

Antimicrobial activity of *Lactobacillus plantarum* strains against *Salmonella* pathogens

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Abstract

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Introduction. Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocin during lactic fermentations. All of these can antagonize the growth of some spoilage and pathogenic bacteria in foods.

Materials and methods. To determine the antimicrobial activity of *Lactobacillus plantarum* D1 and *Lactobacillus plantarum* D2 against *Salmonella* sp. and *Salmonella abony* ATCC 6017, the method of co-culturing was applied. The study was conducted under static conditions at 37±1 °C for 72 hours, taking samples at 0, 12, 24, 36, 48, 60 and 72 h and monitoring the changes in the titratable acidity and the concentration of viable cells of both the pathogens and the *Lactobacillus plantarum* strains.

Results and discussion. In the single-strain cultivation of each *Lactobacillus plantarum* strain and each *Salmonella* strain high concentration of viable cells were achieved by the 24th hour and it was maintained by the end of the culturing. In the co-culturing of each *Lactobacillus plantarum* strain and each *Salmonella* strain, the *Lactobacillus* strain was not significantly influenced by the presence of any of the *Salmonella* strains. But the number of viable cells of the pathogens was greatly reduced, the reduction being strain-specific. In the co-culturing of each *Lactobacillus plantarum* strain and *Salmonella abony* ATCC 6017, the concentration of viable cells of the pathogen strain was reduced by the 60th h. In the co-culturing of each *Lactobacillus plantarum* strain and *Salmonella* sp., the concentration of viable cells of the pathogen strain was reduced by the 72th h. The observed antimicrobial activity was due to a great extent to the acidification of the medium because of the production and accumulation of lactic and other organic acids.

Conclusions. The demonstrated antimicrobial activity is a prerequisite for further research on the probiotic potential of the two *Lactobacillus plantarum* strains for their inclusion in the composition of probiotic preparations and starters for probiotic functional foods.

Introduction

Lactic acid bacteria play an important role in food fermentation processes. Raw foods such as milk, fruits, vegetables or meat are often preserved by lactic acid fermentation. These organisms produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocin during lactic fermentations. All of these can antagonize the growth of some spoilage and pathogenic bacteria in foods [1, 2].

Most of the probiotic lactobacilli in human foods are supplied in highly concentrated forms containing more than 10^{10} cfu/cm³. The ability of *Lactobacillus* strains to adhere to the mucosal surfaces of the intestine and the subsequent long or short term colonization has long been one of the most commonly encountered criteria for the selection of probiotic strains [3, 4, 5].

Enteric disorders are one of the most important problems in the food industry, with salmonellosis and colibacillosis regarded as the major bacterial diseases occurring in human. *Salmonella* and *Escherichia coli* infections range from severe acute disease to mild infections of a chronic nature [6].

The purpose of the present work was to study the antimicrobial activity of *Lactobacillus plantarum* D1 and *Lactobacillus plantarum* D2 against the Gram-positive pathogenic microorganisms *Salmonella* sp. (clinical isolate) and *Salmonella abony* ATCC 6017, that cause toxicoses and toxicoinfections.

Materials and methods

1. Microorganisms

Lactobacillus plantarum D1 and *Lactobacillus plantarum* D2, isolated from salad dressings;

test pathogenic microorganisms *Salmonella* sp. (clinical isolate) and *Salmonella abony* ATCC 6017.

2. Media

2.1. MRS – broth medium

Composition (g/dm³): peptone from casein – 10; yeast extract – 4; meat extract – 8; glucose – 20; K₂HPO₄ – 2; sodium acetate – 5; diammonium citrate – 2; MgSO₄ – 0.2; MnSO₄ – 0.04; Tween 80-1 cm³/dm³; pH = 6.5. Sterilization – 15 minutes at 118 °C.

2.2. LAPTg10 – agar medium

Composition (g/dm³): peptone – 15; yeast extract – 10; tryptone – 10; glucose – 10; Tween 80-1 cm³/dm³, agar – 15. pH=6.6 – 6.8. Sterilization – 20 minutes at 121 °C.

