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# COMPARATIVE ANALYSIS OF THE POTENTIAL PROBIOTIC ABILITIES OF LACTOBACILLI OF HUMAN ORIGIN AND FROM FERMENTED VEGETABLES

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*Abstract* - In this study, twelve strains of *Lactobacillus plantarum* derived from spontaneously fermented vegetables (carrots and beetroot) and traditionally prepared sauerkraut were compared for their potential probiotic abilities with seven *Lactobacillus rhamnosus* strains of human origin. The tested strains were investigated for some technological properties and *in vitro* functional characteristics for potential probiotic strains. Selection probiotic criteria included the ability of the strains to withstand conditions similar to the digestive tract, antimicrobial activity against a wide range of intestinal pathogens and sensitivity to antibiotics. The total acidity in milk was generally higher in intestinal strains in relation to plant strains. The ability of the tested strains to survive simulated gastric conditions showed a greater resistance of the human strains at a low pH 2.5 and the presence of pepsin, while in the presence of bile salts and pancreatin, some intestinal strains were more sensitive compared to plant strains. A wide spectrum of antimicrobial activities was observed in all tested strains. Most of the plant strains were resistant to aminoglycosides and vancomycin but sensitive to ampicillin and penicillin, while only some intestinal strains were resistant to these drugs.

*Key words:* Fermented vegetables, intestine, *Lactobacillus plantarum*, *Lactobacillus rhamnosus,* probiotics.

## INTRODUCTION

Probiotic refers to live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host (FAO, 2006). The health benefits associated with the ingestion of probiotic bacteria include a reduction in colon irritation and constipation, treatment of acute rotavirus diarrhea, inhibition antagonism towards intestinal pathogens, synthesis of B vitamins, lactose maldigestion, cholesterol absorption and inhibition of tumor formation (Ziemer and Gibson, 1998).

In order to provide health benefits, probiotic strains must overcome physical and chemical barriers in the gastrointestinal tract, especially acid and bile stresses (Del Piano et al., 2006). The absence of pathogenicity, the ability to adhere to the gastrointestinal mucosa and the competitive exclusion of pathogens are also criteria that have been used for the selection of probiotics (Ouwehand et al., 2004). The mechanism through which probiotics may antagonize pathogens involves the production of antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide and bacteriocins.

Although *Lactobacillus* spp. have GRAS (generally recognized as safe) status, because of their long and safe use in food fermentation, it is necessary to confirm the safety of these microorganisms intended for use in food production. There is serious concern as to the resistance of probiotic bacteria to antibiotics. Thus, one of the crucial criteria in the selection of potential probiotics is their sensitivity to antibiotics. Probiotic bacteria as well as LAB used in food, may have antibiotic-resistance genes, which can be transferred to pathogenic bacteria that occur in the gastrointestinal tract (Radulović et al., 2012).

There is growing interest in the utilization of *Lactobacillus* strains isolated from both traditional fermented products and humans for their health-promoting effects. Some *Lactobacillus* strains occur naturally in the human intestine. For this reason such intestinal microorganisms are also preferentially developed for commercial use as probiotics. A better resistance of strains of human origin in simulated gastrointestinal conditions compared to strains of dairy origin has been reported (Prasad et al., 1999). However, Haller et al. (2001) showed that the metabolic and functional properties of intestinal lactobacilli are also found in certain bacteria originating in fermented foods. Additionally, similar immunoenhancing properties were described for strains independent of their origin (Dogi and Perdigón, 2006).

The aim of this work was to compare the potential probiotic ability among lactobacilli derived from different fermented vegetables with human-derived lactobacilli. The probiotic properties were comprised of their sensitivity to simulated gastrointestinal tract conditions, antimicrobial activity against a wide range of pathogens and antibiotic resistance.

