EXPLORING DIVERSITY, ANTAGONISTIC PROPERTIES AND PHOSPHATASE ACTIVITY OF ENDOPHYTIC BACTERIA IN *ARTEMISIA* SPP.

S. Hadian[1*], S. Supronienė[1], D. Smith[2]
[1] Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Akademija, Lithuania
[2]McGill University, Quebec, Canada/ Plant Science
*Shervin.hadian@lammc.lt

Introduction:

Endophytic bacteria, which live within plant tissues without causing harm, provide promising solutions for sustainable agriculture. These bacteria can inhibit plant diseases, increase nutrient availability, and improve overall plant health, making them beneficial in integrated pest management and organic agricultural systems. *Artemisia* species, which are known for their medicinal virtues and ability to withstand a wide range of environmental conditions, host a variety of endophytic bacteria with potential agricultural applications. The purpose of this work is to investigate the diversity, antagonistic characteristics, and phosphatase activity of endophytic bacteria isolated from four *Artemisia* spp. tissues, with the goal of contributing to our understanding of plant-microbe interactions and their implications for sustainable agriculture.

Materials and Methods:

➢ Plant Sample Collection

Figure1. *Artemisia* spp. geographical collection place (Kaunas, Kėdainiai, Šiauliai), June 2021.

➢ Isolation endophytic bacteria

Figure 2. Four distinct species of Artemisia plants and an example of bacterial strains exhibiting different colony shapes, colors, and margins.

➢ Molecular identification isolated endophytic bacteria by 16srDNA

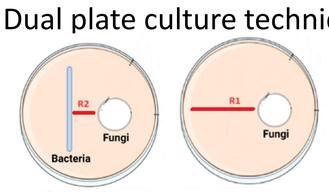
- DNA extraction from bacteria strains (CTAB DNA extraction protocol)
- Taxonomic identification (The bacterial 16S rDNA was amplified by PCR by universal primer pair 27F 5'-(AGAGTTTGATCMTGGCTCAG)- 3', and 1387R 5'-(GGGCGGWGTGTACAAG GC)-3'.

➢ Pathogenic fungi isolation

Pathogenic fungi were obtained from diseased pea roots. Morphological characteristics and molecular identification of the ITS region by ITS3f (5'-GCATCGATGAAGAACGCAGC-3') and ITS4r (5'-TCCTCCGCTTATTGATATGC-3') primers were employed for pathogenic fungi.

➢ Inhibition of fungal growth by isolated strains

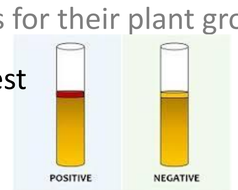
Dual plate culture technique on Potato Dextrose Agar (PDA) medium was used.



(R1-R2)/R1 × 100
R1: Radial growth pathogen in the absence bacteria
R2:Radial growth pathogen in the present bacteria

➢ Evaluation of isolates for their plant growth promotion potential

- Indole production test

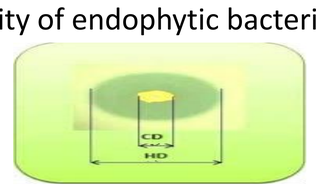


- Nitrogen fixation

The assessment of nitrogen fixation utilizing Ashby's N-free medium (NFM).

- Phosphate Solubilization activity of endophytic bacteria Based on Pikovskaya methods

SI=(CD+HD)/CD
CD: colony diameter
HD: halo zone diameter.



➢ Statistical analyses

Analysis of variance (ANOVA), R studio statistical software (4.2.3), Post hoc test (Tukey's HSD) ($P < 0.05$).

Results:

➢ Isolation endophytic bacteria from *Artemisia* spp.

In total, 61 bacterial endophytes displaying distinct morphologies were isolated. Gram staining revealed that 83% of isolates were Gram-positive. Sixty-one percent of the isolates showed positive catalase activity. Based on 16S rDNA gene, most of strains belonged to the phylum *Proteobacteria* and *Firmicutes* (Figure 3).

➢ Inhibitory effect of endophytic bacteria on the growth of *Fusarium* sp.

AR11 (90.83%) and VR24 (88%) strains isolated from the roots of *A. absinthium* and *A. vulgaris*, respectively, showed the highest inhibitory effect on *Fusarium* spp. growth.

