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Separation and sugar composition of gelatinous sheath carbohydrates produced by *Microcystis*

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Sugar compositions of polysaccharides of the gelatinous sheath produced by colonies and single cells of 3 species of *Microcystis* were compared. Single cells of *M. aeruginosa* without gelatinous sheath were obtained by changing the culture conditions. Single cells of *M. wesenbergii* and *M. viridis* were surrounded by a thin layer of gelatinous sheath, and were unable to form colonies. Carbohydrates of the gelatinous sheath and intracellular carbohydrates were separated by column chromatography on DEAE-Sephadex A-25. Polysaccharides of the gelatinous sheath produced by colonies showed marked heterogeneity in their sugar residues, while those of single cells were mainly composed of glucose. In the gelatinous sheath of colonies, xylose or glucose was abundant in two strains of *M. aeruginosa*, while in *M. wesenbergii* glucose, galactose and xylose were the main constituents and in *M. viridis* mannose was the characteristic major constituent. The relative amounts of the various sugar constituents of gelatinous sheath carbohydrates differed markedly among the three species of *Microcystis* cultured under the same conditions.

Key Index Words: colonies—gelatinous sheath—Microcystis aeruginosa—Microcystis viridis—Microcystis wesenbergii—*single cells—sugar composition.*

Microcystis, belonging to cyanobacteria, often causes waterblooms in eutrophic lakes. *M. aeruginosa, M. wesenbergii* and *M. viridis* are found together in the same lakes, but the dominant species varies depending on lakes, seasons, and years (WATANABE *et al.* 1986; TAKAMURA and WATANABE 1987). A conspicuous feature of *Microcystis* is the gelatinous sheath that surrounds cells. DREWS and WECKESSER (1982) called the structured external layer 'sheath', and the undefined, unstructured zones 'slime'. It is difficult to separate the sheath and the slime, although they can be differentiated microscopically. In this paper, slime and sheath are treated together as 'gelatinous sheath'. SAITO (1985) showed that the properties of the gelatinous sheath of colonies of the three species of *Microcystis* and the rate of cellular dispersion after chlorination differed from each other.

The gelatinous sheath of *Microcystis* was found to be mainly composed of heteropolysaccharides containing uronic acids (AMEMIYA and NAKAYAMA 1986; NAKAGAWA et al. 1987), and its intracellular carbohydrates were composed mainly of glucose polymers (HAMA and HANDA 1982; NAKAGAWA *et al.* 1987). However, details of the differences in the gelatinous sheath of these species remain obscure because it is difficult to isolate the gelatinous sheath without injuring cells.

We, therefore, examined the methods for isolating the gelatinous sheath of the three *Microcystis* species mentioned above (AMEMIYA and NAKAYAMA 1988). Solubilization of the gelatinous sheath was attempted at that time by physical treatments such as heat, shaking and sonication, and by chemical treatment with a chelator or in alkaline solution. Of the three *Microcystis* species, slime of *M. aeruginosa* was solubilized most easily, followed by that of *M. wesenbergii,* although it was partially solubilized, while that of *M. viridis* was only solubilized in an alkaline solution. The sheath of *M. wesenbergii* was almost totally

insoluble by these procedures.

Based on the results of the solubilization experiment, we conducted further detailed investigation on the sugar constituents of polysaccharides from the gelatinous sheath produced by *Microcystis* colonies, and compared them with those of single cells which were unable to form colonies, thereby we expected to reveal possible differences in the characteristics of the gelatinous sheath between colonies and single cells of the same strain. In the present paper, we report the separation of carbohydrates from the gelatinous sheath in addition to intracellular carbohydrates using ion-exchange chromatography, together with determination of their sugar constituents.

Materials and Methods

Cultures and growth conditions

Microorganisms: Unialgal colonies of two strains of *M. aeruginosa* and one strain of *M. wesenbergii* were isolated from a waterbloom in Lake Suwa in August 1980. *M. viridis* NIES-102 was obtained from the National Institute for Environmental Studies. This strain is axenic and composed of single cells which lack the ability to form colonies.

