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Characteristics of *Ralstonia (Pseudomonas) solanacearum* from Gangetic West Bengal

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The bacterial wilt disease of tomato (*Lycopersicon esculentum* Mill.) caused by *Pseudomonas solanacearum* E. F. Smith, is endemic in the low altitude areas of gangetic West Bengal. The disease is wide spread during winter season but sporadic in nature during summer and rainy season. In India the pathogen is serious on solanaceous vegetables including tomato in several states of India. It was confined to South India for many years. A survey was conducted in the gangetic West Bengal to establish the identity and distribution of biotypes. In addition to that standard tests for the identification of the biotypes, different physical tests were carried out to ascertain the possible existence of variation among different strain and the strain of *R. (P) solanacearum* was identified as rare 3 and biotype II.

Key words : *Ralstonia (Pseudomonas) solanacearum*, characteristics of the strain, oxidation of carbohydrates, virulence rating, biotype, solanaceous crops

INTRODUCTION

India is a country with variety of meterological conditions. The bacterial wilt disease of tomato (*Lycopersicon esculentum* Mill.) caused by *Ralstonia (Pseudomonas) solanacearum* occurs from time to time in the different regions of India including West Bengal, but is endemic in the low altitude areas, where the climate is hot and dry ; summers followed by monsoon and cold winters. The disease is wide spread during winter seasons but very sporadic in nature during summer and rainy seasons.

The bacterial wilt disease of tomato caused by *Ralstonia (Pseudomonas) solanacearum* is prevalent in temperate, tropical and sub-tropical regions of the world. (Kelman, 1953 ; Engelbrecht and Pinsloo, 1985). In India the pathogen is serious on several solanaceous vegetables including tomato in several states (Rao and Sohi, 1977). The disease was confined to South India for many years (Gnanamanickam *et al.*, 1979). Sharma and Mukherjee (1970) reported the bacterial wilt of jute (*Corchorous olitorious* and *C. capsularis*) caused by *Pseudomonas solanacearum* and opined that it was of biotype IV.

Das and Chattopadhyay (1955) reported the wilt of eggplant caused by *P. solanacearum* var. *asiaticum*. Addy *et al.* (1980) reported the wilt of tomato plant caused *P. solanacearum* race 1 from Assam and Orissa. Samaddar *et al.* (1998) reported that all the isolates of *P. solanacearum* from eggplant, tomato and potato from different localities of West Bengal were race 1.

Buddenhagen *et al.* (1962) differentiated strains of *Pseudomonas solanacearum* into three races : race 1 affects tobacco (*Nicotiana tabacum* Linn), tomato (*L. esculentum* Mill.), and many solanaceous crops and certain diploid bananas (Moko disease) (Gangadin, 1984) ; race 2, pathogenic on triploid bananas and Heliconias, *Heliconea* spp. ; and race 3 affects potatoes (*Solanum tuberosum* L.) and tomatoes, but is not highly virulent on other solanaceous crops.

Hayward (1964) differentiated strains of the pathogen in four biotypes according to their ability to oxidize three disaccharides (lactose, maltose and

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cellobiose) and three hexose alcohols (mannitol, sorbitol and dulcitol). Biotype I oxidizes none of the carbohydrates, biotype II only the disaccharides, biotype III oxidizes both the disaccharides and hexose alcohols and biotype IV, the hexose alcohols only. Biotype II can, according to Buddenhagen and Kelman (1964) be designated as race 3, the potato race. It is the only grouping which can be correlated with race or pathotype, as defined by other workers Denny and Back (1991) cited genetic evidences that extracellular polysaccharides is a virulence factor of *(Pseudomonas solanacearum)* which was supported by Kao *et al.* 1992 and Xu *et al.*, 1990)

A survey was conducted in the Gangetic West Bengal to establish the identity and distribution of the different biotypes defined by Hayward (1960). In addition to the standard tests for identification of the biotypes, other physiological tests were carried out to ascertain the possible existence of variation among different strains (Bosch *et al.*, 1985 ; Hayward, 1976). Isolates were also compared for virulence in a series of host plants, including tomato (*L. esculentum* Mill), potato (Vasse *et al.*, 1995), Egg plant (*S. melongena* L), Chilli (*C. annuum* Linn), jute (*C. capsularis* Linn) and banana (*Musa* spp.).

