DETECTION OF PLANTARICIN GENES IN STRAINS OF LACTOBACILLUS PLANTARUM – ANTAGONISTS OF PHYTOPATHOGENIC BACTERIA

The aim of investigation was to detect the presence of genes responsible for bacteriocin synthesis in strains of Lactobacillus plantarum with the clear antagonistic effect against the gram-negative phytopathogens. Methods. To reveal eleven genes involved in plantaricin synthesis the polymerase chain reaction was used. To test the ability to synthesize bacteriocins as antagonistic compounds the experiments with the lawns of test-strains Listeria ivanovii INRA, Rhizobium radiobacter C58, Ralstonia solanacearum B-1109-UCM, Erwinia carotovora ZM1, Rhizobium vitis OHY 389, R. vitis OHY 388, R. vitis 379 and R. rhizogenes 15834 were used. Results. In genomes of the tested L. plantarum strains the genes plnD, plnEF, plnG, plnI, plnN were present, but the genes plnA, plnB, plnC, plnW were not revealed. Applying the cultural liquids of lactobacilli on the lawns of the test-strains has shown that the cell-free cultural liquid with the initially low pH (4.1–4.3) caused the zones of growth inhibition on the lawns of all test-strains. The neutralized cell-free cultural liquid did not affect the growth of the test-strains. Conclusion. Although the tested strains L. plantarum ONU 87, L. plantarum ONU 206 and L. plantarum ONU 991 possessed some genes of plantaricin regulon, in the investigations in vitro they caused the inhibition of the phytopathogens and listerias due to the low pH of the cell-free cultural liquids but not due to the synthesis of bacteriocins. The combination of the genes of plantaricin regulon of L. plantarum ONU 206 and L. plantarum ONU 991 resembled that in L. plantarum J23 described in literature.

Key words: Lactobacillus plantarum, antagonists, bacteriocins, phytopathogens.

Lactobacillus plantarum bacteria isolated from plant surfaces and dairy products are characterized by the clear antagonistic activity against some phytopathogens [4]. The ability of L. plantarum to inhibit bacteria and fungi is strain-specific [15].

Antagonistic activity of lactobacilli is the result of the effects of organic acids, hydrogen peroxide, microbial competition and bacteriocin synthesis [14, 16]. Although the bacteriocin action is described in details only as an effect on gram-positive bacteria, the cases of inhibition of gram-negative bacteria are also known in literature [12, 13]. This makes lactobacilli especially perspective for plant protection as the majority of phytopathogens are the gram-negative bacteria.

In our previous investigation, the use of Lactobacillus plantarum ONU 87, Lactobacillus plantarum ONU 206 and Lactobacillus plantarum ONU 991 strains in plant protection on a model of Kalanchoe daigremontiana Mill. was described. Not only the supernatants with low pH but also the supernatant of L. plantarum ONU 206 culture
with pH 6.5 caused the inhibitory effect on tumor formation decreasing the amount of infected samples in 70.6% [8]. Basing on the results of investigation, it was supposed that the inhibitory effect of metabolism products from the cultural supernatant of lactobacilli was caused not only by lactic acid, but also by bacteriocins. Several types of plantaricin regulons are described in literature [6; 11]. The most studied is the regulon of L. plantarum C11. The regulatory operon plnABCD encodes an inducible peptide-pheromone PlnA, a histidine-kinase PlnB and the response regulators PlnC and PlnD. The operons plnEFT and plnJKL encode two-peptide bacteriocins PlnEF and PlnJK and the corresponding immunity proteins. Operon plnGHSTUVW encodes the proteins of ABC transport system necessary for the processing and secretion of a bacteriocin [1; 6; 13].

According to this, the aim of investigation was to detect the presence of genes responsible for bacteriocin synthesis in strains of Lactobacillus plantarum with the clear antagonistic effect against the gram-negative phytopathogens Rhizobium radiobacter, R. vitis, R. rhizogenes, Ralstonia solanacearum, Erwinia carotovora.

