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Extracellular carbohydrate polymers from five desert soil algae with different cohesion in the stabilization of fine sand grain

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Abstract

Extracellular polymeric substances (EPS) from four filamentous cyanobacteria *Microcoleus vaginatus, Scytonema javanicum, Phormidium tenue* and *Nostoc sp.* and a coccoid single-cell green alga *Desmococcus olivaceus* that had been separated from desert algal crusts of Tegger desert of China, were investigated for their chemical composition, structure and physical properties. The EPS contained 7.5–50.3% protein (in polymers ranging from 14 to more than 200 kD, SDS-PAGE) and 16.2–40.5% carbohydrate (110–460 kD, GFC). 6–12 kinds of monosaccharides, including 2-*O*-methyl rhamnose, 2-*O*-methyl glucose, and *N*-acetyl glucosamine were found. The main carbohydrate chains from *M. vaginatus* and *S. javanicum* consisted mainly of equal proportion of Man, Gal and Glc, that from *P. tenue* consisted mainly of arabinose, glucose and rhamnose. Arabinose was present in pyranose form, mainly α-L 1 → 3 linked, with branches on C4 of almost half of the units. Glucose was responsible for the terminal units, in addition of having some units as β1 → 3 and some as β1 → 4 linked. Rhamnose was mainly 1 → 3 linked with branches on C2 on half of the units. The carbohydrate polymer from *D. olivaceus* was composed mainly of β-1 → 4 linked xylose, galactose and glucose. The galactose part was present both in β-pyranose and -furanose forms. Arabinose in α-L-furanose form was mainly present as 1 → 2 and 1 → 2, 5 linked units, rhamnose only as α 1 → 3 and xylose as β 1 → 4. The backbone of the polysaccharide from *Nostoc* sp. was composed of β-1 → 4 linked xylose, galactose and glucose. Most of the glucose was branched on position C6, terminal glucose and 2-*O*-methyl glucose units are also present. The relationship between structure, physical properties and potential biological function is discussed.

Keywords: Carbohydrate polymers; Cyanobacteria; Desert algal crust; *Desmococcus olivaceus*; Extracellular polymeric substances; *Microcoleus vaginatus*; *Nostoc* sp; *Phormidium tenue*; *Scytonema javanicum*; Viscosity

1. Introduction

Algae are ubiquitous photoautotrophic micro-organisms. Although they generally occur in freshwater or marine habitats, they also occupy a variety of terrestrial environments. They commonly grow either on the surface or at a depth of up to several centimetres in soil, they aggregate soil particles and sand grains to form microbial crusts, stabilize soil and reduce erosion. They have been applied as soil conditioners in many countries (Metting, 1981) and have been suggested as biofertilizers as well (Painter, 1993).

Soil algae are known to excrete into the surrounding soil a variety of extracellular low molecular and polymeric substances (EPS) like amino acids, polypeptides, amides, proteins, polysaccharides, vitamins, growth regulators and a number of other compounds not yet fully characterized. These EPSs fulfil a variety of different roles. They both protect algae from harmful effects of toxic substances or unfavourable factor (e.g. desiccation, antibiotics, ultraviolet ray etc.) and directly attach to solid surface and other matrix (De Philippis & Vincenzini, 1998; Stal, 2000). As early as 1964, Moore and Tischer (1964) suggested that algal EPS affected life-support systems. In recent years Painter (1993) has reviewed their roles in reclamation of desert soil. Mazor,

Abbreviations: EPS, extracellular polymeric substances; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; HPAEC-PAD, high pH anion exchange chromatography-pulse amperometric detection.

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Kidron, Vonshak, and Abeliovich (1996) further ascertained their effect on providing nutrient and enhancing moisture in desert microbiological crusts. Owing to the attracting future, a number of algal EPS have been studied. But only a few structural studies have been performed due to the relative high protein content and water insolubility of EPS from soil algae (Bertocchi, Navarini, Cesaro, & Anastasio, 1990; Flaibani, Olsen, & Painter, 1989; Helm, et al., 2000; Huang, Liu, Paulsen, & Klaveness, 1998; Morvan, Gloaguen, Vebret, Joset, & Hoffmann, 1997; Vincenzini, Dephilippis, Sili, & Materassi, 1990; Vincenzini, Dephilippis, Sili, & Materassi, 1993). All attempts to fractionate them into more than a singled entity by salt gradient and enzymatic depolymerization were unsuccessful (Flaibani et al., 1989; Helm et al., 2000; Matulewicz, Percival, & Weigel, 1984). To our knowledge, only a few chemical structures of intact complete EPS (no material present have been removed) has been proposed for soil algae. The lack of structural information has limited the better understanding of their role and possible applications in soil reclamation, desert control and industrial application (Morvan et al., 1997). For this reason it is important to seek for better methods to solve these problems.

