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# Development of a Selective Medium for *Xanthomonas campestris* pv. *translucens*

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# 맥류 세균성줄무늬병균의 선택배양기 개발

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**ABSTRACT : We have developed a selective medium (KM-1) useful for isolation of bacterial leaf streak pathogen of wheat and barley from diseased plant materials and other environment. KM-1 contains 10 g of lactose, 4.0 g of D(+) trehalose, 0.2 g of thiobarbituric acid, 0.8 g each of $K_2HPO_4$ and $KH_2PO_4$, 30 mg of yeast extract, 1 g of $NH_4Cl$, 100 mg of cycloheximide, 8.0 mg of tobramycin, 1.0 mg of ampicillin and 15 g of Bacto agar per liter of distilled water (pH 6.6). Plating efficiency of KM-1 over non-selective Wilbrink's medium was about 1.30 for *Xanthomonas campestris* pv. *translucens* and those of KM-1 over nutrient agar medium for other 12 *Xanthomonas* nomenspecies were also investigated. This medium revealed high selectivity for *X. campestris* pv. *translucens* and against wide spectrum of soil-borne plant pathogens and/or saprophytic bacteria and other microflora associated with infected as well as noninfected leaves. The shelf-life of this medium was proven to be two months or longer at 5°C. The potential usages of this medium for ecological study were also discussed.**

***Key words* : *Xanthomonas campestris* pv. *translucens*, selective medium, KM-1.**

A few media have been described for various xanthomonads (8, 12, 15), but none has been useful for isolation of *Xanthomonas campestris* pv. *translucens*. The medium commonly employed for isolating this pathogen is Wilbrink's agar (4) which is not considered to be selective. SX agar was described in 1974 by Schaad and White (15) for isolation of *Xanthomonas campestris* from soil. A semi-selective medium for isolation of *Xanthomonas campestris* pv. *juglandis* from walnut was developed by Mulrean and Schroth in 1981 (12). Kado and Heskett (8) in 1970 reported that their D-5 medium selectively favors the growth of *Xanthomonas* spp. and *Agrobacterium tumefaciens* while suppressing the growth of *Pseudomonas* spp.

The objective of this study was to develop a selective medium, an essential tool for epidemiological studies of this pathogen. Selective medium development is usually an important step in the scientific study of a bacterial plant pathogen.

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## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this work are listed in Table 1. *Xanthomonas campestris* pv. *translucens*, hereinafter referred to as *X. c. t.* and *Agrobacterium* spp., were maintained on Wilbrink's agar (4); 10 g of sucrose, 5 g of Bacto-peptone (Difco Laboratories, Detroit, MI.), 0.5 g of $K_2HPO_4$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $Na_2SO_3$, and 15 g of bacto-agar (Difco) per liter of distilled water. All other strains of *Xanthomonas* were maintained on nutrient broth (Difco), 5 g of casein hydrolysate (Sigma Chemical Co., St. Louis, MO.), 1 g of yeast extract (Difco), 2.0 g of $K_2HPO_4$, 0.5 g of $KH_2PO_4$, and 15 g of Bacto-agar per liter of distilled water. *Corynebacterium sepedonicum* and *C. insidiosum* were maintained on yeast glucose calcium carbonate agar; 15 g of glucose, 10 g of yeast extract, 50 g of $CaCO_3$, and 20 g of agar per

liter of distilled water. *Bacillus cereus, Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were maintained on nutrient agar (Difco nutrient broth amended with 15 g of Difco agar). *Escherichia coli* was maintained on Luria's agar containing 10 g of casein hydrolysate, 10 g of NaCl, 5 g of yeast extract, and 15 g of Bacto-agar per liter of distilled water. *Pseudomonas* spp. were maintained on King's B agar (10). All strains were transferred regularly and stored at 3~5°C.

