Bacterial Streak and Bulb Rot of Onion: I. A Diagnostic Medium for the Semiselective Isolation and Enumeration of *Pseudomonas viridiflava*

R. Gitaitis, D. Sumner, and D. Gay, Department of Plant Pathology, D. Smittle and G. McDonald, Department of Horticulture, and B. Maw, Department of Biological and Agricultural Engineering, University of Georgia, Tifton 31793; W. C. Johnson III, Nematodes, Weeds, & Crops Unit, USDA ARS, Tifton, GA 31793; and B. Tollner, Department of Biological and Agricultural Engineering, and Y. Hung, Center of Food Safety and Quality, University of Georgia, Athens 30602

ABSTRACT

Gitaitis, R., Sumner, D., Gay, D., Smittle, D., McDonald, G., Maw, B., Johnson, W. C., III, Tollner, B., and Hung, Y. 1997. Bacterial streak and bulb rot of onion: I. A diagnostic medium for the semiselective isolation and enumeration of *Pseudomonas viridiflava*. Plant Dis. 81:897-900.

A semiselective, diagnostic agar medium (T-5) and low temperature incubation technique were developed for recovering *Pseudomonas viridiflava* from the environment or plant material. Medium T-5 contains the following per liter: NaCl, 5.0 g; $\text{NH}_4\text{H}_2\text{PO}_4$, 1.0 g; K_2HPO_4 , 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; D-tartaric acid, 3.0 g; phenol red, 0.01 g; agar, 20.0 g; bacitracin, 10 mg; vancomycin, 6 mg; cycloheximide, 75 mg; novobiocin, 45 mg; penicillin G, 5 mg. The pH is adjusted to 7.4. Antibiotics are added aseptically after autoclaving. *P. viridiflava* recovery from artificially infested, field-soil (Tifton loamy-sand), with a cropping history of no onion production, was high, with a corresponding reduction of 99.99% of nontarget bacteria. However, soils from fields with a long history of onion production, near Vidalia, Georgia, contained significantly larger populations of background microflora that grew on medium T-5. Incubation at 5°C reduced contaminating microflora 1,000- to 10,000-fold with no reduction in recovery of the target organism. However, this low temperature incubation required an increased incubation period of 3 weeks and reduced the level of fluorescence of *P. viridiflava*.

Bacterial streak and rot of onion (*Allium cepa* L.), caused by *Pseudomonas viridiflava* (Burkholder) Dowson, has been a problem in onion-growing areas of Georgia since 1990 (6). Often, lesions initiate in the creases of flagged leaves as water-soaked, tan, oval spots. Lesions expand as dark green, water-soaked streaks down the leaf and darken with age until they are almost black. Soft rot frequently develops at the base of leaves above the neck of the bulb. Inner scales of infected bulbs initially are bright yellow, become reddish-brown with age, and occasionally are speckled with dark, "metallic" green globular material (1 to 3 mm diameter). The disease predisposes onion bulbs to other soft-rot pathogens as well as secondary or opportunistic organisms associated with postharvest decays. The disease is particularly severe under cool, wet conditions

that occur in Georgia in the winter and early spring.

Sources of inocula for recent epidemics in onion have not been identified. *P. viridiflava* has a wide host range that includes alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.) (15), bean (*Phaseolus vulgaris* L.) (4), poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) (22), tomato (*Lycopersicon esculentum* Mill.) (10), and other plants (24). It is not known if the onion pathogen survives in seed, soil, in association with another host, or as a resident on weeds. A laboratory technique for the detection and enumeration of *P. viridiflava* was needed for such epidemiological studies.

Numerous semiselective media, including BCBRVB (17), KBC (16), KFT (1), NPC (18), NPCC (20), and others (2,11,12,21) have been reported for culture of fluorescent pseudomonads. Most of these media are modifications of King's medium B (KMB; 13) and were unsuitable for isolation of *P. viridiflava* from natural sources in Georgia. We describe a semiselective, diagnostic medium that suppresses growth of many nontarget microorganisms and serves as a suitable medium for growth and enumeration of *P. viridiflava*. A preliminary report of this work has been made (8).

MATERIALS AND METHODS

Sources of bacteria. Strains DD2, R2, and 91-1 of *P. viridiflava* were isolated

from onions in Georgia; strains PV 12322 and PV 12323 (original host bean) were obtained from the American Type Culture Collection (Rockville, MD) and strain PV 5777-4 (original host tomato) was obtained from J. B. Jones, University of Florida Gulf Coast Research & Education Center (Bradenton, FL). Bacteria were maintained in 15% glycerol at -73°C for long-term storage and in sterile tap water for routine use. Strains were grown and maintained on KMB (13) unless otherwise indicated.

