Variations of Single-Basidiospore Isolates of Thanatephorus cucumeris

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ABSTRACT

Nineteen single-basidiospore isolates from a parent clone of *Thanatephorus cucumeris* were compared for their variability. The average growth rate at 25 C ranged 5.7-12.8 mm per day and optimum pH for mycelial growth was between 6.5 and 7.2. Isolates varied in their ability to form sclerotia on both potato-dextrose agar and in soil. Isolates varied from avirulent to moderately virulent. Fifteen isolates formed basidia on soil varying in the amount of hymenium produced and the size of basidial structures. Basidial shapes varied from subglobose to oblong and the ratio of average width of basidia to average width of supporting hyphae ranged from 1.5 to 1.9. The latter have ratios similar to those of *Ceratobasidium*.

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Additional key words; soil-over-culture method, sexual recombination.

Accumulated literature on variations of single-basidiospore isolates of *Thanatephorus cucumeris* (Frank) Donk indicate that field isolates of *T. cucumeris* are heterokaryotic (10). Variations after sexual recombination include differences in cultural characteristics (2,8,15), physiological characteristics (1,7,9,15), saprophytic behavior (6,7), fruiting ability (1,3,4,5,8), and pathogenicity (1,3,4,9). Our purpose was to determine the extent of variations in some of these well-known features, and to especially note morphological changes of basidial structures in single-basidiospore isolates obtained from a Taiwan isolate of *T. cucumeris*. The latter parameter could be important in taxonomic determination of the resulting isolates.

MATERIALS AND METHODS.—An isolate of *T. cucumeris* causing disease on kenaf *(Hibiscus cannabinus* L.) in Taiwan was used. Hyphal-tip isolations of the fungus were made and colonies were maintained on potato-dextrose agar (PDA). The fungus formed basidia in 2-4 days on the surface of soil that had been added to growing cultures of the fungus in petri dishes (12).

Single basidiospore isolates were obtained by evenly spacing five small hymenial mats on the lid of a petri dish inverted over 10 ml of 2% water agar. After 24 hours the lid with hymenial mats was removed and replaced with a sterile lid. The plate was kept at room temperature for 10-16 hours and was then examined with a dissecting microscope (× 30). Germinating basidiospores were cut with a sterile needle, and transferred to PDA plates. Plates were further examined microscopically (× 100) to

make certain that each block of agar carried only one spore.

Linear growth rate, effect of pH on mycelial growth, and sclerotium formation were studied on PDA at 25 ± 0.5 C. Sclerotium formation was also evaluated in autoclaved sandy soil. Eighty ml of sterile sandy soil was placed in a 9-cm-diameter petri dish and infested with 30 ml of a macerated hyphal suspension obtained from a 4-day-old culture growing on Czapek's solution. After 30 days, sclerotia of each isolate in an 80-ml subsample of soil from three petri dishes were removed by sieving and counted. Pathogenicity tests were performed in the laboratory and greenhouse. In the laboratory, surface-disinfested (3 minutes in 1% sodium hypochlorite) soybean [*Glycine max* (L.) Merr.] seeds were planted in infested sandy soil in a petri dish. Emergence of soybean seed was determined daily for 6 days. In the greenhouse, a hyphal disk (5-mm diameter) from a 3-day-old colony was placed on a 14-day-old soybean seedling at the soil level. There were 18 plants for each isolate. Disease severity was determined 7 days after inoculation. To compare fruiting ability and basidial structures, isolates were induced to form basidia by the soil-over-culture method (12). The size of hymenium formed on soil was estimated 6 days after hymenium initiation. Three-hundred mature basidiospores and basidia from each isolate were measured.