MATERIALS AND METHODS

Identification tests were carried out in our laboratory from 1984 to 1985 and two strains of *Pseudomonas solanacearum* were isolated from tomato (*L. esculentum* Mill.) of the same loaction (i.e. Midnapore district) of West Bengal (India), but from the two different sources. These strains were isolated on tetrazolium chloride (TZC) (Kelman, 1954) medium and was identified by using standard laboratory techniques (Harrigan and McCance, 1966 ; Hayward, 1964 ; Sands *et al.*, 1980). These included microscopic examination, Gram staining and the following physical tests : the formation of the fluorescent pigments on medium 'A' and 'B' of King *et al.* (1954), indole production, oxidase and catalase activity. Tests for oxidation/fermentation of glucose and other sugars and hexose alcohols, production of a brown diffusible pigment on a tyrosine medium, salt tolerance and production of PHB (Poly-β hydroxybutyric acid) and temperature maxima and minima (Burdon, 1944 ; Katayama *et al.*, 1984). All these tests were repeated five times. Cultures were stored in McCartney bottle in sterilized, distilled water at 30°C (Kelman, 1954).

To determine if differences existed among strains assigned to the same biotype, tests were conducted to determine the ability of strains to oxidize nineteen carbohydrates using the synthetic medium of Ayers *et al.* (1919). The following carbohydrates were included : sucrose, maltose, lactose, cellobiose, mannitol, sorbitol and dulcitol. The carbohydrates were filter sterilized and added to the presterilized medium in capped test tubes to obtain a final concentration of 0.2%. The indicator bromothymol blue (0.008%) was included in the medium to detect acid production by change in colour from green to yellow, compared with an inoculated control without any carbohydrate. Strains were cultured on slants of TZC medium at 30°C for 48 hrs. Bacteria were suspended in sterilized, distilled water untill the absorbance equalled 0.2 (at 620 nm) on the scale of spectrophotometer 10060 Hitachi model. Test tubes were inoculated with 0.05 ml of the suspension and incubated upto 21 days at 30°C.

Plants used in virulence tests were tomato var. Pusa early dwarf (1943, Sutton) ; *Solanum melongena* var Pusa Kranti (1597, Sutton) ; *Capsicum annuum* var. Suryamukhi (1676, Sutton) ; *Solanum tuberosum* var. Kufri Jyoti and Chandramukhi and *Musa* spp. var. local and *Corchorous capsularis* var. JRI 426. Seed potato tubers (French, 1986) and rihozome of banana were planted directly in 10 cm earthen pots.

Seeds of the other plants were sown in seedling trays and were transplanted to pots 3 to 4 weeks after sowing. The root inoculation technique (Winstead and Kelman, 1952 ; Lallmahoned and Ricand, 1978) was used on 11 cm to 14 cm tall plants. By cutting the side roots 30 mm inoculum (with an absorbance value of OD, 1cm, 620 nm of 1.9 corresponding to 8.6 X 10^9 cells/ml) was poured at cut ends of per plant. Uninoculated plants served as control. Two strains selected for virulence tests were strain 1) ICMP-9738 and 2) ICMP-9738 respectively. Both were isolated from tomato but source were different ICMP-9758 from diseased host of tomato and ICMP-9738 from tomato culti-

vated diseased soil. Each isolate was inoculated into a total of at least fifteen plants of each host. Inoculated plants were grown in a glass house at 28°C (day) and 25°C (night). Disease indices were recorded 15 days after inoculation on a scale of 1-5, where 1=no symptoms, 2=wilt of one leaf, 3=wilt of upto half of the leaves, 4=wilt of nearly all the leaves and 5=complete wilt or death (He *et al.*, 1983). Virulence ratings were based on average disease indices according to the scale of He *et al.*, (1983).

The degree of susceptibility of potato, chilli, egg plant, tomato, jute and banana plants to both the isolated strains of *Pseudomonas solanacearum* based on the disease indices 15 days inoculation showed that the strain ICMP-9758 is highly virulent on potato and tomato but mildly virulent on chilli, egg plant and jute and could not induce disease in banana.

RESULTS

Both the strains of *P. solanacearum* were rod shaped and gram-negative. On TZC medium the strain ICMP 9758 produced fluidal, slightly raised concleved colony with pink centres surrounded by the white fluidal mass after 48 hrs of incubation ; but, colonies of strain ICMP 9738 were deep red and afluidal. Both the strains gave a negative reaction for indole production at 24 hrs of incubation ; and at 7 to 14 days of incubation but both the strains produced indole later on. Strain ICMP 9738 produced more indole in presence or absence of tryptophan than the strain ICMP 9758. None of the isolates produced fluorescent pigment on medium A and B of King *et al.* (1954) and positive reaction for starch hydrolysis, but slightly positive reaction to gelatin liquefaction at 14 days incubation. Both the strains were oxidative and Kovac's oxidase positive (1956), produced a brown pigment on a tyrosine medium, and produced catalase. The cells of the fluidal colony type stained more uniformly with Sudan Black B than the nonfluidal cells (Burdon, 1946). The relative mobility of afluidal cell was suprior to that of fluidal cell on the same medium, during the same incubation period in still culture. Salt tolerance varied from 1-2% in the medium GYE (glucose-yeast extract). Based on these results the strains were identified as *Ralstonia (Pseudomonas) solanacearum.*

Assignment of the strain to biotype group is presented in Table 1. Sucrose was oxidized by both the strains. Results of the oxidation of the other carbobydrates are also given in Table 1.

