J. Phycol. **28,** 550–553 (1992)

COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY AND TAXONOMIC ASSIGNMENT OF THE *CHLORELLA* (CHLOROPHYCEAE) STRAINS OF THE CULTURE COLLECTION OF THE UNIVERSITY OF TEXAS AT AUSTIN[1]

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ABSTRACT

The species of the genus Chlorella *exhibit considerable biochemical and physiological differences. Therefore, it is important to select for and utilize in research or biotechnology correctly identified strains of the species having the most favorable properties for the respective project. We examined the* Chlorella *strains of the University of Texas collection at Austin, Texas, according to species-specific chemotaxonomic characters and assigned 58 strains to 10 well-established species (only 17 of these strains were correctly named before).*

Key index words: chemotaxonomy; Chlorella; *Chlorophyta; UTEX culture collection; utilization for research and biotechnology*

The taxonomy of the genus *Chlorella* Beijerinck has been chaotic for a long time. Physiological and biochemical characters are essential for the delimitation of species (Kessler and Soeder 1962, Kessler 1982, 1984). Seventeen taxa characterized thus far combine greatly different physiological properties (Table 1) with only slight morphological differences (cf. Fott and Nováková 1969, Andreyeva 1975). Therefore, it is essential that correctly identified strains be used for physiological or biochemical studies. For biotechnological projects, the species with optimum properties for the respective purpose should be selected (cf. Kessler 1980, 1986).

Our earlier work (cf. Kessler 1982, 1984) was carried out predominantly with *Chlorella* strains from the collection of algae at Göttingen, Germany (SAG), and many strains had to be renamed (cf. Koch 1964, Schlösser 1982). However, many strains available in other culture collections remain unidentified or incorrectly named. Therefore, we examined *Chlorella* strains of the UTEX collection at Austin, Texas (cf. Starr and Zeikus 1987), to provide correctly identified strains for future work.

MATERIALS AND METHODS

We studied the 74 axenic *Chlorella* strains of the Culture Collection of the University of Texas at Austin (UTEX; Starr and Zeikus 1987; cf. Tables 2, 3), excluding the contaminated ("B" or "LB") strains. Our culture medium was that of Kessler and Czygan (1970).

Ten biochemical and physiological characters were used for the identification of the strains (Kessler 1982, 1985, 1987, Kessler and Huss 1990; cf. Table 1). We stress that Table 1 can be applied to identify *Chlorella* strains like a key is used for the traditional identification of organisms. Seven of the characters are easily determined by means of simple growth experiments, i.e. the ability to utilize nitrate, requirements for vitamins B_1 (thiamine, 40 $mg \cdot L^{-1}$) and B_{12} (5 $\mu g \cdot L^{-1}$), the ability to use mannitol (2 $g \cdot L^{-1}$) for growth in the dark, and tolerances of growth to acidity (Kessler 1965), salinity (NaCl; Kessler 1974), and elevated temperatures (Kessler 1985). Two tests require moderate equipment, i.e. manometry for the determination of hydrogenase activity under anaerobic conditions (with nitrite or methylene blue as acceptors; Kessler 1957) and thin-layer chromatography for the detection of secondary carotenoids (astaxanthin and other keto-carotenoids) in nitrogen-deficient cultures (Kessler and Czygan 1965). Only the measurement of the base composition of deoxyribonucleic acid (DNA) requires the application of more sophisticated techniques (Huss et al. 1986). In most cases the determination of some of these characters is sufficient for the unequivocal assignment of a *Chlorella* strain.

RESULTS AND DISCUSSION

Table 1 summarizes the results of our previous work (cf. Kessler 1982, 1984), which was based mainly on the *Chlorella* strains from SAG and included the respective type strains (cf. Fott and Nováková 1969, Huss et al. 1989) for most of the taxa. Fifty-eight *Chlorella* strains from the UTEX collection could be assigned to 10 of these taxa (Table 2). Their biochemical and physiological characters fully agree with those of the respective species as listed in Table 1. This is true not only for the qualitative characters but also for the quantitative ones (limits of growth and GC contents of the DNA), which were found for each strain to be within the limits indicated in Table 1. According to our results, 41 strains had to be renamed; only 17 were correctly identified before (cf. Starr and Zeikus 1987). For example, the 9 strains of *C. vulgaris* Beij. were previously assigned to *C. vulgaris, C. pyrenoidosa* Chick, *C. infusionum* Beij., *C. salina* Butcher, and *C. vannielii* Shih. et Krauss; the 11 strains of *C. protothecoides* Krüger were assigned to *C. protothecoides, C. saccharophila* Krüger (Migula), *C. vulgaris, C. xanthella* Beij., *C. variegata* Beij., and *C. pyrenoidosa.* On the other hand, the strains previously assigned to *C. vulgaris* were found to belong to *C. vulgaris, C. sorokiniana* Shih. et Krauss, *C. kessleri* Shih. et Krauss, and *C. protothecoides,* and those labelled *C. pyrenoidosa* belong to *C. fusca* var. *vacuolata* Shih. et Krauss, *C. fusca* var. *fusca* Shih. et Krauss, *C. vulgaris, C. sorokiniana,* and *C. protothecoides,* i.e. species with greatly different physiological and biochemical characters.

