Chapter 5
Taxonomy and Biodiversity of Sourdough Yeasts and Lactic Acid Bacteria

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5.1 Taxonomy of Sourdough Yeasts and Lactic Acid Bacteria

5.1.1 Taxonomy of Sourdough Yeasts

Yeasts are microscopic fungi that undergo typical vegetative growth by budding or fission resulting in an unicellular appearance and a sexual reproduction without a within or upon which the resulting spores are formed [1]. From the agro-alimentary and scientific point of view, yeasts are among the most important eukaryotes. Yeast species found in sourdough microbial communities share an adaptation to the specific and stressful environment created mainly by a low pH, high carbohydrate concentrations and high cell densities of lactic acid bacteria (LAB). Such adaptations can be found in species located mostly on one branch of the evolutionary tree

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of fungi, which accommodates the ascomyceteous yeasts. Within this branch, recognized by the current classification in the phylum Ascomycota, as the subphylum Saccharomycotina, class Saccharomycetes, order Saccharomycetales [2], sourdough yeasts belong to different genera. The major sourdough yeasts belong to genera that are currently placed in the family Saccharomycetaceae, although family assignment of yeast genera is still difficult because of a lack of informative data. Basidiomycetous yeasts and dimorphic ascomycetes, also adapted to growth in liquid environments by unicellular growth forms, lack the fermentative abilities that are common to the Saccharomycetales and that are important for growth under oxygen limitations. The taxonomy of the Saccharomycetales, classically based on morphology and physiology, is in the process of being adapted to the increasing knowledge of evolutionary relationships reconstructed from gene sequences, in other words, a phylogenetic system of classification is being developed [2]. This implies a number of name changes. The new genus names have the advantage of reflecting the common genetic background of related yeast species, hereby providing an informative classification in contrast to the former largely artificial classification.

An overview of recent name changes restricted to species that have been obtained from sourdough is given here. The name changes most relevant to the yeasts found in sourdough concern the genera *Saccharomyces* Meyen ex Reess and *Pichia* E.C. Hansen emend. Kurtzman. The genus *Saccharomyces* has been limited to the group of species known as *Saccharomyces sensu stricto*, including the type species of the genus, *Saccharomyces cerevisiae,* on the basis of multiple gene sequences [3]. The group of species formerly often addressed as *Saccharomyces sensu lato* has been divided into several genera. The new genus *Kazachstania* is accommodating the former *Saccharomyces exiguus*, *Saccharomyces unisporus,* and *Saccharomyces barnettii* as *Kazachstania exigua*, *Kazachstania unispora*, and *Kazachstania barnettii*, respectively. *Saccharomyces kluyveri* has been assigned to the new genus *Lachancea* as *Lachancea kluyveri*. The genus *Pichia* has been restricted to species closely related to the generic type species *Pichia membranifaciens*, including *Pichia fermentans* [4]. The former genus *Issatchenkia* has been integrated into the newly defined genus *Pichia* as its species are located on the same branch as the type species *P. membranifaciens* on the phylogenetic tree based on multiple gene sequences used for the redefinition of genera. While the species epithet of *Issatchenkia occidentalis* has been preserved in its new name *Pichia occidentalis*, a complete name change of *Issatchenkia orientalis* to *Pichia kudriavzevii* was necessary as the combination *Pichia orientalis* had been used for a different species before. The former species *Pichia anomala* and *Pichia subpelliculosa* were found to be only distantly related to the generic type species *P. membranifaciens* and have therefore been assigned to the newly created genus *Wickerhamomyces* as *Wickerhamomyces anomalus* and *Wickerhamomyces subpelliculosus*. A review of the taxonomic considerations including the earlier genus name *Hansenula* has been given by Kurtzman [5, 6]. Other, only occasionally from sourdough isolated species from the former genus *Pichia* have been reassigned to new genera, while preserving their species epithet and include *Kodamaea ohmeri, Meyerozyma guilliermondii*, *Millerozyma farinosa*, *Ogataea polymorpha*, *Saturnispora saitoi,* and *Scheffersomyces stipitis*.

To determine the entities, or taxa,[1] that deserve attention in the sourdough context, about 40 original publications were reviewed and the repeatedly reported species are listed in Table 5.1. These publications span the time from the early 1970s until present and it is obvious that not all of them are based on identification techniques that are currently considered as the most accurate. However, most of the six regularly (seven or more reports) encountered species *S. cerevisiae* (syn.[2] *S. fructuum*); *Candida humilis* (syn. *Candida milleri*); *P. kudriavzevii* (syn. *I. orientalis*, anamorph *Candida krusei*); *K. exigua* (syn. *S. exiguus*, anamorph *Candida [Torulopsis] holmii); Torulaspora delbrueckii*, anamorph *Candida colliculosa*; and *W. anomalus* (syn. *P. anomala, Hansenula anomala*, anamorph *Candida pelliculosa*) can be distinguished from each other reasonably well by classical methods based on morphology and physiology as used in the reviewed literature up to the late 1990s. However, comparing studies using phenotypic identification techniques (n = 19) with those using DNA-based techniques (n = 23), the incidence of *C. humilis* has increased markedly in the DNA-based studies, probably at the cost of a decreased frequency of detecting *K. exigua*. These two phylogenetically closely related species may be mistaken for each other if using phenotypical identification methods. For example, originally *S. exiguus* was reported from San Francisco sourdough [7], while some strains from this study that were preserved in culture collections, later served for the description of *C. milleri*, currently a synonym of *C. humilis*. The sourdough isolate M14 reported as *S. exiguus* and deposited also as CBS 7901 was suggested to belong to *C. humilis* after DNA-based analyses [29, 50]. The regularity with which *S. cerevisiae, C. humilis, P. kudriavzevii, K. exigua*, *T. delbrueckii*, and *W. anomalus* are encountered in sourdough is an indication of their common association with this substrate. This is not necessarily an exclusive association with sourdoughs and some of the species have to be considered as generalists, able to thrive in a wide range of environmental conditions, as for example *W. anomalus* [51], while a specific ecological niche of the most frequently encountered species *S. cerevisiae* has been elucidated [52]. Other species are less frequently detected in sourdough, namely *Candida glabrata*; *P. membranifaciens* (anamorph *Candida valida*); *Candida parapsilosis*; *Candida tropicalis*; *Candida stellata* (syn. *Torulopsis stellata*); *K. unispora* (syn. *S. unisporus*, *Torulopsis unisporus*); *Kluyveromyces marxianus* (anamorph *Candida kefyr*); *M. guilliermondii* (syn. *Pichia guilliermondii*, anamorph *Candida guilliermondii*); and *Saccharomyces pastorianus*. Finally, 14 species have been mentioned only in single reports and may be considered as rather transient or present fortuitously in the sourdough ecosystem (Table 5.2).

[1] A taxon (plural taxa) refers to a group of individuals that are judged to form a single unit. A taxon may or may not be given name or rank (species, genus, family, etc.). Primarily, the term serves communication about taxonomic units without the necessity or the possibility to be more specific about them.

[2] Synonymous names and those of asporogenic forms (asexual or anamorphic forms, not producing ascospores) are only selectively mentioned if used in the sourdough literature. It is preferable to use the name of the sporogenous form (sexual or teleomorphic form) if it exists and if no strong reasons require the explicit referral to the asporogenous form.

Table 5.1 Overview of original studies that analyzed the yeast diversity of sourdoughs. Only species repeatedly obtained from sourdough are listed. Numbers in species columns indicate the number of obtained isolates. These numbers depend on the isolation method (i.e., one or multiple representatives of the same colony morphotype) and are mostly of value to judge species abundance within a given study. An "x" was used if the isolate number was not specified. Multiple x's indicate the relative abundance of a detected species if mentioned. Studies that report on more than one sourdough may include sourdoughs of differing yeast species composition. However, a more detailed assignment of yeast species to single sourdoughs was not possible as not all studies reported the necessary data. Synonyms or asporogenic forms (anamorphs) are listed only if used in the cited references or if resulting from recent taxonomic changes

Reference	Wheat (W), rye (R), sorghum (S), barley (B), maize (M), spelt (Sp), buckwheat (Bw), teff (T)	Number of analyzed sourdoughs	Phenotypic (P) or molecular (M) identification	Country	***Saccharomyces cerevisiae*** syn. *S. fructuum*	***Candida humilis***	*Candida milleri* syn. of *C. humilis*	***Pichia kudriavzevii*** syn. *Issatchenkia orientalis* Anamorph *C. krusei*	***Kazachstania exigua*** syn. *S. exiguus* Anamorph *C. (Torulopsis) holmii*	***Torulaspora delbrueckii*** Anamorph *C. colliculosa*	***Wickerhamomyces anomalus*** syn. *P. anomala, Hansenula anomala* Anamorph *C. pelliculosa*	***Candida glabrata***	***Pichia membranifaciens*** Anamorph *C. valida*	***Candida parapsilosis***	***Candida stellata*** syn. *T. stellata*	***Candida tropicalis***	***Kazachstania unispora*** syn. *S. unisporus, T. unisporus*	***Kluyveromyces marxianus*** Anamorph *C. kefyr*	***Meyerozyma guilliermondii*** syn. *P. guilliermondii* Anamorph *C. guilliermondii*	***Saccharomyces pastorianus***
[7]	W	5	P	USA		148[a]														
[8]	W	3	P	Italy					x											
[9]	R	2	P	Germany	11			27	4											
[10]	W	3	P	Spain	X															
[11]	R	20	P	Finland	11				6		2				1		1			
[12]	W	2	P	Spain	29														6	
[13]	W, R	10	P	Italy	X		x		x						x					
[14]	S	2	P	Sudan	[d]			x												

[15]	W	12	P	France	220				25	25	27						
[16]	B, M	10	P	Morocco	21[d]		23[b]										
[17]	W	24	P	Italy	51			13	12		1						
[18]	M	10	P	Ghana	x[d]			x						x		x	
[19]	W	7	P	Italy	25												
[20]	R, M, W	33	P	Portugal	27			11		13	5		12			1	
[21]	W	10	P	Italy	x[d]				xx								
[22]	R	5	P, M	Finland	3	10[c]											
[23]	R, M	n.s.	P	Portugal	7					1	2						
[24]	W	5	P, M	Greece	14								25				
[25]	R	2	P	Denmark	x[d]												
[26]	W	25	P	Italy	17			1	1								
[27]	n.s.	14	P, M	Italy	33[d]	2	7	8									
[28]	W	1	M	Italy	[d]	403											
[29]	R	3	P, M	Germany	48		36	50				19					
[30]	n.s.	35	P, M	Italy	118	42	7	8	3								
[31]	W	13	P, M	Italy	58[d]			3		5		2	3				
[32]	W	8	P, M	Italy	6[d]		6										1
[33]	W	1	M	Italy	6[d]												
[34]	n.s.	35	P, M	Italy	102		48	8	3								
[35]	W	1	P, M	Italy	45		36										
[36]	W	1	P, M	Italy	13	27											
[37]	n.s.	7	M	Italy	23	36											2
[38]	W	10	P, M	Italy	37		15										
[39]	W	3	M	Italy	x		xx										
[40]	W, R, Sp	33	P, M	Belgium	102[d]					2	177	62			1		
[41]	W	4	M	Italy	60[d]	18	2										

(continued)

Table 5.1 (continued)

Reference	Wheat (W), rye (R), sorghum (S), barley (B), maize (M), spelt (Sp), buckwheat (Bw), teff (T)	Number of analyzed sourdoughs	Phenotypic (P) or molecular (M) identification	Country	***Saccharomyces cerevisiae***	***Candida humilis***	***Candida milleri***	***Pichia kudriavzevii***	***Kazachstania exigua***	***Torulaspora delbrueckii***	***Wickerhamomyces anomalus***	***Candida glabrata***	***Pichia membranifaciens***	***Candida parapsilosis***	***Candida stellata***	***Candida tropicalis***	***Kazachstania unispora***	***Kluyveromyces marxianus***	***Meyerozyma guilliermondii***	***Saccharomyces pastorianus***
[42]	W	4	P, M	Thailand	4[d]		1						1			1				
[43]	W	9	P, M	Italy	33	3														
[44]	W	15	P	Pakistan	x															
[45]	W	10	P, M	Greece	4[d]															163
[46]	W	20	P, M	Italy	49[d]		10	1		2		5								

[47]	Bw, T	2	M	Ireland	x[d]							x										
[48]	W	7	M	China	43	22	2	2	6	3			2						1			
Total occurrences					***38***	***19***	***14***	***12***	***8***	***7***	***4***	***3***	***2***	***2***	***2***	***2***	***2***	***2***	***2***	***2***	

[a]Originally designated as *S. exiguus*, but in culture collections deposited representative strains were reassigned to *C. humilis*. One of them served as the type strain for the description of the currently not taxonomically recognized population *C. milleri* (NRRL Y-7244, NRRL Y-7245, NRRL Y-7246, NRRL Y-7248; [49]). *C. humilis* and *C. milleri* isolates are listed here in partially separated columns as some studies distinguished them despite their taxonomic synonymy

[b]Labeled as *C. milleri* by the authors, ITS sequence of one strain deposited as CBS 7541 shows intermediate similarities to *C. humilis* and *C. milleri*, while its ITS RFLP HaeIII type is similar to *C. humilis*

[c]Retrospective identification based on the presence of type and reference strains [22]

[d]No baker's yeast added as explicitly mentioned by the authors

n.s. Not specified by the original authors

Table 5.2 Yeast species isolated from sourdough mentioned by single reports. None of these reports refer to these species as the sole or dominating yeast found in sourdough. Species considered as rare contaminants (e.g., *Schizosaccharomyces pombe*, *Rhodotorula glutinis*, *R. mucilaginosa*, *Endomycopsis fibulinger*) or reported as unidentified were not included

Species	Synonyms[a]	Reference
Candida boidinii		[12]
Candida parapsilosis		[42]
Hanseniaspora uvarum		[38]
Kodamaea ohmeri	*Pichia ohmeri*	[23]
Lachancea kluyveri	*Saccharomyces kluyveri*	[20]
Meyerozyma guilliermondii	*P. guilliermondii,* anamorph *C. guilliermondii*	[12]
Ogataea polymorpha	*P. polymorpha*	[10]
Pichia fermentans	Anamorph *C. lambica*	[31]
Pichia occidentalis	*Issatchenkia occidentalis*	[20]
Saccharomyces bayanus	*Saccharomyces inusitatus*	[7]
Saturnispora saitoi	*P saitoi*	[9]
Scheffersomyces stipitis	*P. stipitis*	[42]
Wickerhamomyces subpelliculosus	*P. subpelliculosa, Hansenula subpelliculosa*	[10]
Yarrowia lipolytica		[24]

[a]Synonyms or asporogenic forms (anamorphs) were listed only if used in the cited references or if resulting from recent taxonomic changes

The species complex *C. humilis/C. milleri* frequently detected in sourdough deserves a detailed elucidation. In terms of classification it was placed in the artificial genus *Candida*, because no sexual reproduction could be observed. Phylogenetically, *C. humilis/C. milleri* belongs to the same group as the genus *Kazachstania* [3]. It has, however, not yet been taxonomically placed in this genus, as the phylogenetic reclassification is treating sexually reproducing taxa with priority. The species *C. humilis* has been described based on a yeast strain associated with South African bantu beer, made from kaffir corn (*Sorghum caffrorum*) or finger millet (*Eleusine coracana*) [53]. The species *C. milleri* has been described to accommodate yeast strains isolated from San Francisco sourdough fermentations and initially assigned to *S. exiguus* [7, 49]. The basis for this reassignment were significantly higher guanine-plus-cytosine contents in selected San Franscisco sourdough strains compared to the type and other reference strains of *S. exiguus* and growth stimulation of *C. milleri* by calcium pantothenate. *C. humilis* and *C. milleri* were indicated to be conspecific based on their identical D1/D2 region large subunit (LSU) ribosomal DNA (rDNA) sequences, a locus that in rare cases may not suffice to resolve closely related species [54, 55]. DNA-DNA reassociation of at least 90% between the *C. milleri* type strain CBS 6897 and strain CBS 2664, the type strain of *T. holmii* var. *acidilactici*, a synonym of *C. milleri*, are of interest in this situation and confirms the conspecificity of these strains [54, 56]. Strain CBS 2664 shows ten substitutions in the internal transcribed spacer (ITS) regions of the rDNA, if compared to the type strain of *C. milleri*, indicating the degree of ITS divergence within this species.

In the sourdough context, *C. humilis* is often distinguished from its synonym *C. milleri* by targeting the ITS rDNA region [27]. This is done by restriction fragment length polymorphisms (RFLPs) of the ITS generated by the restriction enzyme *HaeIII* that is recognizing and cutting the nucleotide sequence GGCC. This site is made unrecognizable by a single nucleotide change from C to T (resulting in GGCT) in a population represented by the *C. milleri* type strain. As a result of this single nucleotide substitution the RFLP analysis shows two fragments, in contrast to three fragments for strains with the intact recognition site. A comparison of relevant publicly available ITS sequences (CBS 5658: AY046174, CBS 6897: AY188851, SY13: DQ104399; CBS 2664 and CBS 7541: yeast database at www.cbs.knaw.nl/) shows the transitional position of some strains between the type strain sequences of *C. humilis* and *C. milleri*, especially CBS 7541, indicative of a continuum of ITS sequence variants between both type strains. Therefore, these two taxa might be best considered as populations of one species. Eventual heterogeneity of the species *C. humilis*, especially of applied value, should be documented and accompanied by the deposition of the isolates in public culture collections for further study.

5.1.2 Taxonomy of Sourdough Lactic Acid Bacteria

LAB comprise a heterogeneous group of Gram-positive, nonsporulating, strictly fermentative lactic acid-producing bacteria that play an important role in the organoleptic, health-promoting, technological, and safety aspects of various fermented food products. As a result of natural contamination through the flour or the environment or by deliberate introduction via dough ingredients, a wide taxonomic range of LAB has also been found in sourdoughs. In sourdough environments, LAB live in association with yeasts and are generally considered to contribute most to the process of dough acidification, while yeasts are primarily responsible for the leavening. Although also obligately homofermentative LAB have been isolated from sourdoughs, obligately or facultatively heterofermentative LAB species have the best potential and competitiveness to survive and grow in this particular food environment [57, 58].

