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*Short communication*

# Phenotypic characteristics of *Xanthomonas campestris* pv. *campestris* from Nepal

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**Abstract**

Twenty strains of *Xanthomonas campestris* pv. *campestris* (Xcc) were isolated from two major crucifer-growing valleys, Chitwan and Kathmandu in Nepal and characterized by biochemical and pathogenicity tests. Strains were homogeneous in bacteriological characteristics. The ability of a strain to induce high or low disease severity index (DSI) on three host plants, broccoli, cabbage, and cauliflower, was interpreted as virulence. Strains that were associated with high or low virulence were significantly different ($P > 0.05$). No relationship between virulence and biochemical characteristics was observed.

Black rot, caused by *Xanthomonas campestris* pv. *campestris* is a globally important disease of crucifers (Williams, 1980). The disease can cause significant losses, particularly in warm and humid environments (Schaad, 1976; Williams, 1980). Traditionally, characterization of *X. c.* pv. *campestris* has largely depended on biochemical and physiological tests, pathogenicity, and phage typing. Recently, monoclonal antibodies, and molecular markers have been used to characterize the population biology of the pathogen (Alvarez et al., 1994).

Nepal has a wide range of climates from temperate to sub-tropical. Chitwan and Kathmandu valleys, both are located in the central region of Nepal where most vegetable growers produce crucifer crops because of good economic returns. Although black rot of crucifers in Nepal has been reported as early as 1977 (Shrestha, 1977), characterization of *X. c.* pv. *campestris* strains has not been fully studied. In this article, we report the phenotypic characteristics of *X. c.* pv. *campestris* from Nepal.

During the 1994 and 1995 seasons, samples of plants showing black rot symptoms were collected from commercial fields and home gardens in Chitwan and Kathmandu valleys. Ten fields (4 broccoli, 3 cabbage, and 3 cauliflower), in Chitwan and (1 broccoli, 2 cabbage, and 7 cauliflower) in Kathmandu were sampled. Bacteria were isolated on YDC (1% yeast extract, 2% dextrose, 2% fine-powdered calcium carbonate, and 1.7% agar) medium. Round, convex, and mucoid single colonies were selected and purified by re-streaking on YDC medium. The 20 strains of *X. c.* pv. *campestris* were characterized by the methods of Dye (1962). All strains were positive for levan production, Tween 80 hydrolysis, gelatin liquefaction, alkaline reaction in litmus milk, acetoin production, $H_2S$ production, oxidative metabolism of glucose and grew on 1% tetrazolium salt agar medium. All strains produced acid from dulcitol, DL-arabinose, D(+)galactose, D(+)xylose, maltose, arginine, sorbitol, sucrose and glucose but strains varied in inositol, mannitol, trehalose, lactose, cellobiose, and ribose. All

strains were negative for fluorescent pigment production on agar medium, and tolerated 1% but not 3% or 5% NaCl. Most strains were sensitive to nalidixic acid (30 μg/ml) and tetracycline (30 μg/ml), and resistant to gentamicin (10 μg/ml), erythromycin (15 μg/ml), bacitracin (10 μg/ml), ampicillin (10 μg/ml), cloxacillin (30 μg/ml), cephalexin (30 μg/ml), chloramphenicol (30 μg/ml) and nitrofurantoin (30 μg/ml). The results suggest that the biochemical and physiological characteristics of *X. c.* pv. *campestris* strains from Nepal were highly similar. Our findings agree with previous descriptions (Alvarez et al., 1994; Schaad and Alvarez, 1993; Swings et al., 1993). Minor variation in carbon source utilization has been observed among strains of *X. c.* pv. *campestris* in this study. Thus, more strains of *X. c.* pv. *campestris* should be collected from additional locations and hosts in Nepal in order to determine genotypic and phenotypic diversity in *X. c.* pv. *campestris*.

