Amino acid-converting behaviour of coagulase-negative staphylococci in a rich medium and in fermented sausages

D. A. Stavropoulou[1], H. De Maere[2], A. Berardo[3], S. De Smet[3], L. De Vuyst[1] and F. Leroy[1*]

[1] Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium

[2] Research Group for Technology and Quality of Animal Products, KU Leuven Technology Campus, Ghent, Belgium

[3] Laboratory for Animal Nutrition and Animal Product Quality, Ghent University, Melle, Belgium

*fleroy@vub.ac.be

Abstract – The ability of different coagulase-negative staphylococci (CNS) to convert amino acids into technologically important metabolites was investigated in a rich medium (brain heart infusion, BHI) and in dry fermented sausages. Firstly, 56 CNS strains from five different species were tested for volatile and biogenic amine production after 24 h and 48 h of culturing in BHI. With respect to volatile production, most CNS strains produced 3-methyl butanol, albeit with considerable differences between species and even between strains. In contrast, levels of 3-methyl butanal, which was already present as a background compound in the culture medium, mostly decreased. As to the production of biogenic amines in BHI, 2-phenylethylamine was the most abundant variant, although the average total concentration remained below 100 µM after 48 h of incubation. Next, four CNS strains were selected and applied in sausage fermentations. This led to strain-dependent differences in volatile profiles, with 3-methyl butanol, 3-methyl butanoic acid, and 2-methyl butanoic acid as the most pronounced end-products of amino acid conversions.

Key Words – Volatile compounds, biogenic amines, meat fermentation

I. INTRODUCTION

In sausage fermentation, coagulase-negative staphylococci (CNS) are an important part of the fermentative microbiota and several metabolites, such as volatiles and biogenic amines, are produced through conversion of meat-derived amino acids [1, 2]. *Staphylococcus carnosus* and *S. xylosus* are two CNS species that are frequently added as commercial starter cultures, as they contribute to dry fermented sausage flavour, although their individual involvement may differ. Nevertheless, in spontaneously fermented sausages prepared without the addition of a starter culture, other CNS species are also relevant with respect to aroma development, but their relative contributions are even more uncertain [3]. Besides volatile compounds, amino acid conversion by CNS may lead to biogenic amine production, although a detailed overview of the ability of different CNS species and even strains to generate such compounds is lacking, in contrast to lactic acid bacteria (LAB), for which this is well described [4-6].

In general, the specific amino acid-converting abilities of different CNS need further charting. Therefore, the aim of the present study was to evaluate the ability of different CNS strains to generate volatile compounds and biogenic amines from amino acids and to explore their inter- and intra-species heterogeneity when they grow in a rich medium, as well as to investigate the different capacity of CNS starter cultures to produce volatile compounds during sausage fermentation.

II. MATERIALS AND METHODS

A screening of 56 CNS strains belonging to five different species, namely *S. carnosus* (12 strains), *S. epidermidis* (6), *S. equorum* (16), *S. saprophyticus* (8), and *S. xylosus* (14), was performed in 30 ml of brain heart infusion medium (BHI; Oxoid, Basingstoke, UK) at 30°C for 48 h. Volatiles and biogenic amines were quantified after 24 h and 48 h of culturing by mass spectrometry after their separation through gas chromatography, using static-headspace analysis (SH-GC-MS; Agilent 6890, Agilent Technologies, Santa Clara, CA, USA), and through ultra-performance liquid chromatography (UPLC-

MS/MS; Waters, Milford, MA, USA), respectively. The concentrations of volatiles were calculated using a calibration curve, while the levels of biogenic amines were calculated through standard addition.

