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INTRACELLULAR PROTEIN AND ISOENZYME VARIABILITY OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*

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ABSTRACT

In the current study, usefulness of SDS-PAGE and isoenzyme variability including four enzyme systems were analysed to assess the variability of *Xanthomonas campestris* pv. *campestris* (*Xcc*) and to differentiate this pathovar from four other xanthomonads. Eighteen reproducible bands were produced by SDS-PAGE of intracellular proteins of xanthomonads with 15 electrophoretic phenotypic groups and from the dendogram, *Xcc* were differentiated from other xanthomonads. Electrophoretic phenotypic grouping generated 44 groups for α- and β-esterase, catalase and superoxide dismutase (11 for each enzyme) and β-esterase and catalase were efficient to differentiate various xanthomonads. Analysis based on the banding pattern of four isozymes considered together was also efficient for detection of different xanthomonads. Common and specific loci for various xanthomonads were identified in the SDS-PAGE and isozyme gel which could be used as specific biochemical markers for the detection and identification of the respective pathogens. However, variability based on four isoenzymes together did not correspond with the grouping of *Xcc* isolates based on virulence and geographical location. A number of common and unique loci for SDS-PAGE and isoenzymes were identified which could be used as specific biochemical markers to identify these xanthomonads. SDS-PAGE and isozymes (β-esterase, and catalase) were efficient for differentiating various xanthomonads.

KEY WORDS
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INTRODUCTION

The genus *Xanthomonas* is a diverse and economically important group of bacterial pathogens, belonging to the gamma-subdivision of the Proteobacteria. Black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (*Xcc*) was found to be very extensively occurring, as reported time to time, from different parts of the globe. The disease was reported from West Bengal (Chattopadhyay and Mukherji, 1955), Katrain in Himachal Pradesh (Rao and Srivastava, 1964) and outbreaks were reported in 1969 (Chakravarti *et al*., 1969) and 1973 (Rangaswami and Rajagopalan, 1973) from Udaipur, Rajasthan. Similarly, bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*), bacterial blight of pomegranate caused by *Xanthomonas campestris* pv. *punicae* (*Xcp*) and bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*) are all serious diseases causing severe yield losses all over the world.

Various techniques employed for studying the variability in plant pathogenic xanthomonads such as serology, SDS-PAGE of intracellular proteins, isoenzyme variability, DNA-DNA hybridization, characterization of 16S and 16S-23S intergenic spacer region of ribosomal rDNA, Rep-PCR have generated variable results. Isozyme analysis is a simple, efficient, and inexpensive technique for evaluating the taxonomy, genetics, virulence, and epidemiology of plant pathogens and has become a standard technique for the study of plant pathogens (Micales and Bonde, 1995).

It was reported that, SDS-PAGE profiles of membrane proteins of *Xcc* were distinct from all other bacteria tested including 13 other xanthomonads (Thaveechai and Schaad, 1986). Studies on the intracellular protein and isoenzyme profile for plant pathogenic bacteria are widespread and reliable. Protein and isoenzyme studies are among the quickest and cheapest marker systems to develop and remain an excellent choice for projects that only need to identify low levels of genetic variation (Onasanya *et al*., 2008). Different studies have shown that the enzyme systems in use differ in their value and potential in bacterial diversity analysis, characterization and classification (Quesada *et al*., 2002).

At present little information is available regarding the population structure and variability of *Xcc* in Indian subcontinent (Singh *et al*., 2011). Moreover, the sources of resistance against this economically important pathogen are very limited (Sharma *et al*., 2004). Studying variability within and among different species and pathovars of *Xanthomonas* is prerequisite for developing successful management approach for this group of devastating pathogens. Protein profiling and isozyme analysis are the important tools for variability analysis of xanthomonads with special reference to *Xcc* is the hypothesis for testing in the present study and based on the current knowledge and understanding of the

status of black rot disease, the present study was framed with the objective to elucidate to SDS-PAGE and isozyme profiling for determination of biochemical variability in *Xcc* population collected from Indo-Gangetic regions of West Bengal, and comparing the isoenzyme variability with other xanthomonads infecting non-cruciferous hosts.

