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Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters

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ABSTRACT

The adaptability of lactic acid bacteria (LAB) and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava was investigated using PCR-DGGE and bacteriological culture combined with rRNA gene sequence analysis. Sourdoughs were prepared either from flours of the cereals wheat, rye, oat, barley, rice, maize, and millet, or from the pseudocereals amaranth, quinoa, and buckwheat, or from cassava, using a starter consisting of various species of LAB and yeasts. Doughs were propagated until a stable microbiota was established. The dominant LAB and yeast species were *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus paralimentarius*, *Lactobacillus plantarum*, *Lactobacillus pontis*, *Lactobacillus spicheri*, *Issatchenkia orientalis* and *Saccharomyces cerevisiae*. The proportion of the species within the microbiota varied. *L. paralimentarius* dominated in the pseudocereal sourdoughs, *L. fermentum*, *L. plantarum* and *L. spicheri* in the cassava sourdough, and *L. fermentum*, *L. helveticus* and *L. pontis* in the cereal sourdoughs. *S. cerevisiae* constituted the dominating yeast, except for quinoa sourdough, where *I. orientalis* also reached similar counts, and buckwheat and oat sourdoughs, where no yeasts could be detected. To assess the usefulness of competitive LAB and yeasts as starters, the fermentations were repeated using flours from rice, maize, millet and the pseudocereals, and by starting the dough fermentation with selected dominant strains. At the end of fermentation, most of starter strains belonged to the dominating microbiota. For the rice, millet and quinoa sourdoughs the species composition was similar to that of the prior fermentation, whereas in the other sourdoughs, the composition differed.

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1. Introduction

Bread baking using sourdough is a common practice and has the advantage of improving the nutritional value and sensory qualities of breads, achieving the baking ability of doughs for rye bread production, and increasing the shelf life of breads by delaying the germination of bacterial and mould spores (Hammes et al., 2005). The microbiota of sourdoughs consists of specifically adapted lactic acid bacteria (LAB), mostly lactobacilli, as well as yeasts (Hammes and Gänzle, 1998). Its composition is affected by the endogenous ecological factors which in turn are determined by the flour and process (exogenous) factors. Although the microbiota of traditional wheat and rye sourdoughs has well been characterised, research on the sourdough microbiota underwent a renaissance in the last years, leading to an increase in description of new *Lactobacillus* species, e.g. *L. hammesii* (Valcheva et al., 2005), *L. rossiae* (Corsetti et al., 2005), *L. nantensis* (Valcheva et al., 2006), *L. siliginis* (Aslam et al., 2006), *L. secaliphilus* (Ehrmann et al., 2007), *L. namurensis* (Scheirlinck et al., 2007a) and *L. crustorum* (Scheirlinck et al., 2007b).

Recently, new consumer demands have emerged for food products with improved nutritional value or health benefit, posing new challenges also for the baking industry. Furthermore, baked goods from wheat and rye are problematic for an increasing amount of people suffering from celiac disease. Thus, there is a market for new novel bakery products produced by using alternative cereals like rice, maize, sorghum and millet, or pseudocereals such as buckwheat (*Fagopyrum esculentum* Mönch), amaranth (*Amaranthus caudatus* L.) and quinoa (*Chenopodium quinoa* Willd), and possibly even starchy roots such as cassava (*Manihot esculenta* Crantz). These plants do not contain gluten, the causative agent for celiac disease. Moreover, pseudocereals are rich in proteins, especially in essential amino acids such as lysine (Aufhammer, 2000), which is limited in wheat and rye flour. On the other hand, the use of such alternative flours is restricted due to their low baking quality, as well as the sensory quality of the baked products (Gallagher et al., 2003). Fermentation of such alternative flours may improve both the sensory and baking qualities. First sourdoughs from rice flour are already on the market, but are fermented by applying starters for wheat and rye sourdoughs (Meroth et al., 2004). Meroth et al. (2004) showed that during rice sourdough fermentation, substrate-specific LAB and yeast species establish which are different from the

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common microbiota of wheat and rye sourdoughs. In conclusion, there is a lack of knowledge on the adaptation and competition of LAB and yeasts in sourdough fermentation made from alternative cereals, pseudocereals or cassava, hindering the development of new starters.

