Selective Medium for *Xanthomonas campestris* pv. *pruni*

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ABSTRACT

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A simple selective medium was developed for the detection and isolation of *Xanthomonas campestris* pv. *pruni*. The selectivity and sensitivity of *X. c.* pv. *pruni* selective medium (XPSM) were evaluated using 9 *X. c.* pv. *pruni* strains, 14 strains of eight other *Xanthomonas* nomen species, and one strain each of *Agrobacterium tumefaciens, Corynebacterium michiganense, Erwinia stewartii,* and *Pseudomonas pseudoalcaligenes* subsp. *citrulli*. The plating efficiencies of all *X. c.* pv. *pruni* strains were the same or higher on XPSM than on nutrient agar supplemented with glucose. Two strains of *X. c.* pv. *begoniae* and one strain of *X. c.* pv. *pelargonii* grew on XPSM. However, the plating efficiencies of these strains on XPSM were generally low, and the colony appearance of these strains was clearly distinct from that of *X. c.* pv. *pruni*. Colonies of two strains each of *X. c.* pv. *phaseoli, X. c.* pv. *manihotis, X. c.* pv. *vesicatoria,* and one strain of *P. pseudoalcaligenes* subsp. *citrulli* appeared on XPSM, but growth was suppressed. No colonies of any of the other strains developed on XPSM. *X. c.* pv. *pruni* virulence, sensitivity to lysis by two phages, and ability to produce characteristic yellow, mucoid colonies on nutrient agar supplemented with glucose were not detectably altered after growth on XPSM. Added *X. c.* pv. *pruni* was quantitatively recovered from soil containing about 10^2–10^3 colony-forming units per gram and was recovered from leaf extracts containing less than 10^2–10^3 colony-forming units per milliliter. *X. c.* pv. *pruni* was also readily detected in and isolated from extracts of lesions on naturally infected apricot leaves. Soil and leaf bacteria were generally suppressed on XPSM, and only an occasional fungal colony developed from soil samples on this medium.

Xanthomonas campestris pv. *pruni* (4) is the causal agent of bacterial spot disease of *Prunus* species. Epidemiologic studies have been hampered by the lack of a sensitive, reliable method for specifically detecting the bacterium. A selective medium would be useful for the quantitative detection, isolation, and monitoring of *X. c.* pv. *pruni* in natural situations.

The purpose of this work was to develop and evaluate a selective medium for *X. c.* pv. *pruni*. Selective isolation media for various *Xanthomonas* species have been described (6,7), but none has been useful for *X. c.* pv. *pruni*. Medium D-5 (6) was reported to selectively favor the growth of *Xanthomonas* and *Agrobacterium* spp. while suppressing the growth of *Pseudomonas* spp. However, our experience has been that medium D-5 did not support growth of *X. c.* pv. *pruni* as well as did nutrient agar supplemented with glucose. The plating efficiency of a single strain of *X. campestris* on medium D-5 was 78% relative to general purpose medium 523 (6). Similarly, the plating efficiency of this *X. campestris* strain on medium 523 was about 80% relative to nutrient agar supplemented with glucose (6). A cellobiose-yeast extract agar medium, described as semiselective for *X. c.* pv. *pruni*, was used to monitor leaf surface populations of this bacterium (3); however, no comparative qualitative or quantitative data are available for assessing the selectivity and sensitivity of this medium for *X. c.* pv. *pruni*.

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MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this work are listed in Table 1. *Xanthomonas* spp., except *X. fragariae* strains, were maintained and assayed on Difco nutrient agar supplemented with 2% glucose (NGA). *X. fragariae* strains were maintained and assayed on medium B (5). *Agrobacterium tumefaciens* was maintained and assayed on nutrient agar supplemented with 0.1% yeast extract, 0.5% sucrose, and 0.002 M magnesium sulfate ($MgSO_4$). *Corynebacterium michiganense* was maintained and assayed on medium 523 (6). *Erwinia stewartii* was maintained and assayed on Difco nutrient agar supplemented with 0.5% yeast extract and 0.5% glucose. *Pseudomonas pseudoalcaligenes* subsp. *citrulli* (8) was maintained and assayed on Difco Pseudomonas agar P. All strains were subcultured by transfer to fresh media at 1–2 wk intervals and stored at 3 C.

