Streptomycin Inhibition of Peronospora parasitica and its Host Reversed by Manganese and Calcium

Dorothy McMeekin

Department of Natural Science, Michigan State University, East Lansing 48823.
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ABSTRACT

Spore germination and systemic growth of *Peronospora parasitica* in the cabbage host, the growth of cabbage roots, and the growth of *Pythium aphanidermatum* are inhibited by streptomycin. This inhibition is reversed by calcium and manganese, indicating that the mode of action of streptomycin in these species is similar. Studies on the action of streptomycin in fungi and higher plants should always indicate calcium and manganese levels.

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A few Oomycete species are sensitive to streptomycin in vitro (3, 10). When streptomycin is applied to a host, the growth of *Peronospora* and *Phytophthora* in the host is affected (5, 9). This effect was not observed by Bonde (2). Growth of tomato roots and bacteria is inhibited by streptomycin (4). This inhibition is partially reversed in tomato but not in bacteria by manganese and calcium, indicating that the action of streptomycin in higher plants and bacteria is probably different (4).

This study compares the effect of streptomycin with and without manganese and calcium on the growth of the obligate parasite *Peronospora parasitica* Pers. ex Fr. in cabbage, *Brassica oleracea* var. *capitata*, *P. parasitica* spore germination, cabbage root development, and the growth of the nonobligate parasite *Pythium aphanidermatum* (Edson) Fitzp. The objectives are to see if the action of streptomycin, in stopping systemic infection by *P. parasitica*, can be reversed by calcium and manganese, and to determine if these parasites are closer to higher plants or to bacteria in their response to streptomycin with or without calcium and manganese.

Sixteen different media were prepared. Modified Hoagland's minus Ca^{++} and Mn^{++} was used as a basic medium (6). Streptomycin sulfate (Nutritional Biochemicals Corp.) was added to eight portions to give a 1.37×10^{-4} M concentration. Three portions with and three without streptomycin were supplemented with either $MnSO_4 \cdot H_2O$ or $Ca(NO_3)_2 \cdot 4H_2O$ to give final concentrations of either 1.37, 6.85, or 13.7×10^{-4}M. Modified Hoagland's without streptomycin, calcium, or manganese served as a control.

Danish Ballhead cabbage seeds were surface-sterilized in 10% Clorox (5.25% sodium hypochlorite) for 5 min, and rinsed in sterile distilled water. For root studies, the sterilized seeds were placed on moistened sterilized glass wool on the bottom of covered glass baking dishes at 21 C in the dark for 5 days. Five germinated seeds were transferred to 5-cm petri dishes lined with glass wool, and moistened with 2 ml of the test solutions. The seedlings were incubated in the dark at 15 C, and 9 days later the roots were measured.

Hypocotyls for systemic infection studies were

grown by placing surface-sterilized cabbage seeds on 1% water agar for 7 days. The roots and cotyledons were cut from the hypocotyls. The hypocotyls were pushed through two layers of cheesecloth covering a 5-cm petri dish bottom. They were held in a vertical position with one end of the hypocotyl in 10 ml of the test solution. A 1-mm square piece of cotyledon, infected with *P. parasitica*, was placed on the side of the hypocotyl just above the cheesecloth. The petri dishes were put in a covered glass dish lined with moist paper at 15 C with 5 ft-c of light for 6 days. The hypocotyls were removed from the solutions, placed on slides with cotton blue in lactophenol, and pressed with another slide before a cover slip was applied. The distance between the tip of the hypocotyl that was in the test solution, and the mycelium that had grown from the point of inoculation, measured using X100 magnification, was used as an indication of the compatibility of the systemic fungus and the solution (8).

Spores (200/ml) were placed in the test solutions. Germination counts were made after 24 hr at 15 C using X100 magnification.

Pythium aphanidermatum was grown in 9-cm petri dishes on potato-dextrose agar (PDA) amended to give the concentrations of streptomycin, $MnSO_4$, and $Ca(NO_3)_2$ in the test solutions. Hoagland's solution minus calcium was omitted (6). The plates were inoculated in the center with 2- to 3-mm^2 pieces of PDA containing *P. aphanidermatum* mycelium, and incubated at 21 C. Colony diameter was measured 4 days later.

Bacterial response was determined on nutrient agar inoculated with a X 1,000 dilution of a 24-hr culture of *Bacillus subtilis*. Streptomycin solutions were placed in glass rings (outer diam, 10 mm) on top of the nutrient agar. The experiment was repeated twice with the calcium and manganese in the ring

