Identification of a *Xanthomonas* pathogen of coriander from Oregon

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ABSTRACT

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A coriander seed lot grown in Oregon yielded *Xanthomonas*-like colonies on MD5A agar, at 4.6 x 10^5 CFU/g seed. Colonies were mucoid, convex and yellow on YDC agar. Koch's postulates were completed on coriander. Water-soaked lesions developed on inoculated coriander leaves and turned necrotic in 1-2 weeks, with the growing point killed on some plants. The bacterium also was pathogenic on fennel, lovage and parsnip; but not on dill, celery or parsley. On carrot, isolates occasionally produced very mild symptoms 3-4 weeks after inoculation. The bacteria were motile, Gram-negative, aerobic rods, positive for production of amylase, catalase, xanthomonadins and H_2S from cysteine, but negative for quinate metabolism, oxidase and nitrate reductase. A 3S primer pair for detection of *X. campestris* pv. *carotae* (*Xcc*) amplified a 350 bp fragment of genomic DNA from the coriander isolates and *Xcc*, but rep-PCR fingerprints distinguished the coriander isolates from *Xcc*. This pathogen has not previously been reported in the USA.

INTRODUCTION

Pseudomonas syringae pv. *coriandricola* is the primary bacterial pathogen of concern to coriander and cilantro (*Coriandrum sativum* L.) growers in the US (6). To our knowledge, the only reports of a *Xanthomonas* pathogen of coriander are from India in 1961 (7) and Taiwan in 2004 (4). Srinivasan et al. (7) proposed the name *Xanthomonas campestris* pv. *coriandri* (*Xccor*) to distinguish this pathogen from the carrot pathogen, *X. campestris* pv. *carotae* (*Xcc*). *Xccor* was shown to be pathogenic on coriander and fennel (*Foeniculum vulgare*), but not on anise (*Pimpinella* sp.), caraway (*Carum copticum*), carrot (*Daucus carota*), cumin (*Cuminum cyminum*), or dill (*Peucedanum graveolens* = *Anethum graveolens*) (7). *Xcc* is reported as a pathogen of carrot only (2).

In the spring of 2003, a US seed company requested that a coriander seed lot produced in Oregon be tested for *Xcc*, an established pathogen of carrot in the Pacific Northwest (1). The seed assay was requested for phytosanitary certification of the seed for export. Standard seed assay methods for detection of *Xcc* (3) revealed high numbers of a putative *Xanthomonas* sp. that resembled *Xcc*.

The objectives of this research were to:

1) determine the cultural and physiological characteristics of the US coriander seed strains,
2) evaluate the Umbelliferous host range of these strains, and
3) compare the US coriander strains with carrot seed strains of *Xcc*.

MATERIALS & METHODS

Cultural and physiological characteristics. A seed soaking method was used to extract bacteria from the coriander seed lot produced in Oregon, followed by plating a dilution series of the soakate onto MD5A medium (3). Six strains were selected for cultural and physiological tests (Table 1), and compared with carrot seed strains of *Xcc* from the Pacific Northwest.

Pathogenicity tests. Pathogenicity tests on carrot and coriander seedlings were carried out at the University of Idaho-Moscow using coriander seed strain US-A and *Xccor* strain ATCC 17996. Plants were inoculated by rolling a Q-tip that had been dipped in a suspension of the appropriate bacterial isolate (10^7 CFU/ml) on the leaf surface, and by placing drops of the suspension onto unfurled leaves. Inoculated plants were incubated in a dew chamber for two days, and then moved to a growth chamber. Isolations were carried out from symptomatic and asymptomatic leaves for each treatment.

