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Lactic acid bacteria and their controversial role in fresh meat spoilage

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ABSTRACT

Lactic acid bacteria (LAB) constitute a heterogeneous group that has been widely associated with fresh meat and cooked meat products. They represent a controversial cohort of microbial species that either contribute to spoilage through generation of offensive metabolites and the subsequent organoleptic downgrading of meat or serve as bioprotective agents with strains of certain species causing unperceivable or no alterations. Therefore, significant distinction among biotypes is substantiated by studies determining spoilage potential as a strain-specific trait corroborating the need to revisit the concept of spoilage.

1. Introduction

Food is a susceptible commodity bound to decompose in time (Gram et al., 2002). In general, apart from the physicochemical alterations (e.g., respiration, macromolecule breakdown, lipid oxidation, or other autolytic reactions catalyzed by the endogenous enzymes), food is prone to microbial growth since the composition of nutrients facilitates cell proliferation. Hence, microbial activity leads inevitably to undesirable deterioration accelerating the decay of foodstuffs. Fresh raw meat constitutes a highly perishable ecological niche (Borch, Kant-Muermans, & Blixt, 1996), due to intrinsic parameters and the direct exposure of the carcass to the environment once the natural anatomical barrier of the skin/hide is removed. More in detail, the high water content ($a_w > 0.99$), the pH that corresponds to the optimal range for microbial growth (5.5–6.5), the availability of energy-yielding nutrients (e.g., glucose, ribose, amino acids, and nucleosides) as well as vitamins and minerals, account for meat being a foodstuff with a short shelf-life (Buncic et al., 2014).

Meat is contaminated with microbiota originating initially from the animal and/or the abattoir facilities. Additionally, microorganisms can also derive from the processing environment, whereat carcasses are handled, during transportation and distribution (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Therefore, the initial, diverse microbial community colonizing retail meat encompasses biota with heterogeneous traits that require different environmental conditions to thrive and eventually cause spoilage manifestations (Gram et al., 2002). Meat spoilage is usually caused by Gram negative bacteria (pseudomonads, *Enterobacteriaceae*, *Shewanella putrefaciens*) and several Gram positive (lactic acid bacteria (LAB), *Brochothrix thermosphacta*, clostridia) that dominate under different conditions (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Doulgeraki, Ercolini, Villani, & Nychas, 2012; Nychas et al., 2008).

Currently, the microbial spoilage is defined as the perceivable biochemical alteration occurring on food by the microbiota reaching the highest cell density among the microbial community and thus the alterations are generally attributed to the dominant microbial consortium (Huis in' t Veld, 1996). LAB constitute a group that has been greatly associated with fresh meat and cooked meat products but represents a controversial cohort of microbial species that either contribute to generation of offensive metabolites and the subsequent organoleptic downgrading of meat (Huis in' t Veld, 1996; Labadie, 1999) or serve as bioprotective agents with strains of certain species demonstrating reduced spoilage capacities and inhibitory activity against spoiling microbiota (Chaillou et al., 2014b; Fall et al., 2012; Vasilopoulos et al., 2010). Consequently, this suggests that the presence of high LAB communities does not necessarily result in quality defects. In addition, the intra-species variation in the capability of LAB strains to cause spoilage has been recognized (Björkroth, Vandamme, & Korkeala, 1998; Pothakos, Snauwaert, De Vos, Huys, & Devlieghere, 2014b). Currently, significant distinction among biotypes is substantiated by studies monitoring spoilage potential at strain level corroborating the need to revisit the concept of spoilage, at least in the case of LAB.

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2. Occurrence of LAB in relation to storage conditions

Packaging food under impermeable (plastic) polymer wrapping is a hurdle frequently applied to protect food products from the environment (Cutter, 2002). It can enhance product quality and freshness, while extending the shelf-life of portioned meat (i.e., beef, poultry, pork, lamb etc.) providing convenience (Singh, Wani, Saengerlaub, & Langowski, 2011). Especially modified atmosphere (MA) and vacuum packaging (VP) have become widely implemented food preservation techniques with minimal effect on fresh meat characteristics, and therefore fit well with the recent consumer's preference for additive-free foods (Gould, 1996, 2000). High O_2 levels are required to ensure red meat color by keeping the heme pigment in the oxymyoglobin form and avoid brown discolorations that render the products uninviting for consumption (Lorenzo & Gómez, 2012; McMillin, 2008). Although, high O_2 MAP is widely employed by meat industries, certain LAB species have demonstrated adaptation to oxidative stress and ability to proliferate competitively under a large range of gas combinations (Fig. 1) (Argyri, Doulgeraki, Blana, Panagou, & Nychas, 2011; Faucitano et al., 2010; Jääskeläinen et al., 2013; Kiermeier et al., 2013). Moreover, packaging is very often coupled with low-temperature storage (<7 °C) since it successfully retards microbial growth (Kreyenschmidt et al., 2010; Welch & Mitchell, 2000). Cold-chain maintenance, avoidance of fluctuations or temperature abuse during handling and transportation are crucial for the quality of meat (Nychas et al., 2008; Säde, 2011). The inhibition of deterioration is also more effective at low temperature, since CO_2 solubility to the aqueous phase of the product increases (Kiermeier et al., 2013).

