MXP, a Semi-Selective Medium for *Xanthomonas campestris* pv. *phaseoli*

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ABSTRACT

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A semi-selective medium (MXP) was developed for isolation of *Xanthomonas campestris* pv. *phaseoli* and 'fuscans' strains from common blight-infected dry beans and infested soil. All *X. c.* pv. *phaseoli* strains tested grew on MXP and most *X. campestris* pathovars tested grew equally well. Other phytobacterial pathogens of bean failed to produce colonies on MXP. For recovery from seed, incubation for 6 hr at 28 C in a diluent of 12.5 mM PO_4 + 10 mM $MgSO_4$ + 0.01% Tween 20 resulted in the greatest number of cells of *X. c.* pv. *phaseoli* recovered. Incubating seeds in distilled water resulted in limited or no recovery of *X. c.* pv. *phaseoli*. MXP medium consists of (in grams per liter): K_2HPO_4 (0.8), KH_2PO_4 (0.6), yeast extract (0.7), soluble potato starch (8.0), potassium bromide (10.0), glucose (1.0), and agar (15.0). After autoclaving, chlorothalonil (15 mg/L), cephalexin (20 mg/L), kasugamycin (20 mg/L), gentamycin (2 mg/L), methyl violet 2B (30 μl/L; 1% solution in 20% ethanol), and methyl green (60 μl/L; 1% aqueous solution) were added before pouring the plates.

Common blight, incited by *Xanthomonas campestris* pv. *phaseoli*, including "fuscans" strains, is a common disease of dry bean (*Phaseolus vulgaris* L.) throughout the world (7,8). Disease symptoms are usually distinctive but may closely mimic other bean maladies including drought, chemical damage, physiological browning, halo blight, and other pod and foliar diseases. *X. c.* pv. *phaseoli* is seedborne (2,3,24,28,29,32,35,36,38) and in-field dissemination occurs via active and passive vectors such as animals, machinery, irrigation water, windblown soil and debris (6), and other plant species (1). Control of common blight is principally achieved by planting certified and foundation seed (9,21,22,37). Techniques used for detecting *X. c.* pv. *phaseoli* include immunology (19,33), phage typing (12,16,17,31,36), seedling injections (18,23), grow-out tests (37), and semi-selective media (33).

Selective or semi-selective media have been developed for the *X. campestris* pathovars *campestris* (4,27), *juglandis* (20), *phaseoli* (33), *pruni* (5), and *translucens* (26). Media are also available for *X. fragariae* (14), and for general growth of all *X. campestris* pathovars and species (10,15).

Selective or semi-selective media are advantageous for isolating phytopathogenic bacteria from plant tissue, seed, soil, and water, and are invaluable in epidemiological and etiological research. The medium should be selective at least at the species level. Selectivity for specific pathovars is ideal, but the plating efficiency of some strains may be unacceptable. Semi-selective media are most practical, as it is difficult to develop a medium that restricts growth of all contaminants from the diverse microflora found in seed, plant tissue, soil, and water. The medium preferably would use commonly available ingredients, be easy to prepare, inexpensive, and remain effective after prolonged storage.

The development of the semi-selective medium MXP, and its use in the recovery of *X. c.* pv. *phaseoli* from naturally infested soil and plant debris, and from seed are reported herein.

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MATERIALS AND METHODS

Development of MXP medium. The initial formulation of MXP consisted of (in grams per liter): K_2HPO_4 (0.6), yeast extract (0.1), potato starch (8.0), boric acid (1.1), and agar (15.0) (M. Sasser, *unpublished*). Other amendments included chlorothalonil (Daconil 2787 [40.4% a.i.], Fermenta Plant Protection Co., Painesville, OH), kasugamycin (30 μg/ml), cephalexin (20 μg/ml), and nalidixic acid (4 μg/ml), which were added after autoclaving (121 C for 15 min) and after the medium had cooled to 40 C. After plating *X. c.* pv. *phaseoli* strains on this medium, nearly 50% of the strains failed to grow; the remaining strains grew slowly (6–7 days) and were nearly devoid of yellow pigment. Serious problems also were encountered with contaminants. To enhance growth and ensure semi-selectivity, various antibiotics, stains, chemicals, and dosage rates were evaluated. Criteria for the medium were distinctive colony morphology of *X. c.* pv. *phaseoli*, pigment intensity, high plating efficiency relative to nonselective media, and the inhibition of other fungal and bacterial contaminants commonly found on bean plants, seed, and in soil and debris. Evaluations were based on a representative sample of 25–200 colonies of *X. c.* pv. *phaseoli* per plate per strain. The medium that met most of the above criteria contained (in grams per liter): K_2HPO_4 (0.8), KH_2PO_4 (0.6), yeast extract (0.7), soluble potato starch (8.0), potassium bromide (10.0), glucose (1.0), and agar (15.0). After autoclaving and after the medium had cooled to 40 C, 1 ml each of Daconil 2787 (1.2–38.8 ml of H_2O, containing 15 mg of tetrachloroisophthalonitrile [chlorothalonil]), cephalexin monohydrate (200 mg in 100 ml of H_2O), kasugamycin hemisulfate (200 mg in 100 ml of H_2O), and gentamycin sulfate (20 mg in 100 ml of H_2O) were added to give final concentrations of 15, 20, 20, and 2 μg/ml, respectively. Antibiotics were purchased from Sigma, St. Louis, MO, and filter sterilized before use. Starch hydrolysis zones were easily determined by adding 30 μl/L of methyl violet 2B (1% solution in 20% ethanol) and 60 μl/L of methyl green (1% aqueous solution) before pouring the plates (13). Stains were also obtained from Sigma, and fresh stock solutions were prepared about every 3 mo.

