# APPLICATION OF AUTOCHTHONOUS LACTIC ACID BACTERIA STRAINS FOR ITALIAN FERMENTED SAUSAGES INDUSTRIAL PRODUCTION

Federica Barbieri[1*], Alberto Baños[2], Josè M. Garcia Madero[2], Rudy Magnani[3], Gabriele Gardini[3], Fausto Gardini[1], Chiara Montanari[1] and Giulia Tabanelli[1]

[1]Department of Agricultural and Food Sciences, University of Bologna, Italy

[2]Department of Microbiology, DOMCA S.A.U., Spain

[3]C.I.a.i. Soc. Coop, Via Gambellara 62/A, 40026 Imola (BO), Italy

*Corresponding author email: federica.barbieri16@unibo.it

## I. INTRODUCTION

Meat fermentation is one of the most ancient preservation strategies to assure safety and prolong food shelf-life. The recent introduction of starter cultures, mainly lactic acid bacteria (LAB) and coagulase-negative staphylococci, has become widespread in meat industries, allowing fermentation optimisation and product standardisation, thanks to the technological features of selected strains, improving the quality and safety of final products [1]. Furthermore, a great variability (in terms of microbiota, chemico-physical features and sensory properties) has been observed in fermented sausages obtained through spontaneous fermentation [2]. These products can represent a sources of isolation of potentially new autochthonous starter cultures, able to confer specific organoleptic characteristics to typical productions [3]. Within the framework of the European project BioProMedFood (PRIMA – Section 2 Programme), in this work, after a preliminary screening [4], the most promising LAB strains isolated from spontaneously fermented European sausages were used as starter cultures to industrially produce fermented sausages in order to evaluate their performances and the organoleptic impact on the final product.

## II. MATERIALS AND METHODS

Italian salamis (“Contadino corto”) were produced by CLAI s.c.a. (Imola, Italy), according to their manufacturing protocol (diameter of 65 mm and initial weight of approx. 1 kg). During two industrial trials, different LAB strains were tested as starter cultures: in the first production, *Latilactobacillus sakei* 2M7 and SWO10, *Lactiplantibacillus paraplantarum* BPF2 and *Latilactobacillus curvatus* KN55 were inoculated at a final concentration of about 7 log CFU/g and their performances were compared with those of a commercial starter traditionally used for the same product (Control). The second industrial production was carried out using *Lat. sakei* ZK39, *Companilactobacillus alimentarius* CB22 and again *Ltp. paraplantarum* BPF2 and *Lat. curvatus* KN55 at a final concentration of about 7.5 log CFU/g, to favour a rapid colonisation of the meat batter. A control sample (commercial starter addition) was also produced. Each sample was monitored during fermentation and ripening regarding physico-chemical parameters (pH and water activity) and the principal microbial groups (LAB, staphylococci/micrococci, enterococci, yeasts and *Enterobacteriaceae*), detected through plate counting onto selective media, provided by Oxoid (Basingstoke, UK), incubated according to the manufacturer’s recommendations. Volatile organic compounds of products at the end of ripening were detected through a gas-chromatography-mass spectrometry coupled with the solid-phase microextraction technique (GC-MS-SPME) [2]. Moreover, biogenic amines (BA) content was also assessed through HPLC analysis [2]. Each analysis was performed in triplicate. Samples were also subjected to a consumer test with untrained panellists in order to evaluate the acceptability of the obtained products. Data were analysed through a one-way ANOVA model with Statistica 8.0 software (StatSoft Inc., Tulsa, USA), according to Tukey test ($P \leq 0.05$).

## III. RESULTS AND DISCUSSION

In the first trial all the tested strains showed good growth ability in the meat batter. Indeed, starting from the initial pH value (5.7 ± 0.02), in all samples this parameter dropped to 5.2 ± 0.03 during fermentation

