SECTION A, BIOLOGICAL SCIENCES

SUBSECTION BOTANY

Suitability of Diaporthe phaseolorum for use in Studies of the Physiolgy of Soft-rot Pathogenesis[1]

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Mutant organisms have been useful in working out pathways of fundamental cellular processes (Beadle and Tatum, 1945). Mutants have also been used in studies of the effect of nutrition upon pathogenesis (Garber, 1945 and Keit and Boone, 1945). They should be useful in demonstrating the role of definite extra-cellular enzymes in soft-rot pathogenesis. For this purpose, isolates are needed which vary in their pathogenicity and/or their potential for enzyme production.

Materials and Methods

The fungus used in this study, *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehmeyer, was isolated from birdsfoot trefoil, *Lotus corniculatus* L. This fungus is known to cause a soft-rot of tomato and okra fruits (Luttrell, 1947) and will also cause soft-rotting of potato tubers when introduced into the tuber.

Ultra-violet irradiation of conidia was used as a method of increasing the variability within the parent culture. Conidia were suspended in sterile distilled water and diluted to approximately 1000 conidia per ml. One ml aliquots of this suspension of conidia were distributed evenly over the surface of solidified potato dextrose agar in petri dishes. The dishes containing the conidia were irradiated using a short wavelength UV lamp (Geo. W. Gates Co., type MR4, delivering radiation principally at 2573 A) for 45 to 60 seconds at a distance of eight inches. The lids of the dishes were removed during irradiation. The cultures were then incubated at room temperature for three days, after which single conidial colonies were selected from the relatively few survivors.

The weight of host tissue decayed was used as an index of the virulence of the isolates. A #1 cork borer was used to remove a plug of tissue from firm, nearly ripe tomato fruits. A disk of inoculum, cut from an agar culture with the same cork borer, was inserted into the hole in the fruit, the plug of tissue was replaced and covered with cellophane tape. One week later the resultant decayed tissue was scraped from the fruit onto a previously weighed filter paper. The dry weight of the decayed tissue was then obtained. Potatoes were inoculated similarily, except care was taken to insure placement of the fungus in the center of the tuber.

The activity of the enzymes, pectin polygalacturonase and depolymerase, results in a reduction of the viscosity of pectin sols. The viscosity of a mixture of 0.5 M acetate buffer, 1.5 percent pectin sol and culture filtrate in 3:10:2 ratio, (temperature maintained at 30° C) was measured as rate of flow through a marked pipette. Rate of flow determinations were made at the time of mixing and at measured intervals thereafter.

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Enzyme activity of the filtrate was calculated from the following formula:

$$\frac{t_o - t}{t_o - t_w} \times 100 = \text{activity},$$

where t_o = time of flow of mixture at time zero
t_w = time of flow of demineralized water
t = time of flow of mixture at any subsequent measurement.

The rates of growth of the isolates were measured by obtaining the dry weight of mycelium produced upon the following liquid media:

Dextrose minimal medium

Dextrose	30.0 g
NH_4NO_3	2.0 g
KNO_3	1.0 g
$MgSO_4 \cdot 7\ H_2O$	0.5 g
KCl	0.5 g
K_2HPO_4	0.8 g
$KH_2PO_4 \cdot 3\ H_2O$	0.2 g
Demineralized water	to 1 liter

Pectin minimal medium

The above medium with 30 grams pectin substituted for the dextrose was used as the pectin medium. Multiple vitamin solution (Lamey, *et al*, 1956)

Thiamine HCl	61 mg
Riboflavin	50 mg
Pyridoxine HCl	61 mg
Calcium pantothenate	218 mg
p-amino benzoic acid	50 mg
Nicotinic acid	200 mg
Choline chloride	200 mg
i-inositol	400 mg
Biotin	50 μg
Folic acid	4.5 μg
Demineralized water	to 1 liter

The vitamin solution, when used, was added to the minimal medium at the rate of 5 ml per liter of medium.

Inoculation into the growth flasks was made from vitamin-free, minimal medium. After 10 days, the mycelium was harvested by filtering the liquid medium through previously weighed filter papers. The mycelium and filter paper were then dried for twelve hours in a vacuum drying oven and weighed.

