Interaction between lactic acid bacteria and two species of the *Penicillium roqueforti* group: an *in vitro* and *in vivo* approach

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INTRODUCTION

P. roqueforti s.s. and *P. paneum* are the main toxigenic mould species contaminating silages in temperate climates. They are well adapted to silage conditions, i.e. low O_2 levels, and high levels of CO_2, lactic acid and acetic acid. Interaction between HoLAB and HeLAB on the one hand, and *P. roqueforti s.s.* and *P. paneum* on the other hand was studied *in vitro* in agar medium and *in vivo* using microsilos.

IN VITRO EXPERIMENT

Corn Infusion Agar (CIA) was prepared according to Niderkorn (2007). HoLAB or HeLAB (resp. 1188 and 11A44 from Pioneer Hi-Bred) were incorporated into the CIA at 1.10^6 cfu/ml, and allowed to proliferate anaerobically during seven days at 20°C. Centrally in the medium, 20 µl of conidiospore suspension of *P. roqueforti s.s.* MUCL46746 or *P. paneum* CBS112295 was inserted (5.10^5 spores/ml), prior to the anaerobic incubation or afterwards (n=4). Mould growth was facilitated by aerobic incubation at 20°C for seven days and registered. Roquefortine C (ROC) was quantified on three pooled nine-mm agar plugs; pH, lactic acid and acetic acid were determined on the remaining agar.

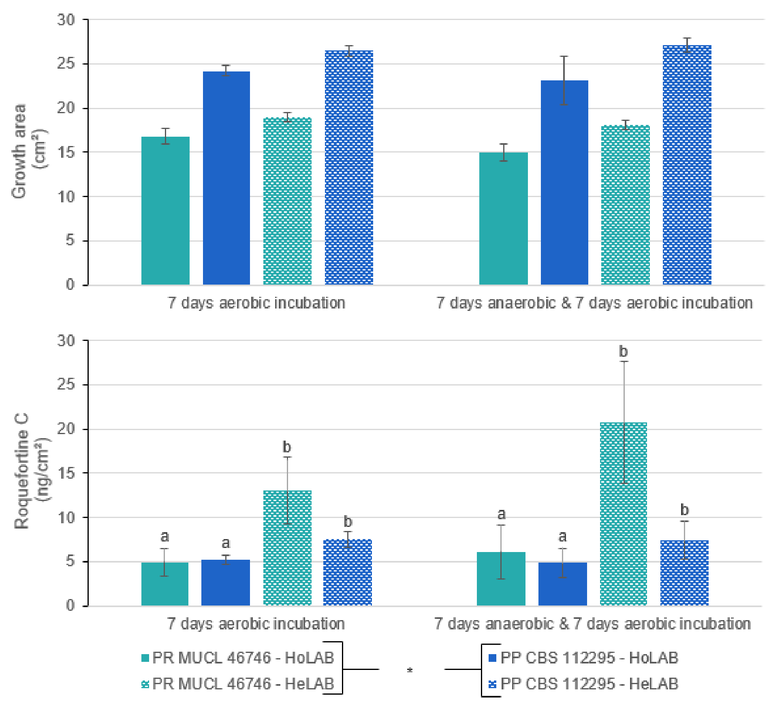

Figure 1. Growth of and roquefortine C (ROC) production by *P. roqueforti s.s.* (PR) MUCL46746 and *P. paneum* (PP) CBS112295.
Mean values per treatment, with error bars representing the standard deviation. No significant interactions between mould, additive and incubation regime were found. Growth was not significantly influenced by additive or incubation regime. ROC production was not significantly influenced by incubation regime, while the effect of additive is indicated by lettercode. For both parameters a significant difference between PR and PP was found, indicated by an asterisk symbol.

P. roqueforti s.s. MUCL46746 exhibited significantly stronger growth than *P. paneum* CBS112295, associated with a higher ROC production.
HeLAB inoculation resulted in increased ROC levels compared to HoLAB.

Both inoculants displayed a different acid production pattern. Compared to non-infected medium, mould growth triggered a reduction of both lactic acid and acetic acid in the medium, demonstrating metabolization.