Plating efficiencies. Cultures of *X. c.* pv. *phaseoli* obtained from various locations throughout the world (Table 1) were used in evaluating growth on MXP medium. Cultures were maintained on yeast extract-glucose-calcium carbonate (YGC) plates (11) at 4 C or on silica gel (30), and also in lyophilization for long-term

storage. One loopful of bacterial cells was added to test tubes containing 5 ml of nutrient broth (NBY) (34) and placed on a rotary shaker (200 rpm) for 12–14 hr at 28 C. The cells were centrifuged at 13,800 *g* (Beckman J2-21, Palo Alto, CA) for 15 min, then resuspended in sterile 12.5 mM PO_4 (pH 7.1), and 10mM $MgSO_4$ buffer (PMS). The cell suspensions were adjusted to an absorbance of 0.1 at 640 nm (Bausch and Lomb Spectronic 20) followed by a 10-fold dilution series with PMS as the diluent and plated for isolated colonies. Plates were incubated at 28 C and the colonies counted after 4 days.

Recovery of *X. c.* pv. *phaseoli* bacteria from inoculated plants. Red kidney beans (cultivar Dark Red Kidney) plants were inoculated when the first trifoliate leaves were fully developed (2–3 wk after planting). A 100-μl suspension of *X. c.* pv. *phaseoli* (2×10^8 colony-forming units (cfu/ml)) was infiltrated into the leaf with a syringe equipped with a 26-gauge needle. The inoculated sites were about 1 cm from the midrib and 1 cm apart. Each leaf received approximately 20 inoculations. Common blight symptoms were evident 10 days after inoculation and the leaves were harvested 14–16 days after inoculation. Approximately 0.5 g of leaf tissue was ground in a sterile mortar containing 5 ml of PMS and an additional 5 ml of PMS was added as a wash after grinding. The homogenate was filtered through two layers of cheesecloth, diluted in PMS, and plated on both MXP and control medium YGC. A minimum of two plates each of MXP and YGC was used for each dilution. The plates were placed in an incubator at 28 C and colonies counted after 4 days.

Recovery of *X. c.* pv. *phaseoli* from plant debris and soil. Soil and bean debris were collected in December 1984 from several locations in Nebraska where common blight had been present during the growing season. The soil was sieved through a 0.85-mm-mesh screen and placed in plastic bags at room temperature. The plant debris was thoroughly shaken to remove adhering soil particles, cut into 1-cm sections, and placed in a paper bag at room temperature. For testing, 1 g of soil was added to 9 ml of PMS and placed on a rotary shaker (200 rpm) for 2 hr at 28 C. One-half gram of plant debris was added to 9 ml of sterile PMS and also placed on a rotary shaker for 2 hr. A 10-fold dilution series was carried out with PMS as the diluent. No disinfectants were used to surface sterilize the plant materials.

Recovery of *X. c.* pv. *phaesoli* from seed. Common blight-infested 'Great Northern' bean seeds (courtesy of J. Steadman, University of Nebraska, and A. W. Saettler, Michigan State University), were ground in a Wiley mill (0.85-mm-mesh screen), placed in plastic bags, and stored at room temperature. The milled seeds (0.5 g) were added to 4.5 ml of diluent (see Results), vortexed, and incubated in stationary cultures at 4 and 28 C for 0, 3, 6, and 24 hr. Before sampling, the mixture was vortexed (Vortex-Genie, Scientific Industries, Bohemia, NY) for approximately 15 sec at a medium setting. The heavy particulates were allowed to settle (3–4 min) and the test sample (0.1 ml) was removed from the supernatant portion, serially diluted in various diluents (see Results), and then plated on YGC and MXP.

