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Identification of the race of *Pseudomonas solanacearum* causing wilt of solanaceous vegetables in West Bengal and its survival

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Pseudomonas solanacarum, the causal agent of wilt of eggplant, tomato, potato and chilli was isolated from different fields of West Bengal. The race status of the isolates was investigated. Pathogenicity and biochemical parameters were used for identificatin of the race status of the isolates. Oxidation of different sugars, sugar alcohols and denitrification properties were examined. The results indicated that all isolates irrespective of the host was race 1. It was of interest tht all the potato isolates from the plains of West Bengal including dooars area were of race 1 and not race 3. The short and the long term survival of the pathogen in the carposphere, in natural soil as well as in weed hosts as primary source of infection was also examined by using a streptomycin and erythromycin resistant double marker strain of the pathogen. The results indicated that race 1 of the pathogen could survive more than six months on seed surface and in soil. Furthermore, the organism could survive both in the rhizosphere, on the rhizoplane and inside the root as symptomless carrier in a large number of weed plants of West Bengal. The epidemiological significance of the survival of race 1 in seed, soil and weed plants has been discussed with a view to evolving management programme for the pathogen.

Key words : Bacterial wilt, race identification, survival of the pathogen

INTRODUCTION

Bacterial wilt incited by *Pseudomonas solanacearum* is by far the most destructive wilt disease of solanaceous vegetables like eggplant, tomato, potato and chilli in West Bengal. The wilt disease causes severe loss of yield and consequent economic loss to the farmers. There are distinct differences in the biochemical and pathogenic properties among isolates of *P. solanacearum* from different hosts leading to establishment of sub-specific categories like races, pathovars or biotypes (Okabe and Goto 1952; Buddenhagen and Kelman, 1964; Hayward, 1964). Buddenhagen *et al.* (1962) separated isolates into 3 races; race 1, affecting solanaceous hosts like tobacco, tomato, eggplant, chilli and other plants; race 2, pathogenic on triploid bananas and Heliconias; and race 3, affecting potato and weakly pathogenic on other solanaceous crops. Hayward (1964) established four biotypes; Biotype I, II and III equivalent to race 2, race 3, and race 1 of Buddenhagen *et al.* (1962) respectively and Biotype IV affecting ginger mostly.

Das and Chattopadhyay (1955) indentified the pathogen causing eggplant wilt as *P. solanacearum* var *asiaticum*. Addy *et al.* (1980) in a study of 30 tomato isolates of the pathogen from Assam and Orissa concluded that all isolates of the pathogen belonged to race 1. The race status of the isolates obtained from different solanaceous hosts of West Bengal is insufficiently known.

Mode of survival of the pathogen in the absence of host plant in nature is quite controversial (Schuster and Coyne, 1974; Hayward, 1991). Several reports claimed survival of the pathogen on seed (Moffett *et al.* 1981), on weed host (Hayward, 1991) and in soil (Hayward, 1991; Scqueira, 1994). Effective control of the vegetable wilt has not yet been found out. Breeding for disease resistance has not been very successful because of the extensive variability of the bacterial strains.

The objectives of this investigations were to identify the isolates obtained from solanaceous host to the sub-specific group(s) and to study the source of primary infection and survival of a marker strain under the agroclimatic conditions of West Bengal. Attempt was also made for managment of the disease.

MATERIALS AND METHODS

The pathogen from wilted eggplant, tomato, and potato was isolated using the specific medium of Karganilla and Buddenhangen (1972; KB medium). The reference isolates of Race 1,2 and 3 were obtained by the courtesy of Rothamsted Experimental Station, England. The morphological properties like staining, presence of flagella, capsule and spore, and biochemical properties like catalase activity, hydrolysis of starch and liquifaction of gelatin were determined following the method of Smith (1946). Acid and gas production by utilization of various carbon sources were determined following the methods of Sands *et al.* (1980) and Haryward (1964).

Double marker strain (S_2E_1) resistant to streptomycin sulfate (2 mg/ml) and erythromycin (1 mg/ml) were developed and isolated following the method of Obaton (1971).

