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BIOLOGICAL CONTROL OF POSTHARVEST BOTRYTIS CINEREA AND PENICILLIUM ON APPLES

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Abstract

Seven microbial strains were selected for their protective activity against *B. cinerea* and *Penicillium* sp. among 329 epiphytic microorganisms isolated from apple fruit surface. Ratio between inoculum concentration of antagonists and pathogens and time between application of the protective yeast and inoculation of the pathogens appeared to be important factors in controlling the level of protection. The application of 10^8 cfu/ml of the most efficient strains K (*Pichia anomala*) or O (*Candida sake*) is required to inhibit completely the lesions of *B. cinerea* inoculated with spore concentrations of 10^5 conidia/ml . Population of K and O in wounds increased at 25°C to reach a maximum after 12h of incubation and a relationship appeared between population size of the biocontrol agent and the level of protection. *In situ* spore germination of *B. cinerea* was markedly reduced on K or O-treated-wounded sites even when pathogen and yeast were applied simultaneously, thus suggesting that an other factor than inhibition of germination may interact in biocontrol effectiveness.

Introduction

The interest for alternative approaches to control diseases appeared since a few years. Progress has been made particularly in the biocontrol of postharvest diseases of fruits (JANISIEWICZ, 1991). The present work is dealing with two postharvest diseases of apples : gray mold caused by *Botrytis cinerea* Pers & Fr. and blue mold caused by *Penicillium* sp. LK. & Thom. Seven strains of yeasts were selected for their protective activity at 5 and 25°C among 329 epiphytic microorganisms (yeast and bacteria) isolated from apple fruit surface (cv Golden Delicious and Jonagold) when applied to wounded apples inoculated 24h later with 50 µl of 10^6 spores/ml of both pathogens (unpublished results).

A more precise screening of antagonistic strains and identifiçation of the major parameters controlling the level of protection constitued a prerequisite in order to approach the mechanism of action of protective yeasts. The second objective of the present study was to determine the effect of biocontrol agents (BCA) on conidial germination of *B. cinerea* with a *in situ* method.

Material and methods

Surface sterilised-Golden Delicious apples were inoculated by depositing 50 µl of *B. cinerea* or *Penicillium* sp. spore

suspension on wound sites (2 wounds of 6 mm diameter and 3 mm deep at the equator per apple) previously treated with 50 µl of yeast suspension (in sterile 0.1% pepton water). Inoculated fruits were incubated on wet filter paper in closed plastic boxes at 25°c and symptom intensity (as measured by the diameter of the lesion) was evaluated 5 days after the inoculation
The protective effect of BCA was studied in relation to the time between antagonist application and pathogen inoculation as also the concentration of yeast cell and pathogen spore suspension.

The ability of the antagonist to colonize the inoculation site was investigated by inoculating wounded fruits (one wound 20 mm in diameter 1 mm deep per appel) with 50 µl of antagonist suspension (10^8 cfu/ml). After increasing incubation times (0 to 72h) wound sites were blended and dilution-plated in triplicate onto PDA for assay of colony formings units (cfu). Parallel with population studies, the level of protective activity against *B. cinerea* was measured with the same experimental protocol.

In order to determine the effect of BCA on conidial germination of *B. cinerea* on apple, inoculum of the pathogen was applied on a transparent menbrane filter (0.2 µm pore diameter) put in the antagonist-pretreated wound sites. 24h after inoculation the percentage of germination of *B. cinerea* was determined under light microscopy.

All experiments were repeated at least once. Factorial analyses of variance for the effect of incubation time and inoculum concentration were performed with models procedure of SAS.

Results and discussion

1. Effect of duration between treatment of antagonistic yeast and inoculation of pathogen and the effect of the yeast cell and pathogen spore concentration on the biocontrol efficacy.

Protective activity of yeasts against *B. cinerea* and *Penicillium* sp. increased with the incubation time between the application of the antagonist and inoculation of pathogen (table 1). The most efficient strains (K and O) reduced significantly the diameter of decay lesion even when inoculation of the pathogen and application of the yeast were performed simultaneously. Application of strains K (*Pichia anomala*) and O (*Candida sake*) gave rise to 90-100% of inhibition of lesion of both pathogen inoculated 48h later (table 1).
There was a quantitative relationship between spore concentration of the pathogens and the amount of antagonist needed for disease control (tables 2). The application of 10^8 cfu/ml of strain K is required to inhibit completely the lesions of *B. cinerea* caused by spore concentration of 10^6 conidia/ml whereas 10^8 cfu/ml of strain O protected fruits inoculated with 10^5 spores of *B. cinerea*/ml.