Culture conditions: Algal cells were cultured in B12 medium (SHIBUKAWA *et al.* 1986) at 25°C and 1500 lux as standard conditions.

Isolation of single cells: Single cells were isolated from two strains of *M. aeruginosa* No. 1 and No. 2 and one strain of *M. wesenbergii*, all of which formed colonies. Details of the isolation method used are described in the Results.

Fractionation of carbohydrates from cell contents and gelatinous sheath

Single cells were precipitated by centrifugation at 10,000 × g for 10 min, followed by two washings with distilled water, and then lyophilized. Colonies were collected by filtration with a Nuclepore filter (1.0 μm), washed with water and lyophilized. Thirty milligrams of dried cells were suspended in 0.05 M Tris buffer (pH 8.0), sonicated at 90 W for 6 min and then centrifuged at 1,500 × g. The precipitate was repeatedly sonicated and centrifuged. The supernatant was applied to a column (2.2 × 35 cm) packed with DEAE-Sephadex A-25, and carbohydrates were eluted with a gradient of sodium chloride in Tris buffer.

Analytical methods

Total organic carbon of algal cells was measured by an element analyzer (Perkin-Elmer, Type 240). Total carbohydrates were determined by the phenol-sulfuric acid method, and uronic acid residues were determined by Dishe's carbazole method modified by GALAMBOS (1967). Monosaccharides were determined according to the method of NAKAGAWA *et al.* (1987). After hydrolysis with sulfuric acid, resulting individual monosaccharides were converted to their respective alditol acetate derivatives, and subjected to gas chromatography on a Hitachi chromatograph (Model 163) with a 2-m glass column. For determination of glucose and galactose, a column was packed with 3% ECNSS-M and heated at 190°C; for other neutral monosaccharides, a column packed with 3% EGSS-X was used and heated at 200°C.

Results

Preparation of single cells and colonies

Single cells were prepared from colonies of two strains of *M. aeruginosa* and a strain of *M. wesenbergii*. For *M. aeruginosa*, single cells are reported to grow faster than colonies (GANF and SCHACHE 1983). Therefore, temperature, light intensity and concentration of sodium nitrate were increased to obtain faster growth. Furthermore, the concentration of calcium and ferric ions was decreased and that of EDTA was increased, unless gelatinous sheath was produced, or extracellularly released material became insoluble gelatinous sheath. So, the colonies of *M. aeruginosa* No. 1 were cultured at 64 (2^6) sorts of combinations of culture conditions, i.e. temperature (25 and 30°C), light intensity

(1500 and 3000 lux), sodium nitrate (100 and 200 mg/*l*), calcium chloride (40 and 10 mg/*l*), ferric citrate (6 and 2 mg/*l*) and EDTA (1 and 3 mg/*l*). Single cells and paired cells undergoing binary division grew with colonies under 29 conditions. The culture conditions and the number of cultures in which single cells appeared from colonies are shown in Table 1. Single cells showed a tendency to grow better at 30°C, 3000 lux, 200 mg/*l* sodium nitrate, and 10 mg/*l* calcium chloride, which are considered to be more favorable for single cells than the standard conditions. After repeated subculture on agar plates, single cells were obtained without gelatinous sheath. When cultured in the standard B12 medium under the standard conditions, they still grew singly.

Since single cells lacking the ability to form colonies became detached during culture of colonies of *M. aeruginosa* No. 2, they were isolated. There was no difference in the colonial form and size of a single cell between *M. aeruginosa* No. 1 and *M. aeruginosa* No. 2.

Single cells of *M. wesenbergii* which lack the ability to form colonies were also obtained during colony culture. However, each of the isolated single cells had a thin surrounding layer of gelatinous sheath. Hence, no single cells lacking gelatinous sheath could be prepared, but single cells with thin gelatinous sheath were treated as single cells of *M. wesenbergii* because they lacked the ability to form colonies.