**Selective Medium (KM-1).** This selective medium for *X. c. t.* contains 10 g of lactose (Sigma Chemical Co., St. Louis, MO.), 4.0 g of D(+) trehalose (Sigma), 0.2 g of thiobarbituric acid (Sigma), 0.8 g of $K_2HPO_4$ and $KH_2PO_4$ (Sigma) respectively, 0.03 g of yeast extract (Difco Laboratories, Detroit, MI.), 1 g of $NH_4Cl$ (Mallinckroft Chemical Works, St. Louis, MO.), and 15 g of Bacto agar per liter of double distilled water. Before adding agar, the ingredients were dissolved completely on a hot plate with stirring bar, and pH was adjusted to 6.6 with 1 N NaOH solution. After autoclaving these ingredients, cycloheximide (Sigma) dissolved in ethanol, tobramycin (Sigma) dissolved in ethanol-water (1:1, v/v), and ampicillin (Sigma) dissolved in ethanol-water (1:1, v/v) with addition of 1 pellet of sodium hydroxide (Mallinckroft Inc., Paris, Kentucky) were added aseptically to the final concentration of 100 μg/ml, 8 μg/ml and 1 μ/ml, respectively.

**Characterization of bacterial isolates from the KM-1 selective medium.** Color, shape, and form of colony were initially used to distinguish *X. c. t.* Selected colonies were also transferred to Wilbrink's agar to observe the rapid development of yellow, mucoid growth, which characterizes xanthomonads. These colonies were further confirmed by an oxidase test and pathogenicity tests.

**Relative colony development of *Xanthomonas campestris* pv. *translucens*.** Single colony culture from Wilbrink's agar was suspended in 5 ml phosphate-buffered saline (PBS) containing 0.85 percent NaCl, 0.57 percent $K_2HPO_4$ and 0.34 percent $KH_2PO_4$ (pH 6.8), after adjusting cell suspensions to contain approximately $10^6$ colony forming units (CFU) per milliliter as determined by a Klett-Summerson photometric colorimeter with the green filter (Model 800-3, Klett Mfg. Inc., NY., U.S.A.). The serial ten-fold dilutions were made on PBS. Using a spinning turntable, one-tenth of a milliliter of diluted cell suspensions was spread with an L-shaped glass rod over the surface of KM-1 medium and Wilbrink's medium in each of three plates. The plates were incubated at 28°C for 5~7 days. Unless otherwise stated, the dilution plating procedure and incubation period are the same throughout this paper.

**Relative colony development of other *Xanthomonas* nomenspecies.** Some strains of *Xanthomonas* spp. other than *X. c. t.* do not grow very well on Wilbrink's agar. Therefore, the relative colony development on KM-1 medium was also compared with nutrient agar amended with 1 percent glucose.

**Spectrum of inhibition.** Isolates were first replica-plated, and then dilution-plated to determine the spectrum of inhibition of this KM-1 medium. The isolates that grew poorly, if at all, on the KM-1 medium by replica plating were selected. Dilution plating was carried out to see how selective this medium was to those bacteria (Table 1).

In order to determine the spectrum of inhibition to soilborne plant pathogenic bacteria and saprophytic bacteria, three strains of *X. c. t.* were mixed with 11 different nomenspecies of bacteria (Table 5). After the dilution plating method was used, the plates were observed in 2~3 days for fast growing organisms and again in 5~7 days for *X. c. t.* at 28°C.

**Selectivity tests.** Selectivity of the KM-1 medium was tested by the dilution plating method of various plant samples, such as 1) old samples naturally infected with *X. c. t.*, 2) fresh samples naturally infected, 3) old barley leaf debris not infected, and 4) soil samples from greenhouse. Old barley leaf debris was rinsed with sterile distilled water to remove soil particles and kept in phosphate-buffered saline (PBS) containing 0.85 percent NaCl, 0.57 percent dibasic potassium phosphate ($K_2HPO_4$), 0.34 percent monobasic potassium phosphate ($KH_2PO_4$), for four hours. Old and fresh samples of naturally infected barley were kept in PBS for the same period of time. No surface sterilization was attempted for any plant materials. The 10 grams of the soil sample from the greenhouse were suspended in 100 ml distilled sterile water and stirred vigorously with a stirring bar for 30 minutes. After 10 minutes settling, the supernatant was centrifuged for 20 minutes at 1,000 g force to remove plant materials and soil particles. The supernatant was centrifuged at 3,000 g for 20 minutes. The pellet was suspended in 3 ml PBS and used as the sample for the dilution plating method in which 0.1 ml of each appropriate dilution in PBS was spread on three replicates of both KM-1 medium and Wilbrink's medium.

