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BIOLOGICAL PROPERTIES OF NITROGEN-FIXING BACTERIA  
ISOLATED FROM RHIZOSPHERE

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**Summary.** Nitrogen-fixing bacteria were isolated from rhizosphere of grape and characterized by the main biological properties: morphology, ability to grow on nutrient media, fatty-acid analysis. Strain was identified as a candidate to *Azotobacter* sp.

*Key words:* nitrogen-fixing microorganisms, fatty-acid profiles, rhizosphere

### **Introduction**

Nitrogen-fixing bacteria are very important for agriculture and biotechnology. *Azotobacter* spp. are the most known microorganisms able to fix nitrogen from atmosphere and thus supplying nitrogen-containing compounds to plants. Biotechnology of improving of plant growth relies on biological preparations containing beneficial microorganisms. Biopreparations based on nitrogen-fixing bacteria are very promising and include easy steps of application.

**Aim.** To isolate nitrogen-fixing microorganisms from rhizosphere of plants.

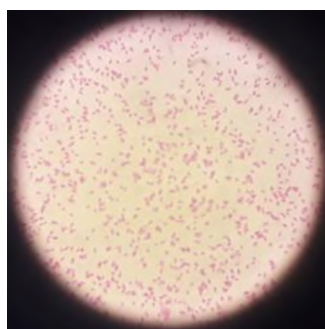
### **Materials and Methods**

Samples were picked from the upper 15 cm layer of soil and used for isolation of nitrogen fixing bacteria. Ashby medium without nitrogen source was used for the

experiment. Only nitrogen-fixing bacteria could grow on Ashby medium. Cultural characteristics were observed after incubation at 25-30°C for 24-48 hours. Bacteria from slimy colonies were characterized using the criteria of Bergey's Manual of Systematic Bacteriology. Identification by fatty-acid analysis with gas chromatography was carried out.

### Results and Discussion

4 strains of nitrogen-fixing microorganisms were isolated on Ashby medium. They formed white slimy mucous colonies. Gram staining indicated the presence of thin Gram-negative rods (Fig. 1). One strain was taken to further analysis.



**Fig. 1. Gram-negative cells of nitrogen-fixing bacteria isolated from grapevine rhizosphere.**

The study of fatty acid profile carried out by gas chromatography (Agilent, USA) allows to determine the composition of cells fatty acid (Table 1).

Table 1

#### **Fatty acid composition of a nitrogen-fixing bacterium isolated from grapevine rhizosphere**

<b>Peak Name</b>	<b>Percent</b>
12:0/ Dodecanoicacid	8.55
13:0/ Tridecanoicacid	0.59
14:0/ Tetradecanoicacid	6.67
15:0 anteiso/12-Methyltetradecanoic acid	0.36
Sum in Feature 2	20.24
Sum in Feature 3	22.72
16:1 w5c/ (11Z)-11-Hexadecenoic acid	0.39
16:0/ Hexadecanoicacid	16.89
15:0 iso 3OH/ 3-Hydroxy-13-Methyltetradecanoic acid	3.97
17:1 w8c/(9Z)-9-Heptadecenoic acid	0.70
17:0 cyclo/ cis-9,10-Methylene-Hexadecanoic acid	6.18

17:0/Heptadecanoic acid	1.14
Sum in Feature 8	11.27
18:1 2OH/ 2-Hydroxyoctadecanoicacid	0.33
Summed Feature 2	20.24
Summed Feature 3	22.74
Summed Feature 8	11.27

Biological properties allowed us to suggest that the isolated strain belongs to *Azotobacter* sp.

### **Conclusion**

The nitrogen-fixing microorganism candidatus to *Azotobacter* genus was isolated from rhizosphere of grape. This strain will be studied further for precise identification and for application in biotechnology of stimulation of plant growth.