A Semiselective Medium for the Isolation of *Pseudomonas syringae* pv. *savastanoi*

G. Surico and P. Lavermicocca

Istituto di Patologia e Zoologia forestale e agraria, Università degli Studi, Facoltà di Agraria, 50144 Firenze, Italy, and Istituto tossine e micotossine da parassiti vegetali del CNR, Bari, Italy.
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ABSTRACT

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A new medium, designated as PVF-1 agar, was developed and tested for isolating *Pseudomonas syringae* pv. *savastanoi* from olive plants under field conditions. It was compared with other conventionally used media, including nutrient-sucrose agar medium (NSA), King's B medium, and the NPC medium of Sands and Rovira. The new medium, a modification of Kado and Heskett's D4, allowed growth of *P. s. savastanoi*, but retained its selectivity against saprophytic bacteria. PVF-1 agar contained (in grams per liter) sucrose (30), glycerol (10 ml), Difco casamino acids (2.5), dipotassium phosphate trihydrate (1.96), magnesium sulfate heptahydrate (0.4), and sodium dodecyl sulfate (0.4). Of the 32 strains of *P. s. savastanoi* from different hosts and geographic areas that were tested, all grew up on PVF-1 agar with variable morphology that paralleled that of the other media. Identification of *P. s. savastanoi* was mainly accomplished by the production on PVF-1 agar of fluorescent pigments. The mean quantitative recovery for all 32 strains tested was approximately 73%. PVF-1 agar, which inhibited a high percentage (90% or more) of the saprophytic bacteria washed from olive tissues, was successfully used to detect epiphytic populations of *P. s. savastanoi* on leaves, stems, and fruits. Isolation from olive and oleander galls was also enhanced in comparison to usual media, including NSA and King's B medium.

Additional keyword: olive knot disease.

Pseudomonas syringae van Hall pv. *savastanoi* incites galls on olive (*Olea europaea* L.) and other plants, including oleander (*Nerium oleander* L.), privet (*Ligustrum japonicum*), ash (*Fraxinus excelsior* L.), and jasmine (*Jasminum* spp.) (1,19,25). Olive galls usually occur on young stems, branches, and twigs. The leaves and fruits are rarely infected, but they usually harbor *P. s. savastanoi* on their surfaces (3,11).

Several semiselective media including M-71 (13), proline agar (14), SNR (20), and BCBRVB (17) have been described for the enumeration of bacteria in the genus *Pseudomonas* (18). In our hands, none works well for *P. s. savastanoi*. A suitable substrate for the isolation of the bacterium from young galls is the nutrient agar medium supplemented with 50 mg of sucrose (NSA), 2.5 μg of crystal violet and 60–75 μg of cycloheximide per milliliter (NSACC) (23). Another medium used for the isolation of *P. s. savastanoi* from olive leaves has been yeast-tryptone-glucose extract agar (4) supplemented with 67 μg of cycloheximide per milliliter. However, both media are generally unsatisfactory for the selective isolation of *P. s. savastanoi* from the host phylloplane due to the presence of a number of other gram-negative organisms that occur in the same habitat (4). Recovery of *P. s. savastanoi* on nonselective media is difficult as the bacterium does not have a distinctive morphology, even though it produces fluorescent pigments. Varvaro (23) developed a semiselective medium, NSA-S, for isolating *P. s. savastanoi* from olive leaf surfaces, although we found that several strains of the bacterium grew poorly, if at all, on NSA-S agar.

The purpose of this study was to develop and evaluate a new medium for isolating *P. s. savastanoi* from naturally infested plant tissue.

MATERIALS AND METHODS

Bacterial strains. All cultures used in this study were obtained from the stock culture collection of the Department of Plant Pathology, University of Bari, and from those maintained at the Istituto tossine e micotossine da parassiti vegetali, CNR, Bari, and at the National Collection of Plant Pathogenic Bacteria, Harpenden, UK. Antagonistic bacteria used to evaluate their in vitro effect on the recovery of *P. s. savastanoi* were isolates from previous research (12). Cultures were stored on nutrient-glycerol agar (NGA) slants at 4 C until tested.

Development of semiselective agar medium. A selective medium developed by Kado and Heskett (9) for *Pseudomonas* spp. was the starting medium. It consisted (in g/L) of glycerol (10 ml), sucrose (10), casein hydrolysate (1), ammonium chloride (5), disodium phosphate (2.3), and sodium dodecyl sulfate (SDS) (0.6).