## MATERIALS AND METHODS

### *Bacterial cultures*

Twelve autochthonous *Lactobacillus plantarum* strains isolated from various fermented vegetables (data not published) and seven *Lactobacillus rhamnosus* strains derived from preterm infants (Bogićević, 2009) were used for examination (Table 1). The strains from fermented vegetables belong to the strains collection of the Department of Industrial Microbiology, Faculty of Agriculture, University of Belgrade, while the human strains belong to the strains collection of the Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade. The tested strains were maintained as a frozen stock at -20°C in Man Rogosa Sharpe (MRS) broth containing 20% (v/v) glycerol, propagated twice in MRS broth at 30°C before use.

### *Technological characteristics of the strains*

The examination of the tested strains for potential application in food production was performed by testing some technological properties: growth at different temperatures (15, 30, 45°C) and survival at 60°C, growth in milk, growth in litmus milk and production of lactic acid.

### *Tolerance to simulated gastrointestinal conditions (gastric and bile salts test)*

Sensitivity to simulated gastrointestinal tract conditions has been tested as described by Doleyres et al. (2004), with certain modification. Briefly, survival of the tested strains in gastric conditions was performed at a low pH 2.5 in the presence of pepsin (0.3%) after 30 min incubation at 37°C, and for duodenal conditions, cell survival in the presence of 0.4% bile salts and 0.2% pancreatin after 60 min incubation at 37°C.

### *Antimicrobial activity*

The antimicrobial activity of the tested plant and human strains was screened using the spot method as described by Bernet et al. (1993). The antimicrobial activities were tested against a range of intestinal pathogens: *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 24433, *Salmonella enteritidis* ATCC 31806, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 19115. The antibacterial activity was related to the inhibition clear zone, which was calculated as the difference between the total of inhibition zone and the diameter of the growth spot of the indicator strains.

*Antibiotic resistance*

The antibiotic resistance patterns of the tested plant and human strains were determined by the disk diffusion method on an MRS agar plate (Bauer et al., 1966). A total of 12 antibiotic substances were tested: tetracycline, chloramphenicol, streptomycin, neomycin, penicillin, ampicillin, kanamycin, trimethoprim-sulfamethoxazole, vancomycin, rifampin, gentamicin and doxycycline (Torlak, Serbia). MRS agar plates were overlaid with 6 ml of top MRS agar (0.6% of agar) inoculated with 0.2 ml of tested lactobacilli. After the top MRS agar hardened, the disks with antibiotics were dispensed onto the plates and incubated under anaerobic condition at 37°C for 24 h. The diameter (mm) of the inhibition zone was measured and the mean of two observations was expressed in terms of resistant (R), moderately susceptible (MS) and susceptible (S).

## RESULTS AND DISCUSSION

*Sensitivity to some technological characteristics*

Comparing the growth at different temperatures, it was observed that all the isolates from fermented vegetables grew at 15 and 30°C, while two human isolates, TMFM and TMFV, showed an ability to grow at 45°C as well as to survive at 60°C (Table 1), which proved them to be good candidates for food application.

The total acidity in milk was generally higher in the intestinal *L. rhamnosus* strains in relation to *L. plantarum* strains from fermented vegetables, while all tested isolates showed coagulation, acidification and reduction of litmus milk (Table 1). In the work of Ayad et al. (2004), it was indicated that most strains of *L. plantarum* isolated from different sources showed a slow acidification rate. The strain of human origin, TMF55, was a fast acidifying culture and produced 0.810 mmol/l of lactic acid (Table 1).

