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Competition for amino acids as a potential mechanism of *Aureobasidium pullulans* against post-harvest apple blue mold

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Abstract: *Aureobasidium pullulans* (strain Ach1-1) has proved to be very effective against *Penicillium expansum* on postharvest wounded apples. In this work, the role of amino acids in its antagonistic activity was investigated. Exogenous application of amino acids into apple wounds had significantly reduced the protective level of strain Ach1-1, the extent of the reduction being dependent on the applied concentration. HPLC analysis of apple amino acids at the wound site during the first 24-hour incubation period r vealed that amino acids, especially serine, glycine and glutamic acid, were more depleted in wounds containing strain Ach1-1 alone or both strain Ach1-1 and *P. expansum* than in wounds inoculated with *P. expansum* alone or untreated wounds. Individual applications of these amino acids, most particularly serine, in apple wounds significantly decreased strain Ach1-1 efficacy against *P. expansum*. It seems thus from our data that competition for amino acids may be an important mode of action of strain Ach1-1 against *P. expansum* and serine one of the most limited amino acids in this competition.

Key words: apple fruits, biocontrol, nutrient competition, *Penicillium expansum*

Introduction

Aureobasidium pullulans strain Ach1-1 was selected for its high biocontrol activity against *Penicillium expansum* on wounded Golden delicious apples (Achbani et al., 2005). In a previous study, competition for nutrients was found to be a main mode of action of this strain (Krimi Bencheqroun et al., 2006). Among apple compounds, amino acids were found to be more involved than sugars and vitamins in its biocontrol activity. The purpose of the present work was to focus on the role of amino acids in the biocontrol activity of strain Ach1-1 against *P. expansum* on harvested apples and to identify those which are the most limited in the mechanism of competition.

Material and methods

Effect of exogenous application of amino acid mixture on the biocontrol activity of strain Ach1-1

The efficacy of *A. pullulans* (strain Ach1-1) against *P. expansum* (strain 880) on apple (cv. Golden Delicious) wounds was assessed as previously described (Jijakli & Lepoivre, 1993) with some modifications (Krimi Bencheqroun et al., 2006). Amino acid solutions were prepared by mixing most of those known to be present in apple tissues at concentrations 2, 10 and 20 times the concentration reported for apple fruits (USDA nutrient database for standard

reference, release 14, 2001). Amino acids were applied (40 µl per wound) one hour after the pathogen inoculation. Controls were treated with the same amino acid solutions in the absence of the antagonist. Lesion diameters and the protective level were estimated according to Krimi Bencheqroun et al. (2006).

Time-course evolution of amino acids in apple wounds

Wounded apples were distributed into four sets: Set 1: non treated apples (control); Set 2: application of strain Ach1-1 alone (antagonist); Set 3: inoculation with *P. expansum* alone (pathogen); Set 4: application of strain Ach1-1 followed by the inoculation with *P. expansum* (antagonist + pathogen). For each set, culture solutions in wounds were extracted by pipeting 0, 4, 6, 14 and 24 hours after the application of the antagonist and their amino acid concentrations were determined by HPLC.

Effect of individual applications of some specific amino acids on the biocontrol activity of strain Ach1-1

Serine, glycine, glutamic acid and alanine were selected for biocontrol assays in wounds apple. The evaluation of individual effects of these amino acids on Ach1-1 efficacy was performed as above at the high concentration (20 times) only.

Statistical analysis

For biocontrol assays, 15 apple fruits were used per treatment (3 wounds per apple). Each test was conducted twice and data were subjected to analysis of variance. Means were separated using the student - Newman - keul's at $P \leq 0.05$. All analyses were performed using the Statistical Analysis System (SAS/STAT) software.

Results

Effect of exogenous application of amino acid mixture on the biocontrol activity of strain Ach1-1

In controls, lesion diameters were increased with increasing amino acid concentrations (Table 1). The application of strain Ach1-1 significantly reduced these infection lesions, the extent of the reduction being dependent on the applied concentration. The higher the amino acid concentration, the lower was the protection level of strain Ach1-1 against *P. expansum*.

Table 1. Effect of exogenous amino acid application in apple wounds on lesion diameter (cm) developed by *P. expansum* strain 880 after 5 days of incubation in the absence (Control) or in the presence of *A. pullulans* strain Ach1-1 (Strain Ach1-1) and on the corresponding levels of protection (%)

	Lesion diameter (cm)		
Treatment	Control	Strain Ach1-1	Protective level (%)
None	1.30 ± 0.04 d	0.12 ± 0.02 g	91.0
Amino acids (2x)	1.60 ± 0.03 c	0.26 ± 0.02 f	83.8
Amino acids (10x)	1.87 ± 0.02 b	0.80 ± 0.03 e	57.5
Amino acids (20x)	2.00 ± 0.02 a	1.34 ± 0.03 d	32.8

Values with the same letter are not significantly different ($P \leq 0.05$).

