Towards morphological variability of symbiotic algae

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Green hydra is the host to unicellular algae in its gastrodermal myoepithelial cells. It is known that different xenobiotics can damage this symbiotic relationship. The effects of sublethal doses of antibiotics on the hydra–alga symbiotic relationship are assessed using cTEM microscopy. Chloroplasts showed changes in thylakoid structures. Pronounced changes in mitochondria of both hydra and algae are noted. In some algae plastoglobules are visible and the number of ribosomes in cytoplasm of algae is changed. Results show that endosymbiotic algae represent a stronger partner with less pronounced damages compared to hydra host in the studied symbiotic model.

Keywords: cTEM microscopy, endosymbiotic algae, green hydra, toxicants, xenobiotics.

GREEN hydra (*Hydra viridissima* Pallas, 1766) is a simple metazoan organism that contains endosymbiotic unicellular green algae within symbiosomes in its gastrodermal myoepithelial cells, forming a stable long-term mutualistic symbiosis[1] (Figure 1). Autotrophic algal symbionts provide not only a significant competitive advantage to the host largely supported by nutrients recycling between the symbiotic partners but the mutualistic association can shift into parasitism under specific conditions[2]. A symbiotic process requires certain prerequisites in order to be established and is maintained through interaction of numerous genes[3] as well as their exchange acquiring new properties in the process. Some studies hypothesize that the symbiotic relationships arose from parasitic ones[4] and symbiotic partners can be periodically or permanently separated and continue to live independently[5].

Hydra is a suitable test organism for research in toxicology, genetics and molecular biology, with green *Hydra* being a particularly suitable model in studies on symbiosis[6–8]. Study of endosymbiosis on the level of organisms can contribute to a better understanding of the endosymbiosis on cellular level and vice versa. Experiments on symbiotic organisms treated with antibiotics, where chloroplasts and mitochondria represent target organelles, provide additional evidence[4]. For more than half a century, antibiotics have been widely used to treat various diseases. Chloramphenicol has disruptive effects on synthesis of proteins in chloroplasts of some microorganisms[9]. Cinoxacin inhibits bacterial DNA synthesis based on the inhibition of DNA gyrase[10]. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV[11]. In this study, the effects of chloramphenicol, cinoxacin, ciprofloxacin on the hydra–alga symbiosis were studied using conventional transmission electron microscopy.

Green hydra specimens used in the experiment were collected from Maksimir Lake in Zagreb, Croatia (N 45°49′39.80″E 16°01′02.50″) and kept in glass dishes in the laboratory conditions at a temperature of 21°C. Photoperiod regime light/dark was 14/10 h. Each experimental group of green hydras consisted of 20 individuals. The first group served as control and was kept in aerated aquarium water. The second group was treated for 24 h with 0.2 mM of aqueous solution of chloramphenicol (‘Pliva’, Zagreb, Croatia). The third group was treated for 72 h with 0.05 mM of aqueous solution of cinoxacin (‘Lilly Deutschland GmbH’, Bad Homburg, Germany). The fourth group was treated for 72 h with 0.15 mM of aqueous solution of ciprofloxacin (‘Bayer AG’, Leverkusen, Germany). Two most damaged individuals of green hydra specimens from each of the four experimental groups were used for cTEM analysis. Immediately after treatment they were fixed in 1% glutaraldehyde (pH 6.9) buffered with 0.01% sodium cacodylate buffer and post-fixed in 1% osmium tetroxide buffered with the same buffer, transferred in acetone and araldite and cut with a glass knife on ultramicrotome. Finally, they were dyed with 4% uranyl acetate and lead citrate[12]. Micrographs were obtained using electronic microscope *Zeiss* EM10A.