*Survival in simulated gastrointestinal condition (gastric and bile salts test)*

Before reaching the intestinal tract, probiotic bacteria must pass through the stomach where the pH can be as low as 1.5 to 2 (Dunne et al., 2001). This acid tolerance helps *Lactobacilli* to reach the small intestine and colon and thus contributes in balancing the intestinal microflora. Comparing the survival of tested strains in a low pH of 2.5, a generally lower survival of isolates from fermented vegetables in relation to intestinal isolates was observed (Fig. 1). Lactobacilli of intestinal origin are considered intrinsically resistant to acid environments and are often employed in fermented foods as probiotics (Tannock, 2004). However, different strains of *L. plantarum* were found to show a high tolerance to the acid condition (pH 2.0) and bile salts. This was observed both for the strains isolated from intestinal samples and for those that were isolated from fermented foods (De Vries et al., 2006). Our study revealed that the strains from fermented vegetables could survive under simulated gastric conditions but in a lower number (with a loss of viability ranging from 2.0±0.2 to 3.85±0.5 $\log_{10}$ CFU $ml^{-1}$) than the strains from human sources (Fig. 1). Acid tolerance of bacteria is important not only for withstanding gastric conditions, but also for their use as dietary adjuncts. It enables the strains to survive for longer periods in acid carrier foods, such as yogurt, without a large reduction of viable cells (Minelli et al., 2004).

Afterwards the plant and human strains were tested for their resistance to simulated duodenal conditions (0.4% bile salts and 0.2% pancreatin). Bile tolerance is considered one of the important properties required for high survival and probiotic activity. Bile plays an essential role in lipid digestion. It emulsifies and solubilizes lipids and functions as a biological detergent (Begley at al., 2006). The antimicrobial nature of bile is mainly because of its detergent property, which dissolves bacterial membranes. There is considerable evidence that upon the deconjugation of conjugated bile salts in the intestine, the free bile acids are toxic for Gram-positive and Gram-negative

**Table 1.** Sources of isolation and some technological properties of plant and human strains.

| No | L. plantarum | Source | Growth at 15 ºC | Growth at 30 ºC | Growth at 45ºC | Survival at 60ºC | Growth in milk coagulation | Growth in litmus milk | Growth in lactic acid (mmol/l) |
|---|---|---|---|---|---|---|---|---|---|
| Plant strains | | | | | | | | | |
| 1. | PF1 | Fermented beet oot | + | + | - | - | - | ACR[b] | 0,360±0.21[c] |
| 2. | PF2 | Sauerkraut | + | + | - | - | +[a] | ACR | 0,720±0.18 |
| 3. | PF3 | Sauerkraut | + | + | - | - | + | ACR | 0,648±0.32 |
| 4. | PF4 | Fermented carrots | + | + | - | - | + | ACR | 0,612±0.29 |
| 5. | PF22 | Sauerkraut | + | + | - | - | - | ACR | 0,351±0.25 |
| 6. | PF23 | Sauerkraut | + | + | - | - | - | ACR | 0,360±0.15 |
| 7. | PF24 | Sauerkraut | + | + | - | - | - | ACR | 0,315±0.21 |
| 8. | PF30 | Fermented beet root | + | + | - | - | + | ACR | 0,540±0.23 |
| 9. | PF31 | Fermented carrots | + | + | - | - | + | ACR | 0,540±0.32 |
| 10. | TP3C | Fermented beet root | + | + | - | - | - | ACR | 0,315±0.50 |
| 11. | TP10 | Sauerkraut | + | + | - | - | + | ACR | 0,540±0.51 |
| 12. | TP11 | Sauerkraut | + | + | - | - | + | ACR | 0,450±0.29 |
| Human strains | | | | | | | | | |
| No | *L. rhamnosus* | | | | | | | | |
| 1. | TMFM | Preterm infant | + | + | + | + | + | ACR | 0,554±0.23 |
| 2. | TMFV | Preterm infant | + | + | + | + | + | ACR | 0,626±0.34 |
| 3. | TMFEM | Preterm infant | + | + | - | - | + | ACR | 0,711±0.45 |
| 4. | TMFEV | Preterm infant | + | + | - | - | + | ACR | 0,765±0.33 |
| 5. | TMF37 | Preterm infant | + | + | - | - | + | ACR | 0,711±0.27 |
| 6. | TMF48 | Preterm infant | + | + | - | - | + | ACR | 0,702±0.15 |
| 7. | TMF55 | Preterm infant | + | + | - | - | + | ACR | 0,810±0.14 |

[a] (+)-coagulation of milk, [b]A-acidification; C-coagulation; R-reduction; [c]mean±standard deviation.

microorganisms, which is one of the antagonistic mechanisms against pathogens in the intestine.