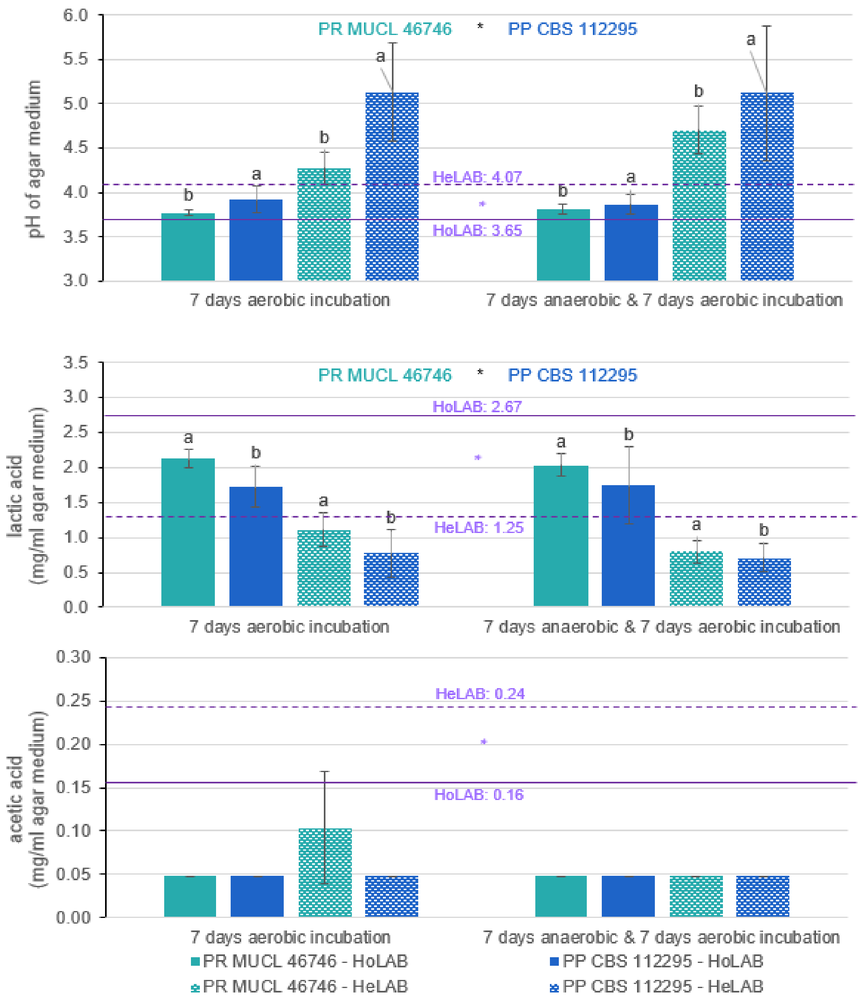

Figure 2. Agar medium: pH, lactic acid content and acetic acid.
Mean values per treatment, with error bars representing the standard deviation. No significant interactions between factors were detected. Incubation regime did not influence any parameter significantly, whereas a significant effect of mould species and additive was detected on pH and lactic acid. Signficant differences between *P. roqueforti s.s.* (PR) MUCL46746 and *P. paneum* (PP) CBS112295 are indicated by black asterisk symbols, while the effect of additive is indicated by lettercode. The parameters observed in HoLAB or HeLAB inoculated CIA (without PR or PP addition) are indicated in purple, with purple asterisk symbols indicating a significant difference between HoLAB and HeLAB inoculation.

IN VIVO EXPERIMENT

A 2.75-liter microsilo experiment was conducted with a mixture of perennial ryegrass and white clover, ensiled at 42% dry matter (DM). The following treatments were compared (n=4):

1) no additive (treated with plain water)
2-4) *P. roqueforti s.s.* MUCL 46746 (PR) infection: no additive – HoLAB – HeLAB
5-7) *P. paneum* CBS112295 (PP) infection: no additive – HoLAB – HeLAB

Mean silo density was 186 kg DM/m³. At opening after 56 days, the DM content, pH and the aerobic stability (3°C temperature rise) were determined, as well as the number of *P. roqueforti* propagules per gram fresh matter (FM).

Table 1. *In vivo* experiment: *P. roqueforti* numbers, dry matter content, pH and aerobic stability.
A significant interaction between infection and additive was only observed for pH. *P. roqueforti* numbers were significantly influenced by additive, while both additive and infection had a significant effect on the dry matter (DM) content at silo opening. Aerobic stability was not influenced by infection nor by additive.

TREATMENTS		*P. roqueforti*		DM at desiling			pH			aerobic stability
Infection	Additive	(log cfu/g FM)	*add.*	(g/kg FM)	*inf.*	*add.*		*inf.**	*add.**	(hours)
negative control	no additive	0.00 (0.00)	*a*	431 (2)	*a*	*a*	4.83 (0.05)	a	-	144 (44)
P. roqueforti s.s. MUCL 46746	no additive	2.08 (0.21)	*a*	413 (1)		*a*	4.84 (0.04)	a	a	128 (58)
	HoLAB	0.22 (0.43)	*b*	403 (3)	*b*	*b*	3.96 (0.02)	a	b	> 175**
	HeLAB	1.43 (0.30)	*ab*	389 (4)		*c*	4.62 (0.01)	a	c	> 175**
P. paneum CBS 112295	no additive	2.40 (0.19)	*a*	397 (3)		*a*	4.62 (0.07)	b	a	> 175**
	HoLAB	0.81 (0.94)	*b*	389 (8)	*c*	*b*	3.98 (0.01)	a	b	> 175**
	HeLAB	1.95 (0.23)	*ab*	378 (3)		*c*	4.58 (0.02)	b	a	> 175**

* Interaction between the factors infection and additive
** No temperature increase of 3°C above ambient temperature observed within 175 hours

HoLAB inoculation significantly reduced *P. roqueforti* numbers compared to the no additive application. HoLAB and HeLAB both significantly reduced the pH of PR-infected silage compared to no additive, with the lowest pH observed for HoLAB. In PP-infected silage, HoLAB significantly reduced the pH compared to no additive and HeLAB. Both inoculants significantly increased the DM content, while the aerobic stability was not significantly influenced.

CONCLUSION

P. roqueforti s.s. and *P. paneum* are well adapted to silage conditions, attributable to their ability to metabolize lactic acid as well as acetic acid. However, HoLAB inoculation significantly reduced *P. roqueforti* numbers *in vivo* compared to no additive application, while HeLAB inoculation resulted in intermediate *P. roqueforti* numbers.

Niderkorn V. (2007) Activités de biotransformation et de sequestration des fusariotoxines chez les bactéries fermentaires pour la detoxification des ensilage de maïs. PhD thesis, Université Blaise Pascal. *In French.*