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EFFECT OF *LACTOBACILLUS PLANTARUM* ON SURVIVAL OF CROWN GALL AGENT AND TUMOUR FORMATION

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Aim. *To study the effect of Lactobacillus plantarum on formation of tumours and survival of Agrobacterium tumefaciens pJZ on surfaces and in tissues of plants.* ***Materials and Methods***. *Test-plants carrots Daucus carota L. cv Carotel, kalanchoe Kalanchoe daigremontiana Raym.-Hamet., tomatoes Solanum lycopersicum L. cv Ballada, grape Vitis vinifera L. cv Pinot noir and Moldova were inoculated with phytopathogenic A. tumefaciens pJZ expressing green fluorescent protein (GFP) and L. plantarum (L. plantarum ONU 12, ONU 311, ONU 355) in a ratio 1:1. Presence of GFP-labelled A. tumefaciens pJZ cells in inoculation sites was detected by epifluorescent microscopy.* ***Results***. *If inoculates contained lactobacilli, amount of plants with crown gall symptoms decreased in 85 – 100%. Mean weight of tumours decreased 2 – 12 times comparing with a control. Microscopy of tissues of symptomless plants showed that in case of treatment with lactobacilli pathogenic agrobacteria survived in 100% of carrots and grapes, 30% of tomatoes, but their populations were smaller than in positive controls inoculated only with phytopathogen and did not cause tumours. Inoculation of grape with a mixture of phytopathogen and lactobacilli resulted in stimulation of plant growth – amount of buds that grew increased in 25,8%, mean length of stems – in 28,3%, mean area of leaves – in 8,6%, that could be hypothesized as an overproduction of plant hormones as a result of possible transformation of plant tissues.* ***Conclusions***. *Antagonistic L. plantarum could suppress attachment and survival of phytopathogenic A. tumefaciens pJZ on surfaces and in tissues of test-plants eliminating population of the pathogen or decreasing its density with the probable attenuation of pathogenic properties, the mechanism of which needs further investigations.*

Keywords: Lactobacillus plantarum, Agrobacterium tumefaciens, survival, antagonism, crown gall, symptomless plants.

Bacteria from *Lactobacillus* genus are representatives of plant normal microbiota, the amount of them on non-damaged plant surfaces don't exceed 1% from total population of microorganisms but significantly increases if tissues are damaged [1, 2, 3]. Wounded plant surfaces are the gates for various phytopathogens, and active proliferation of lactobacilli exactly in wounded sites consuming sap from damaged tissues could be one of the mechanisms against pathogens realized by microorganisms from plant normal microbiota. Inhibition of phytopathogenic bacteria by lactic acid bacteria was described before [4, 5, 6, 7, 8, 9, 10]. But it is still an open question whether the suppression of pathogen occurs on a stage of initial interaction with plant surface, attachment stage or penetration in tissues.

The aim of this work was to study the effect of *Lactobacillus plantarum* on formation of tumours and survival of *Agrobacterium tumefaciens* pJZ on surfaces and in tissues of plants.

Materials and Methods. To study the possibility of inhibition of the phytopathogen *A. tumefaciens,* antagonistic strains *L. plantarum* – ONU 12, ONU 311 and ONU 355, isolated from grape must were used. Lactobacilli were cultivated overnight in MRS (de Man, Rogosa and Sharpe) broth at 37ºC [11] and brought to experiments in concentration 10^8 CFU/ml.

Strain *A. tumefaciens* pJZ carrying plasmid with *gfp* gene was used to study whether pathogenic agrobacteria penetrate tissues infecting test-plants, if they form biofilms on surfaces and if lactobacilli could suppress these processes [12]. Cells expressing this gene fluoresce under epifluorescence microscope with bright light of green color on dark green or red background of plant tissues. Phytopathogenic strain *Agrobacterium tumefaciens* pJZ labeled with GFP was kindly provided by Dr. Clay Fuqua (USA) and Dr. Igor Golovlev (Sweden). Agrobacteria were cultivated in LB (Luria-Bertani) broth at 28ºC and brought to experiments in concentration 10^8 CFU/ml.