For the preparation of dry fermented sausages, lean pork (70.5 %), pork backfat (27.0 %), sodium chloride (2.5 %), sodium ascorbate (500 mg/kg), and sodium nitrite (150 mg/kg) were used. The following five batches were prepared using different CNS starter cultures: (1) *S. carnosus* 833; (2) *S. xylosus* 2S7-2; (3) *S. equorum* DFL-S19; (4) *S. saprophyticus* FPS1; and (5) a batch without added CNS. All batches were inoculated with *Lactobacillus sakei* CTC 494 too. The batches were stuffed into casings and a fermentation followed by a ripening phase was performed for 28 d. The fermentation (first two days) was carried out at 24 °C and at relative humidity of 94 %. During the ripening phase, temperature was set to 12 °C and relative humidity to 82 % for the last two weeks. Samples were taken for analysis after 0, 1, 2, 8, 14, 21, and 28 d and the volatile profiles were determined by solid-phase microextraction followed by gas chromatography coupled to mass spectrometry (SPME-GC-MS).

III. RESULTS AND DISCUSSION

The metabolite data obtained from the screening indicated a considerable heterogeneity among CNS species and strains for both volatile and biogenic amine production.

Concerning volatile production, the leucine-derived metabolites were the most abundant. More specifically, 3-methyl butanol was produced during culturing by most of the strains investigated. This amounted up to 52 µM for *S. xylosus* W1-1 after 48 h of incubation. The average production of 3-methyl butanol by strains of *S. xylosus* was higher than for strains of *S. carnosus*, whereas strains of *S. epidermidis* were very poor producers (Figure 1). The average concentration of 3-methyl butanol increased from 24 h to 48 h for *S. equorum*, *S. saprophyticus*, and *S. xylosus*, in contrast to the production by the two other CNS species. In the initial medium background, traces of 3-methyl butanal, 2-methyl butanal, 2-methyl propanal, and 2.5-dimethyl pyrazine, all potentially derived from amino acids [7], were already present, but only 3-methyl butanal concentrations changed over time. However, only a few CNS strains led to slight increases of the latter compound, whereas the majority of the strains led to a decrease of its concentration or left it unaffected. This was in accordance with former studies, which have reported that 3-methyl butanal is a transient metabolite, produced during the exponential growth phase, which is rapidly oxidized to the corresponding carboxylic acid [8, 9].

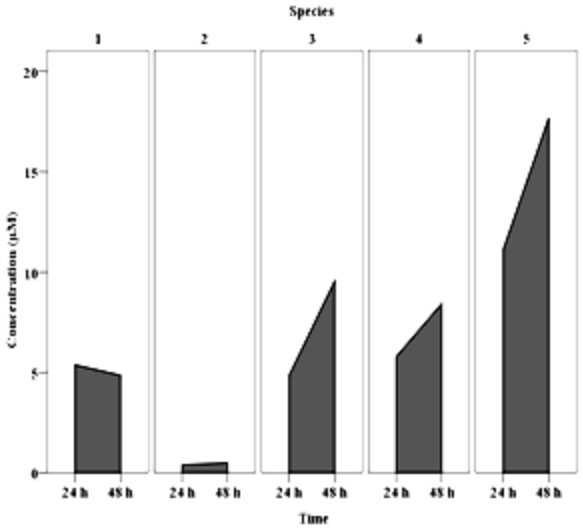

Figure 1. Average 3-methyl butanol concentrations (µM) per *Staphylococcus* species (1: *S. carnosus*, 2: *S. epidermidis*, 3*: S. equorum*, 4: *S. saprophyticus*, and 5: *S. xylosus*) produced in brain heart infusion medium after 24 h and 48 h of culturing.

Regarding the biogenic amine production by CNS, increases in the concentrations of 2-phenylethylamine (PEA), tryptamine, tyramine, histamine, cadaverine, putrescine, and agmatine were found. The commonly targeted biogenic amines in dry fermented sausages, namely tyramine, putrescine, and cadaverine [11], were found on average below 10 µM per species, in contrast to PEA, a biogenic amine usually present in low concentrations in such products [10, 11], which was on average produced over 65 µM by *S. carnosus* strains after 48 h of culturing (Figure 2). Strain dependency of biogenic amine production was found, for instance within the species *S. saprophyticus*, for which the strain FPP1 was the only one producing rather elevated concentrations

of PEA (over 50 µM after 48 h). Nevertheless, the total concentration of biogenic amines did not exceed 100 µM (*i.e.*, 92 µM for *S. epidermidis* after 48 h) and thus was never approaching the toxicity level of 200 mg/kg of meat [12].

