Toxigenic *Fusarium scirpi* in Maize Grain from Midnorthern China

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ABSTRACT

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Fusarium scirpi (CF 12), isolated in 1983 from maize (*Zea mays*) stored by families as a staple food in midnorthern China was identified. This is the first report of this species on maize in China. Extracts of the fungus cultured on a rice medium were toxic to laboratory rats and induced significant chromosome aberrations and cytotoxic effects when added to a culture of V_{79} fibroblasts of Chinese hamster.

Recently, *Fusarium scirpi* Lambotte & Fautrey had been isolated from several substrates, e.g., Burgess et al (1) isolated *F. scirpi* from plant debris washed from soil in Australia, in 1976 and 1977. Since then, this species has been found elsewhere in eastern Australia, in Cape Province of South Africa, and in the Republic of Transkei (1,4). Wollenweber and Reinking (6) described *F. scirpi* as being similar to *F. equiseti* (Corda) Sacc.; however, Burgess et al (1) emended this description. Few have positively identified this species until recently.

In 1983, a strain, which was tentatively identified earlier as *F. equiseti*, was isolated from maize kernels (*Zea mays* L.) on ears stored by a peasant family in the midnorthern part of the People's Republic of China, where maize is a staple food. Experiments were made to identify the species of *Fusarium* and ascertain clastogenicity and toxicity of the fungus.

MATERIALS AND METHODS

Fifty samples of kernels from maize ears stored by 50 families as food supplies in midnorthern China were collected and stored in sterile paper bags at room temperature for 3 wk. These ears had been harvested and stored outdoors over winter. Corn kernels were washed with dilute detergent, surface treated with 75% ethanol for 2 min, cut into small pieces, and placed on a potato-dextrose agar medium (PDA). The PDA medium was prepared from fresh potatoes, dextrose, and Difco Bacto-agar. After cultures were single-spored, they were lyophilized in 0.5 ml of milk and stored at −20 C.

Isolates were identified to species based on morphology of the culture on PDA and on carnation-leaf agar (CLA), using the manual of Nelson et al (5). The 1.2% agar was poured into 60-mm petri dishes, and several sterile fragments of carnation leaves were emersed in the cooled, molten agar (2). Hyphal fragments were transferred from the PDA culture to CLA, and the colonies growing therefrom were identified. These cultures were kept at about 24 C under fluorescent lamps (5,300 lx) for 2–4 wk before being identified.

The extract of *F. scirpi* was prepared from cultures grown on rice. The rice medium was prepared by adding 50 ml of distilled water to 100 g of Chinese machine-polished rice and autoclaving it for 1 hr at 121 C; the rice medium was autoclaved again after 24 hr. Cultures of *F. scirpi* CF 12 that had been maintained on PDA were transferred to the rice medium and incubated at 25 C for 2 wk, then at 10 C for two more weeks. Twenty grams of inoculated rice was ground and extracted with 100 ml of methanol and water (85:15, v/v), shaken for 2 hr, allowed to stand for 10 min, and the precipitate was discarded. The crude extracts were dried in a water bath set at 60 C, then redissolved in dimethylsulfoxide (DMSO). The DMSO solvent was diluted further with Dulbecco modified Eagle medium (DMEM) (Gibco Lab. W/L-glutamine, W/4,500 mg of D-glucose/L), and different amounts of this solvent were added to the cell cultures of V_{79} fibroblasts of Chinese hamster.

The V_{79} cells were cultured in DMEM supplemented with 10% (v/v) new-born calf serum, 100 units of penicillin per milliliter, and 100 μg of streptomycin per milliliter, at pH 7.6. The V_{79} cells were cultured in a CO_2 incubator containing 5% CO_2, at 37 C (3). The V_{79} cells were cultured for 24 hr before, and again 24 hr after, the addition of the crude extract of the isolate. A 96-well microtest tray was used for assaying toxicity; 3×10^3 cells were placed in each well. The final concentration of the *Fusarium* extract was equivalent to 0.002, 0.02, 0.01, 0.2, 1, 2, 10, and 20 mg dry weight of *Fusarium*-rice culture per milliliter of culture medium. The final concentration of DMSO in the cultures varied from 0.00008 to 0.8%. Parallel controls containing 0.00008 and 0.8% DMSO were run for each experiment. Cell morphology and semiquantification were determined using a phase microscope after fixation of material with Formalin and giemsa stain.

