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Taxonomic Studies on Endocytobiotic Chlorophycean Algae Isolated From Different American and European Strains of *Paramecium bursaria*

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Abstract

Endocytobiotic coccoid chlorophycean algae were isolated from different *Paramecium bursaria* Ehrbg. strains (Ciliata) collected at various European and American freshwater sites. The taxonomic characterization of algae by light and electron microscopy, physiology, and G+C-contents of DNA shows that ciliates of different sites harbour also different chlorophycean partners. Some algal strains can be assigned to *Chlorella vulgaris* and the *Chlorella vulgaris*-group, others show some resemblance to the *Chlorella fusca*-group. It is discussed whether *Paramecium bursaria* forms an ecological niche which can be settled by different algal taxa and thus possibly represents a sort of evolutionary resting-place for old or transitional forms as well as for new offsprings at least within the genus *Chlorella*. Isolated chlorellae differ from free-living counterparts only by a pH-dependent excretion of glucose, maltose or fructose, other features of algae don't show any specific adaptation to the symbiotic milieu.

Keywords: *Chlorella*, *Paramecium bursaria*, symbiosis, taxonomy

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1. Introduction

Endocytobiotic associations of fresh-water ciliates and green algae (Chlorophyceae) have been studied repeatedly (for a review see Reisser, 1986) and some of them are well characterized by thorough data on ultrastructure, physiology, and behaviour. Less attention was paid to taxonomic questions. Among ciliates holo-, peri-, and heterotrich hosts are observed and usually only one species of a host genus forms a stable association with algae. Algal partners of different host species have been generally assigned to the genus *Chlorella* (Chlorellaceae, Chlorophyceae) and probably belong to the *Chlorella vulgaris*-group (Reisser, 1984).

Basing on data derived from the best studied systems, i.e. the association of *Paramecium bursaria* Ehrbg. with *Chlorella* sp., the so-called green *Paramecium*, an ecological concept of symbiosis formation was developed (Reisser et al., 1983). According to that model the ciliate host forms an ecological niche which can be settled by all algae meeting its physiological requirements. Thus it could be shown by infection experiments with alga-free *Paramecium bursaria* and different strains of symbiotic and free-living *Chlorella* spp. that ciliates are settled by most algae and that artificially induced symbiotic systems are formed which differ in stability (Reisser, 1987a). Accordingly there are good reasons to assume that also different algal partners may exist in naturally occurring green paramecia. Until now the taxonomy of algal partners has been studied thoroughly only in different ciliate host species but not in the same one (Reisser, 1984). Thus the aim of this paper is to test the validity of the ecological concept of symbiosis formation by checking the taxonomic position of algae isolated from green paramecia of different European and American fresh-water sites by studying their morphological, physiological, and molecular (DNA) features as well as by infection experiments.

2. Materials and Methods

Isolation of algae from *Paramecium bursaria* and subsequent culture under sterile conditions as stock and mass cultures were done according to procedures described by Reisser (1981, 1984). Sources of algae and strain designations are given in Table 1. For culture of green ciliates and protocols of infection experiments see Niess et al. (1982).

Classification of algae by morphological features, i.e. size and shape of cells, shape of chloroplast, presence of pyrenoid, number of autospores, and structure of cell wall, was done according to Fott and Novakova (1969) and

Table 1. Sources and designations of tested algae exsymbiotic from *Paramecium bursaria* and of free-living algae

Alga	Source of *Paramecium bursaria*	Isolated by/in	Reference
Exsymbiotic			
Pbi	Botanical Garden, Univ. Göttingen	Reisser/1974	1
PbAm	pond near Amönau, FRG	Widowski/1986	2
PbBS	pond near Bad Schwalbach, FRG	Widowski/1986	2
PbDu	pond near Düsseldorf, FRG	Widowski/1986	2
PbOp	pond near Opladen, FRG	Widowski/1986	2
PbW	Wards Biological Supply, USA	Reisser/1986	2
1/W	D.S. Weis, USA	Weis/1978	3
NC64A	S.J. Karakashian, USA	Karakashian/1963	4
Free-living			
211–11b *C. vulgaris* Beij. (type strain)	SAG	Beijerinck/1892	5
211–8b *C. fusca* var. *vacuolata* Sh. et Kr. (type strain)	SAG	Emerson/1923	5

C; Chlorella; SAG: Sammlung von Algenkulturen Göttingen, Schlösser (1982); 1: Reisser (1975); 2: this paper; 3: identical with strain 1 of Tab. 1 in Weis (1978); 4: Karakashian (1963), obtained through J.L. van Etten, Univ. of Nebraska, Lincoln, USA; 5: Schlösser (1982).

