Control of Bacterial Blight of Soybean by Bdellovibrio bacteriovorus

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ABSTRACT

Bdellovibrio bacteriovorus, a small, comma-shaped bacterium parasitic on other gram-negative bacteria, was isolated from the rhizosphere of soybean roots. *B. bacteriovorus* isolate Bd-17 inhibited development of local and systemic symptoms of bacterial blight when inoculated onto soybean with *Pseudomonas glycinea* at ratios of 9:1 and 99:1, respectively. Two other *B. bacteriovorus* isolates were less effective in inhibiting bacterial blight. The ability of *B. bacteriovorus* to inhibit bacterial blight was correlated with the average cell burst size of the particular isolate.

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Bdellovibrio bacteriovorus Stolp & Starr is a small, comma-shaped bacterium parasitic on gram-negative bacteria. It attaches to the cell wall of the host bacterium, penetrates and enters the host cell, grows in the form of a long spiral which cleaves into individual *B. bacteriovorus* cells, and the new cells are released upon collapse of the host cell (3). Earlier work concerning *B. bacteriovorus* has dealt primarily with morphological or physiological aspects of parasitism (3, 4, 6, 7, 8). Only a preliminary report on a portion of this work (2) has dealt with *B. bacteriovorus* as a biological control of a bacterial plant disease. The effectiveness of *B. bacteriovorus* in inhibiting the development of bacterial blight of soybean caused by *Pseudomonas glycinea* Coerper was evaluated in this study.

MATERIALS AND METHODS.–*Bdellovibrio bacteriovorus* cultures were isolated from the rhizosphere of soybean roots. Soil that adhered to the roots was suspended in sterilized distilled water and centrifuged at 1,000 *g* for 10 min to remove soil particles. The supernatant liquid was passed through a series of Millipore filters with pore sizes of 3.0, 1.2, 0.65, and 0.45 μm. One-half-ml samples from the 0.65- and 0.45-μm filtrates were mixed with indicator host bacteria (*P. glycinea*) and plated on tris-yeast-peptone agar (TYPA – 0.1 M Tris, pH 7.2; Bacto yeast extract, 0.3%; Bacto peptone, 0.06%; and glucose, 0.3%) using the double-agar-layer technique with the top and bottom layers containing 0.6 and 2.0% agar, respectively (3).

Plaques caused by bacteriophage appeared after incubation overnight and were marked. Plaques that developed 2-3 days later were formed by *B. bacteriovorus. Bdellovibrio bacteriovorus* cells from a single plaque were suspended in 10 ml of sterilized distilled water, passed through a 0.45-μm pore size Millipore filter, diluted, and plated with host bacteria as described above. After this process had been repeated three times, a strain was considered to be pure.

Bdellovibrio bacteriovorus isolates used for inoculations in combination with *P. glycinea* were prepared by growing *B. bacteriovorus* on lawns of *P. glycinea* in double-layer plates for 5 days. The soft agar layer which contained *B. bacteriovorus* and lysed *P. glycinea* cells was removed and suspended in sterilized 0.85% saline. This suspension was filtered through 3.0- and 0.45-μm pore size Millipore filters to remove any remaining *P. glycinea* cells. *Pseudomonas glycinea* was grown on TYPA slants for 48 hr and suspended in 0.85% saline for plant inoculations. Suspensions of *B. bacteriovorus* and *P. glycinea* were adjusted to 10^8 cells/ml for each bacterium for inoculation of soybean leaves.

