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PROBIOTIC PROPERTIES OF STRAINS OF *LACTOBACILLUS PLANTARUM* ISOLATED FROM FERMENTED PRODUCTS

Aim. *To study probiotic properties of the strains Lactobacillus plantarum, isolated from the national fermented foods of plant and animal origin of different regions of Ukraine.* ***Methods.*** *The ability of strains L. plantarum to adhere was studied on the cells of human buccal epithelium. The ability to filmformation of strains L. plantarum was studied in plastic plates. We investigated the survival of L. plantarum in the conditions of the gastrointestinal tract: resistance to lysozyme, gastric juices and bile salts.* ***Result****. Probiotic properties of 24 strains L. plantarum has been studied. These strains have a wide range of antagonistic action on opportunistic microorganisms used and proved to be sensitive to the clinically important antibiotics. Index adhesion of the lactobacilli ranged from 3.63 ± 0.38 to 30.11 ± 1.47 and didn`t depend on the source of strains isolation. Most of the studied strains (79%) had the average ability to form biofilms. Gastric juice was one of the most aggressive factors of the gastrointestinal tract, in its action, growth had 42% of the studied strains. L. plantarum resistant to lysozyme and to 0.3% bile salts have been determined.* ***Conclusions.*** *The probiotic properties of 24 strains of L. plantarum were investigated and analyzed. Selected two strains of L. plantarum 1047s and L. plantarum 691ch, which may be promising for use in oral probiotic preparations.*

Key words: probiotic properties, Lactobacillus plantarum, fermented foods.

Lactic acid bacteria (LAB) of genus *Lactobacillus* is a helpful component of human biological community norm. These bacteria have a high biological activity, which contributes to their widespread use in modern probiotic preparations and foodstuffs [5]. Probiotic bacteria are defined by FAO/WHO as "live microorganisms which when administered in adequate amount confer a health benefit". Probiotic effect of lactobacilli on the human body caused by the inhibition of growth of pathogenic and opportunistic microorganisms. This inhibition could be due to the inhibitory compounds such as organic acids, hydrogen peroxide, bacteriocins, synthesis of the vitamins and enzymes [11]. In addition, they also reduce the risk of gastro-intestinal disorders and prevent diarrhea [9]. To make a probiotic effect, the strain must survive passing through different parts of the gastrointestinal tract (GIT) and have the ability to adhere to intestinal surfaces. Probiotic strains of lactobacilli also have to be sensitive to clinically important antibiotics, including ampicillin, gentamicin, kanamycin, streptomycin and chloramphenicol to assess the safety of their use as food additives [14].

It is known that fermented foods has a positive effect on the host. This effect is provided by autochthonous strains LAB, therefore fermented foods are increasingly seen as a source of probiotic strains LAB in recent years. The use of fermented vegetables as a source of probiotic strains of Lactobacilli, paid much less attention compared to dairy products and this issue is very important for Ukraine. Therefore, the study of probiotic properties of strains of *Lactobacillus plantarum*, isolated from fermented vegetables and their comparison with strains isolated from dairy products is important in terms of the biology of these organisms and is of fundamental and practical importance.

The aim of the present work was to study the probiotic properties of strains of *L. plantarum*, isolated from the national fermented foods of plant and animal origin derived from different regions of Ukraine in the comparative aspect.

Materials and Methods

The 24 strains of *L. plantarum* were the main objects of the present research. These strains were isolated from the national fermented foods of plant (sauerkraut, pickled cucumbers) and animal origin (sour milk, cream, cheese) [3]. Stock cultures were stored in 30% glycerol at -50 °C. Before the experiment the microorganisms were subcultured three times on medium MRS [8].

Adhesion of *L. plantarum* strains to human buccal epithelium cells was studied using method of Brilis and Brilene [1]. Code adhesion of microorganisms was determined – the average number of bacteria in one epithelial cells which is involved in the adhesion.

Monitoring biofilms formation of all LAB was performed as described Rode et al. [15].

Bile tolerance (0.3%) strains of *L. plantarum* was determined according to the protocol proposed by Christiaens and Leer [7].

To determine the stability of *L. plantarum* to the gastric juices there were used overnight culture of each strain, 1 ml of gastric juice (pH 2.0) (CJSC "Biopharma", Ukraine) and incubated at 37 °C for 2 h [12].

Resistance of *L. plantarum* to the action of lysozyme studied by the method of Vizoso Pinto et al. [17].

The statistical analysis of data was performed using the program «STATISTICA 7.0». To assess the reliability of quantitative indicators of differences in different strains there were used Post-hoc-test using the criterion of LSD. The difference was considered significant of $P \leq 0,05$.

