A Differential Medium for Semiselective Isolation of *Xanthomonas campestris* pv. *vesicatoria* and Other Cellulolytic Xanthomonads from Various Natural Sources

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ABSTRACT

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A semiselective medium (CMC-E) was more effective than Tween medium B agar or nutrient agar for recovery of *Xanthomonas campestris* pv. *vesicatoria* from the environment or plant material. The selectivity of CMC-E medium was from the sodium salt of high-viscosity carboxymethyl cellulose as the primary carbon source and gelatin as the sole source of nitrogen. Additional selectivity was from the incorporation of eosin Y, methylene blue, bacitracin, cephalexin, cycloheximide, 5-fluorouracil, neomycin sulfate, and tobramycin. Colonies of cellulolytic *Xanthomonas* spp. on CMC-E were circular, flat, reddish purple, and distinct from those of most other bacterial species because they were in the center of saucer-shaped pits formed in the carboxymethyl cellulose-agar gel. Plating efficiencies for strains of *Xanthomonas* on CMC-E varied from 0 to 111%, but for most strains, the efficiencies were high enough to permit quantitative studies. By use of CMC-E, *X. c. vesicatoria* was recovered from pepper seed, irrigation water, and air samples.

The bacterial spot diseases of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.), caused by sometimes different strains of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, are important in the southeastern United States and continue to reduce yield and quality in both production and vegetable transplant fields in Georgia. Infected transplants may serve as the source of inoculum for northern areas; consequently, the detection of *X. c. vesicatoria* in seed or on transplants results in rejection of certification by the Georgia Department of Agriculture. Although *X. c. vesicatoria* has been reported to survive in association with plant debris (15,17), rotation crops (5), seed (1,7,11), soil (25), volunteer plants (15,25), and weeds (15,18), sources of inocula for recent epidemics in Georgia have not been identified.

Semiselective media have been useful tools for several epidemiologic studies on xanthomonads (2,16,23,26,27), including *X. c. vesicatoria* (1,20). Unfortunately, our results obtained from plating assays of more than 400 different commercial seed lots of tomato and pepper onto semiselective media developed for *X. c. vesicatoria* were inconsistent (R. D. Gitaitis, *unpublished*). In those tests, Tween medium B (20) appeared to be superior to the other media evaluated. It selectively favored the growth of *X. c. vesicatoria* over certain types of microflora in some samples but supported the growth of numerous other bacteria from different samples. Thus, inconsistencies could have been associated with the different microflora that develop because of the different cultivars, extraction methods, geographic locations for seed production, and seed treatments used by the various seed or processing companies. However, before such a hypothesis could be tested, methods were needed to reduce further the interference of nontarget microflora. We describe a semiselective medium that suppresses growth of nontarget microflora, provides a suitable medium for growth, and differentiates colonies of *X. c. vesicatoria* by pit formation.

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MATERIALS AND METHODS

Bacterial strains. Strains of *X. c. campestris* (Pammel) Dowson, *X. c. vesicatoria*, *X. c. vignicola* (Burkholder) Dye, and opportunistic *Xanthomonas* spp. were originally isolated from cabbage, cauliflower, and collards; pepper and tomato; cowpea; and pepper and tomato, respectively (Table 1). Cultures of each strain were maintained in 2 ml of sterile tap water in a screw-cap vial at room temperature or stored for long periods as turbid suspensions in 15% glycerol at −73 C. Strains were subcultured on nutrient agar for production of pure cultures before all tests. Suspensions were prepared from 48- to 72-hr shake cultures in nutrient broth. Cells were harvested by centrifugation at 2,000 *g* for 15 min, and the bacterial pellet was suspended in sterile phosphate buffered saline (PBS) (0.85% NaCl with 0.01 M $KH_2PO_4 \cdot K_2HPO_4$, pH 7.4). Suspensions were adjusted with sterile PBS to 50% transmittance at 600 nm on a spectrophotometer. These suspensions contained approximately 5×10^8 cfu ml^{-1} as determined by standard serial dilution plating on nutrient agar. Serial (1:9) dilutions were made in PBS and 0.1 ml of selected dilutions was spread onto the surface of the test media. Colony counts were made after plates were incubated at 30 C for 7–10 days. The plating efficiency was (cfu on test medium/cfu on nutrient agar) × 100.

