Tween Media for Semiselective Isolation of *Xanthomonas campestris* pv. *vesicatoria* from Soil and Plant Material

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ABSTRACT

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Three semiselective culture media have been developed for the isolation of *Xanthomonas campestris* pv. *vesicatoria* (*X. c.* pv. *vesicatoria*) from soil and plant material. These media contain Bacto peptone, Tween 80, potassium bromide, calcium chloride, and Difco agar. Selectivity is afforded through the use of boric acid, methyl green, and the antibiotics cephalexin, 5-fluorouracil, tobramycin, and cycloheximide. Although elimination of contaminating bacteria is not total, identification of *Xanthomonas* is aided by the use of Tween 80, a fatty acid ester and substrate for lipolytic enzymes of the pathogen. The released fatty acids are precipitated by calcium and form soap crystals around the colonies. Also, production by *Xanthomonas* of a yellow pigment, a dibromomethoxyphenyl polyene, is enhanced by incorporating KBr into the media. The resulting colonies of *Xanthomonas* have a fried-egg appearance. Compared with King's medium B, recovery on Tween media of *X. c.* pv. *vesicatoria* averages more than 90%, as does reduction of contaminating microorganisms from soil and plant samples.

Additional key words: *Capsicum, Lycopersicon*

Xanthomonas campestris pv. *vesicatoria* (Doige) Dye (*X. c.* pv. *vesicatoria*) is the organism responsible for bacterial spot of tomatoes and peppers. In Florida and other states where these crops are important, tremendous yield losses may result from this disease (14). Efficient detection of the pathogen on plants and in the soil is therefore paramount if its ecology is to be studied and control established.

Bacterial populations can be detected and monitored by culturing biological or soil samples on the host of the bacterium (5,7,17) or on selective culture media. Selection takes advantage of the different biological pathways and enzyme systems within species and can involve the use of specific carbon and nitrogen sources to stimulate the growth of one species over that of another. Selection also results when bacterial enzymes react with constituents in the medium to promote a cultural distinction between two species that otherwise appear similar. A third selective force is the addition to a culture medium of chemicals toxic to some species of bacteria. Selection is always a compromise between the reduction of contaminants and the survival of target species.

Several selective and semiselective culture media have been developed for the genera of phytopathogenic bacteria. Species of *Xanthomonas* tend to be more fastidious than those of other genera, and earlier media used specific growth requirements (13,19). Cellobiose or starch proved semiselective as carbon sources in some instances, and dyes were often used to reduce contaminants (6,16). Media were also developed for specific pathovars of *X. campestris* (4,12). However, the media developed previously were not suitable for studying the ecology of *X. c.* pv. *vesicatoria*. They either were not sufficiently selective or they did not support recovery at a sufficiently high level of efficiency. The objective of this work was to develop a selective culture medium that would allow efficient recovery and identification of *X. c.* pv. *vesicatoria* from plant material and soil samples. A preliminary report on this research has been published (9).

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MATERIALS AND METHODS

Media and recovery of xanthomonads. A medium described by Sierra (18) containing the surfactant Tween was adapted for the selective recovery of *X. c.* pv. *vesicatoria* because it already incorporated a form of selectivity based on a cultural distinction among bacterial genera. Because the microflora of leaves and soil are so different and study of both would be required to learn the ecology of *X. c.* pv. *vesicatoria*, several Tween media were tested.

In initial adaptations of the Tween medium, the amount of peptone was varied between 2 and 10 g/L and supplemented with, or replaced by, yeast extract to 2 g/L. The 5 g of NaCl was also partially or totally replaced with an amount of KBr to 10 g/L, keeping the ionic balance of the medium approximately constant. The effects of these changes on the color of *X. c.* pv. *vesicatoria* were monitored. In an attempt to maximize the zones of lipolysis by *X. c.* pv. *vesicatoria*, the pH of the medium was adjusted between 6.0 and 8.0; Tweens 20, 40, 60, and 80 were incorporated; and $CaCl_2$ was varied between 0.1 and 0.5 g/L.

