PROTEOLYTIC AND PHYTASE ACTIVITY IN SOURDOUGH LACTIC ACID BACTERIA

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ABSTRACT

Twenty-six strains of lactic acid bacteria (LAB) isolated from French and Bulgarian sourdoughs were screened for their enzymatic activities, to elucidate their possible roles during the fermentation process. Phytase and phosphatase activities were measured. These activities were determined spectrophotometrically by using p-nitrophenyl phosphate and sodium phytate as substrates. SDS-PAGE and zymograme were performed to prove phytase activities and evaluate molecular weight of purified proteins. The enzyme responsible for these activities was an acid phosphatase, with a molecular mass of 70 kDa. Proteolytic activity were measured with casein and asocasein in model system and also in modified media with gluten as only one nitrogen source. Several strains have shown an interesting combination of proteolytic and phytase activities, suggesting their possible roles during dough fermentation.

Keywords: gluten hydrolysis, phytase, sourdough microflora,

Introduction

Phytases can be derived from a number of sources including plants, animals and microorganisms. Recent research has shown that microbial sources are more promising for the production of phytases on a commercial level and on cereal based foods (1). Endogenous phytase activity may be contained in the wheat and rye flours but the level of it greatly varies with the variety and crop year, and, generally, is considered to be insufficient to significantly decrease the amount of phytic acid (2). Although lactic acid bacteria represent the great part of the sourdough microorganisms and positively influence several dough and bread properties, very few studies have been carried on their phytase activity. Lactobacillus sanfranciscensis is considered as a key sourdough lactic acid bacterium due to its very frequent isolation from sourdoughs and due to its fermentative and enzymatic properties (3,4). In other sourdough strains Lactobacillus plantarum, Lactobacillus acidophilus and Leuconostoc mesenteroides subsp. mesenteroides also have been detected phytase activity (5). Food allergies are of concern to both medical care providers and the general population because of a rapidly increasing prevalence during the past few decades. Wheat flour is largely consumed in the daily diet (e.g., leavened baked goods and pasta) and constitutes one of the most frequent causes of food allergy. It has been shown that gastrointestinal enzymes (pepsin, pancreatin and trypsin) are not able to degrade cereal allergens which reach unaltered the intestine where they elicit the immune response. (6) In this paper we showing the capacity of selected lactic acid bacteria used as starters for the manufacture of sourdough breads to hydrolyze gluten in model system as only one nitrogen source and partial purification and characterization of phytase.

Materials and methods

Bacterial Strains and Culture Conditions

Twenty six strains of sourdough lactic acid bacteria were isolated from sourdoughs from different origin.

Proteolytic assays:

Proteolytic activity was determed using substrate casein according to Kunitz (10).

Proteolytic digestion of gluten

To analyze the proteolytic activity of the strains, all the strains were cultured on Gluten Maltose Broth (GMB) at 30 ˚C for 96 h.. The supernatants of the cell cultures were then analyzed by 12 % SDS-PAGE according to Laemmli (1970) and silver stained by Blum et.al (11).

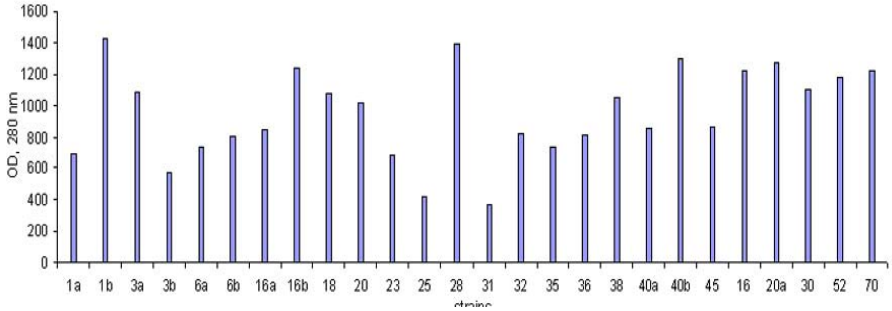

Fig. 1. Proteolytic activity of 26 lactic acid bacteria toward casein

Phytase activity measurement

Preparation of cell free extracts.

Strains were propagated in modified SDB broth (7) supplemented with maltose (27 mM), glucose (55 mM) and Na-phytate (3 mM) or modified MRS-MOPS broth where inorganic phosphate were substituted with MOPS (0.1 M) at 30 ˚C for 48 h. Cytoplasmic extracts were obtained from the cells either by the procedure of De Angelis et al., (1) or by the disruption of the cells with liquid nitrogen.

Phytase activity was measured using a modification of the method of Fiske and Subbarow (8). Phytase activity was also determined by monitoring the rate of hydrolysis of p-nitrophenyl phosphate (p-NPP). The assay mixture contained 200 µl of 200 µM p-NPP (final concentration) in 0.2 M Na-acetate, pH 5.2, and 400 µl of enzyme preparation or cell suspension. The mixture was incubated at 45 ˚C and the reaction was stopped by adding 600 µl of 0.1 M NaOH. The ρ-nitrophenol released was determined by measuring the absorbance at 405 nm.