Preliminary tests comparing growth of six *P. s. savastanoi* strains (PVBa229, PVBa230, NCPPB640, PVBa215, PVBa204, and ITM510) by dilution plating (24) on this medium in comparison to NSA showed recovery of all strains was very poor or completely negative. However, parallel experiments demonstrated that D4 medium had a satisfactory selective action

against saprophytic bacteria normally colonizing olive surfaces. Thus, attempts to increase the usefulness of the medium were made by eliminating, substituting, or changing the levels of some components. Other compounds, obtained from Sigma Chemical Co., St. Louis, MO, were also screened for specific inhibition of microbial contaminants commonly associated with olive tissue. These included (in μg/ml), novobiocin (45), oleandomicin (15 and 50), sodium azide (1 and 5), and penicillin G potassium salt (75,000 units). All compounds were filter sterilized (Millipore, 0.22 μm) and aseptically added, in combination or individually, to the autoclaved test medium before pouring the plates. Other media used for comparison were: NSA, NSACC, NPC medium of Sands and Rovira (16), NSA-S (23), and King's B medium (10) supplemented with 2.5 μg of crystal violet and 75 μg of cycloheximide per milliliter (KBCC). All inoculated media were incubated at 26 C for 3–6 days.

Quantitative recovery. To test the growth and recovery of *P. s. savastanoi* on the semiselective medium, a total of 32 strains of *P. s. savastanoi* were selected. These included 12 olive strains, 13 oleander strains, three privet strains, and four jasmine strains.

All organisms were grown under shake culture in King's B broth at 26 C for 48 hr. Cells were removed from the culture medium by centrifugation (7,000 *g* for 10 min), washed twice in sterile saline (0.85% NaCl), and diluted serially with saline to 10^{-5} and 10^{-6} colony-forming units/ml (cfu/ml). The dilutions were plated by spreading onto three plates each of test medium. NSA plates served as the nonselective medium. All plates were incubated at 26 C and colonies were counted after 3–5 days.

Detection of *P. s. savastanoi* in naturally infested olive leaves, stems, and fruits. Plant samples were obtained from two infected olive orchards located, respectively, at Valenzano and Altamura (Bari) in southern Italy. A group of four trees was sampled quarterly from March to August in 1985 and 1986. On each sampling occasion, 50 healthy leaves were collected from each tree, bulked, and taken immediately to the laboratory. Three random batches of 25 leaves were individually added to 20 ml of 0.05 M phosphate buffer, pH7, in a 100-ml Erlenmeyer flask and shaken at room temperature for 2 hr. One milliliter was removed and diluted serially to 10^{-3} in phosphate buffer. Tenth milliliter aliquots of each bacterial suspension were plated in triplicate onto PVF-1. Twigs (2 cm long internode pieces; 30 pieces/flask) and developing or mature drupes (12–20 per flask) were tested in the same manner as with leaves. Epidermis tissue from olive and oleander galls was removed with a flamed scalpel, and the remaining tissue was ground in a mortar with several milliliter of phosphate buffer. The supernatant was then plated on PVF-1. Other media used for comparison included NSA, NSACC, and KBCC. All agar plates were incubated at 26 C for 3–6 days. The plates then were placed under a long-wave length UV light source. Colonies that fluoresced were tentatively considered as *P. s. savastanoi*. A random selection of isolates were restreaked on NSA and further tested by gram-staining, oxidase production, production of indoleacetic acid (IAA) (2), and pathogenicity to olive plants. Pathogenicity tests were carried out as described (21). Cultures were grown for 2 days on NGA slants and were applied with a wooden applicator directly to wounds made in the bark of 1-yr-old olive stems (cultivar Nocellara del Belice). Inoculated plants were held in a glasshouse in natural light, a temperature of between 20–24 C and 85–95% humidity. Plants were observed for the development of symptoms for up to 60 days after inoculation.

Effect of antagonistic bacteria on the recovery of *P. s. savastanoi*. To determine the effect of antagonists on the recovery of *P. s. savastanoi* from leaves, 75 μl of a suspension of two antagonistic isolates (OTI15 and OEI14: *Pseudomonas* and *Bacillus* spp., respectively) containing 5 cfu each were plated onto NSA medium together with 75 μl of washings from naturally contaminated olive leaves. The plates were incubated as described and the results recorded after 2 and 4 days.

Detection of *P. s. savastanoi* in mixed cultures. Nine epiphytic bacterial strains, OEI16, OTG12, OEI20 (*Bacillus* spp.); OTI33, a nonfluorescent pseudomonad; NEI10A, NT4B, GTG18 (*Xanthomonas* spp.); NT5E, OTI29 (*Erwinia* spp.) (10), and one strain of *P. s. savastanoi* (PVBa229) were grown for 48 hr on NGA slants at 26 C. Bacteria were washed from the medium with sterile distilled water and adjusted turbidimetrically about 1×10^8 cfu/ml. Cell suspensions were individually diluted 1:10 in sterile distilled water and mixed to yield a final ratio of approximately 100:1 total epiphytic bacteria to *P. s. savastanoi*. The bacterial suspension was serially diluted and plated in triplicate on NSA, KBCC, NSACC, and PVF-1 agar plates with a sterile glass rod. The plates were incubated for 5 days at 26 C, after which colony counts and fluorescence were recorded.

