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Biological control of blue mould on apple by a strain of *Candida sake* under several controlled atmosphere conditions

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Abstract

The biocontrol potential of the yeast *Candida sake* (CPA-1) against *Penicillium expansum* decay of apples under several controlled atmosphere conditions was investigated. In a laboratory trial under different commercial cold storage conditions, increasing concentrations of *C. sake* improved decay control. A maximum reduction of decay was achieved at 3% O_2–3% CO_2 atmosphere. It amounted to a 97% lesion reduction after treatment with a suspension containing 2.4×10^6 CFU/ml of *C. sake* (CPA-1). In a semi-commercial trial at 1°C with wounded fruits, the reduction in decay diameter caused by *C. sake* exceeded 80% after 60 days at 21% O_2 and 60% after 120 days of storage under controlled atmosphere conditions. For seven controlled atmosphere conditions studied, a significant influence by *C. sake* on the *P. expansum* decay was observed, and the lesion size was reduced more than 70% by *C. sake* at 10^7 CFU/ml. The populations of *C. sake* (CPA-1) on the apple surface followed the same pattern under all controlled atmosphere conditions studied. They decreased 4–10-fold during the first 2 weeks, followed by an increase to the initial level after 45 days, and thereafter the count remained constant for the period of 90 days examined. This indicated the capacity of *C. sake* (CPA-1) to colonize the surface of apples under various storage conditions. The ability to colonize was even higher in apple wounds.

Keywords: *Candida sake*; *Penicillium expansum*; Biological control; Apple; Controlled atmosphere; Postharvest; Yeast

1. Introduction

Postharvest fruit diseases continue to cause significant losses. Infection of fruits by pathogens can occur either prior to harvest, during harvesting, and subsequent handling, in storage. The development of a fungal disease during the postharvest phase depends on storage conditions, the physiological age of the host, and the defense mechanisms of the host. Postharvest decay can be reduced by minimizing fruit injuries, by maintaining the natural resistance of the host, and by delaying senescence (Shewfelt, 1986). However, these beneficial practices are usually not sufficient to protect the product from fungal infection. Application of synthetic fungicides has been the primary means of controlling postharvest diseases (Eckert and Ogawa, 1988), although the use of chemicals is becoming increasingly restricted

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because of concerns for the environment and health, as well as the cost of developing new pesticides to overcome resistance developed by the pathogens. Biological control using microbial antagonists has been considered as a desirable alternative to the use of such chemicals (Woodhead et al., 1990).

A number of bacteria, filamentous fungi, and yeasts have been isolated and shown to protect fruit against postharvest pathogens (Janisiewicz, 1987; Wilson and Chalutz, 1989; Roberts, 1990). There are several references to using *Candida* strains as antagonists of postharvest diseases (Jijakli et al., 1993; Wilson et al., 1993; Usall, 1995; Viñas et al., 1998). Some antagonists have been tested on a large scale under commercial conditions (Pusey et al., 1988; Hofstein et al., 1994; Chad-Goyal and Spotts, 1997).

The ultimate potential of biological control will depend on its effectiveness and its compatibility with current handling and storage practices such as low temperatures and controlled or modified atmosphere during storage. There are reports suggesting that biocontrol procedures can be integrated into commercial postharvest operations (Pusey, 1994). However, more research is needed to determine the effects of various postharvest practices on the population dynamics and the biological activity of the antagonists to determine the means of manipulating the antagonistic activity of introduced biocontrol agents.

In previous studies, a screening programme was carried out with the aim to identify, from plant surfaces, naturally occurring bacteria and yeasts that would be effective against postharvest diseases in pome fruits. The most effective antagonist was identified as *Candida sake* (Saito & Ota) Van Uden & Buckley (Viñas et al., 1998).

C. sake is an organism ubiquitous in nature and a component of the epiphytic community on mature fruits (Beech and Davenport, 1970; Buhagiar and Barnett, 1971). It has never been found to be associated with warm-blooded animals (Hurley et al., 1987).

More than 50% of apple production in the Ebro Valley (Spain) and in other important producer areas of the world, is stored at lower temperature and under different controlled atmosphere conditions until 10 months, depending on the cultivar and the area practices (Herrero and Guardia, 1991). Thus, biocontrol agents must control the pathogens for a long period in a wide range of temperatures and atmosphere conditions.

The objective of this study was to determine effectiveness of a new biocontrol agent, *C. sake* strain (CPA-1), against the major postharvest pathogen of pome fruits, *Penicillium expansum* (Palazon et al., 1984), under several controlled atmosphere conditions.

2. Materials and methods

2.1. Pathogen

P. expansum Link isolate CMP1 was isolated from apples decayed after several months in storage. 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; 200 ml of extract from boiled potatoes, 20 g of dextrose (Rectapur, 24 379.294, Prolabo, Fontenay S/B, France), 20 g of agar (Prolabo, 20 768.292) and 800 ml of water). To maintain virulence, it was grown once a year on apples and reisolated. The inocula consisted of a conidial suspension of 10^4 conidia/ml. The suspensions were prepared from 10-day-old cultures, using a haemocytometer (Thoma, Brand, Germany).

