SYMBIOSIS (2006) 42, 175–176 ©2006 Balaban, Philadelphia/Rehovot ISSN 0334-5114

Technical note

Detection of aluminum depositions in green and brown hydra

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(Received October 5, 2006; Accepted October 29, 2006)

Abstract

Although aluminum is one of the most abundant chemical elements in the Earth's crust, its effect on living beings was little known until recently. Since the effect of aluminum ions upon green and brown hydras has not been studied yet, with this technical note we showed how aluminum depositions in symbiotic and asymbiotic hydras could be detected, observed and analyzed. Differential eriochrome cyanine staining on hydras has been performed for the first time, using an appropriately modified technique for detecting the aluminum depositions in hydras. This hydra bioassay was shown to be a useful and exact tool for detection of expected results e.g. aluminum depositions in hydra cells.

Keywords: aluminum depositions, brown hydra, green hydra, symbiosis

1. Introduction

Aluminum is one of the most abundant chemical elements in the Earth's crust. Its effect on living beings was little known until recently (Exley, 1995; Khan et al., 2005). Since the effect of aluminum ions upon green and brown hydras has not yet been studied, with this technical note we showed how aluminum depositions in symbiotic and asymbiotic hydras could be detected, observed and analyzed. Hydra is a simple freshwater organism, which comprises two cellular layers separated by mesoglea. Green hydra forms a symbiotic relationship with alga of *Chlorella* genus (Douglas, 1994). Detection of possible aluminum depositions in two cellular and one acellular layer of the symbiotic green and the asymbiotic brown hydra appeared to be a not yet reported issue.

2. Materials and Methods

Aluminum comparative toxicity test on the two hydra species has been performed for the first time, using individuals of green (*Hydra viridissima* Pallas, 1766; strain S1J-J1) and brown (*Hydra oligactis* Pallas, 1766; strain S1M-K1) hydra. They were treated with 9 concentrations of aqueous solution of aluminum sulphate (25, 50, 80, 100, 150, 200, 250, 350 and 500 mg/l; Kemika, Croatia) in laboratory conditions (22.8°C) in subacute exposure for three days and compared to the control groups of organisms.

Differential eriochrome cyanine staining of hydras has been performed for the first time, using an appropriately modified technique. For the preparation of slides, standard histological methods were used. After bringing sections to distilled water, cuts were differentially stained with 0.2% acid eriochrome cyanine (Kemika, Croatia) according to Pearse (1972) and modified as described in this technical note. The 0.2% acid eriochrome cyanine was prepared in 1% aqueous HCl. It was twice filtrated each time before the use. The stain could be stored and used for at least 6 months after preparation. The slides were stained for 22 minutes and afterwards immediately washed out in (running) hot tap water for 5 times e.g. until the preparations became stained in light blue-gray color throughout.

The sections were then dehydrated in series of ascending concentrations of ethanol according to the following procedure: differential staining in acid ethanol (1 ml of concentrated HCl in 100 ml of 70% ethanol) followed by the standard dehydration thorough 80%, 96% and absolute ethanol (once per bath). One series should contain at most 6 slides. It is extremely important to keep the slides in each ethanol concentration for a few moments only

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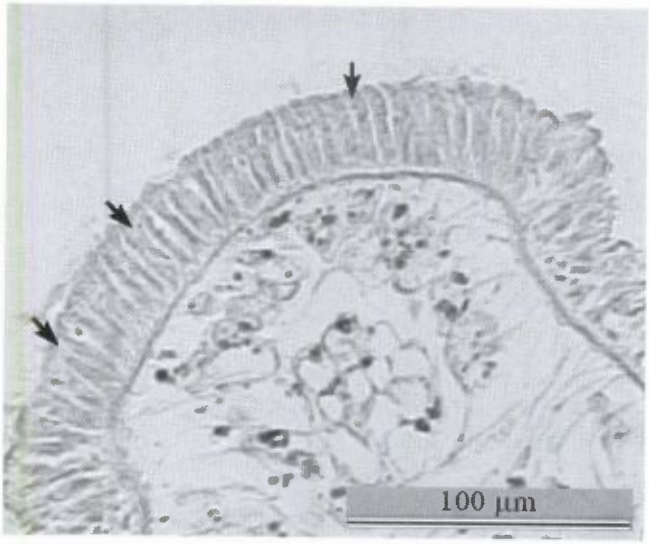

Figure 1. *Hydra oligactis* treated with 100 mg/l aluminum sulphate. Foot of brown hydra with aluminum deposition-containing ectodermal cells. Depositions appeared as pink dots (arrow). 0.2% acid eriochrome cyanine.

before they were transferred to the next ethanol concentration. Finally, they should standardly be immersed in xylol and canada-balsam. Differential staining in 70% acid ethanol removes all the counterstain and, if any, only the aluminum depositions remained.

3. Results

Aluminum depositions appeared pink. Only treated hydras contained aluminum depositions, while the control animals did not contain any aluminum depositions, proving the effectiveness and correctness of the method.

Aluminum depositions were found in the form of single and multiple (2–4 separate) depositions, clusters or huge areas of aluminum depositions. Preliminary results showed aluminum depositions found in cellular layers of hydras only, while mesoglea did not contain any depositions. In symbiotic, green hydras aluminum depositions appeared in gastrodermal cellular layer only, including the endosymbiotic algae *Chlorella*, while in brown hydras aluminum depositions appeared in ectodermal cellular layer only.

4. Discussion

Further investigation on aluminum depositions in symbiotic and asymbiotic hydras should involve morphometrical analysis and determination of the percentage of the cells containing aluminum depositions and measuring the surface area of those depositions in comparison to the total area of cells containing them. It should also answer the question of appearance and purpose of these depositions, as well as their very determined position in cellular layers of the green and brown hydra.

This hydra bioassay was shown to be a useful and exact tool for detection of expected results e.g. aluminum depositions in hydra cells

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