International Journal of Food Microbiology 274 (2018) 52–59

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

The narrowing down of inoculated communities of coagulase-negative staphylococci in fermented meat models is modulated by temperature and pH

Despoina Angeliki Stavropoulou[a,1], Emiel Van Reckem[a,1], Stefaan De Smet[b], Luc De Vuyst[a], Frédéric Leroy[a,*]

[a] Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

[b] Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Ghent, Belgium

ARTICLE INFO

Keywords:
Starter cultures
Meat
Fermentation
Staphylococcus

ABSTRACT

Coagulase-negative staphylococci (CNS) are involved in colour and flavour formation of fermented meats. Their communities are established either spontaneously, as in some artisan-type products, or using a starter culture. The latter usually consists of *Staphylococcus carnosus* and/or *Staphylococcus xylosus* strains, although strains from other CNS species also have potential for application. However, it is not entirely clear how the fitness of alternative starter cultures within a fermented meat matrix compares to conventional ones and how this may be affected by processing conditions. Therefore, the aim of this study was to assess the influence of two key processing conditions, namely temperature and acidity, on the competitiveness of a cocktail of five different strains of CNS belonging to species that are potentially important for meat fermentation (*Staphylococcus xylosus* 2S7-2, *S. carnosus* 833, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus equorum* DFL-S19, and *Staphylococcus saprophyticus* FPS1). To this end, fermented meat models consisting of cured meat batters with initial pH values of 5.3, 5.5, or 5.7 were inoculated with these strains, stuffed in containers, and incubated at 23, 30, or 37 °C. Both the pH level and the temperature influenced the composition of the CNS communities, giving a competitive advantage to the best adapted species. *Staphylococcus xylosus* preferred low temperature and mild acidity, whereas an elevated temperature selected for *S. epidermidis* and a low pH for *S. carnosus*. Under the conditions tested, *S. saprophyticus* and *S. equorum* were outcompeted by the three other CNS species. Hence, CNS communities in fermented meats are not only established based on the initial presence of specific species in the meat batter but also by their subsequent adaptation to the processing conditions during fermentation, potentially overruling the use of starter cultures.

1. Introduction

During meat fermentation, the key bacterial groups are the lactic acid bacteria (LAB), which lower the pH of the meat matrix, and the coagulase-negative staphylococci (CNS), which contribute to colour and flavour of the end-products (Leroy et al., 2006; Sánchez Mainar et al., 2017; Talon and Leroy, 2014). In traditional fermented meat products, which often rely on spontaneous fermentation and are valued for their distinct sensory qualities, a diversified staphylococcal ecosystem has been described (Aquilanti et al., 2016; Chevallier et al., 2006; Fonseca et al., 2013a; Greppi et al., 2015; Iacumin et al., 2006, 2012). Although *Staphylococcus equorum*, *Staphylococcus saprophyticus*, and *Staphylococcus xylosus* are often the main species, a wide range of several other subdominant CNS species have been found. In an industrialised context, starter cultures provide a common and convenient strategy to obtain a uniform end-product quality (Talon and Leroy, 2011). In Europe, starter cultures habitually consist of a restrictive selection of strains of *Lactobacillus sakei*, together with *Staphylococcus carnosus* and/or *S. xylosus* (Leroy et al., 2006; Ravyts et al., 2012). Because it is often supposed that this leads to a limited product differentiation, the use of wild-type strains isolated from traditional fermented meat products has been proposed as an alternative (F. Leroy et al., 2013; S. Leroy et al., 2013; Leroy et al., 2015; Villani et al., 2007). Dedicated *in vitro* studies have been carried out to characterize and

* Corresponding author.
E-mail address: Frederic.Leroy@vub.be (F. Leroy).
[1] Equal contribution.

https://doi.org/10.1016/j.ijfoodmicro.2018.03.008
Received 25 January 2018; Received in revised form 27 February 2018; Accepted 11 March 2018
Available online 12 March 2018
0168-1605/

evaluate strains of CNS that may offer benefits related to, for instance, proteolytic or lipolytic activities, aroma formation, colour generation, or the production of antimicrobials (Babić et al., 2011; Cachaldora et al., 2013; Casaburi et al., 2005; López et al., 2015; Martín et al., 2007; Marty et al., 2012; Mauriello et al., 2004; Papamanoli et al., 2002; Sánchez Mainar et al., 2014, 2016; Stavropoulou et al., 2015; Villani et al., 2007). In addition, autochthonous CNS strains have been tested as potential starter cultures for meat fermentation, including strains belonging to the species *S. equorum* (Bonomo et al., 2011; Fonseca et al., 2013b; Talon et al., 2008; Villani et al., 2007), *S. saprophyticus* (Fonseca et al., 2013b, 2013c), and *S. xylosus* (Casaburi et al., 2008; Di Maria et al., 2002; Gardini et al., 2002; Mauriello et al., 2002). Less commonly isolated CNS species, such as *Staphylococcus epidermidis* (Fonseca et al., 2013b, 2013c), *Staphylococcus succinus* (Talon et al., 2008; Villani et al., 2007), and *Staphylococcus vitulinus* (Casquete et al., 2011; Prpich et al., 2016) have also been explored. However, these added starter cultures have not been monitored in detail during the meat fermentation process, with only a few studies considering their evolution after meat batter inoculation (Di Maria et al., 2002; Fonseca et al., 2013c; Talon et al., 2008). Such analyses may nonetheless be of importance, as the ability of CNS starter cultures to govern the fermentation is not always guaranteed. In the case of a low intrinsic competitiveness of the added starter cultures, the latter risk being outcompeted by a more adapted background microbiota (Fonseca et al., 2013c; Sánchez Mainar and Leroy, 2015; Villani et al., 2007). The fitness of CNS during meat fermentation is a function of the production parameters, although only few studies have examined how CNS communities are shaped by process technology (Janssens et al., 2013). Conditions of potential relevance include, among others, the concentration of curing salt, the fermentation temperature, the degree of acidification driven by the concentration of fermentable carbohydrates, the optional inclusion of moulding or smoking, and the intensity of drying (Leroy et al., 2014).

