ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM BULGARIAN HOMEMADE COW AND SHEEP YOGURTS

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ABSTRACT

In this study, 25 homemade cow and sheep yoghurt samples were collected from different regions of Bulgaria for isolation and screening of lactic acid bacteria for, final pH and growth at different temperature (15^{0}C ,25^{0}C, 37^{0}C, 40^{0}C and 50^{0}C). Totally 19 strains were isolated and identified as *Lactobacillus* spp. based on their growth, Gram stain, catalase and oxidase activity.

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

I. Intoduction

Lactic acid bacteria are industrially important organisms used for the production like yogurt, cheese ,butter milk and kefir. The species used for these applications typically belong to the grop of gram positive bacteria including the genera Lactobacillus, Pediococcus, Leuconostoc and Streptococcus. They are recognized for their fermetative ability and this enhancing food safety improving organoleptic attributes,enriching nutrients and increasing health benefits (Sharma et al., 2012, Steelle et al 2013). Due to the characteristic of milk that is nighey perishable the main purpose of milk fermentation used LAB is to prolong its shelf-life as were as to preserve the nutrition component of milk .It is also recognized that fermentation of milk using LAB undoubtedly produce good quality of products with highly appreciated organoleptic attributes. Recently there is a growing interest to develop a variety of fermented milk products. For other beneficial purposes particularly for health purposes and preventing of toxins produced by food-borne potogenes and spoilage bacteria.(Penesar,2011, Sharma et al., 2012).

II. Material and Methods

1. Samples

A total of 25 homemade cow and sheep yogurt samples from different regions of Bulgaria were collected. All samples were stored at 4^{o}C until their analysis.

2. Isolation of lactic acid bacteria

Ten grams of yogurt 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^{0}C for 24 – 48 h. The colonies which were morphologically different were picked up and inoculated as stab. Totally 19 strains from cow and sheep yogurt 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.

III. Results and Discussion

Nineteen strains isolated from 25 homemade cow and sheep yoghurts were characterized phenotypically. The strains grew anaerobically on selective MRS agar at 42^{o}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 rate of acidification of the medium by the starter microorganisms is an important parameter of the fermentation process. No excessive acidification of the medium is prefered in the production process, as according to Zanatta and Basso (1992) and Beal et.al., (1999), this provides a more homogenous structure of the curd, and higher viscosity of the final product. Over-acidification of the products from other countries could worsen its taste. pH <4.8 is necessary to ensure the formation of a stable gel of coagulated milk protein (Rasic J.L, et.al., (1978). The resistance of the Lactobacilli to the increased acidity of the environment and other adverse factors is related to the synthesis of specific proteins. it has been found that the reduction of pH to 4.75 leads to the expression of 3 (heat shock proteins) protein (GroES, GroEL, and DnaK) in *Lactobacillus bulgaricus* (Lim et al., 2000, and lowering the pH to 4.5, resulting in the synthesis 9 protein (14.1 to 56.2 kDa) with *Lactobacillus acidophilus* (Lorca and Font de Valdez, 2001).

Table 1. Isolated strains from of isolated Bulgarian Homemade Cow and Sheep Yogurts.

Lactobacillus strains	Final pH	Gas from glucose	*Growth at different temperature (^{0}C)*				
			15 ^{0}C	25 ^{0}C	37 ^{0}C	40^{0}C	50^{0}C
K1	**4.08**	-	+	+++	+++	+	+
K2	**4.00**	-	+	+++	+++	+	+
K3	**4.30**	-	+	+++	++	+	-
K4	**4.28**	-	–	+++	+++	++	+
K5	**4.27**	-	+	+++	+++	++	+
K6	**4.40**	-	–	+++	+++	++	+
K7	**4.27**	-	+	+++	+++	++	+
K8	**4.20**	-	–	+++	+	+	+
K9	**4.05**	-	+	+++	+++	++	+
K10	**4.10**	-	–	+++	+++	++	+
O1	**4.39**	-	–	+++	+++	++	+
O2	**4.07**	-	+	+++	+++	++	+
O3	**4.13**	-	–	+++	+++	++	+
O4	**4.28**	-	–	+++	+++	++	+
O5	**4.35**	-	+++	+++	+++	++	+
O6	**4.41**	-	+++	+++	+++	++	+
O7	**4.36**	-	–	+++	+++	++	+
O8	**4.27**	-	–	+++	+++	++	+
O9	**4.24**	-	–	+	+	+	+

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

The ability of the tested strains to survive at different temperatures was tested in the temperature range of 25^{0}C to 42^{0}C. The temperature of the production and the temperature at which the finished product is stored are among the parameters which affect the viability of the bacteria in the final fermented product. In this regard, potentially important in the dairy industry are strains

which are thermally resistant to storage at low temperatures and strongly acidified final product. For this purpose tested strains were grown at temperatures of 15^0C, 25^0C, 37^0C, 40^0C and 50^0C, and before starting the experiment tested strains were three times precultivated at their optimal growth conditions. The results showed that the isolated strains tolerate temperatures from 25^0C to 37^0C shown in Table 1.

The ability to tolerate high and low temperatures is strain specific. It can be successfully used in the steps of the fermentation process of the milk, which require exposure to high temperatures. It is well known that in response to exposure to stress conditions such as heat, shock and cold the defense mechanisms of adaptation of the bacteria are activated. They include the synthesis of specific proteins, increasing the content of specific fatty acids, and increased concentration of unsaturated fatty acids in the membrane phospholipids (Wang Y., et.al., 2005, Melilli C et.al., 2004, Lim, E et.al., 2000, Fernandez Murga et.al., 1999 ,Gomez Zavaglia et.al., 2000) . All these properties, however, are strain specific and they define their specific technnical features.

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