Initially, classification of LAB was based on morphology, ecology, and physiological characteristics [59]. At a later stage, also chemotaxonomical properties such as cellular fatty acid and cell wall composition were included. In LAB as well as in many other bacterial groups, phenotypic characters are often limited in their taxonomic usefulness for discrimination of closely related species and suffer from poor interlaboratory exchangeability. As a result, differentiation of LAB solely based on phenotypic traits is generally only considered reliable at the genus level. The introduction of DNA-based techniques such as genomic mol % GC, DNA-DNA hybridization, and sequencing of ribosomal RNA (rRNA) genes has brought significant changes to LAB taxonomy [60–62]. Especially the use of rRNA gene sequences as evolutionary chronometers has allowed the elucidation of phylogenetic relationships between LAB species. As a result, comparison of 16S rRNA gene sequences

with sequences in public online databases has become a standard approach for identification of unknown LAB isolates. However, the low evolutionary rate of ribosomal genes may compromise differentiation between LAB species exhibiting identical or nearly identical 16S rRNA gene sequences [63–66]. Alternatively, the use of multiple housekeeping genes encoding essential cellular functions has been proposed for sequence-based identification of LAB. For instance, classification of *Lactobacillus* species based on sequence analysis of the housekeeping genes *pheS* and *rpoA* proved to be highly congruent with 16S rRNA gene phylogeny [67].

The LAB species diversity associated with sourdoughs has been reviewed by several authors in recent years [57, 58, 68–70]. On the basis of these reviews, an updated overview of the LAB species most commonly found in fermented sourdough is compiled in Table 5.3. As is the case for many food ecosystems, this overview again highlights that lactobacilli are by far the most frequently recovered LAB species from sourdough ecosystems. The taxonomy of the genus *Lactobacillus* is extremely complex; according to the April 2011 update, at least 171 species names have so far been proposed in this genus (www.bacterio.cict.fr/l/lactobacillus.html). However, as several of the proposed species names have meanwhile been synonymized, the actual number of phylogenetically unique species is lower. In sourdoughs, more than 55 *Lactobacillus* species have been identified, of which the large majority are obligately heterofermentative (Table 5.3). Given the taxonomic complexity of this genus, accurate identification of unknown *Lactobacillus* isolates requires specific expertise, for example the use of methods that offer sufficient taxonomic resolution and the correct interpretation of identification results by comparison with complete and up-to-date databases. In most of the older studies, however, identification of lactobacilli mainly or even exclusively relied on phenotypic approaches with limited taxonomic resolution at species level. Therefore, it is safe to assume that some of the *Lactobacillus* species previously reported in sourdough environments may have been incorrectly identified at the species or even at the genus level. A typical example is the taxonomic situation in the *Lactobacillus plantarum* group where discrimination between the ubiquitous sourdough bacterium *Lb. plantarum* and the phylogenetically highly related *Lactobacillus paraplantarum* and *Lactobacillus pentosus* may be problematic when identification methods with insufficient taxonomic resolution are used. In this regard, Torriani and colleagues [71] were among the first to suggest that sequences of housekeeping genes such as *recA* rather than 16S rRNA gene sequences are recommended to distinguish between members of this phylogenetically tight species group. Likewise, several sourdough isolates initially assigned to *Lactobacillus alimentarius* may in fact belong to the later described and closely related *Lactobacillus paralimentarius* due to phenotypic misidentification [72]. Also in this case, it has been shown that molecular fingerprint- or sequence-based methods are required to differentiate between both species [67, 73]. In *Lactobacillus rossiae*, a remarkable intraspecific heterogeneity leading to the identification of several subspecific clusters based on *pheS* gene sequencing may complicate unambiguous identification of *Lb. rossiae* strains [77]. Finally, nomenclatural issues may also be a cause for taxonomic confusion. Corrections of originally misspelled specific epithets, such as "*Lactobacillus*

Table 5.3 LAB species generally associated with sourdough fermentation or found in fermented sourdoughs[a]

Obligately heterofermentative[b]	Facultatively heterofermentative	Obligately homofermentative
Lb. acidifarinae	*Lb. alimentarius*	*E. casseliflavus*
Lb. brevis	*Lb. casei/paracasei*	*E. durans*
Lb. buchneri	*Lb. coleohominis*	*E. faecalis*
Lb. cellobiosus	*Lb. kimchi*	*E. faecium*
Lb. collinoides	*Lb. paralimentarius*	*Lb. acidophilus*
Lb. crustorum	*Lb. pentosus*	*Lb. amylolyticus*
Lb. curvatus	*Lb. perolens*	*Lb. amylovorus*
Lb. fermentum	*Lb. plantarum*	*Lb. crispatus*
Lb. fructivorans	*Lb. sakei*	*Lb. delbrueckii* subsp. *delbrueckii*
Lb. frumenti	*P. acidilactici*	*Lb. farciminis*
Lb. hammesii	*P. dextrinicus*	*Lb. gallinarum*
Lb. hilgardii	*P. pentosaceus*	*Lb. gasseri*
Lb. homohiochii		*Lb. helveticus*
Lb. kefiri		*Lb. johnsonii*
Lb. kunkeei		*Lb. mindensis*
Lb. lindneri		*Lb. nagelii*
Lb. mucosae		*Lb. salivarius*
Lb. namurensis		*Lc. lactis* subsp. *lactis*
Lb. nantensis		*S. constellatus*
Lb. nodensis		*S. equinus*
Lb. oris		*S. suis*
Lb. panis		
Lb. parabuchneri		
Lb. pontis		
Lb. reuteri		
Lb. rossiae		
Lb. sanfranciscensis		
Lb. secaliphilus		
Lb. siliginis		
Lb. spicheri		
Lb. vaginalis		
Lb. zymae		
Le. citreum		
Le. gelidum		
Le. mesenteroides subsp. *cremoris*		
Le. mesenteroides subsp. *dextranicum*		
Le. mesenteroides subsp. *mesenteroides*		
W. cibaria		
W. confusa		

(continued)

Table 5.3 (continued)

Obligately heterofermentative[b]	Facultatively heterofermentative	Obligately homofermentative
W. hellenica		
W . kandleri		
W. paramesenteroides		
W. viridescens		

E. Enterococcus, Lb. Lactobacillus, *Lc. Lactococcus*, *Le. Leuconostoc*, *P. Pediococcus*, *S. Streptococcus*, *W. Weissella*

[a]Data compiled from [57, 58, 68, 70]

[b]Classification in glucose fermentation types according to Felis and Dellaglio [59]

sanfrancisco" [75] (now *Lactobacillus sanfranciscensis*) and "*Lactobacillus rossii*" [76] (now *Lb. rossiae*), and the synonymization of species, such as the recognition of *Lactobacillus suntoryeus* as a synonym of *Lactobacillus helveticus* [77], can take a while to be introduced in subsequent taxonomic literature.

Triggered by the introduction of molecular DNA-based taxonomic methods in sourdough microbiology and the growing number of 16S rRNA gene sequences in public databases, in recent years many new *Lactobacillus* species have been described which were first isolated from a sourdough environment. Since 2000, 13 new *Lactobacillus* species originally isolated from sourdoughs have been proposed, i.e., *Lactobacillus frumenti* [78], *Lactobacillus mindensis* [79], *Lactobacillus spicheri* [80], *Lactobacillus acidifarinae* [81], *Lactobacillus zymae* [81], *Lactobacillus hammesii* [82], *Lb. rossiae* [76], *Lactobacillus siliginis* [83], *Lactobacillus nantensis* [84], *Lactobacillus secaliphilus* [85], *Lactobacillus crustorum* [86], *Lactobacillus namurensis* [87], and *Lactobacillus nodensis* [88] (Table 5.3). However, many of these species have only been reported once or are rarely isolated from this type of fermented food, and are represented by only a few strains. Therefore, it is not clear which of these species are really typical for sourdough environments and if so, what their geographical distribution is. In fact, only a few *Lactobacillus* species such as the obligately heterofermentative *Lb. sanfranciscensis* and the facultatively heterofermentative *Lb. paralimentarius* seem to be optimally adapted to this specific environment and are rarely isolated from other sources. Other heterofermentative species such as *Lactobacillus brevis* and the facultatively heterofermentative *Lb. plantarum* are also frequently isolated from fermented sourdoughs, but have also been found in many other food and nonfood environments [69].

Although the LAB microbiota of sourdoughs is clearly dominated by lactobacilli, other less predominant or subdominant LAB species may also be found, including members of the genera *Weissella*, *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Streptococcus* (Table 5.3). Of these, specific species of *Weissella*, *Pediococcus,* and *Leuconostoc* are particularly well adapted to survive and grow in plant-derived materials [57, 89]. The taxonomy of the latter three genera is much less complex than in *Lactobacillus*, and their presence in sourdoughs is restricted to only a few species. Weissellas are obligately heterofermentative LAB of which a

number of species produce dextran, the best-documented exopolysaccharide formed by heterofermentative LAB. In sourdoughs, the dextran-producing species *Weissella cibaria* and *Weissella confusa* are most frequently found. Both taxa are positioned together on one of the four phylogenetic branches in this genus based on 16S rRNA gene analyses [90], but can be differentiated using restriction analysis of the amplified 16S rDNA [91], randomly amplified polymorphic DNA-PCR (RAPD-PCR) [92], and PCR targeting the ribosomal ITS [26]. Within the facultatively heterofermentative pediococci, the species *Pediococcus acidilactici* and *Pediococcus pentosaceus* are most commonly found in sourdoughs. Differentiation between these two biochemically and phylogenetically related species can be achieved by fingerprinting methods such as ribotyping [93, 94], restriction analysis of the amplified 16S rDNA (16S-ARDRA) [95], RAPD-PCR [96, 97], and by sequence analyses of the 16S rRNA gene, the ribosomal ITS regions and the heat-shock protein 60 gene [98]. In the obligately heterofermentative genus *Leuconostoc*, the majority of sourdough isolates so far identified belong to *Leuconostoc mesenteroides* and *Leuconostoc citreum.* In the former species, further taxonomic distinction is made at subspecies level between *Le. mesenteroides* subsp. *mesenteroides*, *Le. mesenteroides* subsp. *dextranicum,* and *Le. mesenteroides* subsp. *cremoris* (www.bacterio.cict.fr/l/leuconostoc.html). The three subspecies can be separated by RAPD-PCR fingerprinting [99].

5.2 Microbial Species Diversity of Sourdoughs

5.2.1 Influence of Geography

5.2.1.1 The Origin of Sourdough

Historically, sourdough production started as a *conditio sine qua non* to process cereals for the production of baked goods [100]. Indeed, thousands of years ago the first bread production must have been based on spontaneous wild lactic acid fermentation whether or not associated with yeasts and with little or no leavening. Leavening could not have been very pronounced because of the use of barley (*Hordeum vulgare*) and ancient grains [such as spelt (*Triticum aestivum* subsp. *spelta*), emmer (*Triticum turgidum* subsp. *dicoccum*), and kamut (*T. turgidum* subsp. *turanicum*)] in early times and no addition of yeast for leavening. This form of flat (sour) bread production is still daily practice in many countries of the world, in particular in African countries and the Middle East. Leavening must have been an accidental discovery when yeasts from the air or the flour were allowed to ferment the cereal dough mixture extensively. However, it was only from the late nineteenth century onwards that yeast starter cultures were introduced for bread production from wheat (*T. aestivum* and *Triticum durum*) flour, first by using brewing yeasts as remnants of beer production followed by intentionally produced commercial bakers' yeast, *S. cerevisiae*. Consequently, sourdough bread must have been consumed

for a long time. Also afterwards, bread production in countries relying on cereals other than wheat such as rye (*Secale cereale*) had still to be supported by lactic acid fermentation. Rye bread baking requires dough acidification to inhibit the abundant α-amylase in the rye flour and to make rye starch and pentosans more water-retaining to form a good dough texture since not enough gluten is present in rye. Hence, various (rye) breads from Germany, Central European countries, and Scandinavia are based on sourdough. In the USA, sourdough was the main base of bread supply in Northern California during the California Gold Rush, because of the easy way to store it and to keep it active for daily bread production. Today, San Francisco sourdough bread is commercially produced in the San Francisco area and it remains a part of the culture of the San Francisco bay area. In the early 1970s, the responsible sourdough bacterium was identified as *Lb. sanfrancisco*, now *Lb. sanfranciscensis*, named according to the area where it was discovered [75]. Notice that this LAB species is actually identical to *Lb. brevis* var. *lindneri* (now *Lb. sanfranciscensis*), which was found to be responsible for various sourdough breads produced in Europe [17]. Also, it was shown that these sourdough LAB species occur in a stable association with the yeasts *C. humilis* (syn. *C. milleri*) and *K. exigua* (syn. *S. exiguus*), respectively [7, 101]. Nowadays, sourdough is used for its technological (dough processing and bread texture, flavor, and shelf life) and nutritional effects [57, 58, 69, 102, 103]. Moreover, sourdough products are appreciated for their traditional value, gastronomic quality, and natural and healthy status [104].

5.2.1.2 The Origin of Sourdough Variation

Thanks to the fact that craftsmanship has determined bakery practice for a long time, a huge variety of bakery products, in particular those based on sourdough, exists, which may differ considerably from region to region. Most of these products, including breads, cakes, snacks, and pizzas, originate from very old traditions. For instance, in Italy, numerous different types of sourdough breads exist, often called according to the name of the region, such as Altamura bread and Pugliese bread [101]. Also, seasonal varieties exist, which are traditionally produced on the occasion of religious festivities. For instance, Panettone cake in Milan and Pandoro in Verona are made for Christmas, while Colomba is a Milanese cake made for Easter. Alternatively, sourdough-based products such as crackers, French baguettes, and Italian ciabattas are much more common, although the original sourdough recipe has been replaced by the faster growing bakers' yeast that is in only few cases allowed to ferment longer to enable contaminating LAB to develop for flavor formation (pre-doughs or type 0 sourdoughs). Fortunately, numerous bakery sourdoughs have been kept alive for tens of years through backslopping procedures, i.e., repeated cyclic re-inoculation of a new batch of flour and water from a previous one with a so-called "sour" during refreshment of the flour-water mixture by the baker, thereby assuring the quality of the baked goods produced thereof. It turned out that these traditional sourdoughs harbor a mixture of distinctive yeast and LAB strains, which may be held responsible for the typical organoleptic quality of the breads

made thereof, as backslopping results in a prevalence of the best-adapted strains [57, 68, 69]. This diversity of natural sourdough starters likely accounts for the variety of artisan sourdoughs produced by bakeries, whether or not typical for a certain geographical region. Alternatively, sourdough starter cultures, comprised of one or more defined strains, are commercially available now. In addition to *Lb. sanfranciscensis* strains, commercially available strains of other LAB species include *Lb. brevis*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, and *Lb. plantarum*, albeit that not all strains in use are competitive enough to dominate the sourdough fermentation processes that have to be started up [69]. These starter cultures are used for rapid acidification of the raw materials and flavor formation upon fermentation. Also, industrial manufacturers produce dried sourdough powders that are used as nonliving flavor ingredients in industrial bread production.

5.2.1.3 Region-Specific Sourdoughs and Their Associated Microbiota

Whereas it was initially thought that a relationship could be seen between the presence of certain LAB species and the geographical origin of a particular sourdough, it turned out through systematic and detailed taxonomic investigations that the species diversity of both LAB and yeasts of local sourdoughs has nothing to do with the geography of the sourdough production process (Tables 5.1 and 5.4; 57, 68, 69). For instance, the typical sourdough bacterium of San Francisco sourdough bread of the San Francisco bay area, *Lb. sanfranciscensis*, has been found in various wheat sourdoughs throughout Europe, and hence its (unique) presence should be ascribed to other factors, which are mainly based on the fermentation technology and practical conditions applied [121, 152–154]. In various countries, such as in Italy, several studies have been focused on region-specific sourdoughs (Table 5.4). However, no clear-cut relationship could be shown between for the region typical sourdoughs and their associated microbiota. In contrast, Italian sourdoughs harbor simple to very complex communities of LAB species depending on the final products examined, among which *Lb. brevis, Lb. (par) alimentarius, Lb. plantarum, Lb. sanfranciscensis*, *Lb. fermentum, P. pentosaceus*, and *W. confusa* are widespread. Similarly, Belgian bakery sourdoughs have been analyzed extensively and are characterized by LAB consortia of *Lb. brevis, Lb. hammesii, Lb. nantensis, Lb. paralimentarius*, *Lb. plantarum, Lb. pontis, Lb. sanfranciscensis*, and/or *P. pentosaceus* [105, 106, 155]. Sourdoughs with both stable large and stable restricted species diversities may occur [106]. Also, *Lb. rossiae* seems to have a wide distribution in sourdoughs, as has been shown through its isolation from sourdoughs in Central and Southern Italy, Belgium, and elsewhere [74, 105, 106, 156–158]. LAB species are responsible for the acidification of the dough and contribute to flavor formation. Besides LAB species, a large variety of yeast species are found in sourdough ecosystems (Tables 5.1 and 5.2). *S. cerevisiae* has been found in almost every sourdough study (38 out of 42 reviewed) regardless as to whether or not bakers' yeast is added (14 studies mention *S. cerevisiae*, although they indicate that no bakers' yeast was added). A single sourdough usually harbors only one or two yeast species at a given time, among which *C. humilis* (and *K. exigua*) and *P. kudriavzevii* occur most frequently [40]. Yeasts are responsible for the leavening of the dough and also contribute to flavor formation.