Results and discussion

It was studied the biological activity of the 109 strains of *L. plantarum.* It was found that only 24 strains inhibited all the pathogenic bacteria tested such *Pseudomonas aeruginosa, Staphylococcus epidermidis, Shigella flexneri, Shigella sonnei, Proteus vulgaris, Escherichia coli, Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella enterica, Candida albicans* and were sensitive to clinically

important antibiotics [2, 4]. These strains were selected for further studies on their probiotic properties.

According to FAO / WHO [10] to probiotic strains LAB put forward a number of requirements, including – adhesion to epithelial cells and resistance to gastrointestinal conditions. The ability to adhere to epithelial cells provides colonization resistance of the host. It was shown, that all the investigated strains of *L. plantarum* are able to adhere to the human buccal epithelium. Low index adhesion had 50% of the lactobacilli (from 3.63 ± 0.20 to 9.95 ± 0.61), 37% – average (from 10.20 ± 0.46 to 14.58 ± 1.13). 12.5% of the strains of *L. plantarum* (1047s, 1092s, 743s) showed the high adhesive activity, these strains were isolated from sauerkraut. From this source were isolated as strains that had low index adhesion (4.31 ± 0.28) (Fig. 1).

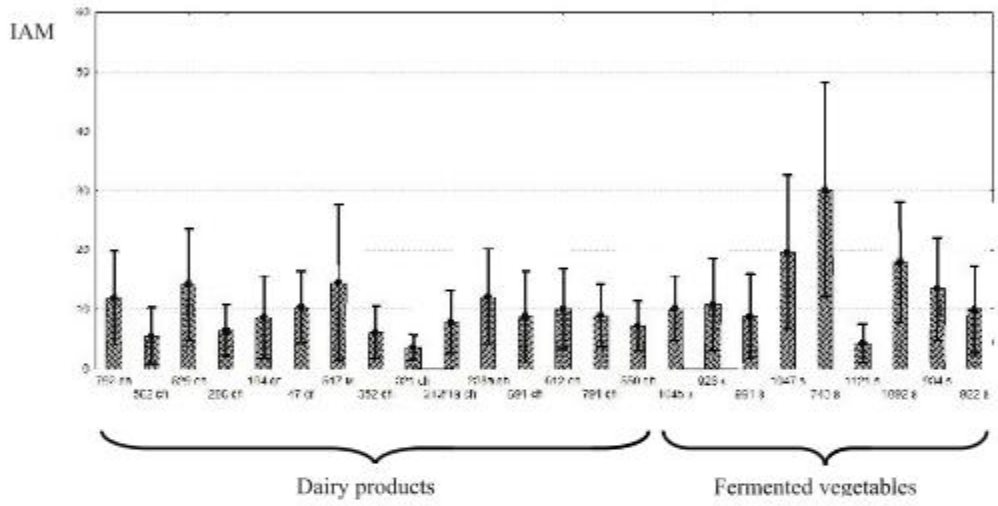

Fig. 1. Index adhesion strains of Lactobacillus plantarum to human buccal epithelium

Note: source of isolation of the strain:"ch" – cheese, "cr" – cream, "m" – sour milk , "s" – sauerkraut, "c" – pickled cucumbers; IAM – index adhesion of the strains.

The obtain results show that the adhesive activity depends on the strain and does not depend upon the source selection. The similar results were highlighted in another study, which shows that the adhesive activity depends on the strain of the microorganism [16].

LAB form biofilm, which ensures their survival in adverse conditions gastrointestinal microorganism of host [13]. Therefore, we have investigated the ability of *L. plantarum* strains to form biofilms (Fig. 2). Four strains of *L. plantarum* (529ch, 1045s, 352ch, 612ch) showed high ability to form biofilms. Many of the investigatied strains (79%) had an average rate of biofilm formation, one strain of *L. plantarum* 517m practically did not show this ability. There was no established relationship between the source of isolation of the strain and its ability to form biofilms, but it should be noted that the two strains isolated from cheese had the highest rate of the formation of biofilm.

All investigated strains of *L. plantarum* proved resistant to 0.3% bile salts in the medium.

