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ORIGINAL ARTICLE

Microbial characterization of sourdough for sweet baked products in the Campania region (southern Italy) by a polyphasic approach

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Abstract The microflora of nine sourdoughs used for sweet bakery products underwent preliminary microbiological characterization using lactic acid bacteria (LAB) and yeast enumeration. Five sourdough samples were submitted for microbial identification by culture-dependent techniques employing 16S and 26S rRNA genes sequencing, as well as a culture-independent technique using PCR-DGGE analysis. The LAB species isolated belonged principally to facultative heterofermentative *Lactobacillus* spp., *Leuconostoc* spp., and *Lactococcus* spp. Yeast strains were identified as *Saccharomyces cerevisiae*, with one exception represented by a strain belonging to *Metschnikowia pulcherrima*. PCR-DGGE analysis allowed the identification of *Streptococcus thermophilus, Lactobacillus sakei*, *Weissella groceries* and *Lactobacillus sanfranciscensis* among lactic acid bacteria and *Saccharomyces cerevisiae* and *Metschnikowia pulcherrima* among yeasts. This polyphasic approach highlighted different levels of biodiversity, from two to eight different typical LAB species, always associated to *Saccharomyces cerevisiae*, that could be selected to be specifically used in naturally fermented brioche and *cornetto* preparation.

Keywords Sweet baked products · Sourdough · LAB and yeast · Polyphasic approach

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Introduction

Traditional sourdough essentially consists of a mixture of flour and water that is fermented by active lactic acid bacteria (LAB) and yeasts that multiply and become dominant. The ripe sourdough microflora is formed by stable associations of lactobacilli and yeasts that may persist for years, although the fermentation process runs under non-aseptic conditions (De Vuyst and Neysens 2005). It has been extensively shown that more than 50 LAB species, especially belonging to the genus *Lactobacillus*, and more than 20 yeast species, mostly belonging to the genera *Saccharomyces* and *Candida*, may be involved during the fermentation steps (Rossi 1996; Stolz 1999).

The use of sourdough during the bakery production improves dough machinability, nutritional properties and organoleptic features and prolongs the shelf-life (Corsetti et al. 1996; Hammes and Gänzle 1998; Salovaara 1998; Lavermicocca et al. 2000; Pepe et al. 2003; Corsetti and Settanni 2007) even if backslopping technology takes a long time and is labor consuming. Small and industrial bakeries have developed Italian sweet leavened baked products from sourdoughs (Panettone cake and Pandoro for Christmas, Colomba and some snacks for breakfast like Brioches and Cornetti). Their production processes share the use of a particular cycle of preparation starting from a sourdough (or *madre*-mother sponge), reproduced continuously over a long period. It consists of a natural mixed culture obtained by spontaneous selection of the original microflora of the flour. Using the culture method, Vernocchi et al. (2004) and Zorzanello and Sugihara (1982) performed microbial characterization of sourdough for Panettone, Pandoro and Colomba, identifying LAB belong-

ing to *Lactobacillus brevis*, *Lactobacillus sanfranciscensis*, *Leuconostoc mesenteroides* and yeast species ascribed to *Torulopsis holmii*, *Candida stellata*, *Candida milleri* and *Saccharomyces exiguus*. The culture-dependent method does not necessarily provide reliable information about the microbial communities although it allows the technological studies of the microbial isolates for future applications (Pepe et al. 2004). The use of a culture-independent molecular approach to identify and characterize the sourdough microflora can decrease the variability and instability of certain phenotypic traits and the dependence on culturing conditions. Indeed, one of the most important features of the molecular methods is the possibility to monitor the presence and persistence of microorganisms in the ecosystem without any cultivation, as well as their rapidity and reliability despite some inherent limitations. PCR-DGGE (denaturing gradient gel electrophoresis) has been successfully applied to the study of the LAB and yeasts composition of fermented cereal-based products (Sandhu et al. 1995; Ben Omar and Ampe 2000; Ampe et al. 2001; Miambi et al. 2002; Meroth et al. 2003a, b, 2004; Gatto and Torriani 2004; Randazzo et al. 2005; Settanni et al. 2006; Van der Meulen et al. 2007; Garofalo et al. 2008). Whereas microflora isolated from bread sourdoughs have been studied, those of sourdough microbiota for sweet baked products have received little attention, especially the investigation by a molecular approach. The aim of this work was to investigate the LAB and yeast populations of different sourdoughs involved in making sweet baked products such as brioches and *cornetto* produced in the Campania region (southern Italy). The identification of the LAB and yeast species was carried out using a polyphasic approach including a combination of phenotypic and genotypic methods (culture-dependent; PCR, encompassing 16S and 26S rRNA genes as well as PCR-DGGE). Optimization of the latter, specifically, for sweet sourdough was also considered.

