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EFFECT OF *LACTOBACILLUS PLANTARUM* ON GROWTH CHARACTERISTICS OF WHEAT IN HYDROPONICS AND SOIL

The aim *was to study the effect of Lactobacillus plantarum on germination and some growth characteristics of wheat Triticum aestivum L. in hydroponics and soil under greenhouse and field conditions.* ***Materials and Methods.*** *Strains of L. plantarum, their mixtures in a ratio 1:1 had been prepared immediately before the experiment, as well as mixed cultures of these strains which had been cultivated together for at least one week and re-inoculated every two days were used. Before grounding, seeds were inoculated with lactobacilli (in concentrations 1%, 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%) for an hour. Seed germination, length of the roots and height of the seedlings were calculated as means with standard errors or confidential intervals.* ***Results.*** *Treatment of wheat seeds with individual L. plantarum ONU 12, L. plantarum ONU 311, L. plantarum ONU 355 strains, their mixed cultures grown together and mixtures has increased seed germination in 6.0–40.0% depending on the conditions of germination and inoculum concentration. Under conditions of hydroponics and soil in the greenhouse the best results were shown by inoculums in concentration 103–106 CFU/ml, and under field conditions – in concentration 10^2–10^4 CFU/ml. Mixtures and mixed cultures of L. plantarum ONU 12 + L. plantarum ONU 311 and L. plantarum ONU 12 + L. plantarum ONU 355 formed developed biofilms on roots of wheat seedlings. Mean height of seedlings increased in 8.0–41.0%, and the length of the roots increased by 2.4 times in hydroponics and in 6.8 –64.5% in soil.* ***Conclusion.*** *The mixtures and mixed cultures of bacteria L. plantarum ONU 12 + L. plantarum ONU 311 and L. plantarum ONU 12 + L. plantarum ONU 355 could stimulate wheat germination and growth and can be used for development of plant growth promoting bacterial preparations.*

Key words: Lactobacillus plantarum, biofilm, inoculum, wheat, growth characteristics, hydroponics.

Modern agriculture experiences the increasing need for environmentally friendly biological preparations. Lactic acid bacteria, in particular lactobacilli, are famous for a wide range of antagonistic properties [1; 5; 9; 11; 16], as well as their ability to stimulate plant growth [3; 4; 8; 13; 15] due to the synthesis of hormones or precursors of growth-promoting hormones [7].

In particular, wheat growth stimulation with the help of biological preparations

might be possible not only in field conditions, but also in hydroponics, including the future possibility of cultivating wheat in artificial conditions, including space stations [12; 14].

Previous studies conducted on wheat seedlings in moist chambers, demonstrated high stimulatory effect of *L. plantarum* strains on germination and growth characteristics of seedlings [3], however further investigations under conditions more close to natural were needed.

The aim of this work was to study the effect of bacteria *L. plantarum* on germination and some growth characteristics of wheat *Triticum aestivum* L. in hydroponics and soil under greenhouse and field conditions.

Materials and Methods

In this investigation, stimulatory activity of the next bacterial strains from the collection of the Department of Microbiology, Virology and Biotechnology of Odesa National I. I. Mechnykov University (ONU) was studied: *L. plantarum* ONU 12, *L. plantarum* ONU 311, *L. plantarum* ONU 355; a mixture of strains *L. plantarum* ONU 12, *L. plantarum* ONU 311, *L. plantarum* ONU 12, *L. plantarum* ONU 355 and mixed cultures of *L. plantarum* ONU 12, *L. plantarum* ONU 355 and *L. plantarum* ONU 12, *L. plantarum* ONU 311. All *L. plantarum* strains were originally isolated from grapes and stored at – 80 °C in 20% glycerol.

Lactobacilli were grown for 24 hours in a liquid MRS medium [6] at 37 ºC. Daily cultures of lactobacilli at the concentration of 10^8 CFU/ml were used to prepare the dilutions: 1%, 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%.

A mixture of strains was created by mixing the overnight cultures of different strains of lactobacilli in a ratio 1:1 and immediately used in the experiment (for dilutions and treatment).

A mixed culture of lactobacilli was created by mixing the overnight cultures of different strains of lactobacilli in a ratio 1:1 and cultivating them together for a week with re-inoculation every two days. For the experiment, a mixed culture was grown overnight to reach the concentration of 10^8 CFU/ml.

