Control of Rice Diseases

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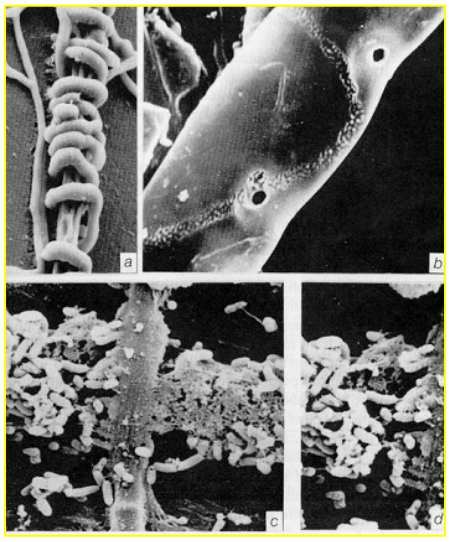
1. Basics

1.1. Historical Background



The science of biological control of plant pathogens is more recent than the biological control of insect pests and weeds. The historical background of biological control of plant pathogens has been through three phases of development.

The first phase is the prehistoric and traditional uses of crop manipulation, which may have led to control by biological means, but the control mechanisms were uncertain.

The second phase is actually the early phase of scientific study spanning over a period of six decades from the 1920s. A considerable amount of information was generated during this period. Major successes in the early part of this period included the use of antagonistic fungi to control damping off in pine seedlings in 1921 and potato scab in 1927, and the control of fungal root disease of cereals by resident micro-organisms in 1931. Substantial research was done in this period on the mechanism and mode of action of biological control agents. The first commercial product *Peniophora* (now *Phlebia*) *gigantea* to control *Fomes* (now *Heterobasidion*) *annosus* on tree stumps was marketed on a limited scale in the early 1960s. The first international conference on the use of biological control was held in 1965, followed by others. Several important books were published and more commercial products were marketed.

The third phase, starting from the later part of 1980s can be considered as period of increased research with reasonable funding.

1.2. Biological Control of Plant Pathogens

The term biological control was first coined by Harry Smith of the University of California in relation to the biological control of insects. He defined biological control as the suppression of insect populations by the actions of their native or introduced enemies. Since then, many definitions have been suggested. Most of these are related to the biological control of insects, weeds or diseases. In generic terms, biological control can be defined as a population-leveling process in which the population of one species lowers the number of another species by mechanisms such as predation, parasitism, pathogenicity or competition. In other words, biological control broadly refers to the use of one living organism to curtail the growth and proliferation of another, undesirable one.

The widely quoted and accepted definition of biological control of disease is:

> **"the reduction in the amount of inoculua or disease-producing activity of a pathogen accomplished by or through one or more organisms."**

A few examples of mechanisms of biological control of fungal plant pathogens are given in Figure 1.

