

## API ZYM ENZYMATIC PROFILE OF LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL BULGARIAN MEAT PRODUCT “LUKANKA”

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### Abstract

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The enzyme potential of lactic acid bacteria is an important factor in the formation of characteristic taste of meat products. The aim of the study is the determination of the enzyme profile of different lactic acid bacteria using API ZYM system. The enzyme activity of 31 strains of *Lactobacillus plantarum*, *Lactobacillus sakei* and *Lactobacillus brevis* isolated during different stages of the fermentation process of the naturally fermented traditional Bulgarian meat product “Lukanka” was determined. It was established that most of the strains showed low lipolytic activity. Only a few strains showed phosphatase and  $\beta$ -galactosidase activity. The amidase activity varied among the strains. Almost all strains possessed high  $\beta$ -glucosidase,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -mannosidase and N-acetyl- $\beta$ -glucosaminidase activity.

*Key words:* Enzyme activity, Lactic acid bacteria, Traditional Bulgarian Meat Product “Lukanka”

### Introduction

Production of naturally fermented sausages is a tradition in the countries of Southern Europe, but also occurs in some Scandinavian countries and Latin America. Specific flavor, odor, color and structure of the sausages are due to the characteristics of the raw meat and spices used, natural microflora including lactic acid bacteria as well as applied technology. During the ripening of dry sausage biochemical and physicochemical processes occur, which alter the composition and the structure of meat to form end products that give specific flavor, taste, color and other features of the product. Enzymatic activity of microflora, including the lactic acid plays an essential role in these processes (Montel et al., 1998; Demeyer et al., 2000; Virgili, 2000; Papamanoli et al., 2003).

### Materials and Methods

#### Microorganisms

Thirty-one strains of lactic acid bacteria (*Lactobacillus plantarum* – 24 strains, *Lactobacillus sakei* - five strains and *Lactobacillus brevis* – three strains) isolated during different stages of the ripening of traditional Bulgarian meat product “Lukanka” were used in this study. The strains were maintained on MRS agar.

#### Enzyme profile

The enzyme activity of the lactic acid bacteria was determined using API ZYM miniaturized test following the manufacturer’s instructions. The API strips were inoculated with 24-h-old cultures grown in MRS broth, and then incubated

at 30°C for 4 h. The evaluation of the activity was carried out on 5-grade scale, according to the intensity of coloration. Twenty enzyme activities were determined.

### Results and Discussion

Several enzyme activities included in lipid, carbohydrate, protein and phosphate metabolism were studied.

The lipase activity (Figure 1) of the strains was very low and this confirmed the result of other authors (Montel et al., 1998). According to Vestergaard and Virgili (2000) and Demeyer et al. (2000) responsible for lipolysis of meat products are mainly muscle lipases and phospholipases, while bacterial enzymes show very low activity in fermentation process of the products.

The activity of valin-arylamidase and cysteine-arylamidase of the most of the tested strains was high whereas

leucine arylamidase was relatively low. Seven strains of *L. plantarum* (S1, S2, S4, S5, S6, S7 and S11) did not show leucine arylamidase activity and the same number of strains showed high activity (Figure 2). Our results are consistent with those of Papamanoli et al. (2003). The degradation of the amino acids, obtained by exopeptidase activity of lactic acid bacteria and muscle aminopeptidases to volatile molecules plays an important role in the formation of characteristic taste of the dried sausages. The studies of Montel et al. (1998) showed that the quantity of the aldehydes, alcohols and acids derived from degradation of leucine, valine, phenylalanine and methionine was very low.

The majority of the studied strains did not show alkaline and acid phosphatase activity

Only a few strains of *L. plantarum* (S3, S10 and S26) and *L. brevis* (S8, S27 and S28) showed one or both activities.

