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LACTIC ACID BACTERIA FROM SPONTANEOSLY FERMENTED RYE SOURDOUGH

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Abstract

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Sourdough represents a complex biological system in which lactic acid bacteria and yeasts exist in symbiotic relationships. Changes that occur in the environment parameters affecting microbial associations lead to the development of specific and characteristic associations of species of each particular case. The aim of this study is to characterize the composition and the growth dynamics of the microflora of rye sourdough samples from the region of Stara Zagora, prepared by mixing of water and flour in the ratio 1:1. The fermentation was carried out at 30°C for 72 hours. During the fermentation the population of the lactobacilli displayed highest growth rate ($0.12.h^{-1}$) at 48 h compared with the growth of streptococci and yeast populations. After 72 hours a reduction of the number of all microbial groups was determined. In the composition of rye sourdough microbiota from the region of Stara Zagora the species of *Lactobacillus spicheri*, *Lactobacillus paralimentarius*, *Lactobacillus kimchii*, *Lactobacillus sanfranciscensis* were detected by phenotypic and molecular methods. This is the first report of these bacteria from the rye sourdough in Bulgaria.

Key words: Lactobacillus, spontaneously fermented rye sourdough, 16S-23S rDNA ITS analysis, genus specific PCR

Introduction

Sourdough is an important fermentation of cereals employed in the manufacture of a variety of products such as breads, cakes and crackers. Spontaneously fermented sourdough is prepared by mixing flour and water in a specified ratio. After incubation at a specific temperature the fermentation caused by the development of a variety of microbial species in the mixture takes place. The metabolic activity of the microbial populations developed spontaneously ensures biological stability of the sourdough by acidification, and forms of specific aroma and a specific texture (De Vuyst et al., 2009; Hammes et al., 2005).

The composition of the microbial community is determined by many factors, among which the most important are the characteristics of the technology, the type and physico-chemical composition of raw material (rye and wheat flour), process parameters (temperature, pH, ratio of mixing flour and water), and the duration of the fermentation process (De Vuyst and Neysens, 2005; Hammes et al., 2005). Sourdoughs, on the basis of the technology applied, have been grouped into three types. Type I sourdoughs are produced with traditional techniques and are characterized by continuous, daily refreshments to keep the microorganisms in an active state at a low temperature (20°C÷30°C). Type II sourdoughs, are semi-fluid silo preparations characterized by long fermentation periods (from 2 up to 5 days) and fermentation temperature sometimes >30°C to speed up the process. Type III sourdoughs are dried preparations containing lactic acid bacteria (LAB) resistant to the drying process (Hammes and Ganzle, 1998; De Vuyst and Neysens, 2005). Unlike type I sourdoughs, doughs of types II and III require the addition of baker's yeast (*Saccharomyces cerevisiae*) as leavening agent.

Studies on the composition of the microflora of the spontaneosly fermented sourdough show that LAB are the dominant group of bacteria (Corsetti and Settanni, 2007; De Vuyst and Neysens, 2005; De Vuyst and Vancanneyt,

2007; De Vuyst et al., 2009). In contrast to the use of mostly homofermentative LAB in the majority of fermented food applications, heterofermentative species play a major role in sourdough fermentation, especially when sourdoughs are prepared in a traditional manner (Corsetti et al., 2003; Corsetti et al., 2001). So far, a few less than 50 different species of LAB isolated from sourdough have been reported (Hammes et al., 2005).

The microbial diversity in the spontaneously fermented sourdoughs and the identification of the dominant species were investigated by phenotypic and molecular genetic methods based on sequence analysis of 16S rDNA genes (Vogelmann et al., 2009), RFLP analysis of the 16S-23S ITS rDNA (Ferchichi et al., 2008; Valcheva et al., 2007) and RAPD-PCR (Moroni et al., 2011; Corsetti et al., 2007; Catzeddu et al., 2006). These results indicate that the sourdough microflora varies considerably depending on the type of feedstock, the physico-chemical composition and physical environment factors (De Vuyst et al., 2014).

