BIOGENIC AMINES PRODUCTION BY *LACTOBACILLUS*, *STAPHYLOCOCCUS* AND *ENTEROCCOCCUS* ISOLATED FROM PORTUGUESE FERMENTED/SMOKED MEAT PRODUCTS

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Abstract – The aim of this study was to evaluate the decarboxylase activity from 38 fermentative bacterial strains, including lactic acid bacteria (LAB) and coagulase-negative *Staphylococci*, in order to characterize and select the strains most suitable for use as safe starter cultures. These strains were previously isolated from traditional Portuguese fermented/smoked meat products, identified by PCR-amplification and characterized according to their technological properties. Biogenic amines were analyzed by RP-HPLC/UV after incubation of the tested strains in decarboxylase synthetic broth enriched with amino acids (histidine, tyrosine, ornithine, phenylalanine, tryptophan and lysine). The data highlights that aminogenic potential for *Enterococcus* was not species-dependent with high values for tyramine and considerable levels of 2-phenylethylamine. Among *Lactobacilli*, the production of tyramine was mainly related to the species of *L. curvatus*. A few strains of *Lactobacilli* also produced putrescine and tryptamine in residual quantities. In contrast, *Staphylococcus* species such as *S. xilosus, S. equorum* and *S. carnosus* showed no decarboxylase activity. In summary, *L. plantarum, L. sakei* and staphylococci isolates from Portuguese meat products seems not to be producers of dangerous biogenic amines and, therefore, suitable for use as starter cultures in meat products without harmful effects on their quality and safety.

Key Words – Biogenic amines, LAB strains, Meat products, Safety

I. INTRODUCTION

Fermented and smoked sausages, in general, are meat products widely consumed in Portugal and like most of protein-rich products and nitrogen compounds, although they are inherently products of low toxicological risk, in certain circumstances may be hazardous, especially, if during processing are introduced or formed undesirable substances. With the exception of physiological polyamines, biogenic amines are mainly produced by microbial decarboxylation of their amino acids precursors in foods. Histamine and tyramine have been the most studied biogenic amines due to the toxicological effects derived from their vasoactive and psychoactive properties [1]. However, the diamines putrescine and cadaverine may potentiate the toxicity of the above amines, and even they might serve as indicators of poor hygienic quality in some foods [2]. The type and amount of BAs depend on several factors, such as of microbial strains, the effect of starter cultures, concentration of free amino acids, and environmental conditions. Many lactic acid bacteria (LAB) belonging to the genera *Lactobacillus*, *Enterococcus*, *Carnobacterium, Pediococcus, Lactococcus* and *Leuconostoc* are able to decarboxylate one or more amino acids [3]. LAB have been used for longer as starters for meat fermentation due to its main roles for lactic acid generation and pH drop but also for improving the sensory quality and the bio-protective effect during processing [4]. Therefore, the objective of this study was to characterize 41 strains of *Lactobacillus*, *Enterococcus* and *Staphylococcus* related to the potential to produce biogenic amines and select the strains that could be used as starter cultures (decarboxylase-negative microorganisms) in fermented/smoked meat products.

II. MATERIALS AND METHODS

A total of 38 fermentative bacterial strains of *Lactobacillus*, *Enterococcus* and *Staphylococcus*, previously isolated and selected according to technological conditions from traditional Portuguese fermented/smoked meat products and identified by PCR-amplification, were analyzed. To assess amino acid decarboxylase capability of bacterial strains, *Lactobacillus* strains were sub-

cultured in MRS broth and *Enterococcus* and *Staphylococcus* strains in Nutrient Standard broth containing 0.1% of the amino acid precursor (L-histidinemonochlorohydrate; L-ornithine monochlorohydrate; L-triptophan free base; L-lysine monochlorohydrate; L-phenylalanine free base; L-tyrosine free base) at 37 °C for 24 h. Afterwards, bacterial strains were placed in an improved decarboxylase medium (screening medium) described by Bover-Cid and Holzapfel [1]. All strains were streaked in duplicate on the decarboxylase medium plates with and without amino acids (as control) and incubated aerobically at 30 °C for 4 days. After incubation, 2 mL of decarboxylase broth were centrifuged (12000 rpm/5 min) for cell precipitation. For the HPLC analysis, 1 mL of 0.1 M HCl was added to 1 mL of supernatant to precipitate proteins, centrifuged (12000 rpm/5 min) and the supernatant was filtered through 0.45µm. Eight biogenic amines (cadaverine, histamine, putrescine, 2-phenilethylamine, tryptamine, tyramine, spermidine and spermine) were detected and quantified by reverse-phase high performance liquid chromatography (RP-HPLC) with UV detection as previously described in Smělá et al. [5] to determine biogenic amines production.

