A Strain of *Serratia marcescens* (Enterobacteriaceae) with High Virulence *Per Os* to Larvae of a Laboratory Colony of the Corn Earworm (Lepidoptera: Noctuidae)[1]

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Abstract An unpigmented strain of the bacterium *Serratia marcescens* Bizio that is highly virulent when fed to larvae from a laboratory colony of the corn earworm, *Helicoverpa zea* (Boddie), was found as a contaminant in a sample of the nuclear polyhedrosis virus of the celery looper, *Anagrapha falcifera* (Kirby), that also infects *H. zea*. High rates of mortality (>70%) were obtained by feeding doses of less than 100 bacterial cells per larva. Previous reports indicated that when insects could be killed with *S. marcescens* administered orally, doses on the order of 10^5 to 10^6 cells per insect were required to obtain comparable levels of mortality. Its virulence may have been increased through unintentional selection during the *in vivo* production of the virus in corn earworm larvae. The insect colony with which high mortality was obtained was compared with another colony, and the *S. marcescens* strain was compared with other strains. Results indicate that the highest levels of mortality are associated with a particular combination of insect colony and bacterial strain. While the potential of this organism as a biological control agent has not been evaluated, these apparently unique interactions could make this system an interesting and potentially useful model for the study of host-pathogen virulence factors.

Key Words *Serratia marcescens,* virulence, corn earworm, *Helicoverpa zea*

Serratia marcescens Bizio (Enterobacteriaceae) is a common, gram-negative, short rod-shaped bacterium that often grows as a saprophyte in soil and water. Many strains of *S. marcescens* produce a red pigment, prodigiosin. These strains are familiar and generally easy to recognize, though other species of bacteria can also produce prodigiosin (Sikorowski et al. 2001). Other strains of *S. marcescens* are unpigmented and more difficult to recognize (Bucher 1963).

Serratia marcescens is known as a potential or facultative pathogen of insects (Bucher 1963, Lysenko 1985, Steinhaus 1959, Tanada and Kaya 1993, Sikorowski et al. 2001). It is highly virulent against many species of insects when introduced into the hemocoel by injection or through wounds. Frequently, injection of 10 or fewer viable cells will kill an insect.

In contrast, *S. marcescens* is usually considered to be at most weakly pathogenic

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when ingested by otherwise healthy insects. Several published studies have indicated that when insects can be killed by ingestion of *S. marcescens*, doses of 10^5 to 10^6 viable cells per insect are required to obtain significant levels of mortality (Ansari et al. 1987, Grimont and Grimont 1978, Iwahana et al. 1985, Ourth 1988, Podgwaite and Cosenza 1976a, b, Steinhaus 1959). More recently, Sikorowski et al. (2001) fed measured doses of *S. marcescens* ranging from 2 to 2×10^8 cells per larva to *Heliothis virescens* (F.) and obtained rates of larval mortality ranging from about 20% at the lowest doses to about 50% at the highest doses.

Herein we report the discovery of a strain of *S. marcescens* that is unlike any previously reported in that it causes high rates of mortality in a laboratory colony of the corn earworm, *Helicoverpa zea* (Boddie), when fed to larvae at extremely low doses. This bacterium was found as a contaminant during bioassays of the nuclear polyhedrosis virus of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV), against *H. zea* (Farrar and Ridgway 1997). This virus is active against *H. zea* and a number of other lepidopterans (Hostetter and Puttler 1991), against which it was being evaluated as a biological control agent. The virus normally requires 6 to 8 d to kill the second-instar larvae that we used in our bioassays (Farrar and Ridgway 1997). However, in tests on one sample of AfMNPV, larvae frequently died 2 to 3 d after being fed foliage treated with dilute suspensions of the virus, which suggested another cause of mortality. These larvae were similar in appearance to larvae killed by the virus; they turned dark and their tissues liquefied. When larvae that died 2 to 3 d after treatment were examined for the presence of bacteria, an unpigmented strain of *S. marcescens* was isolated. The isolation, identification, and characterization of this strain of *S. marcescens* are reported herein, as are tests of its virulence against the corn earworm.

