THE MECHANISM OF ACTION OF THE YAM NEMATODE, 
SCUTELLONEMA BRADYS

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Chromatographic analysis of the incubation solution of S. bradys showed that five amino acids - aspartic acid, phenylalanine, hydroxyinol acetic acid, leucine and isoleucine were discharged by this nematode. Pectinase and amylase activities were detected in homogenates of S. bradys. The absence of the steroid group of compounds in the nematode-infected yam tubers revealed by spectrometric analysis might be disease-related as evidenced by its appearance in the healthy yam tubers.

The dry rot disease of yam tubers (Dioscorea sp.) is closely associated with the yam nematode Scutellonema bradys, which predisposes yam tubers to infection by secondary organisms like fungi and bacteria that cause wet rot (Adesiyan et al. 1975; Bridge, 1973). To date, very little is reported about the role of S. bradys in the dry rot disease of yam tubers. Experiments were therefore designed to study the substances discharged by S. bradys that might cause dry rot in yam tubers. The amino acids discharged by S. bradys were investigated by methods described by Myers & Krusberg (1965). Cellulolytic and pectolytic enzymes were separately assayed by viscometric methods (Levinson & Reese, 1950); amylase and invertase activities were measured by the methods of Bernfeld (1955) and Sumner & Somers (1953). Yam tissue extracts were also examined for other classes of compounds spectrometrically.

MATERIALS AND METHODS

Nematodes were extracted from infected yam tubers using the tray modification of the Baermann funnel method (Whitehead & Hemming, 1963). The nematodes were then surface-sterilized with 0.1% streptomycin sulphate and washed five times with a fine mist of sterile distilled water. The surface sterilized nematodes were incubated at 30° for 48 hours in shaken flasks of distilled water and 1% glucose adjusted with 1 N HCl to pH 5.0. The incubation solution was checked for microbial contamination by pipetting about 1 ml of it into melted potato dextrose agar (PDA) in a test tube, and pouring the mixture into petri dishes and incubating at 30° for 48 hours. Counts of microbial colonies were found to be negligible (less than 200) and the incubation solution was used immediately.

Chromatographic analysis of incubation solution. Solutions in which nematodes were incubated were tested microchemically to determine the classes of compounds
present, about 0.1 ml being used in each test for amino acids, urea and other amides. A two-dimensional ascending chromatogram was used with n-butanol: acetic acid and water (12:3:5) as the first dimension and phenol: water (80:20 w/v) as the second dimension. The papers were left for 24 hours and then sprayed with ninhydrin and dried at 80°-100° for some minutes when most of the amino acids showed up brightly.

*Enzymes of S. bradyi.* About 5 ml of the massed nematodes in 1% sodium chloride solution were homogenized for 30 min in a 15 ml Ten-Broeck ground glass tissue grinder held in an ice bath. Microscopic examination revealed that all the nematodes were macerated. The homogenates were then filtered, and the clear nematode extract was assayed for pectinases, cellulases, amylases and invertases.

**Assay systems for hydrolytic enzymes.** Cellulolytic and pectolytic enzymes were separately assayed by viscometric methods (Levinson et al., 1950). Solutions of 1% carboxymethylcellulose (Cellulose gum Type CM32) and 1% pectin in 0.05 M potassium phosphate buffer, were prepared as substrates and a small amount of toluene was added to inhibit bacterial activity. The pH of the solution for the cellulose gum was 5.0 and that of pectin was adjusted to 6.0. About 10 ml of the substrate were added into a Volac No. 0507 viscosity pipette which was supported in a constant temperature water bath at 27±0.5°. The initial time flow of the substrate alone was measured. Then 3 ml of the enzyme were added and thoroughly mixed with the substrate in the viscosity pipette. Time flow measurements were thereafter made at 5 minute intervals. Extracts from infected yam (from which nematodes had been extracted) clean yam, and 0.1 M NaCl were tested in the same way. The clean yam extract was used as control.

**Relationship between nematode homogenate in ml and percentage drop in viscosity.** The ability of the extracts of nematode homogenate to degrade pectin was tested viscometrically. Five different quantities of the enzyme were used: 2.5, 5, 7.5, 10, and 12.5 ml. About 2.5 ml of the enzyme were pipetted each time into the viscometer pipette containing 10 ml of the substrate mixed thoroughly with the substrate and thereafter viscosity measurements were made at 5 minute intervals.

**Assay systems for amylase and invertase.** The substrates for the amylase and the invertase assays were 1 g of starch in 100 ml 0.02 M potassium phosphate buffer (pH 6.9), and 5 g of glucose in 100 ml 0.02 M potassium phosphate buffer (pH 5.0) respectively. To 2 ml of each of the substrates, 2ml of the enzyme from the nematode extracts and infected yam were separately added and each mixture was incubated at between 24°-28° for 2 hours. Controls of 0.1 M NaCl solution and homogenate boiled for 5 minutes (B' HOM) were incubated along with others. A colorimetric technique using 3, 5 dinitro salicylic acid reagent (D.N.S.A.)