Selective medium. The *X. c.* pv. *pruni* selective medium (XPSM) contained 2 g of alginic acid (Sigma Chemical Co., St. Louis, MO), 0.2 g of 8-azaguanine (Sigma), 2 mg of nicotinic acid (Sigma), and 3 mg of cysteine (Sigma) per liter of basal medium (0.08% monobasic potassium phosphate [KH_2PO_4], 0.08% dibasic potassium phosphate [K_2HPO_4], and 0.01% $MgSO_4$). However, during the development and evaluation of XPSM, the effects of 4 g of alginic acid and 2 g of azaguanine per liter of basal medium were tested. After autoclaving, chlorothalonil (BRAVO 75 WP, Diamond Shamrock Corp., Cleveland, OH 04414), and kasugamycin (Sigma) were added aseptically to final concentrations of 80 μg/ml and 16 μg/ml, respectively. The medium contained 1.5% Difco Bacto-agar.

Dilution plating. Bacteria were grown overnight in shake cultures of Difco nutrient broth supplemented with 0.2% glucose and 0.5% sodium chloride (NaCl) (NGSB) at 27–29 C. Cells were collected by low-speed centrifugation at 5,000 *g* for 15–20 min and suspended in sterile distilled, demineralized water. After adjusting cell suspensions to 70–80% T at 620 nm to contain approximately 1–3 × 10^8 colony-forming units (CFU) per milliliter as determined by standard dilution plating on NGA, serial tenfold

dilutions were made in sterile distilled, demineralized water. One-tenth milliliter of diluted cell suspension was spread, with an L-shaped glass rod, over the surface of 10–20 ml of appropriate media in each of three plates. Plates were incubated at 27 C. Colonies were counted 5–7 days later.

Characterization of bacterial isolates from XPSM. The time required for colonies to develop and colony shape, form, and texture were initially used to distinguish *X. c.* pv. *pruni*. Susceptibility of selected single-colony isolates from XPSM to lysis by pruniphages PP1 and PP3 was determined by spotting droplets of appropriately diluted, phage-containing preparations on the surface of 5 ml of NGA supplemented with 0.5% NaCl seeded with 0.5 ml of cell from an overnight NGSB shake culture at 24 C (2). Selected colonies were also replica transferred to NGA to observe the development of yellow, mucoid growth characteristic of xanthomonads. Finally, identification of *X. c.* pv. *pruni* was confirmed by pathogenicity tests (1).

Recovery of *X. c.* pv. *pruni* from soil. Field-collected soil (Galestown gravelly loamy sand) was suspended in sterile phosphate-buffered saline (PBS) containing 0.8% NaCl, 0.02% KH_2PO_4, 0.1% dibasic sodium phosphate (Na_2HPO_4), and 0.02% potassium chloride (KCl) at the rate of 1 g in 10 ml of PBS and thoroughly mixed. No attempt was made to adjust the moisture content of the soil samples collected at different times. *X. c.* pv. *pruni* (strain XP1) cells collected from overnight NGSB shake cultures at 27 C were adjusted turbidimetrically to contain approximately 1×10^8 CFU/ml. Serial tenfold dilutions were made in PBS. Cell suspension titers were determined by plating 0.1 ml of appropriate dilutions in sterile PBS on three replicate XPSM plates. One milliliter of appropriately diluted cell suspension was added to the soil suspension. After thorough mixing, the mixture was serially diluted, and 0.1 ml was spread, as above, over the surface of XPSM in each of three plates. All plates were incubated at 27 C for 5–7 days.

Recovery of *X. c.* pv. *pruni* from leaf tissue extracts. Composite tissue samples consisted of 15 disks excised with a 5-mm-diameter cork borer from each of two batches of randomly selected leaves from greenhouse-grown Sunhigh peach seedlings. Each composite 15-leaf tissue disk sample was homogenized with a sterile mortar and pestle in 10 ml of sterile PBS. The homogenate was then diluted to 30 ml with sterile PBS. After plating 0.1-ml aliquots of the undiluted extracts on XPSM, *X. c.* pv. *pruni* strain XP1 was added to each extract. After thorough mixing, 0.1-ml aliquots of each mixture were plated on XPSM. All plates were incubated at 27 C for 5–7 days.

Isolation of *X. c.* pv. *pruni* from naturally infected tissue. Naturally infected leaves with characteristic *Prunus* bacterial spot lesions were collected from apricot seedling trees. Individual lesions were excised with a 5-mm-diameter cork borer. Individual lesions or pools of 10 lesions were triturated in 1 ml of sterile PBS per lesion. After serial tenfold dilution in sterile PBS, 0.1 ml of appropriately diluted extract was spread, as above, over the surface of NGA and XPSM. All plates were incubated at 27 C for 5–7 days.