The single cells obtained from *M. viridis* had gelatinous sheath similar to that of single cells of *M. wesenbergii*. These single cells were also classified as single cells because of their inability to form colonies. Preparation of colonies from single cells was made by employing conditions reverse to those under which single cells were prepared from colonies of *M. aeruginosa* No. 1. Accordingly, single cells of *M. viridis* were cultured under $2^4 \times 3^2 = 144$ conditions involving all combinations of the six factors, i.e. temperature (25 and 15°C), light intensity (1500 and 500 lux), sodium nitrate (100 and 30 mg/*l*), EDTA (1 and 0 mg/*l*), calcium chloride (40, 120 and 360 mg/*l*) and ferric citrate (3, 9 and 27 mg/*l*). Small granular colonies and slightly larger rod-shaped colonies were formed under 20 and 21 out of the 144 conditions, respectively. Table 2 summarizes the conditions under which the rod-shaped colonies grew: the temperature was lowered from 25°C to 15°C, the concentration of sodium nitrate was decreased from 100 mg/*l* to 30 mg/*l*, and the concentration of calcium chloride was increased from 40 mg/*l* to 360 mg/*l* for preparation of colonies from

Table 1. Number of cultures in which single cells appeared from colonies of *M. aeruginosa* No. 1 in each condition. Total number of cultures 29.

	Condition*	Number	Condition	Number
Temperature	25℃	11	30℃	18
Light intensity	1500 lux	6	3000 lux	23
Sodium nitrate	100 mg/l	11	200 mg/l	18
Cacium chloride	40 mg/l	12	10 mg/l	17
Ferric citrate	6 mg/l	14	2 mg/l	15
EDTA	1 mg/l	14	3 mg/l	15

* Standard culture conditions.

Table 2. Number of cultures in which rod-shaped colonies of *M. viridis* appeared in each condition. Total number of cultures 21.

	Condition	Number	Condition	Number	Condition	Number
Temperature	25℃	4	15℃	17		
Light intensity	1500 lux	11	500 lux	10		
Sodium nitrate	100 mg/l	7	30 mg/l	14		
Calcium chloride	40 mg/l	3	120 mg/l	3	360 mg/l	15
Ferric citrate	3 mg/l	6	9 mg/l	11	27 mg/l	4
EDTA	1 mg/l	11	0 mg/l	10		

single cells. The single cells were, then, cultured under the above conditions using 1-*l* flasks. The results showed that, although some colonies were formed, single cells grew well. We then substituted the natural samples of *M. viridis* collected from the bloom in Lake Suwa for colonies of this species. These samples were used after partial removal of the gelatinous sheath of *M. aeruginosa* and *M. wesenbergii* by shaking and sonication procedures (AMEMIYA *et al.* 1988).

Organic material and carbohydrates produced in culture

Amounts of grown cells in cultures for 21 days, and their organic and carbohydrate carbon contents are summarized in Table 3. Single cells grew better than colonies during the culture for 21 days. This tendency was especially evident in *M. aeruginosa* No. 2. Similarly the production of carbohydrates was higher in single cells of *M. aeruginosa*, especially in those of *M. aeruginosa* No. 2. Carbohydrate carbon content of cultured cells ranged from 14% to 30% of organic carbon.

Separation of cellular carbohydrate

Separation of carbohydrates present within the cell from those in the gelatinous sheath was performed using ion-exchange chromatography. After the cells had been sonicated and centrifuged, the supernatant was applied to columns of CM-Sephadex A-25 and DEAE-Sephadex A-25. Some of the carbohydates were adsorbed by DEAE-Sephadex A-25. Carbohydrates in single cells were intracellular, while those in colonies comprised intracellular carbohydrates and gelatinous sheath carbohydrates. The carbohydrate fraction eluted in the same volume upon chromatography of extracts from both single cells and colonies was considered to be the intracellular carbohydrate fraction, whereas the carbohydrate fraction found only in colonies was regarded as that from the gelatinous sheath.

