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Comparative study of the ecological niche of *Penicillum expansum* Link., *Botrytis cinerea* Pers. and their antagonistic yeasts *Candida oleophila* strain O and *Pichia anomala strain K*

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Abstract: The protective level of both yeast strains *Pichia anomala* and *Candida oleophila* against *P. expansum* and *B. cinerea* was previously positively correlated with a yeast density superior or equal 10^4 CFU/cm^2 of fruit surface in practical conditions. Trials in laboratory conditions confirmed this observation and highlighted that the protective level might depend on the humidity level on fruit surface. A study of the ecological factors (water activity and temperature) susceptible to influence the yeast density on fruit surface was undertaken. *In vitro*, both yeast strains had a similar ecological niche as compared with that of both wound pathogens of apple. Nevertheless at low water activities and low temperatures, the lag time to start antagonistic yeast growth was higher than that observed for wounds pathogens. The *in vivo* study allowed designing two predicting growth models for describing the yeast density on apple fruit surface according to the relative humidity, incubation temperature and the initial concentration of yeast application. A weak yeast density was observed at low relative humidity (75%). The effect of humidity appeared to be more important than that of incubation temperature. For both pathogens, the *in vivo* study revealed only significant effect of incubation temperature on diameter lesion. The comparison between the *in vitro* and *in vivo* trials underlined that yeasts followed the same growth tendency. In contrast, the growth of pathogens was limited at relative humidity close to saturation (98%). All results suggest the importance to maintain the storage room at saturate relative humidity in order to reduce the losses dues to blue and grey moulds.

Keywords: apples, efficacy, environmental factors, modelling, post-harvest

Introduction

Candida oleophila strain O and *Pichia anomala* strain K are two antagonistic yeasts isolated from Golden Delicious for their biocontrol activity against post-harvest fungal diseases of apple fruits (Jijakli et al., 1993). Several studies were undertaken to determine the mechanisms of action of both antagonistic yeasts (Jijakli & Lepoivre, 1998; Friel et al., 2004). However, little attention was given to the influence of environmental factors like temperature, water activity and relative humidity on *in vitro* and *in vivo* growth of the antagonistic yeast and their pathogenic target. Such study is crucial to determine optimal and detrimental conditions for fungal infection and to assess the ecological fitness of antagonists. Data obtained will influence the bio-pesticide formulation, in order to favour the antagonistic agent. Lahlali et al. (2005) reported that the *in vitro* growth of *P. expansum* was significantly affected by water activity of medium and temperature. They underlined that the effect of water activity was more important than that of temperature.

The present work focused on two major objectives. On the one hand, the influence of water activity (a_w) and temperature was evaluated on the *in vitro* growth rate of both antagonistic strains and both pathogens. On the other hand, similar experimentations were

carried out *in situ* and the establishment of yeast population density on apple fruit surface was evaluated according to the temperature and relative humidity.

Material and methods

Antagonistic strains

P. anomala strain K and *C. oleophila* strain O were cultured at 25°C for three successive generations on Potato Dextrose Agar (PDA) medium with an interval of 24 hours. The final concentrations of both yeasts were adjusted according to D.O. measurement as previously described (Jijakli & Lepoivre, 1998; Jijakli, 1996).

P. expansum strain *vs2* and *B. cinerea* strain V were isolated from decayed apple fruits by the Plant Pathology Unit (Gembloux Agricultural University, Belgium) and stored onto PDA medium at 4°C in darkroom. The conidial suspension was prepared from 9±2 day-old cultures of pathogen cultivated on PDA medium by scraping the surface of the colonies recovered with Tween 20 (0.05%). Spores concentrations were counted with a Bürker's cell and these concentrations were adjusted only with sterile distilled water.

In vitro effect of water activity and temperature on growth rates of post-harvest pathogens of apple fruits and their antagonistic yeasts

The basic medium used was Potato Dextrose Agar (PDA, a_w 0.995) with an a_w of 0.995. The a_w was modified by adding increasing amounts of glycerol, sorbitol, glucose or NaCl (Lahlali et al., 2006) to obtain a_w levels of 0.980, 0.960, 0.930, 0.910 and 0.890 at 5, 15 and 25°C. The a_w of all media was measured with an AquaLab series 3 instrument (Decagon, 950 NE Nelson Court Pullman, Washington 99163). Prepared Petri dishes were inoculated with pathogens (10 µl of a 1×10^6 spores/ml suspensions were deposited in the centre) or antagonist strains (100 µl of 1×10^4 CFU/ml suspensions were plated). Each experiment was carried out in triplicate for each solute-a_w-temperature combination.

