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EFFECT OF NITROGEN NUTRITION ON THE GROWTH OF ENDOPHYTIC BACTERIA IN THE ROOTS OF *VITIS VINIFERA*

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Introduction. The cultivated grape (*Vitis vinifera*) belongs to the *Vitaceae* family and is one of the most economically important woody perennial fruit crops in the world. However the successful cultivation of grapes is hindered by susceptibility to its diseases which can be caused by a complex of pathogens including fungal etiology [2]. The development of measures to protect grapes is relevant and practically significant given that the greening of modern crop production is based on the use of a biological method of protecting plants from diseases. This method consists in the use of potential agents among various groups of microorganisms in order to use them for protection against phytopathogenic fungi [3]. According to Berlanas et al. PGPR-bacteria (plant growth stimulating rhizobacteria) such as *Pseudomonas* and *Bacillus* strains are the main colonizers of winerad roots [1]. The use of beneficial endophytic bacteria to control plant pathogens offers an ecofriendly alternative to pesticides. The search for such endophytic microorganisms, as well as the improvement of knowledge about their biology and cultivation, make it possible to expand the boundaries of the application of the biological method [4]. The study of the physiological and biochemical characteristics of bacteria is of great importance in the development of effective biological preparations.

For normal development and vital activity all microorganisms need nitrogen (nitrate, ammonium forms) since it is part of amino acids and participates in the formation of protein peptide bonds.

The aim of this work is to study the influence of nitrogen nutrition sources and select the optimal source of nitrogen in nutrient media for endophytic bacteria of *Vitis vinifera* roots which exhibit high antagonistic activity against phytopathogenic fungi.

Materials and methods. Endophytic bacteria were isolated from the roots of the Taiga Izumrud grape variety growing on the plantation of the Pinsk Winery. The roots were washed in tap water, immersed in ethanol (70%) for 30 s, surface disinfected in sodium hypochlorite (2% available Cl) for 3 min, and rinsed twice in sterile distilled water. As a control, the last water used to rinse the roots was plated onto potato dextrose agar (PDA). After disinfection, a suspension of mechanically destroyed roots was sown on PDA medium and incubated at 30°C for 48 hours. After that, antagonistic activity was screened against phytopathogenic fungi from the Belarusian collection of non-pathogenic microorganisms (*Fusarium oxysporum* BIM F-609, *Alternaria alternata* BIM F-119, *Cladosporium cladosporioides* BIM F-593, *Aspergillus awamori* BIM F-7, *Penicillium funiculosum* BIM F- 7, *Aspergillus terreus* BIM F-17). To study the sources of nitrogen nutrition a line of solid nutrient media was developed with the following composition: distilled water - 1000 ml, magnesium sulfate – 0,2 g, potassium dihydrogen phosphate - 1 g, sodium chloride - 5 g, glucose - 10 g, agar-agar - 15 g. Nitrogen sources were added to the medium before sterilization in the following amounts: fermented peptone and yeast extract - 10 g each; potassium nitrate, ammonium nitrate, ammonium sulfate, ammonium phosphate – 1 g each. Sterilization of the medium 0,5 atm. 15 minutes.

To determine the optimal source of nitrogen nutrition the cultures isolated from the endosphere of the grape root were transferred to a liquid glucose-peptone medium. The cultures were cultivated at a temperature of 37°C for 24 hours, after which they were diluted to 10^{-6} and inoculated on the above line of the nutrient medium. The seeded cups were placed in a thermostat at 30°C for 48 hours with peptone and yeast extract and 72 hours with mineral salts.

Results. In total 15 strains of bacteria were isolated from the endosphere of grape roots and 3 of them showed high antagonistic activity against collection phytopathogenic fungi. According to the results of identification by macro- and micromorphological features the bacteria were assigned to the genus *Pseudomonas* and designated as P1, P2 and P3. The maximum number of bacterial colonies (P1, P2, P3) was observed on the medium with peptone. On the medium with yeast extract the value of the studied parameter was lower by an average of 12%. The high level of the studied parameters in the variant with peptone, which is a protein hydrolyzate, is probably due to its nutritional properties and the presence of a high proteolytic activity of the studied bacterium. When cultivated on media with inorganic sources of nitrogen it was shown that the strains are able to assimilate both ammonium and nitrate nitrogen, carrying out assimilation nitrate reduction. The results of the study of the growth of strains of endophytic bacteria are presented in the table.

Table – The nature of the growth of the studied bacterial strains of the genus *Pseudomonas* on media with different sources of nitrogen nutrition

Strains	Nitrogen source					
	Peptone	Yeast extract	Potassium nitrate	Ammonium nitrate	Ammonium sulfate	Ammonium phosphate
P1	colonies large beige	colonies large beige	colonies small white	colonies small white	no growth	Colonies small beige
P2	colonies large beige	large white colonies	colonies large beige	colonies small beige	no growth	Colonies small white
P3	colonies large beige	large white colonies	colonies small white	colonies small beige	colonies small white	Colonies small white

The best source of inorganic form of nitrogen nutrition for the studied strains was potassium nitrate and ammonium nitrate. On a nutrient medium with nitrates as the only source of nitrogen there are differences in the growth pattern of the studied species of *Pseudomonas* in terms of color and size of colonies.

P3 on the medium with peptone had large beige matte colonies in places with small folds and on the medium with yeast extract had large white strongly convex crater-like colonies.

Conclusions. Fermented peptone and yeast extract are optimal sources of nitrogen for isolated endophytic bacteria of the genus *Pseudomonas.* In general, the possibility of using nitrogen sources of different chemical structure makes it possible to consider the isolated endophytic bacteria of the genus *Pseudomonas* as an object for the development of domestic biotechnology for obtaining biocontrol agents for diseases of *Vitis vinifera* and other agriculturally important crops.

References

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