**Shelf life of KM-1 medium.** Several packages of

**Table 1.** Strains used for evaluation of medium selective for *Xanthomonas campestris* pv. *translucens*

| Nomenspecies and designation | Source[a] |
|---|---|
| *Agrobacterium tumefaciens* | 1 |
| *Agrobacterium rhizogenes* | 1 |
| *Bacillus cereus* | 2 |
| *Corynebacterium insidiosum* | 2 |
| *Corynebacterium sepedonicum* | 2 |
| *Erwinia carotovora* var. *atroseptica* | 2 |
| *Erwinia carotovora* var. *carotovora* | 2 |
| *Escherichia coli* | 1 |
| *Pseudomonas aeruginosa* | 5 |
| *Pseudomonas fluorescens*, Pf5 | 6 |
| *Pseudomonas phaseoli*, G50 | 7 |
| *Pseudomonas phaseoli*, HB20 | 7 |
| *Pseudomonas savastanoi* | 3 |
| *Pseudomonas syringae*, (8 strains) | 10 |
| *Pseudomonas syringae* pv. *tagetis* | 4 |
| *Pseudomonas putida* | 10 |
| *Rhizobium phaseoli*, 1233 | 3 |
| *Xanthomonas albilineans*, 29184 | 8 |
| *Xanthomonas axonopodis*, 19312 | 8 |
| *Xanthomonas fragariae*, NCPPB 2473 | 8 |
| *Xanthomonas campestris* pv. *begoniae*, 077-3382 | 8 |
| *X. campestris* pv. *carotae*, Floral-1 | 8 |
| *X. campestris* pv. *campestris*, #73 | 8 |
| *X. campestris* pv. *manihotis*, Xm6 | 8 |
| *X. campestris* pv. *pelargonii*, 078-1100 | 8 |
| *X. campestris* pv. *phaseoli*, 86 | 8 |
| *X. campestris* pv. *pruni*, 8D51 | 9 |
| *X. campestris* pv. *translucens*, (11 strains) | 10 |
| *X. campestris* pv. *vesicatoria*, #26 | 8 |
| *X. campestris* pv. *vitians*, 068-790, 7D51 | 9 |
| 068-1406-7D5 | 9 |
| 069-561-7D42 | 9 |

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this medium were stored at 5°C in a cold room from one week to two months. The selectivity of these KM-1 media were checked at intervals up to two months by the dilution plating method using strains listed in Table 1.

## RESULTS

**Relative colony development of *Xanthomonas campestris* pv. *translucens*.** The average number of colonies of 11 strains of *X. c. t.* developed on KM-1 medium was from 0.91 to 2.13 times greater than on Wilbrink's medium (Table 2). Generally, more colonies appeared on the KM-1 medium than on rich and non-selective Wilbrink's medium. The size of colonies was from 1.5~2.0 to 3.5~4.0 mm in 5~7 days with yellowish color. All strains of *X. c. t.* grew well on this selective medium when a loopful of cell suspension was streaked and incubated for 5~7 days.