RESULTS

Efficiency of plating of in vitro cultures. All *X. c.* pv. *phaseoli* strains tested grew on MXP and hydrolyzed starch (Table 1). Plating efficiencies of 10 strains of *X. c.* pv. *phaseoli* ranged from 62 to 96% with an average of 79% (Table 2). In addition to *X. fragariae*, MXP supported growth of all other *X. campestris* pathovars tested except *X. c.* pv. *pruni*, *X. c.* pv. *translucens*, and

TABLE 1. Phytopathogenic bacteria used for evaluating growth and starch hydrolysis on MXP medium

Bacterium and strain designation	Origin	Donor[a]	Growth[b]	Starch hydrolysis[c]
Curtobacterium flaccumfaciens subsp. *flaccumfaciens* NE 59	Nebraska	1	−	NT[d]
Pseudomonas syringae pv. *phaseolicola* 82 H1	Nebraska	1	−	NT
P. syringae pv. *syringae*	Nebraska	1	−	NT
Xanthomonas campestris pv. *alfalfae* KX-1	Kansas	2	+	+
X. c. pv. *begoniae* PDDCC 194	New Zealand	3	+	+
X. c. pv. *campestris* NCPPB 528	England	4	+	+
X. c. pv. *glycines* NE 84	Nebraska	1	+	+
X. c. pv. *hederae* NE	Nebraska	1	+	+
X. c. pv. *holcicola* KS 66	Kansas	2	+	−
X. c. pv. *malvacearum*	Texas	5	+	+
X. c. pv. *oryzae* PXO 61	Philippines	7	−	−
X. c. pv. *oryzae* PXO 86	Philippines	7	−	−
X. c. pv. *phaseoli* NCPPB 1420	Zambia	4	+	+
X. c. pv. *phaseoli* NCPPB 1646	Australia	4	+	+
X. c. pv. *phaseoli* NCPPB 1811	Romania	4	+	+
X. c. pv. *phaseoli* NCPPB 2064	Sudan	4	+	+
X. c. pv. *phaseoli* B-495 (fuscans)	Michigan	1	+	+
X. c. pv. *phaseoli* B-496	Canada	1	+	+
X. c. pv. *phaseoli* B-702	Brazil	1	+	+
X. c. pv. *phaseoli* LB-2	Nebraska	1	+	+
X. c. pv. *phaseoli* V_3S_2 (fuscans)	Dominican Republic	1	+	+
X. c. pv. *phaseoli* V_4S_3 (fuscans)	Dominican Republic	1	+	+
X. c. pv. *phaseoli* DRS-103	Dominican Republic	1	+	+
X. c. pv. *pruni* PDDCC 51	New Zealand	3	−	−
X. c. pv. *vesicatoria* 75-3	Florida	6	+	−
X. c. pv. *translucens* 85-G	Nebraska	1	−	−
X. c. pv. *zinniae* PDDCC 5762	Australia	3	+	+
X. albilineans PDDCC 196	Fiji	3	−	NT
X. axonopodis PDDCC 50	Colombia	3	−	NT
X. ampelina PDDCC 4298	Crete	3	−	NT
X. fragariae PDDCC 6269	New Zealand	3	+	+

[a] 1 = A. K. Vidaver, Department of Plant Pathology, University of Nebraska; 2 = L. E. Claflin, Department of Plant Pathology, Kansas State University; 3 = J. M. Young, Curator, PDDCC, Auckland, NZ; 4 = National Collection of Plant Pathogenic Bacteria, Harpenden Laboratory, Harpenden, England; 5 = L.S. Bird, Texas A&M University; 6 = R. E. Stall, Department of Plant Pathology, University of Florida; 7 = T. W. Mew, IRRI, Manila, Philippines.
[b] Growth is defined as single colony formation within 10 days.
[c] As determined by a clear zone of hydrolysis and/or an application of dilute iodine solution (1.0%) within 10 days.
[d] NT = not tested.

X. c. pv. *oryzae*. *X. albilineans*, *X. axonopodis*, and *X. ampelina* did not grow on MXP, nor did other phytobacterial pathogens of bean, *Curtobacterium* (syn. = *Corynebacterium*) *flaccumfaciens* subsp. *flacccumfaciens*, *Pseudomonas syringae* pv. *phaseolicola*, and *P. syringae* pv. *syringae* (Table 1). Starch hydrolysis by *X. campestris* pathovars other than *X. c.* pv. *phaseoli* was variable.