Materials and methods

Sourdough sampling, microbiological and chemical investigation

Nine sourdoughs samples used to prepare sweet baked products such as brioches and *cornetto* (Italian croissants) were obtained from nine different small bakeries in the Naples area (Table 1). These artisan bakeries used different technologies to produce sweet baked goods in which sourdoughs were continuously propagated by backslopping using mother sponge taken from the preceding fermentation process without the use of starter cultures. The sourdough were stored at 4°C until refreshment to keep the mixed culture in an active state through the fermentation step performed at room temperature (20–30°C) for one night (about 16 h). Generally, sweet dough was produced adding baker's yeast to improve leavening. In our tests, 200 g of sourdoughs were aseptically sampled, immediately transported at 4°C to the laboratory and submitted to microbiological, acidity and molecular analysis. Ten grams of dough samples were homogenized in 90 ml of quarter-strength Ringer solution (Oxoid) by using a Stomacher (Stomacher 400 Circulator; PBI International, Italy) and serially diluted. One hundred microliters of each dilution was spread plated on Chalmers Agar (Pepe et al. 2001). Triplicate plates were incubated in aerobically conditions and in a Gaspak jar using the commercial gas-generating kit (AnaeroGen™, Oxoid) for anaerobic growth. LAB and yeasts enumerations were performed after 4 days of incubation at 30°C.

Total titratable acidity (TTA) and pH were determined by standard methods (AAAC 1975).

Table 1 Microbial contents (Log CFU g^{-1}), pH and total titrable acidity of sourdoughs for sweet baked products analyzed

Sourdough source[a]	Log CFU g^{-1}		pH[d]	TTA[e]
	Bacteria[b]	Yeasts[c]		
I1	3.6	<1	4.51	7.5
I2	2.9	<1	4.55	7.8
I3	3.6	<1	3.76	6.8
I4	3.0	<1	4.68	4.8
I4b	7.9	8.5	4.74	8.0
I4c	7.5	7.2	3.76	6.0
I5	5.2	4.7	3.91	11.8
I6	5.0	8.5	4.63	8.5
I7	7.0	8.0	4.75	4.0

[a] Location of sourdoughs: *I1* Portici (Naples), *I2* Ponticelli (Naples), *I3* Torre del Greco (Naples), *I4* Torre del Greco (Naples), *I4b* Torre del Greco (Naples), *I4c* Torre del Greco (Naples), *I5* Torre del Greco (Naples), *I6* Sorrento (Naples), *I7* Sorrento (Naples)

[b, c] Data are means of triplicate counts on modified Chalmers agar medium ± SD ($0.01 \leq SD \geq 0.02$ and $0.01 \leq SD \geq 0.03$ for bacteria and yeasts, respectively)

[d] Data are means of triplicate analyses ± SD ($0.05 \leq SD \geq 0.3$)

[e] Total titrable acidity measured as ml NaOH 0.1 N/10 g of sample. Data are means of triplicate analyses ± SD ($0.1 \leq SD \geq 0.5$)

