Identification of Fusarium oxysporum f. sp. cubense Race 4 from Soil or Host Tissue by Cultural Characters

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ABSTRACT

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Fusarium oxysporum f. sp. *cubense* race 4 formed laciniated colonies distinct from those of races 1 and 2 on modified Komada's medium (K2 medium) but not on potato-dextrose agar, PCNB agar, Martin's medium, or surfactant agar. Race 4 was detectable by the laciniated colonies recovered from infected host tissue, and also directly from soil on dilution plates. All isolates of *F. oxysporum* obtained from 55 wilted Cavendish banana trees susceptible only to race 4 formed laciniated colonies on K2 medium. None of the isolates obtained from 16 wilted Latundan banana trees susceptible only to race 1 formed laciniated colonies on the same medium. Six formae speciales of *F. oxysporum*, one saprophytic *F. oxysporum* and three other species of *Fusarium* tested did not form laciniated colonies on K2 medium. The recovery of race 4 from experimentally infested soil was about 90% using K2 medium. The population of race 4 in naturally infested soil, determined with K2 medium, ranged from 50 to 650 propagules per gram of air-dried soil.

Previously there were only two races (races 1 and 2) of *Fusarium oxysporum* f. sp. *cubense* (E. F. Sm.) Snyd. & Hans. that caused wilt of *Musa* species (6). The 'Cavendish' banana cultivars grown commercially in Taiwan are highly resistant to these two races. Race 3 of the fungus causes wilt of wild *Heliconia* spp. in Central America (9). In 1967 a new race (race 4) of *F. oxysporum* f. sp. *cubense* capable of attacking Cavendish cultivars was found in the southern part of Taiwan (7). Currently, the wilt is affecting more than 2,300 hectares of banana plantations.

Many selective media are available for isolation of *F. oxysporum* (8). However, none is suitable for selective isolation of *F. oxysporum* f. sp. *cubense* because its identification depends on pathogenicity tests conducted in the fields or tanks (10). A rapid pathogenicity test using small seedlings of *Musa balbisiana* was developed by Stover (5). It was useful for rapid identification of *F. oxysporum* f. sp. *cubense*, but not races of this fungus. We report herein a method for identification of *F. oxysporum* f. sp. *cubense* race 4, isolated from both infected tissue and soil, by cultural characters on an agar medium.

MATERIALS AND METHODS

Two isolates (T and L) of *F. oxysporum* f. sp. *cubense* were isolated from pseudostems of wilted Cavendish and Latundan banana trees, respectively. Their pathogenicity was demonstrated using banana plantlets derived from tissue cultures of differential cultivars (E. J. Sun and H. J. Su, *unpublished*). Isolates T and L were identified as races 4 and 1, respectively. Races 1 and 2 of *F. oxysporum* f. sp. *cubense* supplied by R. H. Stover also were used.

Komada's selective medium (1) was modified to enhance expression of morphological characteristics of race 4 for easy identification. The basal medium contains the following compounds in 900 ml of distilled water: K_2HPO_4, 1 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; FeNaEDTA, 0.01 g; L-asparagine, 2 g; galactose, 10 g (in contrast to 20 g in Komada's original medium), and 16 g of agar. After it was autoclaved, the basal medium was mixed with 100 ml of solution containing the following agents: PCNB (pentachloronitrobenzene, 75% WP), 0.9 g (1 g in original medium); oxgall, 0.45 g (0.5 g in original medium); $Na_2B_4O_7 \cdot 10H_2O$, 0.5 g (1 g in original medium); and streptomycin sulfate, 0.3 g. The medium was adjusted to pH 3.8 ± 0.2 with 10% phosphoric acid. To determine the population of *F. oxysporum* f. sp. *cubense* race 4 in soil, 0.5 ml of diluted soil suspension was spread on the surface of solidified K2 medium in a petri plate. Five plates per treatment were used and colonies were observed after 10-day incubation at 25 C under fluorescent light. The experiments were repeated twice.