Materials and Methods

Insects. All insects were obtained as eggs from stock colonies maintained at the Crop Protection and Management Research Laboratory, USDA-ARS, Tifton, GA (GA colony) or at the Crop Science Research Laboratory, USDA-ARS, Mississippi State, MS (MS colony). Larvae were reared to late first to early second stadium on artificial diet (King and Hartley 1985). When insects of both colonies were being tested, they were reared under identical conditions.

Plants. The host plant was snap bean, *Phaseolus vulgaris* L., cv. 'Roma II'. Plants were grown in a greenhouse at a temperature of 24 ± 3°C and a photoperiod of 16:8 (L:D) (daylight supplemented with low pressure sodium lamps). A commercial potting medium (Pro Mix, Premier Brands Inc., Red Hill, PA) was used. Plants were fertilized weekly (Peters Professional 20-20-20, Grace-Sierra, Milpitas, CA) and were 4 to 5 wk old when used.

Virus. A sample of an aqueous suspension of AfMNPV produced in corn earworm larvae (lot LT-2) was received on 4 Dec 1993 from biosys (Palo Alto, CA; the assets of biosys have since been acquired by Certis USA, Columbia, MD). A second sample (lot Af091295) was obtained from biosys on 22 Apr 1996. Both samples were stored in a refrigerator at 5°C until needed.

Bacteria. For comparison to the *S. marcescens* isolated from diseased insects, two other strains of *S. marcescens*, NIMA (a strongly pigmented strain) and D 1 (a weakly pigmented strain), were obtained from Ken Nickerson [University of Nebraska). NIMA is pathogenic, while D 1 is not pathogenic, when injected into larvae of *Manduca sexta* (L.) (K. Nickerson, pers. comm.].

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Isolation, identification, and characterization of the bacterium. A single corn earworm that had died 2 d after being fed AfMNPV was placed aseptically in a 60-ml sterile Whirl-Pak bag (NASCO, Ft. Atkinson, WI). It was homogenized in 10 ml of sterile deionized water in a Stomacher blender (Seward Laboratory, London, UK) on low for 30 s. Bacteria were plated directly onto L-agar (Miller 1972) and incubated at 30°C for 16 h. Individual colonies were then streaked for isolation. Bacteria from individual colonies were gram stained, and the Biolog system (Biolog, Inc., Hayward, CA; Bochner 1989) was used to identify them. For identification, bacteria were grown on Bugm + 5% sheep blood media (Biolog Inc.). Additional tests on the bacteria included hemolysis on blood agar and production of protease, amylase, lecithinase, DNase and chitinase. Paper disks (BBL, Cockeysville, MD) were used to perform antibiotic sensitivity tests.

The identity of the bacterium isolated from the corn earworm larva was confirmed by a commercial laboratory, Analytical Services, Inc. (Williston, VT). Identification was confirmed by gas chromatographic analysis of fatty acids and by repeating analysis with the Biolog system.

To confirm that the source of the bacteria was the virus stock, a 10 µl aliquot of virus stock LT-2 was plated onto L-agar. Resulting bacterial colonies were streaked for purity, and the Biolog system was used to identify them. The initial attempt to isolate *S. marcescens* from the virus stock was complicated by the presence of other bacteria. Therefore, a selective medium was developed, based on the Biolog profile, that allowed the growth of *S. marcescens*, but not of other bacteria found with the virus. This medium was the defined medium M-9 (Miller 1972), modified by substituting sorbitol for glucose. The Biolog system was again used to identify bacteria grown on this medium.

Tests of virulence. Cultures of BF 1, the *S. marcescens* that had been isolated from a dead *H. zea* larva (see Results), were tested against larvae from the GA colony. The bacterium was cultured on plates of L-agar in 9-cm diam Petri dishes at 30°C for 16 h. Colonies were scraped off the medium and suspended in 10 ml of sterile deionized water. An aliquot of this suspension was removed for determination of bacterial titer. The aliquot was diluted and plated onto L-agar, incubated at 30°C for 16 h, and developing colonies were counted. At the same time, serial dilutions of the suspension were made for testing against larvae. Concentrations were initially based on estimated concentrations of bacteria; when the titers became available, these values were corrected to reflect actual concentrations.

