mota *et al*., 2013) and wavelengths (Ehling-Schulz *et al*., 1997; Chen *et al*., 2009) on EPS yield have been reported. Different light regimens (continuous light and light-dark cycles) do not significantly affect the monosaccharidic composition of EPS (Vincenzini *et al*., 1993; De Philippis and Vincenzini, 1998). However, little information is available about the effects of light intensity on the monosaccharidic composition of EPS.

Nostoc sp. is a exopolysaccharide-secreting cyanobacterium dominant in biological soil crusts (BSCs) (Hu *et al*., 2003). This study aims to investigate the effect of light intensity on EPS component from *Nostoc* sp. and the effect of light intensity on morphological structure of the EPS using atomic force microscopy (AFM). A physicochemical investigation would provide essential information on the function and potential uses of this polysaccharides.

Nostoc sp. (FACHB 892) was separated from BSCs from Tengger Desert, China and conserved in the Institute of Hydrobiology, Chinese Academy of Sciences. Cultures were carried out axenically in 250 ml glass flasks containing 200 ml of BG-11 medium (Rippka *et al*., 1979) at 25±1°C and were continuously illuminated laterally on one side by a combination of cool-white. Light intensity was controlled at 40 and 80 μE/m^2/s with a quantitherm light meter thermomter (Hansatech, UK). Cultures were inoculated at a chlorophyll a concentration of 1.4 μg/ml.

Chlorophyll *a* fluorescence was measured using a plant efficiency analyzer (Hansatech). *Nostoc* sp. was dark-adapted for 15 min before measuring the fluorescence parameter Fv/Fm (Photosystem II activity). The saturating light pulse was 1,500 μE/m^2/s.

After 20 days of growth, the cells in suspension were harvested by filtration and freeze-dried. Biomass measured as dry cell weight. The polysaccharides content released into the culture medium (RPS), in the supernatant, was determined according to the procedure of Chen *et al.* (2003). The polysaccharides formed capsular or slimy layer (CPS) were prepared using the method of Su *et al.* (2008). The amount of total exopolysaccharides (EPS) were the sum of the amounts of RPS and CPS.

Purification of RPS was performed according to the procedure of Hu *et al.* (2003). The filtered supernatants were concentrated in a rotary evaporator (BUCHI Rotavapor R-210, Switzerland) at 35°C. The concentrates were directly

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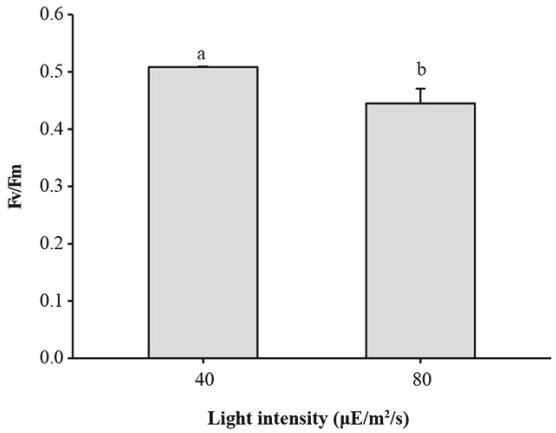

Fig. 1. **Effect of different light intensities on Fv/Fm of *Nostoc* sp.**

placed in a DEAE-Sepharose fast-flow column (5×50 cm). The column was first eluted with distilled water for collection of the neutral polymers, then eluted with 1.0 M NaCl to collect the acidic I polymers, followed by 2.0 M NaCl to get the acidic II polymers. For CPS, the supernatants extracted from dry cells maintained at 80°C for 6 h were prepared using the same method as that of RPS.

Carbohydrate and protein contents of the purified EPS were measured using the phenol-sulfuric acid method (Dubois *et al.*, 1956) and Lowry's method (Lowry *et al.*, 1951), respectively.

Quantitative determination of the carbohydrate composition was carried out according to Hokputsa *et al.* (2003). The trimethysilylated samples were analyzed by gas chromatography (Agilent 6890, USA) with a DB-5 fused silica capillary column. The temperature program was as follows: the initial column temperature of 150°C was held for 1 min; the temperature was then increased to 220°C at a rate of 4°C/min; held at 220°C for 10 min, and then increased again to 250°C at a rate of 10°C/min and held for 5 min. The temperature of the injector was 250°C. The split ratio was 30:1; nitrogen was used as the carrier gas, and the flow rate of N_2 was 0.5 ml/min. The temperature of the flame ionization detector was 280°C.

