STUDY OF THE PROTEOLYTIC ACTIVITY OF STRAINS ISOLATED FROM HOMEMADE BULGARIAN YOGHURT

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ABSTRACT

In this study, 5 homemade cow yoghurt samples were collected from different regions of Bulgaria for isolation and screening of lactic acid bacteria for proteolytic activity. Totally 6 strains were isolated and identified as Lactobacillus spp. based on their growth, Gram stain, catalase and oxidase activity. The isolates were examined for their proteolytic activity. The obtained results show that the strains possess most intense proteolytic activity to α - lactalbumin. 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., proteolytic activity*

I. Introduction

The milk represents a great source of nutrients for an infant, and ensures his survival during the most critical period after his birth. (Jouan P.2002) The fundamental components of the milk are water, carbohydrates, fats, proteins, vitamins and minerals. The development of the diary industry today is directly dependent on the development and expanded knowledge of food microbiology and structure, functional and biological properties of the various components of the milk.

Milk proteins, in particular the ones from the cow milk, are intensively studied over the years. They are represented by casein and whey proteins. The milk proteins are the first foreign proteins which are consumed in large amounts by the children (Järvinen K.-M., *et.al, 2001*) and there is evidence for the occurrence of milk allergy in children under certain conditions.

(Prioult G., *et.al.,*2005, Jakobsson I. *et.al.1979*, Bock S.A. *et al.,* Hùst A. *et.al.,*1988, Saarinen K.M., *et al.,* 1999, Halken S. *et al.,*2000, , Gerrard J.W., *et.al.,*1986). The proteolytic activity of the lactic acid bacteria and their probiotic properties were studied intensively over the last decades. десетилетия (Ehn B.-M., *et.al.,* 2005, Booth, M., *et.al.,* 1990, Wohlrab Y. and Bockelmann, W., 1993, Bockelmann W., *et.al.,* 1996, Law J. and Haandrikman, A., 1997). Due to the fact that milk does not contain sufficient free amino acids and oligo-peptides, (Zourari, Accolas, & Desmazeaud, 1992; Abu-Tarboush, 1996) and the lactic acid bacteria are auxotrophs to many of them, (Kok, J., and W. M. De Vos. 1994) they have a complex system of proteinases and peptidases, which makes their development in milk.

II. Materials and methods

1. Isolation of the microorganisms

Selecting lactobacilli strains of lactic acid homemade products was done following the traditional microbiological approaches. Used medium MRS broth and MRS agar (MRS broth, Merck, KGaA, 64271 Darmstadt Germany), suitable for the isolation of lactobacillaceae strains. After the pre-enrichment of the culture in 0.5% skimmed milk (Merck, KGaA, Darmstadt Germany) for 16-18h and a series of dilutions, the culture was grown on MRS agar medium. From each dish with MRS agar medium were randomly selected colonies of all samples and selected strains K3,K5,K14,K15,K20 and K24 were subsequently cultured in MRS broth 42^0C.

2. Proteolytic activity

Strains were grown in MRS, M17 and Elliker broth. After that they were reinoculated from the liquid medium into MRS, M17 and Elliker agar. Collected cells were resuspended in 0.01 mmol/L K-phosphate buffer (pH 6.5) and inoculated in skimmed goat milk. The samples were incubated at 37 C and 30 C for 20 h. This method is based on the reaction of the free a-amino groups released by hydrolysis of casein with o-phthaldialdehyde (OPA) in the presence of b-mercaptoethanol to form a complex that strongly absorbs at 340 nm. The absorbance of the OPA reagent with aliquot of the control (non-inoculated skimmed goat milk) was substracted from each reading. The results were expressed in mmol/L of a-amino acid. (Church et al 1983).

3. SDS-Page analysis of samples obtained after cultivating in the presence of proteins

The ability of the isolated strains to hydrolyze casein,whey and alpha lactalbumin was checked by culturing them on MRS medium supplemented with the corresponding proteins.

