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Diversity of lactic acid bacteria on organic flours and application of isolates in sourdough fermentation

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Abstract

Organic farming preserves biodiversity and organic products can be the source of many microbial species. The species diversity in organically grown wheat, spelt and rye was investigated in order to find strains suitable for sourdough fermentation. Colonies representing various morphological appearances were isolated and catalase-negative colonies were identified by mass spectrometer Microflex LT ™ MALDI-TOF. The fermentation products (lactic, acetic, formic and phenyllactic acid) were determined by high performance liquid chromatography, while the antifungal activity was determined using an overlay agar method. Wheat flours showed less microbial biodiversity than the rye and spelt flours. The most common genera in the tested flour were Lactobacillus, Pediococcus and Enterococcus. Isolated Lactobacillus farciminis, Pediococcus pentosaceus, Leuconostoc citreum and Lactobacillus brevis showed the best acidification activity. Lactobacillus brevis, Pediococcus pentosaceus, Weissella cibaria and Lactobacillus farciminis showed significant antifungal activity against A. niger 357 and Penicillium sp. 505. The prefermented medium of Lactobacillus farciminis were characterized by high content of lactic and phenyllactic acid.

Keywords: *organic cereals, species diversity, Lactobacillus, sourdough, spelt*

Introduction

Microbial spoilage is the major factor that limits the shelf life of bakery products and causes huge economic losses for both manufacturers and consumers. The most common spoilage fungi in bakery products belong to the genera *Penicillium, Aspergillus, Fusarium, Rhizopus* and *Mucor* (Lavermicocca et al. 2003). Salts of propionic, sorbic and benzoic acids are routinely used to control mold growth in bakery products, but the levels of chemical preservatives permitted in bakery products in Europe have been reduced due to application of EU Directive 95/2/CE (Lavermicocca et al. 2003). In addition, consumers generally perceive natural food preservatives as better than synthetic additives. Within the past decade bakery products sale has been increased in volume and variety due to increased demand for ready to eat food products at reasonable costs. Due to the increase of the production of bread and trader's request for full racks of baked goods throughout the all day, large quantities of unsold bread must be returned to the retailer. If unsold bread may not be returned immediately or if it is kept in bakery for some time before use for breadcrumbs or feed, mold growth and mycotoxins production occur. This bread is as waste disposed in landfill sites where it decomposes to methane and carbon dioxide. Some authors propose bread waste utilization as substrate in solid state fermentation for chemicals or biofuels production (Leung et al. 2012). This could be the ultimate solution, but development of new bakery processes which would ensure better natural protection and extended bread shelf life is required.

Lactic acid bacteria (LAB) have been traditionally used as starter cultures in the food industry in production of fermented dairy, vegetables or meat products as well as in bread production. LAB are the most promising microorganisms in biopreservation, producing different kinds of bioactive molecules, such as organic acids (lactic, acetic, formic, propionic, butyric, phenyllactic acid), antagonistic compounds (carbon dioxide, ethanol, hydrogen peroxide, fatty acids, acetoin, diacetyl), cyclic dipeptides, bacteriocins or bacteriocin-like inhibitory substances that preserve food from spoilage microorganisms (Dalie et al. 2010; Falguni et al. 2010; Lavermicocca et al. 2000; Lavermicocca et al. 2003; Magnusson et al. 2003, Rouse et al. 2008; Valerio et al. 2009, Šušković et al. 2010). Additionally, LAB produce several compounds that contribute to taste, odour, color, and texture of the food. In bread production, the use of LAB has a long tradition, from spontaneous fermentations to the development and use of defined and functional starter cultures. LAB and yeasts form a complex microbial ecosystem – sourdough that affects the microbiological, technological, organoleptic, nutritional and health-promoting characteristics of bread (Gobbetti et al. 2014).

Although a number of studies have identified and characterized LAB of mature sourdoughs, very limited data exists about microbial ecology of flour, especially organic flour, used in the production of baked goods (Alfonzo et al., 2013; Rizzello et al. 2015). Recently, organic and sourdough microbiota diversity was investigated and in some case a comparison between organic and conventional microbial ecosystem was also carried out. Opposites evidences arise. Once a higher diversity of lactic acid bacteria species was found in conventional wheat sourdoughs, while when the diversity of Firmicutes was investigated, organic sourdoughs showed the highest complexity (Pontonio et al., 2016).

