



Biocontrol agents and their mechanism in plant disease management

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Abstract : The environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological control. Biocontrol of plant diseases provides practices compatible with the goal of a sustainable agricultural system. The mechanisms of biocontrol mainly include antibiosis, competition, mycoparasitism, cell wall degrading enzymes, and induced resistance. These mechanisms are probably never mutually exclusive; these terms are meant to organize the examples into general groups to facilitate comparisons. A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people, and the environment.

Keywords : biocontrol, mechanism, plant disease.

(Received : January 2014, Accepted March 2014)

1. Introduction

In plant pathology, the term biological control applies to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations.

More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. National Research Council took into account modern biotechnological developments and referred to biological control as “the use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects, and microorganisms”, but this definition spurred much subsequent debate and it was frequently considered too broad by many scientists who worked in the field (US Congress, 1995). Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. Consequently, understanding the mechanisms of biological control of plant diseases through the interactions between biocontrol agent and pathogen may allow us to manipulate the soil environment to create conditions conducive for successful biocontrol or to improve biocontrol strategies (Fravel, 1988). Recently several methodologies for genetic analysis, such as the approach of mutant analysis, have provided promise for the study of mechanisms of biocontrol agents and their targets (Wilhite et al., 1994). In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In such a scheme, hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism. Biological pest control provides an important route to environmentally harm- less plant protection. This route reduces risks, e. g. of becoming aware too late of unfortunate side effects of plant protection agents. Intense use of active substances can harm beneficial organisms and some- times even promote pests.

2. Interactions contributing to biological control

Odum (1953) proposed that the interactions of two populations be From the plant’s perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions. Mutualism is an association between two or more species where both species derive benefit. Sometimes, it is an obligatory lifelong interaction involving close physical and biochemical contact, such as those between plants and mycorrhizal fungi. However, they are generally facultative and opportunistic. Protooperation is a form of mutualism, but

the organisms involved do not depend exclusively on each other for survival. Many of the microbes isolated and classified as BCAs can be considered facultative mutualists involved in proto-cooperation, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions. Down the spectrum, commensalism is a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited. Most plant-associated microbes are assumed to be commensals with regards to the host plant, because their presence, individually or in total, rarely results in overtly positive or negative consequences to the plant. Neutralism describes the biological interactions when the population density of one species has absolutely no effect whatsoever on the other. Related to biological control, an inability to associate the population dynamics of pathogen with that of another organism would indicate neutralism. In contrast, antagonism between organisms results in a negative outcome for one or both. Competition within and between species results in decreased growth, activity and/or fecundity of the interacting organisms. Biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant. Direct interactions that benefit one population at the expense of another also affect our understanding of biological control. Parasitism is a symbiosis in which two phylogenetically unrelated organisms coexist over a prolonged period of time. In this type of association, one organism, usually the physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable extent. The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol. And, interestingly, host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens through the stimulation of host defense systems. Lastly, predation refers to the hunting and killing of one organism by another for consumption and sustenance.

3. Mechanism of biocontrol agents

Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter.