Kalina and Puncocharova (1987) with log-phase cells. Algae were grown in Erlenmeyer flasks mounted on a shaker under identical culture conditions, i.e. stock culture conditions as described by Reisser (1984) and enriched by the addition of meat extract (0.1%) and soil extract (3.0%).

Physiological features, i.e. activity of hydrogenase, synthesis of secondary carotenoids, tolerance to acid pH or different NaCl- concentrations, production of proteolytic and amylolytic enzymes, thermophily, growth with different C- and N-sources as well as qualitative and quantitative excretion of carbohydrates were determined according to methods described by Kessler (1972, 1982) Kessler and Czygan (1970), Soeder (1962, 1963), and Reisser (1984). In addition, activity of carbonic anhydrase was tested by measuring the time needed for lowering the pH from 8.3 to 7.3 after addition of CO_2-saturated water to algae grown for 4 days at 0.04% CO_2 and then having been suspended in 25 mM Veronal/H_2SO_4-buffer (2°C, 10, 000 lux of Phillips fluorescent bulbs).

UV-resistance of algae was measured by exposing lawns of log-phase algae grown on enriched Bold's basal medium with 1.5% algar in Petri-dishes for different time (5–20 min) and at different distances (30–60 cm) to a UV-tube (Phillips TUV 30W). During the exposure to UV-rays half of the algal lawn was protected against radiation and then Petri-dishes were kept under stock culture conditions. Two weeks later algal colonies in both exposed and protected areas were counted.

For staining of cell walls with Ruthenium Red and light and transmission electron microscopical techniques see Reisser (1984). Scanning electron microscopy was done according to Vobis et al. (1986). For preparation of the algal DNA the cell material was centrifuged and resuspended in 10 ml of 0.01 M Tris-buffer, pH 8.1, containing 1.0% SDS and passed through a french press (110 MPa). The DNA was then extracted and purified as has been described by Zur Hausen et al. (1970). RNAse A and Proteinase K were obtained from Serva and Sigma, respectively.

Melting points of DNA preparations in 0.1×SSC (1.0×SSC: 0.15 M NaCl + 0.015 M trisodium citrate, pH 7.0) were determined at 260 nm with a Gilford spectrometer equipped with a thermoprogrammer. The temperature was raised at a rate of 1°C min^{-1}. *Escherichia coli* B NTCT 10537 DNA was isolated according to Marmur (1961) and included in each run. Two preparations were made from each alga and each preparation was run at least twice. The mol % G+C-contents was calculated from the equation of De Lay (1970) and corrected for the differences obtained between the

Table 2. Morphological features of tested exsymbiotic and free-living algae[a]

Alga[b]	Diameter of cells [μm][c]	Ratio of axes [1:][c]	Shape of plastid[d]	Number of autospores[e]
Exsymbiotic				
Pbi	5.5–6.0	1.00–1.07	saucer	4 (8)
PbAm	5.8–6.7	1.06–1.23	saucer/cup	4 (2)
PbBS	6.5–7.5	1.00–1.12	sucer	4 (2)
PbDu	6.5–9.8	1.15–1.30	parietal*	4/8
PbOp	6.0–9.0	1.18–1.33	parietal*	4/8
PbW	5.0–7.5	1.00	girdle	4/8
1/W	7.0–7.8	1.00	girdle	4/8
NC64A	5.0–6.2	1.22–1.50	girdle	4
Free-living				
211–11b	5.5–6.3	1.00–1.07	cup/girdle	4
211–8b	12.0–16.0	1.00–1.35	parietal*	4/8 (2)

a: data from cells grown under identical culture conditions, see Materials and Methods; b: for strain designations see Table 1, c: ratio of largest to smallest diameter of cell (e.g. spherical cells: 1:1; (a), +/− 5%, d: according to nomenclature of Fott and Novakova (1969), e: () = rarely; *: pyrenoid located in thickening of plastid.

determined mol % G+C of *Escherichia coli* B and 51.6% which is the value given by the Deutsche Sammlung für Mikroorganismen, Mannheim, for this strain (Fahmy et al., 1985; Henssen et al., 1987).