2.2. Yeast

The *C. sake* (Saito & Ota) van Uden & Buckley (CPA-1) suspensions were prepared by growing cultures in nutrient yeast dextrose broth (NYDB; 8 g of nutrient broth (Biokar Diagnostics, BK003 Beauvais, France), 5 g of yeast extract (Biokar Diagnostics, 112002), 10 g dextrose (Rectapur, 24 379.294, Prolabo, Fontenay S/B, France), 20 g of agar (Prolabo, 20 768.292) and 1000 ml of water) for 36–42 h at 25±1°C with shaking at 150 rpm on a orbital incubator (Gallenkamp, Leicester, UK). The medium was then centrifuged (Avanti J-25, Beckman, USA) at $7520 \times g$ for 10 min and cells were resuspended in water. Desired concentrations were obtained by adjusting the suspension according to a standard curve prepared by measuring the transmitt-

ance at 420 nm with a spectrophotometer (Cecil CE 1020; Cecil Instruments, Cambridge, UK).

2.3. Fruit

The apple cultivar Golden Delicious was used in all experiments. Apples were obtained from commercial orchards in Lleida, Catalonia, that used standard cultural practices. The fruits were treated following harvest.

2.4. Laboratory trial for C. sake under commercial atmosphere conditions

As a preliminary test, during the 1994–95 season, following harvest, surface-sterilized Golden Delicious apples were wounded at the stem and calyx ends. The wounds were 3×3 mm^2 and 3 mm deep. Twenty-five μl of *C. sake* (CPA-1) suspensions (7.5×10^5, 1.6×10^6 or 2.4×10^6 CFU/ml) were applied to each wound. This was followed by inoculation with 20 μl of an aqueous suspension of *P. expansum* at 1×10^4 conidia/ml. Twenty apples constituted a single replicate and each treatment was replicated three times. The fruits were stored at 1°C for 60 days under three commercial controlled atmosphere conditions: 21% O_2; 3% O_2–3% CO_2; and 1% O_2–1% CO_2. The infected wounds and the lesion diameters were recorded after this period.

2.5. Semi-commercial trial under cold-storage conditions with wounded fruits

As a verification of previous results, a semi-commercial trial was carried out. Golden Delicious apples were randomly harvested from commercial orchards during the 1995–96 season. Each apple was cut in four locations (midway between the calyx and stem ends) with a sharp palette knife. The cuts were approximately 10 mm long and 2 mm deep. Fruits were submerged for 30 s in an aqueous suspension of *C. sake* at 1.6×10^5 or 1.6×10^6 CFU/ml. After 1 h fruits were submerged again for 30 s in a conidial suspension of *P. expansum* (10^4 conidia/ml). Sixty apples constituted a single replicate and each treatment was replicated three times. The lesion diameters were recorded 60 days after cold storage at 1°C and 21% O_2; or 120 days after storage at 1°C and two controlled atmosphere conditions: 3% O_2–3% CO_2 or 1% O_2–1% CO_2.

2.6. Small-scale trials of the antagonist under seven atmosphere conditions

After two seasons with trials at the most common atmosphere conditions, we studied the effect of seven atmosphere conditions on *P. expansum* development and *C. sake* (CPA-1) effectiveness. During the 1996–97 and 97–98 seasons following harvest, Golden Delicious apples were surface wounded at the equator of the fruit on two sides, 2 mm diameter and 2 mm deep, with a nail. Fruits were submerged for 30 s in an aqueous suspension of *C. sake* at 10^6 or 10^7 CFU/ml. After 1 h the fruits were resubmerged for 30 s in a conidial suspension of *P. expansum* (10^4 conidia/ml). Fifteen apples constituted a single replicate and each treatment was replicated three times. Each replicate were stored in an individual chamber of 200 l volume. The infected wounds and the lesion diameters were recorded 90 days after storage at 1°C and seven controlled atmosphere conditions: 3% O_2–6% CO_2; 3% O_2–3% CO_2; 2% O_2–6% CO_2; 2% O_2–4% CO_2; 2% O_2–2% CO_2; 2% O_2–1% CO_2; or 1% O_2–1% CO_2.