LAB and yeasts isolation

Colonies of LAB and yeasts from sourdough samples I4b, I4c, I5, I6 and I7 were randomly isolated from the higher counting plates on the basis of their dimension, edge, color, elevation, consistency and $CaCO_3$ dissolution halo on modified Chalmers agar (Pepe et al. 2001). After purifica-

tion on the same growth medium, bacterial colonies were first recognised as LAB by assessing their morphology (phase contrast microscopy), Gram reactions and catalase activity. Gram-positive and catalase-negative cocci and rods were sub-cultured and stored at 4°C for the further study.

Likewise, isolate yeast colonies on modified Agar Chalmers, were differentiated on the basis of their macro-morphology and micro-morphology, and purified on the same growth medium.

Identification by the culture-dependent method

Characterization of yeast strains by ITS-RFLP analysis

Preliminarily, yeast strains were grouped according their internal transcribed spacers—restriction fragment length polymorphism (ITS-RFLP). The ITS region (ITS1-5.8S rDRA-ITS2) of yeasts was amplified by using oligonucleotide primers, ITS1 and ITS4, described by White et al. (1990). PCR mixture and conditions were performed as previously reported (Pennacchia et al. 2008). Restriction endonucleases *Hae*III and *Cfo*I were used in the ITS-RFLP to digest 25 μl of the ITS PCR products (30 U in a total volume of 30 μl at 37°C for 5 h). Restricted DNA was resolved by 2% (w/v) agarose–TBE gel electrophoresis.

Identification by sequencing

InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA) was used for DNA extraction from LAB and yeast isolates according to the supplier's recommendations.

Synthetic oligonucleotide primers fD1 and rD1 described by Weisburg et al. (1991) were used to amplify the 16S rRNA gene. PCR mixture and conditions were performed as previously reported (Blaiotta et al. 2003). The D1-D2 domain of 26S rDNA of yeasts was amplified by using oligonucleotide primers NL1 and NL4 (Kurtzman and Robnett 1998). All PCR reactions were performed in a PTC-100 thermocycler (M J Research, Watertown, MA, USA).

Amplified products were resolved on a 1.5% (w/v) agarose–TBE gel electrophoresis, purified by using a QIAquick gel extraction kit (Qiagen, Milan, Italy) and sequenced by using the primer fD1 (Weisburg et al. 1991) for 16S rDNA of LAB and the primer NL1 (Kurtzman and Robnett 1998) for the D1-D2 domain of 26S rRNA of yeasts. The DNA sequences were determined as described by Blaiotta et al. (2003). The sequences were analyzed by MacDNasis Pro v3.0.7 (Hitachi Software Engineering, Olivet Cedex, France) and compared to the GenBank nucleotide data library using the Blast software at the National Centre of Biotechnology Information website (http://www.ncbi.nlm.nih.gov) (Altschul et al. 1997) in order to determine their closest phylogenetic relatives.

PCR-DGGE analysis of sourdoughs

Production of standardized dough

To study the functionality and the efficiency of different protocols for DNA isolation directly from dough samples, preliminary experiments on standardized dough were carried out. For this purpose, lactobacilli (*Lactobacillus brevis* H6, *Lactobacillus plantarum* E5 and *Lactobacillus sanfranciscensis* B9) were grown in MRS broth (Oxoid) and incubated overnight at 30°C. *Saccharomyces cerevisiae* T22 was cultured in Malt extract (Oxoid), for 2 days at 30°C. The cells were then collected by centrifugation (5,000 *g*), washed with sterile distilled water and resuspended to obtain $5\pm0.5\times10^9$ microorganisms/ml (direct microscopic counts). The standardized dough was prepared as previously reported (Coppola et al. 1998). The LAB and yeasts concentration in the final dough was $5\pm0.5\times10^7$ CFU/g. The dough was fermented for 24 h at 30°C and the sourdoughs obtained were used for direct DNA isolation and subsequent PCR-DGGE analysis.

