Chapter 6
Genetics of Lactic Acid Bacteria

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Introduction

Many meat (or fish) products, obtained by the fermentation of meat originating from various animals by the flora that naturally contaminates it, are part of the human diet since millenaries. Historically, the use of bacteria as starters for the fermentation of meat, to produce dry sausages, was thus performed empirically through the endogenous micro-biota, then, by a volunteer addition of starters, often performed by back-slopping, without knowing precisely the microbial species involved. It is only since about 50 years that well defined bacterial cultures have been used as starters for the fermentation of dry sausages. Nowadays, the indigenous micro-biota of fermented meat products is well identified, and the literature is rich of reports on the identification of lactic acid bacteria (LAB) present in many traditional fermented products from various geographical origin, obtained without the addition of commercial starters (See Talon, Leroy, & Lebert, 2007, and references therein). The LAB species that are naturally present in those products and become dominant in the final processing steps essentially belong to *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum*. These are also the three main species that are sold as starters for the fermentation of dry sausages, essentially in Europe, to which should be added the two other pediococci species *Pediococcus pentosaceus* and *Pediococcus acidilactici* (Hammes & Hertel, 1998).

Since the last 20 years, many microbiologists have investigated the physiology of these LAB, in order to understand the mechanisms by which they contribute to the quality of the final product, and to improve their use. Molecular tools were therefore developed, leading to an increase of the knowledge about their genetics. More recently, the genomes of *L. plantarum* WCFS1 (Kleerebezem, et al, 2003), *L. sakei* 23K (Chaillou, et al., 2005), and *P. pentosaceus* ATCC25745 (Makarova, et al., 2006) were entirely sequenced, giving a general overview on the whole genetic repertoire of those bacteria. However, the description and analysis of all

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the genes composing a genome is a hard and tricky task, and authors usually focus on emblematic characteristics of a species when they aim to describe what are the most important genetic traits encoded by a genome. For instance, the genome of *L. plantarum*, a ubiquitous species found in many fermented products but also present in the gastrointestinal tract of mammals and even described as a putative probiotic, was searched mostly for the functions explaining its ubiquity. The genome of *P. pentosaceus* was compared with that of many other LAB, in order to understand evolution and phylogeny of this vast taxonomic group, and finally the *L. sakei* genome was analyzed in order to understand its adaptation to the meat environment.

In this chapter, we will present an overview of the genetic elements known for these species and we will describe, from the three genomes available, some traits that are required for the development of fermented meat products and that are common to *L. sakei*, *L. plantarum*, and *P. pentosaceus*. We will consider *P. pentosaceus* as belonging to lactobacilli, since its genome analysis confirmed that the pediococci should be included in the lactobacilli genera (Makarova et al., 2006).

Mobile Elements and Plasmids in Meat LAB Starters

These genetic elements can be transferred intra- and inter-species and may largely contribute to bacterial genetic evolution and to the acquisition of new functions that will then influence the ability of the host to adapt or to become more competitive to an ecological niche.

Insertion Sequences and Transposons

Transposable genetic elements are ubiquitous, their presence or absence within a genome can vary between individual strains. Transposable elements have impacts on the host genomes by altering gene expression, providing genomic rearrangements, causing insertional mutations and are sources of phenotypic variation. Moreover, numerous systems of bacterial transferable elements such as plasmids, transposons, integrative and conjugative or mobilizable elements, insertion sequences (IS), and prophages are known to promote horizontal gene transfer events and DNA rearrangements. Many of these elements carry accessory genes that encode various functions such as resistance to antibiotics, virulence, and degradation of xenobiotics (Frost, Leplae, Summers, & Toussaint, 2005). Conjugative elements harbor modules involved in their own conjugative transfer, while mobilizable elements carry a module which includes an origin of transfer and one or two mobilization genes, and require the mating machinery provided by the conjugative elements to be transferred. IS contain genes involved in transposition usually edged by two inverted repeat sequences, that allow insertion and excision processes through site-specific recombination. Transposition, performed by transposons or by bacterial IS may lead to the random mutation of chromosomal genes. Twelve IS are found

Table 6.1 Main characteristics of mobile elements in the genomes of LAB meat starters

	Genome size (bp)	Plasmids	Nr of proteins	Nr of prophages	Nr of mobile elements
Lactobacillus sakei 23K	1 884 661	0 (2)*	1886	0 (+1 remnant)	12
Lactobacillus plantarum WCFS1	3 308 274	3	3009	2 (+2 remnant)	15
Pediococcus pentosaceus ATCC25745	1 832 387	0	1757	1	1

* plasmids present in the non cured strain, not yet sequenced.

within the *L. sakei* 23K genome (Chaillou et al., 2005), including five IS*1520*, three IS*Lsa1*, three IS*Lsa2*, and one IS*Lsa3*, belonging to respectively, IS*3*, IS*30*, IS*150*, and IS*4* families (Table 6.1). While only one transposase is present within the genome of *P. pentosaceus*, ATCC 25745 (Makarova et al., 2006), 15 IS are present in *L. plantarum* WCFS1 (Boekhorst et al., 2004). Genetic exchanges between *L. plantarum* and *P. pentosaceus* have been suggested as homologous sucrase genes, sharing 98% nucleic acid identity, were found in the raffinose-sucrose gene clusters that were flanked by IS elements (Naumoff, 2001).

Bacteriophages

Bacteriophages encode genes involved in specific functions like encapsidation, bacterial lysis, recombination, or conjugation that are usually grouped in functional modules. The bacteriophages still cause dramatic damage during fermentation of dairy products.

Despite the increasing number of reports on dairy LAB bacteriophages, information on the meat LAB phages is poorly documented. Very few bacteriophages using *L. sakei*, *P. pentosaceus*, or *L. plantarum* as hosts were isolated from industrial meat fermentation. Basic characteristics of ten virulent phages active on silage-making lactobacilli were investigated (Doi et al., 2003). Morphological properties, host ranges, protein composition, and genome characterization allowed classification into five groups. Morphologically, three phages of group I belonged to the Myoviridae family, while seven other phages of groups II, III, and V belonged to the Siphoviridae family according to the Ackermann classification. The seven phages of groups I, II, and V were active on *L. plantarum* and *L. pentosus*. Phage phiPY4 (group III) infected both *L. casei* and *L. rhamnosus*. Three other *L. plantarum* virulent bacteriophages, phiJL1 (Lu, Altermann, Breidt, Predki, Fleming, & Klaenhammer, 2005; Lu, Breidt, Fleming, Altermann, & Klaenhammer, 2003), phiB2 (Nes, Brendehaug, & von Husby, 1988) and the temperate phage phi g1e (Kakikawa, Yamakawa, et al., 2002; Kakikawa, Yokoi, et al., 2002), have been studied at the morphological, host range, and genome levels. In *L. sakei*

23K, only one remnant prophage is found in the complete genome (Chaillou et al., 2005). One virulent *L. sakei* bacteriophage, PWH2, has been isolated from fermented sausage. Fourteen *L*. sakei strains and five *L. curvatus* strains were tested as potential hosts and only strain *L. sakei* Ls2 was sensitive (Leuschner, Arendt, & Hammes, 1993). These results may explain why no direct proof has ever been shown for a detrimental role of phage attack in meat fermentations.