RESULTS

NSA-S and NPC media, previously formulated for isolation of *P. s. savastanoi* and fluorescent pseudomonads, respectively, were generally unsuitable for supporting the growth of the *P. s. savastanoi* strains tested (Table 1). No growth was observed on D4 medium, while NSACC and KBCC media permitted good recovery of five of the six strains used.

With respect to D4 medium, parallel experiments (results not presented) demonstrated that *P. s. savastanoi* was unable to grow when NH_4Cl was used as the sole nitrogen source. Sucrose and casein hydrolysate at 10 and 1 g per liter, respectively, did not support, in absence of SDS and with NH_4Cl substituted with $(NH_4)_2SO_4$, good growth of the six *P. s. savastanoi* strains. SDS at 0.6 g/L reduced the plating efficiency.

Of the three antibiotics tested, oleandomicin at 15 and 50 mg/L and penicillin G (75,000 units/ml) inhibited growth of several strains. Sodium azide had the same effect at the highest concentration tested (5 mg/L). Only novobiocin (45 mg/L) did not inhibit *P. s. savastanoi*, but because this antibiotic did not significantly improve the selectivity of PVF-1 agar, it was eliminated from the medium. The final composition of the semiselective medium contained the following per liter of distilled water: glycerol, 10 ml; sucrose, 30 g; Difco casamino acids, 2.5 g; $K_2HPO_4 \cdot 3H_2O$, 1.96 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; SDS, 0.4 g; agar, 16 g. The pH was adjusted to 7.1 with HCl.

Differences in morphology and growth of the colonies were apparent among many of the 32 *P. s. savastanoi* strains when they were grown on NSA or PVF-1 media. On PVF-1 medium (Fig. 1) most strains grew slowly and in 6 days produced smooth, relatively small (2–3 mm), grayish-white, slightly raised colonies with entire margins (see ITM311). A few strains, notably PVBa208 and PVBa229, produced brownish-white colonies with wrinkled surfaces. Colonies from strains PVBa204 and PVBa206 were 4–6 mm in diameter, dark in color and with erose to irregular margins. Colonies of PVBa215 and PVBa204 had dull centers. All strains showed a pale-blue fluorescence under UV light after 3–5 days of incubation.

On NSA, all *P. s. savastanoi* strains grew more rapidly than on PVF-1 and in 48–72 hr produced colonies with few apparent

TABLE 1. Quantitative recovery of six representative *Pseudomonas syringae* pv. *savastanoi* strains on five different media[a]

Medium[b]	Recovery compared with NSA(%)[c]					
	PVBa229	PVBa230	NCPPB640	PVBa215	PVBa204	ITM510
NSACC	92.0	90.1	28.5	82.2	86.0	100.0
NSA-S	21.2	0.6	0.0	10.5	50.9	10.2
NPC	69.7	4.7	0.0	79.0	5.4	1.8
KBCC	92.5	92.7	21.1	82.2	71.9	48.2
D4	0.0	0.0	0.0	0.0	0.0	1.1

[a] Average of two replicates; bacteria were grown on NAG slants for 48 hr at 26 C, washed twice with sterile 0.85% NaCl, diluted to 10^{-3} and 0.1 ml plated on triplicate plates of each medium.

[b] NSA: nutrient agar with 5% sucrose; NSACC: NSA plus crystal violet and cycloheximide; NSA-S: NSA plus crystal violet, oleandomicin, penicillin G, and cycloheximide (23); NPC: a semiselective medium for fluorescent pseudomonads (16); KBCC: KB plus crystal violet and cycloheximide; D4: a semiselective medium for *Pseudomonas* spp. (9).

[c] Percent recovery was calculated as: (number of colony-forming units recovered on test media/number of colony-forming units on NSA) × 100.

differences (Fig. 2). They were circular or irregularly round, smooth, 3–5 mm in diameter, gray to pale yellow or grayish-white, slightly raised with entire or irregular margins. Colonies of some strains (PVBa204, PVBa215) had a dull center, but those of strain ITM311 appeared slightly pulvinate.

The plating efficiencies of the 32 strains on PVF-1 were variable, ranging from 8.7 to 112% (Table 2). Eighteen strains (56%) had a percentage of recovery greater than 80%, whereas seven strains (22%) had a percentage of recovery of 50% or less.

