Differentiation of Leaf-Streak and Blight Pathogens of Rice by Immunoelectrophoresis

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ABSTRACT

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Isolates of the leaf-streak pathogen (*Xanthomonas translucens* f. sp. *oryzicola*) produced two overlapping precipitin bands with different electrophoretic mobilities, but the blight bacterium (*X. oryzae*) formed only one precipitin band against homologous antisera. Also, the leaf-streak antigen did not cross-react with the blight pathogen antiserum and vice versa.

Immunoelectrophoresis has been useful for characterizing a mixture of antigens because they can be separated by electrophoretic mobility before diffusion of antibodies against them (1). We used immunoelectrophoresis to differentiate the leaf-streak and blight pathogens of rice.

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MATERIALS AND METHODS

Four isolates of *Xanthomonas translucens* f. sp. *oryzicola* (Fang et al) Bradbury and 12 isolates of *X. oryzae* (Uyeda et Ishiyama) Dowson were selected from stock cultures of Department of Plant Pathology of All-India Coordinated Rice Improvement Project, Hyderabad, India. The cultures were grown and maintained on modified Hayward's medium (sucrose, 20 g; peptone, 10 g; $MgSO_4 \cdot 7\ H_2O$, 0.25 g; $Na_2HPO_4 \cdot 12\ H_2O$, 0.30 g; agar, 20 g; distilled water, 1 L; pH adjusted to 7.0).

Antisera were produced against isolate Pxt_2 of the leaf-streak pathogen and isolate H 384 of the blight pathogen. The bacterial growth from 24-hr-old agar slants was suspended in saline and the protein concentration was adjusted to 675 μg/ml. The bacterial suspensions were mixed with an equal volume of Freund's complete adjuvant, homogenized, and injected subcutaneously in white rabbits at weekly intervals. Rabbits were bled at the end of the fourth week, and antisera titers were determined by tube agglutination tests. Antisera with titers of 2,560 were used in immunoelectrophoretic tests.

Electrophoresis was conducted in agar gel (sodium azide, 0.2 g; NaCl, 8.183 g; KH_2PO_4, 1.36 g; Noble agar, 10 g; distilled water, 1 L) of 3 mm thickness on 2.5×7.5 cm glass slides. Heat-killed (60 C for 1 hr) bacterial suspensions were

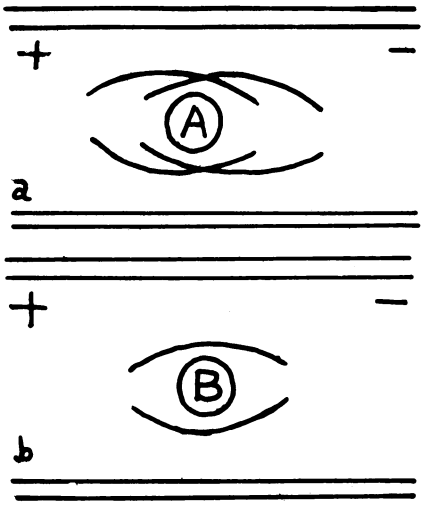

Fig. 1. Immunoelectrophoretic patterns of antigens of the leaf-streak (**A**) and blight (**B**) pathogens of rice tested against homologous antisera (a) and (b), respectively; + and − indicate the polarity of the electric current.

placed in the antigen well (4 mm diameter) at the center and a current of 15 mA/frame was supplied for 2 hr in 0.1 M phosphate buffer, pH 7.0. Antisera were added to parallel troughs that were 1 mm wide and 5 mm from the well. The slides were incubated for 24 hr at 28 ± 1 C.

RESULTS AND DISCUSSION

Isolates of the leaf-streak bacterium produced two overlapping precipitin bands; one electrophoretic component moved toward the anode and the other to the cathode when homologous antisera were used (Fig. 1A). Isolates of the blight bacterium formed only one thick band close to the antigen well when tested with homologous antisera (Fig. 1B). Isolates of the leaf-streak bacterium did not cross-react with antisera to the bacterial blight pathogen and vice versa. Similar electrophoretic patterns were obtained with different isolates of the leaf-streak or blight bacteria.

These results suggest that *X. translucens* f. sp. *oryzicola* is serologically distinct from *X. oryzae*, a conclusion supported by earlier gel-diffusion tests (2). The overlapping of precipitin bands noted with antigens of the leaf-streak bacterium suggests that it may have two serologically similar but not identical antigenic determinants, which are distinguishable only by their electrophoretic mobilities and are inseparable in ordinary gel-diffusion tests. Thus, the immuno-electrophoretic test is useful for differentiating the leaf-streak and blight pathogens of rice, as are enzymatic tests (3).

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LITERATURE CITED

1. Graber, P. 1958. The use of immunochemical methods in studies on proteins. Adv. Protein Chem. 13:1-33.
2. Mahanta, I. C., and Addy, S. K. 1977. Serological specificity of *Xanthomonas oryzae*, incitant of bacterial blight of rice. Int. J. Syst. Bacteriol. 27:383-385.
3. Reddy, O. R., and Ou, S. H. 1974. Differentiation of *Xanthomonas translucens* f. sp. *oryzicola* (Fang et al.) Bradbury, the leaf-streak pathogen, from *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, the blight pathogen of rice, by enzymatic tests. Int. J. Syst. Bacteriol. 24:450-452.