# PROBIOTIC PROPERTIES OF *LACTOBACILLUS CASEI* SSP. *RHAMNOSUS* LBRC11

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**Abstract:**
*Restoring the balance of the intestinal microflora is achieved by consummation of foods and concentrates, containing beneficial bacteria - lactobacilli and bifidobacteria - known as functional foods and probiotics. Not all lactobacilli can be included in the composition of probiotics and probiotic foods, but only those who possess certain properties.*
*Identification of the strain Lactobacillus LBRC11 is achieved through the application of physiological and biochemical (API 50 CHL) and molecular genetic (ARDRA) methods. The antimicrobial activity against saprophytic microorganisms (Bacillus subtilis, Bacillus mesentericus, Aspergillus niger, Penicillium sp., Rhizopus sp., Saccharomyces cerevisiae) is determined using the agar diffusion method and the antimicrobial activity against pathogens (E.coli ATCC 25922, E.coli 8739, Salmonella abony, Salmonella sp., Staphylococcus aureus ATCC 25093) is investigated by carrying out joint cultivation. The profile of antibiotic resistance is determined by the method of diffusion in agar. The enzymatic profile of the strain is studied with the test kit API ZYM.*
*By applying API 50 CHL and ARDRA the strain Lactobacillus LBRC11 is identified as Lactobacillus casei ssp.rhamnosus. The strain possesses antimicrobial activity against the saprophytes Bacillus mesentericus, Aspergillus niger, Penicillium sp., Rhizopus sp. and against all of the pathogens included in the study. Lactobacillus casei ssp.rhamnosus LBRC11 is resistant to 8 of the 22 antibiotics used in the study and is sensitive to 10 of them.*
*The results of the tests on some probiotic properties of Lactobacillus casei ssp.rhamnosus LBRC11 make it suitable for incorporation in probiotics and probiotic foods.*

**Keywords:** *probiotics, ARDRA, cultivation, pathogen, saprophytic microorganism*

## 1. Introduction

Maintaining the balance of the flora in the stomach and the intestines is a necessary condition for good health. Restoring the balance of the intestinal microflora is achieved by consummation of foods and concentrates, containing beneficial bacteria - lactobacilli and bifidobacteria - known as functional foods and probiotics.
FAO defines probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host [1, 2].
The main components of probiotics are lactic acid bacteria (*Lactobacillus, Enterococcus, Pediococcus, Lactococcus, Streptococcus, Leuconostoc*) and bifidobacteria, which are applied in the manufacture of probiotic foods [3, 4, 5, 6, 7], with lactobacilli being the largest part.
Not all lactobacilli can be included in the composition of probiotics and probiotic foods, but only those that possess certain

properties [3,4,5]: to be part of the natural microflora in humans and animals; to have the ability to adhere to epithelial cells or cell lines; to survive under the conditions of the stomach and the intestines, i.e. to survive at acidic pH values in the stomach and to withstand the action of bile [8, 9]; to be able to reproduce in the gastrointestinal tract, predominantly utilizing the substrate to suppress and expel from the biological niche the pathogenic and toxigenic microorganisms; to allow industrial cultivation; to have antimicrobial activity against pathogens and carcinogens; to produce antimicrobial substances; to modulate the immune response and to be safe for clinical and food applications.

**2. Experimental**

1. Media:

1.1. Saline. Composition (g/dm$^3$): NaCl - 5 g; distilled water - 1litre. Sterilization - 20 minutes at 121ºC.

1.2. Liquid LAPTg10 medium. Composition (g/dm$^3$): peptone - 15, yeast extract - 10; tryptone - 10, glucose - 10. The pH is adjusted to 6.6 - 6.8 and Tween 80 - 1cm$^3$/l is added. The medium is sterilized for 20 minutes at 121ºC.

1.3. LAPTg10-agar. Composition (g/dm$^3$): Medium L-broth + 2% agar. Sterilization - 20 minutes at 121ºC.

2.Physiological and biochemical methods

2.1. Determination of the biochemical profile. The determination of the biochemical profile is performed, using the system API 50 CHL (BioMerieux SA, France) for identification of species belonging to the genus *Lactobacillus* on the basis of their ability to utilize 49 carbon sources. Fresh 24-hour culture of the studied strain is centrifuged for 15 minutes at 5,000 x g. The resulting biomass sludge is washed twice with PBS-buffer and resuspended in L resuspension medium, which is an integral part of the used kit. The API strips are placed in incubation boxes and the microtubules are inoculated with the prepared cell suspension and sealed with sterile liquid paraffin. The results are reported on the 24$^{th}$ and on the 48$^{th}$ hour of incubation at optimum temperature for the development of the studied strain. Reporting is done according to colour change in comparison to the control (microtubule 0). Positive results are reported in the case of a colour change from green to bright yellow. The results are processed with apiweb$^R$ identification software.

