Pilot tests of *Candida sake* (CPA-1) applications to control postharvest blue mold on apple fruit

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Received 20 January 2000; accepted 8 June 2000

Abstract

The yeast *Candida sake* (strain CPA-1) was tested as a biocontrol agent of postharvest diseases, primarily blue mold, caused by *Penicillium expansum* on apple fruits. In a semi-commercial trial with non-injured fruits stored in air at 1°C, a concentration of 1.6 × 10⁶ colony forming units/ml (CFU/ml) of *C. sake* reduced the incidence of decayed fruits by more than 70%. Over a period of three seasons, in commercial trials the efficacy of CPA-1 applied in a drench was evaluated and compared with the fungicides imazalil and thiabendazole + folpet. The application of *C. sake* at 10⁷ CFU/ml resulted in a reduction in the incidence of decay to a level equal to that with imazalil (375 ppm) and higher than that with thiabendazole (425 ppm) + folpet (1000 ppm). Population of the biocontrol agent increased on the surface of wounded fruits 5-fold and decreased on the surface of non-wounded fruits more than 10-fold in the first 60 days in storage at 1°C. The viability of *C. sake* was not reduced after 30 min immersion in benomyl, sulfur, flusilazol, ziram, thiabendazole or diphenylamine. Conversely, captan, imazalil, and ethoxyquin decreased *C. sake* viability and would not be compatible with it used at commercial rates. The yeast was able to grow in culture at temperatures from 1 to 34°C. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Biological control; Yeast; *Penicillium expansum*; Postharvest diseases

1. Introduction

Microbial antagonism plays an important role in the natural control of numerous postharvest pathogens of fruits (Janisiewicz, 1987; Wilson and Chalutz, 1989; Roberts, 1990; Lima et al., 1997). Strains of *Candida* spp can control several postharvest fruit diseases (Jijakli et al., 1993; Wilson et al., 1993; Usall, 1995; Viñas et al., 1998) and some bacteria and yeast antagonists have been tested on a large scale under commercial conditions (Pusey et al., 1988; Droby et al., 1993; Chand-Goyal and Spotts, 1997; Arras and Arru, 1999), and in several formulations (Janisiewicz and Jeffers, 1997; Droby et al., 1998). A number of commercial products are available in the USA.
market including Aspire (*Candida oleophila* strain 182, Ecogen Inc., Langhorne, PA) and Bio-save 10 and 11 (*Pseudomonas syringae* strains ESC30 and ESC 11, EcoScience Corp., East Brunswick, NJ).

The ultimate acceptance of biological control will depend on its effectiveness and compatibility with current handling and storage. More research is needed to determine the effects of various postharvest practices (such as washing, waxing, and fungicide residues from preharvest applications) on the population dynamics and biological activity of the antagonist.

In previous studies, a naturally occurring yeast, *Candida sake* (Saito and Ota) van Uden and Buckley (strain CPA-1), was isolated from the surface of apple fruit and exhibited antagonistic activity in vivo against a wide spectrum of postharvest pathogens of pears and apples (Vinás et al., 1998). In this paper we report the results of a large-scale experiment under cold storage and controlled atmosphere conditions and several pilot tests to assess the effectiveness of the yeast antagonist CPA-1. The population dynamics of *C. sake* (CPA-1) on the apple surface, and its tolerance to commercial pre- and postharvest fungicides and antiscald chemicals, was studied.

### 2. Materials and methods

#### 2.1. Fruits, pathogen and yeast isolates

The apple cultivar ‘Golden Delicious’ (*Malus domestica* Borkh) was used in all the experiments. Fruits were obtained immediately after harvest from commercial orchards in Lleida, Catalonia, Spain.

*Penicillium expansum* Link isolate CMP1 was isolated from a decayed apple that had been in cold storage for several months. This isolate was the most aggressive one in our collection and caused the largest lesions on inoculated apples. The fungus was maintained on potato-dextrose agar (PDA) with periodic transfers through apple tissue. The inocula consisted of aqueous conidial suspensions of $10^4$ conidia per ml. The suspensions were prepared from 10-day-old cultures incubated at $25 \pm 1^\circ C$.

