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Structural and functional characterization of galactooligosaccharides in *Nostoc commune*: β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_2$-β-D-1,4-anhydrogalactitol and β-(1→6)-galactofuranosylated homologues

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Abstract—A new class of galactooligosaccharides has been identified from the terrestrial cyanobacterium *Nostoc commune* by MS and NMR techniques. These consist of β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_n$-β-D-1,4-anhydrogalactitols with *n* ranging from 2 to 8, corresponding to compounds designated **1** through **7**. In total these saccharides amounted to ~0.35% of the dry thallus of *N. commune*, while in several other cyanobacteria they were not detected. Possibly they play some role in protection from damage by heat and desiccation as suggested by experiments with heterologous systems. For example, phosphoglucomutase (EC 2.7.5.1) from rabbit muscle was protected against heat inactivation by these oligosaccharides, and α-amylase (EC 3.2.1.1) from porcine pancreas by the oligosaccharides **6** and **7**. The homologues of lower molecular mass, however, enhanced heat sensitivity of α-amylase. The viability of *Escherichia coli* was completely abolished by desiccation, whereas in the presence of **4** survival rates were ~50% of controls not subjected to desiccation. The newly identified saccharides are compared with known galactofuranose-based oligo- and polysaccharides and possible biological functions of them are discussed.

Keywords: Oligosaccharides; Galactofuranose; D-1,4-Anhydrogalactitol; *Nostoc commune*; Enzymes; Heat protection; *Escherichia coli*; Desiccation

1. Introduction

Oligo- or polymers of galactofuranose, often representing constituents of complex macromolecules, are known to occur in bacteria,[1–4] fungi[5–12] and in a green alga.[13] In the pathogens *Mycobacterium tuberculosis* and *Nocardia* sp., they are essential cell-wall components,[3,4] and as a consequence the metabolism of galactofuranose has become a promising target for drug design.[14] Galactofuranans from fungi, for example, from *Penicillium* and *Aspergillus*, may also be contained in the cell wall[6–8,11,12,15] or may appear as excretion products in the growth medium,[5,10] amongst them carolose,[5] to our knowledge the first galactofuranose oligosaccharide described. These saccharides seem to be responsible for the high immunogenicity of cell-wall antigens from pathogenic fungi[12,16] and for toxic effects of phytopathogens.[10]

The glycosidic linkages of galactofuranose units in bacterial cell walls are either β-(1→5) throughout,[1,3] as in the green alga *Trebouxia*,[13] or varied linkage types within the saccharide chain.[2,3] A similar situation is met also with fungal sources; but in addition, cases exist here with the sugar moieties connected exclusively β-(1→6) within the main chain.[7,9,11] Use has been made of the different linkage types found in fungi for chemotaxonomic purposes.[9,11,15]

In this work we report on galactooligosaccharides of the cosmopolitan cyanobacterium *Nostoc commune* forming a thallus consisting of gelatinous aggregations

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of filaments. Analysis of aqueous thallus extracts by NMR spectroscopy revealed a homologous series of β-(1→6)-linked D-galactofuranosides with a terminal 1,4-D-anhydrogalactitol, representing a new group of naturally occurring oligosaccharides. Data on their contents in the thallus are presented, and, in context with their possible ecological relevance, experiments were carried out on their influence on the thermosensitivity of enzymes and on the desiccation tolerance of *Escherichia coli*.

2. Results

2.1. Identification of galactooligosaccharides in *N. commune*

Two successive chromatographic separations yielded novel galactooligosaccharides from aqueous thallus extracts of *N. commune*. In a first step, the extract was fractionated by gel filtration and then, in a second step, each of the fractions were subjected to TLC and stained for sugars. Figure 1 shows the thin-layer chromatogram of those fractions which, as expected from calibration runs of the gel-filtration system, should contain material of relative molecular mass $\geqslant 1000$. Surprisingly, saccharides migrated as fast as the much smaller reference sugars glucose, maltose and maltotriose. The pattern of spots suggested the presence of a series of saccharides that differ by a stepwise increase in molecular mass. Starting with the apparently smallest compound (Fig. 1, fractions 48 and 49), the members of the series were numbered **1** through **7**, proceeding towards greater molecular mass. Generally, preparations of **1** through **4** were done using gel filtration followed by TLC. Compounds **5** through **7**, poorly separable by TLC, had to be purified by repeated gel filtration on appropriate column materials (see Section 4.3.2).

In the first orienting experiments, hydrolyses of some of the unknown saccharides with trifluoroacetic acid yielded galactose as the sole sugar released as indicated by TLC. More specific enzymatic tests with β-D-galactose dehydrogenase proved its identity as D-galactose. A further product of hydrolysis was an aglycon of relatively high TLC mobility ($R_{Gal} = 1.55$), which was not detectable by the routinely used diphenylamine-based spray for sugars but was stained by alkaline $KMnO_4$.