2.2. Determination of the profile of antibiotic sensitivity. The profile of antibiotic resistance is determined by the disk diffusion method of Bauer, Kirby et al. [13]. Fresh 24-hour culture of the tested strain is used to inoculate the plates with LAPTg10-agar. Standard discs impregnated with antibiotics are placed in the plates. The plates are incubated for 48 hours at optimal temperature. The diameters (in mm) of the sterile zones formed around each of the antibiotic discs are recorded. Then they are subjected to the following designations: R - resistant (zone < 8 mm), SR - intermediately sensitive (zone 8-16 mm), S - sensitive (zone > 16 mm).

2.3. Determination of the antimicrobial activity against saprophytic

The studies of Saxelin et al. [10, 11], Donohue & Salminen [12], Salminen et al. [3, 4, 5] indicate that the safety of lactic acid bacteria and bifidobacteria has been shown and strains belonging to the genera *Lactobacillus, Lactococcus* and *Bifidobacterium* are most often with GRAS status (generally recognized as safe).

The purpose of the present article is to investigate the presence of some probiotic properties of the strain *Lactobacillus* LBRC11 - antimicrobial activity against pathogenic and saprophytic microorganisms, antibiotic resistance, the presence of surface layer proteins and determination of the enzyme profile.

microorganisms. To determine the antimicrobial activity of the tested strain against saprophytic microorganisms liquid culture (LC), acellular supernatant without pH adjustment (ASN) and neutralized acellular supernatant (NASN) (pH 6.5), obtained from a 48 hour culture of the tested strain are used. The antimicrobial activity is tested against the following test microorganisms: bacteria - *Bacillus subtilis, Bacillus mesentericus*; yeasts - *Saccharomyces cerevisiae*, molds - *Aspergillus niger, Penicillium* sp., *Rhizopus* sp. Each of the test microorganisms ($10^6$-$10^7$ cfu/$cm^3$) is inoculated in a Petri dish with agar medium and after the hardening of the agar wells (6 mm) are prepared. 0,06 $cm^3$ of KT, ASN or NASN are inoculated in the wells of the plates and the plates with the test microorganisms are incubated at $30^0$C or 37ºC for 24 to 48 hours, and then the inhibition zones in mm are reported.

2.3. Determination of the antimicrobial activity against pathogenic microorganisms. To determine the antimicrobial activity of the studied strain against pathogens a 48 hour culture of the *Lactobacillus* strain is used. In the mixtures 0,5 ml of the suspension of the *Lactobacillus* strain, 0,5 ml of the suspension of the pathogen and 9 ml of culture medium (LAPTg10) are mixed, while in the control of the *Lactobacillus* strain and in the control of the pathogen 9,5 ml of the liquid LAPTg10 medium are mixed with 0,5 ml of the suspension of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and of the suspension of the pathogen respectively. The following pathogens are used: *E. coli* ATCC 25922, *E.coli* 8739, *Salmonella abony*, *Salmonella* sp., *Staphylococcus aureus* ATCC 25093. Joint cultivation of the strain *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and pathogenic microorganism under static conditions in a thermostat at $37^0$C for 60 to 72 hours, taking samples at 0, 12, 24, 36, 48, 60 and 72 h and monitoring the change of the titratable acidity and the concentration of viable cells of both the pathogen and the *Lactobacillus* strain is performed.

2.4. Determination of the profile of enzyme activity. The determination of the profile of enzyme activity is performed, using the test kit API ZYM (BioMericux, France) for semi-quantitative determination of the enzyme profile of the studied strain. Fresh 24-hour culture of the tested strain is centrifuged for 15 minutes at 5000 x g, the obtained biomass precipitate is washed twice and resuspended in API suspension medium. The API ZYM strips are placed in the incubation boxes and the microtubules are inoculated with the prepared cell suspension. The sample is incubated for 4 to 4,5 hours at $37^0$C. After the incubation one drop of reagent A and one drop of reagent B are pipetted into each microtubule. After 5 min staining is reported according to the color scheme described in the manufacturer's instructions. The enzyme activity is determined according to the color scale from 0 (no enzyme activity) to 5 (maximum enzyme activity).