2.7. Population dynamics on the apple surface

The population dynamics of the *C. sake* was examined on the surfaces of Golden Delicious apples. The fruits were wounded at the equator of the fruit on two sides, about 2 mm diameter and 2 mm deep, with a nail. Fruits were submerged for 30 s in an aqueous suspension of *C. sake* at 1×10^7 CFU/ml, then placed on tray packs in plastic boxes and incubated at 1°C under the previous seven atmosphere conditions. Fruits samples were taken to determine the number of colony-forming units of *C. sake* at 0, 15, 45 and 90 days after inoculation. Twenty-five pieces of peel surface of 2.5 cm^2 were removed with a cork bore from each apple and four fruits were selected for each replicate. Peeled surface pieces were shaken in 100 ml sterile phosphate buffer (70 ml 0.2 M KH_2PO_4 (Rectapur, 26 923.298, Prolabo) + 30 ml 0.2 M K_2HPO_4 (Rectapur, 26 930.293, Prolabo) + 300 ml of water) (pH 6.8) on a

rotatory shaker (Rotabit, Selecta, Abrera, Catalonia) for 20 min at 150 rpm and then sonicated (Ultrasons, Selecta, Abrera, Catalonia) for 10 min in an ultrasound bath. This final step was used to improve detachment of micro-organisms from the apple surface

Serial 10-fold dilutions of the washings were made and plated on nutrient yeast dextrose agar (NYDA) containing 0.5 g/l streptomycin sulphate (Merck, 1.10117.0025, Darmstadt, Germany) as a bacteriostat. After incubation at 25°C in the dark for 48 h, the viable colonies per cm^2 of fruit surface were calculated for each sample. There were three replicates per treatment and the experiment was repeated twice.

2.8. Population dynamics on apple wounds

The population dynamics of the *C. sake* was examined on Golden Delicious apples wounds. Fruits were wounded midway between the calyx and stem ends by removing a block of tissue $3 \times 3 \times 3$ mm^3. A 20-μl suspension of *C. sake* at 2.4×10^6 CFU/ml was applied to each wound on the fruits. Fruits were placed on tray packs in plastic boxes and incubated at 1°C at three atmosphere conditions: 21% O_2; 3% O_2–3% CO_2 and 1% O_2–1% CO_2. *C. sake* was recovered from the wounds after incubation for 0, 60, 120 and 180 days (150 days for fruits stored at 21% O_2). Wounded tissue was removed with a cork bore (1 cm diameter by 1 cm deep) and ground with a mortar and pestle in 1 ml of 0.05 M phosphate buffer (pH 6.8). Serial 10-fold dilutions of the slurry were made and the CFU determined as described above. There were three single-fruit replicates per treatment and the experiment was repeated twice.

2.9. Data analysis

Analysis of variance was performed on data from lesion size and percentage of wounds infected. The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) was used (version 6.03; SAS Institute, Cary, NC, USA). The means of lesion diameter and percentage of wounds infected at various antagonist concentrations were separated by using Duncan's multiple range test at $P = 0.05$.

3. Results

3.1. Laboratory trial with C. sake under commercial atmosphere conditions

C. sake was very effective in controlling blue mould lesions. At 3% O_2–3% CO_2, the treatment resulted in 97% decay reduction measured by lesion diameter and the number of infected wounds were reduced for 98–7% at 2.4×10^6 CFU/ml of *C. sake* (Fig. 1). The control of *P. expansum* was significantly improved at the three controlled atmospheres tested, at increasing concentrations of *C. sake*.

3.2. Semi-commercial trial with C. sake under cold-storage conditions with wounded fruits

The lesion size was strongly reduced at all controlled atmosphere conditions tested after treatment with a suspension of 1.6×10^6 CFU/ml of *C. sake* (Table 1). At this concentration and after storing apples for 60 days at 1°C in 21% O_2, decay development was reduced more than 80% and after 120 days at controlled atmosphere conditions the lesion size of *P. expansum* was also reduced by more than 60%. Inoculum concentration of *C. sake* showed a significant effect on lesion development under all three controlled atmosphere conditions tested, and the lesion size decreased as concentration of the antagonist increased from 1.6×10^5 to 1.6×10^6 CFU/ml.

3.3. Small-scale trials of the antagonist under seven atmosphere conditions

The influence of the different controlled atmosphere conditions studied on *P. expansum* severity was only significant in the season 1996–1997 (Table 2), with the highest lesion sizes at 2% O_2–4% CO_2 and 3% O_2–3% CO_2 and the smallest at 3% O_2–6% CO_2, 2% O_2–2% CO_2 and 2% O_2–1% CO_2. Under all seven controlled atmosphere conditions and in both seasons, *C. sake* (CPA-1) was effective in controlling *P. expansum*, and the interaction between treatments and atmosphere conditions was not significant in any of two seasons ($P = 0.05$) (data not shown). The incidence and the severity of *P. expansum* were reduced in both seasons at both tested

