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Exopolysaccharides of *Synechocystis aquatilis* are sulfated arabinofucans containing N-acetyl-fucosamine

Daniela Flamm*, Wolfgang Blaschek

Pharmaceutical Institute, Department of Pharmaceutical Biology, Christian-Albrechts-University of Kiel, Gutenbergstr. 76, 24118 Kiel, Germany

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ABSTRACT

Cyanobacteria are known to be a rather diverse group of organisms regarding e.g. morphology, metabolism and composition of excreted exopolysaccharides (EPS). Considering the high number of known cyanobacterial species the EPS from only a small percentage are investigated in detail. This work examined EPS from the unicellular strains of *Synechocystis aquatilis* and *S. pevalekii* with various methods. The results emphasize the heterogeneity of cyanobacterial EPS. *S. pevalekii* secrets complex hetero-polysaccharides and acidic proteins as proteoglycan-complexes whereas the protein-free but highly sulfated EPS from *S. aquatilis* only consist of 4 dominant monosaccharides. Especially remarkable is the composition of these EPS: an arabinofucan with higher amounts of N-acetyl-fucosamine (FucNAc) and only minor quantities of glucose. Both EPS and the newly found component FucNAc in EPS from *S. aquatilis* extend the possible components of cyanobacterial EPS and the knowledge of heterogeneity of cyanobacterial metabolites.

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1. Introduction

Some cyanobacteria have a long tradition as nourishment in South America, Asia and Africa (Gantar & Svirčev, 2008) and actually they become still more important as dietary supplement, food additive and functional food. Additionally, cyanobacterial exopolysaccharides (EPS) can be used for different industrial applications such as thickener, emulsifying agent or for removing heavy metal ions from solvents and soil (Pereira et al., 2009). Two important structural characteristics are essential for the functionality of EPS: ionic charge and partial hydrophobicity. The former is given by carboxylic groups of uronic acids, pyruvate or sulfate substituents (Pereira et al., 2009) or by acidic amino acids of associated proteins as examples in literature show (Flaibani, Olsen, & Painter, 1989; Marra, Palmeri, Ballio, Segre, & Slodki, 1990; Nakagawa, Takamura, & Yagi, 1987; Schrader, Drews, Golecki, & Weckesser, 1982). Parts of EPS molecules are hydrophobic due to desoxy sugars, acylation or attached protein moieties (Pereira et al., 2009). In three quarters of so far described polymers cyanobacterial EPS have a complex and diverse structure with six or more different monosaccharides. In total, 12 different monosaccharides have been reported as components of cyanobacterial EPS together with a broad range of different linkage types. Various hexoses and pentoses as ketoses and aldoses as well as desoxy, amino and methyl sugars and uronic acids were found. Often glucose is the main component. The structure of cyanobacterial EPS was investigated in detail, however, only in a few cases, although detailed knowledge of them is essential for understanding the chemical and physical properties of these polymers as well as their biological activities (De Philippis & Vincenzini, 1998).

EPS from *Nostoc commune* and *Synechocystis aquatilis* (*S. aquatilis*) had shown an interesting biological activity: inhibition of the classical pathway of the human complement system (Brüll, Huang, Thomas-Oates, & Smestad Paulsen, 2000; Volk, Venzke, Blaschek, & Alban, 2006). The human complement system is part of the innate immune system (Tudoran & Kirschfink, 2012). Defects in this complex system cause rare serious diseases, but it can also participate in the genesis of widespread diseases like asthma. Especially EPS from *S. aquatilis* showed an impressing inhibitory activity (Volk et al., 2006). Nevertheless, a detailed analysis of the structure of these EPS was still missing. Therefore the composition and fine structure of the EPS from *S. aquatilis* were investigated in detail. For comparison and for extending the knowledge of composition of cyanobacterial material the EPS from the closely related *S. pevalekii* was analyzed additionally.

