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13 *Beauveria bassiana*, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens

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Abstract

Microbial control of plant pathogens and insect pests is an important component of efforts to reduce reliance on chemical pesticides and increase sustainability of agriculture. Although the number of registered microbial products has increased in recent years, many potential biopesticides have either not been developed for commercial use, or have had limited success, due to their pathogen or pest specificity, inconsistent performance across environments, or a lack of understanding of the mechanism(s) of biocontrol, resulting in ineffective

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use. Beauveria bassiana is a commercially available entomopathogenic fungus with an extensive host range of insect pests. Isolates of this fungus have been shown to be endophytic in specific crop plants, and in some cases, endophytic colonization has been linked to the ability of the fungus to control insect pests. With few exceptions, research on B. bassiana as a potential biological control for plant pathogens has been limited to in vitro studies on growth inhibition and cell lysis of plant pathogens. The potential of B. bassiana to control important plant pathogens in situ remains largely unknown. We have evidence that B. bassiana 11-98, originally isolated from an infected click beetle in Scott County, Tennessee, U.S.A., can control damping-off of tomato, caused by the soilborne fungal pathogen, Rhizoctonia solani, and can provide protection to cotton against a seedling disease complex in field soils. Furthermore, seed treatment with B. bassiana isolate 11-98 has resulted in endophytic colonization of tomato seedlings in a gnotobiotic system. The mechanism(s) of biocontrol by B. bassiana against plant pathogens, and whether or not endophytic colonization is a critical component of biocontrol are currently being investigated. Development of biopesticides that can control both plant pathogens and insect pests will have enormous value for plant protection in U.S. agriculture. Such biopesticidal organisms would be similar to wide spectrum pesticides with their potential for a significant reduction in pest management costs. However, unlike most chemical products, which have activity only against insects or plant pathogens, these biocontrol agents would have activity against both. Furthermore, disease and pest control with microbial agents offer the environmental advantages associated with biological control.

Introduction

Microbial control of plant pathogens and insect pests has received increasing attention in recent years, with the registration of a variety of commercial products. Although many organisms have been proposed as potential microbial control agents for either plant diseases or insect pests, few have attained commercial success. The specificity of most microbial control agents is partially responsible for their limited commercial use. Expanding the range of potential applications of specific biopesticides would be an important step in increasing their utility in agriculture.

Beauveria bassiana is a soilborne hyphomycetous fungus with entomopathogenic properties. Isolates of this fungus occur worldwide and have an extensive host range of insects at all stages of development. Efficacy against insect pests, such as citrus root weevil, *Artipus floridanus* (1), Colorado potato beetle, *Leptinotarsa decemlineata* (2), European corn borer, *Ostrinia nubilalis* (3,4), greenhouse whitefly, *Trialeurodes vaporariorum* (5), lesser

stalk borer, *Elasmopalpus lignosellus* (6), and sweet potato whitefly, *Bemisia tabaci* (1), has been demonstrated. Bioinsecticides containing *B. bassiana* are commercially available. These products are produced as conidial suspensions and applied as foliar sprays for insect control.

In recent studies (7,8), it has been shown that *B. bassiana* has the potential to control soilborne plant pathogens. By optimizing the biocontrol activity of the entomopathogen *B. bassiana* against plant pathogens, and elucidating its mode of action against disease-causing organisms, the potential of this fungus as a biopesticide could be greatly enhanced.

Enzymes and toxic metabolites produced by *Beauveria bassiana*

Infection of host insects generally occurs via attachment to and penetration of the outer integument. Several types of enzymes are produced by the fungus during degradation of the host insect cuticle. These enzymes are inducible and are regulated by the composition of the insect cuticle (9). The classes of inducible enzymes involved in infection of host insects include chitinases, lipases, and proteases. Germ tubes of *B. bassiana* penetrate the cuticle and epidermis, then grow toward the hemocoel of the host insect. Following penetration of the insect host cuticle, *B. bassiana* reproduces as hyphal bodies within the insect host (10). Hyphal bodies produced *in vivo* lack a well-defined cell wall and are surrounded by a thin fibrillar layer over the plasma membrane (11). In corn earworm (*Heliothis zea*) infected with *B. bassiana*, the fat body begins to show signs of damage 60-70 hours after penetration. At 6-7 days, the gut and Malphigian tubes of the insect can become infected; death and mummification can also occur. Death of infected insects most likely results from nutrient depletion, dehydration, and production of toxins by *B. bassiana* (12).