Table 5.4 Nonexhaustive overview of the species diversity of LAB in sourdoughs made from various flours and of different geographical origins. The method of identification of the LAB species is indicated

Country	Cereal flour type Method of identification	LAB species reported	Reference(s)
Belgium	Wheat Molecular	*Lb. acidifarinae, Lb. brevis, Lb. buchneri, Lb. crustorum, Lb. hammesii, Lb. helveticus, Lb. namurensis, Lb. nantensis, Lb. parabuchneri, Lb. paracasei, Lb. paralimentarius, Lb. plantarum, Lb. pontis, Lb. rossiae, Lb. sakei, Lb. sanfranciscensis, Lb. spicheri, Leuc. mesenteroides, P. pentosaceus, W. cibaria, W. confusa*	[86, 87, 105, 106]
	Rye Molecular	*Lb. brevis, Lb. hammesii, Lb. nantensis, Lb. paralimentarius, Lb. plantarum*	[105, 106]
	Wheat-rye Molecular	*E. mundtii, Lb. brevis, Lb. curvatus, Lb. fermentum, Lb. paracasei, Lb. paralimentarius, Lb. plantarum, Lb. pontis, Lb. rossiae, Lb. sanfranciscensis, P. acidilactici, P. pentosaceus, W. confusa*	[105, 106]
	Spelt Molecular	*Lb. brevis, Lb. curvatus, Lb. hammesii, Lb. nantensis, Lb. paralimentarius, Lb. plantarum, Lb. pontis, Lb. rossiae, Lb. sakei, Lb. sanfranciscensis, P. pentosaceus, W. cibaria*	[105, 106]
	Wheat (laboratory) Molecular	*Lb. fermentum, Lb. plantarum*	[107]
	Spelt (laboratory) Molecular	*Lb. brevis, Lb. paraplantarum, Lb. plantarum, Lb. rossiae*	[107]
	Wheat (laboratory) Molecular + microarray	*Lb. curvatus, Lb. fermentum, Lb. plantarum, P. pentosaceus*	[108]
	Spelt (laboratory) Molecular + microarray	*Lb. fermentum, Lb. plantarum, P. pentosaceus, W. confusa*	[108]
	Rye (laboratory) Molecular	*Lb. brevis, Lb. fermentum, Lb. plantarum, P. pentosaceus*	[109]

China	Wheat Molecular	*Lb. brevis, Lb. crustorum, curvatus, Lb. fermentum, Lb. guizhouensis, Lb. helveticus, Lb. mindensis, Lb. paralimentarius, Lb. plantarum, Lb. rossiae, Lb. sanfranciscensis, Lb. zeae, Lc. lactis, Leuc. citreum, W. cibaria, W. confusa*	[48]
Denmark	Rye Phenotypical	*Lb. amylovorus, Lb. panis, Lb. reuteri*	[25]
Ethiopia	Teff (enjera) Phenotypical	*E. faecalis, Lb. brevis, Lb. fermentum, Lb. plantarum, Leuc. mesenteroides, P. cerevisiae*	[110]
Finland	Rye Phenotypical	*Lb. acidophilus, Lb. casei, Lb. plantarum*	[11]
France	Wheat Phenotypical	*Lb. acidophilus, Lb. brevis, Lb. casei, Lb. curvatus, Lb. delbrueckii* subsp. *delbrueckii, Lb. plantarum, Leuc. mesenteroides* subsp. *dextranicum, Leuc. mesenteroides* subsp. *mesenteroides, P. pentosaceus*	[111]
	Wheat Phenotypical	*Lb. brevis, Lb. curvatus, Lb. plantarum, Lb. rhamnosus, Leuc. mesenteroides, P. pentosaceus*	[112]
	Wheat Molecular	*Lb. frumenti, Lb. hammesii, Lb. nantensis, Lb. panis, Lb. paralimentarius, Lb. pontis, Lb. sanfranciscensis, Lb. spicheri, Leuc. mesenteroides* subsp. *mesenteroides*	[113]
	Wheat Molecular	*Lb. frumenti, Lb. hammesii, Lb. nantensis, Lb.panis, Lb. paralimentarius, Lb. sanfranciscensis, Lb. spicheri, Leuc. mensenteroides* subsp. *mensenteroides*	[114]
	Wheat Molecular	*E. hirae, Lb. brevis, Lb. curvatus, Lb. paracasei, Lb. paraplantarum, Lb. pentosus, Lb. plantarum, Lb. sakei, Lb. sanfranciscensis, Lc. lactis, Leuc. citreum, Leuc. mesenteroides, P. pentosaceus, W. cibaria, W. confusa*	[115]
Germany	Wheat Phenotypical	*Lb. brevis, Lb. buchneri, Lb. casei, Lb. delbrueckii, Lb. fermentum, Lb. plantarums*	[116]

(continued)

Table 5.4 (continued)

Country	Cereal flour type: Method of identification	LAB species reported	Reference(s)
	Rye Phenotypical	*Lb. acidophilus, Lb. alimentarius, Lb. brevis, Lb. buchneri, Lb.casei, Lb. farciminis, Lb. fermentum, Lb. fructivorans, Lb. plantarum, Lb. sanfranciscensis*	[9, 117]
	Rye – use of a starter Phenotypical	*Lb. acidophilus, Lb. alimentarius, Lb. brevis, Lb. buchneri, Lb. casei, Lb. farciminis, Lb. fermentum, Lb. fructivorans, Lb. plantarum, Lb. sanfrancis-censis, Pediococcus* spp.	[118]
	Wheat (Panettone, wheat bread from Germany, Italy, Sweden, and Switzerland) Phenotypical	*Lb. brevis, Lb. casei, Lb. farciminis, Lb. hilgardii, Lb. homohiochii, Lb. plantarum* (spontaneous) *Lb. brevis, Lb. hilgardii, Lb. sanfranciscensis,W. viridescens* (masa madre)	[119]
	Rye Molecular	*Lb. amylovorus, Lb. frumenti, Lb. pontis/panis, Lb. reuteri* (type II)	[120]
	Rye (bran) – use of a starter Molecular	*Lb. mindensis, Lb. sanfranciscensis* (rye, type I) *Lb. crispatus, Lb. fermentum, Lb. frumenti, Lb. panis, Lb. pontis* (rye, type II) *Lb. johnsonii, Lb. reuteri* (rye bran, type II)	[29]
	Rice Phenotypic + molecular	*Lb. paracasei, Lb. paralimentarius, Lb. spicheri*	[80]
	Wheat Molecular	*E. faecium, Lb. casei, Lb. fermentum, Lb. plantarum, Lb. sanfranciscensis, P. pentosaceus, W. confusa*	[121]
	Rye Molecular	*Lb. pontis, Lb. sanfranciscensis*	[121]

	Amaranth (from India, Peru, Mexico, Germany)	*Lb. plantarum, Lb. sakei, P. pentosaceus*	[122]
	Molecular		
	Cereals and pseudocereals	*Lb. fermentum, Lb. helveticus, Lb. paralimentarius, Lb. plantarum, Lb. pontis, Lb. spicheri*	[123]
	Molecular		
Greece	Wheat	*Lb. brevis, Lb. paralimentarius, Lb. sanfranciscensis, Lb. zymae, W. cibaria*	[72]
	Phenotypical + molecular		
Italy	Wheat (panettone)	*Lb. brevis, Lb. plantarum*	[8]
	Phenotypical		
	Wheat (panettone, brioche)	*Lb. fermentum, Lb. plantarum, Lb. sanfranciscensis, Leuc. mesenteroides, Pediococcus* spp.	[13]
	Phenotypical		
	Wheat (Umbria)	*Lb. farciminis, Lb. plantarum, Lb. sanfranciscensis*	[17]
	Phenotypical		
	Pizza from Naples	*Lb. plantarum, Lb. sakei, Leuc. gelidum, Leuc. mesenteroides*	[124]
	Phenotypical		
	Wheat (Molise, Umbria, Venise)	*Lb. sanfranciscensis*	[125, 126]
	Molecular		
	Mother sponges (Lombardy)	*Lb. sanfranciscensis*	[21]
	Molecular		
	Wheat (Apulia)	*Lb. acidophilus, Lb. alimentarius, Lb. brevis, Lb. delbrueckii* subsp. *delbrueckii, Lb. fermentum, Lb. plantarum, Lb. sanfranciscensis*, *Lc. lactis* subsp. *lactis*, *Leuc. citreum, W. confusa*	[26]
	Molecular		
	Wheat (Southern Italy)	*Lb. alimentarius, Lb. brevis, Lb. farciminis, Lb. fermentum, Lb. fructivorans, Lb. planta rum, Lb. sanfranciscensis, W. confusa*	[127]
	Molecular		

(continued)

Table 5.4 (continued)

Country	Cereal flour type / Method of identification	LAB species reported	Reference(s)
	Wheat (Molise) Molecular	*Lb. arizonensis/plantarum, Lb. brevis, Lb. kimchii/paralimentarius, Lb. paraplantarum, Lb. sanfranciscensis*	[33]
	Wheat (Sicily) Molecular	*Lb. casei, Lb. kimchii/alimentarius, Lb. plantarum, Lb. sanfranciscensis*	[128]
	Wheat (Molise) Molecular	*Lb. brevis, Lb. fermentum, Lb. plantarum, Lb. sanfranciscensis*	[129]
	Wheat (Altamura, Apulia) Phenotypical + molecular	*Lb. brevis, Lb. casei, Lb. paracasei, Lb. plantarum, Leuc. mesenteroides*	[130]
	Wheat (Sardinia) Molecular	*Lb. brevis, Lb. pentosus, Lb. plantarum, Lb. sanfranciscensis, W. confusa*	[131]
	Wheat (Abruzzo) Physiological + molecular	*Lb. alimentarius, Lb. brevis, Lb. farciminis, Lb. fermentum, Lb. fructivorans, Lb. frumenti, Lb. hilgardii, Lb. mindensis, Lb. panis, Lb. paralimentarius, Lb. paraplantarum, Lb. pentosus, Lb. plantarum, Lb. pontis, Lb. rossiae, Lb. sanfranciscensis*	[133]
	Wheat (Abruzzo) Molecular	*E. faecium, P. pentosaceus*	[133]
	Wheat (Panettone from Central Italy) Molecular	*Lb. brevis, Lb. farciminis, Lb. plantarum, Lb. sanfranciscensis, Leuc. mesenteroides*	[39]
	Wheat (Cornetto, Basilicata) Phenotypic + molecular	*Lb. curvatus, Lb. paraplantarum, Lb. pentosus, Lb. plantarum, Leuc. mensenteroides, W. cibaria*	[134]
	Wheat – use of a starter Phenotypic + molecular	*Lb. brevis, Lb. paralimentarius, Lb. plantarum, Lb. sanfranciscensis, Lc. lactis, Leuc. durionis, Leuc. fructosus, P. pentosaceus, W. cibaria*	[41]

	Wheat (Marche) Phenotypic + molecular	*Lb. brevis, Lb. casei, Lb. curvatus, Lb. fermentum, Lb. paracasei, Lb. paralimentarius, Lb. plantarum, Lb. rhamnosus, Lb. sakei, Lb. sanfranciscensis, Leuc. citreum, Leuc. pseudomesenteroides, W. cibaria, W. confusa*	[43]
	Wheat – use of a starter Molecular	*Lb. paralimentarius, Lb. plantarum, Lb. rossiae, Lb. sanfranciscensis, Lc. lactis* subsp. *lactis, P. pentosaceus, W. cibaria, W. cibaria/confusa*	[135]
	Barley – use of a starter Molecular	*Lb. alimentarius, Lb. brevis, Lb. paralimentarius, Lb. plantarum*	[136]
	Emmer Molecular	*Lb. plantarum, Lb. rossiae, W. confusa*	[137]
	Spelt Molecular	*Lb. brevis, Lb. curvatus, Lb. plantarum, Lb. sanfranciscensis, P. pentosaceus/acidilactici, W. confusa*	[137]
	Wheat Molecular	*Lb. plantarum, Lb. rossiae, P. pentosaceus*	[138]
	Wheat germ Molecular	*Lb. plantarum, Lb. rossiae, P. pentosaceus, W. confusa*	[139]
Iran	Sangak Phenotypical	*Lb. brevis, Lb. plantarum, Leuc. mesenteroides, P. cerevisiae*	[140]
Ireland	Oat Molecular	*Lb. coryniformis, Leuc. argentinum, P. pentosaceus, W. cibaria*	[141]
	Buckwheat Molecular	*Lb. crispatus, Lb. fermentum, Lb. gallinarum, Lb. graminis, Lb. plantarum, Lb. sakei, Lb. vaginalis, Leuc. holzapfelii, P. pentosaceus, W. cibaria*	[47]
	Buckwheat – use of a starter Molecular	*Lb. amylovorus, Lb. brevis, Lb. fermentum, Lb. frumenti, Lb. paralimentarius, Lb. plantarum, Leuc. argentinum, W. cibaria*	[142]
	Teff Molecular	*Lb. fermentum, Lb. gallinarum, Lb. pontis, Lb. vaginalis, Leuc. holzapfelii, P. pentosaceus*	[47]

(continued)

Table 5.4 (continued)

Country	Cereal flour type Method of identification	LAB species reported	Reference(s)
	Teff – use of a starter Molecular	*Lb. amylovorus, Lb. brevis, Lb. fermentum, Lb. frumenti, Lb. paralimentarius, Lb. plantarum, Lb. pontis, Lb. reuteri, Lb. sanfranciscensis, P. acidilactici*	[142]
Mexico	Maize (pozol) Molecular	*Lb. alimentarius, Lb. casei, Lb. delbrueckii, Lb. lactis, Lb. plantarum, S. suis*	[143]
Morocco	Sourdough ferments, traditional starter sponges Phenotypical	*Lb. brevis, Lb. buchneri, Lb. casei, Lb. plantarum, Leuc. mesenteroides, Pediococcus* spp.	[16]
	Soft wheat flour Phenotypical	*Lb. buchneri, Lb. casei, Lb.delbrueckii, Lb. plantarum, Lb. sanfranciscensis, Leuc. mesenteroides, P. pentosaceus*	[144]
Nigeria	Maize	*Lb. brevis, Lb. casei, Lb. fermentum, Lb. plantarum, Leuc. mesenteroides, P. acidilactici*	[145]
Portugal	Maize (broa, Northern Portugal) Phenotypical	*E. casseiliflavus, E. durans, E. faecium, Lb. brevis, Lb. curvatus, Lc. lactis subsp. lactis, Leuconostoc* spp., *S. constellantus, S. equines*	[21]
Russia	Rye Phenotypical	*Lb. brevis, Lb. fermentum, Lb. plantarum*	[146]
Spain	Wheat Phenotypical	*Lb. brevis, Lb. plantarum*	[10]
	Wheat Phenotypical	*Lb. brevis, Lb. cellobiosus, Lb. plantarum, Leuc. mesenteroides*	[12, 147]
Sudan	Sorghum (kisra) Phenotypical	*Lb. amylovorus, Lb. fermentum, Lb. reuteri*	[14]
	Kisra Molecular	*E. faecalis, Lb. fermentum, Lb. helveticus, Lb. reuteri, Lb. vaginalis, Lc. lactis*	[148]

Sweden	Rye-wheat Phenotypical	*Lb. acidophilus, Lb. brevis, Lb. delbrueckii, Lb. farciminis, Lb. fermentum, Lb. plantarum, Lb. rhamnosus, Lb. sanfranciscensis, W. viridescens*	[149]
	Rye Phenotypical	*Lactobacillus* sp., *P. pentosaceus*	[150]
Thailand	Wheat Phenotypic + molecular	*Lb. casei, Lb. plantarum*	[42]
Turkey	Wheat Phenotypic	*C. divergens, Lb. acetotolerans, Lb. amylophilus, Lb. brevis, Lb. plantarum, Lb. sakei, P. acidilactici, P. pentosaceus, T. halophilus*	[151]
USA	Wheat Phenotypic	*Lb. sanfranciscensis*	[75]
	Wheat Molecular	*Lb. paralimentarius, Lb. plantarum, Lb. sanfranciscensis*	[121]

C. Carnobacterium, E. Enterococcus, Lb. Lactobacillus, *Lc. Lactococcus*, *Le. Leuconostoc*, *P. Pediococcus*, *S. Streptococcus*, *W. Weissella*

Single isolations of yeast and LAB species have caused former misinterpretations of their association with certain sourdough-producing regions, not only because of the random isolation itself but also regarding the single habitat (sourdough) explored. Thus, the association of, for instance, *Lb. spicheri* with German rice sourdoughs [80] might represent accidental discoveries [57, 68, 69]. Instead, the dedicated use of basic raw materials as well as the technological procedures applied rather determines the stability and persistence of the yeast and LAB communities involved in the sourdough fermentation process. Indeed, the presence of *Lb. sanfranciscensis* in wheat sourdoughs, which was for a long time the sole habitat wherein this LAB species could be found [now, it has been detected in rye sourdoughs (Table 5.4) and the insect gut as well [159]], can be ascribed to its selection by the type of technology applied, i.e., backslopping practices, temperature of incubation of the dough, pH of the dough, and/or microbial interactions [113, 121, 135, 160, 161]. However, the use of certain raw materials, encompassing cereal types and other ingredients such as adjunct carbohydrates, salt, yoghurt, herbs, etc., and operational practices, such as dough yield and refreshment times, may be linked to local traditions, may favor particular microorganisms as a result of trophic and metabolic relationships and interactions (both cooperation and antibiosis), and hence may associate specific LAB and/or yeast species with specific geographical regions. For instance, the use of rye may select for amylase-positive homofermentative *Lactobacillus amylovorus*, although higher temperatures cause a shift toward the predominance of heterofermentative lactobacilli [120, 162]. The dominance of mainly heterofermentative LAB species in traditional sourdoughs is caused by a highly adapted carbohydrate metabolism, a dedicated amino acid assimilation, and environmental stress responses [57, 58, 68, 163–166]. In particular, maltose, as the most abundant fermentable energy source in dough, is metabolized via the maltose phosphorylase pathway and the pentose phosphate shunt by strictly heterofermentative LAB species such as *Lb. sanfranciscensis*, *Lactobacillus reuteri*, and *Lb. fermentum*. This efficient maltose metabolism coupled to the use of external alternative electron acceptors such as fructose, together with specific pathways such as the arginine deiminase (ADI) pathway, and various environmental stress responses such as response to acidic conditions, increases its competitiveness in the harsh sourdough environment [167–172]. Moreover, maltose-positive LAB species such as *Lb. sanfranciscensis* often form a stable association with maltose-negative yeast species such as *C. humilis*, thereby preventing competition for the same carbohydrate sources [58, 164, 165]. Also, the production of specific inhibitory compounds, maintained through backslopping, such as the antibiotic reutericyclin produced by *Lb. reuteri*, may favor the dominance of this LAB species, as is the case for certain German type II sourdoughs [173].