Abbreviations: EPS, exopolysaccharides; FucNAc, N-acetyl-fucosamine; GLC, gas liquid chromatography; HV, hydrodynamic volume; IEC, ion exchange chromatography; MW, molecular weight; MWCO, molecular weight cut-off; *S.*, *Synechocystis*; SEC, size exclusion chromatography; TFA, trifluoroacetic acid.

* Corresponding author. Tel.: +49 431 8801145; fax: +49 431 8801102.
E-mail addresses: dflamm@pharmazie.uni-kiel.de (D. Flamm), wbla@pharmazie.uni-kiel.de (W. Blaschek).

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2. Materials and methods

2.1. Material

The cyanobacterial strains *S. aquatilis* Sauvageau SAG 90.79 (*S. aquatilis*) and *S. pevalekii* Ercegovic SAG 91.79 (*S. pevalekii*) from the "Culture Collection of Algae at Goettingen University (SAG)" were cultivated in 10-liter-wide-neck-flasks containing 8 l of mineral medium under constant aeration (1 ml/min) and constant illumination (~150 μmol/m^2 s^{-1}). *S. aquatilis* was cultivated for a period of time of up to 42 days, *S. pevalekii* of up to 63 days. The medium consisted of (mg/l): 500 KNO_3, 50 K_2HPO_4, 5.5 $Na_2EDTA{\cdot}2H_2O$, 4.0 $FeCl_3{\cdot}6H_2O$, 0.4 $MnCl_2{\cdot}4H_2O$, 0.1 $CoSO_4{\cdot}7H_2O$, 0.1 $ZnSO_4{\cdot}7H_2O$, 0.1 $Na_2MoO_4{\cdot}2H_2O$, 0.01 $CuSO_4{\cdot}5H_2O$ and 10% of "Instant Ocean" (Aquatic Systems Inc., Atlanta, USA). Axenic culture was checked routinely by microcopy. Cells were separated by centrifugation (RZB ~15,000) and extracellular proteins were removed by heat denaturation (86 °C, 15 min) and centrifugation. EPS were precipitated with ethanol (final concentration 80%), dialyzed (MWCO 12–14 kDa) against demineralized water and lyophilized.

2.2. Monosaccharide composition

After hydrolysis of ~5 mg EPS with 2.0 molar trifluoroacetic acid (TFA) for 1 h at 121 °C the monosaccharides were derivatized by reduction and acetylation as described by Blakeney, Harris, Henry, and Stone (1983). The resulting alditol acetates were analyzed by gas liquid chromatography (GLC) with flame ionization detection and by GLC-mass spectrometry (GLC-MS) with interpretation of MS-spectra using comparison with reference components (Goellner, Ichinose, Kaneko, Blaschek, & Classen, 2011a).

2.3. Determination of uronic acids and polysaccharide substituents

The content of uronic acids was determined as described by Blumenkrantz and Asboe-Hansen (1973). Acylation of polysaccharides was analyzed using the method of McComb and McCready (1957). Substitution of sugars with pyruvate groups was quantified according to Sloneker and Orentas (1962).

2.4. Elemental analysis, degree of sulfation and amino acid composition

Carbon, hydrogen, nitrogen and sulfur were quantified with a HEKAtech CHNS Analysator (Co. HEKAtech, Wegberg, Germany) by calibration with sulfanilamide using ~2 mg of EPS sample. The content of sulfate groups was calculated from the sulfur concentration using a conversion factor of 2.474. The amount of protein in EPS was calculated by multiplying the nitrogen content, reduced by the nitrogen content from amino sugars, with a conversion factor of 6.25 for *S. aquatilis* (acc. to Kjeldahl, 1883) or of 8.24 for *S. pevalekii*, based on the respective amino acid composition, determined as described by Goellner, Blaschek, and Classen (2010).

2.5. IR-spectroscopy

FT-IR spectra were recorded with a Perkin Elmer instrument (Spectrum 100 FT-IR-Spectrometer; Universal ATR Sampling Accessory).