Test-plants carrots *Daucus carota* L. cv Carotel, kalanchoe *Kalanchoe daigremontiana* Raym.-Hamet., tomatoes *Solanum lycopersicum* L. cv Ballada, grape *Vitis vinifera* L. cv Pinot noir and Moldova were used. Carrots were sterilized and cut on explants according to Ryder et al. (1985) [13]. Explants were inoculated with agrobacteria (positive control), lactobacilli (negative control) and a mixture "phytopathogen:lactobacilli" in a ratio 1:1 (experiment) [14]. Control plants were treated with water (negative control). Overnight cultures of *A. tumefaciens* pJZ and *L. plantarum* were mixed in a ratio 1:1 and immediately after mixing poured on a surface of freshly cut carrot discs, especially thoroughly on cambial ring. Symptoms of crown gall were evaluated after 30 days.

Kalanchoe plants were obtained by planting 6-7 cm cuttings. Rooted cuttings after 3 months were brought to experiment. Inoculation was carried out by injections of 10 µl of overnight cultures of agrobacteria and lactobacilli mixed in a ration 1:1 in stems. Tomato plants were grown up by planting seeds of cv Ballada in non-sterile commercial peat soil "Poliskii universalnii". Germination and cultivation of plants were carried out under green house conditions (18-22°C, light – 12 h). Two-month plants were inoculated with bacterial suspensions of *A. tumefaciens* pJZ by injections in stems. Symptoms of crown gall were estimated after 50 days. Plants were checked for tumours which were cut out and weighed. Studies of carrots explants, tomatoes, kalanchoe were carried out in three independent experiments (60 – 75 plants in each variant).

Freshly cut wooden stems of grape taken from the vineyard in the beginning of March were inoculated by soaking basal ends in bacterial suspensions of agrobacteria, lactobacilli or their mixture for 1 hour. Cuttings soaked in water were the negative controls. After, cuttings were rooted under green house conditions in peat soil without adding of any fertilizers or growth stimulators. After 30 days, the symptoms of crown gall (tumours and necroses) were

detected, and some growth characteristics of plants such as amount of survived cuttings, number of buds that grew, mean length of green stems and mean area of leaves were evaluated by standard methods [15, 16]. Studies on grapes were carried out during 3 years (2016, 2017, 2018), in each variant of independent experiments 75–100 cuttings were used.

Statistical evaluation of morphometric characteristics of plants and amount of galled plants was carried out in Microsoft Excel. Mean values and confidential intervals (95%) were calculated. Significant differences between experimental and controls values were estimated in t-test ($p<0,05$).

For microscopy, thin slices of tumours and plant tissues from inoculation sites were prepared and dyed with 0,1% crystal violet for 40 sec to minimize the natural fluorescence of plant tissues interfering detection of GFP-labelled cells [17]. Optical Carl Zeiss epifluorescence microscope system with 20x planachromat objective and Olympus DCM camera was used. Images of the biofilms on plant roots surfaces were obtained with BP490 filter set, a 505 nm dicroic filter and 530 nm long-pass emitter (EO530).

Presence of *A. tumefaciens* pJZ was detected by bright light green fluorescence of GFP in phytopathogen cells. Single green fluorescent cells and aggregates of bacteria were detected.

Results. Treatment with tested lactobacilli interfered formation of tumours by pathogenic agrobacteria, and level of protection depended on lactobacilli strain and species of test-plant (Table 1).

Amount of crown galled plants decreased in 85 – 100%. If some plants treated with lactobacilli and agrobacteria still exhibited symptoms of the disease, their manifestation were smaller than in positive control inoculated just with *A. tumefaciens* pJZ: mean weight of tumours decreased 2 – 12 times (Table 2).

To find out if pathogenic agrobacteria penetrate test-plants tissues in case of the obvious antagonistic effect of lactobacilli resulted in absence of the disease, microscopy of tissue slices from inoculation sites was carried out. It was found out that in all symptomless carrot explants treated with agrobacteria and lactobacilli, vivid motile cells of the phytopathogen were observed (Fig. 1, B, C, D). But density of their population was much smaller than in explants inoculated only with *A. tumefaciens* pJZ (Fig. 1, A).