Strains of *X. c.* pv. *phaseoli* were tolerant to stains and antibiotics at different levels. The biological stains methyl violet 2B and methyl green supported growth up to 75 μl/L (= 75 μg/L) and 125 μl/L (= 125 μg/L), when tested individually and in combination, respectively. However, the lowest concentrations enabling colony differentiation of three test strains (LB-2, B-495, B-496) were used, namely 30 μl/L of methyl violet and 60 μl/L of methyl green. Strains LB-2, B-495, and B-496 were very sensitive to gentamycin, as 3 μg/ml resulted in a slight decrease in growth, whereas 4 μg/ml inhibited growth altogether. The other antibiotics used in MXP, kasugamycin and cephalexin, were not as toxic; all of the strains tested grew on media containing up to 30 μg/ml.

Comparison of MXP and other semi-selective media. Other media described as being specific for *X. campestris* pathovars were evaluated for growth of *X. c.* pv. *phaseoli*. On XTS medium, formulated for *X. c.* pv. *translucens* (26), very few strains grew and only at low dilutions. On S-M medium, formulated for *X. c.* pv. *campestris* (4), only one strain (LB-2) grew as very tiny colonies after 5 days of growth. No strains grew on XPSM, formulated for *X. c.* pv. *pruni* (5). Media of Dhanvantari, for several pathovars (10), and Trujillo and Saettler (33; specifically formulated for *X. c.* pv. *phaseoli*), enabled all strains tested to grow, but unacceptable problems were encountered with contaminants. D-5 medium for all *X. campestris* pathovars (15) and the Mulrean and Schroth (20) medium for *X. c.* pv. *juglandis* were not evaluated.

Recovery from inoculated plants, debris, and soil. Recovery of cells of *X. c.* pv. *phaseoli* from inoculated greenhouse grown bean plants ranged from nearly 70 to over 100% on MXP when compared with YGC (Table 2). Variability was probably affected by sampling or plating errors (e.g., strain NCPPB 1811). MXP was highly efficient in recovering cells of *X. c.* pv. *phaseoli* from naturally infested soil and debris with minimal or no bacterial or fungal contaminants. Plating efficiencies could not be ascertained because of contaminant overgrowth on YGC. Representative strains were inoculated into 'Red Kidney' bean plants for pathogenic verification. These strains produced typical common blight symptoms.

Recovery from seed. MXP was equally efficient in recovery of cells of *X. c.* pv. *phaseoli* from common blight-infested 'Great Northern' bean seed (Table 3) as from plants, debris, and soil. Plating efficiencies between YGC and MXP could not be determined because of the nearly total bacterial and fungal contaminant growth on YGC (Fig. 1). It was found, however, that *X. c.* pv. *phaseoli* is osmotically sensitive in various diluents. The recovery of cells from milled seeds incubated in sterile distilled water was negligible, whereas the maximum occurred when the diluent consisted of 12.5 mM PO_4, 10 mM $MgSO_4$, and 0.01% Tween 20. Colony counts increased nearly 81% from 0 to 6 hr at 28

TABLE 2. Plating efficiency and recovery on MXP of strains of *Xanthomonas campestris* pv. *phaseoli* from broth cultures and common blight infected plants

Strain no.	Planting efficiency[a] (%)	
	In vitro	Plant tissue
NCPPB 1138	76	89
NCPPB 1420	62	104
NCPPB 1646	93	104
NCPPB 1811	85	68
NCPPB 2064	68	91
B-495	78	86
B-496	80	98
B-702	74	82
LB-2	78	93
V_3S_2	96	102

[a] Plating efficiency = Colony forming units recovered on MXP/colony forming units on YGC × 100. Figures were calculated from the mean number of colonies per plate; minimum of six plates per strain. *Phaseolus vulgaris* 'Red Kidney' bean plants were inoculated with known strains as described in the text.

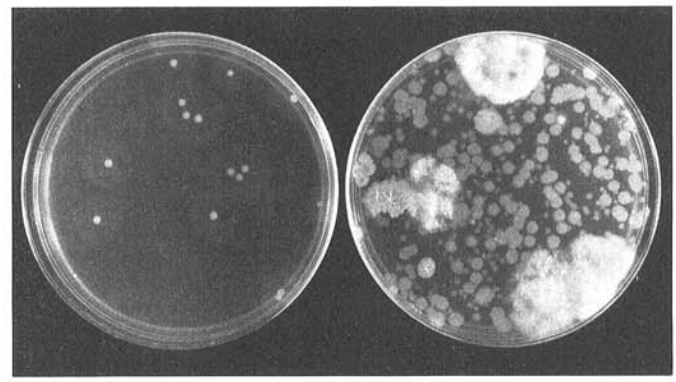

Fig. 1. Comparison of MXP (left) and YGC (right) media after dilution plating a seed sample from a common blight infested field. Plates were incubated at 26 C for 4 days to obtain isolated colonies of *X. c.* pv. *phaseoli*. Translucent areas surrounding colonies of *X. c.* pv. *phaseoli* indicates starch hydrolysis.