MATERIALS AND METHODS

Isolation and maintenance of the pathogen

Small infected leaf tissue pieces were surface sterilized (70 % alcohol for 30 sec), washed thrice in sterile distilled water, teased and a loop-full of this suspension was streaked on potato-sucrose-peptone-agar (PSPA). The bacterial colonies of lemon yellow colour developed after 3-4 days of incubation at 28 ± 1ºC on the streaking line were purified.

Studies on pathogenic variability of *Xcc* isolates

Actively growing 48 h old culture of *Xcc* on yeast-glucose-chalk media were precipitated and resuspended in sterile distilled water to an optical density of 10^7 cfu/ml. Three youngest leaves of thirty days old susceptible cabbage (Green-621) were inoculated by 1 cm incision into the lamina symmetrically to each side of the apex using a sterile scissor dipped in bacterial suspension. Control plants were inoculated with sterile distilled water in the same way. Severity of symptoms was assessed on a six- point scale of 0–9 based on the relative lesion size (Singh *et al*., 2011). For other xanthomonads virulence of the isolates was checked using injection infiltration and leaf tip-cut inoculation (rice).

Bacterial protein extraction

One loop-full of bacterial culture were transferred to 150 ml of Yeast-Glucose-Chalk broth media and incubated for 48 h at 28ºC with shaking at 150 rpm. For SDS-PAGE, whole cell protein was extracted according to Vauterin *et al*. (1991). Native protein was extracted as Kubicek *et al*. (1989) for isoenzyme studies. Protein estimation was performed according to Lowry *et al*. (1951).

Polyacrylamide gel electrophoresis for isozyme profiling

Polyacrylamide gel electrophoresis (PAGE) was performed according to Laemmli (1970). SDS-PAGE, esterase and SOD was run in 10 % PAGE whereas catalase was performed in 8% gel. For each samples, 10 μg protein extract with 1 μl bromophenol blue dye (and total 20 μl) was loaded on the gel. The gel was run for 3-4 hours until the dye front reached the bottom of the gel.

Staining and destaining of SDS-PAGE

Staining and destaining of SDS-PAGE was performed using the protocol of Sadasivam and Manikam (1996). The gel was stored in 5% acetic acid solution at 4°C until photograph was taken in a white background.

Staining of isoenzyme gel

The activity staining for α- and β-esterase were performed according to Mostafa *et al*. (2003). SOD gel was stained according to Madamanchi *et al*. (1994). Catalase gel was incubated in 5 mM H_2O_2 for 20 min in darkness, briefly washed with sterile distilled water and treated with solution containing 2 % ferric chloride and 2 % potassium ferricyanide (Zahrt *et al*., 2001).

Preparation of Zymogram

The relative mobility (Rm, distance travelled by the enzyme / distance travelled by dye front) of each band was calculated and schematic zymograms were prepared.

RESULTS AND DISCUSSION

Isozyme analysis is a simple, efficient, and inexpensive technique for evaluating the taxonomy, genetics, virulence, variability and epidemiology of plant pathogens. Isozyme analysis, together with SDS-PAGE as standard techniques, has been extensively utilized for analysing variability in plant pathogenic organisms. Usefulness of SDS-PAGE of intracellular and outer membrane proteins in variability studies were demonstrated in several instances (Thaveechai and Schaad, 1986; Ojanen *et al*., 1993; Goncalves and Yoko, 2000; Sahin *et al*., 2003). Contradictions regarding the usefulness of SDS-PAGE are also not uncommon in the literature (Vauterin *et al*., 1990; 1991). Therefore, determination of biochemical variability of *Xcc* based on SDS-PAGE and isoenzyme polymorphism and comparison with xanthomonads isolated from other hosts was the focus of the present study.

Based on the virulence studies on cabbage seedlings, *Xcc* isolates were divided into three clusters (Table 1; Fig. 1). Isolate 1.3W was the most virulent and lying separate from all other isolates. Low virulent isolates such as ND, 1, 2, 6 and 3.1 (Sub-group A_2) were also distinct from all other moderate to high virulent isolates.

