A New Semi-Selective Medium for *Xanthomonas campestris* pv. *vitians*, the Causal Agent of Bacterial Leaf Spot of Lettuce

V. Toussaint, MacDonald Campus of McGill University, 21 111 Lakeshore, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada; C. E. Morris, Station de Pathologie Végétale, INRA d'Avignon, 84143, Monfavet, Cedex, France; O. Carisse, Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Gouin Boulevard, St-Jean-sur-Richelieu, Quebec

ABSTRACT

Toussaint, V., Morris, C. E., and Carisse, O. 2001. A new semi-selective medium for *Xanthomonas campestris* pv. *vitians*, the causal agent of bacterial leaf spot of lettuce. Plant Dis. 85:131-136.

A semi-selective medium containing maltose, methyl green, and antibiotics (MMG) was developed for the isolation of *Xanthomonas campestris* pv. *vitians*. The semi-selective medium was evaluated based on plating efficiency of *X. campestris* pv. *vitians* in cell suspensions of pure cultures from leaves and soil. MMG medium allowed recovery of 5.7 to 30.6% of the *X. campestris* pv. *vitians* colonies recovered on nutrient agar and 0.1 to 8.4% of those recovered on 1/10-strength tryptic soy agar. MMG inhibited growth of most background bacteria and allowed reliable identification of *X. campestris* pv. *vitians*. The semi-selective medium contained (per liter) maltose (10 g), tryptone (5 g), K_2HPO_4 (3.5 g), KH_2PO_4 (2.75 g), trace elements (0.02 to 1.0 mg), methyl green (2 ml of a 1% aqueous solution), amoxicillin (32 mg), cephalothin (32 mg), cycloheximide (50 mg), and agar (15 g).

Bacterial leaf spot of lettuce (BLSL) caused by *Xanthomonas campestris* pv. *vitians* is present in several parts of the world and was observed for the first time in Quebec in 1994 (2,12,19,22,25). BLSL is characterized by water-soaked lesions at the margins of leaves, which become black and necrotic in a few days. As the disease progresses, necrotic lesions can develop on other parts of the leaf. When symptoms are limited to older external leaves, the diseased foliage can be removed at harvest. When symptoms are present on younger leaves, however, plants become unmarketable. This disease is thought to be seed-borne, but attempts to reisolate the pathogen from commercial seed lots have failed (11,24). Sahin et al. (15), however, showed that it was possible to produce infected seed from contaminated plants. Management of BLSL with chemical pesticides provides limited control (3,22). Presently,

no bactericides are registered in Canada for controlling bacterial diseases on lettuce.

Information on the ecology of *X. campestris* pv. *vitians* and the epidemiology of BLSL is sparse. *X. campestris* pv. *vitians* probably survives as an epiphyte, but no reports have been published on the subject. The bacterium has been recovered from lettuce debris buried in soil after an overwintering period but it has never been recovered from soil samples without lettuce debris (27). To carry out epidemiological and ecological studies, a detection method must first be developed. The method should allow the detection, identification and reliable quantification of *X. campestris* pv. *vitians* populations.

Semi-selective media permit quantification and can be highly sensitive assays. A semi-selective medium was reported to be as sensitive as polymerase chain reaction (PCR) in detecting *Xanthomonas albilineans* in sugar cane (26). Some semi-selective media are even more sensitive than immunological techniques for detecting low numbers of bacteria (1,26). For *X. campestris* pv. *vitians*, however, none of these methods are available. A semi-selective medium is easy to use and less costly than molecular and immunological techniques. Semi-selective media can often be readily used for diverse samples (soil, water, leaf tissue), whereas molecular and immunological techniques may vary in sensitivity according to sample type.

In preliminary experiments, previously described semi-selective media for different pathovars of *Xanthomonas* were tested but none of them allowed the recovery of

X. campestris pv. *vitians*. Most of the semi-selective media developed for *Xanthomonas* pathovars are based on starch hydrolysis, which leads to clearing of the medium around colonies. The capacity of *X. campestris* pv. *vitians* to hydrolyze starch is poor (4). Consequently, these media are not suitable for studying this bacterium. Considering that *X. campestris* pv. *vitians* populations are often low and that this bacterium grows slower than bacterial saprophytes, it is important to develop a semi-selective medium for *X. campestris* pv. *vitians*. On this new semi-selective medium, it should be easy to recognize *X. campestris* pv. *vitians* but it should also limit the growth of saprophytic bacteria to facilitate enumeration of the pathogen. The objective of this study was to develop a semi-selective medium for the isolation and quantification of *X. campestris* pv. *vitians* from lettuce plants and soil. The medium was also tested for the isolation of *X. campestris* pv. *vitians* from commercial lettuce seeds.