Table 1. Bacterial strains used for evaluation of medium selective for *Xanthomonas campestris* pv. *pruni*

Nomenspecies	Strain designation: Laboratory	Strain designation: Source	Source[a]
Xanthomonas c. pv. *pruni*	XP1	ApB	Original
	XP9		1
	XP10		1
	XP12	417	2
	XP15	420	2
	XP19	926	2
	XP20	2587	2
	XP21	2588	2
	XP22	10016	3
X. c. pv. *begoniae*	XB1	2812	4
	XB2	3499	4
	XB3	3382	4
X. c. pv. *campestris*	XCm1	B-24	5
X. c. pv. *cucurbitae*	XCu1	2493	2
X. c. pv. *manihotis*	XM1	CBB-8	7
	XM6	CIAT-1105	7
X. c. pv. *pelargonii*	XPg1	84	4
X. c. pv. *phaseoli*	XPh1	1811	2
	XPh2	2064	2
X. c. pv. *vesicatoria*	XV1	XV-24	4
	XV2	XV-26	4
X. fragariae	XF3	NCPPB-2473	2
	XF4	ICPB-102	6
Erwinia stewartii	ES1		8
Pseudomonas pseudoalcaligenes subsp. *citrulli*	PPsc1		8
Agrobacterium tumefaciens	AT1	15955	3
Corynebacterium michiganense	CM1	CM9	9

[a] 1 = M. Davis, Rutgers University, New Brunswick, NJ 08903. 2 = National Collection of Plant Pathogenic Bacteria, Harpenden, England. 3 = American Type Culture Collection, Rockville, MD. 4 = M. Sasser, Department of Plant Sciences, University of Delaware, Newark 19711. 5 = N. W. Schaad, Department of Plant Pathology, Georgia Experiment Station. 6 = M. P. Starr, International Collection of Phytopathogenic Bacteria, University of California, Davis. 7 = W. E. Fry, Department of Plant Pathology, Cornell University, Ithaca, NY 14853. 8 = R. Goth, USDA, SEA, NER, HSI, Beltsville, MD 20705. 9 = R. Harris, USDA, SEA, NER, PPI, Beltsville, MD 20705. Originally obtained from M. P. Starr, International Collection of Phytopathogenic Bacteria, University of California, Davis.

Table 2. Comparative colony development of several *Xanthomonas campestris* pv. *pruni* strains on selective (XPSM) and nonselective (NGA) media[a]

Strain	Colonies per plate[b]: NGA	Colonies per plate[b]: XPSM	Plating efficiency[c]	Colony diameter on XPSM (mm)
XP1	33.0 ± 9.0	79.0 ± 19.2	2.4	1.5–2.0
XP9	78.3 ± 30.5	312.0 ± 66.8	4.0	1.0
XP10	8.0 ± 1.4	26.7 ± 2.5	3.9	1.0
XP12	165.7 ± 15.2	141.3 ± 35.9	0.9	1.0–1.5
XP15	14.3 ± 3.2	24.3 ± 1.2	1.7	1.5–2.0
XP19	1.7 ± 2.9	14.0 ± 3.6	8.2	1.0
XP20	118.0 ± 13.0	171.7 ± 23.6	1.4	1.0–1.5
XP21	21.0 ± 6.9	157.7 ± 16.2	7.5	1.0–1.5
XP22	109.5 ± 21.9	299.3 ± 33.8	2.7	1.5–2.0

[a] XPSM = *X. c.* pv. *pruni* selective medium; NGA = nutrient agar supplemented with 2% glucose.
[b] Average number of colonies per plate from three plates, followed by standard deviation. Count was made after incubation for 7 days at 27 C.
[c] Plating efficiency = $\frac{\text{Avg. number of colonies on XPSM/plate}}{\text{Avg. number of colonies on NGA/plate.}}$

RESULTS

Growth of *X. c.* pv. *pruni* on XPSM. In five tests, the average number of colonies of *X. c.* pv. *pruni* strain XP1 that developed on XPSM was 1.8 (range = 1.3–2.4) times greater than on NGA (Table 2). Generally, more colonies of all

nine *X. c.* pv. *pruni* strains tested developed on XPSM than on NGA. After incubation for 5–7 days at 27 C, colonies were 1–2 mm in diameter with entire margins. The colonies were convex, smooth, mucoid and glistening, and opaque. *X. c.* pv. *pruni* colonies were generally largest on XPSM containing 0.4% alginic acid.