2.3. LBG – agar medium

Composition (g/dm³): tryptone – 10; yeast extract – 5; NaCl – 10; glucose – 10; agar – 15; pH = 7,5. Sterilization – 20 minutes at 121 °C.

3. Determination of the antimicrobial activity against pathogenic microorganisms – by co-culturing

To determine the antimicrobial activity of the studied lactobacilli strains against the two pathogens a 48 hour cultural suspension of each *Lactobacillus plantarum* strain was used. Separate cultivation of the two *Lactobacillus plantarum* and the two *Salmonella* strains as well as co-culturing of each of the two *Lactobacillus plantarum* strains and each *Salmonella* strain included in the study were conducted. For the examination of the co-culturing, 0.5 cm³ of the suspension of the *Lactobacillus plantarum* strain, 0.5 cm³ of the

suspension of the *Salmonella* strain and 9 cm³ of culture medium (MRS-broth medium) were mixed. In the control of each *Lactobacillus plantarum* strain and in the control of each pathogen, 9.5 cm³ of the MRS-broth medium were mixed with 0.5 cm³ of the suspension of the *Lactobacillus plantarum* strain or of the suspension of the *Salmonella* strain, respectively. The study was conducted under static conditions in a thermostat at 37±1°C for 72 hours, taking samples at 0, 12, 24, 36, 48, 60 and 72 h and monitoring the changes in the titratable acidity and the concentration of viable cells of both the pathogens and the *Lactobacillus plantarum* strains. The determination of the number of viable cells was done by the spread plate method on LAPTg10-agar (for the enumeration of lactobacilli), on LBG-agar (for the enumeration of pathogens). The titratable acidity was determined according to a standard protocol [7].

Results and discussion

In the study of the antimicrobial activity of the two *Lactobacillus plantarum* against the two *Salmonella* strains by the method of co-culturing, the dynamics of the change in the number of viable cells of both the lactobacilli and the pathogens and in the titratable acidity were monitored (Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7 and Fig. 8).