To reduce numbers of contaminating bacteria on the Tween media, boric acid (0.1–1.0 g/L) and dyes including neutral red, phenol red, eosin, tetrazolium chloride, crystal violet, methyl violet, methyl green, methylene blue, and brilliant cresyl blue (5–15 mg/L) were added to the medium. The effects of several antibacterial compounds on selectivity were also examined including, but not limited to, LiCl, $MnSO_4$, $Na_2Cr_2O_4$, Na_2SeO_3, and Tl_2SO_4 (5–200 mg/L), 0.1% merthiolate tincture (5 μl/L), 2-thiouracil, 5-fluorouracil, 6-azauracil, and 8-azaguanine (5–200 mg/L), ampicillin, cephalexin, dodecyltrimethylammonium bromide, erythromycin, kasugamycin, nalidixic acid, nicotinic acid, oxalinic acid, penicillin G, streptomycin, sulfanilamide, tobramycin, and trimethoprim (0.25–100 mg/L). Control of fungi was tested with chlorothalonil (Bravo) and cycloheximide (20–200 mg/L). All inorganic chemicals and antibiotics were products of the Sigma Chemical Company.

Thirty-nine strains of *X. c.* pv. *vesicatoria* stored in tap water were initially restreaked on NYDA (Difco nutrient agar plus 0.5% Difco yeast extract and 1% dextrose), and suspensions of 0.1 ml from individual colonies that were spectrophotometrically standardized

at about 1×10^3 colony-forming units (cfu) per milliliter were spread on 10 plates of each of the Tween media and KMB (8). Similar suspensions were used to inoculate plates of seven other culture media to compare the efficiencies of recovery. These media included NYDA, ST (19), X-1 (13), D-5 (6), SX (16), and two media developed for *X. c.* pv. *juglandis* (BS) (12) and pv. *pruni* (XP) (4). Strains of *X. c.* pv. *campestris, citri, dieffenbachiea, pelargonii,* and *phaseoli* were also tested. After 4 days of incubation at 28 C, the efficiencies of recovery on the Tween media, relative to KMB, were determined.

Recovery from leaves. About 4 g of leaves from tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum annuum* L.), or nightshade (*Solanum americanum* L.), without symptoms of disease, were shaken in 100 ml of a peptone-phosphate buffer, pH 7.0, containing (per liter) 5.3 g of KH_2PO_4, 8.6 g of Na_2HPO_4, and 1 g of Bacto peptone. In some instances, a small quantity of *X. c.* pv. *vesicatoria* was initially added to the buffer. After 45 min, 0.1 ml from each dilution of 0, 10^{-1}, 10^{-2}, and 10^{-3} was spread over each of 10 plates of Tween B medium and KMB. To concentrate the bacterial cells, 100 ml of wash buffer was also passed through a 0.45-μm filter. This filter was then vortexed 1 min in 5 ml of additional buffer, 0.1 ml of which was subsequently plated on Tween medium. After 4 days of incubation at 28 C, recovery of *X. c.* pv. *vesicatoria* and other bacteria or fungi was recorded and related to the fresh weight of leaves. The identity of the pathovar *vesicatoria* was confirmed by injecting representative colonies into leaves of tomato plants.

Recovery from soil. One gram of moist soil from various locations (with and without added *X. c.* pv. *vesicatoria*) was shaken 30 min in 100 ml of the buffer described earlier, then 0.1 ml from each dilution of 0, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} was spread over each of 10 plates of Tween C medium and KMB. Alternatively, soil suspensions in 100 ml were first centrifuged at $450 \times g$ for 20 min to remove particulate matter. The supernatant was again centrifuged at $4{,}000 \times g$ for 20 min, and the resulting pellet was resuspended in 5 ml of buffer (5). One-tenth milliliter of this concentrated suspension was spread over culture plates to check populations of *X. c.* pv. *vesicatoria* lower than 100 cfu/g of soil. After 4 days of incubation at 28 C, recovery of *X. c.* pv. *vesicatoria* and other bacteria or fungi was recorded and related to the oven-dried weight of the soil.