Table 1: Lesion development (mm) on wounded Golden Delicious apples treated with 50 µl of antagonistic yeast suspension (about 107 CFU/ml) and inoculated with 50 µl of pathogen suspension (106 spores/ml) after different incubation times of the antagonist

	Botrytis cinerea				*Penicillium* sp.			
Incubation time	0 h	12 h	24 h	48 h	0 h	12 h	24 h	48 h
2.13[b]	21,6[a]	9,4[d]	6,9[d]	0,5	20,2	15,6[d]	6,9[d]	5,4[d]
1.58	24,9	12,2[d]	11,2[d]	9,1	16,4[d]	15,6[d]	14,2	9,0
9C5	24,7	19,5	7,0[d]	3,1[d]	20,2	22,6	19,1	10,4
5F2	27,0	13,9	7,1[d]	19,7[d]	18,5	16,7[d]	15,5	4,1[d]
K	10,7[d]	8,6[d]	0,0[d]	0,1[d]	10,6[d]	12,2[d]	3,7[d]	0,0[d]
O	15,2[d]	5,7[d]	4,7[d]	3,0[d]	19,2	18,1	3,1[d]	2,0[d]
9A4	22,0	19,5	14,1[d]	5,4[d]	25,4	22,6	19,2	14,2
control[c]	33,8	25,1	31,9	29,4	23,6	23,4	22,6	19,0

a: Data represent average lesion diameter (mm) measured 5 days after pathogen inoculation.
b: Antagonistic strains.
c: Apples inoculated only with the pathogen.
d: Mean of the antagonist-treated apples is significantly different (p = 0,001) to the control mean according to Dunnett's procedure.
Remark: Data shown for 1 of the 2 trials.

2. Effect of population densities of strains K and O on conidial germination and on level of protective activity against *B. cinerea*.

Population of K and O in wounds increased at 25°C to reach a maximum after 12h of incubation (fig. 1). 72h after their application, the population level was approximately 1 log unit over the initial population level. A close relationship appeared between population of the biocontrol agent and the level of protection. The percentages of reduction of *B. cinerea* inoculated 0, 4, 8, 12, 24 and 48h after the antagonist application were respectively 21.4, 27.2, 38.7, 72.5, 97.3 and 98.1 % on K-treated fruits and 30.9, 35.3, 44.8, 81.8, 98.3 and 100.0 on O-treated fruit.
Spore germination of *B. cinerea* was reduced on K or O-treated-wound sites. After 0, 4, 8, 12, 24 h of incubation of yeasts, spore germinations of *B. cinerea* on membrane filters were 14, 19, 14, 10, 6 % respectively on K-treated apple and 20, 15, 15, 12 and 9 % in case of O application. The conidial germination of control (untreated fruit), after the same incubation times as above described were 58, 73, 83, 60, 45 %.

These overall results suggest that protection effect of yeast was closely related to the colonization. *In situ* spore germination of B. cinerea was markedly inhibited even when pathogen and yeast were applied simultaneously with no subsequent protection, thus suggesting that other factor(s) than inhibition of germination may also interact in biocontrol effectiveness. Additional studies are needed to confirm the *in situ* spore germination and to determine if antifungal metabolites are produced by K or O and/or apple cells in the wound site.

TABLE 2: Lesion development (mm) on wounded Golden Delicious apples inoculated with various spores concentrations of *B. cinerea* or *Penicillium* sp. 24 h after treatment of different concentrations of *Pichia anomala* or *Candida sake*.

	B. cinerea spores concentration (spores/ml)				*Penicillium* sp. spores concentration (spores/ml)			
Yeast concentration (CFU/ml)	10^7	10^6	10^5	10^4	10^7	10^6	10^5	10^4
Pichia anomala								
10^8	3,5[ad]	0,0[d]	0,0[d]	0,0[d]	4,6[d]	0,0[d]	1,1[d]	0,0[d]
10^7	4,7[d]	0,7[d]	1,4[d]	1,6[d]	9,4[d]	2,6[d]	0,0[d]	3,7[d]
10^6	17,0	5,2[d]	10,2[d]	0,0[d]	17,2	10,0	14,1	20,2
10^5	24,4	11,2[d]	5,9[d]	5,0[d]	18,1	12,9[d]	15,4	4,9[d]
control[b]	24,5	28,1	25,0	22,1	21,0	21,4	21,6	19,1
Candida sake								
10^8	9,5[ad]	2,9[d]	0,0[d]	0,0[d]	4,7[d]	6,2[d]	8,5[d]	4,6[d]
10^7	14,2	2,6[d]	3,4[d]	0,0[d]	15,5	4,0[d]	2,1[d]	0,0[d]
10^6	17,1	10,6[d]	9,1[d]	6,7[d]	11,0	15,2	10,1[d]	4,7[d]
10^5	13,2	7,9[d]	2,6[d]	0,9[d]	14,9	16,1	8,4[d]	9,2[d]
control[b]	24,5	28,1	25,6	22,1	21,0	21,4	21,6	19,1

a: Data represent average lesion diameter (mm) measured 5 days after pathogen inoculation.
b: Apples inoculated only with pathogen.
c: Mean of the antagonist-treated apples is significantly different to the control mean according to Dunnett's procedures (P = 0,001)
Remark: Data shown for 1 of the 2 trials

