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Evaluation of fermentation properties of lactic acid bacteria isolated from sourdough

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Abstract

The aim of this work was evaluation of fermentation characteristics of lactic acid bacteria isolated from Slovak bakery sourdough (twenty pure cultures PB1 – PB20). Gram stain, catalase test, carbon dioxide production from glucose, growth at 15 °C and 45 °C and test for fermentable sugar Api 50CH were used for identification of isolates. Three isolates were chosen for evaluation their fermentative characteristic during fermentation process. Amount of colony form units, pH value, titratable acidity and organic acid were established. Results of above mentioned research is design of new bakery starter culture consisted from the combination of all three identified strains.

Key words: bread, fermentation, lactic acid bacteria, sourdough

Introduction

The usage of lactic acid bacteria (LAB) in cereal industry has a long history. LAB is a heterogeneous group of Gram-positive rods and cocci, non-spore-forming. They are aerotolerant, microaerophilic or facultative anaerobic microorganisms. They are mesophilic with optimal temperature of growth between 30 °C and 40 °C, but some strains are able to growth at the temperatures lower than 5 °C or higher than 45 °C (Caplice and Fitzgerald, 1999, Görner and Valík, 2004). For their growth they demand lots of nutritional factors, such as vitamins and minerals (Görner and Valík, 2004, Reddy et al, 2008). To the LAB group belong several genera, namely: *Lactobacillus* sp., *Lactococcus* sp., *Leuconostoc* sp., *Oenococcus* sp., *Pediococcus* sp., *Streptococcus* sp., *Tetragenococcus* sp., *Aerococcus* sp.,

Carnobacterium sp., *Enterococcus* sp., *Vagococcus* sp. and *Weissella* sp. (De Angelis et al, 2007, Reddy et al, 2008).

LAB can be divided to three groups according to utilization of sugars (homofermentative, heterofermentative and facultatively heterofermentative). For homofermentative genera (*Pediococcus* sp., *Streptococcus* sp., *Lactococcus* sp. and some lactobacilli), hexoses are metabolized by enzymes of the glycolytic Embden-Mayerhoff pathway. More than 90 % of substrate is converted to lactic acid during anaerobic metabolism. Heterofermentative genera (*Weisella* sp., *Leuconostoc* sp. and some *Lactobacilli*) metabolize hexoses via Warburg-Dickens (i.e., pentose phosphate) pathway. During this process only 50 % of substrate is converted to lactic acid, the rest is metabolized to acetic acid, formic acid and ethanol. Facultatively, heterofermentative lactobacilli can metabolize hexoses via both pathways, Warburg-Dickens pathway predominates in the deficiency of fermentable sugars (Hutkins, 2006).

Production of fermented food is based on usage of starter cultures. The most important fermented cereal product is bread. The quality of bread depends on several factors. The content of sugars, gluten, minerals, fat and activity of endogenous enzymes are internal factors. Among external factors belong temperature, degree of fermentation, water activity, redox potential and additives (Paramithiotis et al, 2005, Plessas et al, 2008, Stiles and Holzapfel, 1997, Buckenhüskes, 1993).

In bakery technology we can distinguish two types of bread preparing: straight dough production (each component are mixed together at the same time) and sourdough bread preparation. Sourdough is bakery product which typical features are caused by present microflora, lactic acid bacteria and yeasts. Sourdough fermentation is used if the content of gluten in flour is low. The usage of sourdough process has a positive influence on technological, nutritional, sensory properties and extending shelf-life (Dal Bello et al, 2007, Arendt et al, 2007, Kim et al, 2009, Katina et al, 2006). Sourdough application has been extensively increased in the last years due to the consumers demand for food consumption without the addition of chemical preservatives. Several starter cultures have been applied in sourdough bread making targeting the increase of bread self-life and the improvement of sensorial character. Proteolysis activity of LAB improves availability of proteins and amino acids. Fermentation process contributes to the degradation of phytic acid which bind divalent cations (zinc, iron, and calcium) and decreasing their bioavailability. Degradation of phytic