RESULTS.—The average linear growth rate of all single-basidiospore isolates at 25 ± 0.5 C ranged from 5.7 to 12.8 mm per day. Eight isolates grew significantly (P= 0.05) faster than the parent clone and one isolate grew 5.7 mm per day, about one-half the rate of the parent clone. Colonies of the parent clone were appressed, dense, and mealy with light ochraceous to buff secretion droplets. Single basidiospore cultures ranged from those with abundant aerial hyphae to those that were mealy in appearance with sparse aerial hyphae. The secretions varied from a light buff to ochraceous-tawny color.

The optimum pH for mycelial growth of single-basidiospore isolates and the parent clone was between 6.5 and 7.2. Nine single-basidiospore isolates grew better in an alkaline than in an acid medium, a characteristic of the parent clone. However, four isolates grew better on slightly acid media. The remainder showed no specific preference. Single-basidiospore isolates also differed in their ability to form sclerotia on both PDA and in soil.

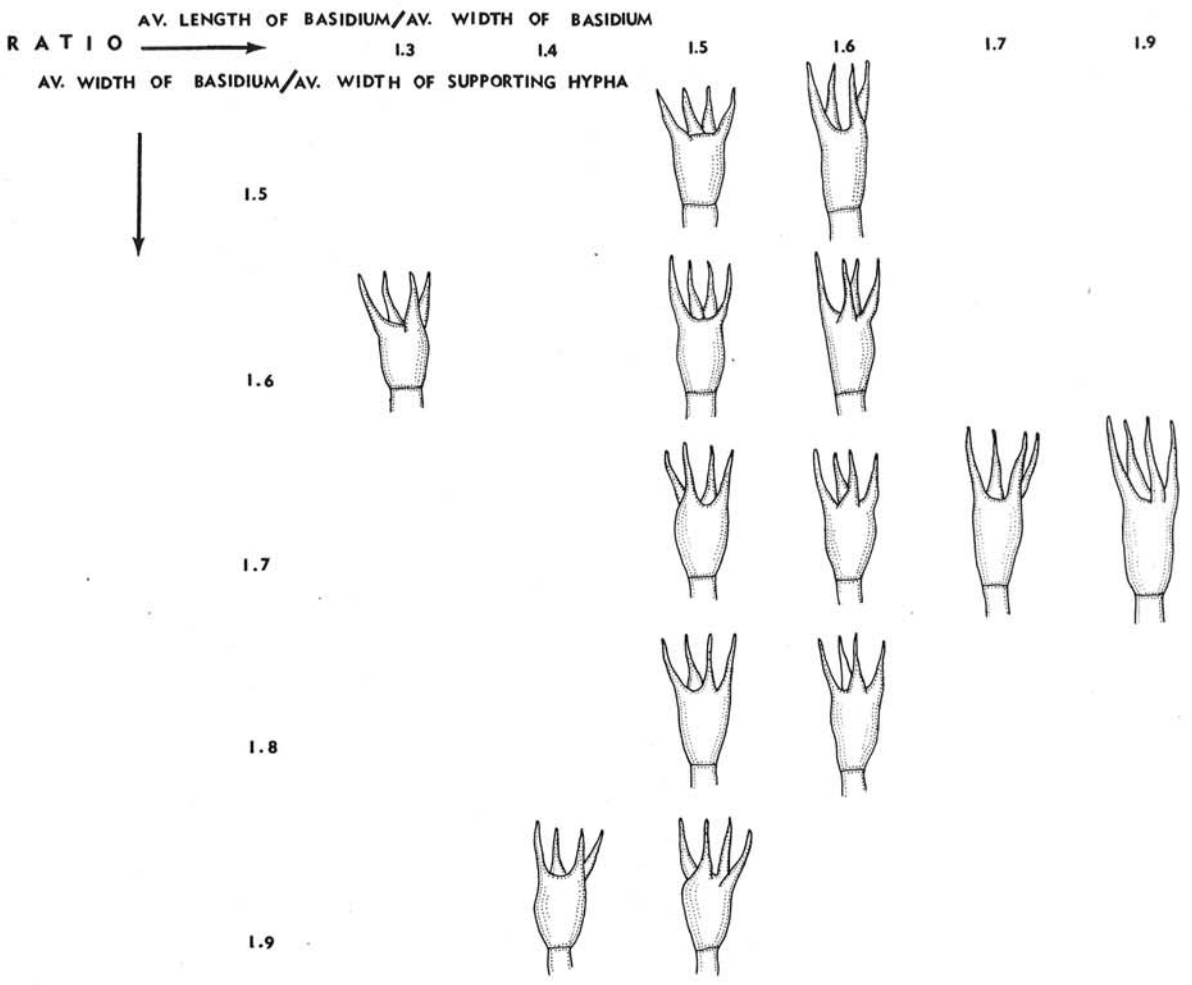

Fig. 1. Variations in shape and attachment of basidia of single-basidiospore isolates of *Thanatephorus cucumeris*.

The results on PDA and soil generally agreed except that three isolates formed sclerotia in soil, but not on PDA. Seventeen single-basidiospore isolates formed fewer sclerotia in soil than did the parent clone. Pathogenicity also varied greatly among isolates. Seven isolates were more virulent on soybean than the parent clone in preemergence rot, but only four isolates showed a higher disease index in seedling infection. In general, isolates highly virulent on seed were highly virulent to seedlings, but exceptions did occur. One isolate was completely nonpathogenic.

Fifteen of 19 single-basidiospore isolates produced basidia on soil. Variations ranged from hymenia covering almost the entire soil surface in a 9-cm-diameter petri dish to only sparse hymenia. These results differed from reports indicating that a majority of single-basidiospore isolates in the first generation failed to fruit (1,3,8,10). However, Flentje et al. (2) pointed out that lack of basidia in some single-basidiospore cultures might be due to environmental, rather than genetic, factors. Although *T. cucumeris* is basically a homothallic fungus (10), there are apparently a number of sterility factors present that prevent the fruiting of single basidiospore cultures (11). The reason for about 20% of the single-basidiospore isolates being sterile is unknown.

