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Microbial Biocontrol of Postharvest Papaya Diseases

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Abstract: The commercial application of biocontrol using epiphytic organisms from fruit has potential to limit postharvest disease losses. Biocontrol in conjunction with other plant extracts and coating is the focus of this project. In the first six months of this project using allied funding, we have screened forty-five epiphytic organisms from papaya fruit. Two yeasts and one bacteria have been shown to be effective in inhibiting Anthracnose development on wounded papaya fruit. We are continuing to screen epiphytic organisms. Selected organisms will be evaluated in our USAID-HortCRSP project with Sri Lanka using plant extracts, coatings and GRAS compounds.

Introduction:

Papaya fruit postharvest losses of up to 75% have been reported to Hawaii shippers by mainland USA wholesalers and retailers. These losses are associated with postharvest disease, often associated with storing color-break fruit for more than three weeks at temperatures of 10ºC or lower and mechanical injury (Paull *et al., 1997*). The major postharvest diseases of papaya are anthracnose (*Colletotrichum gloeosporiades*), Rhizopus, stem end rot and black spot. Postharvest disease has been controlled by hot water dips, hot water spray treatments and fungicides (Couey and Farias, 1979). The usages of postharvest fungicides are being curtailed by the appearance of pathogen resistance, lack of new fungicides and negative public attitude. Physical treatments such as heat (Akamine and Arisumi, 1953) have been used successfully on papaya in Hawaii, however, heat treatments are expensive, can cause injury to the fruit and is sometimes difficult to incorporate into the postharvest handling system. The other potential approach is biocontrol.

The commercial application of biocontrol and its potential have been recently reviewed (Janisiewicz and Korsten. 2002; Fravel, 2005) and has shown potential for papaya (Gamagae *et al., 2004;* Capdeville *et al., 2007*). Capdeville *et al.*, (2007) showed that thirty different yeast isolates inhibited mycelia growth. Subsequent *in vivo* experiment with infected fruit indicated two of the thirty-one were most effective in reducing disease development. The mode of action of these isolates is unknown and no simulated shipping was included in the testing.

This project is developing and evaluating a biological-based approach to postharvest disease control. The epiphytic microorganisms isolated from papaya fruit are being evaluated for their ability to control postharvest disease by their actions as antagonistic microorganisms to the disease organisms. This research follows from our successful isolation of a yeast for pineapple postharvest disease control (Reyes *et al., 2004*). The output from this project would provide a postharvest disease control method for organic papaya production and an alternative to fungicide in conventional production.

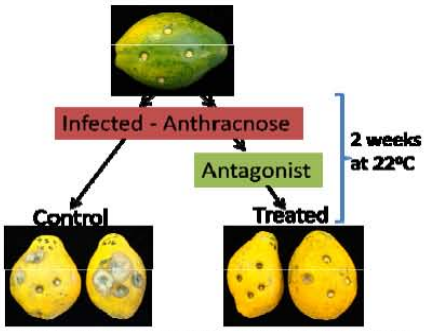

Figure 1. General protocol use to evaluate epiphytic yeast and bacteria isolated from papaya fruit.

Materials and Methods:

C. gloeosporiades was isolated from fruit that developed Anthracnose. Cultures were maintained on potato dextrose agar (PDA) at 4ºC and routinely inoculated, and re-isolated from papaya fruit to maintain pathogenicity.

Microbial isolations were done by washing individual fruit in sterile jars (2 L) with 400 mL of sterile distilled water on a rotary shaker at 100 min^{-1} for 10 minutes. Serial dilutions of the wash water was plated out on PDA and Wort agar (Difco) to determine the best dilutions for microbial counting.

The infection courts were created on papaya fruit by making four wounds (1 mm deep x 7 mm diameter) on the fruit body (Figure 1). The antagonistic bacteria were grown on PDA and the yeast on yeast extract agar medium at 25ºC for one day before being applied to the infection court. A 30 µL drop of each suspension was applied to the infection court at different times (0, 24 and 48 h) after inoculation with (30 µL) of the 10^8 spores/ml *C. gloeosporiades* suspension. Control fruit were treated with sterile distilled water. Fruit were held at 22ºC and evaluated for incidence and severity of disease. Incidence was the percent of wounds that developed disease and severity was disease area size (mm).

Results and Discussion:

We have screened 43 yeasts and two fast-growing bacteria isolated from the surface of papaya grown in a field that has not been sprayed with pesticides. The isolates were evaluated as outlined in Figure 1, with the pathogen, Anthracnose, applied before the antagonist. It is planned to also evaluate the selected isolates against other postharvest disease organisms such as *Rhizopus* and selected pathogens that cause stem-end rot.

Table 1. Effective of four different yeast isolates in inhibiting Anthracnose pathogen development when applied the same day and evaluated 9, 12 and 16 days after pathogen inoculation. The control did not receive the antagonist.

	Incidence (%)					Severity (mm)				
Days	Control	#141	#581	#961	#1061	Control	#141	#581	#961	#1061
9	33	33	25	42	8	6	4	3	6	1
12	58	67	42	67	25	23	12	7	14	5
16	83	67	42	75	50	26	23	14	29	15

Yeast isolates #581 and #1061 were the most effective in limiting Anthracnose incidence and severity when applied the same day as pathogen inoculation (Table 1). Delaying application of the antagonist reduced its ability to inhibit pathogen development (Table 2). The yellow bacteria was a more effective inhibitor than the bacteria whose colony was white, and bacteria were more effective than the yeasts (Table 2). The two Gram⁻ bacteria isolates showed no inhibitory action against other bacteria on the same plate suggesting that it was more effective in competing for nutrients than releasing biostatic compounds.

Table 2. Application of yeast and bacteria antagonists on the same day and four days after pathogen (Anthracnose) application on disease incidence and severity. Evaluated 12 days after pathogen application. The control did not receive the antagonist.

	Control		Yeast #581		Bacteria (Yellow)	
Application	Incidence	Severity	Incidence	Severity	Incidence	Severity
Same day	58	14	42	7	8	1
4 days after	42	12	33	9	42	14

The prevailing view is that competition for nutrients and space are the major mechanism for biocontrol and rapid colonization by the antagonist and is critical. Manipulation of the applied material can therefore play a significant role in the effectiveness of biocontrol. Approaches to increasing effectiveness include combinations of organisms, modified yeasts with antifungal peptide, heat treatments, addition of low dose fungicides, wax coatings, sodium bicarbonate, calcium chloride and other Generally Regarded as Safe (GRAS) nutrient analogs, and integration with other postharvest treatments. Three GRAS compounds are calcium chloride, sodium bicarbonate and chitosan. Sodium bicarbonate fungistatic properties relate to its ability to delay and inhibit spore germination and does not have a persistent effect. Chitosan and its derivatives have shown some postharvest disease control potential with papaya (Hewajulige *et al.*, 2007). We also intend to follow up on published results. Gamagae *et al.* (2004) who found that commercial biocontrol agent, Aspire, gave some control of anthracnose on papaya in conjunction with wax and bicarbonate, with wax alone giving significant disease control and reduced disease severity. It is also crucial in evaluation that biocontrol agents are tested in simulated handling conditions that include a storage and shipping component. This will be the focus of the USAID - HortCRSP with Sri Lanka.

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