The apparent rate of growth and final colony appearance on XPSM were similar with 0.02 or 0.2% 8-azagaunine, but the medium was clearer and the colonies were more easily seen on XPSM containing 0.02% 8-azaguanine. Colonies of *X. c.* pv. *pruni* on XPSM appeared greyish white macroscopically. However, when viewed microscopically against a dark background, the colonies appeared buff with light yellow centers. There was also some variation in the apparent growth rates of strains of *X. c.* pv. *pruni* based on colony size (Table 2). However, all strains grew faster and more extensively on NGA, although with lower plating efficiency, than on XPSM.

Single colonies on XPSM were randomly selected and replica transferred to NGA with sterile toothpicks. All single-colony selections from XPSM produced typical yellow, smooth, mucoid, convex colonies within 2–3 days at 27 C. Similarly, randomly selected single-colony isolates directly from XPSM plates were propagated in NGSB shake cultures at 27–29 C for 18–20 hr. All single-colony selections that were tested were sensitive to lysis by pruniphages PP1 and PP3 (2) and produced characteristic lesions on artificially inoculated Sunhigh peach seedling leaves (1).

Selectivity of XPSM. Among the bacterial strains tested, no colonies of *X. fragariae, X. c.* pv. *cucurbitae, X. c.* pv. *begoniae, X. c.* pv. *campestris, Agrobacterium tumefaciens, Corynebacterium michiganense,* or *Erwinia stewartii* developed on XPSM following dilution plating (Table 3). Colonies of *X. c.* pv. *phaseoli* (strains XPh1 and XPh2), *X. c.* pv. *manihotis* (strains XM1 and XM6), *X. c.* pv. *vesicatoria* (strains XV1 and XV2), and *P. pseudoalcaligenes* subsp. *citrulli* (strain PPsc1) were visible on XPSM only after incubation at 27 C for 7–8 days. In addition, colonies of these strains were minute (0.1–0.5 mm diam), apparently flat, translucent, and difficult to see without the aid of a microscope.

Colonies of *X. c.* pv. *begoniae* (strain XB3) and *X. c.* pv. *pelargonii* (strain XPg1) on XPSM were about 0.5–1.0 mm in diameter. *X. c.* pv. *begoniae* (strain XB1) colonies on XPSM were generally larger (1–2 mm diam) and similar in size and color to XP1 colonies. However, the plating efficiencies of these strains on XPSM were generally less than one, with the exception of *X. c.* pv. *phaseoli* (strain XPh1) and *X. c.* pv. *manihotis* (strain XM1). Colonies of *X. c.* pv. *begoniae* (strains XB1 and XB3) and *X. c.* pv. *pelargonii* (strain XPg1) were only slightly raised or flat, respectively. In addition, none of these three strains was sensitive to lysis by pruniphages PP1 and PP3.

Furthermore, a reaction resembling hypersensitivity occurred within 24–48 hr on Sunhigh peach seedling leaves at inoculation sites following infiltration of about 10^8 CFU/ml. Finally, restricted chlorotic spots developed at similar inoculation sites 7–10 days after infiltration of 10^6 CFU/ml. These spots were not typical or characteristic of lesions produced by *X. c.* pv. *pruni.* Typically, lesions produced by *X. c.* pv. *pruni* strains on Sunhigh peach seedling leaves are readily visible on both upper and lower surfaces. The restricted chlorotic spots at sites inoculated with *X. c.* pv. *begoniae* (strains XB1 and XB3) and *X. c.* pv. *pelargonii* (strain XPg1) were clearly visible only on the upper leaf surface. On the lower leaf surface, areas corresponding to these restricted chlorotic spots were not clearly visible macroscopically.

Recovery of *X. c.* pv. *pruni* from soil. *X. c.* pv. *pruni* (strain XP1) was readily recovered from natural soil to which 10^2 and 10^3 CFU/g were added (Table 4). *X. c.* pv. *pruni* (strain XP1) colonies that developed on XPSM from these soil samples were generally smaller (0.5–1.5 mm diam) than colonies that developed from suspensions of pure culture cells (1–2 mm). The rates of recovery of *X. c.* pv. *pruni* (strain XP1) from 1-g soil samples to which 10^2 and 10^3 CFU were added were nearly quantitative, ranging from 75 to 91%. Colonies of *X. c.* pv. *pruni* were easily differentiated after incubation for 5–7 days at 27 C.