Organic farming is promoted as a sustainable alternative to conventional farming, with positive effects on the diversity of plants and selected microbial and animal taxa. The results of some research confirmed that organic vineyard is natural reservoir of fermentative yeasts unlike conventional one (Tello et al. 2012). Therefore, it can be assumed that the organically grown grain can be the source of LAB with antifungal activity because of the greater contamination of organic grain with molds. Because of that, aim of the study was to determined fermentative and antifungal activities of LAB isolated from organically grown wheat, spelt and rye flour and corresponding sourdough in order to apply these microorganisms as protective starter cultures which can contribute to the reduction or bread spoilage.

Materials and methods

Isolation of LAB

Different wheat, spelt or rye whole grain flour samples (n = 12; 4 wheat, 4 spelt and 4 rye) from Croatia fields and mills were analyzed (Ivanuša, 2013) and used. Flour samples were mixed with sterile tap water (dough yield (DY) 200) and the covered doughs were incubated at 32°C for 18 h. Spontaneous laboratory sourdough fermentations were prepared over a period of 3 days with daily back-slopping. Samples taken before refreshment steps (after 24, 48 and 72 h of fermentation) were plated on de Man-Rogosa-Sharpe agar (m-MRS, with maltose as energy source). After incubation on the agar plates at 32°C for 48 h, catalase-negative colonies were identified by MALDI-TOF MS.

MALDI-TOF MS identification of LAB

For MALDI-TOF MS analysis, samples were prepared using procedure recommended by the manufacturer (Bruker Daltonik, Bremen, Germany). A single colony of fresh MRS agar culture was suspended in 300 µl water and a homogeneous suspension of cells in the tube was generated by vortexing for one minute. The next step was the ethanol/formic acid extraction. Ethanol abs. (900 µl) was added and the suspension was vortexed for one minute. The tube was centrifuged for 2 minutes at 13000 rpm and supernatant was removed. Then, 20 µl 70% formic acid was added to the pellet and mixed very well, following by addition of 20 µl pure acetonitrile. The bacterial extract was obtained after centrifugation at maximum speed (13,000 rpm) for 2 min. Supernatant from previous step (1 µl) was placed onto a MSP 96 polished stainless steel target plate (Bruker Daltonik, Bremen, Germany) and dried on air following by overlay with 1 µl of matrix solution (a saturated solution of alpha-cyano-4-hydroxycinnamic acid, HCCA, in 50 % acetonitrile and 2.5 % trifluoroacetic acid) and drying on air again. MALDI-TOF MS measurements were made using a Microflex LT™ instrument (Bruker Daltonik, Bremen, Germany) with default parameter settings (positive linear mode; laser frequency 60 Hz; ion source 1 voltage, 20 kV; ion source 2 voltage, 16.7 kV; lens voltage, 7.0 kV; mass range, 2,000 to 20,000 Da). For each spectrum, 240 laser shots in 40-shot steps from different positions of the sample spot were accumulated and analyzed (automatic mode, default settings). The instrument was calibrated using a Bruker bacterial test standard. For the identification, the Biotyper software (MALDI Biotyper 3.0 software package, Bruker Daltonics, Bremen, Germany) compares each sample mass spectrum to the reference mass spectra in the database, calculates an arbitrary unit score value between 0 and 3 reflecting the similarity between sample and reference spectrum. Identification criteria used were as follows: a score of 2.300 to 3.000 indicated highly probable species level identification, a score of 2.000 to 2.299 indicated secure genus identification with probable species identification, a score 1.700 to 1.999 indicated probable identification to the genus level, and a score of < 1.700 was considered to be unreliable. The major advantages of MALDI-TOF MS identification compared to conventional microbial identification are its rapidity (~ 1h for 96 samples), simplicity and low cost per analysis.

Sourdough fermentation with selected LAB

Isolated and identified LAB were used for preparing the sourdough. After growth in m-MRS broth (50 mL), the LAB biomass was harvested by centrifugation and used as inoculum for sourdough. Sourdough was prepared by mixing the organic wheat and spelt flour (25 g) with sterile tap water (25 mL) and inoculum of isolated LAB (DY = 200) in sterile beakers. The covered dough was fermented at 32 °C, 18 hours. pH measurement was made by digital pH-meter (Schott).