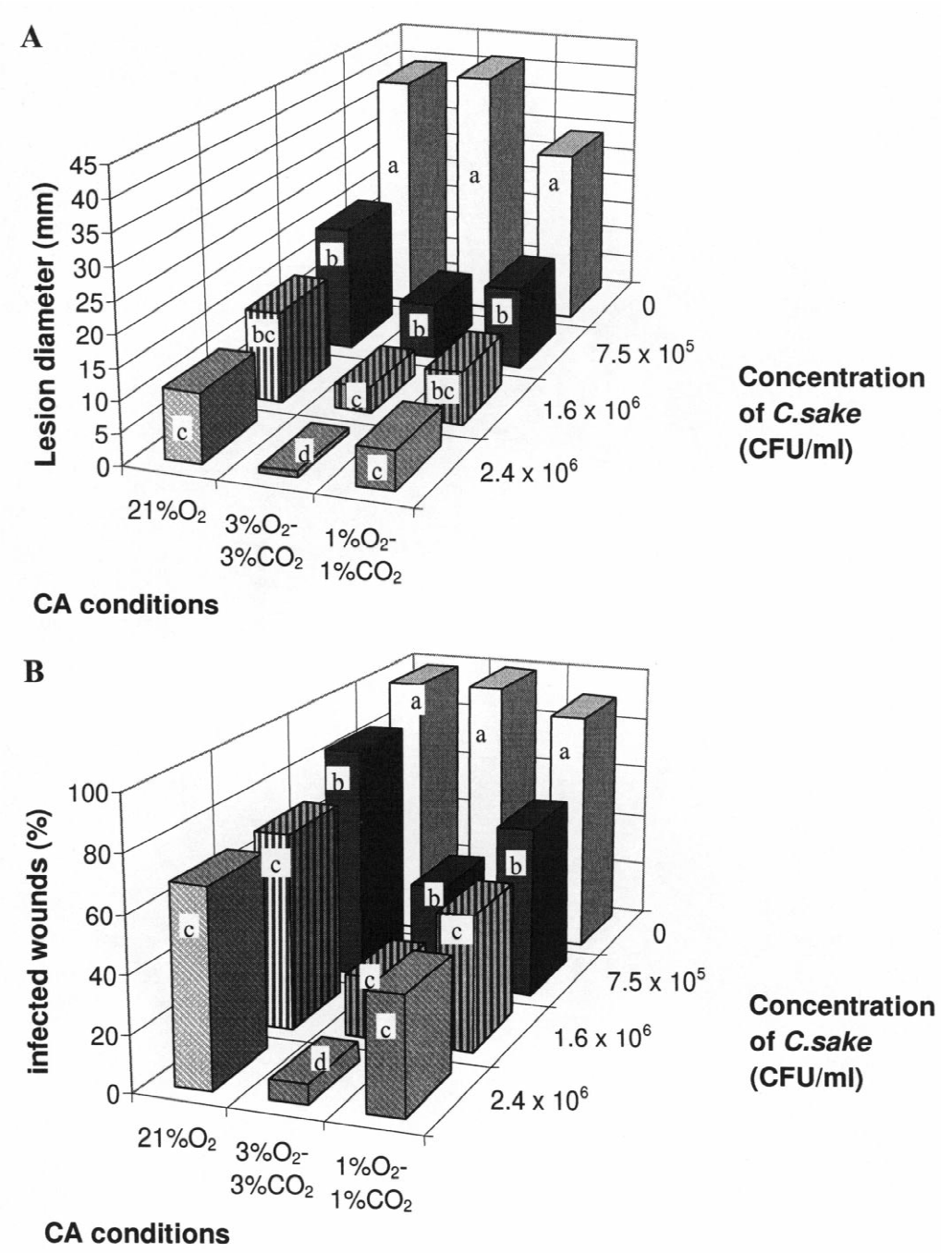

Fig. 1. Effect of *Candida sake* (CPA-1) on lesion diameters (A) or percentage of infected wounds (B) by *Penicillium expansum* on wounded Golden Delicious apples under three commercial controlled atmosphere (CA) storage conditions during 1994–95 season in laboratory trial. Fruits were wounded and inoculated with *C. sake* at the referred doses and *P. expansum* at 10^4 conidia/ml, and stored for 60 days at 1°C at the indicated atmosphere conditions. Different letters in the same atmosphere conditions, indicate significant differences between means according to Duncan's multiple range test ($P = 0.05$).

Table 1
Effect of *Candida sake* (CPA-1) on lesion diameters (mm) caused by *Penicillium expansum* on wounded Golden Delicious apples in a semi-commercial trial

Atmosphere conditions	Concentration of *C. sake* (CPA-1) CFU/ml		
	0	1.6×10^5	1.6×10^6
	Lesion diameters (mm)[a]		
21% O_2	11 a	6 b	2 c
3% O_2–3% CO_2	49 a	34 b	18 c
1% O_2–1% CO_2	48 a	27 b	17 c

[a] Means of 720 lesion diameters on apples (four lesions per fruit). Fruits were wounded and submerged for 30 s with antagonists at referred doses and *P. expansum* at 10^4 conidia/ml, and stored for 60 days at 1°C in 21% O_2 and 120 days at 1°C in 3% O_2 or 1% O_2. Within an atmosphere condition, numbers with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Table 2
Effect of seven atmosphere conditions on *Penicillium expansum* severity and *Candida sake* (CPA-1) effectiveness on wounded Golden Delicious apples