2.6. Conductometric titration

Acidic functional groups of EPS were converted into their protonated form with Amberlite IR 120 (Co. Rohm/Haas, Philadelphia, USA) and titrated with 0.1 molar sodium hydroxide using conductometrical detection with a Seven Easy Instrument (Co. Mettler Toledo, Giessen, Germany) as described by Casu and Gennaro (1975).

2.7. Ion exchange chromatography (IEC) and size exclusion chromatography (SEC)

EPS were fractionated by IEC with Sepharose Q or DEAE Sepharose (Pharmacia Inc., 8 × 1.5 cm column). For elution different concentrations of NaCl (0–2 molar) or 5 molar urea were used stepwise. Fractions were analyzed for carbohydrate content (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956) and protein content (Starcher, 2001).

The molecular weight and hydrodynamic volume were determined by SEC on three different PL aquagel-OH columns in series (PL aquagel-OH mixed, MW range 0.1–1.000 kDa; PL aquagel-OH 40, MW range 10–200 kDa; PL aquagel-OH 20, MW range 0.1–20 kDa; in total good separation between 1 and 1.000 kDa; Polymer Laboratories, Darmstadt, Germany) as described by Goellner, Utermoehlen, Kramer, and Classen (2011b). For calibration pullulan reference compounds (5.9–788 kDa, PL Polysaccharide Standards, Varian Inc., Germany) were used.

2.8. Desulfation

Desulfation of EPS-pyridinium salts from *S. aquatilis* was performed according to Kolender and Matulewicz (2004) with 320 μl chlorotrimethylsilane per 10 mg EPS for 5.25 h at 100 °C. The desulfated EPS were dialyzed (MWCO 12–14 kDa) against demineralized water and lyophilized.

2.9. Analysis of linkage type

Genuine EPS from *S. aquatilis* were converted into their pyridinium salt. Activation and methylation of hydroxyl groups of the polysaccharides from *S. aquatilis* (~1 mg in 500 μl dry dimethyl sulfoxide (DMSO)) were obtained using a sodium hydroxide-DMSO-slurry and stepwise addition of iodomethane: 0.5 ml slurry – 0.05 ml iodomethane – 0.5 ml slurry – 0.2 ml iodomethane. First three additions were followed by a 20 min incubation time each, last addition by a 50 min incubation time. After addition of 5 ml of demineralized water, nitrogen was bubbled through the solution for 15 min and the mixture was dialyzed (MWCO 1 kDa) against demineralized water and lyophilized. The whole process was performed three times.

EPS from *S. pevalekii* were methylated as described by Harris, Henry, Blakeney, and Stone (1984) with freshly prepared dimethylsulfinyl-carbanion (dimsyl-reagent; potassium hydride in DMSO: 80 mg/ml). Samples were dissolved in DMSO (1–10 mg/460 μl) and treated three times alternating with dimsyl-reagent (40, 120 and 400 μl) and iodomethane (10, 30 and 300 μl). Reaction times were 40, 20, 40, 20, 30 and 10 min. Finally, samples were washed with a chloroform/methanol/water (2:1:2) mixture.

Further steps of hydrolysis, reduction and acetylation for methylated EPS from both strains were carried out as described by Harris et al. (1984). Samples were hydrolyzed in 1 ml 2 molar TFA for 1 h at 121 °C and dried. Reduction took place with 2 ml 0.5 molar sodium bordeuteride in 2 molar ammonia at 60 °C for 1 h and stopped by addition of 0.5 ml acetone. Dried samples were acetylated in 0.2 ml glacial acetic acid, 1 ml ethyl acetate and 0.1 ml perchloric acid with 3 ml acetic acid for 5 min at room temperature. Reaction was stopped by addition of 10 ml demineralized water and 0.2 ml 1-methylimidazole. Reaction products were extracted with 2 ml

Table 1
Monosaccharide composition of EPS from *S. aquatilis* and *S. pevalekii*. Mean value (% m/m) of 6 (*S. aquatilis*), respectively, 5 different cultivations (*S. pevalekii*).