MATERIALS AND METHODS

Bacterial strains. The origins of *X. campestris* pv. *vitians* strains used in this study are listed in Table 1. The strain LMG938M3 is a rifampicin-resistant mutant selected from the wild type strain, LMG938 grown on one-tenth-strength tryptic soy agar (1/10-TSA) (3 g of tryptic soy broth [211825, Difco, Sparks, Detroit, MI], and 15 g of agar per liter) supplemented with 100 mg of rifampicin per liter (R-3501, Sigma, Oakville, Canada). Saprophytic bacteria from field-grown lettuce leaves were obtained by dilution plating of leaf washings on 1/10-TSA supplemented with 50 mg of cycloheximide per liter (C-7698, Sigma). To represent a wide diversity of saprophytic bacteria, strains were chosen according to their gram reaction (21) and their color on 1/10-TSA. Strains of *X. campestris* pv. *vitians* were maintained in sterile tap water at room temperature and saprophytes were maintained on 1/10-TSA slants and stored at 4°C. To evaluate the effect of the different ingredients of the semi-selective medium on saprophytic bacteria, all 20 bacterial saprophytes collected from lettuce leaves were mixed in equal parts. *Xanthomonas* strains were tested singly and in mixtures.

Corresponding author: V. Toussaint
E-mail: toussaintv@em.agr.ca

V. Toussaint's present address: Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Gouin Boulevard, St.-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada.

This research was supported by Agriculture and Agri-Food Canada and INRA.

Accepted for publication 18 October 2000.

Publication no. D-2000-1204-01R

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2001.

Development of the semi-selective medium. The different ingredients of the semi-selective medium were chosen based on their capacity to support the growth of *X. campestris* pv. *vitians* and to inhibit the saprophytic populations present on lettuce leaves. Each component was tested separately (carbon sources, antibiotics, growth factors, and dyes).

Carbon utilization. The capacity of *X. campestris* pv. *vitians* and the saprophytes to use different carbon sources was evaluated on solid minimal medium (2.7 g of KH_2PO_4 , 3.5 g of K_2HPO_4 , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g of CaCl_2 , 15 g of agar, 1 ml of a trace elements solution [per liter: 9.5 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 73 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 68 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 10 mg of $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 11 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 10 mg of H_3BO_3], and 1 liter of ddH₂O). The carbon sources tested were added to this minimal medium at a final concentration of 1%. Potential carbon sources were chosen based on the results of a BIOLOG assay (23) which screened 96 different carbon sources. The carbon sources selected were glucose, maltose, sucrose, starch, and cellobiose. Each suspension of *X. campestris* pv. *vitians* and saprophytic bacteria were adjusted approximately to 3×10^9 cells ($A_{590} = 0.2$). The bacterial suspensions tested were first, a mix of equal parts of each of the 14 strains of *X. campestris* pv. *vitians* listed in Table 1; second, a mixture of equal parts of each of the 20 strains of saprophytes isolated from lettuce leaves; and third, pure suspensions of the strains LMG938, VT25, VT100, VT106, and VT107. The growth of bacterial strains on the different carbon sources was determined by dilution plating with three replicates per dilution. Colony-forming units (CFU) were counted after an incubation period of 72 h at 28°C. Plating efficiency was determined for each carbon source relative to the growth of the strains on the minimal medium containing glucose.

Resistance to antibiotics. Twenty-one strains of *X. campestris* pv. *vitians* and 24 lettuce leaf saprophytes were tested for their resistance to antibiotics of the commercial antibiograms ATB UR 14030 and ATB ANA 14260 (bioMérieux, Marcy-L'Étoile, France). Inoculation of the antibiograms was done as described by the manufacturer except that bacterial suspensions were adjusted spectrophotometrically to $A_{590} = 0.2$. Presence of bacterial growth was noted for each antibiotic and for each strain after 1 and 2 days of incubation at 28°C.

