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Review:

Endosymbiotic alga as the stronger evolutionary partner in green hydra symbiosis

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Green hydra (*Hydra viridissima* Pallas, 1766) is a classical example of endosymbiosis. Described ultrastructural features of green hydra symbiosis are extensive widening of perialgal spaces, degradation, loss and fusion of symbiosomes. These changes are considered as defensive and protective mechanisms and the endosymbionts show greater viability than their host. Also, a certain degree of independency was noticed and for the first time endosymbiotic algae from green hydra have been successfully isolated and permanently maintained in pure lab culture. Microscopical and molecular analyses of the isolated endosymbionts resulted in the discovery of two hitherto undescribed endosymbiotic species in green hydra. In final conclusion, endosymbiotic algae from green hydra perform as the "stronger" symbiotic partner in green hydra symbiosis. We keep on searching for more evidence and results.

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Introduction

Symbiotic associations

Symbiotic associations are of wide significance in evolutionary biology. Symbiosis is seen as one of the driving forces in evolution and leads to competitive advantages in particular habitats. It represents a long-term relationship between at least two species. The relationships vary from mutualism to parasitism (Osborne 2007). In mutualism both partners benefit from the relationship and in parasitism only one partner benefits at the cost of the other. It is presumed that symbiotic relationships arose from parasitic attempts. First ideas about symbiosis are known from the time of Herodot, who stated that mutualism is the balance of nature. The term "symbiotismus" was defined by Albert Bernhardt Frank in 1877. The first definition of symbiosis was given by Heinrich Anton de Bary in 1879 according to which symbiosis is the phenomenon where unequal organisms live together (Sapp 1994). Endosymbiosis requires the close physical contact of symbionts, where at least two genomes of different evolutionary origin exist inside the same cytoplasm (Ebringer and Krajčovič 1986, 1994).

Green hydra symbiosis

Green hydra (*Hydra viridissima* Pallas, 1766) is a simple aquatic invertebrate and represents a model for research on the endosymbiotic relationship between two organisms. It is a member of the phylum Cnidaria, class Hydrozoa, order Hydroida, family Hydridae (Holstein and Emschermann 1995). This cnidarian harbors the individuals of photoautotrophic microalgae in its gastrodermal myoepithelial cells and presents a mutualistic symbiosis. This is the only hydra species that is a host to "zoochlorellae". This term was first used by Brandt (1882), besides others, also for endosymbiotic algae from green hydra. Later, Beijerinck (1890) used the term as a synonym for the genus *Chlorella*. He described them as the "green balls" and until today this term denotes all endosymbiotic algae in freshwater invertebrates. Up to 20 individuals of the unicellular green algae occupy about 10% of the cellular volume (Holstein and Emschermann 1995). The adaptation to a new intracellular (endosymbiotic) niche is not easy (Moulder 1985). Each alga is placed into one vacuolar membrane symbiosome. Algae are regularly placed to form columns, one alga above the other, in the basal part of the cell. A once successfully established endosymbiosis disqualifies an endosymbiotic event with other species of algae (Rahat 1991). A constant number of algae in the cell is controlled by inhibition of algal mitosis, expulsion, or digestion of algae (Baghdasarian and Muscatine 2000). The maintenance of symbiosis is bidirectional and strengthens the fitness of the organism. Inside hydra, algae find their protection and habitat and also use the products of the host metabolism (McAuley et al. 1996). Hydra, the heterotrophic host, uses the photosynthate released by the algae. Characteristic of endosymbiotic alga is export of maltose which prevents the fusion of symbiosomes with lysosomes (Hohman et al. 1982). It is important to mention that microalgae have a significant role as primary producers in aquatic ecosystems. Combination of algal partners and selection of host's populations can contribute to better adapted symbionts (Klüetter et al. 2006). In symbiotic associations, size and placement of the symbiotic partners, necessity, duration, stability, mode of maintenance, specificity, recognition, interaction (Smith and Douglas 1987), and integration (Margulis and Sagan 2002) are important.

Besides endosymbiotic, there exist non-symbiotic species of hydras that do not establish symbiosis, and aposymbiotic species that once formed a symbiotic relationship.

Hydras from braueri, oligactis and vulgaris groups in general do not carry algal endosymbionts (Martínez et al. 2010). Symbiotic hydras can better survive starvation than non-symbiotic hydras (Habetha et al. 2003), for example *Hydra oligactis* Pallas, 1766. But the question remained, which symbiotic partner is the „stronger" one in this symbiotic relationship?

Ultrastructural mechanisms

The hydra-alga symbiosis represents the important model for research on host-symbiont specificities in the system invertebrate-alga (Yonge 1958), therefore green hydra is a highly suitable experimental animal (Beach and Pascoe 1998; Kovačević et al. 2005). It has been studied for hundreds of years, with many symbiotic mechanisms described (Dunn 1987; Kalafatić et al. 2001).