Semiselective media composition. Semiselective media KBC (16), KFT (1), BCBRVB (17), and test media T-0 through T-5 were evaluated for growth and enumeration of *P. viridiflava*. In addition to the ingredients in KMB, KBC contains boric acid, 1.5 g; cephalixin, 80 mg; and cycloheximide, 200 mg per liter; KFT contains basic fuchsin, 9 mg and triphenyl-tetrazolium chloride, 0.15 mg per liter; and BCBRVB contains bacitracin, 10 mg; vancomycin, 6 mg; rifampicin, 0.5 mg; cycloheximide, 75 mg; and benomyl, 250 mg per liter.

The composition of media T0 to T5 were based on the utilization of D(-) tartrate (3) and modifications of NPC (18) and BCBRVB (17) and contained the following per liter: NaCl, 5.0 g; $\text{NH}_4\text{H}_2\text{PO}_4$, 1.0 g; K_2HPO_4 , 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; D(-) tartaric acid, 3.0 g; phenol red, 0.01 g; and agar, 15.0 g. The pH was adjusted to 7.4 with 1.0 N NaOH. In addition, T-1 contained penicillin G, 50 mg; novobiocin, 45 mg; and cycloheximide, 75 mg per liter; T-2 contained bacitracin, 10 mg; vancomycin, 6 mg; and cycloheximide, 75 mg per liter; T-3 contained bacitracin, 10 mg; rifampicin, 0.5 mg; and cycloheximide, 75 mg per liter; T-4 contained bacitracin, 10 mg; vancomycin, 6 mg; rifampicin, 0.5 mg; and cycloheximide, 75 mg per liter; and T-5 contained bacitracin, 10 mg; vancomycin, 6 mg; novobiocin, 45 mg; penicillin G, 50 mg; and cycloheximide, 75 mg per liter.

Plating efficiency and selectivity assessments. Thirty milliliters of 24-h-old nutrient-broth, shake cultures were centrifuged at $3,000 \times g$ for 20 min and pellets were suspended in phosphate-buffered saline (PBS) 0.01 M K_2HPO_4 - KH_2PO_4 , 0.85% NaCl; pH 7.3. Bacterial suspensions were adjusted to 50% transmittance at 600

Corresponding author: R. Gitaitis
E-mail: path4@tifton.cpes.peachnet.edu

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the University of Georgia and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Accepted for publication 21 April 1997.

nm using a spectronic 20 (Bausch & Lomb, Rochester, NY) (7). Serial dilutions (1:9) were made in PBS and aliquants (0.1 ml) of selected dilutions were spread on the surface of test media. Colonies were counted after 3 to 5 days' incubation at 25°C. Plating efficiencies were determined by enumerating colonies of *P. viridiflava* on test media, dividing by number of colonies on KMB agar, and multiplying the quotient by 100.

Selectivity was assessed by assaying soil artificially infested with *P. viridiflava*. Soil samples (Tifton loamy-sand [fine loamy, siliceous, thermic Plinthic Kandudults]) were collected from fields in Montgomery, Tift, and Toombs counties, Georgia. The Toombs County site (Site A) had been in an onion-soybean multi-crop, rotation for 5 years and had a history of onions for a minimum of 20 years. The Montgomery County site (Site B) had a history of onion-summer fallow for 5 years. The Tift County site had no history of onion for the past 20 years and was fallow at the time of sampling. Soil cores (80 cores, 2.5-cm diameter, 10 cm deep) were collected from across fields at each site. Soils from each area of a field were mixed thoroughly and divided into two portions for each site. Ten-gram samples from each portion were suspended in separate sterile beakers containing 100 ml of PBS. Aliquants (1.0 ml) of suspensions of *P. viridiflava* were prepared as above and added to one of the soil portions (amended) from each site to produce an estimated population of 10^3 CFU/ml/g of soil. Serial dilutions (1:9) were made in PBS, and aliquants (0.1 ml) from each portion (amended and non-amended) were spread on the surface of test media. Colonies were counted after 3 to 5 days' incubation at 25°C. Tests were replicated three times.

