BACTERIAL LEAF BLIGHT OF RICE. I SEROLOGICAL RELATIONSHIPS BETWEEN VIRULENT STRAIN AND WEAKLY VIRULENT STRAIN OF *XANTHOMONAS ORYZAE.*[1]

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Serological techniques have been adapted for determining the specific toxonomic relationships of plant pathogens (Amos and Burrell, 1967; Burrell *et al.*, 1966; Gooding, 1966; Gooding and Poweres, 1965; and Manning, *et al.*, 1967). It has been proposed that antigen shared by virulent strain might be involved in the pathogensis of disease (Madhosingh, 1964; and Lukezic and DeVay, 1965). Rai and Strobel (1969) demonstrated that phytotoxic glycopeptides produced by *Corynebacterium michiganense* was antigenic. *Xanthomonas oryzae,* a pathogen of bacterial leaf blight on rice plant, has been reported to comprise many strains (Wakimoto, 1960; Kuo, *et al.*, 1968; Chakravarti and Rangarajan 1967; and Shekhawat 1968) based on their physiological properties and virulence. In order to understand the relationship between antigen produced by pathogen and the pathogenicity of pathogen, a highly virulent strain (500) and a weakly virulent strain (507) of *X. oryzae* were selected and their serological relationships were compared by gel-diffusion test. Also a toxic substance isolated from virulent strain was used as an antigen and the antigentic relationships between toxic substance and these two strains of bacteria were studied.

Antisera were prepared by injecting bacterial suspension (3×10^8 bacteria/ml. sterile saline) into the hip muscle of normal New Zealand male rabbits (ca 4 lb). Five injections were given at 6-day intervals. For the initial dose, 0.1 ml of bacterial suspension was injected and the amount was doubled each additional injection untill the dosage reached 1.6 ml per injection. Antiserum was obtained one week following the last injection by sterile cardiac puncture and stored at -15°C for subsequent use. A slight modification of the agar

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double diffusion technique of Ouchterlony (1958) was used. The tests were made in 9 cm Petri dishes containing 20 ml of agar medium which consisted of 1% "Difco agar" (Oxoid) dissolved in 0.14 M NaCl, 0.01 M KH_2PO_4 buffer (pH 7.0) and 0.02% (w/v) sodium azide. Circular reservoirs were made with a 6 mm cork borer. The serum was pipetted into central reservoir, and the bacterial suspensions broken by superbiosonic were placed in six peripheral reservoirs respectively (Fig 1). The Petri dishes were incubated in a humid chamber at 26°C and examined daily for the appearence of precipitin lines.

Result of the gel diffusion test between the antiserum of strain 500, and antigen obtained from strain 500 or 507 is shown in Fig. 1. Five precipitin lines were visible between the antiserum well and the antigen wells of strain 500. Only 3 precipitin lines were observed between the antiserum well of strain 500 and the antigen wells of strain 507. Three common antigens, as indicated in precipitin lines whose ends joined, were evident between strains 500 and 507. However, an intense line near the peripheral well and a small line mixing in three common precipitin lines were observed only between 500 antigen and antiserum but not between 507 antigen and 500 antiserum.

Unpublished experiments demonstrated that virulent strain 500 produced large amount of slime substance which could cause the wilt of rice seedling. On the contrary weakly virulent strain 507 could not produce substance. It is possible that the slime substance of strain 500 might be responsible for the production of those extra precipitin lines in the agar double diffusion test. The slime substance was precipitated with alcohol and then dissolved in distilled water. Same procedure was repeated once and finally the slime substance was dissolved in saline buffer, and then it was used as antigen to react with 500 antiserum. The partially purified toxic substance and 500 antigen were placed in the peripheral wells and 500 antiserum in the central well. As shown in Fig 2 the partially purified toxic substance showed one precipitin line close to the antigen well. The end of the line join to the intense precipitin line which was only detected in 500 antigen-antiserum homologous test. Other 4 precipitin lines could be detected in 500 antigen-antiserum homologous test, but they were too weak to be photographed.

