Computer-Enhanced Image Analysis of Bacterial Polypeptide Patterns on Two-Dimensional Polyacrylamide Gels

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ABSTRACT

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Comparisons of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) profiles of *Erwinia carotovora* pv. *carotovora*, *E. c.* pv. *atroseptica*, *E. chrysanthemi*, *E. rhapontici*, *E. herbicola*, and *E. amylovora* were made by transferring gel images to a Hewlett-Packard 1000F minicomputer interfaced to a Grinnell 270 image processor. Computer-enhanced image analysis produced gel maps that revealed polypeptides common to all bacterial strains studied, as well as polypeptides that were species specific. Although all bacterial strains studied shared a number of polypeptides, significant differences were observed between *E. c.* pv. *carotovora*, *E. c.* pv. *atroseptica*, and other species analyzed, allowing differentiation. Computer-enhanced image analysis increased the sensitivity of protein comparisons on the 2-D PAGE gels more than threefold over visual comparisons.

Additional key words: bacterial soft rot, postharvest.

The resolving power of two-dimensional gel electrophoresis for separating proteins was first demonstrated by O'Farrell in 1975 (8). The first dimension of this method separates polypeptides by charge; the second dimension separates them by mass. With the method, more than 1,000 proteins may be separated on a single gel. The task of analyzing such a number of proteins can be onerous; quantification can be greatly enhanced and simplified using automated procedures.

In our study of *Erwinia* ribosomal proteins, we used two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to compare structural proteins of a number of bacterial strains. We observed significant differences as well as similarities among these strains (5,6). With the potential for comparing large numbers of proteins from many strains using automated analysis techniques, we tested our hypothesis that ribosomal proteins can be used to distinguish *Erwinia* strains, using a computerized data acquisition and analysis system (3).

MATERIALS AND METHODS

Bacterial ribosomes were extracted from 24-hr-old cells of 32 bacterial strains and variants (Table 1) following the procedure of Tissieres et al (11) and Schaad (9) as outlined previously (5,6). Isoelectric focusing (IEF) was carried out overnight for an equivalent of 4,800 volt-hours, then at 800 V for 1 hr according to the method of O'Farrell (8) in a Bio-Rad model 150A gel electrophoresis cell. Gels were removed from glass tubes immediately after IEF and attached to stacking gels of the second dimension with hot 1% agarose. Second dimension 8% sodium dodecyl sulfate-PAGE slab gels were run in a BRL model V162 vertical gel electrophoresis apparatus at 20 mA per gel according to the procedures of O'Farrell (8). Molecular weight protein standards were co-electrophoresed in an outside track of the polyacrylamide gels (4).

Coomassie Brilliant Blue-silver staining and fixation of proteins in the gels were done following the method of Steck et al (10) as modified by Oakley et al (7) and by Moline et al (6). After staining, gels were stored in a solution containing 5% acetic acid and 2% glycerol until photographed and dried. Gels were analyzed according to the method of Hruschka and Massie (4), except that a Grinnell 270 image processor with a television camera was used to digitize the gel images from the negatives, and the data were stored directly on hard disk (1). The digitized gel image showing the most proteins for each bacterial species was chosen as a master and displayed in red on a color monitor. Each image of the remaining strains was then superimposed on that of the master in green, and the position of the camera was manually adjusted to maximize image registration before digitization. In each image, approximately 12 protein spots were selected that provided the clearest match to proteins in the master. The digitized images were then mathematically adjusted for best fit by matching the coordinates of these proteins (5) by a method called rubber sheeting (3). This procedure corrected for slightly different positions of the proteins created by gel-to-gel variations. The computerized analysis then located all proteins common to most of the digitized gels in that species. All proteins visible on each gel were listed, and tables were constructed to show those proteins common to most gels within a species and those common to more than one species. Each rubber sheeted gel was compared with the selected master of that species and other species to help detect similarities and differences among species and pathogens.

RESULTS AND DISCUSSION

Computer-generated gel maps of acidic ribosomal proteins of selected strains of *Erwinia carotovora* pv. *carotovora*, *E. c.* pv. *atroseptica*, *E. chrysanthemi*, *E. rhapontici*, *E. amylovora*, and *E. herbicola* are shown in Figure 1. Abstract composite gel maps showing rectangular areas containing polypeptides common to two or more bacterial strains or species are shown in Figure 2. Sixteen of the 17 *E. c.* pv. *carotovora* strains and variants examined shared 27 polypeptide spots (Fig. 2A); strain C6, which

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did not match, shared only 20 spots. Seven of the eight *E. c.* pv. *atroseptica* strains shared 56 polypeptide spots (Fig. 2B); strain E4, which did not match, shared only 20 spots. When the 16 *E. c.* pv. *carotovora* strains were compared with the seven *E. c.* pv. *atroseptica* strains, 20 common polypeptide spots were matched (Fig. 2C). These digitized gel maps support previous visual comparisons of *E. c.* pv. *carotovora* and *E. c.* pv. *atroseptica* strains (5,6), where the seven numbered polypeptide spots served as the basis for visual comparisons; the remainder were additional matches made possible by computer-assisted imaging.

