A Cultural Variant of a Benomyl-tolerant Strain of Cercospora beticola

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ABSTRACT

A white variant (H1-12/W) developed from a benomyl-tolerant strain (H1-12) of *Cercospora beticola* on potato-dextrose agar containing 100 μg (active ingredient) benomyl/ml. The variant was stable through repeated subculturing and in passage through sugar beet. Benomyl tolerance of H1-12/W was similar to that of H1-12; however, the latter sporulated more prolifically in vitro and in vivo than the variant. H1-12/W was only slightly less virulent than H1-12 in sugar beet, but the incubation period for lesion formation was almost twice as long for H1-12/W as for H1-12. Reisolations from sugar beet infected with H1-12/W yielded cultures identical to H1-12/W, with no reversions to the parental strain.

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The most benomyl-tolerant strain (H1-12) of *Cercospora beticola* Sacc. isolated from sugar beet (5) has shown the same tendency to produce cultural variants previously reported for other isolates of this species (1, 2, 3), especially when grown at high temperatures (26-28 C) on media containing benomyl. One striking nonpigmented variant, H1-12/W, was selected for study of its biology, pathogenicity, virulence, and sensitivity to benomyl.

Strain H1-12/W, when first plated on PDA, developed hyaline hyphae, and a mycelial growth that appeared to be white. Unlike other variants (1), H1-12/W had more aerial mycelia than the normal parent culture. The medium underlying young cultures was pale yellow. Morphological characteristics of the hyphae were similar to those described by La (2). The variant was stable through repeated subculture.

Sporulation of H1-12 and H1-12/W was induced on sugar beet leaf extract agar as described elsewhere (4), except that cultures were initiated with mycelial suspensions (3 ml per 6-cm diameter petri dish) prepared from 10-day-old potato-dextrose agar (PDA) slant cultures of each strain. After 7 days, conidial suspensions of each strain were made (4), and the number of conidia in six subsamples of each suspension was counted with the aid of a hemacytometer. Contrary to other reports of variants (1, 2), H1-12/W sporulated under the conditions described. However, 98% fewer conidia formed, as compared to H1-12 (4.2×10^5 vs. 7×10^3 conidia/cc, respectively; means of three replications). Conidia of the variant were morphologically similar to those of the parent strain, except they were slightly smaller. Conidia of H1-12 measured 49-194 (mean = 118) × 3-5 (mean = 4) μm and had 5 to 20 cells. Those of H1-12/W measured 42-175 (mean = 104) × 2-4 (mean = 3) μm and had 4 to 20 cells.

To confirm benomyl sensitivity of H1-12/W, the variant, its benomyl-tolerant parent culture, and a benomyl-sensitive strain of *C. beticola* (C-1; ATCC 24078) were plated on PDA with 5 μg active ingredient (a.i.) benomyl per ml. Three replications were made. Strains H1-12 and H1-12/W were unaffected by the benomyl, but C-1 failed to grow.

Variants of *C. beticola* are significantly less virulent than normal strains (1, 2, 3). To compare the virulence of H1-12 and that of H1-12/W, each strain was inoculated to 3-month-old sugar beet (highly susceptible cultivar R & G Pioneer) with five replications in a randomized complete block design. Suspensions of approximately 5,000 spores per cc were used as inoculum. Inoculated plants were held in a humidity chamber for 72 hours at 100% relative humidity and 24 to 30 C under continuous fluorescent light (approximately 5,400 lx) and then transferred to a greenhouse bench. Disease severity was rated 0 to 9 (0 = no leaf spots and 9 = complete defoliation), 14 and 28 days after inoculation. After 14 days, plants inoculated with H1-12 had an average disease rating of 5, whereas those inoculated with H1-12/W showed only small brownish flecks and rings and were rated < 1. By 28 days, however, abundant lesions had developed on the plants inoculated with the variant. These lesions were typical of those induced by *C. beticola,* except that their margins were grayish-brown, compared to reddish-brown margins of lesions induced by H1-12. Disease ratings at 28 days were 6 for H1-12 and 4 for H1-12/W. The experiment was repeated with similar results. Thus, although the variant was less virulent than its parent culture, this reduction was due more to an increased incubation period than to a decreased disease severity.

Coons and Larmer (1) reported that a stable white variant failed to induce typical leaf spots in sugar beet, and conidia were not produced on the plant. Leaves of sugar beet infected with H1-12/W and H1-12 from the virulence test were placed in moisture dishes for 48 hours at 25 C. Abundant whitish aerial mycelia developed on H1-12/W lesions, whereas grayish mycelia covered most H1-12 lesions. Strain H1-12 produced abundant conidia under these conditions, whereas H1-12/W produced only a few conidia per lesion. Single-spore and hyphal reisolations from leaves infected with H1-12/W yielded only white cultures. Typical H1-12 was reisolated from leaves inoculated with the parent strain. Thus, H1-12/W did not revert to the parental strain as did variants described by Coons and Larmer (1).

Variants generally are not regarded as important in the origin of new physiologic races of *C. beticola.* Variants normally are much less virulent than parent strains, and they usually fail to produce conidia in culture or in vivo (1, 2, 3). Strain H1-12/W, however, was almost as virulent as its parental source and was capable of some, albeit reduced, sporulation. If such variants develop in nature, they could be another source of benomyl-tolerant

germ plasm in parasexual recombinations with virulent benomyl-sensitive strains. It is still to be determined whether variants can compete with normal strains in nature, and whether parasexualism occurs in *C. beticola*.

A vigorous strain of *C. beticola* with two stable genetic markers, lack of pigmentation and benomyl tolerance, may be useful in heterocaryon or parasexualism studies of this fungus. Cultures of H1-12 and H1-12/W have been deposited in the American Type Culture Collection (ATCC 28058 and ATCC 28059, respectively).

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