

MiniReview

Biocontrol of plant disease: a (Gram-) positive perspective

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Abstract

Biological control offers an environmentally friendly alternative to the use of pesticides for controlling plant diseases. Unfortunately, growers continue to use chemical control over biological agents, and lack of knowledge often contributes to the downfall of a biocontrol agent. Knowledge of the biological environment in which the agent will be used and of how to produce a stable formulation are both critical to successful biocontrol. Certain Gram-positive bacteria have a natural formulation advantage over their Gram-negative counterparts: the spore. Although the Gram-positive bacteria have not been as well represented in the biocontrol literature, their spore-forming abilities and historical industrial uses bode well for biocontrol success. Here we describe several systems utilizing Gram-positive biocontrol agents that have been researched in depth and provide models for the future of biocontrol. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Control of plant disease is a pressing need for agriculture in the 21st century. The increasing demand for a steady, healthy food supply by a burgeoning human population will require controlling diseases that reduce crop yield. Furthermore, increased pressure for food production will intensify the demands on agricultural production systems, which in turn, may increase disease pressure on crop plants. Current practices for controlling plant disease are based largely on genetic resistance in the host plant, management of the plant and its environment, and synthetic pesticides [1]. There is a demand

for new methods to supplement existing disease control strategies to achieve better disease control. Moreover, alternatives to many of the synthetic pesticides currently in use are needed. Many of the synthetic chemicals may lose their usefulness due to revised safety regulations [2–4], concern over non-target effects [5–8], or development of resistance in pathogen populations [9]. Thus, there is a need for new solutions to plant disease problems that provide effective control while minimizing negative consequences for human health and the environment [4,10].

Biological control, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals. The rich diversity of the microbial world provides a seemingly endless resource for this purpose. Increasing the abundance of

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a particular strain in the vicinity of a plant can suppress disease without producing lasting effects on the rest of the microbial community or other organisms in the ecosystem [4,11–13]. Biological control is also likely to be more robust than disease control that is based on synthetic chemicals. The complexity of the organismal interactions, the involvement of numerous mechanisms of disease suppression by a single microorganism, and the adaptedness of most biocontrol agents to the environment in which they are used all contribute to the belief that biocontrol will be more durable than synthetic chemicals [3,4,14].

Despite its appeal and potential, biological control has not made the transition from research plots to farmers' fields very effectively. Quite a few biocontrol agents are currently on the market (see www.barc.usda.gov/psi/bpdl/bioprod.htm, [15]). However, success with biocontrol agents is often unpredictable and too variable for large-scale use. There are many reasons for this, but two common themes are a lack of knowledge of the biological control system and difficulty in obtaining a successful formulation. To determine why biocontrol fails, there must be an understanding of the biocontrol agent and its interactions with the pathogen, the plant, the microbial community, and the environment. Unfortunately, few systems have been studied in detail, and until detailed studies of the whole system are undertaken, we are unlikely to have sufficient insight to avoid failures and reduce variability. Many biocontrol agents are difficult to formulate as products. A number of organisms have been successful in research plots, but scaling up production, providing effective formulation, and producing a stable, inexpensive product has caused them to languish as research marvels that do not reach the marketplace [14].

The sporulating Gram-positive bacteria offer biological solutions to the formulation problems that have plagued biocontrol. In contrast, successful, viable formulations of fluorescent pseudomonads remain a major obstacle for their large-scale use [16]. Sporulating Gram-positive microorganisms, such as *Bacillus* and *Streptomyces*, offer heat- and desiccation-resistant spores, which can be formulated readily into stable products. These spore products can be formulated as a dry powder, while Gram-negative microorganisms, like *Pseudomonas syringae*, are formulated as frozen cell pellets that must be kept on

dry ice until application. There is a substantial base of industrial experience with *Bacillus* and *Streptomyces* spp., which have been used for insect biocontrol, industrial enzyme production, and antibiotic production for many decades. This experience can be applied to the use of members of these same genera for biocontrol to overcome current obstacles to fermentation, formulation, and storage. Some of these organisms have been the subject of intense study at the genetic and biochemical level, providing a basis for study of them as biological control agents. Moreover, a few biological control systems involving Gram-positive bacteria have been studied intensively in the field and laboratory, providing a base of knowledge that can be used to develop the bacteria into effective products and to identify potential sources of failure.