Table 1

Effect of inoculation with *A. tumefaciens* pJZ and *L. plantarum* on tumour formation in test-plants

Strain	Percentage of crown galled plants			
	Carrot	Kalanchoe	Tomatoes	Grape
L. plantarum ONU 12, *A. tumefaciens* pJZ	4,0±0,8%	0	0	5,0±0,7%
L. plantarum ONU 311, *A. tumefaciens* pJZ	8,0±0,6%	0	0	n/t*
L. plantarum ONU 355, *A. tumefaciens* pJZ	15,0±1,2%	9,7±1,8%	0	n/t
A. tumefaciens pJZ (control)	100%	100%	100%	100%

Note: *n/t – non-tested

Table 2

Mean weight of tumour tissues on test-plants, µg

Test-plant	Control	Experiment
Carrot	17,1±2,1	8,0±0,7
Kalanchoe	298,0±11,9	25,4±2,3
Tomatoes	19,3±1,4	0
Grape	915,5±29,8	282,0±18,6

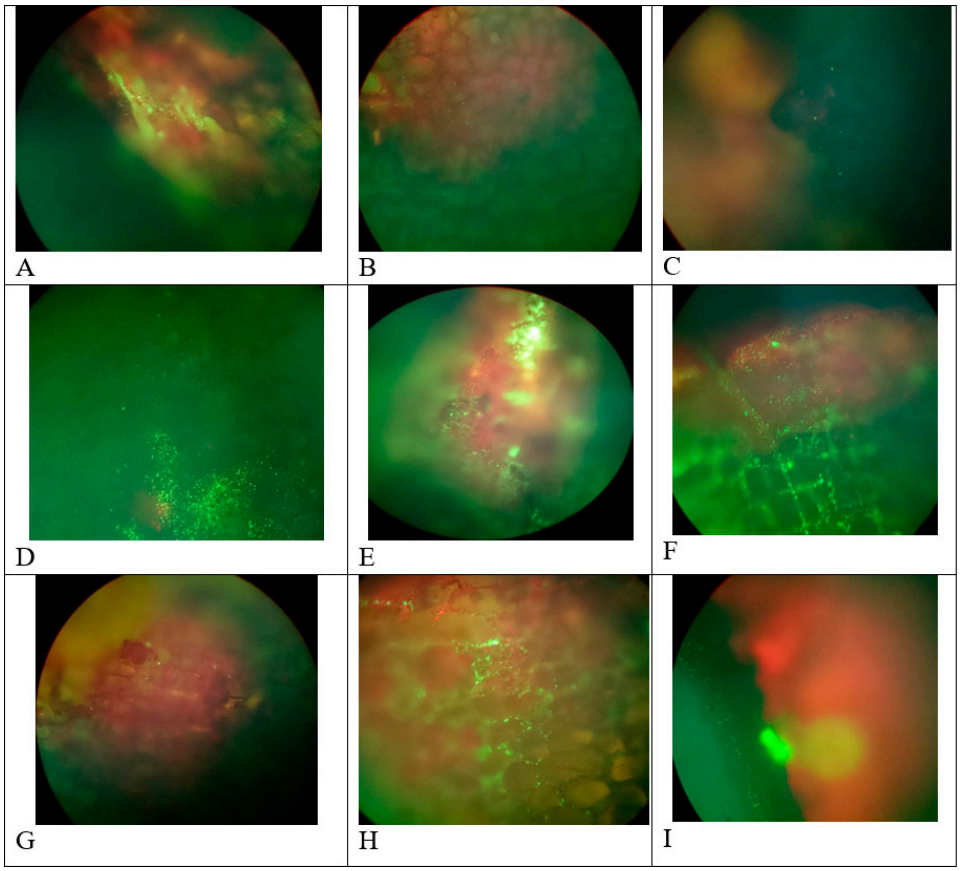

Fig. 1. Microphotographies of tissue slices from carrot explants inoculated with *A. tumefaciens* and *L. plantarum* (600x): sites of inoculation, A – *A. tumefaciens* pJZ (control); B – *L. plantarum* ONU 12 + *A. tumefaciens* pJZ; C – *L. plantarum* ONU 311+ + *A. tumefaciens* pJZ; D – *L. plantarum* ONU 355 + *A. tumefaciens* pJZ; tumours, E - *A. tumefaciens* pJZ (control); F – *L. plantarum* ONU 311 + *A. tumefaciens* pJZ; G - *L. plantarum* ONU 12 + *A. tumefaciens* pJZ; H - *L. plantarum* ONU 355 + + *A. tumefaciens* pJZ; I – ONU 355 + *A. tumefaciens* pJZ – tumour and biofilm.