Figure 1 shows carbohydrates of *M. aeruginosa* No. 1 eluted with 0.05 M Tris buffer stepwise with increasing concentrations of sodium chloride. Most carbohydrates of single cells passed through without adsorption. Little was eluted with the buffer containing sodium chloride at a concentration of 0.1 M. In colonies, the fraction passing through accounted for 45.2% of total carbohydrates, and the fraction eluted with 0.1 M sodium chloride amounted to 3.2%; then, 19.5% of the carbohydrates were eluted

Table 3. Comparison of carbohydrate carbon content in organic carbon between single cells and colonies of three *Microcystis* species after culture for 21 days.

	Algal cells mg/l	Organic carbon mg/l	Carbohydrate carbon mg/l	Carbohydrate carbon content %
M. aeruginosa No. 1				
single cells	60.1	24.1	7.3	30.3
colonies	43.0	17.1	2.4	14.0
M. aeruginosa No. 2				
single cells	82.1	37.2	11.1	29.8
colonies	30.9	14.0	3.5	25.0
M. wesenbergii				
single cells	85.0	33.9	7.0	20.6
colonies	74.1	29.6	8.2	27.7
M. viridis				
single cells	55.3	22.2	4.9	22.1
colonies*	35.5	15.3	4.9	32.0

* Natural colonies collected from a waterbloom in Lake Suwa in August 1986.

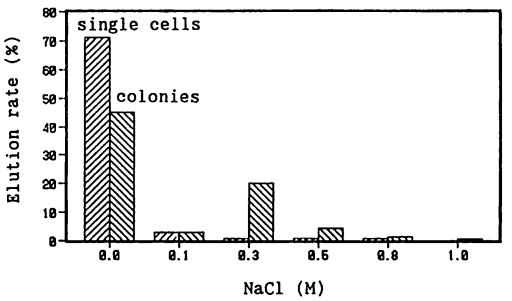

Fig. 1. Carbohydrates eluted stepwise in increasing concentrations of sodium chloride by anion exchange column chromatography (*M. aeruginosa* No. 1).

with 0.3 M sodium chloride, and only 4.7% of the carbohydrates were eluted at sodium chloride concentrations higher than 0.3 M. Accordingly, cellular carbohydrates were found to be divisible into two fractions; intracellular carbohydrates eluted without adsorption to the resin and carbohydrates from the gelatinous sheath eluted with a sodium chloride concentration of at 0.3 M or higher. Some carbohydrates of single cells and colonies, which were eluted with a sodium chloride concentration of 0.1 M, were also fractionated separately so that each of the two appeared as a discrete fraction.

From the results shown in Fig. 1, the method of carbohydrate separation was modified. Figure 2 shows carbohydrate fractions separated into three peaks by the modified method. Fractions A, B and C were eluted with the buffer lacking sodium chloride, the buffer containing sodium chloride at a concentration of 0.1 M, and the buffer with a linear gradient from 0.1 to 0.8 M sodium chloride, respectively. Accordingly, intracellular carbohydrates were contained in fraction A, and carbohydrates of the gelatinous sheath were found in fraction C.

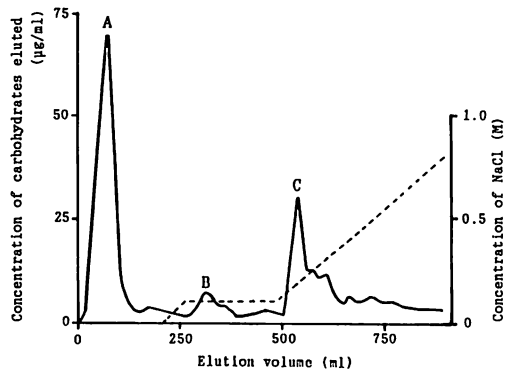

Fig. 2. Separation of carbohydrates in colonies of *M. aeruginosa* No. 1 by anion exchange column chromatography.

