

## ANTIBACTERIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM BULGARIAN HOMEMADE COW AND SHEEP YOGHURTS

Saso Stojanovski<sup>\*1</sup>, Zivko Gacovski<sup>2</sup>, Penka. Moncheva<sup>2</sup>, Valentina Chipeva<sup>2</sup>, Ilia Iliev<sup>2</sup>, Iskra Vitanova Ivanova<sup>1</sup>.

*Bitola University "St. Kliment Ohridski", Faculty of Veterinary*

*<sup>2</sup>Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Microbiology*

*<sup>3</sup>Plovdiv University "Paisii Hilendarski", Faculty of Biology, Department of Biochemistry and Microbiology*

*\* Corresponding author: Saso Stojanovski*

### ABSTRACT

In this study, 18 homemade cow and sheep yoghurt samples were collected from different regions of Bulgaria for isolation and screening of lactic acid bacteria for antimicrobial activity. Totally 17 strains were isolated and identified as *Lactobacillus* spp. based on their growth, Gram stain, catalase and oxidase activity. The isolates were examined for their antibacterial activity against *Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli* using well diffusion method. Six strains showed inhibitory activity against Gram-negative *E. coli* and only one against Gram-positive *B. subtilis* and *B. cereus* using neutralized cell free cultures supernatants. The results of the present study indicate that Bulgarian homemade yoghurts are potential source of functional starter cultures for production of dairy products.

*Key words: homemade yogurt, functional starter cultures, Lactobacillus spp., antibacterial activity*

### I. Introduction

Milk contains many health promoting constituents including immune globulins, bioactive fatty acids and peptides. Apart from this the health attributes of the milk are associated with fermented and probiotic dairy products like cheese, yoghurt and others fermented milk products. Milk has been preserved by fermentation through the action of lactic acid bacteria (LAB).

Yoghurt is perceived as a kind of healthy food which has a low fat and essential value in terms of protein and vitamins ( Akpinar *et al.*, 2011). In Bulgaria, yoghurt is manufactured by using special symbiotic association of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as starter cultures but many people prepare yoghurt at home by simply allowing milk to ferment.

A novel trend in the food industry is to explore the use of functional LAB starter cultures for the manufacture of fermented food. Functional starter cultures possess at least one functional property, which allow improving the quality of the end product. Promising examples are LAB that produces antimicrobial substances such as lactic acid, acetic acid, formic acid, hydrogen peroxide, diacetyl, bacteriocins, or strains with probiotic effects. To develop such cultures, the biodiversity of traditional fermented foods must be analyzed (Ouweh, 2004).

Although, there is a lot of research about antimicrobial activity of LAB, few of them have been studied on homemade yoghurts.

The aim of the present study is to assess antimicrobial activity of lactic acid bacteria from genera *Lactobacillus* isolated from homemade cow and sheep yoghurts collected from different regions of Bulgaria.

### II. Material and Methods

#### 1. Samples

A total of 18 homemade cow and sheep yogurt samples from different regions of Bulgaria were collected. All samples were stored at 4°C until their analysis.

#### 2. Isolation of lactic acid bacteria

Ten grams of yoghurt samples were diluted in 90 ml saline solution, homogenized, serially diluted in the same solution and plated on MRS agar (De Man, Rogosa and Sharpe Agar, Merck

Darmstadt Germany). The plates were then incubated at 42<sup>0</sup>C for 24 – 48 h. The colonies which were morphologically different were picked up and inoculated as stab. Totally 17 strains from cow and sheep yoghurt samples were isolated (Table 1).

### 3. Characterization and identification of isolated lactic acid bacteria

The different pure culture obtained were characterized for their colony morphology, Gram staining, cell morphology, catalase and oxidase reaction using standard protocols.

### 4. Determination of antimicrobial activity

#### 4.1 Test – microorganisms

Antibacterial activity of isolated lactic acid bacteria were screened against Gram-positive and Gram negative foodborne and spoilage bacteria received from National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Bulgaria - *Bacillus subtilis* NBIMCC 1709, *Bacillus cereus* NBIMCC 1085 and *Escherichia coli* NBIMCC 3397. The pure test-cultures were inoculated on Mueller-Hinton agar (Oxoid) as slants cultures and cultivated at 37<sup>0</sup>C for 24 h. Suspensions with density of 0.5 according to McFarland standard were prepared from the overnight cultures and were used for inoculation of agar medium for antibacterial activity screening of the isolated LAB strains.

#### 4.2 Preparation of cell-free supernatants

The selected LAB isolates were inoculated from slants to fresh 250 ml MRS broth and incubated at 42<sup>0</sup>C for 48 h. The culture broths of each isolate were centrifuged at 10,000x g (HEREUS Biofuge Primo R Centrifuge) for 15 minutes. The cell-free supernatants were adjusted to pH 6.0 with 1 mol l<sup>-1</sup> NaOH in order to eliminate possible inhibition effects of organic acids.