Bioassays were conducted with bacteria pipetted onto bean leaf disks so that dosages could be carefully controlled. Dilute suspensions of bacteria (see below for concentrations) were pipetted onto the centers of 6-mm diam bean leaf disks at a rate of 2 µl per disk. For each treatment, 32 disks were placed individually in cells of plastic bioassay trays (16 mm diam by 16 mm deep; Model Bio-BA-128, C-D International, Pitman, NJ) on 1-cm diam disks of moist filter paper. Late first-instar (showing head capsule slippage) to early second-instar larvae were placed individually in the cells. The trays were covered with ventilated clear plastic covers and held at 27°C under a photoperiod of 16:8 h (L:D). Larvae that did not eat 100% of their leaf disks within 48 h, and thus did not ingest the entire dose of bacteria, were excluded from the test. Mortality was recorded at 2 d and surviving larvae were transferred to new bioassay trays, the cells of which each contained about 2 ml of artificial diet (King and Hartley 1985). Larvae were held for 5 d on diet (total of 7 d on foliage and diet); mortality was recorded daily.

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A preliminary test, in which doses of bacteria were not quantified, was done to test the methods. The cause of mortality of larvae that died in the preliminary test was confirmed by isolation and identification of the bacteria from dead larvae as described above.

In the first test, six doses of bacteria, ranged from 5.4×10^2 to 5.4×10^7 (separated by factors of 10) colony-forming units (CFU) per disk (based on measured titers), plus a control (water only), were included. Three additional tests were conducted, which included dosages of: 0; 5; 25; 50; 100; 500; 5,000; and 50,000 CFU per insect (second test); 1.3, 2.6, 6.4, 12.8, 32.0, 64.0, and 128.0 CFU per insect (third test); and 0.6, 1.1, 2.9, 5.7, 14.3, 28.5, and 57.0 CFU per insect (fourth test). Thirty-two larvae per dosage were included.

Tests were divided into two groups for statistical analysis: high doses (first and second tests) and low doses (third and fourth tests). Data were first adjusted for mortality on control treatments (Abbott 1925), normalized by arcsine $\sqrt{\%}$ transformation, then analyzed by analysis of variance (ANOVA) with percentage mortality as the dependent variable and dose (CFU per larva, as determined from measured titers, transformed logarithmically) and test (block) as independent variables (PROC GLM; SAS Institute 1988). Both linear and quadratic effects were evaluated. If no significant quadratic effect was found, the data were reanalyzed and tested for only linear effects. Separate analyses were performed on data from the high and low doses. The times to death of larvae that died were analyzed similarly to the percentages of mortality, except that each larva that died was treated as an experimental unit. Insects that survived the treatments were not included in the latter analysis because, particularly at very low doses, they may not have been infected and thus data from them would provide no information on how long the bacterium requires to kill its host (Farrar and Ridgway 1998).

Comparison of insect colonies. To test whether the pathogenicity of BF 1 to *H. zea* was due to characteristics that might be specific to the insects from the GA colony, these insects were compared with insects from the MS colony. Bioassays of BF 1 against insects from both colonies were conducted as described above, except that titers were determined prior to the test, so that consistent doses were obtained. Doses of 100; 1,000; and 10,000 CFU per insect were included, and the test was replicated four times. Mortality data were adjusted for mortality of control insects of the same colony and normalized as before, then analyzed by factorial ANOVA with insect colony and dose as main effects (PROC GLM; SAS Institute 1988).

To evaluate whether differences found between insect colonies in susceptibility to BF 1 (see Results) were related to general weakness or susceptibility of the larvae to pathogens, bioassays of the virus, AfMNPV (lot Af091295), were also conducted with both colonies. The bioassay was similar to that used in the bioassays of BF 1. To eliminate any mortality caused by any contamination by BF 1, an antibiotic, neomycin sulfate, was added to the suspensions of virus, and to the diet on which the larvae were reared before and after treatment, at a concentration of 0.5 mg/ml (Farrar and Ridgway 1997). Doses of 10, 25, 50, 100, and 500 occlusion bodies (OB) per larva were included, and the test was replicated four times. Mortality was recorded at 10 d. Data were analyzed as with the test of BF 1, except that no control mortality occurred, as well as by probit analysis (PROC PROBIT; SAS Institute 1988).

