Biodegradation of Pharmaceuticals by Bacteria Isolated from Estuarine Environment - Cleanup Technologies through Nature-Based Processes

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Abstract
New sustainable technologies are needed to tackle pharmaceuticals contamination in different environmental compartments. Bioremediation technology, using native microorganisms with capacity for partial or complete elimination of contaminants, can be considered. This work investigated the capability of different bacterial strains to biodegrade paroxetine and bezafibrate, either as a bacterial consortium or as a single strain. These strains were isolated from bacterial cultures previously enriched under static conditions with paroxetine or bezafibrate, using as inoculum an estuarine sediment. All strains were identified through 16S rRNA gene sequencing. Degradation potential was accessed by analyzing pharmaceutical compounds in the culture medium and fluoride ion release (only for paroxetine). The genus *Pseudomonas*, widely reported in biodegradation studies, was predominant among the isolated bacterial strains. Most bacterial strains showed potential to degrade paroxetine (55%-100%) as a single strain in co-metabolism with sodium acetate. Furthermore, bacterial consortia also presented high removal efficiencies (>85%) for paroxetine throughout 4 weeks. For bezafibrate, tests showed a high potential of the bacterial consortia to degrade this compound (>90%). The obtained results highlight the potential of native microorganisms to degrade different pharmaceuticals which should be addressed for future development of bioremediation technologies for the recovery of contaminated environments.
Keywords: Autochthone degrading bacteria; natural communities; bioremediation; pharmaceuticals.

1. Introduction

The consumption of pharmaceuticals has been increasing in the last years and there is an urgent demand for new compounds to tackle old and new diseases. A mixture of different pharmaceuticals, sideways with their active metabolites, are uninterruptedly being released into the environment mainly through wastewaters from urban, hospital, livestock and pharmaceutical manufacturing industries. Recently, several technologies have been developed to remove pharmaceuticals from different matrices but most of them are either expensive or can induce negative impacts on the ecosystems. Thus, bioremediation technologies based on native microbial communities, can be presented as a sustainable alternative. The use of bacterial consortia, instead of individual microorganisms, can have more chances of success (Aissaoui *et al.,* 2017). In fact, different bacteria with different dynamics and functions in the same environment can play an important role in the degradation of the compound due to cooperative or synergistic effects among them (Zhao *et al.,* 2015). Yet, some studies have reported the use of single bacterial strains for the degradation of different pharmaceuticals and some with promising results (Zhang *et al.,* 2012; Lin *et al.,* 2015).
In this context, the main objective of this work was to evaluate the potential of different bacterial strains derived from an estuarine sediment to biodegrade paroxetine (Prx) and bezafibrate (Bzf), either as a bacterial consortium or as a single strain.

2. Methodology

2.1 Biodegradation of Prx and Bzf by bacterial consortium and isolated bacterial strains

Microbial cultures previously enriched with Prx or Bzf were obtained from an estuarine sediment inoculum, under static conditions (Duarte *et al.,* 2019). From each culture, ten bacterial strains were isolated, using two different media (plate count agar and minimum mineral salt medium), and re-assembled into new consortia. For that, a mix of each strain in equal proportions was inoculated, in triplicate, into 60 mL of a liquid mineral-salts medium, starting with an optical density (OD) of 0.1 (λ=600nm). The consortia were fed with 1 mg L^{-1} of Prx or Bzf in cometabolism with sodium acetate (500 mg L^{-1}), in static conditions (the same conditions in which the selected cultures were obtained), to evaluate their potential to degrade the target pharmaceuticals. Cultures were supplemented twice a week with 500 mg L^{-1} of sodium acetate and to ensure the presence of oxygen, each culture was transferred to a new sterilized flask once a week. These experiments were conducted during four weeks, at room temperature and in the dark conditions.

The capacity of isolated strains to degrade the target pharmaceuticals as single strains was also investigated. For that, each strain isolated from each consortium was inoculated, in triplicate, into 60 mL of a liquid mineral-salts medium, starting with an optical density (OD) of 0.1 (λ=600nm). Then, the same procedure described above was applied. Abiotic controls were run in parallel with these experiments.

2.2 Analytical procedures

Bacterial growth was accessed by measuring the solution optical density (OD) at 600 nm. Each week, culture samples were collected and centrifuged at 6500 rpm for further analysis. Removal efficiency of the target pharmaceuticals was accessed by high-performance liquid chromatography with a diode array detector (HPLC-DAD). In addition, fluoride anion concentration was measured as an indicator of Prx defluorination using a fluoride ion-selective electrode. For all the isolated strains, DNA was extracted and identified through 16S rRNA gene sequencing.

3. Results and Discussion

The microbial consortium consisting in the re-assemblage of all strains isolated from the culture pre-enriched with Prx, was able to remove more than 85% of this compound and to completely defluorinate it after 4 weeks of experiment. However, abiotic controls presented around 40% of Prx degradation but no defluorination was observed, meaning that defluorination occurred solely through biotic processes. Regarding each bacterial isolate, removal efficiencies ranged between 55 – 100% wherein defluorination values ranged between 20 – 100%.

Taxonomic identification (Fig. 1) showed that the dominant genus was *Pseudomonas*, however, other genera belonging to the phyla *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were identified.

For Bzf, removal efficiencies higher than 90% were observed for the microbial consortium consisting in the re-assemblage of all strains isolated from the culture pre-enriched with this compound. Taxonomic identification showed that the dominant genus was also *Pseudomonas*, but other genera belonging to *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* phyla were also identified. Data regarding degradation by single bacterial strains is being currently analyzed and further experiments will be conducted to achieve an optimized consortium with the top degrading bacterial strains.

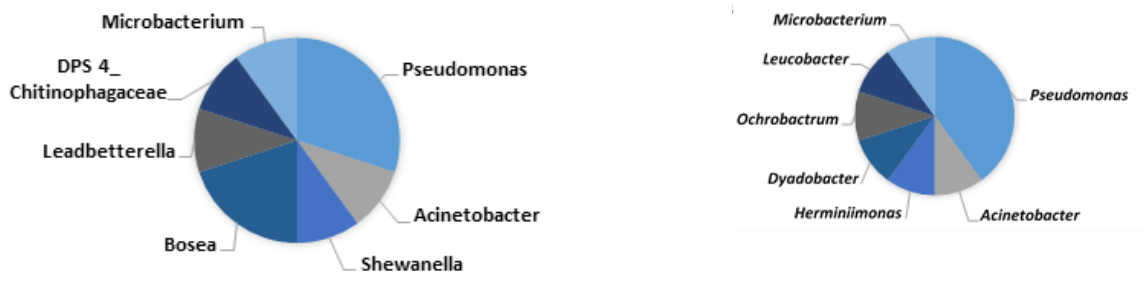

Fig. 1 – Relative abundance of difference bacterial genera among isolated bacterial strains. A – Proxetine bacterial consortium; B – Bezafibrate bacterial consortium.

4. Conclusions

This work showed the potential of native microorganisms isolated from an estuarine sediment to degrade two different halogenated pharmaceuticals. Ongoing experiments will provide more information about which bacterial strains have a key role in the degradation of both tested pharmaceuticals. In addition, an optimization of both degrading consortia will be attempted for future development of bioremediation technologies to recover contaminated environments.

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