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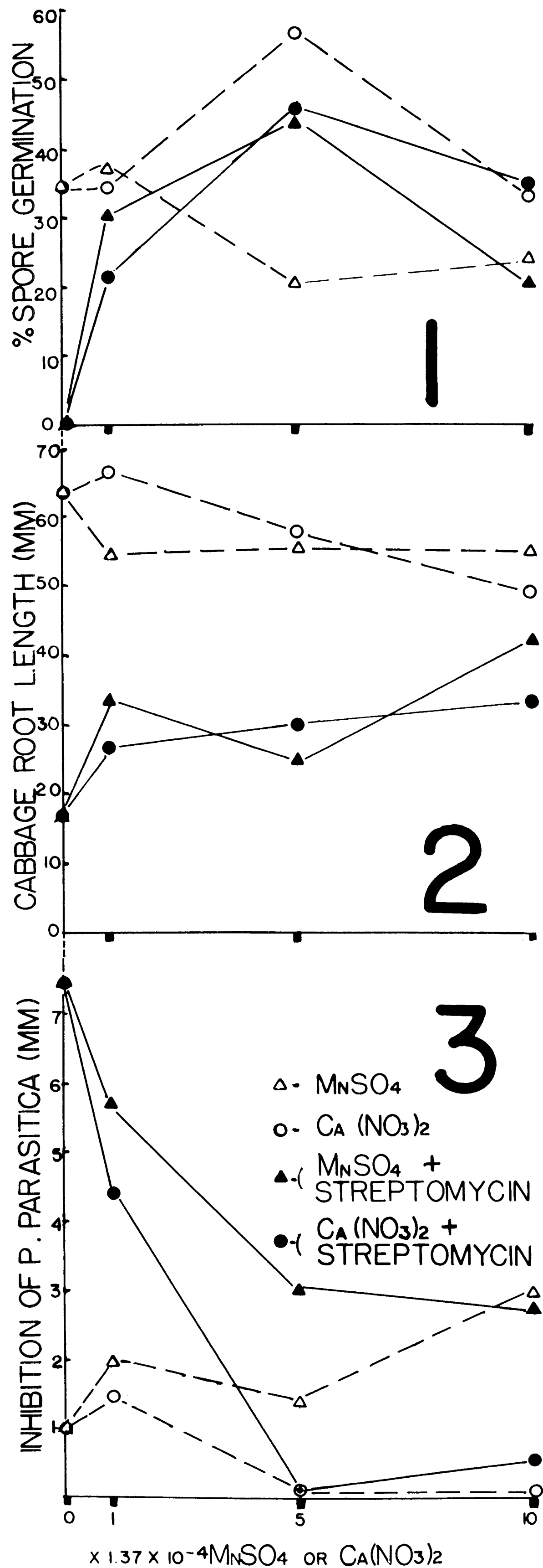

Fig. 1-3. A comparison of the inhibition of spore germination in the parasitic fungus *Peronospora parasitica*, primary root growth in the host cabbage, and systemic growth of the parasite in the host hypocotyl by streptomycin sulfate and the reversal of this inhibition by calcium and manganese. In the three graphs, growth is plotted on the vertical axis, and the concentration of $Ca(NO_3)_2$ or $MnSO_4$ is indicated on the horizontal axis. The solid lines connecting points on the graph indicate that the response of the plant occurred in the presence of 1.37×10^{-4} M streptomycin plus either 0, 5, or 10 X 1.37×10^{-4} M $Ca(NO_3)_2$ or $MnSO_4$. In this case, the zero is a control with streptomycin but free of calcium or manganese. The broken lines connecting points in the graph indicate that the response of the plant occurred in the absence of streptomycin but with either 0, 5, or 10 X 1.37×10^{-4} M $Ca(NO_3)_2$ or $MnSO_4$ present. This control is free of streptomycin, and the zero in this case is free of streptomycin, calcium, and manganese. **1)** Each point represents the percent germinated in a count of 200 spores. **2)** Each point represents the average length of 20 primary roots. **3)** Each point represents the average length of the zone of inhibition of the fungus in 20 host hypocotyls. The zone of inhibition is the distance from the tip of the hypocotyl in the test solution to the closest hypha in the hypocotyl.

with the streptomycin, and twice with the ions in the agar medium. After incubation of the plates at room temperature for 2 days, the width of the zone free of bacterial growth outside the glass ring was measured.

Streptomycin inhibition of *P. parasitica* spore germination, vegetative growth in hypocotyls, and cabbage root growth are reversed by manganese and calcium (Fig. 1-3). A similar reversal occurred with *P. aphanidermatum*. In an average of six replicates, colony diameter was 7.5 cm in the medium with streptomycin, 8.7 cm where manganese or calcium was added to the streptomycin, and 8.6 cm in the control lacking these substances. Also, the *P. aphanidermatum* colony with these ions had more aerial mycelium or a cottony appearance that was lacking in the presence of streptomycin, but without calcium and manganese. The inhibition of *B. subtilis* growth was not reversed by calcium or manganese. The 1.37×10^{-4} M streptomycin produced a 2-mm zone of inhibition, and this zone did not change with the addition of Ca^{++} or Mn^{++} ions to either the agar medium or the ring containing the streptomycin.

There is probably a common mechanism by which streptomycin alters the growth of cabbage, and the fungi *P. parasitica* and *P. aphanidermatum*, since the inhibition is reversed by manganese and calcium. The concentration required to reverse inhibition, 1-5 $\times$ 1.37×10^{-4} M $MnSO_4$ and $Ca(NO_3)_2$ (Fig. 1-3), is in agreement with previous reports (4, 11, 12). Gray (4) found that calcium ions were less effective than manganous ions in reversing streptomycin inhibition, but the results given for spore germination (Fig. 1) and for cabbage root growth (Fig. 2) show that calcium is just as effective as manganese, and Fig. 3 indicates that calcium is more effective in reversing the inhibition of systemic infection by streptomycin. The mode of action of streptomycin in higher plants is unknown. In bacteria, streptomycin prevents protein synthesis (7), but this effect has not been demonstrated in vivo in higher plants (1).

Reports on the effects of streptomycin on plant-pathogenic Oomycetes are contradictory. Grosso (5) sprayed 50-200 μg/ml streptomycin on tobacco leaves and inoculated them with *Peronospora tabicinia*, and Muller et al. (9) placed cut stems of tomato and potato in 40-200 μg/ml streptomycin and inoculated the leaves with *Phytophthora infestans*. Both reports indicate that fungal development was inhibited. Bonde (2) used procedures similar to Muller et al. (9), but he found that streptomycin does not inhibit the development of *P. infestans* in potato. None of these reports (2, 5, 9) indicates available calcium and manganese. The availability of these ions at the infection site could alter the response to low concentrations of streptomycin. Future studies involving the Oomycetes and streptomycin should include information on the presence of calcium and manganese in the medium or the host.

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