Table 1. Composition of Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria*

Component[a]	Concentration for isolation from		
	Fruit (Tween A or B)	Leaves (Tween A or B)	Soil (Tween C)
Peptone	10.00 g/L	10.00 g/L	10.00 g/L
Potassium bromide	10.00 g	10.00 g	10.00 g
Calcium chloride	0.25 g	0.25 g	0.25 g
Boric acid	...	0.30 g	0.60 g
Agar	15.00 g	15.00 g	15.00 g
Tween 80	10.00 ml	10.00 ml	10.00 ml
Cycloheximide	50.00 mg	50.00 mg	50.00 mg
Cephalexin	35.00 mg	65.00 mg	65.00 mg
5-Fluorouracil	12.00 mg	12.00 mg	12.00 mg
Tobramycin	0.40 mg	0.40 mg	...
Methyl green	...	...	10.00 mg

[a] For all media, pH was 7.4. Tween and antibiotics were added aseptically after the media were autoclaved. Calcium chloride refers to anhydrous $CaCl_2$.

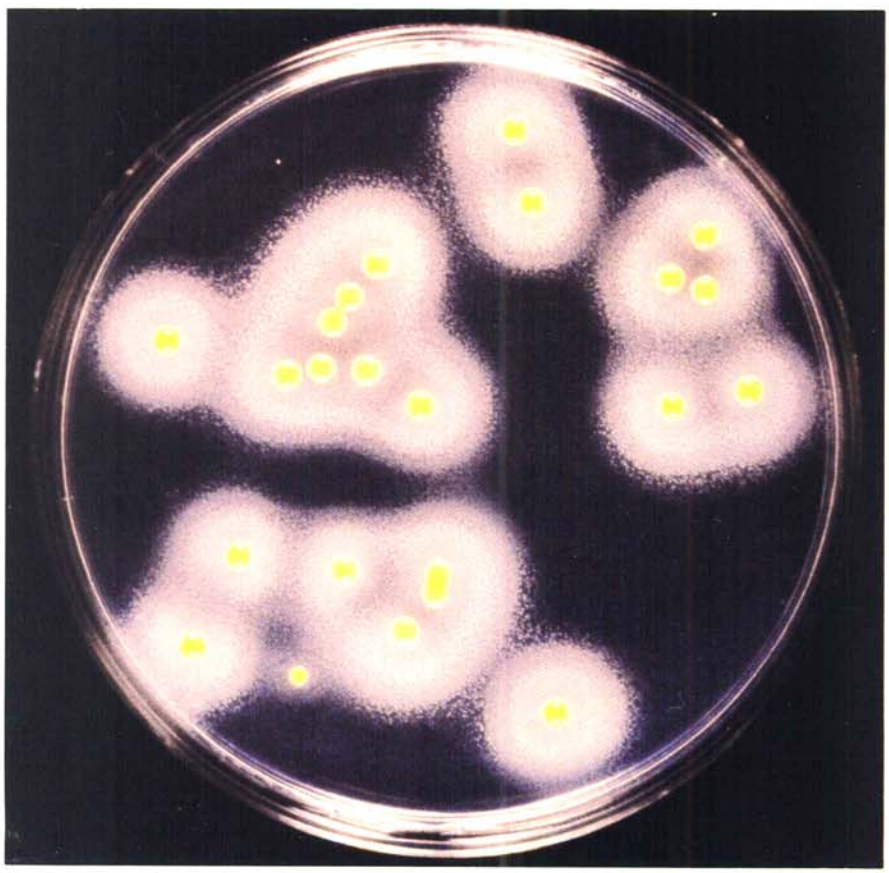

Fig. 1. Pure culture of *Xanthomonas campestris* pv. *vesicatoria* on Tween B medium after 96 hr at 28 C.

RESULTS

Tween media. Three semiselective culture media for the isolation of *X. c.* pv. *vesicatoria* from leaves and soils were developed and tested (Table 1). All three contain (per liter) 10 g of Bacto peptone, which provides all the essential organic requirements for growth; 10 g of KBr, which provides a suitable osmotic environment and the element bromine for enhancement of the xanthomonadin pigment (1); 10 ml of Tween 80 and 0.25 g of anhydrous $CaCl_2$ for demonstration of lipolysis; and 15 g of Difco-Bacto agar. In addition Tween A (a less selective medium for strains less tolerant of boric acid) contains (per liter) the antibiotics cycloheximide at 50 mg, cephalexin at 35 mg, 5-fluorouracil at 12 mg, and tobramycin at 0.4 mg. Tween B, developed for isolations of *X. c.* pv. *vesicatoria* from leaves, additionally contains (per liter) boric acid at 0.3 g, cycloheximide at 50 mg, cephalexin at 65 mg, 5-fluorouracil at 12 mg, and tobramycin at 0.4 mg. Tween C was developed for isolations of *X. c.* pv. *vesicatoria* from soils and contains (per liter) boric acid at 0.6 g, cycloheximide at 50 mg, cephalexin at 65 mg, 5-flurouracil at 12 mg, and the dye methyl green at 10 mg for control of most contaminating microorganisms