A low molecular weight polypeptide (33 kDa) similar to that previously designated No. 1 (5) shares common characteristics in all 25 strains (Fig. 2A and B). Differences in a group of 54 kDa polypeptides (No. 2) and a group of 45–46 kDa polypeptides (Nos. 4 and 5) have been circled. The absence of two 83 kDa polypeptides (No. 3) from all *E. c.* pv. *carotovora* strains (Fig. 2A) and their presence in all *E. c.* pv. atroseptica strains (Fig. 2B) provide a major point for comparison of the two pathovars. Five additional polypeptides common to 17 *E. c.* pv. *carotovora* strains but absent from six *E. c.* pv. *atroseptica* strains are also circled (Fig. 2A).

Comparison of a gel pattern from an unknown bacterium with another gel pattern from a known *Erwinia* strain is not yet possible for the identification of an unknown strain as the full range of variants within a species has not been explored. However, the 16-strain *E. c.* pv. *carotovora* composite electrophoretogram displaying 27 matched polypeptide patterns may prove adequate for such comparative studies. One *E. c.* pv. *carotovora* strain from our collection failed to fit this profile. The electrophoretogram of this strain (strain C6) contained only 100 spots, whereas others contained between 150 and 400 total spots. It is also possible that the atypical electrophoretic pattern may have been a result of the low protein content in the preparation or protein changes occurring during purification or electrophoresis. There are many variables that can affect gel patterns, including protein content, gel thickness, fluctuations in electrical current, and staining (7). Rubber sheeting (3) can correct for some of these inherent gel-to-gel variations; however, some true matches may have been missed because of large gel-to-gel spatial variation. Visual comparisons of *Erwinia* 2-D PAGE gels were made on the basis of seven protein clusters (5,6). Initial computerized comparisons used these seven recognizable gel patterns during rubber sheeting to correct for some gel-to-gel variations. As gels were compared by overlaying them on the video monitor, additional matching polypeptides were recognized. Computer-aided gel analysis has increased the diagnostic sensitivity of the 2-D PAGE gels 300%, providing 27 matching polypeptides among 16 *E. c.* pv. *carotovora* strains, where only seven had previously been identified visually. Part of this difference can be accounted for by the increased sensitivity of the computerized gel analysis. With a threshold set just above grain level of the emulsion on the negative the total volume above threshold could be determined and protein lists created (3). There is a dramatic decrease in sensitivity of prints obtained from the same negatives.

Eighteen polypeptide clusters were matched that were common to all soft-rotting erwinias studied (Fig. 2E). This represents a loss of only two polypeptide patterns from that observed in comparison of *E. c.* pv. *carotovora* and *E. c.* pv. *atroseptica* strains.

TABLE 1. Bacterial strains used for computerized two-dimensional polyacrylamide gel electrophoresis comparisons

Strain	Original host	Source
Erwinia carotovora pv. *atroseptica*		
C2 (master gel)	Potato tuber	W. L. Smith, Jr., collection (WLSC)[a]
E1,E3,E4,E8,E15	Potato stalk	WLSC
E25,E26	Potato tuber	WLSC
E. c. pv. *carotovora*		
C9 (master gel)	Iris rhizome	WLSC
C9R (rough colony variant)	Iris rhizome	WLSC
E32,C7	Iris rhizome	WLSC
C7S (smooth colony variant)	Iris rhizome	WLSC
E40,C6,C3	Celery stalk	WLSC
C3S (smooth colony variant)	Celery stalk	WLSC
C14	Calla rhizome	WLSC
E9,E11	Lettuce leaf	WLSC
E21	Carrot root	WLSC
E22,E31,E60	Potato tuber	WLSC
15713	Potato	ATCC (Type strain)
E. rhapontici		
1025,1026	Rhubarb	Ange, France; J. Louisette
E. chrysanthemi		
11663	Chrysanthemum	ATCC (Type strain)
A17	Sweet potato root	Experiment, GA, N. W. Schaad
M80-1	Sweet potato root	Salisbury, MD, N. E. Moline
E. amylovora		
15580	Pear twig	Kearneysville, WV; T. van der Zwet
E. herbicola		
33243		ATCC (Type strain)

[a]As identified by Burkholder and Smith (2) and maintained at Beltsville, MD.

