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Exploring the metabolic heterogeneity of coagulase-negative staphylococci to improve the quality and safety of fermented meats: a review

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A B S T R A C T

The production of fermented meats, such as fermented sausage, relies on the metabolic activities of lactic acid bacteria and catalase-positive cocci, in particular the group of coagulase-negative staphylococci (CNS). Conventional use of CNS as meat starter cultures usually leads to an appropriate cured colour development based on their nitrate reductase activity, whereas their catalase activity reduces oxidative damage. In addition, CNS metabolism contributes to flavour, although the precise effects are difficult to estimate. There are reasons to believe that these basic technological features of CNS can be further enlarged by exploring their full metabolic potential. Non-negligible differences in metabolism among and within different species of CNS indicate that a rational selection of strains may lead to the development of novel starter cultures with enhanced functionality. Firstly, the use of CNS strains with a superior ability to use alternative energy sources, such as nucleosides or arginine, may improve culture competitiveness and survival. Secondly, cured colour generation could be optimised to lower the amounts of curing salts needed, either by selecting for efficient nitrate-reducing CNS strains or by exploring the potential alternative based on nitric oxide synthase activity. Thirdly, CNS with specific aroma-producing abilities may help to accentuate specific flavours, whereby the selection of wild-type strains from artisan-type fermented sausages seems attractive in the framework of innovation-through-tradition. Finally, bacteriocin-producing CNS strains may offer solutions for bioprotection towards meat pathogens such as *Clostridium botulinum* and *Staphylococcus aureus*. Overall, making use of the metabolic inter- and intraspecies heterogeneity of CNS is promising for the elaboration of healthier, tastier, and safer fermented meats. Yet, the proposed strategies are sometimes still overly theoretical and speculative, requiring further proof-of-principle.

1. Fermented meats in human contemporary diets: origins and trends

Raw meat is highly perishable. Its mild pH (5.5–6.0), high water activity (a_w of 0.99), and rich nutrient content make it prone to microbial deterioration, especially at ambient temperatures. Empirical methods to preserve meat in the absence of a cold chain date back to remote times, in particular relying on the use of salting, drying, smoking, and fermentation. Such products are still popular today as they have distinctive sensory properties and are valued for their contribution to food heritage (Leroy et al., 2013, 2015). The production process of fermented meats inhibits most spoilage microorganisms and food-borne pathogens based on a combination of antimicrobial hurdles, including acidification, the development of anaerobicity, a decreasing water activity during salting and drying, the presence of nitrite and other antimicrobials, optional smoking treatments, and competition with the normal meat microbiota. The microbial communities in fermented meat products generally consist of lactic acid bacteria (LAB), of which the prime role is to cause acidification, in addition to catalase-positive cocci that contribute to the development of colour and flavour, as will be discussed below. In some fermented meats, especially in variants produced in the Mediterranean area, filamentous fungi also play a role in sensory development due to their visual appearance and their proteolytic and lipolytic activities (Leroy et al., 2006; Ravyts et al., 2012).

So-called "traditional" fermented meats are often said to be produced following unique, inherited, and artisan modes of manufacturing that lead to idiosyncratic qualities (Garcia-Varona et al., 2000; Moretti et al., 2004), despite the fact that "tradition" is a problematic concept with a multidimensional and often arbitrary meaning (Geyzen et al., 2012; Leroy et al., 2015). Be that as it may, artisan-type fermented meat production frequently makes use of spontaneous fermentation processes, in which the lead is taken by highly competitive fractions of the "in-house" microbiota. The latter are microorganisms that are naturally present on the raw meat or that originate from the environment (Ravyts et al., 2012; Talon et al., 2007). Together with the intrinsic properties of the raw materials and added ingredients, as well as with the specific process characteristics of the fermentation and maturation

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stages, these naturally present microbiota will shape the quality and safety of the final product. In most industrial production schemes, however, defined starter cultures are commercially purchased and inoculated into the meat batter, as to standardize the production process by overruling the variability of the raw meat composition and the background microbiota (Leroy et al., 2006).

Although fermented meats are age-old products, they have known several important innovations all through history (Leroy et al., 2013, 2015). The fact that tradition and innovation are not a binary reality but rather intertwined categories that are not easy to disentangle is illustrated by the concept of innovation-through-tradition (Geyzen et al., 2012). The latter implies that production characteristics, which are perceived as being of an artisan nature, are (re)introduced in industrial, large-scale production setups to meet urgent consumer demands for “authenticity”, as if it were yielding reassurance in what is perceived to be a threatening global food market. In most cases, such attempts are merely steered by marketing departments and based on the design of “good old-fashioned” packages and product labels. In other cases, however, genuine process adjustments are being made, among which a renewed confidence in the technique of spontaneous fermentation rather than in the use of commercial starter cultures. Due to the convergence of starter cultures worldwide, of which most are now produced by a few multinationals, this factor indeed no longer offers much flexibility for product differentiation. As a hybrid solution, there is an increasing interest in the use of functional meat starter cultures, which are composed of carefully selected strains that can have several utilities, besides the basic acidifying and colour-developing activities of classical starter cultures (Leroy et al., 2006). The use of wild-type strains isolated from spontaneously fermented meats may help in the accentuation of artisan-like flavours. Narratives about such quality-enhancing functionalities are increasingly being used by starter culture producers. As an example, the company *Chr. Hansen* now markets self-styled “traditional cultures” for meat fermentation (Chr. Hansen, 2015).

