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Short communication

Deoxyribonucleic acid reassociation in the taxonomy of the genus *Chlorella*

IV. *Chlorella protothecoides* and its relationship to the genus *Prototheca*

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Abstract. DNA homologies of 14 strains of *Chlorella protothecoides* were determined. All strains are related by a high degree of DNA similarity (96–102% D) with the exception of strain 211–11a which proved to belong to *C. kessleri*. There is, however, no detectable DNA homology with strains of the genus *Prototheca* which is supposed to have evolved from *C. protothecoides* by loss of photosynthetic pigments. Even within *Prototheca* the low degree of DNA similarity indicates a heterogeneity similar to that observed in the genus *Chlorella*.

Key words: *Chlorella protothecoides* – *Prototheca* – Taxonomy – DNA/DNA reassociation – DNA homology – DNA base composition

Chlorella protothecoides can be clearly separated from other *Chlorella* species by its auxotrophy (requirement for thiamine) and mesotrophy (ammonium instead of nitrate is needed as nitrogen source) (Kessler and Zweier 1971). The 14 strains investigated in that study are similar in all biochemical and physiological properties which proved to be of taxonomic significance in the genus *Chlorella* (Kessler 1982a). The tendency towards heterotrophy and the widely held opinion that the heterotrophic colourless alga *Prototheca* has evolved from *Chlorella* by loss of photosynthetic pigments, supported the hypothesis that *C. protothecoides* may have been the progenitor of *Prototheca*. This heterotrophic alga, ubiquitous in sewage, feces and soil (Pore et al. 1983), is interesting in two respects: it can be pathogenic for man and animals (Sudman 1974) and it is able to degrade oil (Walker et al. 1975). Physiological and structural characteristics common to both algae are the requirement for thiamine and ammonium for growth as mentioned above, the polysaccharide composition of the cell walls (Conte and Pore 1973), and most of the chemotaxonomic criteria investigated by Kessler (1977, 1982b) and Kerfin and Kessler (1978) with the exception of an increased acid tolerance of *Prototheca*. As stated by Krüger (1884, 1894), the only significant difference between them is the absence or presence of chlorophyll. To date, however, no molecular data are available which could give information about the assumed phylogenetic relationship.

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In this study we determined DNA homologies of *C. protothecoides* and 5 species of the genus *Prototheca*.

Materials and methods

The 14 strains of *Chlorella protothecoides* and *Prototheca zopfii* 263-1a as well as *P. wickerhamii* 263-11 are the same as those used by Kessler and Zweier (1971) and Kerfin and Kessler (1978). The other *Prototheca* strains are a gift of R.S. Pore.

The media of Kessler and Zweier (1971) and Kessler (1977) were used for growth of *C. protothecoides* and *Prototheca*, respectively. Homogenization of algae and DNA extraction was done as described by Huss et al. (1986). DNA of some strains was further purified by CsCl density gradient centrifugation for 40 h at 40000 rpm and 20° C in a VTi 50 rotor of a Beckman L8-60M ultracentrifuge. Fifty microliters of bisbenzimide (Hoechst dye 33258)/mg DNA were added for better separation of AT- and GC-rich DNA bands (Müller and Gautier 1975) and visualization in UV-light at 312 nm. Dye removal was effected by several extractions of the recovered DNA with CsCl-saturated isopropanol. To remove CsCl, the DNA solutions were dialyzed twice against large volumes of standard saline citrate (0.15 M NaCl + 0.015 M trisodium citrate, pH 7.0). If necessary, DNA was concentrated in dialysis tubes over dry saccharose with subsequent dialysis against an appropriate buffer.

DNA base compositions and DNA homologies were determined according to Huss et al. (1986, 1987a).

Results and discussion

As expected from the results of Kessler and Zweier (1971) and from the observed correlation between uniformity of biochemical and physiological properties and respective DNA homologies in the genus *Chlorella* (Huss et al. 1987b), all strains of *C. protothecoides* with the exception of strain 211-11a are highly related in a phylogenetic sense (Table 2). The observed DNA homologies of 96–102% clearly show *C. protothecoides* to be a well defined species as has already been demonstrated for *C. fusca* var. *vacuolata* and *C. kessleri* (Huss et al. 1987b). Strain 211-11a, however, with a slightly different DNA base composition (Table 1) and a DNA homology of only 34% compared with the type strain 211-7a

which is within the background noise of the method employed (Huss et al. 1986), does not fit in this species. The reexamination of its biochemical and physiological properties showed them all to be characteristic for *C. kessleri*. This was confirmed by DNA hybridization (105 %D with *C. kessleri* 211-11g). The question arises where and when the strain has been confused. Anyway, strain 211-11a now available from the Göttingen collection of algae is *C. kessleri* and not *C. protothecoides*.