The results indicate that a virulent strain and a weakly virulent strain of *X. oryzae* could be easily distinguished by gel diffusion test. Strain 500 antigen-antiserum homologous test produced two more precipitin lines than 507 antigen–500 antiserum heterologous test. One of the extra precipitin lines was caused by a toxic substance which produced only by virulent strain. It appears that the antigen shared by the virulent strain of *X. oryzae* was directly involved in the pathogenesis of bacterial leaf blight of rice plant.

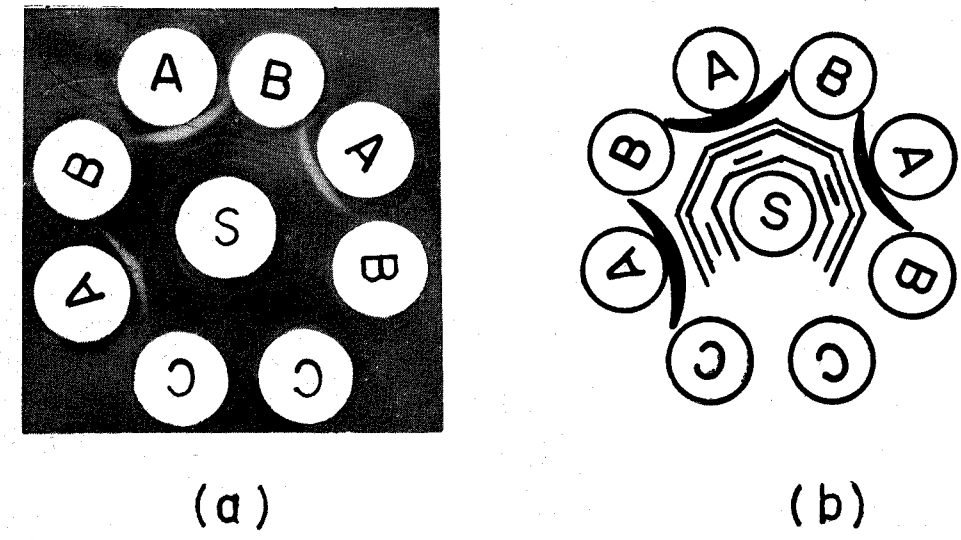

Fig 1. Ouchterlony agar double diffusion comparison of antigen-antisera reactions of strains 500 and 507 of *Xanthomonas oryzae*. The central well contain 500 antiserum(S) and the peripheral wells contains antigens for strain 500(A) strain 507(B) and control(C) respectively.
(a) Immunodiffusion partern (b) Diagramatic drawing of (a).

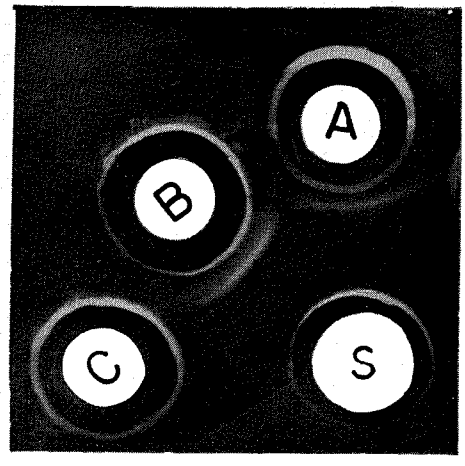

Fig. 2. Ouchterlony agar double diffusion reaction of the toxic substance of *X. oryzae* and antiserum for strain 500. The central well contains antiserum of strain 500(S) and the peripheral wells contain antigens for toxic substance(A) strain 500(B) and control(C).

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Correction from Mr. Hsia Wang.

The authorship of the paper entiled "A study of fruit and seed setting ability and female sterility in the sweet potato *Ipomea batatas* (Lam)" which appeared in the Botanical Bulletin 9(2):139–153 should have been Hsia Wang and Milo Burham. Address of the second author is Department of Horticulture, Louisiana State University, Baton Rouge, La., U. S. A.