DNA isolation from sourdough

Three different methods of DNA extraction were used to obtain the best for DNA isolation directly from sourdoughs: *Wizard* (Promega, Madison, Wisconsin), *Nucleo Spin Food* (Macherey-Nagel, Germany), *DNeasy Plant Mini Kit* (Qiagen). DNA isolation was carried out according to the supplier's recommendations. For all isolation methods 10 g of standardized sourdough (at beginning and after 24 h of fermentation) was diluted (1/10 and 1/100) in quarter-strength Ringer's solution (Oxoid). Two ml of the 10^{-1} and 10^{-2} dilutions were centrifuged at 14,000 *g* for 5 min and the resulting pellets were used for DNA isolations.

The efficiency of the three DNA isolation methods was evaluated considering: (1) quantity of DNA isolated and its quality by means of a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA); (2) DNA amplificability, by PCR of 16S rRNA gene and D1-D2 domain of 26S rRNA (Kurtzman and Robnett 1998), for detection of LAB and yeasts, respectively; and (3) PCR-DGGE analysis of the V3 region of the 16S rRNA and the D1-D2 domain of 26S rRNA for LAB and yeasts, respectively, to detect the biodiversity in the dough samples.

PCR conditions

Primers V3f and V3r, spanning the 200-bp V3 region of the 16S rRNA of *E. coli* (Muyzer et al. 1993) were used for LAB PCR-DGGE analysis. A GC-clamp was added to the forward primer, according to Muyzer et al. (1993). PCR

condition was performed as described by Ercolini et al. (2002).

To analyze the yeast population, the primers NL1 (Kurtzman and Robnett 1998) and LS2 (Cocolin et al. 2000) were used. A GC-clamp was added to the reverse primer, according to Muyzer et al. (1993). PCR mixture and conditions were performed as described by Cocolin et al. (2000).

DGGE analysis

PCR products were analyzed by DGGE according to Muyzer et al. (1993) by using a Bio-Rad DCode Universal Mutation System (Bio-Rad Laboratories). Samples were loaded in a 0.8-mm polyacrylamide gel [8% (wt/vol) acrylamide-bisacrylamide (37:5:1)] by using a denaturant gradient from 15 to 55% (for LAB analysis) and from 20 to 50% (for yeasts analysis) [100% denaturant gradient was 7 M urea plus 40% (wt/vol) formamide] increasing in the direction of electrophoresis. Electrophoresis was performed at 60°C, initially at 50 V (5 min) and then at 200 V (240 min). The gels were stained in an ethidium bromide solution (5 min) and rinsed in distilled water (20 min).

Sequencing of DGGE fragments

The DGGE bands of interest were excised from the gel with a sterile scalpel, disrupted in 20 μl of sterile water and left overnight at 4°C. One μl of the eluted DNA of each DGGE band was re-amplified by using the appropriate primers and the conditions described above. The success of this procedure was checked by electrophoresing 12-μl portions of the PCR products in DGGE gels as described above together with amplified DNA from corresponding sourdough as a control. PCR products, which gave a single band comigrating with the original band, were then purified by QIAquick PCR Purification kit (Qiagen) and sequenced. Sequencing was performed using the reverse primers (V3r and LS2, for bacteria and yeasts, respectively). The partial sequences were compared with the sequences present in public data libraries (GenBank) using the Blast search program in order to determine their closest known relatives.

Statistical analysis

Statistical treatment of data from three independent measurements by means and standard deviation (SD) was performed on each sample.