**Table 2.** Relative colony development of *Xanthomonas campestris* pv. *translucens* on selective (KM-1) and non-selective (Wilbrink's) medium[a]

| Strain | Colonies per plate[b] | | Plating efficiency[c] | Size of colonies on KM-1 |
|---|---|---|---|---|
| | Wilbrink's | KM-1 | | |
| X- 33 | 326.0± 7.0 | 341.5± 0.7 | 1.04 | 2.5~3.0 |
| X- 40 | 138.3±16.6 | 168.0±26.2 | 1.21 | 3.5~4.0 |
| X- 48 | 189.2± 1.7 | 235.7± 9.5 | 1.24 | 1.5~2.0 |
| X- 56 | 389.6±11.9 | 357.3± 6.3 | 0.91 | 3.5~4.0 |
| X- 58 | 160.5±11.3 | 239.0±20.5 | 1.49 | 2.5~3.0 |
| X- 67 | 230.7±17.3 | 416.5±33.2 | 1.80 | 1.5~2.0 |
| X- 87 | 170.0± 7.0 | 363.5±45.9 | 2.13 | 3.5~4.0 |
| X- 90 | 255.5± 7.0 | 275.2±10.9 | 1.07 | 2.5~3.0 |
| X-138 | 278.0±21.9 | 329.0±36.7 | 1.18 | 2.5~3.0 |
| X-140 | 265.0±21.1 | 346.0±21.1 | 1.30 | 2.0~2.5 |
| X-153 | 343.5±16.2 | 348.5±20.5 | 1.01 | 2.0~2.5 |

[a] KM-1 : *X. c.* pv. *translucens* selective medium; Wilbrink's: Dowson, 1957 (4).

[b] Average number of colonies per plate from 3 plates, followed by standard deviation of mean, after 7 days at 28°C.

[c] $\text{Plating efficiency} = \frac{\text{Avg. no. of colonies on KM-1/plate}}{\text{Avg. no. of colonies on Wilbrink's/plate}}$

**Relative colony development of other *Xanthomonas* nomenspecies.** As shown in Table 3, nutrient agar plus glucose is a good medium for most *Xanthomonas* nomenspecies, whereas few of them grew on Wilbrink's. Single strains each of *Xanthomonas axonopodis, Xanthomonas campestris* pv. *campestris* and *X. campestris* pv. *vesticatoria* grew on Wilbrink's medium with colony sizes from 0.5~3.0 mm in diameter. Out of 12 nonemspecies, two strains of *X. campestris* pv. *vitians* 7D5 and 7D52 grew poorly or not at all on the Wilbrink's medium, whereas the strain 7D52 of the same species grew on Wilbrink's medium. However, the number of colonies developed on this medium was about half of those on the nutrient agar.

Whereas one strain each of *Xanthomonas alibili-*

*neans* and *X. fragariae* did not grow on KM-1 medium, one strain each of *X. axonopodis* and *X. campestris* pv. *begoniae* did, even though the plating efficiency of these strains was generally low. One strain out of three strains of *X. campestris* pv. *vitians* did not grow at all, while the two strains of this same nomenspecies grew on KM-1 medium. One strain each of *X. campestris* pv. *carotae, X. campestris* pv. *manihotis, X. campestris* pv. *pelargonii, X. campestris* pv. *phaseoli, X. campestris* pv. *pruni*, and *X. campestris* pv. *vesicatoria* grew on KM-1 medium and the plating efficiency of these nomenspecies was relatively high. However, the growth of *X. campestris* pv. *phaseoli* and *X. campestris* pv. *vesicatoria* was limited and resulted

**Table 3.** Relative colony development of other *Xanthomonas* nomenspecies on the selective (KM-1) and non-selective (WA, NAG) medium