Beauveria bassiana produces several potent toxic metabolites with antibacterial, antifungal, cytotoxic, and insecticidal activities (9,12,13,14,15). These compounds are, in part, responsible for some of its entomopathogenic properties (16,17). These toxic metabolites include beauvericin, bassianolide, oosporein, and cyclosporin A (12). Beauvericin is a cyclic depsipeptide. Both beauvericin and bassianolide are ionophorous secondary metabolites. These toxins dissolve within lipid bilayers and increase the permeability of cell membranes to specific ions. In turn, abnormal ion transport disrupts the function of intact cells or organelles (12). In culture, *B. bassiana* produces oosporein, a red-pigmented dibenzoquinone mycotoxin (1). Oosporein, beauvericin, and bassianolide suppress bacterial growth, thus promoting sporulation of *B. bassiana* on intact insect cadavers (12). Cyclosporin A, an immunosuppressant, is produced by *B. bassiana* in culture, but its activity in

these plant pathogens was delayed and the percentage of conidial germination was reduced (31). *Beauveria bassiana* induced cell lysis of *Pythium ultimum, P. debaryanum* and *Septoria nodorum,* and inhibited their growth, while other phytopathogenic fungi, including *R. solani,* were not inhibited *in vitro* (32). In contrast, culture filtrates of other *Beauveria* isolates inhibited *in vitro* growth of *R. solani* (33). An isolate of *B. bassiana* was one of thirty microorganisms selected for the most effective and consistent biocontrol of take-all disease of wheat (caused by *G. graminis* var. *tritici*) from among 1,800 evaluated in screening assays in pots (29). In greenhouse and field studies, application of *B. bassiana* to onion bulbs significantly reduced infection by *F. oxysporum* f. sp. *cepae* (34).

Control of Rhizoctonia damping-off in tomato with *Beauveria bassiana* isolate 11-98

Rhizoctonia solani is a ubiquitous soilborne fungus that causes disease in a wide range of cultivated plants. In the absence of hosts, the pathogen survives in soil as a saprophyte on dead plant tissues via actively growing mycelium, or as small, loosely formed sclerotia. Damping-off is the most common disease caused by *R. solani*, and results in seeds that fail to germinate (pre-emergence damping-off), or after seedling emergence, lesions are formed at the base of the stem leading to collapse of the stem and death of the seedling (post-emergence damping-off).

In greenhouse studies, *B. bassiana* 11-98 applied as a conidial treatment in 2.5% methylcellulose (MC) solution to tomato seed, provided significant protection against damping-off caused by *R. solani* (7,8). In two trials (Figure 1A and B), using soil infested with *R. solani*, seed treated with *B. bassiana* had significantly greater plant stands than untreated seed. In the second trial (Figure 1B), the percent plant stand from seeds treated with *B. bassiana* and planted in *R. solani*-infested soil was 75%, which was not different from untreated seed planted in pathogen-free soil (82%). In pathogen-free soil, from both trials, the percent plant stand from seeds coated with *B. bassiana* was 89 to 92%. This was similar to the percent plant stand from untreated seeds in pathogen-free soil (82 to 85%).

In a separate greenhouse experiment, seeds of 'Mountain Spring' and 'Mountain Pride' tomatoes, were treated with *B. bassiana* 11-98 conidia in 2.5% MC solution and planted in potting soil infested with *R. solani* (Table 1). Controls in infested soil were: untreated seed or seed treated with 2.5% MC. Additional controls were untreated seed, and seed treated with 2.5% MC (trial 2 only; Table 1) sown in pathogen-free soil. In both trials, for both cultivars in infested soil, seed treated with *B. bassiana* resulted in significantly higher plant stands than untreated seed (Table 1).