The saccharide series was subjected to mass spectrometry. The high-resolution ESI peaks listed in Table 1 are consistent with a homologous series of hexoses with a degree of oligomerization between 3 and 9, each oligosaccharide being attached to a single anhydrohexitol aglycon.

The ^{1}H NMR spectra of D-1,4-anhydrogalactitol, of the shortest member of the homologous series (**1**), of compound **2**, and of the longest member of the series (**7**) are shown for comparison in Figure 2. Already the shorter saccharides exhibited severe signal overlap in

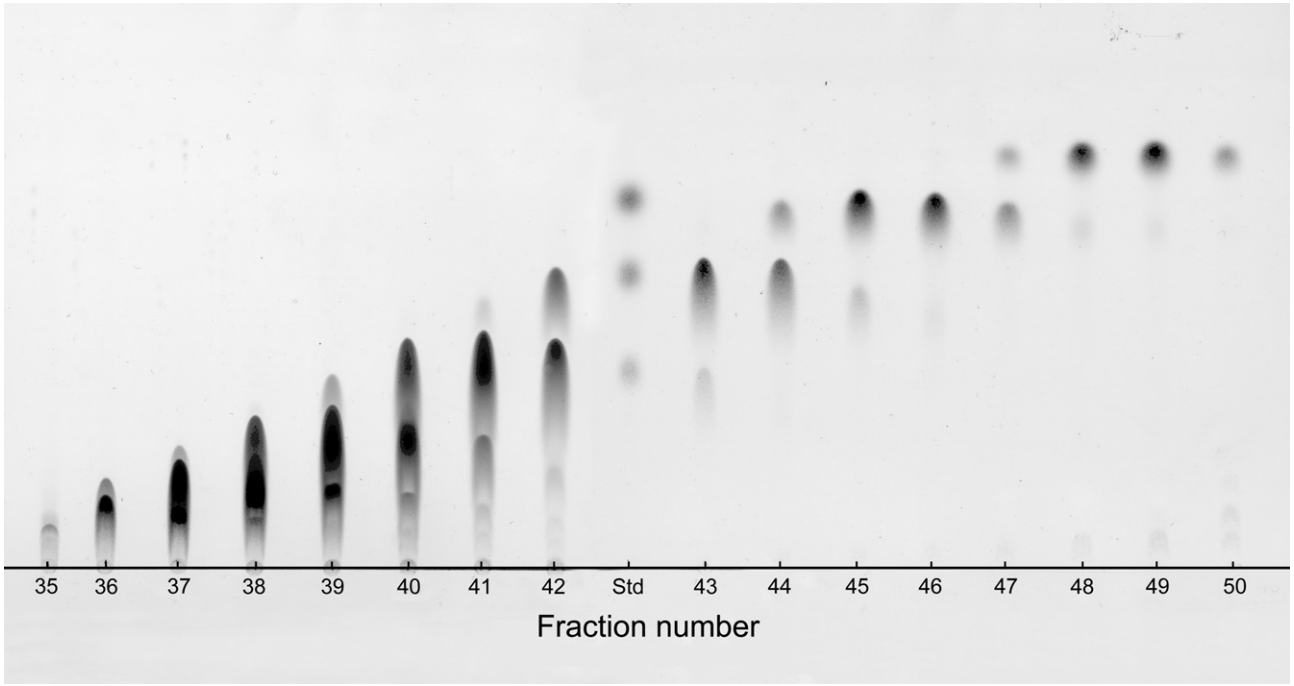

Figure 1. TLC of fractions obtained from a gel-filtration run of an aqueous extract of *N. commune*. An extract of 37 g dried thalli had been separated on a Bio-Gel P-2 column (bed length 133 cm); in calibration runs, fractions 42/43 and 47 corresponded to the reference substances, vitamin B_{12} (M_r 1255) and maltoheptaose (M_r 1152), respectively. Fractions were reduced in volume from 7.0 mL to 300 μL, and aliquots of 2 μL were spotted for TLC. Std: reference sugars glucose, maltose and maltotriose (top to bottom).

Table 1. HRESIMS data of the galactooligosaccharides **1**–**7**

	Formula	Calcd for [M+Na$^+$]	Found for [M+Na$^+$]
1	$C_{24}H_{42}O_{20}$	673.2162	673.2152
2	$C_{30}H_{52}O_{25}$	835.2690	835.2673
3	$C_{36}H_{62}O_{30}$	997.3218	997.3228
4	$C_{42}H_{72}O_{35}$	1159.3746	1159.3745
5	$C_{48}H_{82}O_{40}$	1321.4275	1321.4320
6	$C_{54}H_{92}O_{45}$	1483.4803	1483.4821
7	$C_{60}H_{102}2O_{50}$	1645.5331	1645.5395