The PWH2 bacteriophage belongs to the family of Siphoviridae according to the Ackermann classification (Ackermann, 1987). In the genome of *L. plantarum* WCFS1, two entire prophages and two remnants are present (Ventura, et al., 2003). Two prophages were observed in the *P. pentosaceus* ATCC25745 genome (Table 6.1). Little is known about the mechanism of induction or on the effect of their presence in these genomes except for the three prophages of *P. acidilactici*, which can be induced by classical addition of mitomycin C (Caldwell, McMahon, Oberg, & Broadbent, 1999). The new bacteriophages, named pa97, pa40, and pa42 were characterized morphologically and belong to the family Siphoviridae.

Plasmid Content of the Strains

With the rise of molecular biology of the lactobacilli in the late 1980s, their plasmids have become a subject of study, as a means to characterize strains, to search for plasmid-borne functions, or to characterize replicons for potential use as cloning vehicles. Although many plasmids have been isolated, the global plasmid content of lactobacilli has been the object of relatively few and ancient systematic studies (for a review see Wang & Lee, 1997). As other species of lactobacilli, *L. curvatus*, *L. plantarum*, and *L. sakei* have been reported to commonly contain one to about ten plasmids, whatever the (i.e. isolated from/material) vegetable, meat, or fermented origin of the strains (Liu, Kondo, Barnes, & Bartholomew, 1988; Nes, 1984; Ruiz-Barba, Piard, & Jiménez-Diaz, 1991; Vogel, Lohmann, Weller, Hugas, & Hammes, 1991; West & Warner, 1985). *P. pentosaceus* and *P. acidilactici* have also been reported to commonly harbor plasmids (Graham & McKay, 1985; Pérez Pulido et al., 2006). These plasmids are usually circular and their size may range from less than to 2 kb to more than 60 kb. Mega plasmids, larger than 100 kb have been found in *L. plantarum* (Wang & Lee, 1997), although it rather seems to be a specific trait of *L. salivarius* and a few other species of lactobacilli (Li et al., 2007). Linear plasmids among LAB have been described only in *L. salivarius* to date (Li et al., 2007). Some strains have no naturally resident plasmid.

Circular plasmids can be classified according to their mode of replication (rolling-circle replication characterized by an intermediate single-stranded state or theta-type replication) and the need for both host factors and plasmid-borne determinants. The latter comprise at least a segment of DNA called the origin of replication at which replication initiates and may comprise an additional protein called initiator or Rep protein. Usually, newly sequenced plasmids can be assigned to families by sequence homology. A classification of plasmids has

been tentatively proposed at the website of the Database of Plasmid Replicon (http://www.essex.ac.uk/bs/staff/osborn/DPR_home.htm).

Several plasmids from the five species mentioned above have been isolated and characterized. Among those, the two main modes of replication, rolling-circle, and theta replication exist. At the time of writing, the Genbank database contains the complete sequence for one plasmid of *L. curvatus*, one plasmid of *L. sakei*, 15 plasmids of *L. plantarum*, two plasmids of *P. pentosaceus*, and two plasmids of *P. acidilactici*. Their characteristics are given in Table 6.2. One obvious conclusion is that plasmids belonging to the same family are distributed across species. A picture of plasmid distribution can also be brought by hybridization experiments, which detects homology relationships. For example, it is known that *L. sakei* and *L. curvatus* strains harbor plasmids related to the pLP1 rolling-circle plasmid, originally isolated from *L. plantarum* (Bringel, Frey, & Hubert, 1989) or that pLC2-type plasmids, originally described in *L. curvatus*, are also detected in *L. sakei* (Vogel et al., 1991).

Another characteristic which was revealed by sequence data is the composite nature of plasmids, made of mosaics of segments or clusters of genes from different origins. Typical examples are given by pMD5057 (Danielsen, 2002) and pRV500 (Alpert, Crutz-Le Coq, Malleret, & Zagorec, 2003).

Plasmid-Encoded Functions

So far, only a few functions, other than those involved in replication or transfer, have been identified on these plasmids. Many cryptic plasmids with no characterized phenotype are known in lactobacilli. Among the functions for which the presence of plasmids correlated with a particular phenotype, we can mention cystein transport in *L. sakei* (Shay, Egan, Wright, & Rogers, 1988), β-galactosidase activity in *L. plantarum* (Mayo, Gonzalez, Arca, & Suarez, 1994), maltose utilization in *L. plantarum* and *Lactobacillus* ssp. isolated from fresh meat (Liu et al., 1988) and utilization of various sugars like raffinose, melibiose, maltose, or sucrose in *P. pentosaceus and P. acidilactici* (Gonzalez & Kunka, 1986; Gonzalez & Kunka, 1987; Halami, Ramesh, & Chandrashekar, 2000). Bacteriocin production, that can be chromosomally encoded, has also been reported to be encoded by plasmids in several species (Giacomini, Squartini, & Nuti, 2000; Gonzalez & Kunka, 1987; Kanatani & Oshimura, 1994; Kantor, Montville, Mett, & Shapira, 1997; Miller, Ray, Steinmetz, Hanekamp, & Ray, 2005; Motlagh, Bukhtiyarova, & Ray, 1994; Osmanagaoglu, Beyatli, & Gunduz, 2000; Simon, Frémaux, Cenatiempo, & Berjeaud, 2002; Skaugen, Abildgaard, & Nes, 1997; Van Reenen, Van Zyl, & Dicks, 2006; Vaughan, Eijsink, & Van Sinderen, 2003).

