Ecotoxicology and Environmental Safety 148 (2018) 228–236

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effects of temperature, genetic variation and species competition on the sensitivity of algae populations to the antibiotic enrofloxacin

Andreu Rico[a,*], Wenkai Zhao[b], Frits Gillissen[b], Miquel Lürling[b], Paul J. Van den Brink[b,c]

[a] IMDEA Water Institute, Science and Technology Campus of the University of Alcalá, Avenida Punto Com 2, P.O. Box 28805, Alcalá de Henares, Madrid, Spain
[b] Aquatic Ecology and Water Quality Management group, Wageningen University, P.O. Box 47, 6700 AA, Wageningen, The Netherlands
[c] Wageningen Environmental Research (Alterra), P.O. Box 47, 6700 AA, Wageningen, The Netherlands

ARTICLE INFO

Keywords:
Antibiotics
Green algae
Cyanobacteria
Temperature-dependent sensitivity
Species competition

ABSTRACT

Primary producers are amongst the most sensitive organisms to antibiotic pollution in aquatic ecosystems. To date, there is little information on how different environmental conditions may affect their sensitivity to antibiotics. In this study we assessed how temperature, genetic variation and species competition may affect the sensitivity of the cyanobacterium *Microcystis aeruginosa* and the green-algae *Scenedesmus obliquus* to the antibiotic enrofloxacin. First, we performed single-species tests to assess the toxicity of enrofloxacin under different temperature conditions (20 °C and 30 °C) and to assess the sensitivity of different species strains using a standard temperature (20 °C). Next, we investigated how enrofloxacin contamination may affect the competition between *M. aeruginosa* and *S. obliquus*. A competition experiment was performed following a full factorial design with different competition treatments, defined as density ratios (i.e. initial bio-volume of 25/75%, 10/90% and 1/99% of *S. obliquus/M. aeruginosa*, respectively), one 100% *S. obliquus* treatment and one 100% *M. aeruginosa* treatment, and four different enrofloxacin concentrations (i.e. control, 0.01, 0.05 and 0.10 mg/L). Growth inhibition based on cell number, bio-volume, chlorophyll-a concentration as well as photosynthetic activity were used as evaluation endpoints in the single-species tests, while growth inhibition based on measured chlorophyll-a was primarily used in the competition experiment. *M. aeruginosa* photosynthetic activity was found to be the most sensitive endpoint to enrofloxacin (EC50–72 h = 0.02 mg/L), followed by growth inhibition based on cell number. *S. obliquus* was found to be slightly more sensitive at 20 °C than at 30 °C (EC50–72 h cell number growth inhibition of 38 and 41 mg/L, respectively), whereas an opposite trend was observed for *M. aeruginosa* (0.047 and 0.037 mg/L, respectively). Differences in EC50–72 h values between algal strains of the same species were within a factor of two. The competition experiment showed that *M. aeruginosa* growth can be significantly reduced in the presence of *S. obliquus* at a density ratio of 75/25% *M. aeruginosa/S. obliquus*, showing a higher susceptibility to enrofloxacin than in the single-species test. The results of this study confirm the high sensitivity of cyanobacteria to antibiotics and show that temperature and inter-strain genetic variation may have a limited influence on their response to them. The results of the competition experiment suggest that the structure of primary producer communities can be affected, at least temporarily, at antibiotic concentrations close to those that have been measured in the environment.

1. Introduction

The main sources of antibiotics in the environment are the anthropogenic input from human health care products and medicines, and from agriculture and aquaculture production (Kümmerer, 2009). Since the conventional sewage treatment facilities were never designed to deal with pharmaceuticals and their removal efficiency vary substantially, antibiotic residues have been detected in sewage treatment effluents and downstream areas (Michael et al., 2013; Carvalho and Santos, 2016). Residues from agriculture and aquaculture usually do not undergo any treatment before they are released into the environment. In agriculture, antibiotics are released to soils during the application of sludge or manure fertilisers. Antibiotics may afterwards be leached out or transported with runoff water, contaminating the aquatic environment (Sarmah et al., 2006). In aquaculture, antibiotics can directly enter to the aquatic environment by dissolution from feed pellets or by excretion from the cultured organisms, resulting in high local concentrations in the water column and in adjacent sediments (Rico et al., 2014b; Andrieu et al., 2015). Due to these contamination routes, antibiotics occur in aquatic ecosystems in concentrations

* Corresponding author.
E-mail address: andreu.rico@imdea.org (A. Rico).

http://dx.doi.org/10.1016/j.ecoenv.2017.10.010
Received 18 July 2017; Received in revised form 25 September 2017; Accepted 3 October 2017

Table 1
Algae strains, temperature and enrofloxacin concentrations used in the single-species toxicity tests.

Species	Strains	Strain source	Temperature (°C)	Enrofloxacin concentrations (mg/L)
Scenedesmus obliquus	SAG 276/3a	University of Göttingen (Germany)	20 and 30	0, 24, 48, 80, 96, 120
	UTEX 78	The University of Texas at Austin (USA)	20	
	MPI	Max Planck Institute (Plön, Germany)	20	
Microcystis aeruginosa	PCC 7820	Pasteur Institute (Paris)	20 and 30	0, 0.005, 0.01, 0.05, 0.1, 0.2
	SAG 1785	University of Göttingen (Germany)	20	
	V131	Institute for Biodiversity and Ecosystem Dynamics (University of Amsterdam, the Netherlands)	20	

ranging from ng/L to few µg/L (e.g. Kümmerer, 2009; Bu et al., 2013; Carvalho and Santos, 2016).

