The Development of a Selective Medium for Pseudomonas glycinea

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ABSTRACT

The development and use of an agar medium selective for the growth of *Pseudomonas glycinea* in the presence of bacteria from soybean buds and leaves are described. Selectivity depends on boric acid.

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It is often difficult in the isolation of pathogenic bacteria from healthy or diseased plants in nature to detect the pathogen because of interference from associated microorganisms. Media containing fungicides usually eliminate fungi, but these materials are not effective against the nonpathogenic bacteria. For example, *Pseudomonas glycinea* may be inhibited or overrun by large numbers of many types of bacteria normally inhabiting buds of field-grown soybean (*Glycine max* L.) plants. A selective medium inhibiting or preventing the growth of even some of the unwanted organisms would aid in ecological studies of the pathogen. Such a medium and the manner in which it was developed are described in this paper.

A series of screening tests was employed to identify chemicals that would selectively favor the growth of *P. glycinea* over other bacteria from soybean buds. Since the pathogen grows slowly on semisynthetic agar media, in contrast to many bacteria inhabiting buds, a basal medium rich in natural products was chosen to promote the vigorous growth of *P. glycinea*. Prospective selective inhibitors were tested in this medium with an agar diffusion method that permitted examination of a chemical over a range of concentrations.

The basal medium, designated "TTCC", contained the following ingredients in g/liter: peptone, 10; casein hydrolysate, 1; glucose, 5; cycloheximide ("Acti-dione", Upjohn Co., Kalamazoo, Mich.), 0.05; triphenyl tetrazolium HCl, 0.05; and agar, 20. Five ml of an autoclaved stock solution of the tetrazolium salt was added to the warm, autoclaved medium prior to pouring into petri plates.

Some 350 inorganic and organic compounds were incorporated individually into strips of 1.3- X 7.7-cm blotting paper. Usually 100 mg of a chemical was added to 2 ml of 95% ethanol to reduce microorganisms, and this was then diluted to 10 ml

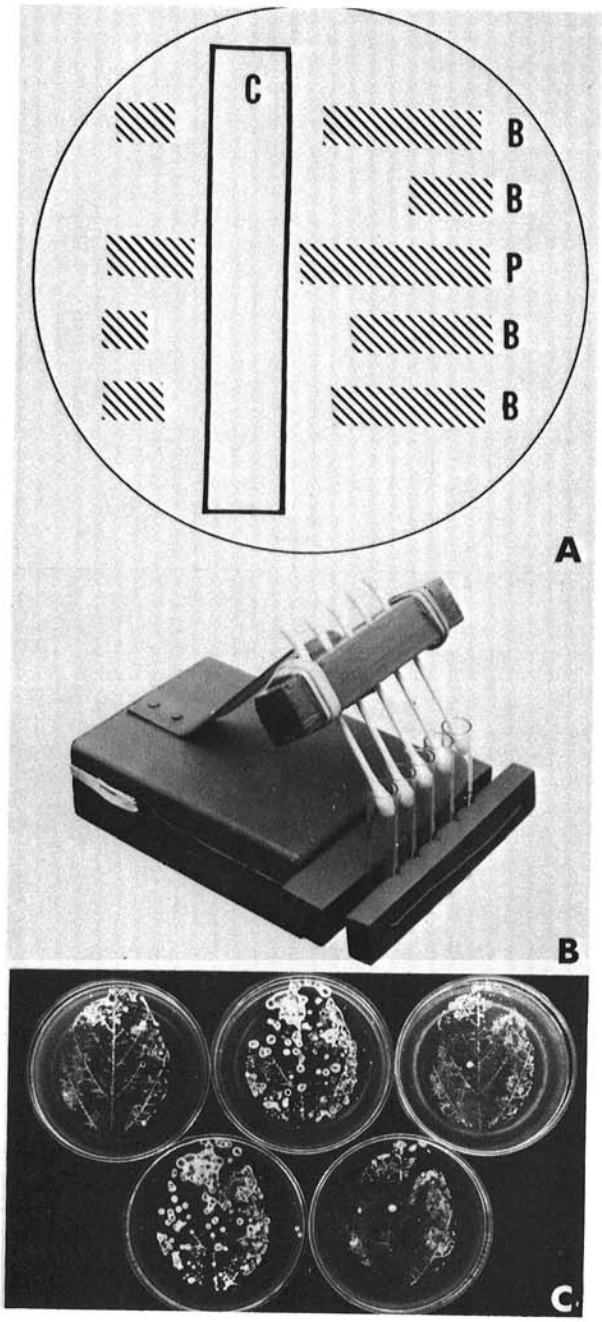

Fig. 1. A) The method used to test the inhibitory action of a chemical diffusing through agar from a paper strip (C) on four isolates of bacteria from soybean buds (B) and on an isolate of *P. glycinea* (P). The bacteria were seeded across the plate in parallel streaks prior to adding the paper containing the chemical. In this example, the chemical showed promise, because growth of *P. glycinea* after 48 hr was inhibited less than that of the bud bacteria. **B)** The device used for streaking plates. **C)** The selective medium, M71, compared with the nonselective medium, TTCC. A leaf naturally lesioned by *P. glycinea* was printed successively on M71, TTCC, M71 (top row, left to right), TTCC, and M71 (bottom row). Most of the small colonies on M71 were the pathogen.

