Enzymatic profile of *Lactobacillus brevis* strains isolated from different sources

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Enzymatic profile of Lactobacillus brevis strains isolated from different sources: *The enzymatic activities of lactic acid bacteria in the composition of sourdough for the production of bread affect the quality of the final product. The enzyme profiles of 3 Lactobacillus brevis strains (L.brevis LBRZ7, L.brevis LBRZ8 and L.brevis X1) using the kit API ZYM are determined. Their proteolytic and amylolytic activities are examined as well. L.brevis LBRZ7, L.brevis LBRZ8 and L.brevis X1 exhibit proteolytic activity as well as esterase lipase, leucine-aminopeptidase, valine-aminopeptidase, cystine-aminopeptidase, acid phosphatase, phosphohydrolase, α-galactosidase and β-galactosidase activity. L.brevis LBRZ7 and L.brevis LBRZ8 also demonstrate proteolytic activity as well as acid phosphatase and β-glucoronidase activity, L.brevis LBRZ7 also has lypolitic activity. L.brevis LBRZ7 exhibits higher amilolytic activity and L.brevis LBRZ8 shows the highest proteolytic activity. The presence of the reported enzymatic activities makes the strains suitable for incorporation in sourdough starters.*

Key words: *Lactobacillus, enzyme profile, API ZYM, amylolytic activity, proteolytic activity, sourdough*

INTRODUCTION

Bakery products have a very short shelf life [1]. The spoiling of bakery products is mainly a result of the growth of molds [13], and the roping of the bread, which is caused by *Bacillus* sp. [3]. Another problem is the reduction of the freshness of bread during storage in parallel to the hardening of the crumb, which leads to loss of acceptable appearance to consumers, a process known as staling of bread [12].

The addition of sourdough is the best approach to prevent bread spoilage that meets consumer's demand for natural foods without additives [3]. Sourdough is a mixture of flour (wheat, rye, rice, etc.) and water, which is fermented by the action of lactic acid bacteria and yeast [6]. There are a number of evidences for the positive effects of sourdough in bread making, such as the improvement of the bread volume and crumb structure [4], the taste [18], the nutritional value [14, 15] and the shelf life [1, 2, 5], slowing the staling process and preventing fungal and bacterial deterioration [7, 11]. These positive effects are associated with the metabolic activity of microorganisms in the composition of sourdough like lactic acid fermentation, proteolysis, production of exopolysaccharides and synthesis of volatile and antimicrobial compounds [1, 3, 17]. With the inclusion of starter cultures, the pH drops much faster, so the whole production process is accelerated, which in turn leads to economic benefits for the manufacturer. The majority of the starter cultures are natural isolates of the desired microorganisms commonly found in the substrates.

In the biotechnology of baked goods there are three sources of enzymes: the endogenous enzymes of flour, the enzymes associated with the metabolic activity of the dominant microorganisms (yeasts and lactic acid bacteria) and the exogenous enzymes, intentionally incorporated in the composition of the dough.

The addition of sourdough affects the effectiveness of the exogenous enzymes during fermentation because of the reduction in pH. Enzymes may be influenced by the metabolic activities of lactic acid bacteria in the composition of the sourdough and to release nutrients or to modify other factors of the environment [16].

The combination of lactic acid bacteria associated with sourdough and exogenous enzymes is of particular importance in modern biotechnology of baked goods [9].

The protein fraction of wheat and rye flour is crucial for the quality of the bread. Protein degradation during the fermentation of sourdough is one of the fundamental phenomena that affect the overall quality of the bread produced with sourdough [10]. In general, lactic acid bacteria play a minor role in the hydrolysis of the protein [19]. Proteolysis provides precursor compounds for the formation of aromatic volatile substances during baking as well as substrates for the microbial conversion of aromatic

amino acids into precursors [18]. The proteins of the gluten in wheat flour define the rheology of the dough, the gasretention ability and the volume of the bread [1].

Primary proteolysis is carried out by the endogenous enzymes of wheat or rye, which are activated by low pH. The further hydrolysis of the peptides to amino acids is carried out by the intracellular peptidases of lactic acid bacteria in a strain-specific manner - the type and amount of the released amino acids depend on the strain [8]. Proteolysis carried out by lactic acid bacteria causes softening of the dough in comparison with chemically acidified doughs [8, 17].

