Comparison and Characterization of Toxin Produced by Helminthosporium sacchari from Australia, Florida, and Hawaii

Gary W Steiner and Ralph S. Byther

Associate Pathologists, Genetics and Pathology Department, Experiment Station, Hawaiian Sugar Planters' Association, Aiea, 96701. Present address of junior author: West Washington Research and Extension Center, Puyallup 98371.

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ABSTRACT

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Culture filtrate of *Helminthosporium sacchari* was obtained from several different sugarcane areas in the world. Samples from Florida and Australia contained a host-specific toxin. Studies involving column, paper, and thin-layer chromotography and host ranges, were conducted with the toxin samples and compared to the host-specific toxin produced in Hawaii by *H. sacchari*. Results indicate that the toxic material in the samples was helminthosporoside.

Eye spot disease of sugarcane, caused by *Helminthosporium sacchari,* is found in most of the major cane-growing areas of the world (4). In 1967, Martin (5) compiled a list of commercial clones grown in various countries and listed the reactions of these clones to several local diseases, including eye spot. Interestingly, some common clones differed considerably in their reaction to eye spot disease in different locations. Different strains of the pathogen and environmental factors were suggested to explain the variation in disease reactions. However, definitive data are not available to substantiate the explanations.

One way to determine the cause for the different reactions would be to test clones in one location with isolates of *H. sacchari* from several different countries. This method was deemed unwise for fear of introducing a new strain of the pathogen into the test location. However, it is now known that the isolate of *H. sacchari* from Hawaii is pathogenic because of its ability to produce a host-specific toxin, helminthosporoside (7, 8, 9). Since the toxin is very stable, it could be sent to a common location for screening clones to eye spot disease. In this paper, we report our results using toxin from three different cane-producing areas.

MATERIALS AND METHODS

Several researchers agreed to cooperate by sending sterile culture filtrate of *H. sacchari* to Hawaii: D. R. L. Steindl, Bureau of Sugar Experiment Stations, Brisbane, Australia; J. L. Dean, USDA Sugarcane Field Station, Canal Point, Florida; M. P. Early, National Sugar Research Station, Kisumu, Kenya; J. A. B. Nolla, University of Puerto Rico, Mayaguez, P. R.; and G. M. Thomson, South African Sugar Association Experiment Station, Mount Edgecombe, Natal, South Africa. To reduce variable factors in medium preparations, a modified Fries medium (3), including 0.1% yeast extract that was dialyzed and lyophilized, was sent to each participant. The pathogen was grown for 20 to 24 days at 24 ± 3 C after which the fungal mat was removed (7). The culture filtrate was treated with an equal volume of chloroform, which stabilizes the toxin and ensures a sterile solution. The culture filtrate-chloroform solutions were sent then to the Experiment Station, HSPA, Honolulu, Hawaii.

Each culture filtrate was treated with acetone and chloroform, and then concentrated to one-fiftieth the original volume using previously published methods (7, 8). In this condition the toxin can be stored indefinitely.

Field and laboratory assays were used to determine toxin activity and selectivity (7). Acetone-chloroform-treated toxin was used.

A Sephadex G-15 column (1.5 × 40 cm) was used in comparing elution patterns of the various toxin samples. Water was used as the elution solution.

Purification of the toxin was accomplished by the methods of Steiner and Strobel (8). Thin-layer chromatography experiments were conducted on silica gel 250 (Quantum Industries). The following solvent systems were used: (a) 1-butanol-acetic acid-H_2O (4:1:5 and 3:1:1, v/v); (b) 1-butanol-acetone-H_2O (4:5:1, v/v); (c) methylethyl ketone-acetic acid-methanol (6:2:2, v/v); (d) hexane-ethyl acetate (85:15, v/v) and propanol-NH_4OH-H_2O (6:3:1, v/v). The toxin was detected by spraying the chromatograms with a chloroform solution saturated with antimony trichloride and heating at 90 C for 3-4 minutes. Toxin appeared as a reddish spot.

The actual weight of pure toxin per milliliter of culture filtrate was determined by G. A. Strobel, Montana State University, Bozeman, using a Cahn electrobalance.

RESULTS

Initially, the laboratory assay was used to determine if

TABLE 1. Comparison of the reaction of several sugarcane clones to *Helminthosporium sacchari* toxin from different areas

Clone	Disease rating[a]			Clone	Disease rating[a]		
	H[b]	F	A		H[b]	F	A
H32-8560	4	4	4	H58-148	1	1	1
H49-5	1	1	1	H58-4392	1	1	1
H50-7209	1	1	1	H58-8029	9	8	9
H53-263	1	1	1	H59-3775	1	1	1
H54-775	5	5	3	H60-1102	9	6	7
H54-2508	2	2	2	H60-6314	1	1	1
H55-8248	3	3	3	H61-5433	1	1	1
H56-5840	1	1	1	H62-4254	3	4	4
H57-1475	1	1	1	H63-2422	1	1	1
H57-1502	1	1	1	H109	8	8	7
H57-5174	1	1	1	Lahaina	9	7	9
				Uahiapele	9	. . .	8

[a]Rating scale: 1 = highly resistant, 9 = highly susceptible.
[b]Toxin from: H = Hawaii, F = Florida, A = Australia.

the culture filtrate contained toxin. Toxic activity was noted from the Florida and Australia samples but not from Puerto Rico, Kenya, and South Africa. Five different clones were used for this evaluation. The two active preparations were compared with a sample from our laboratory. Initial laboratory testing using several clones indicated a difference in toxin titer only. In no case was a clone resistant to one toxin sample and susceptible to the others.