Comparisons of *S. marcescens* strains. To determine if the pathogenicity of BF 1 against insects from the GA colony was due to a general susceptibility to *S. marcescens*, BF 1 was compared with NIMA and D 1. Bioassays were conducted as

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described above. Doses expected to contain 100, 1,000, and 10,000 CFU per larvae were included for each strain; data were adjusted when titers became available. Mortality data were adjusted for control mortality, normalized, and analyzed by factorial ANOVA with strain and dose as main effects. Differences among strains were analyzed by the least significant difference (LSD) test (SAS Institute 1988).

Effect of larval stadium. Most larvae used in the above tests were late first instars, but a few were early second instars that had not apparently fed since molting. To test for possible differences in susceptibility between late first instars and early second instars, 32 late first instars and 32 early second instars of the GA colony were fed doses of BF 1 of 128 CFU per larva. Control larvae of each instar, fed disks treated with water only, were also included. Mortality was recorded at 7 d. Data were analyzed by a Chi-square test; no control mortality occurred.

Results

Isolation, identification, and characterization of the bacterium. Only one type of bacteria, a gram-negative short rod, designated BF 1, was isolated from the insect that died 2 d after being fed AfMNPV. The Biolog system identified it as *S. marcescens*, with a similarity index of 0.899. The distance match between BF 1 and the type strain was 1.395, indicating that BF 1 differed from the *S. marcescens* strains in the data base by less than 2 tests out of the 95 tests performed. It oxidized a variety of carbon sources, including glucose, sucrose, sorbitol, cellobiose, and lactose. It was weakly hemolytic after 3 d of growth. It produced protease, DNase, and chitinase, but not amylase or lecithinase. It produced a distinct odor of indole when grown on complex media. It was resistant to tetracycline, ampicillin, vancomycin, chloramphenicol and rifampicin and sensitive to neomycin and streptomycin.

BF 1 is presently in storage in the laboratory of the author P.A.W.M., USDA-ARS, Insect Biocontrol Laboratory, Beltsville, MD.

The fatty acid profile of BF 1 performed by the commercial laboratory identified it as either *S. marcescens* or *Cedecea davisae*, Grimont et al., with similarity indices of 0.640 and 0.652, respectively. However, the Biolog analysis performed by the same laboratory clearly identified it as *S. marcescens*, with a similarity index of 0.780.

Three distinct types of bacteria were isolated from lot LT-2 of AfMNPV. One, isolated by using a selective medium (M-9 with sorbitol instead of glucose as a carbon source), was identified as *S. marcescens*, apparently identical to BF 1. The other bacteria were identified as *Pseudomonas fluorescens* Migula and *Bacillus* sp. The relative densities of the three species of bacteria were not measured, but *S. marcescens* appeared to be much less numerous than the other species.

Tests of virulence. Only one type of bacteria was isolated from dead larvae from the preliminary test. It was again identified as *S. marcescens*, with a similarity index of 0.974 and a distance of 0.387. This strain, designated BF 1K, was identical to BF 1 in the enzymes that it produced and in its antibiotic sensitivity.

High levels of mortality, $\geq$50%, were observed at every dose of *S. marcescens* tested against the GA colony of the corn earworm (Figs. 1, 2). Because mortality was always $\geq$50%, no probit analyses were attempted. In the two tests that included the higher doses (Fig. 1), significant linear ($F = 9.28$; df = 1, 12; $P = 0.0139$) and quadratic ($F = 6.59$; df = 1, 12; $P = 0.0303$) effects of dose were found; control mortality was 9.4%. In the two tests with lower doses (Fig. 2), only a significant linear effect of dose ($F = 7.94$; df = 1, 13; $P = 0.0167$) was found; no control mortality occurred.

