Screening of bacteria and microalgae for bioremediation of florfenicol in aquaculture water streams

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Introduction

The occurrence of antibacterial agents in natural environment has recently received growing concern due to potential adverse effects on human health and aquatic ecosystems.

Florfenicol (FF) is a synthetic phenicol antibiotic, widely used in veterinary medicine for treating diverse infections, and is inevitably released into the environment, either from uneaten medicated feed or through excretion. 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.

Up to now, removal of FF has been mainly reported using physical-chemical processes. Considering that many species of bacteria and microalgae can grow in a wide variety of wastewaters and industrial effluents and have been proven to be efficient in removing nitrogen, phosphorus and other pollutants, there is a niche opportunity to use those microorganisms in bioremediation processes.

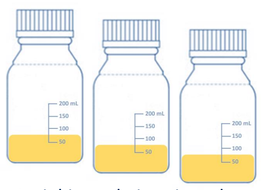
In this study, the potential of bacteria and microalgae, some isolated from a fish farm, to deal with FF was evaluated.

Methods

Bacteria

Sludge from a fish farm and fluoroaromatic degrading bacteria

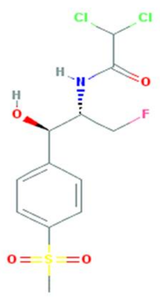
Batch degradation assays



Bacterial inocula in mineral medium with FF and acetate

* FF removal – HPLC
* F^- ion release – Potentiometry
* Cell density - Spectrophotometry

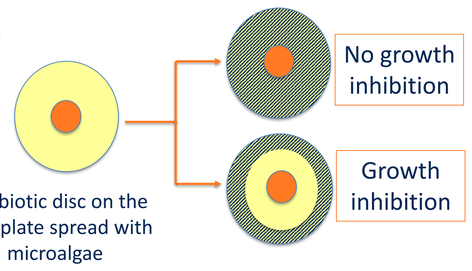
Florfenicol antibiotic



Microalgae

Microalgae isolated from a fish farm

Disk-diffusion assays



Antibiotic disc on the agar plate spread with microalgae

Results & Conclusions

FF bacterial degradation

Screening of bacteria for FF biodegradation

Table 1 – FF removal by the different bacterial inocula at day-30 of incubation

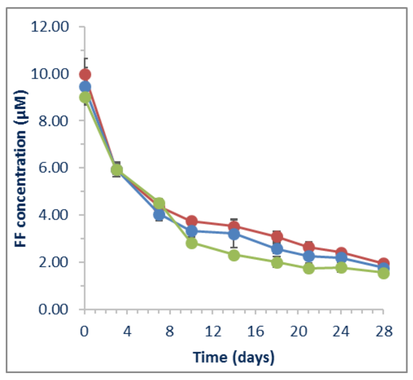
	FF removal (%)
Labrys portucalensis F11	80,4 ± 0,8
Rhodococcus sp. FP1	3,6 ± 1,2
Rhodococcus sp. ED55	2,2 ± 2,1
Sludge from a fish farm	8,8 ± 2,4
Abiotic control	1,8 ± 1,0

Removal expressed as percentage of the initial FF provided (10 µM).
Results represent the mean of triplicates ± SD.

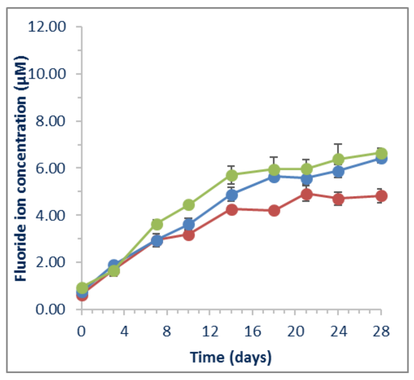
→ ***Labrys portucalensis* F11** able to degrade the antibiotic, initially supplied at 10 µM

Effect of an additional carbon source on FF biodegradation efficiency

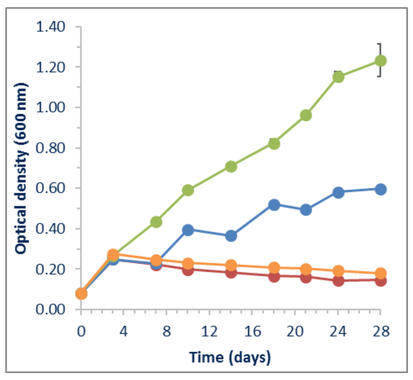
FF consumption



FF defluorination



Bacterial growth



- FF + acetate supplied only at the beginning
- FF + acetate supplied once a week
- FF + acetate supplied twice a week
- acetate supplied only at the beginning

Figure 1 – FF degradation by *L. portucalensis* F11 under different acetate feeding regimes

- Acetate periodic addition improved cell growth but had no effect on FF consumption
- A positive effect of acetate periodic feeding was observed for FF defluorination, which is the enzymatic rate-limiting step of the fluororganic compounds metabolism
- Despite FF antibiotic properties, there was no evidence of toxic effects on F11 cells, whose growth was not affected at the FF supplied concentration.

