SCREENING OF INDUSTRIAL YEASTS FOR BIOCONTROL OF BOTRYTIS STORAGE ROT IN KIWIFRUIT

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Fungicides are a primary means to control postharvest disease, but they have recently come under special scrutiny as posing potential oncogenic risks when applied to process foods. In New Zealand, seven dithiocarbamate fungicides have recently been withdrawn by various manufacturers (NZ Apple & Pear Marketing Board, Circular 1990/1). There are still many pesticides, including fungicides, currently under review by the Environmental Protection Agency of U.S.A. Further, even when acceptable fungicides are applied for the control of postharvest diseases, pathogens often develop resistance to them (Spotts and Cervantes 1986). For these reasons, there is urgent need for new and effective means of postharvest disease control.

Over the past 5 years, we have isolated and identified yeasts, fungi and bacteria with potential as biocontrol agents against postharvest disease fungi including *Botrytis cinerea* Pers. and *Penicillium* spp. We found three isolates of *Trichoderma* spp. that were highly effective against *B. cinerea* in kiwifruit (Cheah *et al.* 1992). However, these fungal isolates produce an active mycotoxin compound, harziano-pyridine (Cutler and Jacyno 1991), so we decided to screen for safe and effective biocontrol agents that do not produce mycotoxins or antibiotics. This paper reports the screening of yeasts, most of whom do not produce antibiotics (Droby *et al.* 1991), for control of botrytis storage rot in kiwifruit (*Actinidia deliciosa* (Chevalier) Liang & Ferguson).

Botrytis cinerea cultures were established on malt extract agar (MEA), each from a single spore isolated from a diseased fruit. Spore suspensions were made by washing spores from 10 to 14-day-old MEA cultures with water. These suspensions were used to inoculate the fruit. Industrial yeast isolates which are used for food processing were obtained from the Processing and Environmental Technology Department, Massey University and the culture collection at Levin Research Centre. Unsprayed kiwifruit (cv. Hayward) growing at Levin Research Centre were picked with pedicels attached. Pedicels were then removed just before inoculation.

Yeast isolates were screened using the method of Roberts (1990). Cultures of individual isolates were grown on nutrient yeast extract dextrose agar (NYDA) for 2 days. Yeast cell suspensions of the isolates were made by taking a loop of culture diluted in sterile distilled water in a test tube, and cell concentrations were adjusted to about 1×10^8 colony forming units (cfu) per ml determined by dilution plating. In initial screening, a 30 μ l droplet of yeast cell suspension was placed at the picking wound of each kiwifruit and then challenge-inoculated with *B. cinerea* spore suspension (10,000 spores/fruit in 30 μ l). Control fruit were not treated with yeasts but received *B. cinerea* spores. Twenty fruit per treatment were used. Treated fruit were held at 22°C. Incidence of botrytis rot was recorded 10 days after treatment.

The six most effective isolates (Table 1) were tested again on kiwifruit using the above methods. After treatment, fruit were randomly arranged in commercial trays held at 22°C for 3 days, then stored at 0°C (ca. 95% RH). Botrytis storage rot was assessed weekly from 6 weeks up to 14 weeks of storage. One hundred fruit per treatment were used. Experiments were repeated four times for each treatment, and data were analysed by analysis of variance using the SAS statistical package.

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

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seeded with 1×10^5 spores/ml of *B. cinerea*. The plates were then incubated for 5 days at 20°C and observed for antibiotic zones around the discs.

Over 120 yeast isolates were screened. Six of these reduced botrytis storage rot incidence from 30% to about 6% (Table 1). Two *Kluyveromyces* isolates were more effective than the other four isolates. None of these yeasts isolates produced zones of inhibition against *B. cinerea* on NYDA, indicating that antibiosis is not an important aspect of their mode of action (Chalutz *et al.* 1988).

TABLE 1: Incidence of botrytis storage rot in kiwifruit treated with different yeast isolates. Picking wounds were inoculated with yeasts then challenge-inoculated with *Botrytis* spores.

Isolate	Yeast	Mean % rotted fruit ¹
K52	<i>Kluyveromyces marxianus</i>	6
K58	<i>Kluyveromyces fragilis</i>	6
H80	<i>Hansenula capsulata</i>	12
K48	<i>Kluyveromyces fragilis</i>	12
S20	<i>Saccharomyces cerevisiae</i>	13
K47	<i>Kluyveromyces fragilis</i>	14
Control	Water	33
LSD (P<0 .05)		3.2

¹ Four replications of 100 fruit per treatment.

Since our industrial yeast isolates are commonly used in food processing (Clark 1990; Mawson 1990), and appear to control botrytis rot, they are probably a very good prospect to gain consumer acceptance. Further work is being carried out to study their mode of action and to enhance their ability to control disease.

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