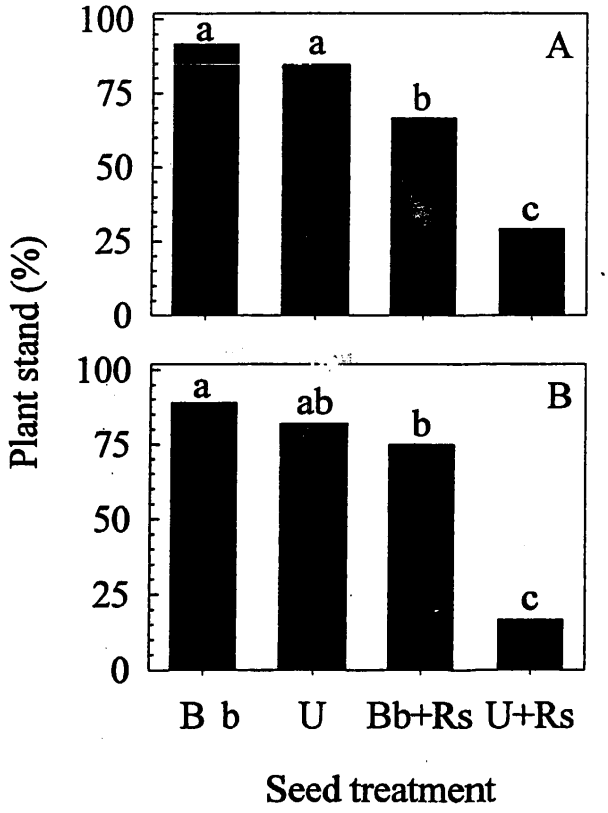


Figure 1. Effect of *Beauveria bassiana* on percent plant stand of tomato in potti soil infested with *Rhizoctonia solani*. 'Mountain Spring' tomato seed were trea with conidia of *B. bassiana* 11-98 and sown in pathogen-infested soil (Bb+F Controls were untreated seeds in pathogen-infested soil (U+Rs); seeds treated with *bassiana* in pathogen-free soil (Bb), and untreated seeds in pathogen-free soil (Within each trial, bars with the same letter are not different according to an F-protec LSD (P = 0.05). A = Trial 1, B = Trial 2. METHODS: *Rhizoctonia solani* was addec potting soil as rice inoculum at 1% w/w. Conidia of *B. bassiana* were produced Sabouraud dextrose yeast agar. Seeds were surface-sterilized in 3% hydrogen perox for 30 min and air-dried. Next, seeds were coated with a conidial suspension of *bassiana* in 2.5% methylcellulose solution at 0.6 mg conidia per seed and air-dried. T final rate of conidia per seed was approximately 6.0 $\log_{10}$ CFU. The four treatme were arranged in a randomized complete block with 72 replicates per treatment, a one plant per replicate. Percent plant stand was determined 35 days after planting.

Table 1. Effect of *Beauveria bassiana* on percent plant stand of two tomato cultivars in potting soil infested with *Rhizoctonia solani*.

Cultivar	Seed treatment[b,c]	Plant stand (%)[a]	
		Trial 1	Trial 2
'Mountain Pride'	Untreated	83 a	97 a
	MC	--	83 a
	B. bassiana + *R. solani*	73 a	75 b
	MC + *R. solani*	53 b	47 c
	Untreated + *R. solani*	47 b	50 c
'Mountain Spring'	Untreated	77 a	82 a
	MC	--	97 a
	B. bassiana + *R. solani*	77 a	62 b
	MC + *R. solani*	17 b	33 c
	Untreated + *R. solani*	27 b	18 c

[a] Percent plant stand was determined 21 days after planting.
[b] *B. bassiana* was applied to seed as a conidial suspension in 2.5% methylcellulose (MC).
[c] Methods: See Figure 1. Treatments were arranged in a factorial design in a split-plot with seed treatment as the main plot and cultivar as the sub-plot. There were 12 replicates of each treatment, and 5 plants per replicate.
[d] Within each trial, for each cultivar, values with the same letter are not significantly different according to an F-protected LSD at $P = 0.05$.

