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EFFECT OF COCULTIVATION ON *LACTOBACILLUS PLANTARUM* STRAINS GROWTH AND ANTAGONISTIC ACTIVITY

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The use of bacterial starters for the production of fermented foods has several advantages over traditional spontaneous fermentation, as it provides a rapid and controlled decrease of pH, improves the microbiological quality of the product, and prolongs the shelf-life. Fermented foods are typically produced using mixed cultures of lactic acid bacteria (LAB) due to the synergism between their constituent bacterial cultures. So, the compatibility of the LAB strains decides the efficacy of a multi-strain starter. The ***purpose*** *of this study was to investigate the effect of the cocultivation of Lactobacillus plantarum strains on the growth, acidification, and antagonistic activity to determine suitable strain combinations for fermented vegetable production.* ***Methods.*** *The effect of cocultivation on growth characteristics of four L. plantarum strains was determined in MRS medium and cabbage-based medium with 2.5 % NaCl. After 8 h of cultivation at 30 °C and 37 °C, the number of viable cells (CFU/ml) and the pH of the medium were determined. The antagonistic activity of monocultures of L. plantarum and their six compositions against opportunistic pathogenic microorganisms was determined by the method of delayed antagonism.* ***Results****. During growth in MRS broth at 30 °C cocultivation of L. plantarum 47SM with L. plantarum 691T or L. plantarum 1047K strains led to enhanced rates of growth compared to the monocultures, suggesting some degree of symbiosis between these strains. Viable cell counts of L. plantarum 47SM, 1047K and 691T strains and ΔpH values of L. plantarum 952K, 1047K, and 691T strains were higher after 8 h growth in the cabbage-based medium at 30 °C compared to MRS broth. Despite the intensive growth of L. plantarum monocultures in cabbage-based medium, a significant decrease of viable cell counts and ΔpH values during cocultivation at 30 °C were found. Cocultivation did not affect the average size of the growth inhibition zones of most of the indicator strains used. However, growth inhibition zones of Shigella flexneri, Escherichia coli, and Proteus vulgaris decreased in some L. plantarum mixed cultures compared to monocultures. Thus, the growth inhibition zones of E. coli and S. flexneri by mixed culture L. plantarum 47 SM+1047K were significantly smaller compared to the growth inhibition zones of L. plantarum monocultures.* ***Conclusions****. Thus, based on the data obtained in present work, we can assume that some of these L. plantarum strains used in the work may be bactericinogenic. Although the four L. plantarum strains studied are compatible when cocultivated in a standard rich MRS medium, the results of cocultivation in a cabbage-based medium with 2.5 % NaCl does not allow to recommend the use of these L. plantarum strains simultaneously in the starter for vegetable fermentation. Further investigation of bacteriocinogenic properties and mechanisms of growth inhibition under cocultivation in vegetable-like conditions are needed, which will allow combining of some of these L. plantarum strains with LAB strains of other species or genera to create multi-starters for vegetable fermentation.*

Keywords: cocultivation, vegetable fermentation, lactic acid bacteria, antagonism.

Fermented vegetables and fruits have long been consumed by populations around the world due to their organoleptic characteristics and health benefits [1]. In the process of fermented vegetable production, one of the key steps is the rapid pH reduction of the raw material, which prevents the development of an undesirable microbiota and provides typical organoleptic characteristics of the final product. The use of bacterial starters for the production of fermented foods has several advantages over traditional spontaneous fermentation, as it provides a rapid and controlled decrease of pH, improves the microbiological quality of the product, and prolongs the shelf-life. Lactic acid bacteria (LAB) play a major role in the fermentation of vegetable raw materials. Due to their wide spectra of biological activity, LAB strains are not only able to accelerate the fermentation process but also provide more beneficial, functional properties to the end-products [2, 3].

