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Triclosan causes toxic effects to algae in marine biofilms, but does not inhibit the metabolic activity of marine biofilm bacteria

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ABSTRACT

Effects of the antimicrobial agent triclosan to natural periphyton communities (biofilms, comprising primarily microalgae and bacteria) were assessed in two independent experiments during spring and summer. For that purpose a semi-static test system was used in which periphyton was exposed to a concentration range of 5–9054 nmol/L triclosan. Effects on algae were analyzed as content and composition of photosynthetic pigments. The corresponding EC50 values were 39.25 and 302.45 nmol/L for the spring and summer experiment, respectively. Effects on periphytic bacteria were assessed as effects on carbon utilization patterns, using Biolog Ecoplates. No inhibition of either total carbon utilization or functional diversity was observed, indicating a pronounced triclosan tolerance of the marine bacteria. In contrast, a small stimulation of the total carbon utilization was observed at triclosan concentrations exceeding 100 nmol/L.

1. Introduction

The antimicrobial agent triclosan (for molecular structure and physico-chemical characteristics, see Table 1 in the Electronic Supplementary Material) is widely used in various consumer products such as toothpastes and soaps, antiseptic cosmetics and toys (Bedoux et al., 2012). Consequently, triclosan is routinely detected in STP effluents, receiving waters and sediments. Typical surface water concentrations range between < 0.001–0.98, 0.012–7.94 and 0.0021–3.53 nmol/L in Europe, North America and Asia respectively (Lyndall et al., 2010; Bedoux et al., 2012). Triclosan concentrations in the marine environment have recently been reviewed by Bedoux et al., who compiled concentrations in the marine environment of up to 0.024, 0.047 and 0.1 nmol/L in European, North American and Asian marine waters respectively (Bedoux et al., 2012).

Triclosan is a broad-spectrum antimicrobial agent, effective against both gram-negative and gram-positive bacteria (Bedoux et al., 2012). Several mechanisms of action have been suggested for triclosan toxicity. It has been demonstrated that triclosan blocks the active site of enoyl-acyl carrier protein reductase (FabI) in bacteria and hence specifically inhibits fatty acid synthesis (Levy et al., 1999; McMurry et al., 1998). Triclosan has also been shown to destabilize membranes (Lygre et al., 2003; Villalaín et al., 2001) and Franz et al. (2008) observed indications that triclosan exposure led to uncoupling of oxidative phosphorylation in microalgae, which previously has also been described for rat liver mitochondria (Newton et al., 2005).

Several authors have studied the acute toxicity on the marine bacterium *Vibrio fischeri* with EC50 values ranging between 183.05 and 1795.95 nmol/L, as reviewed by Bedoux et al. (2012). Chronic studies with freshwater microbial communities revealed higher toxicity values with a LOEC value of 10 nmol/L (Johnson et al., 2009). However, microalgae have been shown to be at least as sensitive as bacteria, with chronic EC50 values ranging from 1.8 to 15 nmol/L for green algae and cyanobacteria and between 65.97 and 1 347 nmol/L for diatoms (Bedoux et al., 2012; Yang et al., 2008).

The aim of this study was to assess the chronic toxicity of triclosan to the algae and bacteria residing in natural marine periphytic biofilms.

Periphyton are biofilm communities that cover submerged surfaces in the aquatic environment. They consist of a variety of autotrophic and heterotrophic species and are responsible for important ecological processes such as primary production and nutrient cycling (Azim et al., 2005). As periphytic organisms grow in a closely confined space in the biofilm, they compete for nutrients, space and light and any change in ecological fitness as a result of an exposure to toxic compounds is likely to not only change the

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overall physiological activity of the biofilm species, but also affect community biodiversity. Communities exposed to toxicants are dominated by more tolerant species (Blanck, 2002).

2. Material and methods

We studied effects on microalgal and bacterial biofilms (periphyton) using the semi-static SWIFT periphyton test (Porsbring et al., 2007) as described in Johansson (2014).Two independent experiments were carried out between 24th of April and 3rd of June 2010. The sampling was performed in the shallow bay (3 m water depth) of Kalvhagefjorden (long 11.4, lat 58.23) near the outermost part of the Gullmar fjord on the Swedish west coast. The site was first described by (Blanck and Dahl, 1996) as a bay with low chemical exposure located in a very sparsely populated area. Hence it can be safely assumed that the microbial communities have not been exposed to triclosan in the past. Previous studies have reported triclosan concentrations below detection limit at reference sites south of our sampling area (Remberger et al., 2002).

Biofilms were established on glass slides in the environment and then transferred to the lab where they were exposed to a concentration series (5–9054 nmol/L) of triclosan. Algal and bacterial members of the periphyton where hence exposed simultaneously to exactly identical triclosan concentrations. The exposure time was 72 and 96 h for determining effects on periphytic bacteria and algae, respectively.