3. Results

Morphology

Morphological features of algae as observed by light microscopy are summarized in Table 2 and illustrated by Fig. 1. The isolated algae showed a coccoid shape, lacked flagellated cells and reproduced only asexually by forming 2–8 autospores. According to morphological features and to the classificatory key given by Fott and Novakova (1969), the American isolates PbW, 1/W, and NC64A belong to *Chlorella vulgaris* Beij., the type strain of which is 211–11b (Schlösser, 1982). Classification of European isolates PbDu and PbOp as belonging to the *Chlorella fusca*-group also seems to be obvious. The taxonomic position of the other European isolates Pbi, PbAm,

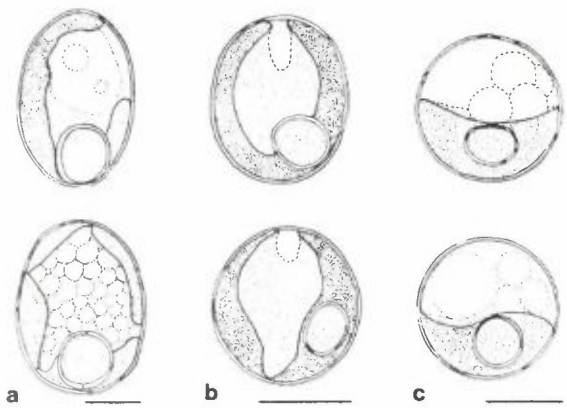

Figure 1. Chloroplast types of symbiotic algae isolated from *Paramecium bursaria* of different collection sites
a: parietal chloroplast in PbDu,
b: girdle-shaped chloroplast in NC64A,
c: saucer-shaped chloroplast in Pbi, for abbreviations see text, bar: 3 μm.

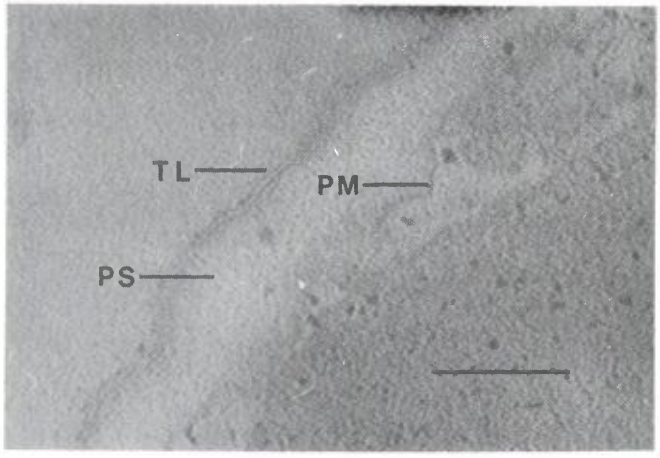

Figure 2. Cell wall of symbiotic alga (PbDu) isolated from *Paramecium bursaria*
TL: trilaminar layer, PS: polsaccharid layer,
PM: plasmalemma, bar: 0.20 μm

and PbBS is less evident. Plastids often are saucer shaped but sometimes also girdle and cup shapes are observed. Since a pyrenoid is always present morphological features of those strains suggest an assignment to the *Chlorella vulgaris*-group (sensu Kessler; see Reisser, 1984) with some closer relationship