Fermentation products determination

High performance liquid chromatography (HPLC) was used for organic acids determination. Carrez reagents were added to the supernatant, and the precipitated proteins were removed by filtration (Lefebvre et al. 2002). Lactic, acetic and formic acids concentrations were quantitatively determined at 210 nm by a ProStar Varian 230 analytical HPLC (USA) with a Varian MetaCarb 67H column (300 × 6.5 mm) heated to 60 ºC in the isocratic mode of elution with 0.005 M sulfuric acid at a constant flow rate of 0.6 mL/min. Phenyllactic acid concentrations were determined also at 210 nm by a ProStar Varian 230 HPLC with a Zobrax 300SBC-18 column (150 × 4.6 mm, 3.5 µm) at 30 ºC. Elution was carried out with 1 mM sulfuric acid and acetonitrile (85:15) as mobile phase at a flow rate of 0.7 mL/min. All samples were analyzed in triplicate.

Antifungal LAB activity

LAB were screened for antifungal activity using dual-culture overlay assay. Mold *A. niger* 357 and *Pencillium* sp. 505 (Collection of microorganisms, Laboratory for General Microbiology and Food Microbiology, Faculty of food technology and biotechnology, Zagreb, Croatia) were used as test microorganisms. An inoculum of individual isolated and identified LAB was transferred to the centre of a m-MRS plate and incubated at 32 °C, for 2 days. After incubation, the plates were overlaid with the test indicator strain inoculated in soft malt extract (0,7%). To prepare the sensitive microbial suspension, mold biomass was scraped from a 5 days malt extract culture plate and suspended in 1 ml of NaCl 0.9% with 0.2% Tween. The number of molds spore was calculated using a Thomao chamber and the suspension was mixed with malt extract in order to obtain 10^5 CFU/ml (Colony Forming Units/ml). The inoculated plates were incubated at a temperature 28 °C for 72 h. After incubation, inhibition clear zone

widths (diameter in mm) were measured. All tests were performed in triplicate.

Results and discussion

Isolation, identification and selection of LAB

In this study the species diversity in organically grown wheat, spelt and rye flours and corresponding sourdough was investigated. Identification was based on mass spectrometry using a Microflex LT ™ MALDI-TOF instrument.

The results presented in Table 1 demonstrate microflora diversity in organic flour and sourdough samples. A total of 42 colonies were isolated and 12 different LAB strains were identified. The most common genera in the tested flours and sourdough were sourdough - specific *Lactobacillus* followed with *Enterococcus* genera which are atypical sourdough LAB but genera associated with cereal grains and flour. Enterococci are abundant due to the use of natural manure containing enterococci, natural inhabitants of the intestine of humans and animals.

To test the robustness of LAB present in different flour samples spontaneous laboratory sourdough fermentations were prepared over a period of 3 days with daily back-slopping.

Only a few strains were found to be competitive and became dominant. The population dynamic in all samples are in accordance with typical "the three phase evolution" (Minervini et al. 2014). The major changes occurred during the first and second days of fermentation (Table 2). In first phase dominated LAB species belonging to the genera *Enterococcus* (*E. durans*, *E. faecium*, *E. gallinarium and E. mundtii*) were detected. These strains predominated during the first days of spontaneous fermentation but did not survive in dough after second day due the dough acidification (the pH value dropped below pH 4.0 after the second day of back-slopping). In second phase adapted sourdough - specific LAB belonging to the genera *Lactobacillus* and *Pediococcus* dominated due to the tolerance to acidic conditions and to different adaptation mechanisms related to carbohydrate and nitrogen metabolism (de Vuyst and Neysens, 2005). *L. plantarum* and *P. pentosaceus* were more acid tolerant and dominated during three days back-slopping fermentation processes in all dough samples. Also, in sourdough produced from wheat, rye and spelt flour, *L. brevis* were found and remained after second and third back-slopping, differently from van der Meulen et al. (2007) in whose research *Lactobacillus brevis* were found only in the stable spelt sourdough together with *L. plantarum*. Weckx et al. (2010) also reported presence of *P. pentosaceus* and L. brevis in addition to *L. plantarum* and *L. fermentum* in stable rye sourdough. *L. brevis* and *L. plantarum* with some other LAB like *L. amylovorus*, *L. alimentarius*, *L. fermentum*, *L. reuteri*, *L. panis*, *L. pontis*, and *L. sanfranciscensis* are the most common strains found in sourdough (De Vuyst et al. 2014).

