Fluorescence on Single-Carbon Sources for Separation of *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *P. viridiflava* on Tomato Transplants

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ABSTRACT

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Pseudomonas syringae pv. *tomato (P. s. tomato)*, *P. syringae* pv. *syringae* (*P. s. syringae*), and *P. viridiflava*, three foliar pathogens of tomato, were readily separated by their differential capacity to fluoresce on iron-deficient Misaghi and Grogan's medium containing sucrose, erythritol, and DL-lactate as single-carbon sources. *P. s. syringae* fluoresced on media containing sucrose, erythritol, or DL-lactate; *P. s. tomato* fluoresced on media containing sucrose but did not fluoresce on erythritol or DL-lactate; *P. viridiflava* fluoresced on media containing erythritol and DL-lactate but not on sucrose. Similar results were obtained when water suspensions from greenhouse-inoculated plants were streaked on the three media. When field samples were plated on media containing sucrose and erythritol, fluorescence occurred on media containing sucrose when *P. s. tomato* was present but occurred on erythritol medium when *P. viridiflava* was present. In a field where both *P. s. tomato* and *P. viridiflava* were present, water suspensions from individual lesions produced colonies that fluoresced on sucrose or erythritol but not on both. In two other fields where only *P. s. tomato* was present, fluorescence of colonies from similar suspensions occurred on media containing sucrose but not on media containing erythritol or DL-lactate. In separate tests in Georgia transplant fields where *P. s. syringae* and *P. s. tomato* were present, 91 and 0% of the lesions infected with *P. s. syringae* and *P. s. tomato*, respectively, produced fluorescent colonies on an iron-deficient medium that contained DL-lactate. Capacity to fluoresce on certain single-carbon media may be a rapid means for differentiating three foliar pathogens that occur on tomato.

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (*X. c. vesicatoria*), has been the most important bacterial-incited foliar disease of tomato in Georgia transplant production fields and fruit production fields in Georgia and Florida. Recently, fluorescent pseudomonads have become common foliar pathogens in tomato fields in Georgia and Florida (7,9) and worldwide (3,6,16,17). Bacterial speck, incited by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, & Wilkie (*P. s. tomato*), was first reported in Florida in 1933 (1) and reached epiphytotic proportions in fruit production fields during the unusually wet winter season of 1978 (13). *P. syringae* pv. *syringae* van Hall (*P. s. syringae*) has become prominent as a leaf spot pathogen in the Georgia tomato transplant fields (4,7). In 1983, another bacterial foliar problem incited by *P. viridiflava* (Burkholder) Dowson caused extensive damage of tomato in southwestern Florida (9). In the tomato transplant industries of Georgia and Florida, rapid and accurate identification of causal organisms of diseases is essential for the efficient operation of a certification program. Determination of which specific fluorescent pseudomonad is responsible for a foliar problem, though not difficult, may require considerable time. Timely transplant shipments require a rapid determination (several days) so that the planting schedule of northern growers is not disrupted. Some progress in rapid separation of *P. s. syringae* and *P. s. tomato* in pure culture and in foliar lesions has been made with indirect immunofluorescence and ice-nucleation activity (8), but improvements still are needed. The purpose of this study was to determine whether different phytopathogenic fluorescent pseudomonads isolated from tomato could be distinguished by fluorescence on media containing single-carbon sources and to determine whether these media can be used for rapid identification of *P. s. syringae*, *P. s. tomato*, and *P. viridiflava* from certain foliar lesions in tomato plants. Although *P. viridiflava* has been found associated with tomatoes in Florida, *P. s. syringae* and *P. s. tomato* are the only fluorescent pathogenic pseudomonads that have been found associated with leaf spots on tomato transplants grown in Georgia or Florida. Therefore, because differentiation of *P. s. syringae* and *P. s. tomato* on tomato transplants is more critical, our emphasis was placed on distinguishing between these two pathogens.

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MATERIALS AND METHODS

Bacterial strains. Forty-two strains of *P. s. syringae*, *P. s. tomato*, and *P. viridiflava* were used. Seventeen strains were *P. s. syringae*, of which 10 were isolated from tomato plants in Georgia. Sixteen strains were *P. s. tomato* from the United States and Canada. Nine strains were *P. viridiflava*, of which seven were isolated from tomato plants from Florida.

All strains were maintained on nutrient yeast-dextrose agar (7) and held at 4–6 C between transfers. For inoculum production, cultures were grown for 24–48 hr at 28 C on medium B of King et al (KMB) (10).

