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# FUNGAL ENDOPHYTIC COMMUNITY ASSOCIATED WITH PEAR TWIGS AND BUDS

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***Abstract***

*This study presents the first screening of the fungal endophytic community associated with pear twigs and buds. Experiments were conducted on collected biological material from the Romanian resistant-disease pear collection. Endophytic fungal community in twigs was represented by Alternaria (35%), Aureobasidium (25%), Cladosporium (16%), Penicillium (5%), Fusarium (4%), Sordaria (4%), Nigrospora (3%), Trichoderma (3%) and Botrytis (0.6%) isolates. Isolates belonging to Alternaria (56%), Aureobasidium (47%), Cladosporium (15%), Fusarium (5%), Trichoderma (4%), Epicoccum (3%), Penicillium (2%) and Nigrospora (2%) genera were detected and identified in buds. Our results highlight the presence of isolates belonging to genera Aureobasidium, Epicoccum, Sordaria, and Trichoderma, which have been described as having antagonistic properties and/or potential to promote plant growth. A better understanding of these endophytic communities in a complex network can bring information on their roles, on their interactions with pear trees and with pathogens, and their mechanism of action.*

***Key words**: fungal endophytes, pear trees, twigs and buds.*

## INTRODUCTION

The microbial component of a plant or the microbiota has important functions in its growth and in maintaining its health (Brader et al., 2017; Lemanceau et al., 2017).

As a part of microbiota, endophytes are microorganisms that live within plant tissues without causing symptoms of disease and are important components of plant microbiomes. They interact with other core microbial groups that colonize plant tissues (Sharma et al., 2021).

As microorganisms have proven their potential in application as biostimulants or biopesticides, there is an increased interest in their integration into protection programs as an alternative to chemical products (Campant et al., 2019; Suciu et al., 2021; Stefan et al., 2021).

Isolation of endophytic fungi and their identification is the first step in studies related to signaling their possible role in the biological control of plant pathogens.

Knowing the community of endophytic microorganisms can bring information about their role, the interaction with plant pathogens and their mechanism of action.

Information about the functionality of plant-microbiome interactions as well as about the factors that are involved in establishing a microbial community can lead to a better understanding of the plant as a meta-organism and how plants benefit from their microbial partners.

The application of a consortium of microorganisms is an approach that is attracting more and more interest. Thus, microorganisms with different properties can be combined, which can be complementary or which can combine different mechanisms of action (stimulation of plant growth, biocontrol of pathogens).

In this context, the aim of this study was to detect and identify the endophytic mycobiota community of pear twigs.

## MATERIALS AND METHODS

Experiments were conducted on Romanian pear disease resistant genotypes, produced at the Voinești Research Station for Fruit Growing and Horticultural Research Station - USAMV Cluj-Napoca, and planted in the Experimental

Orchard of the Faculty of Horticulture, USAMV Bucharest.

Endophytic community associated with pear trees was assessed on pear twigs collected in march 2021 from 'Conference', 'Euras', 'Corina', 'Cristal', and 'Romcor' cultivars.

The twigs were cut into small pieces (0.5 cm), surface sterilized using 70% ethanol for 2 minutes, 4% sodium hypochlorite for 90 seconds and rinsed in water for three times (Ren et al., 2019). The tissues were blotted dried and plated on potato dextrose agar (PDA). Plates were incubated at 22°C until growing of colonies.

Fungal isolates were identified based on their macroscopic (colony color, pigmentation) and microscopic characteristics, the results being expressed as colonization rate (CR, %).

Pure culture of isolates was acquired through subsequent sub-culturing on PDA plates.

## RESULTS AND DISCUSSIONS

The endophyte microbial community associated with twigs is shown in Figure 1. Of the 560 fragments analyzed, 480 were colonized, resulting in a colonization rate of 86%.

The presence of *Alternaria* isolates (35%), followed by *Aureobasidium* (25%) and *Cladosporium* (16%) was detected. Isolates of the genera *Penicillium* (5%), *Fusarium* (4%), *Sordaria* (4%), *Nigrospora* and *Trichoderma* (3%) and *Botrytis* (1%) were also detected and identified.

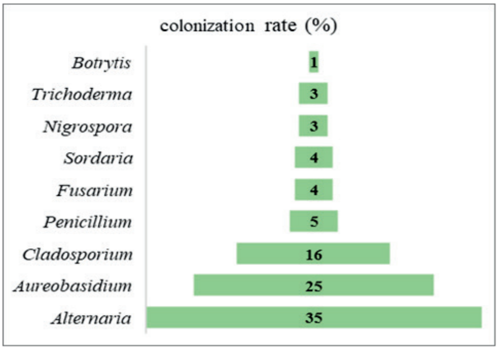


Figure 1. Endophytic community on pear twigs

Among the isolates with antagonistic potential, the presence of those from the genera *Aureobasidium*, *Sordaria*, *Nigrospora* and *Trichoderma* was detected.