The Gram-positive bacteria have received less attention than the fluorescent pseudomonads in the literature on biocontrol, in part, because the Gram-positive organisms have been less tractable for genetic study and less is known about the mechanisms by which they suppress disease [17]. However, their efficacy is striking. Many surveys of soil bacteria have identified strains of *Streptomyces* and *Bacillus* as potential biocontrol agents [18–21].

In this minireview, we seek to highlight the Gram-positive organisms that have potential in biocontrol. We provide examples of Gram-positive bacteria that illustrate certain principles and research approaches. For a more comprehensive treatment of biocontrol, please see one of the recent reviews on the topic [17,22].

2. *Bacillus subtilis* GB03: story of field success

It is probably not a coincidence that among the first successful biopesticides for control of insects and pathogens were members of the genus *Bacillus*. *Bacillus thuringiensis* (Bt) accounts for over 90% of all marketed bio-insecticides and represents a worldwide market of \$110 000 000 annually for control of insects [23]. Bt has been a successful market item for over 25 years [24]. A relative newcomer to the market, Kodiak, is a strain of *Bacillus subtilis* that is highly effective for crop protection from the pathogens *Fusarium* and *Rhizoctonia*, as well as in stimu-

lating plant growth [25,26]. Use of Kodiak has been steadily increasing across the US, and it was used on 2 million hectares of crops in 1994 [26]. The success of these organisms is certainly due, in part, to the ease of formulation and storage of the products. The Gram-positive spore offers a product formulation that has been selected over billions of years of evolution for its robustness and durability. Increased intensity in research on the Gram-positive bacteria associated with plants will provide the potential for a suite of products that may vary in their biological target while sharing a unique formulation.

3. *Bacillus cereus* UW85: story of a system

3.1. Field performance

A system that has been examined at many biological levels – from the molecular to the community interactions – is *Bacillus cereus* UW85 (Fig. 1). The bacterium was initially identified in a collection of rhizosphere isolates as one that suppressed alfalfa

damping off consistently [27]. *B. cereus* UW85 has since proven a reliable biocontrol agent of Phytophthora damping off and root rot of soybeans under diverse field conditions in the upper midwest US [13]. UW85 has been extensively field-tested and is currently in review by the US Environmental Protection Agency for registration as a seed treatment for soybeans. Performance of UW85 has not been reliable in the southern soybean-growing regions (Osburn and Smith, personal communication) and it has been variable on alfalfa (E. Kazmar, personal communication). Therefore, research has focused on the basis for disease control, interaction of UW85 with the plant, and the impact of UW85 on the microbial community in which it must function. The goal of such research is to determine sources of variability in control and develop strategies to improve biocontrol.