Agrobacteria in symptomless explants did not penetrate vessels opposite to tumour tissues in which vessels were tightly filled with phytopathogen cells fluorescing due to *gfp* gene expression (Fig. 1, E). On a figure 1, I, with microphotography of carrot slice treated with a mixture *L. plantarum* ONU 355 and *A. tumefaciens* pJZ a dense, well formed biofilm with developed matrix layer can be seen. Inside the matrix fluorescent cells are observed. It is necessary to point out that if in presence of lactobacilli tumours were still

formed, in their tissues smaller agrobacterial populations were found as it could be seen from fluorescence of single cells (Fig. F, G, H, I), but not the dense aggregates as it was observed in the control not treated with antagonists (Fig. 1, E).

Opposite to carrot, in kalanchoe agrobacteria were not detected in any symptomless plant (Fig. 2, B). It means that on kalanchoe wound surfaces agrobacteria, which did not cause the disease, did not survive when treated with lactobacilli. On tomatoes where 100% protection against tumour formation was observed, the microscopy of tissue slices showed that agrobacteria, opposite to kalanchoe, could survive in inoculation sites when treated with lactobacilli. Fluorescent *A. tumefaciens* pJZ were found in these sites (Fig. 2, D).

In 30% of symptomless tomato plants agrobacteria were found (Table 3), but in other 70% of tomatoes *A. tumefaciens* pJZ cells were not detected in inoculation sites when treated with lactobacilli (Fig. 2, E).