The conditions of hydroponics were modeled using Aquasave S. gel. The test plant used in the research was wheat *Triticum aestivum* L., variety Odeska ozyma. Prior to the experiments, the seed surface was sterilized with 25% hydrogen peroxide during one minute, and washed thrice in sterile distilled water (SDW).

In all the experiments, the seeds were soaked in prepared bacterial suspensions or SDW for 60 minutes.

In the experiments with hydroponics, seeds were later transferred to germinate on Aquasave S gel in glassware. The control group were seeds soaked for 60 min in SDW. Germination was carried out in a greenhouse in Aquasave S gel for the period of seven days.

In the experiments with soil in a greenhouse, commercial soil "Poliskyi Universalnyi" with high content of peat was used; the soil was not sterilized before sowing. Wheat seeds were similarly soaked in bacterial suspensions for one hour and then sowed in plastic cups with soil.

The experiments in the open field were conducted in the south-Ukrainian heavy-bodied low-humus chornozem soil during 2017 and 2018.

In total, three independent experiments of each variant using 240–300 seeds were conducted. After seven days, the growth characteristics of seedlings were measured, namely - mean root length of seedlings and mean seedlings height [2]. Statistical analysis was carried out using the Excel package. The lengths of roots and seedlings were, as quantitative characteristics, expressed as a mean with 95% confidence interval; seed germination was, as a qualitative characteristic, expressed in percentage with the standard error. Significant differences between measurements of control and inoculated seedlings were identified in Student's t-test ($P < 0.05$).

In order to study the ability of bacteria to form biofilms on plant root surface, the seven-day seedlings were washed from gel and soil in distilled water. The biofilm was fixed in 96% ethanol for 15 minutes and stained with 0.1% Acridine Orange solution for 10 minutes. Dyed seedlings were dried on a microscope slide and observed using PrimoStar PC microscope, Carl Zeiss, with x 600 magnification. Microscopy was performed on 10 samples of each variant, with 10 visual fields of each. The biofilms were photographed using Canon EOS 500D camera.

Results and Discussion

The observations of wheat seed germination showed that individual strains of lactobacilli and their mixtures in most cases caused positive effect on germination, increasing the percent of germinated seeds in 6.0–40.0% (Table).

Under greenhouse conditions (both in hydroponics and soil), the effect of seed treatment with lactobacilli was higher due to absence of any additional nutrients in gels and peat soils used for germination of seeds. Metabolites of lactobacilli, dead cells, and residues of the culture medium in which bacteria were cultivated played the role of organic component of the germination substrate.

Stable increasing in germination following treatment with bacteria at all concentrations was observed using the mixtures and mixed cultures of lactobacilli. Consequently, the best combinations were selected and used for the open field experiments – the mixtures of strains *L. plantarum* ONU 12 + *L. plantarum* ONU 311 and *L. plantarum* ONU 12 + *L. plantarum* ONU 355.

In the open field, seed germination in control was higher (22%) than in gel and in peat soil under greenhouse conditions. This is due to the rich composition of organic and inorganic components of the south-Ukrainian heavy-bodied low-humus chornozem of the Odesa region. However, the treatment of seeds with lactobacilli also increased germination in 6–8%. The best concentrations were 0.1–0.01% for *L. plantarum* ONU 12 + *L. plantarum* ONU 311 and 1.0–0.01% for *L. plantarum* ONU 12 + *L. plantarum* ONU 355.

Inoculation with mixtures and mixed cultures of lactobacilli also improved some morphometric characteristics of seedlings in gel. There was a significant increase in mean height of seedlings – following treatment with a mixed culture and a mixture of strains *L. plantarum* ONU 12 + 311 in 37.0–38.1%, and by combinations *L. plantarum* ONU 12 + 355 – in 36.2–41.0% (Fig. 1).