3.2. Interaction with the pathogen

B. cereus UW85 suppresses disease in the laboratory by preventing normal development and infec-

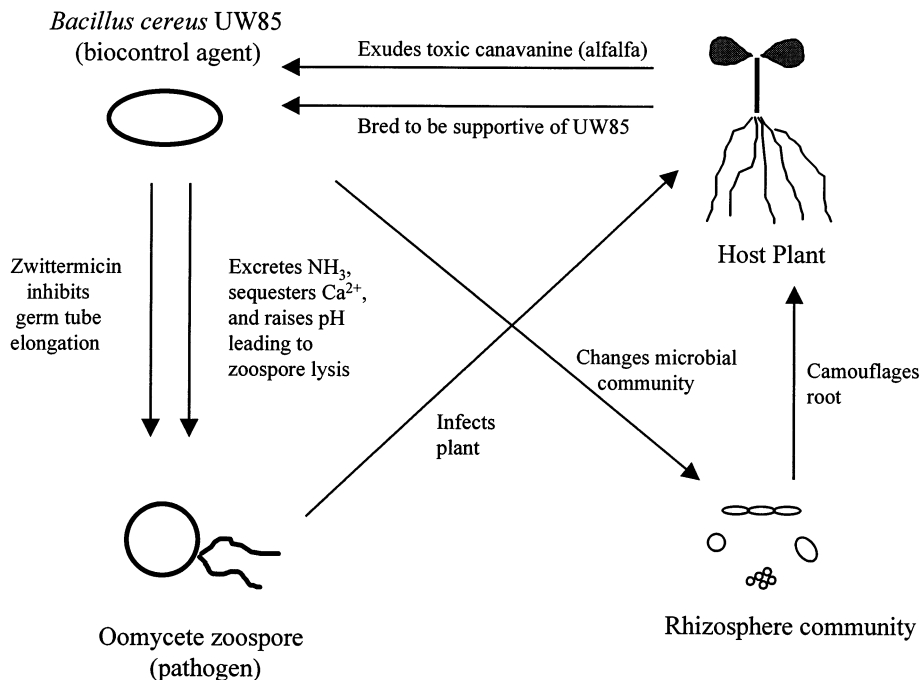


Fig. 1. A working model for biological interactions in the *Bacillus cereus* biocontrol system. We have observed all of these interactions, but we have not determined that all of them play a role in biocontrol. Current research in our laboratory is addressing the role of these interactions in biocontrol.

tion by oomycete pathogens [28]. The bacterium produces two antibiotics, zwittermicin A and kanosamine, which contribute to biocontrol of alfalfa damping off. Kanosamine is an aminoglycoside, and zwittermicin A is an aminopolyol, which is a novel class of antibiotic [29]. The purified antibiotics directly inhibit growth and development of the pathogen and suppress disease, and mutants lacking the antibiotics are reduced in disease suppressiveness [28]. Together these data provide strong evidence that the antibiotics contribute to disease suppression in the laboratory; experiments are needed to determine their role in the field. In addition to antibiotic production, *B. cereus* modifies the ionic composition of the medium in which it grows. It raises the pH, sequesters calcium, and excretes ammonia. This combination is highly toxic to zoospores of oomycete pathogens, causing rapid swelling of the expulsion vacuole, followed by zoospore lysis [30]. The role of these effects in soil is difficult to assess, since *B. cereus* mutants affected in the process do not grow. Thus, it is not known whether zoospore lysis contributes to biocontrol in the field.

3.3. Interaction with the microbial community in the rhizosphere

B. cereus UW85 affects bacterial communities on soybean roots. Introduction of UW85 on soybean seeds greatly altered the composition of culturable communities in some field experiments [12]. This impact of UW85 is surprising, given that it colonizes roots at low population density, although it persists on soybeans throughout the growing season [31]. It is not known whether the alteration of bacterial communities or colonization are required for disease suppressiveness by *B. cereus*, but intriguing hypotheses are suggested by the impact of *B. cereus* on the microbial community in the rhizosphere. Perhaps the effect of *B. cereus* on plant health is exerted through the community by stimulating growth of other bacteria that stimulate root growth, antagonize the pathogen, or induce resistance in the host. Alternatively, *B. cereus* might alter the community in a manner that reduces the attractiveness of the root to the pathogen [32]. These remain testable hypotheses that may explain elements of the complexity of disease suppression.

3.4. Interaction with the host plant

The interaction of *B. cereus* with the host plant has revealed some promising avenues for improving biocontrol. In a study of the impact of plant exudates on antibiotic production by UW85, the alfalfa seed was found to release a potent inhibitor of growth of UW85 [33]. The inhibition is due to canavanine, which is a well-known constituent of legume seeds. Canavanine-resistant mutants of UW85 achieved larger populations surrounding alfalfa seeds than the canavanine-sensitive parent, but populations on the seed surface were not affected by canavanine [34]. These data suggest that large spermosphere populations are not necessary to achieve successful biocontrol in this system, and they highlight the need to consider seed chemistry when designing formulations for biocontrol. In addition to seed inhibitors, there may be other plant traits that could be modified or exploited for biocontrol. Work with tomato indicates that the ability to support biocontrol by UW85 is a heritable trait. Inbred lines of tomato differ significantly in their survival in the presence of UW85, but they do not differ in resistance to the pathogen [35,36]. These inbred tomato lines provide the basis for a breeding program to enhance this trait for practical use as well as the genetic material required for molecular mapping of the loci that contribute to the phenotype [35].