The aim of this work was to study the composition and the growth dynamic of lactic acid microflora from different batches of rye sourdough during spontaneous fermentation.

The aim of this study was to characterize the composition and the growth of lactic acid microflora of rye sourdough at various stages of the spontaneous fermentation.

Materials and Methods

Isolation of sourdough LAB. LAB were isolated from two rye sourdoughs from Stara Zagora bread factory with similar characteristics: dough yield – 100 g; protein content – 14%. Ten grams of each sample were homogenized in 90 ml distilled water and serial dilutions were prepared.

Enumeration of LAB. 0.1 ml of the dilutions was pourplated on MRS-agar, M17-agar and Sabouraud medium. After incubation for 48–72 h at 30°C in anaerobic conditions the total number of lactic acid bacteria and yeasts g^{-1} was estimated.

Acidification of sourdough. The pH changes during the fermentation process were measured by pH meter.

Phenotypic identification of LAB. Distinct colonies which possessed different morphology and that were catalase- and oxydase-negative were picked and after purification procedures were selected. Phenotypic characterization of the isolates was done by carbohydrate fermentation tests, gas production in MRS broth, and growth at 15° and 45°C. Representative isolates were selected for further studies.

Molecular identification of LAB isolates. Genomic DNA was isolated as described by Ferchichi et al. (2008). Amplification of 16S-23S ITS rDNA was carried out with primer pair 16S/4-23/7. The primer pair LBMA-1/R16 was used for genus confirmation of *Lactobacillus* isolates. PCR were performed according to the protocol described by Fechichi et al. (2008). The PCR product was detected by 1,5% agarose gel electrophoresis. Selected lactobacilli were sequenced in Macrogen (Holland). Sequences were analysed by BLAST and compared with available sequences in GenBank.

Results

Acidification during sourdough propagation

The pH changes during sourdough fermentation were followed in dynamics at the 24, 48 and 72nd hours (Figure 1). During the sourdough propagation the average values of ΔpH ranged from 1.4 to 1.8.

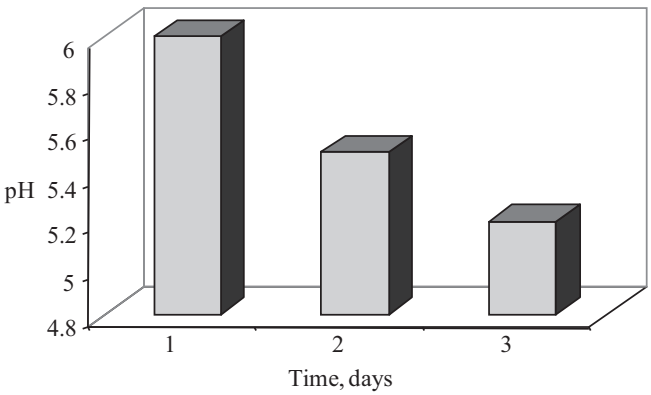

Fig. 1. Change of pH-value of rye sourdough during spontaneously fermentation: *1* – 24 h; *2* – 48 h; *3* – 78 h

Enumeration of LAB

The microflora of the spontaneously fermented rye sourdough was consisted of LAB and yeasts. The enumeration of the two physiological groups showed that LAB was the dominant microflora. The number of LAB determined on M17 medium (lactic acid cocci) increased during the fermentation from $1.8.10^7$ cfu/g at 24 h to $3.9.10^8$ cfu/g at 48 h, and from $1.16.10^8$ cfu/g (24 h) to $2.62.10^{10}$cfu/g (48 h) on MRS medium After 72 h the reduction of number of all groups microorganisms was registered (Table 1). The number of yeasts was not substantially changed during the fermentation process. The growth rate of dominant LAB population and yeasts was highest at 48 h – $0.12.h^{-1}$, $0.11.h^{-1}$ and $0.09.h^{-1}$, respectively (Table 2).

