A Selective Medium for Pectolytic Fluorescent Pseudomonads

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ABSTRACT

A solid agar medium selective for fluorescent pseudomonads also shows whether the organism is pectolytic. The medium, buffered at pH 7.0, contains pectin, proteose-peptone, mineral salts, and the antibiotics novobiocin, penicillin, and cycloheximide. After growth of the organisms and determination of fluorescence, the plates are flooded with hexadecyltrimethylammonium bromide to detect zones of pectolysis. Pectolytic, fluorescent pseudomonads can be plated directly from nature due to the selectivity of the medium.

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Additional key words: Pseudomonas fluorescens, P. marginalis, pectolytic enzymes, selective medium.

The phenotypic variability of the pectolytic, fluorescent pseudomonads is not well defined, nor is the ecology or etiology of these pathogens well understood (8, 13, 14). *Pseudomonas marginalis*, a fluorescent soft-rot pathogen, synthesizes pectolytic enzymes, of which pectate lyase (E.C. 412.99.3) is an important component (2, 3, 9). A few strains designated as *P. fluorescens* which produce pectate lyase have also been implicated in soft-rot disease (1, 3, 17, 18).

Media currently available for the isolation of pseudomonads do not provide information on whether the organism is both pectolytic and fluorescent. A selective medium for fluorescent pseudomonads which shows whether the organism is pectolytic would be most useful in diagnostic plant pathology. Furthermore, the commonly used gel medium described by Starr (15) is not adaptive to a spread plate technique where a solid surface is required.

MATERIALS AND METHODS.–Components of nonselective media which test for pectolytic activity (2) were added to some of the components of Medium B of King et al. (6) necessary for fluorescence. Additionally, three broad-spectrum antibiotics which do not inhibit most fluorescent pseudomonads (11) were also included. The resulting medium permits isolation and enumeration of fluorescent, pectolytic pseudomonads from an environment in which they are greatly outnumbered by nonfluorescent bacteria.

This medium, hereafter referred to as FPA, contains per liter of distilled water, Bacto-Proteose Peptone No. 3 (Difco, Detroit, Mich.), 20 g; citrus pectin (Sunkist Growers, Corona, Calif.), 5.0 g; K_2HPO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 1.5 g; agar (Difco), 15 g. The above components excluding agar are mixed together as a dry powder before distilled water is added. After adjustment of pH to 7.0, the agar is added and the medium is autoclaved and cooled to about 45 C; then 10 ml of a freshly prepared antibiotic mixture is added per liter of medium. Cooling is essential to maintain activity of the antibiotics. Approximately 12 ml of media should be poured into each petri dish, since thicker layers of agar may obscure the detection of pectolytic zones. It is important to use freshly prepared plates because of the instability of the antibiotics.

The antibiotic mixture is prepared as follows: novobiocin (Albamycin, Upjohn, Kalamazoo, Mich.), 45 mg; penicillin G, 75,000 units; and cycloheximide (Acticione, Upjohn), 75 mg; are mixed and sterilized by adding 1 ml of ethanol. Finally, 9 ml of sterile distilled water are added to make a suspension.

All bacteria were cultured for 24 hr in a glucose-mineral broth (16) shaken at 200 oscillations/min at 30 C. Cultures were then streaked on plates or spread in dilution series and incubated at 30 C for 48 to 72 hr. We distinguished fluorescent pseudomonads from nonfluorescent colonies by viewing fluorescence under long wavelength ultraviolet light. The plates are then gently flooded with an autoclaved, aqueous solution (1% w/v) of hexadecyltrimethylammonium bromide (J. T. Baker Chemical Co., Phillipsburg, N.J.) as previously described (2, 5). The solution precipitates the pectin and a clear zone around a colony is indicative of pectate lyase and/or polygalacturonase production. The bacteria remain viable up to 15 min after the plate is flooded, and the colonies that are active should be recovered immediately.

RESULTS.–Phytopathogens and saprophytes were tested on the FPA medium (Table 1). With the exception of one strain of *P. tabaci*, all fluorescent pseudomonads grew on the medium. Furthermore, fluorescent and pectolytic bacteria could be differentiated from all other bacteria.

Over 60 soil samples were examined with the FPA medium. On a nonselective medium, the total microflora ranged from 10^6 to 10^9/g of soil. However, less than 0.1% of the soil microflora grew on the FPA medium. Analysis of storage rot potatoes from several different sources shows that fluorescent pseudomonads can be detected even when they are less than 0.1% of the bacterial population.

Recovery rates on FPA medium were compared to

TABLE 1. Growth, fluorescence, and pectolytic activity of some phytopathogenic and nonphytopathogenic bacteria on FPA agar