Effect of formulation of *Beauveria bassiana* on entomopathogenic activity and survival during storage

The effect of formulations of *B. bassiana* on shelf-life and entomopathogenic activity or storage and introduction as a biocontrol agent of insect pests has been reviewed (4). However, work on formulations for control of plant disease by *B. bassiana* is lacking. Formulation of *B. bassiana* on alginate pellets, a formulation that has been used for production of commercially available plant disease biocontrol agents, has been studied (35). Knudsen et al. (35) formulated *B. bassiana* from liquid culture into alginate pellets. Sporulation of the pellets was greatest when the formulation was supplemented with wheat bran. However, the pellets had excellent shelf life, both with and without bran, and upon rehydration, sporulated profusely after five months of storage at room temperature. Sporulating pellets were placed on wheat seedlings infested with greenbug (*Schizaphis graminum*) and incubated at high humidity. After 9 days, the percentage of live aphids on treated wheat was significantly lower than untreated controls. Secondary spread of the fungus was observed on wheat leaves several centimeters away from the alginate pellets (35).

Formulations of dry mycelia of *B. bassiana* in alginate and cornstarch were evaluated for conidial production after storage and after exposure to

artificial solar radiation, and for infectivity to southern corn rootworm, *Diabrotica undecimpunctata howardi* (36). Production of conidia was highest in a cornstarch-oil formulation, but alginate pellets and cornstarch alone were also effective storage materials. Production of conidia in all three formulations increased after storage for 13 weeks at either room temperature or 4°C. The alginate formulation increased protection of the fungal mycelia from solar light. Mortality to southern corn rootworm adults attributed to mycelium formulated into alginate and cornstarch-oil preparations was comparable to that caused by pure mycelial preparations. In field soil, recovery of populations from commercially formulated conidia of *B. bassiana* was 10 times higher when applied as a granular formulation than when applied as an aqueous suspension of a wettable powder (37).

Formulations of *Beauveria bassiana* for protection of tomato seedlings against Rhizoctonia damping-off

Little information is available on formulations of *B. bassiana* for disease control. In a preliminary greenhouse study, mycelial and conidial preparations

Table 2. Effect of different formulations of *Beauveria bassiana* on percent stand of tomato seedlings in soil infested with *Rhizoctonia solani*.

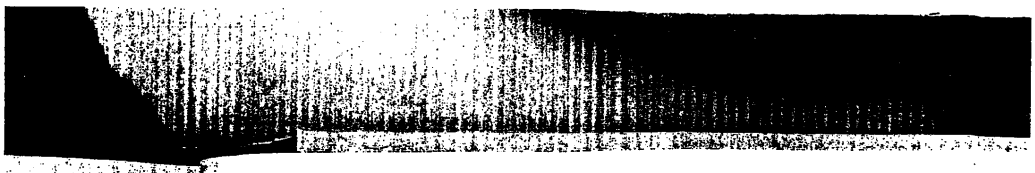
Treatment[c]	Plant stand (%)[a, b]	
	Trial 1[d]	Trial 2[d]
Uninfested control (no *R. solani*)	100 a	80 a
Potting soil with *B. bassiana* mycelia + *R. solani*	75 ab	28 c
Seed with *B. bassiana* conidia + potting soil with *B. bassiana* mycelia + *R. solani*	75 ab	70 ab
Seed with *B. bassiana* mycelia + *R. solani*	75 ab	70 ab
Seed with *B. bassiana* conidia + *R. solani*	63 ab	86 a
Seed with *B. bassiana* conidia (half rate) + *R. solani*	--	68 ab
Control + *R. solani*	38 b	40 c
Methylcellulose (MC) control + *R. solani*	38 b	28 c
Potting soil with dried *B. bassiana* alginate prills + *R. solani*	38 b	76 ab
Seed with *B. bassiana* conidia + potting soil with dried *B. bassiana* alginate prills + *R. solani*	38 b	60 b

[a] Percent plant stand determined at 21 (Trial 1) or 19 (Trial 2) days after seeding.

[b] Within each column, values followed by the same letter are not significantly different according to an F-protected LSD at $P = 0.10$ (Trial 1) or $P = 0.05$ (Trial 2).

