A Pathogenic Pseudomonad from Healthy Field-Grown Soybean Plants

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ABSTRACT

Bacteria resembling *Pseudomonas glycinea* in culture were isolated from buds, pollinated flowers, and young pods of healthy field soybean plants. These organisms, designated "FD", incited a resistance response on leaves of soybean and other beans, and they reduced emergence and produced dwarfed soybean seedlings following hypocotyl or cotyledon inoculation. FD bacteria resembled isolates of *P. syringae* on the basis of pathogenicity and laboratory tests. It is suggested that these organisms colonize the aerial parts of soybean plants. Damage likley would occur only when they are introduced into seedling wounds.

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Additional key words: bacteria, epiphyte, seedling.

Pseudomonas glycinea, the incitant of bacterial blight of soybean [*Glycine max* (L.) Merr.], has been reported to multiply in buds of healthy soybean plants under laboratory conditions (13). The original intent of the work reported in this paper was to search for this pathogen among the diverse bacteria that colonize buds of healthy field-grown plants (2, 9). Instead of *P. glycinea*, however, we found a different pathogenic bacterium, which was designated "FD". This organism is described in this paper. A short account of the work has appeared (12).

MATERIALS AND METHODS.—*Isolation of FD isolates.*—Seven isolates were obtained from macerates of terminal buds (terminal structures <1.5 cm long) from healthy field-grown plants, using a boric acid medium that favored the growth of *P. glycinea* over that of many of the other bacterial residents normally inhabiting buds and leaves of soybean plants in the field [medium M71 (10)]. As explained in Results, seven additional isolates later were obtained from parts of plants other than terminal buds. All samples were collected in early August, 1971, when terminal growth of plants was still active. Fields in which plants were growing were virtually weed-free. Samples were placed over ice when collected and were cultured within 2 hr. Each sample was macerated with a spatula in 2 ml of sterile water and an aliquot of the suspension spread with a loop on the surface of the medium in petri plates. Plates were placed at 24 C and inspected 42-48 hr later.

Bacterial colonies were viewed with a dissecting microscope fitted with light directed tangentially from beneath the plate (13). Stock cultures were made from representative colonies that resembled *P. glycinea*; these were designated FD isolates.

Pathogenicity tests.—Leaf pathogenicity was tested with soybean, snap bean (*Phaseolus vulgaris* L.), and cucumber (*Cucumis sativus* L.) seedlings grown in the greenhouse (21-27 C). The unifoliolate leaves of soybean and snap bean, and the second youngest fully expanded leaves of cucumber were employed. Each was wound-inoculated with ca. 10^8 cells/ml of the test bacterium by means of a cotton swab and Carborundum (11).

Pathogenicity tests were made on cotyledons and hypocotyls of greenhouse-grown soybean seedlings. Seeds were germinated 2 days in damp vermiculite.

Each cotyledon or the hypocotyl of a partly germinated seedling was pierced with a needle bearing ca. 10^7 cells of a test bacterium derived from a 24-hr culture. Radicals of the partly germinated seedling were 2-4 cm long. Inoculated seedlings were replanted in pots containing damp vermiculite or soil. Pots with vermiculite were placed in a chamber containing water-saturated air at 24 C (8); those with soil were put in the greenhouse. Each treatment consisted of 10-30 plants. Data were recorded 10-14 days after inoculation.

Pathogenicity tests with cotyledons and hypocotyls of snap bean and cucumber seedlings were made the same way, except that seedlings were germinated 3-5 days before inoculation. Inoculated seedlings were replanted in pots containing vermiculite and were placed in the chamber.

RESULTS.—*Sources of FD isolates and pathogenicity on soybean leaves.*—Two hundred one terminal buds from healthy plants of a number of soybean cultivars growing in the field were assayed, and colony types resembling *P. glycinea* were found in buds from seven plants (three from the cultivar 'Lindarin', and two each from cultivars 'Monroe' and 'Patterson'). These isolates were designated "FD". Later, additional FD isolates were obtained from the same plants. Three isolates were from withered flowers (presumed by their location on the plant to have been pollinated 1-2 days before collection), three were from small green pods, and one was from a terminal bud on an axillary shoot.

When FD isolates were used to wound-inoculate 'Harosoy 63' soybean leaves, unexpected results were obtained. If these isolates were *P. glycinea* as believed, the typical susceptible disease sequence of yellowing, water-soaking, and necrosis would follow inoculation (11). However, the only symptoms were small necrotic spots, which indicated a resistance rather than the expected susceptible response. These results were obtained with 10 representative FD isolates used in two tests. In addition, the same symptoms were obtained in two tests when Lindarin, Monroe, and Patterson were inoculated. The response was similar to the hypersensitive symptom incited by incompatible *P. glycinea*-cultivar combinations (11), except that necrosis did not develop as rapidly.

These tests demonstrated that the four soybean cultivars tested, including those from which FD isolates were obtained in the field, were not compatible hosts for the FD bacteria. The following questions then arose: (i) were FD isolates *P. glycinea* or a different bacterium and (ii) would FD isolates incite a susceptible response on different soybean tissues; e.g., on seedling tissues?

Additional leaf pathogenicity tests with soybean and other plants.—In these tests, leaves were wound-inoculated with the 10 FD isolates, using 6-10 plants/test. Tests were repeated.

