J. Mycopathol. Res. ***51(2)*** *: 267-272, 2013;* (ISSN 0971-3719)

Biovar distribution of *Ralstonia solanacearum* strains causing bacterial wilt/brown rot of potato in Meghalaya hills

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Received : 12.06.2013 Accepted : 08.09.2013 Published : 28.10.2013

Ralstonia solanacearum, the causal agent of bacterial wilt/ brown rot disease of potato and affects potato production in Meghalaya. Therefore, surveys were conducted to know the exact status of the disease and distribution of biovar of the pathogen in the years of 2011 and 2012. Disease samples were collected from different villages of the East Khasi Hills district of Meghalaya which is the highest potato producing district in the state during summer (February-June) and autumn (July-October) seasons. The disease incidence varied from zero to ten per cent with overall higher incidence in autumn (~0-10%) compared to summer (~0-5%) season. Using biochemical tests, based on the ability to utilize disaccharides and oxidise hexose alcohols, forty four isolates were categorized as biovar 2T, twenty isolates as biovar 2 and three isolates as biovar 3. This study revealed that three biovars are present in the state with highest frequency of biovar 2T followed by biovar 2 and 3 in East Khasi Hills of Meghalaya.

Key words: *Ralstonia solanacearum*, brown rot incidence, biovars, *Solanum tuberosum*

INTRODUCTION

R. solanacearum (Smith, 1896; Yabuuchi *et al.*1995) is a ß-proteobacterium is pathogenic and infects more than 200 plant species belonging to over 50 different botanical families (Denny, 2006). *R. solanacearum* can survive for years in moist soils or water (Alvarez *et al.*, 2008). When the pathogen encounters a susceptible host, it enters the root and colonizes the root cortex, then invades the xylem vessels, and finally spreads rapidly to aerial parts of the plant through the vascular system (Denny, 2006) and latent survival in potato tubers (Sunaina *et al.*, 1989). This pathogen causes bacterial wilt of many plant species in tropical, subtropical and warm temperate regions of the world (Hayward, 1991).

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Historically, *R. solanacearum* has been divided into five races viz. race 1(related to the ability to wilt in members of the family Solanaceae; race 2 (banana); race 3 (potato and tomato in temperate conditions); race 4 (ginger) and race 5 (mulberry) (Buddenhagen *et al.*, 1962), and six biovars based on the ability to metabolize three sugar alcohols and three disaccharides (Hayward, 1964; Hayward, 1991; Hayward, 2000). In India, the disease is endemic in Kerala, Karnataka, western Maharashtra, Madhya Pradesh, North Eastern states of India, Orissa and West Bengal, Chhota Nagpur plateau, Andaman and Nicobar Islands, North-Western Kumaon hills, Nilgiris, Annamalai and Palani hills of Tamil Nadu (Chakrabarti, 2011). In Meghalaya, the disease is known to appear in potato crop in both the seasons in summer as well as in autumn crop. However, the exact status of biovar distribution of *R. solanacearum* strains in the state is not available in the literatures.

Therefore, the present study has been conducted to know the exact status of bacterial wilt/ brown rot and distribution of its biovar in Meghalaya Hills.

MATERIALS AND METHODS

Collection, isolation, maintenance and pathogenicity of Ralstonia solanacearum

The study was conducted at Central Potato Research Station, Upper Shillong (1860 m above MSL and 25.35 ^{0}N and 91.54 ^{0} E) and Central Potato Research Institute, Shimla. Potato plants/ tubers infected with typical symptoms of bacterial wilt disease were collected from various villages of East Khasi Hills district (~1500-1961 m above MSL) of Meghalaya during 2011 and 2012 because it is highest potato growing district of Meghalaya. The samples (stem pieces and tubers) were surface disinfected with 70% ethanol, peeled, sub sampled and macerated in sterile distilled water. Macerates were streaked on Kelman's Triphenyl Tetrazolium Chloride (TZC) agar medium (Kelman, 1954) with slight modification. Plates were incubated at 28±2^o C for 48 to 72 h. This medium helped to distinguish *R. solanacearum* among other bacteria and to distinguish virulent (wild type) colonies from avirulent mutant. Bacterial colonies developing the typical irregular mucoid colonies were again transferred to fresh TZC medium for further purification. One full loop of bacterial culture transferred in 1 ml of double distilled sterile water and stored at 20±2 ^{o} C in BOD (incubator). Pathogenicity of each isolate was tested on potted plants of potato, by stem stab inoculation method (Winstead and Kelman, 1952). The inoculated plants were incubated in poly house conditions and were observed for wilt appearance till 21 days.