Soybean cultivar 'Clark 63' was planted in steam-sterilized soil and grown in the greenhouse at approximately 27 C for inoculations. *Pseudomonas glycinea* cultures were obtained through the courtesy of B. W. Kennedy. Three isolates of *B. bacteriovorus* (Bd-10, Bd-17, and Bd-19) that had similar plaque types on lawns of *P. glycinea* were used for inoculation with *P. glycinea*. Each *B. bacteriovorus* isolate was mixed with *P. glycinea* at ratios of 1:1, 9:1, and 99:1, respectively. These mixtures were used immediately to inoculate Carborundum-dusted leaf surfaces by rubbing with a sterilized cheesecloth pad. *Pseudomonas glycinea* in sterilized 0.85% saline at the same concentrations was used for the control. Each treatment was replicated four times with three plants per replication. The two youngest trifoliates on 21- to 28-day-old soybean plants were inoculated, and disease severity readings were made 7 days after inoculation. Ratings were made on a 0-4 scale as follows: 0 = no symptoms; 1 = 1-25% of the inoculated leaf area with lesions; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100%.

A second study was conducted to determine the effect of *B. bacteriovorus* on development of bacterial blight-induced systemic toxemia (5) in

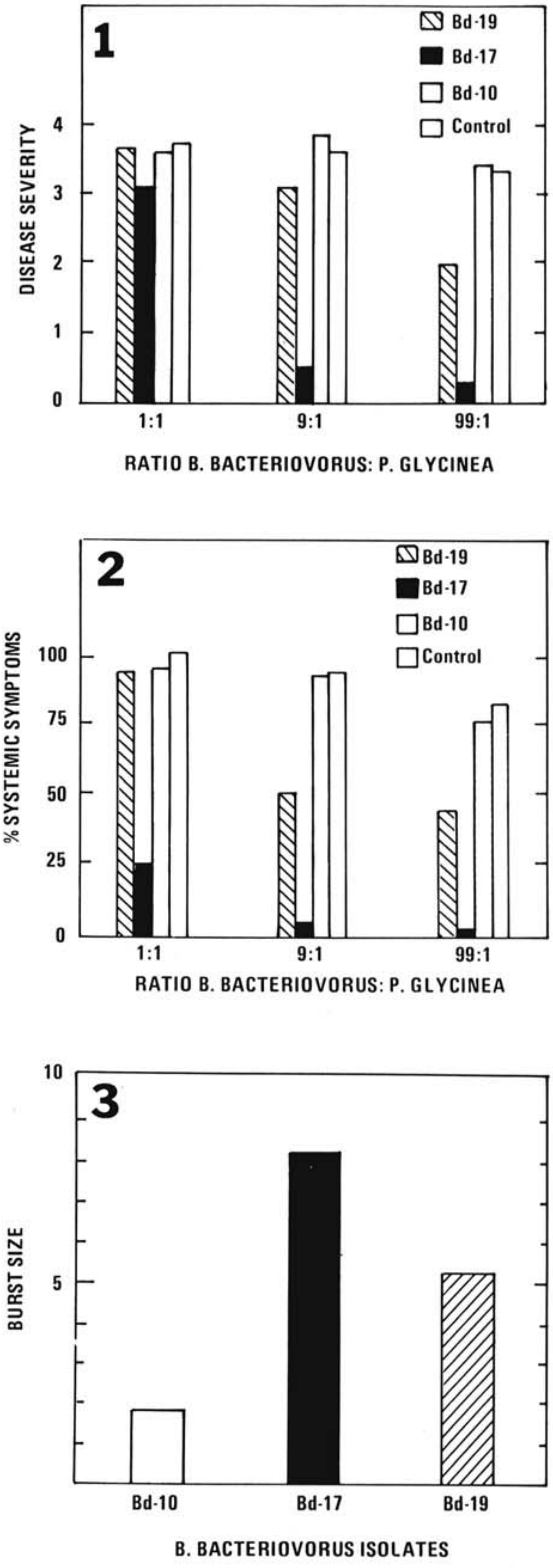

Fig. 1-3. 1) Disease severity on soybean leaves inoculated with mixtures of *Bdellovibrio bacteriovorus* and *Pseudomonas glycinea*. **2)** Percentage of soybean plants showing systemic bacterial blight symptoms when inoculated with mixtures of *B. bacteriovorus* and *P. glycinea*. **3)** Burst size of three *B. bacteriovorus* isolates grown at 30 C on lawns of *P. glycinea*.

noninoculated leaves. The same ratios and cell concentrations of *B. bacteriovorus* and *P. glycinea* and the same inoculation procedures were used as above with one exception; only primary leaves on 10- to 14-day-old soybean plants were inoculated. Readings for systemic toxemia were made 15-20 days after inoculation and were expressed in percentage of inoculated plants showing systemic toxemia.

