EFFECT OF PROCESSING CONDITIONS ON THE COMMUNITY DYNAMICS OF COAGULASE-NEGATIVE STAPHYLOCOCCI DURING MEAT FERMENTATION

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Abstract – A wide variety of coagulase-negative staphylococci (CNS) species can be isolated from traditional fermented sausages. However, it is not entirely clear how the composition of the CNS communities is influenced by specific processing conditions during meat fermentation. 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 CNS strains during meat fermentation (*Staphylococcus carnosus* 833, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus equorum* DFL-S19, *Staphylococcus saprophyticus* FPS1, and *Staphylococcus xylosus* 2S7-2). 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 the aforementioned strains, stuffed in containers, and incubated at 23°C, 30°C, or 37°C. Both pH level and temperature influenced the composition of the CNS communities, giving a competitive advantage to the best adapted species. *Staphylococcus xylosus*, for instance, preferred low temperature and mild acidity, whereas elevated temperature selected for *S. epidermidis* and low pH for *S. carnosus*. Therefore, the CNS communities developing in fermented meats is likely to be ascribed to the initial presence of specific species in the meat batter as well as their subsequent adaptation to the processing conditions during fermentation.

Key Words – starter cultures, fermented meat products, meat microbiology

I. INTRODUCTION

The main microbial groups with technological benefits that prevail during meat fermentation are the lactic acid bacteria (LAB), which contribute to the texture and biosafety of the end-product by lowering the pH, and the coagulase-negative staphylococci (CNS), which are responsible for colour generation through nitrate reductase activity, contribute to flavour formation due to their metabolism of carbohydrates, amino acids, and fatty acids, and exhibit anti-oxidant properties due to their catalase activity [1, 2]. From traditional fermented sausages, which often rely on spontaneous fermentation and are valued for their unique sensory characteristics, a wide variety of CNS species can be isolated [1, 3]. Although the obtained species diversity is known to depend on the raw materials and ingredients used, it is not entirely clear how this is affected by the specific processing conditions that prevail during meat fermentation [4]. Conditions of potential interest are, for instance, the applied concentration of curing salt, the fermentation temperature, the degree of acidification driven by the amount of fermentable carbohydrates, and the intensity of drying. Therefore, the aim of the present study was to assess the influence of two main processing conditions, namely temperature and acidity, on the mutual competitiveness of a cocktail composed of five different CNS strains representing five different species, namely *Staphylococcus carnosus*, *Staphylococcus epidermidis*, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, and *Staphylococcus xylosus*.

II. MATERIALS AND METHODS

Fermented meat models were prepared by mixing minced pork meat with salt (3 %, m/m), sodium nitrate (150 ppm), and ascorbic acid (500 ppm). The meat batter was then divided into three batches, each with a different initial pH of 5.3, 5.5, or 5.7, and inoculated with a cocktail consisting of five

different CNS strains, *i.e.*, *S. carnosus* 833, *S. epidermidis* ATCC 12228, *S. equorum* DFL-S19, *S. saprophyticus* FPS1, and *S. xylosus* 2S7-2, with each strain at the same inoculation level (10^7 log cfu g^{-1}). Subsequently, the batter was stuffed into 60-ml plastic containers and placed into incubators at 23°C, 30°C, or 37°C. Samples were taken immediately after inoculation (day 0) and on days 1, 2, 3, 7, and 14 of the fermentation for plating on mannitol salt agar (MSA) medium. Isolates that were picked up from this agar medium were subjected to $(GTG)_5$-PCR fingerprinting and clustered using BioNumerics 5.1 software, as previously described [5]. The identity of each $(GTG)_5$-PCR fingerprint cluster was further confirmed via sequencing of the *rpoB* gene of the representative isolates.

III. RESULTS AND DISCUSSION

Regarding the CNS species diversity during the different meat fermentations, several trends were found (Figure 1). In general, *S. saprophyticus* and *S. equorum* did not survive beyond the first few days of fermentation and, therefore, seemed not as competitive as the other investigated CNS species when inoculated at the same level. In contrast, *S. xylosus* was present in all meat fermentations and prevailed mostly at low temperature (23°C) and high pH (pH 5.7), which are similar conditions as encountered in Mediterranean-type fermentations where this CNS species is frequently found in industrial practice [6-8]. In contrast, *S. epidermidis* is not usually dominant in spontaneous meat fermentations, but it was very competitive in the present study. It was found throughout all meat fermentations and was often dominant, in particular when the fermentations were performed at middle (30°C) and high (37°C) temperatures. The reason that *S. epidermidis* largely dominated the fermentation at the higher levels of pH and temperature might be due to the fact that this CNS species is frequently isolated from human and mammal skin, where similar conditions apply [9]. Also, the ability of this CNS species to perform well under more acidic conditions might be linked to the presence of arginine deiminase (ADI) activity in the strain applied, which grants it a competitive advantage under acidic stress through the production of ammonia and additional adenosine triphosphate (ATP) [10, 11]. The same might be said for *S. carnosus*, which was also present throughout most of the meat fermentations and prevailed mostly in the fermentations at the lowest pH values. Similar to *S. epidermidis*, the presence of the ADI pathway in the strain applied might have improved its competitive advantage [10, 11].

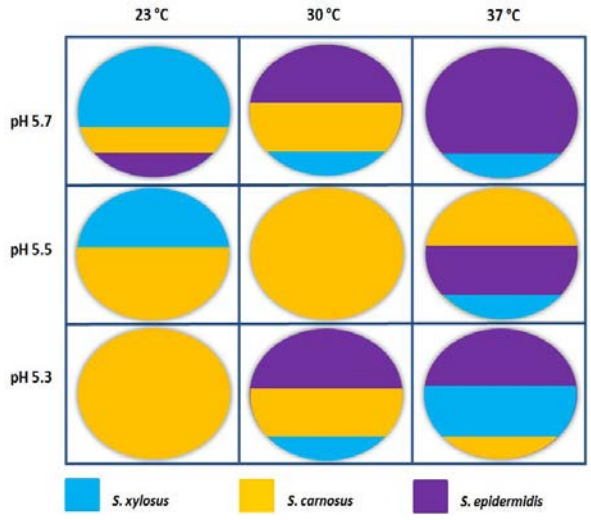

Figure 1. Approximate overview of the CNS communities (*Staphylococcus carnosus*, light grey; *S. epidermidis*, black; and *S. xylosus*, dark grey) present in fermented meat models after two weeks of meat fermentation under different processing conditions of temperature and pH.

IV. CONCLUSION

The present study showed that the fermentation temperature and the acidity influenced the composition of the CNS communities of fermented meats by giving a competitive advantage to the best adapted species. The presence of the ADI pathway could be a crucial factor in the enabling of CNS to survive under acidic conditions, but this requires further investigation. As a result, the CNS communities developing in fermented meats are likely to be ascribed to the initial presence of specific species in the meat batter as well as their subsequent adaptation to the processing conditions during fermentation. Since both factors are highly variable, the exact nature of the CNS community structure displays a large amount of disparity between different fermented meat products

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