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Tolerance of *Colpoda cucullus* resting cysts to ultraviolet irradiation

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ABSTRACT

Resting cysts of the soil ciliate *Colpoda cucullus* Nag-1 were found to be highly resistant to UV light (254 nm); the presumed UV dose required for 99.9% inactivation was 571 mJ/cm^2. On the other hand, in case of vegetative *Colpoda* cells, the presumed UV dose required for 99.9% inactivation was 40 mJ/cm^2). We found that the nuclei of resting cysts were surrounded by auto-fluorescent particles (nuclei-surrounding particles; NSP). The cyst wall components (lepidosomes and ectocyst layer) were also auto-fluorescent. These auto-fluorescent structures may absorb UV light to protect cytoplasmic components.

Keywords: *Colpoda*; resting cysts; UV resistance

INTRODUCTION

Soil protozoa such as *Colpoda* promptly transform into resting cysts that are resistant to desiccation, freezing, high temperature and acid, before the water puddles in which the vegetative forms of protozoa proliferate dry out (Taylor and Strickland, 1936; Maeda *et al*., 2005; Müller *et al*., 2010; Sogame *et al*., 2011). In *Colpoda cucullus* Nag-1, resting cyst formation (encystment) can be induced by suspending the vegetative cells at a high cell density in the presence of Ca^{2+} (Matsuoka *et al*., 2009). On the other hand, excystment is induced by the addition of an infusion of dried wheat leaves or chlorophyllin-Cu (Watoh *et al*., 2003; Tsutsumi *et al*., 2004).

In *C. cucullus* Nag-1, intracellular events such as the degradation of vegetative structures, disappearance of mitochondrial membrane potential, cell cycle arrest, and formation of the cyst wall begin simultaneously in 2-3 h after onset of encystment induction; several days at least are required to complete the resting cyst formation. In the mature resting cyst, the cytoplasmic space—i.e. the space not occupied by mitochondria and nuclei—is occupied by numerous electron-lucent stacked ellipsoidal grains, and the cell is surrounded by a cyst wall composed of a mucous/lepidosome layer, an ectocyst layer and endocyst layers (Kida and Matsuoka, 2006; Funatani *et al*., 2010; Funadani *et al*., 2016). In the present study, we found that the resting cysts of *C. cucullus* Nag-1 were highly resistant to UV light (254 nm), along with evidence suggesting that auto-fluorescent particles surrounding the nuclei may be involved in this UV resistance.

MATERIALS AND METHODS

Tolerance of resting cysts to UV light

Vegetative cells of *Colpoda cucullus* Nag-1 (Funadani *et al*. 2016) were cultured in a 0.05% (w/v) infusion of dried wheat leaves, the cell suspension (approximately 200 cells in 200 μL suspension, at 1 mm depth) was dispensed in watch glasses, and was kept for more than 1 week to naturally encyst under humid conditions. The resting cysts which adhered to the bottom of the watch glass were irradiated with UV light (254-nm light) using a UV sterilizer EG-0003 (Trybest Co., Ltd., Sagamihara, Japan). UV light intensity was determined with a UV photometer SP-82UV (MatherTool Co., Ltd., Nagano, Japan). After UV light irradiation, the medium in the watch glass was discarded, and then a fresh 0.2% infusion of wheat leaves was poured to induce excystment. At 1 day after onset of excystment induction, the resting cysts were randomly chosen, and the number of vacant cysts in which vegetative cells had emerged was counted. The rate of excystment was expressed as a percentage of the total number of tested cells (100–203 cysts).

Tolerance of vegetative cells to UV light

Just-excysted vegetative cells of *C. cucullus* Nag-1 were cultured for several hours in a 0.05% infusion of dried wheat leaves, collected by centrifugation (1500 × g, 2 min), and then suspended in 1 mM Tris-HCl (pH 7.2). For UV light irradiation, 50-cell samples of vegetative cells were transferred into a watch glass using thin glass pipette (200 μL of cell suspension, at 1 mm depth). After UV light irradiation, the cell suspensions were kept for 3 days under humid conditions, and the number of living cells (motile cells and resting cysts) was counted. In order to determine whether or not the resting cysts were alive, the medium was discarded and a fresh 0.2% infusion of dried wheat leaves was poured to induce excystment. The number of vacant cysts were counted at 24 h after onset of excystment induction.