Sourdough metabolite target analysis

Fermentative and antifungal activities of isolated and identified strains were determined in order to select suitable strains for bakery industry and sourdough preparation. Preliminary, sourdough fermentation with all isolates was performed, then one species of each strain was selected, in particular those that rapidly and greatly decreased dough pH. Production of lactic and acetic acid, major metabolites from carbohydrate fermentation during sourdough fermentations as well as formic and phenyllactic acid, compounds with antifungal activity were monitored during wheat and spelt sourdough fermentations initiated with selected starter culture. Eleven LAB were tested: three heterofermentative (*L. brevis*, *Leuc. citerum*, *W. cibaria*), six homofermentative (*L. farciminis, E. durans, E. faecium, E. gallinarium, E. mundtii, P. pentosaceus*) and two facultative heterofermentative strains (*L. graminis* and *L. plantarum*).

The results of the sourdough metabolite target analysis are shown in Table 3. All the test strains were able to decrease the sourdough pH to or below 4.0 after 18 h of fermentation. Spelt sourdough had something higher pH probably due to something lower spelt flour starch concentration (69.42±1,89 % d.m. in wheat versus 61.52±0,22 % d.m. in the spelt) or amylase activity. On the other hand, there were not statistically significant differences in the free fermentable sugars concentration between the wheat and spelt samples (Ivanuša, 2013., data not shown). All of the strains belonging to the genera *Lactobacillus* as well as *P. pentosaceus* acidified the medium around pH 3.5. Sourdough fermented with strains belonging to the genera *Enterococcus*, *Leuconostoc* and *W. cibaria*, except *E. faecium*, had something higher pH till 4.0. The differences in the pH values are the result of differences in the organic acids synthesis between the strains. *L. farciminis*, *L. brevis*, *P. pentosaceus* and *E. faecium* synthesized a satisfying amount of lactic acid. *L. brevis* and *Leuc. citreum* synthesized significant amount of acetic acid while the strains of the genera *Enterococcus* additionally synthesized formic acid. Concentrations of the synthesized organic acids are in accordance with other studies. Hadaegh et al. (2017) detected about 460-2300 mg/100 g lactic, and 3-35 mg/100 g acetic acid in sourdough for toast bread production.

LAB antifungal activity

Molds contaminations in bakery cause huge economic losses and therefore LAB which have the ability to produce inhibitory growth compounds in final bakery products are of particular interest. Because of that, we screened organically grown wheat, spelt and rye grain to find unexplored LAB with antifungal activity. Results of our research confirmed the antifungal activity of *Lactobacillus plantarum, Pediococcus pentosaceus*, *Lactobacillus brevis* and *Weissella cibaria* in decreasing order, and pointed out antifungal activity of *Lactobacillus farciminis* isolated from organic rye flour (Table 4).

In recent years, due to a current focus on healthy food and a healthy lifestyle, the market for organic cereals has grown rapidly. Organic farming avoids the use of synthetically compounded fertilizers, pesticides or growth regulators, and prefers crop rotations, crop residues, animal and green manures, mechanical cultivation and biological pest control. Because of that, one can presume that the organic farming preserves biodiversity and that the organic products can be the source of different microbial species suitable for the food industry. Species diversity in organically grown wheat, spelt and rye showed that wheat flour showed less biodiversity than the rye and spelt flour. Wheat is, after the corn, the most produced crop (Rizzello et al. 2015). The largest quantities of wheat and corn are

farmed conventionally with the use of artificial fertilizers and pesticides causing wheat flour microflora less biodiversity. On the other hand, the production of rye and spelt is not so widespread. The quantities that are produced are intended mainly for human consumption, while wheat is cultivated also for livestock feed. Therefore, the application of protective agents on rye is much smaller.

A very limited data exists about organic farming cereal microbial diversity. Concurrently with our research Rizzello et al. (2015) examined may the organic cultivation of durum wheat reflect the flour-sourdough fermentation. They have examined chemical and technological characteristics as well as bacterial diversity of wheat grown conventionally, or organically with cow manure or green manure and without nitrogen inputs. Contrary to our research, they have examined overall microflora belonging to the *Proteobacteria*, *Firmicutes*, and *Actinobacteria* genera, while we have isolated only a group of microorganisms associated with sourdough. *Pantoea*, *Leuconostoc*, *Pediococcus*, *Pseudomonas*, *Lactobacillus* and *Enterobacteriaceae* family were the main genera found in the dough, but mature sourdoughs were completely and stably dominated by sourdough associated LAB. In accordance with our results, the following species were dominant: *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lecuconostoc citreum* and *Enterococcus lactis*. *Lactobacillus brevis* and *Weissella cibaria* were also isolated. The organic sourdoughs LAB diversity of 16 French organic bakers was investigated by different methods (Michel et al., 2016). The study highlighted that *L. sanfranciscensis* was not the major species in 6/16 sourdough samples and that a relatively high LAB diversity can be observed in French organic sourdough. *L. curvatus*, *L. brevis*, *L. heilongjiangensis*, *L. xiangfangensis*, *L. koreensis*, *L. pontis*, *Weissella* sp. and *Pediococcus pentosaceus* was the most representative species.