The properties of the other 16 strains are shown

[1] Received 18 November 1991. Accepted 2 April 1992.

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TABLE 1. *Biochemical and physiological properties and taxonomy of 17* Chlorella *taxa (strains studied were mainly from the SAG collection at Göttingen).*

Species	Hydrogenase	Secondary carotenoids	NO_3 reduction	Thiamine requirement	B_{12} requirement	Growth on mannitol	Limit at pH	Limit at %NaCl	Limit at °C	DNA mol%GC
C. vulgaris Beijerinck	−	−	+	−	−	−	3.5–4.5	3–4	28–32	58–63
C. sorokiniana Shih. et Krauss	+	−	+	−	−	−	3.5–5.0	<1–3	36–42	62–68, 73–75
C. spec. ("*paramecii*")	+	−	+ (−)	+	+	−	5.5	<1	26–30	66–68
C. lobophora Andreyeva	−	−	+	−	−	−	4.0	1	30	61
C. kessleri Fott et Nováková	+	−	+	−	−	−	2.5–3.0	1–2	34–36	54–57
C. saccharophila (Krüger) Migula	−	−	+	−	−	+	2.0–3.0	3–6	26–30	49–52
C. ellipsoidea Gerneck	−	−	+	−	−	−	2.0–3.0	2	28–30	56–57
C. mirabilis Andreyeva	−	−	+	−	−	−	4.0	<1–1	26–28	56–58
C. minutissima Fott et Nováková	−	−	+	−	−	−	5.5	1	32	46
C. luteoviridis Chodat	−	−	+	−	−	+	3.0	3–5	28	44–45
Chlorella spec. 211-18	+	−	+	−	−	−	5.0	1	28	51
C. homosphaera Skuja	+	+	+	−	−	−	6.0	<1	28	73
C. protothecoides Krüger	−	−	−	+	−	−	3.5–4.0	3–4	28–34	58–62
C. fusca var. *vacuolata* Shih. et Krauss	+	+	+	−	−	−	3.0–3.5	3	32–36	50–52
C. fusca var. *fusca* Shih. et Krauss	+	+	+	−	−	−	4.0	2	32–34	55–56
C. fusca var. *rubescens* (Dangeard) Kessler et al.	+	+	+	−	−	−	4.5	3	30	57
C. zofingiensis Dönz	−	+	+	−	−	−	4.5–5.5	1	28	50, 63

TABLE 2. *Identification of 58* Chlorella *strains from the UTEX collection. Mut. = mutant of strain 1663. The biochemical and physiological properties of all strains of each species were found to be in full agreement with the species-specific characters shown in Table 1.*

New assignment	Strain number	Previous assignment
C. vulgaris	26	*C. pyrenoidosa*
	30	*C. vulgaris* var. *viridis* Chodat
	259	*C. vulgaris*
	265	*C. vulgaris*
	395	*C. pyrenoidosa*
	396	*C. vulgaris* var. *viridis*
	1803	*C. infusionum* var. *actophila* Shih. et Krauss
	1809	*C. salina*
	1811	*C. vannielii*
C. sorokiniana	246	*C. ellipsoidea*
	260	*C. vulgaris*
	261	*C. vulgaris*
	1230	*C. pyrenoidosa*
	1602	*C. sorokiniana*
	1663	*C. pyrenoidosa*
	1664 (mut.)	*C. pyrenoidosa* (mut.)
	1665 (mut.)	*C. pyrenoidosa* (mut.)
	1666 (mut.)	*C. pyrenoidosa* (mut.)
	1667 (mut.)	*C. pyrenoidosa* (mut.)
	1668 (mut.)	*C. pyrenoidosa* (mut.)
	1669 (mut.)	*C. pyrenoidosa* (mut.)
	1670 (mut.)	*C. pyrenoidosa* (mut.)
	1671 (mut.)	*C. pyrenoidosa* (mut.)
	1810	*C. sorokiniana* var. *pacificensis* Shih. et Krauss
C. kessleri	262	*C. vulgaris*
	263	*C. vulgaris*
	397	*C. vulgaris*
	398	*C. vulgaris*
	1808	*C. regularis* var. *umbricata* Shih. et Krauss