3. Genetic methods

3.1. Isolation of total DNA. The isolation of DNA is performed by the method of Delley et al. [14].

3.2. PCR reactions and visualization. All PCR reactions are performed using the PCR kit - Ready To Go$^{TM}$ PCR beads (Amersham Biosciences), in a volume of 25 µl in a Progene cycler (Techne, UK). The resulting products are visualized on a 2% agarose gel stained with ethidium bromide solution (0.5 µg/ml), using an UVP Documentation System (UK).

3.3. 16S rDNA amplification and 16S rDNA ARDRA (Amplified Ribosomal DNA Restriction Analysis). The method ARDRA involves enzymatic multiplication of gene encoding the 16S rRNA, using

primers complementary to the conservative regions at both ends of the 16S rRNA gene and the product of the multiplication is then restricted with restriction enzymes. The resulting profile is highly specific for the particular studied species.

DNA of the studied strain is amplified using universal primers for the 16S rDNA gene - fD1 and rD1 [15]. The amplification program includes: denaturation - 95°C for 3 minutes, 40 cycles - 93°C for 30 s, 48°C for 60 s, 72°C for 60 s, final elongation - 72°C for 5 min. The resulting PCR product from the 16S rDNA amplification of the tested strain is treated with the endonucleases *Eco*RI, *Hae*III and *Hap*II (Boehringer Mannhem GmbH, Germany). Reactions are carried out according to the following quantities: PCR products - 10µl, enzyme solution - 10 µl (1 µl of the respective enzyme, 2 µl buffer, 7 µl $dH_2O$). Incubation for 1 night at $37^0$C is performed. The resulting restriction products are visualized on a 2% agarose gel.

4. Characterization of the surface layer proteins by SDS-PAGE. Untreated bacterial cells or cells treated with 5M LiCl, are resuspended in 1% SDS and are incubated for 30 min at 37ºC to isolate the surface layer proteins. After centrifugation for 5 min at 9000 × g, the supernatant is analyzed using SDS-polyacrylamide electrophoresis [16], using 10% polyacrylamide gel. Protein bands are visualized by staining with Coomassie Blue R-250.

## 3. Results and discussion

1. Physiological and biochemical characteristics of the strain *Lactobacillus* LBRC11

The biochemical profile of the strain *Lactobacillus* LBRC11 is determined using the system for rapid identification API 50 CHL (Biomerieux, France). According to its ability to utilize the 49 carbon sources, included in the test kit (Table 1) the strain belongs to the species *Lactobacillus casei* ssp.*rhamnosus* with a rate of reliability 99.9%.

The strain *Lactobacillus* LBRC11 is subjected to ARDRA-analysis to confirm the results of the species identification obtained by the conventional methods for identification. The results of these genetic experiments are shown in Fig. 1 and Fig. 2. Using the restriction enzymes *Hae* III and *Hap* II the strain's belonging to the species *Lactobacillus casei* ssp.*rhamnosus* is confirmed.