All lactobacilli from fermented vegetables showed high tolerance to 0.4% of bile and 0.2% of pancreatin with a loss of viability ranging from 0.22±0.1 to 2.6±0.6 log $_{10}$ CFU ml$^{-1}$ (Fig. 1). Some tested human strains showed a lower bile tolerance than the plant strains, with a loss of viability of 3.13±0.8 log $_{10}$ CFU ml$^{-1}$ for the TMFEM strain and 3.38±0.9 log $_{10}$ CFU ml$^{-1}$ for the TMF55 strain (Fig. 1). Resistance to bile salts varies a lot among the lactic acid bacteria species and even between strains themselves (Xanthopoulos, 1997). High tolerance to bile salts has been recently reported for some *L. plantarum* strains isolated from fermented olives (Mourad and Nour-Eddine, 2006).

One of the most important characteristic of potential probiotic bacteria is protection against pathogens in the intestinal tract of the host. In this study, *Bacillus subtilis*, *Candida albicans*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli and Listeria monocytogenes* were used as the test bacteria because they are commonly found as food-borne pathogens. The results of antimicrobial activity revealed that lactobacilli from fermented vegetables could inhibit most of the tested pathogenic bacteria, however at different inhibition levels, as shown in Table 2. Most isolates from fermented vegetables showed the highest inhibition level on *E. coli* and *S. enteritidis* with inhibition zones over 9 mm. However, the strains of human origin demonstrated greater antimicrobial activity than the plant

**Table 2** Antimicrobial activity of *L. plantarum* and *L. rhamnosus* strains.

| *L. plantarum* | Inhibition zone (mm) of indicator strains[a] | | | | | |
|---|---|---|---|---|---|---|
| | *B. subtilis* ATCC 6633 | *C. albicans* ATCC 24433 | *S. enteritidis* ATCC 31806 | *Staph. aureus* ATCC 25923 | *E. coli* ATCC 25922 | *L. monocytogenes* ATCC 19115 |
| PF1 | 8±2.1 | - | 5±1.7 | 6±2.2 | 7±2.2 | □ |
| PF2 | 12±1.1 | 2±1.2 | 10±1.6 | 6±1.5 | 12±2.4 | 6±1.9 |
| PF3 | 7±1.4 | 5±2.2 | 9±2.1 | 5±1.9 | 10±2.1 | 7±2.1 |
| PF4 | 8±0.8 | 5±1.6 | 9±2.0 | 5±1.8 | 12±1.8 | 3±2.0 |
| PF22 | 8±1.7 | 4±1.8 | 10±1.6 | 7±1.5 | 10±1.7 | 4±1.1 |
| PF23 | 7±2.1 | 2±2.1 | 7±1.1 | 10±2.1 | 10±2.3 | 8±1.4 |
| PF24 | 6±2.0 | 3±2.4 | 9±1.7 | 4±2.0 | 8±0.8 | 6±1.9 |
| PF30 | 8±1.5 | 2±2.1 | 15±2.1 | 4±2.4 | 10±1.7 | 4±1.3 |
| PF31 | 5±1.7 | 4±1.4 | 10±1.5 | 5±2.2 | 10±2.3 | 4±2.1 |
| TP3C | 6±2.1 | - | 12±1.9 | 4±1.8 | 10±2.1 | 5±2.4 |
| TP10 | 7±2.1 | - | 10±2.1 | 3±0.8 | 11±2.0 | 2±1.5 |
| TP11 | 6±1.6 | - | 10±2.0 | 6±2.1 | 10±1.8 | 6±1.7 |
| L. rhamnosus | | | | | | |
| TMFM | 8±0.6 | - | 20±1.4 | 4±0.4 | 8±0.7 | 25±1.4 |
| TMFV | 9±1.2 | - | 20±0.6 | 7±1.3 | 9±1.2 | 18±1.2 |
| TMFEM | 9±1.7 | - | 10±1.2 | 7±1.3 | 9±1.2 | 23±1.1 |
| TMFEV | 11±2.2 | - | 14±0.8 | 8±1.2 | 11±1.3 | 18±1.4 |
| TMF37 | 11±0.9 | - | 27±1.2 | 8±1.2 | 11±1.2 | 25±1.5 |
| TMF48 | 10±1.2 | - | 17±1.7 | 6±1.2 | 10±1.2 | 24±1.2 |
| TMF55 | 7±1.7 | - | 20±1.4 | 9±0.9 | 7±1.1 | 18±0.3 |