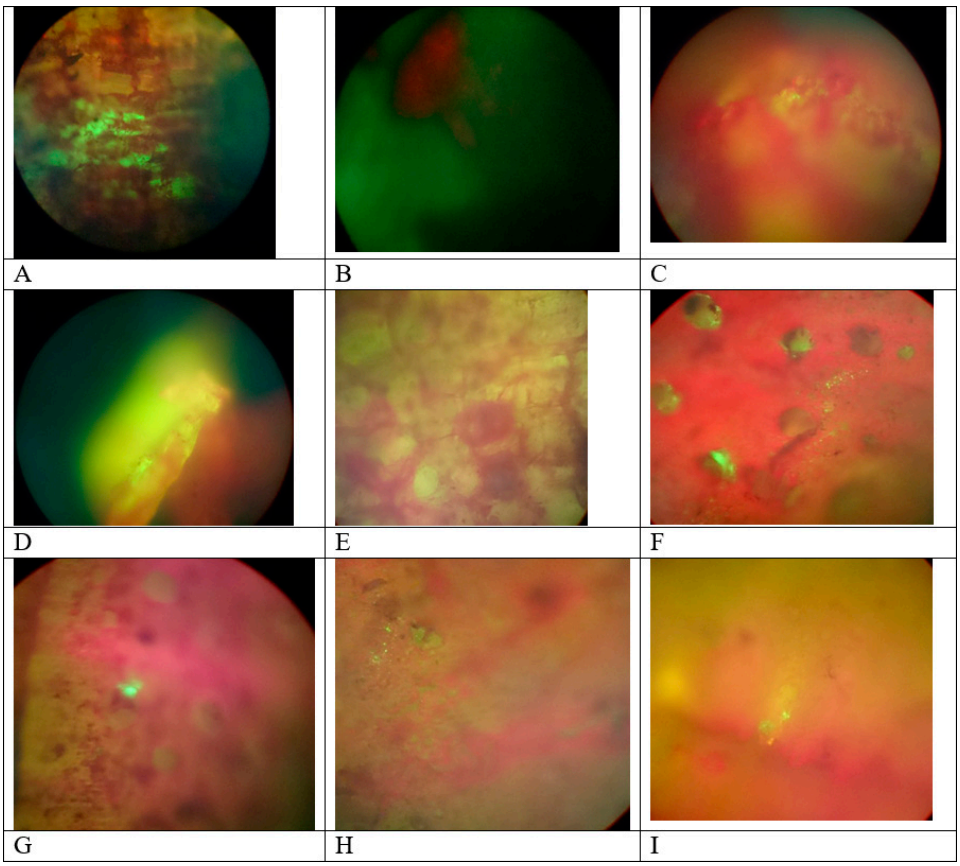

Fig. 2. Microphotographies of tissue slices from kalanchoe, tomatoes and grape cuttings inoculated with *A. tumefaciens* pJZ and *L. plantarum* (600x): tumours, A – *A. tumefaciens* pJZ control, kalanchoe; B – *L. plantarum* ONU 12 + *A. tumefaciens* pJZ, kalanchoe; C – *A. tumefaciens* pJZ – control, tomatoes; site of inoculation, D – *L. plantarum* ONU 355 + *A. tumefaciens* pJZ, tomatoes; E – *L. plantarum* ONU 12 + + *A. tumefaciens* pJZ, tomatoes; vessels of grape, distance from base of cuttings, F – *A. tumefaciens* pJZ, control, 3 cm; G – *L. plantarum* ONU 12 + *A. tumefaciens* pJZ, 3 cm; H – *A. tumefaciens* pJZ, control, 10 cm; I – *L. plantarum* ONU 12 + + *A. tumefaciens* pJZ, 5 cm.

Table 3

Percentage of symptomless plants which tissues after inoculation with agrobacteria and lactobacilli contained *A. tumefaciens* pJZ cells

Test-plant	Percentage of plants sustaining phytopathogen, %
Carrot	100%
Kalanchoe	0
Tomatoes	30,0%
Grape	100%

In grapes, the same as in carrots, pathogenic agrobacteria were found in tissues of all symptomless plants (Table 3, Fig. 2, G, H, I). Microscopy of horizontal cuts taken on various distances from basal ends of the cuttings has shown that bacteria move in vessels 10 cm higher from inoculation site in positive controls inoculated with *A. tumefaciens* pJZ (Fig. 2, H), and 5 cm higher – in cuttings inoculated with a mixture of lactobacilli and agrobacteria (Fig. 2, I). On these distances single cells were detected whereas in inoculation sites and 1 – 3 cm higher xylem vessels were tightly filled with agrobacteria, and in controls phytopathogens penetrate all vessels and nearby tissues (Fig. 2, F), whereas in cuttings treated with lactobacilli the presence of pathogens was restricted only by several xylem vessels (Fig. 2, G). No agrobacteria were found in green shoots.

In grape inoculation with a mixture of agrobacteria and lactobacilli resulted in unexpected stimulation of plant growth: amount of buds that grew increased in 25,8%, mean length of stems – in 28,3%, mean area of leaves – in 8,6% as compared with the negative control – cuttings soaked in water instead of bacterial suspensions (Table 4, Fig. 3, B).

Table 4

Effect of *A. tumefaciens* pJZ and *L. plantarum* on growth characteristics of grape plants

Variant of the treatment	Survived cuttings (%)	Buds that grew (%)	Length of the stem, cm	Leaf area, cm^2
Control (water)	85,2±3,7	70,4±3,1	5,1±0,8	8,4±0,3
A. tumefaciens pJZ	51,3±4,2*	42,2±5,4*	2,2±0,8*	6,6±0,3*
L. plantarum ONU 12	90,0±2,8	77,1±2,1*	6,2±0,3*	8,9±0,2*
L. plantarum ONU 12 + *A. tumefaciens* pJZ	92,1±3,8	96,2±2,8*	7,1±0,4	9,2±0,4*
CFS** *L. plantarum* ONU 12	87,0±3,2	68,4±4,2	5,3±0,3	6,8±0,6*
CFS *A. tumefaciens* pJZ	80,8±2,9	66,4±0,8	5,0±0,4	6,2±0,4*
CFS суміш *A. tumefaciens* pJZ+*L. plantarum* ONU 12	78,8±3,4*	66,4±2,6	5,6±0,8	7,2±0,5*
A. tumefaciens pJZ + CFS *L. plantarum* ONU 12, pH 4,2	70,2±3,2*	82,4±2,2*	6,8±0,5*	8,6±0,6
MRS, pH 4,2	72,8±5,3*	68,4±3,5	3,9±0,3*	5,1±0,5*
LB, pH 6,8	83,7±2,5	71,0±2,1	4,7±0,2	7,6±0,3*
MRS + LB, pH 5,2	86,3±2,9	73,1±5,2	5,4±0,2	7,2±0,4*

Note: * - significantly different from the control, CFS** - cell-free supernatant

Inoculation of grape with agrobacteria caused significantly negative effect: amount of survived cuttings decreased comparing with the control in 34%, amount of buds that grew – in 28,2%, mean length of green stems – in 56,8%, mean leaf area – in 21,4% (Fig. 3, A).