acid is caused by phytases, which are present in raw material (wheat, rye) and there are produced by lactic acid bacteria also (Corsetti and Settanni, 2007, Kotzekidou and Tsakalidou, 2006, Plessas et al, 2011). LAB improves taste and flavour of fermented products via proteolysis and lipolysis activity which cause production of aromatic compounds (acetate, ethanol, diacetyl and acetaldehyde, 3-methyl-1-butanol, 2-phenylethanol). Hydrocolloids improve the volume, texture, and shelf life of bread. Exopolysaccharides produced by LAB during sourdough fermentation can replace hydrocolloids (Leroy and De Vuyst, 2004, Ravyts and De Vuyst, 2011, Galle et al, 2011, Petruláková et al, 2009).

LAB form many antibacterial substances (organic acids, carbon dioxide, ethanol, hydrogen peroxide, diacetyl, antifungal compounds, bacteriocins, antibiotics, fatty acid, phenyllactic acid) which have positive influence on shelf-life of fermented product (Caplice and Fitzgerald, 1999, Navrhus and Sørhaug, 2006, Valerio et al, 2008).

Most of LAB found in sourdoughs belongs to lactobacilli, but *Pediococcus*, *Leuconostoc* and *Enterococcus* was found also. Nearly fifty strains of lactobacilli were isolated from sourdough; however, *Lactobacillus sanfranciscencis* has been found most often (Gül et al, 2005, Paramithiotis et al, 2006, Robert et al, 2005). In the spontaneous rye sourdough ecosystem *Lb. plantarum* and *Lb. fermentum* are dominantly (Weckx et al, 2010).

The object of our work was design of starter culture for bakery industry from their origin and which will able to improve properties of end products (technological properties, sensory value and extension of shelf-life).

Material and methods

Isolation of LAB

LAB were isolated from sourdough obtained from bakery in Považská Bystrica (Slovakia). Ten grams of sourdough were dissolved in ninety millilitres of saline. Relevant decimal dilutions were inoculated on MRS-agar (Merck, Germany) and incubated 24 – 72 hour at 30°C under aerobic and anaerobic conditions. Isolated pure cultures were maintained in MRS broth at 5 °C and in cryo tube under -30 °C also.

Identification of isolates

Isolates was identified according to Gram stain, catalase test, carbon dioxide production from glucose and growth at 15 °C and 45 °C. Isolated strains were also identified with the help of fermentable test Api 50CH. This test is made of strips which include 49 different sugars and

their derivates in micro-chamber. Evaluation of fermentation of sugars is visual (changing of indicator colour in Api 50CHL medium inoculated with isolate).

Fermentation test

During the fermentation tests rye flour T-930 was used. The flour was mixed with sterile water and inoculated with overnight culture of isolated LAB or their combinations. Fermentation was lead at 30°C during 48 hours. Samples for evaluation of colony form units, pH value, titratable acidity and amount of organic acid were collected every 24 hours.

Determination of pH

The pH of sourdough samples was measured by pH-meter CG 843 (Schott, Germany).

Determination of titratable acidity

This parameter was established by visual titration of samples with 0.01 M NaOH with phenolphthalein as a colour indicator. The result was calculated as a content of lactic acid.

Determination of organic acids

The quality and quantity of the produced organic acids (lactic and acetic) were measured by isotachophoretic analysis. One gram of sourdough sample was dissolved in distilled water, centrifuged (15 min, 5000 x g) and filtered. The samples were measured by Isotachophoretic Analyser ZKI 01 (Villanova, Slovakia). The identification of organic acids was evaluated by computer software ITTPro32 according to the RSH value (Relative Step Height) which corresponds to a substance under the same conditions. The amount of substance present in the analyzed sample is proportional to the length of step isotachophoretic record. This dependence has been established by method analytical lines.

