



## Bacterial Bioagents: Mode of Action and Application Methods for Crop Disease Management

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### Abstract

Chemicals used for the management of crop diseases have been shown to be toxic to non-target organisms and may be toxic to humans. Much of modern research in plant pathology aims at finding other environmentally friendly means including use of bioagents for control of plant diseases. A number of bioagents including bacteria of the genera *Pseudomonas*, *Bacillus*, and *Streptomyces* are used for disease suppression. The mechanisms by which bioagents affect the pathogen populations are: (1) direct parasitism or lysis and death of the pathogen, (2) competition with the pathogen for food, (3) direct toxic effects on the pathogen by antibiotic substances released by the antagonist, and (4) indirect toxic effects on the pathogen by volatile substances, such as ethylene, released by the metabolic activities of the antagonist. The bacterial bioagents may be applied through various means like seed treatment, soil amendment, foliar spray *etc.* for management of crop diseases.

**Keywords:** Antibiosis, Bacterial bioagents, *Pseudomonas*, *Streptomyces*

### Introduction

Plant diseases are the major constraint affecting the production and productivity of crops both in terms of quality and quantity. Pesticide application is effective, but their use is being discouraged because it is known to cause serious threat to environment, imbalance in the ecosystem and human health hazards. Eco-friendly approaches have attained importance in modern day agriculture to curtail the hazards of extensive use of toxic chemicals for disease control (Homer, 1988). In recent times, diverse approaches are being used to manage and/or mitigate a variety of pathogens for control of plant diseases. The use of microbial pesticides is one of the best strategies available to combat the diseases in an eco-friendly manner. Modern biological control agents are highly and long-term effects while being friendly to human health and the environment. Biological agents can also be used to treat seeds as effectively as pesticides. Biological control practices for direct protection of plants from pathogens involve the deployment of antagonistic microorganisms at the infection court before or after infection take place. The mechanisms employed by biocontrol organisms in weakening or destroying the plant pathogens they attack

are primarily their ability to parasitize the pathogens directly, production of antibiotics (toxins) against the pathogens, their ability to compete for space and nutrients and to survive in the presence of other microorganisms, production of enzymes that attack the cell components of the pathogens, induction of defense responses in the plants they surround, metabolism of plant produced stimulants of pathogen spore germination, and possibly others. The most commonly used bacterial bioagents are *Agrobacterium radiobacter* K-84, *Pseudomonas fluorescens* and *Bacillus subtilis*. Nowadays, several beneficial bacterial based biopesticides are widely used in agriculture at commercial level. Bacterial bioagents *i.e.*, *Pseudomonas fluorescens*, *Pseudomonas aureofaciens*, *Pseudomonas chlororaphis*, *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus cereus*, *B. pasteurii*, *B. pumilus*, *B. mycoides*, *B. sphaericus*, *B. amyloliquefaciens*, *Burkholderia cepacia*, *Streptomyces lydicus*, *Arthrobacter* sp. and *Agrobacterium radiobacter* suppresses many plant pathogens on diverse hosts and their commercial formulations are also available in the market. This article is written for the detailed information about bacterial bioagents including mode of action and application methods for management of crop diseases.

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## Mode of Action

Biological control agents are the organisms that interact with the components of disease triangle to manage the disease. Various unique and complex mechanisms of action (Junaid et al., 2013) employed by the bacterial biocontrol agents in management of crop diseases are explained under.

### 1. Competition

Both the bio-control agents and the pathogens compete with one another for the nutrients, oxygen, space and other requirements to get established in the environment. This process of competition is considered to be an indirect interaction between the pathogen and the bio-control agent whereby the pathogens are excluded by the depletion of nutrients base and by physical occupation of site. So far, as the competition for nutrients is concerned bio-control agents compete for the rare but essential micronutrients, such as iron and manganese especially in highly oxidized and aerated soils. Iron is required for growth and development of plants and microorganisms. In natural form, iron is present in ferric form, which is insoluble in water and is not utilized by both plants and micro-organisms (Junaid et al., 2013). For example, different strains of *Pseudomonas fluorescens* synthesize i.e., Ferribactin, Ferrichrome, Ferroamine B, Pseudobactin, Pyochelin, Pyoverdine (soluble fluorescent pigment) and *Bacillus subtilis* produce the catecholate siderophores-2 (bacillibactin), 3-dihydroxy benzoate and 2,3-dihydroxybenzoyl glycine. Siderophore is a microbial iron transport agent/ act as chelating agents and extra cellular, low molecular weight compounds. It has micronutrients bindable capacity, specially, it binds the iron molecules and make insoluble form to soluble and also facilitate iron uptake to plants and microorganisms (bioagents); during this process, it is less available/ unavailable to pathogens, so that the disease reduced or controlled.