their ^{1}H NMR spectra. Only three resonances were completely resolved even at 600 MHz for compound **2**. All multiplet intensities measured multiples of one as expected for a non-reducing oligosaccharide with a single anomeric configuration. The multiplet at 5.07 ppm (107.7 ppm ^{13}C) contained all anomeric hydrogens and the Lorentz-to-Gauss transformation estimated an anomeric coupling of less than 2 Hz. Furanosidic rings were identified from the ring ^3J coupling constants (Table 2) and the deshielded C-4 signal at 83 ppm in the ^{13}C NMR spectrum. The ^{13}C resonances were assigned from inverse CH-correlations (Fig. 3). The (1→6)-Gal*f* glycosidic linkage was proven by the C-1–H-6 cross peak in the HMBC spectrum. The β-anomeric configuration caused the intense H-1–H-3 NOE contact in the ROESY spectrum, while the H-1–H-4 NOE contact was nearly absent. The anomeric ^{1}H and ^{13}C chemical shifts are consistent with the literature values for (1→6)-β-Gal*f*.[17] The complete assignment is shown in Table 2. Mass and NMR spectra of the higher homologues up to **7** are consistent with the structure shown in Figure 2, bottom. The well-separated H-2 of

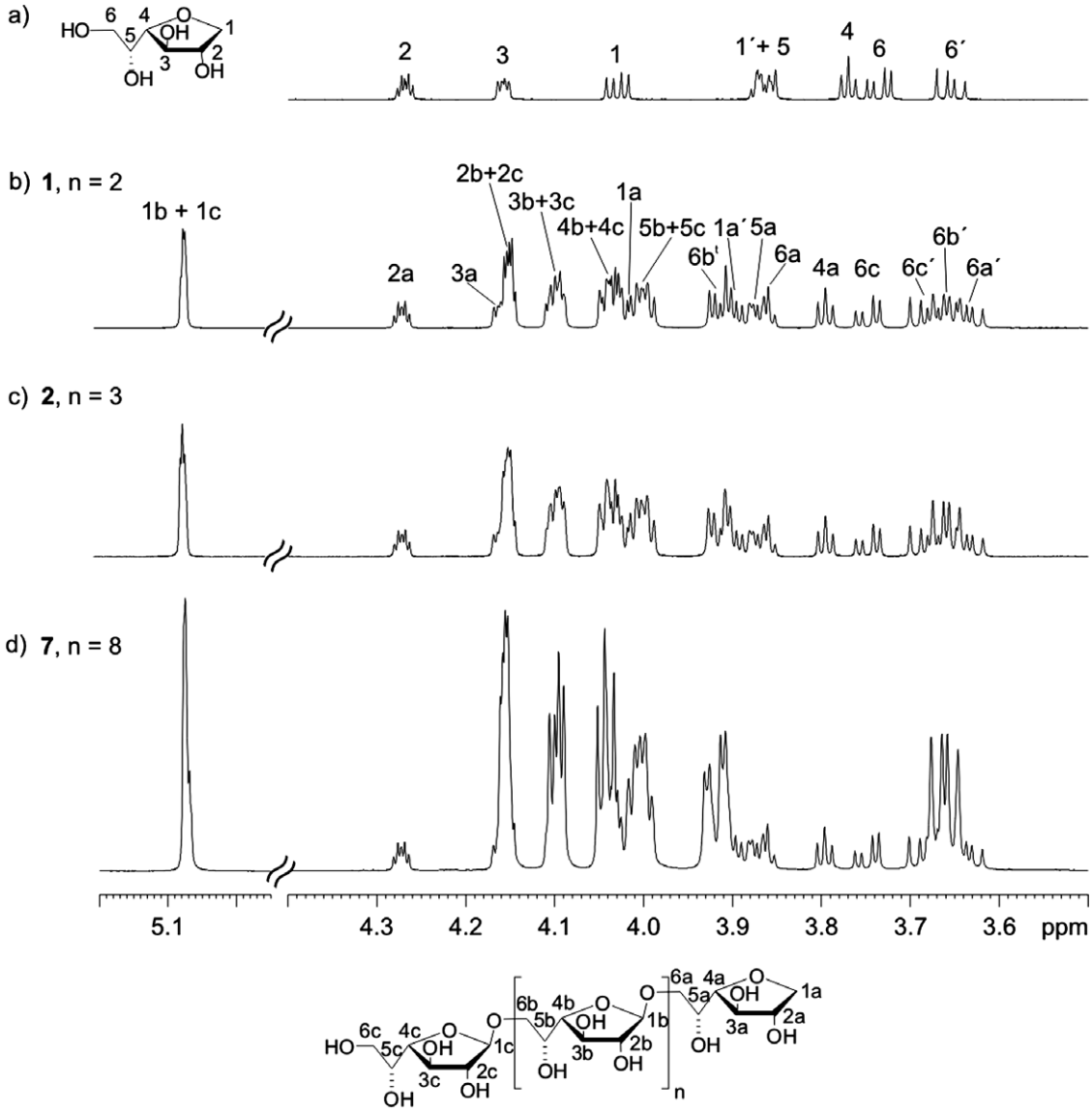

Figure 2. ^{1}H NMR spectra of (a) D-1,4-anhydrogalactitol, (b) **1**, (c) **2** and (d) **7** from *N. commune* at 600 MHz (D_2O, 300 K). Protons have been labelled according to the numbering of the chemical structure given below the spectra.