5.2.2 *Influence of Cereals and Other Raw Materials*

Cereal flours are not sterile. Their microbiological stability is related to their low water activity. As yeasts and LAB naturally occur on plant materials, cereal flours carry both groups of microorganisms and competitive yeast and LAB species reach

numbers above those of the adventitious microbiota upon fermentation of a flour-water mixture [57]. However, whether the type of flour mainly directs the growth of sourdough LAB species remains controversial [25, 26, 47, 89, 105, 107, 121, 123, 135]. For instance, whereas it was assumed that rice sourdough fermentation selects for *Lb. spicheri* [80], this LAB species cannot always be found in rice sourdoughs [123]. This has been ascribed to competitiveness of the microorganisms that are present in the sourdough ecosystem. Indeed, microbial interactions between the spontaneous microbiota and an added sourdough starter culture may lead to the dominance of autochthonous LAB species and/or strains. Among other mechanisms, competitiveness may explain the apparent prevalence of LAB species in specific sourdough preparations, such as evidenced by single reports on *Lb. amylovorus* in rye sourdoughs [120], *Lactobacillus sakei* in amaranth sourdoughs [122], and *Lb. pontis* in teff sourdoughs [47]. Yet, spontaneous sourdough fermentations carried out in the laboratory with flour as the sole nonsterile ingredient indicate that the type and quality (microbiological and nutritional) of the cereal flour used is indeed an important source of autochthonous LAB and yeasts occurring in the ripe sourdoughs [40, 107]. Hence, the flour plays a key role in establishing stable microbial consortia within a short time. In this context, it has been shown that laboratory sourdoughs based on wheat, rye, or spelt, backslopped daily for 10 days at 30 °C, whether or not initiated with a *Lb. sanfranciscensis* starter culture [as tested in the case of wheat sourdough fermentations in the study of Siragusa *et al.* [135]], reach an equilibrium of LAB species through a three-step fermentation process: (1) prevalence of sourdough-atypical LAB species (e.g., *Enterococcus* spp. and *Lc. lactis* subsp. *lactis*); (2) prevalence of sourdough-typical LAB species (e.g., species of *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*); and (3) prevalence of highly adapted sourdough-typical LAB species (e.g., *Lb. fermentum* and *Lb. plantarum*) [107–109, 135]. Indeed, it has been shown that the LAB species *Lb. fermentum* (strictly heterofermentative) and *Lb. plantarum* (facultatively heterofermentative) dominate several sourdough fermentation processes, irrespective of the type of flour or the addition of starter cultures that are not robust enough [47, 108, 123, 136–138, 142, 172, 174, 175]. Concerning yeasts, *C. glabrata* and *W. anomalus* prevail during laboratory sourdough fermentations [40]. Further, it has been shown that previous introduction of flour into the bakery environment helps to build up a so-called house microbiota that serves as an important inoculum for subsequent bakery sourdough fermentations [155]. Indeed, LAB strains adapted to the sourdough and bakery environment (apparatus, air, etc.), which have been shown to be genetically indistinguishable, may be repetitively introduced in consecutive sourdough batches during backslopping. The widespread use of bakers' yeast may be responsible for the prevalence of *S. cerevisiae* in bakery sourdoughs [26, 29, 31, 34–36, 39, 40]. However, there are also indications through reliable molecular data of a large strain diversity of *S. cerevisiae* in single sourdoughs, that suggest an autochthonous wheat flour origin of this yeast species in sourdough too [27, 30, 34]. Supportive of an autochthonous origin of *S. cerevisiae* is also the presence of this species in rye flour [29]. Yet, during laboratory fermentations with flour as the sole nonsterile ingredient and without added bakers' yeast, other species such as *C. glabrata* and *W. anomalus*

emerge [40]. Anyway, the direct environment is another important source of (accidental) contamination of the flour by LAB and yeasts. Consequently, hygienic conditions in the sourdough and bakery environments will play a role as well. Finally, microorganisms occurring on cereals and subsequently in sourdoughs may be of intestinal origin, due to fertilization practices on the grain fields, mouse feces or insects in the flour mills, or fecal contamination of the sourdough production environment [70, 175–178]. It may explain the opportunistic presence of *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lb. reuteri*, and *Lb. rossiae*, which are common gastrointestinal inhabitants.

5.2.3 Influence of Technology

Besides the cereals and other dough ingredients, which are mainly responsible as the source of metabolic activity in the form of flour enzymes and endogenous microorganisms, specific technological process parameters determine the species diversity, number, and metabolic activity of the microorganisms (whether or not added) present in the stable, ripe sourdough. These process parameters include chemical composition and coarseness of the flour, leavening and storage temperature, fermentation time, pH, redox potential, dough yield, refreshment time and number of propagation steps, and interactions between the microorganisms [57, 102, 164, 165, 179].

Different types of sourdough exist, on the basis of the processing conditions and/or technology used for production, with a specific microbiota occurring in each type [57, 180]. Type I or traditional sourdoughs are manufactured by continuous, (daily) backslopping, at ambient temperature (<30 °C), to keep the microorganisms in an active state. Therefore, mother doughs are used as an inoculum for subsequent doughs by addition of the desired amount of dough to a fresh flour-water batch according to defined cycles of preparation. These small-scale sourdough productions are used in traditional (home-made) sourdough bread making. Natural sourdoughs frequently harbor *Lb. sanfranciscensis* and *C. humilis/K. exigua* as prevalent LAB and yeast species, respectively. Type II or industrial sourdoughs are produced through one-step propagation processes of long duration (typically 2–5 days) at a fermentation temperature above 30 °C and with high water content. These large-scale sourdough productions result in semifluid preparations, which are used as dough acidifiers or flavor ingredients. *Lb. amylovorus, Lb. fermentum*, *Lb. pontis*, and *Lb. reuteri* are commonly found in type II wheat and rye sourdoughs. Type III sourdoughs are prepared in dried form to be used as nonliving acidifier supplement and flavor carriers for (sourdough) bread production. In contrast to type I doughs, doughs of types II and III require the addition of baker's yeast for leavening.

Commercially available bulk starter cultures to prepare type II and III sourdoughs aim at standardizing the end products through acidification of and flavor formation in the dough [69, 181, 182]. New trends tend to develop starter cultures that lead to improved functional properties other than acidification and flavor formation, such as texture improvement, antibacterial and antifungal activities, and

health-promoting effects [69, 166]. In this context, strain robustness and fitness towards microbial competitors and environmental conditions should be the driving force in the selection of useful starters for sourdough fermentation processes, as it has been shown that autochthonous strains often emerge [69, 135, 138, 142]. However, studies on the industrial exploitation of sourdough starter cultures are scarce [183, 184]. Recently, the use of starter cultures in type I propagated sourdoughs has been investigated [89, 123, 135, 185]. It is of course well known that the fermentation temperature affects the ratio of lactic acid to acetic acid [185, 187]. In general, homofermentative LAB starter cultures are used at high temperature and for short fermentation times (e.g., 37 °C for 36 h) and heterofermentative LAB starter cultures are used at low temperature and for long fermentation times (e.g., 25 °C for 48 h), resulting in sourdoughs with mainly lactic acid and acetic acid, respectively. However, it would be of great value to know the circumstances for the expression of other functional properties that are of added value to sourdoughs [182].

The fermentation temperature, one of the criteria to distinguish type I and II sourdoughs, is essential for the community dynamics and stability of a sourdough microbiota [29, 160, 175, 179, 185, 188, 189]. For instance, spontaneous wheat sourdough backslopping fermentations (type I) carried out at 23 °C for 10 days select for *Le. citreum* instead of *Lb. fermentum* that prevails at 30 °C and 37 °C [175]. Similarly, rye fermentations initiated with commercial sourdough starter cultures maintain the presence of *Lb. mindensis* and *Lb. sanfranciscensis* at 25 °C (type I), but select for *Lactobacillus crispatus* and *Lb. pontis* at 30 °C and for *Lb. crispatus*, *Lb. frumenti*, and *Lb. panis* at 40 °C (both type II) [184]. Whereas *Lb. sanfranciscensis* prefers long fermentation times at relatively low temperature, conditions that often prevail during type I sourdough preparations, this species grows optimally at 32 °C [159, 189]. However, whereas *C. humilis* grows optimally at 27–28 °C but does not grow above 35 °C [160, 189], the association of *Lb. sanfranciscensis-C. humilis* grows optimally at 25 °C and 30 °C and may explain its stability between 20 °C and 30 °C [160, 190]. The abundance of *Lb. sanfranciscensis* in wheat sourdoughs made at ambient temperature indicates a low competitiveness of other LAB species such as *Lb. fermentum* that prefers higher temperatures for optimal growth. Similarly, temperature may be responsible for a selection toward *Lb. helveticus* during Sudanese sorghum sourdough fermentations, which are carried out at 37 °C [148].

For the growth of sourdough LAB, also the pH plays an important role [160, 179, 188, 189]. For instance, *Lb. sanfranciscensis* cannot grow below pH 3.8–4.0 [160, 189], whereas *C. humilis* is not influenced by the pH [190]. An optimal pH for growth of around 5.0 has been found for *Lb. sanfranciscensis*. This pH value corresponds approximately to that observed during the first stage of dough fermentation. However, the growth of lactobacilli is favored over yeast growth at pH values above 4.5 [160]. Hence, the rate of acidification of the dough may determine the level of *Lb. sanfranciscensis* in the dough. Natural sourdough fermentations displaying higher pH values are often dominated by a different microbiota, encompassing *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Weissella*, which are commonly present in the cereal flour [57, 133] or during the

early fermentation process but die off when a significant pH decrease occurs upon fermentation [89, 108].

Although sourdough fermentation proceeds anaerobically, the presence of oxygen in the beginning of the fermentation and when small amounts of dough (high ratio of surface to volume) are used may favor certain LAB and yeast species [179, 190, 192]. For instance, mild aeration has a positive influence on the competitiveness of *Lb. amylovorus* DCE 471 [179]. Similarly, *P. kudriavzevii* can only grow when enough oxygen is available during fermentation [190]. Further, the ionic strength and salt concentration of the dough affects microbial growth [160, 179, 193, 194]. Similarly, the presence of organic acids in and the buffering capacity of the flour influence growth of both yeasts and LAB [160, 179]. In general, sourdough LAB are acid-tolerant and their growth is favored in the presence of salt, as is the case for, for instance, *Lb. amylovorus* DCE 471 [188, 193]. Alternatively, the growth of *C. humilis* and *S. exiguus* is completely inhibited by 4% NaCl; also, the growth of these yeasts is strongly inhibited in the presence of acetic acid and to a much lesser extent by lactic acid [160, 179].

Whereas backslopping practices select for mainly heterofermentative LAB, the amounts of dough used for backslopping and the frequency of the refreshments determine the community dynamics and stability of the sourdough microbiota as well. The amount of backslopping dough defines the initial pH and in this way influences the growth and acidification rates of the LAB species involved [107, 189, 190]. Also, the amount of backslopping dough determines the dough yield and hence the availability of water (water activity of the dough). Short refreshment times may select for rapidly growing LAB species, which in turn depends on the fermentation temperature and influences the acidification rate. In this regard, *Lb. fermentum* is most competitive at 30 °C and 37 °C with backsloppings every 24 h, while a mixture of *Lb. fermentum* and *Lb. plantarum* prevails at 30 °C with backsloppings every 48 h [175]. This may explain why *Lb. sanfranciscensis* is sometimes missed during laboratory-scale fermentation processes [136, 175]. Also, a short refreshment time seems to favor *C. humilis* during sourdough fermentation compared to *S. cerevisiae* [190].

Finally, interactions between LAB and yeasts are an important aspect for the community dynamics and stability of the sourdough microbiota [50, 57, 165, 189, 195, 196]. Interactions encompass both cooperative and antagonistic ones. During some sourdough fermentation processes yeasts cannot develop at all, perhaps because of inhibition of yeast growth by nutritional competition or the presence of inhibitory compounds [47, 123, 175]. In other processes, mutualistic interactions lead to stable associations, not only between LAB species and yeasts (besides *Lb. sanfranciscensis*/*C. humilis*, also *Lb. sanfranciscensis*/*K. barnettii*, *Lb. plantarum*/*S. cerevisiae*, and *Lb. brevis*/*Candida* spp.) but also among LAB species (e.g., between *Lb. sanfranciscensis* and *Lb. plantarum* or *Lb. paralimentarius*) [40, 41, 58, 69, 142]. Nevertheless, the competitiveness of LAB and yeasts in sourdough seems to be strain-specific and not species-specific, as has been shown for *Lb. sanfranciscensis* [135] and *Lb. plantarum* strains [138] in wheat sourdoughs recently.

5.3 Isolation of Sourdough Yeasts and Lactic Acid Bacteria

5.3.1 Isolation of Sourdough Yeasts

Yeast isolation from mature sourdoughs is relatively uncomplicated as a stable sourdough usually harbors a homogeneous yeast population as part of the resident microbiota. However, the follow-up of different developmental stages of a sourdough or the search for minor components requires a strategy that is optimized towards the detection of subdominant components. Detailed information on food yeast isolation was provided by Deak [197]. Here, only a brief discussion of the currently practiced yeast isolation methods from sourdough is given, including some references to more complete methodological resources. After sample homogenization and dilution, yeast growth is suitably effected on solid media. This sequence of manipulations should be performed with minimal delay to avoid the settling of yeast cells and cell death. Diluents, usually distilled water, peptone water, saline, or Ringer solution, may influence the resulting cell counts, with peptone water having resulted in the highest cell recovery [198]. Overviews of classical growth media and isolation techniques for yeasts and foodborne yeasts are given by Yarrow [199] and Beuchat [200], respectively. The cultivation media used in sourdough analyses are rich media containing complex compounds such as peptone (e.g., Sabouraud agar), tryptone (e.g., Wallerstein Laboratory nutrient agar), yeast extract (e.g., yeast extract peptone dextrose agar), malt (e.g., yeast and malt extract agar, wort agar), and potato infusion (e.g., potato dextrose agar), together with an additional component to inhibit bacterial growth. Most often chloramphenicol is used as an antibiotic that can be added to the medium before sterilization without losing its activity. Acidification of the medium is sometimes used to restrict bacterial growth, while this is known to affect growth of some yeasts (namely of the genus *Schizosaccharomyces*). However, the acidity of the sourdough lets it appear unlikely for acid-sensitive strains to be present in a ripe sourdough. In general, most yeasts show good vegetative growth at room temperature, although some may grow at subzero temperatures and others up to 45 °C [201]. Cardinal growth temperatures of yeasts are species- and strain-specific. The most suitable incubation temperature for sourdough yeasts would be the temperature at which the sourdough is in its most active state. The most frequently applied temperatures are 25–30 °C. First yeast growth can under such conditions usually be observed after 2–3 days, while daily inspection of the plates for 5 up to 10 days is recommended to allow full differentiation of colony morphology and detection of more slowly growing components.

Other than the consideration of growth conditions, the selection of yeast colonies for further characterization and identification is the most important factor influencing the completeness of a diversity survey. Even though sourdough samples often present a homogeneous yeast population, one needs to bear in mind that the colony morphology of different yeast species is often very similar. Each observed morphotype should therefore be sampled more than once. A logical strategy would be to recover a number of colonies of each type that represents a reasonable percentage

of the total number of colonies of that particular morphotype. Each morphoptype's percentage provides important species abundance data in the sourdough if more than one yeast species is isolated. The generation of pure cultures is crucial before characterization and identification of the isolates. The purity should be tested microscopically and on antibiotic-free medium to exclude any carry-over of the accompanying bacterial microbiota.

5.3.2 Isolation of Sourdough LAB

Isolation of LAB from sourdough environments is challenging for three main reasons. First, sourdoughs are complex ecosystems not only in terms of their microbial composition but also in terms of the interactive effects among types of breadmaking processes and ingredients. The utilization of soluble carbohydrates by LAB and, thus, their energy yield are greatly influenced by the associated yeasts and vary according to the type of carbohydrates [195]. However, as many media for selective isolation of LAB incorporate yeast-inhibiting agents such as cycloheximide, pimaricin, and amphotericin B, the trophic interaction between LAB and yeasts is in these cases disturbed, which may affect the recovery potential of LAB strains that strongly rely on this association. Secondly, sourdough fermentation is a dynamic process in which fast-acidifying LAB initially dominate the ecosystem and are then gradually replaced by typical sourdough LAB that largely contribute to the organoleptic and textural properties of the end product. Depending on whether the early subdominant LAB and/or the final dominant LAB are the target of the isolation approach, it may thus be necessary to include multiple samples taken at different time points. Finally, the LAB communities in sourdoughs may consist of metabolically very diverse groups, including obligately homofermentative and facultatively or obligately heterofermentative species. As some of these species have specific growth requirements in terms of the incubation medium and conditions (e.g., temperature, pH, atmosphere, etc.), it seems inevitable that different medium formulations and/or sets of incubation parameters are required to cover the entire metabolic LAB spectrum present in a sourdough sample.

Initially, sourdough LAB were mostly isolated on de Man-Rogosa-Sharpe (MRS) medium [202], which is the general medium used for the isolation and enumeration of lactobacilli from fermented food products. The MRS medium contains glucose as the main carbohydrate source. Triggered by growing insights in the species diversity of sourdough-associated LAB, a number of more specialized media have been developed for the selective isolation of typical sourdough species. For the specific detection of *Lb. sanfranciscensis*, Kline and Sugihara [75] proposed the SourDough Bacteria medium which contains maltose as the carbohydrate source in addition to freshly prepared yeast extract (FYE) to further enhance growth. The Sanfrancisco medium was developed for the isolation and description of *Lb. pontis* and *Lb. mindensis* [79, 203]. This medium contains three carbohydrates (maltose, fructose, and glucose), FYE, cysteine and rye or wheat bran. In parallel to the design of new

media, several authors also described variations of the original MRS medium formulation for isolation of sourdough LAB. Vogel and co-workers [203] proposed a modified MRS medium, referred to as MRS "Vogel", with higher pH value (6.3), whereas the MRS5 medium [185] contains the three major carbohydrates present in the sourdough ecosystem (i.e., maltose, fructose, and glucose) in addition to cystein and a vitamin mixture. In subsequent studies, the MRS5 medium has been successfully used for the isolation of several novel *Lactobacillus* species from sourdough such as *Lb. spicheri* [80], *Lb. namurensis* [87], and *Lb. crustorum* [86]. From these recent descriptions, it thus appears that the use of a modified MRS formulation with a lowered pH (<6.0) and supplemented with an additional carbon source such as maltose and/or fructose as well as with amino acids and vitamins under anaerobic conditions is one of the most successful strategies for the isolation of (new) sourdough LAB species. In a recent study, the qualitative and quantitative performance of 11 elective and selective culture media was compared for isolation of lactobacilli from type I sourdoughs [204]. On the basis of the identification results obtained with protein profiling, the largest species diversity was recovered on maltose-containing MRS medium. However, the fact that MRS5 medium allowed the isolation of a specific (but unidentified) subpopulation only found on this medium indicates that there is no single efficient medium for the recovery of all lactobacilli from type I sourdoughs.