Table 1

Number of lactic acid bacteria and yeasts during the spontaneous fermentation of rye sourdough

Time	Lactic acid bacteria MRS-agar, cfu/g	Lactic acid bacteria M17, cfu/g	Yeasts Sabouraud, cfu/g
0 h	1.16×10^{8}	1.8×10^{7}	1.11×10^{7}
24 h	1.5×10^{8}	2.6×10^{7}	1.46×10^{7}
48 h	2.62×10^{10}	3.9×10^{8}	3.2×10^{7}
72 h	1.05×10^{10}	2.9×10^{8}	3.0×10^{7}

Table 2

Growth parametres of populations of lactic acid bacteria and yeasts during spontaneous fermentation of rye sourdough

Strain	Rate constant, ν, h^{-1}	Generation time, G	Growth rate, μ, h^{-1}
Lactic acid bacteria (MRS-agar)	0.049	20.16	0.12
Lactic acid bacteria (M17)	0.096	10.36	0.11
Yeasts (Sabouroaud)	0.062	16.07	0.09

Characterization of LAB

All colonies which were catalase- and oxidase negative and consisted of Gram-positive, non-spore forming cells were selected. All of the selected cultures that have the ability to grow in anaerobic condition were assumed belonging to LAB. The isolates were separated into eight groups on the base of the colony morphology. The representative isolates from each morphological group were characterized phenotypically. The characteristics of the isolates showed that strains were separated into five groups on the base of the ability to assimilate various substrates (Table 3). The strains from group 4 and 8 were very similar each other as well as those from group 5, 6 and 7. The rest of the group differed significantly among themselves, and from the other groups.

Molecular identification of LAB

The isolates were subjected to identification by molecular method according to the diagnostic algorithm described by Ferchichi et al. (2008). The DNA from the all isolates was amplified with the universal primer pair 16S/4 and 23S/7 for 16S-23S ITS rDNA and the obtained profiles were presented in Figure 2. All strains, except two isolates from the group 2 (strain 2 and strain 12), showed the profile specific for the

Table 3

Biochemical characterization of representative strains from rye sourdough

Source	Strain from group 1	Strain from group 2	Strain from group 3	Strain from group 4	Strain from group 5	Strain from group 6	Strain from group 7	Strain from group 8
Arginine	+	–	+	+	+	–	+	+
Lysine	+	–	+	+	+	+	+	+
Ornithine	+	–	+	+	+	+	+	+
Arabinose	–	–	+	–	+	+	+/–	–
Galactose	+	+	+	–	+	+/–	+	+/–
Glucose	+	+	+	+	+	+	+	+
Maltose	+	–	+	+	+	+	+	+
Saccharose	+	+	+	+	+	+	+	+
Lactose	–	–	+	–	+/–	–	+/–	–
Xylose	+	–	+	–	+	+	+	–
Rhamnose	+/–	–	+/–	–	+/–	+/–	+/–	–
Raffinose	–	–	+	–	+/–	+/–	+/–	–
Trehalose	–	–	–	+	+/–	+/–	+/–	+
Manitol	–	–	+	–	+/–	+/–	+/–	–
Glycogen	–	–	–	–	–	–	–	–
Dulcitol	+/–	–	+	+	+/–	+/–	+/–	–
Esculin	–	–	+	+	+	+	+	+
Inositol	+/–	–	+	+	+/–	+/–	+/–	–
Inulin	–	–	–	–	–	–	–	–