Spontaneous fermentation of cereal substrates obtained especially from rice, maize, sorghum, millet and from cassava is applied all over the world, resulting in a huge variety of traditional products, e.g. Sudanese kisra produced from sorghum, agbelima or fufu produced from fermented cassava doughs, pozol and kenkey produced from fermented maize. Studies on the characterisation of the microbiota of such traditional products revealed a great diversity of LAB and yeasts involved in the fermentation. Dominant LAB were shown to belong to the genera *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Weissella*, dominant yeast genera are *Saccharomyces* and *Candida* (Amoa-Awua et al., 1996, 1997; Ben Omar and Ampe, 2000; Escalante et al., 2001; Hamad et al., 1992, 1997; Hayford and Jakobsen, 1999; Hayford and Jespersen, 1999; Hayford et al., 1999; Jespersen et al., 1994; Mante et al., 2003; Miambi et al., 2003; Obiri-Danso, 1994; Olsen et al., 1995). The most competitive species reported in the literature are *L. plantarum*, *L. fermentum*, *S. cerevisiae* and *I. orientalis*. The microbiota of such traditional fermentations has been studied widely but, to our opinion, not systematically. Often information about fermentation conditions is missing and species identification is often based on physiological criteria only, known to be insufficient for the identification of LAB. Moreover, no data are available about the microbiota of sourdoughs from buckwheat, amaranth, quinoa, oat and barley.

Denaturing gradient gel electrophoresis (DGGE) of PCR generated rRNA gene fragments has recently been shown to be a useful tool for rapid characterisation of the dominating fermentation biota at the species level. PCR-DGGE has successfully been applied not only to characterise the microbiota, but also to monitor the development of its composition during long-term fermentation. Examples of application are malt whisky fermentation (Van Beek and Priest, 2002), Mexican pozol (ben Omar and Ampe, 2000), rye sourdoughs (Meroth et al., 2003a,b), wheat sourdoughs (Randazzo et al., 2005), and rice sourdoughs (Meroth et al., 2004).

In this study, PCR-DGGE and bacteriological culture combined with RAPD-PCR and rRNA gene sequence analysis were used to characterise the adaptability of LAB and yeasts to sourdoughs made from flours of the cereals wheat, rye, oat, barley, rice, maize and millet, of the pseudocereals amaranth, quinoa and buckwheat, and of the starchy root cassava. Fermentations were inoculated with a starter mixture and continuously propagated until a stable microbiota was established. Dominating lactobacilli and yeasts were isolated from the sourdoughs and used as starter organisms in a sourdough fermentation to evaluate their competitiveness in the corresponding fermentation substrate.

2. Materials and methods

2.1. Bacteria, yeasts and culture conditions

The following yeasts were used as reference RH in the DGGE analysis: *Debaryomyces hansenii* CBS 767^T, *Saccharomyces bayanus* CBS 380^T, *Saccharomyces uvarum* LTH H56, *Saccharomyces cerevisiae* CBS 1171^T, *Candida glabrata* DSM 6425, *Saccharomyces servazzii* CBS 4311^T, *Saccharomyces exiguus* CBS 379^T, *Candida humilis* CBS 6897^T, *Dekkera bruxellensis* CBS 74^T, and *Issatchenkia orientalis* CBS 5147^T. The following LAB were used as reference R1 in the DGGE analysis: *Weissella confusa* DSM 20196^T, *Lactobacillus johnsonii* DSM 10533^T, *Lactobacillus fermentum* DSM 20052^T, *Lactobacillus brevis* DSM 20054^T, *Lactobacillus crispatus* DSM 20584^T, *Lactobacillus acidophilus* DSM 20079^T, *Pediococcus pentosaceus* DSM 20336^T, *Lactobacillus farciminis* DSM 20184 ^{T}, *Lactobacillus panis* DSM 6035^T, *Pediococcus acidilactici* DSM 20284^T, *Lactobacillus pontis* DSM 8475^T, *Lactobacillus sanfranciscensis* DSM 20451^T, *Lactobacillus frumenti* DSM 13145^T, *Lactobacillus reuteri* DSM 20016^T, and *Lactobacillus paracasei* DSM 5622^T. For reference R2, the following strains were used: *Lactobacillus amylophilus* DSM 20533^T, *Lactobacillus plantarum* DSM 20174^T, *Lactobacillus paralimentarius* DSM 13238^T, *Lactobacillus amylovorus* DSM 20531^T, *Lactobacillus mindensis* LTH 5527, *Lactobacillus perolens* DSM 12744^T, *Lactobacillus buchneri* DSM 20057^T, *Lactobacillus spicheri* DSM 15429^T, *Lactobacillus fructivorans* DSM 20203^T, and *Lactobacillus ferintoshensis* DSM 15352^T (recently assigned to *L. parabuchneri* by Vancanneyt et al., 2005). Yeasts and LAB were routinely cultured in YG and MRS5 medium as described previously (Meroth et al., 2003a,b).