Effect of growth factors on bacterial growth. The ability of *X. campestris* pv. *vitians* to grow on methionine, yeast extract, and tryptone was determined on the solid minimal medium described above supplemented with 10 g of glucose per liter. The amount of the different growth factors added to the minimal medium was per liter: 0.2 g of methionine, 5 g of yeast extract, or 5 g of tryptone. The bacterial suspensions used were prepared as described previously. The recovery of bacteria from suspensions was accomplished by dilution plating on the media containing none or one of the different growth factors with three replicates for each dilution. CFU on plates were counted after an incubation period of 72 h at 28°C.

Selectivity and differential character of dyes. Methyl green, methyl violet, and a mix of both were tested for their inhibitory effect on saprophytes and on the color of *X. campestris* pv. *vitians* colonies. Dyes were added to the solid minimal medium described above supplemented with 10 g per liter of glucose (per liter: 2 ml of a 1% aqueous solution of methyl green, 1 ml of a 1% methyl violet solution in 20% ethanol, or a mix of 2 ml of methyl green and 1 ml methyl violet solutions). Bacterial suspensions were the same as those described above. The suspensions were dilution plated (1:9) on the different media containing no dye or one of the different dyes with three replicates for each dilution. CFU on plates were counted after an incubation period of 72 h at 28°C.

Evaluation of the maltose, methyl green (MMG) medium. The resulting semi-selective medium was evaluated for the recovery of the *X. campestris* pv. *vitians* in vitro, from lettuce leaves and soil. In a first experiment, the plating efficiency and the accuracy in identifying colonies of *X. campestris* pv. *vitians* on the MMG medium (semi-selective medium) and on D-5 medium (6) was compared to that obtained on 1/10-TSA by dilution plating. The different bacterial suspensions used were the same as the ones described above. Plates were counted after 7 days of incubation at 28°C. A second experiment was performed to compare the recovery rate of the MMG medium, SM medium (4), and tetrazolium chloride (TZC) medium (7) to that of nutrient agar (0001-01-8, Difco). The same bacterial suspensions were used and plates were counted after 7 days of incubation at 28°C.

To evaluate the efficiency of the semi-selective medium in recovering *X. campestris* pv. *vitians* from 4-week-old plants, greenhouse lettuce plants were sprayed with sterile water, a bacterial suspension of LMG938, or a bacterial suspension of LMG938M3. Both suspensions were adjusted to $A_{590} = 0.2$. Inoculated plants were arranged in a greenhouse at $20 \pm 3^\circ\text{C}$ according to a randomized complete block design with a total of three blocks and three treatments per block. After incubation (7 days) three plants per block per treatment were collected. Each plant was weighed and then put in a plastic bag to which 20 ml of 0.1 M phosphate buffer at pH 7.0 (61.5 ml of K_2HPO_4 1 M, 38.5 ml of KH_2PO_4 1 M, and 900 ml of ddH₂O) was added. Plants were macerated in a stomacher (model 400 laboratory blender, Seward Medical, UK) for 2 min at high speed. The size of the total bacterial population and of *X. campestris* pv. *vitians* per gram of lettuce was determined by dilution plating on 1/10-TSA, on the MMG medium, and on 1/10-TSA supplemented with rifampicin at 50 mg per liter. For *X. campestris* pv. *vitians*-like colonies, 25 were randomly sampled and streaked on yeast chalk agar (29). Their ability to induce a hypersensitive reaction on tobacco and their pathogenicity on lettuce were determined by injecting 0.5 ml of bacterial suspension ($A_{590} = 0.2$) into plant leaves.

To evaluate the MMG medium's efficiency in recovering *X. campestris* pv. *vitians* from soil, nine flats were filled with 1.5 kg of muck soil from the Ste-Clothilde experimental farm of Agriculture and Agri-Food Canada, Quebec. Three flats were inoculated with 300 ml of sterile water, three flats with 300 ml of a suspension of LMG938 from a 48-h culture on 1/10-TSA, and three with 300 ml of a suspension of LMG938M3 from a 48-h culture on 1/10-TSA. Both bacterial suspensions were adjusted to $A_{590} = 0.2$. Flats were arranged in a random complete block design in a greenhouse at $20 \pm 3^\circ\text{C}$ and incubated for 4 h. After incubation, three samples of 5 g of soil were taken from each flat and thoroughly mixed with 20 ml of 0.1 M phosphate buffer pH 7.0. Bacterial population size was evaluated by dilution plating on the MMG medium, on 1/10-TSA supplemented with 50 mg of rifampicin per liter and on 1/10-TSA.