The percentage of recovery was increased significantly by decreasing the concentration of SDS to 0.2 g/ L and by substituting casamino acids with 0.1% yeast extract. Unfortunately, this modified formulation of PVF-1 failed to adequately inhibit the growth of saprophytic flora from olive tissues (results not presented). No relationship was observed between the strain source, year of collection, and morphology or plating efficiency. Strains NCPPB640 and NCPPB639, which grew very poorly on PVF-1, have been examined in a previous work (22) for production of plant growth substances (IAA and cytokinins), pathogenicity on olive and oleander plants, and plasmid profile, but no significant differences were observed between them and other *P. s. savastanoi* strains.

There were significant differences among the media in the recovery of *P. s. savastanoi* in the presence of saprophytic bacteria previously isolated from olive leaves (Table 3; Fig. 3B). The inhibition effect of PVF-1 agar averaged 97%. The other two media used in comparison (NSACC and KBCC) had a lower but still satisfactory inhibition effect: about 76% for NSACC and about 81% for KBCC. However, the lower inhibition together with a certain detrimental effect of crystal violet on the growth of some *P. s. savastanoi* strains (Table 1) and, more importantly, the difficulty in identifying *P. s. savastanoi* colonies made these substrates less useful than PVF-1.

P. s. savastanoi was recovered on PVF-1 from all the olive samples analyzed. The colonies of the bacterium were generally easily counted at a dilution of 10^{-1} in the presence of a relatively low amount of saprophytic bacteria. The inhibitory effect of the medium averaged that observed in the experiments with mixed cultures (Table 4). However, in summer, the inhibition efficiency diminished sometimes to about 90% due to the presence on the leaves and stems of many yellow pigmented saprophytic bacteria, many of which grew on PVF-1. On the contrary, *P. s. savastanoi* from drupes or young galls was recovered in almost pure culture in all seasons.

The identification of *P. s. savastanoi* was generally facilitated by the production of fluorescent pigments by the most abundant and morphologically identical colonies observed on PVF-1 agar (Fig. 4). Less frequently encountered fluorescent colonies with a different morphology from that attributed to *P. s. savastanoi* were considered as *P. fluorescens*. Oxidase and pathogenicity tests and IAA production in KB broth confirmed our positive identification of *P. s. savastanoi* at a rate of about 100%.

Antagonism was observed in only two samples plated on media other than PVF-1, but the experimental simulation of *P. s. savastanoi* growing in the presence of small numbers of antagonistic bacteria showed a clear inhibition of *P. s. savastanoi* around antagonistic colonies (Fig. 5). The presence in a given plate of at least 20 antagonistic colonies completely prevented the growth of *P. s. savastanoi*.

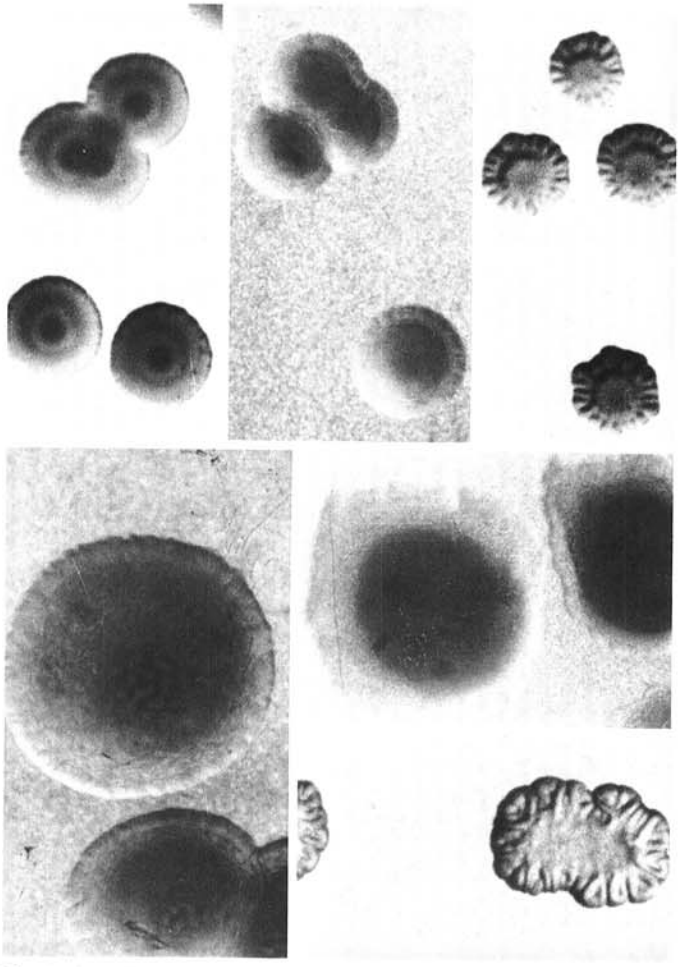

Fig. 1. Colonies of *Pseudomonas syringae* pv. *savastanoi* growing on PVF-1 agar after 6 days at 26 C. Clockwise from bottom right: PVBa208, PVBa204, PVBa215, ITM311, PVBa229, and PVBa206.