The strain CPA-1 of *C. sake* (Saito and Ota) Van Uden and Buckley, obtained from UdL-IRTA Centre, Catalonia, was used in this study. This strain was originally isolated from the surface of a ‘Golden Delicious’ apple, and was previously show to have antagonistic activity against *P. expansum, Botrytis cinerea* and *Rhizopus nigricans* on pome fruits (Usall, 1995; Viñas et al., 1998). Stock cultures were stored at 5°C and sub-cultured on nutrient yeast dextrose agar (NYDA). The cells of strain CPA-1 were grown in 6 l of nutrient yeast dextrose broth (NYDB) medium in a 10 l fermentation vessel of a modular fermenter (Gallenkamp, Leicester, UK) for 35–40 h at $25 \pm 1^\circ C$ and 200 rpm. The medium then was centrifuged at 7520 $g$ for 10 min and the cells were resuspended in 1 l of water. Serial 10-fold dilutions in water were made from this concentrated solution and 0.1 ml was spread in Petri plates with NYDA to determine the number of viable cells. The plates were incubated at $25 \pm 1^\circ C$ in the dark for 48 h and the colonies were counted. The concentrated yeast cell suspension was stored, for not more than 5 days at 4°C and the desired concentrations were obtained by diluting it before use.

#### 2.2. Growth of *C. sake* at several temperatures

The growth of CPA-1 at several temperatures was evaluated on Petri dishes containing NYDA medium. Each plate was inoculated with 100 $\mu l$ of yeast suspension containing about 500 CFU/ml. The plates were incubated for 7 days at 1, 4, 7, 10, 15, 25, 30, 34, 37 and 44 °C, and the presence of yeast colonies was evaluated every day. There were four plates per treatment, and the experiment was repeated twice.

#### 2.3. Semi-commercial trial under cold storage conditions

During the 1994–1995 season, ‘Golden Delicious’ apples were submerged for 30 s in the *C. sake* aqueous suspension containing $1.6 \times 10^4$ or $1.6 \times 10^5$ CFU/ml. After 1 h, the fruits were sub-
merged again for 30 s in a conidial suspension of *P. expansum* (104 conidia/ml).

Sixty apples constituted a single replicate and each treatment was replicated three times. The number of fruits that developed lesions was counted after 8 months in air storage at 1°C.

### 2.4. In vitro screenings of tolerance of *C. sake* to pre- and postharvest fungicides and antiscald products

Since fungicide residues from field applications may affect the activity of *C. sake* (CPA-1), fungicides commonly applied to trees before harvest (benomyl, captan, ziram, sulfur and flusilazol) were tested for their inhibitory action against the yeast. At the same time, compatibility of the antagonistic yeast with the most common antiscald chemicals, used to prevent superficial scald, (diphenylamine and ethoxyquin) and postharvest fungicides (imazalil and thiabendazole) were also studied. Two methods to determine inhibitory activity were carried out as follows.

#### 2.4.1. Inhibition of *C. sake* growth

The paper-disk diffusion method was used to measure inhibition of *C. sake* growth. The cells at 10⁶ CFU/ml were plated on to Petri plates containing NYDA medium. Paper disks, 5.5-mm diameter, were impregnated with 10 μl of several concentrations of the pre- and postharvest fungicides and antiscald products. The disks were allowed to dry and then placed on the surface of each prepared plate. The plates were then incubated at 25°C in the dark for 48 h. The lowest concentration where a zone of inhibition formed was recorded. There were two plates per treatment and the experiment was repeated twice.

#### 2.4.2. Viability of *C. sake* in fungicide and antioxidant solutions

Pre- and postharvest fungicides (1 ml) and antioxidant chemicals at concentrations five and ten times the recommended commercial rate were transferred to glass test tubes containing 9 ml of *C. sake* at 7 × 10⁵ CFU/ml. After 30 min at 25°C, 0.5 ml of each suspension was removed with a pipette and transferred to a glass tube containing 4.5 ml of 0.05 M phosphate buffer (pH 6.8). Serial 10-fold dilutions were made and 0.1 ml was spread onto Petri plates with NYDA medium. The plates were incubated at 25°C in the dark for 48 h and the colonies were counted. The test was repeated twice.