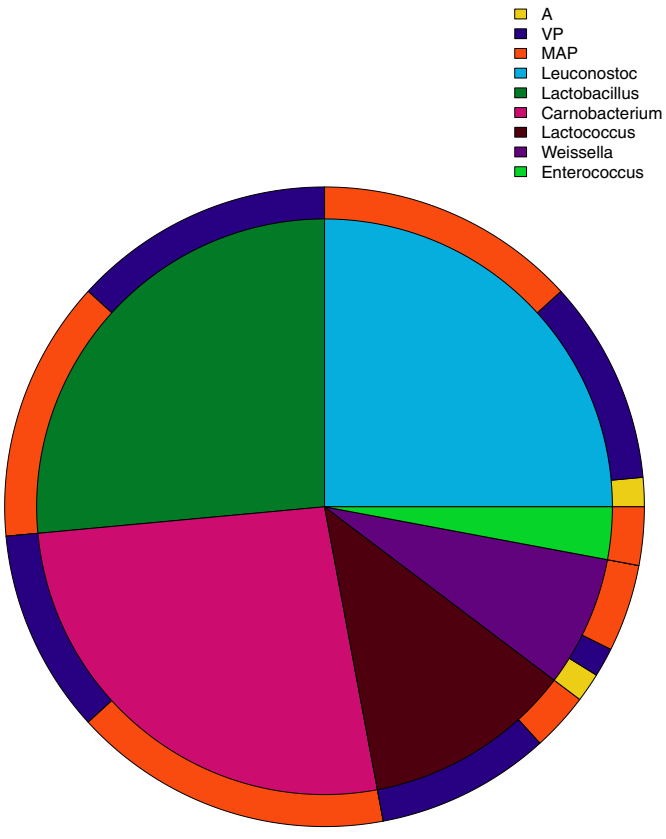

Fig. 1. Occurrence of different LAB genera as dominant members of the microbial community of spoiled meat (inner pie). Packaging technology implemented in every spoilage case (outer ring), A, air; VP, vacuum packaging; MAP, modified atmosphere packaging. Reports used for the construction of the present combination chart range from 1996 to 2014. The species of the major genera are sorted based on number of cases wherein their involvement was documented: *Leuconostoc* (*L. gelidum*, *L. mesenteroides*, *L. carnosum*), *Lactobacillus* (*Lb. sakei*, *Lb. algidus*, *Lb. curvatus*, *Lb. fuchuensis*), *Carnobacterium* (*C. divergens*, *C. maltaromaticum*), *Lactococcus* (*Lc. piscium*, *Lc. lactis*).

However, the combination of these preservation techniques exerts a selection pressure towards psychrotrophic and strictly or facultative anaerobic microbes, like LAB (Fig. 1) since most respiring microbes are inhibited by CO_2 used in the gas composition or accumulating due to microbial activity (Labadie, 1999; Nychas et al., 2008; Pothakos, Snauwaert, De Vos, Huys, & Devlieghere, 2014a). This ecological selection towards cold-acclimatized, CO_2-resistant and aerotolerant LAB has underpinned few species of the spoilage-related core microbiota of meat, which dominate at the end of shelf-life of packaged and chilled-stored foodstuffs (Chaillou et al., 2014a,b). Thus LAB are optimal candidates to develop regardless the packaging system, while their occurrence is dependent on the competitive microbes and the influence of the storage conditions upon the latter.

Apart from the preservation conditions, specific technological practices and types of products, such as marinated, value-added, moisture-enhanced and nonintact meat are more prone to contaminations due to sugar enriched preparations supplemented in/on the meat tissue in order to tenderize or spice it (Sofos & Geornaras, 2010; Vihavainen & Björkroth, 2007). In several cases spoilage of these type of products have been associated with LAB activity (Björkroth, 2005; Schirmer, Heir, & Langsrud, 2009).

2.1. Possible contamination sources of LAB

Numerous LAB taxa, related to food spoilage are ubiquitous in soil and plants (Axelsson, 2004; Chen, Yanagida, & Shinohara, 2005), animal skin and epithelia (Lundström & Björkroth, 2011; Rieder et al., 2012), abiotic surfaces and food processing plants (Bokulich, Bamforth, & Mills, 2012). Nowadays, due to the economical globalization, packaged meat portions are manufactured in processing facilities characterized by significant degree of automation and computerization (Welch & Mitchell, 2000). This systematic food production has corroborated the role of industrial meat-processing environments and technology upon the microbial consortium of the end-product (Audenaert et al., 2010). A reciprocal relation between the carcasses handled in the plant and the actual production environment has been developed. The indigenous microbiota of unprocessed raw meat are introduced in the production facilities and can become resident microbes contaminating meat handling tools and surfaces in the plant wherefrom they are normally transferred to fresh meat, intermediates or final products (De Filippis, La Storia, Villani, & Ercolini, 2013). Few studies have been dealing with the investigation of the environmental origin of LAB found in meat. Cross-contamination of the final retail products by LAB species deriving from the raw materials is speculated on the basis of air- and surface-mediated spreading (Björkroth & Korkeala, 1996, 1997b; Vihavainen & Björkroth, 2009; Vihavainen et al., 2007). Additionally, the conditions characterizing the meat manufacturing installations are favoring the adaptation of psychrotolerant LAB species populations that colonize the plant, thrive under low temperatures and subsequently contaminate the following production batches (Björkroth, 2005; Björkroth & Korkeala, 1997b; Vihavainen & Björkroth, 2009; Vihavainen et al., 2007). This theory of adaptation and survival as house-microbiota has been suggested for other types of foodstuffs highlighting the occurrence of LAB on equipment parts and sites of the premise (Koo et al., 2013; Pothakos et al., 2014c). Therefore, the processing environment can be an important source of LAB contamination for meat, provided that suitable growth conditions and substrate availability are established in processing microclimates that can favor LAB enriched niches that will spread on meat.