Chromosomes were prepared using routine methods (3). The V_{79} cells were cultured in petri dishes for 24 hr before and again 24 hr after adding the crude extract of the isolate. Chromosome structures and numbers were determined in 100 well-spread metaphase figures for each dilution of the extract. Mitosis was ascertained in 1,000 cells of each specimen.

Twenty Wistar rats of 100–250 g body weight were used for the acute toxicity tests. The Chinese machine-polished rice inoculated with *F. scirpi* was cultured for 2 wk at 25 C and then 2 wk at 10 C. Ten rats were given normal animal ration, and 10 were fed with a 1:1 (w/w) mixture of *Fusarium scirpi*-inoculated rice and normal ration. The animal feed (normal ration) consisted of the following ingredients: yeast powder, 500 g; bone powder, 500–1,000 g; flour, 10 kg; sorghum flour, 7.5 kg; wheat bran, 7.5 kg; corn powder, 10 kg; bean cake powder, 7.5 kg; milk powder, 1 kg; salt, 0.5 kg; lysine, 25 g; multi-vitamins, 25 g; fish liver oil, 500 g; eggs, 30. Water was given ad libitum. Body weight was recorded before and at the end of the experiment. The date of death was also noted.

RESULTS AND DISCUSSION

Identification of *F. scirpi*. Thirty-two *Fusarium* isolates were obtained from 50 samples of maize kernels placed on PDA and included *F. moniliforme* Sheldon, *F. semitectum* Berk. & Rav. *F. scirpi*, and other species not identified. Of three isolates of *F. scirpi* one designated CF 12 (China *Fusarium*) was identified and tested further.

The cultural characters of *F. scirpi* observed were similar to those reported by Burgess et al (1). We observed rapid growth on PDA and dense aerial mycelium, which at first was white. A lighter regular rim appeared at the colony margin on the upper surface, but a tan color appeared on the lower surface. On rice, the culture was white, then brown, and the brown color increased in intensity as the culture aged. The macroconidia had characters typical of *F. equiseti*, which undoubtedly led to the earlier identification as this latter species. The elongated apical cells, the characteristic foot cells, the dorsal curvature, and the pronounced cell walls suggested *F. equiseti*. The macroconidia observed on CLA had long apical cells and distinct foot cells (Fig. 1A). Microconidia were abundant and 0–3 septate, and their shapes varied from obovate to fusiform and allantoid (Fig. 1B). Microconidia were abundant and borne on distinctive polyphialides that were short and cross-shaped (Fig. 1C), described by Burgess et al (1), and which influenced the change in identification to *F. scirpi*. Chlamydospores were seen either in chains or in groups (Fig. 1D). Both Burgess et al (1) and Nelson et al (5) described further distinctions between *F. equiseti* and *F. scirpi*. They noted that the principal difference between the two species is that *F. scirpi* produces abundant microconidia of various shapes and sizes on short, truncate, often cross-shaped polyphialides on aerial mycelium. In contrast, *F. equiseti* produces microconidia rarely or not at all, and when they occur they are formed on monophialides. This description confirmed our identification to species as *F. scirpi* Lambotte & Fautrey emend. Burgess, Nelson, Toussoun, and Marasas.