[c] Methods: *Rhizoctonia solani* was added to potting soil as cornmeal:sand inoculum at 4% w/w. Mycelia of *B. bassiana* were produced in a fermenter (1% yeast extract and 1% dextrose), and added to potting soil at 16.4 g mycelia in 250 ml deionized water per liter soil. Conidia of *B. bassiana* were produced on Sabouraud dextrose yeast agar. Tomato seeds, 'Mountain Spring', were treated with 2% MC solution, either alone, or with 10 mg *B. bassiana* mycelia/seed, 1.0 mg *B. bassiana* conidia/seed, or 0.5 mg *B. bassiana* conidia/seed (Trial 2 only), then air-dried. Alginate wheat-bran prills (38) of *B. bassiana* were added to potting soil at 15 g dried prills + 250 ml deionized water per liter soil.

[d] Trial 1 was designed in a RCB with 9 treatments, 8 replicates per treatment, and 1 plant per replicate; Trial 2 was designed in a RCB with 10 treatments, 10 replicates per treatment, and 5 plants per replicate.



of *B. bassiana* were evaluated for control of Rhizoctonia damping-o tomato (8). Under heavy disease pressure, the percent plant stand of treated with either conidia or mycelia alone, or seed treated with co combined with potting soil amended with *B. bassiana* mycelia, ranged 63 to 86% (Table 2). These values were intermediate between the uninfe control (100%) and the infested controls (38%), but not significa different from either in Trial 1. In Trial 2, these treatments were diffe from the infested controls (28 to 40%), but were not different from uninfested control (80%). Potting soil amended with *B. bassiana* mycel infested soil gave inconsistent results with 75 and 28% plant stand in separate trials (Table 2). Application of *B. bassiana* as sodium algir wheat bran prills to potting soil gave inconsistent results, even w combined with *B. bassiana* as a seed treatment. Mycelia of *B. bassiana* v incorporated into the prills immediately prior to drying then added to pot soil 24 h before *R. solani*. More consistent results with alginate prills ma obtained by allowing more time for colonization of the prills by *B. bassi* either prior to drying, or before addition of *R. solani*.

Beauveria bassiana isolate 11-98 as entomopathogen

Beauveria bassiana 11-98 was isolated originally from an infected c beetle (Coleoptera: Elateridae) (39). In addition to direct infection susceptible insect hosts, Wagner and Lewis (25) reported that *B. bassi* can grow endophytically in corn and protect against tunneling of European corn borer, suggesting that ingestion of fungal mycelia metabolites in plant tissue may provide protection from insect herbivory order to investigate the effects of ingested *B. bassiana* 11-98 on insect pe corn earworms (*Helicoverpa zea*) were fed a synthetic diet containing di mycelia of *B. bassiana* (40,41). Mycelia were produced in a fermenter (yeast extract and 1% dextrose solution). Harvested mycelia were dried blended to a fine powder. For diet feeding tests, 16 g artificial t armyworm diet mix (Bio-Serv, Inc., Frenchtown, NJ), 2 g agar, and 82 m deionized water were heated, blended, and cooled. Ground mycelia w added to the diet at 0, 0.1, 0.5, and 5.0% w/v. Neonate corn earworm lar were placed in plastic cups (1 larva/cup) and exposed to 1-cm plugs of d Additional diet was added to the cups as needed. Delayed development high mortality were observed among larvae fed the highest rates (1 and 5 of fungal diet. Weights of surviving larvae and pupae were lower also larvae fed the higher concentrations of mycelia. After 10 days, lar mortality was 100% for the 5% mycelia diet treatment, which v significantly greater than all other treatments (0%).

Regional field trials with *Beauveria bassiana* seed treatments for control of seedling diseases on cotton and snap bean

Beauveria bassiana isolate 11-98 was included as an entry in regional field trials to test biological controls as seed treatments against seedling diseases of cotton and snap bean in 1999. The trials were conducted by participants of Southern Regional Project S-269 (currently S-302). *Beauveria bassiana* was evaluated at 10 field sites in nine U.S. states for protection of cotton against a seedling disease complex (42). Pathogens isolated from seedlings at these sites included *R. solani*, *Pythium* spp., *Thielaviopsis basicola*, and *Fusarium* spp. Twenty-five seed treatments and controls were evaluated. Across all sites, percent plant stand from seed treated with conidia of *B. bassiana* 11-98 was significantly higher than the untreated control and not different from the chemical seed treatment controls (metalaxyl, Vitavax-PCNB, metalaxyl/Vitavax-PCNB) (43). However, the effectiveness of *B. bassiana* 11-98 was variable from site to site. It performed best at two sites in Texas, which were the only sandy loam soils in the test (42). *Rhizoctonia solani* was the most frequently isolated seedling pathogen from the sites in Texas.