It is known that the efficiency of the fermentation process can be enhanced by the use of starter compositions due to the synergism between their constituent bacterial cultures [4]. In our previous studies, we selected four *Lactobacillus plantarum* strains with probiotic properties and demonstrated the effectiveness of inoculation their monocultures for cabbage and cucumbers fermentations, compared with the classical method without the use of a starter [5]. The main condition for using strains in the complex starters is the absence of antagonistic action between them. The **purpose** of this study was to investigate the effect of cocultivation of *L. plantarum* strains on growth, acidification and antagonistic activity to determine suitable strain combinations for a fermented vegetable production.

Materials and Methods. The objects of the study were 4 *Lactobacillus plantarum* strains deposited at the Depositary of Cultures of Microorganisms of Danylo Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine. *L. plantarum* 1047K (IMV B-7566) and 952K (IMV B-7597) strains were isolated from fermented cabbage, strains of *L. plantarum* 47SM (IMV B-7565) and *L. plantarum* 691T (IMV B-7598) – from sour cream and sour milk respectively. These *L. plantarum* strains were selected in our previous studies as promising starter cultures with functional properties for vegetable fermentation [5, 6].

The effect of cocultivation on growth characteristics of *L. plantarum* strains was determined in MRS medium [7] and in cabbage-based medium with 2.5 % NaCl [5]. The media were inoculated with a suspension of an overnight culture of each strain (inoculum volume 1 %) or their compositions (0.5 % of each strain). After 8 h of cultivation at 30 °C and 37 °C, the number of viable cells (CFU/ml) and the pH of the medium were determined. The acid-producing activity was expressed as a value of ΔpH – the difference between the initial pH value of the medium and the value determined after 8 h of cultivation.

The antagonistic activity of *L. plantarum* monocultures and their six compositions against opportunistic pathogenic microorganisms was determined by the previously described method of delayed antagonism [8]. *Shigella flexneri* GISK 337, *S. sonnei* GISK 233169, *Proteus vulgaris* IMV B-905 (ATCC 6896), *Escherichia coli* IMV B-906 (ATCC 25922 (F-50)), *Bacillus cereus* IMV B-908 (ATCC 11778), *Staphylococcus aureus* IMV B-904 (ATCC 25923 (F-49)), *Klebsiella pneumoniae* IMV B-920 (ATCC 10031), *Salmonella enterica* IMV B-921 (NCTC 6017) were used as indicator strains.

A one-way analysis of variance (ANOVA) was carried out with the Statistica 7.0 software (Systat Inc., USA). Results from two independent assays were averaged. Statistical analysis was performed on the differences between the growth characteristics of monocultures and mixed cultures by using the LSD test, and $P < 0.05$ was considered statistically significant.

Results

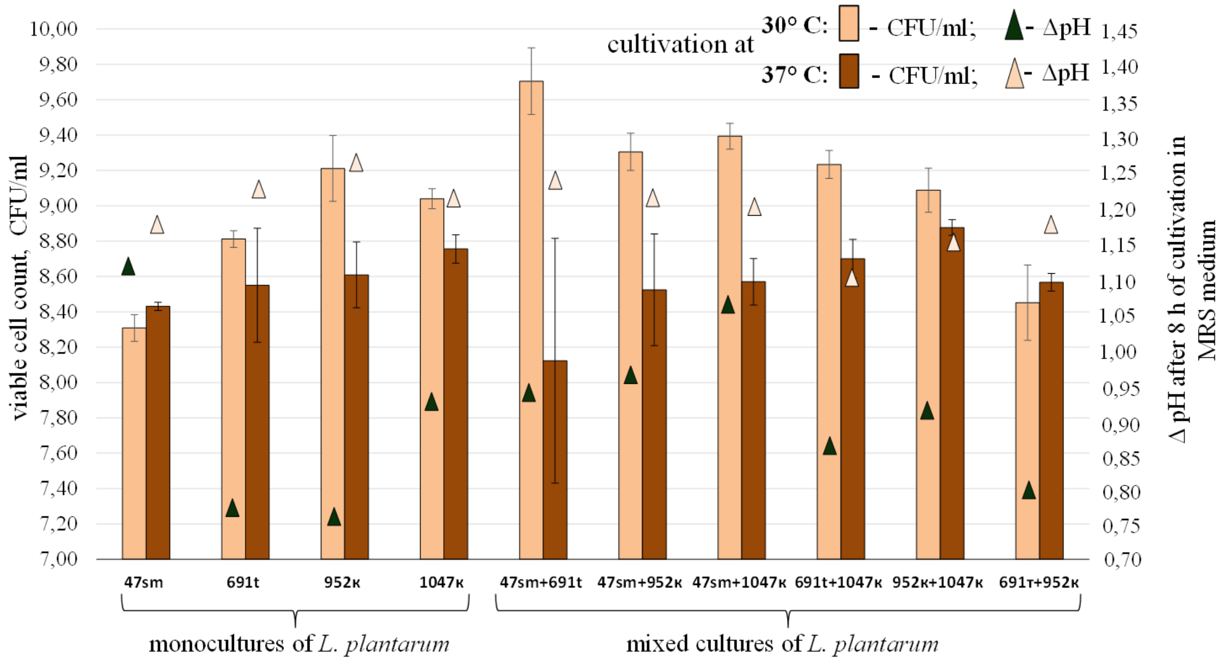
Cocultivation of L. plantarum strains in MRS medium