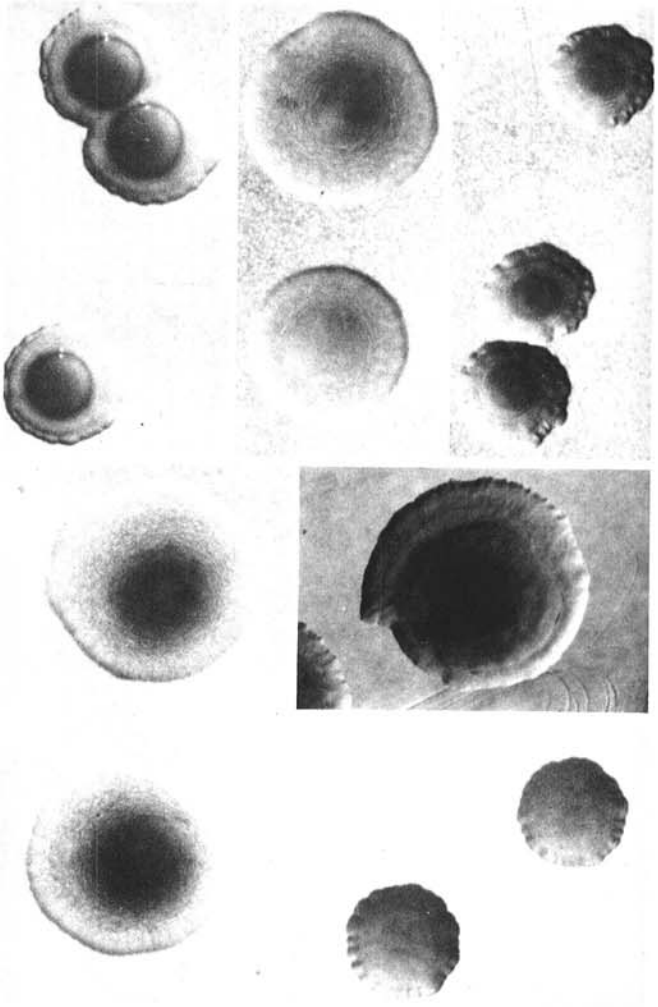

Fig. 2. Colonies of *Pseudomonas syringae* pv. *savastanoi* growing on NSA after 3 days at 26 C. Clockwise from bottom right: PVBa208, PVBa204, PVBa215, ITM311, PVBa229, and PVBa206.

TABLE 2. Quantitative recovery of 32 *Pseudomonas syringae* pv. *savastanoi* strains on PVF-1 agar[a]

Strain	Year collected	Host	Location	Recovery compared[b] with NSA (%)
PVBa229	1968	Olive	Bari, I[c]	82.5
PVBa206	1968	Olive	Ruvo, I	66.7
PVBa204	1968	Oleander	S. Cataldo, I	28.5
PVBa208	1968	Privet	Bari, I	79.4
PVBa224	1975	Olive	Massafra, I	93.5
PVBa218	1968	Privet	Bari, I	52.2
PVBa215	1968	Privet	Bari, I	88.2
ITM311	1982	Oleander	Taranto, I	83.3
NCPPB639	1959	Olive	Yugoslavia	11.1
ITM313	1982	Oleander	Bari, I	30.4
PVBa230	1975	Olive	Bari, I	100.0
ITM407	1983	Olive	Termoli, I	90.3
ITM305	1981	Oleander	California, USA	88.2
ITM19	1984	Jasmine	Greece	104.2
UCD-2	1985	Jasmine	California, USA	91.3
NCPPB640	1959	Oleander	Yugoslavia	8.7
ITM24	1984	Jasmine	Greece	108.0
NCPPB SA-1	1985	Oleander	South Africa	101.0
NCPPB SA-2	1985	Oleander	South Africa	100.0
NCPPB64	1954	Olive	Portugal	78.0
ITM601	1984	Oleander	Sirmione, I	112.0
UCD2015	unknown	Oleander	California, USA	93.2
ITM20	1984	Olive	Greece	86.5
ITM21	1984	Olive	Greece	89.3
ITM510	1983	Oleander	Sibari, I	92.0
ITM4	1984	Oleander	Greece	81.3
ITM603	1985	Olive	Sirmione, I	41.3
ITM413	1983	Oleander	Termoli, I	56.5
ITM410	1983	Olive	Termoli, I	46.0
ITM402	1983	Oleander	Termoli, I	16.4
NCPPB2788	1968	Jasmine	Greece	73.4
NCPPB2780	1967	Olive	France	70.5