Time-course evolution of amino acids in apple wounds

Fig. 1 shows that the concentration of amino acids in apple wounds decreased during apple incubation. The most important decrease was observed in apple wounds treated with strain

Ach1-1 and especially in those containing both strain Ach1-1 and *P. expansum*. The detailed analysis of the different amino acids has shown that serine, glycine and glutamic acid were amino acids whose concentration was largely reduced with incubation time comparatively to the other amino acids (*e.g.* alanine) (detailed data not presented).

Effect of individual applications of some specific amino acids on the biocontrol activity of strain Ach1-1

Lesion diameters of the controls were not significantly affected by the exogenous application of individual amino acids (except in the case of glutamic acid) (Table 2). Strain Ach1-1 significantly reduced lesions developed by *P. expansum* regardless of the amino acid identity. The lowest level of protection was obtained with serine indicating that this amino acid may be the most involved in competition.

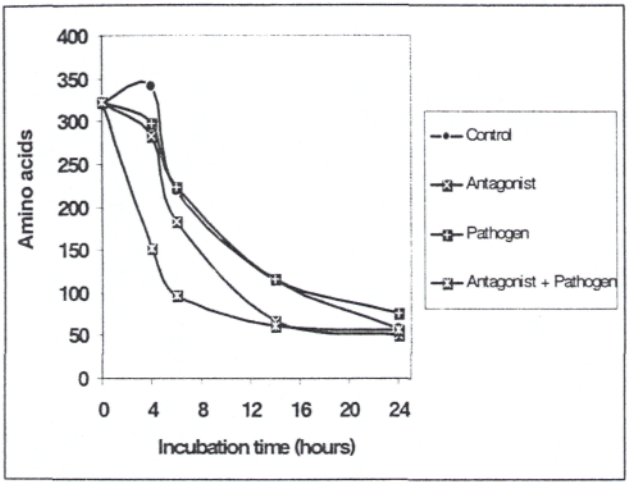

Figure 1. Amino acid concentration (nmol/ml) in apple wounds during the first 24-hour incubation period. Wounds were either non treated (control), treated with strain Ach1-1 alone (antagonist), inoculated with *P. expansum* alone (pathogen) or treated with strain Ach1-1 and then inoculated with *P. expansum* (antagonist + pathogen).

Table 2. Effect of adding specific amino acids in apple wounds on lesion diameter (cm) developed by *P. expansum* strain 880 after 5 days of incubation in the absence (Control) or in the presence of *A. pullulans* strain Ach1-1 (Strain Ach1-1) and on the corresponding levels of protection (%)

	Lesion diameter (cm)		
Treatments	Control	Strain Ach1-1	Protective level (%)
None	2.25 ± 0.03 a	0.28 ± 0.07 f	87.7
Serine	2.15 ± 0.05 a	1.45 ± 0.06 c	32.7
Glycine	2.08 ± 0.04 a	1.19 ± 0.10 d	42.9
Glutamic acid	1.87 ± 0.00 b	0.84 ± 0.10 e	55.1
Alanine	2.07 ± 0.00 a	0.74 ± 0.10 e	64.5

Values with the same letter are not significantly different ($P \leq 0.05$).

Discussion

Recently, we provided *in vitro* and *in situ* evidence that the biocontrol activity of strain Ach1-1 against *P. expansum* essentially relies on competition for nutrients, especially for amino

acids (Krimi Bencheqroun et al., 2006). In this study, we showed that an exogenous application of increasing concentrations of amino acids in apple wounds significantly lowered the biocontrol activity of strain Ach1-1 against *P. expansum* (Table 1) without altering the development of these microorganisms. At the highest concentration (20×), the protective level was reduced by about 64% (32.8 *vs.* 91%), suggesting once again that competition for apple amino acids by strain Ach1-1 plays an important role in suppressing *P. expansum*. This result was confirmed by a time-course analysis of wound amino acids during apple incubation which revealed that these amino acids were more depleted in the presence of strain Ach1-1 than in the presence of *P. expansum* and that the most rapid depletion was obtained in the presence of both agents (Fig. 1). In a previous work, *A. pullulans* was able to assimilate three major amino acids (aspartic acid, serine, and glutamic acid) present in apple juice during 24 hours of incubation (Janisiewicz et al., 2000). In our case, two of these amino acids (serine and glutamic acid) as well as glycine were amino acids whose concentration was the most affected during incubation (data not shown). Based on biocontrol assays, serine seems to be the most limited amino acids as its application strongly lowered the biocontrol activity of strain Ach1-1.

As a conclusion, our data provide strong evidence that competition for apple amino acids would be one of the main mechanisms of action involved in the biocontrol activity of the antagonist *A. pullulans* strain Ach1-1 against *P. expansum* on postharvest apples. Among these amino acids, serine appears to be the most limited nutrient in this competition. The investigation will continue to find out the gene(s) involved in the uptake and the metabolisation of serine by the antagonist cells.

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