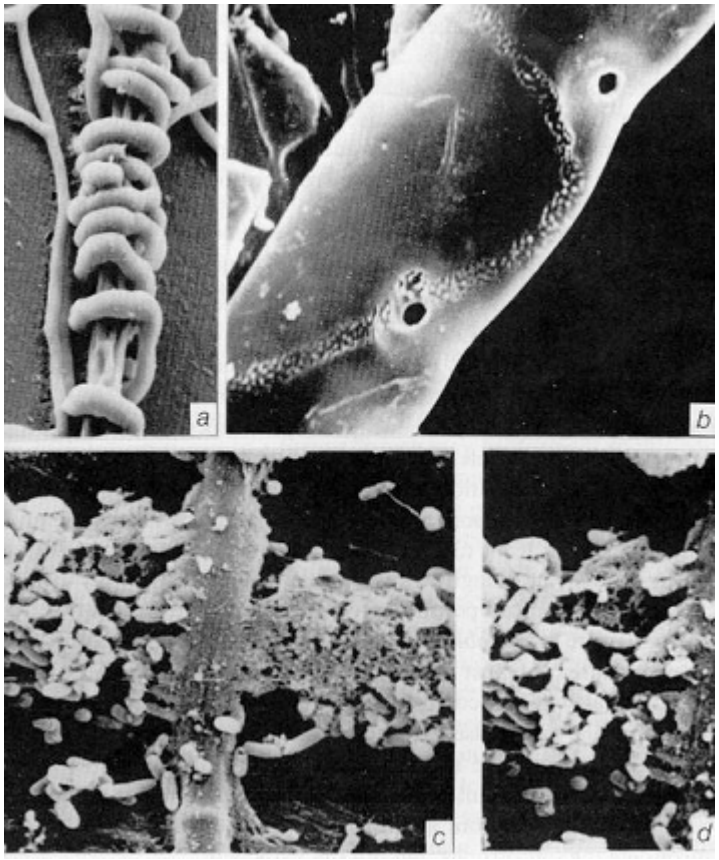


Figure 1. Hyphae of the fungus *Arthrobotrys* coiled around a hypha of a pathogenic fungus *Rhizoctonia* resulting in the death of the latter (1a); the hypha of *Sclerotium* parasitized (revealed by penetration hole) by a parasitic fungus, *Trichoderma* (1b); the hypha of a plant parasitic fungus colonized by bacteria (1c), and only a few fragments of the wall are left (1d)(Campbell 1989).

1.3. Some Common Terms

Before going into further discussion on the biological control of plant pathogens, and rice pathogens in particular, it is important to understand the terms that are commonly used in this field.

1.3.1. Antagonist

An antagonist is an organism that exerts a damaging effect on another organism by the production of lytic enzymes, antibiotics or by competition. Antagonism can be specific or general. Specific antagonism is the reduction of disease or pathogen inocula due to the activity of a particular species or strain of microorganism (Figure 2). General antagonism is the reduction of disease or pathogen inocula due to general microbial activity. In some cases antagonism can be achieved by adding organic matter in the soil.

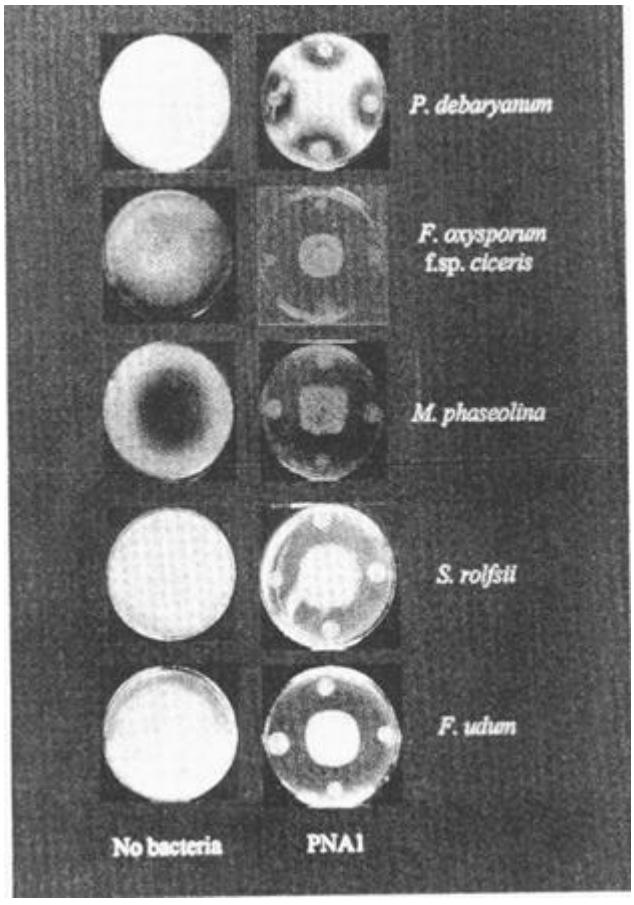


Figure 2. Antagonism of *Pseuedomonas fluorescens* (PNR1) against different soilborne plant pathogens (Desai *et al.* 2003).

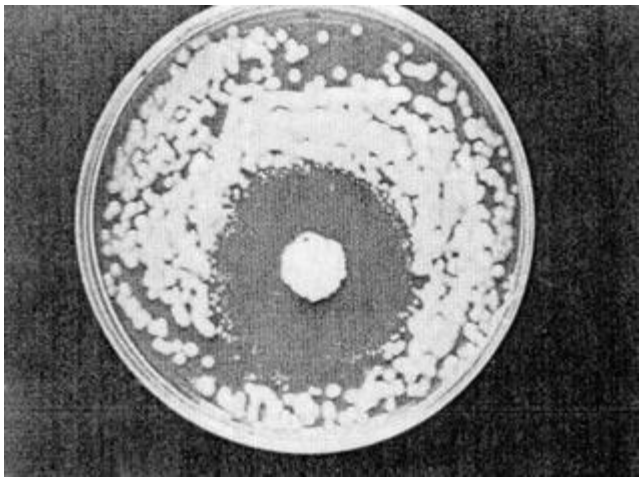


Figure 3. Inhibition of rice bacterial blight pathogen Xanthomonas oryzae pv. oryzae by bacterial antagonists in dual plate assays (Vasudevan 2002).