Peptone, KBr, $CaCl_2$, and when necessary, boric acid are first dissolved in water, and the pH is adjusted to 7.4 with NaOH. Agar is then added, and the

medium is autoclaved for 15 min at 15 lb of pressure. Tween 80 is autoclaved separately, then added immediately after the medium is removed from the autoclave, while it is hot. Leaving a stir bar in the flask of medium while it is autoclaved is recommended because shaking the flask after adding Tween produces bubbles. The medium will immediately appear cloudy after Tween is added but will clear as it cools. Aseptically prepared aqueous solutions of methyl green and the antibiotics are added to the flask of medium 15–30 min before it is poured.

On the Tween media, *X. c.* pv. *vesicatoria* appeared as circular, raised, yellow colonies after 4 days at 28 C, becoming more convex with age (Fig. 1). Colonies of *X. c.* pv. *vesicatoria* were surrounded by zones of white crystals, which were the calcium salts of fatty acids released from Tween by lipolytic enzymes. Production of these enzymes varied among the pathovars and strains; all strains of *X. c.* pv. *vesicatoria* developed white zones around yellow colonies (giving a fried-egg appearance), but the sizes of zones varied. Strains of other pathovars of *X. campestris* also showed variations in the sizes of zones of lipolysis.

Efficiency of recovery of *Xanthomonas*. Compared with KMB, recovery of *X. c.* pv. *vesicatoria* on the Tween media was very efficient. For the 32 Florida strains grown on Tweens A, B, and C, recovery averaged 96, 85, and 99%, respectively (Table 2); for seven strains from other states, recovery averaged 98, 91, and 86%, respectively. Of the 39 strains, however, six were recovered at less than 50% on either Tween B or Tween C or both. Recovery of strains from either pepper or tomato was not significantly different on Tween A or C, but those from pepper were more efficiently recovered on Tween B than were those from tomato. Of the other culture media compared with the Tween media and KMB for their efficiency of recovery of *X. c.* pv. *vesicatoria*, ST was most efficient but was nonselective. Compared with KMB, recovery on ST averaged 99.5%, X-1 averaged 72.8%, NYDA averaged 68.2%, XP averaged 67.2%, D-5 averaged 24.5%, SX averaged 13.5%, and BS averaged less than 1%.

Five other pathovars of *X. campestris* were also grown on the Tween media to test recovery (Table 3). Although pathovars *campestris, citri, dieffenbachiae,* and *pelargonii* were recovered efficiently, growth of pathovar *phaseoli* was poor. In general, recovery on Tweens A and C was better than that on Tween B.

Recovery from leaves. Tween B was specifically developed for isolating *X. c.* pv. *vesicatoria* from leaves. Because leaves from different plants supported diverse communities of epiphytes, the usefulness of the medium varied according to the uses made of it. Of the epiphytes from nightshade that numbered 1.9×10^6 cfu/g of leaf on KMB, 86.2–93.7% were eliminated on Tween B (Table 4). This rate of reduction consequently determined to a large degree the recovery of *X. c.* pv. *vesicatoria* added to flasks of nightshade leaves, which did not have resident populations of this pathogen. With a ratio of total epiphytes to *X. c.* pv. *vesicatoria* lower than 30:1, recovery of *X. c.* pv. *vesicatoria* was greater than 85%; with ratios 10 and 100 times greater, recovery was reduced to between 60 and 70%.