Preparation of semiselective differential medium. Each liter of CMC-E was composed of 1.5 g of KH_2PO_4, 6.0 g of K_2HPO_4, 0.2 g of KCl, 1.0 g of $MgSO_4 \cdot H_2O$, 1.0 g of yeast extract, 26.0 g of high-viscosity carboxymethyl cellulose sodium salt (CMC), 2.0 g of eosin Y, 0.4 g of methylene blue, 5.0 ml of stock solution of minor elements (22), 1 drop of anti-bubble agent (Pourite, Analytical Products, Inc., Belmont, CA), and 3.0 g of agar. The salts, yeast extract, dyes, and Pourite were dissolved in deionized water in a Waring blender set at low speed. The CMC and agar were added to the solution as the blender speed was slowly raised to high speed to prevent lumps. The final preparation was sterilized in an oversized flask (500 ml of medium per 2-L flask) at 121 C for 15 min. Gelatin (2 g L^{-1}) sterilized in a separate 100 ml of the basal medium (except that CMC and agar were omitted), was added aseptically to the hot medium immediately after sterilization. Cephalexin, 64 mg L^{-1}; 5-fluorouracil, 12 mg L^{-1}; bacitracin, 100 mg L^{-1}; and neomycin sulfate, 10 mg L^{-1}, were dissolved in 24, 24, 20, and 10 ml of sterile distilled water, respectively (cephalexin was dissolved in water at 80–90 C). One milliliter of stock solution of tobramycin (0.4 mg ml^{-1}) was added to give a final concentration of 0.4 mg L^{-1}. Cycloheximide (100 mg L^{-1}) was dissolved in 2 ml of ethanol. All antibiotics were

filter-sterilized and aseptically added to the hot basal medium immediately after sterilization. The medium was poured into plates immediately, while still hot, to avoid clumping.

Susceptibility to inhibitors. The antibiotics evaluated for selective growth of *X. c. vesicatoria* were selected based on previous reports (2,16,20) and preliminary tests with BBL Sensi-Discs (Becton Dickinson and Co., Cockeysville, MD). Based on those findings, neomycin sulfate, tobramycin, and kasugamycin were tested at various concentrations. Neomycin sulfate was incorporated in CMC-E at 4, 20, and 40 $\mu g\ ml^{-1}$. Tobramycin was incorporated in CMC-E at 0.04, 0.2, and 0.4 $\mu g\ ml^{-1}$. Kasugamycin was incorporated in nutrient agar at 5, 10, 20, 30, and 40 $\mu g\ ml^{-1}$ and at 20 $\mu g\ ml^{-1}$ in CMC-E, CMC-E less bacitracin and neomycin sulfate, and CMC-E less all antibiotics. Plates were incubated at 30 C for 10 days. Plating efficiencies were calculated as described earlier. All tests were replicated three times with four strains.

Recovery of *X. c. vesicatoria* from seed. Bacteria were extracted from 30 commercial seed lots of pepper by modifications of the methods of Fatmi and Schaad (6). Approximately 10,000 pepper seeds (75 g) and 150 ml of PB-2 buffer (0.05 M $KH_2PO_4 \cdot K_2HPO_4$ plus 0.02% Tween 20, pH 7.4) were placed in a heavy-duty, reinforced, plastic bag and processed for 15 min in a stomacher homogenizer (Tekmar Co., Cincinnati, OH). The liquid phase was poured through a common, wire-mesh kitchen strainer, which had been rinsed in ethanol and air-dried, and then centrifuged at 400 *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 12,000 *g* for 15 min. The resulting pellet from this centrifugation was suspended in 5 ml of PB-2. Serial (1:9) dilutions were made in PBS and 0.1-ml aliquots from the various dilutions (10^0 to 10^{-3}) were spread on the surface of test media. All plates were incubated at 30 C, and colonies were counted after 7–10 days. All tests were replicated three times. Selected colonies were characterized and tested for pathogenicity as previously described (9,21).