Umbelliferous host range. The Umbelliferous host range of US coriander seed isolates and two *Xcc* isolates was tested at the WSU-Mount Vernon REU on carrot ('Red Cored Chantenay'), celery (*Apium graveolens* 'Utah 52-70 improved'), coriander (a proprietary cultivar), dill ('Bouquet'), fennel ('Finocchio'), lovage (*Levisticum officinale* 'HR 1227'), parsley (*Petroselinum crispum* 'Dark Green Italian'), and parsnip (*Pastinaca sativa* 'Andover') (Table 2). An airbrush (12 psi) was used to atomize each bacterial suspension (10^8 CFU/ml) onto the leaves of up to six plants. Control plants were inoculated with phosphate buffer. Inoculated plants were enclosed in a plastic bag for 72 h, uncovered, and placed on a greenhouse bench. Four to five weeks later, three samples of leaf tissue from each treatment were surface-sterilized, rinsed, and cut into pieces in phosphate buffer. The buffer was then streaked onto NSA or YDC agar. Isolates typical of *X. campestris* were tested for starch hydrolysis and quinate metabolism (Table 1). One isolate from each treatment was assayed using a 3S PCR primer pair specific for *Xcc* (5).

Molecular characteristics. Two DNA-based tests were performed to investigate genetic relationships between *Xcc*, *Xccor* ATCC 17996, and the US coriander seed isolates: 1) PCR detection with a 3S primer pair highly specific for *Xcc* (5); and 2) repetitive element PCR (rep-PCR), which provides a DNA fingerprint representative of the genome.

Table 2. Umbelliferous host range of US coriander seed strains of *X. campestris* and carrot seed strains of *X. campestris* pv. *carotae*

Strain of *X. campestris*	Symptoms of infection 25 days > inoculation (# plants symptomatic/# inoculated)							
	Carrot	Celery	Coriander	Dill	Fennel	Lovage	Parsley	Parsnip
US coriander seed strains			1st test (May 2003)					
US-A	+ (1/9)	-	+ (6/6)	-	+ (12/12)	+ (6/8)	-	-
US-B	-	-	+ (6/6)	-	+ (8/8)	+ (3/7)	-	+ (1/18)
US-D	-	-	+ (3/6)	-	+ (8/10)	+ (2/7)	-	+ (1/15)
US-F	-	-	+ (6/6)	-	+ (10/11)	+ (3/8)	-	+ (1/15)
US-G	-	-	+ (6/7)	-	+ (11/12)	+ (3/9)	-	+ (2/15)
US-H	-	-	+ (5/6)	-	+ (10/12)	+ (2/8)	-	-
				2nd test (November 2003)				
US-A	-	-	+ (5/6)	-	+ (5/6)	+ (3/3)	-	-
US-D	-	-	+ (4/6)	-	+ (5/6)	+ (2/3)	-	-
US-H	-	-	+ (6/6)	-	+ (6/6)	+ (2/3)	-	-
X. campestris pv. *carotae*				1st test				
ID-I	+ (12/12)	+ (14/16)	+ (12/12)	+ (1/14)	+ (2/12)	+ (6/6)	+ (13/13)	+ (15/16)
WA	+ (10/10)	+ (5/16)	+ (6/6)	+ (4/18)	-	+ (5/9)	+ (10/12)	+ (1/17)
				2nd test				
ID-I	+ (6/6)	-	-	+ (6/6)	+ (6/6)	-	+ (5/6)	+ (5/6)
WA	+ (6/6)	-	-	+ (6/6)	+ (5/6)	-	+ (3/6)	+ (2/6)
Control	-	-	-	-	-	-	-	-

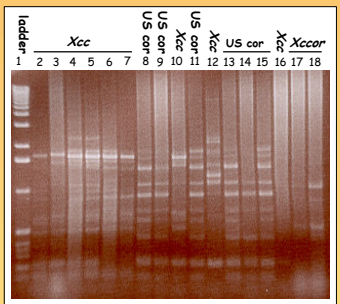

Fig. 3. DNA fingerprints from total genomic DNA of xanthomonads from Umbelliferous plants. Lane 1 = 1 kb ladder. *Xcc* = *X. campestris* pv. *carotae*. *Xccor* = *X. c.* pv. *coriandri* ATCC 17996. US cor = US coriander seed isolates.