As seen in Table 6.2, conjugative or mobilizable elements are also commonly encoded by plasmids (Gevers, Huys, & Swings, 2003). They can, in addition, encode antibiotic resistance genes and metabolic functions which can be transferred between strains present in the bacterial population sharing the same ecological

Table 6.2 Main features of sequenced plasmids isolated from meat LAB starters

Isolated from	Name	Size (kb)	Genbank accession	Function or genes identified (other than those involved in plasmid maintenance)	type of replication and replicon family
L. sakei	pRV500	13	NC_004942	Putative type I restriction modification system	theta; pUCL287
L. curvatus	pLC2	2.5	Z14234	Cryptic	“rolling-circle”; pE194 / pWV01
L. plantarum	p256	7.2	NC_006278	Putative prophage protein, putative cold shock protein	theta; without Rep protein
	pWCFS103	36	NC_006377	Arsenical resistance, DNA-damage-inducible gene, plasmid transfer via conjugation	theta; pLS32
	pLKS	2	AB035265	Phage resistance	theta; pUCL287
	pMD5057	10.9	NC_004944	Tetracycline resistance *tet*(M) gene	
	pLJ42	5.5	DQ099911	Cryptic, mobilization protein	
	pWCFS101	1.9	NC_006375	Cryptic	“rolling-circle”; pC194 / pUB110
	pLP2000	2	NC_003893	Cryptic	
	pLTK2	2,3	NC_002123	Cryptic	
	pM4	3.3	NC_009666	Cryptic, mobilization protein	
	pLP1	2.1	M31223	Cryptic	
	pWCFS102	2.4	NC_006376	Cryptic	“rolling-circle”; pE194 / pWV01
	pPB1	3	NC_006399	Cryptic, mobilization protein	
	pLP9000	9.3	NC_003894	Cryptic	“rolling-circle”; unknown
	pLKL	6.8	AB219181	Phage protein homologs	unknown
	pPLA4	8.1	AF304384	Bacteriocin 423 operon, mobilization protein	unknown
P. pentosaceus	pMD136	19.5	NC_001277	Pediocin A production, putative oxyquinone oxydoreductases, putative ABC transporter, mobilization protein	theta; pWV02
	pRS4	3.5	AJ968953	Mobilization protein	“rolling-circle”; pC194 / pUB110
P. acidilactici	pSMB74	9	NC_004832	Pediocin AcH	theta; pUCL287
	pEOC01	11.7	DQ220741	Streptomycin resistance / erythromycin methylase B	theta; pAMβ1

niche. The possibility of the dissemination of antibiotic resistance by meat lactobacilli has been addressed by Gevers, Danielsen, Huys, and Swings, (2003), who showed that tetracycline resistance plasmids could be transferred from *L. sakei* or *L. plantarum* to other Gram-positive bacteria such as *Enterococcus faecalis* and *Lactococcus lactis* by in vitro conjugation (Gevers, Danielsen, et al., 2003; Gevers, Huys, et al., 2003). This suggests that meat lactobacilli might be the reservoir microorganisms for acquired resistance genes that can be spread among other bacteria.

A number of other plasmid-encoded functions were assigned after plasmid sequence determination, such as putative restriction modification systems or putative stress proteins (see Table 6.2). Thus, plasmids may clearly carry a source of genetic diversity and provide an extension of metabolic properties or additional resistance to environmental conditions.

Vectors and Genetic Tools

Geneticists developed various genetic tools in order to construct mutants, either randomly or in targeted genes, to express foreign genes, or to monitor their expression. Such approaches rely on the capability to transfer DNA molecules, therefore efficient transformation protocols have been developed in parallel.

Electroporation, based on transient pore formation in membranes after applying an electrical field, is the method of choice for gene transfer and protocols have been adapted for each species. Depending on the recipient strains used, they give different amount of transformants, the most efficient ones reaching up to 10^4–10^6 transformants per μg of plasmid DNA (Alegre, Rodriguez, & Mesas, 2004; Aukrust & Blom, 1992; Berthier, Zagorec, Champomier-Vergès, Ehrlich, & Morel-Deville, 1996; Luchansky, Muriana, & Klaenhammer, 1988; Rodriguez, Alegre, & Mesas, 2007).

Conjugation is known to naturally occur in lactobacilli but requires conjugative plasmids. It can be made through the use of broad host-range conjugative plasmids isolated from other bacteria such as pAMβ1 or pIP501 (Gonzalez & Kunka, 1983; Langella, Zagorec, Ehrlich, & Morel-Deville, 1996) either alone or as helpers with mobilizable plasmids. Conjugation has proven useful for plasmid transfer into strains that are poorly transformable by electroporation.

Natural competence and transformation were never reported in LAB, although complete genome sequences revealed the presence of genes homologous to bona fide competence genes involved in DNA transport, in other bacterial groups. The question of naturally occurring gene transfer is of particular interest with food-borne organisms. Plasticity of the chromosomes and plasmids has revealed the relative importance of gene transfer especially among bacteria sharing a same biotope. This may then occur by natural, but not yet characterized competence, stress-induced permeation, plasmid mobilization/conjugation, or phage infection. Indeed, conjugative transfers were detected to occur during sausage fermentation with *L. curvatus* (Vogel, Becke-Schmid, Entgens, Gaier, & Hammes, 1992).

Plasmid vectors have been developed for *Lactobacillus* and to a lesser extent for *Pediococcus*, whose molecular biology is still at its beginning. A non-exhaustive list of vectors and methods used in "meat species" is highlighted here. A comprehensive review of the natural plasmids from LAB and derived vectors was recently published by Shareck, Choi, Lee, & Miguez (2004).

Integrative vectors (also called delivery or suicide vectors), that is those that are incapable of self-replication in the recipient host, are used for the construction of chromosomal mutants. The vector part often comes from widely used plasmids of *Escherichia coli* of the colE1-type, like pRV300 (Leloup, Ehrlich, Zagorec, & Morel-Deville, 1997) or pJDC9 (Chen & Morrison, 1988). Chromosomal integration relies on homologous recombination and allows different kinds of mutations to be obtained: (*i*) inactivation of genes after the simple insertion of the recombinant vector, or (*ii*) point mutations (Stentz & Zagorec, 1999) and deletion mutants (Ferain et al., 1996; Leer et al., 1993; Malleret, Lauret, Ehrlich, Morel-Deville, & Zagorec, 1998; Stentz, Loizel, Malleret, & Zagorec, 2000), for which no exogenous DNA persists. The latter strategy of gene replacement usually involves two successive recombination steps; the first one involves selecting for integration of the entire plasmid, the second one consisting of an excision of plasmid sequences between regions duplicated after integration. In the case of high recombination frequency and provided it can be selected or screened for, a double-recombination event may be obtained in one step (Malleret et al., 1998). Conditionally replicative vectors (replicating only in so-called permissive conditions) are considered as the most effective delivery vectors, especially for low transformable strains, because they allow temporal separation of the transformation and integration steps. Some plasmids have been found to be naturally thermo-sensitive at 42°C, and thermo-sensitive vectors have also been developed (see Shareck et al., 2004). However, it should be noted that this character is strongly dependent on the host and that no thermo-sensitive plasmid has been found effective in mesophilic species, such as a number of *L. sakei* strains, which do not grow over 37°C (Gory, Montel, & Zagorec, 2001).