Scytonema javanicum, *Nostoc* sp., *Desmococcus olivaceus*, *Microcoleus vaginatus* and *Phormidium tenue* are all desert soil algae, they dominate in the crust of the Tegger desert in the following layers from the top surface, respectively, in ca. 0.02–0.05, 0.05–0.1, 0.1–1.0, 1.0–3.0, 3.5–4.0 mm layer from the surface (Hu, Liu, Song, & Zhang, 2002). Among them *M. vaginatus*, *P. tenue*, *S. javanicum* and *Nostoc* sp. all belong to cyanobacteria, the latter two being heterocystous species. *D. olivaceus* is a unicellular green alga, and is a common desert soil green alga with a thick cell wall. *Nostoc* sp. belongs to the thallus flora in taxonomy, but it is also a filamentous species at some stages. It has many special characters and can adapt to different kinds of extreme environments (Dodds, Gudder, & Mollenhauer, 1995; Hu et al., 2002; Potts, 2000). *S. javanicum* is a filamentous diazotrophic species, sometimes growing on the surface of the crusts, even if directly exposed to sunlight, because a special ultraviolet absorbing pigment in the sheath to protect the cells (Dodds et al., 1995; Potts, 2000). *P. tenue* is a filamentous species, often many filaments growing together into a mat. Among these five species, it can stabilize unconsolidated sand grains at the least biomass (Hu et al., 2002). *M. vaginatus* is a filamentous cyanobacterium, single-trichomes at low biomass concentration, 5–115 trichomes rope-like twisted and wrapped in a common thick sheath to form 15–150 μm broad filaments (including sheath) at high biomass concentration. It often combines with sand granules and soil particles under the natural algal crusts, and had the strongest cohesion in stabilization of the soil. On the whole, *M. vaginatus* and *P. tenue* have the strongest cohesion in stabilising fine sand, the diazotrophic species *S. javanicum* and *Nostoc* sp. next to the former two, the coccoid *D. olivaceus* is the weakest one (Hu et al., 2002). The aim of the present study is to determine the chemical properties in composition, structure, molecular weight and viscosity of the EPS, and to discuss the possible relationship between cohesion of each species and the chemical properties of their EPS.

2. Materials and methods

Materials. Five desert soil algae, *S. javanicum*, *Nostoc* sp., *D. olivaceus*, *M. vaginatus* and *P.tenue* isolated from Tegger desert algal crusts of Zhongwei County, Ningxia Hui Autonomous Region of China (37°27′N, 104°57′E) were cultured and their EPS analysed. The descriptions of their natural environments are given (in Hu, Liu, & Song, 2000).

Growth conditions. *M. vaginatus*, *P. tenue* and *D. olivaceus* were, respectively, inoculated in 8L BG11 medium at a temperature of 28 ± 1 °C under continuous white fluorescent tubes at a photon of 80 μmol photon $m^{-2}\,s^{-1}$. *S. javanicum* and *Nostoc* sp. were separately cultured in 5L and 8L $BG11_0$ medium, the temperature and light intensity being the same as in the former experiments. Filaments or colonies of all isolates showed the strong tendency to clump together and do adhere to the vessel walls. For the experiments, a subculture was used. Under sterile conditions, cultures were harvested and homogenized with an all-glass tissue grinder, and stirred strongly by supplying wet air. Growth rates were measured by chlorophyll at a three days interval (Hu et al., 2000). After 60 to 90 days of growth, the cells and suspension were harvested by the following method.

Isolation and purification of extracellular polymeric substance. The culture media were centrifuged to separate algae from the suspension. The Cyanobacteria were freeze dried directly, the green algae were spread into a thin layer on silk and air dried. The suspensions were directly put onto a DEAE-Sepharose fast flow column (50 × 5 cm) with chloride as counter ion at 0.5–1 mL min^{-1}. The column was coupled to a P-1 (Pharmacia) peristaltic pump. The carbohydrate elution profile was determined using the phenol-sulphuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The column was first eluted with distilled water at 2 mL min^{-1}, for collection of the neutral part, 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. All part were dialyzed successively against running tap water and distilled water in a Spectrapor dialysis tube with a molecular weight cut-off of 3500, and finally freeze dried.

Quantitative determination of the carbohydrates composition by methanolysis and GC. The monosaccharide composition was determined by methanolysis using 4 M HCI in methanol at 80 °C for 24 h followed by trimethylsilylation of the resulting methyl glycosides. Identification and quantification of the monosaccharides were performed

on the basis of standard curves of known monosaccharides using mannitol as an internal standard (Barsett & Paulsen, 1992; Reinhold, 1972)

Identification of O-methyl-pentose and -hexose. 5.0 mg 1.0 M NaCl eluents from *M. vaginatus*, *S. javanicum* and *P. tenue* were hydrolysed with 500 μL 2.5 M trifluoroacetic acid (TFA) with argon for 2 h at 100 °C. TFA was removed by a stream of nitrogen. After drying, one half was reduced and converted into the alditol acetates and subjected to GC-MS as described for methylated samples described later.

Carboxyl reduction. Samples were reduced with sodium borodeuteride after activation with carbodiimide as described by Kim and Carpita (1992). The reduction was followed by methylation and GC-MS as described below.

