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0014-4754/84/060561-03$1.50 + 0.20/0

Chloroplast nucleoids in a unicellular hot spring alga *Cyanidium caldarium* and related algae

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Summary. Algal chloroplast nucleoids were compared by epifluorescent microscopy. *Cyanidium caldarium* strain RK-1 or 001 has a rod-shaped chloroplast nucleoid while *Cyanidium caldarium* (*Chroococcidiopsis* sp.) strain M-8 or 002 has a circular chloroplast nucleoid along the periphery of a multilobed chloroplast.

A unicellular eukaryotic hot-spring alga *Cyanidium caldarium* has various features relating it to several phyla such as blue-green algae, green algae, red algae and so on[1]. In addition, some strains such as Allen's strain[2] and strain M-8 from a hot spring of Japan[3] are clearly different from *Cyanidium caldarium* (Tilden) Geitler[4] in cell size, endospore number, fine structure and biochemical properties. Nagashima and Fukuda[3] have proposed that strain M-8 must belong to different genus, *Chroococcidiopsis* Geitler, and also that it may be closely related to primitive Rhodophyta. However, few studies of the organelle DNA of these algae have been made. Kuroiwa et al[5] recently developed an improved method for visualization of chloroplast nucleus (nucleoid) in situ with a DNA-specific dye DAPI (4'6-diamidino-2-phenylindole) by epifluorescent microscopy. In this paper, chloroplast nucleoids of several hot-spring algae are compared by this method.

Materials and methods. Cyanidium caldarium strain RK-1 and *C. caldarium* strain M-8 (named *Chroococcidiopsis* sp. M-8 in the text) were originally isolated from Yumoto-spa, Noboribetsu-spa, Japan, respectively[3]. *C. caldarium* strain 001 and *C. caldarium* strain 002 (named *Chroococcidiopsis* sp. 002 in the text) isolated in Campi Flegrei, Italy, were kindly provided by Prof. R. Taddei and Prof. G. Pinto, Università di Napoli, Italy. RK-1 and 001 strains were cultured autotrophically by

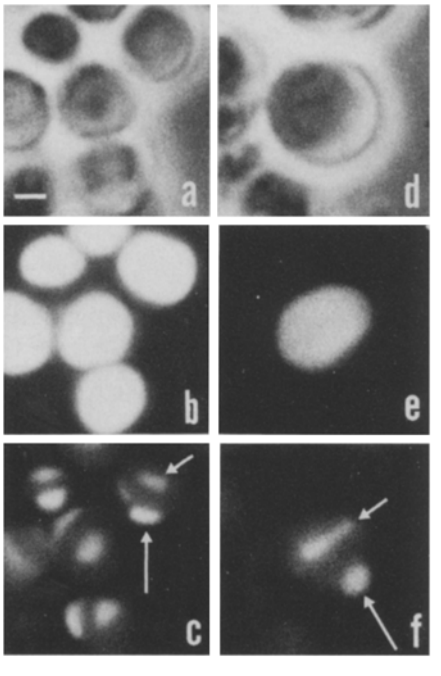

Figure 1. Phase contrast (a, d) and fluorescent micrographs (b, c, e, f) of *Cyanidium caldarium* strain RK-1 (a–c) and strain 001 (d–f) after DAPI staining. Phase contrast (a, d) and fluorescent micrographs with green (b, e) and UV (c, f) lights are taken in the same field in each strain. The ovule chloroplast can be seen in a cell excited with green light (b, e). A spherical cell nucleus (long arrows in c, f) and a rod-shaped chloroplast nucleoid in the chloroplast appears in the cell excited with UV instead of green light (short arrows in c, f). Scale bar, 1 μm.

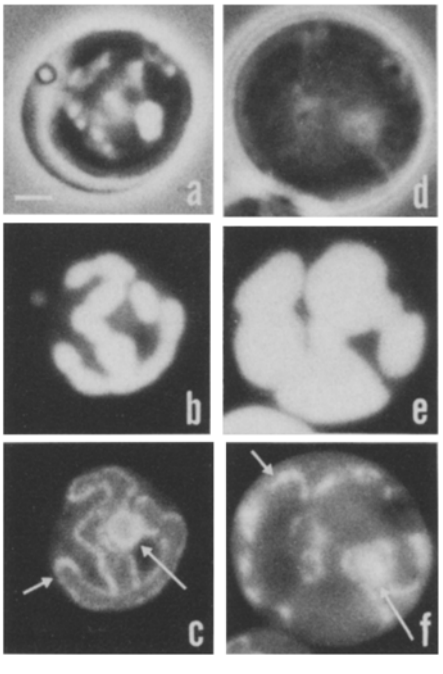

Figure 2. Phase contrast (a, d) and fluorescent micrographs (b, c, e, f) of *Chroococcidiopsis* sp. *(Cyanidium caldarium)* strain M-8 (a–c) and strain 002 (d–f) after DAPI staining. Phase contrast (a, d) and fluorescent micrographs with green (b, e) and UV (c, f) lights are taken in the same field in each strain. The multilobed chloroplast is shown with green light (b, e). A spherical cell nucleus (long arrows in c, f) and a circular chloroplast nucleoid along the periphery of the chloroplast appears with UV instead of green light (short arrows in c, f). Scale bar, 2 μm.