Biovar determination

Biovars of *R. solanacearum* strains were determined by standard procedure (Hayward 1964). The following basal medium was used for biovar identification: $NH_4H_2PO_4$ 1.0 g; KCl-0.2 g; $MgSO_4.7H_2O$-0.2 g; Peptone - 1.0 g; 1% (WV^{-1}) aqueous solution of bromothymol blue- 0.3 ml; agar- 1.5 g; distilled water- 1litre. The pH of the medium was adjusted to 7.1 with 40% (WV^{-1}) NaOH solution before addition of the agar. Five millilitres of a 10% (WV^{-1}) pre-sterilized solution of the sugars (lactose, maltose, cellobiose and trehalose) and sugar alcohols (mannitol, sorbitol and dulcitol) were added to 45 ml of molten cooled basal medium separately. 200 µl of these media added into each well of 96-well micro-titre plates. Hayward's medium without a carbon source and un-inoculated wells served as control. The cultures were incubated at 28 ^{0}C and examined at 3, 7 and 14 days for change of pH (yellow colour).

RESULTS AND DISCUSSION

Incidence of bacterial wilt/brown rots of potato

The present survey was conducted across the potato fields of different villages of East Khasi Hills district of Meghalaya during summer and autumn seasons in 2011and 2012 to know incidence of bacterial wilt/brown rot of potato. Bacterial wilt/ brown rot incidence was highest (6-10%) in Baniun-1 village in most popular variety of potato, Kufri Megha followed by Laitkor peak-2 (6-7%) in unknown local variety. Four to five per cent of wilt incidence was recorded in Mawan-1, Mawklot-1, Mawklot-2 Baniun-2, Mawan-2, and Mawklot-3 in summer season of potato. Zero to two per cent bacterial wilt/brown rot incidence was recorded in rest of the places studied in summer season (Feb-July). Whereas, zero to ten per cent incidence was recorded in Mylliem-1, Baniun-1, Lynkien, Laitkor-2, Mawklot, and Myllium-2 villages, Five to seven per cent incidence was recorded in Mawjrong-1, Laitlyngkot, laitkor-1, 5th mile-1, Mawan, baniun-2, laitkor-2. Rest of the places studied showed less than 5% incidence in autumn crop (July-Oct.) of potato. The present studies indicated that bacterial wilt/brown rot incidence varied from zero to ten per cent with overall higher incidence in autumn season crop (~0-10%) (Table 3) compared to summer (~0-5%) season crop (Table 2).

Table 1 : Biovars differentiation of *R. solanacearum* strains based on the ability to utilize disaccharides and oxidize hexose alcohols

Biochemical Test	Biovar				
	1	2	3	4	5
Cellobiose	-	+	+	-	+
Lactose	-	+	+	-	+
Maltose	-	+	+	-	+
Dulcitol	-	-	+	+	-
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-

Table 2 : Incidence and biovar distribution of *Ralstonia solanacearum* isolates in summer season in East Khasi Hills of Meghalaya