For tests on quantitative recovery of bacteria from pepper seed, known populations of *X. c. vesicatoria* were added to randomly selected seed lots. Aliquots of 0.1 ml of suspensions of *X. c. vesicatoria* prepared as described earlier were added to 75 g of pepper seed in 150 ml of buffer. Populations of *X. c. vesicatoria* ranged from approximately 1×10^1 to 1×10^5 cfu ml^{-1}. Seed samples were processed as described earlier and aliquots of selected dilutions were plated on CMC-E, CMC-E without bacitracin and neomycin sulfate, Tween medium B, and Tween medium B plus bacitracin and neomycin sulfate. Colonies typical of *X. c. vesicatoria* were enumerated, subcultured, and characterized (9,21).

Residual chlorine on seed treated with calcium hypochlorite was neutralized by the addition of 0.2% (w/v) sodium thiosulfate (STS) in PB-2 as described by Maddox and Hubbard (19). Known amounts of *X. c. vesicatoria* prepared as described earlier were added to 150 ml of either PB-2 or PB-2 plus 0.2% STS (final population was approximately 1×10^5 cfu ml^{-1}). Bacteria were exposed to the buffers for 1 hr and then plated on CMC-E and nutrient agar. Tests were replicated three times with four strains.

Recovery of *X. c. vesicatoria* from air samples and irrigation water. Field experiments were conducted at the Blackshank Farm of the Coastal Plain Experiment Station near Tifton, GA. Plots were established on a Fuquay sand soil. Coated tomato seeds (cv. Ohio-7870) were direct-seeded on 14 March 1990 in raised beds (2 × 33.3 m) with four rows spaced 35 cm apart. The experimental design was a randomized complete block with four replications. Each plot consisted of three beds. Recommended seedbed preparation, planting, fertilization, irrigation, and pest control practices were used (8,12,13). Plants were clipped with a PTO-driven, rotary, transplant clipper on a 3- to 4-day schedule begun 45 days after planting.

Greenhouse-grown tomato plants (cv. Ohio 7870) were inoculated with *X. c. vesicatoria* (strain XCV 89-18) by the Carborundum-rub method previously described (8). Plants were placed in a mist chamber for 48 hr and then removed to a greenhouse bench. After symptoms developed, two diseased plants were transplanted to the center of each field plot on 23 April.

Flowable cupric hydroxide (Kocide 606) was applied at 1.12 kg a.i. ha^{-1} with an irrigation-system simulator (3,14,28). Either irrigation water or the cupric hydroxide suspension was applied with the simulator moving at 3.8 m min^{-1} and delivering 0.635 cm of fluid (63,500 L ha^{-1}). Both the irrigation water and cupric hydroxide suspension were collected on 1-m^2 plastic sheets stretched over tubular PVC frames (1 × 1 × 0.5 m) that were placed above transplants in the center of each plot. Approximately 250 ml from each treatment was collected in

Table 1. Plating efficiences for different strains of *Xanthomonas campestris* pv. *vesicatoria* (XCV), *X. c. vignicola* (XVG), *X. c. campestris* (XCC), and opportunistic xanthomonads (CVP)[w] on CMC-E and Tween B media compared with nutrient agar

Strain[x]	Host	Mean percent efficiency	
		CMC-E	Tween B
XCV 89-18 T	Tomato	111[y]	217
XCV 87-2 P	Pepper	103	111
XCV 82-12 P	Pepper	84	2
XCV 71-21 P	Pepper	60	136
XCV 87-13 P	Pepper	54	129
XCV 84-85 P	Pepper	52	123
XCV 83-45 T	Tomato	45	22
XCV 82-23 P	Pepper	40	62
XCV 84-89 P	Pepper	33	71
XCV 87-61 P	Pepper	17	67
XCV 81-11 P	Pepper	3	28
XCV 83-45 T	Tomato	0	60
XVG 5A	Cowpea	98	NT[z]
XVG 80-8	Cowpea	77	NT
XVG 3A	Cowpea	76	NT
XVG 81-27	Cowpea	75	NT
XVG 81-30	Cowpea	74	NT
XVG 81-33	Cowpea	47	NT
XVG 80-4	Cowpea	45	NT
XVG 80-5	Cowpea	33	NT
XVG 80-11	Cowpea	33	NT
XVG 81-35	Cowpea	30	NT
XVG 80-18	Cowpea	23	NT
XCC C-88	Cabbage	88	NT
XCC 81-4	Cauliflower	59	NT
XCC 88-JB	Cabbage	58	NT
XCC 81-2	Cabbage	55	NT
XCC 86-1028-3	Cabbage	48	NT
XCC 87-1	Collards	33	NT
CVP 81-10	Pepper	79	NT
CVP BPR	Tomato	69	NT
$LSD_{0.05}$		30.3	21.5