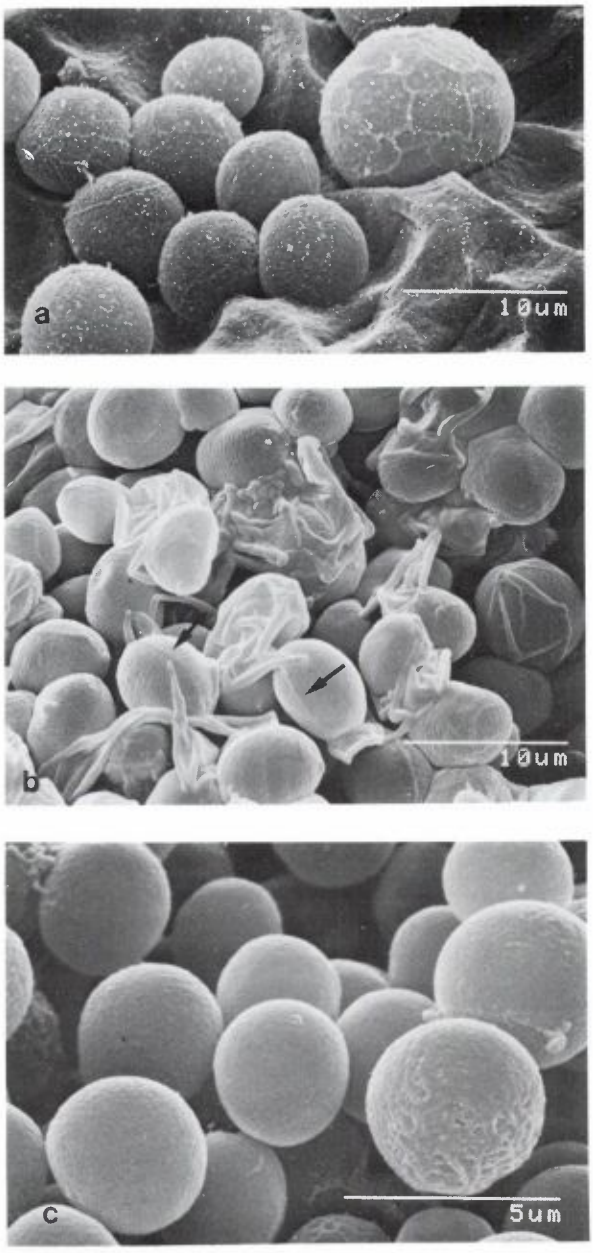

Figure 3. Cell surface structures of free-living algae and symbiotic specimen isolated from *Paramecium bursaria*

a: free-living *Chlorella fusca* var. *vacuolata* (211–8b) with network of ribs on cell surface

b: symbiotic strain PbDu with meridional rib (arrow) on cell surface

c: free-living *Chlorella vulgaris* (211–11b), no formation of ribs on cell surface

to *Chlorella sorokiniana* Sh. et Kr.). An electron microscopic study of cell walls showed for PbDu and PbOp a typical trilaminar structure (TL-layer, Fig. 2; Atkinson et al., 1972) and an inner polysaccharid layer. That TL-layer was also described for *Chlorella fusca* var. *vacuolata* (Atkinson et al., 1972). But whereas the surface of *Chlorella fusca* var. *vacuolata* is characterized by an irregular alignment of ribs, both PbDu and PbOp show only one meridional rib, in few cases two or three ribs. All other tested algae showed no ribs, i.e. a smooth cell wall surface, as is shown by *Chlorella vulgaris* (Fig. 3). Thus it may be concluded from ultrastructural features of PbDu and PbOp that strains are clearly different from *Chlorella vulgaris*. Formation of ribs on cell surfaces suggests a tentative assignment to the *Chlorella fusca*-group. Staining of cell walls with Ruthenium Red showed three different staining-types according to those ones described by Yamada and Sakaguchi (1982). An intense red colour of the cell wall was shown by those algae closely related to *Chlorella vulgaris*, a pink reaction by those ones related to *Chlorella sorokiniana* whereas no reaction was observed with specimen related to the *Chlorella vulgaris*-group (Table 3).

Physiology

Physiological features of algae are shown in Table 3. Classification of symbiotic algae according to Table 3 and to the key given by Kessler (1984) is less obvious than by morphological characteristics. In general, release of organic material is shown by all algae tested, but in symbiotic chlorellae the bulk of released substances is made up by glucose, fructose, and maltose and the amount of excreted material depends on pH, in general being higher at acid pH than at a neutral one whereas in free-living algae release is not significantly dependent on pH under conditions tested. The American isolates release less than 10% of total photosynthetically fixed material at acid pH and thus show a rate similar to the one of the free-living *Chlorella vulgaris*. European isolates — with the exception of PbBS — release significantly more material, e.g. up to 48% by PbAm at pH 4.8. Yet in both groups release is significantly raised by an acidification of the external pH, e.g. in Pbi about 1.6 times, in PbDu and PbOp about 12.0 and 11.2 times, respectively.