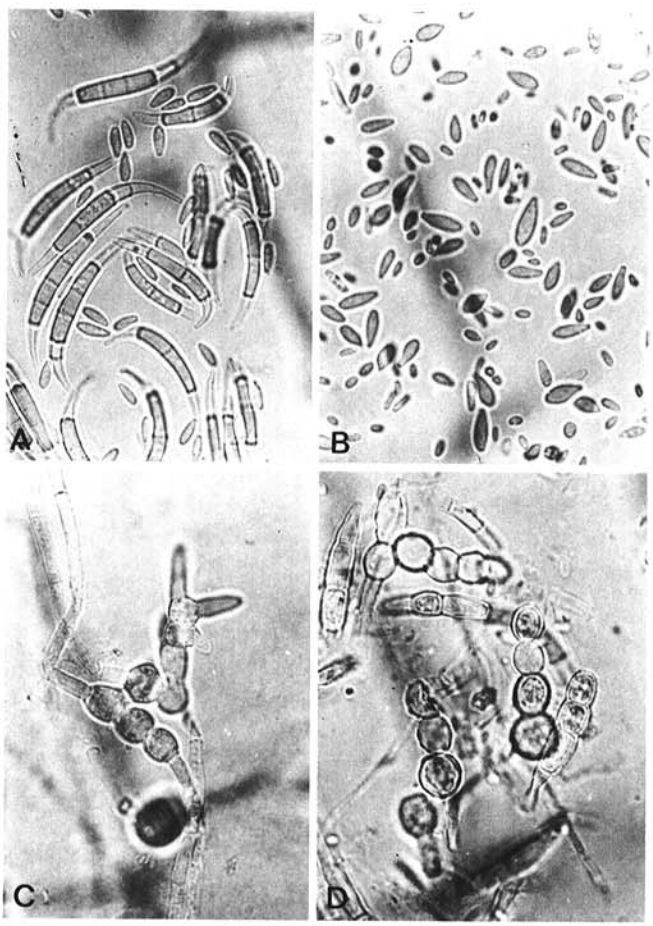

Fig. 1. Spores of *Fusarium scirpi* (CF12) (×1,000) on CLA: **A,** macroconidia produced in sporodochia (×1,000), showing elongated apical cells, foot-shaped cells, and clear-cut septa; microconidia are also visible; **B,** microconidia produced in aerial mycelium showing fusiform, obovoid, and allantoid shapes; **C,** microconidia borne on short and cross-shaped polyphialides, chlamydospores are also visible; and **D,** chlamydospores in chains and groups.

Cytotoxic and chromosomal effects. The toxicity of the extract of *F. scirpi* to the V_{79} cells of the hamster increased with increasing concentrations of the extract of the inoculated rice in the medium, based on four experiments done in a micro-test tray and compared with controls of no DMSO or controls with either of two concentrations (Table 1). This result resembled the effect by the T-2 toxin. At 10 and 20 mg dry weight of *Fusarium*-rice culture per milliliter of culture medium, there were no cells at the bottom of the well in the test tray due to death of all cells.

Various concentrations of the extract from the *Fusarium* rice culture in the medium inhibited the mitotic index on cultured V_{79} cells. The mitotic index in percent for each concentration of the extract in the medium (milligrams of rice dry weight per milliliter of medium) was as follows: 42 (control), 46 (0.06), 32 (0.2), 28 (0.6), 24 (2), 11 (10), and 7 (20). A dose response relationship is apparent. Thus *F. scirpi* (CF12) produced a cytotoxic effect and inhibited mitosis.

The extract from *F. scirpi* grown on rice induced chromosomal aberrations in the V_{79} hamster cells at several concentrations, and a relationship was found between dose and aberration rate (Fig. 2). We conclude that this strain (CF12) produces toxins that have a clastogenic effect.