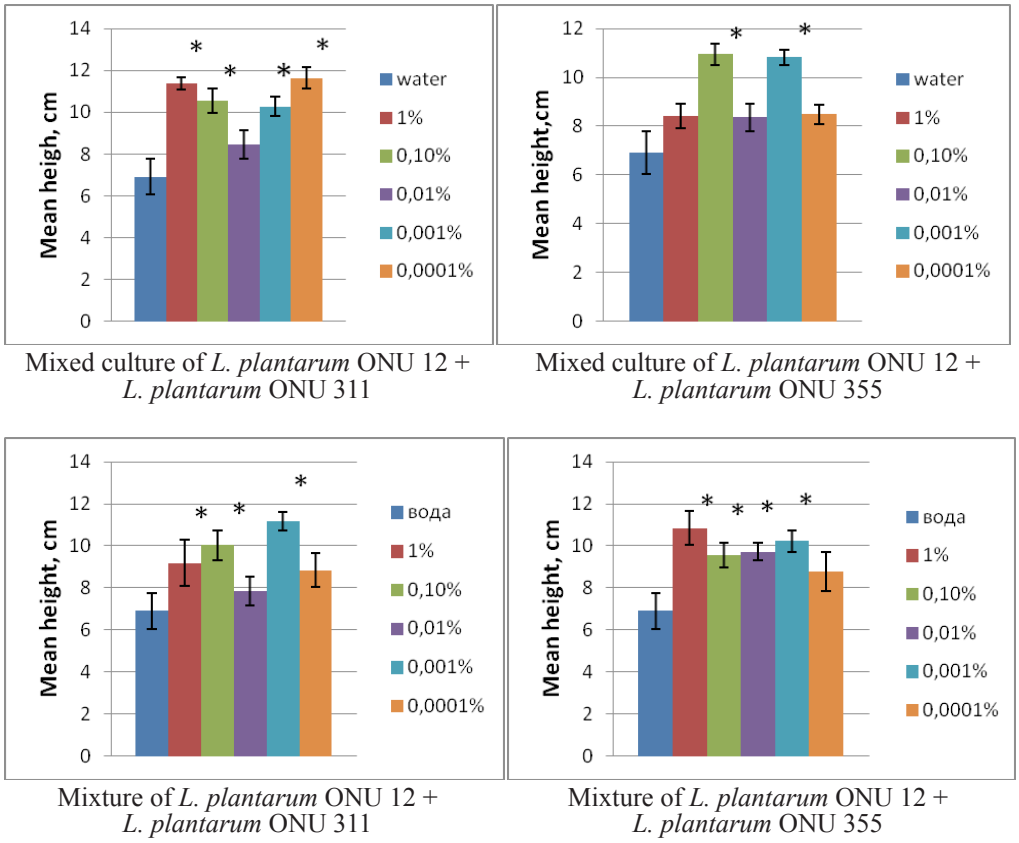
The length of roots of wheat seedlings was the highest as a result of treatment with mixed culture ONU 12 + 311, which yielded 34.1% increase compared to the control, and mixed culture *L. plantarum* ONU 12 + 355 – 2,4-fold increase (Fig. 2).

Table

Germination of wheat seeds following treatment with individual strains of *L. plantarum* and their combinations (data estimated as %)

Conditions of planting	Strain	Final concentration of overnight cultures of lactobacilli				
		1%	0.1%	0.01%	0.001%	0.0001%
Hydroponics	ONU 12	60.0±2.5	80.0±2.1*	70.0±2.4*	60.0±2.5	60.0±2.5
	ONU 311	78.0±2.1*	90.0±1.5*	90.0±1.5*	90.0±1.5*	60.0±2.5
	ONU 355	87.0±1.5*	60.0±2.5	70.0±2.4*	60.0±2.5	80.0±2.1*
	12+311 mixture	70.0±2.4*	100*	90.0±1.5*	80.0±2.1*	75.0±2.4*
	12+355 mixture	90.0±1.5*	70.0±2.4*	80.0±2.1*	80.0±2.1*	90.0±1.5*
	12+311 mixed culture	80.0±2.1	80.0±2.1*	70.0±2.4*	80.0±2.1*	78.0±2.1*
	12+355 mixed culture	80.0±2.1*	80.0±4.4*	80.0±4.4*	70.0±2.4*	78.0±2.1*
	control	60.0±2.5				
Soil under greenhouse conditions	ONU 12	75.0±3.0*	70.0±3.2*	72.0±2.3*	85.0±3.4*	75.0±1.2*
	ONU 311	80.0±2.4*	85.0±1.1*	81.0±1.8*	82.0±3.1*	80.0±4.1*
	ONU 355	65.0±2.2	68.0±1.6	62.0±2.4	75.0±3.0*	78.0±1.2*
	12+311 mixture	75.0±1.5*	78.0±5.1*	76.0±2.2*	80.0±2.8*	86.0±4.3*
	12+355 mixture	67.0±2.2	77.0±1.7*	86.0±2.4*	74.0±3.1*	88.0±1.5*
	12+311 mixed culture	68.0±3.1	80.0±2.1*	72.0±3.8*	69.0±4.3	75.0±1.4*
	12+355 mixed culture	70.0±2.7*	66.0±2.3	71.0±2.4*	82.0±1.3*	88.0±2.6*
	control	60.0±3.4				
Open field	12+311 mixture	84.0±3.2	92.0±1.2*	88.0±1.4*	84.0±1.6	82.0±2.8
	12+355 mixture	90.0±1.1*	90.0±2.6*	92.0±3.1*	86.0±2.2	84.0±1.9
	control	82.0±1.2				