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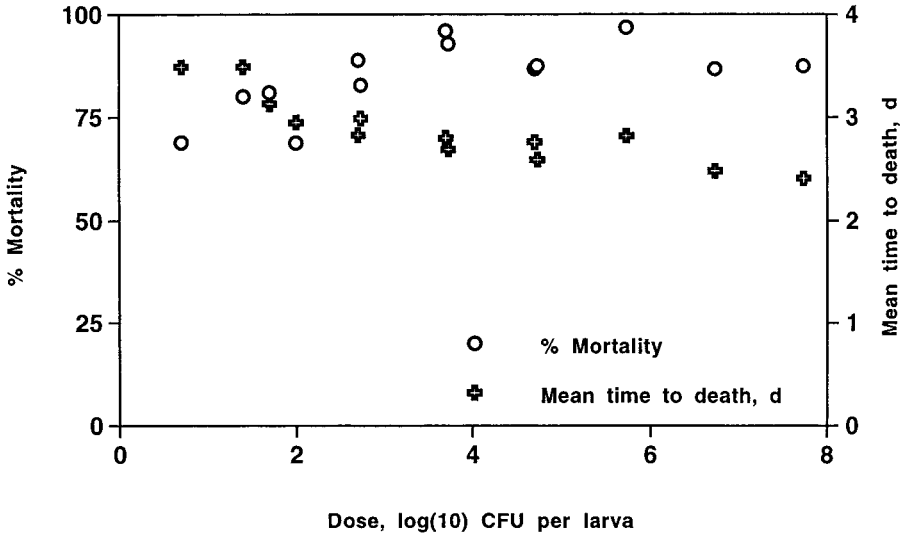

Fig. 1. Mortality and time to death of corn earworm larvae after being fed high measured doses of strain BF 1 of *S. marcescens*. Effects of dose on both mortality and time to death were significant ($P < 0.05$).

Most larvae that died after being fed *S. marcescens* did so between 2 and 3 d after being placed on foliage disks treated with the bacterium (Figs. 1, 2). In the tests of the higher doses (Fig. 1), larvae died sooner as dose increased; the linear effect of dose on time to death was significant ($F = 8.11$; df = 1, 330; $P = 0.0047$), while the quadratic effect was nonsignificant ($F = 2.87$; df = 1, 330; $P = 0.0913$). However, in the tests of the lower doses (Fig. 2), time to death was unaffected by dose (linear: $F = 0.08$; df = 1, 324; $P = 0.7772$; quadratic: $F = 0.09$; df = 1, 324; $P = 0.7631$).

Comparison of insect colonies. Larvae of the GA colony of *H. zea* were significantly more susceptible to BF 1 than were those of the MS colony (Fig. 3). Significant effects of both insect colony ($F = 30.27$; df = 1, 13; $P = 0.0001$) and dose of BF 1 ($F = 10.93$; df = 1, 13; $P = 0.0057$) were found. Control mortality was 7.0% and 2.1% for the MS and GA colonies, respectively.

In contrast, the two insect colonies did not differ greatly in their susceptibility to the virus, AfMNPV. The effect of dose of virus was significant ($F = 257.35$; df = 1, 35; $P = 0.0001$), but the effect of insect colony was nonsignificant ($F = 0.01$; df = 1, 35; $P = 0.9414$). The LD_{50} for the GA colony was 41 OB per larva (95% fiducial limits: 34-51 OB per larva; slope = 1.4452; SE slope = 0.1329; $\chi^2 = 118.18$, $P > \chi^2 = 0.0001$), while that for the MS colony was 60 OB per larva (95% fiducial limits: 44-69 OB per larva; slope = 1.2505; SE slope = 0.1190; $\chi^2 = 110.43$; $P > \chi^2 = 0.0001$). No control mortality occurred in the test of the virus.