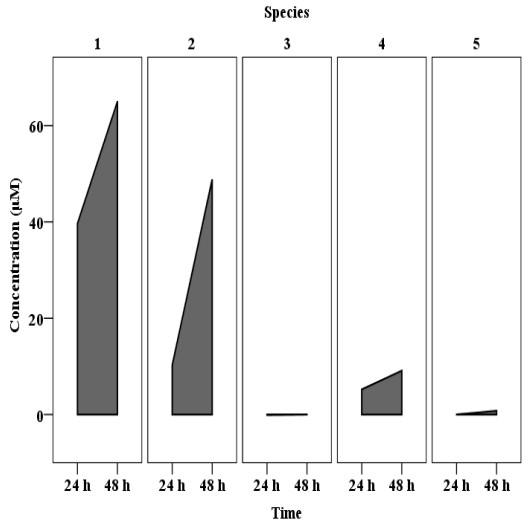

Figure 2. Average PEA concentrations (µM) per *Staphylococcus* species (1: *S. carnosus*, 2: *S. epidermidis*, 3: *S. equorum*, 4: *S. saprophyticus*, and 5: *S. xylosus*) produced in brain heart infusion medium after 24 h and 48 h of culturing.

Concerning the results of the sausage fermentations, the aroma compound analysis indicated differences in amino acid-derived volatile profiles. More specifically, differences in the concentrations of leucine-derived 3-methyl butanol and 3-methyl butanoic acid, isoleucine-derived 2-methyl butanoic acid, valine-derived 2-methyl propanoic acid, and methionine-derived 3-(methylthio)-propanal were found in the five batches made. These metabolites are often present in dry fermented sausages and are considered as volatiles arising from microbial metabolism [13-15]. The production of 3-methyl butanol and 3-methyl butanoic acid differed over time when different CNS starter cultures were applied (Figure 3). The most noteworthy case was the batch inoculated with *S. saprophyticus* FPS1, in which higher concentrations of the carboxylic acids 3-methyl and 2-methyl butanoic acid were produced after both 2 and 21 days. Although application of different starter cultures is known to result in differences in aroma profiles of dry fermented sausages [15, 16], the link with strain-specific variations in volatile production and subsequent inter-conversions of the amino acid-derived compounds needs further exploration (*e.g.*, with respect to the transformation of 3-methyl butanal into 3-methyl butanol and 3-methyl butanoic acid).

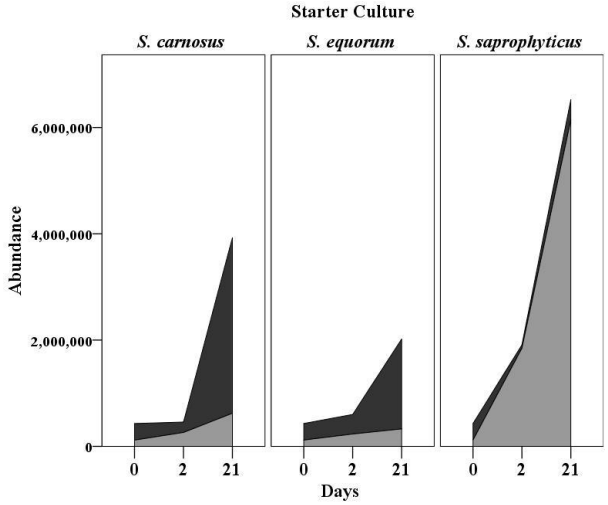

Figure 3. Production of 3-methyl butanol (black areas) and 3-methyl butanoic acid (gray areas) expressed as relative abundance in arbitrary units during sausage fermentation, after 0, 2, and 21 days, for batches inoculated with *Staphylococcus carnosus* 833, *S. equorum* DFL-S19, and *S. saprophyticus* FPS1.

