



Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river



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HIGHLIGHTS

- ▶ We studied the effects of antibiotics detected in river waters on biofilm communities.
- ▶ Biofilm switch to more polluted waters evidenced responses of bacteria communities.
- ▶ Bacteria mortality increased and enzymes activity decreased in switched biofilms.
- ▶ Actinobacteria abundance increased in switched biofilms.
- ▶ Biofilm bacteria responses showed significant correlation with antibiotic levels.

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ABSTRACT

Antibiotics are emerging contaminants, which owing to their bioactivity, may lead to short-term and long-term alterations of natural microbial communities in aquatic environment. We investigated the effects of antibiotics on biofilm bacterial communities in the Llobregat River (Northeast Spain). Three sampling sites were selected: two less polluted sites and one hotspot. River water was collected from each site and used both as inoculum and medium for growing biofilms in independent mesocosms. After 25 d of biofilm colonization, we exposed the colonized biofilms to river waters from the downstream sites (progressively contaminated by antibiotics). A control from each site was maintained where the growing biofilm was always exposed to water from the same site. The bacterial community composition, bacterial live/dead ratio and extracellular enzyme activities of the biofilms were measured before and 9 d after exposing the biofilms to increasing contaminated waters. Sixteen antibiotic compounds were detected in the water from the three sampling sites. At each site, the antibiotics present in the highest concentrations were sulfonamides, followed by quinolones and macrolides. Bacterial communities of biofilms grown with the three river waters differed markedly in their structure, but less so in terms of functional descriptors. After switching the medium water to increasing pollution, biofilms exhibited increased levels of actinobacteria (HGC), a trend that was associated to the higher antibiotic concentrations in the water. These biofilms also showed increased bacterial mortality, and decreased extracellular leucine-aminopeptidase and alkaline phosphatase. There was a significant correlation between antibiotic concentrations and biofilm responses. Our results indicate that the continuous entrance of antibiotics in running waters cause significant structural and functional changes in microbial attached communities.

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1. Introduction

Anthropogenic activities are at the base of increasing levels of priority and emerging contaminants, which mostly derive from

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point and diffuse sources that reach freshwater ecosystems. Due to their strong bioactivity, antibiotics (e.g. β -lactams, quinolones, tetracyclines, macrolides, sulfonamides; Kümmerer, 2009a,b) are among the most worrisome emerging classes of pollutants. Given that 30–90% of any dose of most antibiotics administered to humans and animals is excreted as an active substance (Rang et al., 1999), these drugs and their metabolites are commonly found in

aquatic environments (Gros et al., 2007; Managaki et al., 2007; Luo et al., 2011). Hirsch et al. (1998) detected 18 compounds from four classes of antibiotics in German surface water samples, and Watkinson et al. (2009) detected various antibiotics in 90% of freshwater, estuarine and marine samples in six river catchments.

Antibiotics—either as single compounds or in mixtures—can have numerous detrimental effects on aquatic life, including direct toxicity to aquatic microbes, even at low concentrations (Hernando et al., 2006), accelerated acquisition of antibiotic resistance in several bacterial strains, including pathogens (Kümmerer and Henninger, 2003; Obst et al., 2006), and widespread and persistent contamination of water resources, since their mineralization is essentially due to the presence of microorganisms with specific catabolic activities (Brain et al., 2004; Costanzo et al., 2005; Pomati et al., 2006). Antibiotics are bioactive against natural bacterial communities, and their presence may lead to short-term physiological alterations, including cell death and altered metabolic functions (e.g. biomass production, respiration, and excretion of extracellular enzyme activities), as well as to long-term changes in microbial biomass or in community composition (Bonnineau et al., 2010; Tlili et al., 2010).

To date, most studies concerning the effects of antibiotics in aquatic environments have focused on planktonic microbial communities, while disregarding the response of river biofilms. However, microbial attached communities constitute the major component for the uptake, storage and cycling of carbon, nutrients (Pusch et al., 1998; Battin et al., 1999) and anthropogenic contaminants (Sabater et al., 2007) in many river sections. Biofilms contain bacteria and other heterotrophs (e.g. fungi and protozoa), and autotrophs (e.g. diatoms, green algae and cyanobacteria), all of which are embedded in an extracellular polymeric matrix (Lock, 1993). The mutual benefits and the close spatial relationships between organisms with distinct life-strategies closely mirrors the quality of the surrounding flowing waters, generating a complex micro-ecosystem in which specific metabolic processes and interactions

may occur (e.g. use of algal exudates by bacteria, Murray et al., 1986). The major processes of biofilm bacteria include their capability to mineralize organic molecules, a process in which extracellular enzymes (Pusch et al., 1998; Romaní, 2010; Proia et al., 2012a), and microbial interactions within the biofilm (Rier and Stevenson, 2002; Francoeur and Wetzel, 2003; Rier et al., 2007), play major roles. Considering this complexity, the effects of antibiotics on the structure and function of microbial communities in river biofilms remain unknown.