Methylation and GC-MS spectrometry analysis. Methylation of 1.0 M NaCl eluents and the carboxyl reduced ones and GC-MS analysis of the derived partially methylated alditol acetates were performed as previously described (Barsett & Paulsen, 1992; Paulsen, Aslaken, Freire-Nordi, & Vieira, 1998; Paulsen, Vieira & Klaveness, 1992).

Partial acid hydrolysis. The extracellular polymers were partially hydrolysed with 0.4 M TFA. The 1.0 M NaCl eluates (2.0 mg) were dispersed in distilled water (2.0 mL) by heating. After cooling, TFA was added to a final concentration of 0.4 M and the polymers were hydrolysed at 100 °C for 2.5 h. The hydrolysates were evaporated to dryness at 40 °C under reduced pressure and further evaporated three times with methanol (5 mL). Portions of these oligosaccharide mixtures were used for analysis by HPAEC-PAD as described later.

HPAEC-PAD of oligosaccharide fractionation. High-pH anion exchange chromatography (HPAEC) was carried out as described in Brull, et al. (2000).

Weak acid hydrolysis. 5.0 mg 1.0 M NaCl eluents from *P.tenue* was hydrolysed in 1 to 2 mL of 0.05 M oxalic acid under 100 °C for 2 h, neutralized with $NaHCO_3$, then separated by PD-10 column (Pharmacia Biotech). The carbohydrate elution profile was detected by the phenol-sulphuric acid method. The carbohydrate containing parts were collected and freeze dried. One part was subjected to methanolysis and GC analysis, the other part were used for determination of the molecular weight as given below.

β-elimination of possible O-linked glycoconjugates. The carbohydrate moiety was split off from the protein by alkaline borohydride treatment. The 1.0 mol L^{-1} NaCl eluents (10.0 mg) was heated at 50 °C for 6 h in 10 ml containing 0.125 mol L^{-1} NaOH and 1 mol L^{-1} sodium borohydride. Excess borohydrate was decomposed by addition of 3 mol L^{-1} HCI and removed as methyl borate by five times evaporation with 15 ml aliquots methanol. The hydrolysed materials were fractionated by size exclusion chromatography. The hydrolysed residue was dissolved in 2 ml distilled water, and then eluted on the Bio-Gel P-10 column (37 × 1.6 cm) at 0.3 mol L^{-1} with distilled water. The column was coupled to a P-1 peristaltic pump. The carbohydrate elution profile was detected by the phenol-sulphuric acid method. The carbohydrate moiety was freeze-dried for molecular weight measurement.

Affinity chromatography. 1.0 M eluents of *P.tenue* was subjected to affinity chromatography at room temperature on a Concanavalin A-Sepharose column (Pharmacia, 1 (i.d.) × 2 cm) at a flow rate of 30 mL h^{-1}. Unbound and non-specifically bound components were eluted with Tris-buffer (20 mM Tris, 1 mM $MnSO_4$, 1 mM $CaCl_2$, 0.02% NaN_3, pH 7.40). The samples were dissolved in the same Tris-buffer, filtered through 0.45 mm Millipore filter before application onto the Concanavalin A column. After equilibration of the column with 40 mL Tris-buffer, specifically bound components were eluted with 50 mL of the same Tris-buffer that contained 0.5 mol L^{-1} α-MeMan*p*. 0.5 mL fractions were collected, dialysed (MW cut off 3500) against distilled water and freeze dried. The dried samples were subjected to methanolysis and GC analysis as mentioned earlier.

Relative molecular weight determination. The molecular weight relative to dextran standards was determined by gel filtration chromatography as previously described (Paulsen, et al., 1998). The Superose 6 FPLC column (25 mL) was eluted by distilled water at 30 mL h^{-1}. A Pharmacia LKB pump P-500 was coupled to the FPLC system, fitted with a RID-6A Shimadzu refractive index detector and a Pharmacia LKB Superfrac collector. As standards Dextran B512, Dextran T250, Dextran 70 and Blue Dextran 2000 (Pharmacia Biotech) were used. Absolute molecular weights on further purified materials will follow in the succeeding paper (Hokputsa, Hu, Paulsen, & Harding, 2003).

SDS-PAGE was performed in 0.75 mm × 5 cm × 8 cm homogenous slab gels (4%T, 2.7%C in the stacking gel and 15%T, 2.7%C in the separation gel) using the Bio-Rad (Richmond, USA) Mini-protean® II Electrophoresis Cell. The electrophoresis was performed at 200 V for 40 min. The gels were stained with Coomassie Bright Blue R-250 for 8 min (Laemnli, 1970). The high Molecular weight (45–200 kD) and low Molecular weight (6.5–97.4 kD) protein standards were used (Bio-Rad, Richmond, USA).

Quantitative determination of the protein content. The protein content of the samples was determined by the protein assay of Lowry, Rosebrough, Farr, and Randall (1951) as modified by Petersen (1979).

