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Photobionts Isolated from Some Japanese Species of *Cladonia* (Lichens)

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Abstract

Three species of *Trebouxia* were isolated as photobionts from 10 Japanese species of *Cladonia*. They were *Trebouxia erici*, *T. excentrica* and *T. glomerata*. In this study, photobionts were newly isolated form eight species of *Cladonia*. The results of this and previous studies indicate that a few species of *Cladonia* have two photobionts, which are occurring in individual lichen thalli. However, a single algal partner is restricted to many species of *Cladonia*. *Trebouxia erici*, *T. excentrica*, *T. glomerata* and *T. pyriformis* are the most frequent photobionts of the *Cladonia* species.

Keywords: photobiont, *Cladonia*, lichen, *Trebouxia*, taxonomy

1. Introduction

Cladonia Wigg. (Cladoniaceae) is a cosmopolitan genus of fruticose lichens. About 350 species of *Cladonia* are known of which about 100 species have been reported in Japan. They usually grow on soil, rock and bark from low to high altitudes. Seven species of *Trebouxia* have been reported previously from 36 species of *Cladonia* (Warén 1920; Ahmadjian, 1960; Archibald, 1975; Hildreth and Ahmadjian, 1981; Meisch, 1981; Yoshimura et al., 1987). Although *Cladonia* is a common lichen genus, reports on its photobionts have been very

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few. In the present study, three species of *Trebouxia* isolated from Japanese species of *Cladonia* are described and the diversity of photobionts in *Cladonia* is discussed.

2. Materials and Methods

Photobionts were isolated from 16 species of each of the following 10 species of *Cladonia*.

C. bacillaris (Del.) Nyl.

Hiroshima-ken, Saiki-gun, Miyajima-cho, Alt. 100 m, Dec. 12, 1982: Iguchi 221 (Cul. no. 4125).

C. calycantha Del. ex Nyl.

Hiroshima-ken, Higashi-hiroshima-shi, Alt. 300 m, Dec. 25, 1982: Iguchi 227 (Cul. no. 4131).

C. chlorophaea (Florke ex Somm.) Spreng.

Hiroshima-ken, Yamagata-gun, Togauchi-cho, Sandan-kyo, Alt. 600 m, April 2, 1983: Iguchi 251 (Cul. no. 4156).

C. coniocraea (Florke) Spreng.

Hiroshima-ken, Aki-gun, Fuchu-cho, Mikumari-kyo, Alt. 120 m, Dec. 26, 1982: Iguchi 226 (Cul. no. 4130).

C. crispata Ach.) Flot.

Hiroshima-ken, Saiki-gun, Yuki-cho, Alt. 200 m, Oct. 17, 1982; Iguchi 205 (Cul. no. 4109).

C. floerkeana (Fr.) Somm.

Hiroshima-ken, Hogashi-hiroshima-shi, Hachihonmatsu, Alt. 300 m, Feb. 8, 1983: Iguchi 240 (Cul. no. 4144).
Hiroshima-ken, Higashi-hiroshima-shi, Iida, Alt. 300 m, Sept. 23, 1983: Iguchi 260 (Cul. no. 4233).

C. gracilis (L.) Wild. ssp. *turbinata* (Ach.) Ahti

Nigata-ken, Myoto-kogen-cho, Myoko-kogen, Alt. 1000 m, July 15, 1982: Iguchi 141 (Cul. no. 4049), 142 (Cul. no. 4050).

C. humilis (With.) Laundon

Hiroshima-ken, Yamagata-gun, Togauchi-cho, Sandan-kyo, Alt. 600 m, July 9, 1982: Iguchi 116 (Cul. no. 4040).

C. ramulosa (With.) Laundon

Hiroshima-ken, Yamagata-gun, Togauchi-cho, Sandan-kyo, Alt. 600 m, July 9, 1983: Iguchi 115 (Cul. no. 4030).
Hiroshima-ken, Hiroshima-shi, Senogawa-cho, Alt. 100 m, Feb. 8, 1983: Iguchi 239 (Cul. no. 4143).
Hiroshima-ken, Higashi-hiroshima-shi, Iida, Alt. 300 m, Sept. 23, 1983: Iguchi 262 (Cul. no. 4235).
Yamanashi-ken, Fuji-yoshida-shi, Mt. Fuji, Alt. 2000 m, Oct. 8, 1983: Iguchi 274 (Cul. no. 4247), Iguchi 281 (Cul. no. 4254).

C. squamosissima (Mull. Arg.) Ahti

Kagoshima-ken, Yakushima Isl., Alt. 1497 m, July 26, 1982: Iguchi 124 (Cul. no. 4042).