Cyanobacteria have been demonstrated to be amongst the most sensitive aquatic organisms to antibiotics due to their close physiological characteristics to the target bacteria for which antibiotics have been designed (Van der Grinten et al., 2010). Therefore, antibiotic pollutants in aquatic ecosystems may become an important factor regulating the growth of cyanobacteria, the release of cyanotoxins and the structural composition of the microbial communities. Supporting this, Halling-Sørensen (2000) reported the growth inhibition of five antibiotics on the cyanobacterium *Microcystis aeruginosa* at concentrations that may occur in the environment; while Crane et al. (2006) compared the sensitivity to antibiotics among various algal species and found that cyanobacteria, including *M. aeruginosa*, are more sensitive than other primary producer species.

Ando et al. (2007) investigated the impact of seven antibiotic agents on the growth of eight cyanobacteria species, and suggested to utilise cyanobacteria in ecotoxicity testing because it would enable an easy and highly sensitive assessment of the toxicity of chemicals with antimicrobial mode of action. The relevance of cyanobacteria for ecosystem functioning and its importance as a component of primary producer communities is, however, unknown, especially in environments where primary producers are competing over resources. Together with chlorophytes, cyanobacteria are used as standard test organisms in the risk assessment of antibiotics. A safety factor is applied to the toxicity values obtained from standard laboratory experiments to derive threshold concentrations protective to primary producers under different environmental conditions and to protect the structure of primary producer communities (EMA, 2006a; VICH, 2004). However, the adequacy of these safety factors to properly cover sensitivity differences related to varying environmental conditions (e.g. temperature), genetic variation of populations and species interactions has not been properly evaluated.

The objectives of this study were (1) to evaluate the influence of temperature and strain-based genetic variation on the sensitivity of microalgae to antibiotics, (2) to test whether antibiotic contamination may influence the sensitivity of microalgae populations under different interspecific competition conditions, and (3) to assess whether current safety factors used in antibiotic risk assessment are protective to the sensitivity variations observed in this study. For such purpose several experiments were performed using the cyanobacterium *M. aeruginosa* and the green algae species *Scenedesmus obliquus*. These two species were selected since they are known to occur and coexist in a large number of aquatic ecosystems (Trainor, 1998; Harke et al., 2016) and because they can be successfully cultured in the same laboratory medium (Lürling and Roessink, 2006). The experiments were performed using the antibiotic enrofloxacin (ENR) as model compound. ENR is an antibiotic with widespread use in livestock as well as in aquaculture production (EMA, 2006b; Rico et al., 2013). It belongs to the fluoroquinolone group and acts by inhibiting the DNA gyrase in gram-positive as well as in gram-negative bacteria (Madigan et al., 2003). It shows a relatively high affinity for organic matter and a moderate persistence in aquatic systems (Knapp et al., 2005), and has been monitored in a wide range of surface water environments reaching concentrations up to some µg/L (Heberer, 2002; Rico et al., 2014b).

2. Materials and methods

2.1. Test substance

The commercial product EnFlocin (enrofloxacin-HCl), which is typically used as veterinary medicine and contains 20% of ENR as active ingredient, was used in this study. Stock solutions were prepared in Milli-Q water and preserved at 4 °C until their use in the laboratory experiments.

2.2. Test organisms

We investigated the sensitivity of three different strains of the green algae *S. obliquus* and three strains of the cyanobacterium *M. aeruginosa*. The strains originated from different culture collections (see Table 1). All strains of *S. obliquus* have been subjected to DNA sequencing (SAG 276-3a – GenBank AJ249508.1; UTEX 78 - AF204057.1; MPI: AJ249509.1). Likewise, sequences are available for *M. aeruginosa* SAG 1785 (KM019998.1) and PCC 7820 (DQ786006.1), but not for V131.

Stock cultures were grown in cellulose plug stoppered 300 mL Erlenmeyer flasks containing 150 mL of modified WC (Woods Hole modified CHU10)-medium (Lürling and Beekman, 1999; Supplemental Material Table S1). Stock cultures were maintained in an Orbital Incubator (Sanyo MLR-351H) using a 16:8 h light-dark regime, and a light intensity of 80 µmol quanta $m^{-2}s^{-1}$ and 16 µmol quanta $m^{-2}s^{-1}$ in the light and dark periods, respectively. The Orbital Incubator was set at a velocity of 60 rpm, and all flasks were manually shaken every day. The medium was refreshed every half a month in order to keep the algae populations in the exponential growth phase.

2.3. Single-species toxicity tests

In order to investigate the influence of water temperature on the sensitivity of *S. obliquus* and *M. aeruginosa* to ENR, two different toxicity tests were set-up at 20 °C and 30 °C, using the SAG 276/3a and the PCC 7820 strains, respectively. The toxicity of ENR to the other algae strains used in this study was only investigated at 20 °C, therefore, the sensitivity comparison among strains was performed with the data obtained from the tests performed at 20 °C (Table 1). All toxicity tests were conducted following the recommendations provided by the OECD standard protocol for testing the effects of chemicals on freshwater algae (OECD, 2006). The toxicity tests were set-up in triplicate ($n=3$) with five ENR concentrations and one uncontaminated control (Table 1). The tests units consisted of 100 mL cellulose plug stoppered Erlenmeyer flasks containing 50 mL of modified WC-medium and were maintained using the same light and shaking conditions as described above for the stock cultures. Prior to the start of the experiments, the test flasks were filled with modified WC-medium and autoclaved for 20 min at 120 °C for sterilization. Next, all flasks were transferred into a laminar flow in which vitamins, ENR and algae were added. The initial density of *S. obliquus* and *M. aeruginosa* were set at $2{\cdot}10^5$ cells/mL and $7{\cdot}10^5$ cells/mL, respectively.