TABLE 3. Effect of diluents, incubation time, and temperature on recovery of *Xanthomonas campestris* pv. *phaseoli* from naturally infested 'Great Northern' bean seeds

Time and temperature of seed incubation	Diluent			
	12.5 mM PO_4 + 10 mM $MgSO_4$	12.5 mM PO_4	12.5 mM PO_4 + 10 mM $MgSO_4$ + 0.01% Tween 20	Sterile distilled H_2O
Experiment 1[a]				
0 hr, 28 C	5.4[b]	3.4	10.7	0.6
0 hr, 4 C	4.5	2.4	7.9	0.2
3 hr, 28 C	6.1	3.5	12.9	0.6
3 hr, 4 C	4.6	2.0	9.0	0.2
6 hr, 28 C	10.7	6.2	17.3	0.2
6 hr, 4 C	3.7	2.9	8.8	0.0
Experiment 2[c]				
6 hr, 28 C	123.2 (77.7[d] / 45.4)	2.3	35.6 (34.9 / 0.7)	0.1
6 hr, 4 C	56.8 (11.2 / 45.4)	0.1	0.2	0.2

[a] Seed obtained from J. Steadman, University of Nebraska.
[b] Mean number of *X. c.* pv. *phaseoli* colony-forming units (cfu) × 10^5 per gram of naturally infested bean seed based on five replications.
[c] Seed obtained from A. W. Saettler, Michigan State University.
[d] Figures in parentheses represent a breakdown of recovery for "small" (top number) and "large" *X. c.* pv. *phaseoli* colonies, respectively. All of the small colonies were 'fuscans' strains; however, large colony types may also be 'fuscans' strains. Otherwise, only total colonies were enumerated and represent the mean cfu × 10^5 per 10 MXP plates per gram of seed.

C in all diluents except sterile distilled water. Incubating the seeds for 3 hr resulted in only a 7.4% increase. Incubating the seeds for 6 hr at 4 C resulted in a 21% decrease in colony numbers. In general, incubating the seeds for 24 hr was unsatisfactory, due to the frequency of not recovering *X. c.* pv. *phaseoli*, and increased numbers of contaminants as compared with the 3- and 6-hr incubation periods. Similar results were obtained with common blight infested seeds from different cultivars and locations (L. E. Claflin and A. K. Vidaver, *unpublished*).

MXP could also be used to distinguish between typical *X. c.* pv. *phaseoli* and 'fuscans' strains. Colonies of *X. c.* pv. *phaseoli* were typically mucoid, smooth, viscid, and yellow with pronounced zones of starch hydrolysis, whereas 'fuscans' strains generally were smaller, zones of starch hydrolysis were less, and the colonies tended to have an intense yellow color in the center of the colony (Fig. 2). The 'fuscans' strains required 6–7 days for colony formation, whereas typical colonies of *X. c.* pv. *phaseoli* could be counted after 4 days on MXP. Some 'fuscans' strains were indistinguishable from typical *X. c.* pv. *phaseoli* on MXP. The characteristic brownish pigment of the fuscans strains was not produced on MXP. Therefore, transfer to YGC or other nutrient media was necessary for expression of this trait. Recovery of *X. c.* pv. *phaseoli* 'fuscans' strains from bean seed is detailed in Table 3. Fuscans strains appeared to be more sensitive to detergent than the typical strains of *X. c.* pv. *phaseoli*. A decrease in colony counts of 23% resulted when the diluent consisted of 12.5 mM PO_4, 10 mM $MgSO_4$, and 0.01% Tween 20 compared with the buffer alone. Recovery of *X. c.* pv. *phaseoli* 'fuscans' declined over 500% when the seeds were incubated for 6 hr at 4 C (Table 3).