Isolates	Location	Incidence (%)	Varieties	Source	Biovar
RS-113		0-2	K. Himalini	Stem	2T
RS-114	5th Mile-1	0-2	K. Himalini	Stem	2T
RS-115		0-2	K. Himalini	Tuber	2
RS-116		4-5	Local	Stem	2
RS-117	Mawan-1			Stem	2
RS-118				Tuber	2
RS-119		0-6	Local	Stem	2T
RS-120				Stem	2T
RS-121				Stem	2T
RS-122	Laitkor peak-1			Stem	2
RS-123				Stem	2
RS-124	5th Mile-2	0-2	K Himalini	Tuber	2
RS-125				Tuber	2
RS-126		0-5	Local seed	Stem	2T
RS-127				Stem	2T
RS-128	Lynkien			Tuber	2T
RS-129				Tuber	2T
RS-130	Mawklot-1	4-5	K Jyoti	Tuber	2T
RS-131				Tuber	2
RS-132	Mawkriah west	0-4	Local seed	Stem	2
RS-133				Stem	2
RS-162		0-2	Local seed	Tuber	2T
RS-163	Pomlum	0-2	Local seed	Tuber	2T
RS-164		0-2	Local seed	Tuber	2T
RS-165	Mawklot-2	4-5	K. Giriraj	Tuber	2T
RS-166		4-5	K. Giriraj	Tuber	2T
RS-167	Baniun-1	6-10	K. Megha	Tuber	2T
RS-168	Smit-1	0-4	K. Jyoti	Tuber	2T
RS-169	Baniun-2	3-4	K. Jyoti	Tuber	2T
RS-170	Mawan-2	4-5	Local seed	Tuber	2T
RS-171	Mawklot-3	4-5	Local seed	Tuber	2T
RS-172	Laitkor peak-2	6-7	Local seed	Tuber	2T
RS-173	Smit-2	0-2	K. Jyoti	Tuber	2T

In the North Eastern hill region, especially in Meghalaya, rainfall is very high (~2850 mm) and high soil moisture supports weed growth throughout the year which indirectly provide the food base for survival of the pathogen throughout the year. Besides, temperature is mild in this region. Minimum temperature ranged from 1.2-17.2 ^{0}C and maximum temperature varied 17.3-25.5 ^{0}C which is suitable for survival of *R. solanacearum* in soil. It is highly sensitive to high temperature and cannot withstand exposure to the high soil temperature about 43 ^{0}C (Seneviratne, 1987). As mild temperature prevailed most part of the year, the pathogen survived in the soil and may be one of the reasons for higher incidence in the region in addition to infected seed tubers which is the more efficient means of survival of pathogen (Shekhawat *et al.*, 1982).

Table 3: Incidence and biovar distribution of *Ralstonia solanacearum* isolates in autumn season in East Khasi Hills of Meghalaya

Isolates	Location	Incidence (%)	Varieties	Sources	Biovar
RS-174		0-10	Local seed	Tuber	2T
RS-175	Mylliem-1	0-10	K. Jyoti	Tuber	2T
RS-176	Mawkriah west	3-4	Local seed	Tuber	2T
RS-177	Laitkor peak-1	3-4	Local seed	Tuber	2
RS-178	Baniun-1	6-10	K. Jyoti	Tuber	
RS-179	Mawjrong-1	5-6	Local seed	Tuber	
RS-180	Laitlyngkot	5-7	Local seed	Tuber	
RS-181	Nongpiur	0-6	K Jyoti	Tuber	
RS-182	Nongumlong	0-5	Local seed	Tuber	2T
RS-183		2-5	K Megha	Tuber	2T
RS-184	Thangskning	3-4	Local seed	Tuber	2T
RS-185		3-4	Local seed	Tuber	2T
RS-186	Laitkor-1	6-7	Local seed	Tuber	2T
RS-187		2-3	Local seed	Tuber	2T
RS-188	5th mile-1	6-7	K. Megha	Tuber	2T
RS-189		3-4	K. Jyoti	Tuber	2T
RS-190	Mawan	4-5	Local seed	Tuber	2
RS-191	5th mile-2	0-2	K Jyoti	Tuber	2T
RS-192	Baniun-2	5-7	Local seed	Tuber	2T
RS-193		6-10	Local seed	Tuber	2T
RS-194	Lynkien	6-10	K Jyoti	Tuber	2T
RS-195	5th mile-3	0-1	K Himsona	Tuber	2
RS-196	Mawkriah	3-4	K Jyoti	Tuber	2
RS-197	Mawjrong-2	4-5	K Jyoti	Tuber	2
RS-198	Pomlum	0-3	K Jyoti	Tuber	2T
RS-199		0-3	K Jyoti	Tuber	2T
RS-200	Thangskning	0-4	Local seed	Tuber	2
RS-201	Mawjrong	3-4	Local seed	Tuber	2
RS-202		5-7	K Jyoti	Tuber	2
RS-203	Laitkor-2	5-10	K Jyoti	Tuber	2
RS-204		0-10	K Jyoti	Tuber	3
RS-205	Mawklot	0-10	K Jyoti	Tuber	3
RS-206	Mylliem-2	0-10	K Jyoti	Tuber	3
RS-207	Laitkor peak-2	2-6	Local seed	Tuber	2

