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CLASSIFICATION OF *CHLORELLA* STRAINS BY CELL WALL SUGAR COMPOSITION

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Key Word Index—*Chlorella ellipsoidea, C. fusca, C. kessleri, C. saccharophila, C. sorokiniana, C. vulgaris*; Chlorophyta; anisotropy; cell wall sugar composition; chemotaxonomy; Ruthenium Red staining.

Abstract—Nineteen strains of *Chlorella saccharophila, C. ellipsoidea, C. vulgaris, C. sorokiniana, C. kessleri, C. fusca* and *C. pyrenoidosa* were classified by means of cell-wall sugar composition, stainability with Ruthenium Red and optical anisotropy of the cell walls. The strains tested are divisible into two distinctly different groups by the presence of either glucose and smaller amount of mannose or glucosamine in the rigid wall. *C. saccharophila, C. ellipsoidea, C. fusca* and *C. pyrenoidosa* belong to the group with glucose and mannose, whereas *C. vulgaris, C. sorokiniana* and *C. kessleri* belong to that with glucosamine.

INTRODUCTION

The cell wall components of *Chlorella* reported by many authors [1–8] are quite different. Takeda [8] reported that cell wall properties such as sugar composition, stainability with Ruthenium Red and optical anisotropy are species specific, and thus the eight *Chlorella* strains examined fell into two distinct groups based on the presence/absence of glucosamine in the rigid wall, and further, each group could be classified into sub-groups by the sugar compositions of the hemicelluloses.

In this study, the cell walls of 19 *Chlorella* strains were examined by the same method as in the previous study, and these and the previously studied *Chlorellae* were assigned to species.

RESULTS AND DISCUSSION

The results (Table 1) for the stainability of *Chlorella* cell walls with Ruthenium Red are in agreement with those of Soeder [9].

Anisotropic characters were present in a number of the isolated cell walls, but in strains 211/1a, 20 and 3.80 it was below discrimination. In the rigid wall, the residue remained insoluble after treatment with 0.4 M NaOH at 30° for 4 hr, anisotropy was observed in strains 211-8b, C-104, C-28 and 211-8c.

Cell wall components were analysed after stepwise hydrolyses with 0.5 M H_2SO_4, 72–4% H_2SO_4 and 6 M HCl. The sugars from the hemicellulose were present in the 0.5 M H_2SO_4 hydrolysate, and those from the rigid wall were found either in the 72–4% H_2SO_4 hydrolysate or in the 6 M HCl hydrolysate [8]. The 72–4% H_2SO_4 hydrolysate contained the rigid wall sugars in 12 strains. In each case the sugars found were glucose and smaller amounts of mannose. In the remaining strains the 6 M HCl hydrolysate contained glucosamine (Table 1). This result reinforces the results of the previous report [8].

The hemicellulose sugar compositions of 19 strains are shown in Fig. 1. The results for 211-8b and -8c are in agreement with those of Loos and Meindl [5] and Blumreisinger *et al.* [6]. Strains 211/1a and 20 gave the same result, and their cell walls are the same as those of *C. saccharophila* SAG 211-1a [6, 8] and *C. ellipsoidea* IAM C-87 [7]. The strains 211/1c, 247, 211-9a, 27 and 211-9b have the same hemicellulose sugar compositions. These compositions are also consistent with those of 211-1b, -1c and -1d [8]. The strains 211-11f, 211-8l, 211/9a and 2.80 are similar to each other in hemicellulose sugar composition. The results for 211-11f and -8l are in agreement with those of Blumreisinger *et al.* [6]. Strain 211-11h is characterized by the presence of a large amount of an unknown sugar.

The results obtained in this study and those obtained in a previous study are summarized in Fig. 2. The *Chlorella* strains can be divided into two groups on the basis of their rigid wall components, and then classified into subgroups by the sugar composition of their hemicellulose. The first group which has glucose and mannose in the rigid wall includes *C. saccharophila, C. ellipsoidea* and *C. fusca*. The compositions of the cell walls in the strains 211/1c, 247, 211-9a, 27 and 211-9b are the same along with those in the strains 211-1b, -1c and -1d [8]. The four strains, 211-1b, 211/1b, 246 and C-102 are recorded to be the same [10], but their cell walls gave different results. Only two strains, 211/1b and 246 have the same cell wall composition, but they are separated from this group by the presence of glucosamine in their rigid wall [8], and the strain C-102 is different from the strain 211-1b in its hemicellulose sugar composition [7]. The strains 211/1a and 20 are the same in the characters of cell wall along with the strain 211-1a [8] and C-87 [7], confirming Starr's view [10, 12]. Strain 3.80 is similar to the above four in cell wall chemical composition, but it is different morphologically. Among the strains of *C. saccharophila*, Kessler [13] separated strains 3.80 and 211-1a from 211-1b, -1c, -1d, -9a and -9b on the basis of growth on mannitol and cadmium sensitivity, and classified them into two sub-species, *C. saccharophila* var. *ellipsoidea* and *C. saccharophila* var. *saccharophila*, respectively, following the nomenclature of Fott and Nováková [14]. The