[w] Pectolytic xanthomonads differing from *X. c. vesicatoria* (9).
[x] For most strains, the first two digits indicate year that the strain was isolated originally.
[y] Values are the mean of three plates.
[z] Not tested on this medium.

plastic centrifuge bottles and centrifuged at 12,000 *g* for 10 min. Pellets were suspended in 5 ml of PB-2. Suspensions were serially diluted (1:9) and aliquots of 0.1 ml from selected dilutions were spread on nutrient agar, CMC-E, or Tween medium B. Plates were incubated at 30 C for 10 days. Colonies typical of *X. c. vesicatoria* were enumerated, subcultured, characterized, and tested for pathogenicity (9,21).

Air samples were collected with an Andersen six-stage viable microbial impaction sampler (Andersen Samplers Inc., Atlanta, GA) as previously described (21). The plants were clipped just before the collection of air samples. Air samples also were collected before and after chemigation (14) and irrigation on 2, 11, and 21 May. Plates of the test media were incubated at 30 C for 10 days. Colonies typical of *X. c. vesicatoria* were characterized as described earlier.

RESULTS

Susceptibility to inhibitors. All strains of *X. c. vesicatoria* tested had plating efficiencies of 100% or more when neomycin sulfate was incorporated in CMC-E at ≤20 μg ml^{-1}. However, when the neomycin sulfate concentration equaled 40 μg ml^{-1}, plating efficiencies ranged from 0 to 100% with a mean of 27%. Similarly, all strains had plating efficiencies of 100% or more with tobramycin in CMC-E at ≤0.2 μg ml^{-1}. However, with tobramycin at 0.4 μg ml^{-1}, efficiencies ranged from 0 to 78% with a mean of 30%. Kasugamycin incorporated in nutrient agar at 5, 10, 20, 30, and 40 μg ml^{-1} had no effect on the recovery of *X. c. vesicatoria*. However, when it was incorporated at 20 μg ml^{-1} in CMC-E, CMC-E less bacitracin and neomycin sulfate, and CMC-E less all antibiotics, mean efficiencies were 0, 34, and 100%, respectively.

