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Pathogenic Strains of *Fusarium oxysporum* Fr. Distinguished by their Differential Tolerance to Inhibition by various Actinomycetes

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SUMMARY: Cultures of sixteen different soil actinomycetes were tested for their ability to inhibit the growth of eight pathogenic strains of *Fusarium oxysporum* Fr. on agar media. Three of the actinomycetes did not inhibit growth, and nine inhibited growth of all strains equally, but the other four actinomycetes consistently inhibited the growth of individual strains to different extents. The differences provide an *in vitro* test which distinguishes between certain pathogenic strains of *Fusarium* that are otherwise indistinguishable in culture.

Fusarium oxysporum Fr. is an Imperfect fungus comprising many pathogenic strains which are indistinguishable in culture. The complex system of classification proposed by Wollenweber & Reinking (1935), in which many species were described, was simplified by Snyder & Hansen (1940), who grouped all the members under the name *F. oxysporum* Fr. The many forms of this species cause wilt diseases of several important crops, e.g. banana, tomato, flax and peas. Each pathogenic strain is designated *forma specialis* according to its specific pathogenicity towards a particular host. Within these strains, physiologic races occur, the races differing in their abilities to cause disease in different varieties of the host. Eight members of the species *F. oxysporum* Fr. were grown in culture with sixteen actinomycetes to see whether the actinomycetes affected the growth of the fusarium strains and, if so, whether the inhibition was specific and in any way correlated with the pathogenicity of the fusarium strains.

METHODS

The eight fusarium strains used, with their laboratory numbers and origins, were as follows: 71 B, *Fusarium oxysporum* f. *pisi* race 1, from wilted peas; 72 B, *F. oxysporum* f. *pisi* race 2, from 'near-wilted' peas; 611 A, *F. oxysporum* f. *pisi* race 3 A, from wilted peas (Buxton, 1955); 612 A, *F. oxysporum* f. *pisi*, from wilted peas, equivalent in pathogenicity to 71 B; 51 A, *F. oxysporum* F. (syn. *F. oxysporum* Fr. var. *redolens* (Wr.), n. comb. Gordon, from wilted peas; F 1, *F. oxysporum* f. *gladioli*, yellowing strain from Gladiolus; F 2, *F. oxysporum* f. *gladioli*, yellowing and rotting strain from Gladiolus; 61 A, *F. oxysporum* Fr., non-pathogenic to peas, isolated from soil.

Sixteen actinomycetes, isolated from soil, were tested against the eight fusaria. The tests were made on medium constituted as follows: (g./l. water; $NaNO_2$, 2·0; K_2HPO_4, 1·0; $MgSO_4.7H_2O$, 0·5; KCl, 0·5; $FeSO_4.7H_2O$, 0·01;

Table 1. *Differential tolerance of eight strains of* Fusarium oxysporum *to inhibition by sixteen actinomycetes as shown by the distances (mm.) grown towards the actinomycete colonies*

Isolate number	*Fusarium* species	Origin	Actinomycetes															
			A1	A2	A3	A4	A5	A6	A9	A14	A16	A17	A19	A26	A27	A28	A29	A30
			Distances to nearest 0·5 mm. Low numbers represent severe inhibition.															
71 B	*oxysporum* f. *pisi* race 1	Wilted peas, U.S.A.	18·0	25·0	17·0	8·0	13·0	24·5	22·5	15·0	10·5	13·0	19·0	22·0	24·0	17·0	23·0	23·5
72 B	*oxysporum* f. *pisi* race 2	Wilted peas, U.S.A.	16·5	24·0	14·5	7·5	12·0	23·5	22·0	10·5	11·0	9·0	25·0	15·0	12·5	18·0	18·0	16·0
611 A	*oxysporum* f. *pisi* race 3	Wilted peas, England	19·0	21·0	18·0	8·5	22·5	25·5	22·0	14·0	17·0	13·0	22·5	19·0	19·0	20·0	24·0	23·5
612 A	*oxysporum* f. *pisi* race 1	Wilted peas, England	19·5	27·5	19·0	10·0	16·0	26·0	20·0	11·0	13·0	12·0	20·5	13·5	22·0	14·0	24·0	20·0
51 A	*oxysporum* var. *redolens*	Wilted peas, England	18·5	21·5	16·5	9·0	23·5	26·0	23·5	15·0	20·0	11·0	20·0	21·0	21·0	20·0	24·5	18·0
F 1	*oxysporum* f. *gladioli*	Yellowed gladiolus, England	19·5	24·0	17·0	9·5	19·0	28·0	20·5	13·0	18·0	11·5	20·0	22·0	21·0	21·5	25·0	23·5
F 2	*oxysporum* f. *gladioli*	Yellowed gladiolus, England	20·0	22·5	16·5	9·5	18·0	25·0	22·0	13·0	16·0	11·5	20·5	18·0	20·0	18·5	23·0	21·5
61 A	*oxysporum*	Soil, England	18·5	24·0	21·0	10·5	18·5	25·5	22·0	13·0	20·0	11·0	20·5	17·0	23·5	18·5	21·5	22·0

soluble starch, 10·0; Bacto-tryptone, 1·0; agar, 15; pH adjusted to 7·0 with a concentrated solution of NaOH.