Eighteen reproducible bands of Rf values ranging from 0.26 to 0.70 were produced by SDS-PAGE of intracellular proteins

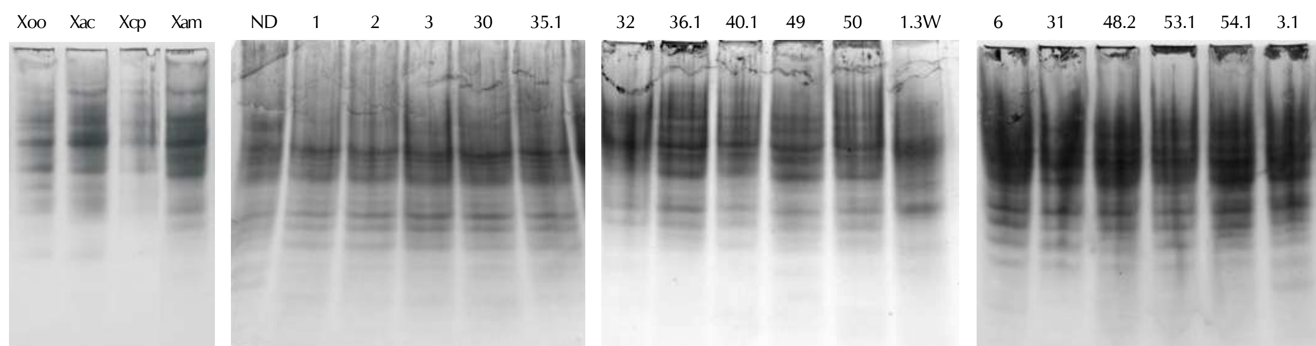

Figure 2: SDS-PAGE protein profiling of different *Xcc* isolates and other *Xanthomonas* sp.

Table 1: Virulence profile of different *Xcc* isolates on cabbage

Sl. No.	Isolates	Place of collection	Host of isolation	Mean virulence on cabbage
Xcc isolates				
1	ND	New Delhi	Cabbage	14.3[c]
2	1	C-block farm, Nadia	Cauliflower	19.7[c]
3	2	Uttar Chandmari, Nadia	Cauliflower	22.3[c]
4	3	Bara Jaguli, Nadia	Cabbage	41.8[b]
5	6	Kankinara, U-24-PGS	Cabbage	18.1[c]
6	30	Neelganj, U-24-PGS	Cauliflower	44.7[b]
7	31	Kantabele, Nadia	Cabbage	54.3[b]
8	32	Shankarpore, U-24-PGS	Cauliflower	41.7[b]
9	35.1	Ghoragacha, Kalyani, Nadia	Cauliflower	50.3[b]
10	36.1	Dasdiya, Nadia	Cabbage	44.2[b]
11	40.1	Ghoja, U-24-PGS	Cauliflower	41.3[b]
12	48.2	Haripara, Nadia	Cabbage	50.2[b]
13	49	Haripara, Nadia	Cauliflower	53.3[b]
14	50	Aiesmali, Ranaghat, Nadia	Cabbage	44.1[b]
15	53.1	Jalpaiguri	Cauliflower	42.0[b]
16	54.1	Bamonpara, Nadia	Cabbage	54.3[b]
17	1.3w	Khaldarpara, Madanpur, Nadia	Cauliflower	83.3[a]
18	3.1	Baganepara, Nadia	Cauliflower	15.3[c]
SEM (±)				5.220
CD				15.002*
Other xanthomonad isolates				
1	*Xac*	Citrus	Mohanpur, Nadia	-
2	*Xoo*-WB	Rice	Mohanpur, Nadia	-
3	*Xam*-ND	Cotton	Collected from IARI, New Delhi	-
4	*Xcp*-ND	Pomegranate	Collected from IARI, New Delhi	-