[a], mean±standard deviation; -, no inhibition zone

**Table 3**. Antibiotic resistance of plant strains.

| Antibiotics | Plant strains | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PF1 | PF2 | PF3 | PF4 | PF22 | PF23 | PF24 | PF30 | PF31 | TP3C | TP10 | TP11 |
| Tetracycline | + | + | + | + | + | + | + | + | + | + | + | + |
| Chloramphenicol | + | + | ++ | + | + | ++ | + | + | ++ | + | + | + |
| Streptomycin | R | R | R | + | + | + | + | + | R | + | + | + |
| Neomycin | R | R | R | R | R | R | R | R | R | R | R | R |
| Penicillin | + | + | + | + | ++ | + | + | ++ | ++ | + | ++ | + |
| Ampicillin | ++ | ++ | ++ | + | ++ | + | + | + | + | + | + | + |
| Kanamycin | R | R | R | R | R | R | R | R | R | R | R | R |
| Trimethoprim sulfamethoxazole | + | + | + | + | R | R | + | + | + | + | R | R |
| Vancomycin | R | R | R | R | R | R | R | R | R | R | R | R |
| Rifampicin | + | + | + | + | + | + | + | + | + | + | + | + |
| Gentamicin | + | + | + | + | + | + | + | + | + | + | + | + |
| Doxycycline | + | + | + | + | + | + | + | + | + | + | + | + |

++: sensitive – with halo of inhibition >10mm; +: moderately sensitive– with halo of inhibition 5-10 mm; R: resistant – with halo of inhibition <5mm

isolates, with the highest inhibitory activity against *S. enteritidis* and *L. monocytogenes* (Table 2). The human isolates demonstrated a lack of inhibitory activity only against *C. albicans* (Table 2). The antimicrobial activity of the tested bacteria may be due to the production of organic acid that lowered the pH of the medium. The probiotic bacteria may also have produced hydrogen peroxide, bacteriocin and

**Table 4.** Antibiotic resistance of human strains.

| Antibiotics | Human strains | | | | | | |
|---|---|---|---|---|---|---|---|
| | TMFM | TMFV | TMFEM | TMFEV | TMF37 | TMF48 | TMF55 |
| Tetracycline | + | ++ | ++ | ++ | ++ | + | ++ |
| Chloramphenicol | + | ++ | ++ | ++ | ++ | + | + |
| Streptomycin | R | + | + | + | ++ | + | R |
| Neomycin | R | R | R | R | R | R | R |
| Penicillin | + | ++ | + | + | ++ | + | R |
| Ampicillin | R | + | + | + | + | + | R |
| Kanamycin | R | R | R | R | R | R | R |
| Trimethoprim + Sulfamethoxazole | R | R | R | R | R | R | R |
| Vancomycin | R | R | R | R | R | R | R |
| Rifampicin | + | + | ++ | ++ | + | + | + |
| Gentamicin | + | + | + | + | + | + | + |
| Doxycycline | ++ | ++ | ++ | ++ | ++ | ++ | + |
| Antibiotics | TMFM | TMFV | TMFEM | TMFEV | TMF37 | TMF48 | TMF55 |

++: sensitive – with halo of inhibition >10mm; +: moderately sensitive– with halo of inhibition 5-10 mm; R: resistant – with halo of inhibition <5mm