5.4 Identification of Sourdough Yeasts and Lactic Acid Bacteria

Traditionally, identification of sourdough microorganisms relied on (selective) culturing, selection and purification of a limited number of isolates, and identification of purified isolates with phenotypic and/or genotypic methods. Although this approach has significantly contributed to our current knowledge of the sourdough-associated yeast and LAB species diversity, the use of culture media holds a number of intrinsic limitations. In a culture-based approach, species with very specific nutrient and growth conditions may only sporadically or even not be recovered which leads to an underestimation of the actual microbial species diversity present in the complex sourdough ecosystem [68]. In contrast, culture-independent techniques that are based on phylogenetic dissection of the metagenomic DNA extracted directly from the sample allow one to unravel the species diversity and dynamics of sourdough yeasts and LAB without the need to isolate and culture its single components. However, also these DNA-based methods have a number of limitations, including poor detection capacity of subdominant species and inadequate taxonomic resolution between phylogenetically closely related species. Depending on the aim of the study, conventional culturing and molecular methods are therefore often combined to obtain a more complete picture of the microbial species diversity of sourdough ecosystems [41, 106].

5.4.1 Culture-Dependent Approaches

5.4.1.1 Yeasts

Identification is the localization of individuals in a classification scheme by means of diagnostic characteristics resulting in the assignment of names. The diagnostic characteristics to be used are provided in the species descriptions and, in the case of yeasts, are collected in the monograph "The yeasts: a taxonomic study," currently in its fourth edition [1], with the fifth edition about to be released [204]. While the fourth edition still included instructions for the phenotypic identification of yeasts [199], the fifth edition reformulates them as phenotypic "characterization" instead [205]. This is a consequence of the need to use DNA-based methods to recognize the since 1998 twofold increased number of yeast species. Nevertheless, the accurate description of fermentation and assimilation abilities as well as other phenotypic characters of yeasts continues to be of interest in technological, ecological and taxonomic frameworks.

Among the DNA-based methods currently applied to yeast identification the partial sequencing of the large subunit (LSU) ribosomal ribonucleic acid genes occupies a key position [206]. The DNA sequences of the variable regions D1 and D2 located at the 5' end of the LSU of virtually all known yeast species are documented in the public databases of the International Nucleotide Sequence Database Collaboration (INSDC, including GenBank, the European Molecular Biology Laboratory, and the DNA Data Bank of Japan). The entries of the three submission hubs are bundled, daily updated, and made available for searches by the NCBI (www.ncbi.nlm.nih.gov/nucleotide/). Few distinct yeast species show no or low sequence divergence in the D1/D2 LSU rDNA, can therefore not reliably be distinguished, and require complementary analyses such as *Saccharomyces bayanus* and *S. pastorianus* [206], *Hanseniaspora meyeri* and *Hanseniaspora clermontia; Hanseniaspora guilliermondii* and *Hanseniaspora opuntiae* [207], *Meyerozyma guilliermondii* and *Meyerozyma caribbica; Trichomonascus ciferrii* and *Candida mucifera; K. marxianus* and *Kluyveromyces lactis* [55], *Debaryomyces hansenii, Debaryomyces fabryi* and *Debaryomyces subglobosus* [208]. Genetic regions that show in most cases larger divergence than the D1/D2 LSU rDNA and for which substantial sequence records have been accumulated include the ITS region of the ribosomal gene cluster. This region is favored by mycologists as the barcoding locus of fungi, although no common threshold value to distinguish intraspecific from interspecific variation can be defined [209, 210]. No systematic evaluation of ITS sequence variation to answer this question for ascomycetous yeast species exists to date. In comparison to sequencing, RFLP analysis of ITS sequences offers simplified access to partial DNA sequence information. The accessed information is determined by the recognition sites of the applied restriction enzymes and typically includes only a few nucleotides, necessitating a range of restriction enzymes to reliably distinguish the species in question.

Single-copy protein-coding gene sequences also accumulate in the public databases and may be used to complement those of D1/D2 LSU and ITS sequences in cases where the ribosomal genes do not allow a conclusive species identification. Such protein-coding genes include the actin gene (*ACT1*) [55], translation elongation factor 1 alpha (*TEF1*), the mitochondrial cytochrome oxidase 2 gene (*COX2*) [211], and the largest and second largest subunits of the RNA polymerase II gene (*RPB1, RPB2*) [212]. In contrast to the multicopy ribosomal DNA (e.g., D1/D2 LSU, ITS), single-copy nuclear genes may be more difficult to amplify, as the design of universal primers effective in phylogenetically distant species is not always possible and as only single primer binding sites are available in a haploid genome in contrast to hundreds of binding sites for ribosomal genes. Highly variable mitochondrial genes (e.g., *COX2*) bear the possibility of having been subject to a different evolutionary path than the nuclear genome, in other words, are prone to potential horizontal gene transfers across species borders. As databases for complementary gene sequences are far more restricted than for the D1/D2 LSU, one needs to assure the existence of reference sequences. Databases that allow searching the available sequences for specific strains, such as the yeast database of the Centraalbureau voor Schimmelcultures, The Netherlands (www.cbs.knaw.nl/yeast/BioloMICS.aspx) and the StrainInfo portal (www.straininfo.net/), Ghent University, Belgium, may be used for the selection of complementary sequencing targets. The comparison of a query sequence with reliable type strain sequences is essential, as type strains are the only valid taxonomic reference for a species. Sequence alignments outside the commonly consulted BLAST (Basic Local Alignment and Search Tool) tabulated results are helpful, because type strain sequences are often not recognizable from the sequence entry title line. It is recommended to include type strain sequences of the phylogenetically most closely related species in these alignments to confirm the differentiation of the species in question by the given DNA region.

While the so far discussed methods use genetic information of few or single loci, the techniques commonly known as DNA fingerprinting exploit genetic information that is distributed throughout the genome. A large variety of protocols exists and the more reproducible among them are based on the specific binding of PCR primers to mini- or microsatellite sequences in contrast to arbitrary binding realised in RAPD-PCR. The primer most frequently applied to sourdough yeasts was derived from a ubiquitous minisatellite sequence found in the protein II gene of the bacteriophage M13 [213]. The primer referred to as M13 results from a consensus sequence of 12 partially incomplete repeats. After its use in a PCR assay as the single primer and visualization of the PCR reaction products by agarose gel electrophoresis, usually species-specific banding profiles based on the different lengths of amplifiable sequences enclosed or flanked by M13 minisatellites are observed. The important influence of experimental factors such as the DNA extraction method and PCR and electrophoresis parameters on the resulting profiles implies the need to include type strains for ideally side-by-side-comparisons if a complete identification is to be performed. However, PCR-fingerprinting without type strains may be used to group larger numbers of isolates and to select those that are representative of each group for identification by DNA sequencing [40].

The species *S. cerevisiae* has been observed to show extensive pheno- and genotypic intraspecies diversity (reviewed in [214]). Part of such strain diversity has been traced by molecular analyses to genomic variability associated with Ty-element insertion sites [215]. Ty elements belong to a group of eukaryotic transposable elements that are also called retrotransposons because of some similarities with retroviruses. Ty-elements are flanked by long terminal repeat (LTR) sequences, in turn formed by repeated delta elements. Delta elements are also found in larger numbers independently from LTR sequences and have been used to design and optimize specific PCR primers to amplify the sequences between two delta elements in *S. cerevisiae*, resulting in an effective strain-typing method for this species [216, 217].

5.4.1.2 LAB

Although largely abandoned and replaced by molecular tools, characterization and identification of sourdough LAB species by phenotypic methods is in some cases still useful, or even mandatory when it concerns new species descriptions. The conventional phenotypic approaches for identification of sourdough LAB species may include physiological and chemotaxonomic tests and determination of major fermentation pathways, carbohydrate utilization patterns, lactic acid configuration, and peptidoglycan types. To determine carbohydrate patterns and enzymatic properties in a faster and more reproducible way, miniaturized biochemical test systems such as the API system (Biomérieux, France) can be used for phenotypic characterization of sourdough LAB species [23]. However, it should be stressed that the identifications obtained by comparison with commercial databases such as those linked to API are only tentative and need verification with other taxonomic methods. A more advanced phenotypic identification approach is offered by chemotaxonomic methods, which are based on the use of analytical methods to detect and characterize one or several chemical cell components. Protein profiling by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been used for identification of LAB isolates recovered from Italian [127] and Greek [72] sourdoughs. SDS-PAGE of cellular proteins generally offers sufficient discrimination of LAB isolates at species level but may fail to discriminate between species in the *Lb. acidophilus* group [218] and the *Lb. plantarum* group [71], both of which are prominent members of sourdough ecosystems. Although not yet evaluated for sourdough LAB, the use of mass spectrometry (MS) methods is probably the most powerful phenotypic approach currently available for classification and identification of bacteria [219]. One of these methods, matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) MS, allows one to measure peptides and other compounds in the presence of salts and to analyze complex peptide mixtures, which makes it an ideal method for measuring nonpurified extracts and intact bacterial cells. The resulting MALDI-TOF MS spectra can be used to generate identification libraries for simple and high-throughput identification of unknown bacterial isolates. De Bruyne and co-workers [220] constructed such identification libraries for the LAB genera *Leuconostoc*, *Fructobacillus,* and *Lactococcus*, and reported that 84% of the

leuconostocs and fructobacilli and 94% of the lactococci were correctly identified at species or subspecies level. Identification accuracies for the former two groups further increased to 94–98% when machine learning was applied, which indicates the important role played by advanced techniques for the analysis of complex MALDI-TOF MS profiles.

Essentially, molecular approaches for identification of sourdough LAB are either DNA fingerprint- or sequence-based. DNA fingerprinting methods rely on the use of restriction enzyme analysis, the use of specific or random PCR primers, or a combination thereof. Ribotyping, one of the first DNA fingerprinting techniques used in bacterial taxonomy [221], relies on a combination of restriction analysis of total genomic DNA and Southern hybridization to visualize a subset of restriction fragments with labeled rDNA probes targeting conserved domains of ribosomal 16S and 23S rRNA encoding genes. Although ribotyping generally provides high discriminatory power at species to subspecies level, it has been used only sporadically as an identification technique for LAB species. In specific cases, ribotyping has proven particularly useful in the classification and identification of sourdough LAB, for example for discrimination between the genomically and phenotypically highly similar sourdough LAB *W. cibaria* and *W. confusa* [63] and for intraspecific differentiation of *Lb. sanfranciscensis* strains from different sourdoughs [121]. Despite its high resolution at species as well as at strain level, amplified fragment length polymorphism (AFLP) fingerprinting was also only used sporadically for LAB identification purposes. Essentially, AFLP combines the power of restriction fragment length polymorphism with the flexibility of PCR-based methods by ligating primer-recognition sequences (adaptors) to the digested DNA [222]. This whole-genome fingerprinting technique has proved useful to support the description of the sourdough species *Lb. hammesii* [82], *Lb. crustorum* [86] and *Lb. namurensis* [87] and for molecular source tracking of *Lb. spicheri*, *Lb. plantarum* and *Lb. sanfranciscensis* in the production environment of artisan sourdough bakeries [155]. In contrast to the aforementioned methods, RAPD-PCR and repetitive DNA element (rep)-PCR are technically less demanding DNA fingerprinting techniques based on a single PCR step. Both methods are fast, relatively inexpensive, and exhibit a high discriminatory power ranging from genus to intraspecific level, which explains their wide application range for the identification and classification of LAB. RAPD-PCR has been used in multiple studies on sourdough LAB for species identification [129], strain differentiation [80, 89, 120, 126, 127, 131, 148, 158] and strain monitoring purposes [70, 122, 135, 138, 223]. The reproducibility of RAPD-PCR is highly influenced by various factors, such as DNA purity and concentration and minimal differences in the PCR temperature programme [224], for which reason this method is less suitable for interlaboratory comparisons. Because of the use of longer PCR primers complementary to bacterial interspersed repetitive DNA elements such as ERIC, BOX, REP or $(GTG)_5$ and higher annealing temperatures, rep-PCR protocols are more robust and display a higher level of reproducibility [225]. rep-PCR using the $(GTG)_5$ primer, i.e., $(GTG)_5$-PCR, has been found particularly useful for differentiation of sourdough LAB at the (sub)species up to the strain level [74, 105, 106, 155, 226, 227]. For high-resolution differentiation of individual

sourdough LAB strains by DNA fingerprinting, however, AFLP fingerprinting and pulsed-field gel electrophoresis (PFGE) are the most powerful. Essentially, PFGE involves the electrophoretic separation of genomic macrorestriction fragments obtained by digestion with rare-cutting enzymes in an alternating electric field. In sourdough studies, PFGE has been used as a typing method to differentiate among strains within *Lb. plantarum* and *Lb. sanfranciscensis* [126, 135, 174].

Sequence-based analysis approaches for identification of sourdough LAB have long relied on the use of 16S rRNA genes, and this has become a standard approach to obtain a first preliminary view of the taxonomic diversity among a set of unknown isolates recovered from a sourdough ecosystem [72, 80, 148]. In many of these studies, only partial 16S rRNA gene sequences are determined and used in comparisons with public sequence databases. In many cases, the use of partial sequences will only allow a tentative identification, of which the reliability is likely to improve when the entire 16S rRNA gene is sequenced [228]. Despite its established use as a standard method for identification of LAB species, 16S rRNA gene sequencing does not allow differentiation of phylogenetically closely related species [63–66, 71]. The growing availability of whole-genome sequences has triggered the search for alternative genes that offer a higher taxonomic resolution than the 16S rRNA gene. The use of protein-encoding genes or so-called housekeeping genes essentially combines the technological advantages of 16S rRNA gene sequencing and the taxonomic resolution offered by a number of fingerprinting methods. Sequencing of one or preferably multiple of these genes as taxonomic markers is a crucial step forward in the development of standardized and globally accessible methods for the identification of LAB. Housekeeping genes such as *pheS* (encoding the phenylalanyl-tRNA synthase) and *rpoA* (encoding the DNA-dependent RNA polymerase alpha-subunit) display higher divergence rates than the 16S rRNA gene, and allow discrimination between closely related LAB species with almost identical 16S rRNA gene sequences [67, 229]. Several studies on sourdough LAB species diversity have used such protein-encoding genes as phylogenetic markers in a single-locus sequence approach. In conjunction with $(GTG)_5$-PCR fingerprinting, the *pheS* gene has been successfully used for the identification of LAB species from sourdough fermentations at laboratory scale [107] and from Belgian artisan bakery sourdoughs and their environment [105, 106, 155], as well as for unraveling the intraspecific diversity in the sourdough species *Lb. rossiae* [74]. Settanni and coworkers [156] used the *recA* gene, encoding a protein essential for repair and maintenance of DNA, in a multiplex PCR assay to discriminate between the phylogenetically highly related *Lb. plantarum*, *Lb. pentosus* and *Lb. paraplantarum* in sourdough ecosystems. The *recA* gene has also been used in combination with the 16S rRNA gene to unravel the identity of LAB isolates recovered during wheat flour sourdough type I propagation [135]. Sequences derived from the *tuf* gene, which encodes the elongation factor Tu, have revealed a higher discriminatory power compared to 16S rRNA gene sequences and have been used to support the delineation of the new sourdough species *Lb. secaliphilus* [85]. Although single-locus sequence analysis approaches are now commonly used within specific LAB groups, it has been argued that the phylogenetic information obtained from only a single gene

may be influenced by lateral gene transfer (LGT) and may lead to incorrect identifications. To compensate for possible LGT events, it has been suggested that multilocus sequence analysis (MLSA) of at least five housekeeping genes from diverse chromosomal loci and with wide distribution among taxa is required to reliably distinguish a species from related taxa [230]. After a more thorough evaluation, however, Konstantinidis and co-workers [231] concluded that three genes are sufficient to anticipate the possible effects of LGT in MLSA-based identification schemes. For LAB, MLSA based on the combined sequence analysis of the genes *atpA, pheS,* and *rpoA* has been successfully explored for species identification of enterococci [229], lactobacilli [67], leuconostocs [232], and pediococci [233]. For sequence-based differentiation of LAB at strain level, multilocus schemes typically include six or seven housekeeping genes. The resulting multilocus sequence typing (MLST) approach has so far mainly been applied to study community structure, evolution and phylogeography of bacterial pathogens [234]. A few MLST schemes have been specifically developed for *Lactobacillus* species, including *Lb. casei* [235, 236], *Lb. plantarum* [237] and *Lb. salivarius* [238].

5.4.2 Culture-Independent Approaches

The first approaches used to identify sourdough microorganisms independent of culturing relied on the use of oligonucleotide probes targeting ribosomal gene sequences specific for individual species or groups of species. The majority of these probe-based methods made use of partial 16S rRNA gene sequences that were identified as molecular signatures unique to specific LAB species [203, 239]. Gradually, the relatively laborious probe hybridizations were replaced by faster community PCR assays using species-specific oligonucleotide primers. The success of both approaches strongly depended on rigorous *in silico* probe or primer design and required in vitro and in vivo validation using taxonomically well-characterized type and reference strains and spiked sourdough samples, respectively. Species-specific PCR primers complementary to signature sequences in the 16S or 23S rRNA gene or in the 16S–23S rRNA intergenic spacer region have been applied for the culture-independent identification of sourdough LAB species [26, 156, 161, 240–242]. By combining multiple sets of primers, several typical sourdough LAB species can be simultaneously detected. In this way, Settanni and co-workers [156] developed a two-step multiplex community PCR assay that enabled rapid identification of up to 16 *Lactobacillus* species in sourdough samples. The introduction of real-time PCR technology has allowed one to further increase the sensitivity of PCR-based identification assays and enables the simultaneous detection and quantification of food microorganisms [243]. For this purpose, SYBR Green-based real-time PCR assays based on the detection of the *pheS* gene have been used for source tracking of *Lb. plantarum* and *Lb. sanfranciscensis* in traditional sourdoughs and their production environments [155].

