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Specificity of *Ustilago maydis* Killer Proteins

Y. KOLTIN[1] AND P. R. DAY

Department of Genetics, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504

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Bacteria and fungi were tested for sensitivity to *Ustilago maydis* killer strains carrying virus-like particles. Various species taxonomically related to *U. maydis* were sensitive.

Virus-like particles are known in over 60 species of fungi (8), but only in the yeast *Saccharomyces cerevisiae* (1) and in *Ustilago maydis* (3, 9) is their presence associated with the excretion of proteinaceous compounds (2, 5) that inhibit or kill sensitive cells. To clarify the biological significance of the killing phenomenon and to see if it could be deployed as a biological control, we have tested the *U. maydis* killer proteins on a wide range of microorganisms.

Three killer specificities (P1, P4 and P6) are known in *U. maydis* (J. E. Puhalla, unpublished data). All three killer strains produce killer materials that are precipitated from culture filtrate by ammonium sulfate and that are nondialyzable, heat labile, and Pronase sensitive. Preliminary tests indicate that the killer materials are proteins or glycoproteins.

Each killer is insensitive to its own killer substance but sensitive to the other two. The sensitivity of bacterial species (Table 1) was tested by replicating the bacterial samples to plates of *U. maydis* complete medium (pH 7.0) (6) on which streaks of the *U. maydis* killer strains had been grown for 24 h at 25 C. Duplicate tests were incubated at 25 and 30 C for 48 h. No inhibition by any of the *U. maydis* killers was noticed among the 51 bacterial specimens tested.

Tests for sensitive fungi were conducted mainly with other members of the ustilaginales. Killer and sensitive yeast strains and several filamentous fungi were also included (Table 1). Each specimen tested was suspended and plated as a lawn in *Ustilago* complete medium; the killer strains, with a sensitive strain as a control, were spotted on the surface (Fig. 1). The plates were incubated for 24 to 48 h at 25 C and scored for inhibition zones surrounding the *U. maydis* colonies. In addition, each specimen was spotted on a lawn of a sensitive strain of *U. maydis* to test for the production of substances inhibitory to *U. maydis*.

The results (Table 1) indicate widespread sensitivity to *U. maydis* killer strains among smuts of grasses. No samples were inhibited by the sensitive strain of *U. maydis*, suggesting that the inhibitory substances produced by killer strains were responsible for the inhibition of other members of the ustilaginales. In a number of samples this inhibition was also observed with ammonium sulfate-precipitated, nondialyzable, cell-free materials from culture filtrates and was abolished by brief heat treatment (75 C, 1 min) and exposure to Pronase (1 mg/ml).

Some samples were sensitive to only one or two of the killer strains. Killer sensitivity is restricted to closely related species since the *U. maydis* killers have no effect on a yeast strain sensitive to the yeast killer protein or on the yeast killer strain. Similarly, the yeast killer does not affect sensitive or killer strains of *U. maydis* in tests at pH 7.0 and 4.7 (4). Other fungi tested, including four species of *Helminthosporium* parasitic on grasses, were also unaffected by the *U. maydis* killer strains.

The results indicate that the killer proteins are unlikely to provide a significant selective advantage to killer strains in competition with other microorganisms. The sensitivity among graminicolous smuts suggests the presence of common receptors in the plasma membranes or cell walls of these species which might be expected if they have a common ancestry. We have no information on the incidence of related virus-like particles within this group. The fact that no specimen of another species proved inhibitory to the sensitive strain of *U. maydis* is of doubtful significance since tests of more than 90 independent collections of *U. maydis* from nature have so far revealed only three with killer activity. The inhibitory capacity of *U. maydis* killer proteins could be employed for the biological control of cereal smuts by the introduction of the informational molecules to various cereal species if these molecules can replicate and express the killing function in the plant cell cytoplasm. The association of ribonucleic acid-dependent ribonucleic acid polymer-

[1] On leave from University of Tel-Aviv, Tel-Aviv, Israel.