Table 2. ^{1}H and ^{13}C NMR data[a] of β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_3$-β-D-1,4-anhydrogalactitol (**2**)

	a		b		c	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
H1 ($J_{1,2}$)	4.03(4.7)	72.2	5.07(2.1)	107.7	5.07(2.1)	107.7
H1′ ($J_{1',2}J_{1,1}$)	3.87(2.7, 9.6)	72.2	—	—		
H2 ($J_{2,3}$)	4.27(2.7)	76.8	4.15(n.d.)	80.9	4.15(n.d.)	80.9
H3 ($J_{3,4}$)	4.15(5.0)	78.5	4.10(n.d.)	76.8	4.10(n.d.)	76.8
H4 ($J_{4,5}$)	3.80(5.0)	83.6	4.04(3.6)	83.0	4.04(3.6)	83.0
H5 ($J_{5,6'}$)	3.86(7.0)	70.3	4.00(7.2)	69.6	4.00(7.3)	69.6
H6 ($J_{5,6}$)	3.90(4.5)	68.6	3.91(3.6)	68.6	3.75(4.5)	62.2
H6′ ($J_{6,6'}$)	3.66(10.8)	68.6	3.63(10.9)	68.6	3.68(11.8)	62.2

[a] 600 MHz, D_2O, 300 K; δ ppm (J = Hz).

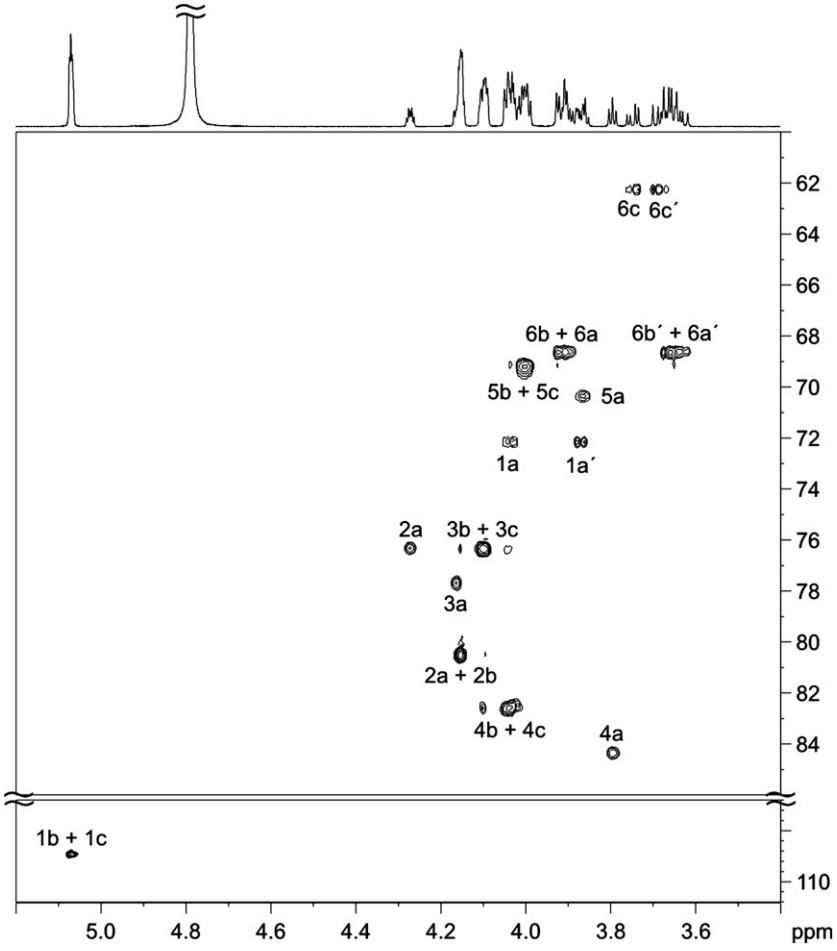

Figure 3. Selected regions of the HSQC spectra of **2**. Signals have been labelled according to the numbering of the chemical structure given below Figure 2.

D-1,4-anhydroglactitol (4.27 ppm) identified the spin system of the aglycon in the TOCSY spectrum. The H-6 protons were shifted upfield towards values identical for the oligo-β-(1→6)-Gal*f* repeating unit. The terminal methylene group of the non-reducing terminus was again separated. Only homooligomers with a well-defined secondary structure are expected to exhibit separated chemical shifts for each building block.[18] The rotable methylene linkage, together with the ring puckering, results in extensive conformational averaging of the title saccharides. The severe signal overlap is a consequence of the largely averaged chemical environment for each monosaccharide building block.