RESULTS AND DISCUSSION

Fusarium oxysporum f. sp. *cubense* race 4 formed laciniated radial colonies on K2 medium that were distinct from those of isolate L and race 1 and 2 (Fig. 1). The number of rays produced per colony ranged from eight to 30. The colonies appeared yellowish when observed from the bottom. The color also was different from other races. On Komada's original medium the growth of race 4 was retarded and the laciniate appearance of the colony was present but not as distinct. The race 4 isolate did not produce laciniated colonies on potato-dextrose agar, PCNB agar (3), Martin's medium (2), or surfactant agar (4), and its colonies were almost in distinguishable from other races. None of the following

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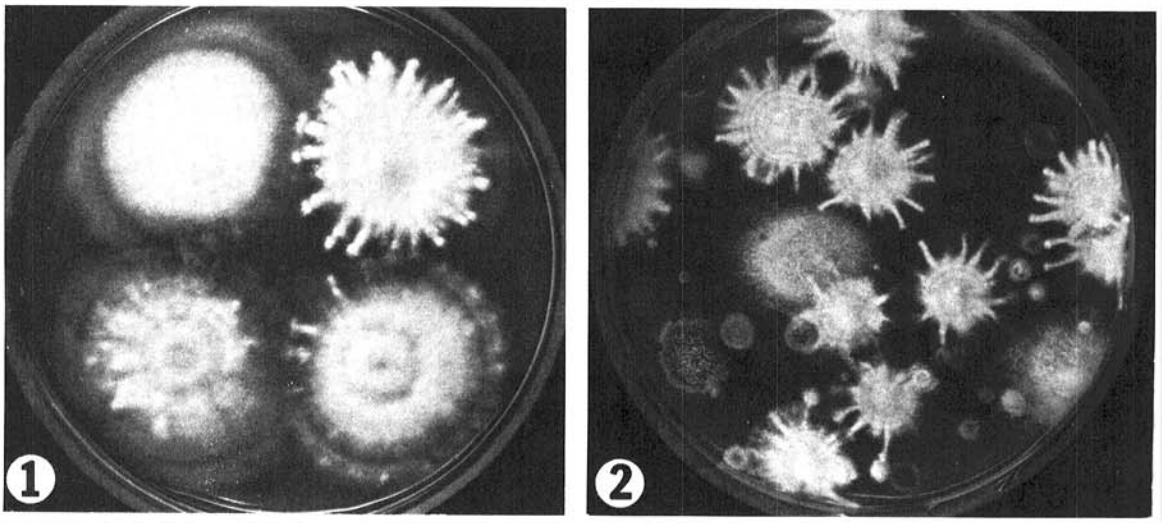

Fig. 1-2. Colony morphology of *Fusarium oxysporum* f. sp. *cubense* race 4 on K2 medium. **1)** Colonies of race 4 (upper right), isolate L (race 1, lower right), race 1 (upper left), and race 2 (lower left) of *F. oxysporum* f. sp. *cubense*. **2)** Colonies of fungi from experimentally infested natural soil. Those colonies of laciniate appearance are race 4 of *F. oxysporum* f. sp. *cubense*.

Fusarium spp. that were tested produced laciniated colonies on K2 medium: *F. oxysporum* f. sp. *lini*, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *batatas*, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *asparagi*, a saprophytic *F. oxysporum*, *F. moniliforme*, *F. roseum*, and *F. solani*. This indicates that the laciniate appearance of the colony on K2 medium is a specific characteristic of *F. oxysporum* f. sp. *cubense* race 4. Cavendish banana cultivars are susceptible only to race 4 (7) and Latundan cultivars are susceptible only to race 1 (6). All isolates of *F. oxysporum* obtained from 55 wilted Cavendish banana trees formed lacinated colonies on K2 medium. However, none of the isolates of *F. oxysporum* obtained from 16 wilted Latundan banana trees formed laciniated colonies on the same medium. These results further support the reliability of the method for identification of *F. oxysporum* f. sp. *cubense* race 4, at least for the isolates known in Taiwan at this time.

The K2 medium also was used to determine population of *F. oxysporum* f. sp. *cubense* race 4 in soil. Conidia of race 4 germinated about 98% on this medium, and the recovery of conidia from experimentally infested soil was about 90% (Fig. 2). When this medium was used to determine race 4 in naturally infested soil, the population of this fungus in 10 soil samples collected from three diseased areas was 55 to 650 propagules per gram of air-dried soil. Race 4 was not detected from any of the 50 soil samples collected from 10 disease-free areas.

All isolates of race 4 of *F. oxysporum* f. sp. *cubense* obtained from wilted Cavendish banana trees were identical in colony morphology. This may reflect the short history of the fungus in Taiwan (7). All of them probably originated from a single mutation. This also may account for the present success of using morphological characteristics for identification of the race. It is possible that a clone of race 4 without laciniated colonies may be found in the future.

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