The evaluated parameters were the radial mycelium growth and the CFU/ml respectively for pathogens and antagonistic strains (Lahlali et al, 2005, 2006). The time required for growth (lag phase) was also recorded in each experiment for each treatment. A second-order polynomial equation was used to fit the square root of the radial growth rate and the logarithm of CFU/ml respectively for pathogens and antagonistic yeasts whatever the solute (Lahlali, 2006).

In vivo effect of relative humidity and temperature on lesion diameters induced by post-harvest pathogens of apple fruits and yeast density

The different values of relative humidity (75, 86.5 and 98%) inside desiccators were controlled using the saturated salt solutions with respect to studied temperatures (5, 15 and 25°C) (Lahlali, 2006; Xu et al., 2001).

Apple fruits Golden Delicious were disinfected by soaking during two minutes in sodium hypochlorite solution (10%) then rinsed twice in sterile water. For both pathogens, disinfected fruits were wounded in two sites at their equatorial zone with a depth of 4 mm and 1-2 mm in diameter. Each wound was inoculated with a suspension of 10 µl of *P. expansum* and *B. cinerea* at 1×10^6 conidia/ml. The inoculated fruits were placed in desiccators in various environmental conditions according to 3^2 full factorial design with three replicates. After 30 days of incubation, the diameter lesion was measured (Lahlali, 2006).

For both antagonistic yeasts, the disinfected apples fruits were subjected to the Box-Benkhen combination with three replicates and three central points. The initial concentration of yeast application (1×10^4, 5×10^7 and 1×10^8 CFU/ml), the relative humidity (75, 86.5 and 98%) and the temperature (5, 15 and 25°C) were evaluated. After 48 hours of incubation, the

recovery of yeast cell on intact fruit surface was performed as previously described (Lahlali, 2006).

Results and discussion

In vitro ***effect of water activity and temperature on growth rate of post-harvest patogens of apple fruits and their antagonistic yeasts***

The growth of pathogens and antagonistic strains was significantly influenced by the water activity of medium. All strains showed a growth rate increase with increasing a_w of medium (Lahlali, 2006).

The results showed that *P. expansum*, *B. cinerea*, *C. oleophila* strain O and *P. anomala* strain K had a similar ecological niche. The only difference was observed from *B. cinerea* which was the sole micro-organism unable to grow at a_w of 0.89. Nevertheless at low water activities and low temperatures, the lag time to start antagonistic yeast growth was higher than that observed for wound pathogens (Table 1). These results support the necessity to apply the antagonists as soon as possible after harvest. This application could be considered as an adequate way for pre-colonizing wounded fruits before the arrival of conidial pathogens.

Table 1. Comparative of a_w minimal for growth and the lag time before growth of wounds pathogens and their antagonistic yeasts

	Minimal a_w value for growth	Lag time at low a_w and temperature
P. expansum	0.91-0.93 (NaCl) or ≤ 0.89 (non-ionic solutes)	++
B. cinerea	0.93 (NaCl) or > 0.89 (non-ionic solutes)	+
P. anomala souche K	0.93 (NaCl) or≤ 0.89 (non-ionic solutes)	-
C. oleophila souche O	0.93 (NaCl) or ≤ 0.89 (non-ionic solutes)	-

Non-ionic solutes (sorbitol, glycerol and glucose), +: short, -: long

These *in vitro* results are in agreement with previous findings describing the growth of both yeast strains on apple fruits in three scenarios reflecting the practical conditions (Lahlali and Jijakli, 2004). Yeast population recovered from apple wounds was highest when wounds were performed on wet apples, as compared to wounds performed on dry apple surface. Therefore, the humidity could be considered as a factor limiting the growth of yeasts, especially in pre-harvest conditions.

In vivo ***effect of relative humidity and temperature on lesion diameters induced by post-harvest pathogens of apple fruits and yeast density***

The *in vivo* results showed a lower yeast density on apple fruits surface when the antagonistic yeasts were applied at a concentration of 10^4 CFU/ml for a relative humidity value rTanging from 75 to 86.5%, and at temperature situated between 5 and 15°C. The table 2 summarizes the regression coefficients, estimated by multiple regressions analysis. For each antagonistic strain, the variation of population density was mainly explained by the quadratic equation model (93.6% for strain O and 95.8% for strain K). The resulting variation (6.4% for strain O and 4.2% for strain K) remained unexplained by the model equation (Box & Draper, 1987). Whatever the strain, the initial concentration of application had a major and significant effect on the yeast density on apple fruit surface followed respectively by the relative humidity and the temperature.