Electron microscopy and fluorescence microscopy

The resting cysts were pre-fixed with a glutaraldehyde (GA) fixative (6% GA, 1% OsO_4, 100 mM cacodylate buffer [pH 7.2], 4 mM sucrose) for 6 h, rinsed in 100 mM cacodylate buffer (pH 7.2), and then post-fixed in a fixative (1% OsO_4, 100 mM cacodylate buffer [pH 7.2], 2 mM sucrose) for 12 h. The post-fixed samples were rinsed in distilled water, dehydrated through a graded ethanol series and then suspended in acetone. The dehydrated samples were embedded in Spurr's resin. Ultrathin sections were stained with 3% uranyl acetate and then with lead citrate (10 min each).

Wet resting cysts aged more than 2 weeks were observed with a fluorescence microscope (BX-50, Olympus) equipped with the filter set WU.

RESULTS AND DISCUSSION

Fig. 1 shows the survival rate (%) of vegetative cells and wet resting cysts of *C. cucullus* Nag-1 which had been exposed to UV light (254 nm). When the vegetative cells were exposed to high doses of UV light, they died soon thereafter (data not shown). Cells that had been exposed to lower doses of UV light died gradually over several days (data not shown). Fig. 1 shows the survival rate of the vegetative cells 3 days after the cells were exposed to UV exposure. In this case, the presumed UV dose required for 99.9% inactivation was 40 mJ/cm^2. On the other hand,

the presumed UV dose required for 99.9% inactivation of the resting cysts was 571 mJ/cm^2, indicating that the resting cysts are highly resistant to UV light.

It has been reported that microorganisms such as viruses, bacterial spores and some protozoa are highly tolerant to UV light. The UV dose required for 99.9% inactivation of infectious pancreatic necrosis virus (IPNV) is 250 mJ/cm^2 (Liltved *et al*., 2005), that of *Bacillus* spores is 50 mJ/cm^2 (Nicholson and Galeano, 2003), and that of *Acanthamoeba* cysts is 130 mJ/cm^2 (Lonnen *et al*., 2014). The resting cysts of *C. cucullus* Nag-1 are even more resistant to UV light than these species. The nature of UV resistance seems to be one of the most important adaptive strategies for inhabitants of the soil surface to survive. To our knowledge, there has been no other report on UV resistance of ciliates.

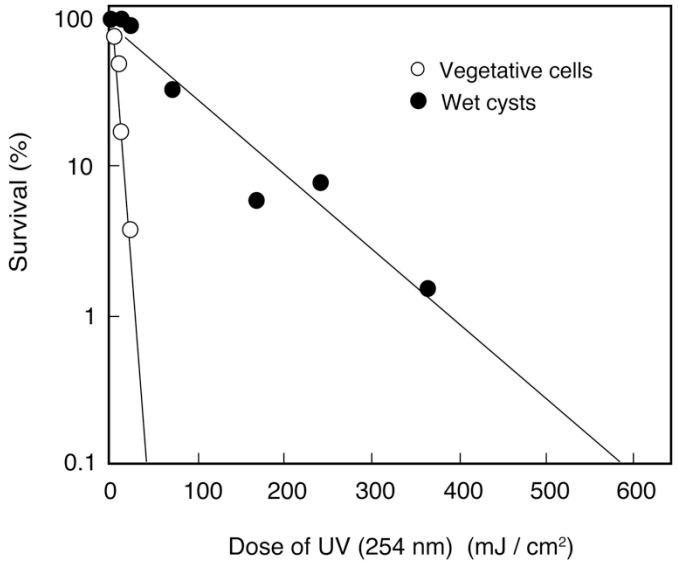

Fig. 1. Inactivation of vegetative cells (○) and 1-week-aged wet resting cysts (●) of *C. cucullus* Nag-1 by irradiation of UV light (254 nm).