The effect of cocultivation on the growth rates of four *L. plantarum* strains was studied in MRS broth, which is commonly used as a growth medium for lactobacilli in laboratory experiments. The results obtained are presented in Fig. 1.

The viable cell counts of mixed cultures *L. plantarum* 47 SM + 691T and *L. plantarum* 47 SM + 1047K in MRS broth at 30 °C was greater than that for each *L. plantarum* monoculture. Despite the increase in the viable cell counts, stimulation of acid production was not observed in mixed cultures. The viable cell count of a mixed culture of *L. plantarum* 691T and 952K strains in MRS broth at 30 °C was lower than that for *L. plantarum* 691T monoculture. Mixed cultures of *L. plantarum* strains did not increase growth and acid production in MRS broth at 37 °C compared with that of each monoculture.

Antagonistic activity of mixed cultures of L. plantarum strains

The *L. plantarum* strains used in this study have a wide range of antagonistic activity against opportunistic bacteria, as shown by us in previous work [8]. The result of the antagonistic action of *L. plantarum* in monocultures and mixed cultures was shown in Fig. 2. Overall, cocultivation did not affect the average size of the growth inhibition zones of most of the indicator strains used. However, growth inhibition zones of *S. flexneri, E. coli*, and *P. vulgaris* decreased in some *L. plantarum* mixed cultures compared to monocultures. Thus, the growth inhibition zones of *E. coli* and *S. flexneri* by mixed cultures *L. plantarum* 47 SM + 952K and *L. plantarum* 47 SM + 1047K were significantly smaller compared to the growth inhibition zones of *L. plantarum* monocultures (Fig. 2). Mixed culture of *L. plantarum* 952K + 1047K less inhibited the growth of *S. flexneri* and *P. vulgaris,* compared to the *L. plantarum* 1047K monoculture. The monoculture of *L. plantarum* 47SM had a larger growth inhibition rate of *P. vulgaris* compared to the mixed cultures of *L. plantarum* 47 SM + + 691T.



F i g. 1. Viable cell counts and pH decrease during cocultivation of *L. plantarum* strains in MRS broth compared to monocultures