Table 1. Physico-chemical properties of *Chlorella* cell walls

Strain	Ruthenium Red staining	Anisotropy		Sugar in rigid wall	
		Whole wall	Rigid*	72–4% H_2SO_4†	6 M HCl‡
211/1a	+	±	—	+	—
20	+	±	—	+	—
3.80	+	±	—	+	—
211/1c	—	+	—	+	—
247	—	+	—	+	—
211-9a	—	+	—	+	—
27	—	+	—	+	—
211-9b	—	+	—	+	—
211-8b	—	+	+	+	—
C-104	—	+	+	+	—
C-28	—	+	+	+	—
211-8c	—	+	+	+	—
211-11f	+	—	—	—	+
211-8l	+	—	—	—	+
211/9a	+	—	—	—	+
2.80	+	—	—	—	+
211-8k	—	+	—	—	+
211/1d	+	—	—	—	+
211-11h	+	—	—	—	+

*Residue remaining after alkali treatment.
†Glucose and mannose.
‡Glucosamine.

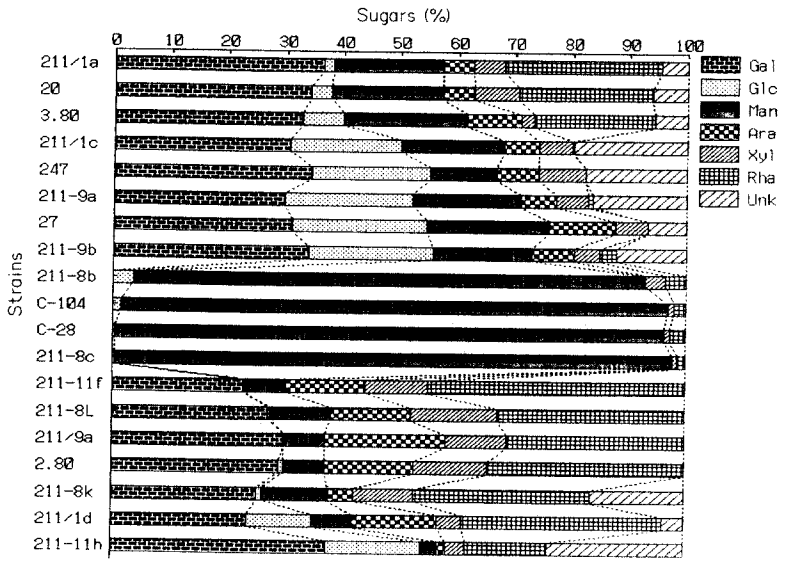

Fig. 1. Comparison of the compositions of the hemicelluloses of the cell walls. Unk indicates unknown carbohydrates.

present study supports this classification. Identity of strains 211-9a and -9b was also shown by Kessler's results on growth temperature [15] and heavy metal resistance [16]. The strains 211-8b, -8c, C-104 and -28 are the same, and their compositions are characteristically different from the other strains. The identity of 211-8b and -8c was also shown by Kessler on the basis of the upper limit of growth temperature [15], GC content of DNA [17] and DNA reassociation [18], and that of 211-8b and C-104 confirmed ref. [10].

The second group which has glucosamine in the rigid wall includes *C. vulgaris*, *C. sorokiniana* and *C. kessleri*. The strains 211-11f, -81 and 211/9a of *C. vulgaris* and *C. saccharophila* 2.80 all have the same cell wall characters, and are similar to strains 211-1e, 30.80 and C-27 [8]. The similarity of strains 211-81, -11f and -1e of *C. vulgaris* was