Antibiotics applied in sublethal doses, as well as some pesticides and herbicides, cause damage to intracellular structures in both the host and endosymbionts[13–15]. It seems that endosymbionts in a certain period can use its host for survival[16]. After treating green hydra with chloramphenicol, electron microscopy identified damage in

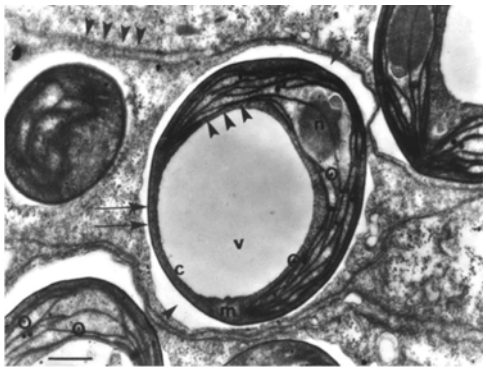

Figure 1. cTEM of gastrodermal layer of control green hydra. Endosymbiotic algae *Chlorella* (2 arrows) within gastrodermal myoepithelial cell of green hydra host (4 arrowheads); symbiosome and perialgal space (arrowhead); vacuole (v), nucleus (n), chloroplast (c, 3 arrowheads), plastoglobules (O), mitochondrion (m) of the algal cell. Bar 1 μm.

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membrane structures of host mitochondria, as well as mitochondria and chloroplasts of symbiotic algae. Membranous structures are present in vacuole. Perialgal space showed decline in some areas of gastrodermal cells compared to the hydras in control group. Formations of high grana with numerous tylakoids were visible in algal cell (Figure 2). After treating green hydra with cinoxacin, damage of mitochondrial membrane structures in green hydra host, as well as damage of mitochondria and chloroplasts in symbiotic algae were identified using electron microscopy. Perialgal space was locally widened or reduced. Mitochondria of treated symbiotic algae were increased in volume compared to the control. Thylakoid

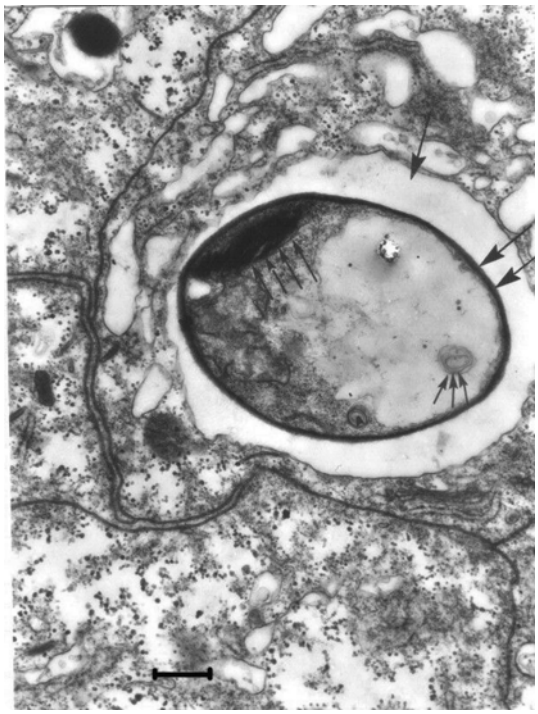

Figure 2. cTEM of gastrodermal layer of green hydra with endosymbiotic alga treated with 0.2 mM of chloramphenicol for 72 h. Green alga (2 arrows) with perialgal space (single arrow); myelin figures present in vacuole (3 arrows) and high grana with numerous tylakoids (4 arrows). Bar 1 μm.

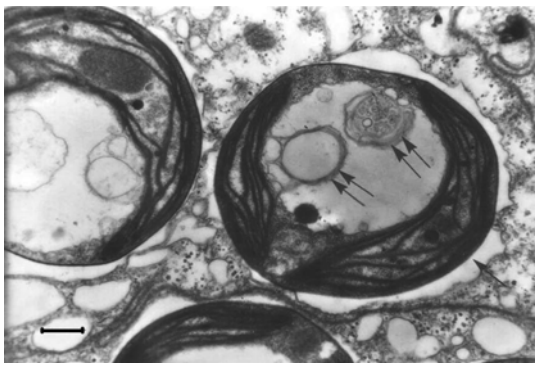

Figure 3. cTEM of gastrodermal layer of green hydra with endosymbiotic algae treated with 0.15 mM of ciprofloxacin for 72 h. Changed perialgal space (single arrow); osmiophilic bodies and membranous structures present in vacuole (2 arrows). Bar 0.5 μm.