Table 1. List of strains studied, their origin and DNA base composition

Species	Strain[a]	Origin	G + C content (mol%)[b]
Chlorella protothecoides	211-7a^T	Göttingen	59.0
Krüger	211-7b	Göttingen	59.9
	211-7c	Göttingen	62.0
	211-7d	Göttingen	59.5
	211-8d	Göttingen	59.4
	211-10a	Göttingen	59.1
	211-10b	Göttingen	60.0
	211-10c	Göttingen	58.4
	211-10d	Göttingen	58.9
	211-10e	Göttingen	58.7
	211-11a[c]	Göttingen	56.9
	211-11i	Göttingen	59.0
	211-13	Göttingen	58.8
	211-17	Göttingen	59.4
C. kessleri Fott and Nováková	211-11g^T	Göttingen	55.1
Protothecа zopfii Krüger	263-1a	Göttingen	69.5
P. wickerhamii Tubaki and Soneda	263-11	Göttingen	60.6
	1283	Pore	60.3
P. moriformis Krüger	1263	Pore	70.7
P. stagnora Cooke	1291	Pore	65.8
P. ulmea Pore	1298	Pore	75.9[d]

[a] Göttingen, Algensammlung des Pflanzenphysiologischen Instituts der Universität, Göttingen, FRG; Pore, R. S. Pore, Morgantown, West Virginia, USA
[b] *Escherichia coli* B (G+C content: 52.0 mol%; Gillis et al. 1970) was used as reference
[c] This strain was found to belong to *C. kessleri*
[d] Nuclear DNA, purified by CsCl density gradient centrifugation as described in Methods
[T] Type strain

It was of further interest in this study to prove the hypothesis that *C. protothecoides* may have been the progenitor of the genus *Prototheca*. As seen in Table 1, however, DNA base compositions of the *Prototheca* strains examined are quite different from that of *C. protothecoides* with the exception of *P. wickerhamii*. But even compared to this species, no significant DNA homology indicating a close relationship of these algae was detected (Table 3). This is also true for the different species of *Prototheca*. DNA base compositions of 60 – 76 Mol% G + C and the low DNA homologies indicate a heterogeneity similar to that observed in the genus *Chlorella*. Only *P. zopfii* and *P. moriformis* share a homology of about 25%, much more than the 6% detected, for example, between the two strains of *P. wickerhamii*.

It has to be emphasized, however, that the technique of DNA/DNA hybridization employed, only reveals close phylogenetic relationships on the species and, in part, on the genus level (Schleifer and Stackebrandt 1983). No higher homologies than those observed in *Prototheca* have been found, for example, between different *Chlorella* species (Huss et al., in preparation). A conclusive insight into the phylogeny of these algae is only possible by comparison of ribosomal RNA sequences (Woese 1987).

In some cases of unusually high amounts of organellar DNA in the nucleic acid preparations of *Prototheca*, nuclear

Table 2. DNA homologies of *Chlorella protothecoides* using the renaturation rate method

Strain	Degree of binding (%D) with 211-7a^T
211-7b	97
211-7c	100
211-7d	102
211-8d	99
211-10a	98
211-10b	96
211-10c	100
211-10d	100
211-10e	97
211-11a[a]	34
211-11i	98
211-13	99
211-17	100

[a] This strain belongs to *C. kessleri*
[T] Type strain

Table 3. DNA homologies of *Prototheca* species and *Chlorella protothecoides* 211-7a^T using the membrane filter method in 3 × SSC/formamide at 45°C under optimal conditions [T_M – 25°C, related to the melting temperature of the (^{3}H)-labelled strain]

