Effectiveness of $CaCl_2$ and Tween 80 in Enhancing Yeast Biocontrol Activity against *Penicillium digitatum* on Tarocco Orange

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Abstract

Postharvest biocontrol activity of $CaCl_2$ and four yeasts against *Penicillium digitatum* was tested on Tarocco oranges. All tested organisms (*Pichia anomala* J121, *Pichia guilliermondii* NRRL Y18314, *Debaryomyces hansenii* DBVPG 4025 and *Saccharomyces cerevisiae* P1.6) significantly reduced mould incidence and severity. Application of $CaCl_2$ enhanced biocontrol efficacy of *P. guilliermondii* and *S. cerevisiae*, while it did not significantly affect biocontrol of *P. anomala* and *D. hansenii*.

Keywords: *Penicillium digitatum*; orange; postharvest; yeast; biocontrol

INTRODUCTION

Moulds caused by *Penicillium italicum* and *P. digitatum* represent the major microbial citrus diseases during storage. The high susceptibility of some citrus species and cultivars, the widespread diffusion of *Penicillia* and their ability to grow at cool storage temperatures requires the use of pesticides to control these diseases coupled with storage techniques. Imazalil (IMZ), thiabendazole (TBZ) and sodium orthophenylphenate (SOPP) act on conidia of the fungi on fruit surface, but have low activity after infection when the mycelium is already inside fruit. Furthermore, the massive use of TBZ and IMZ has lead to selection of resistant fungal phenotypes and although these molecules have low toxicity, they may also remain as residues on fruit surface. These factors have promoted research to develop alternative and integrated methods to control *Penicillium* infection (DROBY *et al*. 1998). Application of Ca^{2+} salts on fruit epicarp seems to play an important role in reducing physiological post-harvest alterations (CONWAY 1982). It has been proposed that application of Ca^{2+} salts increases fruits total calcium content as well as cell wall calcium bonds, through cross-linkages of pectins enhancing their resistance to fungal cell wall degrading enzymes (CONWAY *et al*. 1988). Ca^{2+} salts were proven to be involved in inhibition of postharvest decay of potatoes and apples (MC GUIRE & KELMAN 1986; CONWAY *et al*. 1988). A similar calcium action has been described in the control of *Botrytis* infection on rose flowers (VOLPIN & ELAD 1991), beans and tomatoes (ELAD & VOLPIN 1993), eggplants, peppers and cucumbers (ELAD *et al*. 1993). MC LAUGHLIN *et al*. (1990) demonstrated the role of Ca^{2+} salts in improving the efficacy of yeast biocontrol against *Botrytis* and *Penicillium* rots on apples. Preliminary studies have shown that addition of 2% $CaCl_2$ to *Candida oleophila* enhances the yeast's protection on post-harvest decay of apples (WISNIEWSKI *et al*. 1995). $CaCl_2$ plays a major role in controlling *P. digitatum* moulds on grapefruit (CHALUTZ *et al*. 1992) and on orange cv. Washington Navel (ARRAS *et al*. 2001). The aim of this paper was to assess the influence of 1% $CaCl_2$ + 0.1% Tween 80 on postharvest biocontrol efficacy of different yeasts, some of which are known

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to be active against *P. digitatum*. Tarocco orange was selected as it appears to be the most susceptible Italian citrus variety to green mould.

MATERIALS AND METHODS

Trials were carried out on Tarocco oranges harvested in February and March 2002 from 10 year-old plants in a farm near Catania (Sicily, Italy) where pesticides are not used. Fruits were selected on the basis of uniform ripening and absence of epicarp lesions, and were stored at 5°C until use.