Replicative vectors can be used to easily bring new functions in the cell or to express genes at a high level (expression vectors). Inducible promoters have been used for over-expression (Sørvig, Mathiesen, Naterstad, Eijsink, & Axelsson, 2005) or to modulate functions (Stentz et al., 2000).

Reporter genes are also interesting tools in the molecular biologist's arsenal and comprise *lacZ* (Stentz et al., 2000), *gusA* (Hertel, Schmidt, Fischer, Oellers, & Hammes, 1998) and the Green Fluorescent Protein (GFP) gene (Gory et al., 2001), the activity of the latter being detected by fluorescence, thereby allowing to follow the strains in complex environments. It should be noted that the use of *lacZ* may be hampered by the presence of β-galactosidase activity often existing in a number of lactobacilli.

In a view of food-grade applications, plasmids should not contain DNA originating out of LAB species and in particular should not harbor genes for resistance to antibiotics. In this context, appropriate selective cassettes have been developed; nisin immunity conferred by *nisI* has been used in *L. plantarum* (Takala & Saris, 2002), and selection based on specific metabolic properties could be used

provided the encoding genes can complement a deficient strain as described in Bron, et al., (2002); and in Takala, Saris, and Tynkkynen, (2003).

Random mutagenesis via transposition is still to be developed in meat starter species, this approach also requires thermo-sensitive delivery vectors. Genetic tools have been developed in LAB using IS, such as IS946 or IS*AS*1, in combination with non-replicative vectors to efficiently perform site-directed or random mutagenesis by transposition (Maguin, Prévost, Ehrlich, & Gruss, 1996; Romero & Klaenhammer, 1992). No system based on the IS elements present in their genome have yet been developed for *L. sakei*, *P. pentosaceus*, and *L. plantarum*. Attempts for obtaining random transposition were performed in *L. plantarum* with variable success. Whereas Tn*917* delivered by pTV1Ts essentially targeted the naturally harbored plasmids (Cosby, Axelsson, & Dobrogosz, 1989), pGhost9:IS*S*1 was successfully used at 42°C to obtain a set of random transposition mutants (Gury, Barthelmebs, & Cavin, 2004).

Chromosomal Elements

In addition to the genetic elements mentioned above, that largely participate in the genetic repertoire and biodiversity of bacteria, the chromosomally encoded functions represent the large majority of the genetic elements that characterize a bacterial species and its properties.

The chromosomes of *L. plantarum* WCFS1, *L. sakei* 23K, and *P. pentosaceus* ATCC27745 encode respectively 3009, 1886, and 1757 coding sequences (Table 6.1), showing a large difference between those species. Besides housekeeping functions, the genetic repertoire of those bacteria shows specific features that are common to the three species, and absent from other closely related bacteria (lactobacilli) which do not usually develop in meat products. One can thus hypothesize that these specific features are important for the adaptation to the fermented meat environment. They mostly allow bacteria to grow in meat as a substrate, and to resist the stressing environment that the steps of meat fermentation represent, for instance the presence of high amount of salt, redox potential variations, or temperature changes during the process.

Functions Involved in Growth and Fermentation

The main role of LAB starters during meat fermentation is to degrade sugars to produce mainly lactic acid. Some traditional sausages can be manufactured without the addition of any sugar. In this case, LAB performs fermentation through the utilization of the carbon sources that are naturally present in meat, mainly ribose and glucose. We previously noticed that in *L. sakei*, the gene cluster involved in ribose utilization had a characteristic organization (Stentz & Zagorec, 1999). Indeed, ribose transport was not performed by an ABC transporter as usually observed in other

bacteria. Instead of the *rbsABCD* genes encoding the ribose specific ABC transporter, we found a gene, named *rbsU* that was similar to glucose transporters mediating sugar transport by a facilitated diffusion mechanism. Interestingly, a similar situation is observed in the genomes of *L. plantarum* and *P. pentosaceus*, but also in the genome of *Enterococcus faecalis* V583, a bacterial species that can contaminate meat products. Such a situation is not encountered in other LAB species, the genome of which has been sequenced. It is therefore plausible that such a property, shared by meat-borne bacteria confers them an advantage to compete for carbon sources, useful for energy production and growth. As a comparison, the gene syntheny observed around the *ldhL* gene of *L. sakei*, encoding the unique lactate dehydrogenase in this bacterium, responsible for L-lactate production, is conserved in *L. plantarum* and *P. pentosaceus* but also in other LAB genomes such as those of *L. casei* ATCC334 and *L. brevis* ATCC367, which are not used for meat fermentation. Concerning the capability to ferment sugars, it was previously noticed that *L. plantarum* has the widest range of sugar utilization due to the presence of many sugar transport systems, a characteristic trait of this species that is certainly linked to the many ecosystems it can colonize (Kleerebezem et al., 2003).

Meat is an iron rich medium, especially regarding complexed iron like haemoglobin or myoglobin. Iron metabolism seems to be emblematic of meat LAB species. More particularly, the ability to transport complex iron seems to be restricted to meat lactobacilli; only two other LAB species (*L. casei* and *Lactococcus lactis*) possess such an equipment (Table 6.3). *L. plantarum*, is also the only LAB species possessing a ironIII ABC transporter (AfuA). Moreover, iron dependent transcriptional regulators (Fur family) are also well represented in these meat LAB species. In fact, three are present in the *L. sakei* genome and two in both *L. plantarum* and *P. pentosaceus*. It seems thus likely that the ability to use iron compounds and especially iron complexes could be considered as a kind of meat signature for meat LAB species.