| Nomenspecies and designation | Colonies per plate[a] | | | Plating efficiency[d] | Size of colonies on KM-1 |
|---|---|---|---|---|---|
| | NAG | WA | KM-1 | | |
| *Xanthomonas albilineans*, 29184 | 25.0±13.5[b] | 0[c] | 0 | 0 | 0 |
| *X. axonopodis*, 19312 | 140.6±22.0 | 355.5±63.4 | 29.6± 4.0 | 0.21 | 2.6±3.2 |
| *X. fragariae*, NCPPB 2472 | 77.3±16.0 | 0 | 0 | 0 | 0 |
| *Xanthomonas campestris* pv. *begoniae*, 077-3382 | 113.6± 6.6 | –[e] | 21.0± 2.6 | 0.18 | 0.2~0.5 |
| *X. c.* pv. *carotae*, Floral-1 | 210.6±17.0 | – | 412.0±56.2 | 1.96 | 1.5~2.5 |
| *X. c.* pv. *campestris*, #73 | 157.6± 6.6 | 282.0±63.8 | 180.6±17.2 | 1.14 | 3.0~3.5 |
| *X. c.* pv. *manihotis*, Xm6 | 202.0±24.0 | 0 | 296.0±43.0 | 1.46 | 3.5~4.0 |
| *X. c.* pv. *pelargonii*, 078-1100 | 63.6±12.5 | – | 190.3±32.8 | 2.98 | 2.5~3.0 |
| *X. c.* pv. *phaseoli*, 86 | 97.0± 7.0 | – | 134.0±12.5 | 1.38 | 0.7~1.2 |
| *X. c.* pv. *pruni* 8D51 | 81.3± 7.0 | – | 454.6±46.0 | 5.59 | 2.5~3.0 |
| *X. c.* pv. *vesticatoria*, #26 | 121.6±18.0 | 346.3±15.5 | 293.3±53.2 | 2.41 | 0.2~0.5 |
| *X. c.* pv. *vitians*, 068-790, 7D51 | 191.0±19.0 | – | 287.3± 9.6 | 1.50 | 3.0~3.5 |
| *X. c.* pv. *vitians*, 068-1406, 7D5 | 128.3±12.7 | 0 | 127.6±19.1 | 0.99 | 2.0~3.0 |
| *X. c.* pv. *vitians*, 069-561, 7D52 | 135.3±11.2 | 63.3±10.2 | 0.6± 0.5 | 0.004 | 1.0~1.5 |

[a] Average number of colonies per plate from 3 plates, followed by standard deviation, after incubation at 28°C for 6~7 days. NAG : Nutrient agar amended with 1% glucose. WA : Wibrink's agar.
[b] Very small colonies less than 0.5 mm while colonies of another nomenspecies on NAG were 3.5~6.0 mm in diameter.
[c] No colonies developed at all.
[d] Plating efficiency=Avg. number of colonies on KM-1/avg. number of colonies on NAG.
[e] Microsopic colonies, the sizes being less than 0.1 mm in diameter.

**Table 4.** Colony development of phytopathogenic and saprophytic bacteria on selective (KM-1) or non-selective medium (Wilbrink's) for *Xanthomonas campestris* pv. *translucens*

| Nomenspecies | Number of colonies[a] | | Recovery ratio[c] |
|---|---|---|---|
| | Wilbrink's | KM-1 | |
| *Agrobacterium tumefaciens* | 116.6±26.0 | 0[b] | 0 |
| *Erwinia carotovora* var. *atroseptica* | 19.6± 3.5 | 0 | 0 |
| *Erwinia carotovora* var. *carotovora* | 283.6±13.2 | 0 | 0 |
| *Pseudomonas aeruginosa* | 173.6±14.5 | 0 | 0 |
| *Pseudomonas fluorescens* | 283.3±53.7 | 0 | 0 |
| *Pseudomonas putida* | 288.3±45.3 | 0 | 0 |
| *Pseudomonas savastanoi* | 140.3±11.5 | 0 | 0 |

[a] Average number of colonies per plate from 3 plates, followed by standard deviation, after incubation for 7 days at 28°C.
[b] No colonies developed in 7~9 days at 28°C.
[c] $\text{Recovery ratio} = \frac{\text{Number of colonies on KM-1 medium}}{\text{Number of colonies on Wilbrink's medium}}$

only in small colonies after 5~7 days.

