Rapid Diagonosis of *Pseudomonas avenae* by Pathogenicity and Serology

D.D. Shakya

Instruction Committee, Kirtipur Compus, Tribhuvan University, Kathmandu, Nepal

ABSTRACT

Recently it has been reported the world wide occurrence of bacterial stripe of rice caused by *Pseudomonas avenae* Manns (Shakya *et al* 1985). Approximately 150 isolates had been made from various parts of rice seedlings showing typical bacterial stripe raised from 55 rice seed samples of 29 countries of the world. Identification of bacterium by biochemical test is time consuming. Therefore an attempt was made to identify the isolates by pathogenicity and serology. Each isolate was studied for gram differentiation (Suslow *et al* 1982), oxidase reaction, pathogenicity to rice seedlings (hypodermic injection and clip method) and serology (Ouchterlony gel double diffusion and agglutination).

All pathogenic isolates were gram negative, positive in oxidase and nonfluorescent. Those isolates which were pathogenic to rice seedlings agglutinated strongly (complete clearing with cottony flucose clumps) only with *P. avenae* antiserum. A few isolates showed doubtful agglutination reaction without an appearence of macroscopic flucose cottony clumps. Only the pathogenic isolates produced precipitation bands on Ouchterlony gel double diffusion test. The precipitation bands produced by these isolates were 1 to 3 indicating *P. avenae* is serologically heterogenous. It is suggested that *P. avenae* can be rapidly identified by pathogenicity and serology.

Biological Control of Bacterial Wilt of Tomato by Non-pathogenic Strains of *Pseudomonas glumae*

S. Wakimoto

Faculty of Agriculture, Kyushu University, Japan

Owing to much effort made by many chemists and plant pathologists, various chemicals effective in controlling plant diseases have been developed. By application of such chemicals, the majority of plant diseases caused by fungi are able to be controlled at present. However, most of bacterial diseases and virus diseases are still remained difficult to control by chemicals.

To control these embarrassing plant diseases, some measures based on the cultivation practices have been recommended. Such cultivation practices, however, are not always sufficiently effective.

Since early 1970s when Dr. Kerr and his coworkers reported on the efficacy of the

bacteriocin producing non-pathogenic strain(K84) of *Agrobacterium tumefaciens*(Aoradiobacter K84) on the control of crown gall of peach tree, studies on biological measures to control bacterial diseases have been emphasized in the world. Thereafter, many saprophytic or non-pathogenic bacteria were reported to be effective to suppress fungal or bacterial plant diseases.

On the assumption that plant pathogenic bacteria will be expected tobe a source of the useful agents for biological control due to their higher compatibility in the Laboratory of Plant Pathology, Kyushu University. Among many plant pathogenic bacteria tested, most strains of *Pseudomonas glumae*, the causal bacterium of grain rot of rice, were found to be a producer of antibiotics showing wide activity range against various plant pathogenic bacteria. They produced wide growth inhibition zone on the lawn of such bacteria *A. tumefaciens*, *Corynebacterium michiganense* pv. *michiganense*. *C. m.* pv. *sepedonicum*, *Erwinia carotovora subsp. cayotovora*, *Pseudomonas solanacearum*, *P. syringae* pv. *syringae*, *Xanthomonas campestris* pv. *oryzae* and *X.* c. pv. *citri*.

One of the most important items to be demanded to the biological agents is non-pathogenicity. Therefore, non-pathogenic strains were selected from the strains of *P. glumae* producting anti-*P. solanacearum* substance. Fortunately, since the pathogenicity and antibiotic productivity seem to be controlled by different genes in *P. glumae* strains, the strains having both characteristics of non-pathogenicity and antibiotic productivity were readily selected from stock cultures. Presumably, these strains have lost their pathogenicity without losing antibiotic productivity during successive transfers for preservation in the past. Thus, two strains, 750 and 805, were selected as suitable agent for biological control of bacterial wilt.

According to the procedures shown in Fig. 1, the effect of the two strains on the protection of tomato seedlings from infection with *P. solanacearum* was tested.