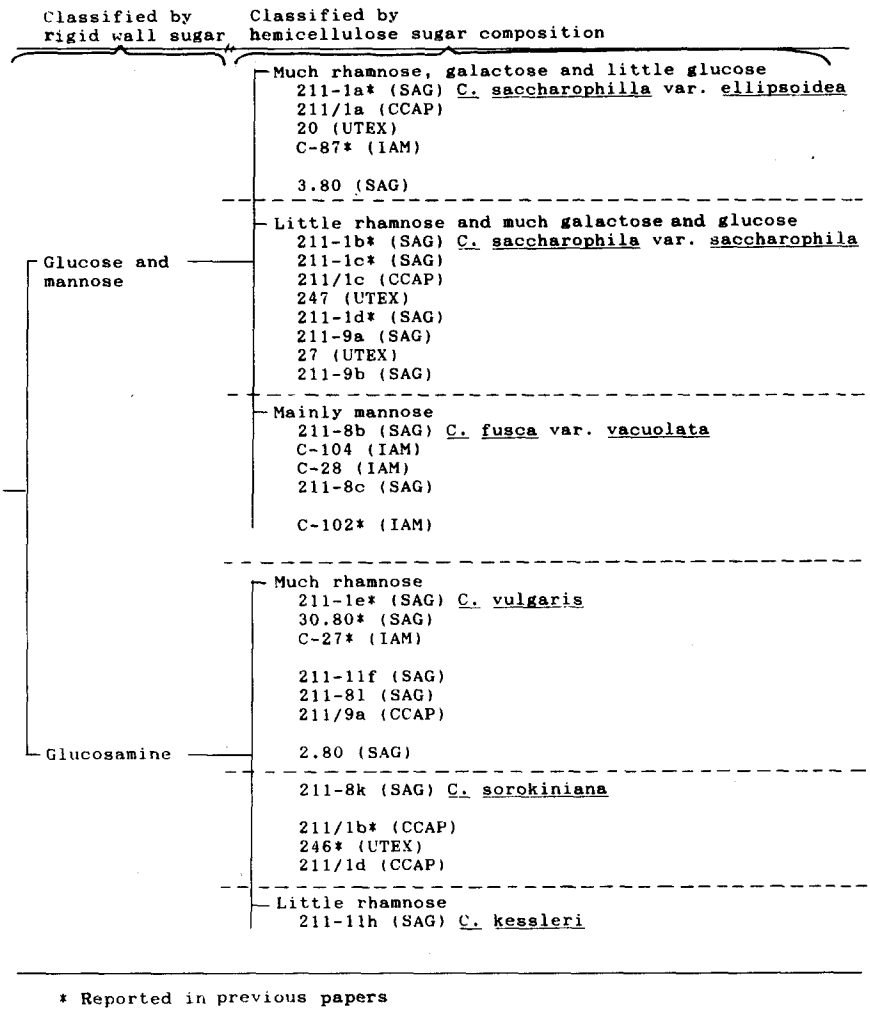

Fig. 2. Classification of the 30 *Chlorella* strains by the sugar compositions of the cell walls.

shown by Kessler [15–17] by GC content of DNA and limits of pH, NaCl concentration, temperature and heavy metal resistance. As seen in Fig. 2, however, some strains of *C. saccharophila* (30.80, 211/1b, 211/1d and 2.80) and *C. ellipsoidea* (C-27 and 246) appeared in this group. The strain 211/1d is recorded to be the same as strain 211-1d [10, 11], but it is separated from *C. saccharophila* because of the presence of glucosamine in the rigid wall. The strain 2.80 has the cell wall characteristic for *C. vulgaris*. Improper classifications were suspected for strains 211/1d and 2.80 in addition to the strains 30.80, C-27, 211/1b and 246 [8]. Recently, E. Kessler (personal communication) found that strain 30.80 has the characters of *C. vulgaris*, and strains 211/1b and 246 those of *C. sorokiniana*. These findings support the present results. For unambiguous classification, cell wall analyses can be effectively used along with the other biochemical characters [19–21].

EXPERIMENTAL

Algal strains. The *Chlorella* strains were obtained from the following algal collection centres: Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, F.R.G. (SAG): *Chlorella saccharophila* 211-9a, 211-9b, 2.80, 3.80, *C. vulgaris* 211-81, 211-11f, *C. sorokiniana* 211-8k, *C. fusca* 211-8b, 211-8c; Culture Centre of Algae and Protozoa, Institute of Terrestrial Ecology, Natural Environment Research Council, Cambridge, U.K. (CCAP): *C. saccharophila* 211/1a, 211/1c, 211/1d, *C. vulgaris* 211/9a; The culture collection of algae, University of Texas at Austin, U.S.A. (UTEX): *C. ellipsoidea* 20, 247, *C. saccharophila* 27; Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan (IAM): *C. pyrenoidosa* C-28, C-104. *C. kessleri* (*C. vulgaris*) 211-11h was kindly supplied by Prof. S. Miyachi of the University of Tokyo.

Culture. The algae were grown by the same method as in the previous study [8].

Analyses. These were performed as described in ref. [8].

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