The Interaction of Four Bacteria Causing Pink Disease of Pineapple with Several Pineapple Cultivars

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ABSTRACT

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Four bacterial isolates representing three genera, *Gluconobacter, Acetobacter,* and *Enterobacter* caused pink disease of pineapple fruit. Each genus exhibited slight differences in symptoms in uncooked fruit. The *Gluconobacter*-type symptom was characterized by a "cantaloupe-like" aroma and a light- to bright-pinkish color of the raw fruit flesh. The *Acetobacter* type was characterized by no aroma and a light- to dark-brown color. The *Enterobacter*-type symptom did not exhibit any pink or brown discoloration of infected tissues prior to cooking. All types produced dark browning of the fruit flesh following cooking. All isolates were consistently reisolated from fruit showing typical symptoms. The *Enterobacter* type was the most virulent. One isolate of the *Gluconobacter* type was highly virulent, but the other isolate was only weakly virulent. Virulence varied significantly relative to harvest period, cultivar, and isolate.

Additional key words: bacterial disease, acetic acid bacteria, bacterial types, cultivar susceptibility.

Bacterial pink disease of pineapple in Hawaii is characterized by a dark-brown discoloration which develops in the fruit flesh during the canning process (8). Prior to cooking, infected fruit either may be symptomless or may vary in color from a light pink or brown color to a dark brown color. Occasionally a "cantaloupe-like" aroma may be associated with diseased fruit. Preliminary physiological testing indicated that the causal organisms are acetic acid bacteria in the genera *Acetobacter* and *Acetomonas* (the latter was changed to *Gluconobacter* in Bergey's 8th edition) (2, 7).

A brief report by Buddenhagen and Dull (2) as well as an earlier unpublished report (Buddenhagen and Smith, Pineapple Research Institute of Hawaii, *unpublished report*) have indicated that strain differences occur. However, identification of strains was difficult prior to the development of a successful field inoculation technique (8). The following study was undertaken to demonstrate the existence of different bacterial types as causal agents of pink disease of pineapple, and to determine if they interacted differently with several pineapple cultivars. Knowledge of this interaction would aid in screening of cultivars for resistance.

MATERIALS AND METHODS

Cultivar culture and test design.—Seven unnamed pineapple cultivars [*Ananas comosus* (L.) Merr.], designated A to F, and X (the commercial cultivar, Smooth Cayenne) from the breeding program of the Pineapple Research Institute of Hawaii were selected on the basis of varying natural or induced (8) pink disease susceptibility. All cultivars were grown by the standard cultural practices for Smooth Cayenne (4). The test was designed for factorial split plot analysis with variables of harvest period, cultivar, and isolate. Test design was a split plot with forcing date as the main plot, cultivar as the subplot, and isolate as the sub-subplot with four replications. Each plot consisted of eight data plants with two plants on each end as buffer plants. Four harvest periods were obtained by chemically forcing (5) mature plants on 2 July, 13 August, 24 September, and 5 November 1973. These flower induction dates resulted in harvest periods of 13 December 1973 to 20 February 1974, 6 February to 10 April, 2 April to 29 May, and 15 May to 16 July 1974.

Origin of isolates and preparation of inoculum.—Four isolates (#180, 303, 295, and 189) were selected based on cultural characteristics. Isolates were tentatively identified (A. C. Hayward *unpublished*) as *Gluconobacter oxydans* (Henneberg) DeLey (#180 and 303) [Synonym: *Acetomonas oxydans* (Henneberg) Shimwell and Carr], *Acetobacter aceti* (Pasteur) Beijerinck (#295) and *Enterobacter agglomerans* (Beijerinck) Ewing and Fife (#189) according to the eighth edition of Bergey's Manual (1). Isolate 180 was obtained from a severely infected fruit from the Pineapple Research Institute of Hawaii at Wahiawa, Oahu, Hawaii. Isolates 303 and 295 were obtained from the stock culture collection of I. W. Buddenhagen, University of Hawaii. Isolate 189 was obtained from diseased fruit from the island of Maui, Hawaii.

