Viscosity Test for Preliminary Identification of Strains of *Xanthomonas campestris*

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ABSTRACT

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A diagnostic test based on the viscosity of suspensions prepared from 6- to 7-day-old cultures differentiated 16 of 17 pathovars of *Xanthomonas campestris* from miscellaneous yellow bacteria. The viscous suspensions of xanthomonads (viscosity presumed to be caused by xanthan gums) required ≥26 sec for 0.9 ml to flow through a 1-ml Pyrex pipet, vs. 5.6 sec or less for other yellow bacteria.

The differentiation of *Xanthomonas campestris* (Pammel) Dowson from other yellowish, mucoid bacteria, such as *Erwinia herbicola* (Lohnis) Dye, that grow on selective and nonselective media is difficult, even for experienced diagnosticians. Physiological tests are useful for identifying xanthomonads, but they are also time-consuming. It would be helpful to have an easy test to tentatively identify pathovars of *X. campestris* for further authentication, especially when isolations are made on rich, multipurpose media and there are many yellow colonies.

We report a simple technique that differentiates many *X. campestris* pathovars and strains from miscellaneous yellow bacteria that commonly grow on isolation media. The test is based on the presence of a viscid substance(s) presumed to be the hetero-polysaccharide xanthan (5), which contributes to the mucoid character of colonies and viscosity of aqueous suspensions of the bacteria.

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MATERIALS AND METHODS

Sixteen known strains and pathovars of *X. campestris*, two strains of *X. albilineans* (Ashby) Dowson, one strain of *X. fragariae* Kennedy and King, one strain of *X. axonopodis* Starr and Garces, and 20 miscellaneous yellow, unidentified bacteria of various physiological and morphological characteristics were used in the experiments. The miscellaneous bacteria were isolated from a variety of diseased plants. The physiological characteristics used for identification of xanthomonads included the oxidase reaction, growth on Hugh-Leifson medium covered with mineral oil and on Tween A medium, induction of the hypersensitive reaction on tobacco (*Nicotiana glutinosa* L.) at 32 C, and use of L-asparagine as a sole carbon and nitrogen source (2–4). Pathogenicity tests using standard techniques were done with unknown strains that were positive for the viscosity test (6).

The viscosity test. Bacteria were cultured on sucrose-peptone agar (SPA), composed of 20 g/L of sucrose, 5 g/L of peptone, 0.5 g/L of K_2HPO_4, 0.25 g/L of $MgSO_4 \cdot 7H_20$, and 20 g/L of agar (2). A turbid suspension (0.1 ml) of bacteria was spread on the medium in 90-mm petri dishes with a sterile glass rod to make a lawn. After incubation at 28 C for 6–7 days, 10 ml of sterile water was added to the bacterial lawn. Bacteria were scraped from the medium with a glass rod and the suspensions poured into 20-ml test tubes and mixed thoroughly with a glass rod. One ml of suspension of a test strain was drawn into a 1-ml disposable serological pipet (Pyrex). The time required for the meniscus to move from zero to the 0.9-ml mark of the pipet after the finger was removed was measured with a stopwatch. This time was related to the viscosity of the suspension. The timing tests were done at room temperature and repeated four times, each with a different replicate. The concentration of the suspension for each strain was determined for one of the four replicates. To learn whether dilution of the suspensions would appreciably affect the results, the above experiment was repeated with two consecutive 1:9 dilutions made with sterile water from each suspension (one replicate).

RESULTS AND DISCUSSION

Suspensions of all strains of *X. campestris*, except *X. c.* pv. *juglandis* (Pierce) Dye, had a discharge time (viscosity reading) of 26 sec or longer (Table 1). Some suspensions were too viscous to flow, and others required several minutes for discharge of 0.9 ml. Consequently, we arbitrarily set 30 sec as the maximum time necessary to record the flow rate. The variation in flow rates among replicates was negligible. *X. c.* pv. *juglandis* was negative, because it does not produce mucoid colonies. It also differs substantially from most other *X. campestris* pathovars on the basis of DNA-DNA homology (D. C. Hildebrand and N. Palleroni, *personal communication*). It is likely that the nature of the xanthan gum may differ among species and some strains. Accordingly,

a mucoidal strain of *X. fragariae* tested negative for the viscosity test. The test was also negative, as expected, for *X. albilineans* and *X. axonopodis* because they do not produce mucoid colonies (2). All strains of *X. campestris* were oxidase negative, did not grow on Hugh-Leifson medium when covered with mineral oil, and grew on Tween A medium. Most, but not all, induced the hypersensitive reaction in tobacco.

The concentrations of the suspensions that were made to remove bacteria from the media were not critical as long as the suspensions were not further diluted. Dilution of initial suspensions 1:9 or 1:99 with sterile water often diminished the viscosity so much that strains of *X. campestris* could not be distinguished from other bacteria in the test (*data not shown*).

Six of the 20 unknown yellow bacteria were positive (discharge time ≥26 sec) in the viscosity test. These strains subsequently were identified as members of *X. campestris* on the basis of standard tests (Table 2) (2). All six strains caused disease in pathogenicity tests on the same species from which they were isolated (Table 2). The viscosity readings for the other strains were under 2 sec. They subsequently were determined not to be strains of *X. campestris* (2).