The background microbial populations in 10-g soil samples dispersed in 100 ml of sterile PBS contained approximately 1–3 $\times$ 10^4 recoverable colony-forming units per milliliter on XPSM. Only an occasional fungal colony from the background microbial population of these soil samples developed on XPSM. Approximately 400–800 colonies of soil bacteria appeared on NGA within 2–3 days at 27 C before colonies of *X. c.* pv. *pruni* developed. However, these background bacterial colonies were suppressed on XPSM and were generally less than 1 mm in diameter. Thus, colonies of *X. c.* pv. *pruni* that developed from soil samples containing about 100–1,000 *X. c.* pv. *pruni* (strain XP1) colony-forming units per milliliter were easily differentiated. Finally, fewer than 100 *X. c.* pv. *pruni* (strain XP1) colony-

Table 3. Comparative colony development of several phytopathogenic bacteria on selective (XPSM)[a] and nonselective media

Bacterium	Strain	Colonies per plate[b] Medium[c]	Colonies per plate[b] XPSM	Plating efficiency[d]	Colony diameter on XPSM (mm)
Xanthomonas c. pv. *pruni*	XP1	195.3 ± 64.2	369.7 ± 188.4	1.89	1.5–2.0
X. c. pv. *begoniae*	XB1	280.3 ± 26.6	242.3 ± 32.0	0.86	1.0–2.0
	XB2	325.0 ± 32.0	...[e]	...	
	XB3	330.0 ± 26.4	52.5 ± 0.7	0.16	0.5–1.0
X. c. pv. *campestris*	XCm1	326.3 ± 184.2	...		
X. c. pv. *cucurbitae*	XCu1	274.3 ± 18.0	...	...	
X. fragariae	XF3	572.0 ± 22.5	...	...	
	XF4	127.7 ± 9.1	...	...	
X. c. pv. *phaseoli*	XPh1	296.0 ± 25.6	368.7 ± 75.8	1.24	0.1–0.5
	XPh2	362.3 ± 24.6	326.3 ± 26.1	0.90	0.1–0.5
X. c. pv. *pelargonii*	XPg1	124.7 ± 22.77	18.3 ± 6.3	0.15	0.5–1.0
X. c. pv. *manihotis*	XM1	180.3 ± 3.8	21.3 ± 24.0	0.12	0.1–0.5
	XM6	191.7 ± 7.5	241.7 ± 88.4	1.26	0.1–0.5
X. c. pv. *vesicatoria*	XV1	192.7 ± 7.6	156.0 ± 44.5	0.81	0.1–0.5
	XV2	292.3 ± 15.5	258.0 ± 45.2	0.88	0.1–0.5
Agrobacterium tumefaciens	AT1	389.0 ± 26.0	...	...	
Corynebacterium michiganense	CM1	203.7 ± 9.1	...	...	
Erwinia stewartii	ES1	169.0 ± 27.0	...	...	
Pseudomonas pseudoalcaligenes subsp. *citrulli*	PPs1	163.0 ± 24.0	75.0 ± 29.7	0.46	0.1–0.5

[a] XPSM = *X. campestris* pv. pruni selective medium.

[b] Average number of colonies per plate from three or six (XP1) plates, followed by standard deviation. Count was made after incubation for 5–7 days at 27 C.

[c] Nutrient agar supplemented with 2% glucose for XP1, XB1, XB2, XB3, XCM1, XCu1, XPh1, XPh2, XPg1, XM1, XM6, XV1, and XV2; medium B (4) for XF3 and XF4; medium 523 (5) for CM1; nutrient agar supplemented with 0.1% yeast extract, 0.5% sucrose, and 0.002 M magnesium sulfate for At1; nutrient agar supplemented with 0.5% yeast extract and 0.5% glucose for ES1; and Pseudomonas agar P for PPs1.

[d] Plating efficiency = $\frac{\text{Avg. no. of colonies on XPSM/plate}}{\text{Avg. no. of colonies on appropriate medium/plate.}}$

[e] ... = No apparent colonies developed on XPSM after incubation for 7–8 days at 27 C.

Table 4. Recovery on XPSM[a] of *Xanthomonas campestris* pv. *pruni* (strain XP1) from soil

Avg. no. of *X. c.* pv. *pruni* CFU added[b]	Avg. no. of *X. c.* pv. *pruni* CFU recovered[c]	Avg. % recovery of *X. c.* pv. *pruni* CFU
$8.5 \pm 1.5 \times 10^4$	$8.8 \pm 2.4 \times 10^4$	103.5
$7.7 \pm 2.9 \times 10^3$	$7.7 \pm 4.6 \times 10^3$	100.0
$7.9 \pm 1.6 \times 10^2$	$5.9 \pm 3.5 \times 10^2$	74.7