Monosaccharide	*S. pevalekii*	*S. aquatilis*
Fucose	9.2 ± 1.4	37.0 ± 4.5
Arabinose	3.2 ± 0.3	30.2 ± 3.4
N-acetyl-fucosamine	–	18.0 ± 4.1
N-acetyl-glucosamine	10.8 ± 4.9	–
Rhamnose	7.4 ± 2.6	0.6 ± 0.5
Xylose	7.9 ± 5.5	0.1 ± 0.3
Galactose	11.4 ± 2.7	2.4 ± 2.2
Glucose	19.9 ± 3.1	10.6 ± 7.7
Mannose	21.3 ± 1.6	0.4 ± 0.4
Unknown sugar	9.1 ± 2.1	0.4 ± 0.4

dichloromethane. The partially methylated alditol acetates were analyzed by GLC-MS according to Goellner et al. (2011a).

2.10. NMR

For ^{13}C, ^{1}H and H,C-COSY NMR spectroscopy a sample of EPS from *S. aquatilis* was partially hydrolyzed in 12.5 mmolar oxalic acid for 5 h at 100 °C. Insoluble components were removed by centrifugation. Possible high molecular weight fragments were precipitated with ethanol (final concentration 80%). Ethanol-soluble fragments were dried, dissolved in D_2O and neutralized. Spectra were recorded at 75 MHz and 300 K (Advanced III 300, Co. Bruker, Rheinstett, Germany). Chemical shifts (δ, in ppm) were calculated using acetone as internal standard (31.45 ppm) and compared with literature (Bilan et al., 2007; Ciancia et al., 2007; Glushka et al., 2003; Goellner et al., 2011b; Hu, Liua, Smestad Paulsen, Petersenc, & Klaveness, 2003; Matulova et al., 2005; Vinogradov, Kaca, Knirel, Rozalski, & Kochetkov, 1989).

3. Results and discussion

3.1. Composition of polysaccharides

The EPS from *S. pevalekii* were composed of 7 different neutral monosaccharides and the amino sugar N-acetyl-glucosamine (Table 1). Hexoses were the main components in these EPS. This complexity is a common characteristic of many cyanobacterial EPS (Pereira et al., 2009). Although EPS from two other *Synechocystis* strains (*Synechocystis* sp. PCC 6714 and 6803) also showed such a complex monosaccharide composition (Panoff, Priem, Morvan, & Joset, 1988), other *Synechocystis* species (*Synechocystis* sp. BASO 444, 511, 507, 670, 672) excreted EPS consisting of less than 4 monosaccharides with glucose, xylose, arabinose and ribose as components (Ozturk & Aslim, 2009; Ozturk, Aslim, & Suludere, 2009). The EPS from *S. aquatilis* were heteropolymers containing 4 dominant monosaccharides as well (Table 1). The identified monosaccharides, however, were untypical for known cyanobacterial EPS, containing appreciable amounts of N-acetyl-fucosamine (FucNAc) besides fucose and arabinose. This amino sugar FucNAc was detectable by GLC as well as TLC and HPTLC (data not shown) and could be finally identified by GLC-MS, IR- and NMR spectroscopy as well as by comparison with an especially synthesized reference compound (Dr. A. Fekete & Prof. Dr. A. Liptak, see Acknowledgment) and a published MS-spectrum (Okutani & Tandavanitj, 1991). Thus also with common analytical methods FucNAc can be detected comparable to detection of the amino sugars glucosamine and galactosamine, which are reported components of cyanobacterial EPS (Pereira et al., 2009). Real identification of FucNAc, however, required some higher methodological efforts. In conclusion and to the best of our knowledge, FucNAc has not been reported before as a component of cyanobacterial polysaccharides and therefore can be pointed out as a new constituent in cyanobacterial EPS. Perhaps it would be beneficial to reanalyze some other cyanobacterial EPS for the presence of FucNAc.