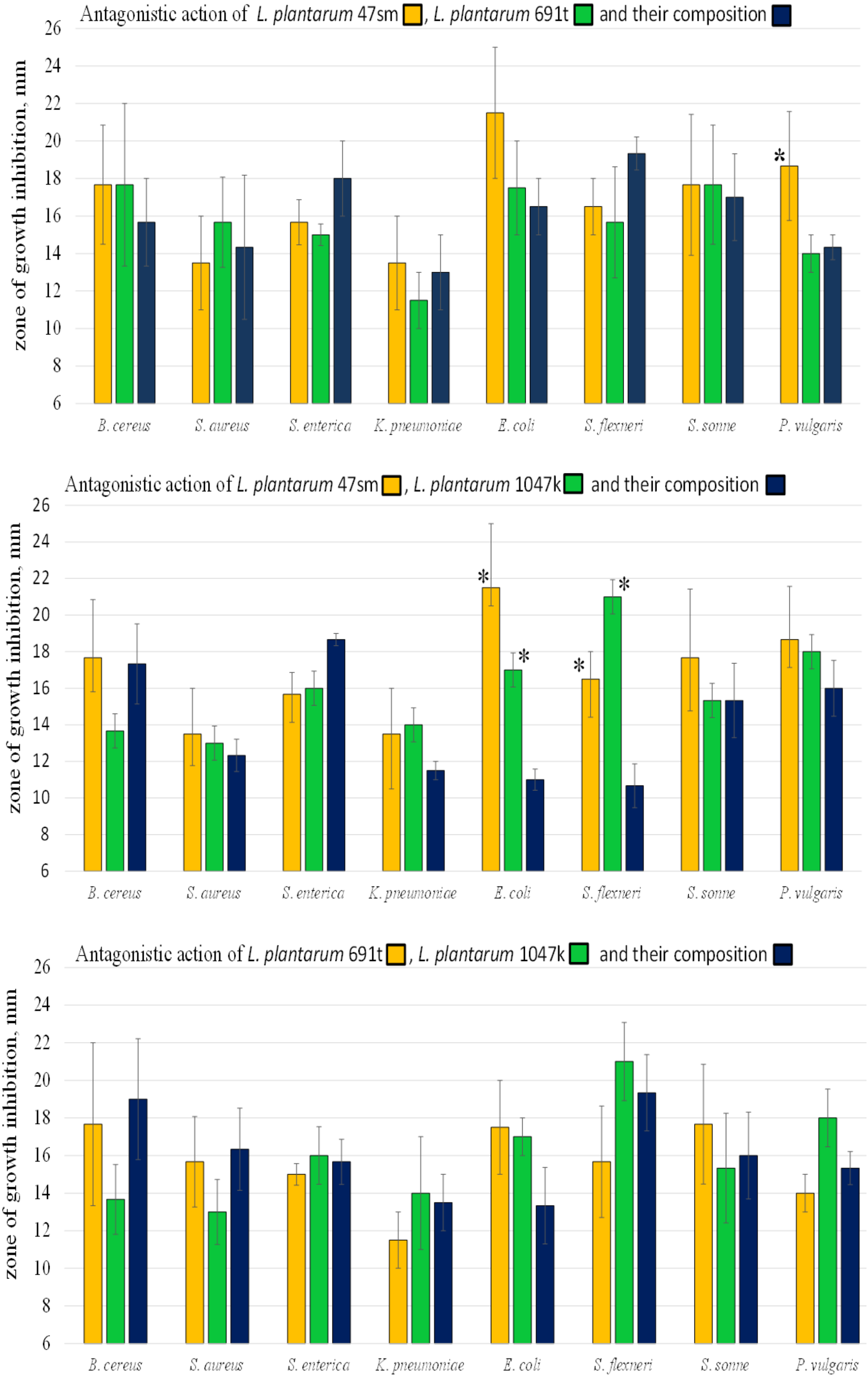
Cocultivation of L. plantarum strains in cabbage-based medium with 2.5 % NaCl

The growth rates and acid production of *L. plantarum* as monocultures and six mixed cultures in the cabbage-based medium with 2.5 % NaCl at different temperatures are shown in Fig. 3. As seen, at 30 °C viable cell counts in mixed cultures *L. plantarum* 691T + 1047K and *L. plantarum* 47SM + 1047K and 691T were higher compared to their mixed cultures with strain *L. plantarum* 952K. The level of acid production in mixed cultures during cultivation in the cabbage-based medium at 30 °C was lower, compared to the monocultures of *L. plantarum* strains.