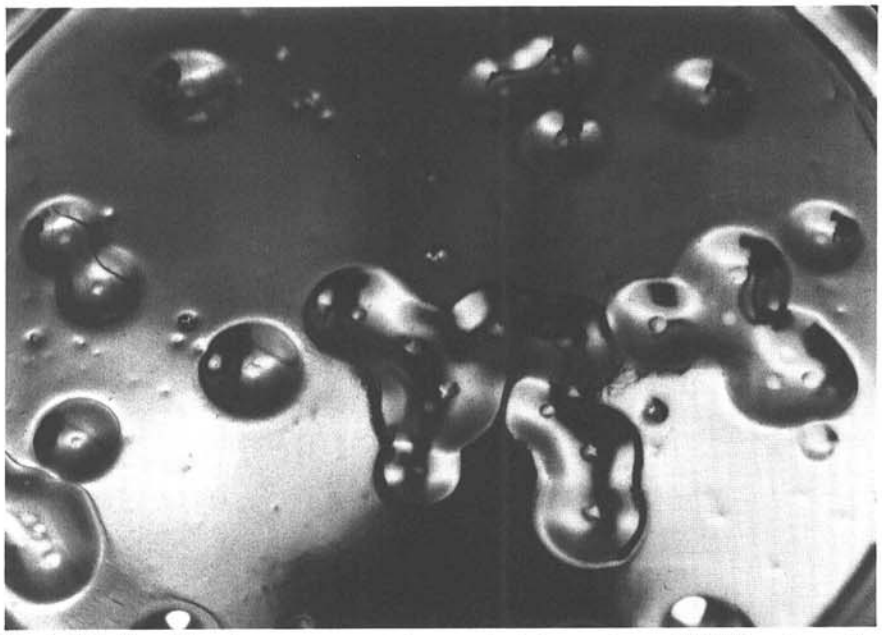

Fig. 1. Colonies of *Xanthomonas campestris* pv. *vesicatoria* growing on CMC-E medium after 5 days. Note the saucer-shaped depressions attributable to cellulolytic activity.

Table 2. Plating efficiencies of *Xanthomonas campestris* pv. *vesicatoria* with nutrient agar (NA) and CMC-E when exposed as a cell suspension[x] for 1 hr in PBT-2 or PBT-2 containing 0.2% sodium thiosulfate

		Plating efficiencies (%)	
Medium	Strain	PBT-2[y]	PBT-2 + 0.2% Na thiosulfate
CMC-E	XCV 89-18 T	86 a[z]	56 b
NA	XCV 89-18 T		55 b
CMC-E	XCV 83-47 T	39 a	8 b
NA	XCV 83-47 T		86 b
CMC-E	XCV 85-275 T	26 a	19 a
NA	XCV 85-275 T		73 b
CMC-E	XCV 82-12 P	84 b	133 a
NA	XCV 82-12 P		155 a

[x] 1×10^5 cfu ml^{-1} of buffer.
[y] PBT-2 (6).
[z] Plate efficiencies based on populations recovered on nutrient agar (NA). Separation of means by Waller-Duncan's multiple range test; means with the same letter within each horizontal row are not significantly different ($P = 0.05$).

Growth of *X. c. vesicatoria* on CMC-E. After 7 days, colonies of *X. c. vesicatoria* and other cellulolytic xanthomonads grown on CMC-E were 1–2 mm in diameter, butyrous, glistening, opaque, and reddish purple. Initially colonies were flat, circular, and had entire margins. After 14 days, colonies were 5–7 mm in diameter, irregular with undulate margins, and either slightly umbonate or convex. In addition, the cellulolytic xanthomonads caused saucer-shaped depressions in the medium (Fig. 1). After prolonged growth, the depressions continued to expand and coalesced to form irregular patterns.

Recovery efficiency of semiselective media. Plating efficiencies on CMC-E for 12 strains of *X. c. vesicatoria* ranged from 0 to 111% and for 12 strains of *X. c. vignicola* ranged from 23 to 98% (Table 1). The mean efficiencies for *X. c. vesicatoria*, *X. c. vignicola*, *X. c. campestris*, and opportunistic xanthomonads were 50, 56, 57, and 74%, respectively. In contrast, with Tween medium B, the mean for *X. c. vesicatoria* was 86%.

Recovery of *X. c. vesicatoria* from pepper seed. *X. c. vesicatoria* was recovered from five, four, and zero of 30 commercial seed lots assayed with CMC-E, Tween medium B, and nutrient agar, respectively. Depending on the sample, CMC-E prevented the growth of 75–95% of nontarget bacteria. All *X. c. vesicatoria* recovered from seed were strict aerobes, positive for the hydrolysis of aesculin, carboxymethyl cellulose, casein, and gelatin; positive for catalase; but negative for pectolytic activity, nitrate reduction, oxidase production, and starch hydrolysis. All strains caused typical bacterial spot symptoms in inoculated greenhouse-grown pepper plants.

Opportunistic xanthomonads also were recovered from three of 30 commercial seed lots only on CMC-E. These strains were similar to *X. c. vesicatoria* except for starch hydrolysis and pectolytic activity. In addition, the opportunists were not pathogenic in pepper plants.