4. Antibiotic mediated suppression

Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. Antibiotics produced by bacteria include volatile antibiotics (hydrogen cyanide, aldehydes, alcohols, ketones, and sulfides) and nonvolatile antibiotics: polyketides (diacetylphloroglucinol; DAPG and mupirocin), heterocyclic nitrogenous compounds (phenazine derivatives: pyocyanin, phenazine-1-carboxylic acid; PCA, PCN, and hydroxyphenazines) (de Souza et al. 2003), and phenylpyrrole antibiotic (pyrrolnitrin) (Ahmad et al. 2008). *Bacillus* strains produce a variety of lipopeptide antibiotics (iturins, bacillomycin, surfactin, and Zwittermicin A). Methods have been developed to ascertain when and where biocontrol agents may produce antibiotics (Notz et al. 2001) but detecting expression in the infection court is difficult because of the heterogeneous distribution of plant-associated microbes and the potential sites of infection. In a few cases, the relative importance of antibiotic production by biocontrol bacteria has been demonstrated, where one or more genes responsible for biosynthesis of the antibiotics have been manipulated. For example, mutant strains incapable of producing phenazines (Thomashow and Weller 1988) or phloroglucinols (Keel et al. 1992) have been shown to be equally capable of colonizing the rhizosphere but much less capable of suppressing soilborne root diseases than the corresponding wild-type and complemented mutant strains. The role of antibiotics in biocontrol has been studied by genetic analysis, e.g., mutants that do not produce antibiotics to demonstrate a correlation between antibiotic productivity and biocontrol activity. For example, a phenazine antibiotic (Phz) produced by *Pseudomonas fluorescens* strain 2-79 has been implicated in control of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Handelsman and Parke, 1989). Among other bacteria, antibiotic agrocin 84 produced by *Agrobacterium radiobacter* strain K84 is one of the best described examples of biocontrol to control crown gall caused by virulent *A. tumefaciens* strains (Kerr, 1989). Several studies have implicated agrocin K84 in the disease control process produced by *Trichoderma viridescens* in the suppression of *Pythium* damping-off of cotton seedlings has also been confirmed recently by mutational analysis (Di Pietro et al., 1993)

5. Competition

This process is considered to be an indirect interaction whereby pathogens are excluded by

depletion of a food base or by physical occupation of site (Lorito et al., 1994). Biocontrol by nutrient competition can occur when the biocontrol agent decreases the availability of a particular substance thereby limiting the growth of the pathogen. Particularly, the biocontrol agents have a more efficient uptake or utilizing system for the substance than do the pathogens (Handelsman and Parke, 1989). For example, iron competition in alkaline soils may be a limiting factor for microbial growth in such soils (Leong and Expert 1989). Some bacteria, especially fluorescent pseudomonads produce siderophores that have very high affinities for iron and can sequester this limited resource from other microflora thereby preventing their growth (Loper and Buyer 1991). Some studies have found siderophores to play little or no role in disease control, particularly with *Pythium* species (Hamdan, et al., 1993). More recently, Leeman et al. 1996 have reported that iron-chelating salicylic acid produced by selected *P. fluorescens* strains at low iron availability may be involved in the induction of systemic resistance to *Fusarium* wilt of radish. Competition for specific substances or stimulants for germination of microorganisms may also occur in soil since most resting structures of microbes cannot germinate without specific stimulants due to soil fungistasis (Ko, and Lockwood 1970). Infection of plants by pathogens occurs only after dormancy is broken in the presence of stimulants from plant hosts. Consequently, microbes including pathogens may compete for specific stimulants of germination that may come from germinating seeds or growing roots. These factors may include fatty acids, or their peroxidation products (Harman and Nelson 1994), or volatile components such as ethanol and acetaldehyde (Gorecki, Harman and Mattick 1985.). Harman et al. 1993 have also proposed that the volatile peroxidation products of unsaturated fatty acids may be the active stimulants for fungal germination

6. Parasitism

This process involves the direct utilization of one organism as food by another (Handelsman and Parke 1989). Fungi that are parasitic on other fungi are usually referred to as mycoparasites (Baker and Cook 1974.). Many mycoparasites occur on a wide range of fungi and some of them have been proposed to play an important role in disease control (Adams, 1990). For example, *Darlucarium* (now *Sphaerellopsidium*) was described by Saccardo as a parasite of some rust fungi, especially *Puccinia* and *Uromyces* (Sundheim and Tronsmo 1988). *Trichoderma lignorum* (*T. viride*) parasitizing hyphae of *Rhizoctonia solani* and suggestion of