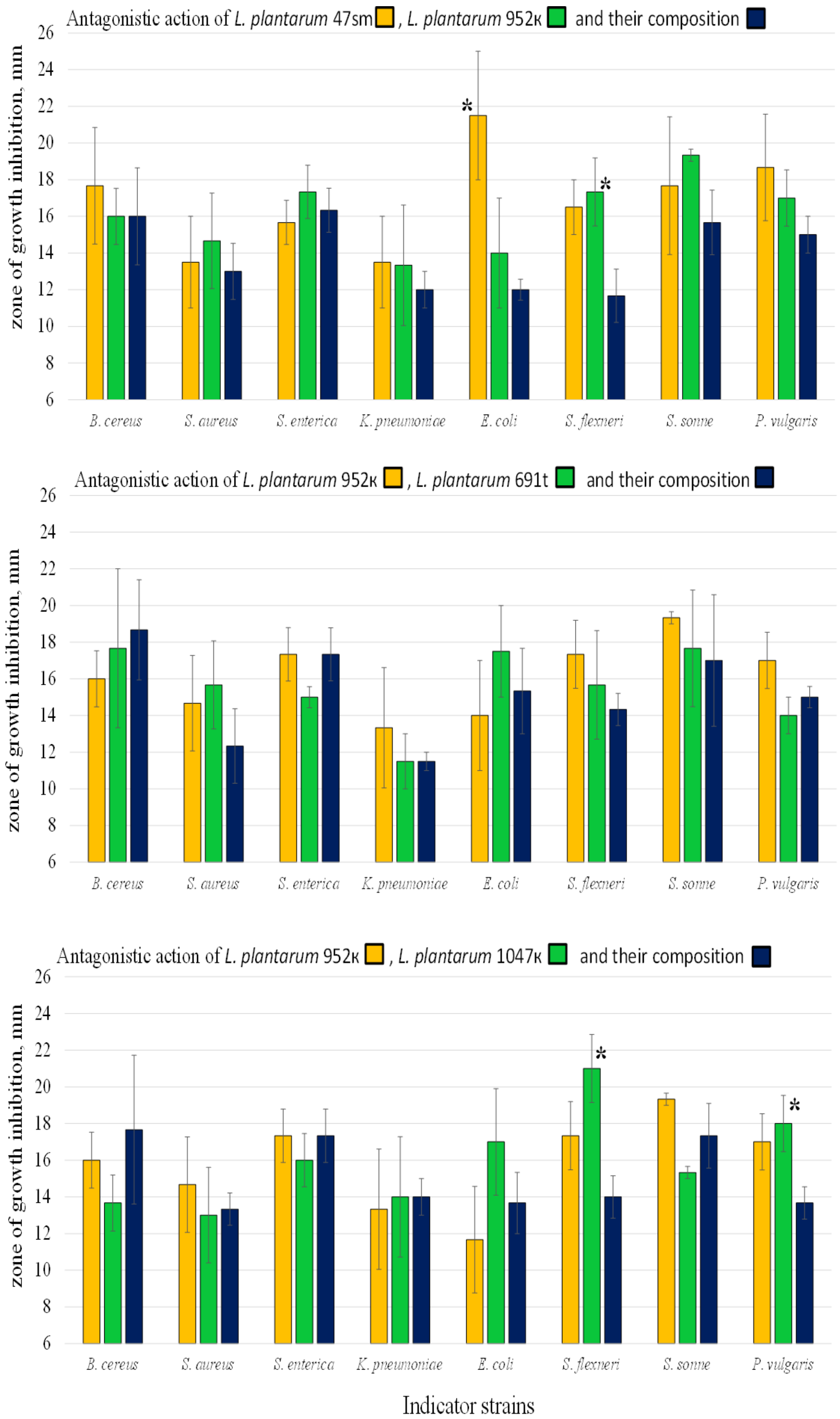
The viable cell count of mixed cultures of *L. plantarum* 47 SM + 95T and *L. plantarum* 691T +952K in the cabbage-based medium at 37 °C was lower than that for *L. plantarum* 691T and 47SM monocultures. During cultivation at 37 °C decreasing of pH in mixed cultures was more rapid than that of monocultures except for the combination of *L. plantarum* 47SM + 691T strains.

