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BRIEF COMMUNICATION

Chemical composition of the cell wall in some green algae species

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Abstract

Cell walls of two strains of *Chlorella vulgaris* (from fresh water, F, and saline water, S) and of *Kirchneriella lunaris* contained various proportion of saccharides and proteins (highest percentage in dry matter was found in *K. lunaris*). They differed also in presence of individual monosaccharides (5 in *C. vulgaris* F, 8 in *C. vulgaris* S and 6 in *K. lunaris*) and amino acids (11 in *C. vulgaris* F, 6 in *C. vulgaris* S and 7 in *K. lunaris*). Common substances were rhamnose, cystine, proline, glutamic acid and leucine.

Takeda and Hirokawa (1978) demonstrated that the cell wall of *Chlorella ellipsoidea* was composed of two major constituents: alkali-soluble hemicellulose and alkali-insoluble rigid wall. The former was composed of neutral sugars, rhamnose, xylose, arabinose, mannose and glucose, and the letter had glucosamine as a main constituent. According to Blumriesinger *et al.* (1983) cell wall of the green algae *Chlorella, Monoraphidium, Ankistrodesmus* and *Scenedesmus* contain 24 - 74 % of neutral sugars, 1 - 24 % uronic acid, 2 - 16 % proteins and 0 - 15 % glucosamine. Main sugars are either rhamnose and galactose or mannose and glucose. Other papers dealing with *Chlorococcales* found galactose, glucose and rhamnose in various proportions (Northcote *et al.* 1958, Becker and Schefner 1964), rhamnose, xylose, arabinose, mannose and galactose (El-Sheikh 1990), mannose and glucose (Northcote *et al.* 1960, Loos and Meindl 1982), glucosamine (Northcote *et al.* 1958, Takeda and Hirokawa 1978) and sporopollenin (Atkinson *et al.* 1972).

Brunner and Loos (1985) isolated 4-0-methyl-D-xylose in an acid hydrolysate from cell wall of *C. vulgaris*. Takeda and Hirokawa (1988 a, b) and Takeda (1991) used sugar composition of cell wall as a criterion for *Chlorella* classification and tested in this way about forty *Chlorella* strains. As concerns amino acids, Northcote *et al.* (1958) identified in cell wall acid hydrolysate from *Chlorella* serine, glycine, glutamic acid, threonine, arginine, lysine histidine, alanine, proline, tyrosine, valine,

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phenyalanine and leucine. El-Sheikh (1990) found alanine, glycine, aspartic acid, glutamic acid, valine and isoleucine. To complete these studies, we compared the cell wall composition of green algae *Kirchneriella* and of two strains of *Chlorella* (from fresh or saline water).

Chlorella vulgaris (from fresh water), *C. vulgaris* (from saline water) and *Kirchneriella lunaris* were isolated from Kafr El-Sheikh Governorate, North Delta region of Egypt. These strains were identified in algal collection culture in Göttingen, Germany. The algae were grown under sterile conditions in 1000 cm^3 glass tube (6.5 cm diameter) or 2000 cm^3 conical flasks) in a medium of Kuhl (1962), which was bubbled with filtered sterilized 3 % CO_2 - air mixture under continuous irradiance 11.2 W m^{-2} at room temperature. After reaching maximum growth, the cells were harvested by centrifugation (3000 *g* for 15 min), then washed, resuspended in distilled water by recentrifuged again. 50 mg dry matter were suspended in 10 cm^3 distilled water, freezed and thawed three time and homogenized with a mechanical homogenizer for 30 min at the highest speed. The homogenate was sonicated in a *Soniprep 150 MSE* (Germany) in ice 2 - 3 times, each for 15 min, and centrifuged at 1000 *g* for 10 min. The resulting pellet was washed with distilled water, extracted at room temperature under stirring with methanol, chloroform/methanol (1:2; v:v), and methanol, 30 min in each solvent. The residue was washed twice with water and incubated at 30 °C in a mixture containing 0.02 M potassium phosphate buffer (pH 6.2), 0.02 % sodium azide, 1 mM calcium chloride and 50 units in 1 cm^3 procine-amylase. After 0.5 to 2 h, starch grains were dissolved as judged from microscopic examination and from tests for saccharides. The residue containing isolated cell walls was washed with distilled water and extracted for 2 min at 100 °C in a solution containing 8 M urea, 2 % sodium dodecyl sulphate and 5 % mercaptoethanol, then washed several times with distilled water, and dried over P_2O_5 at room temperature (Loos and Meindl 1982).