4. *Streptomyces* spp.: story of ecological principles

4.1. Scab-suppressive soil: understanding a system

A well-developed system that has been studied with an ecological approach is suppression of potato scab by *Streptomyces* spp. Scab is caused by *Streptomyces scabies*, which is closely related to the species used for its control. This relatedness of the pathogen and biocontrol agent has led to some remarkable studies of the ecological behavior and interaction of the pathogens and strains that suppress them. Study in this system was initiated with the interesting observation that continuous cropping of potato often led to a natural decline in disease and a scab 'suppressive' soil [37], suggesting that a biological change occurred in the soil. In an effort to de-

termine whether biological factors could be associated with the disease suppressiveness of the soil, Liu et al. [38] isolated two strains of *Streptomyces* sp., designated PonR and PonSSII, from tubers grown in disease-suppressive soil. In a 4-year field trial, the strains significantly reduced the number of scab lesions on tubers in all years.

4.2. Effect of disease severity

Disease pressure is one component of biocontrol that is highly variable and often under-researched. Research of disease pressure is important for assessing the relationship between disease and biocontrol success and learning how well the strains protect at varying levels of disease. And since success is defined in terms of disease reduction, knowledge of variability in disease across a field will contribute to more accurate measurements of biological control success. Scab severity and biocontrol efficacy were carefully monitored for 2 years in a research field [39]. Both levels of scab and suppression of it varied among blocks in the field. Interestingly, biocontrol in a plot was positively correlated between years, suggesting that the biocontrol strains are efficient colonizers, that detrimental effects on the pathogen persist into the next year, or that microsite variation affects control the same way in consecutive years. The natural disease variability present in this research provided an opportunity for studying the interaction between disease severity and disease suppression, and in 1 year of the 3-year study, disease and biocontrol were positively correlated (L. Kinkel, personal communication).

4.3. Effect of suppressive strains: optimizing conditions

In any system, formulation, dose, and application method must be studied to identify the optimal method for introducing the biological control agent into the field. A comprehensive, three-year field trial with scab-suppressive strains was conducted to identify the most effective methods for introducing the control strains. Better control was achieved using repeated application of the suppressive strains each year, larger doses of the suppressive strains, individual strains rather than combinations, and a vermic-

ulite formulation rather than a tuber-dip [39]. All of these data indicated that larger doses of the suppressive strains led to increased biocontrol of scab. A greenhouse study was undertaken to monitor how pathogen populations were affected by the addition of suppressive strains. In this study, there was a positive correlation between pathogen root or soil populations and scab severity, suggesting that disease incidence is driven by the population density of the pathogen [40]. Increasing the inoculum density of the suppressive strain led to an increase in its root populations; however, pathogen root populations were unaffected. Interestingly, soil populations (for both pathogenic and suppressive strains) were more predictive of disease severity than root populations, suggesting that the soil, and not the root, may be the source of tuber inoculum [40].

4.4. Friend or foe: tracking *Streptomyces* strains

One difficulty in *Streptomyces* field research is identifying and tracking strains. Colony morphology is the standard method used to identify *Streptomyces* species; however, this tends to be time-consuming and subjective. Fatty acid analysis is a reliable and feasible method of distinguishing pathogenic *Streptomyces* strains from suppressive strains [41]. Using this powerful method, Bowers et al. [42] examined the ecology of suppressive and pathogenic *Streptomyces* species in soil and monitored the effects that addition of suppressive strains had on the *Streptomyces* community. Following an initial inoculation with suppressive strains, *Streptomyces* populations in soil, on roots, and on tubers were monitored over 2 years. Inoculation did not alter the diversity of the *Streptomyces* community as evidenced by similar numbers of fatty acid strain groupings in both inoculated and uninoculated plots. However, inoculation did decrease the number of pathogen-related isolates at least 85% in the first year of the study and at least 36% in the following year. The number of scab lesions declined with inoculation with the suppressive strains and this decline in lesion number was correlated with the decline in pathogen populations. Due to the relatedness of pathogenic and suppressive strains in this system, it is thought that the addition of suppressive strains leads to scab control through the displacement of pathogenic strains.