Discussion. Fermented foods are typically produced using mixed cultures of LAB. So, the compatibility of the LAB strains decides the efficacy of a multi-strain starter. Besides a standard rich MRS medium, we conducted our study using a cabbage-based medium with 2.5 % NaCl, which is more similar to cabbage fermentation process conditions. It should be noted that the growth characteristics of monocultures were different in these two media used depending on the temperature of incubation. Viable cell counts of *L. plantarum* 47SM, 1047K, and 691T strains and ΔpH values of *L. plantarum* 952K, 1047K, and 691T strains were higher after 8 h growth in the cabbage-based medium at 30 °C compared with MRS broth. Although the viable cell counts were at the same level during growth at 37 °C, the level of acid production was dramatically higher in the MRS medium compared to the cabbage-based medium. A lack of correlation between the cell number and the level of acid formation by LAB strains was shown by authors [9]. Such differences are not unexpected and maybe dye by considerably different in media nutrient composition.

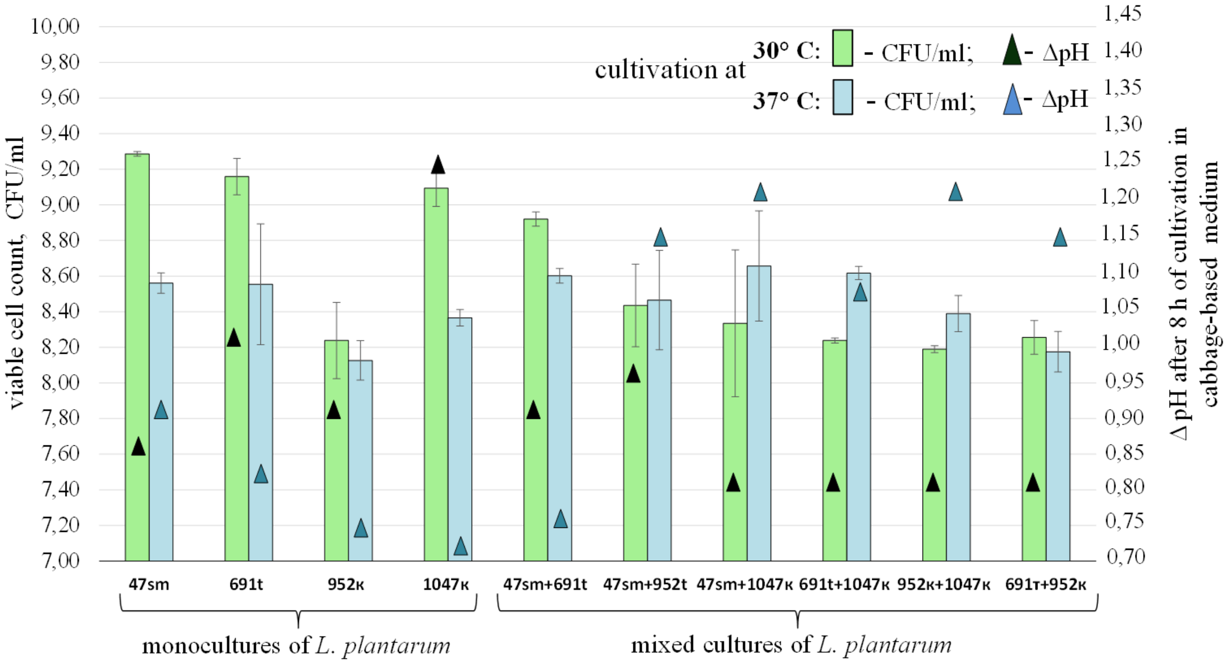
The adaptation of lactic acid bacteria to vegetable environment markedly varied within strains of LAB and poorly investigated compared to other fermented foods, such as dairy products. It was shown by authors, that the growth of *L. plantarum* strains in tomato and carrot juices was similar to growth in the MRS medium [10]. In another