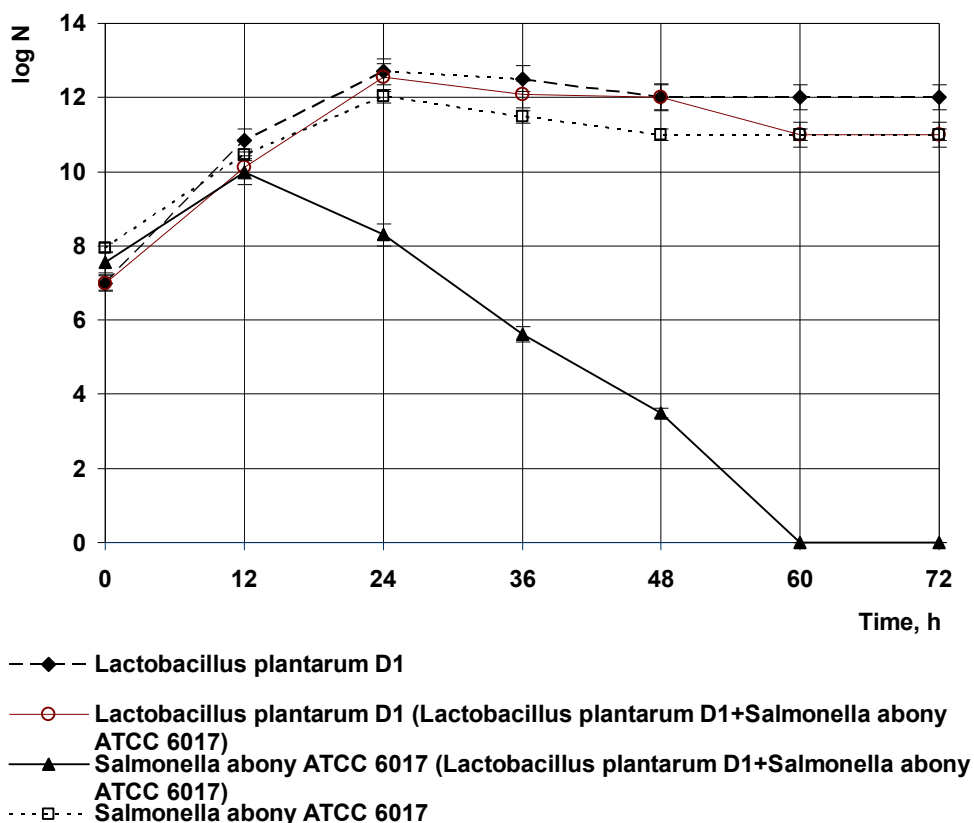


Figure 1. Changes in the number of viable calls of *Lactobacillus plantarum* D1 and *Salmonella abony* ATCC 6017 in single-strain culturing and in a mixed population at 37±1 °C

In the single-strain cultivation of each *Lactobacillus plantarum* strain and each *Salmonella* strain high concentration of viable cells were achieved by the 24th hour and it was maintained by the end of the culturing. In the co-culturing of each *Lactobacillus plantarum* strain and each *Salmonella* strain, the *Lactobacillus* strain was not significantly influenced by the presence of any of the *Salmonella* strains. But the number of viable cells of the pathogens was greatly reduced, the reduction being strain-specific. The obtained results were commensurable with the observations described in [9].

The *Lactobacillus plantarum* and *Salmonella* strains entered the stationary growth phase at the 24th h, reaching maximum concentration of viable cells – above 10^{12} cfu/cm³ (Fig. 1, Fig. 2, Fig. 5 and Fig. 6). Meanwhile the titratable acidity of the medium in the culturing of both the two strains of lactobacilli reached 140°T (Fig. 3, Fig. 4, Fig. 7 and Fig. 8). A similar trend was established in the single-strain culture of the two *Salmonella* strains. Therefore, *Salmonella* sp. Entered the stationary growth phase at the 12th h, while *Salmonella abony* ATCC 6017 entered it at the 24th h, reaching concentrations of viable cells about 10^{12} cfu/cm³ (Fig. 1, Fig. 2, Fig. 5 and Fig. 6).