Viscosity measurement. Kinematic viscosity was measured according to Huang et al. (1998) at 25 ± 0.01 °C with an Ostwald micro-viscometer in an automated measuring unit AVS 310 (Schott-Geräte, Hofheim) by using capillary tube with 1 and 2 mL of sample, the concentration of the polymers was 0.1% w v^{-1}.

NMR. For ^{13}C NMR spectroscopy, broad band decoupled 125,76 MHz ^{13}C NMR spectra were obtained on a Bruker Avance DRX 500 MHz spectrometer equipped with a BBO BB-H-D 5 mm probehead, using a 30° pulse angle and 39 KHz spectral width with 64 K data points. The acquisition time was 0.826 s, and the relaxation delay was

2.5 s (cycle time per scan 3.326 s). For all analyses 50 K (51200) scans with a line broadening of 3.0 HZ and double zero filling (128 K) were used. All spectra were recorded in D_2O at 75 °C.

3. Results

3.1. Productivity of the polymers

Table 1 lists the productivity of EPS of the five species. *M. vaginatus* exhibited the highest productivity, followed by *S. javianicum, Nostoc* sp. and *D. olivaceus. P. tenue* excreted the lowest amount of EPS. The EPSs produced by all organisms were separated into one neutral and two acidic fractions by anion exchange chromatography and the amounts for each fraction is also given in Table 1. 80 ~ 98% of the EPSs were eluted by 1.0 M NaCl, whereas the neutral and acidic II parts only accounted for small proportions. For this reason only the 1 M fractions were further characterized.

3.2. Chemical composition of the 1 M NaCl fractions

The compositions of the 1 M NaCl fractions are reported in Table 2. The EPS from *M. vaginatus* contained ca. 28% carbohydrate and 50% protein. The carbohydrate part was complex, with mannose, galactose and glucose as the most abundant and in almost equal amounts; arabinose, xylose, fucose, rhamnose, 2-*O*-methyl rhamnose, galacturonic acid, glucuronic acid and *N*-acetyl glucosamine were present in smaller amounts, while 2,3-*O*-methyl rhamnose, 3-*O*-methyl rhamnose, 4-*O*-methyl rhamnose and 3-*O*-methyl glucose were identified amongst the trace monosaccharides.

The EPS from *S. javanicum*, contained 50% protein and 17% sugar. As for *M. vaginatus*, mannose, galactose and glucose were the most abundant and present in almost equal amounts. Arabinose, rhamnose, 2-*O*-methyl rhamnose and xylose were present in minor amounts, whereas 2,3-*O*-methyl rhamnose, fucose, galacturonic acid, glucuronic acid and *N*-acetyl glucosamine were identified as trace monosaccharides.

The EPS from *P. tenue* contained 22% protein and 36% carbohydrate. The composition of the carbohydrate part was different from the two samples mentioned above. The main components were arabinose, glucose and rhamnose, respectively, 44, 32 and 10%. Fucose, xylose, mannose and galactose were present in smaller amounts, whereas 3-*O*-methyl rhamnose, 4-*O*-methyl rhamnose, 3-*O*-methyl glucose, 6-*O*-methyl glucose and galacturonic acid were identified as trace monosaccharides.

The EPS from the unicellular green algae, *D. olivaceus*, contained 14% protein and 16% sugar. The total content of carbohydrate and protein was the lowest amongst the five species studied. In the carbohydrate part, galactose and glucose were the most abundant, followed by arabinose and xylose. Rhamnose, fucose, mannose and 2-*O*-methyl glucose were present in minor amounts, while galacturonic acid and glucuronic acid were identified as trace monosaccharides.

The EPS from *Nostoc* sp. contained 8% protein and 41% carbohydrate, the lowest protein and the highest carbohydrate content among the five species. The main components of the present EPS were glucose, xylose, galactose and 2-*O*-methyl glucose, and their molar ratios were, respectively, 2:1:1:0.5. Rhamnose and mannose were only present in small amounts.

3.3. Linkage analyses of the carbohydrate part of the 1 M EPS fractions

The 1.0 M NaCl eluates of the EPS from the five species were methylated, both before and after carboxyl reduction of the polymers, for possible location of the linkage of the uronic acids. The resulting partially methylated alditol acetates from the total procedure were identified by GC-MS.

Methylation results (Table 3) showed a high degree of similarity among the linkage types of the EPSs from *M. vaginatus* and *S. javanicum*. Their main chains were both composed by mannose, galactose and glucose, but owing to their collution it was impossible to identify and quantify the different types of linkages for the hexoses in the polymers. Galactose was responsible for the main part of the end groups, and both $1 \rightarrow 2$, $1 \rightarrow 3$ and $1 \rightarrow 4$ linkages could be identified in the mixture of compounds. Rhamnose was mainly present as $1 \rightarrow 4$ linked units, which also was the case for 2-*O*-methyl rhamnose present in polymers from both species. Glucuronic acid mainly exists as terminal units. ^{13}C NMR gave a complex pattern in the anomeric region, thus it was