1.3.2. Antibiotics

Antibiotics are a substance (toxin or bacteriocin) produced by a microorganism that is damaging to another organism at low concentration.

1.3.3. Avirulence

Avirulence is the lack of ability of a pathogen to cause disease. A strain of a pathogen may be avirulent, even though the species or genus as a whole is pathogenic.

1.3.4. Bacteriocins

Bacteriocins are chemicals, usually proteins, which are produced by microorganisms, and act as antibiotics against closely related species or strains of microorganisms.

1.3.5. Epiphyte

A microorganism living on the surface of plants in a non-parasitic relationship.

1.3.6. *In vitro*

In vitro is a test or procedure that is carried out under laboratory conditions. As, for example, testing an antagonist in a petri dish against a culture of pathogen (Figures 2 and 3).

1.3.7. *In vivo*

In vivo is a test or procedure that is carried out with live host material. As, for example, testing an antagonist against a pathogen that is growing on or in its living host.

1.3.8. Lysis

Destruction, disintegration, dissolution, or decomposition of biological materials. Figure 4 shows lysis of hyphae of the root pathogen *Gaeumannomyces graminis* in the presence of bacteria which produced antibiosis in culture.

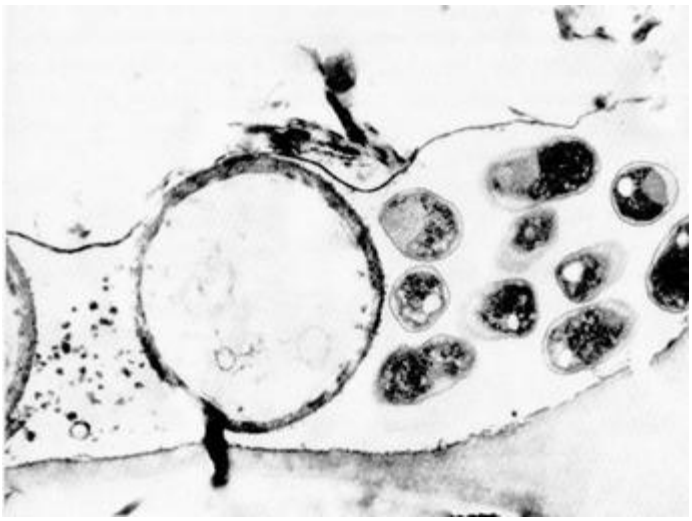


Figure 4. Lysis of hyphae of the root pathogen *Gaeumannomyces graminis* (Campbell 1989).

1.3.9. Natural enemy

In nature, some microorganisms affect or suppress growth of pathogenic microorganisms. These beneficial organisms are collectively called "natural enemies", "biological control agents/biocontrol agents" or "beneficials".

1.3.10. Necrosis

Necrosis is the death, usually of a limited part of a plant, due to a disease.

1.3.11. Pathogen

A pathogen is an organism capable of causing disease in a plant, animal or microorganism.

1.4. Types of Biological Control

Biological control is of three types:

1. Introduction or classical biological control
2. Augmentation biological control
3. Conservation biological control

1.4.1. Introduction or classical biological control

This generally refers to importation, introduction and establishment of a non-native natural enemy population for suppression of non-native or native organisms.

1.4.2. Augmentation biological control

This approach refers to natural enemy populations that are increased through mass culture for periodic release (either inoculative or inundative) and colonization for suppression of native or exotic pests. Inoculative releases are intended to colonize natural enemies early in a crop season so that they and their offspring will provide pest suppression for an extended period. While

inundative releases are intended to provide rapid pest suppression by the released individuals.

1.4.3. Conservation biological control

Conservation biological control refers to the modification of human influences to allow natural enemies to realize their potential to suppress pests. Conservation deals with resident enemy populations. It involves (i) identification and remediation of negative influences that suppress natural enemies, and (ii) enhancement of systems as habitats for natural enemies.