Table 2
Contents of different substituents and protein moiety in EPS (% m/m).

Substituent and protein content	*S. pevalekii*	*S. aquatilis*
Acetyl groups	0.10 ± 0.14 (*n* = 1)	−0.05 ± 0.08 (*n* = 2)
Pyruvate groups	0.26 ± 0.41 (*n* = 3)	2.26 ± 0.49 (*n* = 4)
Sulfate groups	1.09 ± 1.53 (*n* = 4)	18.37 ± 0.97 (*n* = 5)
Uronic acids	0.46 ± 0.35 (*n* = 2)	0.88 ± 0.39 (*n* = 3)
Protein	45.28 ± 6.21 (*n* = 4)	0.96 ± 1.13 (*n* = 5)

n: number of analyzed EPS from different cultivations.

Table 3
Amino acid composition (% m/m) of the protein part of EPS from *S. pevalekii*.

Amino acid	Amount (%)	Amino acid	Amount (%)
Aspartic acid	12.9	Proline	6.2
Glutamic acid	9.0	Isoleucine	5.3
Glycine	8.5	Tyrosine	5.2
Threonine	8.3	Lysin	4.0
Leucine	8.1	Arginine	1.7
Valine	7.6	Methionine	0.4
Phenylalanine	7.5	Histidine	0.2
Alanine	7.4	Tryptophan	0.2
Serine	6.9		

In both EPS, less than 1% of the monosaccharides carried a carboxyl group (Table 2). In comparison with EPS from other cyanobacterial strains and especially with EPS from *Synechocystis* sp. minor amounts as well as concentrations of up to 89% of uronic acids were reported (Ozturk & Aslim, 2009; Ozturk et al., 2009; Panoff et al., 1988). EPS from *S. pevalekii* contained neither pyruvate nor acetate groups whereas EPS from *S. aquatilis* also contained no acylation but 2.3 ± 0.5% pyruvate groups (Table 2). Known concentrations for these two structural features in various cyanobacterial EPS are 0.3 up to 12.9% for acetyl groups and 0 up to 6.2% for pyruvate groups (De Philippis & Vincenzini, 1998; Pereira et al., 2009). Thus both investigated strains in this regard are no exceptions.

The elementary analyses showed additional individual characteristics. The EPS from *S. aquatilis* contained 18.4 ± 1.0% of sulfate groups, whereas the EPS from *S. pevalekii* were only low-sulfated (<1%) (Table 2). The highest sulfate content in cyanobacterial EPS so far known is 19.3% for *Oscillatoria corallinae* CJ1 (Gloaguen, Morvan, & Hoffmann, 1995), but also different EPS free of sulfate groups occur (Pereira et al., 2009). Therefore, EPS from *S. aquatilis* can be described as a highly sulfated polysaccharide.

EPS from *S. pevalekii* had a high concentration of nitrogen according to elemental analyses corresponding to a content of protein of 45.3 ± 6.2% (Table 2). In contrast, EPS from *S. aquatilis* only contained traces of protein (<1%). Little amounts of protein (1–3%) seem to be common for cyanobacterial EPS (De Philippis & Vincenzini, 1998), but also high amounts of protein were detected, e.g. for *Synechocystis* sp. an amount of up to 60% (Panoff et al., 1988). The protein moiety of *S. pevalekii* EPS contained high amounts of the acidic amino acids aspartic- (12.9%) and glutamic acid (9.0%) (Table 3). The presence of these acidic amino acids in EPS from *S. pevalekii* as well as the high degree of sulfation in EPS from *S. aquatilis* were proven by IR-spectroscopy and conductometric titration. A high negative charge of excreted cyanobacterial proteins had also been reported for other strains and seems to be characteristic for organisms from alcaline or high-salt habitats (Flaibani et al., 1989; Grant & Tindall, 1986; Marra et al., 1990; Nakagawa et al., 1987; Reed, 1986; Schrader et al., 1982). By no method (e.g. IEC, SEC, specific precipitation) it was possible to separate the charged protein fraction from the neutral carbohydrate moiety of

Table 4
Linkage type analysis of EPS from *S. pevalekii* arranged according to the amount (% mol/mol).