Statistical analysis. Analyses of variance were conducted on recovery rate (%) with the general linear model (GLM) procedure (Statistical Analysis System Institute Inc., Cary, NC). Differences among treatment levels were determined using the least significant difference (LSD). The arcsin-square root transformation was not used because some values were greater than 100%.

RESULTS

Carbon utilization. There was a significant effect of carbon sources on the

Table 1. Origin of *Xanthomonas campestris* pv. *vitians* strains used in this study

Strain	Origin
3.10.3 L3, 3.10.3 L7	K. Pernezny, Florida
BPIC1788	M. P. G. Psallidas, Greece
CFBP3979	L. Gardan, France
LMG938	LMG Culture Collection
VT14, VT25, VT41	Isolated in Quebec, summer 1996, V. Toussaint
VT100, VT106, VT107, VT112, VT115, VT117	Isolated in Quebec, summer 1998, V. Toussaint

recovery rate of *X. campestris* pv. *vitians* strains ($P = 0.0088$) and saprophytes ($P = 0.0001$). For *X. campestris* pv. *vitians*, more bacteria were recovered on starch and sucrose than on glucose, but the variation among strains was large for these two carbon sources. There was no significant difference in the number of bacteria recovered on cellobiose and maltose as compared to the glucose medium (Fig. 1). For saprophytes, CFU recovery on cellobiose, maltose, and starch was significantly greater than on glucose. On sucrose, however, there was an excessive production of slime by the saprophytic bacteria and by *X. campestris* pv. *vitians*. On maltose, *X. campestris* pv. *vitians* colonies were circular and convex as opposed to the slimy texture and rapid confluence of the colonies on sucrose. On cellobiose and starch, the colonies developed only after 8 days of incubation and were smaller compared to those on media amended with maltose and glucose. Maltose was selected as the main source of carbon because it prevented the formation of slimy colonies and facilitated the enumeration of bacterial colonies.

Effect of antibiotics. Amoxicillin and cephalothin completely inhibited 33% of the strains of saprophytes; whereas, each of these antibiotics inhibited only 5% of the *X. campestris* pv. *vitians* strains tested. The antibiotics vancomycin, gentamycin, and tobramycin used in different semi-selective media for *Xanthomonas* (17) inhibited 44, 91, and 95%, of the *X. campestris* pv. *vitians* strains, respectively.

Effects of growth factors. There was a significant effect of growth factors on the recovery rate of *X. campestris* pv. *vitians* ($P = 0.0001$) and saprophytic bacteria ($P = 0.0001$). For the *X. campestris* pv. *vitians*, recovery rate was significantly lower on yeast extract than on the medium without growth factors. No significant difference in recovery rate was obtained for methionine, but recovery rate was significantly higher on tryptone compared to the medium without growth factors (Fig. 2). For the saprophytes the recovery rates on tryptone and yeast extract were significantly lower than on the medium without growth factors, yet were significantly higher on methionine (Fig. 2).

Effect of dyes. There was a significant effect of dyes on the recovery rate of *X. campestris* pv. *vitians* strains ($P = 0.0001$) and saprophytes ($P = 0.0004$). For *X. campestris* pv. *vitians*, recovery on the medium was significantly reduced by the addition of dyes. The recovery rates were 81.4% on methyl green and 3.8% on methyl violet (Fig. 3). For the saprophytes, recovery was also significantly inhibited by the addition of dyes. The presence of methyl green in the medium, however, gave a greenish color to the colonies of *X. campestris* pv. *vitians* which made them easier to differentiate from other bacteria.