#: Transformed values; *: Significance at 5 % level; Pathogenicity not tested

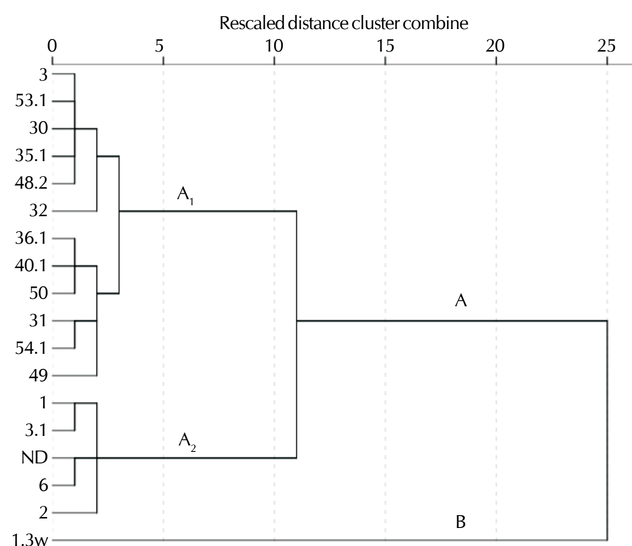

Figure 1: Grouping of *Xanthomonas campestris* pv. *campestris* isolates based on virulence on cabbage

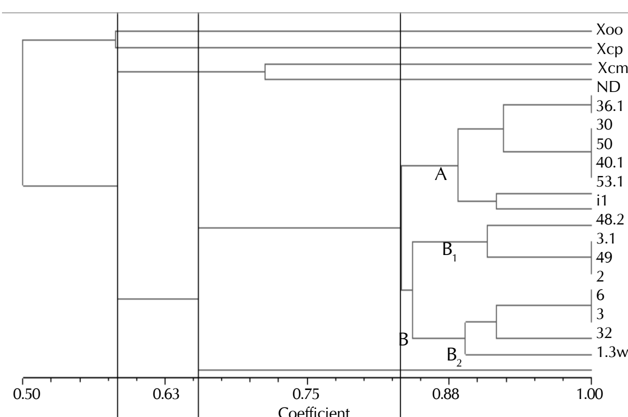

Figure 3: SDS-PAGE protein profiling of different *Xcc* isolates and other *Xanthomonas* sp.

of xanthomonads (Fig. 2; Table 2). Dendogram based on Jaccard's similarity coefficient of SDS-PAGE binary profile of *Xcc* and other xanthomonads shows that there is 50 % similarity present among all the xanthomonad isolates analysed (Fig. 3). However, only *Xcc* isolates exhibit approximately 66 % similarity among them and were separated from other xanthomonads such as *Xoo, Xac, Xcp* and *Xam*. *Xcc* isolates were again classified into three groups based on hierarchical cluster analysis *viz.*, A, B and C. Group C comprises of only one isolate (1.3W) except which other *Xcc* isolates show 84 % of similarity among themselves. Thus, SDS-PAGE could only distinguish one isolate of *Xcc*, namely, 1.3W which was found to be the most virulent isolate on cabbage (Fig. 1 and Fig. 3) and *Xcc* with different level of virulence were grouped in cluster A and B. There was no relationship in SDS-PAGE patterns of *Xcc* isolates with their virulence patterns.

Several protein bands were found to be specific for *Xcc* or other *Xanthomonas* sp. based on SDS-PAGE profile (boxed in Table 2). Thus, bands with Rf values of 0.26 and 0.38 were specific for *Xcc* and 0.57 was for *Xac*. However, bands with Rf values of 0.34, 0.37, 0.48 and 0.56 were all expressed by all the xanthomonads.

Table 2: Electrophoretic phenotypic grouping (EPG) of the isolates of *Xcc* and other xanthomonads based on SDS-PAGE profiles

	S. No.	Rf	*Xoo*	*Xac*	*Xcp*	*Xam*	ND	1	2	3	6	30	31	32	35.1	36.1	40.2	48.1	49	50	53.1	54.1	1.3W	3.1	Total EPG
SDS-PAGE	1	0.26	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	2	0.29	—		—	—			—	—	—	—	—	—	—					—	—	—	—		
	3	0.33		—		—	—					—			—	—	—			—	—	—			
	4	0.34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	5	0.37	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	
	6	0.38		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	7	0.40																					—		
	8	0.42	—		—		|																		
	9	0.43		—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	10	0.48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15
	11	0.51			—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	
	12	0.52	—	—		—																			
	13	0.56	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	14	0.57		—																					
	15	0.59	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	
	16	0.63					—			—		—			—	—		—	—	—		—		—	
	17	0.66		—			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	18	0.70	—	—		—																			
EPG			1	2	3	4	5	6	7	8	7	9	7	10	9	11	12	13	13	9	14	9	15	13	