### 2.5. Commercial trials under controlled atmosphere conditions

During the 1995/1996, 1997/1998 and 1998/1999 seasons, commercial trials were conducted in Lleida (Catalonia). ‘Golden Delicious’ apples were randomly chosen at harvest and stored in bins containing about 1300 fruits each. Fruits were drenched following standard industrial procedures. In the 1995/1996 season, fruits were treated with a solution of imazalil 7.5% (w/v) (Deccozil-S-7.5, Atochem Agri) at a commercial dose (0.5%), and with *C. sake* strain CPA-1 at either 10⁵ or 10⁶ CFU/ml. In the 1997/1998 season fruits were treated with a solution of imazalil (Deccozil-S-7.5, Atochem Agri) at a commercial dose (0.5%), and with *C. sake* strain CPA-1 at either 10⁶ or 10⁷ CFU/ml. In the 1998/1999 season, fruits were treated with a solution of thiabendazole 17% (w/v) + folpet 40% (w/v) (Tebefred, Agridés) at a commercial dose (0.25%), and with *C. sake* strain CPA-1 at 10⁷ CFU/ml. In each season, non-treated bins served as controls. Each treatment was replicated four times. All the fruits were stored at 1°C and 3% O₂ + 3% CO₂ in a commercial room, filled to capacity with similar apples that had received standard commercial treatments (in 1995/1996 and 1997/1998 with imazalil at 0.5%, and in 1998/1999 with thiaben-dazole at 0.25%). After 8, 7, or 4 months in the 1995/1996, 1997/1998, and 1998/1999 seasons, respectively, decayed fruits were counted.

### 2.6. Recovery of biocontrol agent in the commercial trials

Approximately 50 ml samples of the treatment suspension were taken during the application to the fruit to determine the *C. sake* population. After 10-fold dilutions, sample aliquots of 0.1 ml were plated onto Petri plates containing NYDA
medium with 0.5 g/l streptomycin sulphate as a bacteriostat. After incubation in the dark at 25°C for 48 h, the colonies were counted. There were four sample aliquots per treatment.

Population sizes of *C. sake* on the surface of ‘Golden Delicious’ apples were determined during the 1997/1998 season. The biocontrol agent was recovered from the apple surface immediately after drench application and after 7 months in storage. Treated apples were weighed and aseptically peeled. The peel was shaken in 200 ml sterile phosphate buffer (pH 6.8) on a rotatory shaker for 20 min at 150 rpm and then sonicated for 10 min in an ultrasonic bath. This final step was used to increase detachment of microorganisms from the apple surface. Serial 10-fold dilutions of the washings were made and plated on NYDA containing 0.5 g/l streptomycin sulphate as a bacteriostat. After incubation at 25°C in the dark for 48 h the colonies were counted and the colony forming units per gram fresh weight of fruit were calculated for each sample. There were four single fruit replicates per treatment.

### 2.7. Population dynamics on the apple surface at 1°C

Population dynamics of *C. sake* on wounded or non-wounded surfaces of ‘Golden Delicious’ apples were determined. In the 1997/1998 season, two sets of apples were rinsed in fresh water after harvest. One of these sets was cut in four locations (midway between the calyx and stem end). The cuts were approximately 10 mm length and 2 mm deep. All (both sets) were dipped for 30 s in *C. sake* aqueous suspension containing 1 × 10⁶ CFU/ml. Fruits were placed in tray packs in plastic boxes and incubated at 1°C. *C. sake* was recovered as described earlier from the apple surface after 0, 4, 30, 60, and 90 days. The experiment was repeated twice.

### 2.8. Scanning electron microscopy

*C. sake* (isolate CPA-1) at 7.5 × 10⁵ CFU/ml was inoculated into wounds (3 × 3 mm and 3 mm deep) and on the surface of ‘Golden Delicious’ apples. The fruits were incubated at 20 ± 1°C and 75 ± 5% RH for 24 and 72 h. The peel tissue from the wounds and surface was removed and fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.0 for 2–24 h. The samples were washed three times for 30 min with 0.1 M phosphate buffer. The tissue was then dehydrated in an acetone series (30, 50, 70, 95%, and three times 100%) for 10 min each, with critical point drying (CPD) using CO₂ as the drying fluid. Dried tissue was mounted on aluminium studs, coated with gold-palladium, and observed in a DSM 940 A Zeiss scanning electron microscope (SEM).

### 3. Results

#### 3.1. Growth of *C. sake* at several temperatures

After 24 h, strain CPA-1 grew in culture at temperatures of 7–34°C. At 1 and 4°C CPA-1 grew only after 48 h. There were no colonies of CPA-1 on the plates stored at 37°C after 7 days.

#### 3.2. Semi-commercial trials under cold storage conditions

After 8 months of storage at 1°C, *C. sake* at 1.6 × 10⁶ CFU/ml significantly reduced the incidence of decay on non-wounded apples to 2.2% from 8.4% found in untreated control fruits. At 1.6 × 10⁴ CFU/ml of *C. sake* the incidence of decay was reduced to 4.2%, but it was not significant compared to the untreated control.