Rapid acidification during sourdough fermentation is a LAB technological characteristic that is of paramount importance (Corsetti and Settanni, 2007). Also, for bakery industry, it is important to find strains of LAB which are able to reduce pH value on level around 3.5 and produce lactic acid about 2g/100g of sourdough in order to create stable environment in sourdough (Kocková et al. 2011). *L. brevis* is one of the most frequently isolated LAB species in sourdoughs (Corsetti and Settani, 2007) and their role in bread quality and also health benefit due GABA (gamma-aminobutyric acid) synthesis is well recognized (Kim et al. 2009). *Pediococcus* strains are less frequently encountered in sourdough then *Lactobacillus* species. Nevertheless, *P. pentosaceus* is often present in sourdough, especially in combination with *L. plantarum*. In French wheat sourdough these two microorganisms were the main flora (Robert et al. 2009). Sterr et al. (2009) recommended these two strains as a starter culture to make gluten-free sourdough from amaranth. *P. pentosaceus* also inhibits the growth of rope-forming *Bacillus* in wheat bread if 20–30 % of sourdough is added in dough due to pediocine synthesize (Katina et al. 2002). *Lactobacillus farciminis* is strain known as probiotic for animal applications (Simon, 2005). It is also a bacterium that is naturally present in many fermented foods like meat products or traditional Korean dish *kimchi* (Nam et al. 2011). Some natural sourdough contains *L. farciminis* but in contrast to *L. brevis* and other sourdough lactobacilli it has not been explored enough. Our results showed that *L. farciminis*, isolated from organic rye flour, is resistant to low pH and it is stable in an acidic environment after 72 hours of fermentation. Among the tested strains, during fermentation it achieved the lowest pH and synthesized highest concentration of lactic acid. Additionaly, *L. farciminis* synthesized the most phenyllactic acid, especially in wheat. Nevertheless, *Lactobacillus farciminis* is an obligate homofermentative species which does not produce acetic acid and CO_2 that are metabolites with defining relevance for sourdough production and because of that it is suitable for sourdough fermentation only in combination with some other heterofermentative bacteria.

Sourdough application is an innovative technology in bakery which contributes to the development of desired sensorial, nutritional and technological properties of the final product, but also to its microbiological safety. Namely, cereals are often referred as raw materials contaminated with mold (Halt et al. 2004). Generally, molds in cereals fall into two categories, field fungi (genera *Alternaria*, *Fusariuim* and *Cladosporium*) which invade seeds, and plant and storage fungi (genera *Aspergillus* and *Penicillium*) encountered on stored products at moisture conditions. Many results on antifungal activity of LAB have been reported and some genera and species are more or less active than others. The antifungal activity of *Lactobacillus plantarum* (Coda et al. 2011; Magnuson et al. 2003; Lavermicocca et al. 2000), *Pediococcus pentosaceus* (Dalie et al. 2010), *Weissella cibaria* (Rouse et al. 2008), *Lactobacillus rossiae* and *Lactobacillus paralimentarius* (Garofalo et al., 2012) and *Lactobacillus brevis* (Falguni et al. 2010; Tropcheva et al. 2014) has been reported by other authors but our results highlighted the antifungal activity of *L. farciminis*. *L. farciminis* suppressed the growth of *Penicillium* sp. 505 and *Aspergillus niger* 357, contrary to Coda et al. (2011) who investigated 40 different LAB, among other *L. farciminis*, and detected only *L. plantarum* with antifungal properties against *Penicillium roqueforti* DPPMAF1. Recently, Laref et al. (2013) published *L. farciminis* LB53 antifungal activity against *Aspergillus* sp. This LAB were isolated from silage and the large zone of inhibition was observed at 25°C. The authors did not identify compounds with antifungal activity. We have no other information of the results confirming *L. farciminis* antifungal activity. Analyze of the fermentation products of our *L. farciminis* strain shows that it was characterized by low pH dough values, and a high content of lactic and phenyllactic acid. We can assume that phenyllactic acid in synergy with lactic acid has an important role in the antifungal activity. Phenyllactic acid suppresses the fungal growth of strains belonging to *Aspergillus*, *Penicillium*, and *Fusarium* genus (Lvermicocca et al. 2000; Lvermicocca et al. 2003). *Leuc. citreum* and *E. durans* did not show the same results, despite phenyllactic acid synthesis, probably due to the much lower lactic acid concentration. Phenyllactic acid was also synthesized by *L. plantarum* and *P. pentosacesus* in accordance with Lvermicocca et al. (2003) and Yu et al. (2015), but antifungal activity of these strains could be additionally explained by other antifungal compounds (Dalie et al. 2010). Valerio et al. (2016) improve the antifungal activity of eight lactic acid bacterial (LAB) strains by the addition of phenylpyruvic acid (PPA), a precursor of the antifungal compound phenyllactic acid (PLA), to a defined growth medium. In the presence of PPA the inhibitory activity (expressed as growth inhibition percentage) against fungal bread contaminants *Asper-*