2.2. Composition and content of galactooligosaccharides in *N. commune*; occurrence in other cyanobacteria

The newly identified oligosaccharides were quantified by Dionex anion-exchange chromatography of aqueous

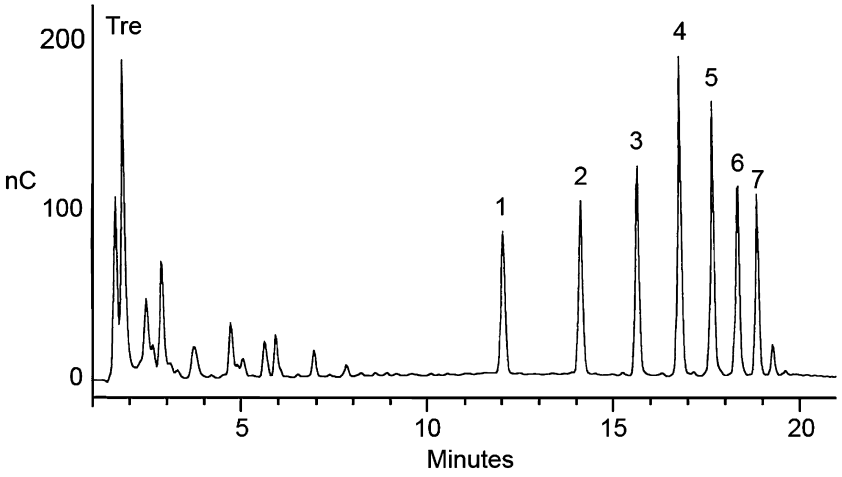

Figure 4. Dionex anion-exchange chromatography of an aqueous, desalted extract of *N. commune*. On top of peaks are indicated the locations of the reference saccharides trehalose (Tre), β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_2$-β-D-1,4-anhydrogalactitol (**1**) and β-(1→6)-galactofuranosylated homologues up to **7**.

thallus extracts. Except for a large peak assignable to trehalose, the elution profiles generally were dominated by the series of galactooligosaccharides, **4** and **5** being most abundant (Fig. 4). The whole series represented about 50% of all detected material. In Table 3, contents in the thallus are listed for each of these oligosaccharides, which in total amounted to ~0.35% of the thallus dry weight.

Besides for *N. commune* collected in the field, analyses with the Dionex system were extended to cyanobacteria grown in liquid medium. For two strains of *N. commune* (SAG 1453-3 and 1453-5), evidence for the occurrence of the galactooligosaccharide series was obtained, showing a relatively high abundance of **3**, whereas **1** was missing (data not shown). From the total of saccharides, however, they comprised only a minor fraction (<8%). For *Nostoc ellipsosporum*, *Anabaena variabilis*, *Scytonema* sp., *Synechocystis* sp., *Oscillatoria chalybea* and *Oscillatoria limosa*, the corresponding saccharide peaks were not detected at all. The occurrence of Gal*f*-oligosaccharides in cyanobacteria, therefore, seems to be restricted to *N. commune*.

Table 3. Contents of galactooligosaccharides[a] and trehalose in the thallus of *N. commune*

Saccharide	Content[b] of saccharide [mg (g thallus dry weight)$^{-1}$]
1	0.37 ± 0.10
2	0.41 ± 0.12
3	0.48 ± 0.14
4	0.69 ± 0.17
5	0.62 ± 0.16
6	0.53 ± 0.12
7	0.41 ± 0.10
Trehalose	0.98 ± 0.21

[a] β-D-Galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_2$-β-D-1,4-anhydrogalactitol (**1**) and its β-(1→6)-galactofuranosylated homologues up to β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_8$-β-D-1,4-anhydrogalactitol (**7**).

[b] Average values ± SEM from Dionex column runs of three independent preparations of aqueous *Nostoc* extract.

2.3. Effect of *Nostoc* galactooligosaccharides on activity of heat-treated enzymes and on viability of desiccated *E. coli* cells

Fructosylated kojioligosaccharides found in several cyanobacteria have been reported to protect α-amylase from heat damage;[19] this stimulated us to look for thermoprotective properties of the *Nostoc* galactooligosaccharides. To this end enzymes were preincubated at elevated temperatures in the presence of saccharides and then tested for activity. In preliminary experiments with hexokinase and glucose-6-phosphate dehydrogenase, no significant action of the *Nostoc* saccharides could be seen; with phosphoglucomutase and α-amylase, however, distinct effects were observed. Phosphoglucomutase was protected from heat inactivation, especially by oligosaccharides **4** and **5** (Table 4); at the highest preincubation temperature (57 °C) these were superior to even trehalose, a well-known heat protectant.[20] With α-amylase, the saccharides showed surprisingly differing actions, depending on chain length. Compounds **6** and **7** proved to be very efficient thermoprotectants, similar to sucrose; the homologues of shorter chain length, however, which were hardly detrimental when preincubated with the enzyme at 0 °C, exerted a strong inhibitory action when present at elevated temperatures of preincubation (Table 5). This discontinuous behaviour upon passing a critical temperature was well reproducible but must remain unexplained.