[a] Average of three replicates: Bacteria were grown on NGA slants for 48 hr at 26 C, washed twice with sterile 0.85% NaCl, diluted to 10^{-3} and 0.1 ml plated on triplicate plates of PVF-1 (the semiselective medium for *P. s. savastanoi*) and nutrient agar with 5% sucrose (NSA).
[b] Percent recovery was calculated as: (number of colony-forming units recovered on PVF-1 number of colony-forming units on NSA) × 100.
[c] I: Italy.

TABLE 3. Evaluation of PVF-1 agar in recovering *Pseudomonas syringae* pv. *savastanoi* strain PVBa229 in the presence of saprophytic bacteria[a]

Medium[b]	Mean number of colonies per plate		Reduction saprophytic bacteria[c] (%)
	P.s. savastanoi	Other epiphytic bacteria	
PVF-1	91.3	2.1×10^2	97.1
NSA	NC[d]	8.7×10^3	...
D4	0.0	2.7×10^2	96.8
KBCC	NC	1.6×10^3	81.4
NSACC	NC	2.1×10^3	75.6

[a] Average of two replicates; bacteria were grown for 48 hr on NGA slants at 26 C, washed from the medium, and mixed to yield a final concentration ratio of approximately 100:1 total saprophytic bacteria to *P.s. savastanoi*. Counts on NSA, KBCC, and NSACC were made on 10^{-4} and converted to 10^{-2} dilution.
[b] PVF-1: the semiselective medium for *P.s. savastanoi*; NSA: nutrient agar with 5% sucrose; D4: a semiselective medium for *Pseudomonas* spp. (9); KBCC: KB plus crystal violet and cycloheximide; NSACC: NSA plus crystal violet and cycloheximide.
[c] The percent reduction of saprophytic bacteria was calculated as: (100—number of colony-forming units recovered on test media × 100/number of colony-forming units on NSA).
[d] NC: not countable due to the high number of other bacteria.

DISCUSSION

The media used to isolate *P. s. savastanoi*, such as NSA or NSACC, are mainly designed to recover and identify *P. s. savastanoi* on the basis of colony morphology (6). Isolation is possible only when populations of *P. s. savastanoi* are much greater than the saprophytic bacteria or if the morphology of colonies of *P. s. savastanoi* are easily differentiated from other bacteria. On the contrary, when the ratio of *P. s. savastanoi* to other bacteria is low, growth of the pathogen on NSA or NSACC can be masked. In addition, because the morphology of *P. s. savastanoi* may be quite variable, a number of biochemical tests must be used to confirm the identification of the bacterium.

PVF-1 agar seems to be a medium that allows the isolation of *P. s. savastanoi* from different sources and in mixture with other bacteria. The selective agent, SDS, has little effect on *P. s. savastanoi*. Other authors (7,15,23) have also found resistance of *P. s. savastanoi* to penicillin G and oleandomicin. Our results indicate that these substances can inhibit the growth of the bacterium. This contrasting finding is probably due to the fact that the above authors used the paper disk assay or heavily streaked the bacteria on the semiselective media to test the antibiotic tolerance. On the contrary, in this study, penicillin G and oleandomicin, as well as the other antimicrobial agents, were included in the test media and bacteria were always spread at an optimum concentration (8).

In spite of the selectivity of PVF-1, a relatively high number of gram-negative bacteria can grow. Fortunately, these can be distinguished from *P. s. savastanoi* by characteristics such as lack of fluorescence under a long-wave length UV light source, rapid growth, and/or morphology.

PVF-1 agar generally prevented growth of 90% or more of the leaf- or twig-associated saprophytic bacteria, depending on the season when plant materials were sampled. The observed variability in the percentage of inhibition on PVF-1, as well as on NSACC and KBCC, was probably due to the fact that populations of epiphytic bacteria, like *P. s. savastanoi*, undergo seasonal

fluctuations on the phylloplane of olive (5,11). Therefore, a decrease in the percentage of inhibition could be observed in those periods when the groups of bacteria capable of growing on PVF-1 are more abundant on olive leaves and twigs. In particular, this was observed in summer, when some yellow bacteria which grow well on PVF-1 were relatively numerous and prevailed over the other epiphytic bacteria. In any case, the usefulness of PVF-1 was greater in late summer, when the density of *P. s. savastanoi* populations on olive phylloplane was very low compared with that of the other bacteria (11).