**Materials and Methods**

Strains from the Collection of Microbiology, Virology and Biotechnology Chair of Odesa National I.I. Mechnykov University – L. plantarum OHY 87, L. plantarum ONU 206, L. plantarum ONU 991 isolated from dairy products, were brought to the assays. To test lactobacilli on the ability for the synthesis of bacteriocins, the test-strain Listeria ivanovii INRA was used because listerias are the classical test-objects for the detection of bacteriocinogenic activity in lactic acid bacteria. The Listeria ivanovii strain was kindly provided by Dr. Thomas Haertle (INRA, Nantes, France). Phytopathogens Rhizobium radiobacter C58, Ralstonia solanacearum B-1109-UCM and Erwinia carotovora ZM1 were kindly provided by the Collection of D.K. Zabolotny Institute of Microbiology and Virology (Kyiv, Ukraine), Rhizobium vitis ONU 389 and R. vitis OHY 388 – by the Collection of Microbiology, Virology and Biotechnology Chair of Odesa National I.I. Mechnykov University, R. vitis 379 and R. rhizogenes 15834 – by the Collection of Microorganisms of Institute of Agricultural Microbiology, Saint-Petersburg, Russia.

Lactobacilli were cultivated overnight at 37 °C in MRS broth [5]. DNA was isolated by the kit “DNA sorb” (ZNII Epidemiology, Russia). The presence of pln locus genes was detected by the method of polymerase chain reaction (PCR): plnA [6; 10], plnB, plnC, plnD [6], plnEF [1; 6], plnJ, plnK [6], plnJ [1; 6], plnG, plnN [6] and the structural plantaricin W gene [1]. Amplification was carried out by the parameters proposed by Ben Omar et al. (2008) [2]. PCR products were detected by electrophoresis in 1.5% agarose. The markers of molecular weight pUC19/MspI (501, 404, 331, 242, 190, 147, 111 b.p.) and pBR322 DNA/Alul (908, 659, 521, 403, 281, 257 b.p.) were used (Fermentas, Lithuania).

To reveal the antagonistic effect, the overnight cultures of lactobacilli were centrifuged and filtered through 0.22 μm Millipore filters. Phytopathogens and listerias were cultivated overnight at 37 °C in LB broth [3], and after used for the preparing of lawns and testing by the well-diffusion method. The cell-free supernatants both with the initial low pH and neutralized with 1 M NaOH were brought to the wells. The zones of growth inhibition were detected after the overnight cultivation at 37 °C.
Results and Discussion

The next results of the search for the genes responsible for bacteriocin synthesis in studied *L. plantarum* strains isolated from the home-made dairy products, were obtained (Tab. 1).

### Table 1

<table>
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<tr>
<th>Strain</th>
<th>plnA</th>
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<th>plnC</th>
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<th>plnG</th>
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<th>plnK</th>
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<tr>
<td><em>L. plantarum</em> ОНУ 87</td>
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<tr>
<td><em>L. plantarum</em> ОНУ 206</td>
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<tr>
<td><em>L. plantarum</em> ОНУ 991</td>
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</table>

As the obtained data show, none of the studied strains possess all the tested genes of plantaricin regulon. Genes of the recognition system responsible for the synthesis of an inducible peptide-pheromone PlnA, a histidine protein kinase PlnB and a response regulator PlnC were absent. Also none of the strains contained the *plnW* gene. This gene was described in literature only for the one strain, and it was found out that this sequence is rare in *L. plantarum* [7]. Thus, our obtained data coincide with the data of literature.

All the strains possessed the genes of plantaricin synthesis *plnEF* and the gene encoding own immunity against it (*plnI*) [13], and that allowed us to suppose that the studied strains isolated from dairy products could produce the bacteriocin (Fig. 1).

In the strains isolated from dairy products, gene *plnK* was revealed only in one strain – *L. plantarum* ОНУ 87, but genes *plnD, plnEF, plnN, plnI* were found in all of the three strains mentioned in the table (Fig. 2).