$\beta$ -galactosidase activity was recorded for three strains

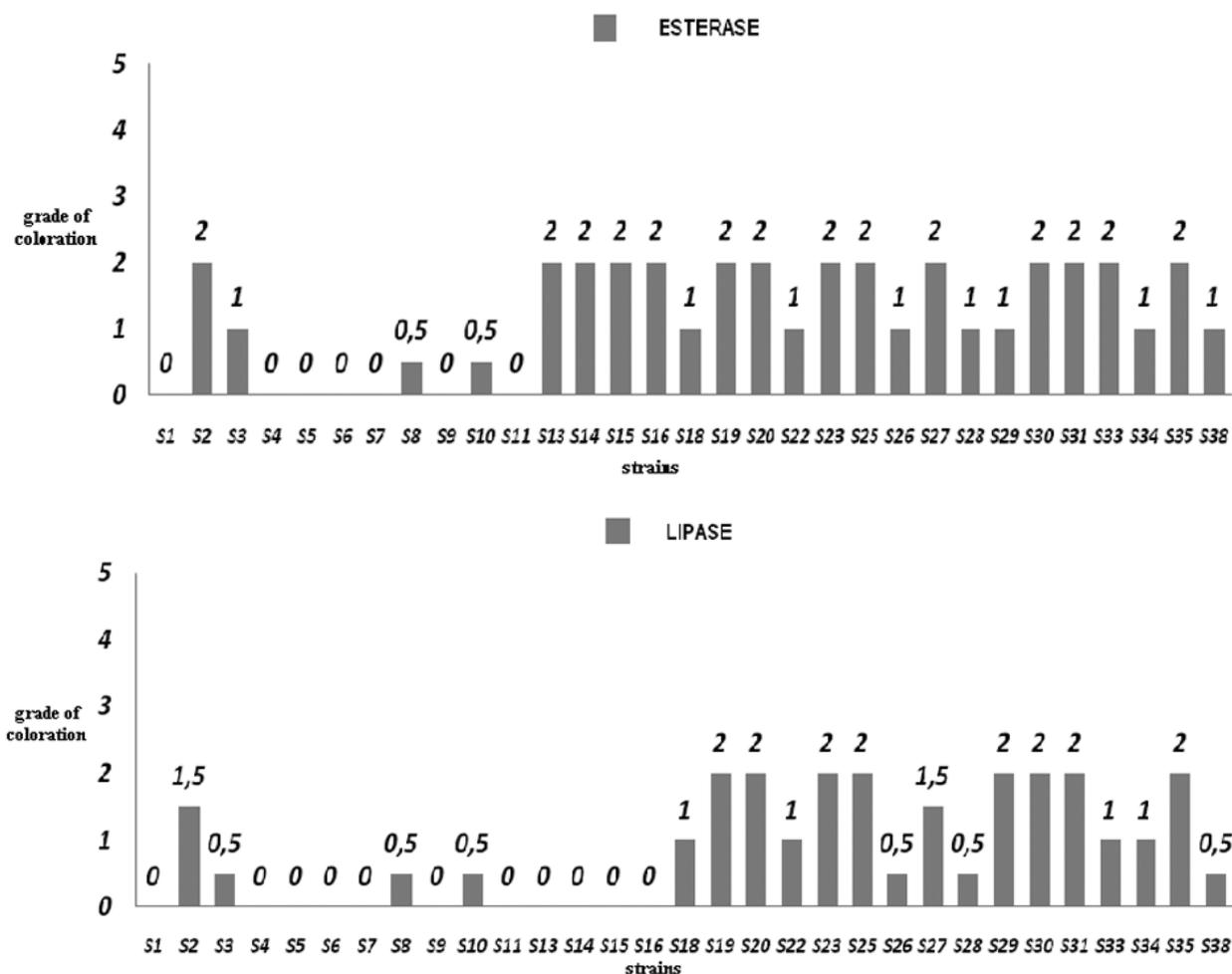


Fig. 1. Lipase and Esterase activity (A and B) of selected strains

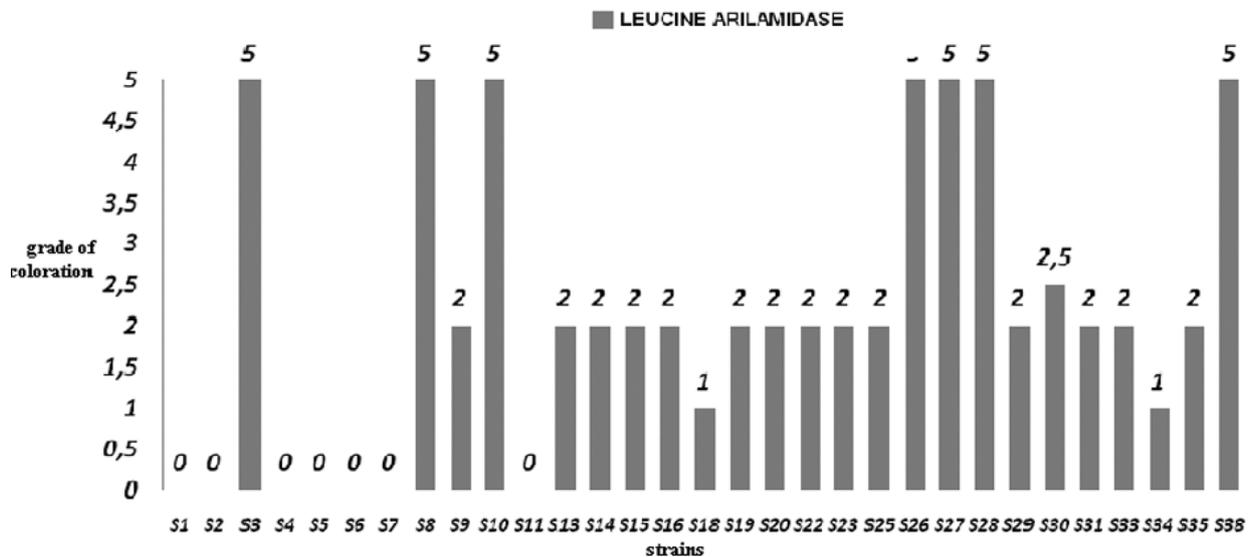
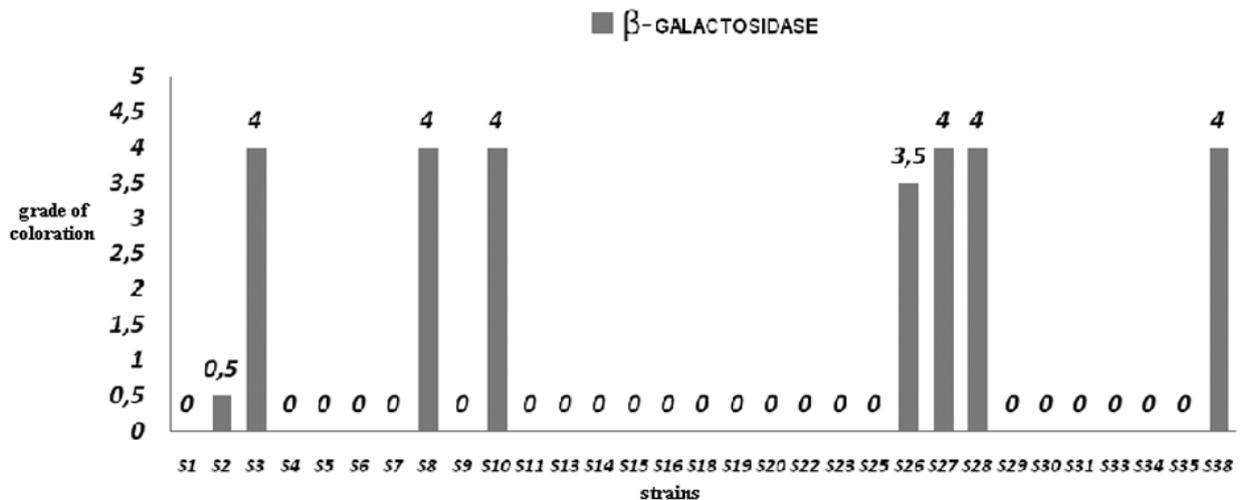


Fig. 2. Leucine arilamidase activity of selected strains

Fig. 3.  $\beta$ -galactosidase activity of selected strains

of *L. plantarum* (S3, S10 and S26), and four strains of *L. brevis* (S8, S27, S28 and S38), whereas 24 strains possessed  $\alpha$ -galactosidase activity (Figures 3 and 4).

Almost all strains showed  $\beta$ -glucosidase activity (except S13, S14, S15 and S16), while the number of the strains with  $\alpha$ -glucosidase activity was significantly lower (seven strains)

The most of the investigated strains possessed high  $\alpha$ -galactosidase (Figure 4),  $\beta$ -glucuronidase (Figure 5),  $\alpha$ -mannosidase and N-acetyl- $\beta$ -glucosaminidase activity. The data obtained confirmed the results of Papamanoli et al. (2003) for these enzyme activities.

The acid production during the fermentation of dry sausage depends on the type and the concentration of sugars added to the meat and on the number of lactic acid bacteria. The level of the produced acids depends on the acid formation activity of the strains. The sour taste of fermented meat products correlates with the amount of acid and is one of the factors determining one of the overall flavors of the sausage (Montel et al 1998). This fact should be taken into account in the selection of starter cultures depending on the user preferences. In the Nordic countries, sourer flavor of raw dried sausages is preferred unlike the South.