Samples for AFM were prepared as follows: the purified EPS fraction was dissolved in ultrapure water (1 mg/ml), stirred and centrifuged for 30 min at 3,000×*g*; the supernatant was diluted to a final concentration of 10^{-3} mg/ml. The diluted EPS solutions were spread on freshly cleaved mica disks and fixed for 2 min, and then dried with N_2 gas. AFM (Agilent 5500, USA) was carried out in air at 25±0.5°C and 50–60% relative humidity. All imagines were obtained in tapping mode. Three samples were text for each figure. The molecular height and length on AFM images were measured using the AFM analysis software.

Analysis of variance was performed using the SPSS 18.0, with the significance level set at 0.05 or 0.01. Values are expressed as the mean±standard deviation (n=3).

After 20 days of culture, the Fv/Fm of *Nostoc* sp. at 40 μE/m^2/s was 1.1-fold higher than that at 80 μE/m^2/s (P<0.05) (Fig. 1). The biomass of *Nostoc* sp. at 40 and 80 μE/m^2/s did not differ significantly (P>0.05) (Table 1). These results suggest that 40 μE/m^2/s was more optimal for *Nostoc* sp. culture compared with 80 μE/m^2/s.

The yields of RPS, CPS and EPS produced by *Nostoc* sp. at 80 μE/m^2/s (134.26, 71.94 and 206.20 mg/g DW for RPS, CPS and EPS, respectively) were all higher than that at 40 μE/m^2/s (97.78, 57.70, and 155.49 mg/g DW) (P<0.01) (Table 1). Our data are in agreement with previous studies that EPS production is enhanced by high light intensities (Friedman *et al.*, 1991; Moreno *et al.*, 1998; Otero and Vincenzini, 2003; Trabelsi *et al.*, 2009; Yu *et al.*, 2010; Mota *et al.*, 2013), suggesting that energy availability significantly affects the EPS biosynthesis of cyanobacteria. Higher light intensity results in higher EPS production, which may constitute mechanism of releasing the excess energy absorbed by the cells (Mota *et al.*, 2013) and increasing CO_2 fixation rate in the cells under high light intensity.

The polysaccharides produced by *Nostoc* sp. were separated into three parts, one neutral and two acidic fractions by anion exchange chromatography. The percentages of each fraction are listed in Table 1. The 2.0 M NaCl eluates (7.36–9.50%) only accounted for a small proportion of the total; thus, were not characterized further. Light intensity did not significantly affect the fractions of RPS or CPS from *Nostoc* sp. The water eluates comprised the main fraction of RPS, whereas the 1.0 M NaCl eluates comprised the main fraction of CPS (Table 1). Results from a previous study revealed that the water eluates comprised the main fraction of CPS in three soil *Nostoc* species (Huang *et al.*,

Table 1. **The amount of EPS from *Nostoc* sp. and the percentage of one neutral and two acidic fractions of purified EPS (% of total purified EPS content) under different light intensities**

			40 μE/m^2/s	80 μE/m^2/s
Biomass (g DW/L)			0.82±0.00	0.83±0.05
Yield (mg/g DW)	RPS		97.78±1.38	134.26±7.03
	CPS		57.70±5.08	71.94±3.95
	EPS		155.49±6.46	206.20±10.98
Percentage (%)	RPS	Water eluates	56.19±1.23	57.97±2.50
		1.0 M NaCl eluates	34.31±1.68	34.67±2.27
		2.0 M NaCl eluates	9.50±0.44	7.36±0.24
	CPS	Water eluates	16.74±0.39	15.30±1.00
		1.0 M NaCl eluates	74.90±1.04	76.40±1.40
		2.0 M NaCl eluates	8.37±0.65	8.30±0.39

DW indicates dry weight of biomass.

Table 2. Monosaccharide composition (% of total carbohydrate content) and the total carbohydrate content and total protein content (% of polysaccharides dry weight) of the purified EPS of *Nostoc* sp. under different light intensities