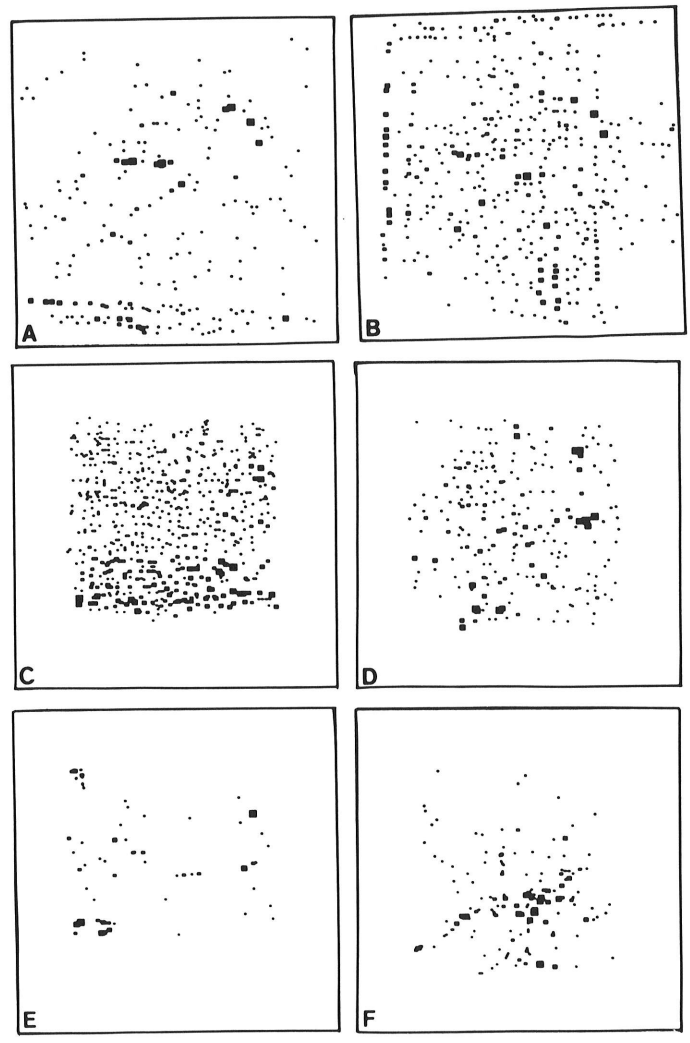

Fig. 1. Computer-generated gel maps of acidic ribosome-enriched proteins of **A,** *Erwinia carotovora* pv. *carotovora*, **B,** *E. c.* pv. *atroseptica*, **C,** *E. chrysanthemi*, **D,** *E. rhapontici*, **E,** *E. amylovora*, and **F,** *E. herbicola*. **A** and **B** represent maps made directly from video images of negatives of gels, **C–F** represent trimmed maps where peripheral polypeptides that tended to smear have been removed. *E. amylovora* and *E. herbicola* gel maps differed so significantly from the soft-rotting erwinias (**A–D**) that no further mathematical comparisons were made with gels from these two species.