Four isoenzymes namely: α-esterase, β-esterase, catalase and superoxide dismutase of *Xcc* were analysed for determination of variability among *Xcc* isolates (Fig. 4). A total of nine isomers of α-esterase were produced by different isolates of *Xcc* and different xanthomonads ranging from Rf values of 0.24 to 0.68 (Table 3, Fig. 4). The dendogram (Fig. 5) generated from the α-esterase binary matrix indicated that there was entirely 100 % divergence present among the xanthomonads studied. This may be due to the presence of entirely unique isoenzymes produced by *Xac* and *Xcp* and almost entirely different bands produced by *Xoo*. However, *Xam* produced isomers common to *Xcc* and thus this isolate could not be differentiated by this isoenzyme pattern from *Xcc*. *Xcc* isolates (including *Xam*) showed a similarity of approximately 47 % among themselves. The isolate *Xoo* produced two almost unique bands (Rf values of 0.24 and 0.3), *Xac* produced one (0.52) and *Xcp* produced one (0.5) unique band (boxed in the Table 3). The isomer of Rf value 0.44 is unique for *Xcc* except one isolate (isolate 32).

Beta-esterase produced ten polymorphic isomers based on which the various xanthomonads could clearly be distinguished (Fig. 4; Table 3). The dendogram generated from the binary matrix of β-esterase (Fig. 5) indicated the existence of high level of overall variability (similarity of only 17 %) among xanthomonads whereas only *Xcc* shared high level of similarity (54.5 %) among themselves. *Xcc* and *Xcp* produced two unique bands of Rf values 0.39 and 0.49, respectively, which could be used as specific biochemical markers for these pathovars (boxed in the Table 3).

Eight polymorphic isomers of catalase were produced by different isolates of *Xcc* and different xanthomonads analysed ranging from Rf values of 0.08 to 0.49 (Fig. 4; Table 3). The dendogram generated from the binary matrix of catalase isoenzyme (Fig. 5) shows that all the xanthomonads could be clearly distinguished based on this isoenzyme banding pattern. Overall similarity of 33 % existed among all the xanthomonads and only *Xcc* isolates shared 63 % similarity among them. Catalase also produced an isomer of *Xac* (Rf value of 0.22) unique from other xanthomonads which could be used as specific biochemical marker for *Xac* (boxed in the Table 3).

Although SOD isoenzyme showed overall variation of 28-100 % among all the xanthomonads and distinguished *Xcc* from *Xoo* and *Xcp*, it could not differentiate *Xac* and *Xam* from *Xcc* (Fig. 4 and 5). SOD produced isomers of Rf values 0.29 and 0.51 common to all the xanthomonads studied and isomer of Rf value 0.62 unique to *Xcp* which could be used as specific biochemical marker for *Xac* (boxed in the Table 3).

The dendogram generated from combined analysis of banding pattern of the four isozymes (α-esterase, β-esterase, catalase and SOD) (Fig. 6) indicated overall similarity of only 17 % among all the xanthomonads and 58 % among only *Xcc*. The average similarity of *Xcc* isolates with *Xoo*, *Xac*, *Xcp* and *Xam* were 17, 43, 28 and 49 %, respectively. Moreover, *Xoo*, *Xac*,

Table 3: Electrophoretic phenotypic grouping (EPG) of different *Xcc* isolates and other xanthomonads based on isoenzyme profiles