The American isolates, PbW, 1/W, and NC64A, seem to be closely related to free-living *Chlorella vulgaris* with NC64A showing the highest degree of identity differing only in a somewhat lower salt tolerance and carbohydrate excretion. Although any hydrogenase activity could not be detected strains PbDu and PbOp show most similarities to free-living *Chlorella fusca* var. *vac-*

Table 3. Physiological and cytochemical features of tested exsymbiotic and free-living algae

Alga*	Hyd	Sec	Gel	Sta	pH	Sal	Tem	Vit	N	Sug	released organic material [%] of totally fixed material at pH 5.9	4.8	RuR
Exsymbiotic													
Pbi	–	–	–	–	4.5	0	–	+	1, 2	G, M	16.47	25.83	+/–
PbAm	/	–	–	+	>5.0	0	–	?	?	G, M	22.17	48.07	+/–
PbBS	+	–	–	+	>5.0	0	–	?	?	G, M	2.65	6.38	+/–
PbDu	–	+	+	+	4.0	1	+	+	1, 2, 3	G, M	1.87	22.47	–
PbOp	–	+	+	+	4.0	1	+	+	1, 2, 3	G, M	2.66	29, 83	–
PbW	–	–	–	+	4.0	3	+	+ –	1, 2	G	4.87	2.96	+
1/W	–	–	–	+	4.0	3	+	+ –	1, 2	G	10.50	7.45	+
NC64A	–	–	–	–	4.0	1(2)	+	+	?	F, G, M	1.71	4.17	+
Free-living													
211–11b	–	–	–	–	4.0	2(3)	+	–	1, 2	–	5.84	4.29	+
211–8b	+	+	+	+	4.0	2(3)	+	–	1, 2, 3	–	0.66	0.79	–

a: for strain designations see Table 1 and Materials and Methods; Hyd: hydrogenase activity; Sec: synthesis of secondary carotenoids; Gel: liquefication of gelatine, i.e. production of proteolytic enzymes; Sta: degradation of starch, i.e. production of amylolytic enzymes; pH; lower level of pH tolerance (growth still possible); Sal: lower level of NaCl tolerance [% NaCl] (growth still possible); Tem: growth possible over 34°C; Vit: requirement of vitamins B, and B_{12}; N: growth with N-source; 1: NH_4Cl; 2: glutamine; 3: glutamic acid; Sug: carbohydrate excreted; F: fructose; G: glucose; M: maltose; RuR: staining of cell wall with Ruthenium Red; ?: could not be tested because of growth only in complex media; /: not tested.

uolata differing besides in hydrogenase activity only in salt tolerance and release of sugars which in those strains is clearly pH dependent. Strains Pbi, PbAm, and PbBS form a third group which is characterized by an extreme NaCl- and temperature-sensitivity. That group may be assigned to the *Chorella-vulgaris*-group, i.e. shows some relationsip to *Chlorella vulgaris* Beij., *Chlorella sorokiniana* Sh. et Kr., *Chlorella lobophora* Andr., and *Chlorella sorokiniana* (Krüg.) Mig. (Reisser, 1984). PaAm is outstanding

Table 4. G+C-contents of DNA isolated from algae exsymbiotic from ciliates, a sponge, and from free-living algae

Alga*	Exsymbiotic from	Reference	G+C-contents (mol %)[b]
Pbi	*Paramecium bursaria*	1	74.4
241.80	*Paramecium bursaria*	2	73.3
PbDu	*Paramecium bursaria*	3	89.0
PbOp	*Paramecium bursaria*	3	89.0
PbW	*Paramecium bursaria*	3	62.5
1/W	*Paramecium bursaria*	4	67.8
Cvi	*Climacostomum virens*	5	67.9
Chi	*Coleps hirtus*	5	67.5
Edi	*Euplotes daidaleos*	5	68.1
Spi	*Stentor polymorphus*	5	69.4
211-40c	*Spongilla* sp.	2, 5	92.1
211–11b	/ (free-living)	2	68.0
211-8b	/ (free-living)	2	57.3

a: for strain designations see Table 1 and Materials and Methods; b: +/− 5%, G+C-contents of *Ecscherichia coli* B NTCT 10537 DNA was measured to be 51.5 mol % (see Materials and Methods); 1: Reisser (1975); 2: Schlösser (1982); 3: this paper; 4: Weis (1978); 5: Reisser (1984).

in this group in releasing nearly 50% of the total photosynthetically fixed carbon.

Other features of algae turned out to be less suitable for a classification. Capacity of heterotrophic growth is obviously not typic for symbiotic algae since all algae tested could grow heterotrophically with glucose. Since the CO_2-supply of symbiotic algae is guaranteed by host respiratory CO_2, lack of carbon anhydrase activity would not be detrimentous but enzyme activity could be detected in both free-living and symbiotic algae. UV-resistance was different among chlorellae but without showing a special pattern for free-living and symbiotic specimen. An exposure period of 20 min and a distance

to the UV-source of 30 cm was lethal to all algae (no colonies counted in exposed area of Petri-dish).