As shown in Fig. 2 and 3, the efficacy of the pretreatment with the suspension of *P. glumae*

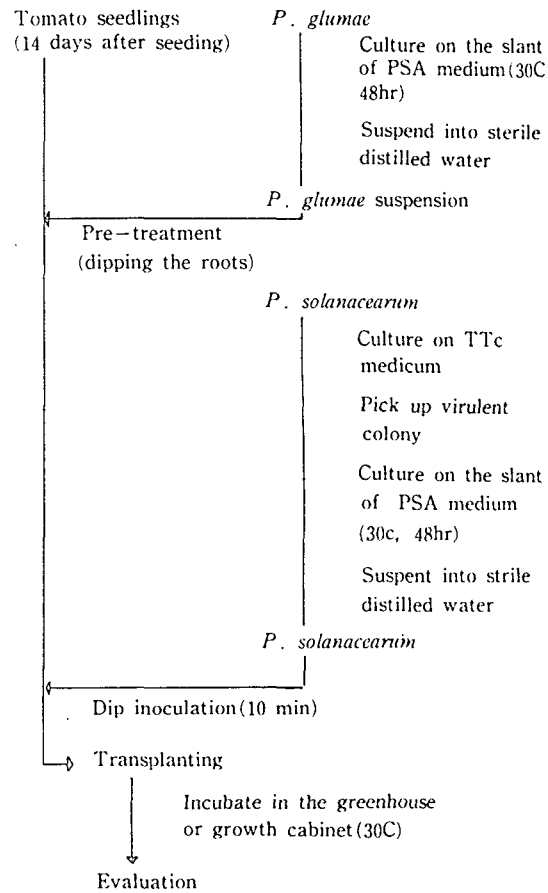

Fig. 1. Method for evaluating biocontrol effect of *P. glumae* on bacterial wilt of tomato seedlings.

depended upon concentration of the bacteria and length of dipping time.

To make clear the mechanisms of the protection effect, various experiments were carried out. The results obtained were summarized as follows:

1. When both cells of *P. glumae* and *P. solanacearum* were mixedly cultured in YPD medium, soil and tomato rhizosphere, the population of *P. solanacearum* were not suppressed.

2. The antibiotic non-producing strains selected from non-pathogenic strains of *P. glumae* also showed remarkable protection effect.

3. The *P. glumae* cells killed by heating, UV irradiation or chloroform treatment also showed marked protection effect as in the case of living cells.