"+" – positive; " +/–" – weak positive; "–" – negative

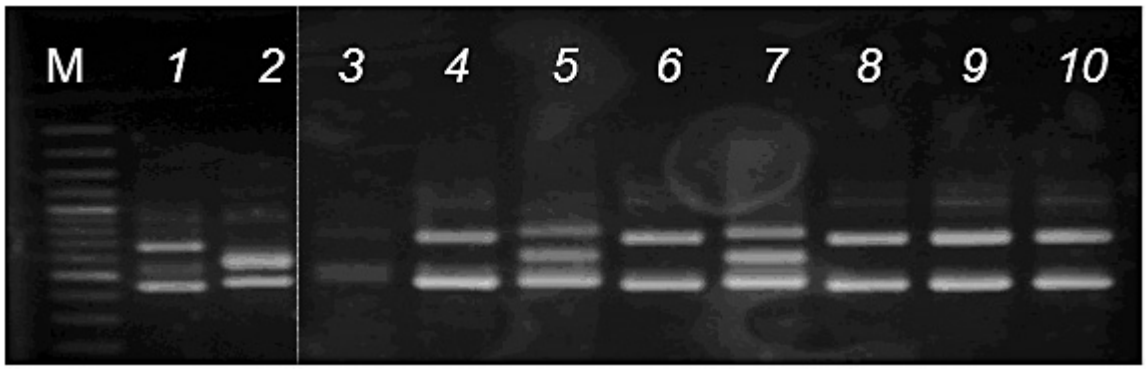

Fig. 2. PCR-amplification of 16S-23S ITRs (16S-4/23S-7 primers) of strains isolated from rey souyrdough. Lane *1* – *L. plantarum* NBIMCC 2972, Lane *2* – *Enterococcus faecalis*, Lane *3* – strain 11, Lane *4* – strain 1, Lane *5* – strain 2, Lane 6 – strain 3, Lane *7* – strain 12, Lane *8* – strain 4 – strain 5, Lane *10* – strain 6, *M* – molecular weight marker (100-bp DNA ladder)

genus *Lactobacillus* (two fragments of 450 and 650 bp). The strains 2 and 12 formed the profile with three fragments (450, 500, 650 bp). This profile was specific for the species *Lactobacillus sanfranciscensis* (Valcheva et al., 2007). The belonging of the all isolates to the genus *Lactobacillus* was confirmed by PCR-amplification using a genus-specific primer pairs LBMA-1/R16.

The DNAs from the some strains were amplified with universal primers for 16S rDNA and sequenced. Obtained sequences were compared with the database in the GenBank and were referred to the following species: strain 1 was 99% similar to *Lactobacillus spicheri*, strains 4 and 8 were 100% identical with *Lactobacillus paralimentarius* and strain 6 was 98% similar to *Lactobacillus kimchii*.

Discussion

From an ecological point of view sourdough was a specific ecosystem with low pH, high content of carbohydrates and protein, limited concentration of dissolved oxygen, which provides development of lactic acid bacteria and yeasts (De Vuyst and Neysens, 2005). The process of spontaneous fermentation of Bulgarian rye sourdough was characterized by slight decrease in pH value (pH 6.0 to pH 5.2) due to its high buffering capacity. The data obtained confirmed the results of De Vuyst et al. (2002), but differed from those of Vogelmann et al. (2009), Moroni et al. (2011).

The microflora of rye sourdough was dominated by lactic acid bacteria. Lactic acid cocci and lactobacilli dominate in the fermented sourdough in the region of Abruzzo (Italy) (Corsetti et al., 2007).We identified four *Lactobacillus* species - *Lactobacillus spicheri, Lactobacillus paralimentarius, Lactobacillus kimchii* and *Lactobacillus sanfranciscensis.* Our results confirmed the data obtained by other authors on the microflora of sourdough (Vogelmann et al., 2009; Catzeddu et al., 2006) who considered a part of these species as endogenous to sourdough. We have not found the spec ies *Lactobacillus plantarum* and *Lactobacillus fermentum* which were dominant in rye sourdough according to Weckx et al. (2010) and Ferchichi et al. (2008). Comparative analysis of the obtained results and the data from other authors suggests that the species composition of lactic acid microflora of fermented sourdough depends on the raw material used, and from the technology applied for the preparation of the sourdough.

Conclusions

Microbial population in spontaneously fermented rye sourdough from the region of Stara Zagora mainly involves species *Lactobacillus spicheri, Lactobacillus paralimentarius, Lactobacillus kimchii* and *Lactobacillus sanfranciscensis.*

References

Corsetti, A., L. Settanni, S. Valmorri, M. Mastrangelo and G. Suzzi, 2007. Identification of subdominant sourdough lactic acid bacteria and their evolution during laboratory-scale fermentations. *Food Microbiol.*, **24**: 592–600.