Bacterial group	Source[a]	Growth on FPA	Fluorescent pigment	Pectolytic activity
Fluorescent pseudomonads				
Pseudomonas fluorescens				
Strain D-5	Wheat rhizosphere CSIRO	+	+	–
Strain D-9	Wheat rhizosphere CSIRO	+	+	–
Strain 33 (Biotype G)	UCBB	+	+	–
Strain 31 (Biotype D)	UCBB	+	+	–
Strain 143 (Biotype F)	UCBB	+	+	–
Strain 54	Potatoes CAES	+	+	–
Strain 55	Potatoes CAES	+	+	–
Strain 56	Potatoes CAES	+	+	–
Strain 57	Soil CAES	+	+	+ slight
Strain 60	Face cream CAES	+	+	–
Strain 61	Face cream CAES	+	+	–
Strain 63	Mosquito CAES	+	+	–
Strain 65	Mosquito CAES	+	+	–
Strain 67	Mosquito CAES	+	+	+
Strain 137	Poison ivy CAES	+	+	+
Strain 138	Poison ivy CAES	+	+	+
Strain 140	Poison ivy CAES	+	+	+ slight
Strain 149	Horse nettle CAES	+	+	–
Strain 62-12	Onion CUPP	+	+	–
Strain 63-111	Onion CUPP	+	+	+
Strain 64-6	Onion CUPP	+	+	+
Pseudomonas putida				
Strain 90 (Biotype A)	UCBB	+	+	–
Pseudomonas marginalis				
Strain 2	UCBB	+	+	+
Strain 9	UCBB	+	+	+
Pseudomonas syringae (and various nomen-species)				
P. mori var. *huszi* Strain 1037	NCPPB	+	+	+
P. mori	NCPPB	+	slight	–
P. phaseolicola Strain G 71	UCDPP	+		–
P. phaseolicola Strain HB 33	UCBB	+		–
P. syringae Strain B 3A	UCDPP	+		–
P. syringae Strain 129	CAES	+	+	–
P. glycinea Strain R 2	UMPP	+		–
P. glycinea Strain R 5	UMPP	+		–
P. tabaci Strain Pt 3	CARDC	+	+	–
P. tabaci Strain Pt 1	OARDC	+	+	–
P. tabaci Strain Pt 112	OARDC	–		
P. tabaci Strain Pt 5	OARDC	+	+	–
P. coronafaciens var. *atropurpurea* 1328	NCPPB	+		–
Nonfluorescent pseudomonads				
Pseudomonas diminuta Strain D 8	CSIRO	–		
Pseudomonas fragi Strain 58	CAES	+	–	–
Pseudomonas cannibina Strain 1437	NCPPB	–		
Other bacteria				
Erwinia carotovora Strain 1	UCBPP	–		
Erwinia carotovora Strain 2	UCBPP	–		
Erwinia carotovora Strain 8061	ATCC	–		
Erwinia aroideae Strain 1	UCPP	–		
Cellulomonas biazotea Strain 163	CAES	+ slight	–	+ slight
Cellulomonas flavigena Strain 165	CAES	+	–	–

[a] ATCC = American Type Culture Collection, Rockville, Md. CAES = The author's collection at the Connecticut Agricultural Experiment Station, New Haven. CSIRO = Division of Soils (A. D. Rovira), Commonwealth Scientific Industrial Research Organization, Glen Osmond, South Australia. CUPP = Department of Plant Pathology (R. S. Dickey), Cornell University, Ithaca, New York. OARDC = Department of Plant Pathology (H. A. J. Hoitink), Ohio Agricultural Research & Development Center, Wooster. UCBB = Department of Bacteriology and Immunology (M. Doudoroff, N. J. Palleroni, & R. Y. Stanier), University of California, Berkeley. UCBPP = Department of Plant Pathology (M. N. Schroth & D. C. Hildebrand), University of California, Berkeley. UCDPP = Department of Plant Pathology (R. G. Grogan & J. DeVay), University of California, Davis. UMPP = Department of Plant Pathology (B. W. Kennedy), University of Minnesota, Minneapolis.

those on the same agar without antibiotics and on Agar F (Difco). *P. marginalis* (strain 2) was spread in dilution series on both media. After 3 days, the number of colonies on each agar agreed within 10%, indicating that the antibiotics and pectin have little if any effect on plating efficiency. Bacteria growing on media containing the antibiotics required 1 day longer to grow than on the same medium without antibiotics.

Using the FPA medium, we assayed for pseudomonads in market vegetables (without surface sterilization) which were approaching the end of their normal shelf life. Pectolytic, fluorescent pseudomonads were found in cauliflower, spinach, and celery.

DISCUSSION.–The medium described in this report (FPA medium) is designed primarily for the detection of pseudomonads which are both pectolytic and fluorescent and are associated with soft rot infection; e.g., pectolytic strains of *P. marginalis* and *P. fluorescens.* With the medium buffered at pH 7.0, pectolysis is probably due to pectate lyase rather than to polygalacturonase, since the latter is reported to be produced and most active at lower pH (4, 7). The FPA medium may be less suitable for detecting pectolytic activity in oxidase-negative pseudomonads (such as *P. mori* and *P. phaseolicola*), since such organisms grow slowly on the medium and reportedly only produce polygalacturonase (4, 7).

Soils generally contain relatively low numbers of fluorescent pseudomonads. Rovira & Sands (10) and Sands & Rovira (12) report that fluorescent pseudomonads comprise no more than 0.06 to 0.27% of the bacterial population in Australian soils, and such data are in agreement with the low numbers we find in Connecticut soils. Since the concentration of such organisms in soil is so low, it would be almost impossible to detect fluorescent and pectolytic pseudomonads without a selective medium.

Although we report the presence of fluorescent and pectolytic organisms on market vegetables, the origin of these isolates cannot be ascertained, and may have resulted from postharvest contamination. As suggested by Misaghi & Grogan (8), it is difficult to ascertain the pathogenicity of such isolates, and a selective medium is certainly no substitute for a valid pathogenicity test. However, the production of pectate lyase is one factor in the soft-rot syndrome (2, 3, 9, 17), and the FPA medium detects such pectolytic activity.

The FPA medium may also be useful for isolating fluorescent pseudomonads with different regulatory mechanisms governing pectate lyase synthesis. It has already been shown that phytopathogens may exhibit a wide variety of regulatory mechanisms (19). The ecological and etiological relationships of pectolytic, fluorescent pseudomonads may be more easily studied with the use of the FPA medium.

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