Isolation and identification of R. solanacearum

In the present investigation, bacterial wilt/ brown rot infected potato stem/ tubers were collected/ obtained from wilt affected areas of East Khasi Hills of Meghalaya. A total of 67 (Table 2 & Table 3) pure strains of *Ralstonia solanacearum* were isolated from infected potato stem/ tuber samples and identified through colony characteristics for distinguishing virulent colonies. Virulent colonies appeared irregular in shape and pinkish centre while avirulent colonies appeared round in shape and red in colour. All isolates of *Ralstonia solanacearum* were tested pathogenic to potato causing bacterial wilt/brown rot symptoms.

Biovar determination

The sixty seven strains were characterized into biovars on the basis of their ability to utilize disaccharides and to oxidize hexose alcohols (Table 1). The result revealed that biovar 2, 2T and 3 were present in East Khasi Hills in Meghalaya (Table 2 & 3). Among sixty seven isolates, forty four (65.6 %) belonged to biovar 2T (i.e. race 3), twenty (29.9 %) to biovar 2 and three (4.5 %) to biovar 3 (race 1) of the pathogen. Earlier studies indicated that Shekhawat *et al.* (1978) recorded that isolates of *P. solanacearum* from different host collected from northeastern region belonged to race 1 and biovar 3 and all isolates were pathogenic to potato, chilli, tomato and brinjal. Race 3 and biovar 2 were obtained only from a few places in the central plains and Deccan plateau (Shekhawat *et al.* 1982). The prevalence of race 1 and biotype 3 infecting potato, tomato, aurbergine (brinjal), chilli, jute and banana from West Bengal reported by Bhattacharya *et al.* (2003). In our present investigation represented that the biovar 2T was most prevalent followed by biovar 2 and biovar 3 in East Khasi hills of Meghalaya (North eastern hill region of India).