Results and discussion

Based on our knowledge, there are few studies on sourdough for sweet baked products as brioches and *cornetto*. Most of them focus mainly traditional Italian sweet baked goods such as Colomba, Pandoro or Panettone, manufactured according to specific procedures often at an industrial level (Foschino et al. 1999; Vernocchi et al. 2004). Moreover, only one (Garofalo et al. 2008), to the

Table 2 Lactic acid bacteria isolated from sourdough samples by culture-dependent analysis

Sourdough samples	16S rRNA sequence analysis		
	Signature of strains [a]	Closest relative species (% identity)	Accession numbers[b]
I4b	78, 77	*Lactobacillus (Lb.) plantarum* (99%)	EF536363
	56A1, 55	*Lactobacillus* sp. (100%)	DQ682968
	62B2, 77A	*Leuconostoc (Ln.) pseudomesenteroides* (99%)	AM491818
	62A, 12, 49B1	*Ln. lactis* 99%	AJ970316
	57B, 57B2	*Lactococcus (Lc.) lactis* subsp. *cremoris* (100%)	AM406671
	47B, 57A, 59B, 59B2, 79	*Lc. lactis* subsp. *lactis* (99%)	DQ171719
I4c	64A1, 68A1, 68A2, 96B, 69B2	*Lb. sakei*/*Lb. curvatus* (99%)	DQ989236/AM113777
	69B, 95A, 68A	*Ln. lactis* (97%)	AJ970316
	68B1, 68B2	*Ln. mesenteroides* (97%)	EF579730
I5	81, 83, 85, 85A, 85B, 83	*Lb. plantarum* (99%)	AL935261
I6	127, 127A	*Lactobacillus* spp. (99%)	DQ682970
I7	117A, 118A	*Lb. curvatus* subsp. *curvatus*/*graminis* (98%)	AY204894/AM113778
	114A, 115A	*Lb. casei* (99%)	CP000423
	116A, 116B	*Lb. coryniformis* subsp. *torquens* (99%)	AB289064
	103, 105	*Leuconostoc* sp. (99%)	EF204331

[a] Representative LAB strains grouped by morphological and physiological characteristics and identified by 16S rRNA gene sequencing

[b] Accession number of the sequence of the closest relative species identified using the Blast software

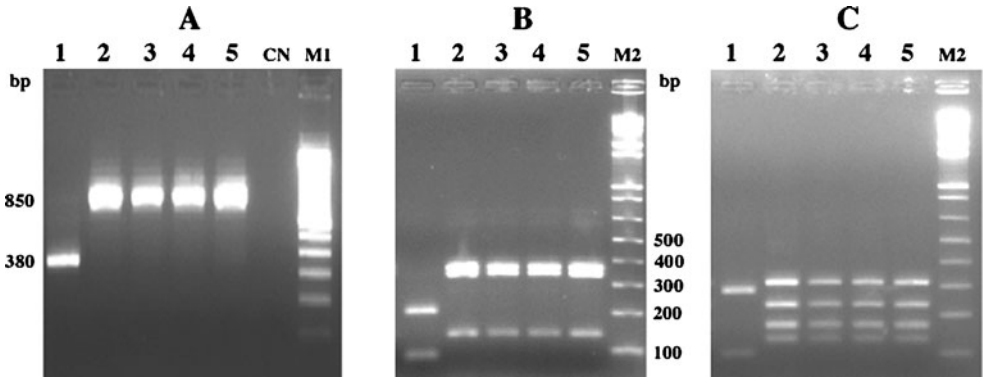

Fig. 1 ITS1- 5,8S- ITS2 (**a**) and ITS1- 5,8S- ITS2-RFLP (*Cfo* I and *Hae* III) (**b c**) patterns of some yeast strains. *Lane 1* strain 138, *lane 2* strain 87, lane 3 strain 100B, *lane 4* strain 61, *lane 5* strain 111. *CN* negative control, *M1* 100 bp DNA Ladder (Invitrogen), *M2* 1 Kb DNA Ladder Plus (Invitrogen)

best of our knowledge, had focused their study on molecular fingerprinting of the cultivable plus non-cultivable microbial population during leavening process of Panettone, and no study has been conducted regarding the microbiological characterization at species level of sourdough for the production of baked goods like *cornetto* and brioche.

