A STRATEGY TO ELIMINATE THE USE OF CHEMICAL ANTIOXIDANTS FOR FERMENTED SAUSAGES PRODUCTION

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I. INTRODUCTION

Researchers and the meat business have been working hard to create new products with better nutritional qualities. The main tactics involve cutting back on or replacing ingredients like sodium chloride, nitrate/nitrite, and saturated fat, which are linked to an increased risk of developing certain diseases as well as including functional ingredients like probiotics and prebiotics. The fundamental role of lactic acid bacteria in fermented meat process is well known and undisputed, but their potential can still be exploded, focusing on strains with particular metabolic peculiarities. In the presence of oxygen and supplementation with heme or heme plus menaquinone, a number of LAB strains can switch from fermentative to respiratory metabolism [1], boosting their antioxidant activity among other functions. Given the foregoing, respiratory metabolism exhibits encouraging potential for the selection and application of a competitive and useful functional starter culture, which is the goal of this study in order to eliminate the use of nitrate/nitrite in fermented sausages production.

II. MATERIALS AND METHODS

129 strains belonging to the *Lacticaseibacillus casei/paracasei* species [2] were first characterized for their capability to grow at different environmental conditions (pH, [NaCl]), safety traits (antibiotic resistance versus 13 antibiotics, haemolysis, antimicrobial properties, biogenic amines production or degradation capabilities (DAB assay and MCO detection), *arcABC* gene presence (involved in the ADI pathway), and their ability to switch to respiratory metabolism. Finally, on the bases of the results, one strain was chosen for *in vivo* trials: production of fermented sausages without nitrate/nitrite using the selected strain adapted under respiratory vs anaerobic conditions. A negative control (no starter addition and sodium nitrate (E252, 150 mg/kg) was also monitored. The evolution of the microflora, pH, Aw, weight loss, colour, synthesis of volatile substances, proteolysis, and strain survival were all specifically assessed. Principal component anlysis (PCA) of volatile compounds was performed.

III. RESULTS AND DISCUSSION

The assessment of the technological capabilities and safety characteristics reveals a considerable degree of heterogeneity in the behaviour of the strains. This demonstrates the strain-specific nature of these abilities. Some strains have demonstrated specific qualities. *L. casei* N2014 displayed MCO capability and possessed the *agdi* and *hdc* genes. *arcC* and *agdi* genes were present in *L. paracasei* B169. The *arcC gene*, the *agdi gene*, and MCO capabilities were present in *L. paracasei B195*. The data of *L. casei* and *L. paracasei* were combined into a single matrix in order to better comprehend the distribution of the various strain characteristics (Fig. 1). The significance of the correlation is shown by the various colours in the heat map. *L. paracasei* TMW 1.1444, TMW 1.1259, and V3 are the strains that are the most distinct when compared to all of the assessed features, however all of the strains are associated in subgroups. Additionally, it is possible to evidence that the strains were grouped in two main clusters (I and II). In Cluster I, there was a correlation between practically all growth factors (pH, [NaCl] and [EtOH]), as well as intrinsic resistance to antibiotics (vancomycin and tobramycin). In Cluster II were grouped strains showing the majority of the antibiotic resistance, MCO, haemolytic

abilities, the presence or absence of the BAs and ethyl carbamate genes, and the production of BAs. In addition, the strains able to grow at pH 3.2 were also contained in this cluster.

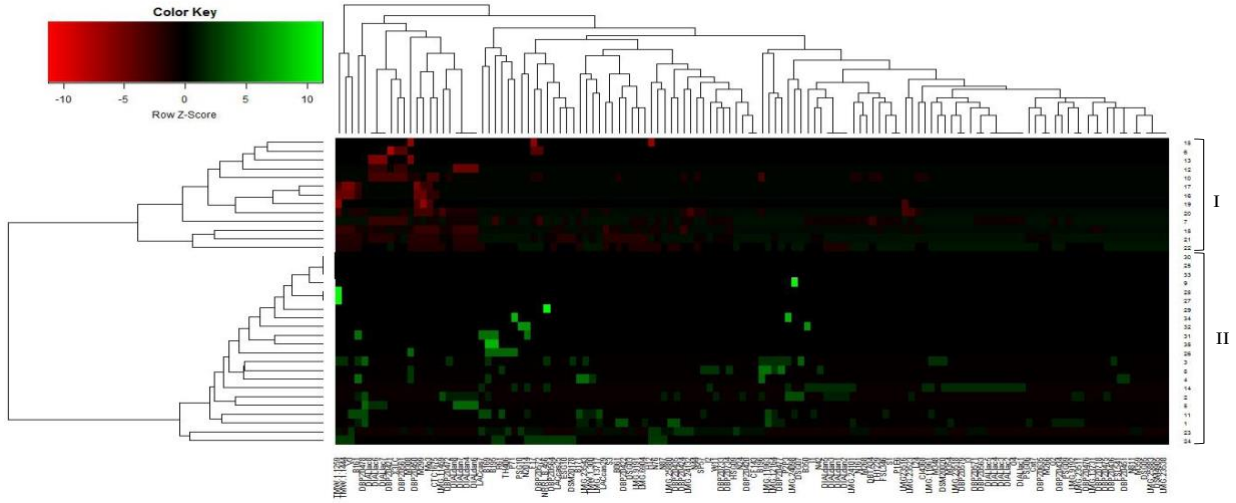

Figure 1. Heat map of *L. casei* and *L. paracasei* strains obtained by the comparison of the several characteristic studied. Each number indicate a characteristic: 1 to 13: antibiotic resistance (cefoperazone 30 µg, cefazolin 30 µg, chloramphenicol 10 µg, clindamycin 10 µg, erythromycin 30µg, kanamycin 30 µg, ofloxacin 5 µg, quinupristin/dalfopristin 15 µg, rifampicin 30 µg, streptomycin 25 µg, tetracycline 10 µg, tobramycin 10µg and vancomycin 30 µg); 14 to 22 growth abilities (pH 3.2, 3.8, 4.2; 4.6; NaCl 2%, 4%, 6.5% and ethanol 12%, 15%); 23 = hemolysis capabilities, 24 = MCO production; 25 to 28 BAs production evaluated by Bover – Cid et al., 1999 method (histamine, tyramine, putrescne or agmantine, cadaverine); 29 to 32 genes involved in BAs production (*tyrdc, odc, agdi, hdc* genes) and 33 to 35 genes involved in ethil carbamate production (*arcA, arcB, arcC* genes).

In vitro and *in vivo* experiments have highlighted the activation of respiratory metabolism in *L. casei* N87, which demonstrated also to be able to carry out sausage's fermentation.
The strain had a distinctive volatile profile that distinguished the sausages from the control. Aw and physic-chemical parameters showed no variations between samples, and weight loss was lower in the inoculated samples in respect to the control (2.5% variation). The colour parameters of the N87 starter culture-inoculated samples did not differ noticeably from the nitrate-added control sample, indicating that *L. casei* N87 was performing a protective function. Additionally, the fermented sausages made with the starter culture exhibited a similar oxidative state to those made with the highest allowed level of nitrate. The proteolysis that occurred during ripening was similar in the two trials.

IV. CONCLUSION

In order to improve the nutritional qualities of fermented sausages (reduction of aldehydes and elimination of nitrates), this study showed that it is possible to eliminate the use of nitrate in the production of fermented sausage using *L. casei* N87 starter culture that has been adapted under respiratory conditions.

REFERENCES

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