F i g. 2. Antagonistic activity of *L. plantarum* monocultures and their compositions towards opportunistic pathogens



F i g. 2. Continuation



F i g. 3. Viable cell counts and pH decrease during cocultivation of *L. plantarum* strains in cabbage-based medium compared to monocultures

study was shown that the viable cell counts for *L. plantarum* strains were significantly higher in cucumber juice than in MRS broth [11].

In mixed starter cultures, it is important that the growth of each lactic acid bacterium is not inhibited by mixing and that the bacteria stimulate each other's growth and acid production.

During growth in MRS broth at 30 °C cocultivation of *L. plantarum* 47sm with *L. plantarum* 691t or *L. plantarum* 1047k strains led to enhanced rates of growth compared with the individual cultures, suggesting some degree of symbiosis between these strains. Stimulatory interactions have been observed between LAB strains belonging to different genera, for example, *Bifidobacterium* and *Lactobacillus* strains [12] or *Lactococcus lactis* [4].

Despite the intensive growth of *L. plantarum* monocultures in the cabbage-based medium, a significant decrease of viable cell counts and ΔpH values during cocultivation at 30 °C were found. Thus, based on the data obtained in the present work, we can assume that some of these *L. plantarum* strains used in the work may be bactericinogenic. This assumption may be supported by the data presented in the literature. As was shown by authors, the induction of bacteriocin production through cocultivation with specific bacterial strains is a common feature in the species *L. plantarum* [13, 14, 15]. The *L. plantarum* CECT4185 strain was able to increase bacteriocin production more than 10 times when cocultured with *L. lactis* IL1403 strain [15]. Furthermore, many bacteriocinogenic LAB have been described whose bacteriocin production could be induced by coculture, including strains of *Lactobacillus salivarius*, *Lactobacillus acidophilus,* and *Enterococcus faecium* [16, 17].

In most cases, high bacteriocin production is associated with intensive bacterial growth [18, 19]. But, as was shown by authors, stress conditions can lead to a much higher bacteriocin production [19]. It was reported by authors that bacteriocin production can be enhanced by NaCl in some LAB strains [20, 21]. *L. plantarum* LPCO10 strain was isolated from olive fermentation brine and its maximum plantaricin S activity is achieved at 2.5 % (w/v) NaCl concentrations in the culture medium [21].

In addition to growth stimulation, the effect of cocultivation on the biological activity of *L. plantarum* strains was shown by authors. Cocultivation of *L. plantarum* DC400 with the other *Lactobacillus* strains markedly increased the capacity to form a biofilm, the level of adhesion to Caco-2 cells and to prevent the adhesion of potential intestinal pathogens [22]. In the present work, the effect of cocultivation on the antagonistic activity of *L. plantarum* strains towards opportunistic pathogens was evaluated. In most cases, there were no significant differences between sizes of growth inhibition caused by *L. plantarum* monocultures and their mixed cultures.

The exception was mixed culture *L. plantarum* 47SM + 1047K, which growth inhibition zones of *E. coli* and *S. flexneri* were smaller compared to monocultures of *L. plantarum* 47SM and 1047K strains. We can speculate, that the cocultivation effects on the spectrum of antimicrobial metabolites produced since it is known that LAB strains can simultaneously produce several bacteriocins [23]. Production of bacteriocins may be a desirable trait in LAB starter for vegetable fermentation [24]. Further investigation of bacteriocinogenic properties and mechanisms of growth inhibition under cocultivation in vegetable-like conditions are needed, which will allow combining of some of these *L. plantarum* strains with LAB strains of other species or genera to create multi-starters for vegetable fermentation.

Conclusions. Although the four *L. plantarum* strains studied are compatible when cocultivated in a standard rich MRS medium, the results of cocultivation in a vegetable-like conditions (cabbage-based medium with 2.5 % NaCl) does not allow to recommend the use of these *L. plantarum* strains simultaneously in the starter for vegetable fermentation.