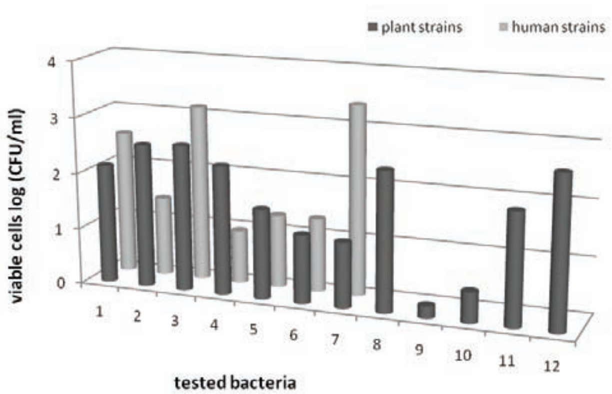


**Fig. 1.** Survival of plant and human strains in simulated gastric condition (pH 2.5, 0.3 % pepsin).

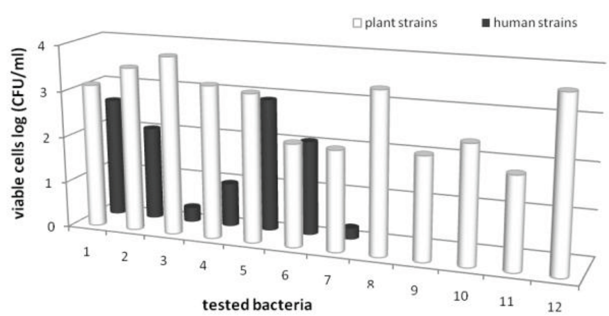


**Fig. 2.** Survival of plant and human strains in simulated intestinal conditions (0.4 % bile salts, 0.2 % pancreatin).

antibacterial compounds or competed for nutrients (Šušković et al., 2010).The difference in antagonistic activity among the tested lactobacilli is probably due to the different intrinsic factors induced by the different origins.

The widespread use of antibiotics for therapeutic and prophylactic purposes and for health promotion in animal husbandry has resulted in an increased number of antibiotic resistant bacteria and most antibiotics became ineffective (Radulović et al., 2012). Therefore, the antibiotic susceptibility of potential probiotic bacteria is one of the crucial criteria for the safe application of probiotics in human consumption since bacteria used as probiotics may serve as hosts of antibiotic resistance genes, which can be transferred to pathogenic bacteria. All strains from fermented vegetables showed resistance to kanamycin, vancomycin and neomycin, whereas strains PF1, PF2, PF3 and PF31 were resistant to streptomycin as shown in Table 3.

Vancomycin is an antibiotic that inhibits the synthesis of peptidoglycan, which is the main component of bacterial cell wall. Salminen et al. (1998)

reported that resistance to vancomycin is commonly found in the genera *Lactobacillus*. In addition, the resistance to aminoglycosides, kanamycin, gentamicin and streptomycin was probably a natural feature of lactobacilli (Hummel et al., 2007)

The isolates from fermented vegetables were susceptible to β-lactam antibiotics, ampicillin and penicillin, as well as to the protein synthesis inhibitor, chloramphenicol, which is in accordance with Kheadr et al. (2004). For trimethoprim-sulfamethoxazole, all human isolates showed resistance, whereas most isolates from plant materials were susceptible to this antibiotic (Table 4).

Resistance of tested strains to trimethoprim was considered to be due to a trimethoprim-insensitive dehydrofolate reductase (Huovinen, 1987). In addition, two strains from human sources (TMFM and TMF55) showed resistance to penicillin, while all strains from fermented vegetables were susceptible to this drug (Table 4). From these results it can be generally concluded that the intestinal isolates showed a higher resistance to antibiotics than the isolates from fermented vegetables. However, this situation is not unusual when considering the fact that the gastrointestinal tract of newborns is inoculated with the mother's microflora.

The differences in antibiotic susceptibility of lactobacilli from different origins indicated the role of sources of bacterial isolation that might have an influence on the genetic level of their antibiotic resistance.

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