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The effect of three different predatory ciliate species on activated sludge microfauna

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Abstract

Bacterivorous ciliates play important roles in the functioning of activated sludge by reducing dispersed bacteria and enhancing flocculation. There are, however, no data on the resistance of this functional group of microorganisms to predation. Our experiment was conducted with activated sludge subsamples subjected to artificially introduced three predatory ciliates species. The two predator species originating from activated sludge were *Oxytricha* sp. and *Spathidium spathula*. *Dileptus margaritifer* was a "foreign" predator species. The latter was introduced to compare its effect with the influence of predators naturally occurring in activated sludge on the ciliates community potentially adapted to certain predation strategies. Results showed that introduction of predatory ciliates into the activated sludge did not significantly change the total abundance of protozoa but rebuilt bacterivorous ciliate communities. Introduced predators significantly affected the most numerous ciliate species from the genera *Epistylis* and *Cyclidium*. In the presence of *D. margaritifer*, the abundance of sessile, colonial ciliates (*Epistylis* sp.) was significantly lower compared to the control treatment and to the treatments with the other predators. The activated sludge ciliate community was the most affected by the introduction of the "foreign" predator – *D. margaritifer*, a large ciliate armed with toxicysts.

Keywords: Biodiversity; Ciliate ecology; Food web; Predation

Introduction

Activated sludge in bioreactors of wastewater treatment plants (WWTP) is a particular type of ecosystem exposed to extreme conditions due to the fluctuating composition of influent, the possible toxic inflow and the WWTP operating process. Bacteria, protists, and small metazoans, such as rotifers, tardigrades and nematodes, exist in the activated sludge under strong selective pressure toward high growth rate, good settling properties and resilience to a rapidly changing environment. The species composition of the activated sludge community and the reciprocal interactions of its components affect the effectiveness of sewage purification.

Among protozoa, which constitute approximately 5% of activated sludge dry mass (Curds 1973), ciliates are the most abundant. Some ciliates were considered to be bioindicators of wastewater treatment effectiveness (Madoni 1994). Correlation of certain ciliate species and their abundance

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with effluent quality was broadly described in the literature (Foissner 2016; Lee et al. 2004; Puigagut et al. 2009; Perez-Uz et al. 2010; dos Santos et al. 2014; Zhou et al. 2006). The ciliate species that occur most frequently in activated sludge have also been identified (Amann et al. 1998; Hu et al. 2013; Madoni 2011; Martin-Cereceda et al. 1996; Martín-Cereceda et al. 2001). Ciliates play an important role in reducing the number of dispersed bacteria (Curds 1963) and in reducing excessive sludge production (Ratsak et al. 1996). Additionally, ciliates contribute to the development of aggregates or flocs formation (Arregui et al. 2008; Curds 1963; Watson 1945). As secondary consumers, they affect bacterial activity, growth rate and species composition. The classic paper by Curds et al. (1968) clearly shows that activated sludge consisting solely of bacteria after artificial elimination of protists functions much less effectively than non-modified sludge. In spite of their practical significance, the interdependencies between activated sludge microorganisms were not thoroughly investigated. They are still often treated as a “black box” by WWTP operators, who usually focus on influent and effluent chemical composition without insight into the “cause and effect” relationship. Previous studies raised the problem of predation in activated sludge only in the context of ciliates being the main consumers of bacteria. Curds and Cockburn (1970) noticed that a high density of predatory suctorians in aeration tanks caused an appreciable reduction in bacterivorous ciliates diversity and consequently, worsened the sludge performance in several WWTPs in the UK. Apart from this study, we do not have relevant information about the way in which predation could affect bacterivorous ciliates, and, even more importantly, whether predation can destabilize activated sludge biocenosis and thus lower effluent quality. During our standard microscopic investigations of the sludge, we frequently observed that omnivorous *Holophrya discolor* digested attached *Epistylis* ciliates and *Arcella* testate amoebae. Several times we also noticed that suctorians captured and digested crawling ciliates, such as *Aspidisca* spp. and *Euplotes* spp. Such observations suggest that predatory ciliates may modify bacterivorous species diversity and indirectly influence bacterial communities.

Although the role of predators in food web of freshwater ecosystems is relatively well known (Calbet and Landry 1999; Sherr and Sherr 2002), there is still limited knowledge of their function in activated sludge. Similarly, the knowledge of prey species resistance to predators is more extensive concerning species present in natural freshwater habitats then those inhabiting semi-natural environment of biological reactors. There were reports showing examples of constitutive and inducible defense in prey ciliates inhabiting natural environments (Altwegg et al. 2004; Fyda 1998; Wiąckowski et al. 2004; Fyda et al. 2009), whereas next to nothing is known of resistance of prey species in activated sludge. The only report concerning the subject is that *Aspidisca* ssp. was highly resistant to ciliates armed with toxicysts, such as *Acineria uncinata*, which was observed during monitoring of activated sludge in a newly opened biological WWTP (Pajdak-Stós et al. 2010).

The aim of this research was to determine how predatory ciliates diversified by mouth size and hunting strategy could modify the diversity of activated sludge microfauna. By introducing a “foreign” predator we wanted to check if the resilience of the bacterivorous ciliate community depends on predator “familiarity”. We hypothesized that the ciliate community would respond with specific adaptations to different strategies of predation.

Material and Methods

Isolation, cultivation and determination of predatory ciliates

Three predatory ciliate species with different predatory strategies were used in this experiment. Predatory ciliates have been cultivated for several years in the Institute of Environmental Sciences laboratory. Two of them, *Oxytricha* sp. and *Spathidium spathula*, were isolated from activated sludge samples, whereas *Dileptus margaritifer* was derived from a small natural pond near Kraków. *Oxytricha* sp. is a facultative predatory ciliate (body length: 130–180 μm), and has a large mouth with an adoral zone of membranes (AZM).

The second obligatory predator, *S. spathula* (body length: 60–72 μm), has rather small mouth (size: 18–28 μm) that is armed with numerous toxicysts that can immobilize or paralyze the prey.

D. margaritifer is the largest known obligatory predatory ciliate (body length with trunk up to 1000 μm) with large cytostome (size: approximately 30 μm), which is armed with toxicysts (Dragesco 1962; Kuhlmann et al. 1980). This species rarely occurs in activated sludge, in percolating filters and in rotating biological contactors (Vďačný and Foissner 2012), but we decided to include it in our experiments to determine the effect of a predator foreign to the protist community.

The clones of the predatory ciliates were started from one individual sampled using a Pasteur pipette, and the cultures were maintained in Petri dishes with Żywiec spring water at 15 °C in Sanyo MLR-350 environmental test chambers. *Tetrahymena* sp. cultures were used as food.

Experimental setup

Activated sludge used in the experiment was sampled from domestic WWTP coded as RAD and located in southern Poland near Kraków. RAD is a small treatment plant with a population equivalent of 765, treating only municipal sewage (average day flow 164 m^3). The plant consists of primary clarifier, four aeration tanks of 30.9 m^3 volume each and secondary clarifier.