From these results, the mechanisms of the

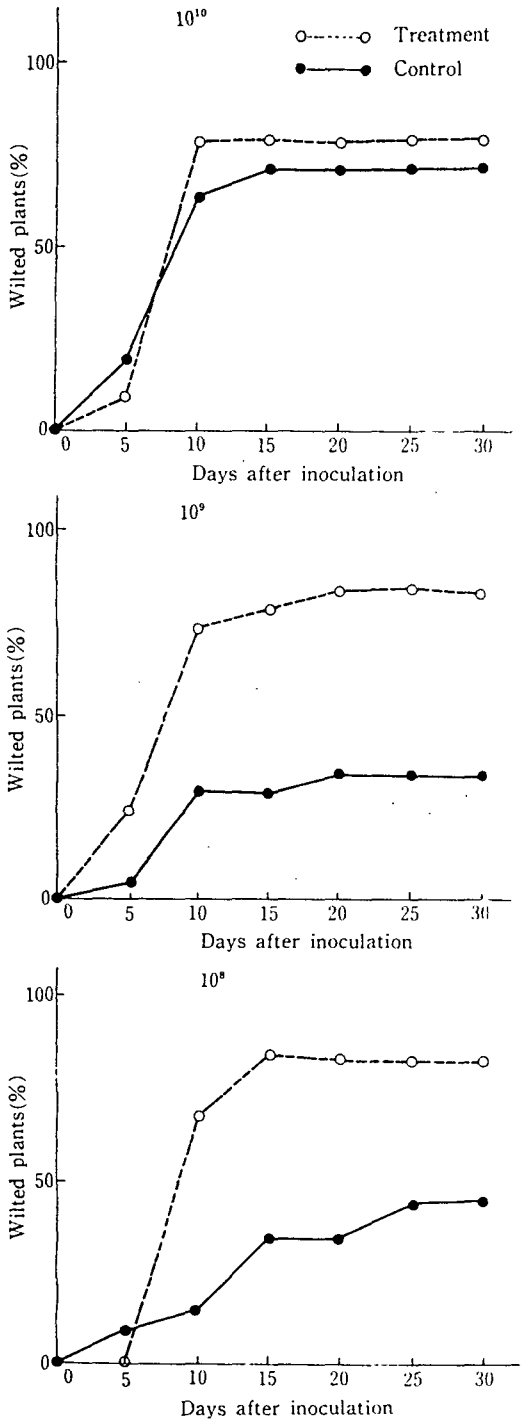

Fig. 2. Protection of tomato seedlings from infection of *P. solanacearum* by dipping in the suspension of *P. glumae*.
First dip : *P. glumae* strain 750(10^{10} cfu/ml, 60min).
Second dip : *P. solanacearum* strain C319(10^{10}, 10^{9}, 10^{8} cfu/ml, 10min).

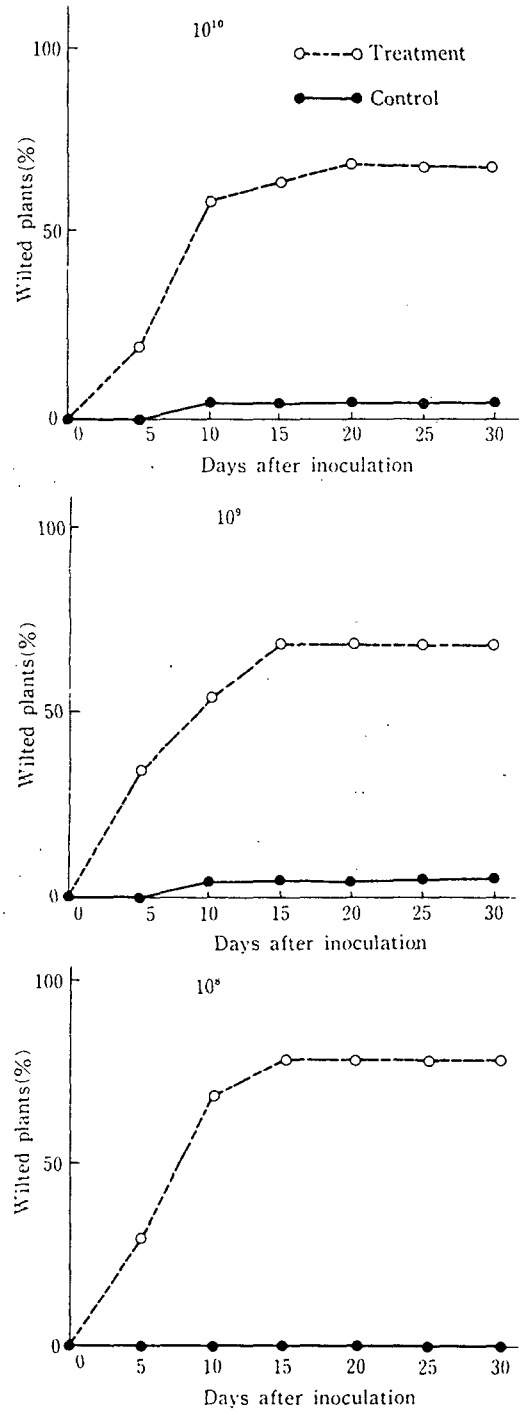

Fig. 3. Protection of tomato seedlings from infection of *P. solanacearum* by dipping in the suspension of *P. glumae*.
First dip : *P. glumae* strain 750(10^{10} cfu/ml, 12hr).
second dip : *P. solanacearum* strain C319(10^{10}, 10^{9}, 10^{8} cfu/ml, 10min).

protection effect of tomato seedling by *P. glumae* from infection with *P. solanacearum* were considered to be caused not only by antibacterial substances produced by *P. glumae* but also by other factors such as occupation of attachment site or induction of resistance in host tissues.

To establish biological method for control bacterial wilt of tomato plants, the longevity of the efficacy should be tested first, and the relationship between the efficacy and environmental conditions such as the inoculum potential, kind of soil, temperature and humidity of soil, etc. should be made clear. These experiments are being done at present in our laboratory.