Linkage type	Amount (%)	Linkage type	Amount (%)
1,3-Glc*p*	11.6	1-Fuc*p*	3.1
1,2-Man*p*	9.9	1,3,4-Fuc*p*	2.9
1,3-Fuc*p*	8.0	1,3-GlcNAc*p*	4.3
1,4-Man/Glc*p*	4.8	1,3,x-GlcNAc*p*	3.8
1-Glc*p*	6.5	x-GlcNAc*p*	2.0
1,3-Gal*p*	6.7	1,3,4-Gal*p*	3.2
1,4-Fuc*p*	3.6	1,2-Rha*p*	2.2
1,2-Xyl*p*	0.1	1,2,6-Gal*p*	0.8
1,4-Rha*p*	4.9	1-Rha*p*	1.5
1,4-Gal*p*	2.5	1,4,6-Man*p*	1.2
1,2,6-Glc*p*	4.9	1,3,4-Glc*p*	0.6
1,3-Ara*p*	3.7	1,3,4-Man*p*	0.6
1-Gal*p*	2.9	1,2,4-Man*p*	0.7
1-Ara*p*	3.0		

x: difinite linkage type unknown.

Table 5
Linkage type analysis of EPS from *S. aquatilis*, after desulfation and partial hydrolysis of these EPS arranged according to the neutral sugars (% mol/mol).

	Amount (%)		
Linkage type	EPS pyridinium-salt	EPS desulfated	EPS partially hydrolyzed
1-Ara*f*	0.7	0.7	0.0
1-Ara*p*	0.7	0.5	2.4
1,3-Ara*p*	1.4	2.0	8.9
1,4-Ara*p*	0.0	1.4	4.3
1,3,4-Ara*p*	31.5	33.3	8.7
1-Fuc*p*	0.0	0.6	0.7
1,3-Fuc*p*	31.4	33.2	31.0
1,3,4-Fuc*p*	11.6	0.0	7.6
1,2,3-Fuc*p*	0.0	5.9	0.0
1-FucNAc*p*	19.8	20.8	16.0
1,4-hexose*p*	2.9	1.6	20.5

EPS from *S. pevalekii*. Both seem to be covalently linked, forming a proteoglycan-complex as main component in EPS from *S. pevalekii*.

The molecular weight (MW) and the hydrodynamic volume (HV) of the sodium salts of the EPS main fractions obtained by IEC were determined. EPS from *S. aquatilis* had a MW of 360 kDa and a HV of 865 kDa, whereas those of EPS from *S. pevalekii* were 570 kDa and 893 kDa, respectively. The higher HV compared to MW indicates a linear shape of both EPS molecules. Compared to literature with MWs of more than 1000 kDa for many cyanobacterial EPS (Pereira et al., 2009) these analyzed EPS molecules are rather small. Measurements with original EPS (without IEC) showed much higher MW-/HV-values (>1000 kDa) indicating the formation of agglomerates of several molecules.

3.2. Analyses of linkage type

EPS from *S. pevalekii* had a complex linkage type profile (Table 4) due to the high number of different monosaccharides. As reported earlier highly complex structures of cyanobacterial EPS complicate the analysis itself and the proposal of a structural model (Pereira et al., 2009). The results for EPS from *S. pevalekii*, at least, confirmed the predominant linearity of the polysaccharide moiety because of the high amount (62%) of linear linkage types. This predominant linearity was also observed in SEC analyses by comparison of molecular weight and hydrodynamic volume results (see Section 3.1). Additionally, it was obvious that the EPS from *S. pevalekii* almost exclusively consisted of pyranosidic monosaccharide units.