Comparison of *S. marcescens* strains. The three strains of *S. marcescens* differed in their virulence against insects of the GA colony (Fig. 4). Significant effects of strain of *S. marcescens* ($F = 74.1$; df = 2, 27; $P = 0.0001$) and dose of bacteria ($F = 8.27$; df = 1, 27; $P = 0.0078$) were found. Control mortality was 2.6%. BF 1 was the

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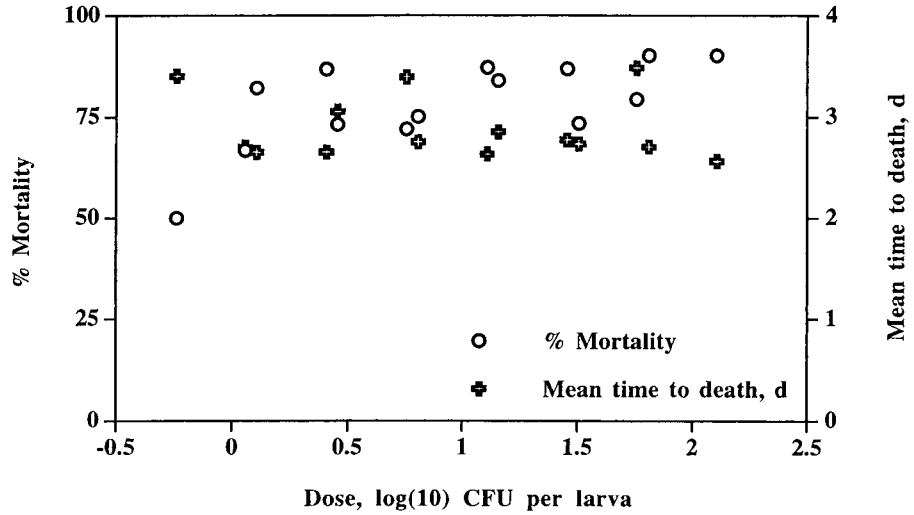

Fig. 2. Mortality and time to death of corn earworm larvae after being fed low measured doses of strain BF 1 of *S. marcescens.* Only linear effects of dose on mortality were significant ($P < 0.05$); effects of dose on time to death were nonsignificant ($P > 0.05$).

most virulent strain; D 1, the least virulent; and NIMA, intermediate in virulence. All differences among strains were significant ($P < 0.05$). Again, because mortality was >50% on most BF 1 treatments, and <50% on all D1 treatments, no probit analyses were attempted.

Effect of larval stadium. Mortality of larvae of the GA colony fed *S. marcescens* as late first instars or as early second instars was 90 and 96%, respectively, at 7 d after treatment, with no significant difference ($\chi^2 = 0.2677$, df = 1, $P > 0.50$) between instars. No mortality was observed in control larvae.

Discussion

Strain BF 1 of *S. marcescens* has pathogenicity *per os* against the GA colony of the corn earworm that is unlike that reported for any other strain of *S. marcescens* against any other insect. Previous reports indicated that *per os* doses of some 10^5 to 10^6 viable cells of *S. marcescens* per insect are needed obtain high rates of mortality in insects. Our data indicate that this strain can kill late first to early second-instar corn earworms of the GA colony with doses under 100 bacterial cells per insect. For comparison, approximately 1000 *Bacillus thuringiensis* Berliner spore equivalents are required to kill 50% of third-instar *M. sexta* larvae (P.A.W.M., unpubl. data). The high level of activity of BF 1 is unusual based on the lack of production of lecithinase, an enzyme which has been implicated in pathogenicity of other bacteria (Rahmet-Alla and Rowley 1989).

