The Killer System in Ustilago maydis: Heterokaryon Transfer and Loss of Determinants

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ABSTRACT

The properties of killer production and resistance to killer are cytoplasmically determined and transmitted at dikaryon formation. A spontaneous mutant cured of killer production could not be reinfected with the killer determinant.

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Additional key words: viruslike particles, cytoplasmic inheritance, mutants.

P1 cells of the corn smut pathogen *Ustilago maydis* (DC.) Cda. release a protein that kills sensitive P2 smut cells (2, 5). We report evidence that the cytoplasmic determinants [I], for killer protein production, and [S], for resistance to killer protein, both carried by P1 (5), are transmitted at dikaryon formation, and that [I] is lost at low frequencies during vegetative growth.

Two other facts are relevant. P2 cells are sensitive or resistant according to whether they carry the alleles *s* or s^+ at a chromosomal locus. Cells of a third class, P3, carry [S], are resistant but produce no killer, and were distinguished from P2s^+ by a breeding test (5). Media have already been described (1, 2, 5).

Compatible P1 [I] [S] and sensitive P2 cells of *U. maydis* were mated on minimal medium containing charcoal (1) and produced an unstable dikaryotic mycelium. The mating was successful because killer protein is not produced on unbuffered minimal medium (2). Tufts of dikaryotic mycelium were transferred to fresh medium without charcoal and the two haploid parental types isolated. These were identified by their mating types and were tested for

TABLE 1. Dikaryons of *Ustilago maydis* and their breakdown products

Parental genotypes	Recovered phenotypes (no. of colonies)
a2bD P2*s* + *a1bI* P1s^+	*a2bD* P1(8) *a1bI* P1(8)
a1bA P3*s* + *a2bD* P1*s*	*a1bA* P1(11) *a2bD* P1(21)
a2bD P2*s* + *a1bA* P3*s*	*a2bD* P3(46) *a1bA* P3(18)

TABLE 2. Cell types recovered from killer (P1) cultures of *Ustilago maydis*

Killer parent	Treatment	Number of colonies tested	Number sensitive (P2)	Number not sensitive not killer (P3)	Number unstable
P1s^+	0	133	0	1 (No. 1355)	0
	Ultraviolet light	186	0	0	0
P1s^a	0	1,200	0	1 (No. 1351)	0
	Ultraviolet light	1,363	0	0	2
	Acriflavine	1,248	0	0	0
	40 C 18 hr	119	0	0	0
	38 C 16 hr	342	0	0	0
P1 (2n)	0	1,154	0	0	0

[a] Culture derived by infecting P2*s* by heterokaryon transfer.

their P phenotype. All cells recovered from P1 + P2 dikaryons were P1 in phenotype.

Similar experiments showed that P1*s* + P3*s* gave both parental mating types that were P1 and that P2*s* + P3*s* gave both parental mating types that were P3 (Table 1). The results fulfill the expectation of a heterokaryon test for cytoplasmic inheritance (3, 4).

P1 cells of several kinds were grown overnight in liquid complete medium at 30 C and plated on double-strength complete medium to give 100 to 200 colonies per plate. After 2 to 3 days' incubation, colonies were transferred to plates of minimal medium overlayered with 3 to 5 ml of minimal agar with 1% glycine containing 10^4 to 10^5 P2*s* cells/ml. After further incubation at 25 C for 24 to 48 hr, halos 1- to 2-mm wide with no P2*s* growth surrounded P1 colonies. Several colonies which did not kill P2*s* cells were rescued, distinguished from the tester P2*s* background cells by their mating type and tested further. Two mutants were recovered, one (No. 1355) from a P1s^+ culture, the other (No. 1351) from a P1*s* culture that had been produced by heterokaryon infection of a P2*s* culture. Attempts to increase the frequency of nonkiller mutants by treatment with ultraviolet light (1% survival of log phase cells), by overnight incubation in acriflavine concentrations (1 μg to 10 μg/ml) that inhibited growth, and by heat treatments, were not successful (Table 2). No mutants were recovered from untreated diploid P1 cells.

Mutant No. 1351 was not sensitive to killer and transmitted [S] to P2*s* by heterokaryon transfer. When No. 1351 was mated with a P1 strain, two colony types were recovered from the resultant dikaryotic hyphae that were, respectively, P3 and P1 in phenotype. Evidently No. 1351 resists infection with [I]. Attempts to confirm that it carries a nuclear gene for resistance were frustrated by our finding that in crosses in corn seedlings between No. 1351 and a compatible auxotrophic P2*s* strain teliospores germinated but did not undergo meiosis.

Mutant No. 1355 carries s^+ and, as expected, was resistant to killer. Attempts to transmit [S] to P2*s* cells were blocked by this mutant's failure to mate on agar media. It formed galls in corn seedlings when inoculated with a compatible haploid strain but, like No. 1351, teliospores did not undergo meiosis.

Our failure to recover P2*s* cells from P1 cells is not likely due to their being killed by the products of surrounding P1 cells since in liquid complete medium no killer substance is produced and low plating densities (<200) allow both cell types to form colonies. The possibility that P1*s* cells might lose [S] but retain [I] was tested by plating cells grown in liquid complete medium on minimal agar at pH 4.0 on which no killer protein is produced. Some 4,200 colonies were replicated to complete medium and pH 4.0 minimal medium and the replicas scanned for colonies that did not grow on complete. The only ones discovered had failed to be transferred. The conditional lethal class was not found. However, this is perhaps not surprising since no P2*s* cells have been recovered either. It seems that the [S] determinant is not readily lost.

These experiments raise the question of the nature of the [I] and [S] determinants. The following paper (6) presents evidence the [S] is a viruslike particle. The nature of [I] remains unknown. It could be a viruslike particle, with many physical properties in common with [S], or it could be a cytoplasmic determinant of another kind.

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