2.2. Diversity of LAB in spoiled meat

A large number of LAB genera and species have been found in spoiled meat products constituting a significant diversity (Fig. 1). Species belonging to the genus *Lactobacillus* (e.g., *Lactobacillus sakei*,

Lactobacillus curvatus, *Lactobacillus algidus*, *Lactobacillus fuchuensis*, *Lactobacillus oligofermentans*) are associated with severe acidification, emission of off-odor compounds and ropy slime in the case of poultry, marinated meat, minced beef and pork stored under vacuum or MAP (Audenaert et al., 2010; Doulgeraki, Paramithiotis, Kagkli, & Nychas, 2010; Jiang et al., 2010; Koort et al., 2005; Lyhs & Björkroth, 2008; Sakala, Hayashidani, Kato, Kaneuchi, & Ogawa, 2002a; Sakala et al., 2002b; Samelis, Kakouri, & Rementzis, 2000). The genus *Leuconostoc* (e.g., *Leuconostoc gelidum*, *Leuconostoc carnosum*, *Leuconostoc mesenteroides*) is very frequently responsible for the production of organic acids (e.g., acetic acid), generation of buttery aroma, formation of slime, blowing of packages and green discoloration in all types of meat and packaging conditions (Diez, Jaime, & Rovira, 2009b; Nieminen et al., 2011; Samelis, Björkroth, Kakouri, & Rementzis, 2006; Susiluoto, Korkeala, & Björkroth, 2003; Vihavainen & Björkroth, 2007, 2009). Moreover, *Carnobacterium* (e.g., *Carnobacterium divergens*, *Carnobacterium maltaromaticum*), is very often encountered in beef, poultry and pork at low O_2 packaging and the inflicted spoilage manifestations can vary (Casaburi et al., 2011; Ercolini et al., 2010a; Laursen et al., 2005; Nieminen et al., 2011; Rieder et al., 2012). Members of the genus *Weissella* (e.g., *Weissella viridescens*, *Weissella* spp.) are more often related to bulging of broiler or cooked meat packages and spoilage of minced meat, especially under vacuum (Diez, Björkroth, Jaime, & Rovira, 2009a; Nieminen et al., 2011; Samelis et al., 2006; Zhang et al., 2012). The genus *Lactococcus*, although it is generally associated with dairy fermentation processes, encompasses meat spoilage species (e.g., *Lactococcus piscium*, *Lactococcus raffinolactis*), which cause alterations mainly in beef stored in MAP or under vacuum (Jiang et al., 2010; Rahkila, Nieminen, Johansson, Säde, & Björkroth, 2012; Sakala et al., 2002a). Also certain species of the genus *Enterococcus* (e.g., *Enterococcus viikkiensis*, *Enterococcus hermanniensis*) have been found in spoiled meat but in lower populations (Björkroth, Ristiniemi, Vandamme, & Korkeala, 2005; Koort, Coenye, Vandamme, Sukura, & Björkroth, 2004).

Overall, LAB do not show affinity to a specific meat substrate but are generally resistant to the currently implemented preservation methods to which they demonstrate tolerance. Still the natural diversity of LAB is considered vast among species, subspecies and strains, since habitation to food or environmental ecosystems propagates adaptation to different conditions. However, there may be a distinction between cold-acclimatized mesophiles and strictly psychrotrophic species based on the temperature range they proliferate (Pothakos, Samapundo, & Devlieghere, 2012; Pothakos et al., 2014a) and the geographical coordinates of occurrence worldwide. Cold-acclimatized, mesophilic LAB (i.e., *Lb. sakei*, *Lb. curvatus*, *L. carnosum*, *L. mesenteroides*, *Carnobacterium* spp., *Weissella* spp.) are generally identified as spoilers in countries with warmer climates (Doulgeraki et al., 2010, 2012; Ercolini, Russo, Nasi, Ferranti, & Villani, 2009; Ercolini et al., 2011) but recently the high underestimation of mesophilic (30 °C) shelf-life parameters towards strictly psychrotrophic (unable to grow at 30 °C) LAB species (i.e., *L. gelidum* subsp. *gelidum*, *gasicomitatum* and *aenigmaticum*, *Lb. algidus*, *Lb. fuchuensis*, *Lc. piscium*) has been highlighted in Northern European countries (e.g., Finland, Belgium) and Japan (Björkroth et al., 2000; Kato et al., 2000; Lyhs, Koort, Lundström, & Björkroth, 2004; Nieminen et al., 2011; Pothakos et al., 2014a; Rahkila et al., 2012; Sakala et al., 2002a,b).