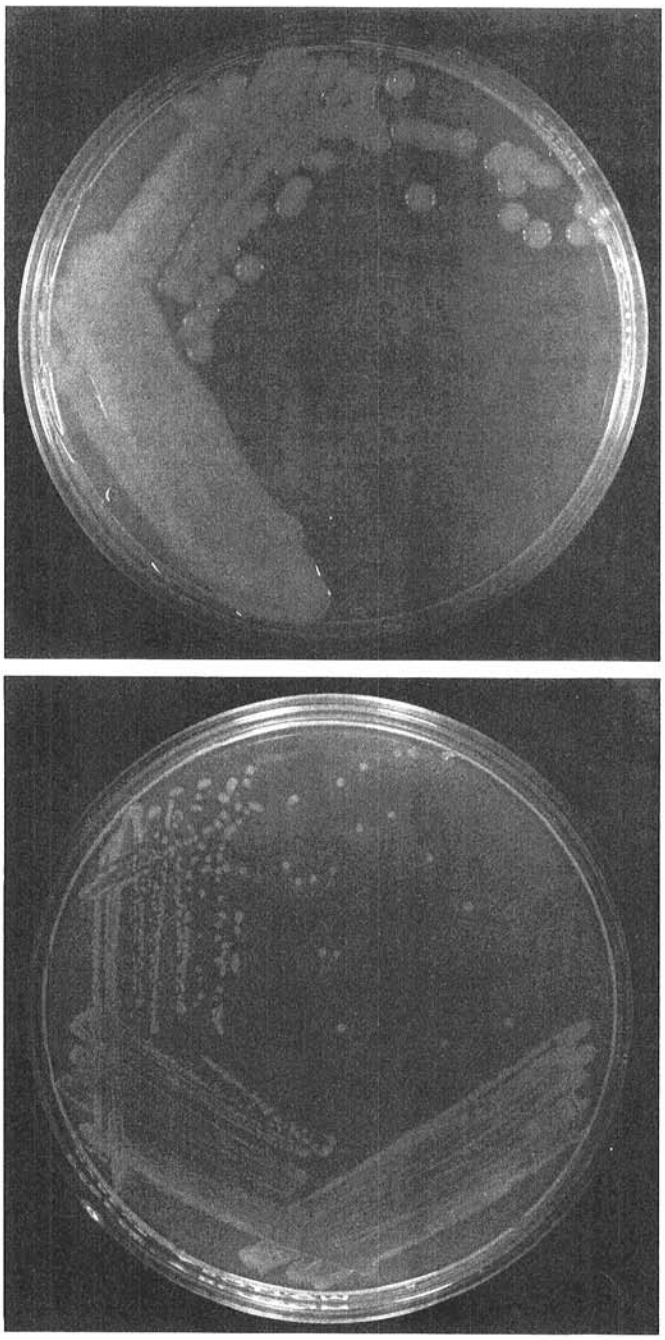

Fig. 2. Illustrative colonies of *Xanthomonas campestris* pv. *phaseoli*. Large colony type (top) and the small colony type (bottom) on MXP. Plates were incubated for five days at 26 C. All small colony types tested were 'fuscans' strains; large colony types sometimes were 'fuscans' strains.

DISCUSSION

A semi-selective medium, MXP, was developed to recover cells of *X. c.* pv. *phaseoli* from plants, plant debris, seed, and soil with a high degree of plating efficiency. Bacterial and/or fungal contaminants failed to grow or their growth was suppressed sufficiently to allow growth and enumeration of colonies of *X. c.* pv. *phaseoli* from debris and soil. In addition to the 11 strains of *X. c.* pv. *phaseoli* listed in Table 2, approximately 50 additional strains have been tested. All of the strains hydrolyzed starch and grew on MXP. However, it is conceivable that some strains will be found that grow very poorly, or not at all, on MXP. In pathovar *X. c.* pv. *campestris*, not all strains hydrolyze starch (N. W. Schaad, *personal communication*), and a similar possibility exists with *X. c.* pv. *phaseoli*. A reduction in xanthomonadin pigment intensity was observed when the number of colonies exceeded 50 per plate. Colony development was inhibited when depth of MXP in plates was 2 mm or less; around 3 mm thickness of the medium was optimum.

Our strains of *X. c.* pv. *phaseoli* were very sensitive to gentamycin. Generally, 2 μg/ml was adequate; our strains tolerated concentrations up to 3 μg/ml but failed to grow at 4 μg/ml. Each new purchase of gentamycin received from the manufacturer was assayed to determine the minimal and maximal dosage rates for efficacy in plating efficiencies and inhibition of contaminants. It is strongly recommended that others using this medium follow this practice. *X. campestris* pathovars apparently have varying degrees of tolerance to gentamycin as the XTS medium for *X. c.* pv. *translucens* (26) specified 8 μg/ml. Other medium components were less critical.