Fractionation of carbohydrates with DEAE-Sephadex A-25

Carbohydrates obtained in each fraction of the three species of *Microcystis* are shown in Table 4. In single cells of *M. aeruginosa* No. 1 and No. 2, about 70% of total carbohydrates were eluted in fraction A, 3–6% in fraction B, and 2–5% in fraction C. Nearly 40 to 45% of carbohydrates present in colonies of *M. aeruginosa* were eluted in fraction A, and about 25% in fraction C, indicating that intracellular carbohydrates and carbohydrates of the gelatinous sheath were present in an approximate ratio of 1.5–1.9 : 1. In *M. wesenbergii* and *M. viridis*, as observed from the thin-layered gelatinous sheath, the percentage of carbohydrates of single cells eluted in fraction C was similar to that of colonies. Intracellular carbohydrates and carbohydrates

Table 4. Separation of carbohydrates by DEAE-Sephadex A-25 (%).

	Fraction			Recovery
	A	B	C	
M. aeruginosa No. 1				
single cells	71.3	3.3	4.7	79.3
colonies	45.2	3.2	24.2	72.6
M. aeruginosa No. 2				
single cells	70.6	5.8	2.0	78.4
colonies	40.9	3.5	26.4	70.8
M. wesenbergii				
single cells	36.5	1.0	34.3	71.8
colonies	32.3	9.4	37.5	79.2
M. viridis				
single cells	37.5	16.4	20.3	74.2
colonies*	48.7	9.2	26.0	83.9

* Natural colonies collected from a waterbloom in Lake Suwa in August 1986.

of the gelatinous sheath fraction of *M. wesenbergii* and *M. viridis* were present in ratios of 1 : 1 and 1.8 : 1, respectively.

Table 5 shows the percentages of uronic acid residues to carbohydrates. Single cells of *M. aeruginosa* did not contain uronic acids in fraction A, whereas single cells and colonies of other species contained uronic acids between 1.6% and 4.0% in this fraction. Uronic acids in fraction B increased from 3.5% to 7.4%, and those in fraction C showed further increases in amounts ranging from 9.1% to 22.7%. Gelatinous sheath fractions contained most of the uronic acids. In fraction C, the content of uronic acids in colonies was higher than that in single cells; the content in colonies of *M. wesenbergii* was highest, amounting to 22.7%. It was found that neutral sugars accounted for most of the carbohydrates in each fraction, exceeding 77.3%.

Sugar composition of the three species

The compositions of neutral sugars in each fraction of the three species of *Microcystis* are shown in Figs. 3–6. Glucose, galactose, mannose, xylose, fucose, rhamnose, ribose, and arabinose were present. However, only the first six monosaccharides are presented graphically, and the last two monosaccharides were not included because they accounted for less than 2% of the total content. In *M. aeruginosa* No. 1 (Fig. 3), the main constituent of intracellular carbohydrate in fraction A was glucose, which amounted to 82% and 65% in single cells and colonies, respectively.

Table 5. Uronic acid content (%) in carbohydrates of each fraction.

	Fraction		
	A	B	C
M. aeruginosa No. 1			
single cells	0.0	7.1	9.1
colonies	4.0	6.6	12.3
M. aeruginosa No. 2			
single cells	0.0	3.5	10.5
colonies	4.5	6.3	16.2
M. wesenbergii			
single cells	1.6	6.5	14.2
colonies	4.0	7.4	22.7
M. viridis			
single cells	3.6	4.3	9.6
colonies*	2.1	7.3	15.5

* Natural colonies collected from a waterbloom in Lake Suwa in August 1986.