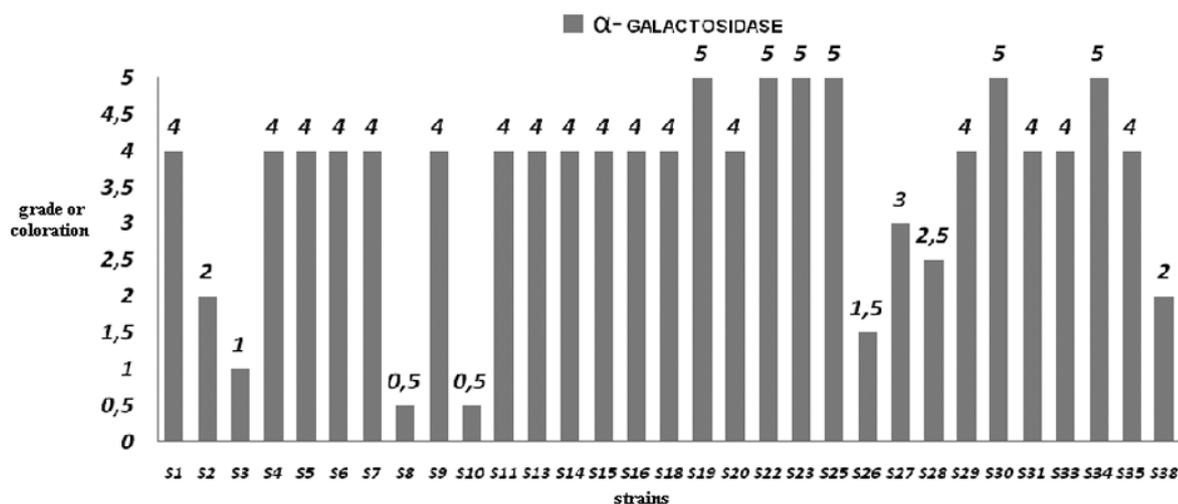


Fig. 4.  $\alpha$ -galactosidase activity of selected strains

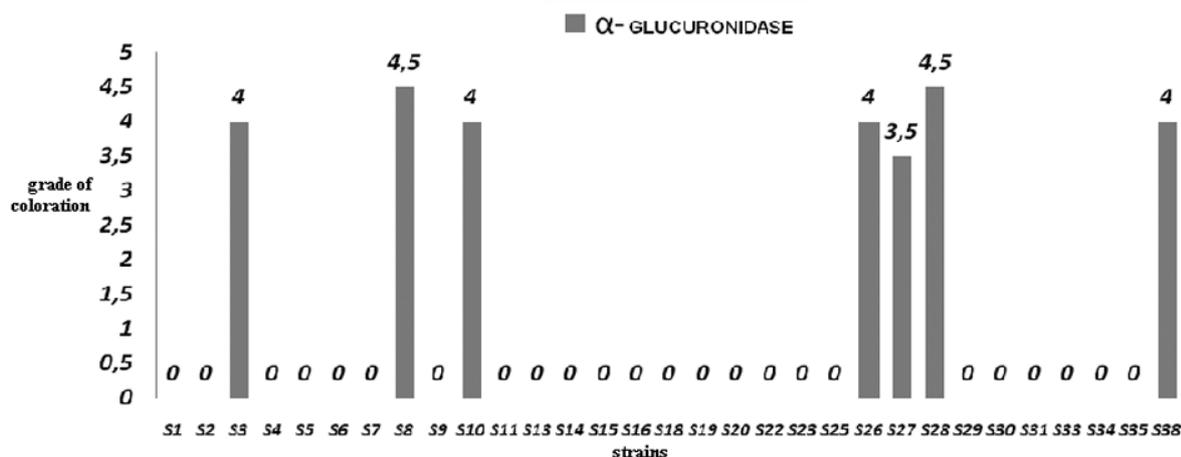


Fig. 5.  $\beta$ -glucuronidase activity of selected strains

## Conclusion

Processes that occur during the ripening of the product are complex and are result from the interaction of the remaining enzymes of muscle and fat tissue and the action of bacterial enzymes, including the enzymes of lactic acid bacteria. It was established that most of the strains showed low lipolytic activity. Only a few strains showed phosphatase and  $\beta$ -galactosidase activity. The amidase activity varied among the strains. Almost all strains possessed high  $\beta$ -glucosidase,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -manosidase and N-acetyl- $\beta$ -glucosaminidase activity.

## References

Demeyer, D., Raemaekers, M., Rizzo, A., Holck, A., De

Smedit, A., ten Brink, B., Hagen, B., Montel, C., Zanardi, E., Murbrekk, E. and Leroy, F. 2000. Control of bioflavour and safety in fermented sausage. *Food international*, **33**: 171–180.

Lücke, F., 1974. Fermented sausages. *Microbiology of Fermented Foods*. Applied Science Publishers, London, pp. 41–49.

Montel, M. C., F. Masson and R. Talon, 1998. Bacterial role in flavour development. Proceedings, 44<sup>th</sup> International Congress on *Meat Science and Technology*, Barcelona, IRTA/*Eurocarne.*, 1, pp. 224–233.

Papamanoli, E., Tzanetakis, N., Lipopoulou-Tzanetaki, E. and P. Kotzekidou, 2003. Characterization of lactic acid bacteria isolated from a Greek dryfermented sausage in respect of their technological and probiotic properties. *Meat Science*, **65**: 859–867.

Vestergaard, C. S., C. Schivazappa and R. Virgili, 2000. Lipolysis in dry-cured ham maturation. *Meat Science*, **55**: 1–5.