THE USE OF POLYACRYLAMIDE GEL ELECTROPHORESIS IN THE IDENTIFICATION OF *XANTHOMONAS FRAGARIAE KENNEDY AND KING*

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ABSTRACT.—Electrophoretic patterns of buffer-soluble proteins extracted from bacterial cells were used as an aid in the precise identification of organisms responsible for the angular leaf spot disease of strawberries. Isolate K-1 was found identical to *X. fragariae* and both of them entirely differed from isolate K-2 in respect of number, position, width, and density of the bands. The usefulness of the technique as an adjunct to other criteria used in the diagnostic investigations and taxonomic studies is stated.

A bacterial disease of strawberry with symptoms suggestive of those caused by *Xanthomonas fragariae* were observed in epiphytotic proportions on variety Dixieland in Kentucky during June, 1965 (Powell and Khare, 1967). Isolations from infected leaves appeared to be easy to obtain but typical symptoms were not obtained from greenhouse inoculations and biochemical tests of the isolate did not conform to those described by Kennedy and King (1962). Further studies showed that a contaminant was involved in the isolation. Thus, two different bacteria, Kentucky-1 (K-1) and Kentucky-2 (K-2) were separated by dilution and studied. K-1 was very similar to *X. fragariae* in physiochemical properties, and when used in pure culture produced fairly typical symptoms of the disease when plants were inoculated. Isolate K-2 differed from K-1 in both cultural and biochemical properties and also proved innocuous to strawberry (Powell and Khare, 1967).

Currently considerable attention is being paid to the use of gel electrophoresis in clinical diagnosis and in numerous other biochemical diagnostic investigations in different fields (Gottlieb and Hepden, 1966; Kates and Goldstein, 1964; Levitron, Myers and Grunbaum, 1964; Macko, Novacky and Stahmann, 1967; Vaughan, Waite, Boulter and Waiters, 1965). Polyacrylamide gel electrophoresis was implemented as an additional tool for the precise identification of bacteria. Electrophoretic patterns of the soluble proteins of K-1 and K-2 were compared with the protein patterns of a pure culture of *X. fragariae* (Minnesota isolate) which was obtained from B. W. Kennedy, University of Minnesota.

MATERIALS AND METHODS

The organisms were purified and cultured on Modified Emerson's medium (MEM) of the following composition (Reinhardt and Powell, 1960):

Yoast extract, 1.0g; peptone, 4.0g; beef extract, 4.0g; dextrose, 1.0g; NaCl, 2.5g KH_2PO_4, 5.0g; K_2HPO_4, 8.0g; agar, 20.0g; distilled water to one liter.

A loop of bacteria from 48 hours-old MEM plates was used to inoculate 50 ml of MEM broth (MEM minus agar), con-

tained in 125 ml Erlenmyer flask. After 12 hours of incubation the inoculum was added to another 2 liter Fernberg flask containing 900 ml MEM broth which was then agitated over a reciprocating shaker (90 cycles/min.) for 48 hours at room temperature (25°-26° C).

Bacteria were harvested by centrifugation at 3500 x G for 20 minutes. The supernatant was discarded. The pellet was washed three times in phosphate buffer (0.1M; pH 7.0) to remove the nutrients. Bacteria were suspended in the chilled buffer, 4 ml, and subjected to sonification (rupturing the cell wall by sonic oscillation) at 0°C for 10 minutes. The extracts were centrifuged at 105,000 x G (Spinco Ultracentrifuge Model L, Rotor No. 40) for one hour at 4° C. The resultant clear supernatant was decanted and the volume of each sample required to give about 125 ug protein for electrophoresis was determined by Lowry's Folin test (Lowry, Rosebrough, Farr and Randall, 1951).

The disc electrophoresis apparatus (Model 12), materials sold as a 600-pack unit No. 400 P, by CANALCO Corporation, Bethesda, Maryland, was used. The procedure was used as described by Davis (1964) with some modifications (Gill and Powell, 1967).