#### 3.3. Effectiveness of *C. sake* in commercial trials under controlled atmosphere

The incidence of decay in untreated fruits was about 1.5% in the 1995/1996 and 1997/1998 seasons, and more than 5% in the 1998/1999 season. Blue mold and *Alternaria* rot were the predominant observed diseases.

All the treatments, except *C. sake* at 8 × 10⁴ CFU/ml, significantly reduced the percentage of infected fruits compared with the untreated control (Fig. 1). In the first and second tests, *C. sake*
at all the applied concentrations, was equal to the most effective fungicide approved for postharvest use on pome fruits in Spain (Fig. 1). In the third test, incidence of decay was reduced by more than 70% with 10⁷ CFU/ml of *C. sake* and this was significantly more effective than the commercial treatment (thiabendazole + folpet) (Fig. 1).

### 3.4. Recovery of biocontrol agent in the commercial trials

In all three tests, the difference between the concentration of cells applied at the start of the trial and that recovered during the trial was similar (Table 1). At both concentrations, recovery of *C. sake* from the apple surface after 7 months was

![Fig. 1. Efficacy of *C. sake* (CS) at different concentrations (CFU/ml) in comparison with imazalil (IZ) and thiabendazole + folpet (TBZ + F) at commercial rates to reduce the incidence of postharvest diseases on ‘Golden Delicious’ apples in commercial trials.](image)

#### Table 1
Concentration of *C. sake* (CFU/ml) applied in commercial trials

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td><em>T</em></td>
<td><em>R</em></td>
<td><em>T</em></td>
</tr>
<tr>
<td>High</td>
<td>10⁶&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 x 10⁵a</td>
<td>10⁷</td>
</tr>
<tr>
<td>Low</td>
<td>10⁵</td>
<td>8 x 10⁴</td>
<td>10⁶</td>
</tr>
</tbody>
</table>

<sup>a</sup> CFU/ml, Colony forming units/ml; *T*, concentration theoretically applied; *R*, concentration after the recovery; – treatment not applied.

#### Table 2
Recovery of *C. sake* (CFU/g) from the surface of ‘Golden Delicious’ apples drenched in commercial trials during the season 1997/1998

<table>
<thead>
<tr>
<th>Applied concentration of C. sake (CFU/ml)</th>
<th>0 months (CFU/g fresh weight of fruit)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>7 months (CFU/g fresh weight of fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁶</td>
<td>1119 ± 64</td>
<td>1933 ± 917</td>
</tr>
<tr>
<td>10⁷</td>
<td>6643 ± 844</td>
<td>8920 ± 2855</td>
</tr>
</tbody>
</table>

<sup>a</sup> Recovery was conducted within 4 h of the application of the treatments.
Table 3
Concentrations of several fungicides and antioxidants that inhibited the growth of *C. sake* (CPA-1)

<table>
<thead>
<tr>
<th>Fungicides and antioxidants</th>
<th>Benomyl</th>
<th>Sulfur</th>
<th>Captan</th>
<th>Ziram</th>
<th>Flus</th>
<th>IZ</th>
<th>TBZ</th>
<th>DPA</th>
<th>Etox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
<td>1500</td>
<td>100</td>
<td>50</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>Maximum&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000</td>
<td>6000</td>
<td>1500</td>
<td>3000</td>
<td>500</td>
<td>1000</td>
<td>2000</td>
<td>2000</td>
<td>4000</td>
</tr>
<tr>
<td>MIC&lt;sup&gt;c&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>1500</td>
<td>2000</td>
<td>–</td>
<td>1000</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ø I (mm)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Flus, flusilazol; IZ, imazalil; TBZ, thiabendazole; DPA, diphenylamine; Etx., ethoxyquin.

<sup>b</sup> Minimum and maximum applied concentrations (ppm).

<sup>c</sup> MIC, Minimum inhibitory concentration (ppm); –, no inhibition in any dose.

<sup>d</sup> Ø I, Diameter (mm) of inhibition area of growth at the MIC, around the paper-disc.