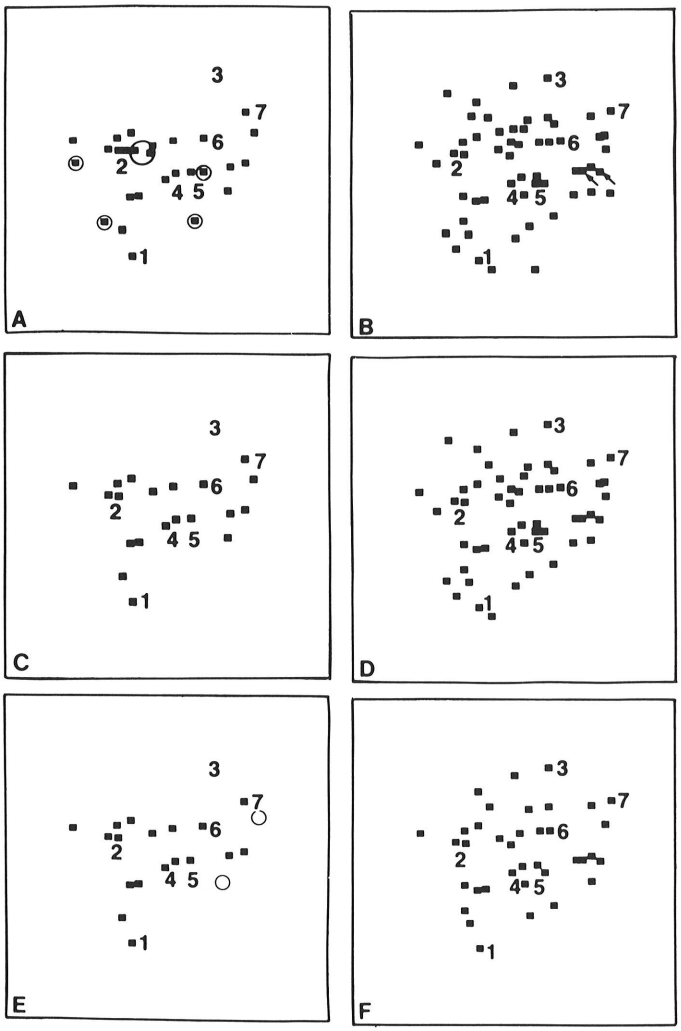

Fig. 2. Abstract computer-generated gel maps showing areas where common polypeptides matched among a number of strains. Seven previously identified protein clusters (5) are numbered and several areas of significance have been circled. **A,** Composite map showing 27 matched polypeptides among 16 *E. c.* pv. *carotovora* strains. Note the absence of a polypeptide cluster adjacent to No. 3. **B,** Composite gel map showing 56 matched polypeptides shared by seven *E. c.* pv. *atroseptica* strains. Note the presence of 2 high MW clusters near No. 3 and the large polypeptide cluster indicated by double arrows. **C,** Composite gel map showing 20 matched polypeptide clusters shared by *E. c.* pv. *carotovora* and *E. c.* pv. *atroseptica* strains. Numbered protein clusters show changes in seven significant areas as well as other matched polypeptides. **D,** Composite gel map showing 52 matched protein clusters common to seven *E. c.* pv. *atroseptica* and three *E. chrysanthemi* strains. **E,** Composite map showing 18 matched polypeptide clusters shared by 16 *E. c.* pv. *carotovora*, seven *E. c.* pv. *atroseptica*, three *E. chrysanthemi*, and two *E. rhapontici* strains. **F,** Composite gel map showing 37 matched polypeptide clusters shared by seven *E. c.* pv. *atroseptica*, three *E. chrysanthemi*, and two *E. rhapontici* strains.

Considering that there were only three *E. chrysanthemi* and two *E. rhapontici* strains examined, this is not surprising. Comparison of *E. c.* pv. *atroseptica*, *E. chrysanthemi*, and *E. rhapontici* shows that a similar drop in number of matched polypeptides occurred. There were 52 matched polypeptides common to *E. c.* pv. *atroseptica* and *E. chrysanthemi* strains and 37 matched polypeptides when comparing *E. c.* pv. *atroseptica*, *E. chrysanthemi*, and *E. rhapontici* strains. This compares with 74 matched polypeptides shared by the three *E. chrysanthemi* and two *E. rhapontici* strains examined.

Where significant biochemical differences have been established between existing species, separation based on structural protein profiles of two or three strains may prove adequate; however, a minimum of 10 or more strains may be necessary to establish valid criteria on which to base most comparisons. Differences could not be demonstrated when comparing the composite master from 17 *E. c.* pv. *carotovora* strains with three *E. chrysanthemi* strains. On the basis of the few strains studied, our *E. c.* pv. *atroseptica* sample appears to share more structural polypeptides with strains of *E. chrysanthemi* and *E. rhapontici* (Fig. 2B, D, and F) than with *E. c.* pv. *carotovora*. Some major points of comparison are the 83 kDa polypeptides (No. 3), an extra component near 73 kDa, a prominent polypeptide cluster at 60 kDa, and a group of polypeptides near 46 kDa.

There is little doubt that computerized assistance can significantly increase the sensitivity of 2-D PAGE analysis. A program using more complex image processing mathematics might find more spots of analytic significance. Although much effort may be involved creating an initial library, in skilled hands additional information can be obtained from 2-D PAGE gels. We have demonstrated that computerized analysis significantly increased the amount of information obtainable from our gels by increasing the number of matched spots threefold over visual comparisons. Identification of individual polypeptides on 2-D gels and elucidation of their function will further enhance the amount of information obtainable.

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