The four isolates were cultured for inocula as described previously (8). A bacterial suspension of each isolate was applied at 1×10^8 cells per milliliter over the flowering inflorescences in the morning with a compressed-air

sprayer at the rate of 25-50 ml per plant. Inoculations were made twice weekly throughout the flowering period which ranged from 3-6 weeks depending on season and cultivar.

Evaluation of disease.—Fruit were harvested when about 50 to 100% of the fruitlets were yellow. The fruit shell was removed and autoclaved for 20-25 minutes at 1.97 kg-force/cm^2 (28 psi), while the fruit cylinder was held for reisolations. Disease incidence was recorded from the cooked fruit shell as percentage of diseased fruit showing symptoms, and severity was scored as: 0 = no fruitlets showing symptoms; 1 = 1-2% of the fruitlets with symptoms; 2 = 3-5%; 3 = 6-10%; 4 = 11-25%; 5 = 26-50%; and 6 = 51-100%. Incidence data were analyzed using a factorial-split plot analysis of variance and Duncan's Bayesian LSD test for significant difference between means (6).

Reisolations.—Infected areas on the autoclaved fruit shells were matched with the respective fruit cylinders. A 1-2 cm^3 portion of the infected fruit tissue which contained nectary and placenta areas (8) was aseptically removed from the cylinder, juice was extracted and streaked on modified yeast extract-dextrose-calcium carbonate media (YDC) (8) or YDC and modified Hoyer's medium (3) containing 5% (v/v) ethanol (if isolate 295 was indicated). Bacterial reisolates were identified by comparison with standard cultures of each isolate. Growth of isolates 180 and 303 was similar on YDC media. Both isolates grew rapidly at 29 C and produced a nondiffusible pigment and a characteristic aroma. However, isolate 180 could be distinguished from isolate 303 by a slightly flattened colony surface. Isolate 295 was distinguished from isolates 180 and 303 by the absence of the aroma, the presence of a very dark brown diffusible pigment, and growth on Hoyer's medium. Isolate 189 was distinguished from the other isolates by a more rapid and luxurious growth on YDC media, with colonies becoming whitish at 48 hours.

RESULTS

Pink disease symptoms in both uncooked and cooked fruit tissues were consistent and similar within and between cultivars. Symptoms induced by isolates 180 and 303 were visible prior to cooking and included slight to moderate water-soaking of fruit flesh with a pink to brown discoloration. A characteristic "cantaloupe-like" aroma was also present. Cooked infected fruit tissue was characterized by a light to dark reddish brown fruitlet discoloration with lighter pigmentation in adjacent fruitlets. In contrast, uncooked fruit flesh infected by isolate 295 was characterized by a brown-to-black discoloration localized in the infected fruitlet. Cooked infected tissues turned dark brown to black. Unlike the other isolates, infections by 189 did not exhibit the above symptoms prior to cooking, but became brown to black following cooking (Fig. 1).

A statistical summary of the effects of the individual variables, harvest period, cultivar, and isolate on the percent diseased fruit is shown in Fig. 2. The percent diseased fruit was significantly greater in the December-February and February-April harvest periods than in the April-May and May-July harvest periods. Cultivar B was the most susceptible, and cultivar X, Smooth Cayenne, was the least susceptible. Other cultivars were intermediate in susceptibility. All isolates were significantly different from each other with 189 being the most virulent and 303 the least.

No significant differences in percent diseased fruit occurred between harvest periods for cultivar A (Table 1). In cultivar B, the lowest disease incidence occurred in the December-February harvest period with the highest in the May-July period. Cultivar C had the highest disease level in the February-April harvest period whereas the lowest levels occurred in the December-February and May-July periods. The highest percent diseased fruit in cultivar D occurred in December-February and February-April harvest periods. In cultivar E, the percent diseased fruit decreased significantly between each harvest period, whereas in cultivar F, the percent diseased fruit decreased significantly only between the last three harvest periods.