Note: * values are significantly different from the control ones ($P < 0.05$).



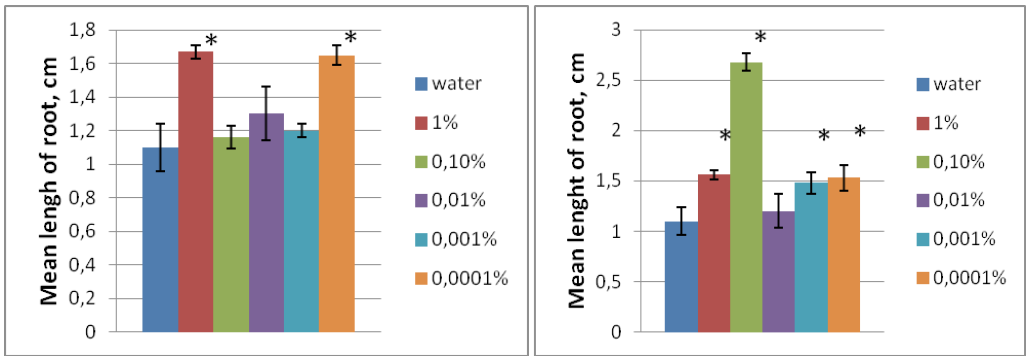
Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311

Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311

Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

Fig. 1. Mean height of wheat seedlings following treatment with mixtures and mixed cultures of lactobacilli in gel: * values are significantly different from the control ones (P < 0.05)



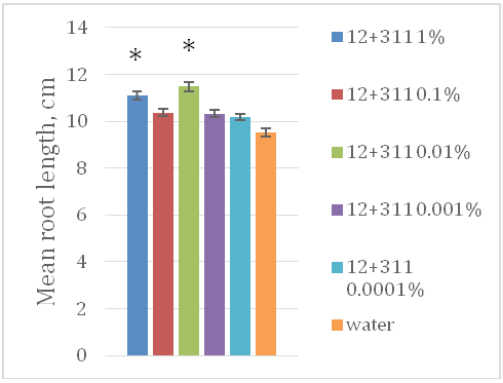
Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311

Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

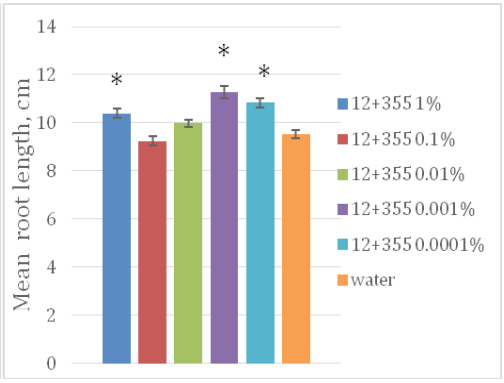
Fig. 2. Mean root length of wheat seedlings following treatment with mixtures and mixed cultures of lactobacilli in gel: * values are significantly different from the control ones (P < 0.05)

Overall, different strains of lactobacilli and their combinations positively influenced the growth of wheat seedlings in gel, causing increase in seedlings height in 36.2–41.0%, and the mean length of the roots increased in 2.4 times. As a rule, the best effect was observed at the concentration of 1.0–0.001%, that is, bacterial suspensions with 10^6–10^3 CFU/ml.

