International Journal of Food Microbiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Pervasiveness of *Staphylococcus carnosus* over *Staphylococcus xylosus* is affected by the level of acidification within a conventional meat starter culture set-up

Despoina Angeliki Stavropoulou[a], Hannelore De Maere[b], Alberto Berardo[c], Bente Janssens[a], Panagiota Filippou[a], Luc De Vuyst[a], Stefaan De Smet[c], Frédéric Leroy[a,*]

[a] Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium
[b] Research Group of Technology and Quality of Animal Products, KU Leuven Technology Campus, Ghent, Belgium
[c] Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Ghent, Belgium

ARTICLE INFO

Keywords:
Staphylococcus carnosus
Staphylococcus xylosus
pH
Meat fermentation
Starter culture
Coagulase-negative staphylococci

ABSTRACT

Staphylococcus carnosus and *Staphylococcus xylosus* are commonly used, individually or in combination, within conventional starter cultures for the purposes of colour and flavour development during meat fermentation. Yet, little is known about the relative importance of both species under different processing conditions. The present study aimed at investigating the competitiveness of *S. carnosus* within a meat starter culture under different acidification profiles. The experimental set-up involved a gradient of decreasing experimental control but increasing realism, ranging from liquid meat fermentation models in a meat simulation medium, over solid mince-based meat fermentation models, to fermented sausage production on pilot-scale level. In general, *S. carnosus* gained a fitness advantage over *S. xylosus* in the most acidified variants of each set-up. In contrast, increasing persistence of *S. xylosus* was seen at the mildest acidification profiles, especially when approximating actual meat fermentation practices. Under such conditions, *S. carnosus* was reduced to co-prevalence in the mince-based meat fermentation models and was fully outcompeted on pilot-scale level. The latter was even the case when no *S. xylosus* starter culture was added, whereby *S. carnosus* was overpowered by staphylococci that originated from the meat background (mostly *S. xylosus* strains). The results of the present study suggested that conventional starter cultures behave differently when applied in different technological set-ups or using different recipes, with possible repercussions on fermented meat product quality.

1. Introduction

The industrial use of starter cultures to produce fermented sausages is widespread as a strategy to improve process control, thereby also standardising the end-product and contributing to biosafety and quality (Ravyts et al., 2012; Talon and Leroy, 2011). Conventional meat starter cultures consist mainly of selected strains of lactic acid bacteria (LAB) for acidification, besides coagulase-negative staphylococci (CNS) for colour and flavour development (Leroy et al., 2006). In European-type fermented sausages, the LAB fraction most often consists of strains of *Lactobacillus sakei*, whereas strains of *Staphylococcus carnosus* and/or *Staphylococcus xylosus* are usually representing the CNS group (Ravyts et al., 2012). The use of *S. carnosus* may be somewhat surprising, as this CNS species is rarely isolated from spontaneously fermented meat products (García-Fontan et al., 2007; Talon et al., 2007). In contrast, *S. xylosus* seems to commonly dominate such artisan-type fermented meat products prepared without starter culture (Aquilanti et al., 2016; Cocolin et al., 2001; García-Varona et al., 2000). Nonetheless, *S. carnosus* is known to be very effective during meat fermentation when added as a starter culture (Leroy et al., 2006). Indeed, *S. carnosus* harbours genes that both reflect adaptation to the meat fermentation process and are relevant to technologically important properties, among others the potential for nitrate and nitrite reduction, carbohydrate degradation, and catalase activity (Sánchez Mainar et al., 2017). Also, genes that are related to pathogenicity and virulence seem to be lacking (Rosenstein et al., 2009), which meets food safety expectations (EFSA, 2005). The genome of *S. carnosus* is one of the smallest among the staphylococcal genomes available, suggesting that this CNS species may have become adapted to domesticated use in meat processing environments (Rosenstein and Götz, 2010).

* Corresponding author.
E-mail address: Frederic.Leroy@vub.be (F. Leroy).

https://doi.org/10.1016/j.ijfoodmicro.2018.03.006
Received 26 December 2017; Received in revised form 27 February 2018; Accepted 7 March 2018

Currently, it is not well known to which degree *S. carnosus* and *S. xylosus* prevail at an individual level under different meat processing conditions. This is not trivial, as both CNS species may not only have different metabolic impacts on fermented meat quality (Sánchez Mainar et al., 2017), but also since fermented meats are produced according to a very wide spectrum of different recipes and processing conditions (Leroy et al., 2015). In general, *S. carnosus* seems to be mostly present in acidic sausages (Aymerich et al., 2003), being a rather acid-tolerant CNS species (Janssens et al., 2012; Ravyts et al., 2010; Stahnke et al., 2002). This acid tolerance contrasts with the behaviour of *S. xylosus*, being more at ease under mildly acidified conditions (Aquilanti et al., 2016; Blaiotta et al., 2004; Fonseca et al., 2013; Pisacane et al., 2015). One should not overlook the fact that the meat starter culture also needs to compete with the background microbiota of the raw meat, of which the community dynamics depend on the applied practices too (Cocconcelli and Fontana, 2014; Janssens et al., 2012, 2013; Leroy et al., 2014; Stahnke et al., 2002).

More insights into the relationship between the technological fitness of conventional starter culture CNS species during meat fermentation and the major processing factors is required, with the level of acidification being a key component of process variation. Heavily acidified fermented meats are representative for the Northern-European type of sausage fermentation, whereas mild acidification is more common in Mediterranean-type variants. Therefore, the aim of the present study was to investigate how the competitive behaviour of *S. carnosus* is affected by acidification in the presence or absence of *S. xylosus*. The methodology involved a gradient of decreasing experimental control but increasing realism, ranging from liquid meat fermentation models in a meat simulation medium, over solid mince-based meat fermentation models, to fermented sausage production on pilot scale.