X. c. vesicatoria was recovered from experimentally contaminated seed on CMC-E, CMC less bacitracin and neomycin sulfate, Tween medium B, and Tween medium B plus bacitracin and neomycin sulfate if bacterial concentrations in the liquid phase of the seed extract before centrifugation were either 1×10^3 or 1×10^5 cfu ml^{-1}. However, when the bacterial concentration was 1×10^1 cfu ml^{-1}, the pathogen was not detected on Tween medium B.

Exposure of three out of four strains of *X. c. vesicatoria* to sodium thiosulfate reduced their viability on both nutrient agar and CMC-E (Table 2). Strains with reduced plating efficiencies after exposure to sodium thiosulfate had mean reductions of 45 and 28% on CMC-E

and nutrient agar, respectively.

Recovery of xanthomonads from air samples and irrigation water. Airborne xanthomonads were recovered on all sampling dates but only with the CMC-E. Mean populations recovered over nontreated plants before irrigation were 36, 133, and 962 cfu m^{-3} of air on 2, 11, and 21 May, respectively, whereas after irrigation, the means were 9, 177, and 424 cfu m^{-3} of air on 2, 11, and 21 May, respectively (LSD [$P = 0.05$] by date was 471). With the plants treated with cupric hydroxide, prechemigation populations averaged 159, 44, and 4,529 cfu m^{-3} of air for 2, 11, and 21 May, respectively, whereas after chemigation, the respective averages were 97, 203, and 327 cfu m^{-3} (LSD [$P = 0.05$] by date was 1,814). *X. c. vesicatoria* was detected in the irrigation water only on 11 and 21 May at populations of $\leq$0.1 and 52 cfu ml^{-1}, respectively. No bacteria were recovered from cupric hydroxide suspensions on any date. In all cases, CMC-E prevented the growth of $\geq$90% or more of the nontarget microbes.

Xanthomonads recovered from air samples on 2 May were all pectolytic, positive for starch hydrolysis, and not pathogenic to pepper. However, on 11 and 21 May, all xanthomonads recovered from both air and water samples were pathogenic in pepper and negative for starch hydrolysis and pectolytic activity on CVP (4).

DISCUSSION

A number of phytopathogenic bacteria, including *X. c. vesicatoria*, display cellulolytic activity (10). Paul et al (24) reported that *X. c. vesicatoria* has high β-glucosidase and endoglucanase (C_x) activity but that exoglucanase (C_1) activity was feeble. They concluded that *X. c. vesicatoria* has the ability to completely degrade cellulose but that it is more efficient in CMC as opposed to filter paper. CMC was chosen as a carbon source for this reason and after a preliminary screening of other compounds including: arabin, cellobiose, dextrin, ethionine, isatin, lyxose, monosodium glutamate, saccharin, salicin, sarcosine, and xylan. CMC as a carbon source and as a gelling agent in a semiselective medium not only allows for selective growth but also differentiates cellulolytic bacteria from noncellulolytic bacteria by the saucer-shaped pits that are formed. This characteristic is similar to the depressions formed in CVP medium (4) by pectolytic *Erwinia* spp. Furthermore, like the recovery of soft rot *Erwinia* on CVP medium, cellulolytic xanthomonads may be detected on CMC-E by the presence of pits even when the ratio of noncellulolytic bacteria to cellulolytic ones is extremely high. In addition, *Xanthomonas* will continue to grow on CMC-E forming both larger diameter colonies and depressions, whereas most saprophytes do not continue to grow or form depressions.

Gelatin was chosen as a nitrogen source after a preliminary screening of KNO_3, NH_4Cl, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, gelatin, and peptone. With gelatin as a sole source of nitrogen, the medium should be selective only for gelatin-hydrolyzing bacteria.

Many of the antibiotics tested in the sensi-disc test were inhibitory to *X. c. vesicatoria*. However, ampicillin, bacitracin, carbenicillin, cephalexin, cloxacillin, methicillin, nafcillin, neomycin sulfate, oxacillin, and penicillin had no effect on the growth of *X. c. vesicatoria*. Bacitracin and neomycin sulfate were selected for incorporation in CMC-E. Cephalexin, cycloheximide, 5-fluorouracil, and tobramycin were selected on the basis of previous research (20). The combination of inhibitors in CMC-E resulted in the reduced plating efficiency for *X. c. vesicatoria* compared with nutrient agar, Tween medium B, or the basal CMC medium (CMC-E without antibiotics).

