OMNI Life Science

Application Note

Toxic Effects of the Antibiotic Ciprofloxacin on Algae Cell Growth

Growth inhibition test with the unicellular green algae R. subcapitata according to OECD guideline 201 using CASY

Sonja Faetsch, Marianne Matzke and Stefan Stolte

Center for Environmental Research and Sustainable Technology UFT, Department Sustainable Chemistry, University of Bremen, Germany

Introduction

Growth assays with unicellular green algae are established tools in ecotoxicology for determining phytotoxic effects of chemical pollutants, such as pesticides, industrial chemicals or pharmaceuticals (1). This study was carried out according to OECD guideline 201 (6) to determine the toxicity of the widely used antibiotic ciprofloxacin (CPX) to the non-target species *Raphidocelis subcapitata*.

The antibiotic ciprofloxacin belongs to the group of fluoroquinolones that inhibit the key bacterial enzyme DNA gyrases, involved in DNA replication and transcription (3, 7). Ciprofloxacin can also have adverse effects on off-site targets such as algal mitochondria and chloroplasts, since they require gyrase activity as well. Fulfilling many essential functions in algae cells, impairment of these organelles results in cell growth inhibition and, ultimately, cell death (4, 7, 8, 9). Inhibition of growth was detected by comparing growth of a series of algae cultures exposed to increasing concentrations of ciprofloxacin with an untreated control culture using CASY.

Methods

Algae Cells

The algae R. subcapitata (SAG strain 61.81) were grown as described in Application Note "Monitoring the Cell Cycle of the Unicellular Green Algae Raphidocelis subcapitata" (OLS) (2).

***Growth inhibition assays with ciprofloxacin (CPX)** were conducted under the same conditions as cultivation. Eight concentrations of ciprofloxacin (1.5 to 5 mg/l) were added to initial cell densities of 3 x10^4 cells /ml. Potassium dichromate was used as reference substance (EC_{50} of 0.9 mg/l). After 72 hours, algae concentrations were measured with CASY using dilution factors of 6 to 201, depending on the algae cell density.*

Data from two independently conducted experiments, each carried out in triplicates, were pooled for data analysis. Inhibition of growth was calculated as the logarithmic increase in cell numbers, over the test duration of 72 hours (according to OECD 201). The EC_{50} (Effective Concentration of test substance that caused a growth inhibition of 50%) was calculated by non-linear regression with GraphPad PRISM, version 5.03

CASY Analysis

All samples were analyzed with CASY (60 µm Capillary; 3x200 µl; 0-20 µm; sample volume 50 µl-2ml) for cell number, cell size and total cell volume. Graphs of the CASY measurements were created with CASYworX 1.2 software (OLS).

Results

Adverse effects on non-target organism

It has been shown recently that the antibiotic ciprofloxacin has ecotoxicological effects on aquatic organisms (5). Here, we demonstrate the application of CASY cell analyzer for toxicity assays of aquatic organisms.

Algae cultures affected by ciprofloxacin

Eight concentrations of ciprofloxacin in the range from 1.5 to 5 mg/l were tested. Algal cell numbers were measured with the CASY system (Fig.1, Table 1) as counts/ml at suitable dilutions. Ciprofloxacin clearly inhibited the growth of the algal cultures and also had an impact on the cell sizes. Cell numbers decreased with increasing concentration of ciprofloxacin (Fig. 2). The EC_{50}, the concentration that inhibits growth by 50%, was determined 3.5 mg/l.