TABLE 1. Cytotoxic effect on V_{79} hamster cells of a crude culture extract from *Fusarium scirpi* (CF12) compared with the effect of the T-2 toxin

Treatment	Concentration	Cytotoxic effect per experiment[a]			
		1	2	3	4
F. scirpi extract[b]	0.002	−	−	−	−
	0.02	−	−	−	−
	0.1	+	+	+	+
	0.2	+	+	++	+
	1	++	++	++	++
	2	++	++	++	++
	10	+++	+++	+++	+++
	20	+++	+++	+++	+++
T-2 toxin[c]	0.01	−	−	−	−
	0.1	−	−	−	−
	1	−	−	−	−
	6	+	+	+	+
	9	++	++	+	+
	18	+++	+++	+++	+++
	32	+++	+++	+++	+++
	64	+++	+++	+++	+++
Control:DMSO[d]	0	−	−	−	−
	0.00008	−	−	−	−
	0.8	−	−	−	−

[a] − = no cytotoxic effect; + = mild, ++ = moderate, and +++ = severe toxic effect.
[b] Concentration as milligrams dry weight of rice culture per milliliter of medium.
[c] Concentration of toxin as nanograms per milliliter of medium.
[d] Concentrations as percent of dimethylsulfoxide in medium.

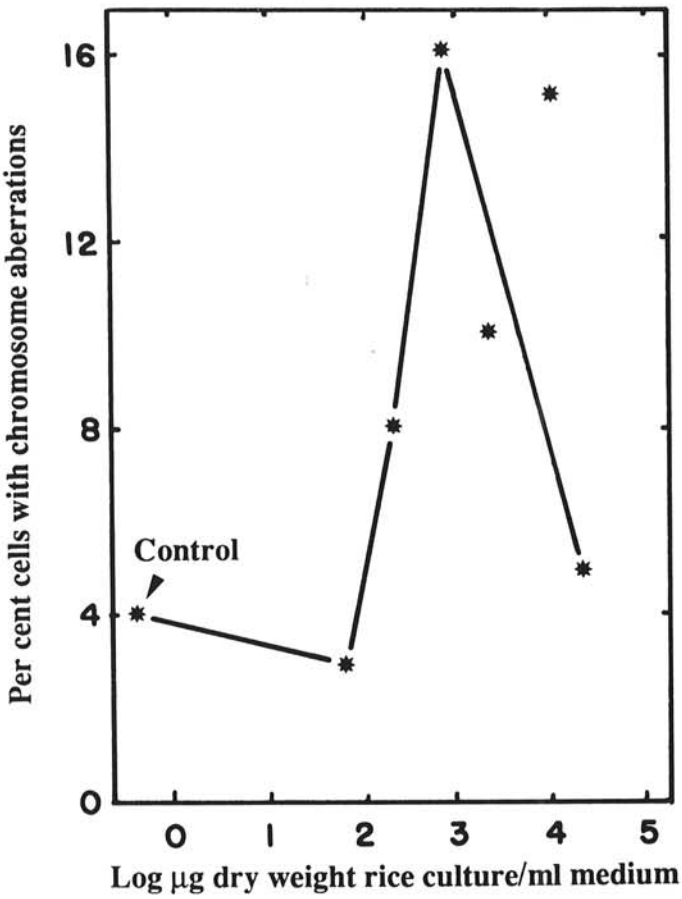

Fig. 2. Dose response relationship of chromosome aberration in cultured V_{79} hamster fibroblast cells with six different concentrations of a crude extract from *Fusarium scirpi* (CF12) in the culture medium.

Toxic effect on rats. Four rats died on the ninth day when fed the *Fusarium*-rice culture following a period of decreasing appetite and a 20% decrease in body weight. Rats in the control group increased body weight by 40% on the ninth day of test. Five rats died after 17 days when fed cultured rice and sustained an average decrease of 18% in body weight during this period. The control group of rats increased body weight by an average of 150%. We concluded that this species can be toxic to animals.

Because this species of *Fusarium* was first isolated from plant debris in soil from Australia, it attracted interest primarily because of its different and characteristic morphology. There is no information regarding its toxigenicity to human beings. This species has been isolated from maize, a staple food of the local residents in midnorthern China, and it is toxic to animals, both in vivo and in vitro. We also showed that the metabolites of this strain induced chromosomal aberrations in animal cells. It deserves further study as to its importance to human health.

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