As for the mechanism for the resistance against UV light in the resting cysts of *C. cucullus* Nag-1, we found that the macro- and micronucleus of the resting cysts were surrounded by fine particles (nuclei-surrounding particles; NSP) (Fig. 2B, C, arrowheads), although there is no NSP in the vegetative cells (Fig. 2A; reproduced from Watoh *et al*. 2005). Fluorescence microscopy showed that NSP was auto-fluorescent (Fig. 3, arrowhead; Fig. 5). The cyst wall components such as lepidosomes and the ectocyst layer were also found to auto-fluoresce (Figs. 4, 5). The fact that these structures (NPS and cyst wall components) emit fluorescence suggests that these structures absorb UV light, and thereby play a role for protection of cellular components.

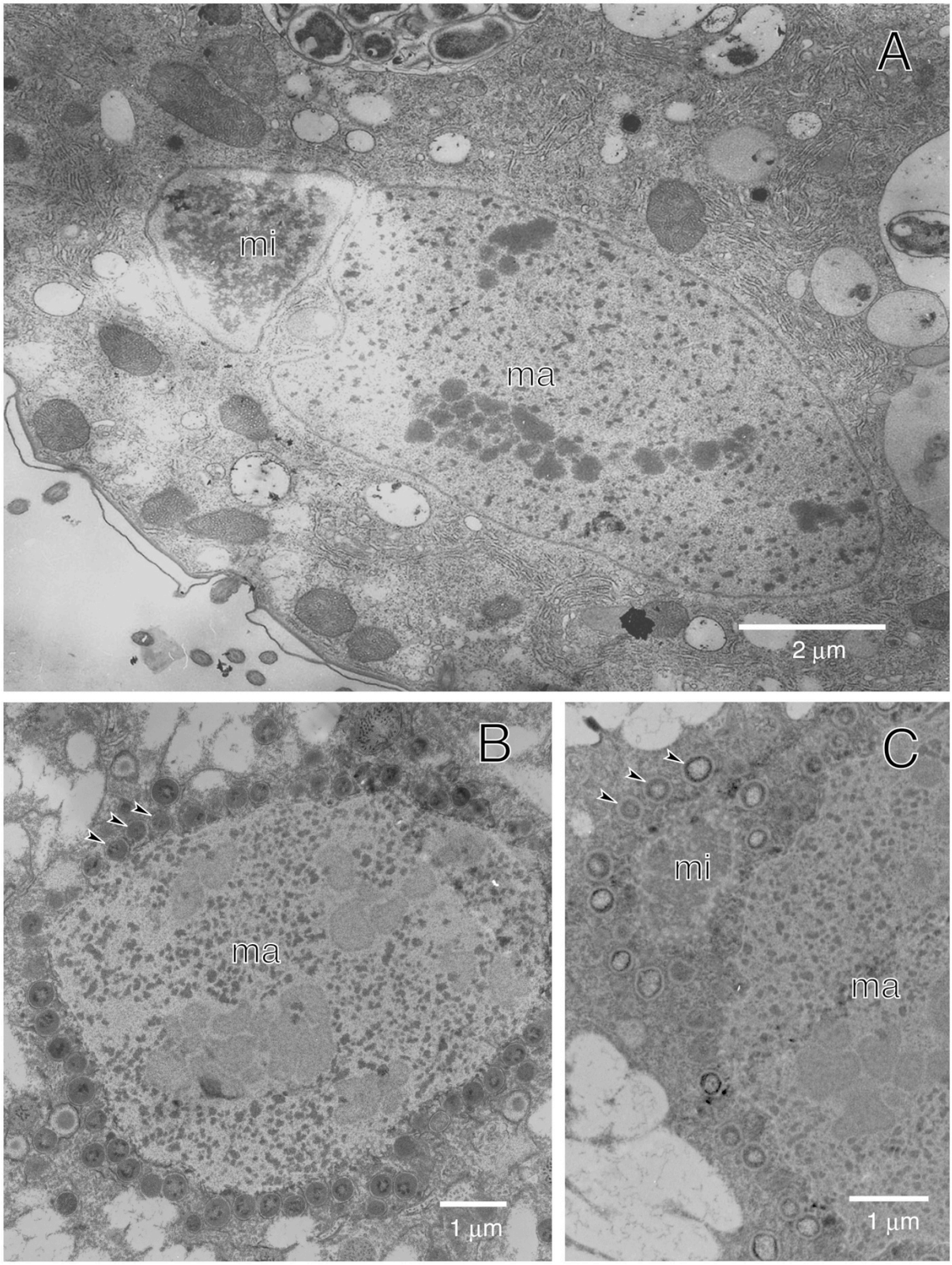

Fig. 2. Electron micrographs of a vegetative cell (A) and the wet resting cysts of *C. cucullus* Nag-1 aged more than 2 weeks (B, C), showing particles surrounding nuclei. ma, macronucleus; mi, micronucleus; arrowheads, particles surrounding nuclei (NSP). An electron micrograph of vegetative cell (A) is reproduced from Watoh *et al*. (2005).

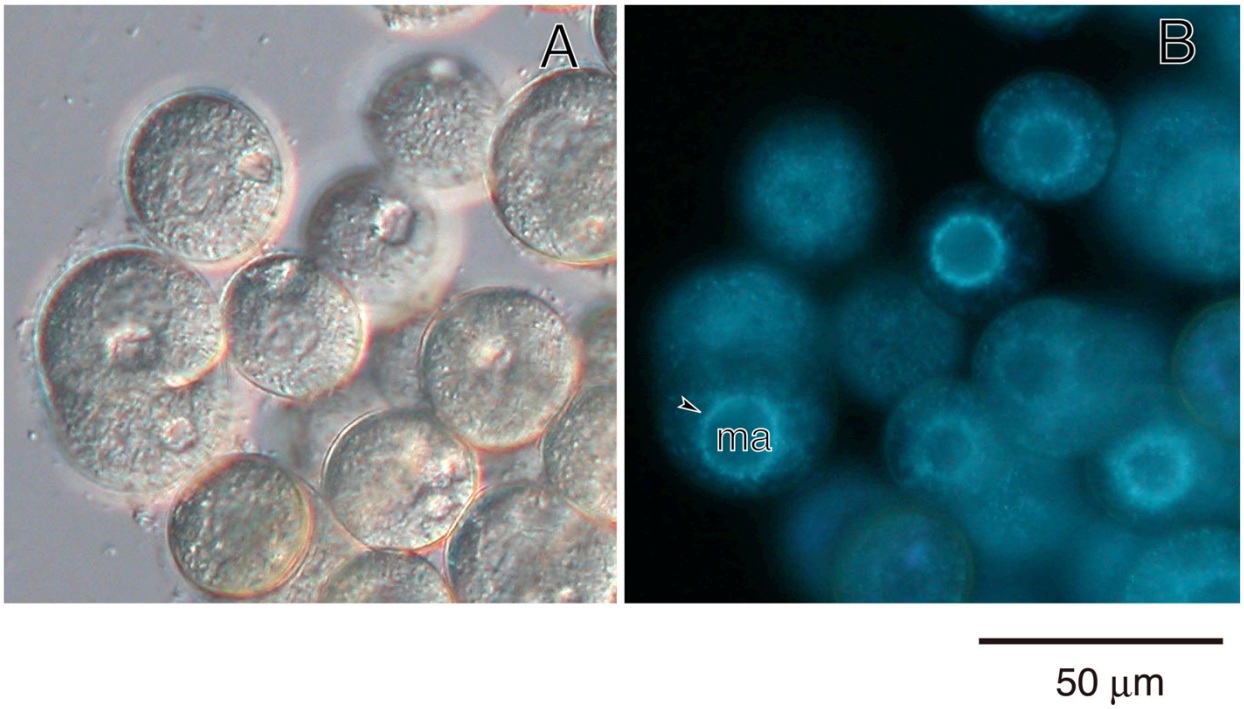

Fig. 3. A Nomarski image (A) of resting cysts of *C. cucullus* Nag-1 aged more than 2 weeks and its fluorescence photomicrograph (B), showing auto-fluorescent particles ('arrowhead') surrounding macronucleus ('ma').