**Table 1:**
**API 50 CHL of the strain *Lactobacillus* LBRC11**

| # | Carbohydrate | LBRC11 |
|---|---|---|
| 1 | Glycerol | +/- (25-30%) |
| 2 | Erythriol | - |
| 3 | D-arabinose | - |
| 4 | L-arabinose | - |
| 5 | Ribose | + (90-100%) |
| 6 | D-xylose | - |
| 7 | L-xylose | - |
| 8 | Adonitol | - |
| 9 | β-metil-D-xyloside | - |
| 10 | Galactose | + (90-100%) |
| 11 | D-glucose | + (90-100%) |
| 12 | D-fructose | + (90-100%) |
| 13 | D-mannose | + (90-100%) |
| 14 | L-sorbose | + (90-100%) |
| 15 | Rhamnose | + (90-100%) |
| 16 | Dulcitol | - |
| 17 | Inositol | + (50%) |
| 18 | Manitol | + (90-100%) |
| 19 | Sorbitol | + (90-100%) |
| 20 | α-methyl-D-mannoside | - |
| 21 | α-methyl-D-glucoside | + (90-100%) |
| 22 | N-acetyl-glucosamine | + (90-100%) |
| 23 | Amigdalin | + (90-100%) |
| 24 | Arbutin | + (90-100%) |
| 25 | Esculin | + (90-100%) |
| 25 | Esculin | + (90-100%) |
| 26 | Salicin | + (90-100%) |
| 27 | Cellobiose | + (90-100%) |
| 28 | Maltose | + (90-100%) |
| 29 | Lactose | + (90-100%) |
| 30 | Melibiose | - |
| 31 | Saccharose | + (50%) |
| 32 | Trehalose | + (90-100%) |
| 33 | Inulin | - |
| 34 | Melezitose | + (90-100%) |
| 35 | D-raffinose | - |
| 36 | Amidon | - |
| 37 | Glycogen | - |
| 38 | Xylitol | - |
| 39 | β-gentiobiose | + (90-100%) |
| 40 | D-turanose | + (90-100%) |
| 41 | D-lyxose | - |
| 42 | D-tagarose | + (90-100%) |
| 43 | D-fuccose | - |
| 44 | L-fuccose | - |
| 45 | D-arabitol | - |
| 46 | L-arabitol | - |
| 47 | Gluconate | + (70-75 %) |
| 48 | 2-keto-gluconate | - |
| 49 | 5-keto-gluconate | - |

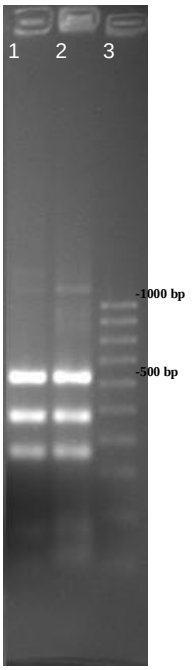


**Figure 1: Restriction profile with *Hae* III**

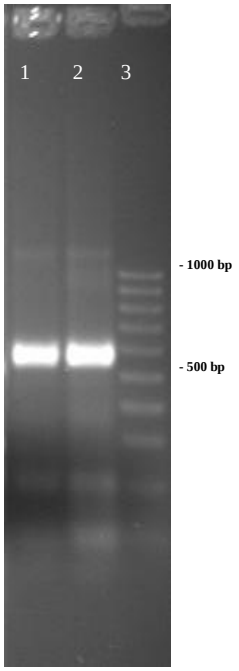


**Figure 2: Restriction profile with *Hap* II**

**1. *Lactobacillus casei* ssp. *rhamnosus* LMG 6400**
**2. *Lactobacillus* LBRC11**
**3. M 100 bp**

Antimicrobial activity of *Lactobacillus casei* ssp. *rhamnosus* LBRC11 against saprophytic microorganisms

The antimicrobial activity of *Lactobacillus casei* ssp. *rhamnosus* LBRC11 against saprophytic microorganisms is studied by the method of diffusion in agar. For this purpose, cultural liquid is used to determine the inhibitory effect of the cells of the strain on the test microorganisms; acellular supernatant obtained from the cultural liquid after centrifugation without pH adjustment (with acidic pH) is used to determine the influence of the acidic pH on the saprophytes. In a parallel experiment the activity of the neutralized acellular supernatant after neutralization to pH 6.5 to eliminate the inhibitory effect of the lactic acid produced by the *Lactobacillus* strain. Test microorganisms belonging to different groups of microorganisms are used: bacteria - *Bacillus subtilis, Bacillus mesentericus*; yeasts - *Saccharomyces cerevisiae*, molds - *Aspergillus niger, Penicillium sp., Rhizopus sp.* Incubation is conducted for 24 to 48 h at 30°C and/or 37°C. The results of the repeated twice experiments are summarized in Table. 2.

**Table 2:**
**Antimicrobial activity of the strain *Lactobacillus casei* ssp. *rhamnosus* LBRC11 against saprophytic microorganisms. The values are in mm. Diameter of the well - 6 mm. CL - cultural liquid; ASN - acellular supernatant without pH adjustment and NASN – neutralized acellular supernatant (pH = 6.5).**