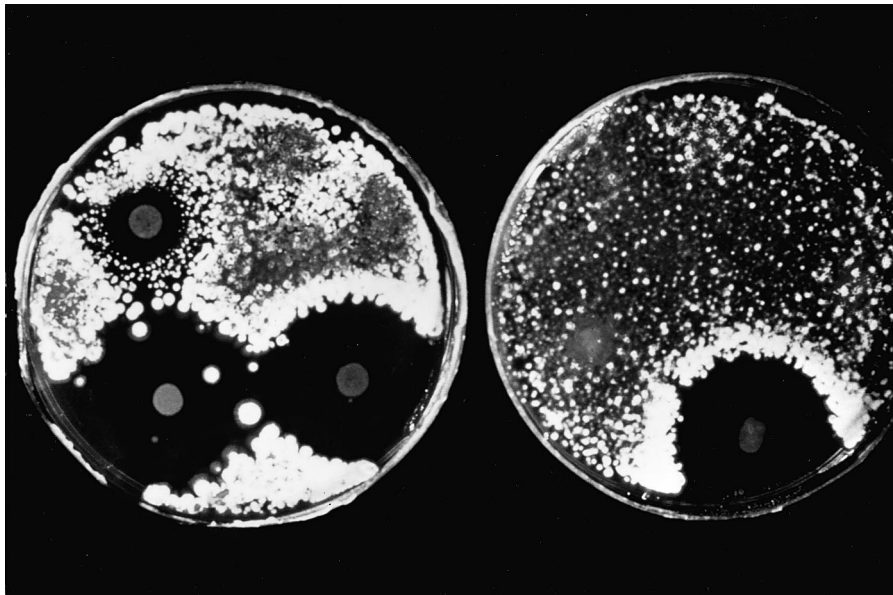


Fig. 2. Antibiotic production by tester strains against sensitive *Streptomyces* strain RB4. Tester strains were spotted on R2 generation media and grown 3 days at 30°C. Tester strains were killed by a 1-h exposure to chloroform vapors. A-15 ml overlay of 1% water agar was added, followed by 100 µl of RB4 culture. Zone sizes were scored after 3–5 days at 30°C. Photo supplied by L. Kinkel and reproduced with permission from [43].

4.5. Strain-specific effects: searching for super-suppressive strains

Equipped with basic information about this system, the Kinkel group set out to isolate new *Streptomyces* strains from several sites to identify strains that are more suppressive than those originally isolated (Fig. 2). Of the 93 strains examined, 22 were more antagonistic in vitro than the standard PonR and PonSSII strains, and none of these was pathogenic. These 22 strains, PonR, PonSSII, and 17 virulent strains were tested for in vitro antagonism in all possible pairwise combinations. Interestingly, suppressive strains were more antagonistic and inhibited more strains than pathogenic strains. Six of the newly acquired antagonistic strains were tested in the field, and all but one significantly reduced the number of scab lesions as compared to the uninoculated control, although none of these new isolates were more effective than PonR or PonSSII [44]. This suggests that suppression of potato scab in the field by *Streptomyces* spp. is not simply related to the in vitro inhibition ability of each strain.

4.6. Communication between strains: power in pairs

The latest approach for improving disease control in this system involves combinations of strains. Recently, Becker et al. [45] unearthed evidence for interspecies communication between *Streptomyces* strains. They found that PonSSII produced antibiotic(s) earlier and at higher levels when sterile culture supernatants from other *Streptomyces* strains were added to the PonSSII growth medium (Fig. 3). The added culture supernatants do not have antibiotic activity, suggesting that the increased inhibition is due to communication between the strains. Homoserine lactone autoinducers were not detected in the *Streptomyces* supernatants using an *Agrobacterium* indicator strain; thus, it is possible that the *Streptomyces* strains are producing autoinducers other than homoserine lactones or communicate with a different signal. The next goal is to develop cocktails containing strains that communicate with each other to maximize antibiotic production and disease control (L. Kinkel, personal communication).

