ANTI-LISTERIAL ACTIVITY OF AUTOCHTHONOUS LACTIC ACID BACTERIA IN FRESH PORK SAUSAGES

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

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

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

*Corresponding author email: chiara.angelucci3@unibo.it

I. INTRODUCTION

Fresh sausages are considered as food that must be cooked before consumption. For this reason, the EU regulation 2073/2005 (2005) does not give any indication concerning the presence pathogens such as *Listeria monocytogenes* and Salmonella in this product, although they can represent a concern for producers. Besides to appropriate hygiene practices during production and high raw material microbiological quality, innovative approaches such as the use of bioprotective strains or bacteriocins have been investigated for the control of *List. monocytogenes* contamination of fresh sausages [1]. Bio-protection can be seen as an alternative to reduce the use of chemical antimicrobials in food. The ideal biopreservation agent should exhibit specific antimicrobial activity against the target pathogenic or spoilage microorganism and it should not negatively affect the intestinal microbiome of consumers [2]. Another key feature that allows the use of bioprotective cultures in food products is their low organoleptic impact on food characteristics [3]. Based on these premises, the aim of this research was to assess the bio-protective potential of certain lactic acid bacteria (LAB) strains isolated from spontaneously fermented sausages.

II. MATERIALS AND METHODS

Within the project BioProMedFood financed in the frame of PRIMA Program, supported by the European Union, different types of spontaneously fermented sausages collected in four European countries were characterized and used as a source of isolation of autochthonous lactic acid bacteria (three from Italy, two from Slovenia, seven from Spain and three from Croatia). About 151 different biotypes (belonging mainly to the species *Latilactobacillus sakei*, *Latilactobacillus curvatus*, *Lactiplantibacillus paraplantarum* and *Pediococcus acidilactici*) were detected and evaluated for their safety and technological aspects. The most promising strains were selected and tested in model systems for their antimicrobial activity against *L. monocytogenes* Scott A belonging to the collection of the Department of Agricultural and Food Sciences (University of Bologna). Firstly, 14 LAB strains were individually inoculated (cell load approx. 6 log CFU/ml) in BHI medium (Oxoid, Basingstoke, UK) together with *L. monocytogenes* Scott A (cell load approx. 3 log CFU/ml). A control inoculated with the pathogen strain alone was also monitored. The data obtained from plate count at different sampling times were modelled through Gompertz equation as modified by Zwietering [4] with Statistica 8.0 software (StatSoft Inc., Tulsa, USA). Moreover, with the same software, data were analysed through a one-way ANOVA model, according to Tukey test ($P \leq 0.05$). The best performing LAB strains were used for a challenge test in laboratory scale-trials against *L. monocytogenes*, inoculated in a fresh sausage batter. In this case the challenge test was repeated twice testing the LAB at two different concentrations (6 or 8 log CFU/ml), to evaluate also their potential organoleptic impact, while the pathogen was always inoculated at a concentration of 3 log CFU/ml. The samples were incubated at 6°C (to simulate a slight thermal abuse) for 12 days and periodically analysed by plate counting on selective media, namely MRS (for LAB) and LSO (for *L. monocytogenes*), both provided by Oxoid (Basingstoke, UK). Each analysis was performed in triplicate.