DNA

Classification of algae by morphological and physiological data was corroborated by a determination of the G+C-contents of algal DNA isolated from selected strains and for reasons of comparison also from further symbiotic chlorellae of different fresh-water ciliates (Table 4; Reisser, 1984). Data support the classificatory scheme derived from both morphological and physiological studies in assigning 1/W and chlorellae isolated from *Climacostomum virens, Coleps hirtus, Euplotes daidaleos*, and *Stentor polymorphus*, i.e. Cvi, Chi, Edi, and Spi, — which are rather uniform (Reisser, 1984) — to *Chlorella vulgaris*. Pbi and 241.80 are closely related. PbOp and PbDu show the same G+C-contents and are clearly different from other chlorellae, interestingly also from *Chlorella fusca* var. *vacuolata*. They show an extremely high G+C-contents which is similar to the one of 211–40c, a *Chlorella* sp. isolated from a freshwater sponge (Reisser, 1984).

Infection

Since infection experiments showed for algae isolated from different *Paramecium bursaria* a great taxonomical difference, in a further series of experiments it was tested whether different symbiotic algae are still able to form a stable symbiotic unit with another *Paramecium bursaria* strain than the one they have been isolated from (cross-infection). Results (Table 5) clearly demonstrate that cross-infection in most cases is possible, i.e. stable new symbiotic associations are formed not only by partners which have lived together originally, but also by newly combined ones.

4. Discussion

Whereas eukaryotic algal partners in marine endocytobiotic associations show a great taxonomic diversity comprising chlorophytes and rhodophytes as well as diatoms and dinoflagellates (Taylor, 1984) the taxonomic position of algae in fresh-water symbiotic systems such as those formed by turbellarians, clams, coelenterates, ciliates, and amoebae is reported to be rather uniform in that algae are classified as belonging to the *Chlorella vulgaris*-group (Douglas, 1987; Reisser, 1984, 1986; Douglas and Huss, 1986). The only exclusion reported is a *Chlorococcum* species in *Hydra magnipapillata* (Rahat and Reich, 1985a). Yet taxonomic studies on the genus *Chlorella* are

Table 5. Infection of European and American strains of alga-free *Paramecium bursaria* with different free-living algae and algae exsymbiotic from European and American strains of green *Paramecium bursaria*

Alga*	Infection of alga-free *Paramecium bursaria* European strain[b]	*Paramecium bursaria* American strain[c]
Exsymbiotic		
Pbi	+	+
PbAm	+	/
PbBS	+	/
PbDu	+	/
PbOp	+	/
PbW	–	–
1/W	–	–
Nc64A	+	+
Free-living		
211–11b	–	/
211–8b	–	/

a: for strain designations see Table 1; b: raised from green *Paramecium bursaria* isolated from a pond in the Old Botanical Garden of Göttingen University by the first author, source of Pbi (see Table 1); c: raised from green *Paramecium bursaria* obtained through Carolina Biological Supply Comp., USA, and isolated by the first author; +: stable symbiotic unit formed, i.e. ciliates contain maximum algal population about 2 weeks after start of the experiment and do not loose them in the dark in an inorganic medium (Niess et al., 1982); –: symbiotic unit not formed; /: experiment not performed.

awkwardly hampered by its lack of sexuality, i.e. the classical species definition is not applicable. Consequently, a lot of efforts aimed at the construction of classificatory schemes based on morphological and physiological data (Fott and Novakova, 1969; Kessler, 1984). Unfortunately, in those schemes the taxonomic significance of some characteristics sometimes is a matter of "taste". Thus recent studies (Huss et al., 1986, 1987a, 1987b) center on the analysis of DNA. Experiments show that in the genus *Chlorella* some species such as *Chlorella fusca* var. *vacuolata* (type strain: 211–8b, Schlösser, 1982) and *Chlorella saccharophila* (type strain: 211–9a, Schlösser, 1982) are clearly dif-