with deionized water. A paper strip was dipped into this solution or suspension, drained, and dried at room temperature. The dry paper was placed on TTCC agar in a petri dish; the surface of the agar had been streaked a few minutes earlier with suspensions of isolate Ohio-40 of *P. glycinea* and four isolates of bacteria from soybean buds as shown in Fig. 1-A. A device employing machine-made cotton tipped swabs ("Q-tips", Chesebrough-Pond's Inc., New York, N.Y.) was used for streaking (Fig. 1-B). After an incubation period of 42-48 hr at 24 C (used in all experiments reported in detail), the distance from the paper each isolate was inhibited was measured. Eight to 12 bud isolates were used for each chemical. Bud isolates originally were obtained from plants grown in water-saturated air (2) or from field plants grown in 1969. Chemicals considered promising were those that inhibited bud isolates more than *P. glycinea* (Fig. 1-A). Tests with these materials were repeated one or more times. Chemicals with consistently good performances were further screened against additional bud isolates.

Boric acid (H_3BO_3) was selected for detailed study as a result of these tests. It was autoclaved in water and incorporated in a range of concentrations into warm TTCC prior to pouring petri plates. The plates were then streaked with test bacteria as before. In comparison with TTCC, 1 g/liter of boric acid in TTCC prevented or sharply reduced the growth of most of the bud isolates; on the other hand, *P. glycinea* was not affected so markedly. The colony diameter of isolate Ohio-40 of the pathogen was only slightly reduced. That of five other isolates was reduced up to 50%. With 2 g/liter of boric acid, colony diameter of all isolates was reduced farther, but growth was not prevented. The medium containing 1 g/liter was taken as a reasonable compromise and was designated M71. The plating efficiency of two isolates of *P. glycinea* was $> 90\%$ on M71 when compared with TTCC. The characteristic color and morphology of *P. glycinea* colonies were the same on TTCC and M71, making it possible to recognize the colony reliably with a dissecting microscope and light directed tangentially from below the plate (2).

To test further the properties of M71, growth of bacteria in macerates of buds from field soybean plants was compared on TTCC and M71. Buds (terminal structures < 1.5 cm long) were collected the first week of August 1971, when plants were actively growing. Each bud was macerated in 2 ml of buffer (g/liter: KH_2PO_4, 3; Na_2HPO_4, 7; and NaCl, 4) with a sterile spatula. A loop (ca. 0.01 ml) of the macerate was placed on a plate of each medium, using a cross-streaking method that spread the macerate to all parts of the plate and which permitted the examination of colony types and a gross estimation of colony numbers.

Macerates from 134 buds from healthy plants of a number of cultivars were analyzed this way. As expected, many types of bacterial colonies were observed, often > 5 types from the same bud. M71, as compared to TTCC, reduced the number of types, the size or numbers of colonies of given types, or combinations of these effects, depending on the bud. For example, categorization of the 134 buds according to the most prominent inhibitory feature was as follows: (i) with 19% of the buds, bacteria grew on TTCC but not on M71; (ii) bacterial types were fewer on M71 than on TTCC with 37% of the buds; (iii) colony size of given types was reduced by M71 with 22% of the buds; and (iv) M71 reduced colony numbers of given types with 8% of the buds. Growth on M71 was no different than on TTCC with 14% of the buds, and with one bud more colony types were found on M71 than on TTCC. *P. glycinea* was not recovered from these buds, but in other tests with buds from blighted rather than healthy plants, M71 greatly aided the detection of the pathogen.

The selective properties of M71 also were shown by prints of leaves bearing *P. glycinea* lesions in nature. Leaves were collected at three times in August and September 1971, and were printed by pressing momentarily to TTCC or M71 in petri dishes. The pathogen usually could be identified readily on M71; in contrast, it often was not seen or was difficult to discern among colonies of other bacteria on TTCC. Selectivity also was demonstrated convincingly by printing the same lesioned leaf alternatively on TTCC and M71 in a series of plates (Fig. 1-C).

M71 has been used to advantage under a variety of conditions. As noted above, the medium did not inhibit all bacteria associated with soybean buds and leaves, and it is possible that boric acid-resistant organisms will constitute the main population of a plant organ under some conditions. We have found that most isolates of *P. glycinea* on M71 are best observed after incubation for ca. 48 hr, because after this time the distinctive colony features of the pathogen may be lost. However, if the presence of slow growing isolates of *P. glycinea* is suspected, observations may be continued.

The selective property of boric acid for certain coliform bacteria was first recognized in 1921 (3), and the chemical now is used in a standard confirmatory test for fecal coliforms in water (1). This use was not known until the present work was completed.

Boric acid merits further study with other bacterial pathogens. Explanatory tests indicated that the diameter of colonies of *P. lachrymans* was only slightly reduced on M71. Plating efficiency was $> 90\%$ (T. D. Miller, *unpublished data*). On the other hand, when compared with TTCC, M71 was inhibitory to most bacteria in macerates from 20 terminal buds from field cucumber (*Cucumis sativus* L.) plants.

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