structures of chloroplasts were visible and plastoglobules in chloroplasts increased in size. Numerous ribosomes were present in cytoplasm. After treating with ciprofloxacin, effects similar to the ones produced by cinoxacin were detected. Perialgal space was visible but reduced. The number of ribosomes was decreased. Membrane structures, similar to osmiophilic structures, and myelin figures were observed in the vacuoles (Figure 3). An increased number of plastoglobules was visible and mitochondria appeared lighter, with matrix less condensed compared to the control individuals. It was found that after treating with certain xenobiotics, vacuolization increases in the host cell; perialgal space increases in size with merging symbiosomes[5]. Less damaged algae survived treatments and after a certain time period re-established endosymbiotic relationship in myoepithelial hydra cells. This leads to the conclusion that symbiotic relations are not fixed and sometimes could be reversible. After treating with antibiotics, endosymbiotic algae have less irreversible damages compared to hydra host, being a more resistant symbiotic partner. It could be assumed that in particular environmental conditions, algae represent more viable and resistant symbionts, and can regenerate and establish a new population. That could be the pattern for appearance of parasitic relations instead of mutualistic ones[2,17]. Symbiotic relationships are successfully maintained as long as stable environmental conditions exist, in which nutrients between the host and endosymbiont are successfully exchanged. In case of instability of environmental conditions, there is a disturbance in symbiotic relationships and mutualism can transform to parasitism. The green hydra host is more sensitive to the effects of different xenobiotics compared to the endosymbiotic green algae. Green algae can successfully outlive the green hydra host and regenerate the incurred damages. Endosymbiotic algae can be successfully maintained in culture in the laboratory conditions using microbiological methods[18]. However, after a period of time, morphological differences between individuals arise. It is noted that coccoid shape transforms to cenobial in the isolated endosymbiotic green algae[19]. We assume that when micro-environmental conditions become unstable, endosymbionts (green algae, stronger partner) can outlive the host (green hydra, weaker partner) and in certain conditions escape from the host, which most probably occurred during coevolution of hydra and algae. Algae can then find a suitable ecological niche and continue to grow and reproduce[20]. As the population grows in numbers, changes in genome can accumulate, resulting in increased variability between individuals which can than lead to the rise of new forms.

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Changes in the seasonal cycle of carbon stocks and fluxes due to fires in the grassland ecosystem of Manipur, North east India

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Fire is a common perturbation in the grassland ecosystems throughout the world. Effect of fire on carbon stock, rate of C-accumulation and soil CO_2 flux have been studied in *Imperata cylindrica–Sporobolus indicus*-dominated grassland community of Manipur, Northeast India. Carbon stock in the vegetation components was estimated to be 12.59 and 12.06 Mg ha^{-1} and soil organic carbon stock was found to be 57.28 and 44.74 Mg ha^{-1} in the control and burnt site respectively. It indicates that fire decreases the carbon stock in the grassland. However in the following year the annual rate of carbon accumulation increased in burnt site (7.94 Mg ha^{-1} year^{-1}) compared to the control site (6.75 Mg ha^{-1} year^{-1}) whereas the annual soil CO_2 flux decreased in the burnt site (4.06 Mg ha^{-1} year^{-1}) in comparison to the control site (7.26 Mg ha^{-1} year^{-1}). Our estimates of carbon budget reveal that the net uptake was 3.88 Mg C ha^{-1} year^{-1} in the grassland ecosystem after the burning treatment. Thus, the annual burning of grassland can cause major changes to carbon stocks and fluxes.

Keywords: Aboveground biomass, belowground biomass, carbon stock, carbon accumulation, soil CO_2 flux.

GRASSLANDS cover about one quarter of the earth's land surface[1] and span a range of climatic conditions from arid to humid. They play an important role in biosphere feedback of atmospheric CO_2 increase and climate change[2]. Grassland ecosystems can contribute to CO_2 mitigation through carbon accumulation in soil[3]. Grassland soils are high in soil organic carbon and contain an extensive fibrous root system, that creates an environment ideal for soil microbial activity[4]. Measurement of CO_2 flux from grassland soils supports their importance in global carbon budget[5].

Grasslands can vary greatly in their degree and intensity of management, from extensively managed rangelands to intensively managed. Anthropogenic land use is now widely considered to either contribute to carbon emissions through degrading land practices or to function as a carbon sink for atmospheric carbon through accumulation in below and aboveground forest and grassland components[6]. This has stimulated research on many different ecosystems regarding global carbon dynamics, and

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