A single streak of an actinomycete was made on a Petri dish containing 10 ml. of the medium; this was then incubated for 5 days at 25°. A streak of a fusarium (as spore suspensions of uniform density) was then made parallel to the actinomycete colony and 30 mm. distance from it. The plates were incubated for 5 more days before the growth of the fusarium towards the actinomycete was measured, these times having been found optimal for showing inhibition.

RESULTS

Three of the actinomycete colonies (A 4, A 14, A 17) strongly inhibited all eight fusaria, six (A 1, A 3, A 9, A 19, A 28, A 30) inhibited all less strongly, and three (A 2, A 6, A 29) allowed them to grow normally, so that the fusarium finally enveloped the actinomycete colony. Four of the actinomycetes (A 5, A 16, A 26, A 27) inhibited different fusaria to different extents. Control streaks of the fusaria grew beyond the 30 mm. lines after 6 days. Strongly inhibited fusarium strains had advancing edges which consisted entirely of tightly packed stunted hyphae, whereas partly inhibited strains had marginal hyphae which branched subnormally. The inhibition shown by actinomycete A 5 (Table 1), identified as a strain of *Streptomyces albidoflavus* (Rossi Doria) Waksman & Henrici, towards strains 72 B (*F. oxysporum* f. *pisi*, race 2) and 611 A (*F. oxysporum* f. *pisi*, race 3 A) is shown in Pl. 1, fig. 1. Zones of inhibition by the same actinomycete on dishes seeded with fusaria are shown in Pl. 1, fig. 2. Culture filtrates of *S. albidoflavus* grown in liquid starch-tryptone medium (constituted as above) were tested against the fusarium strains. Porcelain cylinders containing 0·1 ml. of the filtrate were placed on the surface of dishes containing 10 ml. of starch tryptone agar medium seeded with spores of fusaria. Inhibition differences by the filtrates were analogous to those observed in the streak method and are shown in Pl. 1, fig. 3.

DISCUSSION

There is nothing new in the fact that growth products from actinomycetes should inhibit the growth of fusaria in culture or in the soil. Kublanovskaya (1952) suppressed wilt of cotton, caused by *Fusarium vasinfectum*, and Lachange & Perrault (1953) wilt of flax, caused by *F. lini*, by adding actinomycetes to infected soil. Neither variations in the susceptibility of different pathogenic strains of *Fusarium* nor indeed variations in any strains of wild-type fungus to the action of actinomycete inhibitors, have been previously recorded. Mitchison (1951), however, found isolates of *Mycobacterium tuberculosis* with different abilities to tolerate streptomycin, and Katznelson & Sutton (1953) detected different reactions of strains of *Xanthomonas translucens* to bacteriophage. Such variations are not unexpected, for the ability to grow in the presence of substances which inhibit other organisms in any natural environment has obvious survival value. The plasticity afforded by variations in

reaction by different strains to actinomycete inhibition would be important to heterogeneous soil populations of *Fusarium* spp. The main immediate value of these differences is that they provide a simple *in vitro* method of distinguishing between isolates of *F. oxysporum* that otherwise are distinguishable only by laborious infection tests. Previously, during our experiments concerned with the genetics of pathogenicity in *F. oxysporum*, the only way of distinguishing *in vitro* between isolates used in combined infections was to produce biochemical or morphological mutants by such treatments as irradiation with ultraviolet light. We have found that mutants produced by these treatments are, however, usually decreased in their infectivity. A natural difference in ability to tolerate growth products of an actinomycete provides a test that avoids the complications introduced by artificial mutagenic methods, for the genetical 'markers' concerned with tolerance to inhibition are related to wild-type pathogenicity.

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EXPLANATION OF PLATE

Fig. 1. Differences in tolerance shown by *Fusarium oxysporum* f. *pisi*, race 2 (left) and *F. oxysporum* f. *pisi*, race 3A (right) towards *Streptomyces albidoflavus*. ($\times \frac{2}{3}$.)

Fig. 2. Zones of inhibition of *F. oxysporum* f. *pisi*, race 2 (left) and *F. oxysporum* f. *pisi*, race 3 (right) caused by *S. albidoflavus*. ($\times \frac{2}{3}$.)

Fig. 3. Effect of a culture filtrate of *S. albidoflavus* on three strains of *F. oxysporum*. *a*, *F. oxysporum* f. *pisi*, race 2: strongly inhibited. ($\times \frac{2}{3}$.) *b*, *F. oxysporum* f. *gladioli*, strain F1: mildly inhibited. ($\times \frac{2}{3}$.)

