

# Microbial profiling of a suppressiveness-induced agricultural soil amended with composted almond shells lead to isolation of new biocontrol agents

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**Abstract:** This study focused on the microbial profile present in an agricultural soil that becomes suppressive after the application of composted almond shells (AS) as organic amendments. The role of microbes in the suppression of *Rosellinia necatrix*, the causative agent of avocado white root rot, was determined after heat-treatment and complementation experiments with different types of soil. Bacterial and fungal profiles based on the 16S rRNA gene and ITS sequencing, the soil under the influence of composted almond shells revealed an increase in *Proteobacteria* and *Ascomycota* groups, as well as a reduction in *Acidobacteria* and *Xylariales* (where *R. necatrix* is allocated). Complementary to these findings, functional analysis by GeoChip 4.6 confirmed the improvement of a group of specific probes included in the “soil benefit” category was present only in AS-amended soils, corresponding to specific microorganisms previously described as potential biocontrol agents, such as *Pseudomonas* spp., *Burkholderia* spp. or *Actinobacteria*. Based in such data, a model for the microbial-based suppressiveness is proposed and further isolation of representative microorganisms were performed.

**Keywords:** *Rosellinia necatrix*, organic amendment, biocontrol, *Gammaproteobacteria*

## Introduction

The enhancement of soil suppressiveness using organic amendments has been widely described, especially for soil-borne diseases (Bailey and Lazarovits 2003). The soils that become suppressive soils provide an environment in which plant disease development is reduced, even in the presence of a virulent pathogen and a susceptible host. This phenomenon could be induced as a direct result of the activity of microorganism consortia that are naturally established on soil after application of the amendment (Weller et al., 2002). For this reason, understanding the diversity, composition, structure, function and interactions of microbial communities is crucial to gain insight into the basis for suppressiveness mediated by this organic amendment (Janvier et al., 2007).

Our research interest is focused on the avocado (*Persea americana* Mill.), for which southern Spain is one of the most relevant zones in the Mediterranean area for this crop. In this part of the world, one of the most limiting soilborne diseases affecting avocado trees is white root rot, caused by the fungus *R. necatrix*. Several approaches have been implemented, among them, the use of organic amendments or mulches, such as composted almond shells, which have been previously shown that can affect soil

physicochemical properties and microbial communities (Bonilla et al., 2015; López et al., 2014).

## **Materials and methods**

### ***Soil sampling***

Natural field soil samples allocated underneath of unamended avocado trees (CT) or amended with composted almond shells (AS) were taken to perform the different experiments, as previously described by Vida et al., 2016 from an experimental located at the Experimental Station 'La Mayora' (IHSM-UMA-CSIC, Málaga, Spain) on the coast of the Malaga Province (SE Spain).

### ***Samples processing***

To test the potential role of soil microorganisms in suppressiveness, we prepared three types of processed soils using different treatments: Field soils (raw soils), heat-treated soils and complemented soils (Vida et al., 2016). To isolate *Gammaproteobacteria* strains from rhizosphere and bulk soil, we plated the samples on selective medium (King's B [KB] agar supplemented with antibiotics (Larkin and Honeycutt, 2006)). The most abundant colonies with different morphology (n=267) were isolated and storage at -80°C.

### ***Suppressiveness assays***

Suppressiveness assays against white root rot caused by the virulent strain *Rosellinia necatrix* CH53 were conducted using two different susceptible pathosystems, avocado and wheat (*Triticum aestivum*) using raw, heat-treated soils and combinations (Cazorla et al., 2006; Vida et al., 2016).

### ***Microbial profile analysis***

Composite DNA samples from each soil type (AS and CT) were sent for sequencing to obtain the microbial DNA sequences of the 16S rRNA gene and ITS hypervariable regions. Sequences were analysed using QIIME and CLcommunity<sup>TM</sup> software (ChunLab, Korea). Moreover, additional composite samples of DNA from the different soil samples studied were sent to Glomics Inc (Norman, Oklahoma, USA) for the functional analysis (Tu et al., 2014).

### ***Isolates characterization***

Focused in *Gammaproteobacteria* strains (n=267), antagonistic activity assays (Cazorla et al., 2006), detection by colony blot of biosynthetic genes of antimicrobial compounds and production of exoenzymatic activities were performed. Partial sequencing of 16S rDNA gene, plant growth promotion and biocontrol assays were performed only with some representative selected isolated.

## **Results and discussion**

### ***White root rot suppressiveness assay***

AS field soil samples displayed better suppressive ability than CT field soil samples (data not shown). The disease suppressiveness activity was reduced when AS soil samples were heat-treated (AS<sub>t</sub>). Moreover, suppressiveness was complemented in conductive soils when incorporating AS soil samples. Thus, the microbiota evolved in the composted

almond shells was crucial for suppressiveness because the reduction of the bacterial population after a heat treatment in the organic amendment resulted in a more conducive phenotype. Moreover, total or partial suppressiveness was recovered when these heat-treated soil samples were complemented with a portion of soil influenced by AS.

### Microbial profile analysis

Taking together the results obtained in this work and in previous works related, a theoretical model about the role of the microorganisms in enhancing suppressiveness after amendment with composted almond shells can be proposed (Figure 1).