Functions Involved in Resistance to the Stressing Environment of Fermented Meat Products

Addition of NaCl to fermented meat products, as well as the drying process induce a high osmolarity environment which the bacterial should resist in order to survive in the processed meat. We previously noticed in the genome of *L. sakei* 23K the presence of three gene clusters encoding putative ABC transporters that may be involved in the uptake of osmoprotectants, such as glycine betaine, carnitine, or choline (Chaillou et al., 2005). Interestingly, *Listeria monocytogenes*, a pathogenic Gram-positive bacterium that can contaminate meat products shares the same equipment. However, among those three systems, only the cluster *lsa*0616-0619 was also conserved in *L. plantarum* WCFS1 (corresponding to *lpl*1607-1610) and *P. pentosaceus* ATCC25745 (genes *pepe*1655-1652). The two other osmoprotectant transporters of *L. sakei*, encoded by *lsa*1694-1695 and *lsa*1869-1870 may result from a duplication

Table 6.3 Comparison of redox equipment in *L. sakei* 23K, *L. plantarum* WCFS1, and *P. pentosaceus* 25,745

	L. sakei	L. plantarum	P. pentosaceus	Other LAB
NADH oxidase	2	6	1	*L. casei, L. brevis, L. reuteri, L. sanfransiscensis*
NADH peroxidase	1	1	1	*L. casei*, *L. salivarius, L. brevis, L. acidophilus, L. gasseri*
SOD	1	none	none	*L. casei*
Thiol peroxidase	1	1	1	*L. reuteri*, *L. brevis*, *L. salivarius*, *L acidophilus*, *L. jonhsonii*
NADH, dye-type peroxidases	1	1	none	*L. casei, L. brevis L. salivarius, L. reuteri*
Hydroperoxide resistance	1	none	none	*Lc. lactis*
Catalase	1	1	none	*L. brevis*
CytB5	1	1	none	*L. acidophilus, L. salivarius*
CytP450	1	none	none	None in LAB
Glutaredoxine,	1	1	1	*L. casei, L. salivarius L. brevis, L. delbrueckii*
Glutathione reductase	1	4	1	*L. casei, L. salivarius L. brevis, L. gasseri, L. reuteri*
Glutathion synthase	1	2	none	*L. brevis, L. reuteri, L. salivarius, L. casei*
Thioredoxines	4	4	3	*L. acidophilus*, *L. brevis*, *L. casei*, *L. delbrueckii, L. johnsonii*
Thioredoxine reductases	3	2	2	*L. acidophilus*, *L. brevis*, *L. casei*, *L. delbruecki, L. johnsonii*
Ferric Uptake Regulators	3	2	2	*L. brevis, L. salivarius L. casei*
Iron uptake Complex iron (Fhu)	1	1	none	*Lc. lactis, L.brevis*
Iron III	none	1	none	None in LAB
IronII/IIIMn	1	1	none	*L. casei*
IronII/Mn	3	4	2	*Lc. Lactis, L. johnsonnii, L. casei, L. brevis, Lc. lactis, L. brevis, L. salivarius, L. delbrueckii*
p-coumaric acid decarboxylase	1	1	1	*Lactobacillus* species
Aromatic hydrocarbon decarboxy-lases	2	1	1	*L. reuteri, L. brevis*

as both *L. plantarum* and *P. pentosaceus* had only one additional system (*lpl*0367-0368, and *pepe*0242-0243, respectively) that are equally similar to both the systems of *L. sakei*. Thus, *L. sakei* may be better adapted to high osmolarity than the two other LAB starters.

Meat storage and processing are characterized by numerous changes in oxygen levels, including the use of reducing agents such as nitrate and nitrite in fermented meat products. All the bacterial species present in the meat ecosystem thus have to cope with this main stressing environmental factor in order to grow and survive in fermented meat products. Protection against oxidative damage implies both defense systems against Reactive Oxygen Species (ROS) and repair systems to restore protein functionality or DNA damage. Iron can also enhance oxygen toxicity due to a possible arising of chemical reactions and the consecutive generation of ROS by the Fenton reactions. The emblematic meat species *L. sakei* has previously been reported as a bacterium with complete genetic equipment dedicated to defense against oxidative stress (Chaillou et al., 2005). These features have been searched in the two other meat LAB species for which whole genome sequence was available (Table 6.3). This genome comparison revealed some common features together with species specific traits that could be considered specific for meat LAB starters.

Analysis of the genome content of *L. plantarum* WCFS1, *L. sakei* 23K, and *P. pentosaceus* ATCC25745 shows that the three species harbor a genetic equipment dedicated to protection against oxidative damages. However, this equipment seems to be more similar between *L. plantarum* and *L. sakei, P. pentosaceus* being devoid of the main functions related to peroxide and hydroperoxide resistance. *L. sakei* harbors the most complete equipment for fighting against oxygen toxicity, including, in particular, one superoxide dismutase, a hydroperoxide resistance protein and an uncharacterized NADH oxidase that is unique in LAB. *L. plantarum* is clearly characterized by a high redundancy in glutathion reductases and glutathion synthase, a compound involved in protection against oxidative stress. It appears then that *P. pentosaceus* might be less resistant, than the two other species, to oxidative damage conditions encountered during meat fermentation.

Aromatic compounds can arise from exposure to spices and smoke, that can be used as meat bio-preservation methods, in addition to the fermentation process. Detoxification of such compounds can be achieved by a *p*-coumaric acid decarboxylase (PdcA) and aromatic hydrocarbon decarboxylases. PdcA homologs are largely found in *Lactobacillus* species. Homologs of the aromatic hydrocarbon decarboxylases are present in meat LAB and less represented in other LAB (only *L. brevis* and *L. reuteri*). Remarkably, *L. sakei* is unique among LAB, since it is the only species possessing a specific aromatic hydrocarbon hydroxylase for which no homologous protein is found in other LAB. Moreover, a gene encoding a putative cytochrome P450, whose function is yet unknown, is present only in *L. sakei*.

Conclusion

Genetic studies on LAB emerged a few decades ago, as a mean for microbiologists to better understand the mechanisms of food fermentation, in order to control and improve it or to select the better starter strains. Most of the literature was, for a long time, almost exclusively dedicated to the dairy LAB *Lc. lactis*. However,

although with a certain delay, the genetics of other species, including that of meat LAB starters, gained the interest of the scientific community. The study of the plasmidic genetic elements was relatively poorly developed, mainly aimed as a tool for species characterization or for studies on systematics or classification. The search for bacteriocins, to improve meat safety, led to the isolation of several plasmids but, unfortunately, only the genes responsible for bacteriocin production were characterized, plasmids by themselves being poorly considered. The global conclusion of the genetic knowledge gained from the study of plasmids in LAB in general is that those genetic elements, as other mobile elements, are largely shared and exchanged between strains, species, and genera.

