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Biodegradation potential of bacteria isolated from a fludioxonil – rich fruit processing waste

I. Alexandropoulou[1], Z. Mavriou[1], P. Melidis[1], D. G. Karpouzas[2] and S. Ntougias[1,*]

[1] Laboratory of Wastewater Management and Treatment Technologies, Department of Environmental Engineering, Democritus University of Thrace, Vas. Sofias 12, 67132 Xanthi, Greece

[2] Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, 41500 Larissa, Greece

* Corresponding author: E-mail: sntougia@env.duth.gr, Tel +30 25410 79313

Abstract

The fruit processing industry is among the most dynamic agro-industrial sectors worldwide. To extend the shelf life of fruits during storage, they are treated with post-harvest fungicides. Fludioxonil is a non-systemic phenylpyrrole fungicide, with broad applications in apple and pear possessing agro-industry. However, the biodegradation aspects of fludioxonil remain unknown since no fludioxonil-degrading microorganisms have been isolated yet. In this work, a culture-dependent approach was employed to isolate effective fludioxonil-degraders. The isolated bacteria grown in the presence of fludioxonil were further characterized by 16S rRNA gene sequencing in order to reveal their phylogenetic position. From a phylogenetic point of view, distinct bacterial isolates were identified, possessing the potential of bioremediating fludioxonil-contaminated wastes. It is concluded that the selection pressure applied during long storage of fludioxonil-rich fruit processing waste resulted in the proliferation of a part of microbial community capable of adapting in high concentrations of post-harvest fungicides.

Keywords: *fruit processing waste; fludioxonil; postharvest industry; fungicide-degrading bacteria*

1. INTRODUCTION

The fruit processing industry is considered as a major sector of the agro-industries worldwide. Fungal infection in fruits during post-harvest storage is a major concern of fruit packing industry since fruit postharvest diseases lead to important economic losses. The application of post-harvest fungicides in fruits has prevented such threat, extending the shelf life of fruits during storage. A range of post-harvest fungicides, such as thiabendazole, imazalil and fludioxonil, are commonly applied for the protection of the main fruit crops. In particular, fludioxonil is one of the most commercial post-harvest fruit fungicides [1]. In comparisons to other fungicides, such as imazalil or thiabendazole, fludioxonil is considered as more effective against fungi like *Monilinia* and *Sclerotinia* spp. as well as against certain *Penicillium expansum, P. digitatum, Lasiodiplodia theobromae, Dothiorella dominicana* and *Colletotrichum gloeosporioides*

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strains [2]. Fludioxonil can be used in the pre- and post-harvest treatment of leaves, fruits and seeds [1, 3]. This fungicide can be widely applied during post-harvest processing of a variety of fruits, such as apples, pears, quinces and soft fruits (berries) [4].

The main trait of fludioxonil is its high water solubility (1.8 mg/L) and hydrolytic stability in a broad pH range of 5 to 9 [5]. Fludioxonil is a non-systemic phenylpyrrole, exhibiting a long residual activity [2, 4]. Regarding the toxicological profile of fludioxonil, it is considered as toxic to certain aquatic organisms. Consequently, disposal or leaching of fruit processing wastewater containing high residual concentration of fludioxonil into aquatic habitats can invoke huge damages [6]. Nevertheless, fludioxonil is less toxic than other fungicides, according to US Environmental Protection Agency [7].

During post-harvest treatment of fruits, huge quantities of wastewaters containing high concentrations of residual fungicides are generated by the fruit possessing industries. The complex structure of such antifungal compounds renders obstacles in the waste management of such effluents. A range of chemical oxidation methods, like Fenton, photo-Fenton, O_3/UV, H_2O_2/UV and TiO_2/UV, have been employed in laboratory experiments to mineralize such bio-recalcitrant pesticides [8]. Photolysis is also capable of reducing fludioxonil level in soil [6]. On the other hand, conventional biological systems are not suitable for fludioxonil treatment, since such fungicide is considered as not easily biodegradable [6]. According to Thomas et al. [5], fludioxonil degradation in surface water environments is restricted to the activity of phototrophic microorganisms. However, the biodegradation aspects of fludioxonil in fruit industry wastewater remain unknown since no fludioxonil-degrading microorganisms have been isolated yet. In this work, indigenous bacteria were isolated from fludioxonil-rich residues in order to serve as effective degraders of post-harvest fungicides.