In contrast, the linkage type profile of EPS from *S. aquatilis* was less complex. Again mostly all sugars were found in their pyranosidic form. The genuine EPS, analyzed as pyridinium-salt, mainly consisted of 4 different linkage types (Table 5): 1,3,4-arabinose and 1,3,4-fucose as well as linear 1,3-fucose residues and terminal 1-FucNAc. Despite the high amount of 1,3,4-linked sugars units the molecule seems to be mainly linear according to SEC experiments (see Section 3.1). Desulfation of the EPS (residual sulfate content of 1.8%) resulted in total loss of 1,3,4-fucopyranose and some increase in 1,2,3-Fuc*p*, assumedly a side product of the desulfation by siliation via the desulfation reagent chlorotrimethylsilane. The absence of 1,4-Fuc*p* after desulfation indicated that position 4 of the 1,3,4-fucose residues were sulfated. The slight increase in 1,3-fucopyranose as well as in arabinose linkage types suggested a sulfation of both monosaccharides within the EPS from *S. aquatilis*. This was also confirmed by the molar calculation that showed a too small amount of 1,3,4-fucose for too many sulfate groups (data not shown). Partial hydrolysis of the EPS from *S. aquatilis* with oxalic acid as described for NMR-analysis (see Section 2.10) resulted in a

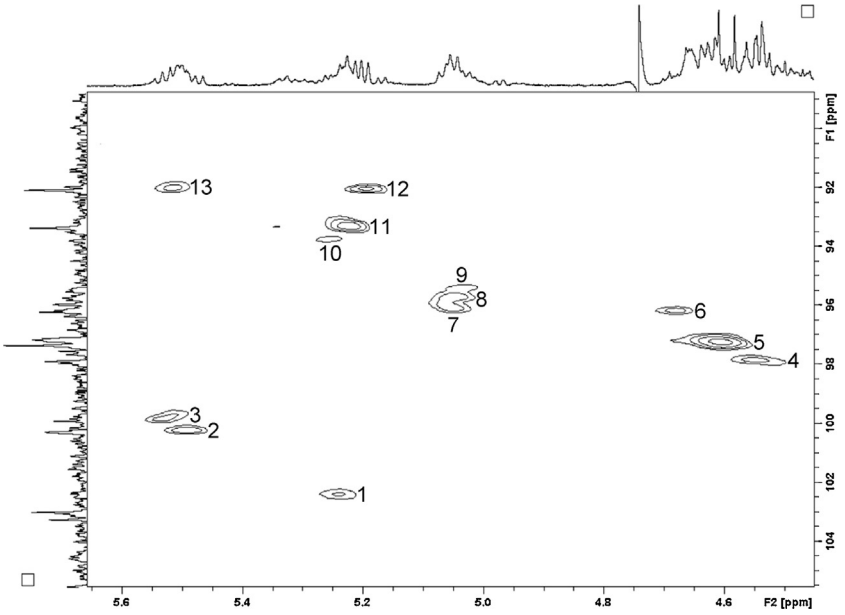

Fig. 1. Region of anomeric carbon signals in H,C-COSY-spectrum of EPS from *S. aquatilis*.

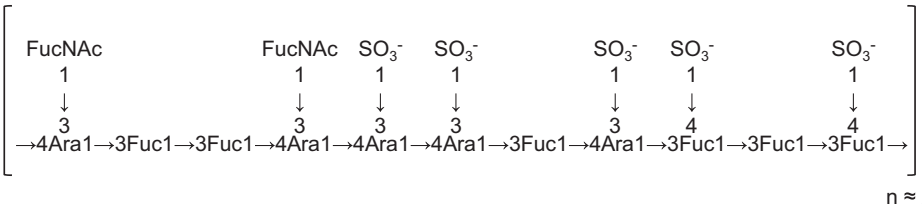

Fig. 2. Possible structural model of sulfated EPS from *S. aquatilis* with β-arabinopyranose and α-fucopyranose residues and FucNAc substitued β-arabinopyranose residues.