Corsetti, A. and L. Settanni, 2007. Lactobacilli in sourdough fermentation. *Food Res. Int.*, **40**: 539–558.

Corsetti, A., M. De Angelis, F. Dellaglio, A. Paparella, P. F. Fox, L. Settanni, and M. Gobbetti, 2003. Characterization of sourdough lactic acid bacteria based on genotypic and cell-wall pro-

tein analyses. *J. Appl. Microbiol.,* **94**: 641–654.

Corsetti, A., P. Lavermicocca, M. Morea, F. Baruzzi, N.Tosti and M. Gobbetti, 2001. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *Int. J. Food Microbiol.,* **64**: 95–104.

Catzeddu, P., E. Mura, E. Parente, M. Sanna and G. Antonio – Farris, 2006. Molecular characterization of lactic acid bacteria from sourdough breads produced in Sardinia (Italy) and multivariate statistical analyses of results. *Syst. Appl. Microbiol.*, **29**: 138–144.

De Vuyst, L., G. Vrancken, F. Ravyts, T. Rimaux and S. Weckx, 2009. Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiol.*, **26**: 666–675.

De Vuyst, L., S. Van Kerrebroeck, H. Harth, G. Huys, H.-M. Daniel and S. Weckx, 2014. Microbial ecology of sourdough fermentations: diverse or uniform? *Food Microbiol.*, **37**: 11–29.

De Vuyst, L., V. Schrijvers, S. Paramithiotis, B. Hoste, B., M. Vancanneyt, J. Swings, G. Kalantzopoulos, E. Tsakalidou and W. Messens, 2002. The biodiversity of lactic acid bacteria in Greek traditional wheat sourdoughs is reflected in both composition and metabolite formation. *Appl. Environ. Microbiol*., **68**: 6059–6069.

De Vuyst, L. and M. Vancanneyt, 2007. Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiol.,* **24**: 120–127.

De Vuyst, L. and P. Neysens, 2005. The sourdough microflora: biodiversity and metabolic interactions. *Trends Food Sci. Tech.*, **16**: 43–56.

Ferchichi, M., R. Valcheva, N. Oheix, P. Kabadjova, H. Prevost, B. Onno and X. Doussert, 2008. Rapid investigation of French sourdough microbiota by restriction fragment lengh polymorphism of the 16S-23S rRNA gene intergenic spacer region. *World J. Microb. Biot.*, **24**: 2425–2434.

Hammes, W., M. Brandt, K. Francis, J. Rosenheim, M. Seitter and S. Vogelmann, 2005. Microbial ecology of cereal fermentations. *Trends Food Sci. Tech.*, 16: 4–11.

Hammes, W. P. and M. G. Gänzle, 1998. Sourdough breads and related products. In: B. J. B. Woods, Microbiology of Fermented Foods (Vol. 1) (pp. 199–216). London: Blackie Academic/ Professional.

Moroni, A., E. Arendt and F. Dal Bello, 2011. Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. *Food Microbiol.*, **28**: 497–502.

Valcheva, R., P. Kabadjova, C. Racman, I. Ivanova, B. Onno, H. Prevost and X. Dousset, 2007. A rapid PCR procedure for the specific identification of *Lactobacillus sanfranciscensis*, based on the 16S–23S intergenic spacer regions. *J. Appl. Microbiol*., **102**: 290–302.

Vogelmann, S., M. Seitter, U. Singer, M. Brandt and C. Hertel, 2009. Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters. *Int. J. Food Microbiol.*, **130**: 205–212.

Weckx, R.,V. Meulen, D. Maes, I. Scheirlinck, G. Huys, P. Vandamme and Luc De Vuyst, 2010. Lactic acid bacteria community dynamics and metabolite production of rye sourdough fermentations share characteristics of wheat and spelt sourdough fermentations. *Food Microbiol.*, **27**: 1000–1008.