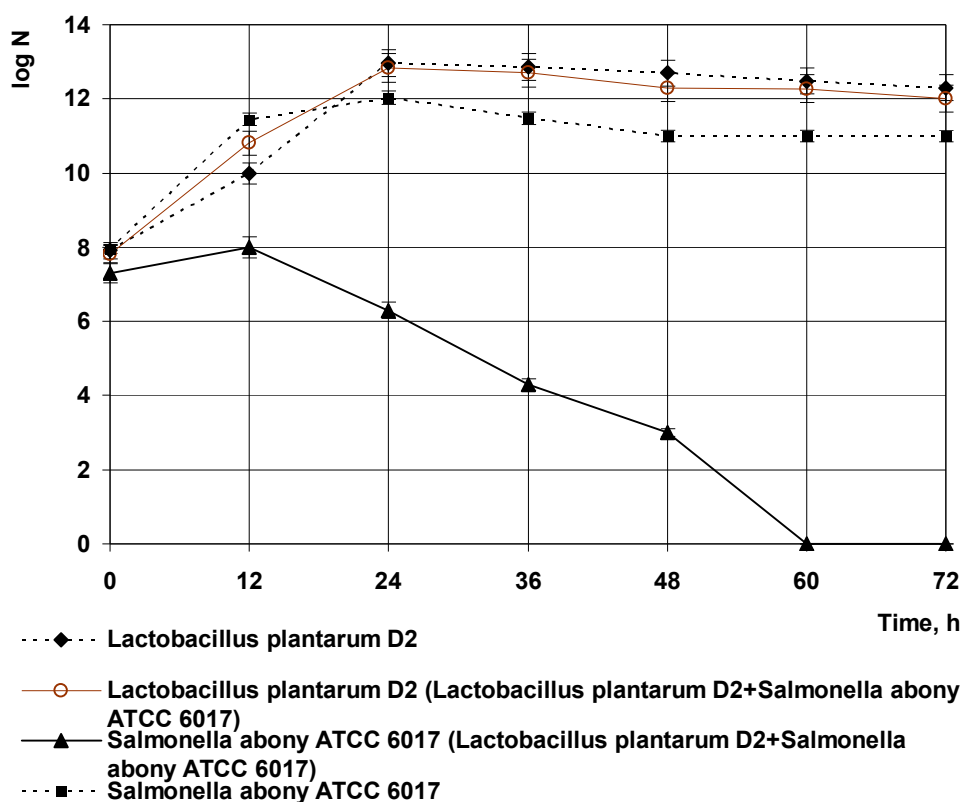


Figure 2. Changes in the number of viable calls of *Lactobacillus plantarum* D2 and *Salmonella abony* ATCC 6017 in single-strain culturing and in a mixed population at 37±1 °C.

In the co-culturing of each *Lactobacillus plantarum* strain and *Salmonella abony* ATCC 6017, the concentration of viable cells of the pathogen strain was reduced by the 60th h (Fig. 3 and Fig. 4).

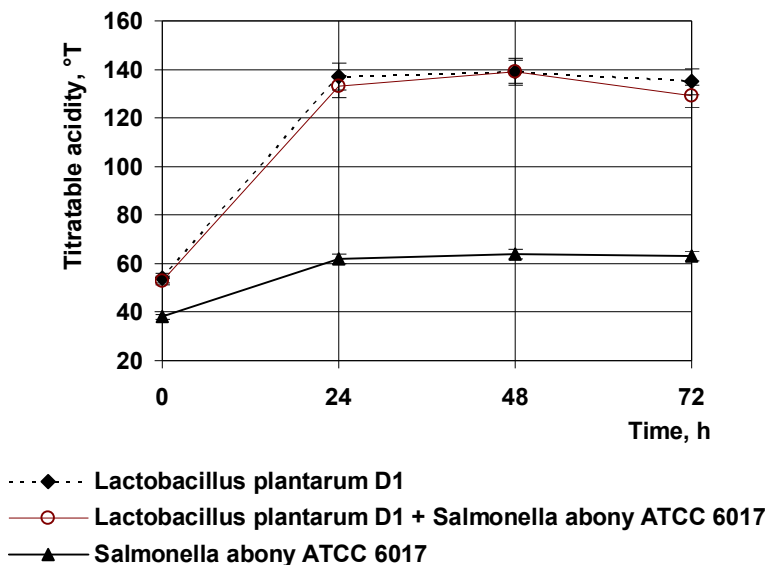


Figure 3. Changes in the titratable acidity of the medium in single-strain culturing and in a mixed population of *Lactobacillus plantarum* D1 and *Salmonella abony* ATCC 6017 at 37±1 °C.

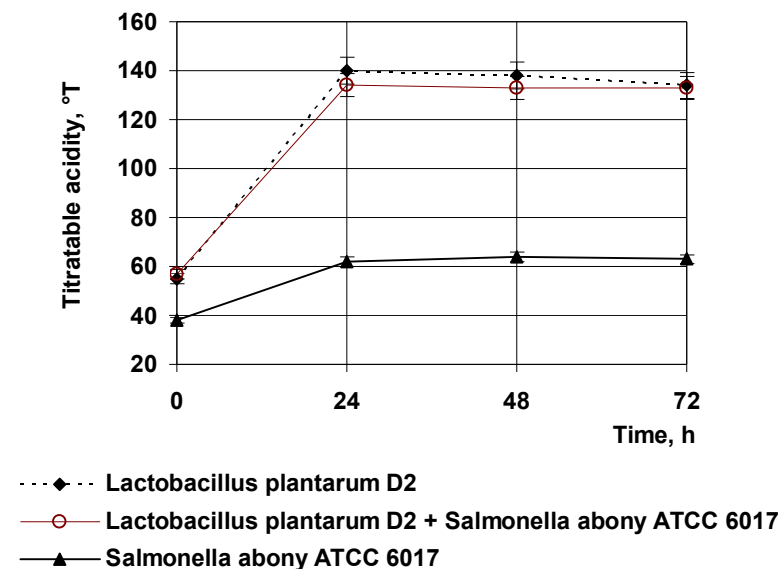


Figure 4. Changes in the titratable acidity of the medium in single-strain culturing and in a mixed population of *Lactobacillus plantarum* D2 and *Salmonella abony* ATCC 6017 at 37±1 °C