2. Materials and methods

2.1. Bacterial strains, media, and inoculum build-up

Strains of *Lactobacillus sakei* (CTC 494) as well as *S. carnosus* (833) and *S. xylosus* (3PA6 or 2S7–2) were used in the present study, either alone or in combination. All strains were present in the collection of the research group of Industrial Microbiology and Food Biotechnology (Vrije Universiteit Brussel, Brussels, Belgium). The LAB and CNS strains were stored at −80 °C in glycerol-containing (25%, v/v) de Man-Rogosa-Sharpe (MRS) medium (Oxoid, Basingstoke, Hampshire, UK) and brain heart infusion (BHI) medium (Oxoid), respectively. For the respective enumeration of LAB and CNS, MRS agar medium (Oxoid) and mannitol salt-phenol-red agar medium (MSA; VWR International, Darmstadt, Germany) were used.

The liquid meat fermentation models were based on a meat simulation medium (MSM), reflecting high contents of salt, lactate and meat-derived peptides, and serving as an approximation of the water phase of fermented sausages (Leroy and De Vuyst, 2005). This medium contained (per litre): 40.0 g of sodium chloride (VWR International), 13.0 g of glucose (VWR International), 11.0 g of bacteriological peptone (Oxoid), 8.8 g of meat extract (Lab Lemco; Oxoid), 6.1 g of calcium lactate.$5H_2O$ (VWR International), 2.2 g of yeast extract (VWR International), 0.2 g of ascorbic acid (Sigma-Aldrich, Saint-Louis, MO, USA), 0.2 g of sodium nitrate (VWR International), 0.038 g of $MnSO_4.5H_2O$ (VWR International), and 1 ml of Tween 80 (VWR International).

For the inoculum build-up, the strains were propagated twice in the appropriate medium and incubated at 30 °C for 12 h. The precultures were then inoculated (1%, *v*/v) into BHI medium (CNS) or MRS medium (LAB) to obtain the final inoculum by propagation at 30 °C for 12 h. The cell pellets were collected by centrifugation (8041 × *g* at 4 °C for 20 min) and used as inoculum for the meat fermentation models and/or sausage preparation after their re-suspension in saline (0.85%, m/v, NaCl; VWR International).

Table 1
Acidification profiles used for the liquid (temperature and pH) and mince-based meat fermentation model experiments (temperature).

Profile	Strong acidification		Mild acidification	
Time (days)	Temperature (°C)	pH	Temperature (°C)	pH
0	25	5.80	20	5.80
1	25	5.60	20	5.80
2	25	5.20	20	5.60
3	20	5.00	18	5.30
4	18	4.90	16	5.25
5	16	4.80	14	5.20
6	14	4.80	14	5.20
7	14	4.80	14	5.20

2.2. Liquid meat fermentation models

All fermentation experiments were performed with a co-culture consisting of L. *sakei* CTC 494, *S. carnosus* 833, and *S. xylosus* 3PA6. Inoculation levels added to MSM were set at about 6 log of colony-forming units (cfu) per ml for L. *sakei* CTC 494 and 5 log cfu per ml for both CNS strains. The fermentations were carried out in 15-litre computer-controlled Biostat C fermentors (Sartorius, Melsungen, Germany) connected to a cryostat (Frigomix 2000; Sartorius) to run them at low temperatures. The fermentors contained 10 l of MSM and were sterilised *in situ* at 121 °C for 20 min. Glucose was sterilised separately and was added aseptically to the fermentors. Moderate agitation (150 rpm; turbine impeller) was maintained to ensure homogeneity of the medium. Two different temperature and pH profiles were implied, representing strong or mild acidification profiles typical for Northern- and Southern-European meat fermentations, respectively (Table 1). The fermentations were followed for 7 days, whereby temperature and pH were controlled on-line with Micro MFCS for Windows NT software (Sartorius), as described previously (Leroy and De Vuyst, 1999). After each sampling at regular time intervals, the sampling valve was sterilised using an external steam generator (J. Strobel & Söhne, Munich, Germany) during 10 min. Every experiment was done in triplicate.

2.3. Solid mince-based meat fermentation models

To validate the findings of the liquid meat fermentation models, a more realistic approach was adopted using solid mince-based meat fermentation models. For the preparation of the meat batter, fresh pork (3 kg) mince was supplemented with 2.5% of sodium chloride (m/m; Merck Millipore, Darmstadt, Germany), 500 ppm of ascorbic acid (Sigma-Aldrich), 200 ppm of sodium nitrate (VWR International), and 200 ppm of $MnSO_4.4H_2O$ (VWR International). Two different batches were prepared by adding different glucose (VWR International) concentrations to achieve a strong (0.7%, m/m) or mild (0.1%, m/m) pH decrease during the fermentation courses. As was the case for the liquid meat fermentation models, the two batches had a different temperature profile (Table 1). The starter culture consisting of L. *sakei* CTC 494, *S. carnosus* 833, and *S. xylosus* 3PA6 was inoculated into the meat batter at a level of approximately 6 log cfu/g. The meat mixture of each batch was then stuffed into 60-ml plastic containers (approximately 100 g per container; VWR International) to enable fermentation in the absence of air. The containers were placed in a water bath coupled to a cryostat (Frigomix 3000T, Sartorius) and the fermentations were followed for 7 days. Bacterial counts and pH measurements (see below) were performed at days 1, 2, 3, 4, and 7. For each time point, three randomly selected containers were selected for the analysis.

2.4. Pilot-scale production of dry fermented sausages

Fermented sausages were produced in a pilot-scale plant at the