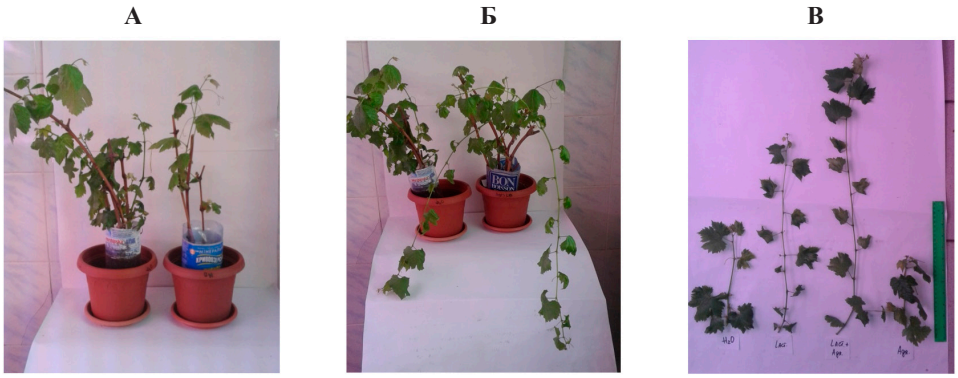

Fig. 3. Grape plants treated in different variants: A – soaked in water, negative control (left), infected with agrobacteria, positive control (right); B – negative control (left), inoculated with a mixture of agrobacteria and lactobacilli (right); C – green stems of grapevine, variants from left to right: negative control, treated with lactobacilli, inoculated with a mixture of lactobacilli and agrobacteria, inoculated with agrobacteria.

Inoculation only with *L. plantarum* ONU 12 improved some plant characteristics as compared with the control (water): amount of buds that grew increased in 6,7%, mean length of stems – in 21,6% (Table 4). Interestingly that lactobacilli in a mixture with agrobacteria increased morphometric characteristics of grape much more. Soaking of cuttings in MRS medium with the same pH as pH of overnight culture of lactobacilli, and in LB medium, treatment with CFS of *L. plantarum* ONU 12 and with a mixture of CFS of *A. tumefaciens* pJZ and CFS of *L. plantarum* ONU 12 did not cause any positive effect on plant growth, opposite – some growth characteristics decreased in 6,4 – 39,0%.

Discussion. The results of investigation indicate that *L. plantarum* actively inhibit *A. tumefaciens* pJZ completely suppressing crown gall symptoms in 85,0 – 100% of test-plants inoculated with a mixture of phytopathogenic agrobacteria and lactobacilli.

Lactobacilli interfered attachment and survival of *A. tumefaciens* pJZ on surfaces and in tissues of test-plants: less density of GFP-labelled agrobacteria was detected by fluorescent microscopy. If in case of treatment with lactobacilli tumours were still formed, their amount and weight were significantly smaller than in controls (2 – 12 times) and inside tumours the populations of agrobacteria were presented by single cells and not by cell aggregates.

Unexpected, vivid agrobacteria survived on surfaces and in tissues of symptomless plants even in case of treatment with antagonistic lactobacilli. Single cells of phytopathogens expressing gene *gfp* were found in inoculation sites (carrot, tomatoes), on plant surfaces as the components of biofilms (carrot

explants) and in vessels on a distance higher from inoculation sites in grape plants. Only in case of kalanchoe, if tumours were not formed, agrobacteria did not survive not on the surfaces nor inside the tissues in inoculation sites that could be explained by the bactericidal metabolites of this plant described in literature [18]. Earlier we have showed by the method of inoculation on nutrient media that in presence of lactobacilli cultivable agrobacteria disappeared from kalanchoe surfaces already on the third day after the treatment of plant [19]. Using in present study GFP-labelled agrobacteria allowed to confirm this fact by microscopy of plant tissues.