[a] XPSM = *X. c.* pv. *pruni* selective medium.
[b] Average number of strain XP1 colony-forming units, followed by standard deviation, per 10 g of soil in 100 ml of sterile phosphate-buffered saline in two or three tests. The actual values were 6.8 ± 1.1, 9.0 ± 0.4, and $9.7 \pm 1.0 \times 10^4$; 4.4 ± 0.4, 9.0 ± 0.4, and $9.7 \pm 1.0 \times 10^3$; and 6.8 ± 1.1 and $9.0 \pm 0.4 \times 10^{-2}$ CFU.
[c] Each value is based on the average number of colonies per plate from three plates, followed by standard deviation, for each of two soil preparations in each test. The actual values were 6.1 ± 0.8, 9.5 ± 0.9, and $10.8 \pm 2.6 \times 10^4$; 3.6 ± 0.9, 6.8 ± 3.9, and $12.7 \pm 2.9 \times 10^3$; and 3.3 ± 5.2 and $8.3 \pm 13.3 \times 10^2$ CFU.

Table 5. Recovery on XPSM[a] of *Xanthomonas campestris* pv. *pruni* from peach leaf extracts

X. c. pv. *pruni* in leaf extract (CFU/ml)	Sample	*X. c.* pv. *pruni* recovered on XPSM (CFU/ml)[b]	Recovery of *X. c.* pv. *pruni* on XPSM (%)
$8.2 \pm 1.2 \times 10^2$	1	$9.8 \pm 1.9 \times 10^2$	120
	2	$9.4 \pm 1.5 \times 10^2$	115
$6.0 \pm 4.2 \times 10^1$	1	$6.0 \pm 1.7 \times 10^1$	100
	2	$8.7 \pm 3.5 \times 10^1$	147
None	1	0	...
	2	0	...

[a] XPSM = *X. c.* pv. *pruni* selective medium.
[b] Each value is the average number of colonies per plate from three plates, followed by standard deviation.

Table 6. Isolation on XPSM[a] of *Xanthomonas campestris* pv. *pruni* from lesions on naturally infected apricot leaves

Test	Extract	Extract[b] dilution assayed	No. of colonies on NGA[c,d] Total	No. of colonies on NGA[c,d] Resembling *X. c.* pv. *pruni*	No. of *X. c.* pv. *pruni* colonies on XPSM[d]
1	A	10^{-2}	88.7 ± 8.1	4.0 ± 2.6	28.0 ± 3.0
		10^{-3}	10.3 ± 5.1	1.7 ± 0.6	0.7 ± 0.6
		10^{-4}	9.0 ± 2.6	0.0	0.3 ± 0.6
	B	10^{-2}	140.7 ± 26.1	4.7 ± 1.5	9.0 ± 3.6
		10^{-3}	14.7 ± 3.2	1.7 ± 0.6	0.0
		10^{-4}	2.3 ± 1.5	0.0	0.0
2	C	10^{-2}	260.3 ± 87.5	109.0 ± 22.8	181.7 ± 43.5
		10^{-4}	0.5 ± 0.7	0.5 ± 0.7	3.0 ± 2.8
	D	10^{-2}	420.0 ± 20.5	1.0 ± 1.7	79.0 ± 40.0
		10^{-4}	73.5 ± 10.6	0.0	2.0 ± 1.4

[a] XPSM = *X. c.* pv. *pruni* selective medium.
[b] Composite ten- and five-lesion samples (tests 1 and 2, respectively) triturated in sterile phosphate-buffered saline at the rate of 1 ml per lesion.
[c] NGA = nutrient agar supplemented with 2% glucose.
[d] Values are average number of colonies per 0.1 ml/sample per plate from two or three plates, followed by standard deviation.

Table 7. Isolation on XPSM of *Xanthomonas campestris* pv. *pruni* from individual lesions on naturally infected apricot leaves[a]

Lesion	Colonies resembling *X. c.* pv. *pruni* on NGA/Total bacterial colonies per plate (no.)	*X. c.* pv. *pruni* colonies on XPSM (no.)
1	2.7 ± 3.0/515.3 ± 87.5	129.7 ± 6.6
2	29.0 ± 19.9/394.0 ± 98.0	209.7 ± 13.4
3	0.7 ± 1.2/N[b]	98.0 ± 5.3
4	107.0 ± 15.7/682.7 ± 101.9	171.0 ± 40.3
5	2.0 ± 3.5/331.3 ± 123.1	197.3 ± 34.2

[a] XPSM = *X. c.* pv. *pruni* selective medium. Values are average number of colonies per plate from three replicate plates, followed by the standard deviation. Each lesion was triturated separately in 1 ml of sterile phosphate-buffered saline (PBS). Each extract was diluted 10^{-3} in sterile PBS before plating on nutrient agar supplemented with 2% glucose and on XPSM.
[b] Colonies were too numerous to count.

forming units added per gram of soil were difficult to identify with certainty by colony appearance only on XPSM when undiluted soil samples were plated. Identity of *X. c.* pv. *pruni* in these cases needs to be confirmed by pathogenicity and/or phage sensitivity tests.