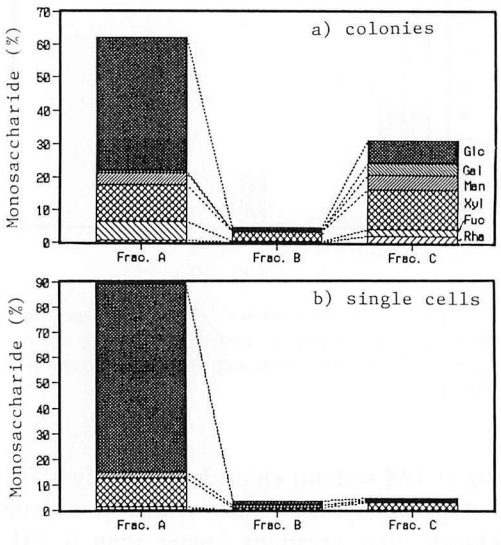

Fig. 3. Sugar composition in three fractions of *M. aeruginosa* No. 1.

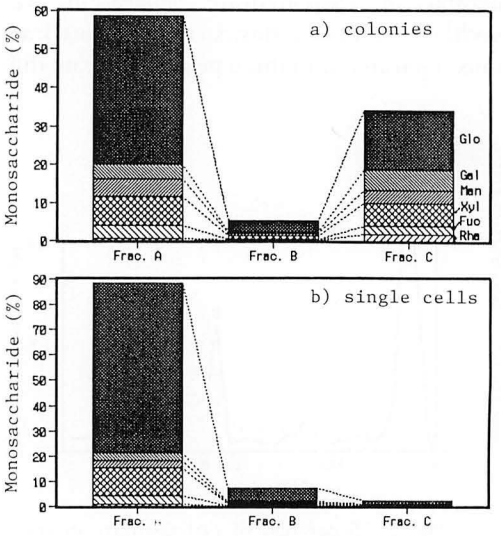

Fig. 4. Sugar composition in three fractions of *M. aeruginosa* No. 2.

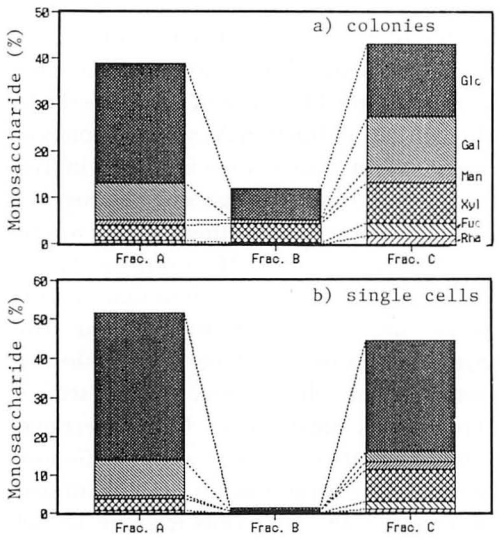

Fig. 5. Sugar composition in three fractions of *M. wesenbergii*.

Carbohydrates of the gelatinous sheath in fraction C consisted of 39% xylose, 22% glucose, 14% mannose, 11% galactose, 7% rhamnose and 6% fucose, suggesting the presence of heteropolysaccharides. Carbohydrates of *M. aeruginosa* No. 2 showed similar compositional patterns to those of *M. aeruginosa* No. 1. However, carbohydrates of the gelatinous sheath were composed mainly of glucose often as much as 45% (Fig. 4). The sugar composition of *M. wesenbergii* is shown in Fig. 5. Intracellular carbohydrates were composed mainly of glucose, accounting for 73% and 64% in single cells and colonies, respectively. The gelatinous sheath fraction contained glucose (36%), galactose (26%) and xylose (20%). In colonies, much galactose was characteristically present not only in the gelatinous sheath fraction but also in the intracellular fraction. In single cells, glucose, the most significant constituent, also comprised more than half of the gelatinous sheath fraction. Figure 6 shows that the carbohydrates of single cells of *M. viridis* consisted of much larger proportions of glucose than those of colonies in fractions B and C. In single cells, glucose accounted for up to 79% of intracellular carbohydrates, and as much as 66% and 62% of the other two fractions, respectively, whereas the sugar contents except glucose were larger than those in colonies. The gelatinous sheath of colonies contained four kinds of residue, i.e. mannose, glucose, galactose and xylose in decreasing order. Mannose was also present in intracellular carbohydrates in large amounts, possibly being a characteristic feature of *M. viridis* colonies.