According to an earlier report (11), *P. s. savastanoi* was recovered not only from leaves but also from stems and fruits. Use of PVF-1 enables one to isolate *P. s. savastanoi* from olive or oleander galls in almost pure culture. Normally, the isolation of *P. s. savastanoi* from fairly young tissues is performed by exposing internal, freshly infected regions and plating a suspension of small pieces cut in distilled water or phosphate buffer. On the other hand, the isolation of *P. s. savastanoi* from old cracked galls is often very difficult due to the presence of a great number of different contaminants (mainly an unidentified yellow pigmented bacterium) and the low viable populations of the pathogen in the diseased tissues. By using PVF-1 it is possible to isolate the pathogen from these galls by carefully removing dirt and debris, homogenizing the remaining tissues in a mortar, and then plating the suspension.

Antagonistic bacteria were generally not a problem on any of the media used. Growth of *P. s. savastanoi* was inhibited only in two of the more than 200 samples of olive tissues tested. However, none of the colonies able to inhibit the growth of *P. s. savastanoi* was capable of growth on PVF-1.

One limitation in the use of PVF-1 is its relative toxicity toward some *P. s. savastanoi* strains. Therefore, it is suggested that the percentage of recovery of the *P. s. savastanoi* populations distributed in a given area or host be determined in advance before extensive usage of PVF-1 agar. The medium developed is simple, inexpensive, and easy to prepare. It can be stored for at least 15 days at 4 C without loss of selective properties. PVF-1 would seemingly be the medium of choice for epidemiological studies involving *P. s. savastanoi.*

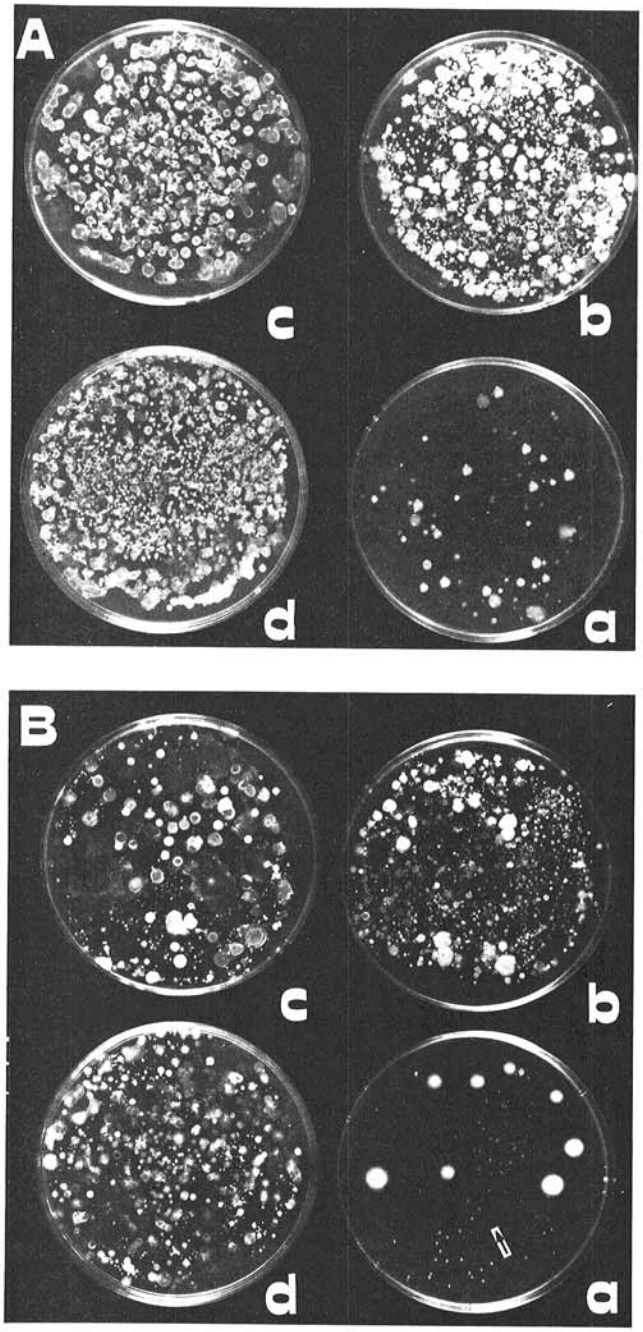

Fig. 3. A, Comparison of D4 (a) with three different media for inhibition of microorganisms from olive leaves. b, NSA; c, KBCC; d, NSACC. **B,** Comparison of PVF-1 (a) with NSA (b), KBCC (c), and NSACC (d) for recovery of *Pseudomonas syringae* pv. *savastanoi*. Bottom right plate shows almost pure *P. s. savastanoi* colonies (arrow). Olive leaves were shaken for 2 hr in 20 ml of phosphate buffer and 0.1 ml of the wash liquid was plated on each medium.