➢ Biochemical properties and Effects effect of the endophytic bacteria on pea plant growth

In this study, 20% of the isolated bacteria exhibited positive results in the indole production test, while 44% demonstrated nitrogen fixation potential. Based on these and other test results, 8 isolated strains were selected to study their effects on pea seed germination and growth promotion. The selected isolates are listed in Table 1.

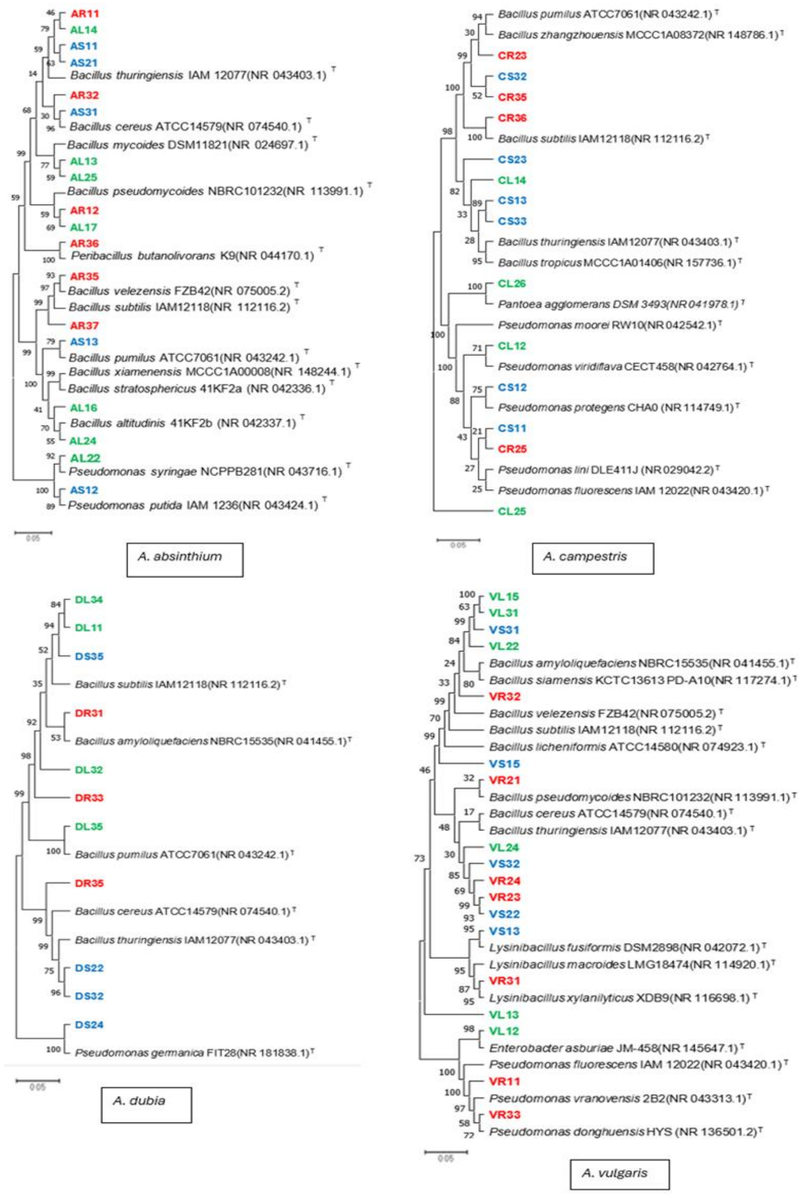


Figure3. Phylogenetic tree reconstruction based on 16S rDNA sequences of isolated endophytic bacteria