III. RESULTS AND DISCUSSION

Table 1 show the amine-forming capacity of each microbial culture performed through qualitative analysis of biogenic amines potentially formed in the screening fermenting broth. Positive reactions, either on decarboxylase plates or in broth, were documented when a purple colour occurred or tyrosine precipitate disappeared around the colonies or in the decarboxylase broth, respectively. Three strains of *L. curvatus* showed a negative response with the screening procedure but these false negatives were shown to be strong tyramine formers. Our results confirm the findings of Bover-Cid and Holzapfel [1] who reported that the screening medium has some limitations in terms of sensitivity in the detection of biogenic amines produced by microorganisms. Compared with the chromatographic analysis, the screening medium allows a preliminary selection of strains with low decarboxylase activity. The majority of strains assessed by RP-HPLC showed tyrosine-decarboxylase activity. The most intensive tyramine producers were *Enterococcus faecium, Enterococcus faecalis* and *L. curvatus* (Figure 1). These results are similar to those of Latorre-Moratalla et al. [6], who also reported high concentrations of tyramine and significant amounts of 2-phenylethylamine. Only a few strains of lactobacilli (*L. curvatus)* produced putrescine and tryptamine in residual quantities. *L. plantarum* and *L. sakei* seems not to be great producers of biogenic amines. Also, *Staphylococcus* species such as *S. xilosus, S. equorum* and *S. carnosus* showed no significant decarboxylase activity.

Table 1 Biogenic amine production of different strains of *Lactobacillus, Enterococcus* and *Staphylococcus* determined by qualitative analysis in the screening medium

Species	Strains	Screening medium with amino acids
S. carnosus	PO6.08	+
S. carnosus	P05.58	+
S. equorum	PO5-74	+
S. xylosus	P06.01	+
S. xylosus	P06.17	+
S. xylosus	P06.102	+
S. xylosus	S2M7	+
S. xylosus	S3B6	+
S. xylosus	S4B8	+
S. xylosus	P06.26	+
L. plantarum	P2B2	-
L. plantarum	P3B6	-
L. plantarum	P3B7	-
L. plantarum	P3B8	-
L. plantarum	S4B6	-
L. plantarum	PO5.15	-
L. plantarum	PO5.50	-
L. plantarum	PO5.51	-
L. plantarum	PO5.67	-
L. plantarum	PO5-28	+
L. sakei	CV2C2	-
L. sakei	CH2C5	-
L. sakei	CH3C7	-
L. sakei	P3B3	-
L. sakei	PO6.23	+
L. curvatus	CH2C3	-
L. curvatus	PO5-119	+
L. curvatus	PO5.4	+
L. curvatus	PO5.108	-
L. curvatus	L3M5	-
L. curvatus	L2B5	+
L. sp	SG2C8	+
E. faecium	3L1.4	+
E. durans	13S2.2	+
E. faecalis	20P2.1	+
E. sp	9CP1Van 2	+
E. sp	12CP1.3	+
E. sp	15S2.2	+

+, positive; -, negative

Recently, Latorre-Moratalla et al. [6] reported that the inoculation of a decarboxylase-negative autochthonous starter culture reduced the biogenic amine accumulation in a different way depending on the species and strains.

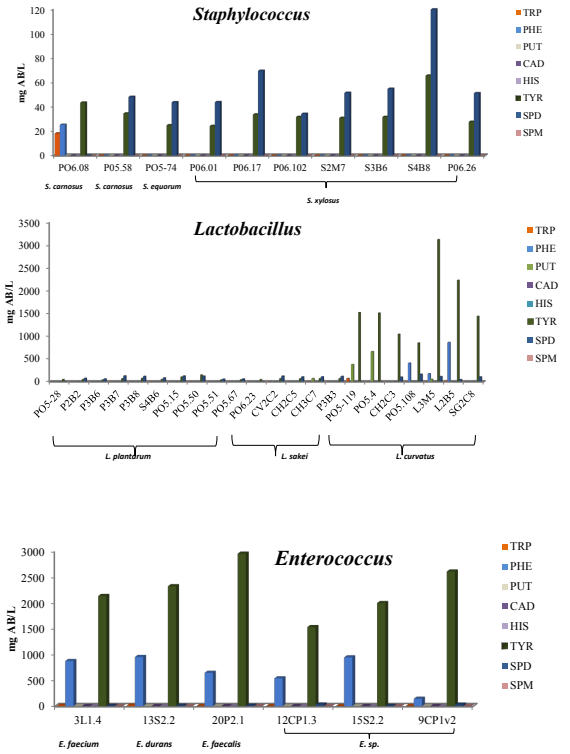

Figure 1. Biogenic amine production of different strains of *Staphylococcus, Lactobacillus* and *Enterococci* determined by RP-HPLC.

IV. CONCLUSION

In summary, *L. plantarum* and *L. sakei* as well as *S. xilosus*, *S. equorum* and *S. carnosus* were the species selected for use as starter cultures in fermented/smoked meat products without any detrimental effect on the quality and safety of the final product.

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