Table 1. Chemical composition of cell wall in three algae species [% of cell wall dry matter]. Two types of independent analyses (1, 2) are presented. F - fresh water, S - saline water

	Cell wall components	*C. vulgaris* F	*C. vulgaris* S	*K. lunaris*
(1)	Hemicellulose	25.00	22.60	23.00
	Rigid wall (alkali-insoluble fraction)	66.60	60.00	70.00
(2)	Saccharides	30.00	35.00	75.00
	Proteins	2.46	1.73	3.96
	Lipids	15.00	10.00	12.50
	Unknown substances	52.54	53.27	8.54

Total saccharides content of the algal cell wall was determined using the phenol sulphuric acid method (Dubois *et al.* 1956). Lipid in the cell wall were determined according to Northcote *et al.* (1958). Total soluble protein content in cell wall was determined according to Lowry *et al.* (1951), amino acids according to Simola (1968). 30 mg of algal cell wall were after extraction of fats treated with 10 cm^3 of 24 % (m/v) KOH at room temperature under nitrogen for 2 h. Solid residue was re-

extracted with further 10 cm^3 of KOH for 2 h. The residue from this extraction was thoroughly washed with water by suspension and centrifuging until the supernatant gave the pH value as the original wash water. The washings and alkali extracts were combined and the white residue (rigid cell wall) was dried over P_2O_5. The alkaline hemicellulose was neutralized at 4 °C with cold acetic acid and then evaporated under reduced pressure to half its volume. It was poured into ethanol (the final concentration of alcohol was 85 %) and the formed precipitate was allowed to settle for 24 h. This precipitate was centrifuged, washed with ethanol, and dried.

Table 2. Monosaccharides and amino acids found in cell wall of three algae species. + present; - absent.

	C. vulgaris F	*C. vulgaris* S	*K. lunaris*
Monosaccharides:			
Rhamnose	+	+	+
Ribose	-	+	+
Glucose	-	+	+
Arabinose	-	+	-
Mannose	-	-	+
Unidentified spots	U1 to U4	U5 to U8	U9 to U10
Amino acids:			
Cystine	+	+	+
Proline	+	+	+
Glutamic acid	+	+	+
Leucine	+	+	+
Histidine	+	-	-
Aspartic acid	-	+	-
Serine	-	-	+
Arginine Hcl	+	-	-
Alanine	-	-	+
Glycine	+	-	-
Unidentified spots	U11 to U14	U12	U12

For analysis of monosaccharides, 4 mg cell wall were hydrolized using 2 cm^3 2 M H_2SO_4 for 6 h at 100 °C, neutralized with $BaCO_3$ and filtered. The filtrate was evaporated till dryness, dissolved in distilled water and chromatographed on *Whatman* No. 1 filter paper using solvent systems butanol-pyridine-water (52 : 32 : 16) and butanol-ethanol-water (52 : 32 : 16). Amino acids were separated on the same band of paper using the solvent system butanol-acetic acid (9 : 1) saturated with water. After 20 d of cultivation, dry matter of alge determined after drying was 0.3 mg cm^{-3} (*C. vulgaris* F), 0.4 mg cm^{-3} (*C. vulgaris* S) or 0.2 mg cm^{-3} (*K. lunaris*). The isolated cell walls were dried over P_2O_5 at room temperature represented 20 (*C. vulgaris* F), 26 (*C. vulgaris* S) or 23 (*K. lunaris*) % of the whole cells. *K. lunaris* contained the highest percentage of saccharides and proteins, while *C. vulgaris* F contained the highest percentage of lipids (Table 1). All the three algal species contained rhamnose, both the *C. vulgaris* S and *K. lunaris* contained three

