

CELL WALL SUGARS OF SOME *SCENEDESMUS* SPECIES

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(Received 13 October 1995)

Key Word Index—*Scenedesmus acuminatus*; *S. acutiformis*; *S. armatus*; *S. costato-granulatus*; *S. falcatus*; *S. obliquus*; *S. producto-capitatus*; *S. wisconsiensis*; algae; cell wall sugar composition; chemotaxonomy.

Abstract—*Scenedesmus acuminatus*, *S. acutiformis*, *S. armatus*, *S. costato-granulatus*, *S. falcatus*, *S. obliquus*, *S. producto-capitatus*, *S. subcapitatus* and *S. wisconsiensis* have a rigid wall constituted of glucose, mannose and galactose, and wall matrices composed of mannose, glucose and galactose in different proportions. Cell walls of all species examined did not stain with Ruthenium Red and showed positive anisotropy.

INTRODUCTION

The genus *Scenedesmus* is morphologically quite diverse as is evident from the monograph of Hegewald and Silva [1]. Kessler [2] has also reviewed some biological and biotechnological problems of this highly variable genus. The chemotaxonomy of *Scenedesmus* has been examined using methods, such as differences in starch hydrolysis [3] and DNA reassociation [4], but were found to be unsatisfactory.

Species and strains of the genus *Chlorella* are morphologically very simple but are diverse in physico-chemical characteristics [5, 6]. The cell wall composition of *Chlorella* species has been shown to be species-specific [7-14]. The author [11] has presented an index of three numerals for the classification of this genus: sugar of rigid wall (1, glucose and mannose; 2, glucosamine), the second, Ruthenium Red stainability (1, plus; 2, minus), and the third, anisotropy (1, plus; 2, minus).

In the present study, cell wall sugar composition of species of *Scenedesmus* from the collection at the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen, were compared for taxonomical purposes in the same way as used for *Chlorella* species [9-11].

RESULTS AND DISCUSSION

Three cell wall characteristics, sugars of the rigid wall, Ruthenium Red stainability and anisotropy of the *Scenedesmus* species examined are shown in Table 1; data for some *Chlorella* species are also listed for comparison. Neutral sugars of the rigid walls were obtained by H₂SO₄ hydrolysis, and glucosamine by 6 M HCl hydrolysis. In *Scenedesmus*, the sugars iden-

tified in their rigid wall were glucose, mannose and galactose; no glucosamine was found. Ruthenium Red did not stain the cell walls of any *Scenedesmus* species. Anisotropy was also observed in the cell walls of all species. Thus, the walls of *Scenedesmus* species were classified as 1.2.1. The uniformity of the cell wall characteristics in the genus *Scenedesmus* is in marked contrast to the diversity shown in the genus *Chlorella* (Table 1).

The sugar constituents of the rigid walls of *Scenedesmus* species are glucose (major), galactose and mannose (Fig. 1). In *Chlorella* species, sugars of the rigid wall comprised either glucosamine or glucose and mannose [11]. In the *Scenedesmus* species examined in this study, none with a rigid wall composed of glucosamine was found but galactose was found in addition to glucose and mannose in the corresponding glucan-type rigid wall. Sugar constituents of the wall matrix were mannose, galactose and glucose (Fig. 2), in contrast to many *Chlorellae*, where matrices were composed of rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose. In the wall matrices of *S. acutiformis*, *S. falcatus*, *S. obliquus* and *S. wisconsiensis* mannose was the major sugar; in those of *S. acuminatus*, *S. armatus* and *S. producto-capitatus*, glucose was dominant. A higher proportion of galactose was detected in *S. subcapitatus*. The author [13] has previously reported that the cell wall of *S. obliquus* 276.3a, does not exhibit anisotropy. Critical re-examination of this species together with others has shown this observation to be incorrect; all *Scenedesmus* were positive.

Kalina and Punčochářová [15] have shown that *C. fusca* var. *vacuolata* differed from other *Chlorella* in the structure of its pyrenoid, which has homogeneous stroma not penetrated by the thylakoid; it was rather similar to that of *Scenedesmus*. This difference was also confirmed by Ikeda and Takeda [16].