Evaluation of the semi-selective medium. The resulting MMG medium was composed of (per liter): 10 g of maltose

(reference M-5885, Sigma), 5 g of tryptone (0.123-17-3, Difco), 3.5 g of K_2HPO_4 (P-3786, Sigma), 2.75 g of KH_2PO_4 (P-5379, Sigma), 1 ml of trace element (as described above), 2 ml of a 1% aqueous solution of methyl green (M-8884, Sigma), and 15 g of agar (0145-07-0, Difco). The pH was adjusted to 7.0, and the medium was autoclaved 20 min at 121°C, 15 psi. After autoclaving and cooling to about 50°C, antibiotics were added. Antibiotics were dissolved in ethanol and added at a final concentration of (per liter): 32 mg of amoxicillin (A-8523, Sigma), 32 mg of

cephalothin (C-4520, Sigma), and 50 mg of cycloheximide (C-7698, Sigma). On MMG, *X. campestris* pv. *vitians* colonies were shiny yellow-green with green centers. Colonies were smooth, circular, and raised (1 to 1.5 mm) with an average diameter of 2 mm. After 4 days incubation, a zone of discoloration of the medium was observed around *X. campestris* pv. *vitians* colonies probably due to the accumulation of the dye by the bacterial colonies. Colonies were easy to identify after 7 days of incubation at 28°C (Fig. 4). Other strains of pathovars of *Xanthomonas* have been

Fig. 1. Recovery rate of *Xanthomonas campestris* pv. *vitians* and saprophytes on different carbon sources. For *X. campestris* pv. *vitians* each bar represents the mean recovery rate (%) (colony forming units [CFU] on carbon source medium/CFU on glucose medium $\times 100$) for each of six strains of *X. campestris* pv. *vitians* (LMG938, VT25, VT100, VT106, and VT107) and a mixture of all 14 of the *X. campestris* pv. *vitians* strains shown in Table 1. The standard error of each mean is indicated.

Fig. 2. Recovery rate of *Xanthomonas campestris* pv. *vitians* and saprophytes on different growth factors. For *X. campestris* pv. *vitians*, each bar represents the mean recovery rate (5) (colony forming units [CFU] on growth factors medium/CFU on glucose medium without growth factors $\times 100$) for each of six pure strains of *X. campestris* pv. *vitians* (LMG938, VT25, VT100, VT106, and VT107) and a mixture of all 14 of the *X. campestris* pv. *vitians* strains presented in Table 1. The standard error of each mean is indicated.

grown on the MMG medium, and overall it was possible to differentiate *X. campestris* pv. *vitians* from others. Tested strains of the pathovars *armoraciae*, *campestris*, *dieffenbachiae*, and *juglandis* of *X. campestris*, grew faster and had a darker green pigmentation than *X. campestris* pv. *vitians* on the MMG medium. *X. campestris* pv. *vesicatoria* grew as fast as *X. campestris* pv. *vitians*, but colonies also showed a darker green pigmentation. Colonies of *X. campestris* pv. *syngonii* were dark yellow, and *X. campestris* pv. *carotae* did not grow on the medium at all. Finally, it was difficult to differentiate *X.*

campestris pv. *alfalfae* from *X. campestris* pv. *vitians*.

The recovery of *X. campestris* pv. *vitians* on MMG was evaluated in vitro, from lettuce plants, and from soil. For the first in vitro experiment, 2.41 and 4.13% of the *X. campestris* pv. *vitians* colonies recovered on 1/10-TSA were recovered on MMG in the first and second trials, respectively (Table 2). *X. campestris* pv. *vitians* strains, however, did not grow well on the D-5 medium. MMG medium allowed recovery of 2.0 and 6.6% of the colonies of saprophytes recovered on 1/10-TSA in the first and second trials, respec-

tively. For the saprophytes on D-5 medium, the recovery rates for the first and second trials were 3.51 and 9.57%, respectively (Table 2). For the second experiment, the recovery rate varied from 5.7 to 149.0% for the MMG medium compared with that of nutrient agar (Table 3).