TABLE 1. *Microorganisms tested for sensitivity to U. maydis killer strains*[a]

Bacteria[b]	No. of specimens	Fungi	No. of specimens	Sensitivity to killers[c]		
				P1	P4	P6
	1	Grass smuts				
Bacillus cereus	1	*Sorosporium consanguineum*	1	+	+	+
B. subtilis	1	*S. consanguineum*	2	+	+	−
Cellulomonas flavigena	1	*Ustilago avenae*	6	+	+	+
C. fimi	1	*U. nigra*	3	+	+	+
C. biozotea	1	*U. bullata*	7	+	+	+
Enterobacter agllomerans	2	*U. bullata*	2	−	+	+
Erwinia carotovora	1	*U. hordei*	16	+	+	+
E. chrysanthemi	1	*U. kolleri*	4	+	+	+
E. atroseptica	1	*U. nuda*	1	+	+	+
E. aroideae	3	*U. sphaerogena*	1	−[d]	−[d]	−
Erwinia sp.	3	*U. striiformis*	1	+	+	+
Escherichia coli	1	*U. tritici*	12	+	+	+
Micrococcus certificans	1					
Proteus vulgaris	3	Other smuts				
Pseudomonas fluorescens	1	*Ustilago scabiosae*	1	−	−[d]	−
P. aeruginosa	1	*U. utriculosa*	1	−	+	−
P. tolaasii	1	*U. vinosa*	1	−	−	−
P. cichorii	1	*U. violaceae*	5	−	−	−
P. phaseolicola	1					
P. tabaci	3	Other fungi				
P. marginalis	1	*Endothia parasitica*	2	−	−	−
P. syringae	18	*Helminthosporium carbonum*	1	−	−	−
Pseudomonas sp.	1	*H. maydis*	1	−	−	−
Serratia marcescens	1	*H. turcicum*	1	−	−	−
Streptomyces albidoflavus		*H. vagans*	1	−	−	−
		Saccharomyces cerevisiae	2	−	−	−
		Schizophyllum commune	2	−	−	−

[a] The sources of *Sorosporium* and *Ustilago* were: *S. consanguineum*, Australia; *U. bullata*, Australia; *U. hordei*, Canada, Ethiopia; *U. striiformis*, Canada; *U. avenae*, Morocco, United States, Canada, Ethiopia; *U. kolleri*, Canada, Ethiopia; *U. nigra*, Canada; *U. nuda*, Canada; *U. tritici*, Germany, Sweden, Denmark, Czechoslovakia, Argentina, Kenya, India; *U. violaceae*, Canada.

[b] No bacteria were sensitive.

[c] +, Inhibition; −, no inhibition.

[d] Slight inhibition.

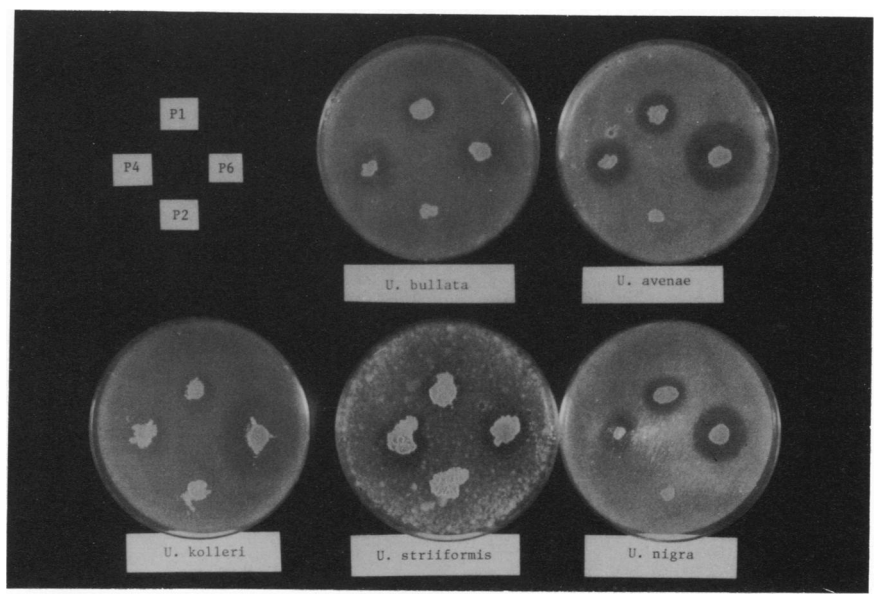

FIG. 1. *Five Ustilago spp. grown as lawns showing inhibition caused by U. maydis killer strains with specificities P1, P4, and P6. P2 is a nonkiller, sensitive strain of U. maydis included as a control. Plates were incubated for 48 h at 30 C.*

ase with viruses of *Penicillium* (7) encourages us to examine this possibility.

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