Science of the Total Environment 571 (2016) 77–81

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short communication

Daphnia as a refuge for an antibiotic resistance gene in an experimental freshwater community

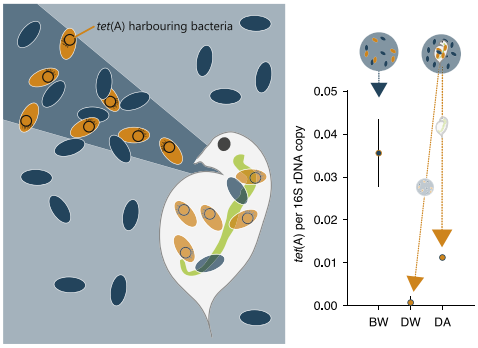
Ester M. Eckert [a], Andrea Di Cesare [a], Birgit Stenzel [a,b], Diego Fontaneto [a], Gianluca Corno [a,*]

[a] Microbial Ecology Group, National Research Council - Institute of Ecosystem Study (CNR-ISE), Largo Tonolli, 50, 28922 Verbania, Italy
[b] Institut für Botanik, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria

HIGHLIGHTS

- The presence of *Daphnia* reduces *tet*(A) abundances in the surrounding water.
- *Daphnia* themselves are, however, carriers of *tet*(A) containing bacteria.
- *Daphnia* contains multiple potential *tet*(A) harbouring bacterial genotypes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 19 July 2016
Accepted 20 July 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Antibiotic resistance
ARGs
Daphnia
Bacterial community
Freshwaters
Tetracycline

ABSTRACT

Mechanisms that enable the maintenance of antibiotic resistance genes in the environment are still greatly unknown. Here we show that the tetracycline resistance gene *tet*(A) is largely removed from the pelagic aquatic bacterial community through filter feeding by *Daphnia obtusa* while it becomes detectable within the microbiome of the daphniids themselves, where it was not present prior to the experiment. We moreover show that a multitude of *Daphnia*-associated bacterial taxa are potential carriers of *tet*(A) and postulated that the biofilm-like structures, where bacteria grow in, may enable horizontal transfer of such genes. This experiment highlights the need to take ecological interactions and a broad range of niches into consideration when studying and discussing the fate of antibiotic resistance genes in nature.

1. Introduction

The contamination of the environment with antibiotic resistance genes (ARGs), prominently through the outflow of wastewater treatment plants, is of increasing concern (Czekalski et al., 2014; Di Cesare et al., 2016). These genes seem to persist in nature, such as in freshwater

* Corresponding author.
E-mail address: g.corno@ise.cnr.it (G. Corno).

http://dx.doi.org/10.1016/j.scitotenv.2016.07.141
0048-9697/

lakes (Di Cesare et al., 2015). The mechanisms that favor the persistence of the various ARGs are still largely unidentified as well as the question to which extent such genes might be transferred back to a clinical setting. In this communication, we intend to draw attention to the potential role of ecological interactions between freshwater bacteria and zooplankton on the persistence of ARGs. More precisely we tested whether the presence of zooplankton may modulate the frequency of ARGs, using the tetracycline resistance gene *tet*(A) as an example, due to the impact of the animal's feeding on the microbial community and the potential attachment of bacteria to the animal's surface and gut.

We designed an experiment where daphniids were added to a Lake Maggiore freshwater bacterial community containing the resistant gene *tet*(A), which is constitutively present within the microbiome of the lake (Di Cesare et al., 2015). Daphniids were chosen as model organisms since they harbor a beneficial and active microbiota, which is composed by both resident and transient bacterial species (Grossart et al., 2010; Eckert and Pernthaler, 2014; Peerakietkhajorn et al., 2015; Sison-Mangus et al., 2015). Moreover, preliminary tests showed that the microbiome of the *Daphnia obtusa* cultures did not harbor *tet*(A) prior to the experiment. Briefly, a grazer-free natural bacterial community was mixed to a 4:1 ratio of natural bacteria to an *E. coli* strain, which was added to easily monitor grazing of daphniids on a specific bacterial gene (*uid*(A)). A single washed daphniid was added to 100 ml of bacterial suspension, in four replicated experiments, whereas the control treatment was composed of triplicated 100 ml bacterial suspension without animals.

2. Results and discussion

After three days of incubation, prokaryotic cell numbers increased by ten and five times in the treatment without and with *D. obtusa*, respectively, with 47% fewer cells counted in the presence of daphniids (Fig. 1). A quantitatively similar reduction was observed for the abundance of *E. coli*, as detected by qPCR of *uid*(A) at the end of the experiment, when they were 43% less abundant in the presence of *D. obtusa* (Fig. S1). Both observations confirm that daphniids actively grazed on bacteria. The relative abundances of *tet*(A) were quantified by qPCR (Table 1) in the water bacterial community with (DW) and without daphniids (BW) and in the daphniids themselves (DA, Fig. 1). In the presence of *D. obtusa* the abundance of *tet*(A) in the aquatic bacterial community were two to three orders of magnitude lower in relative terms (per 16S rDNA gene copy, Fig. 1) and absolute terms (283 ± 148 and 114,614 ± 14,181 copies of *tet*(A) ml^{-1} in DW and BW, respectively).