In the 'Conference' variety (Figure 2), the isolates detected and identified were *Alternaria* (with the highest colonization rate, 38%), *Aureobasidium* (25%), *Penicillium* (12%), *Cladosporium* (10%) and *Fusarium* (5%). Among the isolates with antagonistic potential, we note the presence of those from the genus *Aureobasidium*.

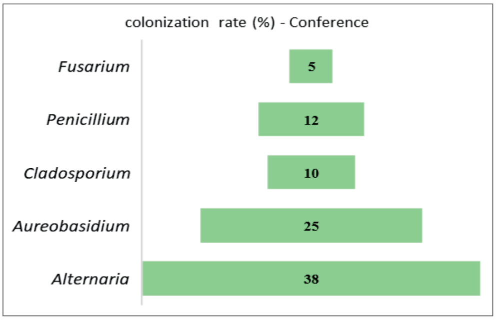


Figure 2. Endophytic community on pear twigs - 'Conference' variety

In 'Euras' variety (Figure 3), the microbial community was also mostly represented by isolates of the genus *Alternaria* (58%). *Cladosporium* (48%), *Aureobasidium* (31%) and *Fusarium* (4%) isolates were also identified.

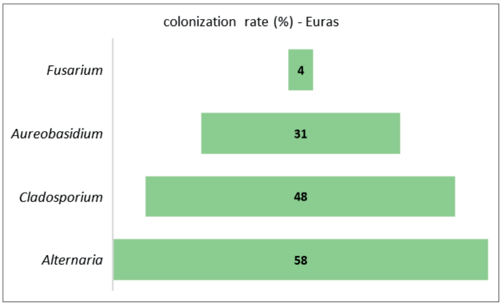


Figure 3. Endophytic community on pear twigs - 'Euras' variety

Among the isolates with antagonistic potential, the presence of those from the genus *Aureobasidium* was detected.

The microbial community identified in 'Cristal' variety is shown in Figure 4. Isolates of the genera *Alternaria* (27%), Aureobasidium (22%), *Cladosporium* (22%) and *Penicillium* (10%) were detected. Among the isolates with antagonistic potential, the presence of those from the genus *Aureobasidium* was detected.

The microbial community identified in 'Romcor' cultivar is shown in Figure 5. In this variety, the presence of *Aureobasidium* isolates with the highest colonization rate (29%) is noted. Isolates of the genera *Alternaria* (16%), *Penicillium* (13%), *Cladosporium* (5%) and *Fusarium* (5%) were also identified.

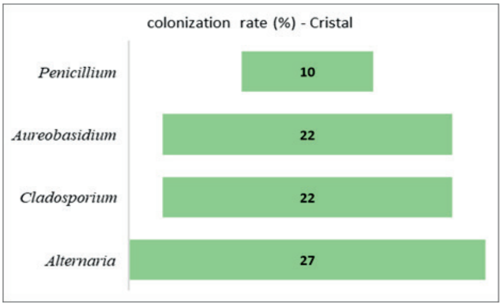


Figure 4. Endophytic community on pear twigs - 'Cristal' variety

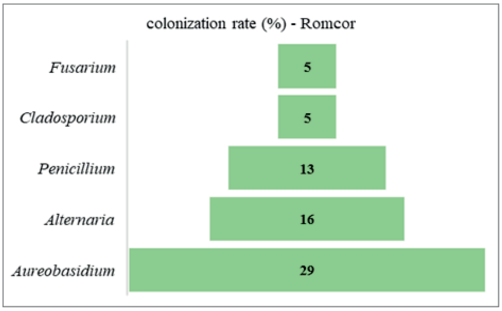


Figure 5. Endophytic community on pear twigs - 'Romcor' variety

In 'Corina' variety, the microbial community was richly represented by isolates of genus *Sordaria*, (32%), a genus known for its species with antagonistic properties (Figure 6).
Isolates of the genus *Alternaria* had a colonization rate of 23%. Isolates of the genera *Cladosporium* (2%), *Fusarium* (3%) and *Botrytis* (5%) were also detected.
Among the isolates with antagonistic potential, *Aureobasidium* (21%), *Trichoderma* (11%) and Sordaria (32%) have been also identified.
Regarding the endophyte microbial community of buds (Figure 7), our results highlight the high colonization rate of *Alternaria* isolates (56%), followed by those of the genera *Aureobasidium* (47%), *Cladosporium* (15%), *Fusarium* (5%), *Trichoderma* (4%), *Epicoccum* (3%), *Penicillium* (2%), and *Nigrospora* (2%).

All the 262 fragments analyzed (131 buds, sectioned) were colonized, resulting in a colonization rate of 100%.
A richly represented microbiota have been observed, with isolates belonging to genera known for their antagonistic potential like *Aureobasidium*, *Trichoderma*, *Epicoccum* and *Nigrospora*.
The presence of *Epicoccum* isolates was detected only at the bud level.