The aforementioned strictly psychrotrophic LAB species have gained great attention in the course of the last ten years as the incidence of involvement in cases of meat spoilage (Jääskeläinen et al., 2013; Kato et al., 2000; Sakala et al., 2002a,b), their severe spoilage potential (Lyhs et al., 2004; Susiluoto et al., 2003), the lack of specificity in terms of substrate or affinity with a specific meat type (Chaillou et al., 2014a,b; Nieminen et al., 2011, 2012a,b; Pothakos et al., 2014a; Säde, 2011) and the competitive growth dynamics (Björkroth, 2005; Rahkila et al., 2012) have prioritized them among all LAB spoilage-related taxa.

3. Adaptation to the meat environment: the case of *L. gelidum*

L. gelidum was described in Shaw and Harding (1989) from vacuum-packed chill-stored beef. Its closest phylogenetic neighbors are *L. inhae*, *L. kimchii* and *L. carnosum* based on the 16S rRNA gene analysis (Rahkila, De Bruyne, Johansson, Vandamme, & Björkroth, 2014). All four species are psychrotrophic and *L. carnosum* has also been related to packaged, cold-stored meat products (Björkroth, Dicks, Endo, & Holzapfel, 2014), whereas *L. inhae* and *L. kimchii* have been associated with the manufacture of kimchi, a traditional Korean fermented vegetable product. Recently, the taxonomic status of *L. gelidum* was revised (Rahkila et al., 2014). *L. gasicomitatum* (Björkroth et al., 2000) was reclassified as *L. gelidum* subsp. *gasicomitatum* comb. nov. and some novel strains isolated from packaged meat products were found to represent a novel subspecies, for which the name *L. gelidum* subsp. *aenigmaticum* subsp. nov. was proposed. The proposal of these two novel subspecies created automatically the subspecies *L. gelidum* subsp. *gelidum* subsp. nov., and thus *L. gelidum* comprises currently three phylogenetically distinct subspecies (Rahkila et al., 2014). Both *L. gelidum* subsp. *gelidum* and *gasicomitatum* are well-known spoilage organisms in packaged refrigerated foods, particularly in meat and meat products (Säde, 2011). Thus far, *L. gelidum* subsp. *aenigmaticum* has not yet been reported as the prevailing community component in any major food spoilage cases.

The typical signs of spoilage caused by this species include formation of dextran (slime), bulging or bloating of packages due to CO_2 formation, acidic or buttery off-odors and green (raw beef) of yellow (German weisswurst) discoloration (as reviewed by Säde, 2011). *L. gelidum* subsp. *gasicomitatum* (Björkroth et al., 2000) was first encountered causing a spoilage problem of MAP, tomato-marinated, raw broiler meat strips in 1997. Clear bulging due to CO_2 formation was noticed in 5 days, even though the manufacturer-defined shelf-life was expected to be 14 days. In meat products, *L. gelidum* has been shown to dominate in MAP marinated broiler breast filet products (Susiluoto et al., 2003) and minced meat (Nieminen et al., 2011) and cause greening and off-odor to value-added MAP, raw beef steaks (Vihavainen & Björkroth, 2007). In addition, *L. gelidum* has recently received international attention as a meat spoilage organism (Chaillou et al., 2014a,b; Pothakos et al., 2014a).

There are certain reasons why meat spoilage by *L. gelidum* started to gain attention for the first time in Finland. One of the key reasons is the fact that already from the beginning on 1990s the Nordic Committee on Food Analysis (NMKL) had recommended incubation temperatures below 30 °C (20 or 25 °C) for enumeration of LAB in meat products. Psychrotrophic leuconostocs do not grow at 30 °C or higher, which had been reported in several taxonomic descriptions of these species. However, the ISO 13721:1995 standard recommends 30 °C as a reference incubation temperature for enumeration of LAB in meat and meat products. In Belgium, high incubation temperatures were highlighted as a reason for underestimating psychrotrophic leuconostocs in 2012 (Pothakos et al., 2012). In addition to the incubation temperatures, molecular methods have been essential for robust identification of *L. gelidum* isolates (Björkroth & Korkeala, 1996, 1997a,b).

Some interesting aspects can be pointed out regarding the emergence of *L. gelidum* during the last 10 years. Increasing implementation of high-oxygen MAP and meat marination can be considered as the two main reasons resulting in the prevalence of *L. gelidum* in meat spoilage LAB communities. *L. gelidum*, especially subsp. *gasicomitatum*, can benefit from the carbohydrates added in the marinades to balance acidic taste. In a metagenomic study comparing microbial communities in marinated and unmarinated MAP broiler meat strips, the results showed that marinade increased the proportions of *L. gelidum* subsp. *gasicomitatum* and *gelidum* in the late shelf-life communities of this meat product (Nieminen et al., 2012a). In order to understand better the growth potential of *L. gelidum* subsp. *gasicomitatum*, the genome of type strain LMG 18811^T was sequenced and the spoilage-associated pathways were predicted (Johansson et al., 2011). Unexpectedly this

organism was found capable of heme-mediated respiration metabolism, as several strains inoculated in meat demonstrated this functionality in situ, suggesting that the heme naturally present in meat enables *L. gelidum* subsp. *gasicomitatum* to respire (Jääskeläinen et al., 2013). Heme-dependent respiration has not been detected functional in the case of the other two subspecies (Rahkila et al., 2014). As a consequence of respiration, substantial amounts of spoilage metabolites acetoin and diacetyl were produced by *L. gelidum* subsp. *gasicomitatum*. Besides the acetoin and diacetyl formation, respiration resulted in higher biomass and increased growth rate indicating that respiration has an early beneficial effect on the growth. Respiration is thus a key factor explaining why *L. gelidum* subsp. *gasicomitatum* is so well adapted in high-oxygen packed meat. If oxygen and heme are not available and the metabolism is based on fermentation, glucose is the most effective carbon source for the growth of *L. gelidum* subsp. *gasicomitatum* (Jääskeläinen et al. 2015) but its fermentation does not lead to formation of acetoin/diacetyl. However, utilization of inosine and ribose resulted in the production of the unwanted buttery odor compounds, acetoin and diacetyl.

