Selecting raw goat milk lactic acid bacteria for sustainable biopreservation?

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INTRODUCTION

In raw milk manufacturing, microbial contamination by spoilage microorganisms could lead to great economic consequences. *Pseudomonas sp.* is one of the most spoilage microorganism causing defects on dairy products. It is a psychrotrophic bacterium that can induce yellow to brown coloration of the rind associated with bad ripening flora development and taste defects.

Objectives

A way to limit *Pseudomonas* proliferation in cheese manufacturing is the use of milk microbial ecosystem.

This project aims to better understand the interactions between interesting lactic acid bacteria (LAB) and *Pseudomonas* to evaluate the capacity of LAB to limit the proliferation of *Pseudomonas* in cheese processing. The interaction between lactic acid bacteria and *Pseudomonas* were studied in planktonic and in biofilm.

MATERIAL & METHODS

1. LAB screening on their ability to inhibit Pseudomonas

150 strains of LAB of different genus (*Lactococcus*, *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Leuconostoc*) were screened on their ability to inhibit a strain of *P. fluorescens* and a strain of *P. koorensis* provided by Vetagro Sup.

a) Cell-cell contact (Fig 1)

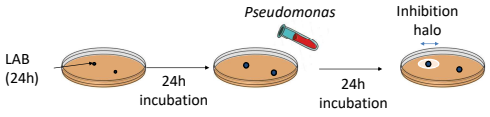


Fig. 1: Spot Test Agar (Grimoud et al., 2010)

b) Supernatant-cell contact (Fig 2)

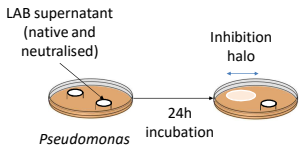


Fig. 2: Well Diffusion Agar (Casla et al., 1996)

c) Culture of *Pseudomonas* in presence of LAB supernatant (Fig 3)

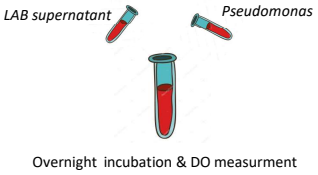


Fig. 3: Inhibition test of supernatant in liquid medium

2. Interactions in biofilm

Dual-species biofilms (co-inoculation of both LAB and *Pseudomonas* and delayed inoculation of *Pseudomonas* on a preformed LAB biofilm)

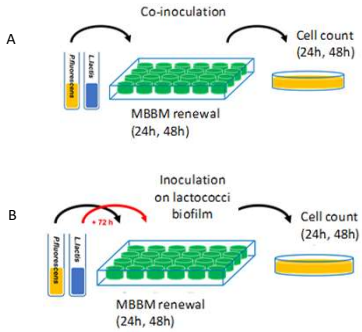


Fig. 4: Dual biofilm: co-inoculation (A) and delayed inoculation (B) (Pecastaings et al., 2010)

RESULTS

- 134/150 LAB strains (121/150 strains) presented inhibitions toward *P. fluorescens* 3.8 (resp. toward *P. koreensis* 15.5)

- No inhibition was observed after contact of native (acidic) and neutralised supernatants of LAB with *P. fluorescens and P. koreensis*

→ Problem of LAB supernatant diffusion in the solid medium?

- After contact of some LAB supernatants with *Pseudomonas* in liquid medium, some inhibitions were observed with native acidic (Fig 5, blue) and neutralised (Fig 5, green) LAB supernatants

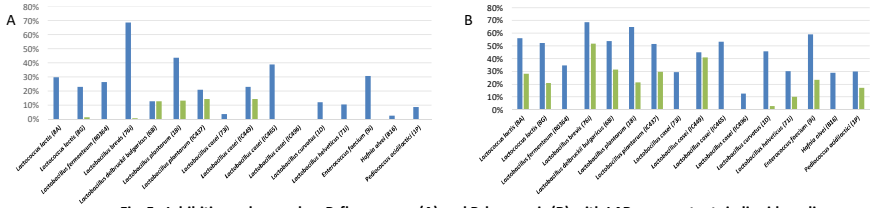
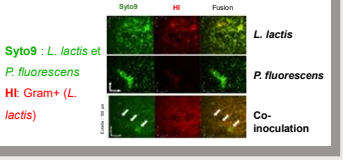


Fig. 5 : Inhibitions observed on P. fluorescens (A) and P. koreensis (B) with LAB supernatants in liquid medium

- LAB strains and *P. fluorescens* coexist in biofilm after co-inoculation and after inoculation of *Pseudomonas* on a pre-formed LAB biofilm (Fig 6).

Fig. 6 : Confocal microscopy observation of biofilms formed after co-inoculation of *P. fluorescens* and *L. lactis*

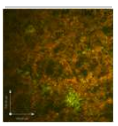


Syto9 : *L. lactis* et *P. fluorescens*
HI: Gram+ (*L. lactis*)

CONCLUSION

Some LAB strains are able to inhibit Pseudomonas mostly by cell contact

Some supernatants (native or neutralised) are active against Pseudomonas (Bacteriocines? Organic acids?)



Pseudomonas and LAB coexist in biofilm

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