**Spectrum of inhibition.** Most of the pseudomonads listed in Table 1 did not grow on the KM-1 medium by replica plating methods or by streaking a loopful of bacterial cell suspensions. Those pseudomonads, *Agrobacterium* sp. and *Erwinia* spp. that did grow on the KM-1 medium by replica plating grew poorly and did not develop colonies on this medium when the bacterial cell suspensions were spread on by the dilution plating method (Table 4).

When the mixture of the 12 nomenspecies of soil-borne plant pathogenic bacteria and soil-borne saprophytic bacteria was serially dilution-plated (Table 5), no colonies appeared on KM-1 medium after 2~3 days incubation at 28°C, whereas 376 colonies were developed on the non-selective Wilbrink's medium. Pure colonies of *X. c. t.*, 1.5~2.0 mm in diameter, appeared in 5~7 days of incubation. Other colonies of unknown classification less than 0.1 mm in diameter also appeared in further incubation. However, they remained as small colonies less than 0.5 mm in diameter even after 15 days of incubation. Selected colonies of either size were transferred to Wilbrink's agar to confirm the virulence to host and the rapid development of yellow color. All the large colonies were proven to be *X. c. t.*

**Selectivity test.** No colonies appeared on KM-1 medium from dilution plating of uninfected barley debris and only three small non-yellow colonies were developed on this medium from greenhouse soil, while 162 and 93 colonies of the unknown contaminants were developed on Wilbrink's medium, respectively (Table 6).

By taking both old and fresh barley leaf samples from infected plants, it was possible to isolate the *X. c. t.* with plating efficiencies of KM-1, as compared to

**Table 5.** Colony development from a mixed inoculum containing several phytopathogenic and saprophytic bacteria on selective (KM-1) and non-selective (Wilbrink's) medium

| Number of days incubated at 28°C | Number of colonies[a] | | Colony size on KM-1 (mm) |
|---|---|---|---|
| | Wilbrink's | KM-1 | |
| 2~3 | 376.6± 6.6[b] | 0 | 0 |
| 5~7 | 602.3±13.6[b] | 71.0±11.8[c] | 1.5~2.0 |

[a] Average number of colonies per plate from 3 plates, followed by standard deviation.

[b] None of the colonies was recognizable as *X. c. t.*

[c] Apparently pure colonies of *X. c. t.* Bacterial inoculum contained approximately equal CFU's of *Agrobacterium tumefaciens, A. rhizogenes, Bacillus cereus, Corynebacterium insidiosum, C. sepedonicum, Erwinia carotovora* var. *atroseptica, E. carotovora* var. *carotovora, Pseudomonas aeruginosa, P. fluorescens, P. putida, P. savastanoi,* and *Xanthomonas campestris* pv. *translucens* (3 strains).

**Table 6.** Relative selectivity of KM-1 medium when plating noninfected samples

| Sample | Number of colonies[a] | |
|---|---|---|
| | Wilbrink's | KM-1 |
| Old leaef | 162.0± 24.0[b] | 0[c] |
| Fresh leaf | 93.0± 2.8[b] | 3.0[d] |

[a] Average number of colonies per plate from 3 plates, followed by standard deviation.

[b] Unknown contaminants by dilution plating method.

[c] No colonies appeared after 5~7 days at 28°C.

[d] Very small colonies less than 0.5 mm in diameter, not recognizable as xanthomonads.

**Table 7.** Relative selectivity of KM-1 medium when plating infected samples

| Sample | No. of colonies[b] on Wilbrink's | | No. of colonies of *X. c. t.* on KM-1 | Plating efficiency[c] | Size of colonies on KM-1 (mm) |
|---|---|---|---|---|---|
| | *X. c. translucens* | Unknown | | | |
| Old leaf[a] | 73.5± 3.5 | 415.5±78.4 | 113.0±16.9[d] | 1.53 | 2.0~2.5 |
| Fresh leaf | 72.0±29.6 | 432.5±85.5 | 81.3±15.6 | 1.13 | 2.0~2.5 |

[a] Barley leaf sample stored in laboratory for 7~8 months.