In a recent study of Rahkila et al. (2015), population structure within 252 *L. gelidum* subsp. *gasicomitatum* strains was determined based on a novel MLST scheme employing seven housekeeping genes. These strains had been isolated from meat and vegetable sources over a time span of 15 years and the findings seem to indicate that certain strains are growing in meat better than in vegetables or contamination sources associated with meat or vegetable processing are different. Since *L. gelidum* subsp. *gasicomitatum* has not been associated with animal carcasses during early stages of slaughtering it is most likely that factors associated with the capability to grow in different foods explain the niche specificity observed. Lastly, the large diversity of biotypes of this subspecies was further documented and the strain-specific ability to adhere on stainless steel and glass food contact surfaces was recently demonstrated (Pothakos, Aulia, Van Der Linden, Uyttendaele, & Devlieghere, 2015).

4. Spoilage potential: production of spoilage-associated molecules by LAB

Determining the spoilage potential of a strain or a microbial group collectively is a very difficult issue. When microbial spoilage occurs as appearance defects (e.g., slime, discoloration) and/or deteriorations related to flavor (i.e., off-odors/tastes), the manifestation is generally attributed to the dominant microbiota, usually referred to as specific spoilage organisms (SSO) (Dalgaard, 1995; Dalgaard, Gram, & Huss, 1993; Huis in' t Veld, 1996). However, other microbial groups may have contributed to spoilage, despite not necessarily having caused unpleasant changes directly by their proliferation or metabolism. In fact, it is more likely that interactions occur among microbial species from different groups that result in generation of spoilage-associated molecules. In addition, further interactions may take place among the produced molecules, thus leading to a quite unpredictable and complicated profile in terms of sensory quality aspects of raw meat (Casaburi et al., 2015). It is therefore more correct in some cases to refer to a "metabiotic spoilage association" in order to describe situations where two or more microbial species contribute to spoilage through exchange of metabolites or nutrients, so the SSO concept should be used to specify a set of organisms that interact to spoil the product (Gram et al., 2002; Jørgensen et al., 2000).

Glucose is the first substrate used by most bacteria in raw meat during chill storage in any conditions (Borch & Agerhem, 1992; Gill, 1983; Nychas, Dillon, & Board, 1988). When glucose is consumed, other substrates such as lactate, gluconate, glucose-6-phosphate, pyruvate, propionate, formate, ethanol, acetate, amino acids, nucleotides, urea and water-soluble proteins can be used by the majority of meat microbiota (Gill, 1986; Nychas, Marshall, & Sofos, 2007). Sugar consumption is of extreme importance for LAB proliferation in meat and for the associated type of spoilage. LAB occurring in meat can be obligate heterofermentative or facultative heterofermentative. The first produce lactic acid, acetic acid, CO_2 and ethanol, while the second give two moles of lactate from glucose. An inducible phosphoketolase activity is expressed in heterofermentative LAB when pentoses are present and it catalyzes their conversion into lactate and acetate without gas formation (Kandler, 1983; Kandler & Weiss, 1986). As it can happen in meat, the ribose fermenting *Lactobacillus* under glucose limitation switch from homofermentative to heterofermentative, determining a significant production of acetic acid (Kandler & Weiss, 1986; Nychas et al., 1988; Borch, Berg, & Holst, 1991; Borch et al., 1996). During aerobic storage of meat, in the presence of low concentration of glucose, spoilage LAB may also use lactate and its precursor pyruvate producing acetate (Samelis, 2006); high acetate concentrations may result in a sharp vinegar-like flavor altering the sensory quality of meat. Other carbohydrates and amino acids present in meat can also trigger LAB growth under glucose limitation. Studies in vitro showed that *Lb. sakei* is able to use arginine to withstand low glucose levels and to release ammonia and biogenic amines such as putrescine and spermine (Labadie, 1999; Montel, Talon, Fournaud, & Champomier, 1991). Heterofermentative LAB such as *Leuconostoc* spp. cannot obtain energy from glycogen, proteinaceous substrates, lactate or fatty acids; however, some species such as *L. gelidum* subsp. *gasicomitatum*, are recognized as potent spoilage agents in meat (Björkroth et al., 2000; Johansson et al., 2011; Susiluoto et al., 2003; Vihavainen & Björkroth, 2007). They have been associated with spoilage in marinated meat products (Björkroth et al., 2000; Nieminen et al., 2012a,b; Susiluoto et al., 2003) where they can find alternative fermentable sugars. This may lead to bulging of packages due to CO_2 formation. In addition, it was recently demonstrated that they can use heme-dependent respiration or use ribose and inosine as alternative carbon sources leading to production of diacetyl, an odor-impact molecule responsible for the buttery and cheesy flavor in spoiled meat (Jääskeläinen et al., 2013, 2015).