Cephalexin at a rate of 20 μg/ml was required to inhibit *Erwinia* sp., especially *E. herbicola*. If problems should occur with *E. herbicola* or other *Erwinia* sp. at this level (20 μg/ml), our strains of *X. c.* pv. *phaseoli* grew at 30 μg/ml without any noticeable effects. Kasugamycin was useful in reducing *Pseudomonas* sp. and other contaminants, and our strains of *X. c.* pv. *phaseoli* grew at levels exceeding 60 μg/ml. Methyl violet 2B and methyl green provided a background that enhanced the zones of starch hydrolysis, as well as inhibiting Gram-positive bacteria (13).

X. c. pv. *phaseoli* survived potassium bromide levels up to 4.5%. Bromine is normally lethal to other bacteria at these concentrations. The xanthomonadin pigment was enhanced at the higher levels. No differences were noted when sodium bromide was substituted for potassium bromide. No detrimental effects were observed during long-term storage; plates that had been stored 2 mo at 4 C were as effective as freshly prepared media.

Initial attempts to recover cells of *X. c.* pv. *phaseoli* from infested bean tissue and seeds using sterile distilled water as the diluent resulted in negligible colony counts. The use of phosphate buffer with $MgSO_4$ greatly enhanced colony numbers from debris and seeds. Similar results were obtained by Schaad and Donaldson (25) when saline was used as the diluent in an attempt to recover *X. c.* pv. *campestris*. They noted that most of the cells were killed in sterile water within 30 min. Pure cultures of *X. c.* pv. *phaseoli* and other *X. campestris* pathovars also showed sensitivity to the diluent similar to bacteria from debris and seed (Claflin and Vidaver, *unpublished*).

MXP is presently being used for epidemiological studies

involving the role of seed transmission of *X. c.* pv. *phaseoli* and monitoring epiphytic populations in naturally occurring populations in bean production areas in Nebraska. MXP would appear to be useful in plant disease diagnostic laboratories and in tests involving seed certification programs for the isolation and identification of *X. c* pv. *phaseoli.*

