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Adaptation of copper community tolerance levels after biofilm transplantation in an urban river

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ABSTRACT

The Water Framework Directive requires the development of biological tools which can act as early-warning indicators of a sudden increase (accidental pollution) or decrease (recovery due to prevention) of the chemical status of aquatic systems. River biofilms, which respond quickly to modifications of environmental parameters and also play a key part in the functioning of aquatic ecosystems, are therefore good candidates to monitor an increase or a decrease of water pollution. In the present study, we investigated the biological response of biofilms transplanted either upstream (recovery) or downstream (deterioration of exposure levels) the urban area of Paris (France). Both modifications of Cu community tolerance levels and of global bacterial and eukaryotic community structure using automated ribosomal intergenic spacer analysis (ARISA) fingerprints were examined 15 and 30 days after the transplantation. Cu tolerance levels of the heterotrophic component of biofilms were assessed using a short-term toxicity test based on β-glucosidase (heterotrophic) activity. Cu tolerance increased for biofilms transplanted upstream to downstream Paris (5-fold increase on day 30) and conversely decreased for biofilms transplanted downstream to upstream (8-fold decrease on day 30). ARISA fingerprints revealed that bacterial and eukaryotic community structures of transplanted biofilms were closer to the structures of biofilms from the transplantation sites (or sites with similar contamination levels) than to biofilms from their sites of origin. Statistical analysis of the data confirmed that the key factor explaining biofilm Cu tolerance levels is the sampling site and not the site of origin. It also showed that Cu tolerance levels are related to the global urban contamination (both metals and nutrients). The study shows that biofilms adapt fast to modifications of their surroundings. In particular, community tolerance varies quickly and reflects the new exposure levels only 15 days after transplantation. Those results support the use of biofilms as reliable early-warning indicators of diffuse urban contamination.

1. Introduction

Over the past decades, human activities in urban areas have contributed to the increase of chemical contamination of aquatic ecosystems (Meybeck, 2003). In particular, urban areas are contaminated by mixtures of chemicals at low, sublethal concentrations (Tusseau-Vuillemin et al., 2007). It is essential to acquire knowledge on the response of aquatic organisms to such chemical contaminations in the field. The Water Framework Directive indeed requires improving both the ecological and the chemical status of aquatic systems before 2015. This consequently means that being able to monitor the biological response of aquatic organisms to a decrease or an increase of pollution levels is a key scientific challenge. It is also important to develop biological tools which can act as early-warning indicators of a deterioration of the chemical status of an aquatic ecosystem.

River biofilms are complex communities of both autotrophic and heterotrophic micro-organisms that develop in a matrix of exopolymers on submerged substrates such as pebbles, wood or sediment. They are typically composed of bacteria, micro-algae, fungi and protozoa. Because they are prompt to respond both structurally (for instance by changes in species composition) and functionally (for instance by changes in photosynthetic activity) to environmental disturbances, they are considered as early-warning indicators of chemical pollution (Sabater et al., 2007). Indeed, the short generation time of biofilm micro-organisms allows a rapid

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response to changing conditions. Moreover, biofilms play an important role in the functioning of aquatic ecosystems as primary producers (autotrophic communities) and also by taking part in the recycling of organic matter (heterotrophic communities). Biofilms are also an important source of food for a range of micro and macro benthic invertebrates and some fish (Burns and Ryder, 2001). Therefore, any modification in biofilms' structures and functioning can further alter the rest of the aquatic ecosystems.

Field transfer of periphytic communities between sites with different contamination levels appears as an efficient way to assess *in situ* effects of chemical contamination. Several authors have investigated changes in diatom species composition (Gold et al., 2002; Morin et al., 2010; Rimet et al., 2005; Rotter et al., 2011), algal community structure (Dorigo et al., 2010a) and algal (Dorigo et al., 2010a; Rotter et al., 2011) or heterotrophic community tolerance (Tlili et al., 2011) after transplantation between polluted and uncontaminated sites. Yet information on the responses of natural biofilms to sudden stress or stress removal, for instance after transplantation, is still scarce (Dorigo et al., 2010b; Rotter et al., 2011), although it might help better understand the link between toxic pressure and biological responses at the community level.

Moreover, little information is usually available on the response of the heterotrophic component of biofilms in case of transplantation or sudden modification of water contamination. Yet aquatic heterotrophic communities might be good indicators of chemical contamination. For instance, Paule et al. (2009) have observed that the bacterial community structure of biofilms was clearly modified by transplantation showing that site characteristics condition the structure of microbial communities. Therefore, heterotrophic communities are likely to respond fast to changes of exposure parameters. However, most studies on biofilms have focused on photosynthetic micro-organisms, although in the last 10 years some studies have included data on bacterial communities (Burns and Ryder, 2001; Dorigo et al., 2010b; Tlili et al., 2011).