1.5. Approaches to Implement Biological Control of Plant Pathogens

There are several different concepts in the biological control of insect pests and plant pathogens. Biological control of plant pathogens and plant parasitic nematodes commonly involves the enhancement or augmentation of antagonists already present on the plant or in the soil but at population too low to provide adequate or timely control. The major concepts involved are:

- introduction of microbes in the phylloplane, rhizosphere or soil
- stimulating indigenous antagonists
- induced resistance
- biorational approaches.

1.5.1. Introduction of microbes in the phylloplane, rhizosphere or soil

Biological protection against infection is accomplished by destroying the existing inocula, by preventing the formation of additional inocula, or by weakening and displacing the existing virulent pathogen population. This is achieved through protection of plant material and roots with biological seed treatments, or suppression of pathogens by the introduction of plant associated antagonists into the rhizosphere.

1.5.2. Stimulating indigenous antagonists

Agents that are closely related to pathogens, such as dsRNA-infected hypovirulent strains. These epiphytes and endophytes may contribute to making soils naturally disease suppressive. Such microbial agents may be stimulated by the addition of organic amendments such as suppressive compost. To facilitate the enrichment, conservation and management of resident soil and plant associated microorganisms following strategies can be used:

- Selective elimination of soil borne plant pathogens and enhancement of antagonists by steaming, sub lethal fumigation, and soil solarization. Management that leads to natural suppression of soil borne pathogens.
- Management that leads to natural suppression of soil borne pathogens.

1.5.3. Induced resistance

Some nonpathogenic microbial agents induce a sustainable change in the plant, resulting in an increased tolerance to subsequent infection by a pathogen. This phenomenon is known as induced systemic resistance. This is

different from systemic acquired resistance, which refers to the host reaction in response to localized infection by pathogens, manifested as broad range of protection against other pathogens.

1.5.4. Biorational approaches

This is a recent approach that combines two major strategies viz. host resistance and biological control agents to reduce or suppress disease incidence. Host resistance and biocontrol agents should complement each other in their activity against pathogens.

2. Biological Control of Rice Diseases

2.1. Introduction

Research on biological control of rice pathogens started recently, mainly in the 1980s. Research is still concentrated on the identification, evaluation and formulation of potential biocontrol agents for deployment. A number of fungus, bacteria, virus, nematode and mycoplasma-like organisms cause disease to rice plants. Among these the fungal diseases viz. blast (*Pyricularia grisea*), brown spot (*Bipolaris oryzae*), stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), bacterial disease such as bacterial blight (*Xanthomonas oryzae pv. oryzae*) and viral disease such as tungro (rice tungro virus) are most important. These diseases are considered as a serious constraint for rice production.

Rice disease management strategies mainly aim at prevention of outbreak or epidemics through the use of host plant resistance and chemical pesticides. The persistent, injudicious use of chemicals has toxic effects on non-target organisms and can cause undesirable changes in the environment. Most of these chemicals are too expensive for the resource poor farmers of Asia, where 90% of the world's rice is grown. Large-scale and long-term use of resistant cultivars is likely to result in significant shifts in the virulence characteristics of pathogens, culminating in resistance breakdown. However, research during the previous two decades indicates another potential option for rice disease management. That is, biological control of rice diseases. Biocontrol assumes special significance being an eco-friendly and cost effective strategy which can be used in integration with other strategies for a greater level of protection with sustained rice yields.

2.2. Biological Control Agents for Rice Pathogens

A diverse group of biocontrol agents--such as bacteria, fungi, viruses--exist in nature. Among them bacterial antagonist are considered ideal candidates because of their rapid growth, ease of handling and aggressive colonizing character. Bacterial antagonists, *Pseudomonas*, and *Bacillus* in particular, are good candidates for biological control (Figure 1 and 2). *Bacilli* are germ-positive endospore-producing bacteria that are tolerant to heat and desiccation; a very good feature required for field application. The pseudomonads are germ-negative rods and have simple nutritional requirements; they are excellent colonizers and widely prevalent in rice rhizosphere.

