

## Current Status and Future Perspective on Enzyme Involving in Biocontrol of Plant Pathogen

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### ABSTRACT

Plant diseases must be controlled to maintain the quality and quantity of food produced by farmers worldwide. Various strategies exist to prevent, reduce, or manage plant diseases. Agronomic and horticultural methods depend on chemical fertilisers and insecticides. These agricultural inputs have contributed significantly to recent gains in crop output and quality. Microbial enzymes help bacteria multiply in a specific habitat by acting as biocatalysts for biochemical processes. It has long been recognised that rhizosphere microorganisms may boost plant development and suppress phytopathogens. Rhizosphere microorganisms may help plants fight phytopathogens in numerous ways. Excreting lytic enzymes is one of the acknowledged biocontrol methods for preventing phytopathogens from surviving in the rhizosphere. To combat phytopathogens, rhizosphere microbes create chitinases, cellulases, proteases, and glucanases. Biological management may soon replace fungicides, say UBC researchers. New molecular approaches are now available to study antagonist-pathogen interactions, rhizosphere antagonist ecology, and biocontrol agent efficacy. Because agro-ecosystems are dynamic structures with numerous factors affecting disease and crop productivity, alternative IPM strategies to manage crop diseases are useful in various environments. Diverse crop systems need IPM management options other than biological control to successfully prevent disease development and yield loss.

**Keywords-** Enzyme, Biocontrol, Plant Pathogen, bacteria, ecosystems, crop diseases, fungicides

### I. INTRODUCTION

The bio control enzymes assist plant growth and survival by using several processes involved in phytopathogen clearance. Environmental damage as a consequence of incorrect agrochemical use, as well as manipulation by a few pesticide opponents, have produced substantial alterations in public attitudes of pesticide use in agriculture. Chemical pesticides are still carefully regulated, and legislation is in place to remove the bulk of harmful chemicals from industry. Furthermore, the scale at which such treatments would have to be done may impede successful chemical application due to the spread of plant diseases in natural settings. As a consequence, a number of pest management professionals have focused their efforts on developing alternative pest and disease control inputs to traditional

insecticides. Alternatives such as biological controls may be applied. Biological controls may take many forms, but for future development and effective application, a deeper knowledge of the dynamic connections that exist between plants, people, and ecosystems is required. Biocontrol is the use of organism species to suppress pathogens and ameliorate illness through the control of plant diseases.

A full description of biological control in plant disease management is provided in this article. Microbial diversity and biological control will be examined as well as the existing and future status of biological control analysis and implementation, as well as the potential for new and effective biological controls for plants to be developed. Biological control has a wide range of definitions, but the basic idea is that it is a technique for reducing illness incidence or severity by influencing microorganisms directly or indirectly. Chemical control

of plant diseases leads in the deposition of hazardous chemical pollutants, which may cause serious ecological problems. Plant pests and microbiological contamination in a number of agricultural crops are now treated using industrial pesticides. Toxic residue from the usage of these chemical fungicides continues to pose a threat to both animals and humans. Because of their enhanced and acute toxicity, synthetic fungicides are prohibited in large quantities in the Western world. A number of pathogens have evolved resistance to chemical fungicides.

This makes treating grain and agricultural plant diseases challenging. Given the harmful effects of traditional fungicides on life-sustaining organism structures, alternative agents for treating pathogenic bacteria are urgently required. Plant disease biological management has long been regarded a viable alternative to chemical control. Biological regulation is the intentional use of live organisms rather than disease-tolerant host plants to control plant disease behaviour and populations. In agriculture, there is an urgent need to minimise or eliminate the usage of synthetic pesticides.

Biological controls may be applied in a variety of ways, but progress and effective implementation need a deeper knowledge of the dynamic connections that exist between plants, people, and the environment. Biocontrol using microbial enzymes has a lot of potential. Plants may use these enzymes to protect themselves against a range of phytopathogens. Preparing bio pesticide products with biocontrol enzyme-producing microbial strains or adding extracellular crude enzyme might boost their application and efficiency. Microbial enzyme-based biocontrol products, on the other hand, are still under investigation and are extremely dependent on application tactics, formulation techniques, and strain types used. The involvement of many putative microbial enzymes in phytopathogen biomicrobiocontrol is discussed in this review.

