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FLORIDOSIDES IN UNICELLULAR HOT SPRING ALGAE

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Key Word Index—*Cyanidium caldarium*; *Chroococcidiopsis* sp.; hot spring algae; chemotaxonomy; low MW carbohydrates; floridoside; *iso*-floridoside; autotroph; heterotroph.

Abstract—*Cyanidium caldarium* strains RK-1, KS-1 and 001 are probably obligate autotrophs, while *Chroococcidiopsis* sp. strains M-8 and 002 (formerly named *Cyanidium caldarium*) are facultative autotrophs. The *Cyanidium* strains contain floridoside (2-*O*-glycerol-α-D-galactopyranoside) and a small amount of *iso*-floridoside (1-*O*-glycerol-α-D-galactopyranoside), both of which are known to be distributed in Rhodophyta. The *Chroococcidiopsis* strains also contain floridoside, but no *iso*-floridoside, under various culture conditions. These results indicate that *Cyanidium* is clearly distinguishable from *Chroococcidiopsis* in *iso*-floridoside content and nutritional properties, and suggest that all strains tested may be closely related to Rhodophyta.

INTRODUCTION

The systematic position of the unicellular hot spring alga, *Cyanidium caldarium* (Tilden) Geitler, is undefined in spite of many studies of its morphological and physiological properties [1, 2]. It was reported in a previous paper [3] that *C. caldarium* strain RK-1 from Yumoto-spa (Japan) may belong to the primitive Rhodophyta because it contains floridoside and its isomer, *iso*-floridoside, contains compounds found in most genera of Rhodophyta as assimilatory products [4, 5]. On the other hand, Kremer and Feige [6] reported that floridoside and *iso*-floridoside are not found in a strain of *C. caldarium* originating from the Yellowstone hot springs (U.S.A.).

It was pointed out by De Luca and Taddei [7] that two algae belonging to different taxa may be mixed in the *Cyanidium* strains from Italian acidic fumaroles. The present authors [8] also reported that *C. caldarium* strain M-8, isolated from Noboribetsu-spa, Japan, is clearly different from *C. caldarium* RK-1 in cell size and endospore number, etc. and is in fact *Chroococcidiopsis* sp. Geitler and Ruttner [9].

In this paper, the nutritional properties and low MW carbohydrates are described for several strains of *C. caldarium* and *Ch.* sp. In addition, the phylogenetic position of these organisms is discussed. A brief report of this work has already been published [10].

RESULTS

Table 1 shows the growth patterns of five strains of hot spring algae from Japan and Italy under a variety of conditions. *C. caldarium* strains RK-1, 001 and KS-1 grew at almost the same rate under L (inorganic medium + light) and LG (glucose + light) conditions. *Ch.* sp. M-8 and 002 (formerly named *C. caldarium*) grew more rapidly under LG and DG (glucose − light) conditions than under L condition. These results suggest that the strains in the first group are obligate autotrophs, while those in the second group are facultative autotrophs, that is, auto-, mixo- and heterotrophs.

Table 1. Growth yields of two hot spring algae in different culture conditions

Alga	Strain	Growth conditions* L	LG	DG
C. caldarium	RK-1	0.31†	0.23	0.0
	KS-1	0.50	0.32	0.0
	001	0.44	0.44	0.0
Ch. sp.	M-8	0.70	13.4	9.2
	002	0.20	12.3	9.5

*The organisms were grown in shake culture for 8 days at 38°. L, inorganic medium + light (2000 lx); LG, 0.5% glucose + light; DG, 0.5% glucose − light.

†Packed cell volume (ml) per 1 l. medium.

The neutral low MW carbohydrate fractions were extracted from the algae and, after trimethylsilylation, analysed by GC. All of the extracts gave a peak corresponding to authentic floridoside (2-*O*-glycerol-α-D-galactopyranoside). However, only the extracts from the *C. caldarium* species gave a peak corresponding to *iso*-floridoside (1-*O*-glycerol-α-D-galactopyranoside). The identities of the peaks were supported by the GC/MS and PC data. Thus, the mass spectrum (20 eV) of TMSi-floridoside showed *m/z* 686 $[M]^+$ ($C_{27}H_{66}O_8Si_6$), 671 $[M-Me]^+$, 581 $[M-HOSiMe_3]^+$, 539, 491 $[M-(HOSiMe_3)\times 2]^+$, 464 and 451, with high intensity ions of *m/z* (rel. int.) 361 (11), 337 (34), 217 (23), 204 (100) $[Me_3SiO{-}CH{=}CH{-}OSiMe_3]^+$ [11] and 103 (10). The mass spectrum (20 eV) of TMSi-*iso*-floridoside was nearly the same as that of TMSi-floridoside except at *m/z* (rel. int.) 337 (78) and 217 (50). At 70 eV, the mass spectrum of TMSi-floridoside [3] was almost the same as that of TMSi-*iso*-floridoside.

Table 2 shows the content of floridoside and *iso*-floridoside in the algae under different growth conditions.

Table 2. Distribution of neutral low MW carbohydrates in two hot spring algae

Alga	Strain	Growth conditions*	Total sugar† (%)	Carbohydrate (%)		
				Flo‡	*iso*-Flo	Other
C. caldarium	RK-1	L	1.01	81.3	8.3	10.4
	KS-1	L	0.46	82.0	14.8	3.0
	001	L	0.90	80.8	8.8	10.9
Ch. sp.	M-8	L	2.54	95.8	0.0	4.2
		LG	0.74	90.6	0.0	9.4
		DG	1.43	88.9	0.0	11.1
Ch. sp.	002	L	2.35	95.0	0.0	5.0
		LG	0.89	83.4	0.0	16.6
		DG	1.27	88.6	0.0	11.4

*See Table 1.
†Glucose equivalents per unit dry wt of algae.
‡Flo, floridoside; *iso*-Flo, *iso*-floridoside; Other, other sugars.