### 2. Antibiosis

Production of low molecular weight compounds or an antibiotic like substances or other chemical metabolites by the microorganism that have a direct effect on the growth of plant pathogen. Bio-agents are known to produce three types of antibiotics viz., non-polar/ volatile, polar/ non-volatile and water soluble. Among all of these the volatile antibiotics are more effective as they can act at the sites away from the site of production (Pal and Gardener, 2006). Different strains of *Pseudomonas fluorescens* produce Phenazine-1-Carboxylic Acid (PCA), Pyrrolnitrin, Pyocinine, Pyoluteorin, Oomycin-A, 2,4-Diacetyl-pholoroglucinol (DAPG), Idionine, etc. *Bacillus subtilis* strains produce Bacillomycin D, Iturin A, surfactin, fengycin and Mycosubtilin; *Bacillus cereus* produces Zwittermycin A; *Agrobacterium radiobacter* produces Agrocine 84; *B. amyloliquefaciens* produces Bacillomycin, fengycin and *Burkholderia cepacia* produces Pyrrolnitrin and Pseudane antibiotic like substances.

### 3. Induced Resistance

Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Plants also possess arrange of active defence apparatuses that are respond to a variety

of chemical stimuli produced by soil and plant associated microbes (PGPR). Such stimuli induce through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. If defence mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced. Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance, wherein plant defences are reconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen or parasite. SAR is a form of induced resistance that is activated throughout a plant after being exposed to elicitors from virulent, avirulent or non-pathogenic microbes or artificial chemical stimuli such as chitosan or salicylic acid. ISR is a resistance mechanism in plants that is activated by infection. Its mode of action does not depend on direct killing or inhibition of the invading pathogen, but rather on increasing physical or chemical barrier of the host plant. Selected strains of plant growth-promoting rhizobacteria (PGPR) suppress diseases by antagonism between the bacteria and soil-borne pathogens as well as by inducing a systemic resistance in plant against both root and foliar pathogens. SAR is triggered by plant pathogens and are mediated by SA-dependent pathway (Singh, 2014) which are activated by certain molecules secreted by microorganism (pathogens) referred as elicitors that leads to expression of defence responses like physical thickening of cell walls by lignification, deposition of callose, accumulation of phytoalexins and synthesis of various proteins produced in plant in the event of a pathogen attack. Infections activate genes that produce PR-proteins. These PR proteins are antimicrobial and cause lysis of invading cells, reinforcement of cell membranes to resist infections or induce localized cell death. ISR is triggered by beneficial microbes living in the rhizosphere which is generally mediated by salicylic acid independent pathway where jasmonic acid and ethylene are the central players and typically functions without PR protein activation. Combination of ISR and SAR can increase protection against pathogens that are resisted through both pathways besides extended protection to a broader spectrum of pathogens than ISR/SAR alone. Some strains of *Pseudomonas fluorescens*, *P. putida* and several specific strains of species *Bacillus* are resulting significant reduction in disease intensity in a diversity of hosts through induce resistance (Chaudhary et al., 2007).

### 4. Lysis

It is one of the mechanisms used by biocontrol agents to control soil-borne pathogens involves the production of cell wall-degrading enzymes or other metabolites. Several microorganisms release lytic enzymes that can hydrolyse 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. Besides production of antibiotics and elicitation of systemic resistance in plant against a variety

of plant pathogenic diseases, biocontrol strains of PGPR viz., *Pseudomonas fluorescens* and *Bacillus* spp. are also capable of producing enzymes like chitinase,  $\beta$ -1,3-glucanase, chitinase, cellulase, and protease having a very strong lytic activity. It exerts a direct inhibitory effect on the hyphal growth of fungal pathogens. Cell wall-degrading enzymes of rhizobacteria affect the structural integrity of the walls of the target pathogen. Other microbial by-products i.e., HCN production by certain bacteria is believed to be involved in the suppression of root pathogens. *P. fluorescens* CHA0 produces antibiotics, siderophores and HCN which suppress the intensity of black rot of tobacco caused by *Thielaviopsis basicola* (Junaid et al., 2013).

