Microorganisms as biocontrol agents against plant pathogens

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Biological control agent

- The terms “biological control” and its abbreviated synonym “biocontrol” have been used in different fields of biology, most notably entomology and plant pathology.
- In entomology, it has been used to describe the use of live predatory insects, entomopathogenic nematodes, or microbial pathogens to suppress populations of different pest insects.
- In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations.
- In both fields, the organism that suppresses the pest or pathogen is referred to as the **biological control agent (BCA)**.

Biological control offers several advantages over other approaches of plant disease management

(1) biological control agents (BCAs) usually target a specific group of pathogens and therefore have fewer negative impacts on the ecosystem as opposed to fungicides, even though some risks of ecological issues should be considered, particularly with the introduction of non-native species.

(2) many BCAs can sustain themselves and keep in place for a longer time without additional efforts to keep the system running.

(3) a documented tradeoff exists between host resistance and agronomic traits.

- BCAs prevent the consistent expression of the host immune system, allowing plants to allocate more energy and resource for agronomic traits important to farmers

Mode of action of biocontrol

1. **Direct mechanism:** Direct lysis or killing of pathogen by biocontrol agent

- Antibiosis
- Parasitism

2. Indirect mechanism: Exclusion of plant pathogen as a result of the presence, activity or products of biocontrol agent.

- Competition
- Induced systemic resistance

Hyperparasitism

- In **hyperparasitism**, the pathogen is directly attacked by a specific BCA that kills it or its propagules.
- In general, there are three major classes of hyperparasites:
  - obligate bacterial pathogens,
  - hypoviruses,
  - facultative parasites
- *Pasteuria penetrans* is an obligate bacterial pathogen of root-knot nematodes that has been used as a BCA.
- **Hypoviruses** are hyperparasites. A classical example is the virus that infects *Cryphonectria parasitica*, a fungus causing chestnut blight, which causes hypovirulence, a reduction in disease-producing capacity of the pathogen.
- There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g. *Coniothyrium minitans*) while others attack living hyphae (e.g. *Pythium oligandrum*). And, a single fungal pathogen can be attacked by multiple hyperparasites.

Antibiotic-mediated suppression

- Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms.
- Most microbes produce and secrete one or more compounds with antibiotic activity.
- In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause.
- To be effective, antibiotics must be produced in sufficient quantities near the pathogen to result in a biocontrol effect.
- Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens.
- The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens.
- Volatile antibiotic, hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations.
- The production of HCN by certain fluorescent pseudomonads is believed to be involved in the suppression of root pathogens.

Table 1. Some of antibiotics produced by BCAs

Antibiotic	Source	Target pathogen	Disease
2, 4-diacetyl-phloroglucinol	*Pseudomonas fluorescens* F113	*Pythium* spp.	Damping off
Agrocin 84	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*	Crown gall
Bacillomycin D	*Bacillus subtilis* AU195	*Aspergillus flavus*	Aflatoxin contamination
Bacillomycin, fengycin	*Bacillus amyloliquefaciens* FZB42	*Fusarium oxysporum*	Wilt
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomyces cochlioides*	Damping off
Gliotoxin	*Trichoderma virens*	*Rhizoctonia solani*	Root rots
Herbicolin	*Pantoea agglomerans* C9-1	*Erwinia amylovora*	Fire blight
Iturin A	*B. subtilis* QST713	*Botrytis cinerea* and *R. solani*	Damping off
Mycosubtilin	*B. subtilis* BBG100	*Pythium aphanidermatum*	Damping off
Phenazines	*P. fluorescens* 2-79 and 30-84	*Gaeumannomyces graminis* var. *tritici*	Take-all
Pyoluteorin, pyrrolnitrin	*P. fluorescens* Pf-5	*Pythium ultimum* and *R. solani*	Damping off
Pyrrolnitrin, pseudane	*Burkholderia cepacia*	*R. solani* and *Pyricularia oryzae*	Damping off and rice blast
Zwittermicin A	*Bacillus cereus* UW85	*Phytophthora medicaginis* and *P. aphanidermatum*	Damping off

Lytic enzymes and other byproducts of microbial life

- Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA.
- Expression and secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly. For example, control of *Sclerotium rolfsii* by *Serratia marcescens* appeared to be mediated by chitinase expression.
- While they may stress and/or lyse cell walls of living organisms, these enzymes generally act to decompose plant residues and nonliving organic matter.
- Furthermore, some products of lytic enzyme activity may contribute to indirect disease suppression.
- For example, oligosaccharides derived from fungal cell walls are known to be potent inducers of plant host defenses.

Competition

- From a microbial perspective, soils and living plant surfaces are frequently nutrient limited environments.
- To successfully colonize the phytosphere, a microbe must effectively compete for the available nutrients.
- On plant surfaces, host-supplied nutrients include exudates, leachates, or senesced tissue.
- Additionally, nutrients can be obtained from waste products of other organisms such as insects (e.g. aphid honeydew on leaf surface) and the soil.
- The most abundant nonpathogenic plant-associated microbes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow.
- In general, soilborne pathogens, such as species of Fusarium and Pythium, that infect through mycelial contact are more susceptible to competition from other soil- and plant-associated microbes than those pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs.
- Biocontrol based on competition for rare but essential micronutrients, such as iron, has also been examined. Iron is extremely limited in the rhizosphere, depending on soil pH. In highly oxidized and aerated soil, iron is present in ferric form, which is insoluble in water (pH 7.4) and the concentration may be as low as 10^{-18} M.
- This concentration is too low to support the growth of microorganisms, which generally need concentrations approaching 10-6 M. To survive in such an environment, organisms were found to secrete iron-binding ligands called siderophores having high affinity to sequester iron from the micro-environment.
- The increased efficiency in iron uptake of the biocontrol agent is thought to be a contributing factor to their ability to aggressively colonize plant roots and an aid to the displacement of the deleterious organisms from potential sites of infection.

Induction of host resistance

- Plants also respond to a variety of chemical stimuli produced by soil- and plant-associated microbes.
- Such stimuli can either induce or condition plant host defenses through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens.
- Induction of host defenses can be local and/or systemic in nature, depending on the type, source, and amount of stimuli.
- The first of these pathways, termed systemic acquired resistance (SAR), is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins.
- These PR proteins include a variety of enzymes some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death.
- A second phenotype, first referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria.
- A number of strains of root-colonizing microbes have been identified as potential elicitors of plant host defenses. Some biocontrol strains of *Pseudomonas* sp. and *Trichoderma* sp. are known to strongly induce plant host defenses.
- A number of chemical elicitors of SAR and ISR may be produced by the PGPR strains upon inoculation, including salicylic acid, siderophore, lipopolysaccharides, and 2,3-butanediol, and other volatile substances.

Microbial metabolites able to induce ISR

- Polyacrylic acid
- Ethylene
- Salicylic acid
- Acetyl salicylic acid
- Amino acid derivatives
- Harpin (*Erwinia amylovora*)

- Stress can induce defense mechanisms against pathogens

Induced systemic resistance- defense responses

Physical thickening of cell walls by;

- Lignification.
- Deposition of callose.
- Accumulation of antimicrobial low-molecular-weight substances (e.g., phytoalexins).
- Synthesis of various proteins (e.g., chitinases, glucanases, peroxidases, and other pathogenesis related (PR) proteins).