Evaluation of antimicrobial activity

Inhibition of tested microorganism was evaluated by well diffusion assay test (Schillinger, Lücke, 1989). Followed indicator strains were used: *Bacillus subtilis* CCM 1718, *Pseudomonas aeruginosa* CCM 1960, *Escherichia coli* CCM 3954 and *Micrococcus luteus* CCM 1048 from Czech Collection of Microorganisms (Brno, CZ), *Staphylococcus aureus* b2 (isolated from Slovak cheese “bryndza”), *Candida albicans* Pn10 and *Candida maltosa* YP1 (both isolated from yogurth) from Department of Nutrition and Food Assesment (FCHPT, STU, Bratislava, SR).

Results and discussion

This work was focused on evaluation of fermentation properties of LAB isolated from sourdough from bakery and their usage as starter cultures for directed fermentation process, which has many advantages for industrial production of bread.

Isolation and identification of lactic acid bacteria

From sourdough we obtained 20 isolates. On the base of Gram stain and morphology observation we found out that nineteen isolates were LAB and one isolate was yeasts. We also did other identification procedures, such as catalase test, carbon dioxide production from glucose, growth at 15 °C and 45 °C. According to the results (Tab. 1) we chose three isolates: PB3, PB7 and PB12.

Table 1. The results of basic identification tests of sourdough isolates.

Strain	G^+/G^-	Morphology	Catalase test	CO_2 production from glucose	Growth	
					15 °C	45 °C
PB 1	+	rod	-	-	-	+
PB 2	+	rod	-	-	-	+
PB 3	+	rod	-	-	+	-
PB 4	+	rod	-	-	+	-
PB 5	+	rod	-	-	-	+
PB 6	Yeast					
PB 7	+	rod	-	-	-	+
PB 8	+	rod	-	-	-	+
PB 9	+	rod	-	-	+	-
PB 10	+	rod	-	-	+	-
PB 11	+	rod	-	+	+	-
PB 12	+	rod	-	+	+	-
PB 13	+	rod	-	+	-	-
PB 14	+	rod	-	-	-	+
PB 15	+	rod	-	-	+	-
PB 16	+	rod	-	-	+	-
PB 17	+	rod	-	-	+	-
PB 18	+	rod	-	-	+	-
PB 19	+	rod	-	-	+	-
PB 20	+	rod	-	+	+	-

Fermentation tests

For evaluation of fermentation characteristics of isolated and identified strains of LAB were realized fermentation tests. We chose the isolates for preparation of starter culture according to pH value, titratable acidity (TTA) and content of organic acids in their fermentation

medium. Titratable acidity and pH value belong to the important indicator of efficiency of fermentation process. These characteristics are result of metabolic activity of strain. For bakery industry and sourdough preparation, it is important to find strains of LAB which are able to reduce pH value on level around 3.3. These bacterial strains can create stable environment in sourdough. From all organic acids, which can be produced during fermentation process, only lactic and acetic acid were measured. The main product of metabolic activity of homofermentative LAB is lactic acid. Heterofermentative LAB can produce acetic acid, ethanol and formic acid and obligate heterofermentative LAB acetic acid and carbon dioxide also. For good sensory quality of bread the strains producing acetic acid are not suitable. The results of fermentation tests are in Figures 1 and 2.

The aim of our research was to find out which isolates (or their combinations) have the best fermentation characteristics. It means good growth features, decreasing of pH value and production of lactic acid. Requirement of bakery industry was to select those which can decrease pH value under 3.3 and production amount of lactic acid is 2g/100g of sourdough. Presence of acetic acid is unfavourable.