FF toxicity to microalgae

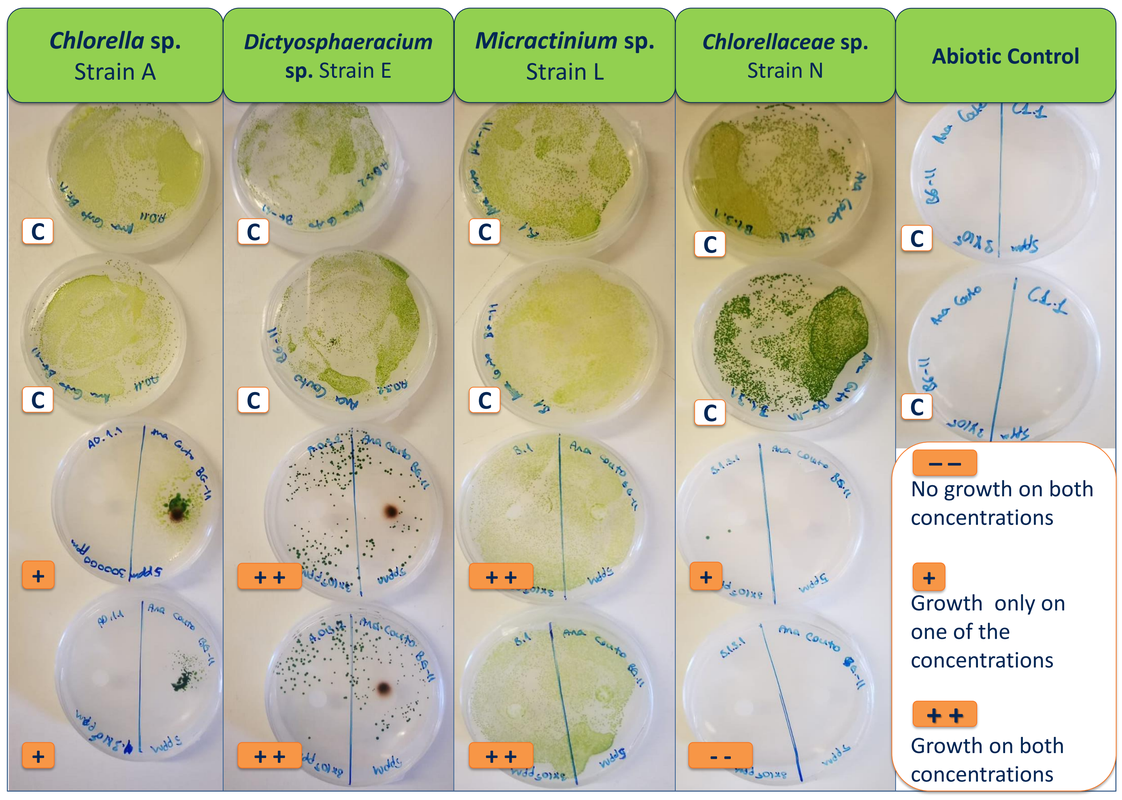


Figure 2 – Disk-diffusion assays to assess FF toxicity to different microalgae. Microalgae growth control without FF **(C)**.
On disk diffusion plates, the left and right disks contain FF at 837 mM (high concentration) and at 12 µM (low concentration), respectively.

- *Dictyosphaeracium* sp. strain E and *Micractinium* sp. strain L were able to grow in the presence of FF, even at high concentrations.
- *Chlorella* sp. strain A was able to grow when FF was added at low concentarions but its growth was inhibited at high FF concentrations.
- The growth of *Chlorellaceae* sp. strain N was inhibited at both concentrations of FF. Nevertheless, a few colonies grew near the high concentration of FF disk.

- *Labrys portucalensis* F11 is a good candidate to recycle this xenobiotic compound back into natural biogeochemical cycles.
- *Dictyosphaeracium* sp. strain E and *Micractinium* sp. strain L ability to cope with FF toxicity is valuable for their application on bioremediation technologies.
- Microorganisms able to deal with FF can contribute for the enhancement of bioremediation processes towards more efficient removal processes.

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