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Short Communication

Species-specific ability of *Chlorella* strains (*Chlorophyceae*) to form stable symbioses with *Hydra viridis*

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Abstract: 46 strains of *Chlorella*, identified by physiological and biochemical characters, were examined for their ability to form stable symbioses with aposymbiotic *Hydra viridis.* It was found to be a species-specific characteristic. Among the 15 taxa studied, only *C. saccharophila* var. *ellipsoidea, C. saccharophila* var. *saccharophila, C. fusca* var. *vacuolata, C. kessleri, C. luteoviridis*, and *C. protothecoides* formed stable symbioses with *Hydra viridis.* Among the 11 known physiological and biochemical characters of these *Chlorella* species, only acid tolerance seems to be correlated with symbiosis: All symbiotic species are capable of growing at or below pH 4.0.

The freshwater polyp *Hydra viridis* owes its green colour to the presence in intracellular vacuoles of a symbiotic alga of the genus *Chlorella* (cf. Smith & Douglas 1987). The native symbiont fails to grow in vitro and thus far could not be further identified. In a study of host/symbiont specificity, Rahat & Reich (1984) introduced free-living *Chlorella* into aposymbiotic *Hydra.* A comparative study with 17 laboratory strains (Rahat & Reich 1985) revealed that 7 of these formed stable symbioses with *H. viridis,* whereas the 10 other strains were expelled or digested by the polyps. Since some of these strains were unidentified, and others of doubtful assignment, it appeared desirable to identify these *Chlorella* strains by physiological and biochemical characters (cf. Kessler 1976, 1982) and to find out whether the ability of symbiosis is a species-specific characteristic. In addition, the discovery of common physiological and biochemical properties of symbiotic *Chlorella* species might lead to a better understanding of the prerequisites for symbiosis and of the microenvironment in the vacuoles of *Hydra.*

In the present work, we have identified the *Chlorella* strains used by Rahat & Reich (1985) and, in addition, have studied the symbiotic properties of 30 *Chlorella* strains previously identified and assigned to 15 taxa by Kessler (1978, 1982, 1987).

Materials and methods

The algae studied were 17 *Chlorella* strains from the Jerusalem laboratory (most of them originally from the Cambridge collection of algae; Rahat & Reich 1985) and 30 strains

Table 1. Identification by physiological and biochemical characters and symbiotic properties of 16 and temperature tolerance of growth, hydrogenase activity, secondary carotenoids, nitrate

Strain	Species	Symbiosis	Limit at pH	Limit at % NaCl
211/8 b	*C. fusca* var. *vacuolata*	+	3.5	3
211/8 p	*C. fusca* var. *vacuolata*	+	3.5	3
211/11 n	*C. fusca* var. *vacuolata*	+	3.0	3
NC 64 A (P)	*C. kessleri*	+	3.0	1
211/7 a	*C. protothecoides*	+	4.0	3
211/11 a	*C. protothecoides*	+	4.0	3
211/6	*C. protothecoides*	+	4.0	3
Fs	*C. protothecoides*	+	3.5	3
211/8 k	*C. sorokiniana*	−	4.0	2
211/11 c	*C. sorokiniana*	−	4.5	1
211/1 e	*C. vulgaris*	−	3.5	3
211/21	*C. vulgaris*	−	4.0	3
CE 76	*C. vulgaris*	−	4.0	3
UTEX 130	*C. vulgaris*	−	4.0	3
NC 64 A (M)	*C. vulgaris*	−	4.0	3
2	*C. vulgaris*	−	4.0	3

studied before in Erlangen (cf. KESSLER 1978, 1987). They were grown in glass tubes (250 ml) under continuous illumination (white fluorescent tubes, 6000 lux) at 25 °C in a water thermostat (Kniese, Marburg-Marbach). The cultures were gassed with air +2% CO_2. The nutrient media were those of KESSLER & CZYGAN 1970 (nitrate, pH 6.4) and KESSLER & ZWEIER 1971 (ammonium + thiamine, pH 6.4; for *C. protothecoides*).

The biochemical and physiological characters studied were (cf. KESSLER 1982): Hydrogenase activity (under anaerobic conditions in the dark), formation of secondary carotenoids (in nitrogen-deficient cultures in the light), ability to utilize nitrate, thiamine requirement, limits of growth in acid media, in media with added NaCl, and at higher temperatures, and base composition of DNA (G + C content; cf. HUSS & al. 1986).

Hydra viridis (Swiss strain) was grown in M solution (LENHOFF & BROWN 1970) under continuous illumination (2500 lux) at 20 °C. The polyps were fed three times a week with freshly hatched larvae of *Artemia* spec. The infection of aposymbiotic *Hydra* with strains of *Chlorella* was carried out according to RAHAT & REICH (1986) with 4 to 5 days old larvae of *Artemia* spec. serving as vectors.

Results and discussion

The 17 *Chlorella* strains whose symbiotic properties had been studied by RAHAT & REICH (1985) were identified by their physiological and biochemical characters (cf. KESSLER 1982) and could be assigned to five species (Table 1, strain 211/9 a discarded). All taxonomic assignments were confirmed also through DNA hybridizations (cf. HUSS & al. 1986). Strain 211/9 a, however, gave inconsistent results: At low pH it exhibited the characteristics of the acid-tolerant *C. saccharophila* (including strongly ellipsoidal cells), whereas when grown at about pH 6 it had the properties of *C. vulgaris*. Thus it appeared to be a mixture of two species and was therefore discarded.