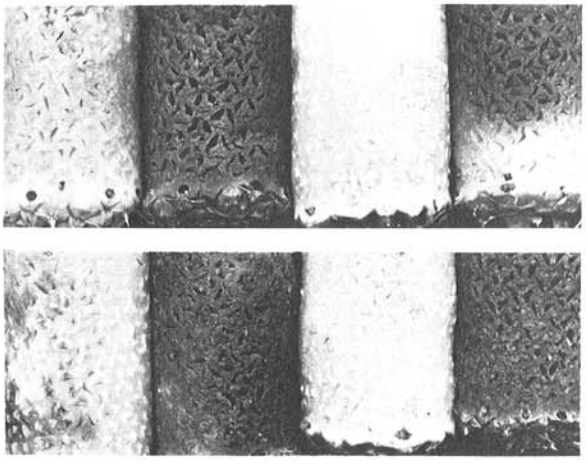

Fig. 1. Pink disease symptoms of four bacterial isolates in uncooked and cooked pineapple fruit (upper left, isolate 180; upper right, 303; lower left, 295; lower right, 189).

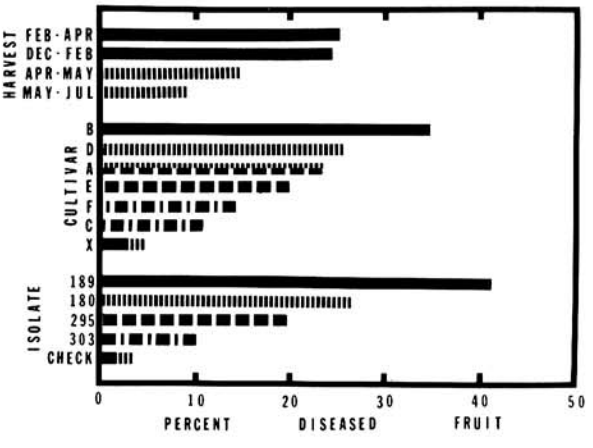

Fig. 2. Significant main effect differences in percent pink-diseased pineapple fruit between harvest period, cultivar, and isolate as determined by Duncan's Bayesian LSD test. Differing bars denote significance ($P = 0.05$).

TABLE 1. Pink disease of pineapple cultivars at various harvest periods

Harvest period	Percent diseased fruit from cultivars[y]:						
	A	B	C	D	E	F	X
Dec. to Feb.	26 a[z]	24 b	6 b	43 a	59 a	24 a	9 a
Feb. to April	20 a	33 ab	26 a	33 a	38 b	24 a	10 a
April to May	29 a	40 a	9 b	14 b	8 c	12 b	2 b
May to July	19 a	43 a	7 b	12 b	1 d	3 c	1 b

[y]Cultivars A to F were unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii; and cultivar X was the commercial cultivar, Smooth Cayenne.

[z]Means in columns followed by the same letter are not significantly different ($P = 0.05$).

TABLE 2. Pink disease of pineapple cultivars inoculated with various bacterial isolates

Isolate	Percent diseased fruit from cultivars[y]:						
	A	B	C	D	E	F	X
Isolate 180	64 a[z]	57 b	7 b	44 ab	22 a	26 a	1 b
Isolate 303	26 b	20 d	3 c	5 c	23 a	7 bc	1 b
Isolate 295	13 c	36 c	12 b	40 b	21 a	11 b	12 a
Isolate 189	31 b	81 a	53 a	57 a	28 a	34 a	32 a
Check	3 d	5 e	1 c	5 c	8 b	3 c	0 b

[y]Cultivars A to F were unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii; and cultivar X was the commercial cultivar, Smooth Cayenne.

[z]Means in columns followed by the same letter are not significantly different ($P = 0.05$).