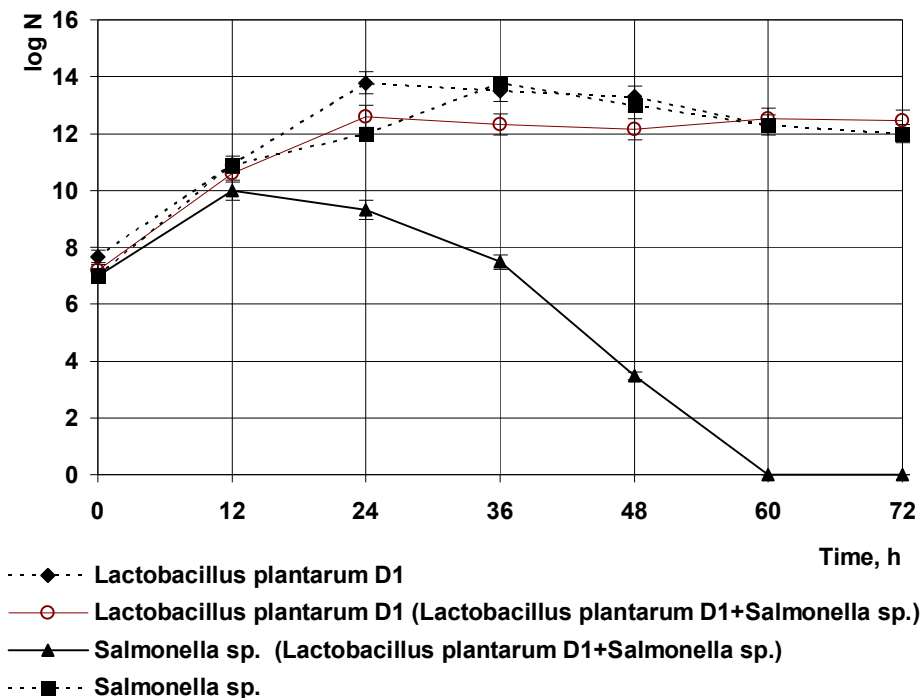


Figure 5. Changes in the number of viable calls of *Lactobacillus plantarum* D1 and *Salmonella* sp. In single-strain culturing and in a mixed population at 37±1 °C.

In the co-culturing of each *Lactobacillus plantarum* strain and *Salmonella* sp., the concentration of viable cells of the pathogen strain was reduced by the 72th h (Fig. 7 and Fig. 8). The two *Salmonella* strains differed in their growth characteristics. In the co-culturing of the two *Salmonella* strains with each of the two *Lactobacillus plantarum* strains, a slight increase in the number of living cells was observed, but it had different behavior depending on the very *Lactobacillus plantarum* strain.

The observed antimicrobial activity of the two *Lactobacillus plantarum* strains included in the present study was due to the production and accumulation of lactic and other organic acids. According to Helander et al. [8], *L. plantarum* produces a variety of low molecular mass compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide and other metabolites. Many of these metabolites have a broad activity spectrum against other species, and their production is largely affected by the food matrix itself. [6].