On artificially inoculated lettuce leaves, the recovery of strains LMG938 and LMG938M3 was possible from all infected lettuce samples. Colonies of *X. campestris* pv. *vitians* were countable after 4 days on MMG medium, but were much easier to differentiate from the saprophytes after 7 days of incubation. From the MMG medium, all isolated colonies which were *X. campestris* pv. *vitians*-like induced an HR in tobacco and were virulent on lettuce. For the plants inoculated with sterile water, 6.6 to 17.4% of the colonies recovered on 1/10-TSA were recovered on MMG medium, but none of the colonies on MMG medium looked like *X. campestris* pv. *vitians* colonies. For the plants inoculated with the wild-type strain, LMG938, 5.6 to 10.6% of the colonies recovered on 1/10-TSA were recovered on MMG medium. For the colonies recovered on MMG medium, 80.0 to 100% of these colonies were *X. campestris* pv. *vitians*-like. It was impossible to determine which of the colonies recovered on 1/10-TSA were *X. campestris* pv. *vitians*. On the plants inoculated with the rifampicin resistant mutant strain LMG938M3, 70.9 to 97.8% of the colonies recovered on 1/10-TSA were recovered on the MMG medium and 82.8 to 100% of the colonies recovered on 1/10-TSA were recovered on 1/10-TSA supplemented with rifampicin. All the colonies recovered on MMG and 1/10-TSA supplemented with rifampicin were *X. campestris* pv. *vitians*-like.

For the soil experiment, the rifampicin-resistant strain LMG938M3 was inoculated at a final concentration of 9.3×10^7 CFU g^{-1} of soil. The recovery was 6.7×10^6 CFU g^{-1} of soil on MMG medium and 8.7×10^6 CFU g^{-1} of soil on 1/10-TSA-rifampicin medium after an incubation period of 4 h. For the wild type strain LMG938, the bacteria were inoculated at a final concentration of 7.2×10^7 CFU g^{-1} of soil and 5.9×10^5 CFU g^{-1} were recovered after 4 h of incubation.

DISCUSSION

MMG medium allowed the recovery of *X. campestris* pv. *vitians* from bacterial suspensions, lettuce leaves and soil samples. As expected, the recovery rate was much lower on MMG medium than on a non-selective medium such as 1/10-TSA. But on a non-selective medium it is nearly impossible to identify colonies of *X. campestris* pv. *vitians* and to quantify bacterial populations. On MMG quantification was possible because most of the saprophytes were inhibited and because of the differential color and shape of the colonies of *X.*

Fig. 3. Recovery rate of *Xanthomonas campestris* pv. *vitians* strains and saprophytes on different dyes. For *X. campestris* pv. *vitians* strains, each bar represents the mean recovery rate (%) (colony forming units [CFU] on dyes medium/CFU on glucose medium without dyes $\times 100$) for each of six pure strains of *X. campestris* pv. *vitians* (LMG938, VT25, VT100, VT106, and VT107) and a mixture of all 14 of the *X. campestris* pv. *vitians* strains presented in Table 1. The standard error of each mean is indicated.

Fig. 4. *Xanthomonas campestris* pv. *vitians* colonies on the MMG medium.

campestris pv. *vitians*. TSA has been reported to be an excellent medium for the recovery of *Escherichia coli* from a wide range of environments (9,14,20), but also for miscellaneous bacteria. The recovery rate on the MMG medium is higher when compared to nutrient agar. The recovery rate of *X. campestris* pv. *vitians* on TZC is high, but saprophytes grow easily on this non-selective medium. For the SM medium, the recovery rate is lower than on MMG (4). On SM, the colonies are very small and no hydrolysis zone is observed around *X. campestris* pv. *vitians* colonies.

Other media for *Xanthomonas* spp. such as SX (16) or FS (30) did not allow the recovery of *X. campestris* pv. *vitians* from soil and plant tissues probably because *X. campestris* pv. *vitians* is inhibited by the methyl violet present in these media. In addition, there was no recovery of *X. campestris* pv. *vitians* strains from bacterial suspensions, lettuce leaves, or soil on D-5 medium. It has already been reported that D-5 is a poor medium for isolation of *Xanthomonas* pathovars (4,17). Moreover, antibiotics such as gentamycin, tobramycin, and vancomycin present in this and other semi-selective media for *Xanthomonas* spp. (5,8,10,13,18,28) inhibit the growth of *X. campestris* pv. *vitians* strains. This could explain, at least in part, why these media were not useful for the isolation of *X. campestris* pv. *vitians*. *X. campestris* pv. *vitians* is a slow grower compared to most saprophytes, hence, a compromise must be made between the selectivity of the medium and the desired recovery rate.