The average burst size for the *B. bacteriovorus* isolates was determined by a single-step growth experiment conducted at 30 C as described by Seidler & Starr (4). They define the average burst size as "the quotient of the titer (in plaque-forming units) at the end of the rise period and the average titer (in plaque-forming units) over the lag period". Thus, the average burst size is simply the average number of *B. bacteriovorus* cells produced per infected *P. glycinea* cell.

RESULTS.–Bacterial blight was inhibited when soybean leaves were inoculated with a mixture of *B. bacteriovorus* isolate Bd-17 and *P. glycinea* at ratios of 9:1 and 99:1. Bd-10 was completely ineffective and Bd-19 was only moderately effective in reducing the severity of bacterial blight (Fig. 1).

Characteristic chlorosis occurred on subsequent trifoliolates when primary leaves of 10- to 14-day-old soybean plants were inoculated with a toxemia-producing strain of *P. glycinea*. When *B. bacteriovorus* isolate Bd-17 was mixed with a toxemia-producing strain of *P. glycinea* and inoculated onto soybean leaves, the development of systemic toxemia was inhibited. Bd-10 showed essentially no inhibition of toxemia and Bd-19 produced less than a 50% reduction in toxemia (Fig. 2).

All three *B. bacteriovorus* isolates produced plaques of similar appearance in vitro on lawns of *P. glycinea*, and all three isolates required approximately the same amount of time for plaque production. No detectable differences were observed among the *B. bacteriovorus* isolates in cell size or shape, motility, or attachment method with phase-contrast microscopy. There was a direct correlation between the average burst size (Fig. 3) and the ability of the *B. bacteriovorus* cultures to inhibit the development of bacterial blight. Therefore, in this study, *B. bacteriovorus* isolates in which a large number of progeny cells were produced per host cell were more effective in inhibiting bacterial blight.

DISCUSSION.–*Bdellovibrio bacteriovorus* isolate Bd-17 inhibits both necrotic lesion development, which is a localized symptom of bacterial blight, and systemic toxemia, a systemic symptom of bacterial blight. Even though *B. bacteriovorus* inhibits systemic symptoms, there is no reason to believe that *B. bacteriovorus* itself is systemic in the plant, since the systemic symptom is thought to be caused by an exotoxin produced by *P. glycinea* (1). *Pseudomonas glycinea* is found infrequently in the region of the soybean plant showing systemic symptoms (5). From these studies it appears that *B. bacteriovorus* is parasitizing *P. glycinea* cells at the site of inoculation–thus inhibiting development of local as well as systemic symptoms of bacterial blight. This does not discount the possibility that *B. bacteriovorus* colonizes growing parts of the plant if a sufficient number of host bacteria are present.

Differences in ability to inhibit bacterial blight were established for the *B. bacteriovorus* isolates. A large cell burst size may be an important factor to consider when trying to find a *B. bacteriovorus* isolate that is effective in reducing the population of *P. glycinea* on soybean, hence preventing the development of bacterial blight. There was, in fact, a close correlation between large cell burst numbers and complete inhibition of bacterial blight. However, factors other than burst size may be involved in determining whether a particular *B. bacteriovorus* isolate will provide effective control of bacterial blight.

The natural involvement of *B. bacteriovorus* in other bacterial plant diseases should be investigated. On the aerial surfaces and within the plant, *B. bacteriovorus* may be important in altering the ecological balance among the resident organisms and thus alter bacterial plant disease development.

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