On nutrient media *L. plantarum* actively inhibited growth of crown gall agent [8, 10]. Significant decrease in symptoms manifestation described in present work indicated the antagonistic effect of lactobacilli also in experiments on test-plants. Lactobacilli are able to attach to plant surfaces [20], so a hypothesis can be proposed that pathogenic agrobacteria in presence of lactobacilli could survive but loose the ability to cause the disease. Probably, some attenuation of the pathogen occurs mechanism of which needs further investigation. Unexpected stimulation of growth characteristics of grape plants could confirm this hypothesis. Bacterial suspensions but not a cell-free supernatant caused stimulation (Table 4), and positive effect could not be explained by the nutrient compounds of the medium or metabolites from overnight cultures. Opposite, nutrient media and CFS decreased some morphometric characteristics of plants. Treatment only with lactobacilli increased amount of buds that grew in 6,7%, and mean length of green stems – in 13%. Effect of lactobacilli mixed with agrobacteria was significantly much more higher: amount of buds that grew increased in 25,8%, mean length of stems – in 28,3%. Moreover, mean area of leaves increased in 8,6%, and plants appeared healthy and well developed though on some especially long stems some young leaves were triangle-shaped (Fig. 3, C), and resembled leaves of plants in case of the overtreatment with growth hormones [21]. In 5% of cuttings inoculated with lactobacilli and agrobacteria, in which tumours were still formed, such stimulation effect was not found.

If cuttings were treated with *A. tumefaciens* pJZ mixed with CFS of *L. plantarum* ONU 12, stimulation effect was the same as in cuttings inoculated with bacterial mixture "lactobacilli:agrobacteria" which indicated that certain effect could be caused not only by the cells of *L. plantarum* but by their metabolites too (Table 5). Antagonistic effect was not caused just by the metabolites of lactobacilli and low pH of medium, but by the presence of antagonistic bacteria: amount of survived cuttings in control variants treated with CFS of lactobacilli decreased the number of survived cuttings in 15% whereas adding of vivid bacteria *L. plantarum* ONU 12 not only prevented manifestation of agrobacterial infection in 95% but also improved the survival of cuttings. So, antagonistic and stimulation effects of treatments with a mixture of lactobacilli and agrobacteria are probably mediated by different mechanisms.

Overproduction of cytokinin was described in 1938 Locke et al. who shown that in case of infection with attenuated agrobacteria a stimulation of buds distal to tumours occurred [22]. In our investigation on grape tumours in majority of cases were not formed but changes in plant morphology typical for overproduction of phytohormones were observed [21]. It is known that

agrobacteria do not produce plant hormones by themselves, overproduction of phytohormones are carried out in plant cells after T-DNA from Ti-plasmid of the pathogen incorporates into plant genome [22]. Probably, in our case transfer of T-DNA with the next transformation of plant cells occurs but tumours are not formed. Indeed, in 2005 Brencis et al. have shown that agrobacteria are able to transform plant cells without the next tumour formation: normal, non-tumour cells of tobacco with T-DNA incorporated in their genome actively produced octopin – a product intrinsic just for the cells transformed by agrobacteria [23]. Unusual stimulation of grape growth with altered morphology of leaves indicates possible transformation of grape cells with T-DNA resulting in overproduction of phytohormones without tumour formation. Probably, certain metabolites of lactobacilli could attenuate *A. tumefaciens* pJZ in such way that agent survives on plant tissues and could even transform plant cells as it possibly occurs on grape, but tumours in majority of cases are not formed. Mechanism of such attenuation needs further investigations on biochemical and molecular biological levels.

Conclusion. Our investigations indicate that antagonistic *L. plantarum* can suppress attachment and survival of phytopathogenic *A. tumefaciens* pJZ on surfaces and in tissues of test-plants eliminating population of the pathogen or decreasing its density with the probable attenuation of pathogenic properties.

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ВПЛИВ БАКТЕРІЙ *LACTOBACILLUS PLANTARUM* НА ВИЖИВАННЯ ТА УТВОРЕННЯ ПУХЛИН ЗБУДНИКОМ БАКТЕРІАЛЬНОГО РАКУ РОСЛИН

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Резюме

Мета. Вивчення впливу бактерій *Lactobacillus plantarum* на утворення пухлин та виживання *Agrobacterium tumefaciens* pJZ на поверхнях та в тканинах рослин. **Матеріали і методи**. Тест-рослини моркви *Daucus carota* L. сорту Каротель, каланхое *Kalanchoe daigremontiana* Raym.-Hamet., томатів *Solanum lycopersicum* L. сорту Балада, винограду *Vitis vinifera* L. сортів Піно чорний та Молдова інокулювали фітопатогенними бактеріями *A. tumefaciens* pJZ, що експресують білок GFP, і лактобактеріями *L. plantarum* (*L. plantarum* ОНУ 12, ОНУ 311, ОНУ 355) у співвідношенні 1:1. Наявність клітин *A. tumefaciens* pJZ у місцях інокуляції враховували за світінням