Table 1
Extracellular polymeric substances (EPS) produced by the five desert soil algae: EPS productivity and fractions

	M.vaginatus	*S. javianicum*	*P. tenue*	*D. olivaceus*	*Nostoc* sp.
Culture medium	BG11	$BG11_0$	BG11	BG11	$BG11_0$
Productivity (mg EPS g biomass dry wt^{-1} d^{-1})	0.20	0.17	0.04	0.09	0.12
Neutral part (mg)	33(13%)	5(6%)	10(6%)	5(2%)	6(2%)
1.0 M NaCl eluates (mg)	202(80%)	70(81%)	135(87%)	250(98%)	265(85%)
2.0 M NaCl eluates (mg)	18(7%)	11(13%)	10(7%)	–	40(13%)

Table 2
Monosaccharide composition expressed as percentage (%) of the single monosaccharide on the total carbohydrate content and total protein contents of the 1.0 M NaCl eluates of the five species

	M. vaginatus	*S. javianicum*	*P. tenue*	*D. olivaceus*	*Nostoc* sp.
Arabinose	9.4	9.6	43.9	13.1	n.d.
Rhamnose	5.5	7.4	10.4	7.0	3.5
2-*O*-methyl rhamnose	2.5	6.0	n.d.	n.d.	n.d.
Fucose	4.4	tr	2.3	1.4	n.d.
Xylose	8.5	6.0	4.7	12.4	20.9
Mannose	21.2	22.9	2.9	5.9	1.6
Galactose	18.3	23.4	1.3	28.8	21.54
Glucose	20.1	24.8	32.5	27.6	44.0
2-*O*-methyl glucose	n.d.	n.d.	n.d.	3.9	8.6
Galacturonic acid	4.6	tr	Tr	Tr	n.d.
Glucuronic acid	3.4	tr	n.d	Tr	n.d.
N-acetyl glucosamine	2.1	tr	1.3	n.d.	n.d.
Total carbohydrate (%)	27.6	16.6	36.1	16.2	40.5
Total protein (%)	50.3	50.2	21.9	14.2	7.5

tr = trace (< 1%), n.d = not detected.

Table 3
Linkage determination of monomers present in the 1.0 M NaCl eluates of EPS of the five desert spoil algae

	M. vaginatus	*S. javianicum*	*P. tenue*	*D. olivaceus*	*Nostoc* sp.
Arabinose Tf	2.2	5.4		4.2	
1 → 2,3 f	2.4	tr			
1 → 3,5f	5.1	4.5			
1 → 2f				4.1	
1 → 2,5f				4.6	
Tp			0.9		
1 → 3 p			25.0		
1 → 3,4 p			17.9		
Rhamnose 1 → 3p	tr	tr	5.5	6.8	3.4
1 → 4p	10.6	13.4			
1 → 2,3p		tr	4.8		
Fucose T p	4.6	tr	tr	tr	
Xylose Tp					1.2
1 → 3p			4.7		
1 → 4p	8.6	6.0		12.4	19.6
Mannose[a]	21.5	22.9			
Tp				5.1	
1 → 2p				tr	
Galactose[a]	18.6	23.4			
Tp	M[b]	M[b]	tr	3.2	
Tf					
1 → 3p			tr	2.1	
1 → 4p				15.0	21.5
1 → 3,6f				1.3	
Glucose[a]	20.4	24.7			
Tp			16.7	7.7	24.9
1 → 3p			6.5		
1 → 4p			8.7	6.9	3.6
1 → 4,6p			tr	15.9	24.1
Glucuronic acid Tp	3.6	tr			

[a] The tri-*O*-methyl alditol acetates of mannose, glucose and galactose could not be separated.
[b] M for galactose, Tp means that galactose was the most abundant endgroup of the hexoses, Tp means terminal group in pyranose form, Tf means terminal group in furanose form; tr means less that 1% present.

Table 4
^{13}C NMR analysis at the anomeric carbons of the polymers isolated from the different organisms

Origin of polymer	^{13}C NMR shifts (anomeric)	Assignments
M. vaginatus	Complex peak pattern in the region 99–107 ppm	The pattern is too complicated for specific assignments
S. javanicum	Complex peak pattern in the region 101,5–110,5 ppm	The pattern is too complicated for specific assignments
P. tenue	105,525	β D 1 → 3 Glcp, β D1 → 4 Glcp
	105,727	β D 1 → Glcp
	107,003	α L 1 → 3,4 Arap
	107,365	α L 1 → 3 Arap
D. olivaceus	103,0 multiple	α L Rhap[a]
	103,8 multiple	β D Glcp[a]
	105,487	β D 1 → 4 Xylp
	105,641	β D Glcp[a]
	105,881	β D 1 → 4 Galp
	106,212	β D Galf[a]
	110,790	α L 1 → Araf
Nostoc sp.	103,624	β D 1 → 4 Galp
	104,066	β D 1 → 4,6 Glcp
	104,305	β D 1 → 4 Glcp
	104,624	β D 1 → 4 Xylp

[a] Type of linkage not assigned.

impossible to assign the different peaks to specific types of linkages for the two polymers (Table 4), thus being possible that both α and β configurations are present.