With leaves from tomato plants, however, for which this medium was originally developed, recovery of added *X. c.* pv. *vesicatoria* was about 100% at a

Table 2. Recovery of *Xanthomonas campestris* pv. *vesicatoria* on Tween media relative to recovery on King's medium B

ID[y]	State	Percent recovery[z] on Tween media			Host
		A	B	C	
61-49	Florida	106 ab	129 a	114 b	Tomato
68-01	Florida	88 ab	82 b	104 a	Pepper
69-23	Florida	88 ab	82 b	92 ab	Pepper
69-27	Florida	82 b	104 a	111 a	Pepper
69-28	Florida	160 a	120 b	117 b	Pepper
71-21	Florida	72 c	78 c	88 b	Pepper
71-34	Florida	94 a	90 ab	79 b	Pepper
75-01	Florida	127 b	148 a	159 a	Tomato
80-01	Florida	58 c	79 b	91 a	Tomato
80-05	Florida	80 b	59 c	64 c	Pepper
81-18	Florida	119 a	77 c	131 a	Pepper
81-23	Florida	55 c	83 b	91 ab	Tomato
81-27	Florida	77 b	47 c	95 a	Tomato
82-11	Florida	141 a	129 ab	120 b	Pepper
82-13	Florida	89 b	88 b	91 b	Pepper
82-15	Florida	96 a	82 b	102 a	Pepper
82-16	Florida	85 b	91 ab	98 a	Pepper
82-17	Florida	93 ab	82 b	98 a	Pepper
83-03	Florida	100 a	103 a	97 a	Tomato
83-06	Florida	142 a	113 bc	123 b	Tomato
83-10	Florida	116 a	109 ab	116 a	Tomato
83-12	Florida	106 a	88 b	109 a	Tomato
83-13	Florida	77 c	0 d	107 a	Tomato
83-14	Florida	91 bc	83 c	110 a	Tomato
83-44	Florida	92 b	104 a	94 b	Pepper
5135	Florida	114 a	114 a	108 ab	Tomato
5612	Florida	75 b	2 c	93 a	Tomato
5613	Florida	101 a	107 a	105 a	Tomato
5909	Florida	119 a	106 ab	117 a	Tomato
5910	Florida	92 a	13 b	10 b	Tomato
6275	Florida	53 b	43 c	41 c	Tomato
6300	Florida	86 b	93 ab	102 a	Tomato
82-23	Georgia	72 b	35 c	97 a	Tomato
82-23	Oklahoma	74 b	58 c	71 b	Pepper
85-01	Michigan	106 a	108 a	0 b	Tomato
1385	Indiana	97 a	96 a	99 a	Tomato
1386	Vermont	103 a	109 a	106 a	Pepper
1389	Pennsylvania	105 a	102 a	105 a	Tomato
1391	New York	131 a	127 ab	121 b	Pepper

[y] In addition to those recovered at GCREC, bacterial cultures were received from R. Stall, R. Dickey, R. Gitaitis, C. Stephens, and G. Lazo.

[z] Average recovery from 10 plates of each medium inoculated with *X. c.* pv. *vesicatoria* between 5×10^1 and 2×10^2 cfu. Within rows, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Recovery of *Xanthomonas campestris* pathovars on Tween media

Pathovar	Number of strains tested	Percent recovery[y] on Tween media		
		A	B	C
vesicatoria	39	96 b[z]	86 c	97 a
campestris	13	107 a	49 c	88 b
citri	5	97 ab	93 b	100 a
dieffenbachiae	5	2 a	81 b	93 a
pelargonii	6	112 a	107 a	111 a
phaseoli	7	20 b	3 c	59 a

[y] Percentage relative to recovery on King's medium B.

[z] Within rows, numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

ratio of total epiphytes to *X. c.* pv. *vesicatoria* of 3,000:1, corresponding to 3.4×10^2 cfu/g of leaf (Table 4). With the sampling methods employed, populations of *X. c.* pv. *vesicatoria* lower than 10^2/g could not be recovered without filtering the wash and concentrating the contaminating species as well. In spite of this, at a ratio of 23,300:1, 65% of the *X. c.* pv. *vesicatoria* applied, corresponding to 27 of 42 cells per gram of leaf, were recovered. Populations of *X. c.* pv. *vesicatoria* from naturally infested tomato and pepper leaves ranged from undetectable numbers after filtering to 3×10^7 cfu/g of leaf and were confirmed by the production of necrotic spots after injection into leaves of tomato. Contaminating species, which on KMB numbered 1.5×10^5 to 2.9×10^7 cfu/g of leaf, were reduced on Tween B from 86.7 to 99.7%.