Table 3. Reisolations from Umbelliferous hosts inoculated with US coriander seed strains of *X. campestris* or carrot strains of *X. campestris* pv. *carotae*

Strain of *X. campestris*	Reisolation of bacteria onto NSA (1st test) or YDC agar (2nd test) media							
	Carrot	Celery	Coriander	Dill	Fennel	Lovage	Parsley	Parsnip
US coriander seed strains				1st test (May 2003)				
US-A	+	-	+	-	+	+	-	-
US-D	-	-	+	-	+	+	-	+
US-G	-	+	+	+	+	+	-	+
				2nd test (November 2003)				
US-A	+/- (1/3)	+/- (1/3)	+ (2/3)	+/- (1/3)	+ (3/3)	+ (3/3)	-	+/- (2/3)
US-D	+/- (1/3)	-	+ (2/3)	-	+ (3/3)	+ (3/3)	+/- (1/3)	-
US-H	+/- (1/3)	-	+ (3/3)	-	+ (2/3)	+ (2/3)	-	+/- (2/3)
X. campestris pv. *carotae*				1st test				
ID-I	+	+	+	+	+	+	+	+
				2nd test				
ID-I	+ (3/3)	-	+/- (1/3)	+ (3/3)	+ (3/3)	+ (2/3)	+ (1/3)	+ (1/3)
WA	+ (3/3)	-	+/- (1/3)	+ (2/3)	+ (2/3)	+ (2/3)	+ (2/3)	+ (2/3)
Control	-	-	-	-	-	-	-	-

'-' = no colonies, '+/-' = 1-3 colonies, '+' = abundant colonies typical of *X. campestris* isolated

Table 1. Characteristics of a US coriander seed strain of *Xampestris campestris* compared to carrot isolates of *X. campestris* pv. *carotae*

Cultural or physiological test	US coriander seed strain A	*X. campestris* pv. *carotae*
Mucoid colonies	+	+
Yellow colonies	+	+
Cell shape & size	Rods (0.7-2.0 um)	Rods (0.7-1.6 um)
Motility	+	+
Gram stain	-	-
Anaerobic	-	-
Amylase (starch hydrolysis)	+	-
Catalase	+	+
Xanthomonadins	+	+
H_2S from cysteine	+	+
Quinate metabolism	-	+
Oxidase	-	-
Nitrate reductase	-	-

RESULTS & DISCUSSION

Cultural and physiological characteristics. Round, yellow, glistening, mucoid, convex colonies characteristic of *X. campestris* were isolated from the US coriander seed lot at 4.6 x 10^5 CFU/g seed. All six coriander isolates displayed the same characteristics as US-A (Table 1), and were identical to *Xcc* except for starch hydrolysis and quinate metabolism (Table 1).

Pathogenicity tests. Strain US-A caused small, irregular, water-soaked lesions emanating from the margins of immature leaves 2-4 days after inoculation. Lesions expanded and turned necrotic (Fig. 1A). After 3 weeks, about 50% of the plants were dead. *Xccor* ATCC 17996 produced similar lesions (Fig. 1B), but symptoms progressed more rapidly than with US-A (93% of the plants were dead 3 weeks after inoculation). Koch's postulates were completed on coriander for US-A and ATCC 17996.

Umbelliferous host range. In both tests, the coriander strains caused symptoms on coriander, fennel and lovage (Fig. 1C-1E), with the most severe symptoms on coriander and fennel (Table 2). Mild symptoms were also observed on a few parsnip plants (Fig. 1F). The coriander strains were re-isolated from all symptomatic plants and a few asymptomatic plants of carrot, celery, dill, parsley and parsnip (Table 3), suggesting the bacteria can persist on some Umbelliferous hosts without causing symptoms. The *Xcc* strains caused symptoms on carrot, dill, fennel, parsley, and parsnip in both tests, and on at least a few plants of each host in the first test (Table 2, Fig. 2). Symptoms were most severe on carrot for both strains. *Xcc* was re-isolated from inoculated leaves of each host, except celery in the second test (Table 3). None of the coriander strains re-isolated from inoculated plants was able to metabolize quinate, but isolates from hosts inoculated with *Xcc* tested positive for quinate metabolism. A 350 bp band was observed for all isolates tested with the 3S primer pair. None of the control plants developed symptoms, nor was *Xanthomonas* isolated from the control plants.