		40-W	40-1	80-W	80-1
RPS	Rhamnose	6.5±0.2	3.5±0.0	9.6±0.2	8.7±0.2
	Xylose	9.1±0.4	6.4±0.1	6.3±0.1	4.6±0.1
	Mannose	4.5±0.1	2.2±0.0	8.3±0.1	3.6±0.0
	Galactose	10.5±0.2	12.4±0.1	13.1±0.0	16.1±0.1
	Glucose	63.2±0.9	71.5±0.0	54.2±0.3	59.4±0.2
	Galacturonic acid	3.7±0.1	3.9±0.0	5.2±0.1	7.7±0.2
	Glucuronic acid	2.4±0.0	-	3.3±0.0	-
	Total carbohydrate	58.6±1.3	52.8±2.2	62.2±4.5	38.0±2.2
	Total protein	11.3±1.2	11.5±0.9	16.1±0.5	19.2±1.7
CPS	Rhamnose	1.5±0.0	13.8±0.1	-	10.8±0.1
	Xylose	-	5.4±0.0	1.2±0.0	6.0±0.1
	Mannose	2.2±0.0	6.9±0.1	1.9±0.0	7.3±0.2
	Galactose	23.4±0.1	12.5±0.3	22.2±0.0	13.8±0.5
	Glucose	62.9±0.2	49.8±0.4	63.9±0.0	53.5±0.7
	Galacturonic acid	4.0±0.0	3.3±0.0	4.4±0.0	2.9±0.1
	Glucuronic acid	6.3±0.7	8.4±0.5	6.3±0.0	5.7±1.5
	Total carbohydrate	56.4±2.3	29.8±2.1	66.6±5.3	29.6±2.1
	Total protein	18.7±1.4	38.0±1.7	15.3±1.1	48.3±1.9

40-W, water eluates from 40 μE/m^2/s; 40-1, 1.0 M NaCl eluates from 40 μE/m^2/s; 80-W, water eluates from 80 μE/m^2/s; 80-1, 1.0 M NaCl eluates from 80 μE/m^2/s.

1998). Hu *et al.* (2003) reported that the 1.0 M NaCl eluates were the main fraction of RPS from a soil algae *Nostoc* sp. In the present study, the difference in the main fractions between RPS and CPS suggests that the physiological properties between RPS and CPS were different.

The contents of purified EPS carbohydrates and proteins are shown in Table 2. For RPS, light did not significantly affect the carbohydrate content in the water eluates ($P>0.05$); whereas the content of carbohydrate in 1.0 M NaCl eluates at 40 μE/m^2/s was 1.4-fold higher than that at 80 μE/m^2/s ($P<0.01$). And the RPS protein contents at 80 μE/m^2/s (16.1% of polysaccharides and 19.2% of polysaccharides for water eluates and 1.0 M NaCl eluates, respectively) were higher than that at 40 μE/m^2/s (11.3%, 11.5%) ($P<0.01$) (Table 2). For CPS, the carbohydrate content of water elutes at 80 μE/m^2/s was 1.2-fold higher than that at 40 μE/m^2/s ($P<0.05$); whereas the carbohydrate contents were similar for the said light intensities in 1.0 M NaCl eluates ($P>0.05$). And the protein content of 1.0 M NaCl eluates at 80 μE/m^2/s was 1.3-fold higher than that at 40 μE/m^2/s ($P<0.01$) (Table 2). Thus, the EPS protein content at 80 μE/m^2/s was generally higher than that at 40 μE/m^2/s in similar fractions ($P<0.01$).

The principal components of EPS are polysaccharides or proteoglycans, wherein the protein part is covalently linked to carbohydrate (Hu *et al.*, 2002). In this study, the rate of nitrate assimilation and metabolism in *Nostoc* sp. may be higher at higher light intensity. Furthermore, proteins can be glycosylated to form glycoproteins, which can be translocated to the cell surface or into the surrounding media