## II. MICROBIAL ENZYMES

In a number of sectors, microbial enzymes offer a lot of biocatalytic potential. Since the beginning of time, microbial enzymes have been employed to make wheat, wine, vinegar, pickles, and curd. Microbial enzymes have aroused the industry's attention due to their longevity, simplicity of processing, and strong biocatalytic activity. Advances in fermentation technology have assured that enterprises have a stable supply of microbial enzymes.

"Pharmaceuticals, baking, dairy, beverage, feed, biopolymer, paper and pulp, fibre, textile, cosmetics, detergents, organic synthesis, and waste treatment are among sectors that use microbial enzymes. Global enzyme demand was \$5.5 billion in 2018, and is expected to reach \$7 billion by 2023. Many of them help in the decomposition of organic materials, the biotransformation of complex organic compounds, and the control of phytopathogens in ecosystems".

### *Chitinase*

Chitinase is a hydrolytic enzyme that may destroy the chitin found in pathogens including insects, fungus, and insect larvae. Chitinase is generated naturally by fungus, bacteria, yeasts, plants, actinomycetes, arthropods, and humans. Endo chitinases and exochitinases are the two forms of chitinases that act differently. By randomly cleaving internal sites across the length of the molecule, endo chitinase creates dimer diacetyl-chitobiose and N-acetyl glucosamine multimers like chitotriose. Exochitinases are split into two categories: (1) -1,4-glucosaminidases, and (2) chitobiosidases, which cleave non-reducing ends of chitins in a stepwise manner, which convert oligomers generated by Endo chitinases into monomers of N-aldehyde monomers.

Chitinases are produced by bacteria primarily to break down chitin for use as an energy source, although certain bacterial chitinases have shown promise as biological control agents against a variety of phytopathogenic fungi-caused plant diseases. *Serratia marcescens*, *Aeromonas punctata* and *A. hydrophila*, *Bacillus pumilus*, *Bacillus thuringiensis*, *Bacillus licheniformis*, and other bacteria may generate chitinases. Furthermore, the fungus *Humicola grisea*, *Rhizomucor miehei*, and *A. flavus* have been discovered as possibilities for producing high chitinase titres.

Chitinolytic enzymes, such as chitinases, such as Chitinase hydrolysis, have been shown to be the most promising solutions for maintaining plant disease. Chitinases have a role in plant immunity as well as plant growth and development. The present plant pathogenesis scenario focuses on the generation of disease-resistant transgenic plants by integrating chitinases expressing genes from any species into any plant in order to promote disease resistance in plants. Another research found that *coralloccoccus sp.* produced the chitin hydrolase C till, which degraded chitin into N-acetylated chitohexose and reduced magnaporthe oryzae development in a dose-dependent manner.

### *Cellulases*

Cellulase is a cellular enzyme generated by fungi, bacteria, and/or protozoas that aids in the breakdown of cellulose or other similar polysaccharides into monosaccharides or simple sugars (e.g., Beta-glucose), shorter polysaccharides, or oligosaccharides by hydrolyzing 1,4-beta-D-glycoside bonds (e.g., hemicellulose, lichenin and cereal beta-D-glucanes). Based on the sort of reaction it conducts, cellulase is classified into three categories. Cellulases are classified as endocellulases, exocellulases (cellobiohydrolases), or beta-glucosidases, based on the kind of reaction they catalyse. *Fusarium oxysporum*, a pathogenic fungus, attacks and disintegrates cellulolytic enzymes separated by the host plant's main and secondary cellular barriers. Degraded goods may enter the sweat stream, obstructing capillaries and causing pain. Fusariosis is a disease that affects several economically important cultures

(cucurbits, sweet potatoes, and tomatoes). The degradation of soil detritus by exo-cellulases by saprophytes of this fungus is connected to lignin and complicated carbon hydrates. As a consequence, fungal cellulases are preferred over bacterial cellulases in biotechnology applications owing to their greater capacity to penetrate celluloses. The extracellular cellulolytic enzyme generated by this fungus was effectively used in biocontrol of the soilborne phytopathogen *Phytophthora parasitica*. Some yeast strains have recently been revealed to be capable of biocontrolling phytopathogens by producing cellulases. The *Wickerhamomyces* yeast, as well as the bacterium and fungus *Actinomycetes* cellulase, have showed biologic activity against the *B. cinerea* and *Penicillium digitatum* infections in vitro and in vivo. Plant pathogens have been identified in biocontrol. *Streptomyces rubrolavendulae* S4 has been shown to have antagonistic effect in the fungal pathogen *P. aphanidermatum*, producing unhealthy damping of plants.

