# T1-P-38 Sessile oak rhizobacteria with plant growth-promoting potential *in vitro*

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**KEYWORDS:** rhizobacteria; sessile oak; growth promotion; *in vitro*

**INTRODUCTION:**

The rhizosphere is a complex habitat with great bacterial heterogeneity, and their activity, diversity, and dynamics are dictated primarily by plant root exudates. Plant growth-promoting rhizobacteria (PGPR) can be distinguished as a specific functional group that elevates plant characteristics and performance by direct and indirect mechanisms and are often members of genus *Bacillus* and *Pseudomonas*. PGPR of oak species in Serbia is poorly understood. Sessile oak (Quercus petraea (Matt.) Liebl) is autochthonous and one of Serbia's most significant forest species due to its economical, technical, ecological, and cultural importance. It is one of the most abundant tree species with a percentage of 7.7% in the growing stock (186.179 ha), and within the belt of its stands, there are 23 different forest types. The main problems of present sessile oak forests are continuous intensive decline, the domination of coppice forests (74.1%), age, and physiological susceptibility to (a)biotic stressors, which all guide to smaller seed yield and difficult natural regeneration. An additional problem is the low percentage of artificial reforestation success.

**OBJECTIVES:**

The objectives of this research were the isolation of bacteria of the genus *Bacillus* and *Pseudomonas* from the sessile oak rhizosphere, the in vitro investigation of their plant growth-promoting potential, and molecular identification of the most competent plant growth promoters.

**METHOD / DESIGN:**

The bacteria of the genus *Bacillus* and *Pseudomonas* were isolated by culturing methods from the sessile oak rhizosphere samples from mountain Rudnik where it naturally occurs. The Gram, catalase, and oxidase tests were performed, as did the fluorescent pigment production for potential pseudomonads. In addition, its plant growth-promoting abilities (IAA synthesis, siderophore production, and phosphate solubilization) were investigated *in vitro*. The selected isolates were molecularly identified based on the 16S rRNA gene sequence.

**RESULTS:**

A total of 179 bacteria were isolated from sessile oak rhizosphere samples, 75 of them being putative *Bacillus* and 48 putative *Pseudomonas* species. Of the total isolates, 155 of them were IAA producers, 81 siderophore producers, and 90 isolates were capable of phosphate solubilization. Fourteen most competent PGPR isolates were moleculary identified based on the 16S rRNA gene sequence as *Lysinibacillus parviboronicapiens, Viridibacillus arvi, Viridibacillus arenosi, Brevibacterium frigoritolerans, Peribacillus simplex, Rahnella variigena, Pseudomonas koreensis, Pseudomonas helmanticensis, Serratia quinivorans, Pseudomonas vancouverensis and Pseudomonas migulae*.

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**CONCLUSIONS:**

The four isolates, out of 179 isolated and investigated, were selected as potential sessile oak plant growth promoters for two of three tested features, being *Viridibacillus arvi, Pseudomonas migulae, Pseudomonas koreensis and Pseudomonas helmanticensis*. Further research is needed to confirm the plant growth-promoting potential of the bacterial isolates *in vivo*.

## T1-P-39 Dibutyl phthalate induces migration and angiogenesis of Ea.Hy926 human endothelial cells through Gper/Erk1/2 signaling

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**KEYWORDS:** dibutyl phthalate; human endothelial cells; cell migration; angiogenesis; ERK1/2

**INTRODUCTION:**

Cell adhesion and migration represent two opposing but intricately balanced functions of endothelial cells (ECs) important in maintaining the stability of the endothelium and during wound healing and angiogenesis. Regulation of cell adhesion and migration involves coordinated events including activation of a number of signaling pathways, cytoskeleton rearrangement, and surface integrin redistribution. Dysregulation of angiogenic factors and increased angiogenesis seem to play a key role in the pathophysiology of various diseases in humans such as tumor growth, progression and metastasis, as well as in many other non-malignant diseases but has also been demonstrated in atherosclerotic plaques as an important factor in early plaque development, intraplaque hemorrhage, plaque instability and rupture, and eventually, acute cardiovascular events. Although epidemiological studies suggest a possible association between exposure to dibutyl phthalate (DBP), a man-made chemical widely used in many industrial and consumer products, and cardiovascular diseases (CVDs), the impact that DBP exerts on EC migration and angiogenesis remains unclear.

**OBJECTIVES:**

Here, we sought to examine cell adhesion to extracellular matrix (ECM), migration, and angiogenesis after acute exposure of human vascular ECs to DBP and investigate the molecular events and signaling pathways involved in these processes.

**METHOD / DESIGN:**

EA.hy926 cells were exposed to either vehicle (0.05% DMSO – control) or three concentrations of DBP ($10^{-6}$, $10^{-5}$, and $10^{-4}$ M DBP in 0.05% DMSO) for up to 48 h. Cell viability was monitored using the alamarBlue™ assay. The adhesion assay on gelatin-coated cell culture plates was used to investigate the effect of DBP exposure on cell adhesion to the ECM. Cell migration was assessed using the wound-healing ("scratch") assay. Angiogenesis was assessed by monitoring endothelial tube formation in the growth factor-reduced ECM membrane-loaded cell culture plates. Gelatin zymography was used to detect the latent and activated forms of matrix metalloproteinases (MMPs) in cell culture media. Quantitative real-time PCR was used to determine mRNA expression levels, whereas Western blotting was employed to investigate protein expression. When indicated, pharmacological inhibitors were added 45 min prior to treatments and were present for the entire duration of the treatments. All results were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) with Dunnett's multiple comparison post-hoc test. A $p$ value of $< 0.05$ was considered significant.

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