2.2. Sourdough fermentations and sampling

For fermentation I, eleven sourdough batches were prepared by using water and wholemeal flours of wheat, rye, oat, barley, rice, maize, millet, amaranth, quinoa, buckwheat, and cassava (dough yield 200). All batches were inoculated with 1% of baker's yeast and 10% of a starter mixture consisting in equal parts of the following starters: rye sourdough I, rye sourdough II, commercial rye full sour, rice starter, sorghum starter, teff starter, and cassava starter (see also Table 1). Rye sourdough I, a rye full sour was obtained from a local bakery and rye sourdough II was produced by inoculating rye dough with a

Table 1
Characterization of the baker's yeast and various starters of the starter mixture used to inoculate sourdoughs of fermentation I

Baker's yeast or starter	Species detected		Cell counts (CFU/g)	
	LAB (% of total LAB)	Yeasts (% of total yeasts)	LAB	Yeasts
Rye sourdough I	*L. pontis* (70) *L. brevis* (20) *L. plantarum* (10)	*S. cerevisiae* (100)	2.9×10^9	2.8×10^7
Rye sourdough II	*L. fermentum* (100)	*S. cerevisiae*[a]	1.2×10^9	$<10^3$
Commercial rye full sour	*L. plantarum*[b,c] (0.0001) *L. fermentum*[b,c] (0.0001) *L. paralimentarius*[b,c] (0.0001) *Acetobacter* spec.[b] (100) *L. sanfranciscensis*[a,d] *L. pontis*[a] *Lc.*[5] *lactis*[a] *L. acetotolerans*[a,d]	*C. humilis*[a]	5.0×10^7	$<10^2$
Rice starter	*L. paracasei*[b] (50) *L. paralimentarius* (50) *L. spicheri* (<1)	*P. anomala*[b] (100)	1.0×10^9	2.8×10^5
Sorghum starter	*L. helveticus*[d] (50) *L. pontis* (50)	*I. orientalis* (100)	1.6×10^9	1.9×10^7
Teff starter	*L. fermentum* (50) *L. plantarum*[b] (50)		5.0×10^8	$<10^3$
Cassava starter	*L. fermentum* (45) *L. plantarum* (45) *L. casei* group[b] (10)	*I. orientalis*[b] (70) *Torulaspora* spec.[b] (20) Unknown yeast[b] (10)	2.0×10^6	5.3×10^5
Baker's yeast	*L. plantarum* (70) *L. curvatus* group (<1) *L. brevis*[b] (10) *Lc.*[e] *lactis*[b] (10) *Le.*[f] *paramesenteroides*[b] (10) *L. fermentum*[a]	*S. cerevisiae* (100)	3.7×10^8	8.9×10^9

[a] detected by PCR-DGGE only.
[b] detected by bacteriological culture only.
[c] detected on the 10^{-1} dilution agar plates as large colonies overgrowing the small and colourless colonies of *Acetobacter*.
[d] sequencing of DGGE bands revealed similarities of less than 97%.
[e] *Lactococcus*.
[f] *Leuconostoc*.

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