To avoid the degradation of antibiotics and dyes, the MMG medium must be

stored under cold, dark conditions. When it is stored at room temperature in the presence of light, the color of the medium changes and colonies of *X. campestris* pv. *vitians* are less typical and become harder to identify. The MMG medium is easy to prepare. Stock trace minerals and dye solutions can be prepared in advance and stored at 4°C in dark conditions. The colonies of *X. campestris* pv. *vitians* are easily recognizable because of their color and their shape on the MMG medium. As for any semi-selective medium, reference strain should always be included in the test to ensure proper identification of the bacteria of interest. Moreover, no UV light or reactive is required for counting colonies, which makes this medium easy to use. Finally, MMG medium will also be a useful tool for diagnosing BLSL, and for recovering bacteria from lettuce plants, infested soil, or other sources of inoculum.

LITERATURE CITED

1. Alvarez, A. M., and Lou, K. 1985. Rapid identification of *Xanthomonas campestris* pv. *campestris* by ELISA. Plant Dis. 69:1082-1086.
2. Burkholder, W. H. 1954. Three bacteria pathogenic on head lettuce in New York State. Phytopathology 44:592-596.
3. Carisse, O., Ouimet, A., Toussaint, V., and Philion, V. 2000. Evaluation of the effect of seed treatments, bactericides, and cultivars on bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians*. Plant Dis. 84:295-299.
4. Chun, W. W., 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. Clafin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology 77:730-734.

6. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Phytopathology 60:969-976.
7. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on tetrazolium medium. Phytopathology 44:693-695.
8. Kim, H. K. 1982. Epidemiological, genetical, and physiological studies of the bacterial leaf streak pathogen, *Xanthomonas campestris* pv. *translucens* (J. J. R.) Dowson. Ph.D. diss. Montana State Univ., Bozeman.
9. McClery, D. R., and Rowe, M. T. 1995. Development of a selective plating technique for the recovery of *Escherichia coli* O157:H7 after heat stress. Lett. Appl. Microbiol. 21:252-256.
10. McGuire, R. G., Jones, J. B., and Sasser, M. 1986. Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. Plant Dis. 70:887-891.
11. Ohata, K., Serizawa, S., Azegami, K., and Shirata, A. 1982. Possibility of seed transmission of *Xanthomonas campestris* pv. *vitians*, the pathogen of bacterial spot of lettuce. Bull. Nat. Ins. Agric. Sci. 36:81-88.
12. Pernezy, K., Raid, R. N., Stall, R. E., Hodge, N. C., and Collins, J. 1995. An outbreak of bacterial spot of lettuce in Florida caused by *Xanthomonas campestris* pv. *vitians*. Plant Dis. 79:359-360.
13. Randhawa, P., and Schaad, N. W. 1984. Selective isolation of *Xanthomonas campestris* pv. *juglandis* from walnut buds and catkins. Phytopathology 74:268-272.
14. Rocelle, M., Clavero, S., and Beuchat, L. R. 1995. Suitability of selective plating media for recovering heat- or freeze-stressed *Escherichia coli* O157:H7 from tryptic soy broth and ground beef. Appl. Environ. Microbiol. 61:3268-3273.
15. Sahin, F., and Miller, S. A. 1997. Identification of the bacterial leaf spot pathogen of lettuce, *Xanthomonas campestris* pv. *vitians*, in Ohio, and assessment of cultivar resistance and seed treatment. Plant Dis. 81:1443-1446.
16. Schaad, N. W. 1972. A new medium for the isolation of *Xanthomonas campestris*. (Abstr.) Phytopathology 62:787.
17. Schaad, N. W. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria. American Phytopathological Society, St. Paul.
18. Schaad, N. W., and White, W. C. 1985. A selective medium for soil isolating *Xanthomonas campestris* pv. *translucens* from wheat seeds. Phytopathology 75:260-263.
19. Schroth, M. N., Thompson, J. P., Bardin, R., and Greathead, A. 1964. A new disease in California: Bacterial spot of lettuce. Calif. Agric. 18:2-3.
20. Silk, T. M., and Donnelly, C. W. 1997. Increased detection of acid-injured *Escherichia coli* O157:H7 in autoclaved apple cider by using nonselective repair on trypticase soy agar. J. Food Protec. 60:1483.