In addition to the above-mentioned demand for traditional qualities, a trend for healthier meat products has emerged (Jimenez-Colmenero et al., 2001). Meat is often negatively perceived by consumers because of an assumed link with (colon) cancer, cardiovascular diseases, and obesity, even if the causality of such associations is under debate (Binnie et al., 2014; Klurfeld, 2015). With respect to processed meats, concerns related to colorectal cancer development are somewhat more pressing, as the World Cancer Research Fund explicitly discourages their consumption (Demeyer et al., 2008). Although the mechanisms to substantiate the alleged contribution to cancer development are still under investigation, several hypotheses have been formulated among which the involvement of carcinogenic *N*-nitroso compounds in the gastrointestinal tract. Meat products that are manufactured using nitrate and/or nitrite as curing agents could increase exogenous exposure to nitrosamines and other *N*-nitroso compounds, as well as their precursors (Chan et al., 2011). However, added nitrite (or its precursor nitrate, which is converted into nitrite by nitrate reductase activity of the catalase-positive cocci in the meat; see below) is a valuable ingredient, as it contributes to the tanginess of the flavour, inhibits pathogenic bacteria, and leads to the production of nitrosomyoglobin. The latter is the desirable and relatively stable red colour of fermented meat products (Sánchez Mainar & Leroy, 2015). Yet, the highest daily intake of nitrite (about 93%) is from endogenous origin, due to its presence in human saliva, whereas nitrate intake is mostly (about 87%) ascribed to the consumption of (leafy) vegetables (Sindelar & Milkowski, 2012). It has even been suggested that dietary intake of nitrate and nitrite may contribute to human health, especially in patients with insufficient nitric oxide (NO) bioavailability. Humans produce the latter molecule in vivo, as it is involved in signalling, blood pressure regulation, neurotransmission, and immunological defences, whereby a lack of NO can lead to severe health dysfunctions, such as hypertension, atherosclerosis, and thrombosis (Moncada et al., 1991). Regardless of the above, a market trend for “clean” and “organic” labelling is noticeable. Manufacturers thus welcome any search for alternatives to preserve the colour, flavour, and shelf-life in the absence of added curing agents (Sebranek & Bacus, 2007). A pragmatic approach consists of so-called “indirect” or “natural curing’, by which vegetable derivatives such as celery powder are used because of their naturally high levels of nitrate, together with the addition of a nitrate-reducing starter culture of catalase-positive cocci to facilitate in situ generation of nitrite during manufacturing (Sebranek & Bacus, 2007). This is, of course, a pseudo-solution, so that more innovative routes are needed. Other strategies that could enhance the perceived healthiness of fermented meats include the use of starter cultures that are either probiotic or lead to the production of health-improving compounds, such as conjugated linoleic acid (De Vuyst et al., 2008; Gorissen et al., 2015; Leroy et al., 2006). Such strategies may nevertheless encounter important technological, legislative, and marketing bottlenecks (De Vuyst et al., 2008; Gorissen et al., 2012).

To sum up, fermented meats have a long history since they were originally developed for preservative purposes and remain very popular up till present. This is not only to be ascribed to their sensory appeal and convenience (Leroy & Degreef, 2015), but also to their function in the creation of (cultural) identity (Leroy et al., 2015). Yet, mass-produced products suffer from a poor market image, as they can be perceived by consumers as uniform, bland, and even unhealthy (Geyzen et al., 2012; Leroy et al., 2013, 2015). Several marketing and technological strategies are being developed in an attempt to counter this problem. The present review focuses on the potential role of microorganisms in such strategies, in particular with respect to the meat-associated group of coagulase-negative staphylococci (CNS), being the major group within the community of catalase-positive cocci. Besides their conventional use in meat starter cultures, some novel approaches to their metabolic potential in view of the development of tastier and healthier meat products will be addressed. It is important, however, to point out that quality and safety are also largely dependent on the nature of the raw materials and processing applied. It remains therefore to be established still to which degree the rational use of CNS starter cultures may overcome and correct for this variability.

2. Coagulase-negative staphylococci in fermented meats

2.1. Diversity and competitiveness in the fermented meat matrix

Spontaneous meat fermentation leads to a variety of different bacteria, among which the LAB are ubiquitous (Ravyts et al., 2010). *Lactobacillus sakei* is the most commonly encountered LAB species, followed by *L. curvatus* and *L. plantarum*. Within the group of catalase-positive cocci, the species diversity can be considerably larger. *Staphylococcus xylosus* is frequently the most dominant species in European-style traditionally fermented sausages (Blaiotta et al., 2004; Cocolin et al., 2011; Comi et al., 2005; Talon et al., 2007), but *S. saprophyticus* and *S. equorum* can also dominate the staphylococcal microbiota depending on the type of product (Blaiotta et al., 2004; Talon et al., 2007). In addition, a large variety of subdominant fractions can be found, such as *S. carnosus*, *S. epidermidis*, *S. haemolyticus*, *S. pasteuri*, *S. sciuri*, *S. succinus*, *S. vitulinus*, and *S. warneri* (Blaiotta et al., 2004; Garriga & Aymerich, 2007; Iacumin et al., 2006; Janssens et al., 2012; Lebert et al., 2007; Mauriello et al., 2004; Talon et al., 2007). In industrialized products, both *S. carnosus* and *S. xylosus* are commonly used as starter cultures due to their nitrate-reducing ability and aroma production (Leroy et al., 2006). Besides CNS, kocurias can be part of the catalase-positive coccal microbiota too, of which *Kocuria varians* is the most frequently found representative, often used as a starter culture for similar reasons (Cocconcelli, 2007).

Overall, the CNS species diversity of a fermented meat product is difficult to predict, as it is influenced by a combination of complex factors, including the muscle type used, the ingredients added, and the processing conditions applied. Yet, some rough patterns of microbial tuning and