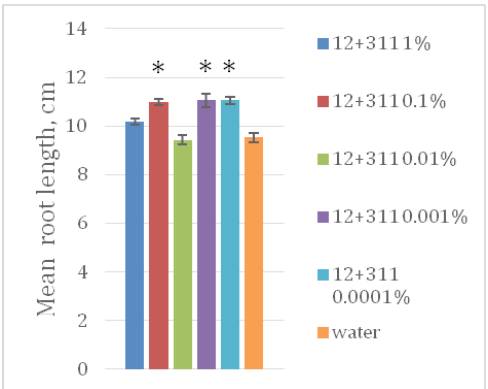
In peat soil under greenhouse conditions, the mean length of seedlings after the treatment with the mixture of strains *L. plantarum* ONU 12 + 311 increased in 8.4–20.4%, with mixed culture of *L. plantarum* ONU 12 + 311 – in 6.8–15.9%, with mixed culture of *L. plantarum* ONU 12 + 355 – in 4.6–17.6% (Fig. 3). For the mixed culture of *L. plantarum* ONU 12 + 355 dilutions in the range of 0.001–0.0001% (10^3 –10^2 CFU/ml) yielded better results, and mixed culture of *L. plantarum* ONU 12 + 311 at all concentrations increased the mean root length. In case of mixtures, the results varied depending on both the combination of lactobacilli and their concentration (Fig. 3).



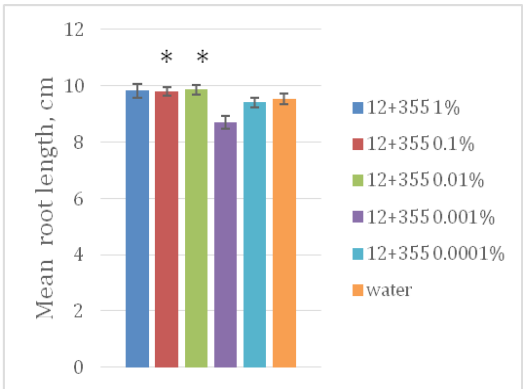
Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311



Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355



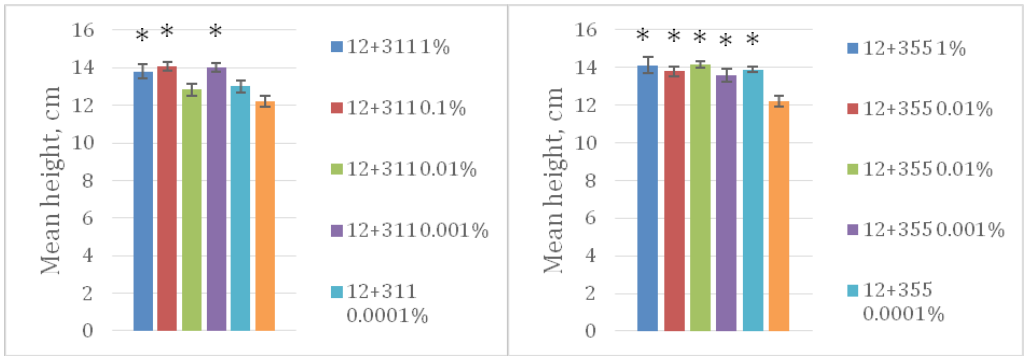
Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311



Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

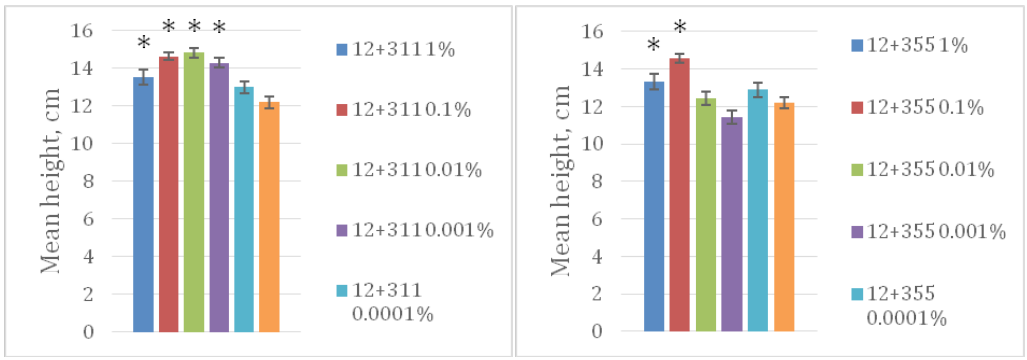
Fig. 3. Mean root length of wheat seedlings following treatment with the mixtures and mixed cultures of lactobacilli in soil under greenhouse conditions: * values are significantly different from the control ones (P < 0.05)

Treatment with bacterial suspensions also had a significant effect on average seedlings height (Fig. 4).



Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311

Mixed culture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355



Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311

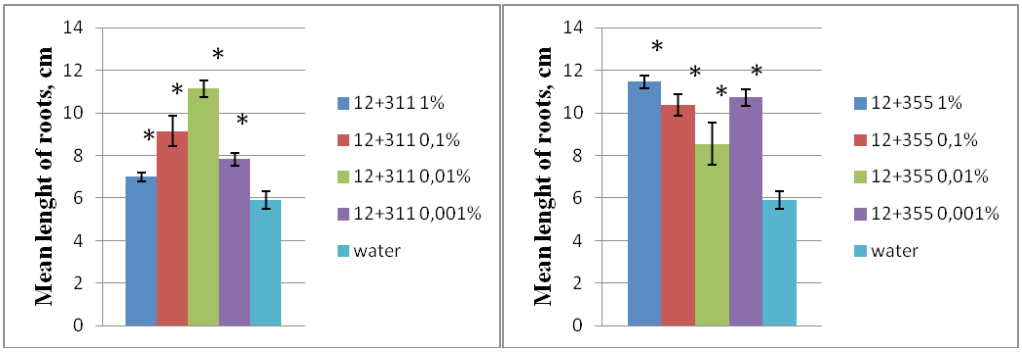
Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

Fig. 4. Mean height of wheat seedlings following treatment with mixtures and mixed cultures of lactobacilli in soil under greenhouse conditions: * values are significantly different from the control ones (P < 0.05)

Treatment with the mixture and mixed culture of *L. plantarum* ONU 12 + 311 increased the mean seedlings height in 6.6 – 21.5%, and inoculation of seeds with the mixture of strains *L. plantarum* ONU 12 + 355 yielded a 12.0–22.8% increase (Fig. 4). The highest values were observed at concentrations 1.0–0.01% (from 106 to 10^4 CFU/ml).

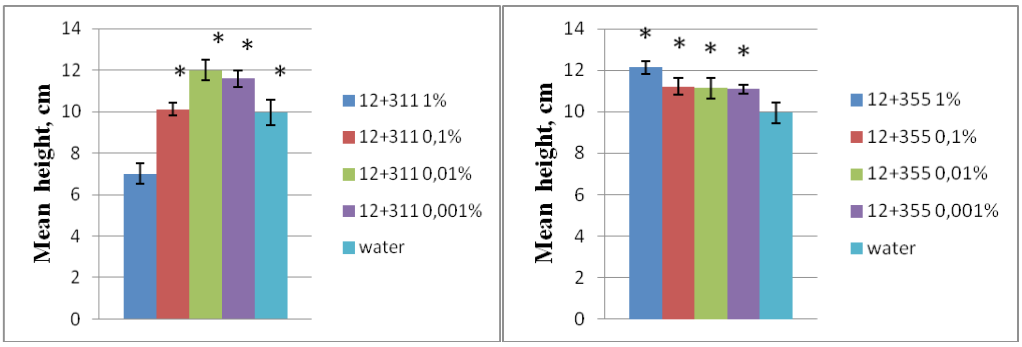
In the open field, the influence of lactobacilli was slightly different, namely - while all concentrations of both mixtures positively influenced the mean root length (Fig. 5), mixture of *L. plantarum* ONU 12 + 311 at the concentration of 1% had an inhibitory effect on the seedlings height (Fig. 6).

This fact probably could be explained by overproduction of phytohormones by the mixture of lactobacilli strains in soil and direct interaction with soil microbiota. Other concentrations of inoculums increased the average height of seedlings in



Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311 | Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

Fig. 5. Mean root length of wheat seedlings following treatment with the mixtures and mixed cultures of lactobacilli in the open field: * values are significantly different from the control ones (P < 0.05).



Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 311 | Mixture of *L. plantarum* ONU 12 + *L. plantarum* ONU 355

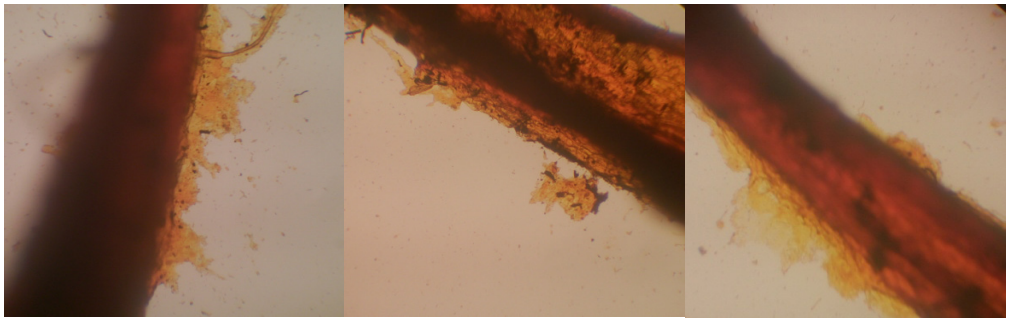
Fig. 6. Mean height of wheat seedlings following treatment with the mixtures and mixed cultures of lactobacilli in the open field: * values are significantly different from the control ones (P < 0.05)

8.0–17.9%, and the length of the roots – in 15.7–64.5% with the best concentrations ranging from 0.1–0.001% (10^4–10^2 CFU/ml) (Fig. 6).

In general, the obtained data indicate that, in order to improve growth characteristics of wheat seedlings in the open field, lower concentrations of lactobacilli suspensions are sufficient than for greenhouse conditions and hydroponics.

The bacteria of both mixed cultures of *L. plantarum* ONU 12 + *L. plantarum* ONU 355 and *L. plantarum* ONU 12 + *L. plantarum* ONU 311 were able to form biofilms on roots of wheat seedlings. The biofilms consisted of well-developed microcolonies and matrix, lacked gaps and occasionally had slight ruptures (Fig. 7).

The results obtained from using mixed cultures of *L. plantarum* strains ONU 12 + *L. plantarum* ONU 355 and *L. plantarum* ONU 12 + *L. plantarum* ONU 311



A B C

Fig. 7. Colonization of roots of the seedlings by mixed cultures of *L. plantarum* ONU 12+311 (A) and *L. plantarum* ONU 12+355 (B, C) (x600)

for treatment of wheat seeds indicate that one hour is sufficient time for lactobacilli to attach to seeds and consequently colonize the seed surface.

High increase in morphological characteristics of plants indicates the stimulatory potential of the studied strains of lactobacilli, which could be explained by the synthesis of auxin hormones or their precursors, as described in the literature [7].

Treatment with lactobacilli improved germination of wheat seeds in 10.0–40.0% in hydroponics and in soil under greenhouse conditions, and in 6.0–8.0% in the open field. Under the influence of the mixtures of *L. plantarum* ONU 12 + *L. plantarum* ONU 311 and *L. plantarum* ONU 12 + *L. plantarum* ONU 355, mean height of seedlings increased in 34.2–41.0% in hydroponics, in 6.6–22.8% in soil under greenhouse conditions, and in 8.0–17.9% in the open field. Following treatment with lactobacilli, the increase in mean root length of wheat seedlings ranged from 34.1% to 2.4 times in hydroponics, from 6.8 to 20.4% in soil under greenhouse conditions, and from 15.7 to 64.5% in the open field. The best concentrations of bacterial mixtures for seed treatment with subsequent germination in hydroponics and soil were 10^3–10^6 CFU/ml, and for open field conditions – 10^2–10^4 CFU/ml. Bacteria of *L. plantarum* mixture of ONU 12 + *L. plantarum* of ONU 311 and *L. plantarum* of ONU 12 + *L. plantarum* of ONU 355 formed developed biofilm on roots of wheat seedlings. Thus, for stimulation of wheat growth mixtures *L. plantarum* ONU 12 + *L. plantarum* ONU 311 and *L. plantarum* ONU 12 + *L. plantarum* ONU 355 should be used with the best concentrations of bacterial mixtures for seed treatment with subsequent germination in hydroponics and soil 10^3–10^6 CFU/ml, and for open field conditions – 10^2–10^4 CFU/ml.