Whereas probe- and primer-based identification approaches offer the specificity and selectivity required to detect and monitor specific LAB in sourdough samples, they were not designed to offer a complete picture of the predominant LAB species diversity or to reveal new or unknown LAB species diversity in sourdough ecosystems. In contrast, community fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) and temporal temperature gradient gel electrophoresis (TTGE) do not require prior knowledge of the ecosystem's diversity and are universally applicable to study the species diversity and dynamics of complex bacterial communities in food environments [244]. The universal use of DGGE fingerprinting is based on the sequence-dependent separation of a mixture of equally sized PCR amplicons generated from a common taxonomic marker such as the 16S rRNA gene. For the design of PCR primers, the V1, V3, and V6-V8 hypervariable regions of the 16S rRNA gene are most commonly used. Taxonomic information on individual members of the sample community can be obtained by band position analysis provided that an identification database is available, clone library analysis, sequencing of excised and purified DGGE bands or hybridization using species-specific probes. Major drawbacks of DGGE fingerprinting include its inability to detect subdominant (i.e., <1%) community members and the fact that a single strain or species may be represented by multiple bands in the DGGE profile due to heterogeneous rRNA operons and/or heteroduplex molecules. Either using universal or group-specific 16S rRNA gene primers, DGGE has been widely applied to inventorize LAB communities in sourdoughs [41, 47, 106, 128, 245, 246] and to investigate the dynamics, adaptation, and source of predominant sourdough LAB communities [34, 39, 80, 107, 123, 142, 155, 185]. Likewise, primers targeting the 26S LSU rDNA have been used for DGGE fingerprinting analysis of sourdough yeast communities [29, 46]. To maximally cover the microbial species diversity present in a sourdough ecosystem, a number of DGGE studies have combined the use of 16S and 23S rRNA gene primers to determine in parallel the predominant LAB and yeast composition of sourdough samples [33, 39, 41, 47, 123, 142, 245]. Compared to DGGE, TTGE has been used to a much lesser extent for culture-independent analysis of the sourdough microbiota [112]. In many of the cited studies, the sequence heterogeneity of the multicopy 16S rRNA gene is mentioned as an important limitation in DGGE, as this may lead to an overestimation of the LAB species diversity. The degree of overestimation can be estimated by scoring individual bands by position analysis with a reference database and/or by band sequencing. Alternatively, single-copy genes that do not exhibit this heterogeneity such as *rpoB* have been evaluated for DGGE fingerprinting of LAB species during food fermentations [247].

Microarray technology represents one of the most recent culture-independent approaches to study the diversity and identify individual members of the sourdough microbiota. Phylogenetic microarrays, containing partial 16S rRNA gene sequences as targets, are ideally suited for this purpose but are currently not available for sourdough microbiota. Alternatively, a functional gene microarray can be used when the original annotation information allows one to link the responding oligonucleotides to the original species. Weckx and co-workers [108, 109] used a LAB functional gene

microarray to analyze time-related RNA samples that represented the metatranscriptome of sourdough fermentations maintained by daily backslopping. The resulting set of hybridization data allowed one to monitor the LAB community dynamics in the sourdough ecosystem and to identify the major LAB species that contributed to the establishment of a stable ecosystem through its three successive phases.

References

1. Kurtzman CP, Fell JW (eds) (1998) The yeasts: a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 111–947
2. Suh S-O, Blackwell M, Kurtzman CP, Lachance M-A (2006) Phylogenetics of Saccharomycetales, the ascomycetous yeasts. Mycologia 98:1006–1017
3. Kurtzman CP (2003) Phylogenetic circumscription of *Saccharomyces, Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma* and *Zygotorulaspora*. FEMS Yeast Res 4:233–245
4. Kurtzman CP, Robnett CJ, Basehoar-Powers E (2008) Phylogenetic relationships among species of *Pichia, Issatchenkia* and *Williopsis* determined from multigene sequence analysis, and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov. and *Wickerhamomyces* gen. nov. FEMS Yeast Res 8:939–954
5. Kurtzman CP (2011) Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. Antonie van Leeuwenhoek 99:13–23
6. Daniel HM, Redhead SA, Schürer J, Naumov GI, Kurtzman CP (2012) Proposals to conserve the name Wickerhamomyces against Hansenula and to reject the name Saccharomyces sphaericus (Ascomycota: Saccharomycotina). Taxon 61 (2):459–461.
7. Sugihara TF, Kline L, Miller MW (1971) Microorganisms of the San Francisco sour dough bread process. Appl Microbiol 21:456–458
8. Galli A, Ottogalli G (1973) The microflora of the sour dough of “Panettone” cake. Ann Microbiol 23:39–49
8. Spicher G, Schröder R, Schöllhammer K (1979) Die Mikroflora des Sauerteiges. Z Lebensm Unters Forsch 169:77–81
10. Barber S, Báguena R, Martínez-Anaya MA, Torner MJ (1983) Microflora of the sour dough of wheat flour bread. I. Identification and functional properties of microorganisms of industrial sour doughs. Rev Agroquím Tecnol Aliment 23:552–562
11. Salovaara H, Savolainen J (1984) Yeast type isolated from Finnish sour rye dough starters. Act Aliment Pol 10:241–246
12. Barber S, Báguena R (1988) Microflora of the sour dough of wheat flour bread. V. Isolation, identification and evaluation of functional properties of sourdough’s microorganisms. Rev Agroquím Tecnol Aliment 28:67–78
13. Galli A, Franzetti L, Fortina MG (1988) Isolation and identification of sour dough microflora. Microbiol Aliment Nutr 6:345–351
14. Hamad SH, Böcker G, Vogel RF, Hammes WP (1992) Microbiological and chemical analysis of fermented sorghum dough for Kisra production. Appl Microbiol Biotechnol 37:728–731
15. Infantes M, Schmidt JL (1992) Characterization of the yeast flora of natural sourdoughs located in various French areas. Sci Alim 12:271–287
16. Boraam F, Faid M, Larpent JP, Breton A (1993) Lactic acid bacteria and yeasts associated with traditional Moroccan sour-dough bread fermentation. Sci Alim 13:501–509
17. Gobbetti M, Corsetti A, Rossi J, La Rosa F, De Vincenzi S (1994) Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of Central Italy. Ital J Food Sci 6:85–94

18. Obiri-Danso K (1994) Microbiological studies on corn dough fermentation. Cer Chem 71:186–188
19. Iorizzo M, Coppola R, Sorrentino E, Grazia L (1995) Micobiological characterization of sourdough from Molise. Industrie Alimentari XXXIV:1290–1294
20. Almeida MJ, Pais CS (1996) Characterization of the yeast population from traditional corn and rye bread doughs. Lett Appl Micobiol 23:154–158
21. Foschino R, Terraneo R, Mora D, Galli A (1999) Microbial characterization of sourdoughs for sweet baked products. Ital J Food Sci 11:19–28
22. Mäntynen VH, Korhola M, Gudmundsson H, Turakainen H, Alfredsson GA, Salovaara H, Lindström K (1999) A polyphasic study on the taxonomic position of industrial sour dough yeasts. Syst Appl Microbiol 22:87–96
23. Rocha JM, Malcata FX (1999) On the microbiological profile of traditional Portuguese sourdough. J Food Prot 62:1416–1429
24. Paramithiotis S, Muller MRA, Ehrmann MA, Tsakalidou E, Seiler H, Vogel R, Kalantzopoulos G (2000) Polyphasic identification of wild yeast strains isolated from Greek sourdoughs. Syst Appl Microbiol 23:156–164
25. Rosenquist H, Hansen A (2000) The microbial stability of two bakery sourdoughs made from conventionally and organically grown rye. Food Microbiol 17:241–250
26. Corsetti A, Lavermicocca P, Morea M, Baruzzi F, Tosti N, Gobbetti M (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
27. Pulvirenti A, Caggia C, Restuccia C, Gullo M, Giudici P (2001) DNA fingerprinting methods used for identification of yeasts isolated from Sicilian sourdoughs. Ann Microbiol 51:107–120
28. Gullo M, Romano AD, Pulvirenti A, Giudici P (2003) *Candida humilis* – dominant species in sourdoughs for the production of durum wheat bran flour bread. Int J Food Microbiol 80:55–59
29. Meroth CB, Hammes WP, Hertel C (2003) Identification and population dynamics of yeasts in sourdough fermentation processes by PCR-denaturing gradient gel electrophoresis. Appl Environ Microbiol 69:7453–7461
30. Solieri L, De Vero L, Pulvirenti A, Gullo M (2003) A phenotypical and molecular study of yeast species in home-made sourdoughs. Industr Aliment XLII:971–978
31. Succi M, Reale A, Andrighetto C, Lombardi A, Sorrentino E, Coppola R (2003) Presence of yeasts in southern Italian sourdoughs from *Triticum aestivum* flour. FEMS Microbiol Lett 225:143–148
32. Foschino R, Gallina S, Andrighetto C, Rossetti L, Galli A (2004) Comparison of cultural methods for the identification and molecular investigation of yeasts from sourdoughs for Italian sweet baked products. FEMS Yeast Res 4:609–618
33. Gatto V, Torriani S (2004) Microbial population changes during sourdough fermentation monitored by DGGE analysis of 16S and 26S rRNA gene fragments. Ann Microbiol 54:31–42
34. Pulvirenti A, Solieri L, Gullo M, De Vero L, Giudici P (2004) Occurrence and dominance of yeast species in sourdough. Lett Appl Microbiol 38:113–117
35. Vernocchi P, Valmorri S, Gatto V, Torriani S, Gianotti A, Suzzi G, Guerzoni ME, Gardini F (2004) A survey on yeast microbiota associated with an Italian traditional sweet-leavened baked good fermentation. Food Res Int 37:469–476
36. Vernocchi P, Valmorri S, Dalai I, Torriani S, Gianotti A, Suzzi G, Guerzoni ME, Mastrocola D, Gardini F (2004) Characterization of the yeast population involved in the production of a typical Italian bread. J Food Sci 69:M182–M186
37. Lombardi A, Zilio F, Andrighetto C, Zampese L, Loddo A (2007) Micobiological characterization of sourdoughs of Veneto region. Industr Aliment XLVI:147–151
38. Restuccia C, Randazzo C, Pitino I, Caggia C, Fiasconaro N (2007) Phenotypic and genotypic characterization of lactic acid bacteria and yeasts from sourdough for Panettone production. Industr Aliment XLVI:1231–1236
39. Garofalo C, Silvestri G, Aquilanti L, Clementi F (2008) PCR-DGGE analysis of lactic acid bacteria and yeast dynamics during the production processes of three varieties of Panettone. J Appl Microbiol 105:243–254

40. Vrancken G, De Vuyst L, Van der Meulen R, Huys G, Vandamme P, Daniel HM (2010) Yeast species composition differs between artisan bakery and spontaneous laboratory sourdoughs. FEMS Yeast Res 10:471–481
41. Iacumin L, Cecchini F, Manzano M, Osualdini M, Boscolo D, Orlic S, Comi G (2009) Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. Food Microbiol 26:128–135
42. Luangsakul N, Keeratipibul S, Jindamorakot S, Tanasupawat S (2009) Lactic acid bacteria and yeasts isolated from the starter doughs for Chinese steamed buns in Thailand. LWT- Food Sci Technol 42:1404–1412
43. Osimani A, Zannini E, Aquilanti L, Mannazzu I, Comitini F, Clementi F (2009) Lactic acid bacteria and yeasts from wheat sourdoughs of the Marche region. Ital J Food Sci 21:269–286
44. Saeed M, Anjum FM, Zahoor T, Nawaz H, Rehman SU (2009) Isolation and characterization of starter culture from spontaneous fermentation of sourdough. Int J Agric Biol 11:329–332
45. Paramithiotis S, Tsiasiotou S, Drosinos E (2010) Comparative study of spontaneously fermented sourdoughs originating from two regions of Greece: Peloponnesus and Thessaly. Eur Food Res Technol 231:883–890
46. Valmorri S, Tofalo R, Settanni L, Corsetti A, Suzzi G (2010) Yeast microbiota associated with spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the Abruzzo region (Italy). Antonie van Leeuwenhoek 97:119–129
47. Moroni AV, Arendt EK, Dal Bello F (2011) Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. Food Microbiol 28:497–502
48. Zhang J, Liu W, Sun Z, Bao Q, Wang F, Yu J, Chen W, Zhang H (2011) Diversity of lactic acid bacteria and yeasts in traditional sourdoughs collected from western region in Inner Mongolia of China. Food Contr 22:767–774
49. Yarrow D (1978) *Candida milleri* sp. nov. Int J Syst Bacteriol 28:608–610
50. Gobbetti M, Corsetti A, Rossi J (1994) The sourdough microflora. Interactions between lactic acid bacteria and yeasts: metabolism of carbohydrates. Appl Microbiol Biotechnol 41:456–460
51. Daniel HM, Moons MC, Huret S, Vrancken G, De Vuyst L (2011) *Wickerhamomyces anomalus* in the sourdough microbial ecosystem. Antonie van Leeuwenhoek 99:63–73
52. Sampaio JP, Gonçalves P (2008) Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus*. Appl Environ Microbiol 74:2144–2152
53. Nel EE, van der Walt JP (1968) *Torulopsis humilis*, sp. n. Mycopathol Mycol Appl 36:94–96
54. Meyer SA, Payne RW, Yarrow D (1998) *Candida* Berkout. In: Kurtzman CP, Fell JW (eds) The yeasts: a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 454–573
55. Daniel HM, Meyer W (2003) Evaluation of ribosomal RNA and actin gene sequences for the identification of ascomycetous yeasts. Int J Food Microbiol 86:61–78
56. Vaughan-Martini A (1995) *Saccharomyces barnetti* and *Saccharomyces spencerorum*: two new species of *Saccharomyces sensu lato* (van der Walt). Antonie van Leeuwenhoek 68:111–118
57. De Vuyst L, Neysens P (2005) The sourdough microflora: biodiversity and metabolic interactions. Trends Food Sci Technol 16:43–56
58. Corsetti A, Settanni L (2007) Lactobacilli in sourdough fermentation. Food Res Int 40:539–558
59. Orla-Jensen S (1919) The lactic acid bacteria. Fred Host and Son, Copenhagen
60. Felis GE, Dellaglio F (2007) Taxonomy of lactobacilli and bifidobacteria. Curr Iss Intest Microbiol 8:44–61
61. Schleifer KH, Ludwig W (1995) Phylogeny of the genus *Lactobacillus* and related genera. Syst Appl Microbiol 18:461–467
62. Stiles ME, Holzapfel WH (1997) Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol 36:1–29
63. Björkroth KJ, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel WH, Korkeala HJ, Vandamme P (2002) Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. Int J Syst Evol Microbiol 52:141–148
64. Cachat E, Priest FG (2005) *Lactobacillus suntoryeus* sp. nov., isolated from malt whisky distilleries. Int J Syst Evol Microbiol 55:31–34

65. Leisner JJ, Vancanneyt M, Lefebvre K, Vandemeulebroecke K, Hoste B, Vilalta NE, Rusul G, Swings J (2002) *Lactobacillus durianis* sp. nov., isolated from an acid-fermented condiment (tempoyak) in Malaysia. Int J Syst Evol Microbiol 52:927–931
66. Yoon JH, Kang SS, Mheen TI, Ahn JS, Lee HJ, Kim TK, Park CS, Kho YH, Kang KH, Park YH (2000) *Lactobacillus kimchii* sp. nov., a new species from kimchi. Int J Syst Evol Microbiol 50:1789–1795
67. Naser SM, Dawyndt PSR, Hoste B, Gevers D, Vandemeulebroecke K, Cleenwerck I, Vancanneyt M, Swings J (2007) Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. Int J Syst Evol Microbiol 57:2777–2789
68. De Vuyst L, Vancanneyt M (2007) Biodiversity and identification of sourdough lactic acid bacteria. Food Microbiol 24:120–127
69. De Vuyst L, Vrancken G, Ravyts F, Rimaux T, Weckx S (2009) Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. Food Microbiol 26:666–675
70. Ehrmann MA, Vogel R (2005) Molecular taxonomy and genetics of sourdough lactic acid bacteria. Trends Food Sci Technol 16:31–42
71. Torriani S, Felis GE, Dellaglio F (2001) Differentiation of *Lactobacillus plantarum, L. pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with *recA* gene-derived primers. Appl Environ Microbiol 67:3450–3454
72. De Vuyst L, Schrijvers V, Paramithiotis S, Hoste B, Vancanneyt M, Swings J, Kalatzopoulos G, Tsakalidou E, Messens W (2002) The biodiversity of lactic acid bacteria in Greek traditional sourdoughs is reflected in both composition and metabolite formation. Appl Environ Microbiol 68:6059–6069
73. Gevers D, Huys G, Swings J (2001) Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. FEMS Microbiol Lett 205:31–36
74. Scheirlinck I, Van der Meulen R, Vrancken G, De Vuyst L, Settanni L, Vandamme P, Huys G (2009) Polyphasic taxonomic characterization of *Lactobacillus rossiae* isolates from Belgian and Italian sourdoughs reveals intraspecific heterogeneity. Syst Appl Microbiol 32:151–156
75. Kline L, Sugihara TF (1971) Microorganisms of the San Francisco sourdough bread process. II. Isolation and characterization of undescribed bacterial species responsible for the souring activity. Appl Microbiol 21:459–465
76. Corsetti A, Settanni L, van Sinderen D, Felis GE, Dellaglio F, Gobbetti M (2005) *Lactobacillus rossii* sp. nov., isolated from wheat sourdough. Int J Syst Evol Microbiol 55:35–40
77. Naser SM, Hagen KE, Vancanneyt M, Cleenwerck I, Swings J, Tompkins TA et al (1980) *Lactobacillus suntoryeus* Cachat and Priest 2005 is a later synonym of *Lactobacillus helveticus* (Orla-Jensen 1919) Bergey *et al*. 1925 (Approved Lists 1980). J Syst Evol Microbiol 2006(56):355–360
78. Müller MRA, Ehrmann MA, Vogel RF (2000) *Lactobacillus frumenti* sp. nov., a new lactic acid bacterium isolated from rye-bran fermentations with a long fermentation period. Int J Syst Evol Microbiol 50:2127–2133
79. Ehrmann MA, Müller MRA, Vogel RF (2003) Molecular analysis of sourdough reveals *Lactobacillus mindensis* sp. nov. *Int*. J Syst Evol Microbiol 53:7–13
80. Meroth CB, Hammes WP, Hertel C (2004) Characterisation of the microbiota of rice sourdoughs and description of *Lactobacillus spicheri*, sp. nov. Syst Appl Microbiol 27:151–159
81. Vancanneyt M, Neysens P, De Wachter M, Engelbeen K, Snauwaert C, Cleenwerck I, Van der Meulen R, Hoste B, Tsakalidou E, De Vuyst L, Swings J (2005) *Lactobacillus acidifarinae* sp. nov. and *Lactobacillus zymae* sp. nov., from wheat sourdoughs. Int J Syst Evol Microbiol 55:615–620
82. Valcheva R, Korakli M, Onno B, Prévost H, Ivanova I, Ehrmann MA, Dousset X, Gänzle MG, Vogel RF (2005) *Lactobacillus hammessi* sp. nov., isolated from French sourdough. Int J Syst Evol Microbiol 55:763–767
83. Aslam Z, Im WT, Ten LN, Lee MJ, Kim KH, Lee ST (2006) *Lactobacillus siliginis* sp. nov., isolated from wheat sourdough in South Korea. Int J Syst Evol Microbiol 56:2209–2213
84. Valcheva R, Ferchichi MF, Korakli M, Ivanova I, Gänzle MG, Vogel RF, Prévost H, Onno B, Dousset X (2006) *Lactobacillus nantensis* sp. nov., isolated from French wheat sourdough. Int J Syst Evol Microbiol 56:587–591