In a similar test at nine field sites in different U.S. states, *B. bassiana* seed treatment was evaluated for control of seedling diseases of snap bean. Across all sites, percent stand count from seed treated with *B. bassiana* was not different from the untreated control. It was ineffective at all sites tested (44).

Viability of *Beauveria bassiana* conidia on cotton and snap bean seed

For the field tests on cotton and snap bean (described above), we treated seed with *B. bassiana* in 2% MC solution in bulk, then the seed were air-dried. Treated seed were then delivered by overnight express mail to all other cooperators. Upon delivery to cooperators, there were 5.9 $\log_{10}$ CFU per seed for both cotton and snap bean (Table 3 [45]). At planting, seed from each field site was shipped to a single location and assayed for populations of *B. bassiana*. Across all planting sites, populations at planting were an average 5.8 $\log_{10}$ CFU per seed for cotton and 6.0 $\log_{10}$ CFU per seed for snap bean. None of the planting sites had population counts of *B. bassiana* on cotton seed that differed significantly from populations at delivery (range = 5.5 to 6.0 $\log_{10}$ CFU), indicating that populations of *B. bassiana* conidia on cotton seed were uniform and stable at planting across the planting sites. However, 33% of the snap bean planting sites had population counts that differed significantly from delivery populations.

Table 3. Populations of *Beauveria bassiana* on cotton and snap bean seeds.[a]

Crop	Log_{10} CFU per seed			Site differences (%)[e]	CV (%)[f]
	Delivery[b]	Planting[c]	Range[d]		
Cotton[g]	5.9	5.8	5.5-6.0	0	3.5
Snap bean[g]	5.9	6.0	5.1-6.2	33	1.9

[a] Compiled from Elliott et al. (45).
[b] Mean value (4 replicates) at delivery of bulk treated seed to cooperators.
[c] Mean value at planting across all locations.
[d] Range of mean values for planting locations.
[e] Percentage of locations that had seed mean values at planting that were significantly different (P = 0.05) from those at delivery of the bulk treated seed, according to Dunnett's t-test.
[f] Coefficient of variation for seed population values at planting.
[g] Methods: Conidia were produced on Sabouraud dextrose yeast extract agar and suspended in 2% MC solution at 20 mg conidia/ml suspension. Seed was not surface-sterilized before treatment. The conidial suspension was mixed with cotton or snap bean seed at 500 ml/kg seed, and treated seed were air-dried.

Detection of *Beauveria bassiana* in tomato tissues

Detection of *B. bassiana* from plant tissues is accomplished most often by plating samples on selective culture medium (3,21,46), a method that relies on the absence of other organisms that might out-compete *B. bassiana* in culture. The polymerase chain reaction (PCR) and gel electrophoresis have been used to detect *B. bassiana* in the cadavers of migratory grasshoppers (47). Species detection and identification in more complex systems containing multiple organisms may be accomplished with the PCR and internally transcribed spacer (ITS) region primers. PCR products can be characterized with gel electrophoresis. The advantage of this approach is that species identification can be confirmed by sequencing the PCR product.

Whereas ribosomal RNA gene sequences overall are conserved among taxonomically related organisms, sequences that lay between rRNA genes (ITS) are often highly variable, even among organisms that are closely related. In a preliminary test, we used a pair of PCR primers (ITS1 and ITS4; [48]) to amplify *B. bassiana* DNA (40,41). In this study, 'Mountain Spring' tomato seeds were surface-sterilized (95% ethanol for 1 min, 0.04% sodium hypochlorite for 5 min, 95% ethanol for 1 min), then treated with conidia of *B. bassiana* in 2% MC (0.5 mg conidia/seed). Controls were surface-sterilized, untreated seeds. Seeds were planted in sterilized vermiculite and grown for two weeks under gnotobiotic conditions. DNA was isolated from a pure culture of