III. RESULTS AND DISCUSSION

Concerning the trials in model systems, the final cell loads of *L. monocytogenes* in the presence of LAB were generally lower if compared to the control (7.5-8.0 *vs.* 8.7-8.9 log CFU/ml), but in some cases the growth rates were similar, with values ranging from 0.13-0.20 log CFU/ml*h. The lag phase duration was more variable, and ranginged from 4 to 10 h in relation to the tested LAB strains. Some strains were more effective in reducing *List. monocytogenes* growth kinetics: in particular, *P. acidilactici* SCT9 significantly reduced the growth rate, even if the final cell load was similar to the samples in which the other LAB were added. The most interesting results were obtained with *Ltp. paraplantarum* BPF2, that completely inhibited *L. monocytogenes* showing a bacteriostatic effect (no data modelling was possible), and with *P. acidilactici* ST6. In particular, this latter induced an initial decrease of *L. monocytogenes* cell culturability (reduction of cell load of about 2 log units), after which (about 34 h of lag phase) the pathogen was able to restart its grow, reaching a final cell load of about 4 log CFU/ml. Based on these results, these three LAB strains were selected to perform the challenge tests.
The results showed that the LAB strains, when inoculated at 6 log CFU/g, were not able to control the growth of the target pathogen: indeed, *L. monocytogenes* cell counts remained stable in the first 3 days of storage, and then constantly increased up to 6 log CFU/g, independently of the presence of bioprotective cultures. Conversely, when the LAB inoculum was higher, an initial decrease of *L. monocytogenes* was observed within 3 days of storage. Then, the pathogen restarted its growth, even if with slower kinetics and reaching final cell loads lower with respect to the control (Table 1).

Table 1 Cellular load of *Listeria monocytogenes* ScottA (log CFU/g) in fresh sausages during storage at 6°C, inoculated with LAB inoculum at 8 CFU/g. Standard deviation is also reported and for each sampling time different letters indicate significant differences ($P \leq 0.05$) according to ANOVA.

	T0	T1	T3	T6	T10	T13
Control	3.45 ± 0.24	3.32 ± 0.31^{a}	3.40 ± 0.28^{a}	4.39 ± 0.21^{a}	5.68 ± 0.19^{a}	6.57 ± 0.16^{a}
P. acidilactici ST6	3.45 ± 0.08	2.72 ± 0.16^{b}	1.10 ± 0.41^{b}	2.76 ± 0.22^{b}	4.42 ± 0.18^{b}	4.60 ± 0.14^{b}
Ltp. paraplantarum BPF2	3.45 ± 0.09	2.74 ± 0.14^{b}	1.54 ± 0.35^{b}	3.51 ± 0.21^{c}	3.17 ± 0.15^{c}	3.30 ± 0.19^{c}
P. acidilactici SCT9	3.45 ± 0.24	2.90 ± 0.31^{ab}	2.41 ± 0.25^{c}	3.87 ± 0.33^{c}	4.15 ± 0.21^{b}	5.08 ± 0.19^{d}

IV. CONCLUSION

The results demonstrated that the LAB strains tested in model systems reduced the growth performances of *L. monocytogenes* with different mechanisms. In the challenge test in real system, the LAB inoculum at 6 log CFU/g did not produce any inhibitory effect against this pathogen, while more promising results were obtained with a higher inoculum, which resulted in a reduction in the growth kinetics of the target pathogen and final values of 1.5-3 logarithmic cycles lower than the control. This research allowed to select some LAB strains as potential bioprotective cultures, endowed with low acidification potential organoleptic impact. This could be therefore a suitable strategy to improve the safety of fresh meat products.

REFERENCES

1. Woraprayote, W., Yuwares, M., Supaluk, S., Adisorn, S., Soottawat, B., & Wonnop, V. (2016). Bacteriocins from lactic acid bacteria and their applications in meat and meat products. Meat Science 120, 118-132.
2. Barcenilla, C., Miroslav, D., Lopez, M., Prieto, M., & Alvarez-Ordoñez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: A sistematic review. Meat Science, 183: 108661.
3. Pedonese, F., Torracca, B., Mancini, S., Pisano, S., & Turchi, B. D. (2020). Effect of a Lactobacillus sakei and Staphylococcus xylosus protective culture on Listeria monocytogenes growth and quality traits of Italian fresh sausage (salsiccia) stored at abusive temperature. Italian Journal of Animal Science, 19:1, 1363-1374.
4. Zwietering, M.H., Jongenburger, I., Rombouts, F.M., and van't Riet, K. (1990). Modeling of the bacterial growth curve. Applied and Environmental Microbiology, 56: 1875-1881.