IV. CONCLUSION

Both volatile and biogenic amine production by CNS was species- and even strain-dependent. Yet, time effects as well as flavour thresholds (for volatiles) and toxicity levels (for biogenic amines) have to be taken into consideration when evaluating these features among potential CNS starter culture strains. Such differences in amino acid metabolism by CNS were shown to lead to differences in aroma formation during actual meat fermentations. Thus, the results obtained showed relevance for starter culture selection and development, involving both quality and safety issues.

ACKNOWLEDGEMENTS

The authors acknowledge their financial support of the Research Council of the Vrije Universiteit Brussel(OZR, SRP, IRP, HOA, and IOF projects), the Hercules Foundation (project UABR 09/004), and the Research Foundation-Flanders (project FWOAL632). The

authors also wish to thank Wim Borremans and Tom Balzarini (Vrije Universiteit Brussel, Belgium) for their technical support.

REFERENCES

[1] Leroy, F., Verluyten, J. & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. International Journal of Food Microbiology 106: 270-285.

[2] Montel, M.C., Masson, F. & Talon, R. (1998). Bacterial role in flavour development. Meat Science 49: 111-123.

[3] Ravyts, F., De Vuyst, L. & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentation. Engineering in Life Science 4: 356-367.

[4] Bover-Cid, S. & Holzapfel, W.H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. International Journal of Food Microbiology 53: 33-41.

[5] Bover-Cid, S., Izquierdo-Pulido, M. & Vidal-Carou, M.C. (1999). Effect of proteolytic starter cultures of *Staphylococcus* spp. on biogenic amine formation during the ripening of dry fermented sausages. International Journal of Food Microbiology 46: 95-104.

[6] Latorre-Moratalla, M.L., Veciana-Nogués, T., Bover-Cid, S., Garriga, M., Aymerich, T., Zanardi, E., et al. (2008). Biogenic amines in traditional fermented sausages produced in selected European countries. Food Chemistry 107: 912–921.

[7] Ardo, Y. (2006). Flavour formation by amino acid catabolism. Biotechnology Advances 24: 238-242.

[8] Beck, H.C., Hansen, A.M. & Lauritsen F.R. (2004). Catabolism of leucine to branched-chain fatty acids in *Staphylococcus xylosus*. Journal of Applied Microbiology 96: 1185-1193.

[9] de Vos Petersen, C., Beck, H.C. & Lauritsen F.R. (2004). On-line monitoring of important organoleptic methyl-branched aldehydes during batch fermentation of starter culture *Staphylococcus xylosus* reveal new insight into their production in a model fermentation. Biotechnology and Bioengineering 85: 298-305.

[10] Latorre-Moratalla, M.L., Bover-Cid, S., Veciana-Nogues, M.T. & Vidal-Carou, M.C. (2012). Control of biogenic amines in fermented sausages: role of starter cultures. Frontiers in Microbiology 3, article 169.

[11] Suzzi, G. & Gardini, F. (2003). Biogenic amines in dry fermented sausages: a review. International Journal of Food Microbiology 88, 41-54.

[12] Nout, M.J.R. (1994). Fermented food and food safety. Food Research International 27, 291-298.

[13] Herrenz, B., de la Hoz, L., Hierro, E., Fernandez, M. & Ordóñez J.A. (2005) Improvement of the sensory properties of dry-fermented sausages by the addition of free amino acids. Food Chemistry 91: 673-682.

[14] Marco, A., Navarro, J.L. & Flores M. (2004). Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). Food Chemistry 84: 633-641.

[15] Ravyts, F., Steen, L., Goemaere, O., Paelinck, H., De Vuyst, L. & Leroy, F. (2010). The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages. Food Microbiology 27: 945-954.

[16] Olesen P.T., Meyer A.S. & Stahnke L.H. (2004). Generation of flavour compounds in fermented sausages - the influence of curing ingredients, *Staphylococcus* starter culture and ripening time. Meat Science 66: 675-687.