Seed samples for seed pathological studies were obtained from local markets of districts of Nadia, Hooghly, North and South 24-Parganas, Howarah, Murshidabad, Jalpaiguri and Coochbehar. For tomato and eggplant 100 seeds from a sample were shaken with 10 ml of sterile distilled water for 1 h and known volume of this washing was plated on KB medium. Results are expressed as number of colony forming unit (CFU) per seed. For potato, 5 g tissue slice was cut from a tuber and shaken with 10 ml of sterile distilled water for 1 h and known volume of the washing was plated on KB medium. Results are expressed as CFU/5 g of tuber sample. Data are average of three washing samples of each seed sample of different host species.

For survival on seeds known number of marker strain was placed on the surface of the healthy, non-infested seeds of host plants and incubated at different temperature and relative humidity (%) conditions in controlled cabinets. At intervals the samples of seeds were taken out, washed as described previously and the washings plated on potato-dextrose agar medium supplemented with streptomycin (2 mg/ml) and 1 mg/ml of erythromycin PDASE). The surviving cells were expressed as number of CFU or log number of cells recovered per 100 seeds (eggplant and tomato) and per 5 g tissue (potato) of host plants.

For survival in weed hosts, nonsymptomatic weeds occurring commonly in and around the solanaceous vegetable fields were carefully dug out with intact roots and rhizosphere soils. Soil dilution plate technique using the KB medium was employed for isolation of

the pathogen from the rhizosphere soil. Root samples were surface sterilised with 0.5% NaOCl and 1 cm pieces were placed on KB medium. Presence or absence of the pathogen was exressed as + or - sign respectively.

For soil survival studies, 30 g portion of air dried soils of the experimental plots of Kalyani University (pH 6.2) were taken in 250 ml glass bottles. The bottles were either supplemented with different amendments or not and inoculated with required volumes of cell suspension of the double marker strain of the pathogen, adjusted to 60% water holding capacity and incubated at 30°C in the dark. Soil samples were taken out at intervals and the number of surviving cells were determined by soil dilution plate method using PDA supplemented with streptomycin (2 mg/ml) and erythromycin (1 mg/ml).

RESULTS AND DISCUSSION

Isolation and identification : All isolates were rod shaped, motile and gram negative. All virulent isolates produced fluidal colonies with pink centres on tetrazolium chloride (TZC) medium (Kelman, 1959) within 48 h. All local isolates were comparable to the reference isolate of tomato obtained from Rothamsted, England (Table 1).

For race determination utilization of sugars, sugar alcohols and cross inoculation ability of the isolates were compared with that of reference isolates obtained from Rothamsted. Results (Table 2) indicated that all isolates from eggplant, tomato and potato were similar to race 1 (biovar 3). In contrast, the races 3 (=potato strain) and 2 (= banana strain) could not use sugar alcohols and had poor or no denitrification ability.

Previously the eggplant isolate from West Bengal was considered as *P. solanacearum* var. *asiaticum* (Das and Chottopadhyay, 1955). Most systematists, however, considered the pathogen attacking solanaceous vegetables including potato in the tropical regions of Asia and Americans as race 1 (Hayward, 1994, French 1994). The potato strain (race 3) of the pathogen is considered to be restricted to cool high hilly regions of the world (Hayward, 1991; French, 1994) including India (Shekhawat, 1976). All isolates from eggplant, tomato and potato tested in the study obtained from different parts of West Bengal including the plains of North Bengal districts were found to be of race 1. The race status of Darjeeling and adjacent hilly regions, however, had not been examined in this study.

Survival on seed : The pathogen (race 1) has been found to be occurring on the carposphere of 62, 70 and 72% of the seed samples of eggplant, tomato, and potato respectively obtained from the market dealers. The number of CFU per seed for eggplant and tomato varied from 40 to 1000, whereas for that of potato ranged 700-6000/5 g of tissue surface area (Table 3). Surface sterilization of the seeds with 250 mg/ml of streptomycin could effectively eradicate the pathogen from the seeds. The results suggested that the pathogen is externally seedborne. Similar results were reported by Moffett *et al.* (1981) using the tomato isolate. Several authors, however, failed to detect the presence of the pathogen in seeds of tomato and chilli previously (Sekhawat *et al.*, 1979).