Table 2. Coefficients significance of Box-Behnken (1960) experimental design adopted for estimating the population density of strain O or strain K on apple fruit surface, obtained after multiple regression analysis

		Antagonistic yeast	
		Strain O	Strain K
Response mean	β_0	3.57***	3.90***
T	β_1	0.65***	0.87*
RH	β_2	0.72***	1.02***
Con	β_3	1.23***	1.58***
T^2	β_{11}	-0.046^{ns}	-0.072^{ns}
RH^2	β_{22}	0.18^{ns}	-0.092^{ns}
Con^2	β_{33}	-0.92***	- 1.13**
T×RH	β_{12}	0.31***	- 0.02***
T×Con	β_{13}	-0.11^{ns}	-0.23^{ns}
RH×Con	β_{23}	-0.20*	- 0.30**

T: temperature; RH: relative humidity and Con: initial concentration of application. *** Highly significant; * Significant.

Focusing on both pathogens, it appeared that incubation temperature had a significant impact on the development of lesion diameter. In opposite, there was no significant effect of relative humidity. The part of variation explained by both studied factors is 52 and 55% respectively for *P. expansum* and *B. cinerea*. The highest lesion diameters were observed at relative humidity of 86.5%.

Table 3. coefficients significance of the 3^2 full factorial adopted to study the combined effect of relative humidity and incubation temperature on the lesion diameter of *P. expansum* and *B. cinerea*.

		Pathogenic fungi	
		P. expansum	*B. cinerea*
Response mean	β_0	34.66*	27.23*
T	β_1	3.96*	3.28*
HR	β_2	1.06^{ns}	-0.51^{ns}
T^2	β_{11}	-0.30^{ns}	-4.27^{ns}
HR^2	β_{22}	-7.79*	-11.08*
T x HR	β_{12}	2.71^{ns}	0.56^{ns}

* Significant

Both antagonistic yeasts presented similar behaviour during *in vitro* and *in situ* experimentations. In the opposite, the growth of pathogens was highly influenced by water activity during *in vitro* experimentations and by temperature during *in vivo* conditions. This result can indicate that the relative humidity of the wound inside fruits is sufficient to support the growth of both fungi, suggesting that after infection, the progress of the decay will not be limited by the variation of relative humidity. The *in vivo* growth of pathogens was also reduced at relative humidity close to saturation (98%) as compared to relative humidity

ranging from 80 to 92%. All results support the importance to maintain the storage room at saturate relative humidity in order to reduce the losses dues to blue and green moulds and to create the optimal conditions of antagonistic yeasts proliferation.

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References

Box, G.E.P. & Draper, N.R. 1987: Empirical Model Building and Response Surfaces. New York, USA: John Wiley: 688 pp.

Jijakli, M.H. 1996: Etude des propriétés antagonistes de deux souches de levures vis-à-vis de *Botrytis cinerea* Pers. Sur pommes en conservation. Ph.D thesis. Fac. Uni. Sci. Agro. Gembloux (Belgium): 173 pp.

Jijakli, M.H. & Lepoivre, P. 1998: Characterization of an exo-ß-1,3-glucanase produced by *Pichia anomala* strain K, antagonist of *Botrytis cinerea* on apples. Phytopathology 88: 335-345.

Jijakli, M.H., Lepoivre, P., Tossut, P. & Thonard, P. 1993: Biological control of *Botrytis cinerea* and *Penicillium sp.* on postharvest apples by two antagonistic yeasts. Med. Landbouw. Uni. Gent. 58/3b:1349-1358.

Lahlali, R. 2006: Etude et modélisation du comportement écologique de *Botrytis cinerea* Pers., *Penicillium expansum* Link. Et de deux levures antagonistes *Candida oleophila* (souche O) et *Pichia anomala* (souche K) vis-à-vis de ces pathogènes des pommes en post-récolte. Ph.D thesis. Fac. Uni. Sci. Agro. Gembloux (Belgium): 155 pp.

Lahlali, R., Friel, D. & Jijakli, M.H. 2006: Effect of incubation temperature and relative humidity on lesion diameter of *Botrytis cinerea* Pers. and *Penicillium expansum* Link. Commun. Agric. Appl. Biol. Sci., Ghent University 71/3b:1159-1166.

Lahlali, R., Serrhini, M.N., Friel, D. & Jijakli, M.H. 2006: *In vitro* effects of water activity, temperature and solutes on the growth rate of *P. italicum* Whmer and *P. digitatum* Sacc. J. Appl. Microbiol. 101: 628-636.

Lahlali, R. & Jijakli, M.H. 2004: Evaluation of population density of *Pichia anomala* strain K and *Candida oleophila* strain O and their protection against *Penicillium expansum* Link on apples. IOBC/WPRS Bull. 27 (8): 340-346.

Lahlali, R., Serrhini, M.N. & Jijakli, M.H. 2005: Studying and modelling the combined effect of temperature and water activity on the growth rate of *P. expansum*. Int. J. Food Microbiol.103: 315-322.

Xu, X.M., Guerin, L. & Robinson, J.D. 2001: Effects of temperature and relative humidity on conidial germination and viability, colonization and sporulation of *Monilia fructigena*. Plant Pathol. 50: 561-568.