inoculating soil with Trichodermaspores to control damping-off of citrus seedling was reported by Weindling and Fawcett in 1936. This and other Trichoderma species were observed to parasitize *Rhizoctonia bataticola* and *Armillaria mellea* (Baker and Cook, 1974). Generally, mycoparasitism can be described as a four-step process (Chet, 1987): The first stage is chemotropic growth. The biocontrol fungi grow tropistically toward the target fungi that produce chemical stimuli. For example, a volatile or water-soluble substance produced by the host fungus serves as a chemo attractant for parasites. The next step is recognition. Lectins of hosts (pathogens) and carbohydrate receptors on the surface of the biocontrol fungus may be involved in this specific interaction (Inbar and Chet 1994). The third step is attachment and cell wall degradation. Mycoparasites can usually either coil around host hyphae or grow alongside it and produce cell wall degrading enzymes to attack the target fungus (Chet, 1987). These enzymes such as chitinases and β -1,3-glucanase may be involved in degradation of host cell walls and may be components of complex mixtures of synergistic proteins that act together against pathogenic fungi (Di Pietro, et al, 1992). The final step is penetration. The biocontrol agent produces appressoria-like structures to penetrate the target fungus cell wall (Chet, 1987). In hyperparasitism, the pathogen is directly attacked by a specific BCA that kills it or its propagules.

7. Induction of systemic resistance

The inducible resistance in plants to a variety of pathogens is known as systemic acquired resistance (SAR). SAR may be induced by inoculating plants either with a necrogenic pathogen or nonpathogen or with certain natural or synthetic chemical compounds (Lam and Gaffney 1993). These defense responses may include the physical thickening of cell walls by lignification, deposition of callose, accumulation of antimicrobial low-molecular-weight substances (e.g., phytoalexins), and synthesis of various proteins (e.g., chitinases, glucanases, peroxidases, and other pathogenesis-related (PR) proteins) (Hammerschmidt, et al, 1984). This defense system is also triggered when plants are colonized by plant growth-promoting rhizobacteria (Sticher, et al., 1997) and a few binucleate *Rhizoctonia* (BNR) AG-K (Poromarto, et al., 1988). Recently, many strains of PGPR have been shown to be effective in controlling plant diseases by inducing plant systemic resistance (Liu, et al., 1995). Plants colonized by these strains are more resistant to foliar diseases, even though the PGPR is present only on roots (Wei, 1996). The chemical

compounds that induce resistance of plants to pathogens may include polyacrylic acid, ethylene, salicylic acid and acetyl salicylic acid, various amino acid derivatives, the herbicide phosphinotricin, and harpin produced by *Erwinia amylovora* (Sequeira, 1983). It is known that stress can induce defense mechanisms against pathogens (Maurhofer, et al., 1994). However, the hypothesis should be proved by genetic analysis such as heterologous expression, which shows that inducing ability may be transferred to other potent strains as an additional complementary mode of action, and gene mutation, which knocks out the ability and leads to less disease control.

8. Endophytic Streptomyces and their potential for biocontrol

Endophytic micro-organisms have received considerable attention for their potential as biocontrol agents of fungal plant pathogens. Varied enzyme production may result in new biochemical characteristics and in part be responsible for the inherent biodiversity of endophytic micro-organisms. Among the lytic enzymes evaluated as source of biocontrol agents, chitinases have been studied largely because these enzymes are produced by a variety of endophytic micro-organisms (El-Tarabily and Sivasithamparam 2006). Many species of bacteria, fungi and plants produce chitinolytic enzymes, which can vary both within and among microbial species, depending on the number, types and positions of discrete binding and catalytic domains (Kobayashi et al. 2002). Chitinases are divided into three categories according to their enzymatic function: exochitinase, endochitinase and chitobiase, which constitute a complex of different degradation enzymes (Vyas and Deshpande 1989). One possible explanation for fungal inhibition is the action of chitinases in fungal cell walls, acting as plant protective agents (Inbar and Chet 1991). However, chitinases are not fully effective in all circumstances owing to different environmental conditions (Gohelet et al. 2006).