TABLE 2. *Continued.*

New assignment	Strain number	Previous assignment
	2228	*C. vulgaris*
	2229	*C. vulgaris*
C. saccharophila	247	*C. ellipsoidea*
	2469	*C. saccharophila*
C. ellipsoidea	20	*C. ellipsoidea*
C. luteoviridis	21	*C. luteoviridis*
	22	*C. luteoviridis*
	23	*C. luteoviridis* var. *lutescens* Chodat
	24	*C. miniata* (Nägeli) Oltmanns
	28	*C. variegata*
	248	*C. luteoviridis*
	257	*C. variegata*
	258	*C. variegata*
C. protothecoides	25	*C. protothecoides*
	27	*C. saccharophila*
	29	*C. vulgaris*
	31	*C. xanthella*
	249	*C. protothecoides*
	250	*C. protothecoides*
	255	*C. variegata*
	256	*C. variegata*
	264	*C. vulgaris*
	411	*C. protothecoides*
	1806	*C. pyrenoidosa* var. *chick* Shih. et Krauss
C. fusca var. *vacuolata*	251	*C. pyrenoidosa*
	252	*C. pyrenoidosa*
	1801	*C. emersonii* var. *globosa* Shih. et Krauss
C. fusca var. *fusca*	343	*C pyrenoidosa*
C. zofingiensis	32	*C. zofingiensis*

TABLE 3. *Properties of 16 unidentified* Chlorella *strains from the UTEX collection.*

Previous assignment	Strain number	Hydrogenase	Secondary carotenoids	NO_3 reduction	Thiamine requirement	B_{12} requirement	Growth on mannitol	Limit at pH	Limit at %NaCl	Growth at 34° C	DNA mol%GC	Remarks
C. miniata	490	+	−	+	−	−	−	4.5	1	(+)	63.7	Unidentified
Chlorella sp.	636	−	−	+	+	+	−	5.5	1	−	49.3	Unidentified
C. anitrata	1798	+	+	+	−	−	−	4.5	<1	+	66.8	Unidentified
C. anitrata var. *minor*	1799	+	+	+	−	−	−	4.5	<1	+	67.1	Unidentified
C. glucotropha[a]	1802	+	+	+	−	−	−	4.0	<1	+	70.7	Unidentified
C. nocturna[a]	1804	−	−	+	−	−	−	4.5	1	(+)	63.4	Unidentified
C. parva[a]	1805	−	−	+	−	−	−	4.0	1	+	78.1	Unidentified
C. regularis var. *minima*[a]	1807	+	+	+	−	−	−	3.5	1	+	78.5	Unidentified
C. minutissima	2341											Does not grow
Chlorella sp.	2168	+	+	+	−	−	−	4.0	1	+	50.6	*Scenedesmus* ?
Chlorella sp.	2248	+	+	+	−	−	−	3.5	1	(+)	55.7	*Scenedesmus* ?
Chlorella sp.	580										49; 61	2 strains ?
Chlorella sp.	820										48; 61	2 strains ?
Chlorella sp.	838										49; 69	2 strains ?
C. autotrophica var. *atypica*[a]	1800										47; 60	2 strains ?
C. minutissima	2219										67; 81	2 strains ?

[a] Shih. et Krauss.

in Table 3. They cannot be identified with any of the *Chlorella* species of Table 1. Strains 1798 and 1799 (*C. anitrata* Shih. et Krauss and *C. anitrata* var. *minor* Shih. et Krauss) are apparently identical. *Chlorella* sp. 2168 and 2248 seem to belong to the genus *Scenedesmus* Meyen. Strain 2341 does not grow under our culture conditions and cannot therefore belong to *C. minutissima* Fott et Nováková. The remaining strains 580, 820, 838, 1800, and 2219 appear to be mixtures of two different strains, as indicated by the biphasic melting behavior of their respective DNAs.