Incubation at different temperatures was evaluated to reduce numbers of colonies of nontarget, saprophytic microorganisms. Aliquants of dilutions of known populations of *P. viridiflava* were spread on KMB and T-5 and incubated at 30, 25, 15, 10, and 5°C. Plating efficiencies were determined as above. All tests were replicated three times.

Identification of bacteria. Colonies picked from test media were characterized by gram-reaction (23), production of a water-soluble, fluorescent pigment on KMB (13), ice nucleation activity (9), utilization of DL-lactate (5,9), D-tartrate (3,5), sucrose (3,5), presence of oxidase

(5,14), and absence of arginine dihydrolase (14). Ability to rot onion was evaluated by aseptically slicing peeled onion bulbs, stabbing the tissue with a bacteriological loop covered with bacteria, incubating them at 28°C, and observing for soft rot symptoms after 24 to 72 h.

Table 2. Recovery of a known population ($\sim 10^3$ CFU/ml) of *Pseudomonas viridiflava* (pv. 91-1) on King's medium B (KMB) and T-5 semiselective agar from six different soil samples^a from Montgomery, Tift, and Toombs counties^b, Georgia

Medium	Soil	Source	Pv. 91-1 population recovered ^{c,d}
KMB	A	Tift	NE
	B	Tift	NE
	C	Tift	NE
	D	Tift	NE
	E	Toombs	NE
	F	Montgomery	NE
T-5	A	Tift	2.5×10^3
	B	Tift	1.6×10^3
	C	Tift	1.5×10^3
	D	Tift	5.9×10^3
	E	Toombs	NE
	F	Montgomery	NE

^a 10 g nonsterile soil; 100 ml PBS (0.01 M K_2HPO_4 - KH_2PO_4 , 0.85% NaCl; pH 7.3); and 1.0 ml of a bacterial suspension containing 10^5 CFU of pv. 91-1/ml PBS (final approximate concentration of 10^3 CFU/ml) were mixed and agitated on a rotary shaker at 150 rpm for 10 min.

^b Cropping history of soils from Tift County did not include onions, whereas the histories of soils in Montgomery and Toombs counties included continuous cropping of onions for 10 and 16 years, respectively.

^c Values represent recovered populations of *P. viridiflava* determined by serially diluting soil-bacterial suspensions in PBS to 10^{-1} , and 0.1 ml from the source and 10^{-1} dilutions were spread plate on each medium. Values are the mean of three replications. NE = no estimate due to interference by abundant growth of nontarget microorganisms.

^d Two representative CFU were picked from each plate of T-5 ($n = 48$) and were streaked to obtain pure cultures on plates of KMB and then were characterized for oxidase, arginine dihydrolase, ice nucleation and utilization of dl-lactate, D(-) tartrate, and sucrose.

Table 3. Effect of incubation temperature (°C) on growth of *Pseudomonas viridiflava* on King's medium B (KMB) and T-5 semiselective agar

Strain	Medium	Log ₁₀ population of <i>P. viridiflava</i> ^a				
		30°C	25°C	15°C	10°C	5°C
DD2	KMB	8.9	8.9	8.8	8.8	8.8
DD2	T-5	8.6	8.8	8.7	8.9	8.8
R2	KMB	8.7	8.8	8.8	8.9	8.9
R2	T-5	8.8	8.8	8.7	8.8	8.7
91-1	KMB	8.9	8.9	8.9	8.9	8.9
91-1	T-5	8.8	8.9	8.9	8.8	8.8

^a Bacteria were grown in nutrient broth for 24 h, harvested by centrifugation, resuspended in PBS (0.01 M K_2HPO_4 - KH_2PO_4 , 0.85% NaCl; pH 7.3), adjusted to 50% transmittance at 680 nm, serially diluted in PBS to 10^{-7} , and 0.1 ml from the 10^{-5} , 10^{-6} , and 10^{-7} dilutions were spread plate on each medium. Values represent log₁₀ estimates of the population of bacterial suspensions incubated at the indicated temperature and are the means of three replications from the 10^{-6} and 10^{-7} dilutions.

