Pergamon

0031-9422(95)00279-O

Phytochemistry, Vol. 40, No. 2, pp. 457–459, 1995

0031-9422/95 $9.50 + 0.00

CELL WALL COMPOSITION AND TAXONOMY OF SYMBIOTIC *CHLORELLA* FROM *PARAMECIUM* AND *ACANTHOCYSTIS*

HIROSHI TAKEDA

Department of Biology, Faculty of Science, Niigata University, Niigata 950-21, Japan

(*Received* 19 *January* 1995)

Key Word Index—*Chlorella*; symbiotic *Chlorella*; Chlorophyceae; *Paramecium bursaria*; *Acanthocystis*; chemotaxonomy; cell wall composition.

Abstract—*Chlorella* strains from six Japanese and one Chinese strain of *Paramecium bursaria* and those of the stock strains isolated from *Paramecium bursaria* and *Acanthocystis turfacea* have a rigid cell wall composed of glucosamine. The cell walls are not stained with Ruthenium Red and have no anisotropy. From the analysis of the sugar composition of the matrix, the symbiotic *Chlorella* may be new species, most closely related to *C. kessleri*.

INTRODUCTION

Taxonomy of *Chlorella* has been advanced by many biochemical and physiological studies [1–3]. Cell wall sugar composition of *Chlorella* is species-specifically diverse and is used for chemotaxonomic purposes [4–6]. Takeda has made a taxonomical index composed of three numerals of cell wall characteristics, expressed by 1 or 2. viz., sugar of rigid wall is either (1) glucose and mannose or (2) glucosamine, Ruthenium Red stainability of the cell wall is (1) plus or (2) minus, and anisotropy of the cell wall is (1) positive or (2) negative [7].

Some strains of endosymbiotic *Chlorella* have been studied [8–11], but their taxonomy is as yet unsatisfactory. Reisser isolated the auxotrophic *Chlorella* from *Paramecium bursaria* and showed that it was different from known *Chlorella* species, belonging to the group of *C. vulgaris* f. tertia Fott et Nováková and *C. vulgaris* Beijerinck [8]. Endosymbiotic *Chlorella* strains from *Paramecium* and *Acanthocystis* were characterized by the presence of hydrogenase activity, no formation of secondary carotenoid, no growth on mannitol, requirements for vitamins B_1 and B_{12}, GC content of DNA, limits of growth at pH, salt tolerance and temperature, heterogeneous utilization of inorganic nitrogen sources and their morphological features [10, 11]. Three European virus-sensitive symbiotic *Chlorella* Pbi, PbA and PbBS were isolated and shown to have rigid glucosamine wall and the sugar composition of the matrix was characteristic for glucosamine-containing cell walls of non-symbiotic *Chlorella* spp. belonging to *C. vulgaris*, *C. sorokiniana* and *C. kessleri*; these *Chlorella* differed from that of the American endosymbiotic association [12].

In the present study, the sugar composition of cell walls of *Chlorella* strains from six Japanese strains and one Chinese strain of *Paramecium bursaria* and those of stock strains of *Chlorella* isolated from *Paramecium bursaria* and *Acanthocystis turfacea* were studied for their taxonomical significance.

RESULTS AND DISCUSSION

In all the endosymbiotic *Chlorella* strains examined (Nn7, MitB, T31, CT39, T316, SO5, Cs2, 211-6 and 3.83), the taxonomical indices were the same, 2.2.2 (Table 1), i.e. the sugar of rigid wall was glucosamine, the cell wall was not stained with Ruthenium Red and showed negative anisotropy, suggesting that they belonged to *C. kessleri* [7]. The possibility of *C. vulgaris* (index 2.1.2) was excluded because of the lack of staining with Ruthenium Red.