Lack of genes *plnA, plnB, plnC* and *plnK*, but the presence of *plnD* allows to suppose that strains *L. plantarum* ОНУ 206 and *L. plantarum* ОНУ 991 are similar to the strain *L. plantarum* J23 described in the literature possessing operon *plnC8IF-NC8HK-D* encoding an inducible peptide PLNC8IF, a histidine kinase PLNC8HK and response regulator PlnD instead of the regulatory operon *plnABCD*. Besides, this strain lacks the gene *plnK* [11].

The presence of genetic sequences indicates the potential ability of *L. plantarum* strain to produce plantaricin, but in some cases a strain possessing all necessary genes does not secrete active bacteriocin in a medium, or its synthesis is non-regulatory [9]. To check the ability to synthesize bacteriocins as antagonistic compounds, tests on the lawns of indicator-strains were carried out. Applying cultural supernatants of the studied strains of *L. ivanovii* INRA, *Rhizobium radiobacter* C58, *Ralstonia solanacearum* В-1109-UCM, *Erwinia carotovora* ZM1, *Rhizobium vitis* ОНУ 389, *R. vitis* ОНУ 388, *R. vitis* 379 and *R. rhizogenes* 15834 we found out that only the supernatants with initial low pH (4.1–4.3) caused zones of growth inhibition on the lawns of all indicator-strains.

Neutralized cultural supernatant did not affect the growth of test-strains. This allowed us to make a conclusion that inhibition of phytopathogens and listerias was the result of low pH of cultural supernatants.

Fig. 2. Electrophoregram of amplification products of PCR with primers to gene **plnK** (amplicon size 246 b.p., M – marker of molecular weight pBR322 DNA/AluI, Fermentas, Lithuania) with DNA of the strains *Lactobacillus plantarum* (1 – strain ONU 87; 2 – strain ONU 206; 3 – strain ONU 991); gene **plnJ** (amplicon size 475 b.p., M – marker of molecular weight pBR322 DNA/AluI, Fermentas, Lithuania): 4 – strain ONU 87; 5 – strain ONU 991; 6 – strain ONU 206)
Thus, the presence of genetic sequences indicates the potential ability of the strain to synthesize bacteriocins but did not allow to make a preliminary conclusion about the active production of antagonistic substances into a medium.

Although the tested strains *L. plantarum* ONU 87, *L. plantarum* ONU 206 and *L. plantarum* ONU 991 possessed some genes of plantaricin regulon, in investigations *in vitro* they caused the inhibition of the phytopathogens and listerias due to the low pH of the cell-free cultural liquids but not due to the synthesis of bacteriocins.
ВИЯВЛЕНИЕ ГЕНОВ ПЛАНТАРИЦИНОВ У ШТАММОВ

**LACTOBACILLUS PLANTARUM** – АНТАГОНИСТОВ ФИТОПАТОГЕННИХ БАКТЕРІЙ

**Реферат**

Целью исследования было выявление наличия генов синтеза бактериоцинов у штаммов Lactobacillus plantarum, которые имеют выраженный антагонистический эффект против грамнегативных фитопатогенов.

**Методы.** Для выявления одиннадцати генов, задействованных в синтезе плантарицинов, использовали полимеразную цепную реакцию. Для проверки способности к синтезу бактериоцинов как антагонистических веществ осуществляли тестирование на газонах тест-штаммов Listeria ivanovii INRA, Rhizobium radiobacter C58, Ralstonia solanacearum B-1109-UCM, Erwinia carotovora ZM1, Rhizobium vitis OHU 389, R. vitis OHU 388, R. vitis 379 и R. rhizogenes 15834.


**Вывод.** Исследованные штаммы L. plantarum OHU 87, L. plantarum OHU 206 и L. plantarum OHU 991, хотя и содержали ряд генов плантарицинового регулона, в опытах in vitro вызывали угнетение роста фитопатогенов и листерий за счет низких значений рН культуральной жидкости, а не в результате синтеза бактериоцинов. По составу генов плантарициновой регулы штаммов L. plantarum OHU 206 и L. plantarum OHU 991 был ближе к описанному в литературе штамму L. plantarum J23.

**Ключевые слова:** Lactobacillus plantarum, антагонисты, бактериоцины, фитопатогены.

**LITERATURE**


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