білка GFP методом епіфлуоресцентної мікроскопії. **Результати**. Кількість рослин з симптомами бактеріального раку за присутності в інокуляті лактобацил зменшувалася на 85 – 100%. Середня маса пухлин зменшувалася у 2 – 12 разів у порівнянні з контролем. Мікроскопування зрізів безсимптомних рослин показало, що за обробки лактобацилами патогенні агробактерії виживали у 100% рослин моркви і винограду, 30% рослин томату, але їх популяції були значно меншими, ніж у позитивних контролях, інокульованих лише фітопатогеном, і пухлини під їх впливом не утворювалися. На винограді інокуляція сумішшю фітопатогена і лактобацил призвела до стимуляції росту, яка проявлялась у збільшенні кількості бруньок, що розпустилися, на 25,8%, середньої довжини пагонів – на 28,3%, середньої площі листків – на 8,6%, що могло бути свідченням надпродукції рослинних гормонів внаслідок ймовірної трансформації рослинних клітин. **Висновки**. Антагоністи *L. plantarum* здатні пригнічувати адгезію та виживання фітопатогенних *A. tumefaciens* pJZ на поверхнях і в тканинах тест-рослин, елімінуючи популяцію патогена або зменшуючи її щільність з ймовірною атенуацією властивостей, механізм якої потребує подальших досліджень.

Ключові слова: Lactobacillus plantarum, Agrobacterium tumefaciens, виживання, антагонізм, бактеріальний рак рослин, безсимптомні рослини

ВЛИЯНИЕ БАКТЕРИЙ *LACTOBACILLUS PLANTARUM* НА ВЫЖИВАНИЕ И ОБРАЗОВАНИЕ ОПУХОЛЕЙ ВОЗБУДИТЕЛЕМ БАКТЕРИАЛЬНОГО РАКА РАСТЕНИЙ

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Резюме

Цель. Изучение влияния бактерий *Lactobacillus plantarum* на образование опухолей и выживание *Agrobacterium tumefaciens* pJZ на поверхностях и в тканях растений. **Материалы и методы**. Тест-растения моркови *Daucus carota* L. сорта Каротель, каланхое *Kalanchoe daigremontiana* Raym.-Hamet., томатов *Solanum lycopersicum* L. сорта Баллада, винограда *Vitis vinifera* L. сортов Пино черный и Молдова инокулировали фитопатогенными бактериями *A. tumefaciens* pJZ, экспрессирующими белок GFP, и лактобактериями *L. plantarum* (*L. plantarum* ОНУ 12, ОНУ 311, ОНУ 355) в соотношении 1:1. Наличие клеток *A. tumefaciens* pJZ в местах инокуляции учитывали по свечению белка GFP методом эпифлуоресцентной микроскопии. **Результаты**. Количество растений с симптомами бактериального рака в случае присутствия в инокуляте лактобацилл уменьшалось на 85 – 100%. Средняя масса опухолей уменьшалась в 2 – 12 раз по сравнению с контролем. Микроскопирование срезов безсимптомных растений показало, что в случае обработок лактобациллами патогенные агробактерии выживали в 100% растений моркови и винограда, 30% растений томатов, но их популяции были значительно меньшими, чем в положительных контролях, инокулированных только фитопатогеном, и опухоли под их влиянием не образовывались. На винограде инокуляция смесью фитопатогена и лактобацилл привела к стимуляции роста, которая проявлялась в увеличении количества распустившихся почек на 25,8%, средней длины побегов – на 28,3%, средней площади листьев – на 8,6%, что могло быть свидетельством сверхпродукции растительных

гормонов вследствие вероятной трансформации растительных клеток. **Выводы**. Антагонисты *L. plantarum* способны угнетать адгезию и выживание фитопатогенных *A. tumefaciens* pJZ на поверхностях и в тканях тест-растений, элиминируя популяцию патогена или уменьшая ее плотность с возможной аттенуацией свойств, механизм которой требует дальнейшего изучения.

Ключевые слова: Lactobacillus plantarum, Agrobacterium tumefaciens, выживание, антагонизм, бактериальный рак растений, безсимптомные растения

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