Table 4
Viable cells of *C. sake* (CPA-1) after 30 min of immersion in fungicide or antioxidant solutions

<table>
<thead>
<tr>
<th></th>
<th>High doses</th>
<th>Low doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (ppm)</td>
<td>VC (CFU/ml)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water</td>
<td>–</td>
<td>(6.1 \times 10^5)</td>
</tr>
<tr>
<td>Benomyl</td>
<td>500</td>
<td>(5.7 \times 10^5)</td>
</tr>
<tr>
<td>Sulfur</td>
<td>4000</td>
<td>(4.7 \times 10^5)</td>
</tr>
<tr>
<td>Captan</td>
<td>1500</td>
<td>(5.7 \times 10^4)</td>
</tr>
<tr>
<td>Ziram</td>
<td>3000</td>
<td>(5.6 \times 10^5)</td>
</tr>
<tr>
<td>Flusilazol</td>
<td>50</td>
<td>(5.0 \times 10^5)</td>
</tr>
<tr>
<td>Imazalil</td>
<td>400</td>
<td>0</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>1000</td>
<td>(5.5 \times 10^5)</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>1000</td>
<td>(6.4 \times 10^5)</td>
</tr>
<tr>
<td>Ethoxyquin</td>
<td>2000</td>
<td>(8.3 \times 10^2)</td>
</tr>
</tbody>
</table>

<sup>a</sup> VC, Viable cells of *C. sake* (CPA-1).

similar or even greater than the recovery just after application (Table 2). Although the higher applied concentration was 10-fold higher than the low, the recovered population was only six times smaller immediately after application, and this difference was reduced to 4.6-fold after 7 months.

### 3.5. In vitro screening of tolerance of *C. sake* to pre- and postharvest fungicides and antioxidant products

#### 3.5.1. Inhibition of growth of *C. sake* by fungicides and antioxidant products

Only captan, ziram and imazalil were inhibitory to *C. sake* and the minimum doses required were 1550, 2000, and 1000 ppm, respectively. The inhibition zone around the paper-disc ranged from 2 to 3 mm for all three compounds (Table 3).

#### 3.5.2. Viability of *C. sake* in the fungicide and antioxidant solutions

Viability of *C. sake* was not reduced after 30 min of immersion in the preharvest fungicides benomyl, sulfur, flusilazol, or ziram at the tested doses, but the growth of *C. sake* was reduced by captan (Table 4). The high dose of captan reduced the number of live cells of *C. sake* 10-fold compared with water.

Imazalil was inhibitory to *C. sake*. The number of live cells was reduced to 0 at 400 ppm (commercial dose) and 100-fold at 200 ppm. In contrast, thiabendazole did not show any effect in *C. sake*.
sake viability. Of the two chemicals commonly used to prevent superficial scald, only ethoxyquin reduced the C. sake viability after 30 min of immersion. The number of viable cells of C. sake were two and three log units lower at 1000 and 2000 ppm ethoxyquin, respectively, compared with water.

3.6. Population dynamics on the apple surface at 1°C

Population of C. sake in the wounded fruits stored at 1°C decreased more than 3-fold 4 days after the application and then began to increase (Fig. 2). The maximum population was recovered 60 days after the yeast application. On non-wounded fruits, the initial population recovered was lower than that in the wounded fruits. The populations of C. sake decreased 10-fold during the first 4 days, and then remained stable until the end of the experiment.

3.7. Scanning electron microscopy

C. sake strain CPA-1 grew rapidly inside wounds made on ‘Golden Delicious’ apples. After incubation at 20°C for 24 h the yeast colonized most of the inner surface of the wound (Fig. 3).

The extensive growth of C. sake (CPA-1) in wounds contrasts with its limited growth on the surface of the apples. This could be attributed to lower nutrient content and lower water activity on the surface of the apple skin.
4. Discussion

Previously, we studied the control of the major postharvest diseases on apple during cold storage and under several controlled atmosphere conditions using the strain CPA-1 of *C. sake* (Viñas et al., 1998; Usall et al., 2000). In this report, those studies were expanded to evaluate the effectiveness of biological control of postharvest diseases of apple with CPA-1 under commercial packinghouse conditions.

Usall and Viñas (1989) showed that only 0.2% of the total fungi isolated in eight orchards in Lleida, Catalonia, was of the genus *Penicillium*. Conversely in two packinghouses in the same area, the genus *Penicillium* represented 65% of the total fungi isolated (Viñas et al., 1991). Wounds are the major point of entry for *P. expansum* (Spotts and Cervantes, 1986; Viñas, 1990). In our semi-commercial trials, with non-injured fruits and dipped for 30 s in a conidial suspension of *P. expansum*, 8% of the fruit were rotten after 8 months in air storage at 1°C. These results indicate that the fruits have natural wounds and the potential risk of decay is very high if *P. expansum* is present in packinghouses.