ferent by morphology and physiology but show rather similar G+C-contents, i.e. 51.6 mol % for 211–8b and 52.0 mol % for 211–9a (Huss et al., 1987a; Kessler, 1984). On the other hand strains such as PbOp and PbDu and 211–8b show a lot of common morphological and physiological features but differ tremendously in G+C-contents (Table 4). Probably more reliable information is obtained from studies on the homology of DNAs from different strains (Huss et al., 1986, 1987a, 1987b). Nevertheless, available data as e.g. G+C-contents which varies from 43.0 mol % to 79.0 mol % (Hellmann and Kessler, 1974) cast some doubt on the monophyly of the genus *Chlorella* and suggest an artificially constructed taxon of coccoid asexual algae lacking formation of planospores and showing many traits of convergency. Accordingly, Kalina and Puncochorova (1987) have proposed to classify the former *Chlorella fusca* var. *vacuolata* (211–8b) as *Graesiella vacuolata* (Sh. et Kr.), Kal. et Punc., comb. nov. (subfamily Scotiellocystoideae Fott 1976, Chlorellaceae), on basis of morphological and ultrastructural features. In the present study it was tested whether in green paramecia of different collection sites different algal taxa exist, rather than to give a thorough discussion on the validity of the species definition in the the genus *Chlorella.*

Since also free-living algae excrete organic substances (Table 3; Fogg, 1966) obviously the only physiological feature characterizing endocytobiotically living chlorellae is excretion of carbohydrates such as glucose, maltose, and fructose by a pH-dependent mechanism. Other features such as heterotrophy — as is suggested by Rahat and Reich (1985b) for the *Hydra viridis-Chlorella* sp.-system — or requirement of vitamins or special N-sources, exoenzymes, tolerance to acid pH or NaCl and even the amount of excreted sugars are not important for symbiosis formation. These observations corroborate former reports on artificially induced stable endocytobiotic associations between alga-free *Paramecium bursaria* and different kinds of free-living *Chlorella* taxa (Reisser, 1987a), as well as experiments supporting the assumption that *Paramecium bursaria* takes up symbiotic partners not primarily because an additional food supply is required but because of a deficiency in its recognition- and immune-system (Reisser, 1987b). Probably the primary advantage in symbiosis formation is due to the alga which compensates the disadvantage of sugar release in a free-living status by settling a special habitat in which all algae which meet its requirements will survive. That ecological model of symbiosis formation is also discussed for the *Hydra-viridis-Chlorella* sp.-system (Rahat, 1985). Therefore, it is not surprising that in green paramecia collected from different sites also different alga taxa

are observed, which can form stable new symbiotic associations with other *Paramecium bursaria* strains than the ones they have been isolated from.

In this paper it could be shown for the first time that besides *Chlorella vulgaris* and related forms also other taxa of chlorophycean coccoid algae, tentatively assigned to *Chlorella* sp., can live endocytobiotically. In *Paramecium bursaria-Chlorella* sp.-systems hosts collected from different sites can harbour also different *Chlorella* spp. some of which may be assigned to *Chlorella vulgaris* and the *Chlorella vulgaris*-group — as was also reported for *Chlorella* isolates from American *Paramecium-bursaria*-strains by Douglas and Huss (1986) — but others belong to clearly different *Chlorella* taxa which can hardly be assigned to free-living counterparts, but show some similarities to *Chlorella fusca* var. *vacuolata* and the *Chlorella fusca*-group (sensu Kessler, Kessler (1982)).

Some of the symbiotic taxa cannot be assigned to already described free-living *Chlorella* taxa. Thus some of the most conspicuous symbiotic forms are strains PbDu and PbOp which seem to be identical. They show some relationship by morphology and physiology to the free-living *Chlorella fusca* var. *vacuolata* (according to Kalina and Puncocharova (1987): *Graesiella vacuolata*, subfamily Scotiellocystoideae within the Chlorellaceae), but differ from it significantly by G+C-contents and alignment of cell surface structures (ribs, Fig. 3). A comparatively high G+C-contents was measured in a symbiotic *Chlorella* strain isolated from a sponge (Table 4; 211–40c), which is clearly different from PbDu and PbOp by morphological and physiological features (Reisser, 1984). Thus PbDu and PbOp could be defined as symbiotic members of the Scotiellocystoideae differing from free-living representatives of the taxon by excretion of carbohydrates as is also the case in the *Chlorella vulgaris*-group.

Studies show that a typical symbiotic *Chlorella* strain common to all *Paramecium bursaria* strains does not exist. Possibly the endocytobiotic habitat formed by the ciliate host represents a niche where old (transitional ?) forms as well as new offsprings of *Chlorella* sp. which have no contemporary free-living counterparts have found a sort of evolutionary resting-place where they can persist without being in competition with free-living forms.

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