85. Ehrmann MA, Brandt M, Stolz P, Vogel RF, Korakli M (2007) *Lactobacillus secaliphilus* sp. nov, isolated from type II sourdough fermentation. Int J Syst Evol Microbiol 57:745–750
86. Scheirlinck I, Van der Meulen R, Van Schoor A, Huys G, Vandamme P, De Vuyst L, Vancanneyt M (2007) *Lactobacillus crustorum* sp. nov., isolated from two traditional Belgian wheat sourdoughs. Int J Syst Evol Microbiol 57:1461–1467
87. Scheirlinck I, Van der Meulen R, Van Schoor A, Cleenwerck I, Huys G, Vandamme P, De Vuyst L, Vancanneyt M (2007) *Lactobacillus namurensis* sp. nov., isolated from a traditional Belgian sourdough. Int J Syst Evol Microbiol 57:223–227
88. Kashiwagi T, Suzuki T, Kamakura T (2009) *Lactobacillus nodensis* sp. nov., isolated from rice bran. Int J Syst Evol Microbiol 59:83–86
89. Corsetti A, Settanni L, Valmorri S, Mastrangelo M, Suzzi G (2007) Identification of subdominant sourdough lactic acid bacteria and their evolution during laboratory-scale fermentations. Food Microbiol 24:592–600
90. Björkroth J, Dicks LMT, Holzapfel WH (2009) Genus III. *Weissella*. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, The Firmicutes. Springer, Dordrecht/New York, pp 643–654
91. Villani F, Moschetti G, Blaiotta G, Coppola S (1997) Characterization of strains of *Leuconostoc mesenteroides* by analysis of soluble whole-cell protein pattern, DNA fingerprinting and restriction of ribosomal DNA. J Appl Microbiol 82:578–588
92. Nigatu A (2000) Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate *Lactobacillus plantarum*, *Lact. fermentum*, *Lact. rhamnosus*, *Lact. sake*, *Lact. parabuchneri*, *Lact. gallinarum*, *Lact. casei*, *Weissella minor* and related taxa isolated from *kocho* and *tef*. J Appl Microbiol 89:969–978
93. Satokari R, Mattila-Sandholm T, Suihko ML (2000) Identification of pediococci by ribotyping. J Appl Microbiol 88:260–265
94. Barney M, Volgyi A, Navarro A, Ryder D (2001) Riboprinting and 16S rRNA sequencing for identification of brewery *Pediococcus* isolates. Appl Environ Microbiol 67:553–560
95. Rodas AM, Ferrer S, Pardo I (2003) 16S-ARDRA, a tool for identification of lactic acid bacteria isolated from grape must and wine. Syst Appl Microbiol 26:412–422
96. Nigatu A, Ahrne S, Gashe BA, Molin G (1998) Randomly amplified polymorphic DNA (RAPD) for discrimination of *Pediococcus pentosaceus* and *Ped. acidilactici* and rapid grouping of *Pediococcus* isolates. Lett Appl Microbiol 26:412–416
97. Mora D, Fortina MG, Parini C, Daffonchio D, Manachini PL (2000) Genomic subpopulations within the species *Pediococcus acidilactici* detected by multilocus typing analysis: relationships between pediocin AcH/PA-producing and non-producing strains. Microbiology 146:2027–2038
98. Dobson CM, Deneer H, Lee S, Hemmingsen S, Glaze S, Ziola B (2002) Phylogenetic analysis of the genus *Pediococcus: Pediococcus claussenii* sp. nov., a novel lactic acid bacterium isolated from beer. Int J Syst Evol Microbiol 52:2003–2010
99. Pérez G, Cardell E, Zárate V (2002) Random amplified polymorphic DNA analysis for differentiation of *Leuconostoc mesenteroides* subspecies isolated from Tenerife cheese. Lett Appl Microbiol 34:82–85
100. Hutkins RW (ed) (2006) Microbiology and technology of fermented foods. Blackwell Publishing, Iowa, pp 1–473
101. Ottogalli G, Galli A, Foschino R (1996) Italian bakery products obtained with sour dough: characterization of the typical microflora. Adv Food Sci 18:131–144
102. Hammes WP, Brandt MJ, Francis KL, Rosenheim J, Seitter MFH, Vogelmann A (2005) Microbial ecology of cereal fermentations. Trends Food Sci Technol 16:4–11
103. Arendt EK, Ryan LAM, Dal Bello F (2007) Impact of sourdough on the texture of bread. Food Microbiol 24:165–174
104. Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78
105. Scheirlinck I, Van der Meulen R, Van Schoor A, Vancanneyt M, De Vuyst L, Vandamme P, Huys G (2007) Influence of geographical origin and flour type on diversity of lactic acid bacteria in traditional Belgian sourdoughs. Appl Environ Microbiol 73:6262–6269

106. Scheirlinck I, Van der Meulen R, Van Schoor A, Vancanneyt M, De Vuyst L, Vandamme P, Huys G (2008) Taxonomic structure and stability of the bacterial community in Belgian sourdough ecosystems as assessed by culture and population fingerprinting. Appl Environ Microbiol 74:2414–2423
107. Van der Meulen R, Scheirlinck I, Van Schoor A, Huys G, Vancanneyt M, Vandamme P, De Vuyst L (2007) Population dynamics and metabolite target analysis of lactic acid bacteria during laboratory fermentations of wheat and spelt sourdoughs. Appl Environ Microbiol 73:4741–4750
108. Weckx S, Van der Meulen R, Allemeersch J, Huys G, Vandamme P, Van Hummelen P, De Vuyst L (2010) Community dynamics of sourdough fermentations as revealed by their metatranscriptome. Appl Environ Microbiol 76:5402–5408
109. Weckx S, Van der Meulen R, Maes D, Scheirlinck I, Huys G, Vandamme P, De Vuyst L (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
110. Gashe BA (1985) Involvement of lactic acid bacteria in the fermentation of tef (*Eragrostis tef*), an Ethiopian fermented food. J Food Sci 50:800–801
111. Infantes L, Tourneur C (1991) Survey on the lactic flora of natural sourdoughs located in various French areas. Sci Alim 11:527–545
112. Gabriel V, Lefebvre D, Vayssier Y, Faucher C (1999) Characterization of microflora from natural sourdoughs. Microbiol Alim Nutr 17:171–179
113. Ferchichi M, Valcheva R, Prevost H, Onno B, Dousset X (2007) Molecular identification of the microbiota of French sourdough using temporal temperature gradient gel electrophoresis. Food Microbiol 24:678–686
114. Ferchichi M, Valcheva R, Oheix N, Kabadjova P, Prevost H, Onno B, Dousset X (2008) Rapid investigation of French sourdough microbiota by restriction fragment length polymorphism of the 16S-23S rRNA gene intergenic spacer region. World J Microbiol Biotechnol 24:2425–2434
115. Robert H, Gabriel V, Fontagné-Faucher C (2009) Biodiversity of lactic acid bacteria in French wheat sourdough as determined by molecular characterization using species-specific PCR. Int J Food Microbiol 135:53–59
116. Spicher G (1959) Die Mikroflora des Sauerteiges. I. Mitteilung: Untersuchungen über die Art der in Sauerteigen anzutreffenden stäbchenförmigen Milchsäurebakterien (Genus *Lactobacillus* Beijerinck). Zeitblatt fur Bakteriologie II Abt 113:80–106
117. Spicher G, Schröder R (1978) Die Mikroflora des Sauerteiges. IV. Mitteilung: Untersuchungen über die Art der in "Reinzuchtsauern" anzutreffenden stäbchenförmigen Milchsäurebakterien (Genus *Lactobacillus* Beijerinck). Z Lebensm Unters Forsch 167:342–354
118. Spicher G (1984) Weitere Unterschungen über die Zusammensetzung und die Variabilität der Mikroflora handelsüblicher Sauerteig-Starter. Z Lebensm Unters Forsch 178:106–109
119. Spicher G (1987) Die Mikroflora des Sauerteiges. XXII. Mitteilung: Die in Weizensauerteigen vorkommenden Lactobacillen. Z Lebensm Unters Forsch 184:300–303
120. Müller MRA, Wolfrum G, Stolz P, Ehrmann MA, Vogel RF (2001) Monitoring the growth of *Lactobacillus* species in a sourdough fermentation. Food Microbiol 18:217–227
121. Kitahara M, Sakata S, Benno Y (2005) Biodiversity of *Lactobacillus sanfranciscensis* strains isolated from five sourdoughs. Lett Appl Microbiol 40:353–357
122. Sterr Y, Weiss A, Schmidt H (2009) Evaluation of lactic acid bacteria for sourdough fermentation of amaranth. Int J Food Microbiol 136:75–82
123. Vogelmann SA, Seitter M, Singer U, Brandt MJ, Hertel C (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
124. Coppola S, Pepe O, Masi P, Sepe M (1996) Characterization of leavened doughs for pizza in Naples. Adv Food Sci 18:160–162
125. Zapparoli G, De Benedictis P, Salardi C, Veneri G, Torriani S, Dellaglio F (1996) Lactobacilli of sourdoughs from Verona bakery: a preliminary investigation. Adv Food Sci 18:163–166

126. Zapparoli G, Torriani S, Dellaglio F (1998) Differentiation of *Lactobacillus sanfranciscensis* strains by randomly amplified polymorphic DNA and pulsed-field gel electrophoresis. FEMS Microbiol Lett 166:325–332
127. Corsetti A, De Angelis M, Dellaglio F, Paparella A, Fox PF, Settanni L, Gobbetti M (2003) Characterization of sourdough lactic acid bacteria based on genotypic and cell-wall protein analyses. J Appl Microbiol 94:641–654
128. Randazzo CL, Heilig H, Restuccia C, Giudici P, Caggia C (2005) Bacterial population in traditional sourdough evaluated by molecular methods. J Appl Microbiol 99:251–258
129. Reale A, Tremonte P, Succi M, Sorrentino E, Coppola R (2005) Exploration of lactic acid bacteria ecosystem of sourdoughs from the Molise region. Ann Microbiol 55:17–22
130. Ricciardi A, Parente E, Piraino P, Paraggio M, Romano P (2005) Phenotypic characterization of lactic acid bacteria from sourdoughs for Altamura bread produced in Apulia (Southern Italy). Int J Food Microbiol 98:63–72
131. Catzeddu P, Mura E, Parente E, Sanna M, Farris GA (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
132. Valmorri S, Settanni L, Suzzi G, Gardini F, Vernocchi P, Corsetti A (2006) Application of a novel polyphasic approach to study the lactobacilli composition of sourdoughs from the Abruzzo region (Central Italy). Let Appl Microbiol 43:343–349
133. Corsetti A, Settanni L, Lopez CC, Felis GE, Mastrangelo M, Suzzi G (2007) A taxonomic survey of lactic acid bacteria isolated from wheat (*Triticum durum*) kernels and non-conventional flours. Syst Appl Microbiol 30:561–571
134. Zotta T, Piraino P, Parente E, Salzano G, Ricciardi A (2008) Characterization of lactic acid bacteria isolated from sourdoughs for Cornetto, a traditional bread produced in Basilicata (Southern Italy). World J Microbiol Biotechnol 24:1785–1795
135. Siragusa S, Di Cagno R, Ercolini D, Minervini F, Gobbetti M, De Angelis M (2009) Taxonomic structure and monitoring of the dominant population of lactic acid bacteria during wheat flour sourdough type I propagation using *Lactobacillus sanfranciscensis* starters. Appl Environ Microbiol 75:1099–1109
136. Zannini E, Garofalo C, Aquilanti L, Santarelli S, Silvestri G, Clementi F (2009) Microbiological and technological characterization of sourdoughs destined for bread-making with barley flour. Food Microbiol 26:744–753
137. Coda R, Nionelli L, Rizzello CG, De Angelis M, Tossut P, Gobbetti M (2010) Spelt and emmer flours: characterization of the lactic acid bacteria microbiota and selection of mixed starters for bread making. J Appl Microbiol 108:925–935
138. Minervini F, De Angelis M, Di Cagno R, Pinto D, Siragusa S, Rizzello CG, Gobbetti M (2010) Robustness of *Lactobacillus plantarum* starters during daily propagation of wheat flour sourdough type I. Food Microbiol 27:897–908
139. Rizzello CG, Nionelli L, Coda R, De Angelis M, Gobbetti M (2010) Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. Food Chem 119:1079–1089
140. Azar M, Ter-Sarkissian N, Ghavifek H, Ferguson T, Ghassemi H (1977) Microbiological aspects of Sangak bread. J Food Sci Technol 14:251–254
141. Hüttner EK, Dal Bello F, Arendt EK (2010) Identification of lactic acid bacteria isolated from oat sourdoughs and investigation into their potential for the improvement of oat bread quality. Eur Food Res Technol 230:849–857
142. Moroni AV, Arendt EK, Morrissey JP, Dal Bello F (2010) Development of buckwheat and teff sourdoughs with the use of commercial starters. Int J Food Microbiol 142:142–148
143. Escalante A, Wacher C, Farres A (2001) Lactic acid bacterial diversity in the traditional Mexican fermented dough pozol as determined by 16S rDNA sequence analysis. Int J Food Microbiol 64:21–31
144. Faid M, Boraam F, Zyani I, Larpent JP (1994) Characterization of sourdough bread ferments made in the laboratory by traditional methods. Z Lebensm Unters Forsch 198:287–291
145. Edema MO, Sanni AI (2006) Micro-population of fermenting maize meal for sour maize bread production in Nigeria. Nig J Microbiol 20:937–946

146. Kazanskaya LN, Afanasyeva OV, Patt VA (1983) Microflora of rye sours and some specific features of its accumulation in bread baking plants of the USSR. In: Holas J, Kratochvil F (eds) Developments in food science, vol 5B, Progress in cereal chemistry and technology. Elsevier, London, pp 759–763
147. Barber S, Báguena R (1989) Microflora de la masa madre panaria. XI. Evolución de la microflora de masas madre durante el proceso de elaboración por el sistema de 'refrescos' sucesivos y de sus correspondientes masa panarias. Revista de Agroquímica y Tecnologia de Alimentos 29:478–491
148. Hamad SH, Dieng MC, Ehrmann MA, Vogel RF (1997) Characterization of the bacterial flora of Sudanese sorghum flour and sorghum sourdough. J Appl Microbiol 83:764–770
149. Spicher G, Lönner C (1985) Die Mikroflora des Sauerteiges. XXI. Mitteilung: Die in Sauerteigen Schwedischer Bäckereien vorkommenden Lactobacillen. Z Lebensm Unters Forsch 181:9–13
150. Lönner C, Welander T, Molin N, Dostálek M, Blickstad E (1986) The microflora in a sour dough started spontaneously on typical Swedish rye meal. Food Microbiol 3:3–12
151. Gül H, Özçelik S, Sağdıç O, Certel M (2005) Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. Proc Biochem 40:691–697
152. Gobbetti M, Corsetti A, Rossi J (1996) *Lactobacillus sanfrancisco,* a key sourdough lactic acid bacterium: physiology, genetic and biotechnology. Adv Food Sci 18:167–175
153. Foschino R, Arrigoni C, Picozzi C, Mora D, Galli A (2001) Phenotypic and genotypic aspects of *Lactobacillus sanfranciscensis* strains isolated from sourdoughs in Italy. Food Microbiol 18:277–285
154. Picozzi C, Bonacina G, Vigentini I, Foschino R (2001) Genetic diversity in Italian *Lactobacillus sanfranciscensis* strains assessed by multilocus sequence typing and pulsed-field gel electrophoresis analyses. Microbiology 156:2035–2045
155. Scheirlinck I, Van der Meulen R, De Vuyst L, Vandamme P, Huys G (2009) Molecular source tracking of predominant lactic acid bacteria in traditional Belgian sourdoughs and their production environments. J Appl Microbiol 106:1081–1092
156. Settanni L, Van Sinderen D, Rossi J, Corsetti A (2005) Rapid differentiation and *in situ* detection of 16 sourdough *Lactobacillus* species by multiplex PCR. Appl Environ Microbiol 71:3049–3059
157. Settanni L, Valmorri S, van Sinderen D, Suzzi G, Paparella A, Corsetti A (2006) Combination of multiplex PCR and PCR-denaturing gradient gel electrophoresis for monitoring common sourdough-associated *Lactobacillus* species. Appl Environ Microbiol 72:3793–3796
158. Di Cagno R, De Angelis M, Gallo G, Settanni L, Berloco MG, Siragusa S, Parente E, Corsetti A, Gobbetti M (2007) Genotypic and phenotypic diversity of *Lactobacillus rossiae* strains isolated from sourdough. J Appl Microbiol 103:821–835
159. Groenewald WH, Van Reenen CA, Todorov SD, Du Toit M, Witthuhn R, Holzapfel WH, Dicks LMT (2006) Identification of lactic acid bacteria from vinegar flies based on phenotypic and genotypic characteristics. Am J Enol Viticult 57:519–525
160. Gänzle MG, Ehmann M, Hammes WP (1998) Modeling of growth of *Lactobacillus sanfranciscensis* and *Candida milleri* in response to process parameters of sourdough fermentation. Appl Environ Microbiol 64:2616–2623
161. Picozzi C, D'Anchise F, Foschino R (2006) PCR detection of *Lactobacillus sanfranciscensis* in sourdough and Panettone baked product. Eur Food Res Technol 222:330–335
162. Leroy F, De Winter T, Foulquié Moreno MR, De Vuyst L (2007) The bacteriocin producer *Lactobacillus amylovorus* DCE 471 is a competitive starter culture for type II sourdough fermentations. J Sci Food Agricult 87:1726–1736
163. Hammes WP, Stolz P, Gänzle M (1996) Metabolism of lactobacilli in traditional sourdoughs. Adv Food Sci 18:176–184
164. Gobbetti M, De Angelis M, Corsetti A, Di Cagno R (2005) Biochemistry and physiology of sourdough lactic acid bacteria. Trends Food Sci Technol 16:57–69
165. Gänzle MG, Vermeulen N, Vogel RF (2007) Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. Food Microbiol 24:128–138
166. Gänzle MG (2009) From gene to function: metabolic traits of starter cultures for improved quality of cereal foods. Int J Food Microbiol 134:29–36