	S. No.	Rf	*Xoo*	*Xac*	*Xcp*	*Xam*	ND	1	2	3	6	30	31	32	35.1	36.1	40.2	48.1	49	50	53.1	54.1	1.3W	3.1	Total EPG
α-esterase	1	0.24	—																					—	11
	2	0.30	—											—											
	3	0.38				—	—	—		—	—	—				—	—	—	—	—	—	—		—	
	4	0.41				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	5	0.44					—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	
	6	0.50			—																				
	7	0.52		—																					
	8	0.65						—	—	—	—	—		—		—	—	—		—	—	—			
	9	0.68				—	—						—	—					—					—	
EPG			1	2	3	4	5	6	7	6	6	6	8	9	1	6	6	6	5	6	6	6	10	11	
β-esterase	1	0.07		—			—							—											11
	2	0.12	—	—				—	—	—		—				—	—	—		—	—	—			
	3	0.22	—	—				—	—	—	—	—	—	—		—	—	—		—	—				
	4	0.27											—												
	5	0.39					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	6	0.40		—			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	7	0.43		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	8	0.45		—	—																				
	9	0.49			—																				
	10	0.54			—	—	—							—											
EPG			1	2	3	4	5	6	6	6	7	6	8	9	10	6	6	6	10	6	6	11	10	10	
Catalase	1	0.88	—	—	—		—	—	—	—	—	—	—	—			—	—		—	—	—	—	—	11
	2	0.12				—	—			—				—	—	—	—	—	—	—		—			
	3	0.22		—																					
	4	0.25	—		—	—																			
	5	0.38	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	6	0.42		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	7	0.46		—		—	—	—	—	—		—	—					—						—	
	8	0.49				—	—		—	—	—	—			—	—	—	—	—	—	—	—	—	—	
EPG			1	2	3	4	5	6	7	5	8	7	6	9	10	10	11	5	10	11	8	11	8	7	
SOD	1	0.12		—	—		—	—			—		—					—		—	—	—	—	—	11
	2	0.15	—			—																			
	3	0.22		—		—	—	—	—	—		—	—		—			—			—	—		—	
	4	0.29	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	
	5	0.32	—																						
	6	0.34				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	7	0.37	—		—																				
	8	0.40		—		—	—	—	—	—		—		—	—	—	—	—	—	—	—	—		—	
	9	0.43	—																						
	10	0.51	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	11	0.62			—																				
EPG			1	2	3	4	5	5	6	6	7	6	8	9	6	9	9	5	9	10	5	5	11	5	

Xcp and *Xam* isolates shared high level of variability among themselves, i.e., 21, 27, 30 and 26 % similarity, respectively.

Xcc isolates could be classified into four groups based on the banding pattern of these four enzymes (Fig. 6). Group A contain

a single isolate 32 indicating wide level of divergence of this isolate with other *Xcc*. Group B and group C contain two isolates each. Group D contains all other *Xcc* isolates and may further be sub-grouped in D_1 containing four isolates and D_2 containing 9 isolates.

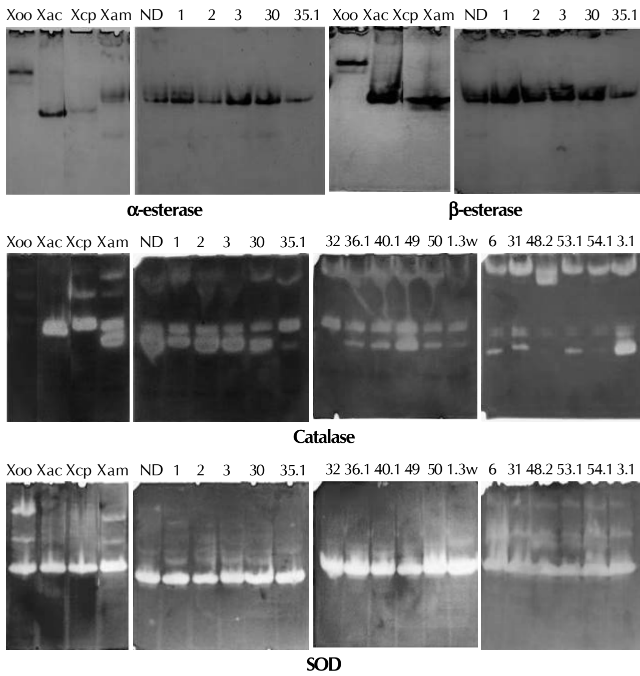

Figure 4: Grouping of *Xcc* isolates based on isoenzyme patterns (α-esterase, β-esterase, catalase and superoxide dismutase

The results indicated that individually various isoenzyme variability pattern of *Xcc* were not being able to distinguish various pathogenic groups of *Xcc*. However, biochemical variability of *Xcc* isolates based on all isoenzymes considered together roughly explained the virulence pattern of *Xcc* on cabbage. With the exception of isolate 6, 32 and 2, isoenzyme grouping could classify other 15 isolates of *Xcc* according to their virulence.