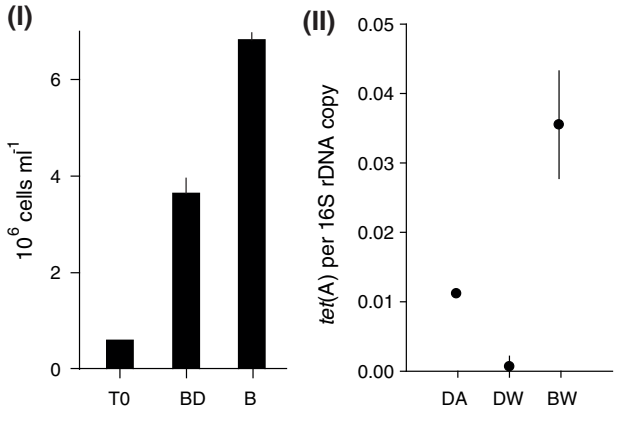


Fig. 1. (I) Bacterial abundance, as determined by SYBR green staining and flow cytometric cell counts; at the beginning of the experiment (T0) and after three days without grazing (B) and with daphniids (BD); the standard deviation of each treatment is reported as a vertical line above the bar. (II) relative abundances (dot = average, vertical line = standard deviations) of the antibiotic resistance gene *tet*(A) as determined by qPCR after 3 days of incubation in the water of the daphniid-free treatment (BW), the water of the daphniid enriched treatment (DW) and the daphniids themselves (DA).

These results indicating that the reduction of *tet*(A) was over-proportional compared to the general reduction in cell numbers and of *uid*(A), meaning that either *tet*(A) containing bacteria grew less in the communities surrounding daphniids than in those without daphniids, or that the animals' grazing selectively reduced *tet*(A)-containing bacteria. However, *tet*(A) could still be detected within the daphniids in similar relative abundance as found in the water of the *Daphnia*-free treatment. Interestingly, *tet*(A) was thus preserved in the bacterial community incorporated into the daphniids through filtration of the water, either because of growth of such bacteria in the animal, or because of horizontal gene transfer of *tet*(A) within the daphniid microbiome where the gene was still detectable after three days.

We analyzed the composition of the bacterial communities attached to the four daphniids used in the experiment by sequencing their 16S rDNA gene, to evaluate whether any bacterial taxa might be a potential carrier of *tet*(A). The daphniid microbiome was composed by a total of 82 OTUs (excluding the *Escherichia/Shigella* sequences, since the added strain and the resident *E. coli* strains could not be distinguished) (Fig. 2). The community was dominated by the orders Burkholderiales, including the genus *Limnohabitans*, and Flavobacteriales, which largely correspond to bacteria commonly associated with various *Daphnia* species (Grossart et al., 2009; Qi et al., 2009; Freese and Schink, 2011; Eckert and Pernthaler, 2014). Comparing the list of genera found on daphniids in this experiment to an updated and curated list of *tet*(A)-harboring bacterial species (Chopra and Roberts, 2001; Roberts, 2015), several of the abundant and rare *Daphnia*-associated bacteria were potential gene-carriers, namely: *Flavobacterium, Pseudomonas, Klabsiella, Citrobacter, Acinetobacter, Serratia* and *Rhizobiom* (Fig. 2 and Table S1).

In this experiment, the presence of *Daphnia* resulted in lower abundances of *tet*(A) in the water; the gene, however, was still found in the microbiome of the animals themselves suggesting a potential persistence or proliferation within the *Daphnia* microbiota (Fig. 1). The *tet*(A) containing bacteria taken up by *D. obtusa* might form part of the flexible microbiota of the daphniids, which is thought to augment the genetic richness of the holobiont (genes of the animal and associated microbes) (Zilber-Rosenberg and Rosenberg, 2008; Shapira, 2016). Additionally, compared to the free-water, resistant bacteria, deriving e.g. from WWTP-effluents that are thus less adapted to the natural conditions in open waters, might have a selective advantage when attaching to a surface, such as an animal's skin or gut, as often observed on biofilm-like structures (Hall-Stoodley et al., 2004). This last observation is confirmed by the low abundances of *tet*(A) containing bacteria in the free-water in the presence of *Daphnia*, and their concomitant survival in or on the animals where ecological conditions are modified by the large nutrients recirculation and the availability of a hard substrate offered by the animal structures. It is noteworthy that the very abundant genus *Flavobacterium*, which contains many opportunistically-growing taxa and potential fish pathogens (e.g. Nematollahi et al., 2003; Neuenschwander et al., 2015), is a potential *tet*(A) carrier. The vicinity of bacteria associated to the animals might allow the natural microbiota of daphniids to horizontally acquire genes from allochthonous bacteria that enter the daphniids through the animal's feeding activity (Allen et al., 2010), and the gut of daphniids might form a hot-spot for horizontal gene transfer, as has been observed in other invertebrates (Dillon and Dillon, 2004). Furthermore, *Daphnia* migrate substantially, which might enable such resistant bacteria to spread to more distant areas (Grossart et al., 2010).

This study suggests that the reservoir or vector function of *Daphnia* might form one of the many so far overlooked distribution opportunity for bacteria related to interactions with other organisms The ample knowledge on *Daphnia* ecology, physiology and genetics (Miner et al., 2012) makes these organisms very promising candidates to study the persistence of specific genes in a food web context. Moreover, daphniids are now part of the non-mammalian model organisms chosen by the US National Institute of Health (National Institutes of Health, 2016), thus research on this organism is of great relevance.