Photobionts were isolated by the following method. Small fragments of podetia from each specimen were washed by tap water or sonicator and then ground between two glass slides. The suspension consisting of photobiont cells and fungal hyphae was spread on sterile 1.5% agar plates of Bold's Basal Medium (BBM) as modified by Bischoff and Bold (1963). After about one month in culture, algal colonies became visible under a stereo microscope. Axenic cultures of isolated photobionts were obtained by the spray method (Wiedeman et al., 1964). Then they were cultured on BBM agar slants under standard conditions (20±1°C), 35 μmole photons $m^{-2}sec^{-1}$ by cool-white fluorescent tube on a diurnal cycle of 12 hr light and 12 hr dark) for two to four weeks and then their morphorogical characters and life cycles could be observed. Light microscopy was carried out using a Nikon OPTIPHOT-X2F Microscope or a Nikon Nomarski interference X2F Microscope. A dilute aqueous methylene blue solution was used to determine the presence of gelatinous sheaths and an Azocarmin G solution was used for observations of pyrenoids.

Algal strains isolated in this study are deposited in the Botanical Institute, Hiroshima University (CCHU).

3. Results and Discussion

One or two algal strains were isolated from each of the *Cladonia* specimens. A total of sixteen strains was obtained. Based on their morphological characteristics, all of them were identified as algal species belonging to the genus *Trebouxia*. Consequently, they were compared with the descriptions and figures of *Trebouxia* by Ahmadjian (1960), Archibald (1975), Hildreth and Ahmadjian (1981) and Gärtner (1985). *Trebouxia* strains isolated in this study were also compared with living strains obtained from the Culture Collection of Algae at the University of Texas (UTEX). As a result, 14 of the strains were identified as *Trebouxia erici* Ahmadjian, one strain was *T. excentrica* Archibald and one strain was *T. glomerata* (Warén) Ahmadjian.

1. Trebouxia erici Ahmadjian *Amer. J. Bot.* 47: 680, figs 6, 10, 15 (1960)

Vegetative cells in the log phase cultures spherical to subspherical 10–13 μm in diameter with 1 μm cell walls in thickness (Figs. 1–3). In the stationary phase cultures, vegetative cells with cell walls, about 1.5–2.0 μm in thickness. Gelatinous matrices not observed. Chloroplast massive, axial containing one central pyrenoid without starch grains.

Asexual reproduction by aplanospores and zoospores. Aplanosporangia usually with 64 spores. Zoospores pear-shaped, wall-less about 7 μm in length, about 3 μm in width, with two anterior flagella of equal length, an anterior nucleus, and a minute stigma in the middle parietal portion, without distinctive contractile vacuoles.

Sexual reproduction not observed.

Specimens examined: Iguchi 115, 134, 141, 142, 205, 226, 227, 239, 240, 251, 260, 262, 274 and 281.

Culture number: 4039, 4042, 4049, 4050, 4109, 4130, 4131, 4143, 4144, 4156, 4233, 4235, 4247 and 4254 (CCHU).

Trebouxia erici was first isolated from *Cladonia cristatella* by Ahmadjian (1960). Meisch (1981) also reported it from *Cladonia coniocraea, C. grayi, C. squamosa* and *C. subulata.* Takeshita et al. (1991) isolated this species from *Cladia aggregata* collected in Japan.

In the present study, this species was isolated for the first time from *Cladonia calycantha, C. chlorophaea, C. crispata, C. floerkeana, C. gracilis* ssp. *turbinata, C. ramulosa* and *C. squamosissima.*