N. commune is an organism able to survive in the anhydrobiotic state,[21] and perhaps this is facilitated by

Table 4. Activity of phosphoglucomutase after preincubation at various temperatures in the presence of galactooligosaccharides[a] from *N. commune* and of trehalose

Saccharide added	Preincubation temperature[b]		
	0 °C	53 °C	57 °C
	Activity (%)[c]		
None	100.0	44.8	10.5
2	98.8	66.8	41.4
3	77.2	65.9	57.3
4	83.2	71.6	69.4
5	84.5	69.8	64.2
6	100.9	60.8	19.3
7	91.8	54.7	17.8
Trehalose	102.0	62.9	19.4

[a] β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_3$-β-D-1,4-anhydrogalactitol (**2**) and its β-(1→6)-galactofuranosylated homologues up to β-D-galactofuranosyl-(1→-6)-[β-D-galactofuranosyl-(1→6)]$_8$-β-D-1,4-anhydrogalactitol (**7**).

[b] For preincubation, phosphoglucomutase dissolved in 0.36 M ammonium sulfate containing 5% (w/v) saccharide was filled in 2-μL capillaries and enclosed in 1.5-mL polypropylene reaction vessels. The vessels were kept submerged in a water bath for 30 min at the indicated temperatures and put on ice. The contents of the capillaries were injected into prepared 1-mL cuvettes to start the phosphoglucomutase assay.

[c] Activity of 100% corresponded to the formation of 0.037 μmol glucose-6-phosphate · min^{-1}. The data are mean values from 2–6 experiments SEM being 8.2 percent units or less.

Table 5. Activity of α-amylase after preincubation at various temperatures in the presence of galactooligosaccharides[a] from *N. commune* and of sucrose

Saccharide added	Preincubation temperature[b]			
	0 °C	53 °C	56 °C	59 °C
	Activity (%)[c]			
None	100.0	74.9	44.7	6.5
2	94.8	56.1	12.9	0.5
3	86.3	27.1	0.3	0.1
5	91.7	27.6	1.8	0.4
6	96.9	77.8	61.8	26.1
7	97.9	80.6	62.3	23.5
Sucrose	96.1	85.5	64.3	27.1

[a] β-D-galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_3$-β-D-1,4-anhydrogalactitol (**2**) and its β-(1→6)-galactofuranosylated homologues up to β-D-galactofuranosyl-(1→-6)-[β-D-galactofuranosyl-(1→6)]$_8$-β-D-1,4-anhydrogalactitol (**7**).

[b] α-Amylase dissolved in 10 mM imidazole/HCl, pH 6.8, containing 2.5 mM NaCl, 0.5 mM $CaCl_2$, 0.01% NaN_3 (w/v), 0.3% BSA (w/v) and 5% saccharide (w/v) was filled in 2-μL capillaries and preincubated analogous as described for phosphoglucomutase (Table 2).

[c] Activity of 100% corresponded to the formation of 0.94 μmol maltose equiv h^{-1}. The data are mean values from 2 to 6 experiments SEM amounting to 5.4 percent units or less.

the presence of its oligosaccharides. This idea seems plausible since for *E. coli* increased rates of survival upon drying have been found when the solutes sucrose or trehalose had been added,[22,23] and furthermore when high intracellular levels of trehalose had been induced.[24] To test whether the *Nostoc* galactooligosaccharides in any way might be of importance in desiccation tolerance, cell suspensions of *E. coli* were dried in the absence and presence of oligosaccharide **4** and tested after five days for viability. At the highest concentration investigated, the saccharide had a preserving function similar to trehalose that ranged between that of sucrose and trehalose (Table 6). The maximum percentage of survival (~50%) achieved with **4** compares well to the highest reported rates[25,26] which, however, pertain to longer storage times (up to six weeks) not tested here.

Table 6. Survival of *E. coli* after 5 days of desiccation in the absence and presence of various saccharides

Saccharide added	Survival (% of control without desiccation)[a]		
None	0.0 ± 0		
	Concentration of saccharide added (%, w/v)		
	20	10	5
4[b]	52.5 ± 9.7	29.9 ± 2.7	10.4 ± 4.4
Trehalose	53.3 ± 9.5	13.3 ± 2.3	3.0 ± 1.5
Sucrose	72.6 ± 8.9	52.7 ± 12.2	22.1 ± 7.0

[a] Control corresponds to colony counts obtained with freshly harvested *E. coli*, without addition of saccharides. The data are average values ± SEM from 4 to 5 experiments.

[b] β-D-Galactofuranosyl-(1→6)-[β-D-galactofuranosyl-(1→6)]$_5$-β-D-1,4-anhydrogalactitol.