The *L. plantarum* strains isolated from salad dressings reduced the amount of viable cells of the two *Salmonella* strains in a mixed population (*Lactobacillus plantarum* and *Salmonella* strain) in the present in vitro study. The obtained results confirm the research by Denkova R. et al., 2013 [9]. But *Lactobacillus plantarum* D1 and *Lactobacillus plantarum* D2 reduced the number of living cells of *Salmonella abony* NTCC 6017 by the 60th h, while *Lactobacillus plantarum* X2 (isolated from spontaneously fermented sourdough) could not do so even by the 72nd h. On the other hand, *Lactobacillus plantarum* F3 (isolated from spontaneously fermented sourdough) and *Lactobacillus plantarum* LBRZ12 reduced the

Salmonella abony NTCC 6017 living cells by the 60th h, in compliance with the results reported in the present manuscript. Hence, *Lactobacillus plantarum* D1 and *Lactobacillus plantarum* D2 possess higher antimicrobial activity against *Salmonella abony* NTCC 6017 than *Lactobacillus plantarum* X2 [9]. *Lactobacillus plantarum* F3 (isolated from spontaneously fermented sourdough) managed to suppress all *Salmonella* sp. (clinical isolate) cells by the 72nd h, which is in compliance with the obtained results for the same pathogen in its co-culturing with *Lactobacillus plantarum* D1 and *Lactobacillus plantarum* D2. But *Lactobacillus plantarum* X2 and *Lactobacillus plantarum* LBRZ12 demonstrated lower antimicrobial activity against *Salmonella* sp. (clinical isolate) – the number of viable pathogen cells by the 72nd h was 10^3 cfu/cm³ [9]. After additional research on the probiotic properties of the two *Lactobacillus plantarum* strains, they can be included in the composition of probiotic preparations and starters for functional probiotic foods and beverages. This in turn would ensure the microbiological safety of the foods and beverages. Moreover, upon intake the high concentration of viable cells of lactobacilli will provide the necessary beneficial flora to maintain the balance in the gastrointestinal tract and perform its inherent preventive role.

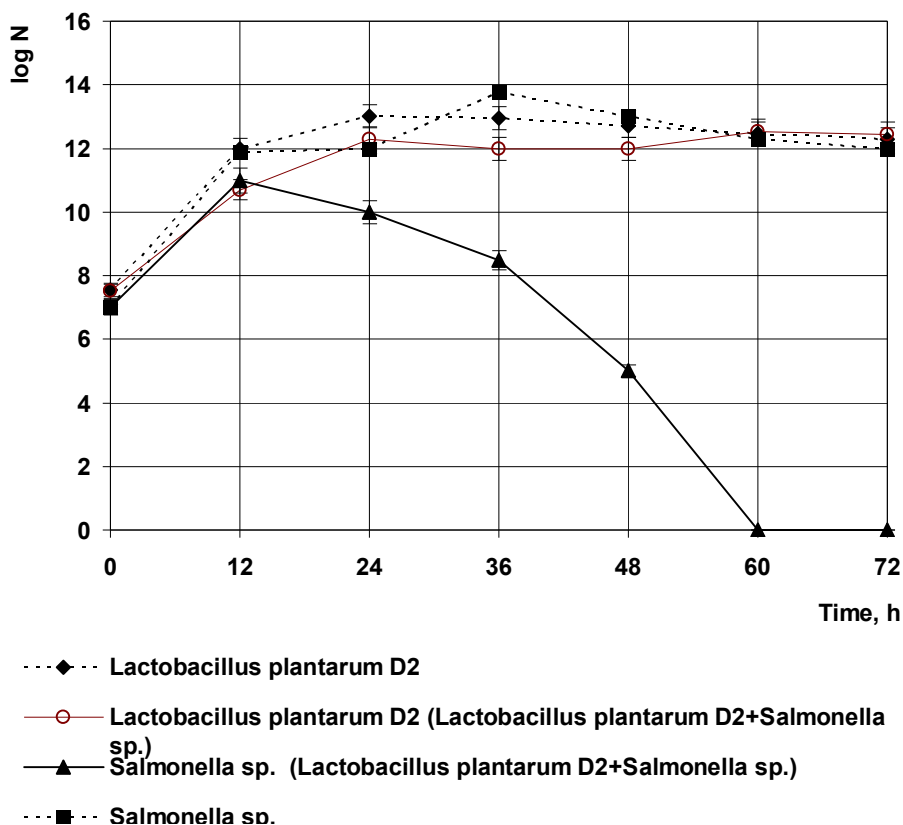


Figure 6. Changes in the number of viable calls of *Lactobacillus plantarum* D2 and *Salmonella* sp. In single-strain culturing and in a mixed population at $37 \pm 1^\circ\text{C}$.