None of the FD isolates was categorized as a described race of *P. glycinea*. Only the necrotic resistance response was incited on cultivars used by Cross et al. (3) for race differentiation. A necrotic response also was obtained with the 'Red Mexican', 'Red Kidney', and 'Bountiful Green' cultivars of *P. vulgaris*. With some isolate-cultivar combinations, the response was not visible until 7-10 days after inoculation. A necrotic response also was incited on mung bean (*P. aureus* Roxb.) and large-seeded lima bean (*P. limensis* Macf. 'Fordhook 242') leaves. The isolates incited a collapse and whitening of inoculated areas of leaves of cucumber ('National Pickling'). The symptoms on bean leaves, especially on lima bean, indicated that FD isolates did not fall within the *P. phaseolicola* nomenspecies (18).

Hypocotyl and cotyledon tests.—Symptoms resulting from cotyledon inoculation of Harosoy 63 and Monroe soybean seedlings with two representative FD isolates were similar in chamber and greenhouse tests, except that symptoms were more pronounced in the chamber. Inoculation reduced emergence (usually >50%) and emerged seedlings usually were smaller than control seedlings. Cotyledons of plants that did not emerge were badly damaged, and those of emerged plants bore lesions that usually enlarged, especially in the humid air of the chamber. Lesions had black centers 3-6 mm in diam and were surrounded by a zone of yellow tissue. In contrast, inoculation of cotyledons with *P. glycinea* resulted in little germination reduction or dwarfing of seedlings. Typical water-soaked lesions bearing *P. glycinea* exudate were produced at the points of inoculation. These results were based on six tests, three in the greenhouse and chamber, and three in the chamber.

When the hypocotyls of Harosoy 63 and Monroe soybeans were inoculated with the representative FD isolates in two tests, emergence also was markedly reduced and emerged plants were dwarfed.

TABLE 1. Responses of FD isolates and other pseudomonads in differentiation tests

Bacterium and number of isolates studied	Arginine dehydrogenase	Fluorescence	Gelatin liquefaction	Hypersensitivity of tobacco	2-Keto-gluconate	Nitrate reduction	Oxidase	Potato soft rot
FD isolates (3)	–	+	+	+[a]	–	–	–	–
P. glycinea (3)	–	+	–	–	–	–	–	–
P. putida (2)	+	+	–	–	+	+	+	–
P. syringae (3)	–	+[b]	+	+	–	–	–	–
P. viridiflava (2)	–	+	+	+	–	–	–	+

[a] One isolate was +.
[b] One isolate was –.

Hypocotyls bore collapsed brown lesions. *P. glycinea* incited similar but less marked symptoms.

The same types of tests with the two representative FD isolates were used with Red Kidney snap beans and cucumber. When cotyledons were inoculated, seedling emergence was reduced (usually >50%) and a large lesion was produced at the point of inoculation. In contrast, *P. glycinea* incited a small lesion and seedlings were otherwise normal. Hypocotyl inoculations with the FD isolates reduced emergence or resulted in dwarf seedlings of both plant species.

Since other tests (see below) suggested that FD isolates were similar to *P. syringae*, three isolates of this species (two from *P. vulgaris* and one from *Pisum sativum* L.) were compared with the two representative FD isolates in cotyledon and hypocotyl tests with Monroe soybeans and Red Kidney snap beans. All five isolates produced similar symptoms, as described above for FD isolates.

Other tests.—The two FD isolates examined were motile, gram-negative short rods with lophotrichous flagellae, properties shared by *P. glycinea* and most other pseudomonads. Common laboratory tests (15) were made with FD isolates, *P. glycinea, P. putida, P. viridiflava* (1), and *P. syringae* (Table 1). FD isolates were most similar to *P. syringae*, based on gelatin and potato tuber reactions. These same FD isolates were employed by D. C. Hildebrand in studies of growth on various substrates useful in distinguishing species of pseudomonad pathogens (7). The isolates keyed out to be representatives of *P. syringae* (D. C. Hildebrand, *unpublished data*).

DISCUSSION.—Apparently, the FD bacteria were growing harmlessly in association with the field soybean plants from which they were isolated. These plants were in two widely separated fields, suggesting that this organism may be distributed generally in buds and other young parts of healthy plants. Young soybean parts also were habitats for other diverse types of bacteria associated with this plant (15). It is possible that, at times, FD bacteria may multiply sufficiently to incite a resistant response in soybean, provided they gain entry to the plant. We suspect that this would not be often and probably would escape notice. On the other hand, if a number of FD cells did come in contact with a wound on the cotyledon or hypocotyl, seedling losses would be expected. If this is happening in the field, the extent of the damage is not known.

FD bacteria probably are disseminated by rain and dew. It is also possible that from their bud habitat they are deposited on other plant parts as the bud unfolds. If they eventually became associated with the seed, they could by this manner be transferred to the next plant generation and may cause seedling damage.

Our studies indicate that FD bacteria resembled isolates of *P. syringae*. This pathogen possesses a wide host range, is nutritionally versatile in comparison with many other pathogenic pseudomonads (15, 17), and is capable of producing a number of bacteriocins (19). Moreover, *P. syringae* has been isolated from the surface of several species of healthy plants (4, 5, 6, 16), and we demonstrated that this pathogen multiplied in healthy snap bean buds (14). All of that work, plus that reported here, suggests that, in nature, *P. syringae* and related bacteria are distributed widely on healthy plants.

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