3. Discussion

The oligosaccharides identified in the present study are characterized by a chain of variable length made up of β-(1→6)-linked D-galactofuranoses with a terminal β-(1→6)-linked D-1,4-anhydrogalactitol. Partial structures of these oligosaccharides have become known through chemical syntheses or by isolation from natural sources. 1,4-Anhydrogalactitol has been synthesized,[27,28] as well as the β-(1→6)-linked galactofuranosyl hexasaccharide.[29] In nature, to the best of our knowledge, D-1,4-anhydrogalactitol has not been encountered yet nor have free oligosaccharides with β-(1→6)-linked galactofuranose units. Saccharides of the latter type, however, have been reported as building blocks of fungal cell walls, forming, for example, a saccharide backbone in a complex glycoprotein[7] or a polysaccharide strand of high molecular mass in *Fusarium*.[11]

The galactooligosaccharides of *N. commune* are reminiscent of those excreted by *Penicillium charlesii* since these are of comparable size and are made up also of galactofuranoses. These saccharides, however, have β-(1→5) linked units, and, they are extracellular products. In *N. commune*, the location should be intracellular since leaching of thalli with water for 24 h did not result in

significant carbohydrate release (data not shown). Taken together, it becomes evident that *N. commune* contains unique galactofuranose oligosaccharides defined by the combined characters of size, linkage type, aglycon and intracellular location. Thus, these compounds extend the array of cyanobacterial carbohydrates of low-molecular-mass, so far known to consist of trehalose, sucrose, glucosylglycerol and fructosylated kojioligosaccharides.[19]

What could be the biological functions of the galactooligosaccharides in *N. commune*? The drastic changes in temperature and hydration to which this cyanobacterium is subjected in nature suggest a preserving function against these stresses, especially in view of known protective properties of solutes against desiccation[22,24] and heat damage.[19,20] In agreement with such an idea is the much lower content of these saccharides in cells kept in liquid culture at moderate temperatures as compared to field material that is exposed to environmental stress. A further hint is the preventive action of the *Nostoc* galactooligosaccharides against desiccation damage in *E. coli* and against heat inactivation of a phosphoglucomutase (see Section 2.3). Definite proof for their relevance in coping with physicochemical stresses should be obtained through studies with knock-out mutants for their biosynthesis.

4. Experimental

4.1. Organisms and growth conditions

Field materials of *N. commune* were collected in the vicinity of Regensburg, Germany. From *Sammlung von Algenkulturen* (SAG, Göttingen, Germany) were obtained two strains of *N. commune* (SAG 1453-3 and SAG 1453-5), *N. ellipsosporum* (SAG 1453-7), *Oscillatoria chalybea* (SAG 1459-2) and *O. limosa* (SAG 42.87). Sources of *A. variabilis*, *Scytonema* sp. and *Synechocystis* sp. are listed in a previous study.[19] *Synechocystis* sp. and the *Oscillatoria* species were grown in BG11 medium,[30] and the *Nostoc* strains and *A. variabilis* in BG11 medium with $NaNO_3$ replaced by 1 mM NaCl. Further culture conditions are described elsewhere.[31] *E. coli* DH5α was grown to early stationary phase in Luria–Bertani (LB) broth with NaCl concentration increased to 0.77 M. Cells were harvested by centrifugation, resuspended in 1% NaCl (w/v) to a cell density of about 9.2×10^8 CFU mL^{-1} and kept on ice until used for drying experiments.

4.2. Chromatographic techniques

For gel filtration, materials Bio-Gel P-2 and P-4 (Bio-Rad, Hercules, CA, USA) were used with columns and elution conditions according to Fischer et al.[19] TLC was carried out as previously,[19] except that for visualization of D-1,4-anhydrogalactitol alkaline $KMnO_4$ was used. High-performance anionic-exchange chromatography (Dionex) was performed as described by Fischer et al.[19] with the elution programme modified to a gradient from 128 mM NaOH, 75 mM NaOAc to 15 mM NaOH, 450 mM NaOAc in the course of 20 min.

4.3. Isolation of oligosaccharides

4.3.1. Preparation of aq extracts of cyanobacteria. Thalli of free-living *N. commune* were washed several times with tap water to remove adhering earth and plant debris, then twice with deionized water. The thalli finally were then spread out for drying. The dried material (typically batches of 20 g) was added to a 20-fold amount of boiling water, brought back to boiling, and further extracted for 10 min in a boiling water bath. The slurry was poured onto a sieve, the swollen thalli were gently pressed, and the resultant extract was centrifuged for 20 min at 27,000*g*. The supernatant was treated with a mixed-bed and an anion exchanger (Serdolit MB and DEAE52-Cellulose Servacel, respectively; Serva, Heidelberg, Germany) to remove salts and coloured material. After reduction in volume (to 1:30) under reduced pressure, followed by one or two freezing–thawing cycles, further insoluble material appeared and was removed by centrifugation and filtration. From cultured cyanobacteria, aq extracts were obtained as given by Fischer et al.[19] Finally, the extracts were subjected to ultrafiltration through membranes with cut-off >3 kDa (Pall Life Sciences, Ann Arbor, MI, USA). To check for artifacts due to the extraction by hot water, a protocol was tried using organic solvents at −20 °C, followed by phase separation at 20–22 °C.[32] This procedure produced no significant difference in saccharide patterns as analyzed by gel filtration and TLC.