The matrix sugar compositions of all *Chlorellas* from Japanese *Paramecium bursaria* strains were the same, showing high percentage of rhamnose and galactose (Fig. 1). *Chlorella* from Chinese *Paramecium* strain Cs2 differed little from those from the Japanese strains in matrix sugar composition (Fig. 1). The sugar compositions of the matrix in *Chlorella* spp. 211-6 and *C.* spp. 3.83 were different from each other, and also from those from Japanese and Chinese *Paramecium bursaria* (Fig. 1).

Fucose is reported to be the characteristic sugar in the matrices of *C. fusca* var. *vacuolata* and *C. kessleri* [7]. However, fucose was not detected in the matrices of all Chlorellae examined in this study (Fig. 1) in contrast to its presence in Pbi [12].

Chlorella from both Japanese and Chinese *Paramecium bursaria* and also *Chlorella* spp. 211-6 and 3.83 have the taxonomical index of 2.2.2. At present, this index is reported to include only one species, *C. kessleri* [7]. There may be more than one species in the taxon with an index 2.2.2, because index 1.2.1 includes *C. saccharophila*, *C. luteoviridis*, *C. fusca* var. *vacuolata* and *C. minutissima*,

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

Strains	Sugar of rigid wall: Glucose and mannose	Sugar of rigid wall: Glucosamine	Ruthenium Red staining	Anisotropy	Taxanomic index [7]
Nn7	–	+	–	–	2.2.2
MitB	–	+	–	–	2.2.2
T31	–	+	–	–	2.2.2
CT39	–	+	–	–	2.2.2
T316	–	+	–	–	2.2.2
SO5	–	+	–	–	2.2.2
Cs2	–	+	–	–	2.2.2
211-6	–	+	–	–	2.2.2
3.83	–	+	–	–	2.2.2

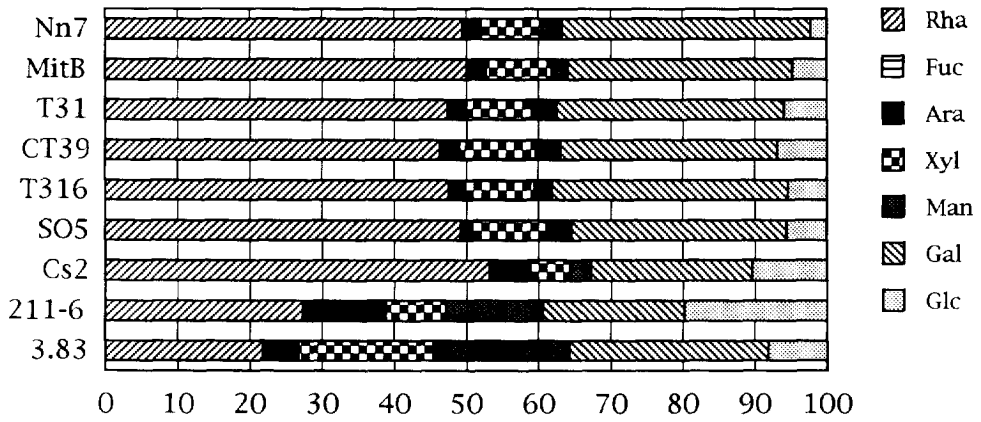

Fig. 1. Comparison of sugar compositions of matrices in endosymbiotic *Chlorella*. Rha, Fuc, Ara, Xyl, Man, Gal and Glc are rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose, respectively. Sugar composition is expressed as per cent of total sugars.

and index 1.2.2 includes *C. zofingiensis* and *C. protothecoides* [7].

Paramecium bursaria Nn7, MitB, T31, T316, SO5 were collected from far distant locations of Japan. But *Chlorella* strains from respective *Paramecium bursaria* strains had the same taxonomical index and matrix sugar composition, suggesting the existence of the same species. *Chlorella* strains from Japanese and Chinese *Paramecium bursaria* strains are clearly different from *Chlorella* Pbi in matrix sugar composition [12]. However, there is one example of an endosymbiotic *Chlorella* with no fucose. The endosymbiotic *Chlorella* NC64A from American *Paramecium bursaria* strains has no fucose in the matrix and the matrix sugar composition is different from those of Pbi, PbA and PbBS [12]. It is interesting to note that all endosymbiotic *Chlorella* so far studied have a rigid wall composed of glucosamine.