gillus niger and *Penicillium roqueforti* significantly increased and was associated to PLA increase. In some species, a phenyllactic acid was detected only in spelt dough. These results can be explained by higher protein content in spelt (data not shown) and different content of specific amino acid. Amino acid spelt analysis showed that spelt had more phenylalanine than the wheat sample (5,4 g/100 g protein in spelt versus 4,5 g/100 g in wheat, Bonafacciaa et al. 2000). Phenyllactic acid was produced by LAB strains through phenylalanine degradation, which may explain the higher phenyllactic acid concentration in spelt samples. *L. brevis* has also shown strong antifungal activity due to high acetic acid concentration which is more effective than lactic acid (Lind et al. 2005). Although Rizzello et al. (2011) reported high formic acid antifungal activity against *Penicillium roqueforti*, tested strains from the *Enterococcus* genus which produce high concentration of formic acid did not suppress mold growth.

Conclusions

A number of studies considered the use of LAB metabolites to inhibit fungal contamination during bread storage. Despite numerous studies, microbial spoilage of bread in industry remains unsolved. Because of that, the selection and addition of novel isolates of LAB may be the key to reducing the use of chemicals, enhancing nutrients and extending the shelf life of bakery products.

The purpose of this research was to determine the composition of LAB in different organic flours in the light of the great interest in finding microbial consortia suitable for sourdough fermentation and able to protect the bread from spoilers, particularly molds. The results showed that the organic flour samples had a rich microflora. A total of 12 different LAB were isolated. Wheat flours showed less biodiversity than the rye and spelt flours. The most common genera in the tested flours were *Lactobacillus* and *Enterococcus*. Isolated *Lactobacillus farciminis, Pediococcus pentosaceus, Leuconostoc citreum* and *Lactobacillus brevis* showed the best acidification activity. *Lactobacillus brevis, Pediococcus pentosaceus, Weissella cibaria* and *Lactobacillus farciminis* showed significant antifungal activity against *A. niger* 357 and *Penicillium* sp. 505.

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Table 1. *Total of identified cultures during three days of spontaneous lactic-acid fermentation and respective flours from which they were isolated.*

Species diversity	Wheat flour	Spelt flour	Rye flour	Number of isolates	MALDI TOF MS score value
Lactobacillus brevis	x	x	x	4	2.145 – 2.401
Lactobacillus curvatus		x		7	1.738 – 2.138
Lactobacillus farciminis			x	1	1.998
Lactobacillus graminis	x	x		2	1.884
Lactobacillus plantarum	x	x	x	5	1.701 – 2.031
Leuconostoc citreum		x	x	4	1.977 – 2.151
Pediococcus pentosaceus	x	x	x	14	2.008 – 2.277
Enterococcus mundtii			x	1	2.201
Enterococcus faecium	x			1	2.236
Enetrococcus gallinarium		x		1	2.016
Enterococcus durans		x		1	2.203
Weissella cibaria			x	1	1.993

A score value: 2.300 - 3.000 highly probable species level identification; 2.000 - 2.299 secure genus identification with probable species identification; 1.700 - 1.999 probable identification to the genus level; score of < 1.700 was considered to be unreliable.