Our results have shown that the genus *Chlorella*, in its traditional sense, is biochemically very heterogeneous (Kessler 1982, 1984). This is further supported by the GC values of the DNA, which cover a very large range from 44 to 75 mol% G+C (cf. Table 1). Indeed, our work on DNA hybridization (Kerfin and Kessler 1978, Huss et al. 1986, 1987, 1989) and ribosomal ribonucleic acid sequencing (Huss and Sogin 1990, Huss et al., unpubl.) shows that there are deep evolutionary gaps between some of the *Chlorella* species. Thus, the first group of five species indicated in Table 1 represents taxa closely related to *C. vulgaris* (the "true" *Chlorella* species, as *C. vulgaris* is the type species of the genus), whereas the third group of four taxa is more closely related to the genus *Scenedesmus* (cf. Huss et al. 1989, Huss and Sogin 1990, Kessler 1991). The phylogenetic position of the species of the second group is still under investigation. It is now evident that, within this heterogeneous group, *C. saccharophila* and *C. ellipsoidea* should be regarded as different species rather than as varieties of *C. saccharophila* (Kessler 1987, Huss et al. 1987, 1989, unpubl.).

Finally, we stress again that, in addition to their taxonomic and systematic consequences, our results are significant for future utilization of *Chlorella* in basic physiological and biochemical research as well as for biotechnological projects. The data assembled in Table 1 can be used for the selection of suitable species for many kinds of projects. Table 4 gives additional and more specific recommendations concerning the suitability of some *Chlorella* species for certain problems in basic and applied research (cf. Kessler 1989).

TABLE 4. *Suitability of* Chlorella *species for research and biotechnology.*

Problem or purpose	Species
Hydrogenase and H_2 metabolism	*C. fusca* var. *vacuolata*
Secondary carotenoids	*C. fusca* var. *vacuolata*, *C. zofingiensis*
Acid tolerance	*C. saccharophila*, *C. ellipsoidea*
Salt tolerance	*C. saccharophila*, *C. luteoviridis*
Temperature tolerance	*C. sorokiniana*
Cadmium sensitivity	*C. ellipsoidea*
Mass culture (MC) under normal conditions	*C. fusca* var. *vacuolata*, *C. vulgaris*, *C. kessleri*, *C. sorokiniana*
MC in acid water	*C. saccharophila*, *C. kessleri*
MC in salt water	*C. saccharophila*, *C. vulgaris*
MC at elevated temperatures	*C. sorokiniana*, *C. fusca* var. *vacuolata*, *C. kessleri*
Production of H_2	*C. fusca* var. *vacuolata*

We express our gratitude to Professor R. C. Starr and Dr. J. A. Zeikus for kindly providing the *Chlorella* strains of the UTEX culture collection and to Mrs. Edith Weitemeyer and Mrs. Gudrun Steingräber for excellent technical assistance.

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J. Phycol. **28,** 553–558 (1992)

CYCLOMORPHOSIS IN *SCENEDESMUS ARMATUS* (CHLOROPHYTA): AN ORDERED SEQUENCE OF ECOMORPH DEVELOPMENT[1]

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ABSTRACT

Scenedesmus armatus *(Chod.) Chod. growth and morphology were monitored in medium 7 (oligotrophic) and Bristol's medium (eutrophic); cultures in both media were incubated at 10 and 22° C. Growth rate at 10° C was reduced, i.e. only one doubling in 7 days in medium 7 and 2.3 doublings in Bristol's, compared to 4.3 and 6 doublings at 22° C over the same period. Unicells as well as cells of colonies were larger at the cold temperature. The lengths of cells were not significantly different regardless of temperature or medium, but cell width was markedly increased at the lower temperature. Additionally, an arcuate, eight-celled, multispined ecomorph, which resembled several previously described taxa, was produced at 10° C. It becomes a component of a previously published ordered sequence of ecomorph development for this species.*

Based on data now accumulated in both the laboratory and the field, these temporal changes are interpreted to be a cyclomorphosis, driven by a coupling of nutrient availability and temperature. Within the addition of new cold temperature (spring) ecomorphs, the ordered sequence of ecomorphs for S. armatus *is a succession from unicells to multispined eight-celled colonies to quadricaudate colonies, ending with acaudate four-celled ecomorphs.*

Key index words: Chlorophyta; cyclomorphosis; ecomorph; phenotypic plasticity; Scenedesmus armatus; *temperature*

[1] Received 18 December 1991. Accepted 7 April 1992.