If one's primary objective is the detection of cellulolytic xanthomonads in seed or the environment, then relatively low plating efficiencies are acceptable. The mean plating efficiency for *X. c. vesicatoria* was 50% for CMC-E compared with NA. However, the value of a semiselective medium is its ability to inhibit nontarget microbes while permitting growth and differentiation of a reasonable proportion of the target species. The CMC-E medium is adequate for most quantitative and epidemiologic studies, particularly for controlled experiments where strains with high plating efficiencies are used. However, for quantifying wild-type strains, certain ones may not be recovered or will be recovered at less than representative numbers. Consequently, the concentration of certain inhibitors may need to be reduced.

In particular, we found that tobramycin and neomycin sulfate were critical to plating efficiencies. However, even without tobramycin and neomycin sulfate, the medium still retains substantial selectivity, and the formation of saucer-shaped depressions allows for the differential detection of cellulolytic bacteria. This aspect was particularly advantageous in the recovery of xanthomonads from air and water samples taken from field plots, where the xanthomonads were not detected with NA or Tween medium B because of the presence of large populations of saprophytic bacteria. However, with CMC-E, cellulolytic xanthomonads were isolated from depressions. When numerous microbial contaminants are on the surface of CMC-E medium, xanthomonads are rarely recovered in a pure culture, some contaminants may grow on by-products formed by the cellulolytic activity and develop in depressions as "secondary" inhabitants. But such bacteria do not grow well in the absence of cellulolytic bacteria, and when colonies in depressions in primary plates are streaked on fresh CMC-E plates, the cellulolytic xanthomonads are easily detected.

The recovery of *X. c. vesicatoria* from pepper seed artificially infested with small populations of the pathogen demonstrates that the selectivity and sensitivity of CMC-E is sufficient for seed assays. Although results were similar with CMC-E and Tween medium B in tests of 30 commercial seed lots, an infestation in one additional lot was detected with CMC-E. Moreover, the distinctive pitting on CMC-E allowed for a fast and relatively easy search of the plates for colonies of the pathogen. Thus, relatively high plating efficiencies are not necessarily required for detection of target bacteria. In contrast, using hypochlorite solutions to reduce surface populations of microflora on seed may create a problem with the detection of xanthomonads. Residual hypochlorite and chlorine may directly affect the viability of xanthomonads in seed extracts. The inactivation of the chlorine with sodium thiosulfate also may reduce plating efficiencies for xanthomonads. These reductions occurred on both nutrient agar and CMC-E. Consequently, the advantage of testing chlorine-treated seed, namely a reduction of saprophytes on seed surfaces, may be offset by reduced plating efficiencies for some strains when exposed to sodium thiosulfate.

As expected, no bacteria were recovered directly in cupric hydroxide suspensions, whereas both opportunistic and pathogenic xanthomonads were detected in irrigation water collected below the simulator. This supports previous findings (21) that irrigation washes bacteria from the air and demonstrates the potential role of irrigation in the dissemination of bacteria after clipping. Unlike the previous test however, there was no consistent pattern of reduced numbers of bacteria in the air after irrigation or chemigation. Only opportunistic xanthomonads were detected on the first sampling date, apparently because the disease had not begun to spread widely in the plot and relatively small populations of *X. c. vesicatoria* were present. However, as disease incidence increased, as many or more bacteria were recovered above plants sprayed with cupric hydroxide as above control plants. If aerosolized bacteria are produced either from epiphytic populations or from clipping of leaves with lesions, higher numbers would have been expected over control plants. However, it is premature to draw conclusions from this preliminary study concerning the effect that overhead

irrigation and chemigation may have on bacterial aerosols. The simulator delivers water much lower to the ground compared with either center pivots or solid-set sprinklers currently used by commercial transplant growers. It is likely that this affects the number of bacteria washed from the air. Furthermore, bacteria may have moved in a laminar flow of air into the space above treated plants because of the close proximity of control plots. Further research is required to answer these questions.

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