Molecular characteristics. The 3S primer pair directed amplification of the target ~350 bp DNA fragment from genomic DNA of known strains of *Xcc* from carrot seed and from *Xccor* and the US coriander seed strains. DNA fingerprints generated by rep-PCR indicated the US coriander seed strains comprised a homogeneous group distinguishable from strains of *Xcc* and *Xccor* (Fig. 3). The results suggest these strains represent a group of genetically distinct xanthomonads that are pathogenic on Umbelliferous plants and detected with the 3S primer pair.

CONCLUSIONS

- This is the 1st report of strains of *X. campestris* pathogenic on coriander in the US.
- Preliminary results indicate the US coriander seed strains are distinct from *Xccor* (6) and *Xcc*.
- The US coriander strains caused severe symptoms on coriander and fennel. Lovage was moderately susceptible. A few parsnip plants and one carrot plant developed mild lesions. Symptoms were not observed on dill, celery or parsley.
- Greenhouse inoculations indicated *Xcc* can be pathogenic on several Umbelliferous hosts besides carrot, although conditions may have been more conducive for infection than occurs in nature.
- The 3S PCR primer pair used to detect *Xcc* on carrot also detected *Xccor* and the US coriander strains.
- Rep-PCR fingerprinting differentiated US coriander strains from *Xccor* and *Xcc*.
- Taxonomic relationships among these strains need further investigation. This represents another example of genetic heterogeneity among xanthomonads infecting common host plants.

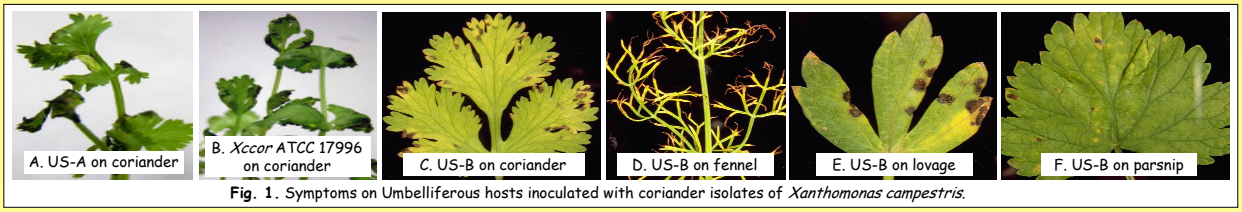

Fig. 1. Symptoms on Umbelliferous hosts inoculated with coriander isolates of *Xanthomonas campestris*.

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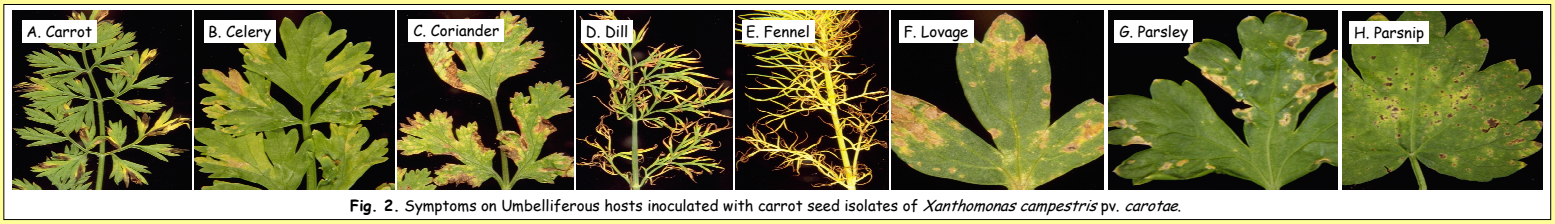

Fig. 2. Symptoms on Umbelliferous hosts inoculated with carrot seed isolates of *Xanthomonas campestris* pv. *carotae*.