Biochemical Identification of the Two Races of Radopholus similis by Polyacrylamide Gel Electrophoresis[1]

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Abstract: Analysis of proteins of the banana and citrus race of *Radopholus similis* was carried out by several different types of polyacrylamide gel electrophoresis. These included standard slab gel, SDS slab gel, gradient slab gel, and two-dimensional slab gel electrophoresis. A major band difference was detected between the two races by slab gel electrophoresis. However, several other poorly resolved but consistent bands of high molecular weight proteins near the gel origin also were considered as diagnostic. Resolution of protein bands was greatly improved by SDS and gradient slab gel electrophoresis, but no differences could be detected among the proteins resolved between the two races with these techniques. Two-dimensional gels revealed a large number of proteins, but background staining obscured them hindering interpretation. When nematode races were reared on three different host plants, no differences in protein patterns were detected between them, indicating host preferences does not play a role in determining the types proteins occurring in these nematodes. *Key words:* proteins, nematode races, burrowing nematode, biosystematics.

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Techniques that include different types of polyacrylamide gel electrophoresis can be useful in establishing a biochemical basis for defining systematic relationships of organisms (16). The analysis of proteins by polyacrylamide gel electrophoresis is sometimes useful in distinguishing species. These types of comparisons have been used successfully to identify genera and species of nematodes (4,7,15,22). However, these techniques rarely distinguish between races within a nematode species (6).

The number of proteins that can be observed on a gel are increased by treating extracts with detergents, such as sodium dodecyl sulfate (SDS). However, these denatured proteins generally reveal only differences between distantly related species or species with major developmental differences (21).

Two-dimensional electrophoresis has been developed recently for protein separation, and it may become valuable in establishing genetic relationships (20). All classes of proteins can be separated by this technique. Since the technique is sensitive enough to separate large numbers of proteins, it should have value as a method of comparing closely related species and populations (3). Also, the technique is amenable for detecting extremely low quantities of proteins by ultrasensitive reagents such as silver nitrate staining (10).

The objective of this study was to evaluate several electrophoretic techniques in order to establish a biochemical basis for the separation of the banana and citrus races of *Radopholus similis* (Cobb) Thorne 1949.

MATERIALS AND METHODS

Six populations of the citrus race and ten populations of the banana race of *Radopholus similis* (Cobb) Thorne were investigated (14). Nematodes were obtained from either carrot disc cultures or from callus tissue cultures (13). Plant callus tissues were grown from germinated okra (*Hibiscus esculentus* L. var. Clemson spineless), alfalfa (*Medigo sativa* L.), or mung bean (*Phaseolus aureus* Roxb.). Plant callus tissue medium was aseptically prepared as described by Krusberg and Blickenstaff (18).

Sample preparation for polyacrylamide slab gels: Samples were prepared as previously described by Huettel et al. (14), except 30 mg powdered sucrose and glass beads were added to the homogenization buffer (5).

Sample preparation for SDS, gradient SDS, and two-dimensional polyacrylamide slab gels. Nematodes collected from tissue

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culture were centrifuged for 30 sec at ca. 1,200 *g* in a Beckman Microfuge, the supernatant removed, and 40 μl of buffer (8) added. The nematodes were homogenized as described previously (14) and the homogenate boiled for 2 min in a water bath. Homogenates were centrifuged for 30 sec at ca. 1,200 *g* and the supernatant loaded onto the gels (ca. 20–30 μg protein per sample). The same procedure was used for two-dimensional electrophoresis, except ampholines were added to the homogenization buffer (8).

Polyacrylamide gel electrophoresis: Vertical polyacrylamide gels were run in a slab gel unit modified at the University of Florida but similar to commercially available units. Slab gels were prepared as modified from Davis (5). An acrylamide/bis, 19:1 preparation was used in place of acrylamide and bis/acrylamide. SDS polyacrylamide slab gels were made as described by Douglas et al. (8). Gradient gels were prepared by mixing a 7.5% acrylamide/bis and 20% acrylamide/bis solution with a Gilson Minipulse 2 Gradient pump. Two-dimensional polyacrylamide gels were prepared according to the methods of Douglas et al. (8).

Staining methods: Proteins separated on the polyacrylamide gels were fixed and stained by placing the gels in either 50% trichloroacetic acid (TCA) plus 0.1% Coomassie blue solution or ultrasensitive silver nitrate stain (10). Malate dehydrogenase and α + β esterase enzymes were stained as described by Bush and Huettel (2).

Population analysis: Coefficients of similarity (CS) were calculated for protein bands observed on polyacrylamide gels (19). Each band observed on a gel was assigned a numerical position. If a band was observed in the same relative position from either the banana and citrus races, it was given the same number. Different band positions were numbered consecutively from the origin. To determine the CS value, the number of bands determined to be common in both races was divided by the total number of bands detected in both races. The reference values for the positions of each band (E_f) were calculated as the ratio of its movement of the band to that of the tracking dye.

RESULTS AND DISCUSSION

Protein banding patterns on polyacrylamide slab gels indicated distinct differences between the two races. The citrus race had a band located at E_f = 0.62 that was not observed in the banana race, and the band at E_f = 0.78 was stained darker than in the banana race (Fig. 1). This may indicate a quantitative difference in faster migrating bands between the two races. In addition, poorly resolved but consistent band differences occurred between the two races in high molecular weight proteins near the gel origin. These types of differences in protein patterns on polyacrylamide gels have been observed among many species of nematodes (1,7,12,15).