#### Proteases

Proteases are ubiquitous enzymes that are required for life to exist. Protein peptide linkages are hydrolyzed, releasing the peptide or amino acid. As a result, proteolytic cleavage has a considerable impact on protein behaviour as an irreversible post-translation modification. Proteases have the ability to breakdown, halt, and eliminate proteins from cells. Proteases are divided into two categories based on the sort of reaction they perform: (1) exopeptidases, and (2) endopeptidases, which “cleave amino acids internally, which remove amino acids from the amino terminal, or carboxy-terminal protein ends”. Proteases are divided into two categories depending on the catalytic process they perform. Among fungi, a large-scale study of *T. harzianum* biocontrol properties has been conducted. Proteases and chitinases were the two primary *Trichoderma* enzymes involved in plant pathogen biocontrol. Several *Trichoderma* strains have been shown to produce extracellular proteases that are resistant to “*Fusarium* sp., *Colletotrichum* sp., *Gloeocercospora* sp., and *Botrytis* sp. Entomopathogenic fungal proteases are also being studied for insect control. Entomopathogenic fungus extracellular proteases are quickly degraded by protein insect cuticle, making them useful bioagents for preventing crop loss from insect assaults. In recent years, researchers have looked at recombinant proteases with improved antifungal efficacy against *Penicillium expansum*, *B. cinerea*, *Monilinia fructicola*, and *A. Alternata*”.

### III. IN BIOCONTROL, KEY MICROBIAL ENZYMES

Such enzymes may breakdown or lyse the cell walls of phytopathogens. This action is seen often in the rhizosphere, where PGPM repels or kills phytopathogens by secreting lytic enzymes, while also indirectly promoting plant growth and production. Microbial

hydrolases and other lytic enzymes have been demonstrated to have biocontrol functions against a number of phytopathogens after extensive research. Biocontrol enzymes are a group of “fungal and bacterial enzymes that may inhibit or alter cell wall production, perforate cell membranes, or disintegrate a host or plant pathogen's cell wall”. Microbial biocontrol enzymes are characterised in terms of their function and processes.

### IV. PLANT DISEASE BIOLOGICAL CONTROL METHOD

#### 1. *Suppressive soil*

For example, in receptive soils, *Fusarium oxysporum* grows rapidly and causes severe soil conditions, but in other soils, it develops slowly and causes moderate ailments. The mechanisms by which soils suppress pathogenic pathogens are complex, including biotic, abiotic, and pathogen-dependent factors. However, they seem to function in the majority of instances, owing to the presence of one or more pathogen-fighting microbes in certain soils. Antibiotics, lytic enzymes, nutritional competition, and overt pathogen parasitisation are all examples of antagonists that keep pathogens from reaching big enough populations to cause severe illness. Bacteria from the genera *Pseudomonas* and *Streptomyces*, as well as *Trichoderma*, *Penicillium*, and *Sporidesmium* have been demonstrated to suppress disease in suppressive soils. Suppressive soil may reduce disease levels by introducing pathogen antagonistic microorganisms, as opposed to favourable soil. Infesting root-red oomycete holes with papaya seedlings planted on suppressive soil in orchard soil *Phytophthora Palmivora*, for example, was utilised to treat papaya root rot caused by *Phytophthora*. After many years of severe illness, however, sustained cultivation in favourable soil with growing concentrations of pathogen-fighting microorganisms eventually leads to disease decrease. As damped *Rhizoctonia*, continuous production of wheat or cucumber promotes declines in the usage of wheat and cucumber, respectively. Similarly, the continual growth of the 'Crimson Sweet' watermelon variety leads *Fusarium* antagonistic species that cause *Fusarium* wilt watermelon to decline rather than flourish. Future disease production is inhibited on such soils. Total suppression is reduced when soil is pasteurised for 30 minutes at 60°C, revealing hostile microorganisms. The soil is suppressed when suitable crops are put into the soil as extra additions.