Electrophoretic phenotype grouping was generated for SDS-PAGE (Table 2) and for the four isoenzymes considered together (Table 3). SDS-PAGE profile generated 15 phenotype groups and a total 44 phenotypic groups were identified for the four enzymes analysed. Each enzyme produced 11 phenotypic groups and *Xcc* isolates could be best differentiated from other xanthomonads based on the banding pattern of β-esterase and catalase.

SDS-PAGE banding pattern has no correlation with the virulence pattern of the isolates. However, correlation study of pathogenic variability with the banding pattern of different isoenzymes has identified a catalase isomer of 0.46 Rf value negatively correlated with the virulence pattern with 5 % level of significance indicating absence of this isomer from the virulent isolates of *Xcc*. The inability of these four isoenzymes to distribute *Xcc* isolates clearly to virulence groups may be

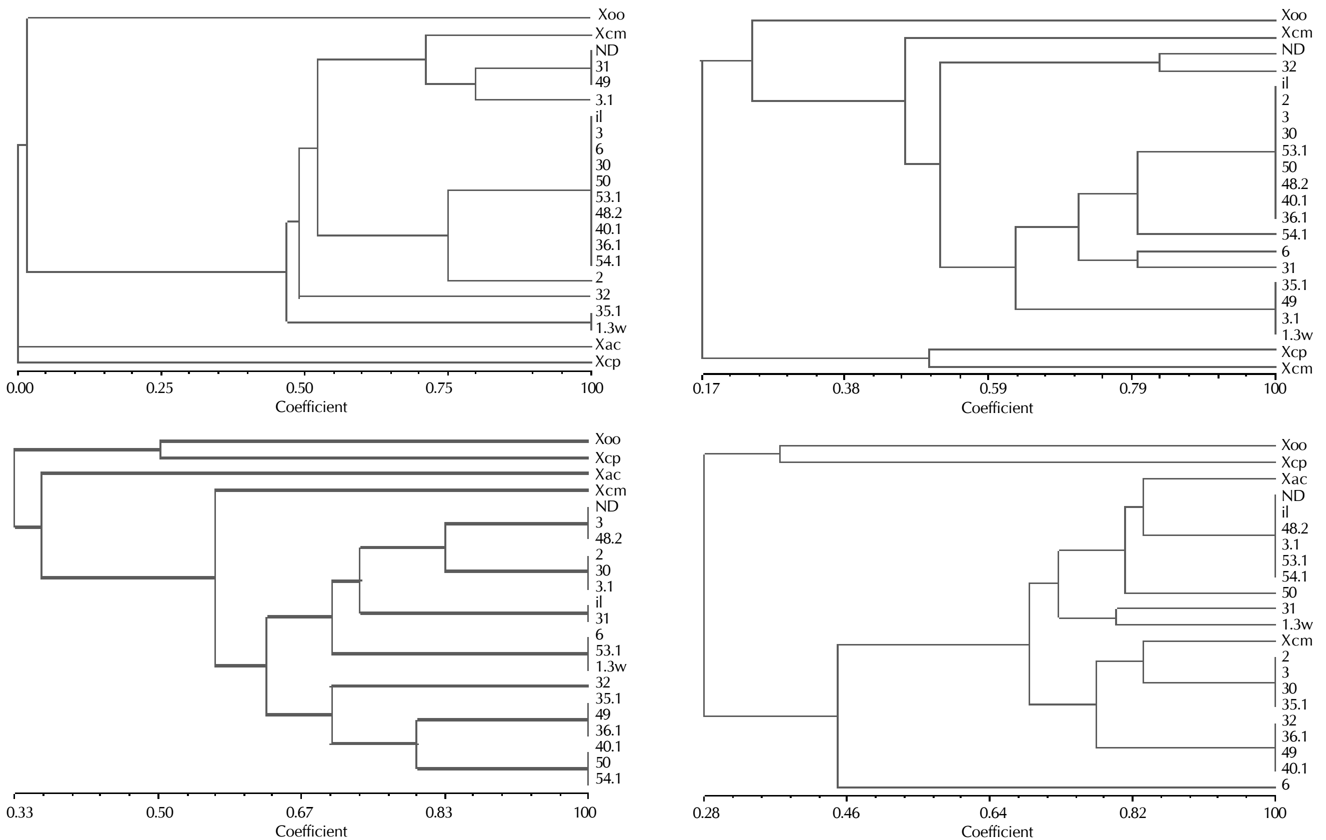

Figure 5: Grouping of *Xcc* isolates and other xanthomonads based on isoenzyme patterns (α-esterase, β-esterase, catalase and superoxide dismutase)