Evaluation of media for growth and fluorescence. Various single-carbon sources used for differentiation of fluorescent pseudomonads (4,14) were tested using two basal media. Mannitol, D(−)tartrate, erythritol, sucrose, DL-lactate, inositol, β-alanine, and sorbitol were added at a concentration of 0.2% (w/v) separately to the mineral basal medium of Misaghi and Grogan (12), modified by eliminating iron to enhance fluorescence and using Noble agar instead of oxoid agar. Strains of the three fluorescent pseudomonads were tested on the media. Plates of the media were streaked with a bacteral suspension (10^8 colony-forming units [cfu] per milliliter) from a culture (28–48 hr) on KMB. Inoculated plates were placed at 25 C and were checked daily for growth and fluorescence.

Evaluation of selected media for detecting *P. s. syringae*, *P. s. tomato*, and *P. viridiflava* in leaf tissue from artificially inoculated tomato plants. FM 6203 tomato plants were inoculated separately with one isolate of *P. syringae*, *P. s. tomato*, or *P. viridiflava*. Five plants were inoculated with each bacterium by gently misting or wounding the foliage as reported previously (7,9). After 10 days, 25 lesions were collected at random from the five plants inoculated with each of the three pathogens. Healthy tissue sections were also collected from uninoculated plants. Individual lesions and healthy tissue sections (control) were macerated in sterile distilled water with dissecting

Table 1. Growth and fluorescence of nine phytopathogenic pseudomonads when grown on a mineral-base medium containing various carbon sources[a]

Bacteria	No. of isolates tested	No. of strains with positive reactions							
		Mannitol	D(−)Tartrate	Erythritol	Sucrose	DL-Lactate	*m*-Inositol	β-Alanine	Sorbitol
Pseudomonas syringae pv. *syringae*	8	8 (8)[b]	4 (4)	8 (8)	8 (8)	8 (8)	8 (8)	0	8 (8)
P. syringae pv. *tomato*	11	11 (11)	11 (2)	0	11 (11)	0	11 (11)	0	11 (11)
P. viridiflava	9	9 (9)	9 (9)	9 (9)	0	9 (9)	9 (9)	0	9 (9)

[a] Basal medium was Misaghi and Grogan's (11), modified by eliminating the iron source. The carbon sources were added at the rate of 0.2%, and plates were streaked with a loop.

[b] The first number in each pair represents the strains that grew, and the number in parentheses represents those that fluoresced. Zero indicates no growth.

Table 2. Growth and fluorescence of bacterial colonies from macerated tomato tissue from fields in Florida on three media containing different single-carbon sources[a]

Field number	No. of samples	Growth and fluorescence			Bacteria identified as cause
		Sucrose	Erythritol	DL-Lactate	
1	8	0	6	ND	*Pseudomonas viridiflava*
2	8	0	5	ND	*P. viridiflava*
3	8	2[b]	3[b]	ND	*P. viridiflava* and *P. syringae* pv. *tomato*
4	8	8	0	ND	*P. syringae* pv. *tomato*
5	10	10	0	0	*P. syringae* pv. *tomato*
6	10	9	0	0	*P. syringae* pv. *tomato*

[a] Basal medium was Misaghi and Grogan's (11), modified by eliminating the iron source.

[b] Lesion extracts that fluoresced on sucrose were not the same lesion extracts that fluoresced on erythritol. ND = Not done.

needles, allowed to stand for 20 min, then streaked on KMB and on modified Misaghi and Grogan's medium containing erythritol, sucrose, D(−)tartrate, or DL-lactate. Plates were incubated at 25 C and observed daily for 4 days for fluorescence intensity. Typical colonies were selected, and the identity of each isolate was determined by routine tests (4,7).

Evaluation of selected media for detecting *P. s. tomato, P. s. syringae,* and *P. viridiflava* in lesions from field-grown tomatoes. Six tomato fields in southwestern Florida with leaf spot problems known to be caused by fluorescent pseudomonads were selected to evaluate the single-carbon source media. Fields designated 1 and 2 had a known problem with *P. viridiflava*, and fields 3, 5, and 6, with *P. s. tomato*. Field 4 had both *P. viridiflava* and *P. s. tomato*. Eight and 10 lesions were collected at random from fields 1–4 and 5–6, respectively. The lesions were macerated and streaked as described earlier. The basal medium was modified Misaghi and Grogan's as described earlier. Sample suspensions from all fields were streaked on media containing sucrose or erythritol. In addition, samples from two fields were also streaked on medium containing DL-lactate. Plates were incubated at 25 C and observed for growth and fluorescence daily for 4 days. Representative colonies were transferred and tested further to confirm their identities.