In all of the strains floridoside constituted some 80–95% of the neutral low MW carbohydrate fraction. No detectable amounts of sucrose and reducing sugars, such as glucose, galactose and fructose, were present. A small amount of *iso*-floridoside (8–15%) was contained in *Cyanidium* RK-1 [3], KS-1 and 001, but not in *Chroococcidiopsis* M-8 and 002.

DISCUSSION

It was found that floridoside is the main low MW carbohydrate in both *C. caldarium* and *Ch.* sp. (Table 2). In view of the presence of this carbohydrate in many red algae [5], we can postulate that both algae may be closely related to Rhodophyta. This idea is supported by the recent data about the ferredoxin amino acid sequence of *C. caldarium* (Allen's strain 1355-1) [12, 13] which may be the same as *Ch.* sp.

Kremer and Feige [6] pointed out that *Cyanidium* may be an endocyanome because it contains fructose and glucose, but no floridoside (heteroside) as photoassimilatory products of the alga. However, as far as the low MW carbohydrates are concerned (Table 2), the *Cyanidium* and *Chroococcidiopsis* strains used in the present paper are not similar to blue-green algae (Cyanophyceae) or endocyanome [3]. In any case, further studies may be needed to establish endosymbiosis theory [14] on *Cyanidium*.

These two algae are, however, different from each other in *iso*-floridoside content and nutritional properties (Tables 1 and 2). In contrast to autotrophic *Cyanidium*, the ability of heterotrophic growth of *Chroococcidiopsis* is consistent with that of *Cyanidium*, Allen's strain (1355-1) [15], and *Cyanidium* form B [16].

These chemical and nutritional differences and the morphological comparison between the two genera [8], indicate that these algae must not have the same name, *C. caldarium*. There have been many studies reported on *Cyanidium*, however, the *Cyanidium* strains used are mixtures of *C. caldarium* and *Ch.* sp. [8] (or *Protococcus sulphurarius* [17]).

Ch. sp M-8 and 002 always contain floridoside not only in autotrophic, but also heterotrophic conditions (Table 2). This suggests that floridoside may play a role in osmotic balance, at least in *Chroococcidiopsis*, as in the case of red algae [18].

EXPERIMENTAL

Algal material. Cyanidium caldarium (Tilden) Geitler strain RK-1 was isolated by Fukuda [19] from Yumoto-spa, Nikko, Japan. *C. caldarium* strain KS-1 was isolated by Nagashima from Kusatsu-spa, Gumma, Japan. *C. caldarium* strain 001 and *Chroococcidiopsis* sp. strain 002 (originally named *Cyanidium caldarium* 002) isolated from Campi Flegrei, Italy, were kindly provided by Professors R. Taddei and G. Pinto, Università di Napoli, Italy. *Chroococcidiopsis* sp. strain M-8 (originally named *Cyanidium caldarium* M-8) isolated from Noboribetsu-spa, Hokkaido, Japan, was kindly provided by the algal collection of the Institute of Applied Microbiology, the University of Tokyo, Japan.

Culture. Algae were cultured autotrophically in basal inorganic medium [15], pH 3.0, at 38° with continuous aeration (5% CO_2 in air) under fluorescent light (2000 lx) for several weeks. They were also cultured at 38° by shaking 500 ml flasks containing 250 ml basal medium in the light (2000 lx) (L), or in basal medium with a supplement of 0.5% glucose in the light (LG) or in the dark (DG).

Growth yield. After the algae had been grown in shake culture for 8 days under various conditions, the packed cell vols. (ml) per 1 l. medium were measured with a haematocrit.

Isolation of neutral low MW carbohydrate. After 1 or 2 weeks cultivation, algal cells were harvested by centrifugation and washed with H_2O (× 3). The gram fr. wt (dry wt) of algae were: *Cyanidium caldarium* RK-1, 8.81 (1.14); *C. caldarium* 001, 13.0 (3.12); *C. caldarium* KS-1, 8.71 (2.10); *Chroococcidiopsis* sp. M-8, L-cell 6.18 (1.08), LG-cell 11.5 (1.91), DG-cell 4.19 (0.70); *Chroococcidiopsis* sp. 002, L-cell 9.52 (2.00), LG-cell 5.0 (0.68), DG-cell 2.60 (0.81).

Neutral low MW carbohydrates were extracted and fractionated from the algae by the methods in ref. [3].

Chromatography. PC was carried out by the method of ref. [3]. Authentic floridoside was isolated from a calcareous red alga *Serraticardia maxima* [20], and authentic *iso*-floridoside was isolated from the red alga *Porphyra yezoensis* by cellulose CC [20]. TMSi derivatives [21] of low MW carbohydrates were analysed by GC: dual FID; glass columns (2 m × 2.6 mm) packed

with 3% OV-17; N_2 60 ml/min; injector and detector temps., 250°. RR_ts of the TMSi derivatives of standards on 3% OV-17 at 200° were; inositol 1.00 (4.16 min); floridoside, 2.34; *iso*-floridoside, 2.65. Temp. programmed from 150 to 200° at 5°/min; inositol, 1.00 (10.6 min); glycerol, 0.089; galactose, 0.65, 0.70 and 0.77; floridoside, 1.57; *iso*-floridoside 1.70. GC/MS: 20 or 70 eV; glass column (2 m × 3 mm) packed with 5% OV-17; isothermal, 200°; injector temp., 270°; ion source, 240°; molecular separator, 250°.

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