The strains were cultured at in the presence of 0.05% the studied proteins. Before the experiment, test strains were cultivated in MRS-broth for 16-18 hours 42^0C. From the pre-culture 10% inoculum of the test medium was inoculated and the strain was cultivated on 42^0C in thermostat. Sampling (30µl of the supernatant) was separated by 90µl sample buffer for SDS-PAGE. The thus prepared samples were handled thermally at 100°C for 3 min, and then were analyzed by electrophoresis using SDS-PAGE.

SDS-PAGE was performed on a 12% separating gel, and 3% concentrating gel by HOEFER Mighty Small SE 245. Samples are treated to 10 mA in the concentrating gel and 20 mA in a separating gel, and then were stained for 1 hour in a staining solution of Coomassie Blue R-250 and discolored for 2 hours in destaining solution.

III. Results and Discussion

The obtained results have shown that the studied strains have relatively intense proteolytic activity (Fig1) .The highest activity was found for strains K14, K15. The electrophoretic analysis confirmed that the used strains have pronounced proteolytic activity when cultured in the presence of lactalbumin and in the presence of caseins (Fig 2)and (Fig.3).The intensity of the obtained bands was less in the case of – lactalglubin .This is an good indentification of the proteolytic capacity of the studied strains.

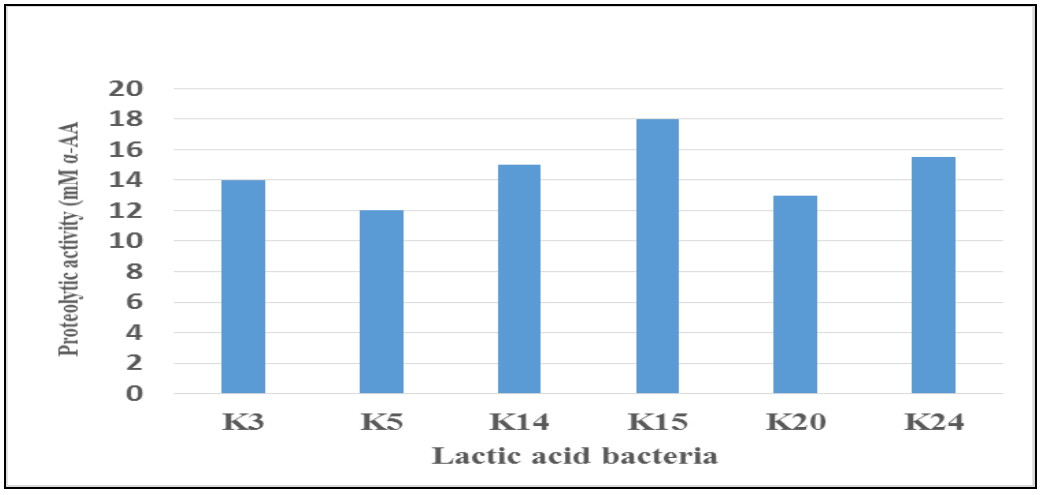

Figure 1. Proteolytic activity of selected LAB strains cultivated in goat milk.