The polyacrylamide slab gels were stained also for α + β esterase and malate dehydrogenase activities. One distinct band was resolved for esterase from both the banana and citrus races; however, a weak second band was visible for the citrus race. The isozymes for malate dehydrogenase were diffuse and smeared and could not be interpreted.

The number of protein bands resolved by SDS and SDS gradient polyacrylamide slab gels was 40 and 46, respectively,

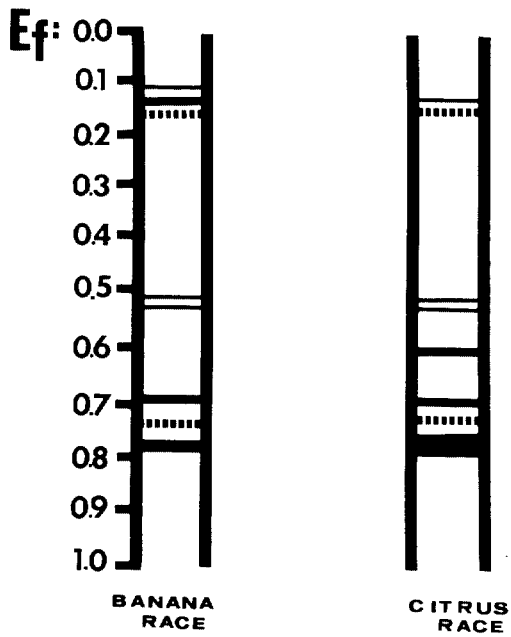

Fig. 1. E_f values of the proteins from the banana and citrus races *Radopholus similis* on polyacrylamide slab gels.

whereas only eight bands were resolved by standard polyacrylamide slab gels (Figs. 1, 2). Good resolution of the middle and lower molecular weight proteins was obtained by gradient gels, whereas the resolution of most higher molecular weight proteins was poor. The reverse was true in SDS polyacrylamide gels. However, no differences were detected between the citrus and banana races of this nematode by this technique. Friedman et al. (9) were able to identify several strains of *Caenorhabditis elegans* and *C. briggsae* from protein patterns obtained from SDS polyacrylamide gels. In general, however, such closely related species are not usually separable by this technique.

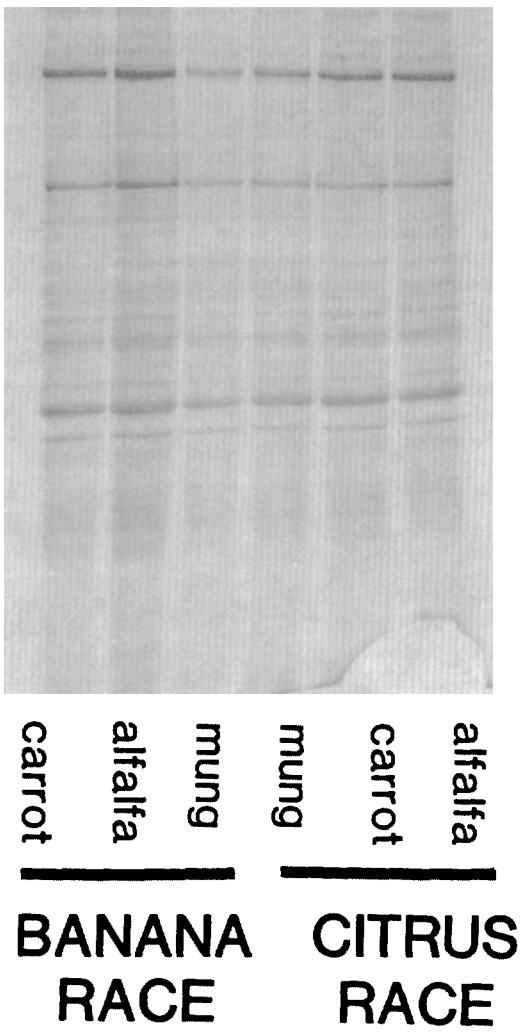

Fig. 2. Gradient SDS polyacrylamide gel patterns of proteins from populations of the citrus and banana races of *Radopholus similis* from Florida on mung bean, alfalfa, and carrot tissues.

Resolution of proteins in the two-dimensional gels was poor. Apparently, the protein concentration was too low to detect the individual components with Coomassie blue stain. Many protein spots were stained with silver nitrate, but intense background staining obscured them. Therefore, no conclusions could be drawn. It appears that relatively large quantities of concentrated proteins are necessary to obtain results with this method.

A CS value of 0.77 was obtained for proteins in this study. This is very close to the CS value for the enzymes observed on starch gels for *R. similis* (15). The CS value, based on protein band differences found in other species of nematodes, indicates that gene flow does not occur between the two races.

Comparison of protein patterns resolved on SDS gradient gels from nematodes reared on mung bean, alfalfa, or carrot revealed no influence of plant diet on the protein patterns as resolved by any of the electrophoretic procedures employed (Fig. 2). Greet and Firth (11) and Ishibashi (17) reported that the host influences the patterns of proteins on polyacrylamide gels. However, it is assumed that host material in the gut of these nematodes was so minute that it did not influence the protein patterns. Furthermore, these results indicated that no detectable induction of unique enzymes or other proteins occurred that could be detected.

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