Development of a Selective Medium for Pseudomonas solanacearum

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ABSTRACT

A selective medium consisting of a simplified basal medium plus antimicrobial compounds was developed for isolation of *Pseudomonas solanacearum* from the soil. Colony counts of *P. solanacearum* on this medium were reduced by 22-33%, as compared to a 65-99% reduction of other soil bacteria. Variation in efficiency occurred with different soil types and with the same soil held at different soil moisture tensions.

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Pseudomonas solanacearum E. F. Smith survives in soil for varying periods of time (6). Most reports have been based on incidence of disease in crops replanted in field studies. The many conflicting conclusions from such studies may be due to wide variability of soil factors and complexity of strains (1). Valid information of soil factors influencing survival of *P. solanacearum* is lacking. This can be attributed to lack of direct efficient methods of isolation from soil, especially when the bacterium is present at low levels.

Selective media have been developed for enumeration of bacteria in the genus Pseudomonas (5, 8, 9); however, they are not useful for detection of *P. solanacearum* in soil. Jenkins et al. (4) detected *P. solanacearum* at a concentration of 2.5×10^6 cells/cc soil by direct isolation, and at 2.5×10^4 cells/cc soil using tomato plants as indicators, a method requiring 21 days. With a serological method requiring 3 days, they detected *P. solanacearum* from artificially infested soil at a concentration of 2.5×10^4 cells/cc soil. However, this method detects dead as well as live cells. Hayward (3) provided the basis for carbon source selectivity by determining the capacity of many isolates of *P. solanacearum* to oxidize various disaccharides and hexose alcohols.

A medium which allows direct isolation of *P. solanacearum* from soil, especially at low levels, would be an extremely useful tool in survival studies. Subsequently, studies involving epidemiology as well as over-all ecology of the disease would be greatly faciliated.

MATERIALS AND METHODS.—*Basal medium.*—A nonselective synthetic medium designated as BBM developed by Buddenhagen & Berger (*unpublished data*) for all strains of *P. solanacearum* was the starting medium. It consists of 1 liter distilled water, 5 g glucose, 0.12 g KNO_3, 0.19 g $(NH_4)_2SO_4$, 0.16 g $MgSO_4 \cdot 7H_2O$, 0.19 g $CaSO_4$, 2 g L-glutamic acid, 0.05 ml metal stock solution [0.616% $MnSO_4 \cdot H_2O$, 1.1% $ZnSO_4 \cdot 7H_2$), 0.176% $FeSO_4(NH_2)_2SO_4 \cdot 6H_2O$, 0.0286% $CoSO_4 \cdot 5H_2O$, 0.0286% $CuSO_4 \cdot 5H_2O$, 0.01144% H_3BO_3, and 0.0000128% KI]. The medium was adjusted to pH 7.2 ± 0.1 with KOH and buffered with 1 ml of 0.0002 M K_2HPO_4 and 1 ml of 0.0002 M KH_2PO_4. Attempts to increase usefulness of the medium were made by eliminating, substituting, or changing levels of some components.

Soil plating.—The inhibitory effect of the developed selective medium on other soil bacteria was tested by the standard method of surface plating dilutions from three soils. The Hawaiian soil types used were Lualualei of the Lualualei series, Dark Magnesium Clay Great Soil group, Manoa of Manoa series, and Wahiawa of Wahiawa series, the latter two belonging to Low Humic Latosol Great Soil Group.

In all tests, tomato isolate P-28 from Hawaii was used.

RESULTS.—*Basal medium.*—To reduce growth of other soil bacteria but retain distinguishing colony characteristics of *P. solanacearum*, BBM, the starting medium, was simplified. With sucrose as the carbon source, $CaSO_4$ eliminated, and 0.005% of 2, 3, 5-triphenyltetrazolium chloride (TZC) incorporated into BBM, an experimental basal medium (EBM) resulted. EBM was further modified so that fewer soil bacteria could grow by changing carbon source and the levels of some components. These modifications resulted in the final modified basal medium (MBM) consisting of 1 liter distilled water, 2.5 g mannitol, 1 g glutamic acid, 0.05 ml metal stock solution, 0.16 $MgSO_4 \cdot 7H_2$), and 5 ml of 1% TZC solution. The pH and buffers in BBM were retained. Colonies of *P. solanacearum* on MBM were smaller and less fluidal than colonies developing on TZC medium (0.1% peptone, 0.05% dextrose, 1.5% agar, and 0.005% TZC compound).