The spoilage of fresh meat is mostly caused by the presence of volatile organic compounds (VOCs) of microbial origin that are responsible for off-odors. The volatile fraction of the microbial catabolites includes: organic acids, volatile fatty acids, ethyl esters, sulfur compounds, ketones, aldehydes, alcohols, ammonia and other molecules. A list of VOCs detected in meat during storage in different packaging conditions and when LAB were inoculated or identified as possible spoilers is reported in Table 1. All these molecules will potentially affect the sensory quality of both fresh and cooked meat. The principal odor descriptors used for the organoleptic assessment of meat that spoiled under conditions that LAB constitute the main spoilers (i.e., under oxygen limitation) are dairy/cheesy character and fermented meat odor especially after the first week of storage when acetoin and volatile organic acids tend to accumulate (Casaburi et al., 2015). Depending on their olfactory thresholds, the masking as well as synergic effects among different volatile or volatile/non-volatile compounds, they can cause off-odors that will render meat spoiled (Casaburi et al., 2015). LAB can produce mainly acids from their main metabolic pathways, however, production of alcohols, aldehydes and sulfur compounds has been reported when meat-colonizing LAB such as carnobacteria are found in spoiled meat along with other Gram-negative bacteria or other spoilers such as *B. thermosphacta*, especially when meat is stored in air compared to vacuum packaging (Casaburi et al., 2011, 2015; Ercolini et al., 2009).

Additionally, the production of exopolysaccharides when sugars are available in the matrix like in the case of marinated meat can result in formation of ropy, viscous slime that contributes to a significant downgrading of meat quality for which lactobacilli and leuconostocs are usually responsible (Björkroth & Korkeala, 1997a; Lyhs et al., 2004).

5. Are all strains of a spoilage-related LAB species equally capable to determine perceivable alteration? Role of strain-level characterization and impact of sensory analysis

The spoilage potential of a microorganism is determined by its ability to produce the metabolites that are associated with the spoilage.

Table 1
Most commonly identified VOCs in meat during storage when LAB were found or deliberately inoculated.

Compounds	Odor descriptors[a]	Meat source	Storage condition[b]	LAB identified/inoculated	Reference
Alcohols					
Butanol	Fruity	Spoiled meat	VP	*C. divergens*,	Hernandez-Macedo et al. (2012)
		Inoculated pork	A + VP	*C. maltaromaticum*, *Lact. lactis*[c] *Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
3-Methyl-1-butanol	Fermented, fusel, alcoholic, pungent, etherial	Inoculated beef	A	*C. maltaromaticum*	Casaburi et al. (2011)
		Spoiled meat	VP	*C. divergens*, *Lact. lactis*[c]	Hernandez-Macedo et al. (2012)
		Spoiled meat	MAP	*Carnobacterium* spp.[c]	La Storia et al. (2012)
1-Octanol	Waxy, green, citrus, aldehydic and floral with a sweet, fatty, coconut nuance	Inoculated beef	A	*C. maltaromaticum*	Casaburi et al. (2011)
1-Octen-3-ol	Mushroom, earthy, green, oily, vegetative and fungal	Inoculated beef	VP	*C. maltaromaticum*[c]	Ercolini et al. (2009)
		Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
		Spoiled meat	MAP	*C. maltaromaticum*[c]	Ercolini et al. (2011)
		Spoiled meat	A + MAP	*C. maltaromaticum*[c]	La Storia et al. (2012)
2-Octen-1-ol	Green vegetable	Inoculated beef	VP	*C. maltaromaticum*[c]	Ercolini et al. (2009)
		Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
2-Ethyl-1-hexanol	Citrus fresh floral oily sweet	Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
		Spoiled meat	VP	*C. maltaromaticum*[c]	Ferrocino et al. (2013)
2,3-Butanediol	Fruity creamy buttery	Spoiled meat	VP	*C. maltaromaticum*, *C. divergens*[c]	Ferrocino et al. (2013)
1-Hexanol	Pungent, etherial, fusel oil, fruity and alcoholic, sweet with a green top note	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Inoculated beef	A		Casaburi et al. (2011)
		Spoiled meat	MAP	*C. maltaromaticum* *Carnobacterium* spp.[c]	La Storia et al. (2012)
Heptanol	Musty, pungent, leafy green, with vegetative nuances	Inoculated beef	A	*C. maltaromaticum*	Casaburi et al. (2011)
Phenylethyl alcohol	Floral rose dried rose flower rose water	Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
		Spoiled meat	A	*Carnobacterium* spp.[c]	La Storia et al. (2012)
Aldehydes					
Hexanal	Fresh green fatty aldehydic grass leafy fruity sweaty	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Inoculated beef	VP	*C. maltaromaticum*[c]	Ercolini et al. (2009)
Nonanal	Waxy aldehydic rose fresh orris orange peel fatty peely green cucumber	Inoculated beef	A	*C. maltaromaticum*	Casaburi et al. (2011)
		Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Inoculated beef	A + VP		Casaburi et al. (2011)
		Spoiled meat	VP	*C. maltaromaticum* *C. maltaromaticum*, *C. divergens*[c]	Ferrocino et al. (2013)
Heptanal	Fresh aldehydic fatty green herbal wine-lee ozone	Spoiled meat	VP	*L. sakei*, *C. divergens*[c]	Hernandez-Macedo et al. (2012)
		Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Inoculated beef	A	*C. maltaromaticum*	Casaburi et al. (2011)
Benzaldehyde	Strong sharp sweet bitter almond cherry	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
		Spoiled meat	VP	*L. curvatus*, *L. sakei*, *L. algidus*, *C. divergens*[c]	Hernandez-Macedo et al. (2012)
Ketones					
Acetoin	Buttery creamy dairy milky fatty sweet	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Inoculated beef	A + VP		Casaburi et al. (2011)
		Inoculated meat	A	*C. maltaromaticum*	Dainty, Edwards, and Hibbard (1989)
		Spoiled meat	A + MAP	*C. maltaromaticum*,	Ercolini et al. (2011)
		Spoiled meat	A + MAP	*C. divergens*[c] *C. maltaromaticum*[c] *Carnobacterium* spp.[c]	La Storia et al. (2012)
Diacetyl	Strong butter sweet creamy pungent caramel	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
3-Octanone	Musty, mushroom, ketonic, moldy and cheesy fermented with a green, vegetative nuance	Inoculated beef	A	*C. maltaromaticum*	Casaburi et al. (2011)
2-Butanone	Acetone-like ethereal fruity camphor	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Spoiled meat	VP	*L. fuchuensis*, *L. sakei*, *Lact. piscium*[c]	Hernandez-Macedo et al. (2012)
2-Heptanone	Cheesy, creamy, fruity spicy sweet herbal coconut wood	Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
Esters					
Ethyloctanoate	Fruity wine waxy sweet apricot banana brandy pear	Inoculated beef	VP	*C. maltaromaticum*[c]	Ercolini et al. (2009)
		Spoiled meat	A + MAP	*Carnobacterium* spp.[c]	La Storia et al. (2012)
Ehylhexanoate	Sweet fruity pineapple waxy green banana	Inoculated beef	VP	*C. maltaromaticum*[c]	Ercolini et al. (2009)
		Spoiled meat	A	*Carnobacterium* spp.[c]	La Storia et al. (2012)
Ethyldecanoate	Sweet waxy fruity apple grape oily brandy	Spoiled meat	A	*Carnobacterium* spp.[c]	La Storia et al. (2012)