Table 1. Recovery efficiency of representative strains of *Pseudomonas viridiflava* on nine different media (KBC [16], KFT [1], BCBRVB [17], and T-0 through T-5) compared with King's medium B (KMB; 13)

Strain	Recovery compared with KMB (%) ^a							
	KBC	KFT	BCBRVB	T-0	T-1	T-2	T-3	T-4
DD2	0.0	18.0	78.0	91.2	50.3	31.2	76.1	23.0
R2	0.0	0.0	6.6	129.8	94.2	7.3	95.0	1.1
91-1	0.0	2.1	52.9	97.4	59.5	27.9	103.5	19.6
12322	0.0	76.8	71.2	39.0	85.2	27.3	67.6	39.3
12323	0.0	24.6	7.8	43.5	47.6	28.5	53.4	41.2
5777-4	0.6	42.1	36.8	108.0	64.6	28.6	85.6	49.7
Mean	0.1	27.3	42.2	85.2	66.9	25.1	80.2	29.0

^a Represents mean of three replicates. Bacteria were grown in nutrient broth for 24 h, harvested by centrifugation, resuspended in PBS (0.01 M K_2HPO_4 - KH_2PO_4 , 0.85% NaCl; pH 7.3), adjusted to 50% transmittance at 680 nm, serially diluted in PBS to 10^{-6} , and 0.1 ml from the 10^{-4} , 10^{-5} , and 10^{-6} dilutions were spread plate on each medium. Plating efficiencies were determined by enumerating colonies of *P. viridiflava* on test media, dividing by number of colonies on KMB agar, and multiplying the quotient by 100.

Data analysis. In all experiments, data were analyzed by PROC GLM and PROC STEPWISE statistical analysis programs using SAS software (19). The Waller-Duncan multiple range test ($P \leq 0.05$) was used as a means separation test. Data were transformed as necessary [$\log_{10}(x+1)$] for analysis.

RESULTS AND DISCUSSION

Medium development and plating efficiencies. Growth efficiencies of different strains of *P. viridiflava* on KFT, KBC, BCBRVB, and test media T-0 through T-5 varied (Table 1). Most strains did not grow well on KBC, and the range of growth efficiency on KFT ranged from 0.0% (onion strain R2) to 76.8% (ATCC 12322), and on BCBRVB from 6.6% (onion strain R2) to 78.0% (onion strain DD2). In general, onion strains DD2, R2, and 91-1 grew more efficiently on T-5 (mean = 90.3%) than did ATCC strains (mean = 57.4%). All strains displayed less efficient growth on media containing both D(-) tartrate and rifampicin at 0.5 µg/ml.

Evaluation of recovery of *P. viridiflava* from soil. *P. viridiflava* was not recovered from any soil samples plated on KMB or from nonamended soils on semiselective agar T-5 (Table 2). The bacterium was recovered on T-5 medium at the expected concentration from all four inoculum-amended soil samples from Tift County (Fig. 1). This medium was selected for further evaluation because it was the most selective of those media that did not significantly affect growth efficiency and *P. viridiflava* raised the pH of the medium and produced a diagnostic pink zone around individual colonies. However, when tested with the two soils from sites A and B (Montgomery and Toombs, counties), the abundant growth of nontarget microflora prevented the easy detection of colonies of *P. viridiflava* (Table 2). Medium T-5 was developed in Tift County and originally was tested with soils that had no history of onion production. The two soils from which *P. viridiflava* was not recovered were from commercial sites with a history of continuous onion production. The different locations, different management practices, and different cropping histories most likely explain the differences in the microflora of these two soils. In all instances, bacterial identities were confirmed by a negative Gram, oxidase, and arginine dihydrolase reactions, slow and very weak utilization of sucrose, utilization of dl-lactate and D(-) tartrate, positive ice nucleation, and ability to rot onion.

Low temperature incubation. Observations of liquefaction of onions stored under refrigeration caused us to hypothesize that incubation at 5°C would support growth of *P. viridiflava* and inhibit competing microflora (8). Plating efficiencies were not significantly different when *P. viridiflava* strains DD2, R2, and PV 91-1

were grown on KMB and T-5 media at temperatures ranging from 30 to 5°C (Table 3). Incubation at 5°C increased the time of growth to achieve a colony large enough for recognition and enumeration from 3 to 5 days at 30°C to 16 to 20 days. In addition, fluorescence was much weaker in plates of cultures grown at the lower temperatures. However, when a final concentration of 10^1 or 10^2 CFU of *P. viridi-*

flava per ml were placed in soil suspensions from Site A and assayed at both 30 and 5°C, the pathogen was recovered only at 5°C. The bacterium was detected 100% of the time when initial levels of inoculum were 10^2 CFU/ml/10 g soil (Fig. 2), but only 33% of the time when bacterial populations dropped to 10^1 CFU/ml/10 g soil (Table 4). This level of sensitivity should be adequate for most epidemiologi-

Fig. 1. Comparison between semiselective medium T-5 (upper) and standard growth medium King's Medium B (lower) for the isolation and enumeration of *Pseudomonas viridiflava* from nonsterile, artificially-infested soil. Darker areas surrounding CFU of *P. viridiflava* on medium T-5 are dark pink and contrast with the lighter, pale orange color of areas with no growth, or around some nontarget microflora.