LAB and yeast enumerations of each sourdough shown in Table 1 revealed two types of sourdoughs for sweet bakery goods. One of these (samples I4b, I4c, I6 and I7) had high yeasts levels (yeast viable counts from 1.5×10^7 to 3.2×10^8 CFU/g; $0.01\leq SD\geq 0.03$) dominating the fermentation overhanging the indigenous LAB flora (LAB viable counts from 1.1×10^5 to 7.0×10^7 CFU/g; $0.01\leq SD\geq 0.02$). A second type contained only low viable counts of LAB ranging from 8.0×10^2 to 1.8×10^5 CFU/g (samples I1, I2, I3, I4, and I5; $0.01\leq SD\geq 0.02$) and the ye asts were counted at a low level (5.0×10^4 CFU/g; $0.01\leq SD\geq 0.03$) or not detected (< 10 CFU/g). Other authors also found low LAB counts in wheat sourdough of central and southern Italy (Gobbetti et al. 1994) as well as high viable counts of yeast (Succi et al. 2003). According to Collar et al. (1994) and Wehrle and Arendt (1998), the pH and TTA values of sourdough analyzed was variable: in fact, values varying between 3.76 and 4.75, even if six out of nine samples showed a pH higher than 4.5 ($0.05\leq SD\geq 0.3$), whereas the TTA values ranged from 4.0 to 11.8 ml of NaOH 0.1 N and seven out of nine samples showed a TTA of more than 6 ($0.1\leq SD\geq 0.5$). The amount and the number of specific homofermentative and heterofermentative LAB strains affected the acidification properties of different dough (Coppola et al. 1996, 1998). Five sourdoughs (I4b, I4c, I5, I6 and I7) fermented at room temperature (20–30°C) for one night (about 16 h), showing higher LAB and yeast counts, were evaluated with regard to the fingerprinting of their microbial population.

On the basis of the different cultural and physiological characteristics, we divided 84 LAB and 49 yeast isolates into different homogeneous groups. From these were chosen representative strains (from two to six for each group) of LAB (Table 2) and yeasts (one for each ITS-RFLP groups; Fig. 1) that were identified by 16S rRNA and 26S rRNA partial gene sequencing, respectively. Modified Chalmers Agar is very suitable for the separate counting of mixed LAB populations (*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Lactobacillus sanfranciscensis* and *Enterococcus faecalis*) and yeast species, with an acceptable recovery in comparison to other media (Pepe et al. 2001; Picozzi et al. 2005). The species that we detected by culture-based method in the sweet sourdough belonged principally to different facultative heterofermentative lactobacilli that dominated the sourdough process as also detected by Ricciardi et al. (2005) and Catzeddu et al. (2006) in southern Italian sourdoughs.

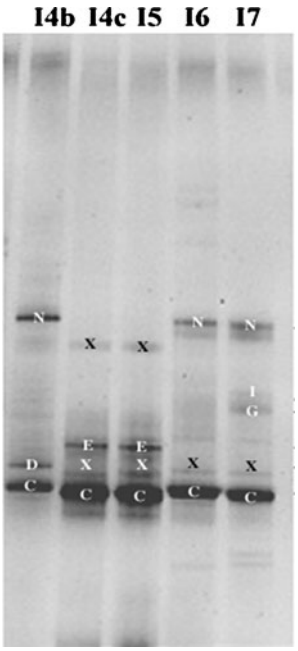

Fig. 2 PCR-DGGE of 16S rRNA V3 region profiles of the DNA of 5 different sourdoughs (I4b, I4c, I5, I6 and I7) obtained by Nucleo Spin Food protocol. Each band was identified by a letter: *C* uncultured bacteria/cereal mitochondrial DNA, *D Streptococcus thermophilus*, *E Lactobacillus sanfranciscensis*, *G Lactobacillus sakei, I* uncultured bacteria, *N Weissella cibaria*, *X* bands that after purification and sequencing yielded inconclusive results