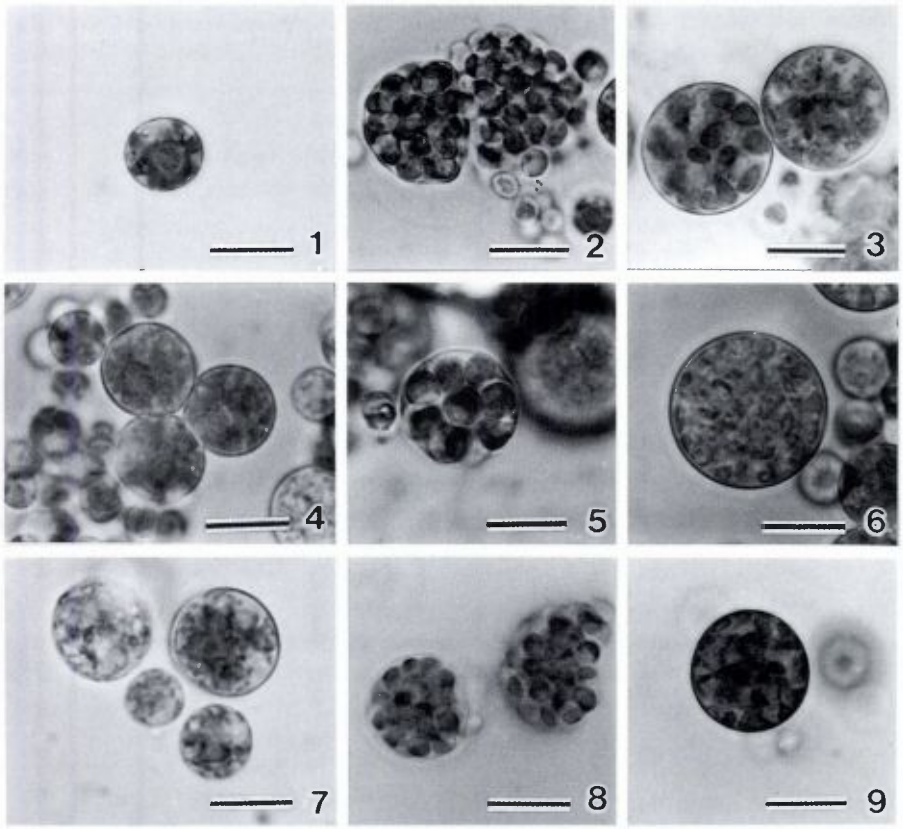

Figure 1–3. *Trebouxia erici* Ahmadjian (1) Young vegetative cell; (2) Aplanosporangia, (3) Zoosporangia. Scale bars = 10 μm.

Figure 4–6. *Trebouxia excentrica* Archibald (4) Mature and young vegetative cells; (5) Aplanosporangium; (6) Zoosporangium. Scale bars = 10 μm.

Figure 7–9. *Trebouxia glomerata* (Warén) Ahmadjian (7) Mature and young vegetative cells; (8) Aplanosporangia; (9) Zoosporangium. Scale bars = 10 μm.

2. Trebouxia excentrica Archibald, *Phycologia* 14: 128, fig. 7 (1975)

In the log phase of cultures, vegetative cells consisting of mostly spherical, infrequently ellipsoidal, 10–15 μm, rarely to 20 μm in diameter, with cell walls

to 1 μm in thickness (Figs. 4–6). Gelatinous matrices not observed. Chloroplast axial, with deep incisions, excentric in one half of cell, containing one large pyrenoid without starch grains.

Asexual reproduction by aplanospores and zoospores. Aplanosporangia with maximum of 64 spores. Zoospores pear-shaped, wall-less, about 7 μm in length, about 3 μm in width, with two anterior flagella of equal length, with posterior nucleus. Distinctive contractile vacuoles and stigma not observed.

Sexual reproduction not observed.

Specimens examined: Iguchi 221.

Culture number: 4125 (CCHU).

Trebouxia excentrica was originally described by Archibald (1975) based on the strain isolated from *Stereocaulon dactylophyllum* var. *occidentale*. Afterwards Hildreth and Ahmadjian (1981) isolated this species from *Cladonia bacillaris, C. subtenuis, C. leporina, Huilia tuberculosa, Lecidea metzleri* and *Lepraria* sp. Meisch (1981) also reported it as photobionts of *Evernia divaricata, E. prunastri, Usnea dasypoga, U. florida U. rigida*. Recently, Yoshimura et al. (1987) isolated this species from *Cladonia vulcani*.

3. Trebouxia glomerata (Warén) Ahmadjian, *Amer. J. Bot.* 47: 679, figs. 9, 10, 15, 17 (1960)

In the log phase cultures, vegetative cells spherical to oviform, 7–13 μm in diameter with about 1 μm cell walls in thickness, rarely 20 μm in diameter, oviform (Figs. 7–9). In stationary phase cultures, cell walls not thickened markedly. Gelatinous matrices were not observed. Chloroplast, massive, axial with shallow incisions, often appeared as small plates, with one small, indistinct, central pyrenoid without starch grains.

Asexual reproduction aplanospores and zoospores. Aplanosporangia with maximum 64 spores. Zoospores pear-shaped, wall-less about 5 μm in length, about 2 μm in width, with anterior flagella of equal length, with posterior nucleus and a minute stigma in anterior portion, without distinctive contractile vacuoles.

Sexual reproduction not observed.

Specimen examined: Iguchi 116.

Culture number: 4040 (CCHU).

Trebouxia glomerata originally was described by Warén (1981–1919) as *Cystococcus glomeratus*. Afterwards, Ahmadjian (1960) transferred this species to the genus *Trebouxia*. Warén (1918–1919) isolated this species from *Cladonia coccifera, C. cornuta, C. deformis, C. gracilis* var. *chordalis, C. macilenta* and

C. rangiferina. Ahmadjian (1960) reported it as photobionts of *Stereocaulon pileatum* and *S. saxatile* (= *S. evolutoides*). Hildreth and Ahmadjian (1981) isolated this species from *Cladonia boryi* and *Huilia albocaerulescens*.