and then rose to reach values about 5.4 ± 0.02. Moreover, the tested strains showed a higher persistence during ripening with respect to the control, in which a reduction of LAB counts was recorded after 20 days from production (from 9.1 ± 0.1 to 7.9 ± 0.1 log CFU/g). However, the massive proliferation of LAB and corresponding acidification was not able to control the growth of enterobacteria: indeed, this microbial group (including both spoilage and pathogen species) was present in the meat batter at a concentration of 3.2 ± 0.2 log CFU/g and rapidly grew in the first 10 days, reaching values ranging from 4.4 ± 0.2 to 5.6 ± 0.1 log CFU/g in all the samples, with the exception of the control (below the detection limit of 1 log CFU/g). Based on these results, a second industrial production was carried out with the aim to test other LAB strains, to optimise their performances and guarantee the quality of the final product. Results are reported in Table 1. Noteworthy, enterobacteria remained stable during the whole process and decrease in sample with BPF2, that is also able to reduce the accumulation of tyramine in the final product. Finally, after a consumer test with untrained panellists, KN55 and BPF2 resulted samples with the higher rates in terms of odour and taste, being in some cases more appreciated than the control sample.

Table 1 Microbial counts, pH evolution and tyramine content detected in different samples. Standard error is also reported.

| | Time (days) | Control | BPF2 | KN55 | CB22 | ZK39 | SE | P-values |
|---|---|---|---|---|---|---|---|---|
| Lactobacilli (log CFU/g) | 0 | 7.79 | 7.72 | 7.60 | 7.41 | 7.67 | 0.05 | 0.1950520 |
| | 5 | $9.10^{a}$ | $8.12^{b}$ | $9.07^{a}$ | $8.37^{c}$ | $8.01^{d}$ | 0.28 | 0.0000004 |
| | 11 | $9.17^{a}$ | $8.39^{bc}$ | $9.10^{a}$ | $8.60^{b}$ | $8.21^{c}$ | 0.13 | 0.0007681 |
| | 20 | $8.73^{ab}$ | $8.43^{b}$ | $8.97^{a}$ | $8.45^{b}$ | $8.06^{c}$ | 0.10 | 0.0008402 |
| | 45 | $7.62^{a}$ | $8.50^{b}$ | $9.06^{c}$ | $9.02^{c}$ | $7.69^{a}$ | 0.23 | 0.0023132 |
| Enterobacteria (log CFU/g) | 0 | 2.01 | 2.05 | 1.98 | 2.00 | 2.14 | 0.03 | 0.6567829 |
| | 5 | 2.24 | 2.20 | 2.19 | 2.03 | 1.70 | 0.03 | 0.1229269 |
| | 11 | 1.92 | 1.87 | 1.87 | 2.02 | 1.72 | 0.04 | 0.2415989 |
| | 20 | 1.69 | 1.50 | 1.84 | 1.65 | 1.75 | 0.06 | 0.4706551 |
| | 45 | $2.02^{a}$ | $1.24^{b}$ | $2.10^{a}$ | $2.42^{c}$ | $1.59^{b}$ | 0.15 | 0.0233874 |
| pH | 0 | 5.83 | 5.80 | 5.83 | 5.82 | 5.83 | 0.005 | 0.3530392 |
| | 5 | 5.31 | 5.30 | 5.31 | 5.33 | 5.32 | 0.003 | 0.2499101 |
| | 11 | 5.36 | 5.35 | 5.34 | 5.34 | 5.35 | 0.003 | 0.0602145 |
| | 20 | 5.43 | 5.41 | 5.42 | 5.43 | 5.41 | 0.002 | 0.1960214 |
| | 45 | $5.47^{a}$ | $5.51^{b}$ | $5.57^{c}$ | $5.48^{ad}$ | $5.50^{bd}$ | 0.009 | 0.0000021 |
| Tyramine (mg/kg) | 45 | $194.7^{a}$ | $62.3^{b}$ | $157.2^{a}$ | $185.9^{a}$ | $160.7^{a}$ | 16.35 | 0.0057183 |

*for each sampling time different letters indicate significant differences ($P \leq 0.05$) according to ANOVA

## IV. CONCLUSION

In conclusion, with the aim to test some LAB strains as new starter cultures for fermented meat industry, the industrial trials performed in this research allowed to identify some good candidates. In particular, *Lat. curvatus* KN55 was characterised by a similar behaviour with respect to the control, but with a higher persistence during ripening. Moreover, *Ltp. paraplantarum* BPF2 can be also considered promising, due to its ability to reduce the BA content of the final product and its positive effect of the sensorial properties.

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