further monosacharides. The number of unidentified monosaccharides was 2 to 4, they were different in different algae species (Table 2). Four common amino acids found in the hydrolysate of cell walls of three algae species were cystine, proline, glutamic acid and leucine (Table 2). In addition, *C. vulgaris* S contained one, *K. lunaris* two, *C. vulgaris* F three other identified and one (*C. vulgaris* S, *K. lunaris*) or four (*C. vulgaris* F) unidentified spots (Table 2). The differences in alkali-soluble and alkali-insoluble fraction of cell wall (hemicellulose and rigid wall) were small (Table 1), the insoluble fraction prevailed in all three species.

References

Atkinson, A.W., Jr., Gunning, B.E.S., John, P.C.C.: Sporopolleninin cell wall of *Chlorella* and other algae: Ultrastructure, chemistry and incorporation of ^{14}C acetate, studied in synchronous culture. - Planta **107**: 1-32, 1972.

Becker, M.J., Schefner, A.M.: Thin layer and paper chromatographic analysis of the carbohydrates in the cell wall of *Chlorella pyrenoidosa* 7 - 11- 05. - Nature **202**: 803-804, 1964.

Blumreisinger, M., Meindl, D., Loos, E.: Cell wall composition of chlorococcal algae. - Phytochemistry **22**: 1603-1604, 1983.

Brunner, B., Loos, E.: Identification of 4-0-methyl-D-xylose as a constituent of the cell wall of *Chlorella vulgaris*. - Plant Sci. **42**: 147-151, 1985.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. - Anal. Chem. **28**: 350-356, 1956.

El-Sheikh, M.M.M.: The systematic position of some algal taxa and the effect of some environmental factors on cell wall composition. - Ph. D. Thesis. Fac. Sci., Tanta Univesity, Tanta 1990.

Kuhl, A. : Beiträge zur Physiologie und Morphologie der Algen. - Fischer-Verlag, Stuttgart 1962.

Loos, E., Meindl, D.: Composition of the cell wall of *Chlorella fusca*. - Planta **156**: 270 - 273, 1982.

Lowry, O.M., Rosenbrough, N.J., Farr, L.A., Randall, R.J.: Protein measurements with the Folin phenol reagent. - J. biol. Chem. **193**: 265 - 275, 1951.

Northcote, D.H., Goulding, K.J., Horne, R.W.: The chemical composition and structure of the cell wall of *Hydrodicton africanum* Yaman. - Biochem. J. **77**: 503 - 503, 1960.

Northcote, D.H., Goulding, K.J., Horne, R.W.: The chemical composition and structure of the cell wall of *Chlorella pyrenoidosa*. - Biochem. J. **70**: 391-397, 1958.

Simola, L.K.: A serological comparison of the seed proteins of the genus *Lathyrus* and certain other genera of the *Papilionaceae*. - Flora **B 158**: 645-658, 1968.

Takeda, H.: Sugar composition of the cell wall and the taxonomy of *Chlorella* (*Chlorophyceae*). - J. Physiol. **27**: 224-232, 1991.

Takeda, H., Hirokawa, T.: Studies on the cell wall of *Chlorella*. I. Quantitative changes in cell wall polysaccharides during the cell cycle of *Chlorella ellipsoidea*. - Plant Cell Physiol. **19**: 591 - 598, 1978.

Takeda, H., Hirokawa, T.: Studies on the cell wall of *Chlorella*. V. Composition of the cell wall chemical composition in strains of *Chlorella ellipsoidea*. - Plant Cell Physiol. **25**: 287 - 295, 1984.

Takeda, H., Hirokawa, T.: Classification of *Chlorella* strain by means of the sugar components of the cell wall. - Biochem. Syst. Ecol. **16**: 367 - 371, 1988a.

Takeda, H., Hirokawa, T.: Classification of *Chlorella* strains by cell wall sugar composition. - Phytochemistry **27**: 3823-3826, 1988b.

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