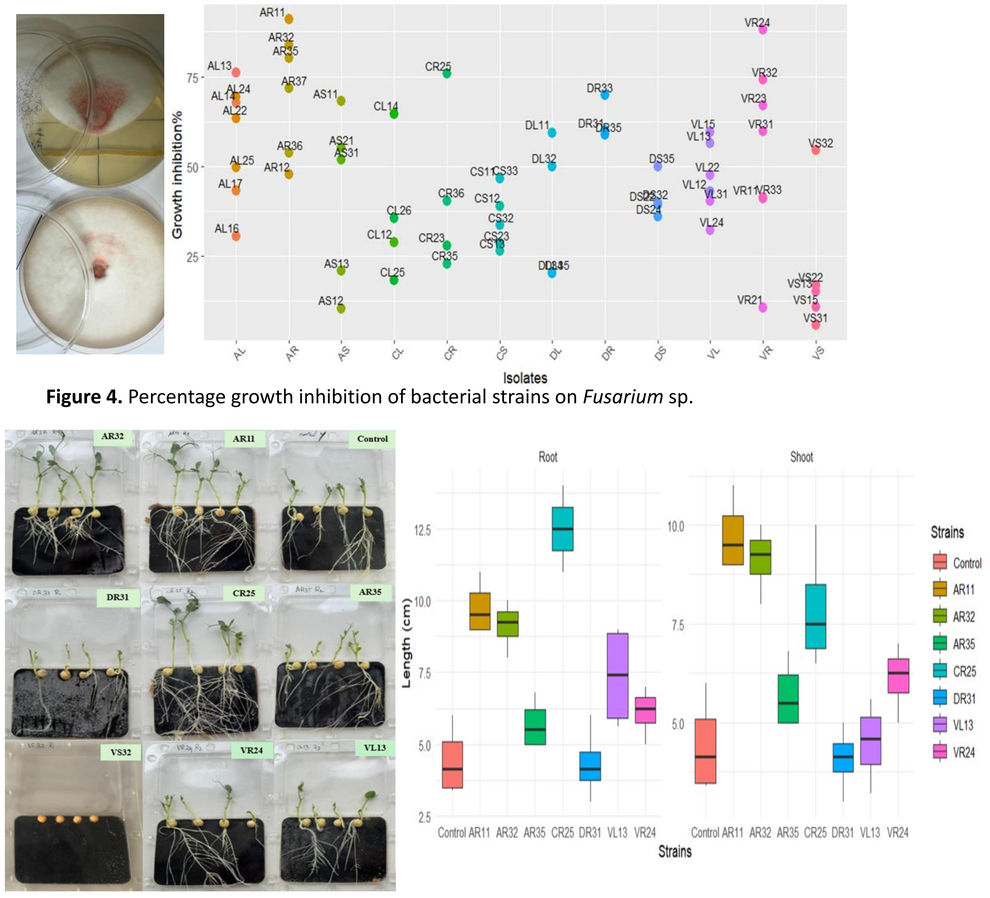


Figure 4. Percentage growth inhibition of bacterial strains on *Fusarium* sp.

Figure5. Effect of different endophytic bacteria strains on pea plant root and shoot growth after 10 days.

Table 1. Characteristics of selected endophytic bacteria

Isolate	plant species, Parts	related species	Similarity	Growth inhibition	PSI	Indole production test	Nitrogen fixation test
AR11	A. absinthium, Root	Bacillus thuringiensis IAM12077	100%	90.83%	2.93	+	+
AR32	A. absinthium, Root	Bacillus cereus ATCC14579	99.57%	83.8%	2.7	-	+
AR35	A. absinthium, Root	Bacillus velezensis FZB42	100%	80.8%	2.33	-	+
VR24	A. vulgaris, Root	Bacillus thuringiensis IAM12077	99.91%	88%	-	-	+
VL13	A. vulgaris, Leaf	Bacillus cereus ATCC14579	99.98%	56.36%	2.5	-	+
VS32	A. vulgaris, Stem	Bacillus cereus ATCC14579	99.91	54.43%	2.3	-	+
CR25	A. campestris, Root	Pseudomonas fluorescens IAM 12022	100%	75.86%	2.1	+	+
DR31	A. dubia, Root	Bacillus amyloliquefaciens NBRC15535	100%	60%	2.6	-	+

Conclusions

This study underscored the significant pathogen inhibition and plant growth enhancement potential of endophytic bacteria isolated from different *Artemisia* species. The diversity of endophytic bacteria, including strains of *Bacillus* and *Pseudomonas*, highlights their functional importance in promoting plant health. The growth inhibition activity against *Fusarium* c.f. *oxysporum* by strains like *B. thuringiensis*, *B. cereus*, *B. velezensis, B. amyloliquefaciens*, and *Pseudomonas fluorescens* underscores their role as effective biocontrol agents. Furthermore, the biochemical properties, including phosphate solubilization, nitrogen fixation, and indole production, particularly by strain AR11, demonstrate their potential as plant growth-promoting agents. AR11 emerged as a promising candidate for further investigation as a microbial biofertilizer, potentially offering an environmentally friendly alternative to chemical pesticides for crop protection.