Bio-preservation of pork carcasses/muscles tissues by lactic acid bacteria

S. Christieans & M. Rivollier*
ADIV, 10 rue Jacqueline Auriol, ZAC Parc Industriel des Gravanches, 63039 Clermont-Fd cedex 2 France
*E-mail : marina.rivollier@adiv.fr

Abstract

The study, described below, is included in TRUEFOOD program (Traditional United Europe Food) whose purpose is to improve quality and safety and introduce innovation into Traditional European Food production systems through research, demonstration, dissemination and training activities. Concerning the biopreservation, our work consisted in improving microbial safety of traditional food products of animal origin through the bio-preservation of pork muscles by lactic acid bacteria (LAB). The antagonistic effect of eight LAB strains was evaluated against *Listeria monocytogenes* and *Staphylococcus aureus* in pork muscles matrix. They were inoculated in standard meat or axenic meat in combination with four conditions of storage (under air or vacuum, at 3°C or 8°C). Results showed that four of them are potentially interesting to reduce pathogens: one *L. farciminis* and three *Lactobacillus sakei*. To allow a good inhibition of LAB, the optimal conditions -method and density of inoculation- were studied. Two methods were tested: soaking during 15, 30 and 60 seconds and spraying. From these experiments, it was concluded that a high inoculum level of LAB (10 $^{6/8}$cfu/cm²) allowed a better inhibition, and that the two methods of inoculation (spraying or soaking for 30s) were equivalent and efficient to inhibit pathogens.

Introduction

The aim of this study was to select antagonistic lactic acid bacteria (LAB) strains to inhibit pathogenic strains in order to bio-preserve raw pork meat. This work was declined in two main steps:

- 1: To evaluate the effectiveness of eight lactic acid bacteria (LAB) strains and to select the LAB strains with a significant antagonistic activity against pathogens.
- 2: To determine the optimum conditions of LAB strains inoculation (inoculum method and density) to inhibit *Listeria monocytogenes* and *Staphylococcus aureus* in a pork muscle matrix.

Material and methods

Selection of antagonistic LAB strains from raw pork muscles

Eight LAB strains (Table 1) were tested against *L.monocytogenes* and *S.aureus* in two pork muscles types: standard meat or axenic meat (to eliminate the influence of natural flora) and in combination with four conditions of storage (under air or vacuum, at 3°C or 8°C). A strain was considered as inhibitory when Δ log was higher than 0.5 log compared to the control (when the pathogenic strain was inoculated alone). The link with the acidification of meat was also evaluated.

Influence of antagonistic LAB strains, inoculums methods and density on the population of both LAB and pathogen strains during the pork meat storage

For a good inhibition of pathogens, the best implantation of lactic acid bacteria was evaluated. Two methods of meat inoculation were tested: 1) spraying on the surface of meat and 2) soaking of meat in a solution of lactic acid bacteria. Concerning the second method, three soaking times were tested: 15 seconds, 30 seconds and 1 minute.

The influence of the inoculation method was carried out on two LAB strains (*L. sakei*: IM8 and *L. farciminis*: Lf) selected due to their high antagonistic effect showed following the previous experiments. These LAB strains were tested at 3 different inoculum levels: 10^4, 10^6 and 10^8 cfu/cm^2 with 3 replicates.

Indeed, to evaluate the effect of the inoculum level and inoculation method of LAB on the behavior of *L. monocytogenes*, each LAB was tested in the presence of *L. monocytogenes* (B23) inoculated at one fixed level (2 log/cm²).

For each LAB strain (IM8 and Lf), the trials realized for studying these two parameters (method of inoculation and inoculum level) were:

"LAB strain alone", "*L. monocytogenes* B23 alone" and "LAB strain+ *L. monocytogenes* B23".

Table 1. Strains tested for their antagonistic activities

Strains	Code	Origin	Description	Source
Lactobacillus sakei	**IM8**	Dry fermented sausage	*In vitro* antagonistic activity	ADIV
Lactobacillus sakei	**DM2**	Dry fermented sausage	*In vitro* antagonistic activity	ADIV
Lactobacillus sakei	**DM3**	Dry fermented sausage	*In vitro* antagonistic activity	ADIV
Lactobacillus sakei	LB1	Dry fermented sausage	Acidifying substrate activity	ADIV
Lactobacillus sakei	*L. sakei* K23	Fresh Meat	Complete genome sequenced	INRA FLEC unit (Jouy en Josas)
Lactococcus lactis	LLO	Fish	Starter culture for fish biopreservation	Bioceane society
Pediococcus acidilactici R1001	Pa		**Food additive**	**Lallemand SA**
Lactobacillus farciminis R1127	**Lf**		**Starter culture**	**Lallemand SA**
Listeria monocytogenes	B23.1	Raw pork muscle	1/2a serotype	ADIV
Staphylococcus aureus		Reference strain	CIP65.8.807	Pasteur Institut

Results and Discussions

Selection of antagonistic LAB strains from raw pork muscles

According to feasible conditions for manufacturers, 3°C under air packaging, two *Lactobacillus sakei* strains (DM2 and DM3) caused pork meat samples acidification, and permitted to reduce *S. aureus* counts and growth.
At 3°C, *Lactobacillus farciminis* showed a significant inhibitory effect against *S. aureus* growth under vacuum conditioning or under air. This bacterial inhibition can be also connected with the pH decrease of meat during storage.
The effectiveness of antagonistic lactic acid bacteria *Lactobacillus sakei* IM8 strain against *L. monocytogenes* was observed in two cases:

- In standard pork muscles, in vacuum packaged at 3°C.
- In axenic pork muscle, in vacuum packaged at 8°C.

During storage at 8°C, *Lactobacillus farciminis* showed a significant inhibiting activity against *L. monocytogenes*.
Thus, four strains are potentially interesting: one *Lactobacillus farciminis* and three *Lactobacillus sakei* (DM2, DM3, and IM8).

Influence of antagonistic LAB strains, inoculums methods and density on the population of both LAB and pathogen strains during the pork meat storage

A high inoculum level of specific LAB strains ($10^{6\text{-}8}$cfu/cm²) allowed a better inhibition against *L. monocytogenes* B23 since the first day of storage as compared with low level (10^{4}cfu/cm²).
The two methods of inoculation tested (spraying and soaking 30s) were equivalent to inhibit *Listeria monocytogenes* B23.

Conclusions

Taking account of the results concerning the eight LAB strains tested, four strains are potentially interesting: one *Lactobacillus farciminis* and three *Lactobacillus sakei* (DM2, DM3, and IM8).

The optimum conditions of LAB strains inoculation (inoculum method and density) to inhibit *Listeria monocytogenes* and *Staphylococcus aureus* in a pork muscle matrix were:

- A high inoculum level of specific LAB (10^{6-8}cfu/cm²) allowed a better inhibition against *L. monocytogenes* B23 since the first day of storage as compared with low level (10^{4}cfu/cm²).
- The two methods of inoculation tested (spraying and soaking 30s) were equivalent to inhibit *L.monocytogenes* B23.
- The two LAB tested (*Lactobacillus sakei* IM8 and *Lactobacillus farciminis* Lf) showed a significant reduction of *L. monocytogenes* B23.

A next step will permit to verify if biopreservation through LAB strains selected is valid in dry sausages process. If so, a clarification will be done concerning the parameters of biopreservation (strain, method of inoculation, density ...) in SMEs (small to medium size enterprises).

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