Table 1 (*continued*)

Compounds	Odor descriptors[a]	Meat source	Storage condition[b]	LAB identified/inoculated	Reference
Volatile fatty acids					
Acetic acid	Pungent acidic cheesy vinegar	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Spoiled meat	VP	*L. curvatus*, *L. sakei*, *Lact. piscium*[c]	Hernandez-Macedo et al. (2012)
Butanoic acid	Sharp acetic cheese butter fruit	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Spoiled meat	VP		Ferrocino et al. (2013)
		Inoculated beef	A + VP	*C. maltaromaticum*/-	Casaburi et al. (2011)
		Spoiled meat	A + VP + MAP	*C. divergens*[c]	Ercolini et al. (2011)
				C. maltaromaticum	
				Carnobacterium spp.[c]	
Hexanoic acid	Sour fatty sweat cheese	Inoculated pork	A + VP	*Lc. gelidum* subsp. *gasicomitatum*	Jääskeläinen et al. (2013)
		Spoiled meat	VP		Ferrocino et al. (2013)
		Inoculated beef	A + VP	*C. maltaromaticum*/*C. divergens*[c]	Casaburi et al. (2011)
		Spoiled meat	VP + MAP		Ercolini et al. (2011)
				C. maltaromaticum	
				Carnobacterium spp.[c]	
Nonanoic acid	Waxy dirty cheese cultured dairy	Spoiled meat	VP	*C. maltaromaticum*/*C. divergens*[c]	Ferrocino et al. (2013)
		Inoculated beef	VP		Casaburi et al. (2011)
				C. maltaromaticum	
Sulfur compounds					
Dimethyl sulfide	Sulfury onion sweet corn vegetable cabbage tomato green radish	Inoculated beef	A + VP	*C. maltaromaticum*	Casaburi et al. (2011)
		Spoiled meat	A + MAP	*Carnobacterium* spp.[c]	Ercolini et al. (2011)
		Spoiled meat	VP	*L. curvatus*, *L. sakei*[c]	Hernandez-Macedo et al. (2012)
Methyl thioacetate	Sulfurous eggy cheese dairy vegetable cabbage	Spoiled meat	VP	*L. curvatus*, *L. sakei*[c]	Hernandez-Macedo et al. (2012)

[a] Odor descriptors are from http://www.thegoodscentscompany.com.
[b] Storage in air (A), vacuum (VP) and modified atmosphere packaging (MAP).
[c] LAB found in association with other non LAB spoilage-associated bacteria.

As in any other environment, adaptation to the fresh meat environment, with respect to the specific nutrient and growth (storage) conditions, as well as the competiveness against the other resident microbiota are strain-specific traits. It is therefore likely that not all the strains of a LAB species recognized as spoiler, can be equally capable of an offensive metabolic activity that can lead to alterations. Most of the studies involving meat spoilage report on the occurrence of LAB or other presumptive spoilage microbiota but strain-dependence of the phenomenon is not considered.