| Saprophytic microorganism | | LBRC11 | | |
|---|---|---|---|---|
| | | CL | ASN | NASN |
| *Aspergillus niger* $1,2x10^7$cfu/cm$^3$ | $30^0$C | 10 | 9 | 9 |
| | $37^0$C | 16 | 10 | - |
| *Rhizopus* sp. $1,8x10^7$cfu/cm$^3$ | $30^0$C | 14.5 | 11 | 8 |
| *Penicillium* sp. $5,2x10^7$cfu/cm$^3$ | $30^0$C | 10 | 8 | 8 |
| *Bacillus subtilis* $1,9x10^7$cfu/cm$^3$ | $30^0$C | - | - | - |
| | $37^0$C | - | - | - |
| *Bacillus mesentericus* $4x10^6$cfu/cm$^3$ | $30^0$C | 10 | 10 | 9 |
| | $37^0$C | 12 | 10 | 10 |
| *Saccharomyces cerevisiae* $9,2x10^6$cfu/cm$^3$ | $30^0$C | - | - | - |
| | $37^0$C | 15 | - | - |

*Lactobacillus casei* ssp.*rhamnosus* LBRC11 possesses antimicrobial activity against *Rhizopus* sp. and *Penicillium* sp., and the cultural liquid has greater antimicrobial activity than the acellular supernatant. The strain exhibits antimicrobial activity against *Aspergillus niger* and *Bacillus mesentericus*, both at $30^0$C and $37^0$C; the antimicrobial activity of the cultural liquid is higher than that of the supernatant. The cultural liquid of the strain exhibits antimicrobial activity against *Saccharomyces cerevisiae* only at $37^0$C, which means that the suppression of the test organism is due to competition for nutrients and/or places of attachment. The strain does not possess antimicrobial

activity against *Bacillus subtilis* at $30^0$C or at $37^0$C.

Determination of the presence of surface layer proteins

The strain *Lactobacillus casei* ssp.*rhamnosus* LBRC11 does not possess surface layer proteins.

Antibiotic resistance

Knowledge on the antibiotic resistance of the lactobacilli with probiotic potential is essential. It can be seen as an important criterion for the selection of probiotic cultures due to the possibility of conducting a combined therapy with antibiotics and probiotics in order to restore the normal microflora of the gastrointestinal tract and/or the urogenital tract [17].

For this purpose, 22 antibiotics with different mechanisms of action most frequently used in medical practice are selected and the sensitivity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 is determined. The results of the diffusion method of Bauer, Kirby et al. [13] for 24-48 hours are summarized in Table 3.

*Lactobacillus casei* ssp.*rhamnosus* LBRC11 is resistant to 8 out of the 22 antibiotics used in the study - penicillin, ampicillin, oxacillin, amoxicillin, vancomycin, cefemandole, chloramphenicol and nalidixic acid. Intermediate sensitivity is observed to four antibiotics - gentamicin, streptomycin, kanamycin and tobramycin. The strain shows sensitivity to 10 out of 22 antibiotics - azlocillin, piperacillin, tetracycline, doxycycline, clindamycin, amikacin, rifampin, lincomycin, erythromycin and ciprofloxacin. It is noteworthy that *Lactobacillus casei* ssp.*rhamnosus* LBRC11 is resistant to 6 out of 8 antibiotics inhibiting the synthesis of the cell wall, but it is sensitive to 7 out of 12 antibiotics that inhibit protein synthesis, and to other 4 of this group it shows intermediate sensitivity.

**Table 3:**
**Antibiotic sensitivity of the strain *Lactobacillus casei* ssp.*rhamnosus* LBRC11. Legend: R-resistant, SR – intermediately sensitive (zone 7-16 mm), S - sensitive (zone > 16 mm)**

| Mechanism of action | Antibiotic | | Concentration | LBR C11 |
|---|---|---|---|---|
| Inhibition of the synthesis of the cell walls | Penicillin | P | 10 E/disc | R |
| | Azlocillin | Az | 75 µg/disc | S |
| | Piperacillin | P | 100 µg/disc | S |
| | Ampicillin | A | 10 µg/disc | R |
| | Oxacillin | O | 1 µg/disc | R |
| | Amoxicillin | Ax | 25 µg/disc | R |
| | Vancomycin | V | 30 µg/disc | R |
| | Cefamandole | Cm | 30 µg/disc | R |
| Inhibition of the protein synthesis | Tetracycline | T | 30 µg/disc | S |
| | Doxycycline | D | 30 µg/disc | S |
| | Gentamicin | G | 10 µg/disc | SR |
| | Streptomycin | S | 30 mg/disc | SR |
| | Clindamycin | Cl | 2 µg/disc | S |
| | Kanamycin | K | 30 µg/disc | SR |
| | Tobramycin | Tb | 10 µg/disc | SR |
| | Amikacin | Am | 30 µg/disc | S |
| | Rifampin | R | 5 µg/disc | S |
| | Lincomycin | L | 15 µg/disc | S |
| | Chloramphenicol | C | 30 µg/disc | R |
| | Erythromycin | E | 15 µg/disc | S |
| Inhibition of the synthesis of DNA and/or the cell division | Nalidixic acid | Nx | 30 µg/disc | R |
| | Ciprofloxacin | Cp | 5 µg/disc | S |