Source of filter-bound DNA	Degree of binding (%D) with (^{3}H)-labelled DNA from:					
	263-1a	1263^N	1291^N	263-11	1283^N	211-7a^T
P. zopfii 263-1a	100	14	10	9	7	6
P. moriformis 1263^N	36	100	12	12	13	9
P. stagnora 1291^N	21	12	100	10	12	8
P. wickerhamii 263-11	12	6	6	100	6	10
1283^N	13	8	9	6	100	4
P. ulmea 1298^N	14	8	8	12	6	12
C. protothecoides 211-7a^T	11	6	5	16	16	100

[N] Nuclear DNA, purified by CsCl density gradient centrifugation as described in Methods
[T] Type strain

DNA was additionally purified by CsCl density gradient centrifugation and separated from what was assumed to be mostly leucoplast DNA. It was ascertained by an unpublished observation that small amounts of chloroplast DNA normally present in our nucleic acid preparations of *Chlorella* do not markedly change the homology values obtained with purified nuclear DNA. There is, however, a tendency to slightly higher values in the presence of chloroplast DNA due to the more conserved character of this DNA species.

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References

Conte MV, Pore RS (1973) Taxonomic implications of *Prototheca* and *Chlorella* cell wall polysaccharide characterization. Arch Mikrobiol 92:227–233

Gillis M, De Ley J, De Cleene M (1970) The determination of molecular weight of bacterial genome DNA from renaturation rates. Eur J Biochem 12:143–153

Huss VAR, Dörr R, Grossmann U, Kessler E (1986) Deoxyribonucleic acid reassociation in the taxonomy of the genus *Chlorella*. I. *Chlorella sorokiniana*. Arch Microbiol 145:329–333

Huss VAR, Schwarzwälder E, Kessler E (1987a) Deoxyribonucleic acid reassociation in the taxonomy of the genus *Chlorella*. II. *Chlorella saccharophila*. Arch Microbiol 147:221–224

Huss VAR, Hehenberger A, Kessler E (1987b) Deoxyribonucleic acid reassociation in the taxonomy of the genus *Chlorella*. III. *Chlorella fusca* and *Chlorella kessleri*. Arch Microbiol 149:1–3

Kerfin W, Kessler E (1978) Physiological and biochemical contributions to the taxonomy of the genus *Prototheca*. II. Starch hydrolysis and base composition of DNA. Arch Microbiol 116:105–107

Kessler E (1977) Physiological and biochemical contributions to the taxonomy of the genus *Prototheca*. I. Hydrogenase, acid tolerance, salt tolerance, thermophily, and liquefaction of gelatin. Arch Microbiol 113:139–141

Kessler E (1982a) Chemotaxonomy in the *Chlorococcales*. In: Round FE, Chapman DJ (eds) Progress in phycological research, vol 1. Elsevier Biomedical Press B.V., Amsterdam, pp 111–135

Kessler E (1982b) Physiological and biochemical contributions to the taxonomy of the genus *Prototheca*. III. Utilization of organic carbon and nitrogen compounds. Arch Microbiol 132:103–106

Kessler E, Zweier I (1971) Physiologische und biochemische Beiträge zur Taxonomie der Gattung *Chlorella*. V. Die auxotrophen und mesotrophen Arten. Arch Mikrobiol 79:44–48

Krüger W (1884) Kurze Charakteristik einiger niederer Organismen im Saftflusse der Laubbäume. Hedwigia 33:241–266

Krüger W (1894) Beiträge zur Kenntniss der Organismen des Saftflusses (sog. Schleimflusses) der Laubbäume. Parts I and II. Zopf's Beitr Physiol Morphol nied Organ (Leipzig) 4:69–116

Müller W, Gautier F (1975) Interactions of heteroaromatic compounds with nucleic acids. A*T-specific non-intercalating DNA ligands. Eur J Biochem 54:385–394

Pore RS, Barnett EA, Barnes WC Jr, Walker JD (1983) *Prototheca* ecology. Mycopath 81:49–62

Schleifer KH, Stackebrandt E (1983) Molecular systematics of prokaryotes. Ann Rev Microbiol 37:143–187

Sudman MS (1974) Protothecosis. Amer J Clin Path 61:10–19

Walker JD, Colwell RR, Vaituzis Z, Meyer SA (1975) Petroleum-degrading achlorophyllous alga *Prototheca zopfii*. Nature 254:423–424

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

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