We had two objectives in this study: to investigate any structural and functional modifications of biofilm bacterial communities along an antibiotic pollution gradient in the River Llobregat (Spain); and to evaluate bacterial responses to biofilm translocation in river waters containing different levels of antibiotic contamination. We hypothesized that biofilm communities inhabiting environments with lower levels of antibiotics would be the most affected ones upon translocation to more polluted waters, and that this effect would manifest itself in community composition changes. We tested this hypothesis through translocation experiments performed under controlled conditions. Field translocation has been previously used for assessing the effects of metal pollution and industrial discharge on river biofilms (Ivorra et al., 1999; Victoria and Gómez, 2010; Tlili et al., 2011). In the present study, we analyzed the response of biofilms grown in water from different river sites before and after the exposure to water from sites with higher levels of antibiotics.

2. Materials and methods

2.1. Study site

The Llobregat is a typical Mediterranean river that flows from the Pyrenees to the south of Barcelona (Catalonia, Spain). The Llobregat 165 km long and drains a catchment area of 4948 km² (Marcé et al., in press). Its water flow is characterized by high variability, including periodic flood and drought events (Ricart et al., 2010). The mean annual precipitation in the area is 3330 hm³, and the River has an annual average discharge of 693 hm³ (Ginebreda et al., 2010). Nearly 30% of the discharge flowing in the Llobregat basin is used for drinking water (Muñoz et al., 2009). Together with its two main tributaries, the Cardener and the Anoia Rivers, the Llobregat is a paradigm of overexploited Mediterranean rivers (Muñoz et al., 2009; Marcé et al., in press). The middle-lower part of the river is densely populated and subjected to major industrial, agricultural and urban activities. In fact, its watershed is located in a densely inhabited area (>3 million people) and receives significant inputs of industrial and urban wastewaters (~137 hm³ annually, Ginebreda et al., 2010) as well as surface runoff from agricultural areas (Kuster et al., 2008). Moreover, salt inputs deriving from salt mines in the Cardener watershed cause an increase in water salinity downstream, worsening the poor conditions of the lower part of the river. For the present study, we chose three sampling sites located in the middle-lower part of the Llobregat River (Fig. 1): two of them were upstream of the entrance of the Anoia River (Castellbell, Mina de Terrassa), and another was located downstream of its junction (Sant Joan Despí).

2.2. Experimental design and biofilm translocation

The two upstream sampling sites, Castellbell (A) and Mina de Terrassa (B), were selected as the less polluted sites, and the one downstream, Sant Joan Despí (C), as the pollution hotspot. River water was collected three times weekly from the three sites, and then used as inoculum to grow biofilms in 18 independent mesocosms. Biofilms were colonized on glass slides (1 cm² each) placed

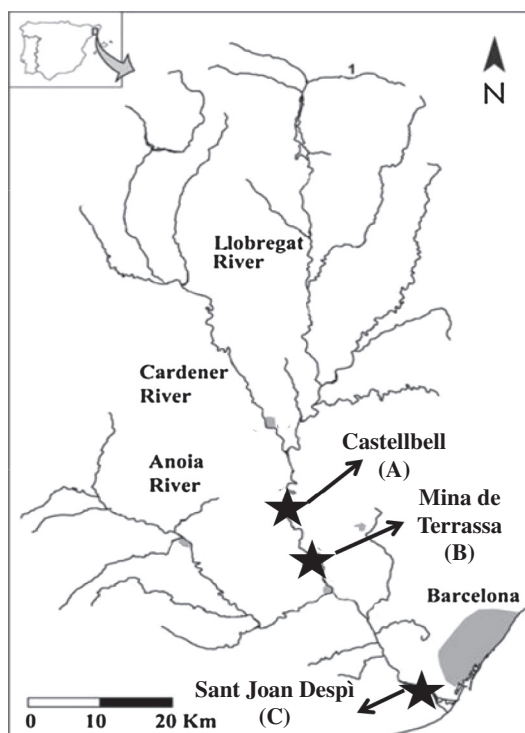


Fig. 1. Study area with selected sampling sites (denoted with stars) in the Llobregat River.

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