The EPS from *P. tenue* was composed mainly by arabinose, glucose and rhamnose. Arabinose was present in pyranose form, mainly 1 → 3 linked, with branches on C4 in almost half of the units. Glucose is responsible for the terminal units, in addition to having some units as 1 → 3 and some as 1 → 4 linked. Rhamnose was mainly 1 → 3 linked with branches on C2 on half of the units. ^{13}C NMR showed 4 peaks in the anomeric region (Table 4). The peaks at δ107.36 and δ107.00 are most probably due to α L 1 → 3 and 1 → 3,4 linked arabinose in pyranose form (Bock & Pedersen, 1983), δ105.72 represent β D glucopyranosyl as terminal units and δ105,52 (a double peak) represents both βD1 → 3 and 1 → 4 linked glucopyranosyl units (Olafsdottir & Ingolfsdottir, 2001).Weak acid hydrolysis of the polymer followed by analysis in the Dionex system at the conditions given earlier, gave rise to one peak approximately at the elution time of a heptamer with maltose configuration, which probably is a glucose-oligomer.

For *D. olivaceus*, the structure of EPS was special; the main chain was composed by 1 → 4 linked xylose, galactose and glucose, the latter with a high degree of branches on C6. The galactose part was present both in the pyranose and furanose forms, as shown by the presence of both types of terminal units. Arabinose in the furanose form was mainly present as terminal units, 1 → 2 and 1 → 2, 5 linked, rhamnose only as 1 → 3 linked and xylose 1 → 4 linked units. ^{13}C NMR (Table 4) gave peaks at positions that indicate that galactose, both in furanose and pyranose forms, was β-linked, as were glucose and xylose, while both rhamnose and arabinose were α L types (Olafsdottir & Ingolfsdottir, 2001).

The EPS from *Nostoc* sp. had a backbone structure most probably composed by 1 → 4 linked xylose, galactose and glucose. Glucose represented the main part of the end groups and the branch points on C6 of 1 → 4 linked units. The 2-*O*-methyl glucose present in this molecule is most probably present either among the terminal or the branching units. ^{13}C NMR (Table 4) gave shifts in the region δ103,62-104,62, indicating that all units are β linked (Perlin & Casu, 1982).

Viscosity. The 1.0 M NaCl eluates from the EPS of the five species were separately redissolved in distilled water at 25 °C, and made into 0.1% concentration. Because of too low viscosity for measurement with other instruments, their kinematic viscosities were determined (Table 5). Although their viscosities were generally low, differences are found between the different species when measured under the same temperature and concentration. The EPS obtained from *D. olivaceus* exhibited the highest viscosity among the five polymers, followed in turn by *S. javanicum*, *Nostoc* sp. and *P. tenue*, whereas *M. vaginatus* appeared to be the lowest.

Molecular weight. The molecular weights of the carbohydrate containing polymers (Table 6) were determined by size exclusion chromatography using a Superose 6 column fitted into a FPLC system of Pharmacia as described in Section 2. The EPS from the *Nostoc* sp. exhibited the highest MW, followed by *M. vaginatus*, *D. olivaceus* and *P. tenue*, that showed the same molecular mass. After treatment with alkali for a possible cleavage of carbohydrate linked to protein, the MW did not change for the EPS obtained from *M. vaginatus* and *D. olivaceus*, while it was somewhat lower in the case of EPSs from *Nostoc* sp. and *P. tenue*. The molecular size of the EPS from *P. tenue* was

Table 5
Kinematic viscosity of the polymer preparation (0.1%) of the five desert soil algae at 25 ± 0.01 °C

M. vaginatus	*S. javianicum*	*P. tenue*	*D. olivaceus*	*Nostoc* sp.
0.9434 ± 0.0011	1.0278 ± 0.0028	0.967 ± 0.005	1.1474 ± 0.0009	1.0149 ± 0.0001

Means are given with standard error.

Table 6
Molecular weight (kDa) of the carbohydrate containing parts of the 1 M eluates determined by gel filtration chromatography on a Superose 6 column

	M. vaginatus	*S. javianicum*		*P. tenue*	*D. olivaceus*	*Nostoc* sp.
1.0M NaCl eluates	380	110	380	380	380	460
After β-elimination	380	63	170	310	380	380
After oxalic acid hydrolysis	n..d.	n.d.	n.d.	380	n.d.	n.d.

n.d. = not determined; Dextrans were used as standards.

not reduced after hydrolysis with oxalic acid, indicating that the arabinose is present in the polymer as pyranose units. The monosaccharide composition was also similar to that before the weak acid hydrolysis. A possible separation of the 1 M polymer from *P. tenue* was attempted by affinity chromatography on a ConA column with no success. This indicates that the polymeric material consists of covalently linked arabinose, glucose and rhamnose. The eluate of the EPS of *S. javianicum* gave two carbohydrate containing peaks by gel filtration. After a possible β-elimination, two peaks were still obtained, but they were both about half in size of the original peaks, indicating the presence of glycoproteins.