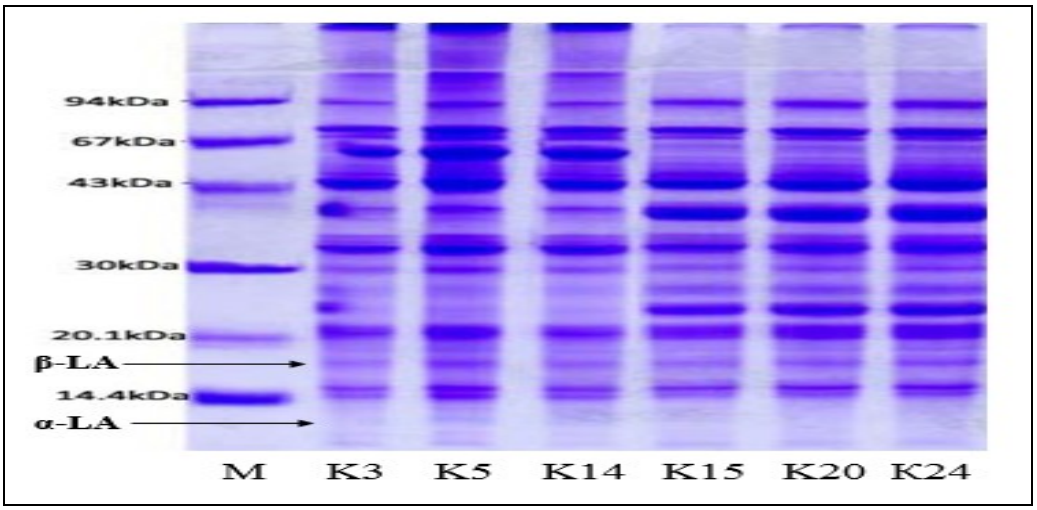

Figure 2. SDS-PAGE of the test strains cultivated in the presence of caseins

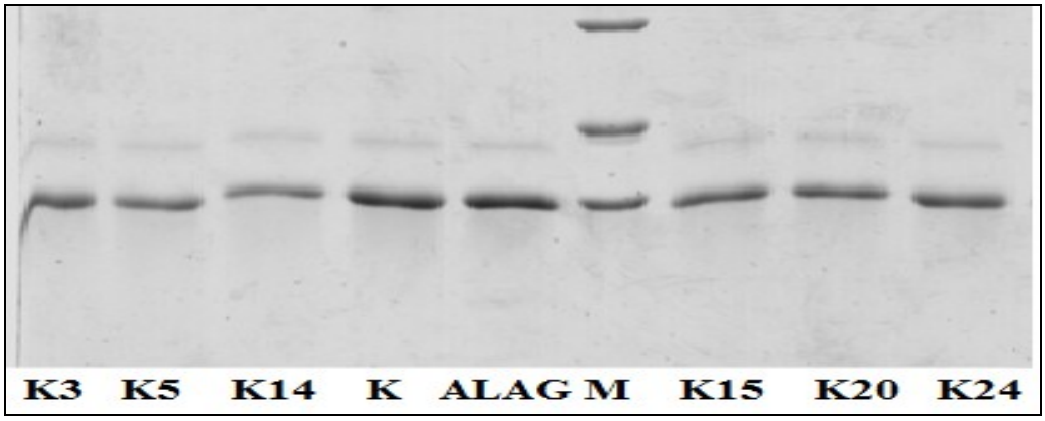

Figure 3 SDS-PAGE of studied strains cultivated in the presence of ALAG

It is well known that whey proteins (ALAC and BLG) are potent allergens (Wal, 1998). They represent peptides with a compact structure. It is believed that the most powerful of these is the protein BLG. (Halken &Host, 1998). Betalaktoglobulinat is globular protein and its sustainable spatial conformation shows high resistance to digestion, which in a way explains his great allergenicity (Prioult G., et.al.,2005). It has been found that hydrolysis of BLG by digestive enzymes reduce its allergenicity (Asselin, Hebert, & Amiot, 1989), but also masks hidden allergenic peptides, which are recognized by specific IgE from sera of allergic patients (Selo et al. , 1999). Three tryptic peptides from BLG were identified as the main allergen epitopes (41-60; 102-124; 149-162) (Selo et al., 1999). In the digestive tract these peptides may become targets for endogenous peptidases of the bacteria colonizing probiotic intestine. (Pessi, Sutas, Marttinen, & Isolauri, 1998).

Besides the hydrolysis of milk proteins during digestion, they can also be hydrolyzed by proteinases of starter cultures during the fermentation process, which creates further opportunity to reduce their allergenicity is (Cross, M. et.al.2001). Furthermore, hydrolysis of milk proteins during digestion, they can be hydrolyzed by proteinases of starter cultures during the fermentation process, which creates further opportunity to reduce their allergenicity is (Cross, M. et.al.,2001).

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