The semi-commercial trial was severe because the fruits were dipped in a suspension of *P. expansum* at a relatively high concentration. However, the incidence of decayed fruits was reduced from a control level at 8.4–2.2% in apples treated with a *C. sake* suspension containing $1.6 \times 10^6$ CFU/ml.

To be commercially acceptable, the application of a biocontrol agent must be compatible with packinghouse operations. Apple fruits are drenched after harvest with a fungicide and a chemical to prevent superficial scald in storage. To substitute the fungicide for the antagonist would be ideal, because the drencher was used successfully to apply CPA-1 in this study. Samples of yeast suspension taken before and after passage through the drencher showed that CFU/ml did not change (data not shown), and the recovery of the biocontrol agent after the commercial drench application was the same after dipping during 30 s in aqueous suspension. CPA-1 was compatible with diphenylamine, which is typically used as an antiscald agent on apples, and with the drenching process.

Sugar and Spotts (1999) reported that two registered biocontrol products (Bio-save and Aspire) combined with 100 ppm of TBZ in commercial trials were as effective as TBZ at 569 ppm in controlling blue mold of pears. In our packinghouse trial during the 1998/1999 season, CPA-1 alone was more effective than 425 ppm TBZ + 1000 ppm folpet in controlling decay in apples. Because CPA-1 is compatible with this fungicide it might be also possible to apply the antagonist mixed with a low dose of TBZ.

Monitoring populations of CPA-1 applied on ‘Golden Delicious’ apples showed that it colonised and survived on wounded and non-wounded fruits stored at 1°C. On wounded fruits, the population increased more than 10-fold in the first 60 days. This suggests that the yeast only grows in wound sites, which are the major point of entry for *P. expansum* (Viñas, 1990). Lima et al. (1998) obtained similar results with two yeasts applied to apples stored at 4°C.

The ability of CPA-1 to grow in culture at low temperatures (including 1°C, the lowest temperature tested) is in agreement with their high efficacy against postharvest diseases during cold storage, and its capacity to grow on apple surfaces and to rapidly colonise the wound sites at 1°C (Viñas et al., 1998).

Strain CPA-1 was compatible with the fungicides commonly applied to apple trees except captan and ziram. However, preharvest sprays with captan and ziram could have little or no effect on the *C. sake* applied after harvest, because they have no systematic activity and most of the surface residues will be removed by weathering and postharvest washing.

Although strain CPA-1 demonstrated excellent potential, it is important that evaluation of this strain in a product formulation be carried out to better predict its effectiveness in commercial use. Formulation may improve or reduce efficacy. Janisiewicz and Jeffers (1997) showed that wettable power formulations of *Pseudomonas syringae* strains ESC-10 and ESC-11 were less or equally effective as the original fresh cells of the antagonist for control blue mold and grey mold on apples.
C. sake is also ubiquitous in nature and a major component of the epiphytic community on mature fruits (Beech and Davenport, 1970). It is a very suitable organism for postharvest disease control as C. sake has never been found to be associated with warm-blooded animals (Hurley et al., 1987) and a temperature of 37°C was lethal for this antagonist. Also, the acute oral toxicity of C. sake was determined by the ‘Centre d’Investigació i Desenvolupament Aplicat’ (Barcelona, Catalonia, Spain), calculating the rat oral median lethal dose (LD₅₀). This work showed that the LD₅₀ of C. sake strain CPA-1, is higher than 1.7 × 10¹⁰ CFU/kg of live weight of Wistar rat and at this concentration no mortality or alterations in the tested animals were observed (data not shown).

The concentration of the antagonist needed to obtain control was low enough to be practical for commercial use. Effectiveness was satisfactory at the low temperatures usual in cold storage, and with typical controlled atmosphere mixtures. The facilities used commonly in the packinghouses for these treatments are well suited for use with C. sake. This makes this antagonist more promising, since additional investments in equipment for the application of alternative chemicals will not be needed.

Acknowledgements

This work was funded by the Spanish Agency Comisión Interministerial de Ciencia y Tecnología (CICYT), and had the financial help of SIPCAM INAGRA S.A. We wish to thank Dr Joseph L. Smilanick (USDA, California) for valuable comments on the manuscript.

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