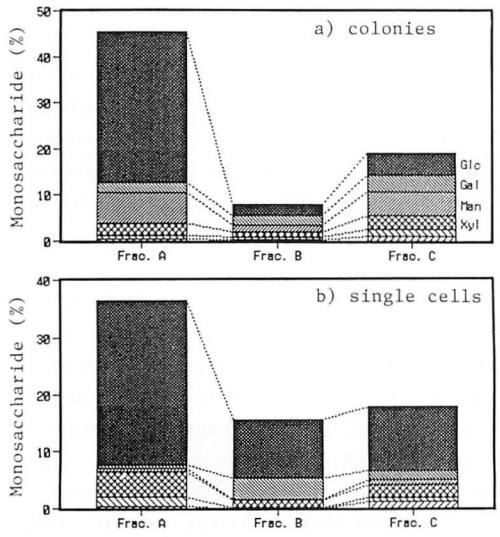

Fig. 6. Sugar composition in three fractions of *M. viridis*.

The sugar compositions of gelatinous sheath carbohydrates of the three species of *Microcystis* are shown comparatively in Fig. 7. Xylose or glucose was abundant in the gelatinous sheath carbohydrates from the two

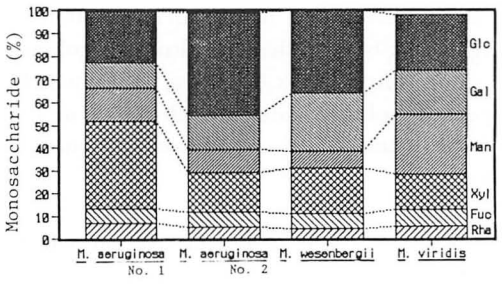

Fig. 7. Sugar composition of gelatinous sheath carbohydrates of three species of *Microcystis*.

strains of *M. aeruginosa*, while in *M. wesenbergii* glucose, galactose and xylose were abundant, and in *M. viridis* mannose was the major constituent except for glucose.

Discussion

Mutual transformation between colonies and single cells

Colonies may clearly lose their gelatinous sheath under certain culture conditions, and in fact light intensity is known to affect the appearance of the gelatinous sheath. Although colonies are present in nature, subculture during colony preservation often results in the occurrence of single cells (KRUGER and ELOFF 1979). OLSEN and GANF (1986) indicated that photosynthetic activity affected the synthesis of proteins and carbohydrates. Under conditions where synthesis of proteins is higher than that of carbohydrates, the increase in light intensity, water temperature and nitrogen concentration might induce an increase in cell growth. Therefore the formation of gelatinous sheath containing much carbohydrates is assumed to be difficult. PARKER (1982) suggested that *Microcystis* formed single cells in low concentrations of cations, and that culture with addition of a chelator could result in disappearance of the gelatinous sheath. A decrease in the concentration of calcium ion to one-quarter is considered to result in predominance of single cells. In contrast, gelatinous sheath might be produced under the reverse conditions. When the calcium concentration was increased nine times that of the normal B12 medium, colonies tended to grow. However, it seemed to be more difficult to produce a shift from single cells to colonies. Culture of colonies without single cells may be obtained by repeated isolation and subculture of several colonies under the conditions mentioned above.