#### 2. *Biofumigation or Biodisinfection*

After new organic matter is absorbed, biological soil disinfection, which is better suited to cooler regions, relies on plastic ground tape. The processes that underpin this freshly developed approach are unclear. Pathogenic fungi are inactivated or killed as a consequence of organic soil fermentation, which produces toxic chemicals and anaerobic conditions. Biofumigation is the use of hazardous substances found in certain plant species

and is based on existing processes. The use of large amounts of organic matter in the development of anaerobic conditions, which are principally responsible for pathogen eradication, is known as "bio-disinfection." Many Brassicaceae plants include the chemical molecule family of glucosinolates, which may be degraded into dangerous chemicals such as thiocyanates by a set of related enzymes. These compounds, which are comparable to certain organic fumigants, act as biocides to battle a wide range of soilborne plant infections. Because brassicas are used as animal feed, plant growers have typically chosen cultivars with reduced glucosinolates to avoid difficulties. Chemicals found in the Alliaceae family of plants have direct or indirect effects on pests and diseases. Volatiles such as thiosulfins and zwiebelanes are produced and converted into disulfides during the decomposition of garlic, onion, and leek tissue. Aside from the toxic effects of these chemicals, huge amounts of organic molecules absorbed by soil were anaerobic, which are dangerous for many insecticides and microorganisms that need aerobic life conditions.

### 3. Biopesticides

Plant diseases have a big impact on agricultural yield and storage. Farmers largely rely on chemical pesticides to keep illness under control or prevent it from spreading. This class of pesticides, with their great efficacy and simplicity of application, has the potential to harm the ecosystem and contaminate our food supply with chemical residues. As a consequence, individuals and government officials are increasingly demanding that chemical pesticides be reduced. Biological control by naturally hostile microbes has shown to be a viable technique in this area. Microorganisms and biochemicals "including plant products such as essential oils and other synthetic substances such as chitin and Chitosan" are the two most common types of biopesticides. These biopesticides have numerous advantages in terms of sustainability, mode of operation, and toxicity.

### 4. Plant pathogen microbial control

Several microorganisms, including fungi, bacteria, and viruses, naturally manage plant pathogens.

Traditional and conservation biological management techniques use any of these. Pneumoparasites and plant pathogens attack fungi and oomycetes. Increased removal of conventional fungicides following government studies of their protection is driving the development of microbial biopesticides for plant pathogens; however, the worldwide prohibition of methyl bromide, previously used as a soil sterilant but gradually phased out due to its link to ozone depletion, is equally important. Biopesticides have just recently been marketed as plant pathogenic parasitic nematode control agents. Only a few beneficial control medical medicines have been openly promoted since the mid-1990s. In the year 2000, there were around 80 medications on the market or close to it. Microorganisms employed for plant disease biocontrol have a wide range of MOA. Antibiotics and plant pathogens share an environment and direct communication channels. Antibiotics and other secondary metabolites that harm the target illness are examples of interaction mechanisms. Parasitic competition for space, water, or food is another. There is also an indirect effect, in which a disease control agent induces the plant to build a resistance response. In addition to the plant itself, a low-virulent plant pathogen strain might operate as an "inducer" for this form of control, new species of microbe, or natural product. This is in sharp contrast to a new microbial insect management technique that focuses only on using virulent parasites to eradicate insect pests.

Many plants pathogen microbial antagonists work in a number of ways to stop the target pest from growing. Soilborne plant pathogenic fungi, for example, are controlled by a range of fungal control agents called Trichoderma. Trichoderma species may "parasitize soil-borne plant pathogenic fungi, produce antibiotics and fungal cell-wall-degrading enzymes, compete with soil-borne pathogens for carbon, nitrogen, and other resources, and boost plant growth by producing auxin-like chemicals. The fungus Trichoderma is a common soil fungus that flourishes in the rhizosphere". Trichoderma's many modes of action give various benefits in terms of disease control (Table 1), as it has effective control in a number of conditions.

**Table 1: Interspecies antagonistic interactions in plant pathogen biological control**

Type	Mechanism	Examples
Direct antagonism	Hyper parasitism/predation	Some lytic and nonlytic mycoviruses Quisqualis ampelomyces Enzymogenes of Lysobacter Penetrans Pasteuria Virens Trichoderma
Mixed-path antagonism	Antibiotics	Phenazines and cyclic lipopeptides 2,4-diacetylphloroglucinol
	Lytic enzymes	Chitinases, Proteases and Glucanases
	Unregulated waste products	Hydrogen cyanide, ammonia, and carbon dioxide
	Physical/chemical interference	Pore blockage in the soil Consumption indicates germination Molecular crosstalk is perplexing.