ВПЛИВ СУМІСНОГО КУЛЬТИВУВАННЯ НА РІСТ ТА АНТАГОНІСТИЧНУ АКТИВНІСТЬ ШТАМІВ *LACTOBACILLUS PLANTARUM*

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Резюме

Використання бактеріальних заквасок для виготовлення ферментованих продуктів має ряд переваг над традиційним методом спонтанного бродіння, оскільки забезпечує швидке та контрольоване зниження pH, покращує мікробіологічну якість продукту та подовжує термін його зберігання. Ферментовані продукти зазвичай виготовляють із використанням змішаних заквасочних препаратів завдяки синергізму між їх складовими культурами молочнокислих бактерій. Отже, сумісність між штамами молочнокислих бактерій є основою ефективності багатокомпонентних заквасок. **Метою** роботи було дослідження впливу сумісного культивування на ріст, кислотоутворення та антагоністичну активність штамів *Lactobacillus plantarum* для оцінки можливості використання їх комбінацій для виготовлелння ферментованих овочів. **Методи.** Вплив сумісного культивування на ростові характеристики чотирьох штамів *L. planrtarum* був визначений в середовищі MRS та капустяному середовищі з 2.5 % NaCl. Після 8 год культивування при 30 °C і 37 °C визначали кількість колонієутворюючих одиниць (КУО/мл) та pH середовищ. Антагоністичну активність монокультур штамів *L. plantarum* та їх шести композицій щодо умовно-патогенних мікроорганізмів визначали з використанням методу відстроченого антагонізму. **Результати**. При рості в MRS бульйоні при 30 °C сумісне культивування штаму *L. plantarum* 47CM з штамами *L. plantarum* 691T чи *L. plantarum* 1047K призводило до підсилення росту в порівнянні з їх монокультурами, що свідчить про наявність певних симбіотичних відносин між цими штамами. Кількість клітин (КУО/мл) штамів *L. plantarum* 47 CM, 1047K і 691T та показники ΔpH штамів *L. plantarum* 952K, 1047K і 691T були вищими через 8 год росту при 30 °C в капустяному середовищі в порівнянні з MPC бульйоном. Не дивлячись на інтенсивний ріст монокультур штамів *L. plantarum* в капустяному середовищі, при сумісному культивуванні в даному середовищі за температури 30 °C спостерігали значне зниження кількості клітин та показників ΔpH. Сумісне культивування не впливало на розмір зон затримки росту більшості індикаторних штамів умовно-патогенних мікроорганізмів. Однак зони пригнічення росту *Shigella flexneri*, *Escherichia coli* і *Proteus vulgaris* були меншими у деяких змішаних культур *L. plantarum.* Так, зони затримки росту *E. coli* і *S. flexneri* змішаною культурою *L. plantarum* 47CM+1047K були достовірно менші в порівнянні з зонами затримки росту монокультур *L. plantarum* 47CM і 1047K. **Висновки**. Отже, виходячи з отриманих в даній роботі даних, ми можемо зробити припущення, що деякі з використаних в дослідженні штамів *L. plantarum* можуть бути бактеріоциногенними. Не дивлячись на те, що при сумісному культивуванні в стандартному багатому середовищі MRS чотири вивчених штами *L. plantarum* є сумісними, результати сумісного культивування в капустяному середовищі з 2,5 % NaCl не дозволяють рекомендувати ці штами для одночасного використання у заквасці

для сквашування овочів. Необхідними є подальші дослідження бактеріоциногенних властивостей та механізмів пригнічення росту за умов сумісного культивування в овочевому середовищі, що дозволить комбінувати дані штами *L. plantarum* з МКБ інших видів чи родів для створення багатокомпонентних заквасок для ферментування овочів.

Ключові слова: сумісне культивування, сквашування овочів, молочнокислі бактерії, антагонізм.

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