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Bacterial degradation of the veterinary antibiotic florfenicol

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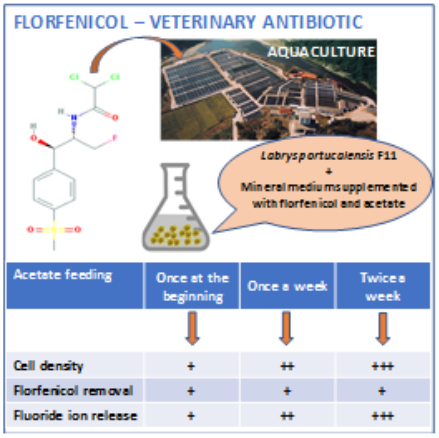

Florfenicol (FF) is an antibiotic largely used in aquaculture and its presence in their aqueous streams can affect the treatment process. Bacteria can play an important role in the cleanup of contaminated sites due to their ability to degrade an impressive variety of pollutants, using them as carbon and energy source. This study aimed to investigate the degradation of FF by a single bacterial strain, *Labrys portucalensis* F11. Biodegradation of FF was assessed in batch mode in the presence of a conventional carbon source and the bacterium was capable to remove ca. 80% of the FF supplied. When acetate was periodically added, although the cell growth was improved, no effect on the uptake of FF was observed but its defluorination was greatly enhanced.

Introduction

Florfenicol (FF) is a synthetic phenicol antibiotic, widely used in veterinary medicine. It is one of the few approved antibiotics for use in aquaculture during both production and processing operations, mainly to prevent and treat bacterial diseases [1,2]. Therefore, this antibiotic is inevitably presented in the effluents from fish farms and eventually directly discharged in surface waters. The accumulation of pharmaceuticals in natural environment due to its extensive use is causing an increasing concern due to potential adverse effects on human health and aquatic ecosystems.

Up to now, removal of FF has been mainly reported using physical-chemical processes [3–5] but its removal by biological processes is scarce. Through biodegradation microorganisms are able to transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles [6]. Bioremediation has been considered the strategy of choice to reduce the risk of hazardous chemicals. Therefore, microorganisms able to deal with FF can contribute for the development and enhancement of bioremediation processes towards more efficient biological removal processes.

The main aim of this study was to investigate FF degradation by a previously isolated bacterial strain, *Labrys portucalensis* F11, capable to degrade other pharmaceuticals. The effect of the periodic feeding with a conventional carbon source on the FF degradation by this bacterium was also investigated.

Methods

Florfenicol degradation. The degradation of FF was tested at a concentration of 10 µM with the addition of a supplementary carbon source, sodium acetate, at 5.9 mM. Assays with a periodic feeding with the same concentration of sodium acetate were also established. Cells of *L. portucalensis* F11 previously grown on NA plates were inoculated to an OD600 of ca. 0.1 into 250 mL flasks containing 75 mL of mineral medium supplemented with FF. Experiments were performed in triplicate under sterile conditions. Control assays without inoculation and controls inoculated with autoclaved (i.e. non-viable) *L. portucalensis* F11 cells were performed to evaluate abiotic degradation and adsorption. A control for cell growth was established with the same concentration of acetate but without FF addition.

All suspensions were incubated for 28 days, at 25 °C, with constant shaking (120 rpm) and protected from light, to avoid FF photodegradation. Samples were collected twice a week to determine FF removal, fluoride release and cell density.

Analytical methods. FF removal was evaluated by HPLC-DAD [7]. The concentration of fluoride ion was measured with a fluoride electrode as previously described by Amorim et al. [8]. Growth was monitored spectrophotometrically by measuring the optical density at 600 nm.

Results

The ability of *L. portucalensis* F11 to degrade FF in the presence of an additional carbon source was evaluated. When acetate was added only at the beginning of the experiment, at the end of 28 days, strain F11 was able to remove about 80% of the FF initially supplied. Considering the reaction stoichiometry, the fluoride release corresponded to 40% of the total amount of consumed substrate. The growth pattern observed for cultures supplied solely with acetate was similar to the one for the cultures supplied simultaneously with acetate and FF. This result revealed that despite FF antibiotic properties, there was no evidence of toxic effects of FF on *L. portucalensis* F11 cells, whose growth was not affect at the supplied concentration. Moreover, FF degradation started from the beginning of the experiment, showing that the bacterium was able to degrade the pollutant without the need of any pre-induction.

Periodic feeding (once and twice a week) with the same concentration of acetate was carried out to investigate if a higher cell mass would improve the biodegradation efficiency of the target compound. In the same period of time, approx. 80% of the initial FF was consumed. Although the presence of a conventional carbon source that could be easily metabolised by strain F11 improved cell growth, FF degradation was not enhanced. Nevertheless, a positive effect of acetate supplementation was observed for FF defluorination. The release of fluoride when acetate was supplied once and twice a week corresponded to 73 and 80% of the total amount of consumed substrate, respectively. The higher availability of a

second carbon source to supply energy to the cells may be a critical factor for biodegradation. The release of the halogen substituent is a critical step in the biodegradation of halogenated compounds. The positive effect of acetate periodic feeding on the dehalogenation indicates that the presence of the additional carbon source could have contributed to circumvent the enzymatic rate-limiting step of FF metabolism.

Conclusion

In this study, FF biodegradation by *L. portucalensis* F11 was observed in the presence of an additional carbon source. Periodic feeding with the additional carbon source did not improve the removal of FF but it had a positive effect on the defluorination of the target compound. An improvement of about 24 and 30% in the fluoride release extent in the cultures fed once and twice a week respectively, with acetate was observed as compared to the culture fed only at the beginning

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