Table 1. Chemical and microscopical properties of cell walls of *Scenedesmus* and *Chlorella* species

Species (strain)	Sugar of rigid wall		Ruthenium Red staining‡	Anisotropy§	Index
	72%–4% H ₂ SO ₄ *	6 M HCl†			
<i>S. acuminatus</i> (38.81)	+	–	–	+	1.2.1
<i>S. acutiformis</i> (276-12)	+	–	–	+	1.2.1
<i>S. armatus</i> (276-4d)	+	–	–	+	1.2.1
<i>S. costato-granulatus</i> (18.81)	+	–	–	+	1.2.1
<i>S. falcatus</i> (2.81)	+	–	–	+	1.2.1
<i>S. obliquus</i> (276-1)	+	–	–	+	1.2.1
<i>S. obliquus</i> (276-3a)	+	–	–	+	1.2.1
<i>S. obliquus</i> (276-3b)	+	–	–	+	1.2.1
<i>S. producto-capitatus</i> (21.81)	+	–	–	+	1.2.1
<i>S. subcapitatus</i> (53.80)	+	–	–	+	1.2.1
<i>S. wisconsinensis</i> (22.81)	+	–	–	+	1.2.1
<i>C. fusca</i> var. <i>vacuolata</i> (211-8b¶)	+	–	–	+	1.2.1
<i>C. saccharophila</i> (211-1b¶)	+	–	–	+	1.2.1
<i>C. zofingiensis</i> (211-14¶)	+	–	–	–	1.2.2
<i>C. kessleri</i> (211-11h¶)	–	+	–	–	2.2.2
<i>C. vulgaris</i> (211-1e¶)	–	+	+	–	2.1.2

*Glucose, galactose and mannose.

†Glucosamine.

‡Cell wall of living cell.

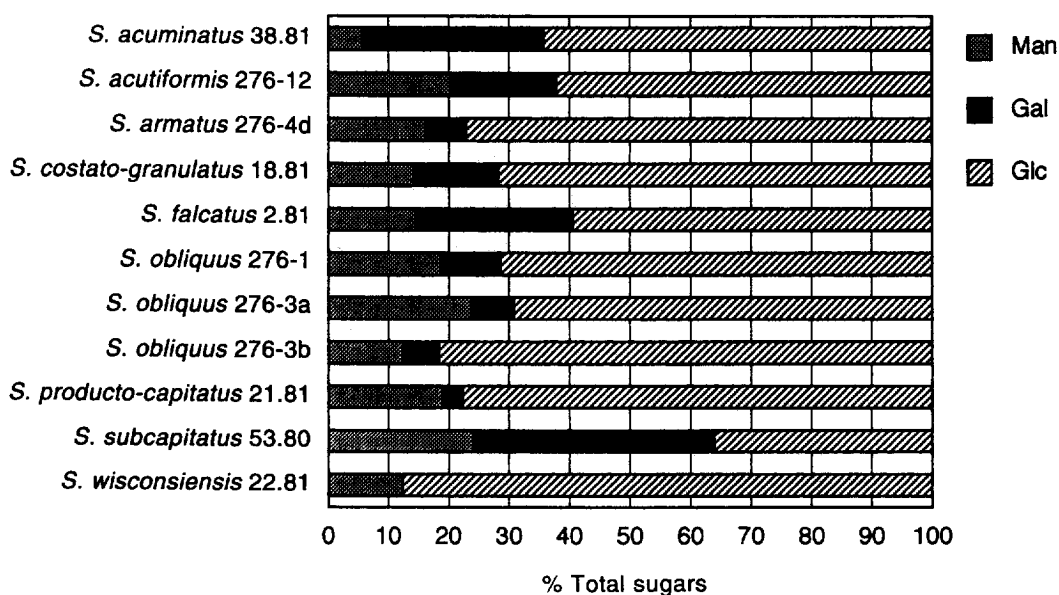
§Isolated cell wall.

||Three numerals: sugar of rigid wall, Ruthenium Red stainability, anisotropy.

¶Reported previously.