Fig. 2. Recovery of *Pseudomonas viridiflava* from artificially amended, nonsterile soil with a cropping history of onion-soybean, multi-crop rotation for 5 years and continuous onion cultivation for a minimum of 20 years. An essentially pure culture of *P. viridiflava* was recovered on semiselective medium T-5 at 5°C (upper), compared with the highly diverse microflora obtained on the same medium at 30°C (lower).

Table 4. Recovery of known populations of *Pseudomonas viridiflava* (pv. 91-1) on T-5 semiselective agar at 5 and 30°C from amended field soil

Log ₁₀ CFU/ml added ^a	Incubation temperature (°C)	% Recovery ^b
1	5	33
1	30	0
2	5	100
2	30	0

^a Bacterial suspensions were amended to soil from Toombs County, Georgia that had been used to grow onions for 20 consecutive years to give a final concentration of 10¹ or 10² CFU/ml.

^b Recovered populations of *P. viridiflava* were determined by serially diluting soil-bacterial suspensions in PBS to 10⁻⁴, and 0.1 ml from all dilutions were spread plate on each medium. Recovery efficiencies were determined by enumerating colonies of *P. viridiflava* on test media, converting the value to CFU/ml and making an estimate of population of bacteria in the soil, dividing the estimate by the expected bacterial population, and multiplying the quotient by 100. Values are the mean of three replications.

Table 5. Differences in populations (log₁₀ + 1) of fluorescent pseudomonads and total bacteria recovered from soil on T-5 semiselective agar when incubated at 5 and 30°C

Soil ^a	30°C		5°C		Difference	
	FP ^b	Total ^b	FP	Total	FP	Total
1	3.00 ^{c,d}	6.00	2.00	3.45	1.00	2.55
2	1.00	5.79	0.00	0.00	1.00	5.79
3	2.48	5.81	0.00	0.00	2.48	5.81
4	3.47	6.04	0.00	1.95	3.47	4.09
5	3.30	6.08	0.00	1.00	3.30	5.08
6	3.30	5.82	0.00	0.00	3.30	5.82
7	3.00	5.98	0.00	0.00	3.00	5.98
8	2.31	5.75	0.00	0.00	2.31	5.75
9	5.08	5.71	4.74	4.74	0.34	0.97
10	5.30	5.34	1.60	2.71	3.70	2.63
11	4.60	5.08	0.00	2.86	4.60	2.22
12	3.90	4.61	1.30	2.11	2.60	2.50
Mean	3.40	5.67	0.80	1.57	2.32	4.10

^a 10 g nonsterile soil and 100 ml PBS (0.01 M K₂HPO₄-KH₂PO₄, 0.85% NaCl; pH 7.3) were mixed and agitated on a rotary shaker at 150 rpm for 10 min.

^b FP = Populations of fluorescent pseudomonads; Total = total count of all bacteria.

^c Values represent recovered populations of bacteria determined by serially diluting soil-bacterial suspensions in PBS to 10⁻⁴, and 0.1 ml from all dilutions were spread plate on T-5 semiselective agar. Values were transformed to log₁₀ + 1 and are the mean of three replications.

^d Six representative CFU were picked from each plate of T-5 and were streaked to obtain pure cultures on plates of KMB and then were characterized for oxidase, arginine dihydrolase, ice nucleation and utilization of dl-lactate, D(-) tartrate, and sucrose.

cal studies. When an additional 12 soil samples were collected from Toombs County, processed, and incubated on T-5 at 5°C, the results were a 10,000-fold reduction of total background microflora and a 10-fold reduction of nontarget, fluorescent colonies compared to similar plates incubated at 30°C (Table 5). As before, *P. viridiflava* was not detected in any of the nonamended samples, but only in the artificially infested soils.