[b] Average number of colonies per plate from 3 plates, followed by standard deviation.

[c] $\text{Plating efficiency} = \frac{\text{Avg. number of colonies on KM-1 medium per plate}}{\text{Avg. number of colonies on Wilbrink's medium per plate}}$

[d] Bacterial colonies of unknown classification with diameters <0.1 mm appeared in 3~4 days growing to <0.3~0.5 mm even after 5~7 days incubation at 28°C. The colonies of *X.c.t.* appeared later but developed to large colonies, 2.0~2.5 mm in diameter in 5~7 days.

Wilbrink's of 1.53 and 1.13, respectively (Table 7). On the rich Wilbrink's medium, numerous unknown contaminants, in addition to fluorescent pseudomonads, were encountered. However, on the KM-1 medium only very small colonies started to appear in 3~4 days when incubated at 28°C. These colonies remained at less than 0.1~0.3 mm in diameter even in 5~7 days incubation. *X. c. t.* colonies appeared later but developed into large colonies, 2.0~2.5 in diameter within 5~7 days at 28°C. Selected colonies of either size were transferred to the rich medium to observe rapid development of yellow color. All the large colonies and a few (1 in 30) of the small colonies were confirmed to be *X. c. t.*

**Shelf life.** This medium was proven to be still effective as a selective medium even after two months of storage at 5°C.

## DISCUSSION

A few selective media have been described for isolation of *Xanthomonas* spp. from nature. None of them is satisfactory for isolation of *X. c. t.* The selective medium designated as KM-1 for this bacterium has been developed to provide sensitive and reliable detection of this bacterium from nature.

Generally, the bacteria comprising the genus *Xanthomonas* grow slower than any of the other plant pathogenic bacteria and the saprophytic bacteria included in this study. The selectivity of this KM-1 medium is mainly based on choice of carbon sources and the use of a combination of antibiotics.

**Choice of components of KM-1.** Dowson (3) reported that all the species of his newly created genus *Xanthomonas* utilized lactose as a carbon source. Thus, lactose is one of the carbon sources that appears to be promising as a selective medium component for *Xanthomonas*. Trehalose, a twelve-carbon sugar, is also a good nutritional substrate for the *Xanthomonas campestris* group (6). *Pseudomonas fluorescens* also utilizes trehalose (11, 16), but is not used by any of the members of the *Pseudomonas syringae* group, *Pseudomonas chichorii* (14), or *Pseudomonas putida*, or *Pseudomonas aeruginosa* (16). The combination of these carbon sources provides selectivity against *Pseudomonas syringae*, which has been recognized as a common contaminant in the isolation of *X. c. t.* from diseased material ever since 1936 (7). Thiobarbituric acid is another component of selective value. Ammonium chloride as a nitrogen source is useful in enhancing the yellowish colony color, a key visual characteristic of these xanthomonads. Yeast extract is a necessary source of nutritional enrichment, permitting these xanthomonads to grow at reasonably fast rates. The basal medium of KM-1 without antibiotics, exhibits fair selectivity against the fluorescent, oxidase negative, "syringae group" of pseudomonads from cereals.

The key problem in developing the selective medium for *X. c. t.* was that saprophytes grow more rapidly and are, in fact, more resistant to most antibiotics. Most plant pathogenic pseudomonads and other plant pathogenic bacteria grow faster than this pathogen in most of the media. We found that fast growing contaminant bacteria can be suppressed by using diluted concentrations of either penicillin or ampicillin, however, this pathogen is more tolerant of ampicillin than penicillin. In both cases, the mechanism of action of the above two antibiotics inhibits actively growing cells. A search for several other antibiotics with different modes of action was initiated. Tobramycin was found very useful to suppress the fast growing pseudomonads, especially the ubiquitous saprophytic fluorescent, oxidase-positive, nutritionally versatile pseudomonads relatively resistant to other antibiotics. Cycloheximide, a broad spectrum antifungal agent, was also added.