The aim of this study was to assess the effects of two main processing conditions, namely temperature and acidity, on the prevalence of a cocktail of CNS composed of five different strains representing five different species in a meat model system.

2. Materials and methods

2.1. Bacterial strains and inoculum build-up

Five different CNS strains were used in this study, namely *S. carnosus* 833, *S. epidermidis* ATCC 12228, *S. equorum* DFL-S19, *S. saprophyticus* FPS1, and *S. xylosus* 2S7-2. These strains have been isolated from fermented meat products previously (Montel et al., 1992; Ravyts et al., 2010), except for *S. epidermidis* ATCC 12228 of which the source is unknown (Zhang et al., 2003). The choice of the CNS species was based on either their application as conventional starter cultures (*S. carnosus* and *S. xylosus*) or their natural prevalence during spontaneous fermentation (*S. equorum*, *S. saprophyticus*, and *S. xylosus*). Hereby, *S. xylosus* stands out, as it belongs to both categories. Also, a strain of *S. epidermidis* was included to represent a species that is only occasionally encountered in fermented meats. All strains were present in the culture collection of the research group of Industrial Microbiology and Food Biotechnology (Vrije Universiteit Brussel, Brussels, Belgium) and were stored at −80 °C in glycerol-containing (25%, v/v) brain heart infusion (BHI) medium (Oxoid, Basingstoke, Hampshire, UK). Their authenticity was confirmed on species level by sequencing of the *rpoB* gene from genomic DNA (Braem et al., 2011). For the inoculum build-up, the strains were propagated in BHI medium twice and incubated at 30 °C for 12 h. The precultures were then inoculated (1%, v/v) into BHI medium at 30 °C for 12 h to obtain the final inoculum. A different volume of the final culture was used to obtain inoculation levels within the same order of magnitude for all strains [about 7 log colony forming units (CFU)/g]. The cell pellets were collected by centrifugation (8041 × *g* at 4 °C for 20 min) and used as inoculum for the meat model experiments after re-suspension in saline (0.85%, m/v, NaCl; VWR International, Darmstadt, Germany).

2.2. Meat model preparation and experimental set-up

A meat batter was prepared by mixing fresh pork mince (around 9 kg, obtained from a local butcher), sodium chloride (3.0%, m/m; VWR International), sodium nitrate (150 mg/kg; VWR International), and ascorbic acid (500 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). To evaluate the impact of the initial pH, this mixture was divided into three parts. A first part had a pH of 5.7 (the original pH of the meat batter), whereas the pH of the two other parts was adjusted with a 70% lactic acid solution (Sigma-Aldrich) to either pH 5.5 or pH 5.3. The meat batter was inoculated with a cocktail of all five CNS strains, each member being present at approximately the same concentration [10^7 colony-forming units per g (cfu/g)]. Finally, the meat mixture was divided over 60-ml plastic containers (VWR International), containing about 100 g per container (closed lid) to fill up the volume and enable fermentation in the absence of air. To simulate potential variations at the level of the fermentation stage according to temperature, the containers were placed into three different incubators, namely at 23, 30, and 37 °C. Together with the variation in initial pH of the meat batter, this set-up resulted in a combination of nine different temperature-pH scenarios. The fermentation was followed for 14 days and meat samples were taken for analysis right before (0) and after (0′) inoculation, and after 1, 2, 3, 7, and 14 days of incubation. For each time point, two randomly selected containers were singled out. One sample per container was taken for bacterial enumeration (duplicate data). For the pH measurement, two measurements per container were performed in two different locations of the meat batter (quadruplicate data).

2.3. pH measurement

After sampling, the pH was measured directly in the meat batter with a DY-P10 pH meter (Sartorius, Göttingen, Germany) equipped with an insertion pH probe (VWR International).

2.4. Enumeration and isolation of microorganisms

Twelve grams of meat sample was aseptically transferred into a stomacher bag (Seward, Worthington, UK) together with 108 ml of maximum recovery diluent [saline and 0.1% (m/v) bacteriological peptone (Oxoid)]. This mixture was homogenized at maximum speed for 2 min in a Laboratory Blender Stomacher 400 (Seward). Appropriate decimal dilutions in saline were prepared and spread on mannitol salt-phenol-red agar (MSA; VWR International) and de Man-Rogosa-Sharpe (MRS; Oxoid) agar, which were incubated at 30 °C for 48–72 h, to enumerate presumptive CNS and LAB, respectively. The bacterial counts were calculated from media containing 30 to 300 colonies, of which 10–30% were randomly selected and picked up to follow the CNS and LAB communities. The colonies obtained from MSA and MRS agar were transferred into BHI and MRS medium, respectively, which were incubated overnight at 30 °C to obtain grown cultures for DNA extraction as well as for storage at −80 °C in cryovials containing 25% (v/v) of glycerol.

2.5. Classification and identification of bacterial isolates through $(GTG)_5$-PCR fingerprinting of genomic DNA

Genomic DNA extraction from cell pellets, obtained by microcentrifugation at 13,000 rpm of 1.5 ml of an overnight culture, was performed with a Nucleospin 96 tissue kit (Macherey Nagel, Düren, Germany), according to the manufacturer's instructions. Prior to extraction, all cell pellets were washed with Tris-ethylene diaminotetraacetic acid (EDTA)-sucrose buffer [TES buffer; 50 mM Tris base