Recovery of *X. c.* pv. *pruni* from leaf extracts. *X. c.* pv. *pruni* was recovered quantitatively on XPSM from extracts of Sunhigh peach seedling leaf tissue containing approximately 60 and 820 CFU of *X. c.* pv. *pruni* (strain XP1) per milliliter (Table 5). In another test, the average rate of recovery of *X. c.* pv. *pruni* (strain XP1) on XPSM from similar duplicate extracts containing 9×10^1, 9×10^2, and 9×10^3 CFU/ml was 92, 110, and 106%, respectively. The generally high apparent recovery rates for *X. c.* pv. *pruni* (strain XP1) from these extracts may be the result of some growth-stimulating factors in peach leaf tissue.

Isolation of *X. c.* pv. *pruni* from naturally infected tissue. *X. c.* pv. *pruni* was readily detected in extracts of lesions from naturally infected leaves (Table 6). More *X. c.* pv. *pruni* colonies developed on XPSM than on NGA in all cases. *X. c.* pv. *pruni* populations associated with the randomly collected samples at different times during the summer were not systematically monitored quantitatively. However, composite 10-lesion extracts contained approximately 1.7 and 3.8×10^5 total recoverable bacterial colony-forming units per milliliter on NGA. When 10^{-2} and 10^{-3} dilutions of these preparations were plated on NGA, 0.03–16.5% of the colonies resembled *X. c.* pv. *pruni*. However, of 10 randomly selected, single-colony isolates, only six were sensitive to lysis by pruniphages PP1 and PP3. No colony-forming units resembling *X. c.* pv. *pruni* were detected on NGA in 10^{-4} dilutions of these preparations.

In contrast, a few colonies of *X. c.* pv. *pruni* were detected on XPSM in a 10^{-4} dilution of one of the two preparations. All of the colonies that developed on XPSM were similar in appearance. In addition, each of 10 randomly selected, single-colony isolates on XPSM was sensitive to lysis by pruniphages PP1 and PP3. In a second test, the average titers of total recoverable bacteria from extracts of two composite five-lesion samples were 1.0–2.6 and $4.2–73.5 \times 10^5$ CFU/ml. On NGA, 109 (42.1%) and 1 (0.40%) colonies from these two extracts were identified as *X. c.* pv. *pruni* based on colony appearance. From the same two extracts, 182 and 79 *X. c.* pv. *pruni* colonies developed on XPSM, respectively.

Isolation of *X. c.* pv. *pruni* from individual lesions on naturally infected apricot leaves using NGA was variable (Table 7). The presence of naturally occurring, rapidly growing bacteria in these samples necessitated diluting the

extracts to be assayed at least 1,000-fold. As with the composite lesion samples, isolation of *X. c.* pv. *pruni* on NGA was generally low, ranging from 0–15.7% of the total recoverable bacteria. The total number of bacteria in each of these lesion extracts was more than 3×10^6 CFU/ml on NGA. In contrast, each of these lesion extracts contained 100–200 *X. c.* pv. *pruni* colony-forming units per milliliter when plated on XPSM. No other colonies of bacteria or fungi associated with these lesions developed on XPSM.

Swabbings from the surfaces of infected and symptomless peach and apricot leaves were also streaked on XPSM. All of the colonies that developed were identified as *X. c.* pv. *pruni* based on colony appearance after replica transfer to yeast extract-dextrose-calcium carbonate agar (7). No fungal colonies developed from these samples of surface swabbings on XPSM.

DISCUSSION

The medium XPSM is a relatively simple, inexpensive one that is potentially useful for the selective isolation of *X. c.* pv. *pruni*. The plating efficiencies of several strains of *X. c.* pv. *pruni* on XPSM were all higher than on NGA. The virulence, phage sensitivity, and yellow pigment production capability on NGA of the *X. c.* pv. *pruni* strains tested here were not altered detectably after growth on XPSM. Except for yellow pigmentation, other qualitative cultural characteristics such as colony shape and texture were similar to those of colonies on NGA. However, 5 or more days were generally required for adequate growth. *X. c.* pv. *pruni* identification can be easily verified by phage sensitivity and pathogenicity tests.