The enzyme profile of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 is studied by determining the presence of a set of 19 enzymes involved in the system for semi-quantitative determination of key enzyme activities API ZYM (BioMerieux, France). The results of these studies are shown in Table 4.

*Lactobacillus casei* ssp.*rhamnosus* LBRC11 possesses the following enzymes: alkaline phosphatase, esterase, esterase-lipase, lipase, leucine-aminopeptidase, valine-aminopeptidase, cysteine-aminopeptidase, chymotrypsin, acid phosphatase, phosphohydrolase, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, alpha-glucoseaminidase and alpha-fucosidase (Table 4).

**Table 4:**
**Enzyme profile of *Lactobacillus casei* ssp.*rhamnosus* LBRC11**

| | Enzyme | Activity* LBRC11 |
|---|---|---|
| 1 | Control | - |
| 2 | Alkaline phosphatase | 3 |
| 3 | Esterase | 3 |
| 4 | Esterase-lipase | 2 |
| 5 | Lipase | 0.5 |
| 6 | Leucine-aminopeptidase | 5 |
| 7 | Valine-aminopeptidase | 5 |
| 8 | Cysteine-aminopeptidase | 3 |
| 9 | Trypsin | - |
| 10 | Chymotrypsin | 2 |
| 11 | Acid phosphatase | 4 |
| 12 | Phosphohydrolase | 2 |
| 13 | α-galactosidase | 3 |
| 14 | β-galactosidase | 5 |
| 15 | β-glucuronidase | 1 |
| 16 | α-glucosidase | 3 |
| 17 | β-glucosidase | 5 |
| 18 | α-glucoseaminidase | 3 |
| 19 | α-manosidase | - |
| 20 | α-fucosidase | 4 |

*the enzyme activity is determined according to the colour scale from 0 (lack of enzyme activity) to 5 (maximum enzyme activity)

The antimicrobial activity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against pathogenic microorganisms is examined. In co-cultivation of the *Lactobacillus* strain with *E.coli* ATCC 25922 in an incubator under static conditions an increase of the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and of the pathogen *E.coli* ATCC 25922 in the first 24 hours is observed; the rates of the increase are commensurable. Then the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 remains unchanged while the one of the pathogen reduces rapidly, reaching 0 on the 60$^{th}$ hour (Fig. 3).

In determining the inhibitory action of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against *E.coli* 8739 an increase in the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and of the pathogen *E.coli* ATCC 25922 in the first 24 hours is established, but the rate of the increase in the *Lactobacillus* strain is higher than that of the pathogen, in contrast to the results obtained with *E.coli* ATCC 25922. Then the concentration of viable cells of the *Lactobacillus* strain remains the same while the concentration of viable cells of the pathogen decreases rapidly, reaching 0 on the 60$^{th}$ hour, as observed in *E.coli* ATCC 25922 (Fig. 4).