Cultivar A was significantly susceptible to all isolates, but it was significantly more susceptible to 180 than to 189, 295, and 303 (Table 2). Isolate 295 was the least virulent. Cultivar B was also significantly susceptible to all isolates, but was significantly more susceptible to 189 and less susceptible to 303. Cultivar C was significantly susceptible only to isolates 180, 295, and 189 and most susceptible to 189. Cultivar D was similar to cultivar B in that greatest susceptibility was to isolate 189. Cultivar E was equally susceptible to all isolates. Cultivar F was similar to C and D except equally susceptible to 180 and 189. Cultivar X, Smooth Cayenne, was significantly susceptible only to isolates 295 and 189.

No significant interactions occurred between harvest period and isolate.

In general, percent reisolation of bacterial isolates was 70% or greater for isolates 180, 295, and 189 with the exception of the February-April harvest period for isolate 295, in which the percent reisolation was only 53 (Table 3). The reisolation of isolate 303 was 60% or less in the last three harvest periods.

Disease severity generally correlated with percent diseased fruit in that the greatest severity occurred at high disease incidence levels.

TABLE 3. Percent reisolation of bacterial isolates from pineapple fruit showing pink disease symptoms in four harvest periods

Bacterial isolate	Harvest period	Total isolations	Reisolation (%)
180	Dec-Feb	44	98
	Feb-Apr	34	88
	Apr-May	28	96
	May-July	21	81
303	Dec-Feb	22	86
	Feb-Apr	14	50
	Apr-May	15	60
	May-July	3	33
295	Dec-Feb	30	90
	Feb-Apr	17	53
	Apr-May	18	72
	May-July	10	90
189	Dec-Feb	51	98
	Feb-Apr	27	100
	Apr-May	21	100
	May-July	36	97

DISCUSSION

The symptomatology of the four bacterial isolates is of particular economic interest. When pink disease occurs in fruit to be canned, the infected fruit must be removed from the packing lines prior to canning. Fruit infected with isolates 180, 303, and 295 exhibit symptoms that facilitate removal from the packing lines (e.g., aroma and pigmentation). However, fruit infected with isolate 189 or slightly infected with isolates 180 or 303 (see Fig. 1, upper right) do not exhibit symptoms prior to cooking, and infected fruit can only be identified through postcanning quality control programs at considerable added expense. In fresh fruit production, the reverse is true. The 180, 303, and 295 isolates are more important economically since severely infected fruit are discolored and unappealing, whereas fruit infected by isolate 189 are symptomless.

The use of the field induction technique described previously (8) has demonstrated the varying virulence of the four isolates used in the present study and their interaction with cultivars. The high virulence of the 180 isolate and the generally low virulence of the 303 isolate demonstrates that strains exist within the *Gluconobacter oxydans* species. Also, the high virulence of *Acetobacter aceti* (isolate 295) and *Enterobacter agglomerans* (isolate 189) demonstrates that pink disease is caused by different bacterial genera.

The reisolation frequency of inoculated isolates was greatest with the most virulent isolates. The natural frequency of these isolates is unknown at this time. Preliminary evidence suggests that the *Gluconobacter* types (isolate 180 and 303) occur most frequently in Hawaii.

Cultivar susceptibility was variable. One cultivar was more susceptible to one bacterial isolate than another, and another cultivar (E) was equally susceptible to all isolates. Also, all cultivars did not follow the Smooth Cayenne (cultivar X) pattern of greatest natural disease incidence in the February-April period (8). Cultivar B was of particular interest in this respect since disease incidence was high in the May-July harvest period.

In conclusion, breeding for pink disease resistance will be complex since different pathogen genera as well as

strains can cause pink disease. The interaction of cultivars with isolates of bacteria that cause pink disease and with harvest period demonstrates the need for seasonal screening of cultivars in a breeding program as well as the use of several isolates to establish true resistance or susceptibility.

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