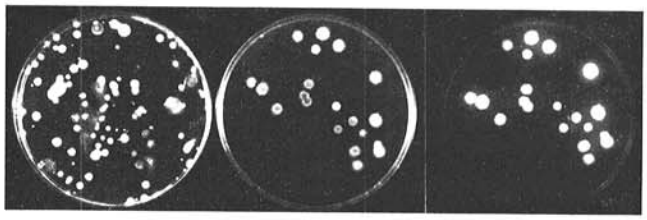

Fig. 4. Comparison between NSA (left) and PVF-1 agar (middle) for recovery of *Pseudomonas syringae* pv. *savastanoi* from naturally contaminated olive twigs. PVF-1 plates are also photographed under long-wave UV light (right). Olive twigs were washed in 20 ml of phosphate buffer and the wash liquid was diluted 10^{-2} before plating.

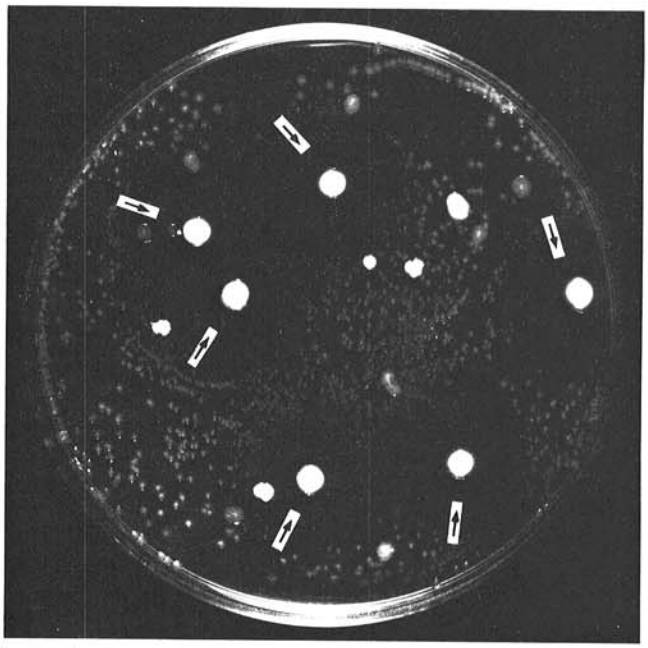

Fig. 5. Effect of antagonistic bacteria on the recovery of *Pseudomonas syringae* pv. *savastanoi* on NSA. The plate was spread plated with 0.15 ml of a mixed culture of *P. s. savastanoi* and antagonistic bacteria previously isolated from leaf washings. Note the inhibition of the pathogen around the larger antagonistic colonies (arrows).

TABLE 4. Evaluation of PVF-1 agar in recovering *Pseudomonas syringae* pv. *savastanoi* and other epiphytic bacteria from naturally contaminated olive leaves, twigs, and fruits[a]

Season	Dilution	Sample	Mean number of colonies per plate				
			P.s. savastanoi	Other epiphytic bacteria			
			PVF-1	PVF-1	NSA	NSACC	KBCC
Late spring	10^{-2}	Leaves	1.4	1.5 (90.6)[c]	16.0	8.3 (48.1)	6.3 (60.6)
		Twigs	1.9	2.9 (94.3)	51.3	14.3 (72.1)	10.0 (80.5)
		Fruits	5.6	1.6 (97.5)	65.0	16.0 (75.4)	17.0 (73.5)
Late summer	10^{-1}	Leaves	2.7	6.7 (92.7)	91.4	18.2 (80.1)	14.7 (83.9)
		Twigs	1.6	39.3 (94.7)	741.3[d]	85.8 (88.4)	37.4 (95.0)
		Fruits	1.1	0.4 (99.5)	78.7	14.3 (81.8)	12.7 (83.9)

[a] Average of three replicates; olive tissues (25 leaves; 30 2-cm long internode pieces or 12–20 fruits) were washed with 20 ml of phosphate buffer. Serial 10-fold dilutions were made to 10^{-3} and assayed by plating 0.1-ml samples on triplicate plates of PVF-1, NSA, NSACC, and KBCC.

[b] PVF-1: the semiselective medium for *P.s. savastanoi*; NSA: nutrient agar with 5% sucrose; NSACC: NSA plus crystal violet and cycloheximide; KBCC: KB plus crystal violet and cycloheximide.

[c] Figures in parentheses are percent reduction of epiphytic bacteria calculated as: (100—number of colony-forming units recovered on test media ×100/ number of colony-forming units on NSA).

[d] Count was made on 10^{-2} and converted to 10^{-1} dilution.

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