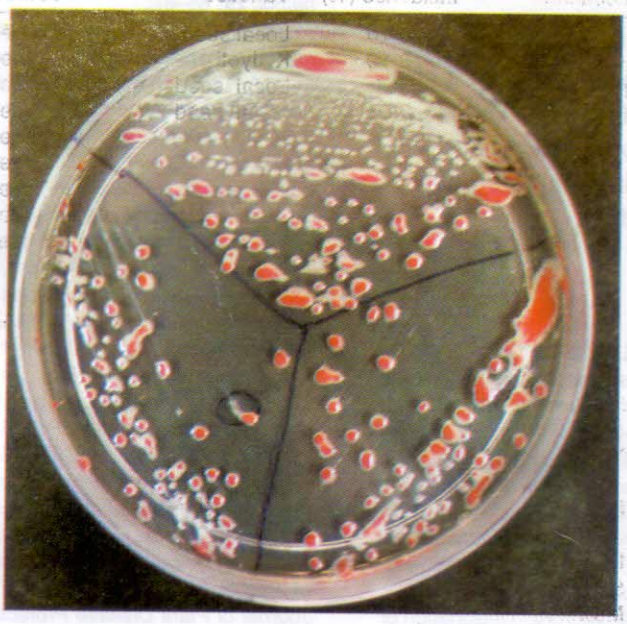
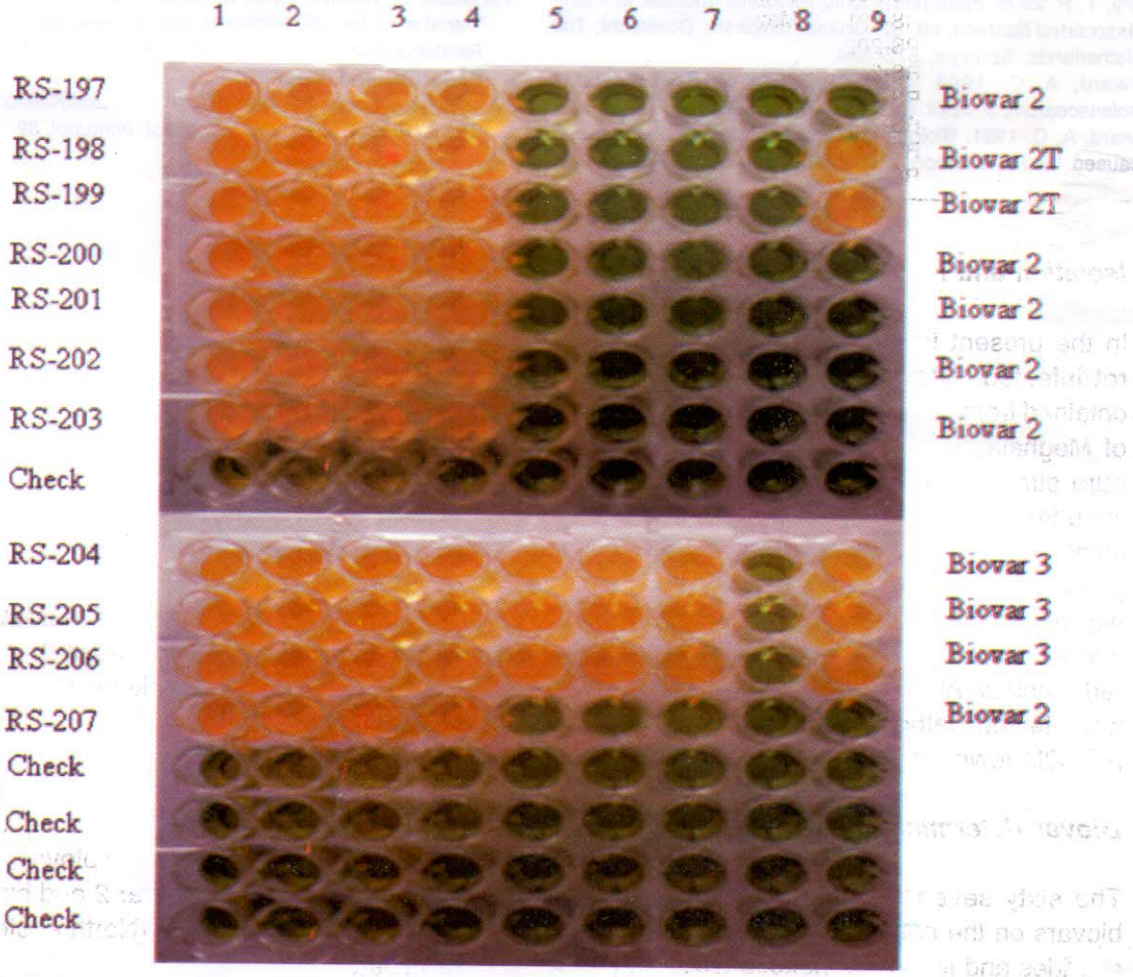


Fig. 1 : Morphological characteristics of *Ralstonia solanacearum* (virulent colony- irregular in shape with pinkish centre)



1= Glucose; 2=Maltose; 3=Lactose; 4=Cellobiose; 5=Manitol; 6=Sorbitol; 7=Dulcitol; 8=Check; 9=Trehlose

Fig. 2 : Micro titre plate showing biovars of *R. solanacearum* isolates

ACKNOWLEDGEMENT

The authors are grateful to the Director, Central Potato research Institute, Shimla (H.P.), The Head, Central Potato Research Station, Shillong and The Head, CPRI, Shimla for providing facilities and encouragement during investigation.

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