Six replications were observed in each run of each isolate, and several runs were made from each protein preparation. The experiments were repeated with fresh, and frozen extracts prepared at entirely different times.

Results and Discussion

Investigations were first conducted on the reproducibility of the fractionation method. Electrophoretic protein patterns of each isolate examined were found identical when tested under standardized conditions regardless of whether the extract was fresh or frozen. The reproducibility of the patterns was further checked and found consistent when an extract preparation of a different culture of the same isolate but grown at different times was employed. This substantiates the earlier studies (Chang, SRB and Steward, 1962; Steward and Barber, 1964) that showed reproducibility of the protein patterns of *Neurospora crassa*.

Data (Fig. 1 and 2), based on number, position, density, and width of the band, demonstrated that isolate K-1 was similar to Minnesota isolate (*X. fragariae*) in its electrophoretic pattern. Also, both of them differ entirely from isolate K-2 in this respect. Some similarities in the protein patterns, band P, Q, and

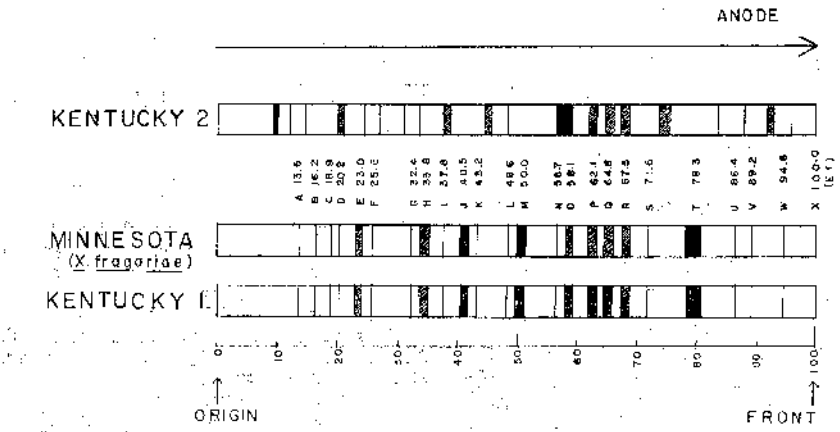

Figure 1. Diagramatic presentation of the electrophoretic patterns of buffer-soluble protein extracts of isolates K-1 and K-2 compared with a Minnesota isolate (*X. fragariae*).