TABLE 1. Tolerance of *Pseudomonas solanacearum* to different antimicrobial compounds, as measured by colony formation

Compound tested	Maximum noninhibitory concentration (μg/ml)
Antibacterial	
Ampicillin	~ 2.5
Bacitracin	>100.0
Cellocidin	< 2.5
Chloromycetin	15.0
Kanamycin	< 2.5
Laurusin	75.0
Neomycin	25.0
Pencillin G	2.5
Tyrothricin	50.0
Vancomycin	75.0
Antifungal	
Actidione	>100.0
Captan	10.0
Mycostatin	>100.0
Myprozine	>100.0
PCNB	>100.0

Effect of antimicrobial agents on growth and recovery of P. solanacearum.—The basal medium used was EBM, since MBM was not yet developed during the assay. Tolerance of *P. solanacearum* to different antimicrobial compounds, as measured by ability to form typical colonies on agar, was determined (Table 1). Potentially useful compounds were then combined at levels *P. solanacearum* could tolerate. A synergistic effect was observed, precluding use of some and necessitating use of reduced levels of other compounds when in combinations. Seven compounds were determined to be the best set of inhibitors. There were, in μg/ml, bacitracin 50, chloromycetin 5, penicillin G 1, tyrothricin 20, vancomycin 10, actidione 50, and captan 10. The incorporation of this combination of inhibitors to the final modified basal medium (MBM) comprised the final selective medium (SM).

In one test, incorporation of this combination of antimicrobial compounds to EBM caused a 33% reduction in the number of colonies of *P. solanacearum* recovered. However, colonies were smaller and less fluidal than on other media, enhancing countable colonies per unit area. In another test, colony recovery of *P. solanacearum* on SM was 37% less than on TZC control.

Efficiency of SM in inhibiting general soil bacteria and in recovering P. solanacearum from soil.—Performance of SM was compared with TZC agar medium, the medium commonly used for *P. solanacearum*; with BBM, the initial synthetic medium; and with MBM, the final basal medium but lacking the antimicrobial compounds. Colony counts of general soil bacteria on SM compared to TZC, BBM, and MBM were reduced by 98-99%, 94-99%, and 34-79%, respectively (Fig. 1). In an experiment dealing with different moisture tensions, however, inhibition of soil bacteria on SM ranged from 65 to 95%, as compared to counts on MBM with greater reduction as moisture levels decreased.

In another test, an appropriate soil dilution was mixed with a dilution of *P. solanacearum* to obtain a 1:10 ratio of *P. solanacearum* to other soil bacteria. At 10^{-3} dilution on SM, percentage reduction of soil bacteria ranged from 90 to 98% using TZC and BBM as controls. MBM caused a reduction of 30 to 57% compared to TZC controls. When plated at 10^{-3}, numbers of colonies of *P. solanacearum* recovered did not vary greatly among the three soils, but varied

TABLE 2. Evaluation of SM (final selective medium) in recovering *Pseudomonas solanacearum* and inhibiting soil bacteria from naturally infested Wahiawa soil

Medium	Number of colonies per plate[a]	
	P. solanacearum	Other soil bacteria
TZC	50	490
BBM	110	380
MBM	50	380
SM	95	35

[a] Average of three replicates; counts on first three media were made on 10^{-4} dilution and converted here to 10^{-3} dilution.

[b] TZC = Tetrazolium chloride medium; BBM = Buddenhagen and Berger medium; MBM = modified basal medium; SM = selective medium.

greatly at 10^{-2} dilution, with very few obtained from Lualualei soil and many from Wahiawa soil (Fig. 2). Evidently, the large colonies of a bacterium tolerant to the antimicrobial agents in SM, which predominated in plates from Lualualei soil at low dilution, inhibited the growth of *P. solanacearum*.

The effectiveness of SM was tested on naturally infested Wahiawa soil. There was considerable inhibition of other soil bacteria on SM (93% reduction as compared to TZC control); hence, colony counting at 10^{-3} dilutions was feasible only on SM (Table 2).

DISCUSSION.—The effectiveness of any selective medium is influenced by many factors. Efficiency of such a medium may vary with soil type and even with same soil held at different moisture tensions. A major reason may be the differences in bacterial microflora in the different soil samples. In our tests, a single antagonistic bacterial species resistant to the antimicrobial agents was present only in one soil. It has been stressed that soil bacteria are not uniformly distributed in single soil profiles (2). With another bacterial pathogen, *Agrobacterium tumefaciens*, a selective medium developed in California (10), was not useful in Australia (7). Our results with one strain of *P. solanacearum* were variable even among Hawaiian soils. It appears, therefore, that performance of a selective medium depends on types of pathogen strains present in a region as well as on the soil itself and on the many factors which affect the microflora of the soil. For instance, mannitol, the carbon source chosen, is appropriate only for isolates of *P. solanacearum* classifiable in Hayward's biotypes 3 and 4 (3). Where other biotypes unable to use mannitol occur, a different carbon source would be required. Although efficiency of this selective medium depends on the microflora and other factors of the test soil, it was useful in estimating populations in several soils. We believe it may be more useful in studying *P. solanacearum* populations with appropriate change of carbon source for specific strains.

