POSTHARVEST BIOCONTROL OF *PENICILLIUM* ROT OF LEMONS WITH INDUSTRIAL YEASTS

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Penicillium digitatum Sacc., which causes lemon fruit rot, accounts for most decay losses during postharvest storage in New Zealand (Sale 1993) and worldwide (Bancroft *et al.* 1984). Overseas, postharvest application of the fungicides benomyl and thiabendazole has provided excellent control of fungicide-sensitive strains of *P. digitatum* and *P. italicum* Wehm. on lemons during storage and marketing (Eckert 1978). Unfortunately, widespread and intensive use of these fungicides has resulted in selection of *Penicillium* isolates that are resistant to these fungicides (Eckert 1982). These fungicide resistance problems, and public awareness of pesticide residues on food, have stimulated efforts to find alternative means of controlling postharvest rots of citrus.

Biological control of postharvest diseases of fruit using antagonistic micro-organisms isolated from plant tissues has been successfully achieved with lemons (De Matos 1983; Wilson and Chalutz 1989). This paper reports the screening of industrial yeasts for control of *Penicillium* rot in lemons.

Isolates of yeasts (Table 1) used in food processing were obtained from the Department of Processing and Environmental Technology, Massey University, Palmerston North and the culture collection at Levin Research Centre. Inoculum *P. digitatum* cultures were established on malt extract agar (MEA) from spores isolated from a diseased fruit. Spore suspensions (10^4 spores/ml) were made by washing spores from a 10 - 14-day-old MEA culture with water. These suspensions were used to inoculate unsprayed lemons (cv Genoa) from a Levin home garden for the tests.

Yeast isolates were screened by a modification of the screening method used by Roberts (1990). Cultures of individual isolates to be tested were grown on nutrient yeast dextrose agar (NYDA) for 2 days. Yeast cell suspensions of the isolates were made by diluting a loop of the culture in sterile distilled water and the cell concentrations were adjusted to about 10^8 colony-forming units (cfu/ml), determined by dilution plating. Similar concentrations of cell suspensions were also made for TV1, ST1 and LN4 (Table 1).

Lemons which had been washed in 70% alcohol for about 1 min and dried, were wounded at four sites on the side of the fruit with a dissecting needle. The wounds were about 4 mm wide x 3 mm deep. The wounds were filled with the cell suspensions (20 µl) of test organisms. After the wound site had dried, each wound was challenged with 20 µl of *P. digitatum*. Control fruit were treated with water. Treated fruit were placed in covered plastic containers with moistened paper towels to maintain high humidity. Five fruit per treatment were used and the experiment was repeated five times. Disease assessments were made after 4 days incubation at 20°C by counting the number of fruit showing *Penicillium* rot. Data were analysed with analysis of variance using the SAS statistical package.

Of 150 industrial yeast isolates screened, we found seven isolates which reduced *Penicillium* rot, with one *Saccharomyces cerevisiae* Meyer ex Hansen. isolate (S20) and three *Kluyveromyces* isolates (K51, K52 and K58) providing complete control (Table 1). Other biocontrol agents (TV1, ST1 and LN4) were not as effective.

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TABLE 1: Incidence of *Penicillium* rot on lemon fruit treated with yeast isolates and other potential biocontrol agents.

Isolates	Biocontrol Agent	Mean % rotted fruit
S20	<i>Saccharomyces</i> sp	0 c ¹
K51	<i>Kluyveromyces</i> sp	0 c
K52	<i>Kluyveromyces</i> sp	0 c
K58	<i>Kluyveromyces</i> sp	0 c
H80	<i>Hansenula</i> sp	5 c
K48	<i>Kluyveromyces</i> sp	10 c
H78	<i>Hansenula</i> sp	35 b
TV1	<i>Trichoderma</i> sp	50 b
ST1	<i>Streptomyces</i> sp	85 a
LN4	<i>Bacillus</i> sp	100 a
Control	Water	95 a

¹ Means followed by the same letter are not different (P=0.05).

To determine whether potential yeast antagonists produced antibiotics, discs of agar (1 cm diam.) containing the yeast isolates (Table 1) were placed on NYDA plates seeded with 1×10^7 spores/ml of *P. digitatum*. The plates were then incubated for 4 days at 20°C and any zones of inhibition around the discs noted.

None of the seven potential antagonistic yeasts produced zones of inhibition against *P. digitatum* on NYDA media.

In this study, we found one *S. cerevisiae* isolate and three *Kluyveromyces* spp. isolates to be highly effective for control of *Penicillium* rot in lemons. This is the first report of effective control of postharvest disease using industrial yeast isolates. As none of these yeast isolates showed evidence of antibiosis toward *P. digitatum*, it seems unlikely that antibiosis is an important aspect of their mode of action (Chalutz *et al.* 1988). In a separate experiment, a study on mode of action indicated that K52 is an effective competitor against *Botrytis cinerea* Pers. for glucose in NYDA culture medium (Cheah, unpublished data). Droby *et al.* (1989) found that an effective yeast isolate, *Debaryomyces hansenii* did not produce antibiotic substances in culture, but showed that competition for nutrients may play a role in biocontrol of *P. digitatum* on grapefruit. Since all these yeast isolates are used in food processing (Clark 1990) and appear to control botrytis storage rot in kiwifruit (Cheah and Hunt 1994), they are likely to gain consumer acceptance as biocontrol agents for control of fruit storage rots.

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