EXPERIMENTAL

Paramecium bursaria strains, Nn7, MitB, T31, CT39, T316, SO5 and Cs2 were provided by Prof. I. Ūsuki, Niigata University, Japan. These were collected from different locations in Japan (Nn7: Nagaoka, Niigata pref.; MitB: Mito, Ibaraki pref.; T31: Tsukuba, Ibaraki pref.; SO5: Suwa, Nagano pref.) and China (Cs2: Yangzhou, China) (CT39: a progeny of Cs2 × T151, a strain of Tsukuba).

A drop of culture suspension of *Paramecium bursaria* was dild to obtain a single protozoa cell, which was dried instantly. The ruptured cell was added immediately to a drop of culture medium for *Chlorella* (MCCa, nitrate as nitrogen source [7]) and the suspension spread on an agar slant. After incubation for 2 weeks many colonies appeared and single colonies were mass-cultured autotrophically according to the ref. [7]. *Chlorella* from more than 10 colonies were the same, suggesting that the endosymbiotic *Chlorella* is homogeneous.

Chlorella spp. 211-6 (endosymbiont of *Paramecium bursaria*) and 3.83 (endosymbiont of *Acanthocystis turfacea*) were from Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen. These were cultured on ammonium medium [13], with the addition of thiamine and vitamin B_{12} [10].

Algal cells were harvested and prepd according to ref. [7]. Cell walls were hydrolysed in 2 M TFA to provide

the sugars of the matrix and the rigid wall, the residue from TFA hydrolysis, was hydrolysed in 6 M HCl and 72% H_2SO_4–4% H_2SO_4, respectively to yield the constituent sugars of the rigid wall [7]. Neutral sugars were determined by GC as their corresponding alditol acetates. Glucosamine was detected by high voltage paper electrophoresis according to ref. [7]. Ruthenium Red staining of cell walls was carried out on living *Chlorella* cells and anisotropic properties tested on isolated cell walls according to ref. [7].

Acknowledgements—The author expresses his gratitude to Profs I. Ūsuki, Niigata University, Japan, and U. G. Schlösser, Göttingen University, Germany for their kind gifts of biomaterials.

REFERENCES

1. Kessler, E. (1982) *Prog. Phycol. Res.* **1**, 111.
2. Kessler, E. (1984) *Systematics of Greeen Algae* (Special issue vol. 27) (Irvine, D. E. G. and John, D. M., eds), p. 391. Academic Press, London.
3. Kessler, E. (1992) *Naturwissenschaften.* **79**, 260.
4. Blumreisinger, M., Meindl, D. and Loos, E. (1983) *Phytochemistry* **22**, 1603.
5. Takeda, H. (1988) *Biochem. Syst. Ecol.* **16**, 367.
6. Takeda, H. (1993) *J. Plant Res.* **106**, 195.
7. Takeda, H. (1991) *J. Phycol.* **27**, 224.
8. Reisser, W. (1975) *Arch. Microbiol.* **104**, 293.
9. Reisser, W., Vietze, S. and Widowski, M. (1988) *Symbioses* **6**, 253.
10. Kessler, E. and Huss, V. A. R. (1990) *Bot. Acta* **103**, 140.
11. Reisser, W. and Widowski, M. (1992) in *Algae and Symbioses* (Reisser, W., ed.), p. 21. Biopress, Bristol.
12. Kapaun, E., Loos, E. and Reisser, W. (1992) *Phytochemistry* **31**, 3103.
13. Kessler, E. and Zweier, I. (1971) *Arch. Mikrobiol.* **79**, 44.