Recovery from soil. Populations of bacteria and fungi in sand, loam, and muck soils, some artificially mixed with *X. c.* pv. *vesicatoria*, numbered between 5×10^6 and 7.8×10^7 cfu/g dry weight on KMB (Table 5). Contaminating species were reduced in nearly every instance above 90% on Tween C, and recovery of *X. c.* pv. *vesicatoria* was also usually higher than 90% except when populations of the pathogen were lower than 10^4 cfu/g of soil. Below this number, concentrating the soil wash became necessary, and large numbers of contaminating species, though reduced above 99%, frequently overwhelmed the selective components of the medium. Recovery of *X. c.* pv. *vesicatoria* was consequently reduced, often below 50%, but the pathogen could still be detected at numbers 1/100 of that possible using KMB. It was not recovered from unamended soils.

Table 4. Recovery of *Xanthomonas campestris* pv. *vesicatoria* (XCV) from leaves of nightshade, tomato, and pepper, and reduction of contaminating bacteria on Tween B medium (TWB) relative to recovery on King's medium B (KMB)

Plant[a]	XCV added (cfu/g)	Total population on KMB (cfu/g)	Xanthomonads recovered on TWB (cfu/g)	XCV recovered on TWB (%)	Reduction of contaminants on TWB (%)
Nightshade	5.8×10^4	1.9×10^6	5.0×10^4	86.7	86.2
	5.7×10^3	1.9×10^6	3.6×10^3	64.0	93.7
	6.4×10^2	1.9×10^6	4.4×10^2	68.6	87.3
Tomato	4.3×10^3	9.8×10^5	4.3×10^3	100.6	99.1
	3.4×10^2	9.8×10^5	3.9×10^2	114.6	98.5
	4.2×10^1	9.8×10^5	2.7×10^1	64.8	99.0
	0	9.8×10^5	Not detected	NA[b]	99.7
	0	1.5×10^5	Not detected	NA	91.6
	0	2.2×10^7	6.0×10^6	NA	95.8
	0	1.6×10^7	6.7×10^4	NA	95.6
	0	2.2×10^7	1.9×10^3	NA	98.1
	0	6.3×10^7	3.0×10^7	NA	98.6
	0	1.8×10^7	2.7×10^5	NA	98.1
	0	2.9×10^7	1.7×10^4	NA	97.6
	0	3.7×10^6	3.0×10^3	NA	72.3

[a] Leaf assay: four replicates of 10 fresh leaflets were each weighed, then washed separately 45 min in peptone-phosphate buffer, pH 7.0, with and without the addition of an XCV culture (strain 75-1). Aliquots were serially diluted, and 0.1 ml was spread over the surfaces of culture dishes. Colony counts were averaged and related to the fresh weight of leaves.
[b] Not applicable.

Table 5. Recovery of *Xanthomonas campestris* pv. *vesicatoria* (XCV) from soil and reduction of contaminating bacteria on Tween C medium (TWC) relative to recovery on King's medium B (KMB)

Soil type[a]	XCV added (cfu/g)	Total population on KMB (cfu/g)	XCV recovered on TWC (%)	Reduction of contaminants on TWC (%)
Sand	8.7×10^6	7.2×10	93.99	87.44
	8.7×10^5	9.0×10^6	110.01	92.34
	3.2×10^4	1.1×10^7	97.04	98.20
	3.6×10^3	1.5×10^7	99.50	98.20
	4.0×10^3	5.0×10^6	35.10	99.49
	4.0×10^2	5.0×10^6	42.93	99.66
Loam	3.2×10^6	8.8×10^6	96.50	99.07
	4.2×10^5	6.0×10^6	128.31	98.14
	4.0×10^3	7.3×10^6	25.00	99.72
	4.0×10^2	7.3×10^6	12.63	99.77
Muck	4.4×10^6	5.4×10^7	113.64	98.16
	9.7×10^5	7.8×10^7	86.01	97.84
	7.3×10^5	2.6×10^7	118.77	93.14

[a] Soil assay: 1 g of fresh soil was mixed with XCV (strains 75-1, 81-18, 83-44, or 6300) and shaken for 30 min in peptone-phosphate buffer, pH 7.0. Aliquots were serially diluted, and 0.1 ml was spread over the surfaces of culture dishes. Colony counts were averaged and related to the weight of soil, which had been oven-dried.