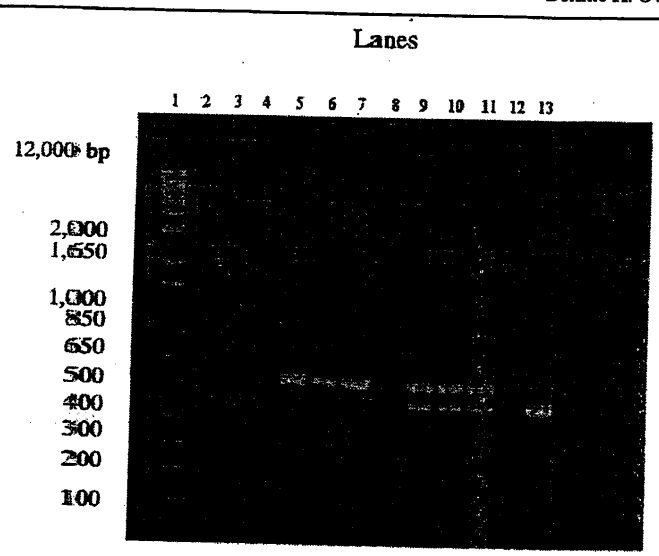


Figure 3. Agarose gel electrophoresis of ITS-specific PCR products from tomato tissue grown from seed treated with conidia of *B. bassiana*. Lane 1: 1 Kb Plus DNA Ladder (Invitrogen Corp., Carlsbad, CA). Lanes 2, 3, 4: Blank. Lanes 5, 6, 7: Tomato shoot tissue from untreated seed, one band at 650 bp. Lane 8: Blank. Lanes 9, 10, 11: Tomato shoot tissue from seed treated with conidia of B. bassiana, bands at 650 (upper) and 550 (lower) bp. Lane 12: Blank. Lane 13: Pure culture of *B. bassiana*, one band at 550 bp. Methods: DNA was isolated using a Puregene kit (Gentra Systems, Minn., MN).

B. bassiana, and from entire tomato seedling tissue excised above the soil line and grown from untreated seed, and from seed treated with *B. bassiana*. After amplification, the PCR products were electrophoresed through 1.5% agarose. A single band of the expected size (550 bp; [49]) was detected when DNA from a culture of *B. bassiana* isolate 11-98 was amplified (Lane 13; Figure 2). A larger single PCR product, corresponding to the expected size of tomato rRNA amplicons (650 bp; [50]), was generated from tissue of tomato seedlings from untreated seed (Lanes 5, 6, and 7; Figure 2). Two bands, each corresponding in mobility to the band from either *B. bassiana* or tomato, were found for seedlings from seed treated with *B. bassiana* (Lanes 9, 10, and 11; Figure 2). The bands from lanes 5-7, 9-11, and 13 were excised and sequenced. Based on DNA sequence analysis, the lower band from treated plants had 100% sequence identity with the band from *B. bassiana*, while the upper band had 100% sequence identity with the band from untreated tomato. Several significant alignments were obtained when the sequences of these two PCR products were compared with GenBank sequence data using the BLAST

protocol (51). For the band from *B. bassiana*, 23 of the top 24 alignments (97-100% identity) were isolates of *B. bassiana*. For the band from tomato, the first 10 alignments (85-90% identity) were *Lycopersicon esculentum* and other species of *Lycopersicon*. These preliminary data provide evidence that *B. bassiana* is an endophyte of tomato. Investigations are underway to determine the significance of endophytic colonization in the biocontrol of *R. solani* of tomato by *B. bassiana*.

Summary

Integrating microbial control methods for management of both plant diseases and insect pests is an important step in the development of sustainable systems that reduce our reliance on chemical pesticides for crop production. Isolates of the entomopathogenic fungus *B. bassiana* have deterious effects on growth of plant pathogens *in vitro*, and *B. bassiana* isolate 11-98 can protect seedlings against soilborne pathogens *in situ*, suggesting that this isolate is a candidate for development as a dual function biopesticide. The dual function of microorganisms against both insect pests and plant pathogens is an integrated approach to pest management that has not yet been exploited, and will lead to new strategies in development of ecologically based pest management systems.

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