CHARACTERIZATION OF THREE BACTERIOPHAGES OF *XANTHOMONAS ORYZAE* (UYEDA ET ISHIYAMA) DOWSON[1]

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Introduction

Bacterial leaf blight of rice plant caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson is a wide-spread disease in Taiwan. For forecasting the occurrence of the disease, the population of bacteria in the rice field has been indirectly measured by the phage titer. The study on this relationship it is necessary to understand the strains of phage in the rice field. The phages collected from different part of this island were isolated and classified into several strains based on their plaque morphology and host specificity. Among these isolates three morphologically distinctive strains were obtained. Since they are different from other strains of *X. oryzae* phage deseribed by other workers (Wakimoto 1960a, Wakimoto 1960b), their properties and relationship are extensively studied and reported in this paper.

Materials and Methods

Bacterium, phage and medium: Xanthomonas oryzae (Uyeda et Ishiyama) Dowson used in these studies was strain 500 of our institute. Phages Xp10, Xp20 and Xf were isolated on the 500 of *X. oryzae* from the water of rice field. The isolation and propagation of phages from a single plaque assay were carried out as described by Adams (1959). The medium (PS) consisting of potato, 200g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5g; $Na_2HPO_4 \cdot 12H_2O$, 2.0g; peptone, 5.0g; sucrose, 15.0g in 1 L of distilled water was used for routine cultivation. This medium was satisfactory for the isolation and propagation of phages. Phage adsorption and one step growth experiment were also conducted in the same medium.

Growth and partial purification of phage: For electron microscopic examination

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and nucleic acid determinations, high-titer preparations of phage, free from bacterial debris were required. All phages were propagated in bacteria which were grown exponentially in PS medium and resuspended in the same medium at final concentration of 3×10^8/ml. The bacteria were inoculated with a phage multiplicity of 2 and the infected cultures were continuously shaken at 30°C for 5 to 8 hours. Then the phage lysate were centrifugad at 8000 × G for 20 minutes to remove the bacteria and debris. The supernatants were centrifuged at 20,000 rpm (Spinco L No. 30 rotor) for 60 minutes. The resulting pellets were resuspended in 0.1*M* phosphate buffer (pH6.5), The same procedure was repeated once for further clarification. Highly purified preparation of Xf phage could be obtained by passing lysate through bacterium-proof millipore filter. This procedure eliminated large amount of bacterial debris.

Assay: The standard two-layer agar technique for plaque forming units (PFU) was used (Adams 1959). The bottom layer was PS medium plus 1.5% agar, and the top layer was PS medium plus 0.8% agar. Indicator bacteria were on overnight culture grown in PS medium.

Phage antisera: Antisera for the phages were prepared in rabbits by the use of three subcutanceous injection of partial purified phage. Each time each animal was injected with 2 ml of antigen containing 10^{10} PFU/ml of phage in 0.01 *M* saline phosphate buffer (pH7.0). injections were given at 5 day intervals. Trial antiserum was collected at one week intervals after the final injection. The K value of the antisera and the cross neutralization was examined according to Adams' method (1959).

Thermal inactivation: Phage suspension (3×10^7 PFU/ml in PS) was heated in a water bath at 60°C for various periods and assayed for survival. The heat stability of phages at 40° to 100°C for 10 minutes was also examined under the same condition.

Adsorption of phages to bacteria: A method modified from that described by Adams (1959) was used to determine adsorption rates. Logarithmically growing bacteria were mixed with phage in a ratio of less than one phage per bacterium. Samples were withdrawn from the aerated mixture at intervals, freed from bacterial cells by centrifugation at 8000 × G and assayed for free phage.

One step growth experiment: Growth characteristics were determined by one-step experiment (Adams 1959). The host bacteria were grown with aeration to a final concentration of about 5×10^8 colony-forming units per ml. The multiplicity of infection in the adsorption tubes was always less than one. Adsorption was allowed to proceed for 5 minutes, a sample of the adsorption mixture was then exposed to antiserum action for 7 minutes and dilutions were made into growth tubes. The growth tubes were shaken in a water bath at