Ultrastructural modifications of green hydra symbiosis in an unfavorable environment comprise extensive widening of perialgal spaces (partial or complete), which leads to degradation and loss of symbiosomes, followed by fusion of symbiosomes and perialgal spaces, resulting in up to three algae per vacuole. These changes perform as protective mechanisms in this green hydra symbiosis and point towards a certain degree of endosymbiont independency. Reassembly of the symbiosis is achieved by forming the regular algal columns in gastrodermal myoepithelial cells (Kovačević et al. 2007). At least during a short period the endosymbiotic alga might convert the mutualistic relationship into a form of parasitism, whereby it creates a favorable environment for survival, while the host remains less viable. Although a mutualistic symbiotic relationship seems stable, it could be disturbed by the "conflict of interests" of symbiotic partners in an unfavorable environment. This could lead to parasitism or termination of symbiosis (Kovačević et al. 2006; Margulis and Sagan 2002; Puce et al. 2007). This is an example of evolutionary reversal, a rare and important event in biological evolution.

Ecotoxicological view

The research of the effect of xenobiotics, e.g., the herbicide norflurazon on green and brown hydra underline the notion that symbiotic hydras are better adapted to unfavorable environment than hydras which do not undergo symbiosis. But, what about the relation hydra-alga and the effect of herbicide upon them? Special emphasis is given on the bleaching effect. Norflurazon was expected to lead to bleaching of the newly formed buds (i.e., of the endosymbiotic algae contained in the new buds). However, there is no bleaching detected. Algae retain their green color throughout the experiment. After a recovery period, the green hydra individuals re-establish regular endosymbiosis with endosymbiotic algae again (Kovačević et al. 2009a). Thus, algae exert features of either resistance to the herbicide or very complex preadaptations to it in this endosymbiosis. The treatment of non-symbiotic algae with norflurazon caused bleaching (Kovačević et al. 2008). The treatment with aluminium salts (unpublished data) showed that endosymbiotic algae can survive higher concentrations than the host, i.e., representing the stronger symbiotic partner. Algae showed higher capability of survival in unfavorable environment than hydra. It might be considered that algae are less dependent on products of hydra metabolism, than hydra is dependent on the products of algal metabolism.

Isolation of endosymbionts

After noticing certain signs of algal independency, the possibility to isolate the endosymbionts from green hydra was researched. The prevailing opinion was that endosymbiotic algae from green hydra belong to the genus *Chlorella* (Friedl 1997) and that algae isolated from hydra could not be maintained in stable long-term culture (Huss et al. 1993/1994; Habetha and Bosch 2005).

Very recently, endosymbiotic algae from green hydra were successfully isolated and permanently maintained in clean lab culture on the surface of a deep stock agar (Kovačević et al. 2010a). Algae have been maintained for more than 5 years, and momentarily the 99th generation of algae has been cultivated in a stable and clean culture.

Diversity of endosymbionts

The phylogeny of isolated endosymbiotic algae was reconstructed on the basis of the 18S rRNA gene and their taxonomical status clarified. The use of reliable and modern phylogenetic methods (Bayesian analysis, maximum likelihood and maximum parsimony analysis) revealed highly similar positions of taxa on trees (Kovačević et al. 2010a). Different genera of unicellular green algae could be present as endosymbionts in green hydra, one at the time (Kovačević et al. 2009b). Recent molecular phylogenetic analyses of endosymbiotic algae from green hydra showed that they are not closely related to the genus *Chlorella* (Trebouxiophyceae) and resulted in the discovery of two hitherto undescribed endosymbiotic species (genera *Desmodesmus* and *Mychonastes*). Therefore, the clear conclusion is that endosymbiotic algae from green hydra have a polyphyletic origin. An unsystematic name for all endosymbiotic algae isolated from green hydra with the characteristic of permanent stable lab growth is given as *Chlorella zagrebiensis* group (Kovačević et al. 2010a).

Further prospects

In general, microorganisms are not prepared to an intracellular lifestyle and they have to adapt to the new endosymbiotic niche. The main goal of these associations is to achieve a level of integration where the endosymbiont becomes an organelle in a long-term process (Kovačević et al. 2009c). In the green hydra endosymbiotic relationship the alga has not (yet) been turned into an obligate endosymbiont. It might be that hydra as a host served as some kind of vector for diversification of endosymbionts. Lateral gene transfer could play an important role in this process (Habetha and Bosch 2005). This could be beneficial for both organisms and also the process of coevolution might drive speciation.

Further prospects on this subject include ultrastructural investigations, further morphological and morphometrical characterization of endosymbiotic algae once they become aposymbiotic and their isoenzyme analyses (Kovačević et al. 20010b), in comparison to their free-living relatives. The quick, simple and indicative Chlorella test (Kovačević et al. 2008) could serve as a possible tool for establishing the basic level of fitness of various endosymbiotic algae from green hydra and their free-living relatives. Preliminary results have shown the obvious difference in fitness between aposymbiotic microalgae and their free living relatives, as well as differences within the two groups themselves. By comparison of symbionts and free-living relatives in general, we could obtain clearer insights into this complex endosymbiosis.

Final conclusion

Endosymbiotic algae act as the "stronger" symbiotic partner in green hydra symbiosis and could be permanently isolated from its host. Green hydra symbiosis revealed that hydra-alga association biodiversity is much more complex than previously thought.

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