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ВПЛИВ *LACTOBACILLUS PLANTARUM* НА РІСТ ПШЕНИЦІ НА ГІДРОПОНІЦІ ТА У ҐРУНТІ

Реферат

Мета. *Вивчити вплив L. plantarum на проростання і деякі ростові характеристики пшениці Triticum aestivum L. в умовах гідропоніки, ґрунту у теплиці та відкритого ґрунту.* ***Методи.*** *Використовували окремі штами L. plantarum, їх суміші у співвідношенні 1:1, приготовлені безпосередньо перед експериментом, а також змішані культури бактерій цих штамів, які культивували разом щонайменше протягом тижня з пересівами кожні два дні. Насіння перед висівом інокулювали різними розведеннями добових культур лактобацил (1%, 0,1%, 0,01%, 0,001%, 0,0001%, 0,00001%) впродовж години. Розраховували середні значення, стандартні похибки або довірчі інтервали таких показників, як схожість насіння, довжина коренів та висота паростків.* ***Результати.*** *За обробки бактеріями окремих штамів L. plantarum ОНУ 12, L. plantarum ОНУ 311, L. plantarum ОНУ 355, їх змішаними культурами та сумішами спостерігалося покращення схожості на 6,0–40,0% в залежності від умов пророщування та концентрації інокулюму. Для умов гідропоніки та закритого ґрунту найкращими концентраціями інокулюмів були 10^3–10^6 КУО/мл, а для умов відкритого ґрунту – 10^2–10^4 КУО/мл. Бактерії сумішей і змішаних культур L. plantarum ОНУ 12 і L. plantarum ОНУ 311 та L. plantarum ОНУ 12 і L. plantarum ОНУ 355 утворювали розвинену біоплівку на коренях сіянців пшениці. Середня висота паростків збільшувалася на 8,0–41,0%, а довжина коренів – у 2,4 рази у випадку росту на гідропоніці та на 6,8–64,5% в умовах ґрунту.* ***Висновок.*** *Бактерії сумішей бактерій L. plantarum ОНУ 12 і L. plantarum ОНУ 311 та L. plantarum ОНУ 12 і L. plantarum ОНУ 355 здатні стимулювати пророщування насіння і ріст рослин пшениці та можуть бути застосовані для розробки рістстимулювальних мікробних препаратів.*

Ключові слова: Lactobacillus plantarum, біоплівка, інокулюм, пшениця, ростові характеристики, гідропоніка.

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ВЛИЯНИЕ *LACTOBACILLUS PLANTARUM* НА РОСТ ПШЕНИЦЫ НА ГИДРОПОНИКЕ И В ПОЧВЕ

Реферат

Цель. *Изучить влияние L. plantarum на прорастание и некоторые ростовые характеристики пшеницы Triticum aestivum L. в условиях гидропоники, почвы в теплице и в открытом грунте.* ***Методы.*** *Использовали отдельные штаммы L. plantarum, их смеси в соотношении 1:1, приготовленные непосредственно перед экспериментом, а также смешанные культуры бактерий этих штаммов, которые культивировали вместе, по меньшей мере неделю с пересевами каждые два дня. Семена перед высевом инокулировали разными разведениями суточных культур лактобацилл (1%, 0,1%, 0,01%, 0,001%, 0,0001%, 0,00001%) в течение одного часа. Рассчитывали средние значения, стандартные ошибки или доверительные интервалы таких показателей, как всхожесть семян, длина корней и высота проростков.* ***Результаты.*** *При обработке бактериями отдельных штаммов L. plantarum ОНУ 12, L. plantarum ОНУ 311, L. plantarum ОНУ 355, их смешанными культурами и смесями, наблюдалось улучшение всхожести на 6,0–40,0% в зависимости от условий проращивания и концентрации инокулюма. Для условий гидропоники и закрытого грунта наилучшими концентрациями инокулюмов были 10^3–10^6 КОЕ/мл, а для условий открытого грунта – 10^2–10^4 КОЕ/мл. Бактерии смесей и смешанных культур L. plantarum ОНУ 12 и L. plantarum ОНУ 311, L. plantarum ОНУ 12 и L. plantarum ОНУ 355 формировали развитую биоплёнку на корнях сеянцев пшеницы. Средняя высота проростков увеличивалась на 8,0–41,0%, а длина корней – в 2,4 раза в случае роста на гидропонике и на 6,8–64,5% в условиях грунта.* ***Вывод.*** *Бактерии смесей L. plantarum ОНУ 12 и L. plantarum ОНУ 311, L. plantarum ОНУ 12 и L. plantarum ОНУ 355 способны стимулировать прорастание семян и рост растений пшеницы, и быть применены для разработки ростстимулирующих микробных препаратов.*

Ключевые слова: Lactobacillus plantarum, биоплёнка, инокулюм, пшеница, ростовые характеристики, гидропоника.

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