Only a weak, though statistically significant, dose response was seen. This weak dose response is consistent with that of Sikorowski et al. (2001) in a similar test,

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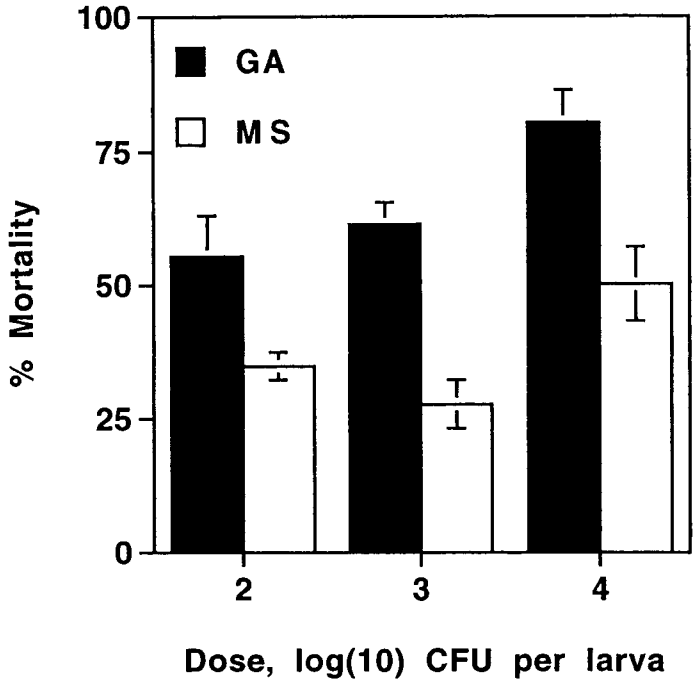

Fig. 3. Mean (±SE) mortality of larvae of GA and MS colonies of the corn earworm after being fed measured doses of strain BF 1 of *S. marcescens.* Effects of both dose and colony were significant ($P < 0.05$).

except that overall larval mortality in their test (about 20 to 50%) was much lower than that in our tests (50 to 97%). A poor relationship between dose of *S. marcescens* and insect mortality is not unusual. The reasons that this relationship is poor are unknown, but may be related to limits in the ability of the bacterium to breach host defenses, such as physical barriers in the gut or cellular or humoral immune responses (Sikorowski et al. 2001).

Koch's postulates (Thomas 1974) were fulfilled for strain BF 1 of *S. marcescens* in the GA colony of the corn earworm. It was isolated from diseased insects from virus bioassays, cultured, administered to healthy insects, and recovered from insects that died. Further bioassays of cultured bacteria (Figs. 1, 2) confirmed that *S. marcescens* was the cause of the early deaths of larvae in the virus experiments (Farrar and Ridgway 1997).

Results of comparisons of the two colonies of *H. zea*, and of the three strains of *S. marcescens*, suggest that highest levels of activity are associated with an interaction of BF 1 and the GA colony. The GA colony is not highly susceptible to all pathogens, or even to all strains of *S. marcescens.* Similarly, BF 1 is not as active against at least one other colony, MS, of *H. zea* as it is against the GA colony. Nevertheless, activity of BF 1 against the MS colony was still greater than would be expected, based on published literature, for orally administered *S. marcescens.*

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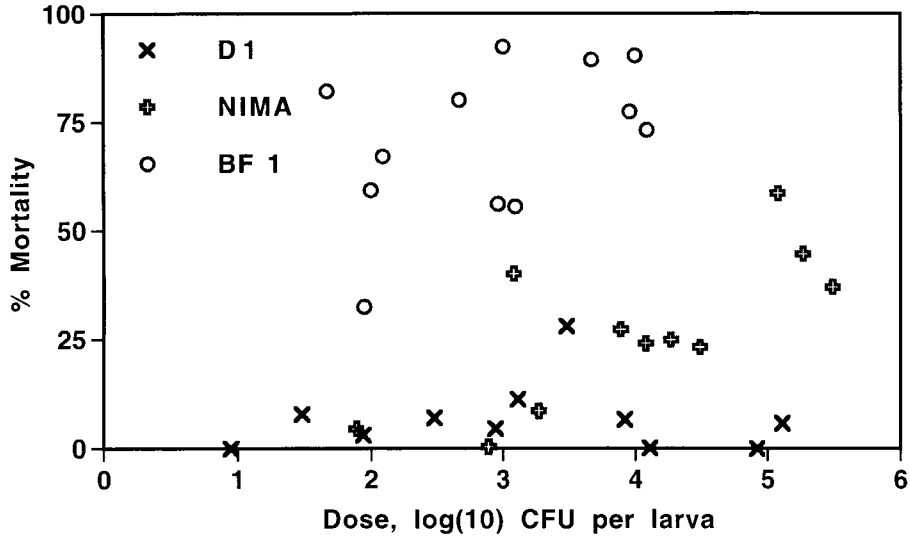

Fig. 4. Mortality of corn earworm larvae after being fed measured doses of three strains of *S. marcescens*. All differences among strains were significant ($P < 0.05$), as were effects of dose ($P < 0.05$).