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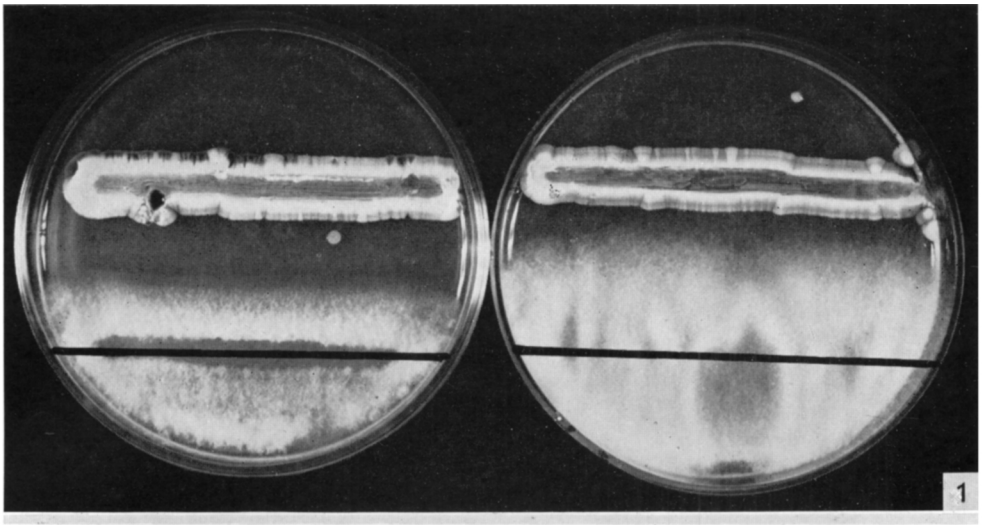

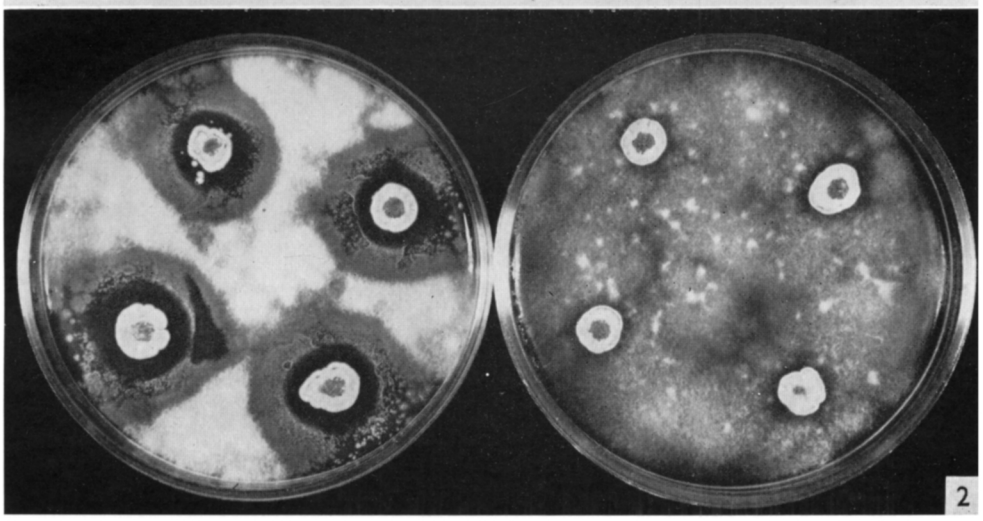

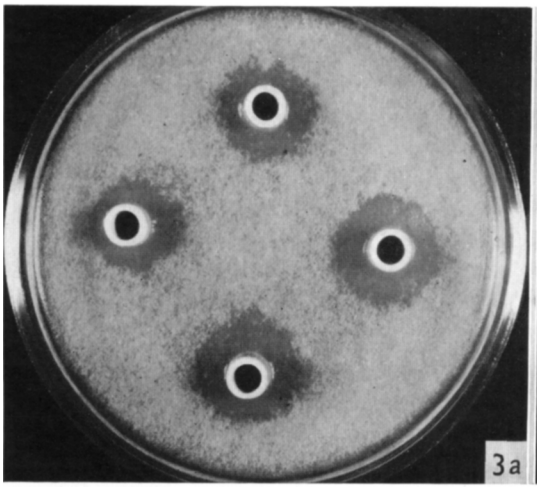

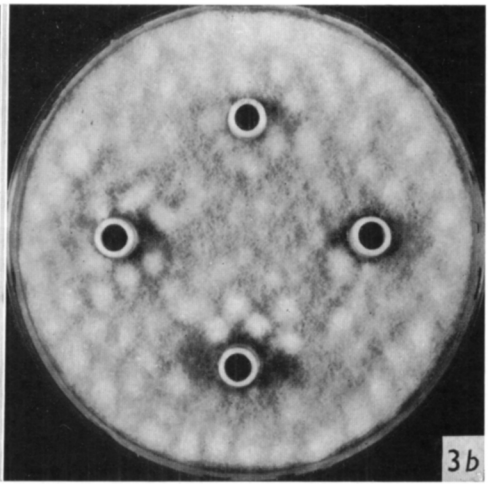

E. W. Buxton and M. G. Richards—Inhibitory distinction by actinomycetes. Plate 1

(*Facing p.* 102)