The aim of the present work is the determination of the enzymatic profiles of 3 strains of *Lactobacillus brevis*, isolated from different sources - *Lactobacillus brevis* LBRZ7, *Lactobacillus brevis* LBRZ8 and *Lactobacillus brevis* X1.

MATERIALS AND METHODS

Microorganisms

The studies in this work are performed with 3 *Lactobacillus brevis* strains: *Lactobacillus brevis* LBRZ7 and *Lactobacillus brevis* LBRZ8 (isolated from fermented vegetables) and *Lactobacillus brevis* X1 (isolated from naturally fermented sourdough).

Media:

Sterile skimmed milk with titratable acidity 16-18ºT. Composition (g/dm^3): skimmed milk powder (Scharlau). Sterilization - 15 minutes at 118ºC.

Saline solution. Composition (g/dm^3): NaCl - 5. Sterilization - 20 minutes at 121ºC.

LAPTg10-broth. Composition (g/dm^3): peptone - 15, yeast extract - 10; tryptone - 10, glucose - 10. pH is adjusted to 6.6 - 6.8 and Tween 80 - 1cm^3/dm^3 is added. Sterilization - 20 minutes at 121ºC.

LAPTg10-agar. Composition (g/dm^3): LAPTg10-broth +2% agar. Sterilization - 20 min at 121ºC.

msLAPTg10-agar. Composition (g/dm^3): peptone - 15, yeast extract - 10; tryptone - 10, soluble starch - 10, Tween 80-1 cm^3/dm^3, agar - 20. pH = 6.6 - 6.8. Sterilization - 20 min at 121ºC.

Examination of the profile of enzymatic activity

The system API ZYM (BioMericux, France) is used for semi-quantitative examination of the enzyme profile of the studied strains. Fresh 24-hour culture of each strain is centrifuged for 15 min at 5000xg, the resulting biomass sludge is washed twice with PBS-buffer and resuspended in API suspension medium. The API ZYM strips are placed in the incubation boxes and the wells are inoculated with the prepared single strain cell suspension. The samples are incubated for 4 hours at 37°C. Then one drop of reagent A and reagent B are added to each well. After 5 min staining is recorded as described in the color scheme in the manufacturer's instructions. Enzyme activity is determined according to a color scale from 0 (no enzyme activity) to 5 (maximum enzyme activity).

Determination of the presence of amylolytic enzymes

msLAPTg10-agar medium is poured in Petri dishes (15 cm^3 medium in each dish) and after the hardening of the medium in each Petri dish 6 wells are made. Fresh 24-hour culture of each of the tested strains is pipetted in the well, each strain being pipetted in quadruplicate. The results (the diameters of the sterile zones in mm) are recorded at the 48th hour.

Determination of the presence of proteolytic enzymes

To a sterile melted LAPTg10-agar medium is added warm sterile skimmed milk (10 cm^3 skimmed milk to every 100 cm^3 of sterile melted LAPTg10-agar) and the resulting well homogenized medium is dispensed in Petri dishes (15 cm^3 in each Petri dish). In each dish after the hardening of the medium 6 wells are made. Fresh culture in the exponential growth phase (a 24-hour culture) of each strain is used. Of each single strain cultural suspension are prepared 3 samples: CS (cultural suspension) - 24hour cultural suspension of the strain; ASN (acellular supernatant) - it is prepared by centrifugation of

the cultural suspension and the resulting supernatant is transferred to a new sterile tube; CSSS (cellular suspension in saline solution) – it is obtained by centrifugation of the cultural suspension of the strain and the resulting sludge is washed once with saline solution, followed by resuspension of the biomass sludge in a saline solution to the initial volume of the corresponding sample. Each sample is done in quadruplicate, and the results (the diameter of the sterile zones in mm) are recorded at the 48th hour of incubation at optimum temperature for the development of the studied strains.

RESULTS AND DISCUSSION

The enzymatic profile of three strains of *Lactobacillus brevis* is determined: *Lactobacillus brevis* LBRZ7, *Lactobacillus brevis* LBRZ8 and *Lactobacillus brevis* X1, using the kit system for qualitative and semi-quantitative detection of enzymatic activity API ZYM. The results of these studies are shown in Table 1.