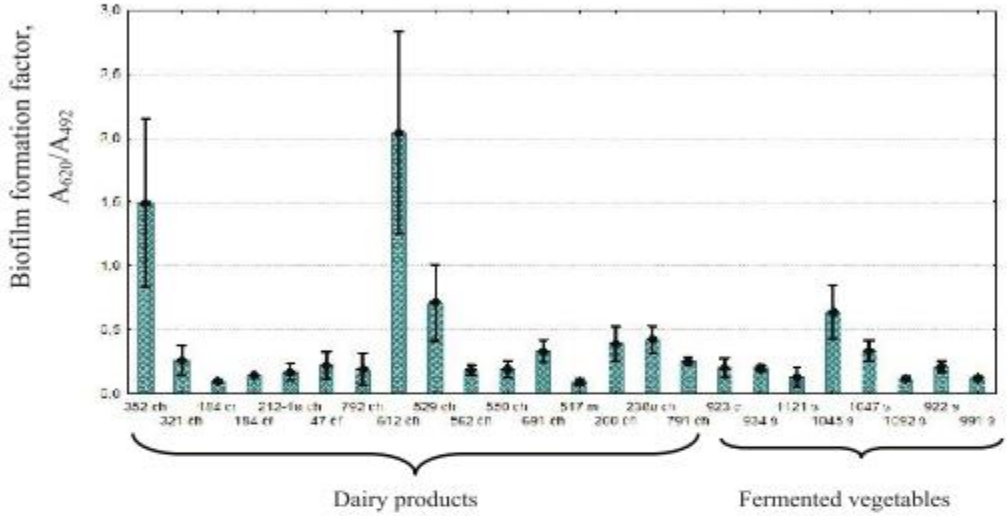

Fig. 2. Biofilm-forming ability of the strains of *Lactobacillus plantarum*

Note: source of isolation of the strain:"ch" – cheese, "cr" – cream, "m" – sour milk , "s" – sauerkraut, "c" – pickled cucumbers.

Gastric juice was the most aggressive factor in the gastrointestinal tract. It was found that 58% of strains of L. plantarum were inhibited by the action of the gastric juice for 2 hours. The other strains had different degrees of survival (Fig. 3). According to the literature, acceptable level of survival of LAB in conditions of the gastrointestinal tract is at least 1×10^4 CFU/ml [18]. Among the strains, only two answered the following criteria. They were isolated from a variety of sources: *L. plantarum* 1047s – with sauerkraut, *L. plantarum* 691c – cheese (Fig. 3).

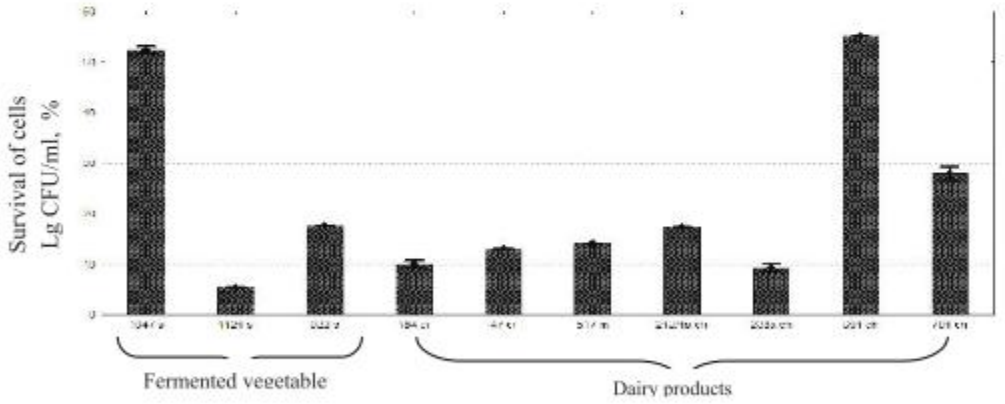

Fig. 3. Tolerance of *Lactobacillus plantarum* strains to intestinal juice

Note: source of isolation of the strain:"ch" – cheese, "cr" – cream, "m" – sour milk , "s" – sauerkraut.

There was no any relationship between the source of isolation of the strains and their sensitivity to the action of gastric juice.

We also investigated sensitivity of lactobacilli to the action of enzyme –lysozyme (Table).

Table

Stability of *Lactobacillus plantarum* strains to the action of lysozyme

Strains	Control, CFU/ml×10⁹	Experiment, CFU/ml×10⁹
352 ch	1,79±0,06*	1,57±0,23
791 ch	1,10±0,01*	0,50±0,02*
934 s	0,89±0,04*	0,82±0,07
562 ch	0,86±0,06*	0,50±0,02*
321 ch	1,78±0,02*	1,72±0,29
1121 s	5,70±0,16	0,37±0,04
1047 s	1,79±0,16	1,55±0,04*
550 ch	1,64±0,29	0,59±0,02*
184 cr	2,47±0,03*	1,79±0,01*
212/1a ch	1,28±0,09*	1,10±0,01*
691 ch	1,85±0,29*	1,35±0,03*

Note: * – significant difference ($p \leq 0,05$),
source of isolation of the strain: «ch» – cheese, «s» – sauerkraut, "cr" – cream.