Controlled atmosphere conditions	Concentration of *C. sake* (CFU/ml)					
	0	10^6	10^7	0	10^6	10^7
	Season 1996–97			Season 1997–98		
	Lesion diameters (mm)[a]					
2% O_2–4% CO_2	35 a	24 a	15 a	30 a	23 a	15 ab
3% O_2–3% CO_2	33 a	25 a	14 a	28 a	20 a	10 b
2% O_2–6% CO_2	31 ab	23 ab	11 a	29 a	17 a	11 b
1% O_2–1% CO_2	24 bc	12 bc	7 b	37 a	25 a	19 a
3% O_2–6% CO_2	19 cd	8 cd	1 c	25 a	21 a	9 b
2% O_2–2% CO_2	18 cd	11 cd	3 bc	25 a	23 a	13 b
2%O_2–1%CO_2	15 d	7 d	3 bc	29 a	24 a	12 b

[a] Means of 90 lesion diameters on apples (two lesions per fruit). Fruits were wounded and submerged for 30 s with *C. sake* (CPA-1) at referred doses and *P. expansum* at 10^4 conidia/ml, and stored for 60 days at 1°C and indicated controlled atmosphere conditions. Values in columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

concentrations of *C. sake* (Fig. 2). As the concentration of the antagonist increased, control of *P. expansum* was also increased and in 1996–1997 season, the lesion size and the infected wounds were reduced by *C. sake* at 10^7 CFU/ml more than 70 and 55%, respectively (Fig. 2).

3.4. Population dynamics of C. sake under different atmosphere conditions

3.4.1. On apple surface

Populations of *C. sake* on apple surface showed the same pattern under all seven controlled atmosphere conditions (Fig. 3). During the first 2 weeks, the population decreased from 4- to 10-fold compared to the ones applied initially. By day 45 the population increased around the initial level, the highest was found at 1% O_2–1% CO_2 and the lowest at 3% O_2–3% CO_2. From this moment yeast population remained stable until the last sampling time at 90 days.

3.4.2. On apple wounds

Populations of *C. sake* in apple wounds were similar under all three tested storage conditions (Table 3). After the first 60 days at 1°C, the populations recovered ranged from 5- to 14-fold higher than the ones applied initially, and the lowest level was found at 21% O_2. By day 120, the population remained stable in the 3% O_2–3% CO_2 atmosphere and increased for the other two. At the last sampling time (150 days for 21% O_2 and 180 days for 3% O_2–3% CO_2 and the 1% O_2–1% CO_2), the populations of *C. sake* achieved similar levels of population at the three studied conditions. This level was about 20-fold bigger when compared to the initial applied concentration.

4. Discussion

In a previous study, we reported that *C. sake* (CPA-1) effectively controlled the major postharvest diseases on apples at cold storage temperature and during the simulated shelf life (Viñas et al., 1998).

Practically no experiments simulating biocontrol efficacy under low oxygen conditions of postharvest diseases on fruits have been reported (Jeffers and Wright, 1994; Lurie et al., 1995; Chad-Goyal and Spotts, 1997). In the present study *C. sake* increased or retained effectiveness in controlling *P. expansum* in controlled atmosphere storage compared with air, as shown in the laboratory trial. Similar results were obtained by Lurie et al. (1995) testing a *Candida oleophila* strain against *P. expansum* in nectarine