4.3.2. Preparation of oligosaccharides. Oligosaccharides from the aq extract of *N. commune* were isolated by gel filtration and TLC analogously to the procedure described in detail for fructosylated kojioligosaccharides.[19] The aq extract of *N. commune* was applied to a gel-filtration column (Bio-Gel P-2, bed length 133 cm) and eluted with water. Fractions were lyophilized, and, for a preliminary screening, aliquots were analyzed by TLC on silica gel sheets. After staining, the fractions proving relevant for preparation of individual oligosaccharides were pooled and completely subjected to TLC. After elution of the respective bands from the sheets with 50% (v/v) EtOH, drying under reduced pressure and dissolving in water, residual colloidal silica gel was removed by centrifugation (150,000*g*, 1 h), and the supernatant was applied to a smaller gel-filtration column (Bio-Gel P-2, bed length 108 cm) that was eluted with water. The peak fractions of purified

saccharide were detected by testing for carbohydrate with the phenol–sulfuric acid method. With these procedures, separation of compounds **1** through **4** (partially also **5)** was possible. For isolation of **5** through **7**, the first run on the Bio-Gel P-2 column fractions were pooled containing saccharides greater than **4**, reduced in volume and separated on a Bio-Gel P-4 column (bed length 191 cm) with water as eluent. Fractions were collected and tested for carbohydrate. The relevant fractions of each peak were pooled, reduced in volume, and, for a last purification step, loaded on the Bio-Gel P-4 column operated as previously. With the procedures described, average yields of lyophilized material of **1** through **7** were 0.14, 0.22, 0.27, 0.61, 0.36, 0.48 and 0.52 mg (g thallus dry weight)$^{-1}$, respectively. In analyses with the Dionex anion-exchange system (see Section 4.2) impurities were found totalling $\leqslant$11% of the preparations' saccharide content.

4.4. Preparation of the aglycon (D-1,4-anhydrogalactitol) from a *Nostoc* galactooligosaccharide

A total of 10 mg of **4** were hydrolyzed in 1 mL of 0.5 M trifluoroacetic acid for 1 h at 95 °C. Upon cooling 3 mL of MeOH were added, and the acid was removed by a stream of N_2 with several cycles of evaporation and MeOH addition. D-1,4-Anhydrogalactitol was then isolated from the hydrolysate by TLC, followed by gel filtration on Bio-Gel P-2.

4.5. Structural identification

A total of 4.45 mg of **2** were dissolved in 0.6 mL of D_2O. All NMR spectra were calibrated to acetone, which was added in trace amounts as internal standard. Pulsed-gradient NMR measurements were recorded at 600 MHz (^{1}H). The aglycon, D-1,4-anhydrogalactitol, **1**, **2** and **7** were characterized by homo- and heteronuclear NMR spectroscopy. The ^{1}H NMR data of D-1,4-anhydrogalactitol were consistent with the literature values.[27]

4.6. Measurement of enzymatic activities

β-D-Galactose dehydrogenase was used from the test combination of lactose/D-galactose supplied by R-Biopharm AG, Darmstadt, Germany. Phosphoglucomutase was purchased from Boehringer, Mannheim, Germany, and was assayed at 29 °C by glucose-6-phosphate-dependent NADP reduction in a coupled optical test according to Bergmeyer et al.[33] Care was taken to ensure linear kinetics and proportionality between activity and enzyme concentration. The activity of α-amylase from porcine pancreas (Boehringer, Mannheim, Germany) was measured spectroscopically via liberation of coloured fragments from the insoluble substrate Amylose-Remazol Brilliant Blue R.[34] Details of the procedure have been described elsewhere.[19]

4.7. Drying of *E. coli* and determination of viability

All operations were carried out between 20 and 23 °C unless indicated otherwise; 10 μL of oligosaccharide solution or water, containing 1.5% (w/v) polyvinylpyrrolidone (Type K30; Roth, Karlsruhe, Germany) were mixed with 1 μL of bacterial suspension in a concave recess (1.5 cm diameter) of a microscopic slide and were covered with a coverslip, without contact to the suspension. After incubation for 1 h the coverslip was removed, and the slide was transferred to a desiccator, which was evacuated for 1 h in the dark with P_2O_5 as desiccant. After storage under vacuum at 4 °C for 5 days, the slide was taken out and kept for 1 h in the dark in a moist chamber to gently rehydrate the cells. These were suspended and washed into a plastic tube by two rinses of 0.5 mL LB medium, followed by short centrifugation of the slide for complete sampling of the liquid. To assess the rate of survival, appropriate dilutions were plated on LB agar, and counts were taken of colonies appearing after a 14–16 h incubation at 37 °C. Freshly harvested *E. coli* suspended in 1% NaCl was plated similarly and served as control.

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