As the 1 M fractions had not been separated further, proteins were present in the EPS samples as seen from Table 1. Determination of the sizes of the proteins was performed by SDS-PAGE in homogenous slab gels followed by staining of gel with CBB (Laemnli, 1970). Table 7 reflects the concrete differences of the five species. It was clearly seen that *M. vaginatus* and *S. javanicum* gave rise to five bands, whereas *P. tenue*, *D. olivaceus* and *Nostoc* sp. only gave one. The bands were all weak and broad, indicating that the protein part was covalently linked to polydisperse carbohydrate moieties. Clear pictures of the gel were impossible to obtain due to the low solubility of the polymers in the application solutions.

Table 6 showed that EPS from *S. javanicum* gave two peaks on Superose 6 gel filtration that contained carbohydrate. β-elimination gave the same result, but with approximately half of the MW as the original. The sugar composition of the two peaks from the original EPS was determined and the results (Table 8) showed only minor differences in the carbohydrate composition of the two products, apart from the fact that 2-*O* methyl rhamnose only was present in the lowest molecular weight material.

4. Discussion

Productivity of exopolymers. Culture condition (nutrition, light and temperature etc.), growth stage, age of culture all affect the productivity of algal exopolymers (De Philippis & Vincenzini, 1998). In this study, we chose BG11 for all non-nitrogenous species, $BG11_0$ for all diazotrophic strains, collected them at the stationary phase, and the same light and temperature conditions. Thus the differences among the three non-nitrogen-fixing species and between the two nitrogen fixing species can be

Table 7
Molecular weight of the protein components of the1.0 M NaCl eluates determined by SDS-PAGE electrophoresis

Molecular weight (kDa) (approximate)	*M. vaginatus*	*S.javanicum*	*P.teune*	*D.olivaceus*	*Nostoc* sp.
>200					+
200		+			
116–200	+			+	
116		+			
97	+	+			
66–97			+		
45	+	+			
14–45	+				
14	+	+			

Table 8
Monosaccharidic composition of the two carbohydrate fractions obtained by gelfiltration of the1.0 M NaCl eluate of *S.javanicum*

	Arabinose (%)	Rhamnose (%)	Mannose (%)	Galactose (%)	Glucose (%)	2-*O*-methyl rhamnose (%)
110 kD	10.3	4.8	32.5	28.5	23.9	8.6
380 kD	8.6	2.6	25.3	19.2	44.2	n.d.

n.d = not detected.

considered as species differences. Results showed (Table 1) that among the three non-nitrogenous species *P. tenue,* which had highest affinity to the soil grains, produced the least amount of EPS, *M. vaginatus* with maximal strength produced highest amounts and *D. olivaceus* with minimal strength produced less amounts. *S. javanicum* secreted much more EPS than *Nostoc* did, both being nitrogen-fixing species (Hu et al., 2002). It was clear that the algal ability for stabilization of the sand grains was basically agreement with the productivity of their EPS. The percentages of neutral parts in the exopolymers were fully consistent with the observed order of strength in stabilization of fine sand grains (Hu et al., 2002). We suggest that further detailed analysis should include the neutral parts in order to better the understanding of the phenomenon of the ability of the organisms to bind the soil or sand grains.

Structure and cohesion. *M. vaginatus* and *S. javanicum* are two filamentous cyanobacteria with different cohesion in stabilization of unconsolidated sand grains, but their EPSs were similar both in protein content, in monosaccharides composition and linkage types. But differences do exist. First the relationship between sugar and protein is somewhat different. In the EPS from *M. vaginatus*, sugar (380 kD) and protein are tightly bound together to form proteoglycan or glycoprotein, possibly *N*-linked, whereas in *S. javanicum* there may exist some products that are *O*-linked, this linkage being alkali labile. This is only based on the determination of the change in MW. Second, the EPS from *S. javanicum* contained two carbohydrate containing fractions, of which the part having the lower molecular weight (MW 110 kD) contained all the 2-*O*-methyl rhamnose present in the EPS. SDS-PAGE also showed slight differences between these to samples. *M. vaginatus* had relatively higher amount of galacturonic acid, glucuronic acid and *N*-acetyl glucosamine than the EPS of *S. javanicum*. Methylation results indicate that the EPS from *M.vaginatus* and that of *S. javanicum* were quite similar in the linkage pattern. These structural features were closely related to their ability in stabilization of the sand soil. We also noted that the molecular sizes of the proteins from the different sources were quite different. Therefore, we suggest that the proteins and also the amino acid moiety should be further studied.