A distinction among different LAB species can be made based on the documented reports concerning their involvement in spoilage cases in order to facilitate greater resolution within the LAB group. Apparently, there are inherently spoilage-capable species (e.g., *L. gelidum*, *Lb. algidus*), which are consistently described as SSO in meat, since they predominate at the end of storage demonstrating high growth dynamics and in every case contribute to meat downgrading exhibiting a standard spoilage pattern (Björkroth et al., 2000; Kato et al., 2000; Nieminen et al., 2011; Santos et al., 2005; Vihavainen & Björkroth, 2007). Secondly, species have been reported to encompass strains that have been substantiated as potent spoilers or bioprotective agents (e.g., *Lb. sakei*, *Lc. piscium*, *L. carnosum*) highlighting the intra-species diversity with distinct characters (Chaillou et al., 2014b; Fall, Leroi, Chevalie, Guérin, & Pilet, 2010; Laursen et al., 2005; Vasilopoulos et al., 2010). Lastly, an interesting minority exists comprising ambiguous members of the LAB group (e.g., *Carnobacterium* spp.), which often constitute spoilers, albeit inflicting unperceivable alterations in meat (Casaburi et al., 2011).

Hence, in order to evaluate whether different biotypes belonging to the same species are able to cause spoilage manifestations, a systematic strain-level characterization is needed. Such characterization should include not only molecular typing methods distinguishing different clones within a given taxon but also a spoilage-oriented "functional characterization" delineating the spoilage potential of distinct strains in meat. This functional characterization should be conducted in situ in meat, considering different storage and packaging conditions and should at least include testing of (i) growth capabilities and dynamics in meat matrix, (ii) competitiveness against other bacteria and (iii) production of spoilage-related molecules. The screening should also include a wide number of strains and a satisfactory intra-species diversity, in order to infer robust evidence about the phenotypic heterogeneity related to spoilage. Finally, such monitoring tests should be coupled to sensory evaluation in order to provide a more complete insight into the effect LAB have in meat.

Several studies have produced extensive lists of VOCs linked to microbial meat spoilage; however, the presumptive sensory impact of each of these compounds was not assessed. Despite the nutritional and safety issues linked to spoilage, the organoleptic attributes determining the acceptability of the product by the consumers constitutes a crucial matter. Cooking and culinary practices significantly modify the volatile composition and the olfactory profile of meat; however, previous studies showed that some alterations in raw meat resulted in post-cooking differences (Resconi et al., 2012). During chill-storage in aerobic conditions, VP and MAP, a wide range of VOCs can be produced by spoilage microbiota both in naturally contaminated samples and in meat model systems (Casaburi et al., 2011; Ercolini et al., 2009, 2010a; Ferrocino et al., 2013; Nychas et al., 2007, 2008) causing changes in texture and the development of slime, off-odor, or any other characteristic rendering meat undesirable for consumption.

Studying the correlation between VOC concentrations and descriptive sensory analysis (QDA) scores by means of multivariate statistical analysis is decisive for determining the actual spoilage potential of a bacterial group and for highlighting the contamination thresholds leading to perceivable spoilage. However, such approaches are rarely considered because sensory evaluation of raw meat is more complicated than for cooked or cured meat, and other foodstuffs (Casaburi et al., 2015).

The systematic functional characterization approach for meat spoilers has been followed in few cases (Casaburi et al., 2011, 2015; Ercolini et al., 2010a,b). As far as LAB are concerned, *C. maltaromaticum* strains were shown to produce many VOCs in meat in absence of competitive microbiota, however, a trained sensory panel did not identify a negative impact on the overall sensorial profile suggesting a negligible role in meat spoilage (Casaburi et al., 2011). These results

further underpin that some LAB species may be fit and competitive in the meat environment outgrowing the rest of the microbiota and potentially producing VOCs but the final evaluation concerning the state of acceptability is subject to sensorial tests.

6. Conclusions

Food is a binary term with biological and cultural substance. Regarding the aspect of human biology and nutrition, food corresponds to organic material that provides the organism with essential nutrients, whereas from a cultural perspective, social anthropology denotes it as a basic cognitive process (Mintz & Du Bois, 2002). Spoilage by definition is a recognition and interpretation of sensorial stimuli, and therefore subjected to individual human perception, nonetheless correlated to microbiological thresholds, chemical concentrations and microbial community patterns.

Currently, the role of LAB in fresh meat spoilage is still controversial. On one side, several species are able to dominate the meat system in VP and MAP storage conditions and can release odor-impact molecules, which may alter the sensory profile of raw or cooked meat, albeit this is not the case in all documented reports. The spoilage character of some LAB taxa is ambiguous and probably correlated with specific spoilage-associated capacities of individual strains that cannot be attributed collectively to the respective species. Moreover, for the LAB with negligible role in sensory spoilage, a bioprotective function in meat can be hypothesized as they can provide favorable antagonistic activity against other undesired microorganisms.

For the aforementioned reasons, the study and interpretation of LAB spoilage in meat, which constitutes a complicated, multifactorial phenomenon are challenging and in order to be described, in depth understanding of its complexity is demanded, as well as combination of different disciplines and tools.

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