Comparison of sugar composition of colonies and single cells

When carbohydrates from the cells were separated into three fractions using an anion-exchanger, fractions A and C were found to be different not only in uronic acid content but also in sugar composition. In colonies, glucose accounted for most of the intracellular carbohydrates in fraction A, several monosaccharides except glucose were increased in fractions B and C, and the degree of heterogeneity in the amounts of constituent sugars was increased. With axenic *M. aeruginosa* K-3A, NAKAGAWA *et al.* (1987) reported that the constituent sugar unit of intracellular carbohydrates was mostly glucose, while the carbohydrates of slime were composed of heterogeneous sugar units. Our experimental findings after separation by anion-exchanger were in approximate agreement with their results. In single cells forming no colonies, glucose was found in the majority of intracellular carbohydrates, and more than 50% of carbohydrates were glucose even in fractions B and C of cells with layered thin gelatinous sheath. The thin layered gelatinous sheath of single cells contained less heterogeneous polysaccharides, and the uronic acid residues were fewer in the gelatinous sheath of single cells than in the gelatinous sheath of colonies. Glucose is considered to play a central role in carbohydrate metabolism in single cells. The present results suggest that single cells and colonies may differ in their mode of carbohydrate synthesis.

Although colonies with gelatinous sheath produced less organic material and carbohydrates than did single cells, the existence of apparently highly heterogeneous polysaccharides might have significance in the physiology of *Microcystis*.

Sugar composition of the gelatinous sheath of three species

TAKAMURA *et al.* (1986) reported that rhamnose and xylose were abundant among the sugars present in the slime of axenic *M. aeruginosa* K-3A, and that glucose or xylose was abundant in the gelatinous sheath of other cyanobacteria. In the present study, xylose and glucose were shown to be the main sugar constituents of the gelatinous sheath of

M. aeruginosa No. 1 and No. 2, respectively. In addition, the amounts of galactose and mannose were higher in the gelatinous sheath of *M. wesenbergii* and *M. viridis*, respectively.

M. wesenbergii and *M. viridis* can be identified easily because their formation of colonies is a relatively consistent feature. However, *M. aeruginosa* shows various characteristics, and has to be reclassified on the basis of cell size, cellular arrangement, colony shape, staining of the gelatinous sheath by alcian blue, chemical properties and so on. WATANABE *et al.* (1986) showed that *M. aeruginosa* can be further divided into *M. aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae*. In the case of *M. aeruginosa*, the difference in carbohydrate composition of gelatinous sheath between the two strains might be due to the difference in the level of forma. In the present study, the sugar composition of gelatinous sheath differed among the three species of *Microcystis* cultured under the same conditions. In general, the properties of polysaccharides are known to depend on the nature of their constituent sugars, particularly the mode of their arrangement, linkage configuration and position. It is suggested that these features of the main polysaccharides of the gelatinous sheath differ to a great extent among the three species of *Microcystis* examined in the present study.

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雨宮由美子・中山大樹：*Microcystis* 属藍藻の生産する粘質鞘多糖の分離と糖組成

Microcystis 属藍藻について，*M. aeruginosa* 2 株，*M. wesenbergii* 1 株，*M. viridis* 1 株のコロニー株と群体を作っていない単細胞株を用い，コロニー株と単細胞株の生産する糖の組成を調べ，粘質鞘の糖組成の特徴を明らかにした。超音波処理により各株の糖を可溶化し，陰イオン交換クロマトグラフィー (DEAE-Sephadex A-25) を用いて，粘質鞘多糖と細胞内多糖の分離を行った。細胞内多糖は吸着せずに溶出する画分に，粘質鞘多糖は 0.1–0.8 M NaCl の濃度勾配で溶出する画分に分離された。細胞内構成糖はグルコースが大半を占めて種による差はなかったが，粘質鞘はヘテロ多糖であり，種により特徴がみられた。粘質鞘は，*M. aeruginosa* ではグルコースの多い株とキシロースの多い株があり，*M. wesenbergii* ではグルコース，ガラクトース，キシロースを多く含み，*M. viridis* ではマンノースを多く含んでいた。（400 山梨県甲府市武田4–3–11　山梨大学工学部環境整備工学科）