Chlorella strains from the Jerusalem laboratory (Rahat & Reich 1985). Acid tolerance, salt tolerance, reduction, thiamine requirement, and base composition of DNA (% guanine + cytosine)

Limit at °C	Hydrogenase	Second. carot.	NO_3 red.	Thiam. requ.	DNA % GC
34	+	+	+	–	51.5
34	+	+	+	–	51.5
36	+	+	+	–	51.5
36	+	–	+	–	57.3
32	–	–	–	+	61.2
30	–	–	–	+	61.2
30	–	–	–	+	60.8
34	–	–	–	+	60.4
40	+	–	+	–	64.8
38	+	–	+	–	64.8
32	–	–	+	–	62.1
28	–	–	+	–	62.3
28	–	–	+	–	61.9
30	–	–	+	–	62.5
28	–	–	+	–	61.7
30	–	–	+	–	61.7

Table 2. Symbiotic properties of 30 previously identified *Chlorella* strains. ^{T} Type strain

Species	Strains	Symbiosis
C. saccharophila var. *ellipsoidea*	211-1 a^{T}, 3.80	+
C. saccharophila var. *saccharophila*	211-1 d, 211-1 f, 211-9 a^{T}, 211-9 b	+
C. fusca var. *vacuolata*	211-8 b^{T}	+
C. kessleri	211-11 g^{T}	+
C. luteoviridis	211-2 a^{T}	+
C. protothecoides	211-7 a^{T}	+
C. sorokiniana	211-8 k^{T}, 211-11 d, 211-11 k, 211-32, 211-34, C-1.1.8, Sless 1, 1-9-30, 211-40 a, 211-40 b, 211-40 c, Prag A 14	–
C. vulgaris	211-11 b^{T}	–
C. spec.	211-30	–
C. fusca var. *fusca*	343^{T}	–
C. fusca var. *rubescens*	232/1^{T}	–
C. zofingiensis	211-14 a^{T}	–
C. minutissima	C-1.1.9	–
C. spec.	211-11 r	–
C. homosphaera	211-8 e (Ca.)^{T}	–

The ability to form a stable symbiosis with aposymbiotic *Hydra viridis* is restricted to *C. fusca* var. *vacuolata* (including also strain 211/8 b, in contrast to an earlier observation [Rahat & Reich 1985]), *C. kessleri*, and *C. protothecoides* (Table

Table 3. Physiological and biochemical properties of *Chlorella* species able and unable to form gelatin, hydrolysis of

Species	Symbiosis	Limit at pH	Limit at % NaCl	Limit at °C	Hydrogenase
C. sacch. var. *ellipsoidea* (GERNECK) FOTT & NOVÁKOVÁ	+	2.0–3.0	2	30	–
C. sach. var. *saccharophila* [(KRÜGER)MIGULA] FOTT & NOVÁKOVÁ	+	2.0–3.0	4	28	–
C. fusca var. *vacuolata* SHIHIRA & KRAUSS	+	(3.0–)3.5	3	34	+
C. kessleri FOTT & NOVÁKOVÁ	+	3.0	2	34	+
C. luteoviridis CHODAT	+	3.0	5	28	–
C. protothecoides KRÜGER	+	(3.5–)4.0	3	30	–
C. sorokiniana SHIHIRA & KRAUSS	–	4.0–5.0	2	40	+
C. vulgaris BEIJERINCK	–	(3.5–)4.0	3	30	–
C. spec. 211-30	–	4.0	<1	26	–
C. fusca var. *fusca* SHIHIRA & KRAUSS	–	4.0	2	34	+
C. fusca var. *rubescens* (DANGEARD) KESSLER & al.	–	4.5	3	30	+
C. zofingiensis DÖNZ	–	5.5	1	28	–
C. minutissima FOTT & NOVÁKOVÁ	–	5.5	1	32	–
C. spec. 211-11 r	–	4.0	<1	28	–
C. homosphaera SKUJA	–	6.0	<1	28	+

1). It should be stressed that strain CE 76, which is supposed to be the native symbiont of *Hydra viridis* isolated by JOLLEY & SMITH (1978), clearly belongs to *C. vulgaris* (cf. also DOUGLAS & HUSS 1986) and is unable to form a stable symbiosis with aposymbiotic *Hydra* (Table 1). We therefore assume that this strain represents a surface contamination of *Hydra* rather than the native symbiont which cannot be grown in vitro so far.

After these results it was desirable to base our conclusions on a wider range of strains. Therefore, 30 *Chlorella* strains which had been assigned before to 15 species (KESSLER 1982, 1987), were similarly examined for their symbiotic properties (Table 2). The ability to form a stable symbiosis appeared again as a species-specific character, with *C. saccharophila* var. *ellipsoidea, C. saccharophila* var. *saccharophila,* and *C. luteoviridis* as additional symbiotic species.

stable symbioses with *Hydra viridis*. For explanation, see Table 1; in addition, liquefaction of starch, lactate fermentation

Second. carot.	NO_3 red.	Thiam. requ.	Gelat. liquef.	Starch hydrol.	Lact. ferm.	Growth on mannitol
−	+	−	−	+ −	+	−
−	+	−	−	+ −	−	+
+	+	−	+	+	+	−
−	+	−	−	−	−	−
−	+	−	−	−	−	+
−	−	+	−	−	+	−
−	+	−	−	− +	+	−
−	+	−	−	−	+	−
−	+	−	−	+	−	−
+	+	−	+	−	+	−
+	+	−	−	−	+	−
+	+	−	−	−	+	−
−	+	−	−	−	−	−
−	+	−	−	+	+	−
+	+	−	−	−	−	−

Table 3 summarizes the 6 symbiotic and the 9 non-symbiotic *Chlorella* species and their physiological and biochemical characters. Obviously, among all the properties known so far it is only acid tolerance that shows a correlation with symbiosis: All *Chlorella* species which are able to form stable symbioses with *Hydra viridis* are capable of growing at or below pH 4.0, i.e., in a rather acid environment. The possible implications of this result will be discussed elsewhere.

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