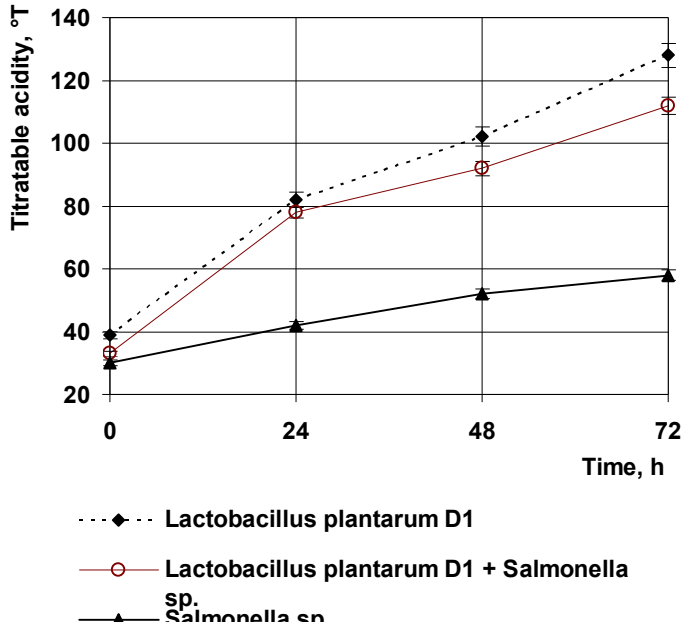


Figure 7. Changes in the titratable acidity of the medium in single-strain culturing and in a mixed population of *Lactobacillus plantarum* D1 and *Salmonella* sp. at 37 ± 1 °C.

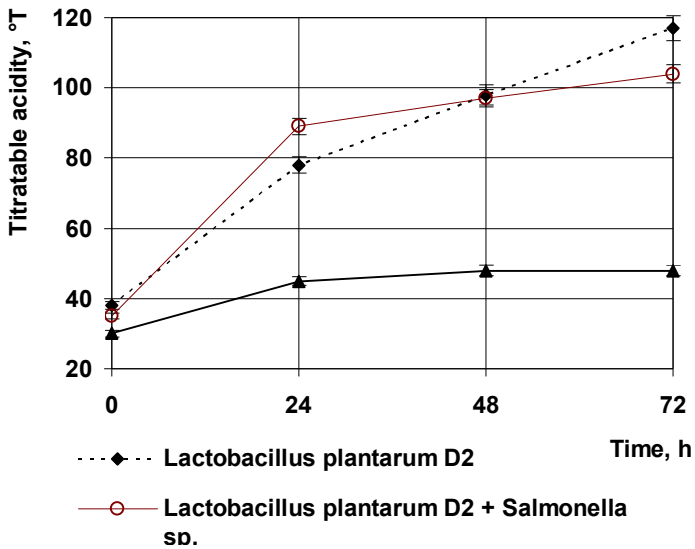


Figure 8. Changes in the titratable acidity of the medium in single-strain culturing and in a mixed population of *Lactobacillus plantarum* D2 and *Salmonella* sp. At 37 ± 1 °C.

Conclusion

Lactobacillus plantarum D1 and *Lactobacillus plantarum* D2 maintained high concentrations of viable cells in single-strain culturing and in co-culture at a temperature of $37\pm 1^\circ\text{C}$. Both *Lactobacillus plantarum* strains inhibited significantly the growth of the two *Salmonella* pathogens. The observed antimicrobial activity was due to a great extent to the acidification of the medium because of the production and accumulation of lactic and other organic acids. The demonstrated antimicrobial activity is a prerequisite for further research on the probiotic potential of the two *Lactobacillus plantarum* strains for their inclusion in the composition of probiotic preparations and starters for probiotic functional foods.

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