References :

- [1] Ahmad F, Ahmad I, Khan M.S 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* 163:173–181
- [2] Adams, P. B. 1990. The potential of mycoparasites for biological control of plant diseases. *Annu. Rev. Phytopathol.* 28:59-72.

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- [3] Baker, K. F., and Cook, R. J. 1974. *Biological Control of Plant Pathogens*. Am. Phytopathol. Soc., St. Paul. MN. 433 pp.
- [4] Chet, I. 1987. Trichoderma application, mode of action, and potential as biocontrol agent of soil-borne pathogenic fungi. Pages 137-160. in: *Innovative Approaches to Plant Disease Control*. I. Chet, ed., John Wiley, New York .
- [5] De Souza J.T.A, Arnould C, Deulvot C, Lemanceau P, Gianinazzi-Pearson V, Raaijmakers J.M 2003. Effect of 2,4-diacetylphloroglucinol on Pythium: cellular responses and variation in sensitivity among propagules and species. *Phytopathol.* 93:966–975
- [6] Di Pietro, A. 1993. Chitinolytic enzymes produced by *Trichoderma harzianum* : antifungal activity of purified endochitinase and chitobiosidase. *Phytopathol.* 83:302-307.
- [7] El-Tarabily, K.A. and Sivasithamparan, K. (2006) Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Soil Biol. Biochem.* 38: 1505–1520.
- [8] Fravel, D. R. 1988. Role of antibiosis in the biocontrol of plant diseases. *Annu. Rev. Phytopathol.* 26:75-91.
- [9] Gohel, V., Singh, A., Vimal, M., Ashwini, P. and Chhatpar, H.S. (2006) Bioprospecting and antifungal potential of chitinolytic microorganisms. *Afr. J. Biotechnol.* 5: 54–72.
- [10] Gorecki, R. J., Harman, G. E., and Mattick, L. R. 1985. The volatile exudates from germinating pea seeds of different viability and vigor. *Can. J. Botany.* 63:1035-1039 .
- [11] Hamdan, H., Weller, D. M., Thomashow, L. S. 1991. Relative importance of fluorescent siderophores and other factors in biological control of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79 and M4-80R. *Appl. Environ. Microbiol.* 57:3270-3277.
- [12] Hammerschmidt, R., Lamport, D. T. A., and Muldoon, E. P. 1984. Cell wall hydroxyproline enhancement and lignin deposition as an early event in the resistance of cucumber to *Cladosporium cucumerinum*. *Physiol. Plant Pathol.* 24: 43-47 .

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- [13] Handelsman Jo., and Parke, J. L. 1989. Mechanisms in biocontrol of soilborne plant pathogens. Pages 27-61.in: Plant-Microbe Interactions, Molecular and Genetic Perspectives, Vol. 3. T. Kosuge, and E. W. Nester, eds., McGraw-Hill, New York.
- [14] Harman, G E., and Nelson, E. B. 1994. Mechanisms of protection of seed and seedlings by biological control treatments: Implications for practical disease control. Pages 283-292.in: Seed Treatment: Progress and Prospects. T. Martin, ed., BCPC, Farnham, UK.
- [15] Inbar, J., and Chet, I. 1994. A newly isolated lectin from the plant pathogenic fungus *Sclerotium rolfsii*: purification, characterization and role in mycoparasitism. *J. Microbiol.* 140:651-657.
- [16] Kerr, A. 1989. Commercial release of a genetically engineered bacterium for the control of crown gall. *Agric. Sci.* 2:41-48
- [17] Keel, C. Voisard, C., Berling, C. H., Kahir, G., and Defago, G. 1989. Iron sufficiency is a prerequisite for suppression of tobacco black root rot by *Pseudomonas fluorescens* strain CHA0 under gnotobiotic conditions. *Phytopathol.* 79:584-589.
- [18] Ko, W. H., and Lockwood, J. L. 1970. Mechanism of lysis of fungal mycelia in soil. *Phytopathol.* 60:148-154.
- [19] Kobayashi, D.Y., Reedy, R. M., Bick, J. and Oudemans, P.V. (2002) Characterization of a chitinase gene from *Stenotrophomonas maltophilia* strain 34S1 and its involvement in biological control. *Appl. Environ. Microbio.* 68: 1047–1054
- [20] Lopez, J. E., and Buyer, J. S. 1991. Siderophores in microbial interactions on plant surfaces. *Molec. Plant Microbe Interact.* 4:5-13.
- [21] Lam, S. T., and Gaffney, T. D. 1993. Biological activities of bacteria used in plant pathogen control. Pages 291-320.in: Biotechnology in Plant Disease Control. I. Chet, ed., John Wiley, New York.
- [22] Leong, S. A., and Expert, D. 1989. Siderophores in plant pathogen interactions. Pages 62-83.in: Plant-Microbe Interactions, Molecular and Genetic Perspectives, Vol. 3. T. Kosuge, and E. W. Nester, eds., McGraw-Hill, New York.