Table 3 Analysis of PCR-DGGE of the bands obtained DNA isolated directly from sweet sourdough samples

Band[a]	Closest relative species (% identity)	Accession Numbers[b]	I4b	I4c	I5	I6	I7
	Bacteria						
C	Uncultured bacterium clone H6 (98%)[c]	EF 599659	X	X	X	X	X
D	*Streptococcus thermophilus* (99%)	DQ001071	X				
E	*Lactobacillus sanfranciscensis* (97%)	AB289299		X	X		
G	*Lactobacillus sakei* (99%)	CR936503					X
I	Uncultured soil bacterium clone 4S2 (100%)	EU052112					X
N	*Weissella cibaria*	EU099612	X			X	X
	Yeast						
1	*Metchnikovia pulcherrima* (98%)	EF564397			X		
2	*Saccharomyces cerevisiae* (100%)	EF192587	X	X	X	X	X

[a] Bands, see Figs. 2 and 3

[b] Accession number of the sequence of the closest relative species identified using the Blast software

[c] The band was also closest related to mitochondrial cereal DNA (98%)

As reported in Table 2, the culture-based method allowed the identification of from one (samples I2, I3, I4, I5 and I6) to five species (sample I4b) of LAB. In particular, according to Zotta et al. (2008) who isolated LAB from wheat sourdough for the production of Matera bread, the sweet sourdoughs analyzed were characterized by the presence of *Lactobacillus plantarum* and/or *Lactobacillus sakei/curvatus* associated with LAB species belonging to the genus *Leuconostoc*; typical obligate heterofermentative lactobacilli dominate the sourdough ecosystem after 4–6 days of fermentation.

The number and the intensity of bands varied among samples when bacterial DNA was investigated by PCR-DGGE analysis (Fig. 2) even if, in accordance with the findings of Scheirlinck et al. (2008) and Iacumin et al. (2009), a small number of bands visualized indicated few bacteria species. Moreover, different species with respect to those found by culture-dependent analysis were assigned. Sourdoughs I4b and I7, whose fingerprints had at least 3 bands, showed more variability. On the 16S rRNA V3 region PCR-DGGE fingerprints, it was possible to detect bands most closely related to different LAB species, namely *Streptococcus thermophilus* (band D; sample I4b), *Lactobacillus sakei* (band G; sample I7), *Weissella cibaria* (band N; samples I4b, I6 and I7) and *Lactobacillus sanfranciscensis* (band E; samples I4c and I5) (Table 3). *Lactobacillus sanfranciscensis* was detected in the sample I5 (Table 3) in association with *Lactobacillus plantarum* (Table 2) as previously reported by Foschino et al. (1999) and Garofalo et al. (2008) in sourdough for traditional sweet baked goods. The inability to isolate this species with the culture method could be due to the reduction of its metabolism when associated with *Sacharomyces cerevisiae* (Iacumin et al. 2009). The visualization of DNA bands ascribed to *Lactobacillus sanfranciscensis* can be explained by the amplification of DNA released from lysed cells stressed by metabolic or osmotic factors during sourdough fermentation. PCR-DGGE analysis was able to show the presence of *Streptococcus thermophilus*, not very frequently isolated from this kind of samples. Moreover, all our PCR-DGGE fingerprints showed an intense band related to uncultured bacteria (98% identity) and a mitochondrial cereal DNA (98% identity). Similar results were obtained by Scheirlinck et al. (2008), who found a PCR-DGGE band