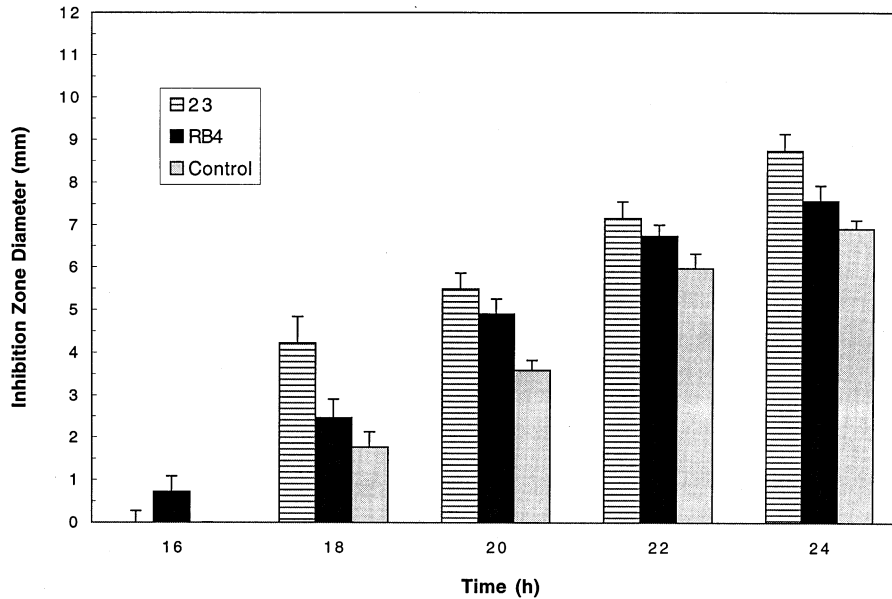


Fig. 3. Effects of strains 23 and RB4 CM on antibiotic production by PonSSII. CM or plain broth (10 ml) were added to PonSSII cultures after 8 h of incubation. Averages of replicate ($n=6$ or 8) flasks are shown. Bars represent standard error. This figure is based on Figure 1 in [45] with permission.

5. Conclusions

The need for biopesticides is intensifying in modern agriculture, but product development lags, in part due to the barriers to effective formulation of cheap, stable products. Many of the troublesome formulation problems associated with Gram-negative bacteria are overcome by packaging biocontrol activity in a Gram-positive spore. However, research on the spore-forming bacteria associated with plants is a field that has received little attention compared with the intensive study of the Gram-negative bacteria. In-depth study of Gram-positive bacteria, such as *Bacillus cereus* and *Streptomyces* spp., has led to biological paradigms that differ from those emerging from the study of organisms such as *Pseudomonas* and *Rhizobium*. With *Bacillus cereus*, large populations on the root are not necessary to achieve disease control, and with both *Bacillus* and *Streptomyces*, interactions leading to control are more complex than simple models based on single characteristics, such as population size or in vitro inhibition.

Many of the biocontrol organisms mentioned in this minireview employ antibiotic production as a mechanism of disease control. Any time an antibiotic

is used, careful measures must be taken to delay the development of antibiotic resistance. Indeed, studies have demonstrated that the use of antibiotics in animal feed leads to antibiotic resistance in humans [46]. Even with this caution in mind, antibiotics produced by biocontrol microorganisms are likely to produce minute amounts of antibiotic, compared with amounts of chemical fungicides used to control disease, and microbes will deliver the antibiotic to the exact location needed, as opposed to the blanket approach used with fungicides [47]. An increased research focus on the Gram-positive bacteria associated with plants will enhance our understanding of the complex interactions between these organisms and generate useful products for management of pests and pathogens in the agroecosystem.

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