DISCUSSION

All microorganisms possess traits that can be used to distinguish them, and often, artificial culture media can capitalize on these characteristics to facilitate selection. KMB and CVP are particularly useful for identifying, respectively, fluorescent pseudomonads (8) and pectolytic erwiniae (3). SX agar (16) is similarly helpful in isolating many starch-hydrolizing xanthomonads. Many isolates of *X. c.* pv. *vesicatoria* do not hydrolize starch, however, and growth on SX is variable (2). The culture media described here instead afford identification of pathovar *vesicatoria* by the use of KBr to enhance production of the yellow dibromo-methoxyphenyl polyene pigment (1) and Tween 80 with $CaCl_2$ to demonstrate lipolysis. Unlike starch hydrolysis, lipolysis seems to be a more constant characteristic of this pathovar (20). All stock strains of *X. c.* pv. *vesicatoria* grown on Tween media were lipolytic, although with a few, zones of soap crystals indicating enzyme activity remained slight after 4 days. By increasing the amount of calcium in the media, these zones could be made more distinct. Because all strains isolated from field material were highly lipolytic, $CaCl_2$ at 0.25 g/L was considered sufficient.

Of the antimicrobial constituents of the Tween media, boric acid prevents the growth of many bacteria from soil and of a few inhabiting plant material. Cephalexin eliminates several other species but was included specifically for the yellow *Erwinia herbicola*. Tobramycin is very effective against pseudomonads that inhabit plant material but also is effective against *X. c.* pv. *vesicatoria*. The combination of tobramycin and boric acid is more toxic to *X. c.* pv. *vesicatoria* than either of them alone but is justified by the contribution to the reduction in contaminants. The concentration of tobramycin, therefore, is critical. Fluorescent pseudomonads are effectively eliminated by 5-fluorouracil, and its concentration at 12 mg/L partially offsets the low level of tobramycin. Cycloheximide inhibits fungi; its level in the media can be increased above 100 mg/L if necessary without adversely affecting recovery of xanthomonads.

Still, the compromise with selection for recovery of *X. c.* pv. *vesicatoria* extends to some genera that would be considered contaminants. The bacteria most likely to interfere are species of *Bacillus* and *Flavobacterium* and nonfluorescent pseudomonads. Numbers of *Flavobacterium* can be reduced by adding dodecyltrimethylammonium bromide to

the media, to 200 mg/L, but the compound reduces the pigmentation of *Xanthomonas*. Without this compound, the twin characteristics of *X. c.* pv. *vesicatoria* (yellow pigment and lipolysis) should prevent misidentification. However, high populations of *Bacillus* or a *Pseudomonas* species can inhibit growth of the xanthomonads. Such antagonisms on a selective medium have also been reported for *X. c.* pv. *translucens* (15).

Although one must be aware of the problems that are possible with a few contaminants, the benefits of the Tween media are significant. Ecological studies, which previously depended on antibiotic-resistant, mutant strains of *X. c.* pv. *vesicatoria*, now can be performed with wild populations of the pathogen (10,11). Depending on the numbers of other epiphytes, these populations of *X. c.* pv. *vesicatoria* can be 1/100 to 1/1,000 of that previously detected. This is especially beneficial when seeds are being assayed for the pathogen, and filtration to concentrate *X. c.* pv. *vesicatoria* (and possible contaminants as well) is required.

The Tween media could also prove useful in other situations with other pathovars of *X. campestris*. Each pathovar is somehow different and should be expected to respond differently to culturing on the Tween media, but four of the five pathovars tested were recovered above 90% on at least one of the three media. As with any medium, one must initially screen a bacterial strain to determine its suitability, but these media themselves may be adapted to suit the selectivity for a particular pathovar by altering levels of boric acid and antibiotics. The primary constituents used for colony differentiation are the substrates for pigment production and lipolytic activity by the bacteria.

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