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EFFECT OF *LACTOBACILLUS PLANTARUM* ON PHYTOPATHOGENS AND PLANT GROWTH

Treatments with Lactobacillus plantarum can be recommended for the protection of plants against crown gall and soft rot (caused by Agrobacterium tumefaciens and Erwinia carotovora, respectively). Soaking of seeds in cell free suspensions of lactobacilli as well as in bacterial suspensions of Lactobacillus increased germination of tomatoes in 12-16%, treatment of wheat seeds with 1% of bacterial suspensions - in 20,0 - 36,0%. Mean length of wheat seedling roots increased in 9,0 - 22,4%, mean height of the wheat plants - in 19,2 - 28,0%. Further studies of L. plantarum and their metabolites for possible application in organic agriculture are needed.

First evidences of successful treatments of plants and soil with lactobacilli had been described in 1980s (Visser et al. 1986; Higa and Kinjo 1989) but till now there are few publications on this topic in scientific literature. Our investigations during last several years have shown the high potential of application of lactic acid bacteria for organic agriculture (Limanska et al., 2013).

The aim of our investigation was to study the antagonistic and stimulation activity of 31 *Lactobacillus plantarum* strains.

Methods. Lactobacilli were grown overnight on MRS medium at 37°C (de Man et al., 1960) and phytopathogens *Agrobacterium*, *Ralstonia* and *Erwinia* - on LB medium at 28°C (Bertani, 1952). The prevention of crown gall infection was studied by wounding kalanchoe and tomato plants and carrot explants with a mixture "phytopathogen:lactobacilli" in 1:1 ratio. The effect on *Ralstonia* was studied by soaking roots of tomato seedlings in a "phytopathogen:lactobacilli" mixture. Inhibition of erwinias was revealed on carrot explants. To study the stimulation of plant growth, wheat and tomatoes seeds were used. Wheat grains were soaked in 1% of overnight suspension of *L. plantarum* for 1 hour and after - put in Petry's dishes or planted in pot soil under the greenhouse conditions. After 5 days, germination, mean lengths of the plants and their roots were evaluated. The effects of *Lactobacillus* cell suspension and their cell-free cultural liquid were compared on tomato plants. In this case, cells washed three times from the cultural liquid and diluted in sterile distilled water till the initial concentration of the overnight suspensions were used as a control.

Results and Discussion. All tested strains of *L. plantarum* showed antagonistic activity against soft rot (*Erwinia carotovora*), crown gall (*Agrobacterium tumefaciens*) and wilt (*Ralstonia solanacearum*) agents on the nutritional medium. *In vivo* lactobacilli suppressed crown gall infection in kalanchoe plants in 86,7% to 100%, and in 32,0% to 100% - in carrot explants. It was the effect of low pH of cultural liquid that explained the antagonistic properties of the investigated strains of *L. plantarum*. The majority of *L. plantarum* strains (33,3% i 53,3%) caused the inhibition of soft rot agent at average and high level. Opposite, wilt agent was more resistant to lactobacilli. The majority of *Lactobacillus* strains – 80% - exhibited the low level of *Ralstonia solanacearum* inhibition. Thus, the antagonistic activity against different phytopathogens was the strain-specific capability. Application of *L. plantarum* can be recommended for the protection of plants against crown gall and soft rot, but not against bacterial wilt.

Treatment of wheat seeds with *L. plantarum* 1% suspensions resulted in increasing of the mean length of seedling roots in 9,0 - 22,4%, and mean height of the wheat plants - in 19,2 - 28,0%. Germination was also improved - in 20,0 - 36,0% *in vitro* in Petry's dishes and in 24,0 - 36,0% in pot soil. Cell free liquid of overnight *Lactobacillus* cultures increased the germination of tomato seeds almost the same well as bacterial suspensions did (12% and 16%, respectively). This means

that the metabolites of lactobacilli also have the positive effect on plant germination. The further study of *L. plantarum* metabolites and their possible application in organic agriculture is needed.

The possible reason why lactobacilli are not so widely used in organic agriculture as they could be used - is their strong dependence on rich nutrient media. That's why their rate of survival in soil and on epiphytic surfaces remains questionable. Our investigations have shown that bacteria *Lactobacillus plantarum* could survive on the surfaces of test plants (tomatoes, kalanchoe, grape) at least for one month (Korotaieva et al., 2015). Studies of Hoda et al. (2011) on tomatoes under the field conditions and our experiments on tomatoes and kalanchoe in a greenhouse showed 2-3 months of survival of lactobacilli in soil (unpublished data). So, these terms are sufficient for application of biological preparation - plants and/or soil should be treated with bacterial suspensions several times during the season. The next question which can throw some doubts - the success of competition with other representatives of plant microbiota with higher rates of multiplication under the limited nutritional conditions. Our investigations have shown the complete eradication of the phytopathogen *Agrobacterium tumefaciens* C58 from the surfaces of test plants on the third day of the experiment in case when a mixture of *L. plantarum* and agrobacteria were applied (Korotaieva et al., 2015). Lactobacilli are not so popular biocontrol and biostimulation agents as *Pseudomonas* and *Bacillus* yet - but these lactic acid bacteria have a very important advantage which no other microorganisms have: they possess GRAS status (Generally Recognized As Safe) which means that are absolutely safe to human and animals. That's why lactobacilli without any doubts can be used in organic agriculture.

Conclusion. *L. plantarum* are the perspective microorganisms for plant growth stimulation and protection against phytopathogens. Further studies of *L. plantarum* and their metabolites for possible application in organic agriculture are needed.

References

1. Bertani G (1951) Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J Bacteriol* 62: 293-300.
2. De Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. *J Appl Bacteriol* 23:130-135.
3. Higa T, Kinjo S (1989) Effect of lactic acid fermentation bacteria on plant growth and soil humus formation. In: Proceedings of 1th Int. Conf. on Kyusei Nature Farming, Khon Kaen, Thailand.
4. Hoda AH, Yomna AM, Shadia MA (2011) *In vivo* efficacy of lactic acid bacteria in biological control against *Fusarium oxysporum* for protection of tomato plant. *Life Science J* 8:462-468.
5. Korotaieva NV, Limanska NV, Basiul OV, Sergeeva Zh, Ivanytsia VO (2015) The survival of lactobacilli and agrobacteria introduced into plant phyllosphere. *Studia Biologica* 9: 23-30.
6. Limanska N, Ivanytsia T, Basiul O, Krylova K, Biscola V, Chobert J-M, Ivanytsia V, Haertle T. (2013) Effect of *Lactobacillus plantarum* on germination and growth of tomato seedlings. *Acta Physiol Plant* 35: 1587-1595.
7. Visser R, Holzapfel WH, Bezuidenhout JJ, Kotze JM (1986) Antagonism of lactic acid bacteria against phytopathogenic bacteria. *Appl Environm Microbiol* 52:552-555.