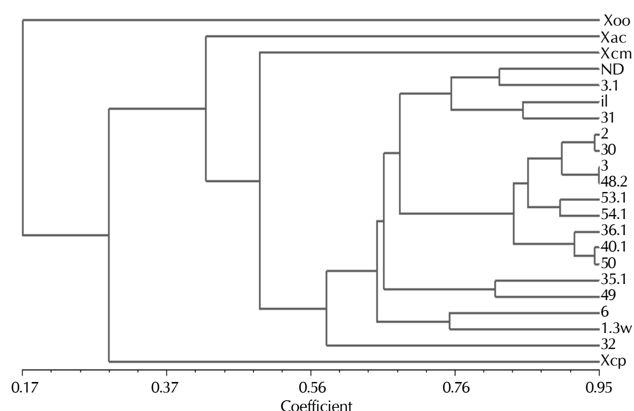

Figure 6: Grouping of different *Xcc* isolates and other xanthomonads based on α-esterase, β-esterase, catalase and SOD isoenzymes together

because these enzymes might not be involved in any pathway related to pathogenicity in this bacterial pathogen (i.e., virulence may be relatively easily transferable added advantage in same or similar genetic background of the pathogen). Therefore, although these enzymes may distinguish different xanthomonads from diverse sources and host origin, a number of isoenzyme banding patterns should be pooled together considering large number of isolates for getting relationship with the pathogenic variability of *Xcc*. Moreover, the unique loci obtained in this study for various xanthomonads may be useful as biochemical markers for the detection of xanthomonads and for the selection of virulent isolates of *Xcc*.

Wide range of variability both in SDS-PAGE pattern and isoenzyme profile were demonstrated as in the case of other studies. Although SDS-PAGE grouping could differentiate five different xanthomonads, there was no relationship in SDS-PAGE patterns of *Xcc* isolates with their virulence patterns. Therefore, SDS-PAGE pattern could not explain pathogenic variability of *Xcc*. However, separation of *Xoo, Xac, Xcp and Xam* from rest of the *Xcc* isolates by SDS-PAGE banding pattern supported the use of protein profiling technique to distinguish the different species of *Xanthomonas*. The extent of polymorphism in isolates of *Xcc* using only four isozymes demonstrates the usefulness of this technique in investigating its genetic diversity analysis. However, protein and isoenzyme analysis of West Bengal isolates of *Xcc* has indicated that the diversity among the isolates did not correlate with their geographical origin or host of origin. In similar kind of works, Mohammadi *et al*. (2001) were able to differentiate Asiatic (A) and atypical Asiatic (aA) forms of *Xac* on the basis of superoxide dismutase (SOD) and esterase (EST) banding patterns. No isozymes were found by Kubicek *et al*. (1989) in the citrus canker groups of strains that distinguished any of the forms of citrus canker. Three enzyme systems of *Xanthomonas axonopodis* pv. *mangiferaeindicae* namely esterase (EST), phosphoglucomutase (PGM) and superoxide dismutase (SOD) were analysed by Some and Samson (1996) and four groups of strains were identified.

Analysis of isozyme banding pattern has also been utilized in case of other plant pathogens. α- and β-esterase has been utilized for differentiating *Alternaria* species in ornamental plants (Ambesh *et al.*, 2014) and in potato, tomato, chilli and brinjal isolates (Marak *et al.*, 2014) and the isolates were differentiated according to the host of origin. In another experiment by Pawar and Ingle (2014), maximum PPO and esterase activities was observed in highly and moderately pathogenic isolates of *R. bataticola*, respectively, and variation among isolates was identified using SDS-PAGE, esterase, PPO and peroxidase isozymes. Thus, usefulness of isozyme variability is a valid tool to differentiate various species and pathovars of plant pathogens including xanthomonads in which β-esterase and catalase individually and four isozymes including α-esterase and SOD were able to differentiate five species and/or pathovars.

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