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Biodegradation potential of bacteria capable of growing in an imazalil-rich wastewater

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Introduction

Imazalil is a systemic imidazole fungicide, with a half-life in soil of four to five months (USEPA 2002). Although it is stable for at least 8 weeks in neutral to acidic aqueous solutions, it decays when it is exposed to high temperature and light.

Imazalil is applied to suppress a range of fungi affecting fruits, vegetables and ornamentals. Moreover, imazalil is widely used as postharvest fungicide for the protection of fruits like citrus and bananas in order to prevent storage decay (Sepulveda et al. 2015). Several technologies, like dipping, spraying, waterfalling or candle mixing (Erasmus et al. 2011; Pérez et al. 2011; Altieri et al. 2013), are employed for imazalil application in fruits and vegetables. Imazalil mode of action includes the inhibition of ergosterol biosynthesis in certain fungi, like *Penicillium* spp. On the other hand, imazalil exhibits toxicity against various aquatic organisms, like zebrafish (Jin et al. 2016).

Moreover, the wide use of imazalil as post-harvest fungicide in fruit processing industry has resulted in the production of high-strength imazalil-containing wastewaters. However, the recalcitrant nature of such effluents resists biodegradation in the conventional activated sludge systems (Santiago et al. 2018). A range of chemical methods have been recently applied at laboratory scale to treat these recalcitrant effluents, like Fenton and advanced oxidation processes (Santiago et al. 2018). However, such approaches have not been adopted for full-scale applications since the cost of chemicals minimize their applicability.

On the other hand, microorganisms that are specialized in the degradation of imazalil are limited, a fact that restricts the adoption of biological methods to face the treatment of imazalil-rich effluents generated by the fruit processing agro-industry. Thus, the scope of this work was to isolate and molecularly identify novel microbiota capable of growing in synthetic wastewater containing high concentration of imazalil in order to serve as starter culture in biological systems treating such fungicide-rich effluents.

Materials and methods

To obtain effective imazalil-degrading microbiota, the residue of a storage tank receiving imazalil-rich effluent was served as the inoculum. To isolate potential imazalil-degrading microbiota, ten-fold dilution plating was performed in defined medium consisting of commercially available imazalil formulation. In particular, the dilution series were performed in a solution consisting of the appropriate nitrogenous inorganic compounds, phosphate salts and trace elements (in the absence of any carbonaceous compound). The aforementioned solution was also used for the preparation of solid media, where 1.7 w/v bacteriological agar served as the solidifying agent. After sterilization and prior agar solidification, 250 mg/L imazalil was added to serve as the carbon source.

To phylogenetically characterize the isolated microorganisms, DNA was extracted by using the Macherey-Nagel "NucleoSpin Tissue" kit. The genomic DNA obtained from the microbial isolates was amplified by using universal primers for the 16S rRNA gene, as previously described (Remmas et al. 2018). In brief, a thermocycling protocol consisting of a primary denaturation step of 2 min at 94°C, followed by 35 cycles of 30 sec denaturation at 94°C, 30 sec primer annealing at 52°C and 90 sec DNA extension at 72°C, was implemented. A final DNA elongation step at 72°C for 10 min was carried out to accomplish the PCR reaction. All sequencing reactions were performed at Eurofins Genomics (Germany) by using universal 16S rRNA gene primers.

Results and concluding remarks

In total, 20 bacterial strains capable of growing in imazalil-containing medium were isolated. Performance of phylogenetic analysis showed that they were representatives of the order *Rhizobiales* (class *Alphaproteobacteria*) and the family *Pseudomonadaceae* (class *Gammaproteobacteria*), which both include various effective degraders. Therefore, it is concluded that the selection pressure applied during storage of imazalil-rich fruit processing waste resulted in the proliferation of a well-adapted bacterial community capable of coping with the severe conditions established by the accumulation of post-harvest fungicides.

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