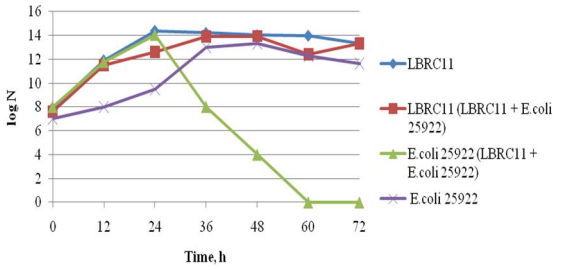


**Figure 3: Antimicrobial activity of *Lactobacillus casei ssp.rhamnosus* LBRC11 against *E.coli* ATCC 25922**

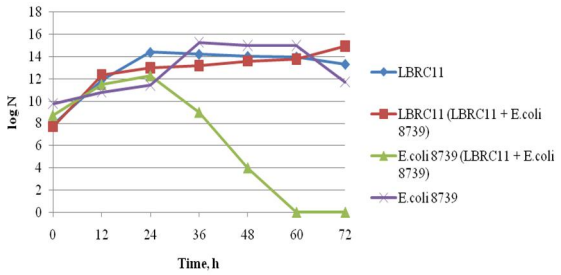


**Figure 4: Antimicrobial activity of *Lactobacillus casei ssp.rhamnosus* LBRC11 against *E.coli* 8739**

In tracing the changes in the titratable acidity it is noticeable that both the two controls of the pathogens are with significantly lower titratable acidity in comparison to the control of *Lactobacillus casei ssp. rhamnosus* LBRC11 and to the titrable acidity of the mixtures (*Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *E.coli* ATCC 25922; *Lactobacillus casei* ssp. *rhamnosus* LBRC11 and *E.coli* 8739), and the values of the titratable acidity of the mixtures are

lower for each hour than the values of the control of the *Lactobacillus* strain. It is noted that during the first 24 hours the titratable acidity of the mixture *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *E.coli* 8739 has higher values than the mixture *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *E.coli* ATCC 25922, while on the 48$^{th}$ hour the acidity of the mixture *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *E.coli* 8739 is lower than that of the mixture *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *E.coli* ATCC 25922, and this trend is maintained until the 72$^{th}$ hour (Fig. 5).

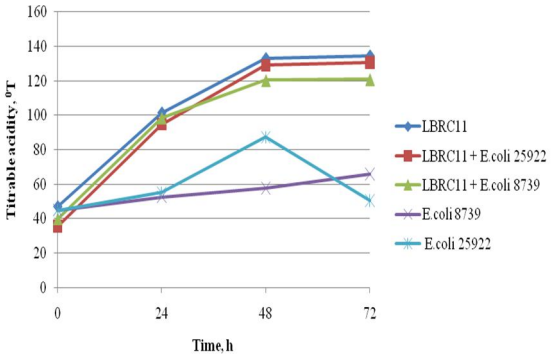


**Figure 5: Change in titrable acidity in testing the antimicrobial activity of the cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against the cells of *E.coli* 8739 and *E.coli* ATCC 25922**

In studying the inhibitory activity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against *Salmonella abony* an increase in the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and of the pathogen *Salmonella abony* during the first 12 hours is observed, and the rates of the increase are commensurable. Then the concentration of viable cells of the *Lactobacillus* strain remains the same while the one of the pathogen decreases more slowly up to 24$^{th}$ hour, after which the rate of reduction in the number of viable cells increases significantly and the concentration of viable cells reaches 0 by the 60$^{th}$ hour (Fig. 6).

In co-cultivation of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *Salmonella* sp. an increase in the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and of the pathogen *Salmonella* sp. in the first 12 hours is established, but the rate of the increase of the *Lactobacillus* strain is lower than that of the pathogen. Then the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 continues to increase while that of the pathogen decreases, reaching $10^4$ cfu/cm$^3$ on the 60$^{th}$ hour (Fig. 7).

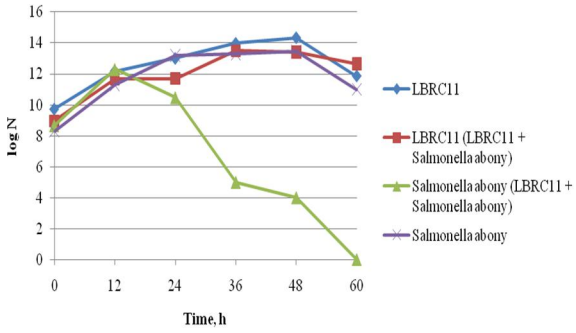


**Figure 6: Antimicrobial activity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against *Salmonella abony***

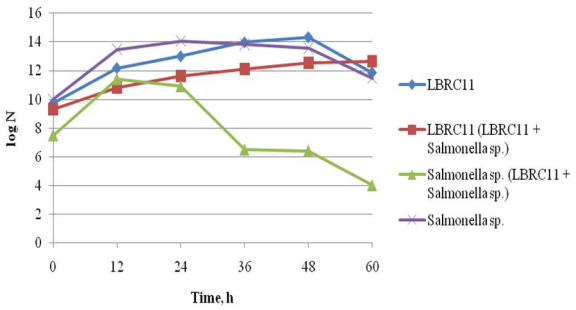


**Figure 7: Antimicrobial activity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against *Salmonella* sp.**

The change in titratable acidity indicates that the values for the titrable acidity of the controls of both the two pathogens are significantly lower than the values of the control of the *Lactobacillus* strain and

those of the mixtures (*Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *Salmonella abony*; *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *Salmonella* sp.). It is noted that during the first 24 hours the change in the titratable acidity of both the two mixtures is commensurable, while on the 48$^{th}$ hour the acidity of the mixture *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *Salmonella abony* is lower than that of the mixture *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *Salmonella* sp., and this trend is maintained up until the 60$^{th}$ hour (Fig. 8).