Although colonies of two strains of *X. c.* pv. *begoniae* (XB1 and XB3) and one strain of *X. c.* pv. *pelargonii* (XPg1) developed on XPSM, the plating efficiencies of these strains were much lower on XPSM than on NGA. These three strains were clearly distinguished from *X. c.* pv. *pruni* strains by colony appearance. No colonies of a third strain of *X. c.* pv. *begoniae* (XB2) developed on XPSM following dilution plating. Colonies of two strains each of *X. c.* pv. *vesicatoria* (XV1, XV2), *X. c.* pv. *phaseoli* (XPh1, XPh2), and *X. c.* pv. *manihotis* (XM1, XM6), and one strain of *P. pseudoalcaligenes* subsp. *citrulli* (PPsc1) developed on XPSM following dilution plating. However, these colonies were only 0.1–0.5 mm in diameter and were not apparent until 7–8 days after plating. In addition, the plating efficiencies of all but XPh1 and XM6 were less on XPSM than on NGA or Pseudomonas agar P. The strains of *X. fragariae, X. c.* pv. *campestris, X. c.* pv. *cucurbitae, A. tumefaciens, C. michiganense,* and *E. stewartii* used here did not grow on XPSM.

Because of its simplicity, sensitivity, and selectivity, XPSM should be useful for selective and quantitative isolation of *X. c.* pv. *pruni* from natural sources. In addition, XPSM is a potential tool for epidemiologic studies when it is necessary to monitor *X. c.* pv. *pruni* populations associated with a wide variety of natural sources.

Based on dilution plating of *X. c.* pv. *pruni* (strain XP1) on XPSM, fewer than 100 CFU/ml in Sunhigh peach seedling leaf extracts and 100–1,000 CFU/g of a single type of field-collected soil (Galestown gravelly loamy sand) were detected. The recovery rate of *X. c.* pv. *pruni* from these soil samples containing approximately 10^2–10^3 added XP1 colony-forming units per gram was variable but generally quantitative on XPSM. No quantitative data on the viability of *X. c.* pv. *pruni* in soil are available. However, the diverse microbial populations in soil were useful to evaluate the selectivity of XPSM. Although many soil bacteria grew on XPSM, none was fast-growing. Only an occasional slow-growing fungal colony from soil samples appeared on XPSM. On XPSM, colonies of *X. c.* pv. *pruni* (strain XP1) from soil samples were generally smaller than those that developed from pure culture cell suspensions. Soil samples may contain some factors that slightly suppress *X. c.* pv. *pruni*.

Another important beneficial aspect of XPSM was the nearly complete growth failure of any other bacteria or fungi from lesions on naturally infected leaves, as well as from symptomless leaves in the field, that might occur in association with *X. c.* pv. *pruni*. It is not known whether bacteria and fungi from these sources were inhibited or greatly suppressed. However, samples were taken from only a limited number of natural sources. Additional samplings need to be made to confirm the apparent high degree of selectivity of XPSM. Nevertheless, leaf and soil samples to be assayed for *X. c.* pv. *pruni* (strain XP1) on NGA had to be diluted at least 100-fold to reduce the number of background colonies before colonies of *X. c.* pv. *pruni* could develop.

Further modification in the composition of XPSM might result in more rapid growth of *X. c.* pv. *pruni* and development of yellow, mucoid colonies characteristic of most xanthomonads. Ideally, any modification should not result in reduction of the sensitivity and selectivity of XPSM.

The plating efficiencies of all the *X. c.* pv. *pruni* strains tested on XPSM were generally high. There were distinct differences in the growth rates, based on colony size of nine strains. Therefore, it is possible that XPSM as used here might not be optimally useful for all strains of *X. c.* pv. *pruni*. However, only minor modifications in the composition of XPSM may be required to meet other specific needs.

LITERATURE CITED

1. Civerolo, E. L. 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. Phytopathology 65:258-264.
2. Civerolo, E. L. 1976. Phage sensitivity and virulence relationships in *Xanthomonas pruni*. Physiol. Plant Pathol. 9:67-75.
3. Dhanvantari, B. N., Dirks, V. A., and Brown, R. J. 1978. Effectiveness of antibiotics for control of bacterial spot of peach in southwestern Ontario. Can. J. Plant Sci. 58:953-959.
4. Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A., and Schroth, M. N. 1980. International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Rev. Plant Pathol. 59:153-168.
5. Hazel, W. J., and Civerolo, E. L. 1980. Procedures for growth and inoculation of *Xanthomonas fragariae*, causal organism of angular leaf spot of strawberry. Plant Dis. 64:178-181.
6. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolating *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas*. Phytopathology 60:969-976.
7. Schaad, N. W., and White, W. C. 1974. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology 64:876-880.
8. Skerman, V. B. D., McGowan, V., and Sneath, P. H. A. 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30:225-420.