The viscosity test appears to be an excellent test to add to the assemblage of methods, such as fatty acid profiles and presence of xanthomonadins, for identifying certain *X. campestris* pathovars. It requires no sophisticated instrumentation and little calibration. It is not even necessary to measure the concentration of the suspension when the prescribed culturing method is used. The test was positive for 16 of 17 known pathovars of *X. campestris* (Table 2). It is expected that this ratio would be similar upon testing the numerous other pathovars in this species. In contrast to most diagnostic tests used for identifying xanthomonads, this one appears to be singularly diagnostic for strains that produce the viscous xathan gum. For example, the tobacco hypersensitivity test is cultivar-sensitive and is influenced by the temperature at which plants grow before and after inoculation (3). The oxidase reaction is often inconclusive for xanthomonads, and weakly positive reactions are common (1). Even the measurement of absorption spectra of crude pigment extracts can lead to misdiagnoses (R. Stall, *personal communication*). Since completing these experiments, we have tested numerous yellow bacteria in our diagnostic laboratory. None has given a viscosity reading above 25 sec that was not subsequently identified as a xanthomonad.

Table 1. Viscosity of suspensions of known pathovars and species of *Xanthomonas*

Culture no.[y]	Name	Viscosity[z] (sec)	Suspension[z] (cfu/ml)
XA 123	*Xanthomonas albilineans*	5.4	5×10^{10}
XA 144	*X. albilineans*	1.2	2×10^{11}
XA 131	*X. axonopodis*	0.9	4×10^{10}
UC 855	*X. campestris* pv. *campestris*	>30.0	1×10^{12}
XC 145	*X. c.* pv. *celebensis*	>30.0	3×10^{10}
UC 867	*X. c.* pv. *incanae*	26.4	1×10^{10}
UC 873	*X. c.* pv. *juglandis*	5.6	2×10^{11}
UC 1060	*X. c.* pv. *juglandis*	2.2	1×10^{11}
UC 874	*X. c.* pv. *malvacearum*	>30.0	2×10^{10}
UC 876	*X. c.* pv. *malvacearum*	>30.0	2×10^{11}
XO 111	*X. c.* pv. *oryzicola*	>30.0	4×10^{9}
UC 131	*X. c.* pv. *pelargonii*	>30.0	9×10^{12}
UC 878	*X. c.* pv. *pelargonii*	>30.0	4×10^{10}
UC 879	*X. c.* pv. *pelargonii*	>30.0	2×10^{11}
UC 880	*X. c.* pv. *phaseoli*	>30.0	1×10^{11}
UC 673	*X. c.* pv. *pruni*	27.0	2×10^{9}
XT 129	*X. c.* pv. *secalis*	>30.0	1×10^{10}
UC 886	*X. c.* pv. *vesicatoria*	>30.0	1×10^{12}
UC 895	*X. c.* pv. *vitians*	>30.0	1×10^{11}
UC 861	*X. fragariae*	0.8	4×10^{10}

[y] From the collection of Plant Pathogenic Bacteria, University of California, Berkeley.
[z] Viscosity and cfu/ml readings represent the mean of four trials; concentration of suspension was determined for one replicate.

Table 2. Viscosity and other characteristics of initially unknown yellow gram-negative bacteria

Culture no.	Plant source	Viscosity[y] (sec)	Suspension[y] (cfu/ml)	Physiological characteristics[x] OX	HL	Growth on Tween A	HR	Use of asparagine
875-3	Alder	0.9	2×10^{9}	−	+	−	−	
119-7	Alder[z]	>30.0	6×10^{11}	−	−	+	+	−
948-3	Cactus	1.7	2×10^{7}	−	+	−	−	
638-5	Geranium	0.9	2×10^{9}	−	−	−	−	
723	Hebe	0.9	4×10^{9}	−	−	−	−	
632-1	Hyacinth[z]	>30.0	5×10^{11}	−	−	+	−	−
597-2	Ivy	0.8	2×10^{11}	−	−	+	−	
446-3	Ivy[z]	>30.0	3×10^{12}	−	−	+	+	−
138	*Malus sylvestris*[z]	0.8	6×10^{10}	−	+	−	−	
351-1	Manzanita[z]	>30.0	3×10^{12}	−	−	+	+	−
417	Oleander	0.8	4×10^{10}	−	+	+	−	
695	Passiflora	0.6	1×10^{11}	−	−	−	−	
91-2	Geranium	0.8	4×10^{10}	−	−	+	−	
604-4	Petunia	0.6	7×10^{10}	−	−	−	−	
626-1	Stock[z]	>30.0	2×10^{10}	−	−	+	+	−
668-5A	Strawberry	1.0	2×10^{10}	−	−	+	−	
668-5B	Strawberry	1.1	2×10^{11}	−	−	+	−	
ML5	Sugar Beet[z]	0.9	1×10^{11}	+	−	−	−	
447-2	Tomato	0.7	3×10^{11}	−	+	+	−	
58	Zinnia[z]	>30.0	1×10^{12}	−	−	+	+	−

[x] OX = Oxidase test; HL = Hugh-Leifson medium, covered with mineral oil; HR = hypersensitive reaction of *Nicotiana glutinosa* at 32 C; use of asparagine as sole carbon and nitrogen source.
[y] Viscosity readings represent the mean of four replicates. Concentration of suspension was determined for one of the replicates.
[z] Bacteria from these sources subsequently were identified as: alder = *Xanthomonas campestris* pv. unnamed; hyacinth = *X. c.* pv. *hyacinthi*; ivy = *X. c.* pv. *hederae*; *Malus sylvestris* = *Erwinia herbicola*; manzanita = *X. c.* pv. unnamed; stock = *X. c.* pv. *incanae*; sugar beet = *Pseudomonas fluorescens*; zinnia = *X. c.* pv. *zinniae*.

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