It is evident from the data that the double marker strain of race 1 of the pathogen could successfuly survive on the seeds of eggplant, tomato and potato (Table 4), when eggplant

Table 1 : Typical morphological and biochemical properties of eggplant, tomato and potato isolates of *Pseudomonas solanacearum*

Characters	Isolates from			Reference isolate from Tomato[a]
	Eggplant (58)	Tomato (45)	Potato (32)	
Gram staining	-	-	-	-
Presence of flagella	+	+	+	+
Presence of capsule	-	-	-	-
Fluidal colony on TZC medium	+	+	+	+
Hydrolysis of starch	-	-	-	-
Liquifaction of gelatin	-	-	-	-
Kovac's oxidase test	+	+	+	+
Growth on D_4 medium	-	-	-	-
Oxidation of Carbohydrates				
Ribose	+	+	+	+
Glucose	+	+	+	+
Fructose	+	+	+	+
Maltose	+	+	+	+
Lactose	+	+	+	+
L-arabinose	-	-	-	-
Sucrose	+	+	+	+
Cellulose	+	+	+	+

[a]Reference isolate by courtesy of Rathoamsted Experimental Station, England.

Table 2 : Biovar determination of the pathogen isolated in and around Kalyani compared with reference isolate

Characters	Biovar 1[a] (Race 2)	Biovar 2[a] (Race 3)	Biovar 3[a] (Race 1)	Biovar 4[a] (Race 4)	W.B. isolates from		
					Eggplant	Tomato	Patato
Oxidation and acid production from							
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Lactose	-	+	+	-	+	+	+
Maltose	-	+	+	-	+	+	+
Cellobiose	-	+	+	-	+	+	+
Mannitol	-	-	+	+	+	+	+
Sorbitol	-	-	+	+	+	+	+
Dulcitol	-	-	+	+	+	+	+
Denitrification (Nitrate oxidation)	±	-	+	+	+	+	+
Pathogenic to	NT[b]	Potato solana-ceae	Tomato, Eggplant, Potato	NT	Eggpl-ant, Tomato, Potato	Eggp-, lant, Tomato Potato	Eggpl-nt, Tomato Potato

[a]Reference isolate from Rothamsted Experimental Station, England

[b]Not tested

Table 3 : Occurrence of *Pseudomonas solanacearum* on the carposphere

Eggplant seeds	62% positive
Number of CFU/seed	40-900
Tomato seeds	70% positive
Number of CFU/seed	70-1000
Potato tuber	72% positive
Number of CFU/5 g tuber tissue	700-6000

Table 4 : Survival of *P solanacearum* on seeds of host plants kept at 30°C and RH 60-65% after collection

Seeds on samples	Number of CFU recovered (x 10^2) per 100 seeds[a] or per 5g[b] of tissue after days of incubation			
	0[c]	14	28	35
Eggplant	800	500	100	20
Tomato	1000	400	50	30
Potato (tuber)	2000	1000	400	100

[a]CFU/100 seeds was counted for eggplant and tomato seeds
[b]CFU/5 g of tissue was counted for potato tuber
[c]Initial CFU determined before the start of the experiment

Table 5 : Survival of double marker strain (S_2E_1) of the pathogen on seeds of eggplant at different temperature and relative humidity (%) conditions

Incubation temperature (°C)	RH(%)	Log_{10} umber of CFU recovered/100 seeds after days of inoculation and incubation				
		0	15	30	60	90
20	50	10.7	10.6	8.7	6.7	1.6
	62	10.7	10.8	8.8	6.9	1.8
30	50	10.7	11.0	8.9	6.9	4.9
	62	10.7	11.2	9.1	7.0	5.1
35	50	10.7	10.5	8.7	-	-
	62	10.7	10.6	8.7	-	-
40	50	10.7	10.2	-	-	-
	62	10.7	10.4	-	-	-

Table 6 : Survival of the marker strain of the pathogen on eggplant seeds kept at 30°C and low humidity conditions

RH(%)	Log_{10} number of CFU recovered/100 seeds after days of incubation			
	0	14	28	42
1.7	7.3	6.5	6.4	4.2
16.9	7.3	7.3	5.9	4.8
33.8	7.3	7.4	6.6	5.5
50.0	7.3	6.0	4.9	4.0

Table 7 : Effect of soil amendment with supplements on the survival of the pathogen