Enzyme characterization

The apparent molecular mass of the purified enzyme was estimated by sodium dodecyl sulphate (SDS)-PAGE according to Laemmli (1970). The gel contained 12% acrylamide (separation distance, 10 cm; gel thickness, 1 mm). The proteins were stained with Coomassie brilliant blue R-250. Molecular mass marker proteins (low range 10–200 kDa, Invitrogen) were used as references. The zymogram was prepared by incubation the gel first in 1% Triton X-100 for 1h at room temperature and then in 0.1 M sodium acetate buffer (pH 4.5) for 1 h at 4 ˚C. Phytase activity was detected by incubating the gel for 16 h in a 0.1 M sodium acetate buffer (pH 4.5) containing 0.4% (w/v) Na-phytate. Activity bands were visualized by immersing the gel in a 2% (w/v) aqueous cobalt chloride solution. After a 5 min incubation at room temperature, the cobalt chloride solution was replaced with a freshly prepared solution containing equal volumes of a 6.25% (w/v) aqueous ammonium molybdate solution and 0.42% (w/v) ammonium vanadate solution. Phytase activity was evident as zones of clearing in an opaque background (Bae et al., 1999) (9).

Results and Discussion

The proteolytic activity of twenty six strains isolated from sourdoughs was analyzed. The obtained results are shown on **Fig. 1**. From the presented data is clear that the different strains showed different activity toward casein (5mg/ml) using spectrophotometric assay. The highest digestion was found for strains 1a, 23, 25, and 31.

In accordance with the received results we have tried to use these strains for proteolysis of gluten. The same strains were cultivated in medium containing gluten 2 %. The residue quantity of gluten was determined by electrophoresis analysis (**Fig.2**).

The visualization of the bands was done by silver staining method. It could be conclude that all of the strains digested gluten to different extent according the received clear bands. Our results we considered to be quite preliminary due to the specifics of the work with glutens containing protein components with different molecular masses.

Only for several lactic acid bacteria isolated from sourdough *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Leuconostoc mesenteroides* subsp. *mesenteroides* have been detected phytase activity. The following experiments in our work were connected with the determination of phytase activity and its visualization. After screening procedure only six of twenty six analyzed strains were found detectible Na-phytate activity. The work was performed using cell free extracts.

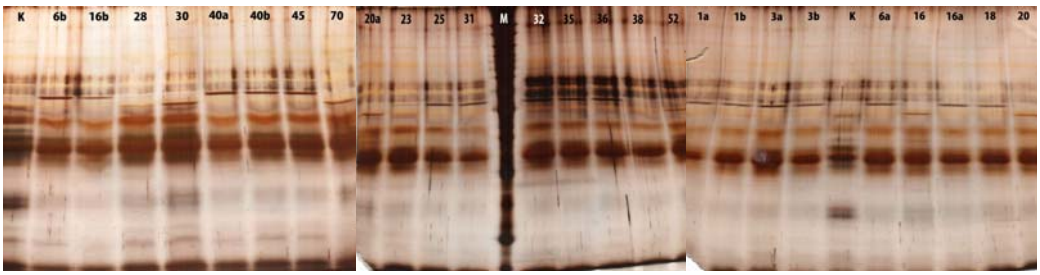

Fig. 2. SDS-PAGE of residual gluten in culture medium

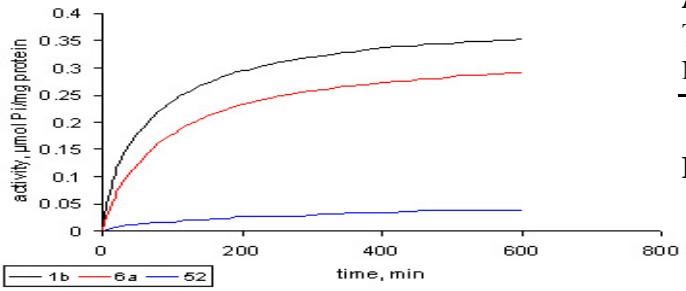

Fig. 3. Kinetics of phytase activity using the Na-phytate

On **Fig.3** were shown the kinetics of phytase activity using the Na-phytate. As it is seen from the results the highest activity was detected for strains 1b and 6a.

The characterization of the molecular mass was done by SDS-PAGE (**Fig.4a**). The single bands running at an apparent molecular mass of 60 kDa was found. As shown by zymogram, the phytase activity correspond to the protein bands located at 60 kDa.

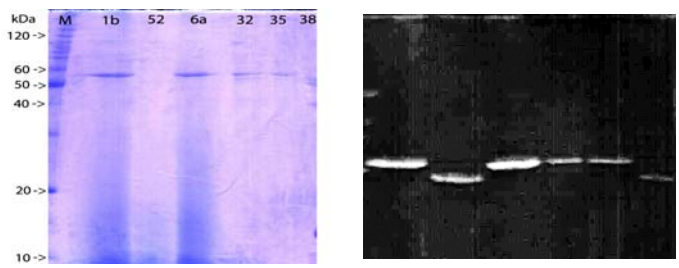

Fig. 4a, 4b SDS-PAGE (4a) for the intracellular phytase from strains 1b, 52, 6a, 32, 35, 38. M-molecular markers and (4b) zymograme developed for phytase activity. Lanes: 1-1b,2-52,3-6a,4-32,5-35,6-38.

Conclusion

From the obtained results we may conclude that the studied 26 strains isolated from sourdoughs had good capacity to hydrolize gluten. Six of them had phytase activity. Microbial phytases are considered of a great value in upgrading the nutritional quality of plant foods.

Acknowledgments

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