2. MATERIALS AND METHODS

2.1 Isolation procedure

The dilution plate method of using 10-fold dilution series was employed to isolate bacteria capable of growing in the presence of fludioxonil. A solution made by mineral salts and trace elements was prepared for the performance of dilution series. The residue that was remained in a storage tank receiving fludioxonil-rich wastewater (generated from the post-harvest processing of fruits) was used as the inoculum. An aliquot of 0.2 ml from each dilution was inoculated on agar plates containing 250 mg/L fludioxonil. The inoculated plates were incubated at 28°C for a period of 2 weeks and single colonies were obtained and transferred into fresh media. Purity of the obtained colonies was examined against a Zeiss optical microscope.

2.2 DNA extraction from microbes

Genetic DNA from the bacterial isolates obtained was extracted by using the Macherey-Nagel Nucleospin Tissue kit, following the instructions recommended by the manufacturer. The DNA recovery was then examined against a 100-bp ladder on 1.2% w/v agarose gel in the presence of UV light to estimate DNA concentration and purity. The extracted DNA was stored at -20 °C for further analysis.

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2.3 PCR amplification of the 16S rRNA gene and sequence analysis

Universal primers, i.e. pA (5′-AGA GTT TGA TCC TGG CTC AG-3′) and pH (5′-AAG GAG GTG ATC CAG CCG CA-3′), targeting almost the entire 16S rRNA gene, were used in order to identify the phylogenetic position of the bacterial isolates obtained. A 50 μl amplification reaction mix was prepared, containing 30 ng genomic DNA (for each strain), 10x Kapa Biosystems PCR buffer (USA), 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.5 μM each of primer pA and pH. The amplification was initiated by adding 2.5 U DNA Taq polymerase (Kapa Biosystems). PCR reactions were performed in a TaKaRa Dice TP600 thermocycler (Japan), by employing a denaturation step of 2 min at 94°C, 35 cycles of 30 sec denaturation at 94°C, 30 sec primer annealing at 55°C and 90 sec DNA elongation at 72°C [9]. A final extension step at 72°C for 10 min was included to end the amplification reactions. PCR products were purified through the use of a commercially available kit (NucleoSpin Gel and PCR Clean-up, Macherey-Nagel, Germany), following the manufacturer's instructions. Sequence reactions were carried out at Eurofin genomics (Germany). The closest phylogenetic relatives of the bacteria isolated in the current study were identified by using the BLASTN software.

3. RESULTS AND DISCUSSION

The bacterial strains obtained in the current study were isolated by using a fludioxonil-rich medium in order to be proliferated the growth of effective fungicide degraders. These indigenous bacterial isolates were further characterized by 16S rRNA gene sequencing in order to identify their phylogenetic position. Performance of phylogenetic analysis revealed that they belong to distinct taxa of the classes *Alphaproteobacteria* and *Betaproteobacteria*. The members of the first operational taxonomic unit (OTU#1) were placed within the family *Sphingomonadaceae*, order *Sphingomonadales*, whereas the isolates belonging to the second OTU (OTU#2) were members of the order *Rhizobiales*. The bacterial isolates placed in the third OTU (OTU#3) belonged to the family *Alcaligenaceae* (order *Burkholderiales*) within the *Betaproteobacteria*.

Members of the families *Sphingomonadaceae* and *Alcaligenaceae* have been reported to be effective degraders [10,11], possessing thus the potential to degrade recalcitrant compounds like post-harvest fungicides. In addition, representatives of the order *Rhizobiales* exhibit the potential to degrade fungicides [12].

4. CONCLUSIONS

It is concluded that the indigenous bacteria isolated in the frame of this research work are capable of degrading fludioxonil-rich wastewaters that are generated by the fruit processing industry after post-harvest treatment of crops with fungicides. Moreover, these bacterial isolates can serve as specialized microbiota in immobilized cell bioreactors to depurate the high fungicide content of the fruit processing wastewaters. Such bioengineeering approach will reduce significantly the environmental footprint of the fruit packing industry.

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