P. tenue is a special filamentous cyanobacterium with respect to stabilization of sand soil (Hu et al., 2002). It is a little weaker than *M. javanicum* in cohesion, but can stabilize sand surface at least biomass. The carbohydrate part of the EPS from *P. tenue* was mainly composed of three monomers, i.e. arabinose, glucose and rhamnose, in which arabinose constituted the highest amount of all monosaccharides, and they are all present in the pyranose form only. The glucose part form probably heptamers as shown by analysis in the Dionex system. Arabinose is present as α L-linkages while the glucose is β D-linked and accounted for 44%. This polymer is a novel type of polymer, compared to polymers from other cyanobacteria (De Philippis & Vincenzini, 1998; Flaibani et al., 1989; Helm et al., 2000; Huang et al., 1998; Sutherland, 1994). With arabinose as a highly branched structure and also in pyranose form, and the relatively high amount of rhamnose, both being more hydrophobic in nature than other monosaccharides, these structures may be closely related to the biological function of the polymer in nature. As known for most benthic cyanobacteria, the cohesion mechanism with the matrix is due to the surface hydrophobicity of exopolymers (Fattom & Shilo, 1984). This phenomenon is probably a feature of this genus, and may be this is the reason why the cohesion of *P. tenue* just next in order to *M. vaginatus* is the case among the five species we have studied.

D. olivaceus is the weakest species in stabilization of the sand grains, and its EPS also had a very special composition and structure as galactose is present both in the furanose and pyranose form. All hexoses are β D-linked, while rhamnose and arabinose are α L-linked. The protein may be bound with the sugar part as in proteglycans of *M. vaginatus*. The polysaccharides were mainly composed of galactose and glucose, but xylose and arabinose were also present in substantial amounts.

Nostoc sp. is a species with weak cohesion in stabilization of the sand grains (Hu et al., 2002), sugar and protein of the EPS were probably not bound in the same molecule, but the EPS had the highest molecular weight of both sugar part (460 kD) and protein part (>200 kD) among the five species studied. The monosaccharide composition was relatively simple, basically only four monomers. The molar ratio of glucose: galactose: xylose: 2-*O*-methylglucose was app. 2:1:1:0,5, the backbone is 1 → 4 linked galactose, xylose and glucose, with some branches on position 6 of some of the1 → 4 linked glucose molecules. All linkages are in the β configuration. The backbone of the EPS from the different strains of *Nostoc sp.* studied has much similarity; thus the structures of their EPS are also comparable. This means that the molecular weight of the EPS was not fully in agreement with the cohesion of stabilization sand soil.

In all, the five desert soil algae have interesting carbohydrate structures compared with other algae. As already noted, any correlation between chemical compositions and physical properties of polysaccharide is generally difficult to predict in the absence of information about the secondary and tertiary structure of the macromolecules. In spite of this, some relations can be seen. First their EPS contain relative high amounts of protein that may be due to the presence of high molecular weight proteoglycans. Second, partly methylated monomers were found in four of the polymers. Finally different monosaccharides and linkage types are present in the polymers, and it appears that the more the amount of variety, the stronger the stabilization of sand grains. It is also interesting to note that the EPS from *D. olivaceus* and *Nostoc* sp., that is weak in cohesion, had polymers with similarities, they have little substitutions, and similar backbones, whereas the strong species in cohesion had a higher degree of substitutions

and different structures of the main chains (*M. vaginatus* and *P. tenue*).

Viscosity and structure. Generally viscosity is positively related to molecular mass or solution concentration. In this study the results were not in agreement with this pattern. The most interesting thing is that *D. olivaceus* with the lowest content of sugar and protein had the highest viscosity, whereas *M. vaginatus* with the highest sugar and protein content exhibited the lowest viscosity. We suggest that the major reason is the difference of the polymeric structure. But viscosity behaviour can also be affected by pH, inorganic salt, charged components (i.e. amido, carboxyl acetyl). Viscosity does not depend on just the primary structure and molecular mass of the polymers, but also comprehensive tridimensional structures of the matrix and the molecular shape. Comparing with the results from Huang et al. (1998), the viscosity of the EPSs from *N. commune* and *N. flagelliforme* are a little higher than that of the EPS from *Nostoc* sp. at the same concentration and temperature. The possible reason is that their polymers had been deproteinated before measurement, but in some species the presence of protein appears to be necessary for having high viscosity values (De Philippis & Vincenzini, 1998). Therefore, it is impossible to explain the relationship of viscosity and primary structure without conformational behaviour of the repeating units.

Solubility of exopolymers. The solubility of EPS from the five species, were similar in behaviour with those from the soil algae that Flaibani et al. (1989) studied. They were all water insoluble if collected by using ethanol precipitation. If EDTA was added or extraction by heating was used, the solubility was largely improved, but the polymers could not be fully dissolved. The insolubility of these polymers in the native state may be due to their competitive affinity with metal ions (like calcium ions). Under natural environment they often grow in high salt concentration and in an alkaline biotope (pH 8–10), where calcium is the most abundant metal ion. Another important reason is that hydrophobic groups may be affected by the different extraction methods. Ethanol precipitation will affect polymer conformation, particularly dependent on the number and distribution of hydrophobic groups. In this study the polymers were not precipitated by ethanol, thus they should for this reason have retained the innate molecular structure and conformation, so they should have been easily dissolved in water solution.

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