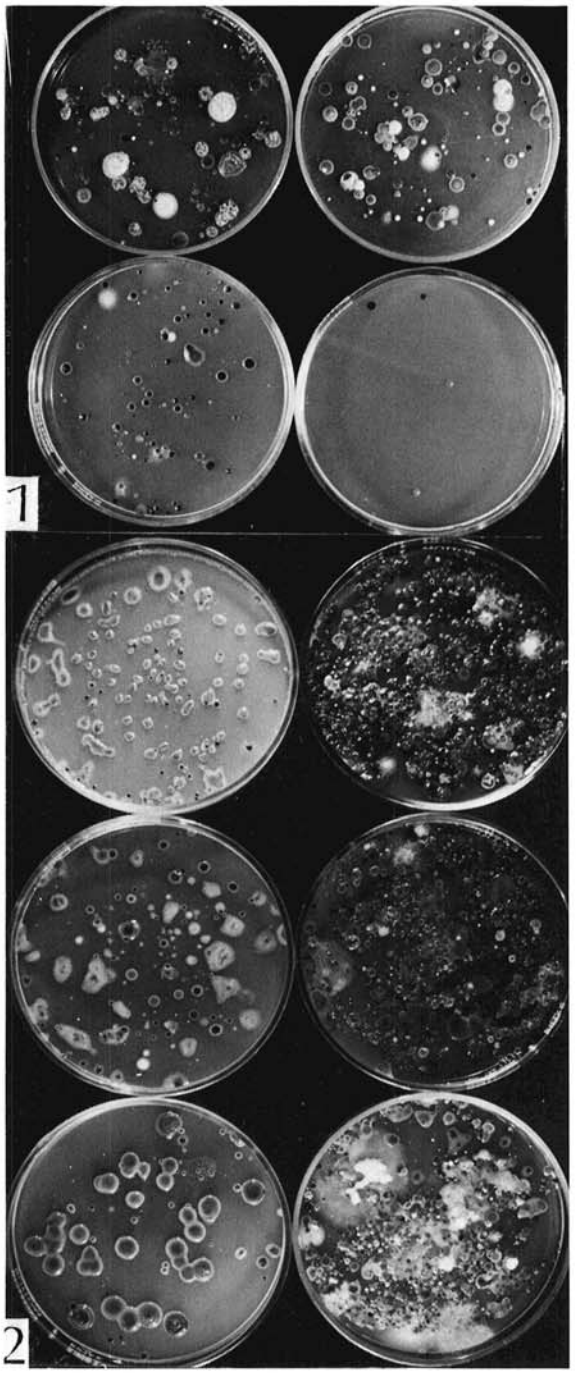

Fig. 1-2. 1) Comparison of four media in inhibiting microorganisms from Wahiawa soil. TZC (Tetrazolium chloride medium) and BBM (Buddenhagen and Berger medium) (above) and MBM and SM (below). The final selective medium (SM) differs from the modified basal medium (MBM) only in addition of antimicrobial compounds. **2)** Comparison of SM (left) with TZC (right) in recovery of *Pseudomonas solanacearum* from three soils. Wahiawa (upper), Manoa, and Lualualei soil (lower). Upper left plate shows almost pure *P. solanacearum* colonies inhibited by a single predominating soil bacterium.

LITERATURE CITED

1. BUDDENHAGEN, I. W. 1965. The relation of plant-pathogenic bacteria to the soil, p. 269-284. *In* K. F. Baker & W. C. Snyder [ed.]. Ecology of soil-borne plant pathogens. Univ. Calif. Press, Berkeley, Los Angeles.
2. CLARK, F. E. 1967. Bacteria in soil, p. 15-49. *In* A. Burgess & F. Raw [ed.]. Soil Biology. Academic Press Inc., London.
3. HAYWARD, A. C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27:265-277.
4. JENKINS, S. F., D. J. MORTON, & P. D. DUKES. 1967. Comparison of techniques for detection of Pseudomonas solanacearum in artificially infested soil. Phytopathology 57:25-27.
5. KADO, C. I., & M. G. HESKETT. 1970. Selective media for isolation of Agrobacterium, Corynebacterium, Erwinia, Pseudomonas, and Xanthomonas. Phytopathology 60:969-976.
6. KELMAN, A. 1953. The bacterial wilt caused by Pseudomonas solanacearum. North Carolina Agr. Exp. Sta. Tech. Bull. No. 99. 194 p.
7. KERR, A. 1969. Crown gall of stone fruit. I. Isolation of Agrobacterium tumefaciens and related species. Australian J. Biol. Sci. 22:111-116.
8. MASUROVSKY, E. B., S. A. GOLBLITH, & J. VOSS. 1963. Differential medium for the selection and enumeration of members of the genus Pseudomonas. J. Bacteriol. 85:722-723.
9. MOUSTAFA, F. A., G. A. CLARK, & R. WHITTENBURY. 1970. Two partially selective media; one for Pseudomonas morsprunorum, Ps. syringae, Ps. phaseolicola, Ps. tabaci and one for Agrobacteria. Phytopathol. Z. 67:342-344.
10. SCHROTH, M. N., J. P. THOMPSON, & D. C. HILDEBRAND. 1965. Isolation of Agrobacterium tumefaciens-A. radiobacter group from soil. Phytopathology 55:645-647.