The phylogenetic relation between *Scenedesmus* species and *C. fusca* var. *vacuolata* has also been studied by molecular methods, such as DNA/DNA hybridization [4] and small subunit rRNA. Using 16s-like rRNA coding region sequences, Huss and Sogin [17] demonstrated that *C. fusca* belong to a group that includes the genus *Scenedesmus*. The cell wall indices of both *Scenedesmus* and *C. fusca* var. *vacuolata* are

1.2.1. The major sugar of the wall matrix of both *S. obliquus* 276.3a [13] and *C. fusca* var. *vacuolata* 211-8b [10] is mannose. *Chlorella* species belonging to the group 1.2.1, include *C. saccharophila*, *C. luteoviridis*, *C. fusca* var. *vacuolata* and *C. minutissima* [11]. Cell wall compositions support the close relationship between *Scenedesmus* species and *C. fusca* var. *vacuolata* [15–17].

Fig. 1. Sugar composition of rigid walls in some *Scenedesmus* species.

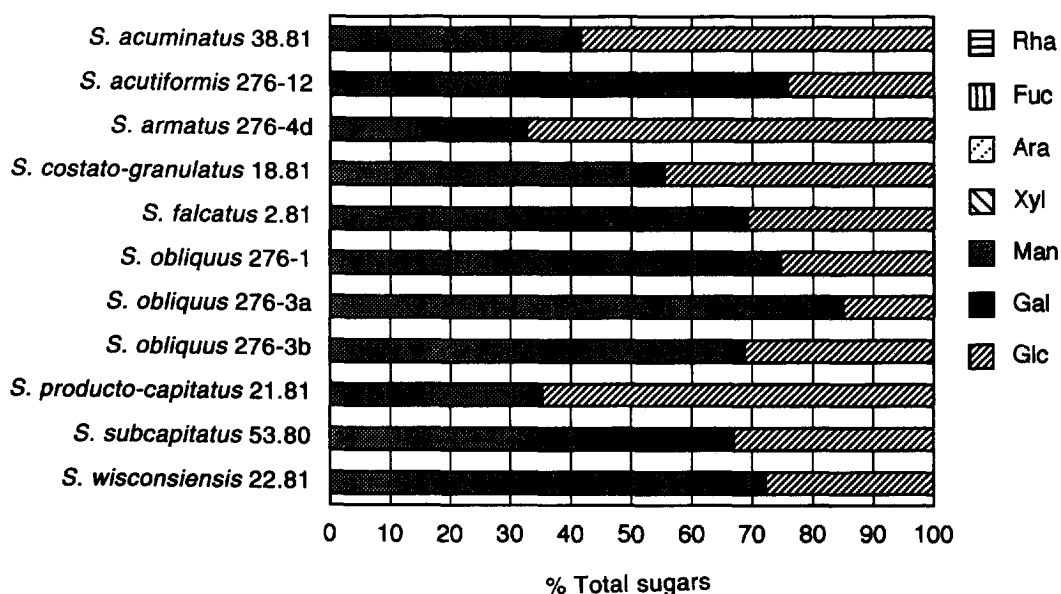


Fig. 2. Comparison of sugar composition of wall matrices in some *Scenedesmus* species.

EXPERIMENTAL

Algal strains. *S. acuminatus* 38.81, *S. acutiformis* 276-12, *S. armatus* 276-4d, *S. costato-granulatus* 18.81, *S. falcatus* 2.81, *S. obliquus* 276-1, 276-3b, *S. producto-capitatus* 21.81, *S. subcapitatus* 53.80, *S. wisconsiensis* 22.81 were obtained from the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, Germany; *S. obliquus* 276-3a was a gift from Prof. Kessler, University of Erlangen-Nürnberg, Germany.

Culture. Algae were grown as described in ref. [11] using the culture medium CA of IAM [20 mg $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 100 mg KNO_3 , 50 mg NH_4NO_3 , 30 mg β -glycerophosphate disodium salt, 20 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10 μg vitamin B_1 , 0.1 μg vitamin B_{12} , 0.1 μg Biotin, 194 μg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 82 μg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 10 μg ZnCl_2 , 4 μg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 8 μg Na_2MoO_4 , 1.5 μg $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 7.02 mg $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 6.6 mg $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 400 mg HEPES and 1 l dist. H_2O].

Analyses. These were performed as described previously in ref. [11].

Acknowledgement—The author thanks Prof. U. G. Schlösser, Universität Göttingen, Germany, for generous gifts of *Scenedesmus* species and for valuable discussions.

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