The mechanism by which BF 1 kills larvae of the GA colony has not yet been determined. Two possible factors are being investigated, however. Hurst et al. (2000) isolated three genes, located on a plasmid, that seem to be responsible for pathogenicity of a related bacterium, *Serratia entomophila* Grimont et al., to grass grubs, *Costelytra zealandica* (White). They further reported that these genes show significant sequence similarity to those that produce insecticidal toxins in *Photorhabdus luminescens* (Thomas and Poinar) Boemare et al., a bacterium associated with entomopathogenic nematodes. A polymerase chain reaction method to detect these genes in *P. luminescens* and other bacteria is presently being developed by our colleagues in the Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD (M. Blackburn, pers. comm.). If it is successful, we plan to use this method to test BF 1. In addition, a recent preliminary gelatin zymogram test indicated that one or more proteases produced by BF 1 are particularly active (M. Blackburn, pers. comm.). Kaska (1976) reported that extracellular proteases produced by a strain of *S. marcescens* were toxic when injected into larvae of the greater wax moth, *Galleria mellonella* (L.). Further investigations of the role of proteases in the pathogenicity of BF 1, including comparisons with strains that are not highly pathogenic *per os*, are planned.

No difference in susceptibility between late first instars and early second instars was seen. Therefore, it seems unlikely that the bacterium entered the hemocoel of the larvae through any breaks in the cuticle, or unhardened cuticle, at molting. This lack of difference is thus consistent with oral pathogenicity rather than pathogenicity through introduction into the hemocoel as has been previously reported in *S. marcescens*.

It is possible only to speculate on the origin of this strain of *S. marcescens*. The

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virus with which it was found was produced in corn earworm larvae. In the production process, insects that are killed by virus are harvested, material taken from them is fed to healthy insects, and the cycle is repeated. The outward appearance of dead larvae killed by the virus is similar to that of larvae killed by *S. marcescens*, so larvae killed by *S. marcescens* could have been harvested along with those killed by the virus. While in the laboratory larvae killed by the bacteria can easily be distinguished from those killed by the virus, workers harvesting the cadavers may not have been trained to recognize the difference. Repeated passage of the bacterium through insects along with the virus could have selected for increased virulence against the corn earworm. If true, this scenario would illustrate the possibility of increasing the virulence of insect pathogens by selection.

Evidence indicates that contamination by *S. marcescens* of stocks of AfMNPV may have been confined to lot LT-2. A sample of AfMNPV produced in larvae of the tobacco budworm, *Heliothis virescens* F., lot Af052595, was received on 6 July 1995 from biosys (now Certis USA, Columbia, MD). Bioassays of this material indicated no evidence of early mortality caused by *S. marcescens* (Farrar and Ridgway 1999).

Pathogenicity of the other bacteria that were found in stock LT-2 of AfMNPV, *P. fluorescens* and *Bacillus* sp., against insects has not been evaluated. However, the high levels of mortality obtained at extremely low doses of BF 1 would indicate that it alone could account for all of the unusually early mortality observed in tests of the virus against *H. zea.*

The potential of BF 1 as a biological control agent has not been evaluated, though if susceptibility to BF 1 were limited to laboratory colonies of *H. zea*, it would probably be of little value for biological control. Testing against field collected *H. zea* and other species would be needed to fully evaluate this potential use, however. A commercial product, Invade, based on *S. entomophila*, has been produced for control of grass grubs (Jackson et al. 1992). While it may not be of immediate interest from a pest management standpoint, the apparently unique interactions of BF 1 and the GA colony could make this system an interesting and potentially useful model for the study of host-pathogen virulence factors.

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