Treatments	Number of CFU/g of soil (x 10^8) after days of inculation			
	0	14	21	28
Control soil	8	64	24	1.0
Soil plus				
Urea 0.05%	8.0	52	14	8.0
0.10%	8.2	50	9	0.5
Flyash 0.05%	8.0	76	35	1.2
0.10%	8.2	74	18	0.7
Bleaching 0.05%	7.8	28	12	0.4
Powder 0.10%	7.8	10	2	0.1
W+FA+BP (0.05%+0.05%+0.05%)	7.8	0.6	0.15	ND[a]

[a]: ND = Not detected

Table 8 : Survival of the pathogen in the rhizosphere and inside the root of weed plants

Plant species	Isolated from	
	Exorhizosphere	Endorhizosphere
Solanum nigrum	+	+
S. anguivi	+	-
S. torvum	+	+
S. xanthocarpum	+	+
Croton bonplandianum	+	+
Euphorbia hirta	+	-
Colocasia esculenta	+	+
Porfulaca oleracea	+	+
Curcuma amada	+	+
Phyllanthus niruri	+	-
Parthenium hysterophorus	+	-

seeds, following inoculation with the marker strain were incubated at different temperature and humidity conditions (Table 5), significant number of CFU of the marker strain of the pathogen could be recovered from the carposphere after 3 months of incubation. High cell number on the seeds were detected even after 6 months of incubation (results not shown). It was of interest that high population of the pathogen persisted on eggplant seeds incubated at low temperature and at low RH(%) conditions (Table 6). The use of double marker strain provided direct proof of the persistence of race 1 on the carposphere of eggplant seeds for a prolonged period. At high temperature (40°C) the population, however, declined rapidly and became zero after 28 days of incubation. Evidently, despite the prevalent occurrence of race 1 in tropical countries, the organism cannot tolerate high soil temperature conditions.

Survival in soil : The double marker strain of race 1 was introduced to natural and sterilized soils under laboratory conditions. In natural soil it could survive for about 4 months, whereas it survived in sterilized soil for more than one year provided water holding capacity was maintained at 60% (Fig. 1). The results indicated influence of soil biota on the population density and persistence of introduced cells of race 1 in soil environment. Large number of microorganisms antagonistic to *P. solanacearum* race 1 have been isolated from the Kalyani soil and the possible biocontrol potential of these isolates would be presented in another report.

Amendment of soil with ammonium sulphate caused significant stimulation of growth and survival of race 1 in soil. In contrast, urea and potassium nitrate were significantly inhibitory to the growth and survival of the test marker strain in soil (Fig. 2). In soils amended with urea, the test organism did not grow at all and after 7 days of incubation could not be detected by soil plating technique. The results suggested successful eradication of the pathogen by the application of high dose (1%) of urea to soil. In soils amended with nitrate, population of the pathogen did not increase and the initial population introduceed declined rapidly to zero level after two to three weeks. Reduction of wilt of tomato supplied with nitrate fertilizers (Kelman, 1950) and that of tobacco by urea (Smith, 1944) have been reported.

Effects of multiple amendment of soil on the population density and corresponding inoculum potential were tested using urea, flyash and active bleaching powder. The results (Table 7) showed that urea (U) flyash (FA) and bleaching powder (BP), when used in combination each at a concentration of 0.05%, the race 1 was completely eradicated from the soil after three weeks of incubation.

Survival in weed hosts : A total of 58 weed host growing in or around the fields cultivated with solanaceous vegetables were examined for the occurrence of the pathogen in their rhizosphere soil, on the rhizoplane and inside the root. Eleven weed hosts (Table 8) were found to be non-symptomatic carrier of the pathogen either in the rhizosphere or inside the root. Apparently, the pathogen can survive well in the rhizosphere or in the roots of several weed host.

The results of this study clearly indicated that race 1 of *P. solanacearum* can survive for a long time in seeds of solanaceous vegetables as well as in soil and the primary source of inoculum in nature is provided by either the seed or the soil or both. The results further showed that the pathogen can survive in the rhizosphere of several weed hosts which can provide the primary inoculum round the year. Longtime crop rotation, use of urea as N-source along with other supplements such as flyash and bleaching powder causing control of the disease need field experimentation.

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