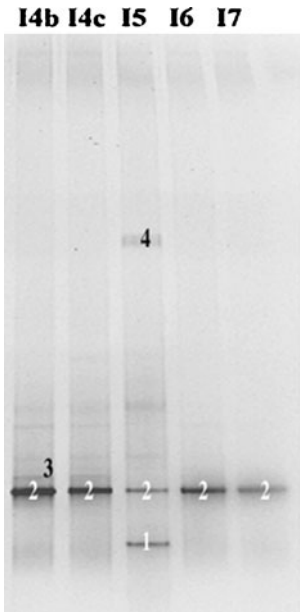

Fig. 3 PCR-DGGE of 26S rRNA profiles of 5 different sourdoughs (I4b, I4c, I5, I6 and I7). Bands are identified by numbers: *1 Metschnikowia pulcherrima*, *2 Saccharomyces cerevisiae*, *3* and *4* bands that after purification and sequencing yielded inconclusive results

related to a mitochondrial cereal DNA in more than 50% of their sourdough samples analyzed. Finally, purification and sequencing of some faint bands (Fig. 2, bands X) yielded inconclusive results.

All yeast strains showed the same ITS pattern with one band at 850 bp, with the exception of strain 100B, isolated from the sourdough I6 that showed one band at 400 bp (Fig. 1a). Similar results were obtained analyzing the *Hae* III and *Cfo* I ITS-RFLP profiles (Fig. 1b, c). From the D1-D2 domain of 26S rRNA sequencing, strains showing ITS at 850 bp were identified as *Saccharomyces cerevisiae* (100% identity), while the strain 100B (ITS at 400 bp) was identified as *Metschnikowia pulcherrima* (100% identity). DGGE fingerprints obtained by analyzing the amplified 26S rRNA fragments with primers NL1 and LS2 confirm the results obtained by the culturing method showing a very low diversity in the yeast population. In fact, only 4 bands could be visualized in the DGGE gel of which there was two main bands with a good intensity (Fig. 3, bands 1 and 2) that could be referred to *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*, respectively (Table 3). This finding illustrates the intrinsic limitation of DGGE analysis in visualizing only the predominant species of a microbial community (Muyzer and Smalla 1998). On the other hand, community analyses based on PCR-DGGE allow the most representative species to be detected, owing to competition among heterogeneous templates (Meroth et al. 2003a; Ercolini 2004). *Saccharomyces cerevisiae* occurs very often in sourdough for sweet leavened baked products because, in common practice, it is introduced through the addition of baker's yeast to the first refreshment to speed up the last leavening step (Garofalo et al. 2008). In the dough I4b, we found *Saccharomyces cerevisiae*/*Lactobacillus plantarum*/*Leuconostoc mesenteroides*, reported as the best association for a good production of aroma and to reach a high level of phytate biodegradation in wheat dough (Coppola et al. 1998; Chaoui et al. 2003; Pepe et al. 2003; Anastasio et al. 2010). *Metschnikowia pulcherrima*, isolated in the sample I6, was also found in the sourdough I5 by PCR-DGGE analysis of another main band with a good intensity (Table 3; Fig. 3, band 1). The occurrence of *Metschnikowia pulcherrima* in sourdough habitat has not been reported elsewhere. Its presence could be due to a contamination of the bakery environmental by ingredients (fruits, honey or sugars). In fact, it was previously isolated from some fruits like grapes by Senses-Ergul et al. (2005). After purification and re-amplification of some faint bands (Fig. 2, bands X, and Fig. 3, bands 3 and 4), we were not able to obtain amplification products. In conclusion, the polyphasic approach used allowed us to discover different levels of biodiversity in the sweet sourdoughs analyzed. The use of the PCR-DGGE analysis confirmed that it should be added to culturing methods since additional LAB species were observed during the study. Suitable polyphasic methods that combine both approaches improve assessment of food bacteria diversity. This overcomes the limitations of the use of both approaches allowing the obtaining a more comprehensive overview of the microbial community in foods. Indeed, some samples showed, in association with *Saccharomyces cerevisiae*, from two to eight different LAB species that can be considered well-adapted species in the sweet sourdough samples analyzed.

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