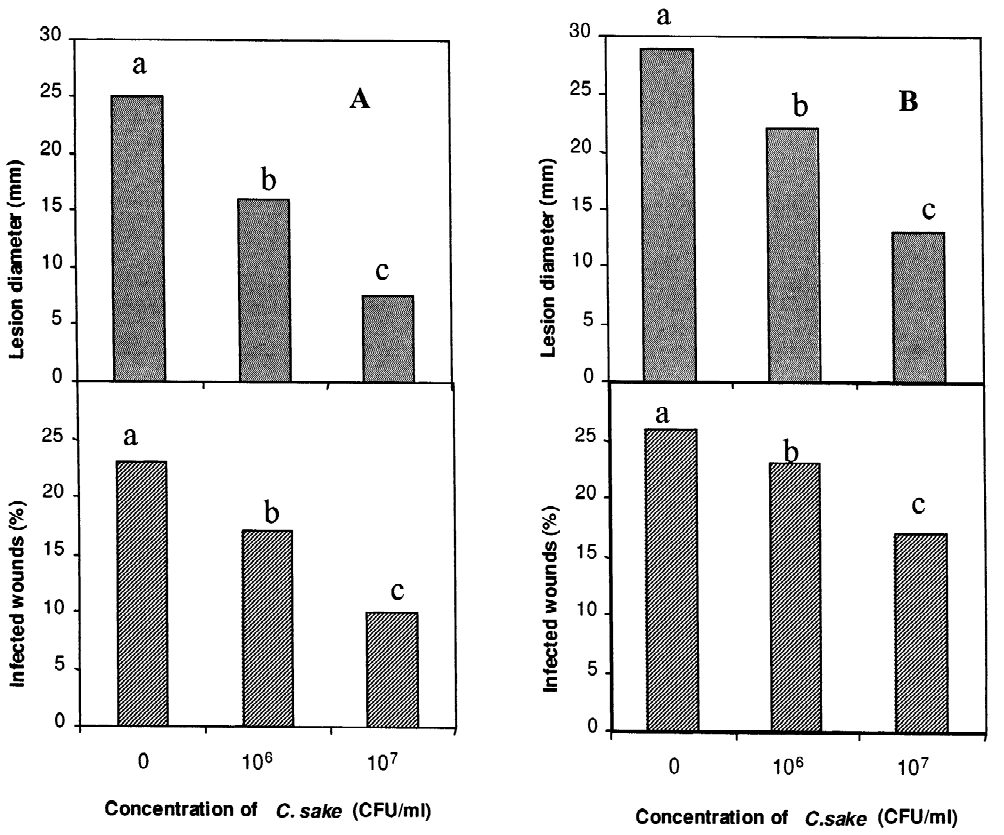

Fig. 2. Effect on *Penicillium expansum* decay of Golden Delicious apple by antagonist strain *Candida sake* (CPA-1). Fruits were wounded and submerged during 30 s with antagonist at referred doses and *P. expansum* at 10^4 conidia/ml, and stored for 90 days at 1°C and under seven controlled atmosphere conditions. (A) Season 1996–1997; (B) season 1997–1998. Values are means of the seven atmosphere conditions of three replicates of 15 fruits of two wounds each. Columns followed by the same letter (a–c) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

fruits and by Chad-Goyal and Spotts (1997) with three yeast strains against *P. expansum* in pears at 1% O_2–0.01% CO_2. In the laboratory and semi-commercial trials, the *C. sake* strain (CPA-1) effectively controlled *P. expansum* under all three storage conditions tested. In the laboratory trial, where results were obtained after the same storage periods, the lesion diameters were smaller in controlled atmosphere storage regimes. This may indicate a negative effect of the low oxygen on the growth of *P. expansum* or a better maintenance of natural resistance of fruits to decay under these conditions. The last idea was also introduced by El-Goorani and Sommer (1989). In the semi-commercial trial, however, results could not be compared between air and controlled atmosphere conditions, as they were obtained after different storage periods.

The different influence of seven studied controlled atmosphere conditions on *P. expansum* severity was not clear, because it was only significant in one of the two studied seasons. In contrast the control of blue mould with *C. sake* and the population dynamics of the yeast, were not different in any seasons by the seven studied controlled atmosphere conditions, so it could be used effectively in a wide atmosphere conditions range.

C. sake (CPA-1) was very effective in colonising apple wounds at low temperature and under commercial atmosphere conditions used in our area. The population of *C. sake* in apple wounds increased