massive decrease in the hydrodynamic volume (<3 kDa), but only in a moderate loss of sulfate groups (remaining content of 18.4%). The strong increase in 1,4-hexose expressed some undermethylation of EPS fragments according to our experience. But more important, hydrolysis resulted in an impressing reduction of 1,3,4-Ara*p* and the formation especially of 1,3-Ara*p* and smaller amounts of 1,4-Ara*p*, indicating 1,4-linked arabinose residues as important components in the backbone of the mainly linear molecule. Branching points in the molecule not explained by the sulfation pattern could be explained by binding of single FucNAc residues in position 3 of arabinose.

NMR-analysis of EPS from *S. aquatilis* demonstrated an α-configuration for fucose as well as β-arabinose residues (δ of anomeric carbon atoms 91.95–102.38 ppm) (Fig. 1). Furthermore, the pyranosidic form of the sugar monomer and mostly all found linkage types from the methylation analyses could be confirmed. Different methods, like solvolysis for desulfation (data not shown), caused a partial hydrolysis of the polymer to a hydrodynamic volume of about 3 kDa or a degree of polymerization of ~13. Besides this, NMR-analysis of the polymer hydrolyzed with oxalic acid showed 13 signals of anomeric carbons. In conclusion, these results points to a repeating unit of approximately 13 sugars for the EPS from *S. aquatilis*.

All these results of linkage type and NMR analyses of EPS from *S. aquatilis* led to the proposed structure model shown in Fig. 2. The basic structure of the molecule, linear with side chains of only single sugar units, is most likely similar to that of *Oscillatoria planktothrix* FP1 (Silipo et al., 2010). Of greater interest, however, is the structural accordance in linearity, linkage types of fucose residues, and the sulfation pattern of the EPS from *S. aquatilis* with fucoidan, a class of polysaccharide from brown algae (Li, Lu, Wie, & Zhao, 2008) and a pronounced biological activity of both molecules in the human complement system (Li et al., 2008; Volk et al., 2006).

4. Conclusion

Cyanobacterial EPS are known to be often acidic polymers (Pereira et al., 2009). EPS can complex ions from the habitat by negatively charged functional groups and thus stabilize the slimy layer especially in alkaline surroundings (Grant & Tindall, 1986; Reed, 1986). The EPS from *S. pevalekii* are of negative charge due to the high content of aspartic and glutamic acid in the protein part of its proteoglycan-complexes whereas the EPS from *S. aquatilis* are highly sulfated. Although the evolutionary aim may be the same, the two strains use different ways to achieve it. Interestingly, EPS from the cyanobacterial genus *Synechocystis* are known to secrete rather diverse polymers ranging from glucans with a high amount of uronic acids to complex hetero-polysaccharides with up to seven different monomers (Ozturk & Aslim, 2009; Ozturk et al., 2009; Panoff et al., 1988) and highly sulfated arabinofucans. The present results underline the heterogeneity of EPS: complex polysaccharides from *S. pevalekii* and simpler but so far unknown composed EPS from *S. aquatilis*. Summarizing the new components of this EPS and the enlarged and confirmed composition compared to Volk et al. (2006), three characteristics must be pointed out. Firstly, as already mentioned, the EPS are highly sulfated. Secondly, FucNAc was found for the first time in cyanobacterial polysaccharides, and, finally, an arabinofucan without higher amounts of glucose had never been reported before for cyanobacterial EPS. Merely *Phomidium tenue* showed a similar composition of neutral sugars – but with glucose as second most common sugar, low amounts of fucose only and without FucNAc (Hu et al., 2003). These results for EPS from *S. aquatilis* and *S. pevalekii* extend the knowledge of possible components of cyanobacterial EPS and their structure.

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