The 20 strains of *X. c.* pv. *campestris* were evaluated for their virulence on three crucifers: cabbage (*Brassica oleracea* L. var. *capitata* L., cv. Copenhagen Market), broccoli (*Brassica oleracea* L. var. *italica* L., cv. Black Brussels), and cauliflower (*Brassica oleracea* L. var. *botrytis* L., cv. Kathmandu local). Seedlings were first raised in nursery beds. Twenty-day-old seedlings were planted in 100-cm plastic buckets containing one plant per bucket. Each plastic bucket was filled with a mixture of farm soil and farmyard manure at a ratio of 1 : 1. N–P–K was applied at the rate of 80–60–40 kg/ha (10-g mixtures of N–P–K per bucket) as the basal dose in the form of ammonium sulfate, super phosphate, and muriate of potash. Plants were irrigated whenever necessary

*Table 1.* Virulence[a] of *X. c.* pv. *campestris* strains after artificial inoculation on broccoli, cabbage and cauliflower in Nepal

| Location | Original host | Strain | Virulence[b] on | | |
|---|---|---|---|---|---|
| | | | Broccoli | Cabbage | Cauliflower |
| Chitwan valley | Broccoli | Xcc4 | 6.9 bcd[c] | 8.7 a | 7.0 bcd |
| | Broccoli | Xcc36 | 6.53 cd | 7.1 c | 7.0 bcd |
| | Broccoli | Xcc81 | 8.9 a | 7.9 bc | 7.8 a |
| | Broccoli | Xcc86 | 6.5 d | 5.3 d | 5.7 e |
| | Cabbage | Xcc11 | 7.1 bc | 8.1 ab | 5.2 ef |
| | Cabbage | Xcc37 | 8.6 a | 7.5 bc | 7.1 bcd |
| | Cabbage | Xcc77 | 5.7 e | 7.5 bc | 7.3 abc |
| | Cauliflower | Xcc40 | 8.9 a | 7.8 bc | 7.4 ab |
| | Cauliflower | Xcc79 | 5.9 e | 7.0 c | 7.6 ab |
| | Cauliflower | Xcc94 | 7.0 bcd | 7.4 bc | 7.5 ab |
| Kathmandu valley | Broccoli | Xcc102 | 4.0 g | 3.9 e | 2.4 h |
| | Cabbage | Xcc103 | 4.4 fg | 7.2 c | 4.8 fg |
| | Cabbage | Xcc106 | 7.0 bcd | 5.3 d | 5.4 e |
| | Cauliflower | Xcc100 | 5.4 e | 4.7 d | 7.3 abc |
| | Cauliflower | Xcc101 | 7.3 b | 7.2 c | 6.7 dc |
| | Cauliflower | Xcc104 | 4.8 f | 5.5 d | 2.3 h |
| | Cauliflower | Xcc105 | 2.2 h | 8.9 a | 4.5 g |
| | Cauliflower | Xcc108 | 4.2 g | 5.4 d | 4.7 fg |
| | Cauliflower | Xcc109 | 6.7 bcd | 7.6 bc | 6.6 d |
| | Cauliflower | Xcc110 | 4.5 fg | 5.2 d | 5.8 e |

[a] Virulence, expressed as disease severity index (DSI), was calculated for each strain according to the 0–9 scale of Alvarez et al. (1994), where 0–1 = non-pathogenic; 1.1–3 = weakly virulent; 3.1–6 = moderately virulent; 6.1–8 = virulent; and 8.1–9 = highly virulent.
[b] Virulence of *X. c.* pv. *campestris* strains was evaluated on broccoli, cabbage and cauliflower in the greenhouse at 28 °C. Each bucket consisted of a single plant of each host. Plants were inoculated 45 days after sowing. For each strain–host combination, four leaves from each plant per bucket were rated individually 28 days after inoculation. The experiment was designed as a completely randomized block design (RCBD) with three replications.
[c] In each column, means followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test (DMRT).

and top-dressed with urea at the rate of 50 kg/ha 20 days after planting.