Results

Data of three isolates which differ in pathogenicity are reported. In rotting tomato fruits, isolate A was approximately four times as effective as isolate C while isolate B was little more effective than C. (Fig. 1) Similar results were obtained from the inoculation of potatoes. Potato tuber tissue was rotted most effectively by isolate A. Isolate A was about two times as effective as isolate B, which was little more effective than isolate C. (Fig. 2)

The isolates differed little in enzyme activity of their filtrates (which were from minimal-pectin medium). Activity of the filtrates, calculated from measurements made after fifteen minutes reaction, were as follows: isolate A, 75; isolate B, 60; and isolate C, 67. (Fig. 3)

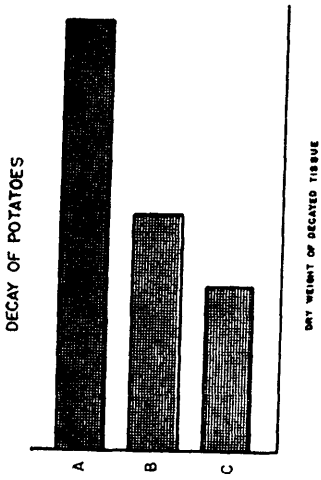
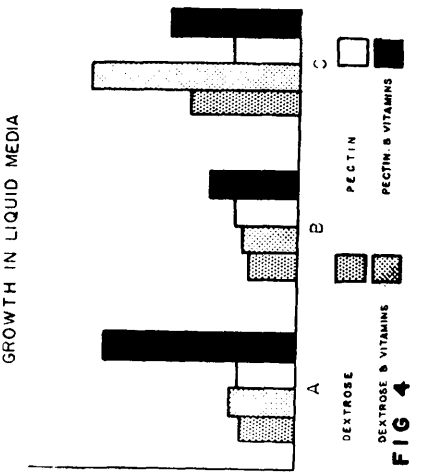


FIG 2



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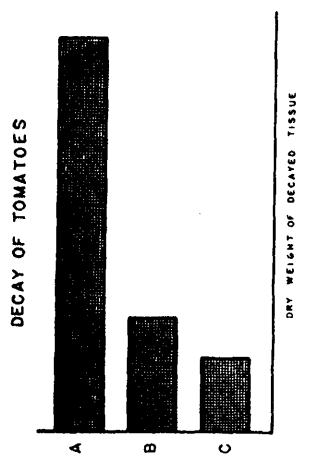

FIG 1

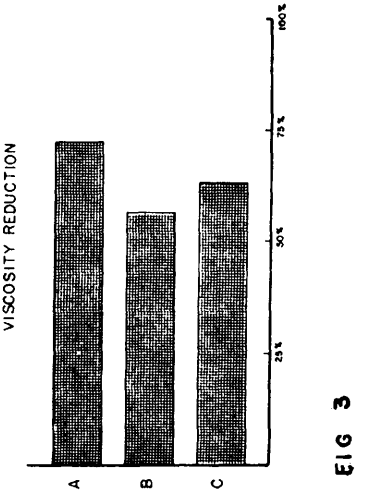

FIG 3

Fig. 1. Relative dry weights of tomato tissue decayed by three isolates of *Diaporthe phaseolorum* var *sojae*.

Fig. 2. Relative dry weights of potato tissue decayed by three isolates of *Diaporthe phaseolorum* var *sojae*.

Fig. 3. Viscosity reduction of a mixture composed of 3 parts 0.5 M acetate buffer, 10 parts 1.5 percent pectin sol and 2 parts culture filtrate during 15 minutes reaction.

Fig. 4. Weight of mycelium produced by three isolates in four different media after 10 days.

The most striking variability of the isolates appeared in their growth rates. When cultured for 10 days in liquid minimal medium without vitamin amendment, isolate C produced nearly twice as great a weight of mycelium as isolates A or B. Addition of multiple vitamin solution to the medium had little effect upon isolates A or B, but increased the growth rate of C, resulting in an overall growth of approximately three times that of A or B. When the same medium was used except that pectin was substituted for the dextrose, weight of mycelium produced by the three isolates did not differ significantly. However, when the vitamin solution was added to the pectin medium, isolate A made signicantly more growth than isolates B or C and isolate C produced a greater weight of mycelium than isolate B. (Fig. 4)

Conclusions

The isolates selected from single conidial colonies surviving UV irradiation vary in pathogenicity as measured by decay of tomato fruits and potato tubers brought about by inoculation with these isolates. These isolates also vary in growth rate and are affected differently by amendment of the medium with vitamins. Small differences in enzyme activity of isolate filtrates were also found. Pathogenicity could not be explained by differences in growth rate or variation in enzyme activity of the isolates.

The fact that these closely related isolates differ in pathogenicity and in physiology should make them convenient tools for further study of soft-rot pathogenesis.

LITERATURE CITED

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