In the 1990s, molecular biologists started to develop tools and methods to genetically modify strains, as a basis to gain knowledge on the metabolism of LAB, and with the dream to control and orientate metabolic fluxes for improving fermentation processes. However, the use of genetically modified organisms in food, especially in fermented food that are eaten raw, i. e., with living microorganisms, such as fermented meat is not acceptable for the consumer. The use of genetics for LAB thus stayed just as a tool for laboratory studies to generate information.

In the mean time, genomics emerged and an increasing number of bacterial genomes were sequenced and analyzed each year during the last decade. After the sequencing of bacterial species, which were considered as models by the microbiologists, many pathogenic species were also sequenced, and finally, the genomes of most of the LAB used for food fermentation have been sequenced in the last years. The genome sequence of *L. plantarum* was published in 2003, that of *L. sakei* in 2005, and that of *P. pentosaceus* in 2006. Consequently, the delay traditionally observed with the knowledge of meat LAB compared to dairy LAB stopped.

Whole genome analysis revealed specific features of the various species. The comparison between several species and genera of LAB confirmed the status of *P. pentosaceus* as belonging to the *Lactobacillus* genus (Makarova et al., 2006). A careful analysis of the genome features common to meat LAB has still to be performed. However, some characteristic traits have already been detected: (*i*) all specifically show a metabolic capability oriented to the meat environment, which is not present in other LAB. This is exemplified by the use of ribose or iron, two nutrients present in meat and absent or substituted by other components in other materials that are also commonly fermented such as milk or vegetables; (*ii*) all show, with some species differences, the ability to resist the stressing environment that characterizes meat fermentation, such as oxidative or salt stresses or detoxification of molecules issued from spices or smoking.

References

Ackermann, J. W. (1987). Bacteriophage taxonomy in 1987. *Microbiology Sciences, 4*, 214–218.

Alegre, M. T., Rodriguez, M. C., & Mesas, J. M. (2004). Transformation of *Lactobacillus plantarum* by electroporation with in vitro modified plasmid DNA. *FEMS Microbioogy Letters, 241*, 73–77.

Alpert, C. A., Crutz-Le Coq, A.-M., Malleret, C., & Zagorec, M. (2003). Characterization of a theta-type plasmid from *Lactobacillus sakei*: A potential basis for low-copy vectors in lactobacilli. *Applied and Environmental Microbiology, 69*, 5574–5584.

Aukrust, T., & Blom, H. (1992). Transformation of *Lactobacillus* strains used in meat and vegetable fermentation. *Food Research International, 25*, 253–261.

Berthier, F., Zagorec, M., Champomier-Vergès, M., Ehrlich, S. D., & Morel-Deville, F. (1996). High-frequency transformation of *Lactobacillus sake* by electroporation. *Microbiology, 142*, 1273–1279.

Boekhorst, J., Siezen, R. J., Zwahlen, M. C., Vilanova, D., Pridmore, R. D., Mercenier, A., et al. (2004). The complete genomes of *Lactobacillus plantarum* and *Lactobacillus johnsonii* reveal extensive differences in chromosome organization and gene content. *Microbiology, 150*, 3601–3611.

Bringel, F., Frey, L., & Hubert, J. C. (1989). Characterization, cloning, curing, and distribution in lactic acid bacteria of pLP1, a plasmid from *Lactobacillus plantarum* CCM1904 and its use in shuttle vector construction. *Plasmid, 22*, 193–202.

Bron, P. A., Benchimol, M. G., Lambert, J., Palumbo, E., Deghorain, M., Delcour, J., et al. (2002). Use of the *alr* gene as a food-grade selection marker in lactic acid bacteria. *Applied and Environmental Microbiology, 68*, 5663–5670.

Caldwell, S. L., McMahon, D. J., Oberg, C. J., & Broadbent, J. R. (1999). Induction and characterization of *Pediococcus acidilactici* temperate bacteriophage. *Systematic and Applied Microbiology, 22*, 514–519.

Chaillou, S., Champomier-Vergès, M. C., Cornet, M., Crutz Le Coq, A.-M., Dudez, A.-M., et al. (2005). Complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nature Biotechnology, 23*, 1527–1533.

Chen, J. D., & Morrison, D. A. (1988). Construction and properties of a new insertion vector, pJDC9, that is protected by transcriptional terminators and useful for cloning of DNA from *Streptococcus pneumoniae*. *Gene, 64*, 155–164.

Cosby, W. M., Axelsson, L. T., & Dobrogosz, W. J. (1989). Tn917 transposition in *Lactobacillus plantarum* using the highly temperature-sensitive plasmid pTV1Ts as a vector. *Plasmid, 22*, 236–243.

Danielsen, M. (2002). Characterization of the tetracycline resistance plasmid pMD5057 from *Lactobacillus plantarum* 5057 reveals a composite structure. *Plasmid, 48*, 98–103.

Doi, K., Zhang, Y., Nishizaki, Y., Umeda, A., Ohmomo, S., & Ogata, S. (2003). A comparative study and phage typing of silage-making *Lactobacillus* bacteriophages. *Journal of Bioscience and Bioengineering, 95*, 518–525.

Ferain, T., Hobbs, J. N., Jr., Richardson, J., Bernard, N., Garmyn, D., Hols, P., et al. (1996). Knockout of the two *ldh* genes has a major impact on peptidoglycan precursor synthesis in *Lactobacillus plantarum*. *Journal of Bacteriology, 178*, 5431–5437.

Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution. *Nature Reviews Microbiology, 3*, 722–732.

Gevers, D., Danielsen, M., Huys, G., & Swings, J. (2003). Molecular characterization of *tet*(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. *Applied and Environmental Microbiology, 69*, 1270–1275

Gevers, D., Huys, G., & Swings, J. (2003). In vitro conjugal transfer of tetracycline resistance from *Lactobacillus* isolates to other Gram-positive bacteria. *FEMS Microbiology Letters, 225*, 125–130.

Giacomini, A., Squartini, A., & Nuti, M. P. (2000). Nucleotide sequence and analysis of plasmid pMD136 from *Pediococcus pentosaceus* FBB61 (ATCC43200) involved in pediocin A production. *Plasmid, 43*, 111–122.

Gonzalez, C. F., & Kunka, B. S. (1983). Plasmid transfer in *Pediococcus* spp.: Intergeneric and intrageneric transfer of pIP501. *Applied and Environmental Microbiology, 46*, 81–89.