**Selectivity and usage of this medium.** Other xanthomonads grow on KM-1 medium and, since they are not believed to be found in cereal grains, do not present problems. Those bacteria capable of growing on KM-1 are: one strain out of three strains of *Xanthomonas campestris* pv. *vitians*, and one strain each of *X. campestris* pv. *carotae*, *X. campestris* pv. *campestris*, *X. campestris* pv. *manihotis*, *X. campestris* pv. *pelargonii*, *X. campestris* pv. *phaseoli* and *X. campestris* pv. *vesicatoria*. The plating efficiency of these nomenspecies, with the exception of *Xanthomonas campestris* pv. *vitians*, was relatively high. The potential use of the KM-1 medium for these nomenspecies remains unknown until many strains representing each species are tested and shown to grow adequately on this medium. One strain of *X. campestris* pv. *pruni* grew very well on this KM-1 medium with a very high plating efficiency. Again, many strains should be tested with this medium along with XPSM medium by Civerolo *et al.* (2), in order to determine the potential use of this medium as a selective medium for this nomenspecies.

This KM-1 medium has high selectivity against the soil-borne plant pathogenic bacteria, such as *Agrobacterium tumefaciens, Agrobacterium rhizogenes, Erwinia carotovora* var. *atroseptica, Erwinia carotovora* var. *carotovora, Corynebacterium insidiosum, Corynebacterium sepedonicum,* and also the common soil-borne saprophytic bacteria such as *Bacillus cereus, Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Pseudomonas putida*. This KM-1 medium may be a useful tool to study the ecology and epidemiology of this pathogen (13). It has a relatively long shelf-life of, at least two months when refrigerated, high selectivity against soil samples and barley leaf debris, and a high recovery ratio of the several strains of *X. c. t.*, compared to the rich but non-selective medium of Wilbrink's (4).

This medium may be a valuable tool to detect and prove the quantitative distribution of this pathogen on plants, and insects or in raindrops as an ice nucleation active agent (9). All xanthomonads recognized at present are plant pathogens found only in association with living plants or with plant material (5, 6). This medium could be a tool to detect and identify new saprophytic or avirulent xanthomonads. *X. c. t.* is known as a poor survivor in soil unless it is combined with plant or plant debris (1). This medium could reveal a quantitative fluctuation of bacterial cells in the soil, and may, if present, also enable one to find a bacterium-vector relation.


## 요 약

맥류세균성 줄무늬병균의 선택배양기(KM-1)를 개발하여 이병식물체 및 토양으로부터 *Xanthomonas campestris* pv. *translucens*를 선택적으로 분리할 수 있는 효율성을 검토하였다. KM-1배양기의 구성성분은 증류수 1 L당 lactose 10 g, D(+)trehalose 4.0 g, thiobarbituric acid 0.2 g, $K_2HPO_4$ 및 $KH_2PO_4$ 각각 0.8 g, yeast extract 30 mg, $NH_4Cl$ 1 g, cycloheximide 100 mg, tobramycin 8.0 mg, ampicillin 1.0 mg 및 Bacto agar 15 g이며 1 N NaOH로 pH 6.6으로 조절하였다. *X. c. t.*의 균주별 KM-1의 배양효율은 비선택성 농후배지인 Wilbrinks agar에 비하여 1.30정도였으며, 기타 토양전염성식물병원세균 *Agrobacterium tumefaciens, Agrobacterium rhizogenes, Erwinia carotovora* var. *atroseptica, Erwinia carotovora* var. *carotovora, Corynebacterium insidiosum,* 및 기타 토양생존 부생세균 *Bacillus cereus, Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Pseudomonas putida* 등의 생장을 완벽하게 억제하였다. KM-1의 저장기간(shelf-life)도 5°C에서 2개월 동안 선택성을 유지하였다. 따라서 본 병원균의 전염원의 생존 등 발생생태연구에 활용될 수 있는 가치가 충분히 인정되었다.


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