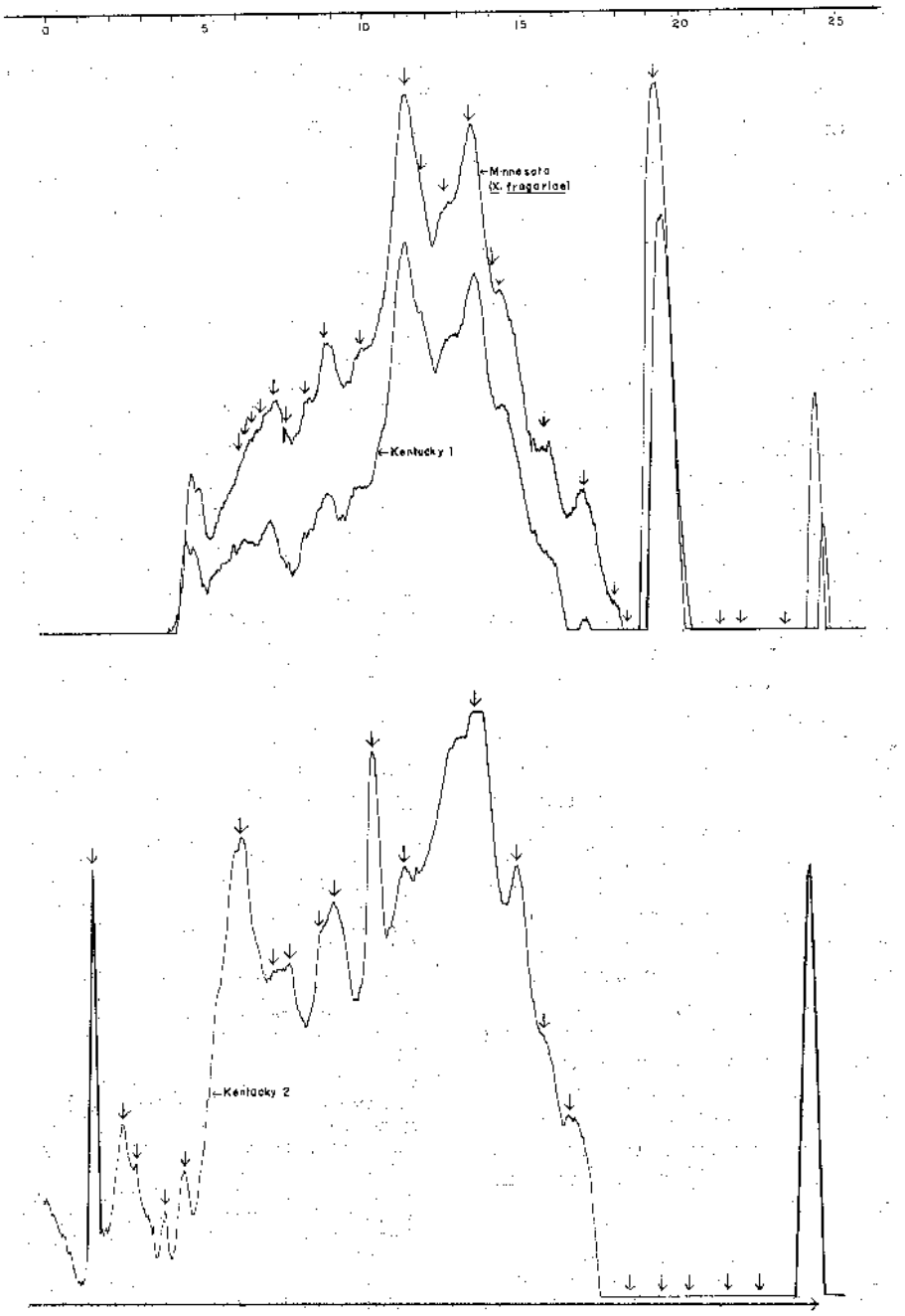

FIGURE 2. Top: A composite of microdensitometric scans of electrophoretic

separation of the buffer-soluble proteins of isolate K-1 compared with Minnesota isolate (*X. fragariae*).

Below: A microdensitometric scan of the buffer-soluble proteins of K-2 isolate bearing little similarities with the other two isolates.

R, appeared identical within the three isolates. However, this does not warrant considering K-2 as another isolate of *X. fragariae*. Identical or nearly identical protein patterns of several isolates within the species have been reported in *Agrobacterium* (Huisingh and Durbin, 1965), *Erwinia amylovora* (Smith, 1967) and in fungi (Clare and Zentmyer, 1966; Durbin, 1966; Gill and Powell, 1967). In contrast to the above results Gottlieb and Hepden (1966) observed variations in the electrophoretic behavior of isolates of a single species of *Streptomyces*. It is strongly emphasized that only organisms in pure culture should be tested by this technique.

The observations presented in this paper support the fact that K-1 is another isolate of *X. fragariae* whereas K-2 is a different bacterium and is a contaminant bearing no resemblence to *X. fragariae* physiologically, pathologically, (Powell and Khare, 1967) or electrophoretically.

The consistent reproducibility of this technique, the identical behavior of the isolates within a species, *X. fragariae*, and the differences detected between species suggest that polyacrylamide gel electrophoresis is a useful technique for the precise identification of phytopathogenic bacteria and it should prove a useful adjunct in taxonomic investigations.

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