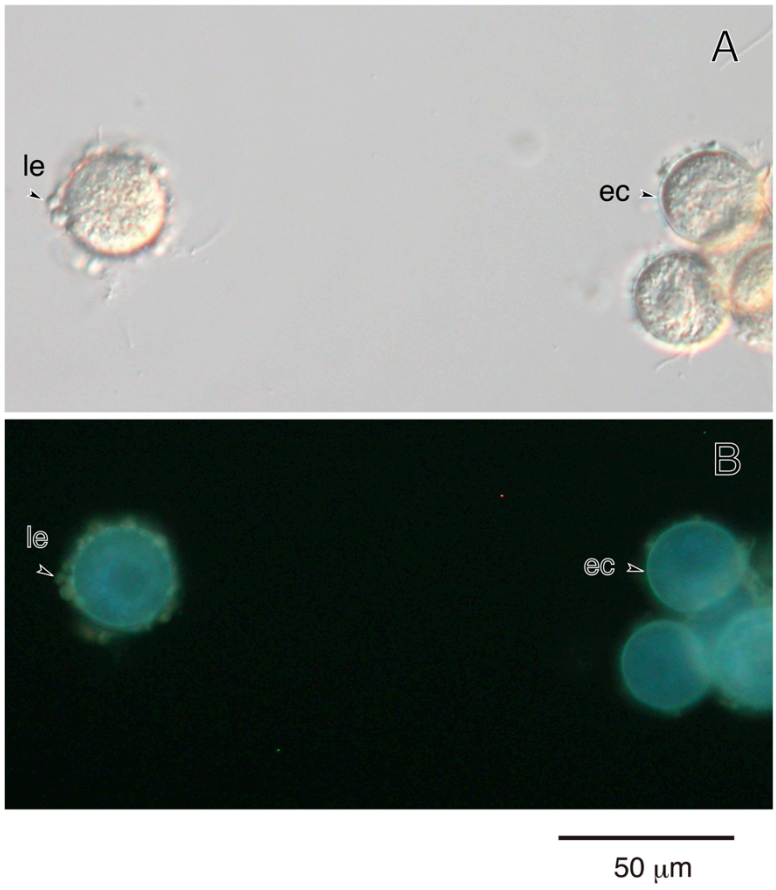

Fig. 4. A Nomarski image (A) of resting cysts of *C. cucullus* Nag-1 aged more than 2 weeks and its fluorescence photomicrograph (B), showing auto-fluorescent cyst wall components. le, lepidosome; ec, ectocyst layer.

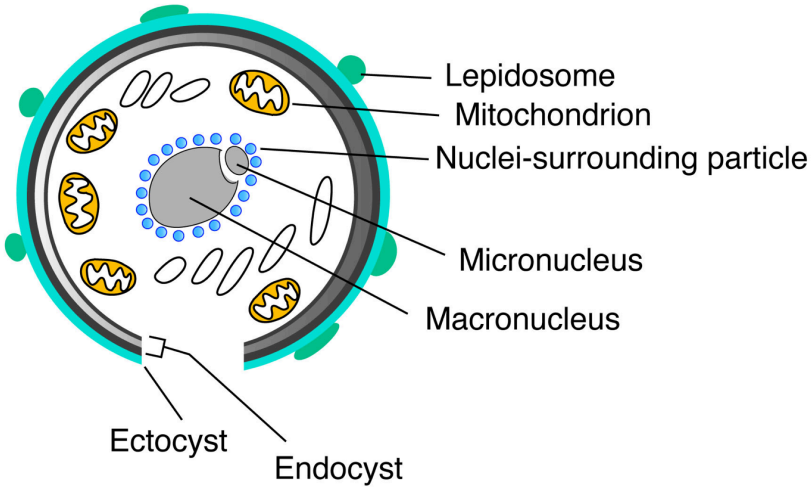

Fig. 5. A schematic diagram of the resting cyst of *C. cucullus* Nag-1, showing auto-fluorescent components (lepidosome, ectocyst layer and nuclei-surrounding particles).

CONFLICT OF INTEREST

All authors declare no actual or potential conflicts of interest.

SUBMISSION DECLARATION AND VERIFICATION

This manuscript is approved by all authors, and if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

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