Gonzalez, C. F., & Kunka, B. S. (1986). Evidence for plasmid linkage of raffinose utilization and associated alpha-galactosidase and sucrose hydrolase activity in *Pediococcus pentosaceus*. *Applied and Environmental Microbiology, 51*, 105–109.

Gonzalez, C. F., & Kunka, B. S. (1987). Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. *Applied and Environmental Microbiology, 53*, 2534–2538.

Gory, L., Montel, M. C., & Zagorec, M. (2001) Use of Green Fluorescent Protein to monitor *Lactobacillus sakei* in fermented meat products. *FEMS Microbiology Letters, 194*, 127–133.

Graham, D. C., & McKay, L. L. (1985). Plasmid DNA in strains of *Pediococcus cerevisiae* and *Pediococcus pentosaceus*. *Applied and Environmental Microbiology, 50*, 532–534.

Gury, J., Barthelmebs, L., & Cavin, J. F. (2004). Random transposon mutagenesis of *Lactobacillus plantarum* by using the pGh9:IS*S1* vector to clone genes involved in the regulation of phenolic acid metabolism. *Archives in Microbiology, 182*, 337–345.

Halami, P. M., Ramesh, A., & Chandrashekar, A. (2000). Megaplasmid encoding novel sugar utilizing phenotypes, pediocin production and immunity in *Pediococcus acidilactici* C20. *Food Microbiology, 17*, 475–483.

Hammes, W. P., & Hertel, C. (1998). New development in meat starter culture. *Meat Science, 49*, S125–S128.

Hertel, C., Schmidt, G., Fischer, M., Oellers, K., & Hammes, W. P. (1998). Oxygen-dependent regulation of the expression of the catalase gene *katA* of *Lactobacillus sakei* LTH677. *Applied and Environmental Microbiology, 64*, 1359–1365.

Kakikawa, M., Yamakawa, A., Yokoi, K. J., Nakamura, S., Taketo, A., & Kodaira K. (2002) Characterization of the major tail protein gpP encoded by *Lactobacillus plantarum* phage phi gle. *Journal of Biochemistry, Molecular Biology, and Biophysics, 6*, 185–191.

Kakikawa, M., Yokoi, K. J., Kimoto, H., Nakano, M., Kawasaki, K., Taketo, A., et al. (2002). Molecular analysis of the lysis protein Lys encoded by *Lactobacillus plantarum* phage phi g1e. *Gene, 299*, 227–234.

Kanatani, K., & Oshimura, M. (1994). Plasmid-associated bacteriocin production by a *Lactobacillus plantarum* strain. *Bioscience Biotechnology Biochemistry, 58*, 2084–2086.

Kantor, A., Montville, T. J., Mett, A., & Shapira, R. (1997). Molecular characterization of the replicon of the *Pediococcus pentosaceus* 43200 pediocin A plasmid pMD136. *FEMS Microbiology Letters, 151*, 237–244.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., et al. (2003). Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proceedings of the National Academy of Science USA, 100*, 1990–1995.

Langella, P., Zagorec, M., Ehrlich, S. D., & Morel-Deville, F. (1996). Intergeneric and intrageneric conjugal transfer of plasmids pAMβ1, pIL205 and pIP501 in *Lactobacillus sake*. *FEMS Microbiology Letters, 139*, 51–56.

Leer, R. J., Christiaens, H., Verstraete, W., Peters, L., Posno, M., & Pouwels, P. H. (1993). Gene disruption in *Lactobacillus plantarum* strain 80 by site-specific recombination: isolation of a mutant strain deficient in conjugated bile salt hydrolase activity. *Molecular and General Genetics, 239*, 269–272.

Leloup, L., Ehrlich, S. D., Zagorec, M., & Morel-Deville, F. (1997). Single cross-over integration in the *Lactobacillus sake* chromosome and insertional inactivation of the *ptsI* and *lacL* genes. *Applied and Environmental Microbiology, 63*, 2127–2133.

Leuschner, R. G. K., Arendt, E. K., & Hammes, W. P. (1993). Characterization of a virulent *Lactobacillus sake* phage PWH2. *Applied Microbiology and Biotechnology, 39*, 617–621.

Li, Y., Canchaya, C., Fang, F., Raftis, E., Ryan, K. A., van Pijkeren, J. P., et al. (2007). Distribution of megaplasmids in *Lactobacillus salivarius* and other lactobacilli. *Journal of Bacteriology, 189*, 6128–6139.

Liu, M. L., Kondo, J. K., Barnes, M. B., & Bartholomew, D. T. (1988). Plasmid-linked maltose utilization in *Lactobacillus* ssp. *Biochimie, 70*, 351–355.

Lu, Z., Altermann, E., Breidt, F., Predki, P., Fleming, H. P., & Klaenhammer, T. R. (2005). Sequence analysis of the *Lactobacillus plantarum* bacteriophage PhiJL-1. *Gene, 348*, 45–54.

Lu, Z., Breidt, F. Jr., Fleming, H. P., Altermann, E., & Klaenhammer, T. R. (2003). Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, phiJL-1, from a cucumber fermentation. *International Journal of Food Microbiology, 84*, 225–235.

Luchansky, J. B., Muriana, P. M., & Klaenhammer, T. R. (1988). Application of electroporation for transfer of plasmid DNA to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria*, *Pediococcus*, *Bacillus*, *Staphylococcus*, *Enterococcus* and *Propionibacterium*. *Molecular Microbiology, 2*, 637–646.

Maguin, E., Prévost, H., Ehrlich, S. D., & Gruss, A. (1996). Efficient insertional mutagenesis in lactococci and other Gram-positive bacteria. *Journal of Bacteriology, 178*, 931–935.

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., et al. (2006). Comparative genomics of the lactic acid bacteria. *Proceedings of the National Academy of Science USA, 103*, 15611–15616.

Malleret, C., Lauret, R., Ehrlich, S. D., Morel-Deville, F., & Zagorec, M. (1998). Disruption of the sole *ldhL* gene in *Lactobacillus sakei* prevents the production of both L- and D-lactate. *Microbiology, 144*, 3327–3333.

Mayo, B., Gonzalez, B., Arca, P., & Suarez, J. E. (1994). Cloning and expression of the plasmid encoded beta-D-galactosidase gene from a *Lactobacillus plantarum* strain of dairy origin. *FEMS Microbiology Letters, 122*, 145–151.

Miller, K. W., Ray, P., Steinmetz, T., Hanekamp, T., & Ray, B. (2005). Gene organization and sequences of pediocin AcH/PA-1 production operons in *Pediococcus* and *Lactobacillus* plasmids. *Letters in Applied Microbiology, 40*, 56–62.