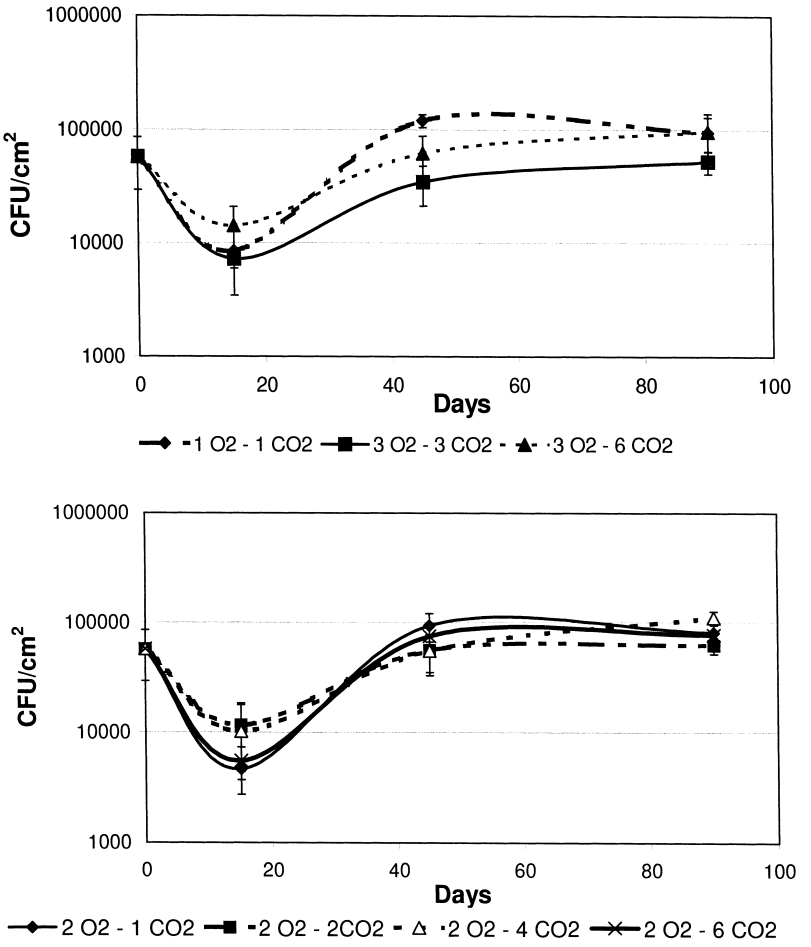

Fig. 3. Recovery of *Candida sake* from Golden Delicious apples surface. Fruits were wounded and submerged during 30 s with *C. sake* at 10^7 CFU/ml and stored for 90 days at 1°C and under seven controlled atmosphere conditions. Fruits samples were removed at various times to recover *C. sake* and the viable colonies per cm^2 of fruit surface were calculated. Bars represent the standard deviation.

Table 3
Population of *Candida sake* from wounds of Golden Delicious apples through time under several atmosphere conditions and 1°C[a]

Gas levels	Population and standard error of *C. sake* (CFU/wound), after several days since time of application									
	0 days		60 days		120 days		150 days		180 days	
	Pop.[b]	S.E.[c]	Pop.	S.E.	Pop.	S.E.	Pop.	S.E.	Pop.	S.E.
21% O_2	5×10^4	5×10^3	2.5×10^5	4×10^4	1.7×10^6	2×10^5	9×10^5	8×10^4	–[d]	
3% O_2–3% CO_2	4×10^4	5×10^3	5.0×10^5	7×10^4	2.5×10^5	2×10^4	–		7.8×10^5	6×10^4
1% O_2–1% CO_2	4×10^4	4×10^3	5.6×10^5	6×10^4	2.5×10^6	3×10^5	–		1.0×10^6	2×10^5

[a] Values are the mean populations per wound from tree apples.
[b] Pop., population of *C. sake*.
[c] S.E., standard error.
[d] –, not determined.

from 5- to 14-fold after 60 days under these storage conditions in apple wounds. At the end of the experiment, lower antagonist populations were found at 3% O_2 than 1% O_2, but in all three storage conditions yeast population increased about 20-fold over the initial level. This could indicate that there is no negative effect of low concentrations of oxygen on *C. sake* growth. In contrast the population of *C. sake* on the apple surface (including wounds) was very similar of the initial level after 90 days in seven studied controlled atmosphere conditions. This suggests that yeast growth occurs only in the wound sites which are the major point of entry for *P. expansum* (Viñas, 1990).

To confirm the potential of an antagonist for commercial application, it is necessary to conduct trials simulating commercial conditions, such as the semi-commercial trials done in the present work. The biocontrol potential of the antagonists is related to the number of viable cells (McLaughlin et al., 1990; Mari et al., 1996; Chad-Goyal and Spotts, 1997; Viñas et al., 1998), and to achieve effective control, it is sometimes necessary to use very high antagonist concentrations. Our results under semi-commercial conditions confirm the usefulness of *C. sake*, applied at a concentration of 1.6×10^6 CFU/ml, in controlling blue mould in commercial storage conditions. Pilot trials under usual packinghouse conditions were conducted in order to assess the antagonist's potential as a commercial product. Results show that its effectivity equals the one of the most effective commercially authorised fungicide for postharvest of pome fruits (unpublished data).

Many authors have suggested (Spotts and Cervantes, 1986; Viñas, 1990) that pathogens infect fruit during postharvest treatments. Spores are ubiquitous and are deposited, especially from the atmosphere in the cold storage chambers, efficiently from water in drenches, and by contamination from fruits and bins spread through water by contact. Therefore the application of the yeast antagonist and the pathogen through dipping is close to commercial practice.

The mechanism of biocontrol has not been fully determined, but the strain *C. sake* (CPA-1) does not produce any antibiotics (Usall, 1995). Effective nutrient competition and site exclusion may play major roles in biological control of *P. expansum*, since the yeast can colonize apple wounds readily. Studies are now in progress to examine scale-up fermentation on cheap industrial waste materials, formulations and shelf-life of *C. sake* (CPA-1) in order to produce a commercial product for practical application in packinghouses.

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References

Beech, F.W., Davenport, R.R., 1970. The role of yeast in cider-making. In: Rose, A.H., Harrison, J.S. (Eds.), The Yeasts, Vol. 3, Academic Press, London, pp. 73–146.

Buhagiar, R.W., Barnett, J.A., 1971. The yeast of strawberries. J. Appl. Bacteriol. 34, 727–739.

Chad-Goyal, T., Spotts, R.A., 1997. Biological control of post-harvest diseases of apple and pear under semi-commercial and commercial conditions using three saprophytic yeasts. Biol. Control 10, 199–206.