Table 1 : Oxidation of carbohydrates[a] by strains of *Ralstonia Pseudomonas solanacearum* from Gangetic West Bengal.

Sources of Carbohydrate	Strain ICMP 9758	ICMP 9738
Maltose	+	+
Lactose	+	+
Cellobiose	+	+
Mannitol	—	—
Sorbitol	—	—
Dulcitol	—	—
Sucrose	+	+
Trehalose	—	—
Ribose	—	—

[a] Based on Stanier *et al.* (30).

Table 2 : Virulence rating[a] of the Gangetic West Bengal *R. (P.) solanacearum* on six hosts

Host	Strain ICMP 9758	ICMP 9738
Potato	H[b]	H[b]
Tomato	H	H
Eggplant	M	M
Banana	O	O
Chilli	L	L
Jute	L	L

[a] Results based on average disease indices on a scale of 1-5 of 15-20 plants after 40 days of inoculation.

[b] H = high (4.1-5.0). M = medium (2.6-4.0) ; L = low (1.1-2.5) ; O = none (0-1.0).

A rapid wilting of the foliage occurred after inoculation. Wilt leaflets and leaf stalks were curled downwards. A brown discolouration of the water conductiong tissue was observed when the stem was cut accross. A milky exudate was also appartent when the cut stem was placed in water (Vock, 1978). Results of the virulence tests are given in Table 2. Both the strains were differentiated as biotype II. One of which was designated by ICMP as ICMP-9758 (strain 1) which showed no virulence type of pathogenicity on banana but was highly virulent on tomato and potato, mildly virulent on jute and chilli. The other ICMP-9738 (strain2) could not cause any disease to any hosts.

Therefore, the strain 1 may be the virulent form of *Pseudomonas solanacearum* where as strain 2 is the avirulent form of it.

DISCUSSION

Although bacterial wilt caused by *Ralstonia (Pseudomonas) solanacearum* has been known in India for more than 22 years, but the characteristics of the strains of the pathogen occurring in the gangetic West Bengal have not previously been determined, with one exception, that the strains isolated from the diseased host plants were representative of biotype II. Biotype-II in India is mainly found in the temperate regions. The tendency to isolate biotype-II from tomatoes reflect the practice of cultivating tomatoes in the cooler regions or during the cool season in the warm regions. Biotype-II was isolated from diseased tomatoes grown in the field with a history of early summer potato production. There are few production where the two crops are grown together. Tomatoes are widely grown in this area where the pathogen is considered to be endemic. Although tomato has been produced for a number of years in this area, the disease was only recently occurred (Katayama *et al.*, 1984) and the identity of the pathogen on this crop was confirmed.

According to the race identification system of Buddenhagen *et al.* (1962), the isolated strains probably belong to race-3, as the disease has not yet been reported on banana in West Bengal. Therefore, based on the results of the virulence tests of the two representative strains, isolates of biotype-II can be designated as race-3.

No physiological differences were observed between the strains when reisolated from diseased inoculated potato and tomato plants, jute, egg plant, chilli and banana. Both the strains of biotype-II failed to oxidizre trehalose and ribose. Strains 1 and 2 identified as biotype-II, as defined by Hayward, but these differed from Hayward biotype-II in its ability to produce indole at 7 to 14 days incubation and slight gelatin liquefaction.

Since this strains found with this particular characteristics may be a mutant. Bacterial wilt due to pathogen *Ralstonia (Pseudomonas solanacearum)* was greatly influenced by the season and age of the crop at the time of inoculation. The wilt induced, was observed more in summer followed by monsoon and winters season. This may be due to rapid multiplication and colonization of the casual bacterium on one hand and susceptibility of the host on the other, resulting in quick and more wilting. Probably high temperature is favourable for multiplication (Kelman, 1953). Higher wilting at elevated temperature due to bacterial wilt pathogen has also been observed by earlier workers (McCarter *et al.*, 1971 ; Rao, 1976 ; Rana Devi and Menon, 1980). As in the case of bacterial diseases, loss in yield was more when young plants were inoculated with *Ralstonia (Pseudomonas) solanacearum* in all seasons.

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