#### 4.3 Antimicrobial activity

The agar well diffusion method was used to determine antimicrobial activity of the isolated LAB strains according to NCCLS (National Committee for Clinical Laboratory Standards (2002). Prepared suspension of each test-microorganism was used to inoculate Mueller-Hinton agar, which was then poured into sterile Petri dishes (20 ml) and stored at 4<sup>0</sup>C for 1 h to solidify. Six millimeters wide wells were cut, using sterile metal borer. Aliquots of 50 µl from culture supernatants of the isolated LAB were poured in each well. The plates prepared in duplicate were kept at 4<sup>0</sup>C for 2 h to allow diffusion and then incubated at 37<sup>0</sup>C for 24 h. After incubation antimicrobial activity was determined by measuring the diameter (in millimeters) of the inhibition zones around the wells.

### III. Results and Discussion

Seventeen strains isolated from 18 homemade cow and sheep yoghurts were characterized phenotypically. The strains grew anaerobically on selective MRS agar at 42<sup>0</sup>C optimal temperature and produced white, shiny colonies. All of them were Gram positive, non-spore forming, catalase and oxidase negative, rod shaped bacteria which indicate that the strains are related to the genera *Lactobacillus*.

The antagonistic properties of the LAB isolates against *B. subtilis*, *B. cereus* and *E. coli* were examined using neutralized cell-free supernatants by agar well diffusion method. Five LAB strains namely O42, K7, K3.1, K3.2, K4.1, K4.2 isolated from cow yoghurt and one LAB strain O42 isolated from sheep yoghurt showed moderate inhibitory effect against Gram-negative *E. coli*. The same results have been reported for *L. bulgaricus* strains isolated from Turkish homemade yoghurts (Akpınar *et al.* 2011). The authors claimed that *L. bulgaricus* strains exhibited antimicrobial activity against *E. coli*.

Only one strain K27 isolated from cow yoghurt showed inhibitory effect against Gram-positive *B. subtilis* and *B. cereus*.

The results for final pH of fermentation broth and antimicrobial activity of neutralized cell-free supernatants of isolated LAB were summarized in Table 1.

Table 1. Antibacterial activity of cell-free supernatants of isolated *Lactobacillus* strains

<i>Lactobacillus</i> strains	Final pH	Antibacterial activity (mm inhibition zone) of neutralized cell-free supernatants		
		<i>B. subtilis</i>	<i>B. cereus</i>	<i>E. coli</i>
K7*	4.08	-	-	15
K3.1	4.00	-	-	15
K3.2	4.30	-	-	14
K4.1	4.28	-	-	15
K4.2	4.27	-	-	13
K27	4.40	24	26	-
O42**	4.27	-	-	16

\*K-LAB strains isolated from cow yoghurt; \*\*O-LAB strains isolated from sheep yoghurt

The results suggest that other compounds different from organic acids take part in the inhibition of selected test-bacteria and this inhibition maybe due to the production of hydrogen peroxide or bacteriocin-like substances. Only a few bacteriocins of LAB with activity against Gram-negative bacteria have been reported (Aslim *et al.* 2004; Sumathi and Reetha, 2012). Miteva *et al.* (1998) reported 36 *Lactobacillus delbrueckii* strains isolated from the ELBY *Bulgaricum* collection with broad spectrum of inhibitory activity.

In conclusion, Bulgarian homemade yoghurts are potential source of *Lactobacillus* strains with antibacterial activity, but further investigation is needed to establish the nature of the inhibitory molecules producing by these strains.

### References

1. Akpinar A., Yerlikaya O. and Kiliç S. (2011) Antimicrobial activity and antibiotic resistance of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from Turkish homemade yoghurts. African Journal of Microbiology Research, 5(6): 675-682.
2. Aslim B., Yuksekdağ Z., Sarıkaya E. and Beyaltı Y. (2004). Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products LWT. Food Science and technology, 1:1-4
3. Miteva V., Stefanova T., Budakov I., Ivanova I., Mitev V., Gancheva A. et al. (1998). Characterization of bacteriocins, produced by strains from traditional Bulgarian dairy products. Systematic and Applied Microbiology, 21(1):235-244.
4. NCCLS, Document M2 (2002). Performance Standards for Antimicrobial Disk Susceptibility Tests. 4<sup>th</sup> ed. 10:7.
5. Ouweh G. and Vest, A. (2004). Anti-microbial components from lactic acid bacteria, In Salminen, S, and Von A. (eds). Lactic Acid Bacteria: Microbiology and Functional Aspects. 2<sup>nd</sup> edition, Marcel Dekker Inc., New York&: 139-160.
6. Sumathi V. and Reetha D. (1012). Screening of Lactic Acid Bacteria for Their Antimicrobial Activity against Pathogenic Bacteria. International Journal of Pharmaceutical & Biological Archives, 3(4): 802-808.