In a separate test, leaf spot samples were collected from 80 tomato transplant fields (three samples per field) in southern Georgia. The samples contained lesions suspected of being caused by *P. s. tomato* or *P. s. syringae* but not *P. viridiflava*. The only likely phytopathogenic fluorescent pseudomonads associated with leaf spots in the tomato transplant industry in Georgia are *P. s. syringae* and *P. s. tomato* (8). In preliminary tests, fluorescence on DL-lactate effectively separated *P. s. syringae* and *P. s. tomato*. Lesion extracts were plated on a mineral basal medium (1 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KH_2PO_4, 1 g of $(NH_4)_2HPO_4$, 1.5% Bacto agar, 20 ml of 0.04% phenol red [Sigma], and 980 ml of water, pH 7.1) containing the DL-lactate syrup and on KMB. After 24 hr, both media were evaluated for fluorescent bacteria. A representative fluorescent colony was picked from one plate per sample. The colony was tested for utilization of erythritol and sucrose (5), oxidase reaction (7), ice-nucleating activity (7), and arginine dihydrolase activity (15). On the basis of a previous study (8), these tests were found sufficient for identifying *P. s. syringae* and *P. s. tomato* in naturally occurring lesions in Georgia.

RESULTS

Evaluation of media for growth and fluorescence. The various strains of fluorescent pseudomonads differed in their capacity to grow and fluoresce on different media containing single-carbon sources (Table 1). When various carbon compounds were added to iron-deficient Misaghi and Grogan's basal medium, only strains of *P. s. syringae* and *P. viridiflava* fluoresced on all media that they utilized (Table 1).

Evaluation of selected media for detecting *P. s. syringae, P. s. tomato,* and *P. viridiflava* in leaf tissue from artificially inoculated plants. Water suspensions from macerated tomato tissue artificially inoculated with *P. s. syringae* produced fluorescent colonies on iron-deficient Misaghi and Grogan's medium that contained sucrose, erythritol, or DL-lactate. Suspensions from lesions caused by *P. s. tomato* resulted in both growth and fluorescence on sucrose. No growth occurred on erythritol or DL-lactate, whereas growth occurred on all plates of D(−)tartrate, but no fluorescence was observed. Suspensions from lesions caused by *P. viridiflava* resulted in growth and fluorescence on erythritol and DL-lactate, but no growth occurred on sucrose medium.

Evaluation of selected media for detecting *P. s. syringae, P. s. tomato,* and *P. viridiflava* in lesions from field-grown tomatoes. When isolations were made from field 1, where *P. viridiflava* was the known pathogen, six of eight lesions produced fluorescent colonies on erythritol but not on sucrose (Table 2). Isolates on erythritol were identified as *P. viridiflava*. Five of eight macerated lesions from field 2, where *P. viridiflava* was also present, produced fluorescent growth on erythritol, but none produced fluorescent growth on sucrose. The isolates that fluoresced on erythritol were identified as *P. viridiflava*. *X. c. vesicatoria* was isolated from three lesions. In field 3, where *P. viridiflava* and *P. s. tomato* were both confirmed, two lesions yielded bacteria that fluoresced on sucrose and three lesions produced fluorescent colonies on erythritol. The bacteria that fluoresced on erythritol were identified by bacteriological tests (9) as *P. viridiflava*, whereas those that fluoresced on sucrose were identified by bacteriological and pathogenicity tests (7) as *P. s. tomato*. In fields 4–6, where *P. s. tomato* was the known pathogen, water suspensions from lesions produced fluorescent colonies on sucrose but not on erythritol. All of the isolates on sucrose were confirmed as *P. s. tomato* by pathogenicity and bacteriological tests (7). D(−)Tartrate medium gave variable results when evaluated in fields 5 and 6.