Variations in the morphology of basidial structures were also great. Most basidia had four sterigmata, but a few had only one to three. Length of basidiospores ranged 4.7-10.5 μm, with the averages for different isolates ranging 7.4-8.5 μm. Width of basidiospores ranged 3.0-7.5 μm, with the averages for different isolates between 4.3 and 5.6 μm. In spite of the significant differences ($P = 0.05$) among basidiospore measurements, their shapes were almost identical. They were oblong to ellipsoid with one side flattened, or slightly broad ovoid, with a truncate apiculus. The ratio of average length to average width of spores was 1.5 - 1.8. Basidial measurements also varied; lengths ranged 6.7-18.0 μm, averaging between 10.9 and 14.8 μm. Widths ranged 3.7-9.9 μm with averages between 7.0 and 8.4 μm. Differences in basidial measurements among single-basidiospore isolates were statistically significant ($P = 0.05$). Since the ratio of average length to average width varied greatly (1.3-1.9 μm), the shapes of basidia were different from one isolate to another; they varied from subglobose to oblong (Fig. 1). Width of subhymenial mycelia ranged from 3.0 to 7.5 μm, with averages of 4.3-5.2 μm. Basidia of some isolates were broadly attached and not much wider than the supporting hyphae, with the ratio of average width of basidia to average width of supporting hyphae of only 1.5. This condition was similar to the description of *T. cucumeris* given by Warcup and Talbot (14). However, basidia of other isolates were more constricted at the base and narrowly attached to supporting hyphae with a ratio of 1.9 for average width of basidia to average width of supporting hyphae. Such basidia were easily confused with *Ceratobasidium,* which was described as "basidium abruptly narrowed at the attachment, 2-3 times the width of the supporting hyphae" (13). Since the variation in basidial structures were so evident, we suggest that even the use of basidial characteristics is not sufficient for the identification of *T. cucumeris.* Characteristics such as multinucleate hyphal cells, anastomosis tests, and the type of subhymenial branching also need to be considered.

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