It was established that this enzyme had no any significant effect on the growth of the most of lactobacilli. However, the concentration of CFU/ml of *L. plantarum* strains as 791ch, 1121 s, 550 ch, 184 cr halved decreased in comparison to controls.

So, as a result of being explored and analyzed the probiotic properties of 24 strains of *L. plantarum*. Most of the studied strains had high biological activity, but proved susceptible to the action of gastric juice, so they can be promising for the development of probiotics for oral cavity [6]. Thus, based primarily from the ability of lactobacilli to withstand harsh conditions of the gastrointestinal tract, we have selected two strains of *L. plantarum* 1047s and *L. plantarum* 691ch, which may be promising for use in oral probiotic preparations.

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ПРОБИОТИЧЕСКИЕ СВОЙСТВА ШТАММОВ *LACTOBACILLUS PLANTARUM*, ИЗОЛИРОВАННЫХ ИЗ ФЕРМЕНТИРОВАННЫХ ПРОДУКТОВ

Реферат

Цель. *Изучить пробиотические свойства штаммов L. plantarum, изолированных из национальных ферментированных продуктов животного и растительного происхождения.* ***Методы***. *Способность L. plantarum к адгезии изучали на клетках буккального эпителия человека. На пластиковых планшетах исследовали способность лактобацилл к пленкообразованию. Изучали выживание L. plantarum в условиях желудочно-кишечного тракта (ЖКТ): устойчивость к лизоциму, желудочному соку и солям жёлчных кислот.* ***Результаты***. *Штаммы L. plantarum имели широкий спектр антагонистической активности к использованным условно-патогенным микроорганизмам (УПМ), а также были чувствительны к клинически важным антибиотикам. Индекс адгезивности колебался от 3,63 ± 0,38 до 30,11 ± 1,47, и не зависел от источника выделения лактобацилл. У большинства штаммов (79%) обнаружили среднюю способность к формированию биопленки. Желудочный сок оказался одним из агрессивных факторов желудочно-кишечного тракта, при его действии выживало 42% штаммов. Установлено, что L. plantarum устойчивы к действию лизоцима и 0,3% солей жёлчных кислот.* ***Выводы***. *Исследованы и проанализированы пробиотические свойства 24-х штаммов L. plantarum. Отобрано два штамма L. plantarum 1047к и L. plantarum 691m, которые могут быть перспективными для использования в составе пероральных пробиотических препаратов.*

Ключевые слова: *пробиотические свойства, Lactobacillus plantarum, ферментированные продукты.*

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ПРОБІОТИЧНІ ВЛАСТИВОСТІ ШТАМІВ *LACTOBACILLUS PLANTARUM*, ІЗОЛЬОВАНИХ З ФЕРМЕНТОВАНИХ ПРОДУКТІВ

Мета. *Вивчити пробіотичні властивості штамів Lactobacillus plantarum, ізольованих з національних ферментованих продуктів рослинного та тваринного походження, відібраних з різних регіонів України.* ***Методи.*** *Здатність штамів L. plantarum до адгезії вивчали на клітинах букального епітелію людини. На пластикових планшетах досліджували здатність лактобацил до плівкоутворення. Досліджували виживання L. plantarum в умовах шлунково-кишкового тракту: стійкість до лізоциму, шлункового соку та солей жовчних кислот.* ***Результати***. *Штами L. plantarum мали широкий спектр антагоністичної дії щодо використаних умовно патогенних мікроорганізмів та виявились чутливими до клінічно важливих антибіотиків. Індекс адгезивності лактобацил коливався в межах від 3,63±0,38 до 30,11±1,47 та не залежав від джерела виділення. Більшість досліджуваних штамів (79%) мали середню здатність до формування біоплівки. Шлунковий сік виявився одним з найагресивніших чинників шлунково-кишкового тракту, при його дії ріст мали 42% штамів. Встановлено, що L. plantarum стійкій до лізоциму та 0,3% солей жовчних кислот.* ***Висновки***. *Досліджено та проаналізовано пробіотичні властивості 24 штамів L. plantarum. Відібрано два штами L. plantarum 1047к та L. plantarum 691т, які можуть бути перспективними для використання у складі пероральних пробіотичних препаратів.*

Ключові слова: Пробіотичні властивості, Lactobacillus plantarum, ферментовані продукти.

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