Lactobacillus brevis LBRZ7, *Lactobacillus brevis* LBRZ8 and *Lactobacillus brevis* X1 are characterized by esterase lipase, leucine-aminopeptidase, valine-aminopeptidase, cysteine-aminopeptidase, acid phosphatase, phophohydrolaze, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase activity. *Lactobacillus brevis* LBRZ7 and *Lactobacillus brevis* LBRZ8 also possess basic phosphatase and β-glucuronidase, while *Lactobacillus brevis* LBRZ7 exhibits lipase activity as well (Table 1).

Table 1. Enzymatic profile of strains of *Lactobacillus brevis* - *Lactobacillus brevis* LBRZ7, *Lactobacillus brevis* LBRZ8 and *Lactobacillus brevis* X1.

	Enzyme	Activity* *Lactobacillus brevis* LBRZ7		Activity* *Lactobacillus brevis* LBRZ8		Activity* *Lactobacillus brevis* X1	
1	Control	-		-		-	
2	Alcaline phosphatase	3		1		-	
3	Esterase	0.5		-		0,5	
4	Esterase-lipase	2		1		0,5	
5	Lipase	1		-		-	
6	Leucine aminopeptidase	5		4,5		3,5	
7	Valine-aminopeptidase	5		2		3	
8	Cysteine aminopeptidase	3		0,5		0,5	
9	Trypsin	-		-		-	
10	Chymotrypsin	-		-		-	
11	Acid phosphatise	5		4		3	
12	Phosphohydrolase	1		0,5		0,5	
13	α-galactosidase	5		5		2,5	
14	β-galactosidase	5		5		5	
15	β-glucoronidase	2		0,5		-	
16	α-glucosidase	5		4		4	
17	β-glucosidase	5		5		5	
18	α-glucosaminidase	-		-		-	
19	α-manosidase	-		-		-	
20	α-fucosidase	-		-		-	

* the enzymatic activity is determined according to a colour scale from 0 (no enzymatic activity) to 5 (maximum enzymatic activity)

Lactobacillus brevis X1 does not possess amylolytic activity, but the other two strains - *Lactobacillus brevis* LBRZ7 and *Lactobacillus brevis* LBRZ8 exhibit amylolytic activity and that of *Lactobacillus brevis* LBRZ7 is more pronounced (Table 2).

Lactobacillus brevis LBRZ7, *Lactobacillus brevis* LBRZ8 and *Lactobacillus brevis* X1 possess proteolytic activity (Table 3). The data obtained for the diameters of the hydrolysis zones of the cultural suspension (CS), acellular supernatants (ASN) and the cell suspensions in saline solution (CSSS) show that the observed proteolytic activity is due to

both the inducible expression of proteolytic enzymes by the cells of the lactobacilli strains and the acidification of the medium as a result of the production of lactic acid and other organic acids by the tested strains.

Table 2. Amylolytic activity of the *Lactobacillus brevis* strains. d_{well} = 6mm.

Strain	Amylolytic activity, mm
Lactobacillus brevis LBRZ7 $9,3x10^{11}cfu/cm^3$	10
Lactobacillus brevis LBRZ8 $5,7x10^{12}cfu/cm^3$	8
Lactobacillus brevis X1 $1,2x10^{13}cfu/cm^3$	-

Table 3. Proteolytic activity of the *Lactobacillus brevis* strains. d_{well} = 6mm. CS (cultural suspension) ASN (acellular supernatant) and CSSS (cellular suspension in saline solution).

Strain	Proteolytic activity, mm	
Lactobacillus brevis LBRZ7 $9,3x10^{11}cfu/cm^3$	CS	9,5
	ASN	9,5
	CSSS	9
Lactobacillus brevis LBRZ8 $5,7x10^{12}cfu/cm^3$	CS	11
	ASN	9
	CSSS	9
Lactobacillus brevis X1 $1,2x10^{13}cfu/cm^3$	CS	9
	ASN	9
	CSSS	9

CONCLUSION

Each manufacturer strives to achieve an optimal balance between quality and cost, while maintaining the traditional taste and meeting the new requirements for bread and healthy diet. The overall goal is improving the organoleptic parameters - color, volume, characteristic taste and maximum shelf life. This requires the use of sourdough containing homo- and heterofermentative mesophilic lactic acid bacteria, whose metabolic activities have beneficial effects on these parameters. The enzymatic profiles of the 3 studied strains of *Lactobacillus brevis* make them suitable for incorporation in sourdough starters for bread production.

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This paper has been reviewed