Table 2. Plate counts and plating efficiency for *Xanthomonas campestris* pv. *vitians* and saprophytes in the first *in vitro* experiment

Strain	Trials	Colonies per plate ^a			Plating efficiency ^b	
		1/10-TSA	D-5	MMG	D-5	MMG
Mixture of <i>X. campestris</i> pv. <i>vitians</i>	1	2,710.3	0.0	65.3	0.0	2.4
	2	4,376.7	0.0	180.7	0.0	4.1
Mixture of saprophytes	1	703.3	24.7	14.0	3.51	2.0
	2	850.0	81.3	55.7	9.57	6.65

^a Plates counts adjusted to the same dilution. 1/10-TSA = one-tenth-strength tryptic soy agar; D-5 = medium from Kado and Heskett (6); MMG = maltose methyl green medium (semi-selective medium for *X. campestris* pv. *vitians*).

^b Plating efficiency = (mean # of CFU on semi selective medium/mean # of CFU on 1/10-TSA) × 100.

Table 3. Plate counts and plating efficiency for *Xanthomonas campestris* pv. *vitians* and saprophytes in the second *in vitro* experiment

Strain	Colonies per plate ^a					Plating efficiency ^b			
	NA	1/10-TSA	MMG	SM	TZC	1/10-TSA	MMG	SM	TZC
Mixture of <i>X. campestris</i> pv. <i>vitians</i>	45	73	13	4	45	162.2	28.8	8.9	100
LMG938	77	1,185	170	295	11	1,539.0	220.8	383.1	14.2
VT25	989	4,810	56	59	872	486.3	5.7	5.9	88.2
VT41	320	1,162	98	40	150	363.1	30.6	12.5	46.9
Mixture of saprophytes	142	320	10	33	55	225.3	23.8	23.2	38.7

^a Plates counts adjusted to the same dilution. NA = nutrient agar, 1/10-TSA = one-tenth-strength tryptic soy agar, MMG = maltose methyl green medium (semi-selective medium for *X. campestris* pv. *vitians*), SM = starch-methionine medium (4), and TZC = tetrazolium chloride medium (7).

^b Plating efficiency = (mean # of CFU on semi selective medium/mean # of CFU on 1/10-TSA) × 100.

21. Suslow, T. V., Shroth, M. N., and Isaka, M. 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology* 72:917-918.
22. Toussaint, V. 1999. Bacterial leaf spot, a new disease of lettuce in Quebec caused by *Xanthomonas campestris* pv. *vitians*. *Phytoprotection* 80:121-125.
23. Toussaint, V., Morris, C. E., and Carisse, O. 1998. Identification, resistance to antibiotics, and utilization of carbon sources by *Xanthomonas campestris* pv. *vitians* strains isolated from different lettuce growing areas. Abstract 3.3.36 in: Abstracts of invited and offered papers, 7th Int. Congr. Plant Pathol., Edinburgh, Scotland.
24. Umesh, K. C., Koike, S. T., and Gilbertson, R. L. 1996. Association of *Xanthomonas campestris* pv. *vitians* with lettuce seed. (Abstr.) *Phytopathology* 86:S3.
25. Wallis, F. M., and Joubert, J. J. 1972. Bacterial leaf spot of lettuce in Natal. *Phytopathology* 4:137-138.
26. Wang, Z. K., Comstock, J. C., Hatzoloukas, E., and Schaad, N. W. 1999. Comparison of PCR, BIO-PCR, DIA, ELISA, and isolation on semiselective medium for detection of *Xanthomonas albilineans*, the causal agent of leaf scald of sugarcane. *Plant Pathol.* 48:245-252.
27. Wellman-Desbiens, E. 1998. Études sur les sources de contamination par les bactéries responsables de la nervation noire du chou et de la nécrose marginale de la laitue. M.Sc. Diss. Sherbrooke University, Sherbrooke.
28. Williford, R. E., and Schaad, N. W. 1984. Agar medium for selective isolation of *Xanthomonas campestris* pv. *carotae* from carrot seeds. (Abstr.) *Phytopathology* 74:1142.
29. Wilson, E. E., Fakhery, M. Z., and Fredrickson, D. L. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. *Phytopathology* 57:618-621.
30. Yuen, G. Y., Alvarez, A. M., Benedict, A. A., and Trotter, K. J. 1987. Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris* pv. *campestris*. *Phytopathology* 77:366-370.