Title image: *Photomicrograph of R. subcapitata by the author*

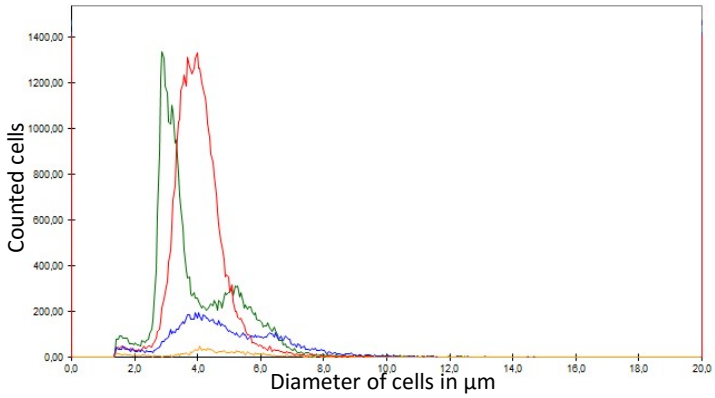

Fig. 1: **Algal cell numbers* and cell sizes affected by ciprofloxacin.**
Results of four out of eight measured concentrations are shown. **Green:** The control without ciprofloxacin shows highest number and smallest cell size. The cell division was almost completed with a majority of autospores (3µm) released from mother cells (approx. 6 µm). The peak shoulder between 4.5 and 5 µm represents mother cells that have not released all four of them yet.
Increasing concentrations of ciprofloxacin:
Red: Algae were incubated with 3.5mg/l, resulting in 41% inhibition of growth. **Blue**: at 4.5mg/l, the inhibition is already about 75% and at 5mg/l (**yellow**) cells were difficult to detect an inhibition at about 100%.
Interestingly, CASY revealed that a majority of the treated cells have a diameter of about 4µm which may indicate a delayed cell division.
*: The figure shows cell counts as determined with CASY, based on varying dilutions of algal samples, as indicated in table 1.

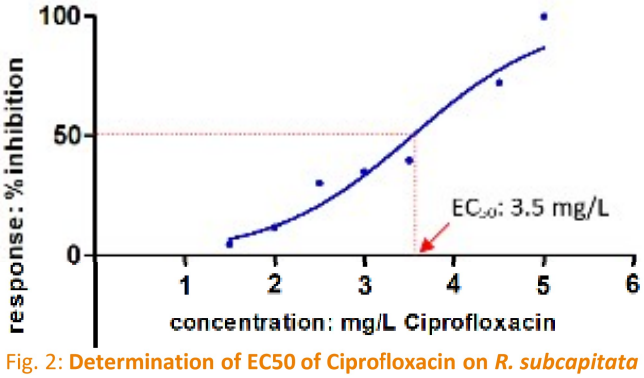

Fig. 2: **Determination of EC50 of Ciprofloxacin on *R. subcapitata***
The effective concentration that inhibits growth by 50% was calculated to be 3.5mg/l.

Summary

Algae cultures growth pattern and Inhibition

CASY is a fast, reproducible and uncomplicated system for simultaneously determining **cell numbers** (counts/ml), **biovolume** (volume/ml) as well as the mean and average **cell size** and **volume distributions** of algae cultures. This allows for a comprehensive analysis of growth inhibiting effects of chemical pollutants in ecotoxicity testing, with a more differentiated interpretation of test results and conclusions on a chemical's mode of action.

Ciprofloxacin affected the cell cycle

In the absence of Ciprofloxacin, the cells had grown and were already in the middle of the cell division process (Fig. 1, green). Hence, the concentration had reached $6.0x10^6$ cells/ml. In the presence of 3.5mg/l of the antibiotic, the concentration was only $8.2x10^5$ cells/ml (Fig. 1, red). Additionally, the sizes of the treated cells were bigger than the control, suggesting that ciprofloxacin had affected the speed of cell growth and consequently mother cells had not reached the size of 6 µm where they start cell division.

Table 1. **Growth Inhibition of Algal Samples through Ciprofloxacin (CPX)**

Sample (Fig.1)	CPX (mg/l)	counted cells (in 600µl)	Dilution factor	Cells/ml	Growth inhibition (%)
green	control	35922	101	6.046.805	-
red	3.5	44466	11	815.216	41
blue	4.5	12851	6	128.514	75
yellow	5	1985	6	19.848	100

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Contact

Sonja Faetsch
Department of Applied Aquatic Toxicology
Hamburg University of Applied Sciences
email: sonja.faetsch@haw-hamburg.de