Twenty-three clones, differing in their reaction to helminthosporoside, were selected for field testing. All clones resistant to pure helminthosporoside were resistant to the three toxin preparations used (Table 1). Clones that were susceptible and intermediate reacted in a similar fashion to toxins from Australia and Florida. These data suggest the three toxin preparations were similar.

Confirmation of these results was obtained by chromatographic methods. After treating the culture filtrate with acetone, chloroform, and butanol, 1-ml samples were eluted from a Sephadex G-15 column. The peak areas of toxin activity were similar; however, toxin concentration was different for all three preparations (Fig. 1). The actual amount of pure toxin per ml of crude culture filtrate was calculated to be 33, 18.5, and 10.8 μg from Australia, Hawaii, and Florida, respectively.

All toxin samples were purified and R_f values compared using paper and thin-layer chromotography (Table 2). These results indicate that the purified toxins are similar.

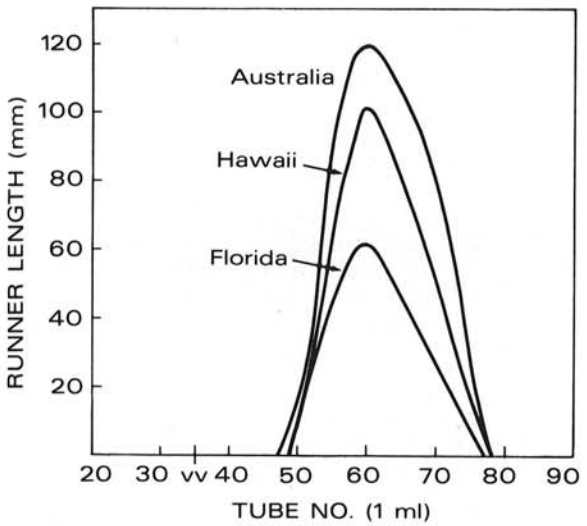

Fig. 1. Column chromatography elution pattern from a Sephadex G-15 column of 1.0-ml *Helminthosporium sacchari* toxin samples.

DISCUSSION

The similarity of reaction of several clones, elution

TABLE 2. Chromatographic R_f values of helminthosporoside (from *Helminthosporium sacchari*) from three different geographic areas

	Solvent[a]	R_f values		
		H[b]	F	A
a.	1-Butanol:acetic acid: H_2O (4:1:5, v/v)	0.31	0.29	0.30
b.	1-Propanol:NH_4OH:H_2O (6:3:1, v/v)	0.84	0.88	0.87
c.	1-Butanol:acetic acid: H_2O (4:1:5, v/v)	0.29	0.29	0.29
d.	1-Butanol:acetic acid: H_2O (3:1:1, v/v)	0.50	0.50	0.50
e.	1-Butanol:acetone:H_2O (4:5:1, v/v)	0.39	0.39	0.39
f.	Methyl ethyl ketone: acetic acid:methanol (6:2:2, v/v)	0.41	0.41	0.41
g.	Hexane:ethyl acetate (85:15, v/v)	0	0	0
h.	NH_4OH:propanol:H_2O (3:6:1, v/v)	0.59	0.59	0.59

[a]Solvents a and b were used on descending paper chromatography and c through h were used with thin-layer plates.
[b]Toxin from: H = Hawaii, F = Florida, A = Australia.

patterns from Sephadex G-15, and R_f values using several solvent systems all suggest that the toxic factor in the culture filtrate from Florida and Australia is helminthosporoside. The R_f values obtained in 1-butanol-acetic acid-H_2O (4:1:5, v/v) and 1-propanol-NH_4OH-H_2O (6:3:1, v/v) are similar to those obtained using authentic helminthosporoside (8).

In a study of this type, additional samples from other foreign countries would be desirable. Culture filtrate from three countries contained no toxin, probably due to contamination of the medium during the 20-day incubation period. We have noted that little or no toxin is produced when *H. sacchari* is contaminated. Another explanation would be that the pathogen has lost its ability to produce toxin. We suggest in this case a loss of virulence since toxin is necessary for the runner symptom to be produced. *Helminthosporium sacchari* is not located in the runner area but confined to the lesion area, hence the need for toxin (6, 7). Scheffer and Pringle (6) have shown in detail that toxin is necessary for *H. victoriae* and *H. carbonum* to be pathogenic. Based on these data, we would conclude that the strain of *H. sacchari* in Australia, Florida, and Hawaii is the same.

Although most clones react the same in various locations, there are some discrepancies. If the strain of the pathogen is the same in these areas, other explanations for the differences would be necessary. We have found that the susceptible reaction of clones to helminthosporoside can be quickly changed to a resistant reaction merely by raising the temperature (1). Clones reacted differently when their reaction was determined in the morning following a "cool" evening than when determinations were made in the evening following a "hot" day (2). We have observed that the pathogen generally fails to produce runner symptoms during the hot summer months, but runners 3 ft long are not uncommon on the same clones during the winter months. Our data show that the pathogen produces toxin during the different periods but the clone fails to show symptoms. The different reaction of clones to *H. sacchari* observed in other countries may be due to the time of year that the clones were tested, not to different strains of the pathogen. Our limited sampling of toxins suggests this conclusion.

The concentration of toxin produced in culture was different for all samples tested. This also has been observed in various Hawaiian isolates. The reason for similar reactions of the 23 clones tested in the field was the adjustment of the concentration of the filtrate to give similar reactions among clones. If the toxins were different, we would expect a clear resistant or susceptible reaction and not minor changes.

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