- [23] Liu, L., Kloepper, J. W., and Tuzun, S. 1995b. Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathol.* 85:843-847.
- [24] Lorito, M., Harman, G. E., Hayes, C. K., Broadway, R. Di Pietro, A., Lorito, M., Hayes, C. K., Broadway, R. M., and Harman, G. E. 1993. Endochitinase from *Gliocladium virens*: isolation, characterization and synergistic antifungal activity in combination with gliotoxin. *Phytopathol.* 83:308-313.
- [25] Maurhofer, M., Keel, C., Schnider, U., Voisard, C., Haas, D., and Defago, G. 1992. Influence of enhanced antibiotic production in *Pseudomonas fluorescens* CHA0 on its disease suppressive capacity. *Phytopathol.* 82:190-195.
- [26] Milgroom, M. G., and Cortesi, P. 2004. Biological control of chestnut blight with hypovirulence: a critical analysis. *Annu. Rev. Phytopathol.* 42:311-338.
- [27] Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D., and Defago, G. 2001. Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathol.* 91:873-881.
- [28] Odum, E. P. 1953. *Fundamentals of Ecology*. W. B. Saunders, Philadelphia / London.
- [29] Sticher, L., Mauch-Mani, and Metraux, J. P. 1997. Systemic Acquired resistance. *Annu. Rev. phytopathol.* 35 : 235 - 270 .
- [30] Sequeira, L. 1983. Mechanisms of induced resistance in plants. *Ann. Rev. Microbiol.* 37:51-79.
- [31] Sundheim, L., and Tronsmo, A. 1988. Hyperparasites in biological control. Pages 53-69. in: *Biocontrol of Plant Diseases*. K. G. Mukerji, and K. L. Garg, eds., CRC Press, Boca Raton, FL.
- [32] Thomashow, L. S., Weller, D. M., Bonsall, R. F., and Pierson, L. S. III. 1990. Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *pseudomonas* in the rhizosphere of wheat. *Appl. Environ. Microbiol.* 56:908-912.
- [33] US Congress Office of Technology Assessment. 1995. *Biologically-based technologies for pest control*. OTA-ENV-636. US Government Printing Office, Washington, DC.

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- [34] Vyas, P. and Deshpande, M. V. (1989) Chitinase production by *Myrothecium verrucaria* and its significance for fungal mycelia degradation. *J. Gen. Appl. Microbiol.* 35: 343–350.
- [35] Weindling, R., and Fawcett, H. S. 1936. Experiments in the control of *Rhizoctonia* damping off of citrus seedling. *Hilgardia* 10: 1-16.
- [36] Wilhite, S. E., Lumsden, R. D., and Straney, D. C. 1994. Mutational analysis of gliotoxin production by the biocontrol fungus *Gliocladium virens* in relation to suppression of *Pythium* damping-off. *Phytopathology* 84 : 816 - 821.