All strains of *X. c.* pv. *campestris* were revived on YDC agar slants. A 3-day-old culture of each strain was used to prepare inoculum. Fully expanded leaves of each plant were inoculated with bacterial suspension grown on YDC medium ($10^8$ cfu/ml) 45 days after sowing. Each plant was inoculated at the base of a newly emerged-leaf with a 10-ml syringe (approximately 1 ml inoculum per plant). The inoculated plants were kept in the greenhouse at 28°C and covered with plastic up to seven days. The experimental unit consisted of one plant per bucket and four leaves from each plant were rated individually 28 days after inoculation. The experiment was designed as a completely randomized block design and each strain–host combination was replicated three times. The strains collected from both Chitwan and Kathmandu valleys were tested in the same experiment and the entire experiment was performed twice. Virulence was calculated for each strain according to the 0–9 disease severity index (DSI) of Alvarez et al. (1994), where 0–1 = non-pathogenic; 1.1–3 = weakly virulent; 3.1–6 = moderately virulent; 6.1–8 = virulent; and 8.1–9 = highly virulent. Disease severity data for each strain–host combination were subjected to one-way analysis of variance (ANOVA). Means were separated by the Duncan multiple range test (DMRT) ($P < 0.05$).

Although strains from both valleys were virulent (DSI ranged 2.2–8.9) on the three host plants used and were significantly different ($P > 0.05$), relative virulence ratings produced by the strains from Chitwan were higher than the strains from Kathmandu (Table 1). In Nepal, many growers produce their own crucifer seeds. The seed sources are generally uniform within the area but differ between Chitwan and Kathmandu and it appears that a clonal population has developed in Chitwan that is more virulent than the population in Kathmandu. The bacterium has also been found to be highly pathogenic on several crucifer plants under different conditions (Alvarez and Cho, 1978; Alvarez et al., 1994; Schaad and Alvarez, 1993). More importantly, the two valleys Chitwan and Kathmandu have similar agro-ecologies to disease-free areas. Thus, there is a risk that disease will spread into new areas from these valleys with seeds. Periodic surveys will have to be undertaken to monitor the spread of the disease within the country.

In conclusion, the 20 strains of *X. c.* pv. *campestris* isolated in Nepal appear phenotypically similar but pathogenicity is variable. The virulent strains described in this paper will be useful for testing and screening germ plasm for resistance to *X. c.* pv. *campestris*. Since the bacterium is highly variable, the use of a multi-faceted programme that consists of pathogen-free seeds, resistant cultivars and crop-rotation may help in controlling black rot of crucifers in Nepal.

## References


Alvarez AM, Benedict AA, Mizumoto CY, Hunter JE and Gabriel DW (1994) Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. Phytopathology 84: 1449–1457

Alvarez AM and Cho JJ (1978) Black rot of cabbage in Hawaii: inoculum source and disease incidence. Phytopathology 68: 1456–1459

Dye D (1962) The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N Z J Agric Sci 5: 393–416

Schaad NW (1976) Control of black rot of cabbage. Georgia Agriculture Experimental Station Res Bull 187: 13

Schaad NW and Alvarez AA (1993) *Xanthomonas campestris* pv. *campestris*: cause of black rot of crucifers. In: J.S. Swings and E.L. Civerolo (eds) *Xanthomonas* (pp 51–55) Chapman and Hall, London

Shrestha K (1997) A list of seed-borne organisms in Nepal. Nepalese J Agric 12: 217–224

Swings J, Vauterin L and Kersters K (1993) The bacterium *Xanthomonas*. In: J. Swings and E.L. Civerolo (eds) *Xanthomonas* (pp 121–156) Chapman and Hall, London

Williams PH (1980) Black rot: A continuing threat to world crucifers. Plant Dis 64: 736–742