LITERATURE CITED

1. Cafati, C. R., and Saettler, A. W. 1980. Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli.* Plant Dis. 64:194-196.
2. Cafati, C. R., and Saettler, A. W. 1980. Transmission of *Xanthomonas phaseoli* in seed of resistant and susceptible phaseolus genotypes. Phytopathology 70:638-640.
3. Cafati, C. R., and Saettler, A. W. 1980. Effect of host on multiplication and distribution of bean common blight bacteria. Phytopathology 70:675-679.
4. Chun, W. W. C., and Alvarez, A. M. 1983. A starch-methionine medium for isolation of *Xanthomonas campestris* pv. *campestris* from plant debris in soil. Plant Dis. 67:632-635.
5. Civerolo, E. L., Sasser, M., Helkie, C., and Burbage, D. 1982. Selective medium for *Xanthomonas campestris* pv. *pruni.* Plant Dis. 66:39-43.
6. Claflin, L. E., Stuteville, D. L., and Armbrust, D. V. 1973. Wind-blown soil in the epidemiology of bacterial leaf spot of alfalfa and common blight of bean. Phytopathology 63:1417-1419.
7. Commonwealth Mycological Institute. 1971. Distribution maps of plant diseases. Map No. 401, Edition 2. Commonw. Mycol. Inst., Kew, Surrey, England.
8. Commonwealth Mycological Institute. 1973. Distribution maps of plant diseases. Map No. 402, Edition 2. Commonw. Mycol. Inst., Kew, Surrey, England.
9. Copeland, L. O., Adams, M. W., and Bell, D. C. 1975. An improved seed programme for maintaining disease-free seed of field beans (*Phaseolus vulgaris*). Seed Sci. Technol. 3:719-724.
10. Dhanvantari, B. N. 1981. Semi-selective media for detection and monitoring of some *Xanthomonas campestris* pathovars. Proc. Int. Conf. Plant Pathol. Bact., 5th. Cali, Colombia 1:135-136.
11. Dowson, W. J. 1957. Plant Diseases Due to Bacteria. 2nd ed. Cambridge University Press, London. 232 pp.
12. Ednie, A. B., and Needham, S. M. 1973. Laboratory test for internally-borne *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* in field bean seed. Proc. Assn. Official Seed Analysts 63:76-82.
13. Fung, D. Y. C., and Miller, R. D. 1973. Effect of dyes on bacterial growth. Appl. Microbiol. 25:793-799.
14. Hazel, W. J., Civerolo, E. L., and Bean, G. A. 1980. Procedures for growth and inoculation of *Xanthomonas fragariae*, causal organism of angular leaf spot of strawberry. Plant Dis. 64:178-181.
15. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology 60:969-976.
16. Katznelson, H., and Sutton, M. D. 1951. A rapid phage plaque count method for the detection of bacteria as applied to the demonstration of internally-borne bacterial infections of seed. J. Bacteriol. 61:689-701.
17. Katznelson, H., Sutton, M. D., and Bayley, S. T. 1954. The use of bacteriophage of *Xanthomonas phaseoli* in detecting infection in beans with observation on its growth and morphology. Can. J. Microbiol. 1:22-29.
18. Lahman, L. K., and Schaad, N. W. 1985. Evaluation of the "dome test" as a reliable assay for seedborne bacterial blight pathogens of beans. Plant Dis. 69:680-683.
19. Malin, E. M., Roth, D. A., and Belden, E. L. 1983. Indirect immunofluorescent staining for detection and identification of *Xanthomonas campestris* pv. *phaseoli* in naturally infected bean seed. Plant Dis. 67:645-647.
20. Mulrean, E. N., and Schroth, M. N. 1981. A semi-selective medium for the isolation of *Xanthomonas campestris* pv. *juglandis* from walnut buds and catkins. Phytopathology 71:336-339.
21. Nebraska Crop Improvement Association. 1985. Pages 22-24 in: Nebraska Seed Certification Standards. Nebraska Crop Improvement Association, IANR, UN-L, Lincoln, NE. 52 pp.
22. Ralph, W. 1977. Problems in testing and control of seed-borne bacterial pathogens: A critical evaluation. Seed Sci. Technol. 5:735-752.
23. Saettler, A. W. 1971. Seedling injection as an aid in identifying bean blight bacteria. Plant Dis. Rep. 55:703-706.
24. Saettler, A. W., and Perry, S. K. 1972. Seed-transmitted bacterial diseases in Michigan Navy (pea) beans, *Phaseolus vulgaris*. Plant Dis. Rep. 59:378-381.
25. Schaad, N. W., and Donaldson, R. C. 1980. Comparison of two methods for detection of *Xanthomonas campestris* in infected crucifer seeds. Seed Sci. Technol. 8:383-391.
26. Schaad, N. W., and Forster, R. L. 1985. A semi-selective agar medium for isolating *Xanthomonas campestris* pv. *translucens* from wheat seeds. Phytopathology 75:260-263.
27. Schaad, N. W., and White, W. C. 1974. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology 64:876-880.
28. Schnathorst, W. C. 1954. Bacteria and fungi in seeds and plants of certified bean varieties. Phytopathology 44:588-592.
29. Schuster, M. L., Coyne, D. P., Nuland, D. S., and Smith, C. C. 1979. Transmission of *Xanthomonas phaseoli* and other bacterial species or varieties in seeds of tolerant bean (*Phaseolus vulgaris*) cultivars. Plant Dis. Rep. 63:955-959.
30. Sleesman, J. P., and Leben, C. 1978. Preserving phytopathogenic bacteria at −70C or with silica gel. Plant Dis. Rep. 62:910-913.
31. Sutton, M. D., and Katznelson, H. 1953. Isolation of bacteriophages for the detection and identification of some seed-borne pathogenic bacteria. Can. J. Bot. 31:201-205.
32. Sutton, M. D., and Wallen, V. R. 1970. Epidemiological and ecological relations of *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* on bean in Southwestern Ontario, 1961–1968. Can. J. Bot. 48:1329-1334.
33. Trujillo, G., and Saettler, A. W. 1979. A combined semi-selective medium and serology test for the detection of *Xanthomonas* blight bacteria in bean seed. J. Seed Technol. 4:35-41.
34. Vidaver, A. K. 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of the carbon source. Appl. Microbiol. 15:1523-1524.
35. Wallen, V. R., and Sutton, M. D. 1965. *Xanthomonas phaseoli* var. *fuscans* (Burkh.) Dowson in white-seeded dwarf bean seed stocks. Ann. Appl. Biol. 60:305-312.
36. Wallen, V. R., and Sutton, M. D. 1965. *Xanthomonas phaseoli* var. *fuscans* (Burkh.) Starr and Burkh. on field bean in Ontario. Can. J. Bot. 43:437-446.
37. Webster, D. M., Atkin, J. D., and Cross, J. E. 1983. Bacterial blights of snap beans and their control. Plant Dis. 67:935-940.
38. Weller, D. M., and Saettler, A. W. 1980. Evaluation of seedborne *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* as primary inocula in bean blights. Phytopathology 70:148-152.