Effects on bacteria were assessed using Biolog Ecoplates™ (purchased from Dorte Egelund ApS, Roskilde, Denmark). These 96-well plates, pre-loaded with 31 different carbon-sources and a tetrazolium dye, provide information on functional diversity and total metabolic activity of the bacteria growing in them. Optical densities were measured over 96 h at 595 nm (absorbance of the oxidized tetrazolium dye) and 700 nm (in order to correct for turbidity). Total content and relative fractions of photosynthetic pigments were used as a measure of algal biomass and community structure (Porsbring et al., 2007). For further details see Johansson (2014).

Triclosan (⩾97%, Sigma–Aldrich Sweden AB, Stockholm Sweden) was dissolved in methanol (Lichrosolv purity, VWR international AB, Göteborg, Sweden) to a final concentration of 9 mmol/L. This stock solution was then diluted in methanol, a dilution factor of 2.3, resulting in one stock solution for each final test solution. To prepare test solutions, 200 μL of each stock solution was pipetted into a 250 mL Pyrex flask and the methanol was let to evaporate before 200 mL nutrient amended GF/F filtered sea water (0.7 mmol/L PO_4^{2-} and 8 mmol/L NO^{3-}), which had been collected the day before the start of the experiment at the periphyton sampling site, was added. New test solutions were prepared daily, followed by vigorous shaking at 4 °C in the dark for at least 12 h prior to use.

A concentration range of 5–9054 nmol/L final concentration was used in the present experiment, which was based on previous rangefinding experiments and was tailored toward describing the full concentration–response curve for effects on algae, the more sensitive organism group, see below. 10 concentrations were tested in total and every second concentration up to 1000 nmol/L was tested in triplicate (11, 26, 60, 322 nmol/L was only tested once), while higher concentrations (1 700, 3 900 and 9 054 nmol/L) were only tested once.

2.1. Data analysis – Biolog Ecoplates

The optical densities measured for each Biolog Ecoplate were analyzed in accordance with Johansson (2014). Here we only report on the background corrected average carbon utilization (AWC), as no significantly toxic effects on bacteria were observed (see below).

2.2. Data analysis – pigment composition

Effects on pigment content were expressed as percent inhibition compared to the arithmetic mean of the untreated controls.

In addition to investigating changes on total pigment content, we performed nonlinear nonmetric multidimensional scaling (nMDS) with all the individual pigments detected in each experiment. nMDS is an ordination method that condenses a multidimensional data structure into a 2-dimensional plot. The distance between two points in an nMDS plot reflects the multivariate dissimilarity between those samples (Clarke, 1999). This was performed using Manhattan Distance for describing the dissimilarity between pairs of samples.

3. Results and discussion

Triclosan effects on the heterotrophic and the phototrophic part of biofilm communities were investigated in two independent experiments during spring and summer 2010. Effects on the algal part of the periphyton communities will be discussed first, followed by an analysis of the effects on bacteria.

The tested concentration range (5–9 054 nmol/L) describes the full concentration response curve for periphytic algae in both experiments (Fig. 1 and Table 1). The algae were more sensitive toward triclosan in the spring experiment (EC50 of 39.25 nmol/L) compared to the summer experiment (EC50 of 302.45 nmol/L). Due to the well-known shortcomings of classic NOEC determinations (e.g. Warne and van Dam, 2008), we instead used the lower 95% confidence belt of the EC10 as an estimate for the first toxic effects, which were observed at 10.81 and 32.74 nmol/L in the spring and summer, respectively (Table 1). The difference in sensitivity between spring and summer periphyton is most likely caused be different thicknesses of the biofilms of both experiments, indicated by the 60% higher chl a content of the summer periphyton, as well as a 20% higher catabolic activity of the bacteria isolated from the summer periphyton. A thicker biofilm would lead to a decreased exposure of the individual algae that are embedded in the biofilms as well as a possibility for enhanced bacterial degradation.

Additionally, the relative pigment composition also indicates that the species composition of the spring and summer periphyton was slightly different. Fig. 2 shows that the summer communities contained slightly elevated relative amounts of fucoxanthin, diadinoxanthin and diatoxanthin, which are abundant pigments in

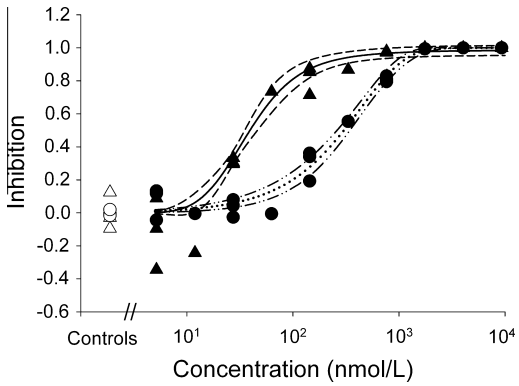

Fig. 1. Total pigment content after 96 h exposure to triclosan in spring (Δ) and summer (○), filled symbols represent triclosan treatments, open symbols the controls. Lines give the corresponding Weibull fits.