<b>Indirect antagonism</b>	Competition	Consumption of exudates/leachates Physical niche occupation scavenging siderophore
	Induction of the host resistance	Contact with the cell walls of fungi Pathogen-associated molecular patterns detection Induction by phytohormones

### 5. Plant Growth Promoting Rhizobacteria (PGPR)

Commercially accessible PGPR bioinoculants come in a number of formats. Bioprotectants, which inhibit plant disease; biofertilizers, which promote nutrient acquisition; and biostimulants, “Bioinoculants such as *Bacillus*, *Paenibacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, and *Agrobacterium*”, which create phytohormones are some of the names given to them. Inducing systemic resistance, generating siderophores, and utilising antibiotics are all ways to combat plant disease. With “biofertilizers, you may increase seed nitrogen intake from nitrogen-fixing bacteria (*Azospirillum*) and iron uptake from siderophile bacteria (*Pseudomicrobium*)”. Inoculants are now distributed via peat, granular, oil, and wettable powder formulations. The extent to which they can colonise the rhizosphere is a key factor in growth promotion. Several recent research have contributed in the development of novel biofertilizers based on natural antibacterial chemicals produced by a range of antagonists.

## V. ADOPTION, DEVELOPMENT, AND RESEARCH IN BIOCONTROL

In the 1970s, biological control was an academic field, but it has now evolved into a discipline that relies on both public and private funding. These developments in computer technology have made it easier for researchers to understand the role of biocontrol agents as well as the diseases they protect against and their host plants. Biological controls and the conditions under which they are most effective will be better understood if certain research questions are addressed. Despite the fact that most illnesses can be controlled by a variety of biocontrol methods, commercial implementation has been hampered by a variety of obstacles. Biological controls' affordability, simplicity of use, effectiveness, and efficiency are all relevant factors to take into account, but only in comparison to other disease prevention methods. There are several “illnesses that can be controlled by cultural practises (such as sanitation, soil planning, and water management) and host resistance. Only when standard agronomic practises fail can biological protection methods be used. The most effective and efficient biological control has been obtained when environmental conditions are most predictable and biocontrol agents can colonise infection courts”. Biological control agents that function as bioprotectants have been effective in controlling monocyclic, soilborne, and postharvest illnesses. Relevant applications targeting specific diseases have been created for high-value crops

(such as fireblight, downy mildew, and a number of worm infections).

## VI. CONCLUSION

Biological disease management is a promising strategy for controlling plant diseases. Meanwhile, it encourages actions that support the long-term goals of the agricultural system. Effective biocontrol requires a thorough grasp of cropping methods, disease epidemiology, biocontrol organism biology, ecology, and population dynamics, as well as “the interactions between these factors. Understanding the causes or behaviours of antagonist-pathogen interactions will be one of the most important tasks, as it will give a reasonable framework for the selection and development of more effective biocontrol drugs”. In recent years, novel molecular methods have expanded our knowledge of the biological control of plant diseases. Biological management might be an alternate technique for controlling plant diseases in the near future. For analysing antagonist-pathogen interactions, antagonist ecological properties in the rhizosphere, and optimising the efficiency of bacterial, fungal, and viral biocontrol agents, new molecular approaches have been developed. Other IPM management techniques other than biological control should be studied and used in order to effectively minimise disease development and agricultural yield loss.

## FUTURE ASPECTS

Biocontrol enzymes are essential items for preventing plants from using harmful phytopathogens. Despite the fact that biocontrol enzymes are not well known for their manufacture and commercial use in the same way as industrial enzymes are, their use in the future, especially in the generation of biocontrol products, may be extended. The manufacturing of biocontrol enzymes is hampered by a lack of efficient strains, expensive research expenditures, poor formulation design, and instability under diverse situations. Hydrolytic enzymes may be produced at a reduced cost by using agro-waste and animal material. According to a research published in the journal *Applied and Environmental Microbiology*, genetic engineering is more effective than physical and chemical techniques in boosting enzyme production efficiency. The *S. griseorubens* E44G strain's chitinolytic activity was increased 1.39-fold when the recombinant gene P2 was inserted. Fungal biopesticides may benefit from improved genetics of empathogenic fungal enzymes. However, in order to enhance biocontrol microorganism performance,

a few factors still need to be explored and improved. New fungal/bacterial plant pathogen strains and routes are highly varied, and their harmful nature changes depending on host plants, therefore the search for new and novel biocontrol microbes with distinct mechanisms is critical.

Finally, microbial enzymes with biocontrol properties considerably improve plant defence against phytopathogens. Furthermore, given certain regulatory restrictions such as certification and safety evaluation, the use of microbial enzymes in the development of next-generation biocontrol agents with host-specific and broad-spectrum activity might be expanded.

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