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Application of Two-Dimensional Pulsed Field Electrophoresis for Determining Molecular Karyotypes

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Abstract

Two-dimensional pulsed field electrophoresis can be used when determining molecular karyotypes of lower eukaryotes. Using this method it is possible to relate chromosomal bands separated under distinct electrophoretic conditions without blotting or hybridization. An example from the fungus *Colletotrichum gloeosporioides* is used to illustrate this method.

Methods

Chromosomal DNA preparations of *C. gloeosporioides* were made from spores or protoplasts according to the method of Orbach *et al.* (1988), except agarose blocks were set in a commercial sample mold (Bio-Rad). Both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* DNA samples were prepared according to Vollrath and Davis (1987). Gel electrophoresis was performed using the contour-clamped homogeneous electric field-dynamically regulated CHEF-DR® II system[3]. The running buffer was 0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA, pH 8.0 (Maniatis *et al.* 1982) and was replaced every 48 hours during extended electrophoretic runs. Buffer temperature was maintained at 14 °C with constant buffer circulation. All separations were carried out on 14 x 12.7 x 1 cm Molecular Biology Certified agarose (Bio-Rad) gels under the following conditions; (1: 130 h, 60 min switch time, 40 V, 0.5% agarose), or (2: 15 h, 60 sec switch time then 9 h 90 sec switch time, 200 V, 1% agarose). Gels were stained with ethidium bromide (1 µg/ml) for 15-20 min and destained in distilled water for at least 1 h. For two-dimensional electrophoresis a gel from condition 1 was stained with ethidium bromide (1 µg/ml) and a lane (4 mm width) cut out after visualization under long-wave UV light (366 nm). The excised lane was embedded in a 1% agarose gel and re-electrophoresed in the second dimension using condition 2 with fresh *C. gloeosporioides* and *Sa. cerevisiae* chromosomes as standards.

Results

Electrophoretic Karyotypes

To resolve chromosomal DNA of *C. gloeosporioides* a number of electrophoretic conditions were tested (see Methods). These conditions resolve all the chromosomal bands that we have visualized for sar[1]. The bands obtained with this isolate fell into two classes. A group of bands of molecular weight lower than 1.3 Megabase pairs (Mb) when compared with the *Sa. cerevisiae* standards were observed, and these were termed mini-chromosomes. Other bands of higher molecular weight (> 1.3 Mb), termed maxichromosomes, were sized by reference to *Sa. cerevisiae* and *Sc. pombe* chromosomes. Using electrophoretic condition 1 (see Methods), three chromosomes were resolved in the size range 4.7 - > 6 Mb and two minichromosomes in the size range 550–1200 Kb were observed for this isolate (Fig. 1A). Separation of *C. gloeosporioides* chromosomes under running condition 2 (Fig. 1C) resulted in resolution of two minichromosomes in the size range 550-600 Kb and two bands in the size range 1-2 Mb. It was unclear whether bands x and y identified using condition 1 (A) corresponded to bands I and II observed in gels from condition 1 (C). Separating bands in the second dimension (B) revealed that bands I and II resolved under condition 2 (C) were confined to band y while band x corresponded to a previously unidentified chromosomal band. Lane D is the *Sa. cerevisiae* size standards.

When determining the electrophoretic karyotype of an organism it is often necessary to use two or more separate running conditions[2] and it can be difficult to identify a particular chromosomal band separated in each gel unless blotting and hybridization is carried out[2]. Two-dimensional pulsed field electrophoresis should increase the speed and accuracy of electrophoretic karyotyping in lower eukaryotes.

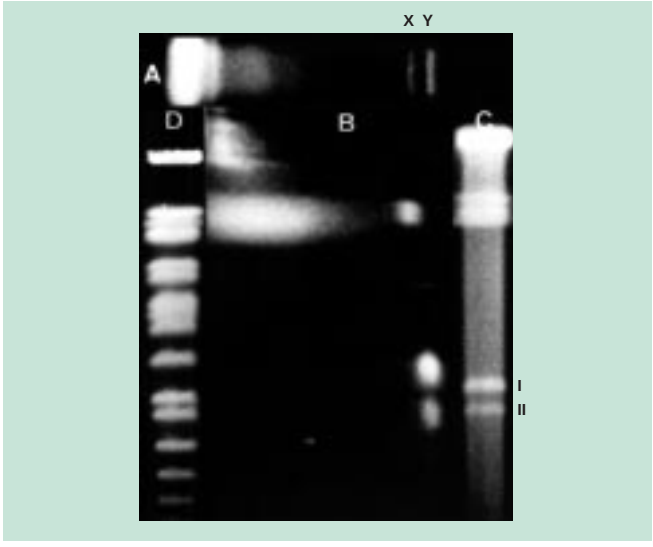

Fig. 1. Two-dimensional electrophoresis of chromosomes of the plant pathogen *C. gloesporioides*. A. condition 1 only, **B.** condition 1 then condition 2, **C.** condition 2 only, **D.** yeast standards, condition 2 only.

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