### Methods of Application

**1. Soil Application:** 2-2.5 kg bacterial formulation (powder formulation) or 500-1000 ml (liquid formulation) is added in 25-50 kg farm yard manure (FYM). Mixed thoroughly, cover with jute bag/ sugarcane leaves/ paddy straw and kept for 2-3 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Maintain optimum moisture for better multiplication of bioagents. Apply well decomposed bacterial based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/ pit/ pot and at the time of transplanting/ sowing. This mixture is sufficient for one acre of land.

**2. Cutting/ Seedling's Root Dip Application:** Mix 10 g bacterial formulation (powder formulation) or 10 ml (liquid formulation) in one litre of water and dip the cuttings and roots of seedlings for about 30 minutes before transplanting. Root dipping is effective against soil borne diseases.

**3. Nursery Bed Treatment:** 500 g bacterial bioagents (powder formulation) mix in 10-15 kg well decomposed FYM/ compost/ vermicompost and broadcast in a one-acre area at evening time and at proper moisture conditions.

**4. Soil Drenching:** 1-2 kg bacterial formulation mix in 200 litre of water and drench the soil in one acre area or 10 g or 10 ml litre<sup>-1</sup> of water bacterial bioagents is sufficient for soil drenching. Maintain optimum soil moisture while applying.

**5. Horticultural Crops:** 50-100 g bacterial formulation mix in sufficient quantity of FYM/ compost/ vermicompost/ field soil and apply the mixture plant<sup>-1</sup> in effective root zone of fruit tree. Doses will change in depending upon age of the plant.

**6. Foliar Application:** 10 g litre<sup>-1</sup> of water bacterial formulation (powder formulation) or 10 ml litre<sup>-1</sup> of water (liquid formulation) sprays uniformly after 35-40 days of transplanting (particularly in cereals, pulses and oilseeds) on diseased plants at cooler hours. 2-3 spray are required depending upon the disease incidence at 10-12 days intervals.

**7. Seed Bio-priming:** It is a process of biological seed treatment. In this process, involves slurry treatment of seeds with bioagents in the presence of gum arabica, jaggery or FYM powder. Dissolve 100 g jaggery in one litre of water and prepare solution there after add bacterial formulations @ 10 g kg<sup>-1</sup> seed in this solution and properly mix it. Next day required quantity of seeds is mix properly

with culture medium. Use polythene bags for filling treated seed, heaped, covered with moist sack of jute and incubate at approximately 25-32 °C for 48 hours to maintain high humidity. Bioagent adhering to surface of seed grows and form a protective covering on seed coat during this period. This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence. Seed biopriming is beneficial for tomato, brinjal, chickpea and soybean crops.

**8. Seed Treatment:** Seed treatment is a process like vaccination applied in animal as well as human. In broad terms, it provides protection to seeds and plants and improves the establishment of healthy crops. Use for bigger seeds treatment @ 8-10 g bacterial formulation per kg seed while small seeds @ 6-8 g kg<sup>-1</sup> seed before sowing. Mix the required quantity of seeds with bacterial formulation and ensure uniform coating followed by drying of coated seeds for 20-30 minutes in the shade prior to sowing. Seed treatment is highly effective against seed and soil borne diseases.

**9. Seed material treatment:** Apply @ 10 g or 10 ml bacterial formulation with one litre of water for the treatment of seed material like sugarcane setts, banana suckers, turmeric, ginger rhizomes and potato tubers before sowing for about 30 minutes. Shade dries the seeds for 20-30 minutes before sowing is necessary.

### Conclusion

Application of bacterial bioagents for crop disease management is safer both for the environment and the humans. These biocontrol agents avoid problems of resistance and also induce systemic resistance in the crop species against the invading pathogens. Bacterial bioagents do not cause any toxicity to the plants; rather it increases crop yields by enhancing the root and plant growth through the encouragement of beneficial microflora in rhizosphere. It also helps in the mobilization of plant nutrients and makes it available to the plant. So, application of bacterial bioagents as prophylactic or curative measure can control the crop diseases effectively without damaging the environment.

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