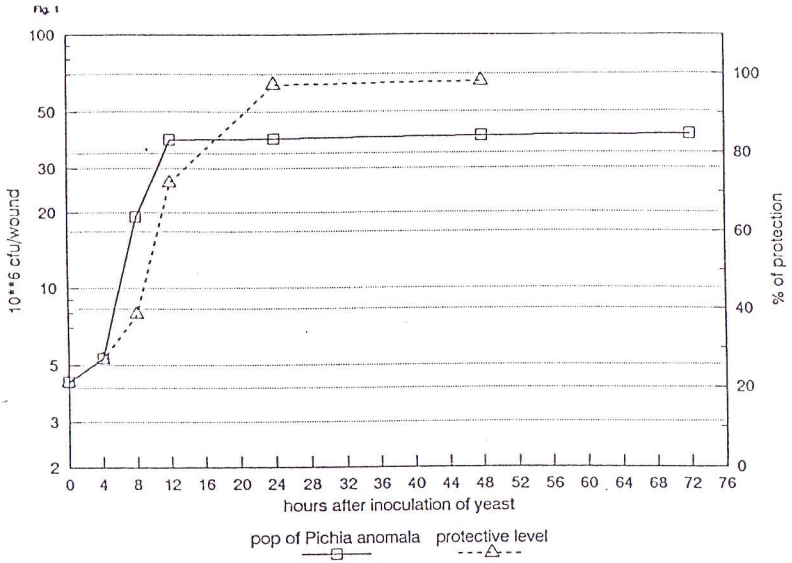

Fig. 1 : Effect of population densities of *Pichia sake* on level of protective activity against *B. cinerea*.. Data for population densities represent mean number of colonies from three trials (one wound site/trial). Each wound was triplicate-plated. Data for protective level represent the mean % of protection (as compared to the control which was not treated with yeast before inoculation of *B. cinerea*) from 2 trials (6 wounds/trial). Bars represent standard error of the mean.

Reference

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ENHANCING BIOCONTROL OF POSTHARVEST DISEASES OF APPLE WITH NITROGENOUS COMPOUNDS.

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Summary

Biocontrol of postharvest diseases of apple is emerging as a promising alternative to fungicidal treatment. However, implementation of this method of control will largely depend on its reliability and economics. Applications of higher concentrations of the antagonists to fruit increase reliability of control but reduce the economics. Thus, a method was developed to enhance biocontrol of Penicilium expansum by adding selected nutrients which stimulate the antagonist on fruit and eliminate the need to apply higher concentrations of the antagonist.

Introduction

In the past decade great progress has been made in biocontrol of postharvest diseases of pome fruit. Private industry has begun exploring commercial potential of some biocontrol agents. Two factors, reliability and economics, will have great impact on the feasibility of using this method of control. A number of factors influence the effectiveness of biocontrol agents on fruit, they include: concentration of a pathogen propagules, fruit maturity, cultivar, wound type, postharvest treatments, and storage conditions. Higher concentrations of the antagonist are required to achieve acceptable control on some cultivars, on more mature fruit, and on fruit with severe wounds. However, the use of higher concentrations of antagonists increases cost. The great advantage of a postharvest biocontrol system is that the physical and chemical environment can be manipulated to enhance the effectiveness of the antagonists. The objective of this work was to enhance biocontrol of Penicillium expansum on mature apple by stimulating population of the antagonist Pseudomonas syringae (L-59-66) with nutrients.

Methods

1. Effect of nutrients on antagonist and pathogen.

Several carbohydrates and nitrogenous compounds were tested for their effect on growth of the antagonist, Pseudomonas syringae (L-59-66), and on germination, germ tube and radial growth of Penicillium expansum. The tests for utilization of these compounds by the antagonist were conducted in microtiter plates containing basic minimum salt medium with single nitrogen or carbon source, and tetrazolium dye. The antagonist suspension was applied to the plates and incubated for 24 hr at 24 C. Then absorption by the wells was determined with a plate reader at 590 nm.