In conclusion, semiselective agar T-5 was useful for recovering low concentrations of *P. viridiflava* from a microbially diverse soil habitat. Selectivity was further enhanced by incubating samples at 5°C. Habitats such as soil and seeds will support diverse populations of bacteria and other microflora, illustrated by the differences in recovery of *P. viridiflava* from soils with and without a cropping history of onions. Research on using semiselective medium T-5 to survey the environment of the onion-growing region of Georgia for survival of *P. viridiflava*, to study the population dynamics of the pathogen throughout its

life cycle, and to characterize the microflora associated with postharvest diseases is ongoing.

LITERATURE CITED

- Bashan, Y., and Assouline, I. 1983. Complementary bacterial enrichment techniques for the detection of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in infested tomato and pepper seeds. *Phytoparasitica* 11:187-193.
- Bashan, Y., Okon, Y., and Henis, Y. 1982. A note on the new defined medium for *Pseudomonas tomato*. *J. Appl. Bacteriol.* 52:297-298.
- Billing, E. 1970. *P. viridiflava* (Burkholder, 1930; Clara 1934). *J. Appl. Bacteriol.* 33:492-500.
- Burkholder, W. H. 1930. The bacterial diseases of bean: A comparative study. Mem. 127 N.Y. Agric. Exp. Stn. Ithaca.
- Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R., and Phillips, G. B., eds. 1981. Manual of Methods for General Bacteriology. American Society for Microbiology. Washington, DC.
- Gitaitis, R. D., Baird, R. E., Beaver, R. W., Sumner, D. R., Gay, J. D., and Smittle, D. A. 1991. Bacterial blight of sweet onion caused by *P. viridiflava* in Vidalia, Georgia. *Plant Dis.* 75:1180-1182.

- Gitaitis, R. D., Sasser, M. J., Beaver, R. W., McInnes, T. B., and Stall, R. E. 1987. Pectolytic xanthomonads in mixed infections with *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper transplants. *Phytopathology* 77:611-615.
- Gitaitis, R. D., Sumner, D., Smittle, D., Gay, D., Maw, B., Hung, Y., and Tollner, B. 1992. A semiselective agar medium for identification and isolation of *P. viridiflava*, causal agent of bacterial blight of onion. Pages 23-25 in: Proceedings National Onion Research Conference. Dec. 4-5, 1991, Savannah, GA.
- Jones, J. B., Gitaitis, R. D., and McCarter, S. M. 1986. Fluorescence on single-carbon sources for separation of *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *P. viridiflava* on tomato transplants. *Plant Dis.* 70:151-153.
- Jones, J. B., Jones, J. P., McCarter, S. M., and Stall, R. E. 1984. *P. viridiflava*: Causal agent of bacterial leaf blight of tomato. *Plant Dis.* 68:341-342.
- Kado, C. I., and Heskitt, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology* 60:969-976.
- Katoh, K., and Itoh, K. 1983. New selective media for *Pseudomonas* strains producing fluorescent pigment. *Soil Sci. Plant Nutr.* 29:525-532.
- King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.
- Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. *J. Appl. Bacteriol.* 29:470-489.
- Lukezic, F. L., Leath, K. T., and Levine, R. G. 1983. *P. viridiflava* associated with root and crown rot of alfalfa and wilt of birdsfoot trefoil. *Plant Dis.* 67:808-811.
- Mohan, S. K., and Schaad, N. W. 1987. An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P. s. pv. phaseolicola* in contaminated bean seed. *Phytopathology* 77:1390-1395.
- Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1980. *Pseudomonas*. Pages 36-44 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
- Sands, D. C., and Rovira, A. D. 1970. Isolation of fluorescent pseudomonads with a selective medium. *Appl. Microbiol.* 20:513-514.
- SAS Institute. 1985. SAS User's Guide: Statistics Version, 5th edition. SAS Inst. Inc., Cary, NC.
- Simon, A., Rovira, A. D., and Sands, D. C. 1973. An improved selective medium for isolating fluorescent pseudomonads. *J. Appl. Bacteriol.* 36:141-145.
- Surico, G., and Lavermicocca, P. 1989. A semiselective medium for the isolation of *Pseudomonas syringae* pv. *savastanoi*. *Phytopathology* 79:185-190.
- Suslow, T. V., and McCain, A. H. 1981. Greasy canker of poinsettia caused by *P. viridiflava*. *Plant Dis.* 65:513-514.
- Suslow, T. V., Schroth, 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.
- Wilkie, J. P., and Dye, D. R. W. 1973. Further hosts of *P. viridiflava*. *N.Z. J. Agric. Res.* 16:315-323.