167. Dal Bello F, Walter J, Roos S, Jonsson H, Hertel C (2005) Inducible gene expression in *Lactobacillus reuteri* LTH5531 during type II sourdough fermentation. Appl Environ Microbiol 71:5873–5878
168. Hüfner E, Britton RA, Roos S, Jonsson H, Hertel C (2008) Global transcriptional response of *Lactobacillus reuteri* to the sourdough environment. Syst Appl Microbiol 31:323–338
169. Vrancken G, Rimaux T, De Vuyst L, Leroy F (2008) Kinetic analysis of growth and sugar consumption by *Lactobacillus fermentum* IMDO 130101 reveals adaptation to the acidic sourdough ecosystem. Int J Food Microbiol 128:55–66
170. Vrancken G, Rimaux T, Weckx S, De Vuyst L, Leroy F (2009) Environmental pH determines citrulline and ornithine release through the arginine deiminase pathway in *Lactobacillus fermentum* IMDO 130101. Int J Food Microbiol 135:216–222
171. Vrancken G, Rimaux T, Wouters D, Leroy F, De Vuyst L (2009) The arginine deiminase pathway of *Lactobacillus fermentum* IMDO 130101 responds to growth under stress conditions of both temperature and salt. Food Microbiol 26:720–727
172. Weckx S, Allemeersch J, Van der Meulen R, Vrancken G, Huys G, Vandamme P, Van Hummelen P, De Vuyst L (2011) Metatranscriptome analysis for insight into whole-ecosystem gene expression during spontaneous wheat and spelt sourdough fermentations. Appl Environ Microbiol 77:618–626
173. Gänzle MG, Vogel R (2003) Contribution of reutericyclin production to the stable persistence of *Lactobacillus reuteri* in an industrial sourdough fermentation. Int J Food Microbiol 80:31–45
174. Pepe O, Blaiotta G, Anatasio M, Moschetti G, Ercolini D, Villani F (2004) Technological and molecular diversity of *Lactobacillus plantarum* strains isolated from naturally fermented sourdoughs. Syst Appl Microbiol 27:443–453
175. Vrancken G, Rimaux T, Weckx S, Leroy F, De Vuyst L (2011) Influence of temperature and backslopping time on the microbiota of a type I propagated laboratory wheat sourdough fermentation. Appl Environ Microbiol 77:2716–2726
176. Du Toit M, Dicks LMT, Holzapfel WH (2003) Identification of heterofermentative lactobacilli isolated from pig faeces by numerical analysis of total soluble cell protein and RAPD patterns. Lett Appl Microbiol 37:12–16
177. Park SH, Itoh K (2005) Species-specific oligonucleotide probes for the detection and identification of *Lactobacillus* isolated from mouse faeces. J Appl Microbiol 99:51–57
178. De Angelis M, Siragusa S, Berloco M, Caputo L, Settanni L, Alfonsi G, Amerio M, Grandi A, Ragni A, Gobbetti M (2006) Selection of potential probiotic lactobacilli from pig feces to be used as additives in pelleted feeding. Res Microbiol 157:792–801
179. Neysens P, De Vuyst L (2005) Kinetics and modelling of sourdough lactic acid bacteria. Trends Food Sci Technol 16:95–103
180. Böcker G, Stolz P, Hammes WP (1995) Neue Erkentnisse zum Ökosystem Sauerteig und zur Physiologie der Sauerteig-typischen Stämme *Lactobacillus sanfranciscensis* und *Lactobacillus pontis*. Getreide Mehl und Broth 49:370–374
181. Vogel RF, Ehrmann MA, Gänzle MG (2002) Development and potential of starter lactobacilli resulting from exploration of the sourdough ecosystem. Antonie van Leeuwenhoek 81:631–638
182. Ravyts F, De Vuyst L (2011) Prevalence and impact of single-strain starter cultures of lactic acid bacteria on metabolite formation in sourdough. Food Microbiol 28:1129–1139
183. Brandt MJ (2007) Sourdough products for convenient use in baking. Food Microbiol 24:161–164
184. Carnevali P, Ciati R, Leporati A, Paese M (2007) Liquid sourdough fermentation: industrial application perspectives. Food Microbiol 24:150–154
185. Meroth CB, Walter J, Hertel C, Brandt M, Hammes WP (2003) Monitoring the bacterial population dynamics in sourdough fermentation processes by using PCR-denaturing gradient gel electrophoresis. Appl Environ Microbiol 69:475–482
186. Decock P, Cappelle S (2005) Bread technology and sourdough technology. Trends Food Sci Technol 16:113–120
187. Katina K, Heinio R-L, Autio K, Poutanen K (2006) Optimization of sourdough process for improved sensory profile and texture of wheat bread. Food Sci Technol 39:1189–1202

188. Messens W, Neysens P, Vansieleghem W, Vanderhoeven J, De Vuyst L (2002) Modeling growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471 in response to temperature and pH values used for sourdough fermentations. Appl Environ Microbiol 68:1431–1435
189. Brandt MJ, Hammes WP, Gänzle MG (2004) Effects of process parameters on growth and metabolism of *Lactobacillus sanfranciscensis* and *Candida humilis* during rye sourdough fermentation. Eur Food Res Technol 218:333–338
190. Vogelmann SA, Hertel C (2011) Impact of ecological factors on the stability of microbial associations in sourdough fermentation. Food Microbiol 28:583–589
191. Valmorri S, Mortensen HD, Jesperen L, Corsetti A, Gardini F, Suzzi G, Arneborg N (2008) Variations of internal pH in typical Italian sourdough yeasts during co-fermentation with lactobacilli. Food Sci Technol 41:1610–1615
192. Martínez-Anaya MA, Llin ML, Macias MP, Collar C (1994) Regulation of acetic acid production by homo- and heterofermentative lactobacilli in whole-wheat sourdoughs. Z Lebensm Unters Forsch 199:186–190
193. Neysens P, Messens W, De Vuyst L (2003) Effect of sodium chloride on growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471. Int J Food Microbiol 88:29–39
194. Simonson L, Salovaara H, Korhola M (2003) Response of wheat sourdough parameters to temperature, NaCl and sucrose variations. Food Microbiol 20:193–199
195. Gobbetti M (1998) The sourdough microflora: interactions of lactic acid bacteria and yeasts. Trends Food Sci Technol 9:267–274
196. Gobbetti M, Corsetti A, Rossi J (1994) The sourdough microflora – interactions between lactic acid bacteria and yeasts: metabolism of amino acids. World J Microbiol Biotechnol 10:275–279
197. Deak T (2003) Detection, enumeration and isolation of yeasts. In: Boekhout T, Robert V (eds) Yeasts in food, 1st edn. Behr's Verlag, Hamburg, pp 39–68
198. Mian MA, Fleet GH, Hocking AD (1997) Effect of diluent type on viability of yeasts enumerated from foods or pure culture. Int J Food Microbiol 35:103–107
199. Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts: a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 77–100
200. Beuchat LR (1993) Selective media for detecting and enumerating foodborne yeasts. Int J Food Microbiol 19:1–14
201. Arthur H, Watson K (1976) Thermal adaptation in yeasts: growth temperatures, membrane lipid, and cytochrome composition of psychrophilic, mesophilic, and thermophilic yeasts. J Bacteriol 128:56–68
202. de Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135
203. Vogel RF, Böcker G, Stolz P, Ehrmann M, Fanta D, Ludwig W, Pot B, Kersters K, Schleifer KH, Hammes WP (1994) Identification of lactobacilli from sourdough and description of *Lactobacillus pontis* sp. nov. Int J Food Microbiol 44:223–229
204. Vera A, Rigobello V, Demarigny Y (2009) Comparative study of culture media used for sourdough lactobacilli. Food Microbiol 26:728–733
205. Kurtzman CP, Fell JW, Boekhout T (2011) The yeasts: a taxonomic study, vol 1-3, 5th edn. Elsevier, Amsterdam
206. Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73:331–371
207. Cadez N, Poot GA, Raspor P, Smith MT (2003) *Hanseniaspora meyeri* sp. nov., *Hanseniaspora clermontiae* sp. nov., *Hanseniaspora lachancei* sp. nov. and *Hanseniaspora opuntiae* sp. nov., novel apiculate yeast species. Int J Syst Evol Microbiol 53:1671–1680
208. Groenewald M, Daniel HM, Robert V, Poot GA, Smith MT (2008) Polyphasic re-examination of *Debaryomyces hansenii* strains and reinstatement of *D. hansenii, D. fabryi* and *D. subglobosus*. Persoonia 21:17–27

209. Bergerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal barcoding and rapid identification procedures. Appl Microbiol Biotechnol 87:99–108
210. Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecies ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evol Bioinf 4:193–201
211. Kurtzman CP, Robnett CJ (2003) Phylogenetic relationships among yeasts of the '*Saccharomyces* complex' determined from multigene sequence analyses. FEMS Yeast Res 3:417–432
212. Tsui CKM, Daniel HM, Robert V, Meyer W (2008) Re-examining the phylogeny of clinically relevant *Candida* species and allied genera based on multigene analyses. FEMS Yeast Res 8:651–659
213. Vassart G, Georges M, Monsieur R, Brocas H, Lequarre AS, Christophe D (1987) A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235:683–684
214. Landry CR, Townsend JP, Hartl DL, Cavalieri D (2006) Ecological and evolutionary genomics of *Saccharomyces cerevisiae*. Mol Ecol 15:575–591
215. Carreto L, Eiriz MF, Gomes AC, Pereira PM, Schuller D, Santos MAS (2008) Comparative genomics of wild type yeast strains unveils important genome diversity. BMC Genomics 9:524
216. Ness F, Lavallée F, Dubourdieu D, Aigle M, Dulau L (1993) Identification of yeast strains using the polymerase chain reaction. J Sci Food Agric 62:89–94
217. Legras J-L, Karst F (2003) Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. FEMS Microbiol Lett 221:249–255
218. Gancheva A, Pot B, Vanhonacker K, Hoste B, Kersters K (1999) A polyphasic approach towards the identification of strains belonging to *Lactobacillus acidophilus* and related species. Syst Appl Microbiol 22:573–585
219. Sauer S, Kliem M (2010) Mass spectrometry tools for the classification and identification of bacteria. Nature Rev Microbiol 8:74–82
220. De Bruyne K, Slabbinck B, Waegeman W, Vauterin P, De Baets B, Vandamme P (2011) Bacterial species identification from MALDI-TOF mass spectra through data analysis and machine learning. Syst Appl Microbiol 34:20–29
221. Grimont F, Grimont PAD (1986) Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann Inst Pasteur Microbiol 137B:165–175
222. Janssen P, Coopman R, Huys G, Swings J, Bleeker M, Vos P, Zabeau M, Kersters K (1996) Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. Microbiology 142:1881–1893
223. Settanni L, Massitti O, Van Sinderen D, Corsetti A (2005) In situ activity of a bacteriocin-producing *Lactococcus lactis* strain. Influence on the interactions between lactic acid bacteria during sourdough fermentation. J Appl Microbiol 99:670–681
224. Olive DM, Bean P (1999) Principles and applications of methods for DNA-beased typing of microbial organisms. J Clin Microbiol 37:1661–1669
225. Versalovic J, Schneider M, De Bruijn FJ, Lupski JR (1994) Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods Mol Cell Biol 5:25–40
226. Bounaix M-S, Robert H, Gabriel V, Morel S, Remaud-Siméon M, Gabriel B, Fontagné-Faucher C (2010) Characterization of dextran-producing *Weissella* strains isolated from sourdoughs and evidence of constitutive dextran sucrase expression. FEMS Microbiol Lett 311:18–26
227. Bounaix M-S, Gabriel V, Robert H, Morel S, Remaud-Siméon M, Gabriel B, Fontagné-Faucher C (2010) Characterization of glucan-producing *Leuconostoc* strains isolated from sourdough. Int J Food Microbiol 144:1–9
228. Stackebrandt E, Goebel BM (1994) A place for DNA-DNA reassociation and 16S ribosomal RNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849
229. Naser SM, Thompson FL, Hoste B, Gevers D, Dawyndt P, Vancanneyt M, Swings J (2005) Application of multilocus sequence analysis (MLSA) for rapid identification of *Enterococcus* species based on *rpoA* and *pheS* genes. Microbiology 151:2141–2150

230. Stackebrandt E, Frederiksen W, Garrity GM, Grimont PAD, Kampfer P, Maiden MCJ, Nesme X, Rossello-Mora R, Swings J, Truper HG, Vauterin L, Ward AC, Whitman WB (2002) Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 52:1043–1047
231. Konstantinidis KT, Ramette A, Tiedje JM (2006) Toward a more robust assessment of intraspecies diversity, using fewer genetic markers. Appl Environ Microbiol 72:7286–7293
232. De Bruyne K, Schillinger U, Caroline L, Boehringer B, Cleenwerck I, Vancanneyt M, De Vuyst L, Franz CMAP, Vandamme P (2007) *Leuconostoc holzapfelii* sp. nov., isolated from Ethiopian coffee fermentation and assessment of sequence analysis of housekeeping genes for delineation of *Leuconostoc* species. Int J Syst Evol Microbiol 57:2952–2959
233. De Bruyne K, Franz CMAP, Vancanneyt M, Schillinger U, Mozzi F, de Valdez GF, De Vuyst L, Vandamme P (2008) *Pediococcus argentinicus* sp. nov. from Argentinean fermented wheat flour and identification of *Pediococcus* species by *pheS*, *rpoA* and *atpA* sequence analysis. Int J Syst Evol Microbiol 58:2909–2916
234. Achtman M (2008) Evolution, population structure, and phylogeography of genetically monomorphic bacterial pathogens. Ann Rev Microbiol 62:53–70
235. Cai H, Rodriguez BT, Zhang W, Broadbent JR, Steele JL (2007) Genotypic and phenotypic characterization of *Lactobacillus casei* strains isolated from different ecological niches suggests frequent recombination and niche specificity. Microbiology 153:2655–2665
236. Diancourt L, Passet V, Chervaux C, Garault P, Smokvina T, Brisse S (2007) Multilocus sequence typing of *Lactobacillus casei* reveals a clonal population structure with low levels of homologous recombination. Appl Environ Microbiol 73:6601–6611
237. de las Rivas B, Marcobal A, Muñoz R (2006) Development of a multilocus sequence typing method for analysis of *Lactobacillus plantarum* strains. Microbiology 152:85–93
238. Raftis EJ, Salvetti E, Torriani S, Felis GE, O'Toole PW (2011) Genomic diversity of *Lactobacillus salivarius*. Appl Environ Microbiol 77:954–965
239. Ehrmann M, Ludwig W, Schleifer KH (1994) Reverse dot blot hybridization: a useful method for the direct identification of lactic acid bacteria in fermented food. FEMS Microbiol Lett 117:143–150
240. Müller MRA, Ehrmann MA, Vogel RF (2000) Multiplex PCR for the detection of *Lactobacillus pontis* and two related species in a sourdough fermentation. Appl Environ Microbiol 66:2113–2116
241. Valcheva R, Kabadjova P, Rachman C, Ivanova I, Onno B, Prevost H, Dousset X (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
242. Zapparoli G, Torriani S (1997) Rapid identification and detection of *Lactobacillus sanfrancisco* in sourdough by species-specific PCR with 16S rRNA-targeted primers. Syst Appl Microbiol 20:640–644
243. Levin RE (2004) The application of real-time PCR to food and agricultural systems: a review. Food Biotechnol 18:97–133
244. Ercolini D (2004) PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. J Microbiol Meth 56:297–314
245. Palomba S, Blaiotta G, Ventorino V, Saccone A, Pepe O (2010) Microbial characterization of sourdough for sweet baked products in the Campania region (southern Italy) by a polyphasic approach. Ann Microbiol. doi:10.1007/s13213-010-0140-2
246. Reale A, Di Renzo T, Succi M, Tremonte P, Coppola R, Sorrentino E (2011) Identification of lactobacilli isolated in traditional ripe wheat sourdoughs by using molecular methods. World J Microbiol Biotechnol 27:237–244
247. Rantsiou K, Comi G, Cocolin L (2004) The *rpoB* gene as a target for PCR-DGGE analysis to follow lactic acid bacterial population dynamics during food fermentations. Food Microbiol 21:481–487