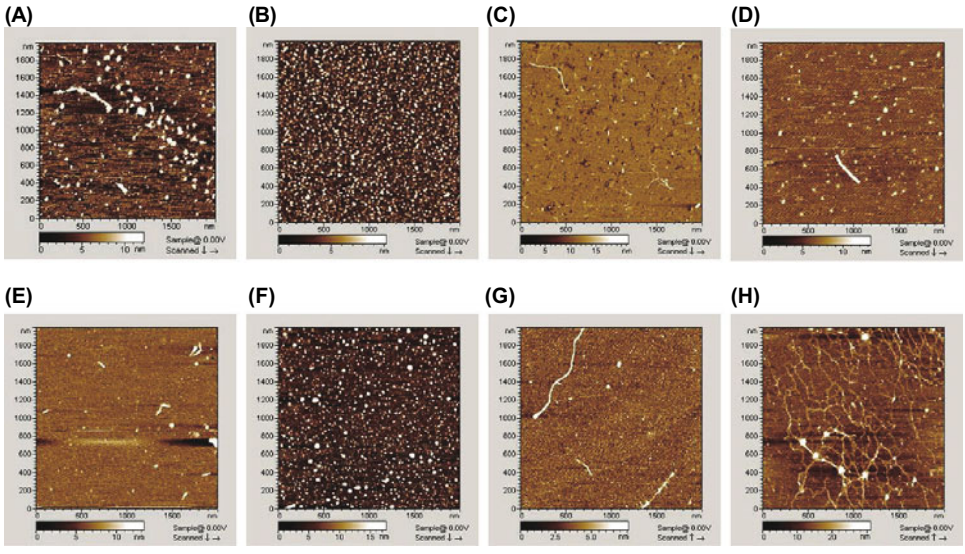

Fig. 2. AFM images of purified EPS from *Nostoc* sp. under different light intensities (image size = 2×2 μm). For RPS: (A) 40-W, (B) 40-1, (C) 80-W, (D) 80-1. For CPS: (E) 40-W, (F) 40-1, (G) 80-W, (H) 80-1. 40-W: water eluates from 40 μE/m^2/s; 40-1: 1.0 M NaCl eluates from 40 μE/m^2/s; 80-W: water eluates from 80 μE/m^2/s; 80-1: 1.0 M NaCl eluates from 80 μE/m^2/s.

Table 3. Molecular sizes of EPS molecules by AFM

		Spherical lump	Chain		Net work
		Height range (nm)	Length range (nm)	Height range (nm)	Height range (nm)
RPS	40-W	8.11–15.88	280–630	10.14–17.23	-
	40-1	6.08–11.15	-	-	-
	80-W	4.95–16.17	420–520	4.29–8.58	-
	80-1	6.42–16.56	400–400	14.53–18.25	-
CPS	40-W	5.07–9.8	-	-	-
	40-1	9.46–28.05	-	-	-
	80-W	4.05–17.91	780–1040	6.08–17.23	-
	80-1	-	-	-	6.76–15.54

40-W, water eluates from 40 μE/m^2/s, 40-1: 1.0 M NaCl eluates from 40 μE/m^2/s; 80-W, water eluates from 80 μE/m^2/s; 80-1, 1.0 M NaCl eluates from 80 μE/m^2/s.

(Pereira *et al.*, 2009). Thus, the protein content of EPS was higher at high light intensity.

Light intensity did not significantly affect the monosaccharide composition of RPS or CPS (Table 2). In the carbohydrate part of RPS or CPS, glucose and galactose were the most abundant, rhamnose, xylose, mannose, galacturonic acid, and glucuronic acid were present as minor components. Different light regimens did not affect the monosaccharidic composition of EPS (Vincenzini *et al.*, 1993; De Philippis and Vincenzini, 1998). We found that light intensity also did not significantly affect the quality of the polymer described above. For most algal strains, RPS and CPS contain the same sugar composition, suggesting that RPS could originate from CPS (Li *et al.*, 2001; Bellezza *et al.*, 2003; Pereira *et al.*, 2009; Yoshimura *et al.*, 2012). Our results are in agreement with previous studies.

The topographical AFM images of EPS are presented in Fig. 2, and the molecular sizes of EPS are shown in Table 3. For RPS, the height of short chain in water eluates at 40 μE/m^2/s (10.14–17.23 nm) was higher than that at 80 μE/m^2/s (4.29–8.58 nm) (P<0.01) (Fig. 2A, 2C, and Table 3). And in 1.0 M NaCl eluates, the short chains were observed at 80 μE/m^2/s, compared with that at 40 μE/m^2/s (Fig. 2B, 2D and Table 3). For CPS, the long chains were observed at 80 μE/m^2/s, compared with the corresponding 40 μE/m^2/s results in similar fractions (Fig. 2E, 2F, 2G, 2H, and Table 3). Clearly, the 1.0 M NaCl eluates at 80 μE/m^2/s appeared as networks, which were thought to contain long chains (Fig. 2H).

Different EPS molecules have different topographical structures. Pletikapic *et al.* (2011) confirmed the surface morphology of EPS isolated from *Cylindrotheca closterium* appeared as networks at 10 μg/ml. The AFM image of EPS from *Amphidinium carterae* appeared as different particle sizes, with 181 nm average roughness (Mandal *et al.*, 2011). The present study on the EPS from *Nostoc* sp. under different light intensities showed spherical lumps, chains and networks. From the AFM images (Fig. 2), the topographical structures of the exopolysaccharides in similar fractions under different light intensities were evidently different, suggesting that light intensity affected the physicochemical properties of EPS from *Nostoc* sp.

In conclusion, the results obtained in this work show that higher light intensity lead to higher EPS yield produced by *Nostoc* sp. The analysis of the purified EPS revealed seven types of monosaccharide, in which glucose and galactose were the most dominant. Moreover, the contents of EPS carbohydrate and protein in similar fractions were different, suggesting light intensity affects the EPS components. The AFM results further indicate that light intensity affects the physicochemical properties of EPS from *Nostoc* sp.

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