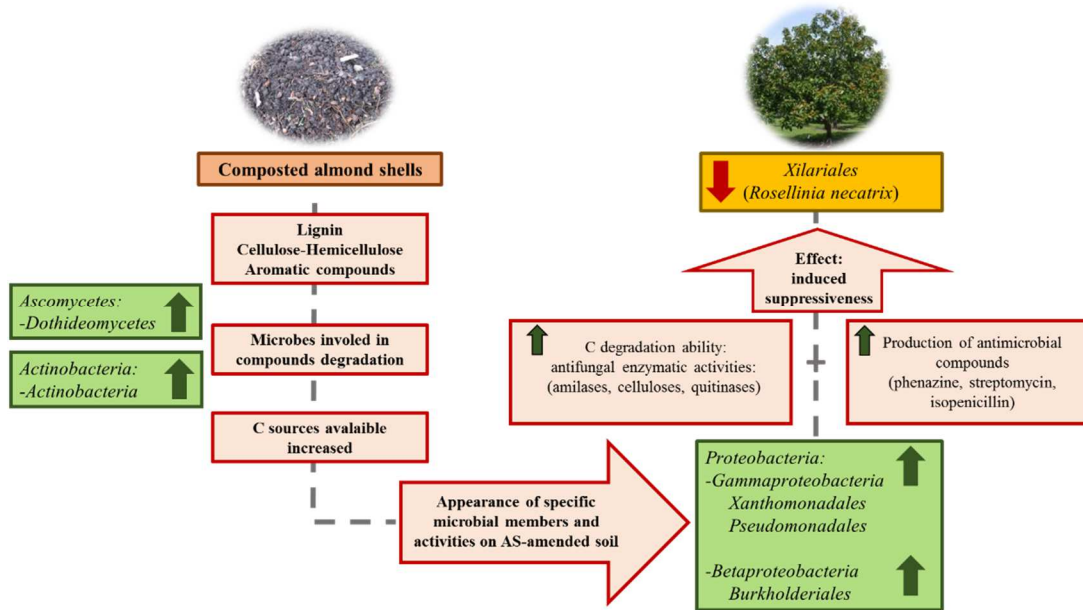


Figure 1. Hypothetical mode of action of almond shells amendment.

Briefly, soil amendment with composted almond shells resulted in an extra input of organic matter rich in lignin that could be initially degraded by fungal members of the community (such as *Dothideomycetes*) and Actinobacteria. Lignin degradation would produce a progressive release to the soil of more simple compounds. Those released compounds lead to an increase in carbon sources available, such as cellulose, hemicellulose and aromatic compounds. At this point, some *Proteobacteria* already present in the soil (mainly *Gammaproteobacteria* and *Betaproteobacteria*) could take advantage metabolizing that available organic matter, thus slightly enhancing their population. A selected group of enhanced microorganisms harbor, among other, genes involved in antifungal enzymatic activities and production of antimicrobial compounds that could have an effect on the interaction with other microbes. This resulting modified microbiota after addition of composted almond shells could be more active against some groups of phytopathogenic fungi (as *Xilariales*, where *Rosellinia necatrix* is included) finally showing a phenotype of induced suppressiveness effect.

### Selection of new biocontrol agents

Finally, the isolation of *Gammaproteobacteria* strains from a suppressive soil could represent a strategy for selecting microorganisms with biocontrol abilities. Thus, some isolates have been obtained, and displayed combined plant growth promoting activities

or antagonistic interactions with the biocontrol activity. The main genera selected correspond to *Serratia* spp., *Pseudomonas* spp. and *Stenotrophomonas* spp

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## References

- Bailey, K.L. and Lazarovits, G. (2003) Suppressing soil-borne diseases with residue management and organic amendments. *Soil Tillage Res.* 72, 169–180. doi:10.1016/S0167-1987(03)00086-2.
- Bonilla, N., Vida, C., Martínez-Alonso, M., Landa, B. B., Gaju, N., Cazorla, F. M., et al. (2015). Organic amendments to avocado crops induce suppressiveness and influence the composition and activity of soil microbial communities. *Appl. Environ. Microbiol.* 81, 3405–3418. doi:10.1128/AEM.03787-14.
- Cazorla, F. M., Duckett, S. B., Bergström, E. T., Noreen, S., Odijk, R., Lugtenberg, B. J. J. et al. (2006). Biocontrol of avocado dematophora root rot by antagonistic *Pseudomonas fluorescens* PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. *Mol. Plant. Microbe. Interact.* 19, 418–428. doi:10.1094/MPMI-19-0418.
- Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T. and Steinberg, C. (2007). Soil health through soil disease suppression: Which strategy from descriptors to indicators?. *Soil Bio. Biochem.* 39, 1-23. doi:10.1016/j.soilbio.2006.07.001.
- López, R., Burgos, P., Hermoso, J. M., Hormaza, J. I., and González-Fernández, J. J. (2014). Long term changes in soil properties and enzyme activities after almond shell mulching in avocado organic production. *Soil Tillage Res.* 143, 155–163. doi:10.1016/j.still.2014.06.004.
- Tu, Q., Yu, H., He, Z., Deng, Y., Wu, L., Van Nostrand, J. D., et al. (2014). GeoChip 4: a functional gene-array-based high-throughput environmental technology for microbial community analysis. *Mol. Ecol. Resour.*, 1–15. doi:10.1111/1755-0998.12239.
- Vida, C., Bonilla, N., de Vicente, A. and Cazorla, F.M. (2016) Microbial profiling of a suppressiveness-induced agricultural soil amended with composted almond shells. *Front. Microbiol.* 7:4. Doi:10.3389/fmicb.201600004
- Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M. and Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* 40, 309–348. doi:10.1146/annurev.phyto.40.030402.110010