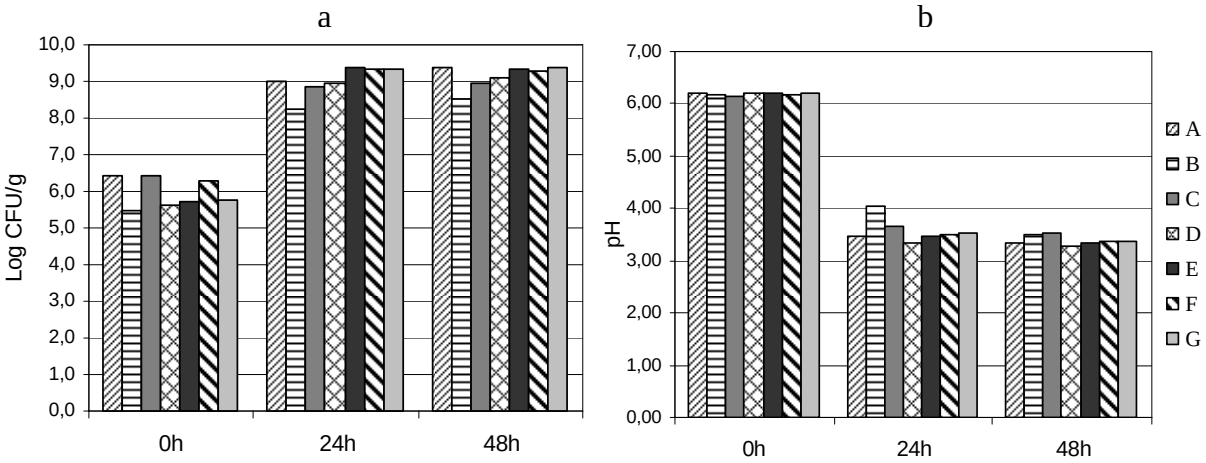

Fig. 1. Evaluation of cell population (a) and changes in the pH (b) in the sourdough during fermentation with selected isolates of lactic acid bacteria and their combinations. **A** – isolate PB3, **B** – isolate PB7, **C** – isolate PB12, **D** – combination of isolates PB3 and PB7 (1:1), **E** – combinations of isolates PB3, PB7 and PB12 (2:1:1), **F** - combinations of isolates PB3, PB7 and PB12 (1:1:1), **G** – combination of isolates PB3 and PB7 (2:1).

As shown Fig. 1a all bacterial strains and their combinations tested showed good growth in sourdough at 30 °C. At the beginning we inoculated sourdough from 5.48 – 6.41

log cfu/g. Population of LAB strains reached amount from 8.52 to 9.40 log cfu/g after 48 hours fermentation process.

The pH value (Fig. 1b) dropped from 6.14-6.21 to 3.27-3.53 during fermentation period. Only in sourdoughs inoculated with isolate PB3, combination of isolates PB3 and PB7 and combination of isolates PB3, PB7 and PB12 (in ratio 2:1:1) were the pH value in level 3.3.