Table 2. *Bacterial community dynamics during laboratory back-slopped organic wheat, spelt and rye sourdough fermentations*

Flour	Microorganisms	Sourdough		
		24 h	48 h	72 h
Wheat	*Lactobacillus brevis*		x	x
	Lactobacillus graminis			x
	Lactobacillus plantarum	x	x	x
	Enterococcus faecium	x		
	Pediococcus pentosaceus		x	x
Spelt	*Lactobacillus brevis*		x	x
	Lactobacillus curvatus	x	x	
	Lactobacillus graminis	x		
	Lactobacillus plantarum		x	x
	Leuconostoc citreum	x		
	Enetrococcus gallinarium	x	x	
	Enterococcus durans	x	x	
	Pediococcus pentosaceus	x	x	x
Rye	*Lactobacillus brevis*		x	x
	Lactobacillus curvatus			x
	Lactobacillus farciminis			x
	Lactobacillus plantarum		x	x
	Leuconostoc citreum	x	x	
	Enterococcus mundtii	x		
	Pediococcus pentosaceus		x	x
	Weissella cibaria	x	x	

Table 3. *Physico-chemical characteristics of organic wheat and spelt sourdoughs started with selected lactic acid bacteria, after 18h fermentation at 32 °C.*

LAB	pH		Lactic acid (mg/g dough)		Acetic acid (mg/g dough)		Formic acid (mg/g dough)		Phenyllactic acid (μg/g dough)	
	Wheat	Spelt	Wheat	Spelt	Wheat	Spelt	Wheat	Spelt	Wheat	Spelt
L, brevis	3,40	3,68	15,05±0,38	13,54±0,29	1,55±0,12	1,56±0,07	0,10±0,04	trace	ND	ND
L, farciminis	3,27	3,35	17,54±0,24	20,41±0,22	0,11±0,09	0,25±0,03	ND	trace	9,58±0,11	39,30±0,44
L, graminis	3,60	3,72	8,93±0,32	11,75±0,19	0,40±0,08	0,28±0,04	3,73±0,12	4,44±0,23	ND	ND
L, plantarum	3,64	3,60	13,45±0,13	13,80±0,28	0,56±0,10	0,40±0,11	0,60±0,11	0,32±0,13	ND	6,80±0,12
Leuc, citreum	3,85	4,00	8,01±0,09	10,79±0,33	1,84±0,13	1,63±0,14	0,13±0,05	0,23±0,08	ND	6,91±0,08
E, durans	3,88	3,96	7,72±0,32	9,56±0,22	trace	ND	3,88±0,14	3,52±0,14	ND	9,31±0,18
E, faecium	3,63	3,70	15,70±0,26	15,90±0,19	0,30±0,07	0,20±0,06	0,31±0,06	0,11±0,06	ND	ND
E, gallinarium	3,82	3,90	8,04±0,12	11,04±0,14	trace	trace	2,89±0,32	2,93±0,27	ND	ND
E, mundtii	3,96	4,02	7,46±0,27	8,77±0,44	trace	ND	3,48±0,12	3,53±0,32	ND	ND
P, pentosaceus	3,44	3,51	15,53±0,08	17,35±0,22	0,45±0,05	0,38±0,08	0,27±0,11	0,13±0,05	ND	trace
W, cibaria	3,81	4,06	6,59±0,11	8,72±0,46	0,77±0,09	0,53±0,07	trace	trace	ND	ND

ND - not detected, trace - concentrations under the limit of quantification
The data are the means of two independent experiments and two samples from each experiment ± standard deviations.

Table 4. *Antifungal activity of LAB on MRS agar against Penicillium sp. 505 and A. niger 357 tested by overlay assays*

LAB	Inhibition	
	Pencillium sp.	*A. niger*
L. plantarum	+++	+++
P. pentosaceus	+++	+++
L. brevis	++	++
L. farciminis	+	++
W. cibaria	-	+
E. faecium	+	+
L. graminin	-	-
Leuc. citreum	-	-
E. durans		-
E. gallinarium	-	-
E. mundtii	-	-

-: without *any inhibition zone; +: 4 mm < zone < 8 mm; ++: 8 mm < zone < 12 mm; +++: 12 mm < zone: diameters of the LAB colonies incubated at 32 °C, for 2 days were around 4 mm*