Eckert, J.W., Ogawa, J.M., 1988. The chemical control of post-harvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. Annu. Rev. Phytopathol. 26, 433–469.

El-Goorani, M.A., Sommer, N.F., 1989. Effects of modified atmospheres on postharvest pathogens of fruits and vegetables. In: Janick, J. (Ed.), Horticultural Reviews, AVI, Wesport, CT, pp. 412–461.

Herrero, A., Guardia, J., 1991. Conservación de Frutos. Manual Técnico, Mundi Prensa, Madrid, 409pp.

Hofstein, R.S., Friedlender, T., Chalutz, E., Droby, S., 1994. Large-scale production and pilot testing of biological control agents for postharvest diseases. In: Wilson, C.L., Wisniewski, M.E. (Eds.), Biological Control of Postharvest Diseases: Theory and Practice, CRC Press, Boca Ratón, FL, pp. 89–100.

Hurley, R., de Louvois, J., Mulhall, A., 1987. Yeast and human and animal pathogens. In: Rose, A.H., Harrison, J.S. (Eds.), The Yeasts, Vol. 1, Academic Press, New York, pp. 207–281.

Janisiewicz, W.J., 1987. Postharvest biological control of blue-mould on apples. Pythopathology 77, 481–485.

Jeffers, S.N., Wright, T.S., 1994. Biological control of postharvest diseases of apples: progress and commercial potential. New Engl. Fruits Meet. 100, 100–106.

Jijakli, M.H., Lepoivre, P., Tossut, P., Thomard, P., 1993. Biological control of *Botrytis cinerea* and *Penicillium* sp. on postharvest apples by two antagonistic yeasts. Med. Fac. Landbouww. Univ. Gent 58 (3b), 1349–1358.

Lurie, S., Droby, S., Chalupowicz, L., Chalutz, E., 1995. Efficacy of *Candida oleophila* strain 182 in preventing *Penicillium expansum* infection of nectarine fruits. Phytoparasitica 23, 231–234.

Mari, M., Guizzardi, M., Pratella, G.C., 1996. Biological control of gray mould in pears by antagonistic bacteria. Biol. Control 7, 30–37.

McLaughlin, R.J., Wisnisiewski, M.E., Wilson, C.L., Chalutz, E., 1990. Effects of inoculum concentration and salts solutions on biological control of postharvest diseases of apples with *Candida* sp. Phytopathology 80, 456–461.

Palazón, I., Palazón, C., Robert, P., Escudero, I., Muñoz, M., Palazón, M., 1984. Estudio de los problemas de la conservación de peras y manzanas en la província de Zaragoza. p 68. Diputación Provincial Zaragoza. Publicación n. 990. Institución Fernando El Católico.

Pusey, P.L., 1994. Enhancement of biocontrol agents for postharvest diseases and their integration with other control strategies. In: Wilson, C.L., Winiewski, M.E. (Eds.), Biological Control of Postharvest Diseases. Theory and Practice, CRC Press, Boca Ratón, FL, pp. 77–89.

Pusey, P.L., Hotchkiss, M.W., Dullmage, H.T., Baumgardner, R.A., Zehr, E., Reilly, C.C., Wilson, C.L., 1988. Pilot tests for commercial production and application of *Bacillus subtilis* (B-3) for postharvest control of peach brown rot. Plant Dis. 72, 622–626.

Roberts, R.G., 1990. Postharvest biological control of grey mould of apple by *Cryptococcus laurentii*. Phytopathology 80, 526–530.

Shewfelt, R.L., 1986. Postharvest treatment for extending the shelf life of fruits and vegetables. Food Technol. 40, 70–80.

Spotts, R.A., Cervantes, L.A., 1986. Population pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. Plant Dis. 70, 106–108.

Usall, J., 1995. Control biològic de *Penicillium expansum* en postcollita de fruita de llavor. Universitat de Lleida. Catalonia, 194 pp.

Viñas, I., 1990. Principios básicos de la patología de post-cosecha. Frut 5, 285–292.

Viñas, I., Usall, J., Teixidó, N., Sanchis, V., 1998. Biological control of major postharvest pathogens on apple with *Candida sake*. Int. J. Food Microbiol. 40, 9–16.

Wilson, C.L., Chalutz, E., 1989. Postharvest biological control of *Penicillium* rot of citrus with antagonistic yeast and bacteria. Sci. Hortic. 40, 105–112.

Wilson, Ch.L., Wisniewski, M.E., Droby, S., Chalutz, E., 1993. A selection strategy for microbial antagonists to control postharvest diseases of fruits and vegetables. Sci. Hortic. 53, 183–189.

Woodhead, S.H., O'Leary, A.L., O'Leary, D.J., Rabatin, S.C., 1990. Discovery, development and registration of a biocontrol agent from an industrial perspective. Can. J. Plant Pathol. 12, 328–331.