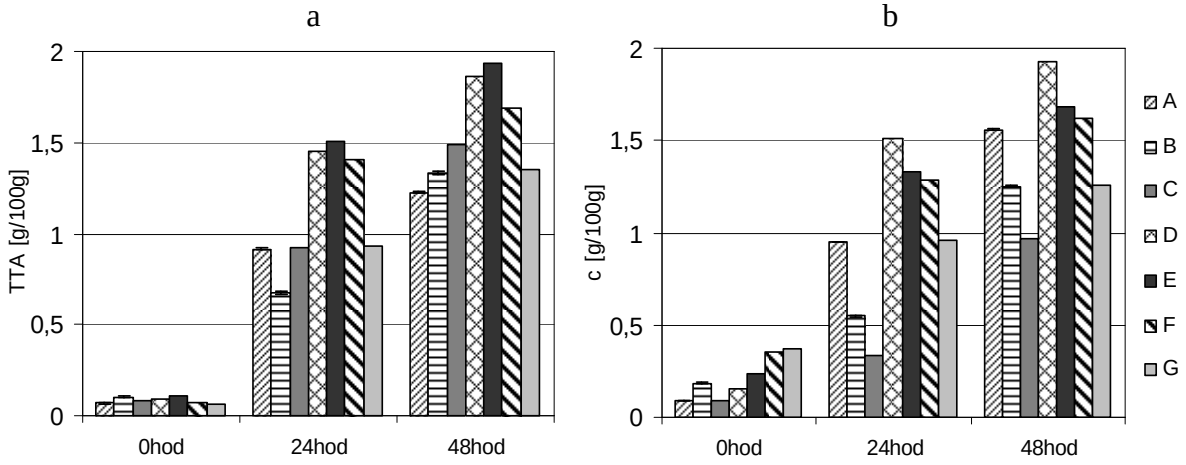

Fig. 2. Changes in the level of titratable acidity (TTA) (a) and lactic acid (b) in sourdough during fermentation with selected isolates of lactic acid bacteria and their combinations.
A – isolate PB3, **B** – isolate PB7, **C** – isolate PB12, **D** – combination of isolates PB3 and PB7 (1:1), **E** – combinations of isolates PB3, PB7 and PB12 (2:1:1), **F** - combinations of isolates PB3, PB7 and PB12 (1:1:1), **G** – combination of isolates PB3 and PB7 (2:1).

Value of TTA (Fig. 2a) in sourdoughs after fermentation was from 1.23 to 1.93 g/100g and amount of lactic acid (Fig. 2b) produced by LAB ranged from 0.97 to 1.93 g/100g. The best results we achieved in sourdough inoculated with combination of isolated strains (PB3 and PB7; and combination of PB3, PB7 and PB12 in ratio 2:1:1), in which the requirement on pH value was fulfilled.

According to these results we recommend for sourdough bread preparation the usage in practice combinations of isolates PB3 and PB7 and combination of isolates PB3, PB7 and PB12 (in ratio 2:1:1).

Antimicrobial activity

LAB plays a significant role in microbial safety of fermented food. This is reason why they are used as natural preservatives. Antimicrobial activity is an important feature for selection

LAB for design starter cultures for industry. Bread can be attacked by filamentous fungi (from cereals), yeasts and bacteria. The filamentous fungi (*Penicillium*, *Aspergillus*, *Fusarium*) and species of the genus *Bacillus* cause the main loses in bread industry. *Bacillus subtilis* and *Bacillus licheniformis* are the main bacteria caused ropy spoilage of bread. The deterioration of bread texture is due to slime being formed as a result of the combined effect of the proteolytic and amylolytic enzymes produced by *B. subtilis* or *B. licheniformis*. This effect economical losses and can cause illness if bacteria are present at concentrations more than 10^8 cfu/g (Valerio et al., 2008). The results of the antimicrobial activity are summarized in Tab. 2.

According to results (Tab. 2), no tested strain had antimicrobial activity towards all indicator strains. Weakest antimicrobial activity was observed in isolate PB12, which suppress only growth of *Micrococcus luteus* CCM 1048. Isolate PB3 had an antimicrobial activity against *M. luteus* CCM 1048 and *Pseudomonas aeruginosa* CCM 1960. Largest spectrum of antimicrobial activity had isolate PB7. It inhibited growth *Bacillus subtilis* CCM 1960, *Escherichia coli* CCM 3954, *M. luteus* CCM 1048 and *P. aeruginosa* CCM 1960.

Table 2. Antimicrobial activity of lactic acid bacteria isolated from sourdough.

Indicator strain	PB3	PB7	PB12
***Bacillus subtilis* CCM 1960**	-	+	-
***Micrococcus luteus* CCM 1048**	++	++	++
***Staphylococcus aureus* b2**	-	-	-
***Escherichia coli* CCM 3954**	-	+	-
***Pseudomonas aeruginosa* CCM 1960**	+	+	-
***Candida maltosa* YP1**	-	-	-
***Candida albicans* Pn10**	-	-	-

Conclusion

The aim of present work was isolation of lactic acid bacteria from sourdough achieved from industrial bakery in Považská Bystrica and their identification. From sourdough were obtained 20 isolates. On the base of identification tests three isolates (PB3, PB7 and PB12) were chosen. These strains as well as their combinations were tested during fermentation process. According to the results the best combinations were chosen: PB3 and PB7 in ratio 1:1) and PB3, PB7 and PB12 (in ratio 2:1:1). The second mentioned combination is used in ADIVIT

Nitra co. for preparation of commercial bakery starter cultures. Antimicrobial activity of lactobacilli species was monitored, too. The best antimicrobial activity was proved by isolate PB7. In the near future, form of starter culture (a suitable carrier) and the conditions for its preservation and use in the bakery industry will be proposed.

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