Motlagh, A., Bukhtiyarova, M., & Ray, B. (1994). Complete nucleotide sequence of pSMB74, a plasmid encoding the production of pediocin AcH in *Pediococcus acidilactici*. *Letters in Applied Microbiology, 18*, 305–312.

Naumoff, D. G. (2001). Beta-fructosidase superfamily: Homology with some alpha-L-arabinases and beta-D-xylosidases. *Proteins, 42*, 66–76.

Nes, I.. F. (1984). Plasmid profiles of ten strains of *Lactobacillus plantarum*. *FEMS Microbiology Letters, 21*, 359–361.

Nes, I. F., Brendehaug, J., & von Husby, K. O. (1988). Characterization of the bacteriophage B2 of *Lactobacillus plantarum* ATCC 8014. *Biochimie, 70*, 423–427.

Osmanagaoglu, O., Beyatli, Y., & Gunduz, U. (2000). Cloning and expression of a plasmid-linked pediocin determinant trait of *Pediococcus acidilactici* F. *Journal of Basic Microbiology, 40*, 41–49.

Pérez Pulido, R., Abriouel, H., Ben Omar, N., Lucas López, R., Martínez Canamero, M., & Gálvez, A. (2006). Plasmid profile patterns and properties of pediococci isolated from caper fermentations. *Journal of Food Protection, 69*, 1178–1182.

Rodríguez, M. C., Alegre, M. T., & Mesas, J. M. (2007). Optimization of technical conditions for the transformation of *Pediococcus acidilactici* P60 by electroporation. *Plasmid, 58*, 44–50.

Romero, D. A., & Klaenhammer, T. R. (1992). IS946-mediated integration of heterologous DNA into the genome of *Lactococcus lactis* subsp. *lactis*. *Applied and Environmental Microbiology, 58*, 699–702.

Ruiz-Barba, J. L., Piard, J. C., & Jiménez-Díaz, R. (1991). Plasmid profiles and curing of plasmids in *Lactobacillus plantarum* strains isolated from green olive fermentations. *Journal of Applied Bacteriology, 71*, 417–421.

Shareck, J., Choi, Y., Lee, B., & Miguez, C. B. (2004). Cloning vectors based on cryptic plasmids isolated from lactic acid bacteria: Their characteristics and potential applications in biotechnology. *Critical Reviews in Biotechnology, 24*, 155–208.

Shay, B. J., Egan, A. F., Wright, M., & Rogers, P. J. (1988). Cystein metabolism in an isolate of *Lactobacillus sake*: Plasmid composition and cystein transport. *FEMS Microbiology Letters, 56*, 183–188.

Simon, L., Frémaux, C., Cenatiempo, Y., & Berjeaud, J.-M. (2002). Sakacin G, a new type of antilisterial bacteriocin. *Applied and Environmental Microbiology, 68*, 6416–6420.

Skaugen, M., Abildgaard, C. I., & Nes, I. F. (1997). Organization and expression of a gene cluster involved in the biosynthesis of the lantibiotic lactocin S. *Molecular and General Genetics, 253*, 674–686.

Sørvig, E., Mathiesen, G., Naterstad, K., Eijsink, V. G., & Axelsson, L. (2005). High-level, inducible gene expression in *Lactobacillus sakei* and *Lactobacillus plantarum* using versatile expression vectors. *Microbiology, 151*, 2439–2449.

Stentz, R., Loizel, C., Malleret, C., & Zagorec, M. (2000). Development of genetic tools for *Lactobacillus sakei*: Disruption of the β-galactosidase gene and use of *lacZ* as a reporter gene to study regulation of the putative copper ATPase, AtkB. *Applied and Environmental Microbiology, 66*, 4272–4278.

Stentz, R., & Zagorec, M. (1999). Ribose utilization in *Lactobacillus sakei*: Analysis of the regulation of the *rbs* operon and putative involvement of a new transporter. *Journal of Molecular Microbiology and Biotechnology, 1*, 165–173.

Takala, T. M., & Saris, P. E. (2002). A food-grade cloning vector for lactic acid bacteria based on the nisin immunity gene *nisI*. *Applied Microbiology and Biotechnology, 59*, 467–471.

Takala, T. M., Saris, P. E., & Tynkkynen, S. S. (2003). Food-grade host/vector expression system for *Lactobacillus casei* based on complementation of plasmid-associated phospho-beta-galactosidase gene *lacG*. *Applied Microbiology and Biotechnology, 60*, 564–570.

Talon, R., Leroy, S., & Lebert, I. (2007). Microbial ecosystems of traditional fermented meat products: the importance of indigenous starters. *Meat Science, 77*, 55–62.

Van Reenen, C. A., Van Zyl, W. H., & Dicks, L. M. (2006). Expression of the immunity protein of plantaricin 423, produced by *Lactobacillus plantarum* 423, and analysis of the plasmid encoding the bacteriocin. *Applied and Environmental Microbiology, 72*, 7644–7651.

Vaughan, A., Eijsink, V. G., & Van Sinderen, D. (2003). Functional characterization of a composite bacteriocin locus from malt isolate *Lactobacillus sakei* 5. *Applied and Environmental Microbiology, 69*, 7194–7203.

Ventura, M., Canchaya, C., Kleerebezem, M., de Vos, W. M., Siezen, R. J., & Brüssow, H. (2003). The prophage sequences of *Lactobacillus plantarum* strain WCFS1. *Virology, 316*, 245–255.

Vogel, R. F., Becke-Schmid, M., Entgens, P., Gaier, W., & Hammes, W. P. (1992). Plasmid transfer and segregation in *Lactobacillus curvatus* LTH1432 *in vitro* and during sausage fermentations. *Systematics and Applied Microbiology, 15*, 129–136.

Vogel, R. F., Lohmann, M., Weller, A. N., Hugas, M., & Hammes, W. P. (1991). Structural similarity and distribution of small cryptic plasmids of *Lactobacillus curvatus* and *Lactobacillus sake*. *FEMS Microbiology Letters, 68*, 183–190.

Wang, T. T., & Lee, B. H. (1997). Plasmids in *Lactobacillus*. *Critical Reviews in Biotechnology, 17*, 227–272.

West, C. A., & Warner, P. J. (1985). Plasmid profiles and transfer of plasmid-encoded antibiotic resistance in *Lactobacillus plantarum*. *Applied and Environmental Microbiology, 50*, 1319–1321.