Exposure of biofilms to a toxicant leads to the disappearance of sensitive components (species, genotypes and phenotypes) which are gradually replaced by more tolerant ones (Blanck, 2002). These selection, adaptation and acclimatation processes are at the basis of the pollution-induced community tolerance (PICT) approach as they lead to an increase of the global community tolerance (Blanck et al., 1988). As a community approach, PICT provides relevant ecological information regarding biological responses to chemical contaminations. Indeed, effects measured at the community level include interactions between assemblages of various populations and integrate variations in terms of sensitivity between community organisms (McClellan et al., 2008).

Community tolerance is assessed by the use of short-term toxicity tests based on the inhibition of physiological activities (typically photosynthesis to evaluate the tolerance of autotrophic communities as discussed by Bérard et al. (2002)). A new short-term toxicity test has recently been developed using β-glucosidase activity as an endpoint (Fechner et al., 2010a). It proved to be efficient to detect tolerance acquisition of the heterotrophic component of metal-exposed periphyton (Fechner et al., 2011b). Tlili et al. (2010) also used β-glucosidase activity to study pesticide-tolerance acquisition of biofilms exposed in microcosms.

The aim of this study was to evaluate the dynamics of metal tolerance of biofilms transplanted either upstream or downstream an urban area (Paris, North of France). The potential to either recover from (communities transplanted upstream) or adapt to (communities transplanted downstream) urban stress was investigated both in terms of heterotrophic community tolerance (using β-glucosidase activity) and global community structure. Copper was chosen as one of the major metals present in the mixture of contaminants in the river (Fechner et al., 2011a). Biofilms transplanted upstream to downstream the urban area were expected to acquire tolerance to copper and inversely, communities transplanted downstream to upstream were expected to either maintain or lose their relatively high tolerance levels. Automated ribosomal intergenic spacer analysis (ARISA) fingerprinting was chosen to visualize similarities between bacterial and eukaryotic community structures of both transplanted and original biofilms.

2. Materials and methods

2.1. Sampling sites and water chemistry

Periphytic communities were collected (and/or transplanted see below in Section 2.2) on three sites located in the Seine river basin (North of France): site A (Marnay), which is located upstream from the urban area of Paris in the Seine river, site B1 (Saint-Maurice) which is located in the urban area of Paris, upstream from Paris in the Marne river and approximately 170 km downstream from site A, and site B2 (Triel) which is located in the Seine river downstream from Paris and the sewer treatment plant Seine-Aval (which receives most of Paris wastewater, nominal capacity 8×10^6 inhabitants) and 90 km downstream from site B1. The urban area of Paris is characterized by intense human activity which explains the diffuse urban contamination gradient (increase of metals, nutrients and organic pollutants) upstream to downstream the Seine river basin (Priadi et al., 2011). Physico-chemical parameters of the river water (concentrations of HCO_3^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, NH_4^+, NO_2^-, PO_4^{3-}, NO_3^- and SiO_2) were measured on days 0, 15 and 30 at sites A, B1 and B2 (August 2009). Mean total and dissolved metal concentrations (Cu, Cd, Ni, Zn, Pb, Cr, Co Mn) were obtained with several individual samplings over the period corresponding to the transplantation experiment (samplings on 22nd July, 19th August and 29th September 2009) at sites A and B2. DGT-labile metal concentrations were measured after a one-month immersion of three DGT devices at the sampling sites. As regards site B1, metal concentrations (total, dissolved and labile) were measured before (21st January 2009) and after (22nd September 2009) the experiment: a mean value of each metal concentration was thus used to characterize metal contamination at site B1.

2.2. Biofilm transplantation and sampling

Biofilms were collected on low density polyethylene (LDPE) plastic membranes (30 cm × 10 cm) that were vertically attached to plastic crates as in Fechner et al. (2010a). For transplantation, communities were collected at sites B1 and A (after 23 and 34 days of colonization respectively). At the beginning of the experiment (day 0), 5 colonized-LDPE membranes were collected at site A, immersed in a clean plastic container (previously washed with 10% HNO_3) filled with river water and transported in a cooler, and were taken to site B2 where they were attached to another plastic crate and immersed in the river (Fig. 1). Inversely, 5 biofilm-colonized LDPE membranes were collected at site B1 and transported to site A (day 1). Five other biofilm-colonized membranes were left at both sites. Transplanted communities will thereafter be called TA (transplanted from A to B2), and TB1 (transplanted from B1 to A); communities collected on sites A and B1 will be called A and B1. After 15 and then 30 days of transplantation (days 15 and 30), biofilms were collected at sites A, B1 and B2 (both transplanted and original communities). After collection, plastic membranes were transported back to the laboratory in 250 mL glass-bottles placed in a cooler within the day. They were then hand-scraped to remove periphyton and make biofilm suspensions in Montdore (Grand Barbier, Mont-Dore, France) mineral water (Fechner et al., 2010a). Aliquots of the suspensions were then assigned to various analyses for periphyton characterization or tolerance measurements.