In this study, this species was isolated for the first time from *Cladonia humilis*.

Photobionts reported from *Cladonia* including the results from this study are listed in Table 1. From this list, it is recognized that seven species of *Trebouxia* have been isolated from 35 species of *Cladonia* world-wide. Formerly, Chodat (1913) had reported three species of *Trebouxia* (as *Cystococcus*) from *Cladonia* species but these species were indistinct owing to descriptions based on mainly colony feature or color of strains isolated. It is difficult for us to confirm those species within our present taxonomical understanding of micro coccoidal green algae. We consider mainly the detailed morphological characteristics of the cells and their life cycles. Therefore, we have not enclosed Chodat's species in our list.

It is known that lichen species of the same genera often have the same algal species as photobiont, for example, twenty-six species of Japanese Graphidaceae have the same algal partner, *Trentepohlia lagenifera* (Nakano, 1988) and twelve species of Japanese *Pertusaria* have only *Trebouxia potteri* as photobiont (Takeshita et al., 1989). *Trebouxia erici* was isolated from 12 species of *Cladonia* and *T. pyriformis* from 12 species. From these facts, it was considered that lichenization of some *Cladonia* species is restricted to a specific algal partner (Table 1).

On the other hand, it has been reported that lichenization of some lichen species is not restricted to a specific algal partner. Friedel and Gärtner (1989) isolated two algal partners (*Trebouxia gigantea* and *T. showmanii*) from *Diploschistes ocellatus* and *D. scruposus* respectively. Furthermore, Takeshita et al. (1991) found that *Cladia aggregata* had two algal partners (*Trebouxia erici* and *T. glomerata*). From the the list of photobionts of *Cladonia* (Table 1), both *Cladonia ramulosa* and *C. squamosa* had two species of *Trebouxia* as algal partner; these were *Trebouxia erici* and *T. pyriformis*. Both *C. coccifera* and *C. deformis* also had two species of *Trebouxia*: *T. glomerata* and *T. pyriformis*. Thus, it seems that the lichenization of some *Cladonia* species is less specific and not restricted to a unique algal partner.

In the genus *Cladonia*, as a whole, most species seem to be lichenized by a specific algal partner and *Trebouxia erici*, *T. excentrica*, *T. glomerata* and *T. pyriformis* seem to be the favoured photobionts.

Table 1. List of photobionts from *Cladonia* species including both those reported previously and those isolated in the present study

Photobiont	Lichen	Reference
Trebouxia erici	*Cladonia calycantha*	present study
	C. chlorophaea	present study
	C. coniocraea	Meisch (1981)
		present study
	C. crispata	present study
	C. cristatella	Ahmadjian (1960)
	C. floerkeana	present study
	C. gracilis ssp. *turbinata*	present study
	C. grayii	Meisch (1981)
	*C. ramulosa**	present study
	*C. squamosa**	Meisch (1981)
	C. squamosissima	present study
	C. subulata	Meisch (1981)
T. excentrica	*C. bacillaris*	Hildreth and Ahmadjian (1981)
		present study
	C. leporina	Hildreth and Ahmadjian (1981)
	C. subtenuis	Hildreth and Ahmadjian (1981)
	C. vulcani	Yoshimura et al. (1987)
T. gelatinosa	*C.* sp.	Wang-Yang (1970)
T. glomerata	*C. boryi*	Hildreth and Ahmadjian (1981)
	*C. coccifera**	Warén (1920)
	*C. cornuta**	Warén (1920)
	*C. deformis**	Warén (1920)
	C. gracilis var. *chodalis*	Warén (1920)
	C. humilis	present study
	C. macilenta	Warén (1920)
T. impressa	*C. cornuta**	Wang-Yang (1970)
	C. furcata	Wang-Yang (1970)
T. magna	*C.* sp.	Archibald (1975)
T. pyriformis	*C. bellidiflora*	Meisch (1981)
	C. chlorophaea	Meisch (1981)
	*C. coccifera**	Meisch (1981)
	*C. deformis**	Meisch (1981)
	C. furcata	Meisch (1981)
	C. pleurota	Meisch (1981)
	C. pyxidata	Meisch (1981)
	*C. ramulosa**	Meisch (1981)
	*C. squamosa**	Archibald (1975)
	C. stellaris	Meisch (1981)
	C. uncialis	Meisch (1981)
	C. verticillata	Meisch (1981)

* Species of *Cladonia* for which two species of *Trebouxia* photobiont have been recorded as photobionts.

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