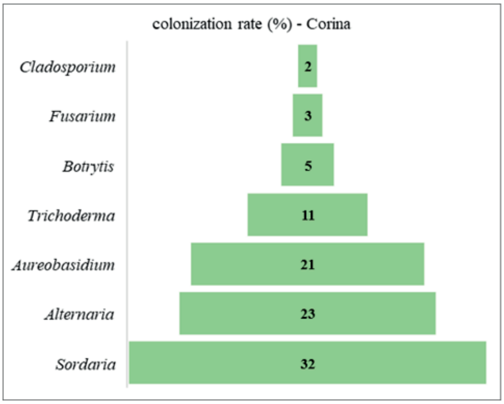


Figure 6. Endophytic community on pear twigs - 'Corina' variety

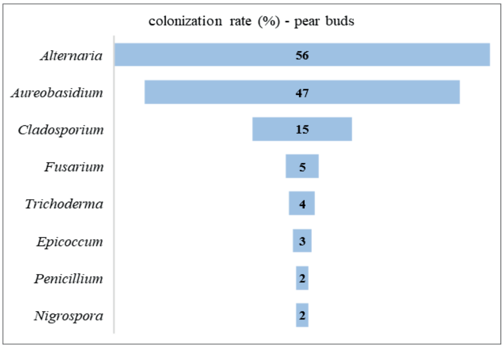


Figure 7. Endophytic community on pear buds

Aspects of the morphology of the detected and identified colonies are presented in Figures 8 and 9.
In endophytic community of pear twigs and buds our results highlight the most abundant phylum as *Ascomycota*. Among the most abundant ascomycetes we reported the *Alternaria* spp. isolates (*Pleosporaceae* family). Previous studies have shown that endophytic trees community mainly consist of Ascomycota and to a lesser extent, Basidiomycota and

Zygomycota (Muller et al., 2016; Sun & Guo, 2012).

A comprehensive view of the endophytic fungal community of pear trees was reported (Fei Ren et al., 2019). Each investigated organ (flower, leaf, fruit, stem and root) harbored a unique fungal assemblage, with *Ascomycota* the most abundant phylum.

The high abundance in all samples of *Aureobasidium* and Sordaria in twigs from 'Corina' variety may indicate an active role. Further studies are needed for the cultivation of endophytic fungal strains with biocontrol effect as well as ecological and functional roles.

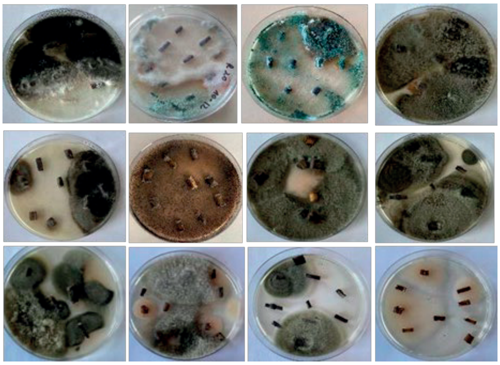

Figure 8. Colonies morphology of endophytic community on pear twigs

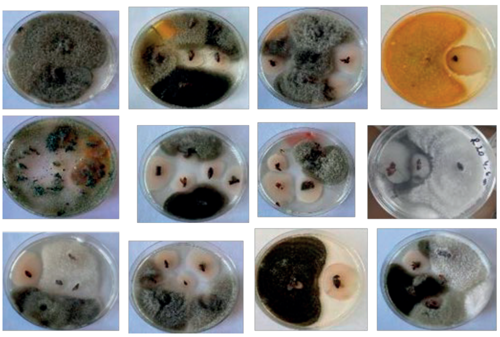

Figure 9. Colonies morphology of endophytic community on pear buds

## CONCLUSIONS

This study presents the first screening of the fungal endophytic community associated with pear twigs and buds. The endophyte fungal community of twigs was represented by isolates from the genera *Alternaria*, *Aureobasidium*, *Botrytis*, *Cladosporium*, *Fusarium, Penicillium*, *Nigrospora, Sordaria*, and *Trichoderma*. Isolates belonging to the genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Fusarium*, *Trichoderma*, *Epicoccum*, *Penicillium* and *Nigrospora* were detected and identified in the buds.

Antagonistic species belonging to the genera *Aureobasidium* (*A. pullulans*), *Epicocum* (*E. purpurascens*), *Sordaria* (*S. fimicola*) and *Trichoderma* (*T. harzianum*) were detected and isolated. The presence of these communities with antagonistic properties is an advantage for plants. Knowledge of the endophytic community can bring information on their role, interactions with plant pathogens and their mechanism of action.


## ACKNOWLEDGEMENTS

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