D. hansenii DBVPG 4025 (isolated from grapes) was obtained from Dipartimento di Biologia Vegetale di Perugia; *P. anomala* J121 (isolated from airtight stored grain) from Department of Microbiology – Uppsala Genetic Centre (Sweden); *P. guilliermondii* NRRL Y18314 (isolated from the surface of lemons) was from ARS Culture Collection – Illinois (WILSON & CHALUTZ 1989); *S. cerevisiae* was isolated from sour-dough (Sweden). Yeasts were cultured on YPDA for 48 h growth cycles. *P. digitatum* was obtained from rotting orange surface and cultured on PDA and Malt-Agar. Conidia were scraped from a 6–10 days old M. A. plate and filtered through sterile gauge. Conidial concentration was titred by a haemocytometer and adjusted to 10^5 conidia/ml. Groups of five Tarocco orange fruits were washed with tap water and their surfaces disinfected by dipping in 70% ethanol and air dried. The epicarp was wounded with a conic needle around the button (4 wounds per fruit 1.5 mm wide and 3 mm deep). Each fruit was dipped in a suspension containing 10^8 cfu/ml of the yeast in water or in 1% $CaCl_2$ + 0.1% Tween 80 and air dried. After one hour, fruits were sprayed with the spore suspension. In standard reference controls fruits were treated with sterile water (negative) and conidia suspension only (positive). The oranges were placed in the dark at 22°C with 95% R. H. and infection monitored daily. Symptoms were scored when positive control reached 100% infection (after six days). Spread and severity of the mould were evaluated.

Each treatment was performed on five fruits and was replicated three times. The experiment was repeated three times. Inhibition was expressed as a percentage of infected wounds compared to the untreated control as (n° infected wounds in control – n° infected wounds in the treatment) × 100/n° infected wounds in control. Severity per wound was scored using a symptom ladder ranging from 0 to 2 (where 0 = no symptoms, 1 = rind softening, 2 = presence of mycelium on the fruit surface). The sum of the four values for each fruit was recorded as severity of infection per fruit ranging from 0 to 8. Results from the three replications were averaged and variance analysed (Table 1).

RESULTS AND DISCUSSION

All treatments differed significantly from the negative and the positive controls, both regarding inhibition of the pathogen and severity of infection. Treatment with 1% $CaCl_2$ + 0.1% Tween 80 reduced inhibition by 54% and symptoms by over 50%. *P. guilliermondii* NRRL with 1% $CaCl_2$ + 0.1% Tween 80 determined major inhibition (80%), while *S. cerevisiae* P1.6 alone was the least effective (40%). Application of 1% $CaCl_2$ + 0.1% Tween 80 significantly improved biocontrol efficacy of *P. guilliermondii* and *S. cerevisiae* (+18% and +28%, respectively).

In agreement with the literature (DROBY *et al*. 1997; CHALUTZ *et al*. 1992), the results revealed enhanced inhibition when *P. guilliermondii* was applied with $CaCl_2$. Different interactions among calcium ions, host, pathogen and yeast may cause the beneficial effect of combining $CaCl_2$ and the yeast. Application of 1% $CaCl_2$ + 0.1% Tween 80 did not significantly

Table 1. Yeast biocontrol activity against *Penicillium digitatum* on Tarroco orange

Strain	Severity[a]		Inhibition (%)	
	H_2O	$CaCl_2$ + Tween 80	H_2O	$CaCl_2$ + Tween 80
D. hansenii DBVPG 4025	2.89 de	2.24 def	61 cd	65 bcd
P. guilliermondii NRRL Y18314	2.60 de	1.20 f	62 bc	80 a
S. cerevisiae P 1.6	4.07 bc	1.93 ef	40 e	68 bcd
P. anomala J121	1.76 ef	2.38 de	73 ab	63 bcd
No yeast	7.33 a	2.93 cd	0 f	54 de

a: scored on a 0–2 range scale per wound × 4 wounds per fruit. Values with the same letter were not significantly different according to LDS (Least Significant Difference) test with $P = 0.1$

affect the efficacy of *D. hansenii* and *P. anomala* and this behaviour may be related to differences in yeasts' Ca^{2+} regulation metabolism. Maintenance of low free cytosolic Ca^{2+} is essential for normal cell function (RASMUSSEN & RASMUSSEN 1990). DROBY *et al*. (1997) reported that in yeast the ability to grow in presence of high Ca^{2+} concentration is dependent on the correct function of intracellular regulatory systems (as the exchanger H^+/Ca^{2+}). DI SILVESTRO *et al*. (2000) reported that *P. guilliermondii* NRRL Y18314 and *P. anomala* J121 showed the best colonisation of Tarocco fruit epicarp and wounds. Thus, the high inhibition activity of these yeasts may be related to their fitness and competition for nutrients. High yeast population on fruit surface may jeopardise commercialisation of the product. Use of Ca^{2+} salts may dramatically decrease the yeast's concentration 10 to 100 fold (MC LAUGHLIN *et al*. 1990; DROBY *et al*. 1997), and achieve the same level of protection.

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