In southern Georgia, 240 lesions were assayed for *P. s. syringae* or *P. s. tomato* (Table 3). Of those, *P. s. syringae*-type colonies were isolated from 207 of the lesions. Extracts from 187 of the 207 lesions produced growth that fluoresced on the DL-lactate medium. Thus 90% of the *P. s. syringae*-type isolates produced fluorescent colonies on DL-lactate. Of the

Table 3. Fluorescence of bacterial colonies from 240 macerated tomato lesions from transplant fields in southern Georgia

Identity of bacteria[a]	Leaf samples (no.)	Fluorescence on		Utilization of		INA[b]	Oxidase	Arginine dihydrolase
		DL-Lactate	KMB	Sucrose	Erythritol			
P. s. syringae	183	+[c]	+	+	+	+	−	−
Not determined	15	−	−	...[d]	...	...	...	...
P. s. syringae	10	−	+	+	+	+	−	−
	4	−	+	+	+	−	−	−
	3	+	+	+	+	−	−	−
P. s. tomato	6	−	+	+	−	−	−	−
X. c. vesicatoria	18	−	−	...	...	...	...	...
Not determined	1	+	+	+	+	−	−	−
No. of lesions	240	187	207	207	201	193	1	1

[a] Based on physiological tests. *P. s. syringae* = *Pseudomonas syringae* pv. *syringae*, *P. s. tomato* = *P. syringae* pv. *tomato*, and *X. c. vesicatoria* = *Xanthomonas campestris* pv. *vesicatoria*.
[b] Ice-nucleating activity.
[c] + = Isolates positive for this particular reaction; − = isolates negative for this reaction.
[d] ... = Reaction not determined.

remaining 240 lesions, *P. s. tomato* was isolated from six. Lesions from which *P. s. tomato* was isolated did not fluoresce on the DL-lactate medium. Eighteen lesions from which *X. c. vesicatoria* was isolated did not fluoresce on DL-lactate medium.

DISCUSSION

In our studies, fluorescence on media containing single-carbon sources was useful for separating the three fluorescent pseudomonads. In vitro tests showed that sucrose and erythritol or DL-lactate are the most useful carbon sources for distinguishing the three pathogens. All strains of *P. viridiflava* fluoresced only on erythritol or DL-lactate. All strains of *P. s. tomato* fluoresced on sucrose, whereas *P. s. syringae* fluoresced on all three media. As observed previously (15) and substantiated in this study, the ability of a particular organism to fluoresce is inhibited in synthetic media where the organic compound is not utilized. This inability to fluoresce on media containing carbon sources not utilized by the bacterium proved useful for separating the fluorescent phytopathogenic pseudomonads present in leaf spots of tomato.

D(−)Tartrate, a carbon source generally considered useful in separating these organisms, gave variable results with *P. s. tomato* and is not considered useful in separating strains by fluorescence. All strains of *P. s. tomato* grew on D(−)tartrate, but most strains did not fluoresce on that carbon source. Meyer and Abdallah (11) showed that fluorescence by *P. fluorescens* was affected by the carbon source used. The bacterium fluoresced when grown on succinate but not when citric acid or malic acid was used in the medium. Their work suggests that Fe^{+++} concentration was the major factor regulating pigment production. Cells grown on succinate were iron-deficient because they required more iron than cells grown on citric or malic acid. Perhaps, different iron requirements by cells grown on the various media in our study explains the differences in fluorescence among the strains. In any case, it is important to determine the ability of the bacterium to fluoresce on a particular carbon source before utilization of that compound as a differential.

Isolations made from greenhouse- and field-grown plants confirmed the usefulness of fluorescence on single-carbon media for separating the three tomato pathogens. Water suspensions from lesions containing *P. s. syringae* consistently fluoresced on sucrose, erythritol, and DL-lactate. Lesions with *P. s. tomato* yielded fluorescent colonies on sucrose, but no fluorescence occurred on erythritol or DL-lactate. Fluorescent colonies grew on media containing erythritol and DL-lactate, but no growth occurred on sucrose when macerated tissue containing *P. viridiflava* was streaked.

Use of media with single-carbon sources, especially those containing sucrose, erythritol, or DL-lactate, will reduce the time required for diagnosis of transplant diseases caused by fluorescent pseudomonads. These media are semiselective because the pseudomonads, as well as saprophytes, differ in their capacity to utilize or fluoresce on the carbon substrates. Use of these media in initial isolations will allow elimination of organisms that cannot grow or fluoresce. The only problem anticipated is the presence of saprophytic fluorescent pseudomonads that may also grow and fluoresce on one or more of the media. In such a situation, however, isolation of *P. s. tomato* would be quite unlikely. Developing lesions from young tissue should be used to avoid saprophytic contamination.

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