

Y. Kononiuk, I. Marynova, N. Adarma, G. Stepaniuk, V. Bratkevich,

V. Tverdokhlib, O. Basiul, N. Limanska

COMPLEX INVESTIGATIONS OF PLANT MICROBIOTA REPRESENTATIVES

Odessa National I.I. Mechnikov University,

Dvorianska str., 2, Odessa 65082; e-mail: limanska@gmail.com

Summary. Phytopathogenic bacteria - crown gall agents - were isolated from grape plants. Antagonistic bacteria - also plant dwellers - were used to struggle these pathogens. *Lactobacillus plantarum* effectively inhibited crown gall agents and survived in soil at least for 45 days. Lactobacilli could interfere pathogenesis of the disease on a step of pathogen attachment. Together with *Bacillus megaterium*, lactobacilli showed stimulation effect on plant growth.

Key words: *Lactobacillus plantarum*, *Agrobacterium tumefaciens*, antagonism, survival

Complex investigations of microbiota of plants are carried out in Department of Microbiology, Virology and Biotechnology of Odessa National I.I. Mechnikov University. On the first stage of our research we isolate microorganisms from their natural environment - from plant vessels or plant surfaces. Our latest investigations included isolation of bacteria - representatives of endophytic microbiota of grape. We found out that the amount of microorganisms inhabiting plant vessels reached from $(1,5 \pm 0,2) \times 10^2$ to $(6,1 \pm 0,7) \times 10^4$ CFU/g.

Bacteria formed colonies of various morphology on nutrient media - round, slimy, with smooth edge, colorless or pinkish or white. 15,4 % of microorganisms from grape vessels were represented by yeasts, 38,5 % - by gram-positive bacteria and 46,1 % - by gram-negative bacteria. Using polymerase chain reaction assay, we found out the presence of pathogenic *Agrobacterium vitis* - crown gall agent - in 7,4 % of the tested grape plants cultivated on the south of Ukraine.

Next stage of our investigations included the application of strong bacterial antagonists in protection of plants against pathogenic agrobacteria. First, we used *Lactobacillus plantarum* strains because of their ability to inhibit a wide range of microorganisms [1, 2, 3].

We inoculated soil with lactobacilli to find out if they can survive in soil and still possess their antagonistic abilities. If a primary concentration of *L. plantarum* suspension $1,2 \times 10^9$ - $1,6 \times 10^9$ CFU/g was added to soil, lactobacilli survived at least 45 days in amount of 18 ± 1 - 37 ± 5 CFU/g in soil without plants and 37 ± 2 - 720 ± 20 CFU/g in soil of plant rhizosphere.

Lactobacilli isolated from soil after their adding there, had the same antagonistic activities as the museum strains. Thus, they inhibited 94 % of plant microbiota representatives, 57 % - gram-negative, 37 % - gram-positive. Only 6 % of microorganisms isolated from plants were resistant to antagonistic action of lactobacilli.

All studied museum strains of *L. plantarum* inhibited tumour formation in 93,3 - 96,7% of carrot explants. Using strain of *Agrobacterium tumefaciens* possessing GFP plasmid, we could find out that lactobacilli interfered crown gall pathogenesis on a step of attachment.

When test plants were treated with lactobacilli, less population of pathogen survived on plant surface. Moreover, agrobacteria could not penetrate the vessels of plants.

Lactobacilli also showed stimulating activity increasing plants growth in hydroponics and in soil. They could improve germination of treated seeds in 5 - 25%.

It was possible to create a consortium containing *L. plantarum* and *Bacillus megaterium*, which formed biofilms with well developed matrix on wheat seeds.

Literature

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