the same methods described previously[6]. M-8 and 002 strains were cultured in shaking flasks containing Allen's medium[2] with added glucose (0.5% final) under 2,000 lux fluorescent light at 38 °C. These materials were collected by centrifugation, washed with distilled water, then fixed in 0.6% glutaraldehyde dissolved in buffer-S[5] and stored at 4 °C. They were mixed in equal quantities with DAPI dissolved in buffer-S on slide glass and squashed gently against the samples. The stained samples were observed with an Olympus BHS-RFK epifluorescence microscope equipped with phase contrast objectives. They were excited with 200 W Hg lamp through a green filter (560 nm) or a UV filter (350 nm) in combination with a 420 nm suppression filter. Phase-contrast, green-excited and UV-excited fluorescent micrographs were taken in the same field in each strain with Fuji Neopan 400 black and white films.

Results and discussion. Figure 1a and 1d show phase contrast micrographs of *Cyanidium* RK-1 and 001 strains, respectively. They are 2–4 μm in diameter and grow by formation of 4 endospores[3]. Under green light excitation, only 1 ovule chloroplast emitting red fluorescence, which occupied the greater part of the cell, was seen per cell in each strain (fig. 1b and 1e). When the cells were excited with UV instead of green light, one bluish white rod-shaped chloroplast nucleoid appeared in the center of a pink chloroplast adjacent to a bluish white nucleus (fig. 1c and 1f).

The rod-shaped chloroplast nucleoids were observed at in every stage of the cell cycles of 2 strains. The central part of the *Cyanidium* chloroplast where a chloroplast nucleoid occurs, appears to correspond to the electron opaque region of electron micrographs of its thin sections[3]. Figure 2a and 2d show phase contrast micrographs of *Chroococcidiopsis* M-8 and 002 strains which are 9–11 μm in diameter and multiply by formation of 4, 8, 16 and 32 endospores[3]. In electron micrographs of thin sections of strain M-8[3] it sometimes seemed that there were several chloroplasts per cell. However, fluorescent microscopic observation with green light excitation (fig. 2b and 2e) indicated that only a single multilobed chloroplast was present per cell and surrounded a vacuole. When glucose was eliminated from the culture medium, the chloroplast occupied a greater part of the cytoplasm than when cells were grown in complete culture medium, in each strain. When the cells were excited with UV light, a circular white nucleoid of the chloroplast could be observed along the periphery of the pink chloroplast, in addition to a bluish white nucleus (ca. 1.7 μm in diameter) (fig. 2c and 2f). The chloroplast nucleoid looked like a chain of small spherical particles as in the case of a brown alga *Ectocarpus indicus*[7]. This observation is consistent with the previous one[8] as to *'Cyanidium caldarium'* strain M-8, and with that by electron microscopy, where an electron-opaque region is recognized inside the peripheral girdle lamella of the chloroplast of strain M-8[3] or strain 002 (= forma B)[9]. These results confirm the previous conclusion that M-8 and 002 strains are very different from *Cyanidium* RK-1 and 001, and must belong to a different genus *(Chroococcidiopsis)*[3]. Kuroiwa et al[8] have proposed that chloroplast nucleoid structure may be classified into at least 5 types as a result of observation on various algal and higher plants by fluorescent microscopy. According to their view, *Cyanidium* RK-1 and 001 may belong to the CN-type in which *Glaucocystis nostochinerum* (Glaucophyta) and *Acetabularia calyculus* (Chlorophyta) are included. On the other hand, *Chroococcidiopsis* M-8 and 002 may belong to CL-type in which some brown algae such as *Sphacelaria* and some diatoms such as *Melosira* are included. The chromophyta *Olisthodiscus luteus* also possesses a ring-shaped chloroplast nucleoid[11]. Therefore, these 2 hot spring algae may be considerably distant from each other phylogenetically, and *Cyanidium* may be more primitive than *Chroococcidiopsis* in its chloroplast nucleoid structure.

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0014-4754/84/060563-02$1.50 + 0.20/0

Karyotypes of three species of Caviinae (Rodentia, Caviidae)[1]

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Summary. Chromosomes of *Cavia aperea aperea* (2n = 64; FN = 116 *Galea spixii* (2n = 64; FN = 118) and *Kerodon rupestris* (2n = 52; FN = 92) are described with data on banding patterns. Comparisons with karyotypes of others species of Caviinae are taken into consideration.

The subfamily Caviinae is composed of 4 essentially South American genera; *Cavia, Galea, Microcavia* and *Kerodon*. Chromosomal data available for this subfamily consist of conventional stained karyotypes of 7 species and 2 subspecies[2–12] plus banding patterns of some of these taxa[8–12]. From these data characteristic features shared by these species can be observed; for example a large number of biarmed autosomes (more than 80% of the complement); biarmed Xs in all species; a considerable karyotypical symmetry; a high frequency of species with an identical diploid number of 64 (only 2 species behave differently: *Galea musteloides,* 2n = 68 and *Kerodon rupestris,* 2n = 52). Our investigation provides a description of the karyotype of subspecies *Cavia aperea aperea,* with G and C banding patterns, not published so far, and additional data about karyotypes of *Galea spixii* and *Kerodon rupestris*. The taxon *Cavia aperea* has a wide range of geographical