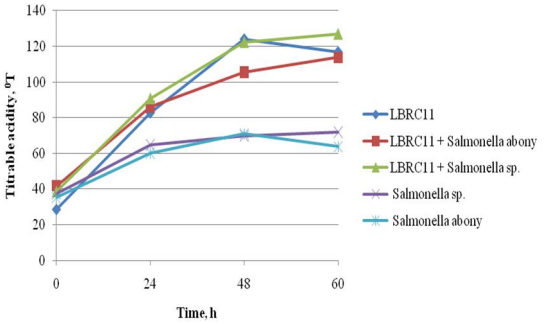


**Figure 8: Change in the titrable acidity in testing the antimicrobial activity of the cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against the cells of *Salmonella abony* and *Salmonella* sp.**

In studying the antimicrobial activity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against *Staphylococcus aureus* ATCC 25093 an increase in the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 and of the pathogen during the first 24 hours is observed, the growth rate of the *Lactobacillus* strain is higher than that of *Staphylococcus aureus* ATCC 25093. Then the concentration of viable cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 continues to grow at a slower rate, while that of the pathogen decreases slowly, reaching $10^5$ cfu/cm$^3$ on the 72$^{nd}$ hour (Fig. 9).

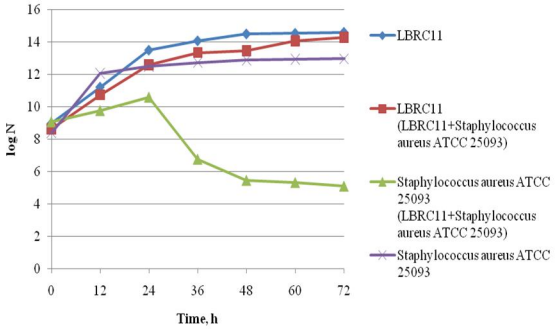


**Figure 9: Antimicrobial activity of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against *Staphylococcus aureus* ATCC 25093**

The data, reflecting the changes in the titratable acidity shows that the titratable acidity of the control of the pathogen is significantly lower than the titrable acidity of both the control of the *Lactobacillus* strain and of the mixture (*Lactobacillus casei* ssp.*rhamnosus* LBRC11 and *Staphylococcus aureus* ATCC 25093) (Fig. 10).

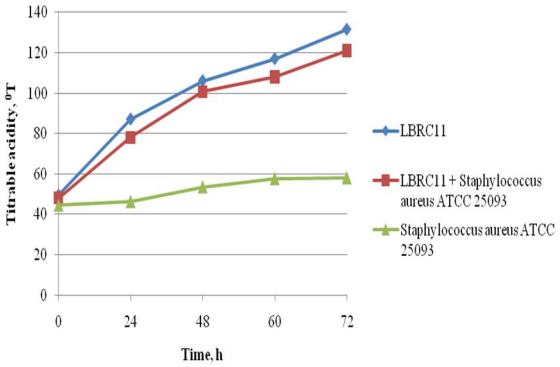


**Figure 10: Change in titrable acidity in testing the antimicrobial activity of the cells of *Lactobacillus casei* ssp.*rhamnosus* LBRC11 against the cells of *Staphylococcus aureus* ATCC 25093**

The strain *Lactobacillus casei* ssp.*rhamnosus* LBRC11 exhibits antimicrobial activity against all of the pathogens included in the study.

## 4. Conclusion

The strain *Lactobacillus* LBRC11, identified by contemporary physiological and biochemical and molecular genetic methods as a representative of the species *Lactobacillus casei ssp.rhamnosus*, has good antimicrobial activity against saprophytic and pathogenic microorganisms. The results of the testings on the possession of some probiotic properties make the strain *Lactobacillus casei ssp.rhamnosus* LBRC11 suitable for inclusion in probiotic preparations for prevention and treatment of various conditions.

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