The fluorescent and nonfluorescent strains of a number of antagonistic bacteria associated with upland and lowland rice rhizosphere soils have been found effective *in vitro*, greenhouse and the field against *R. solani* (sheath blight). As many as 23 bacterial antagonists belonging to the genera *Bacillus, Pseudomonas, Serratia*, and *Erwinia* have been found to inhibit mycelial growth of *R. solani*, while a few of them also inhibit growth of other fungal pathogens like *Sclerotium oryzae* (stem rot), *B. oryzae* (brown spot), *P. grisea* (blast), *Sarocladium oryzae* (sheath rot) and *Fusarium fuijkuroi* (bakanae). Laboratory studies also revealed that a large number of bacterial strains possess the ability to protect rice plants from diseases such as blast, sheath

blight, sheath rot, and stem rot. About 40 bacterial isolates antagonistic to the rice sheath blight pathogen have been identified.

Important fungal antagonists include *Trichoderma* spp., *Penicillium, Myrothecium verrucaria, Chaetomium globosum* and *Laerisaria arvalis*. The important biocontrol agents of major rice diseases are listed in table 1.

Table 1. Major rice diseases and their biocontrol agents (source Vasudevan et al 2002).

Disease	Causal organism	Biocontrol agent
Blast	*Pyricularia grisea* (Cooke) Sacc.	*Pseudomonas fluorescens*
Brown spot	*Bipolaris oryzae* (Breda de Haan) Shoemaker	*Pseudomonas sp.* *P. aeruginosa* *Bacillus* sp. *B. subtilis*
Bacterial blight	*Xanthomonas oryzae pv. oryzae* (Ishiyama) Swing et al.	*Bacillus* sp.
Sheath blight	*Rhizoctonia solani* Kuhn	*P. fluorescens* *P. putida* *Bacillus* sp. *B. subtilis* *B. laterosporus* *B. pumilus* *Serratia marcescens* *Pseudomonas sp.* *P. aeruginosa*
Sheath rot	*Sarocladium oryzae* (Sawada) W. Gams & D. Hawksworth	*P. fluorescens* *B. subtilis* *P. aeruginosa* *Pseudomonas sp.*
Stem rot	*Sclerotium oryzae* Cattaneo	*P. fluorescens* *P. aeruginosa* *B. subtilis* *B. pumilus*
Tungro	Rice tungro virus Vector - *Nephotettix* spp.	*P. fluorescens* (for vector)

2.3. Potential of Biocontrol Agents in Rice Disease Management

The success of potential biocontrol agents in disease suppression depends on a suitable form for application to the plant system. Biocontrol agents can be applied either by direct inoculation (dipping seeds in culture, aerial spraying or spreading it in sowing furrows by a drip system) or by the use of various solid-phase inoculants. The effects of some of the biocontrol agents on the pathogens and/or plant are summarized in the following:

- Bacterium *P. fluorescens* applied (prior to pathogen inoculation) against several rice pathogens to the seed and rice plants can reduce disease severity by 20-42% in a greenhouse and the field. Such bacterization of rice plants can enhance plant height, number of tillers and grain yield by 3 to 160%.
- Seed treatment by antagonistic bacteria can reduce bakanae disease (*Fusarium fujikuroi*) by 72-96%.
- Different species of *Bacillus*--applied to rice plants as a seed treatment before sowing, root dip prior to transplanting, and two foliar sprays prior to inoculation--can suppress bacterial blight pathogen by up to 59%, resulting in a two-fold increase in plant height and grain yield.
- Recent laboratory study demonstrated that *P. fluorescens* have insecticidal effect on the rice tungro virus disease vector *Nephotettix virescens*. Bacterial strains of *Pf*7-14 and *Pp*V14i can cause about 90% mortality if they feed on treated rice leaves for 7 days.

2.4. Mechanism of Pathogen Suppression

Understanding the mechanism of disease pathogen suppression is essential for the successful deployment of biological control as a disease management strategy. Rice disease suppression by biocontrol agents is governed by a multitude of factors. The influence of these factors varies with the type of biocontrol agent and the nature of the pathogen targeted for control. Currently research is underway to improve understanding of the mechanism of rice disease suppression by various agents. The available information is summarized below.

2.4.1. Mechanisms of sheath blight pathogen suppression

- Acidic soil of pH 5.0 and boron toxicity favor biocontrol of rice sheath blight by antagonistic bacteria.
- Bacterial treatment offers greater protection in direct seeded rice than transplanted rice.
- *Trichoderma* spp. elicits biocontrol mainly by being mycoparasites and by being aggressive competitor of the pathogen. Some species of *Trichoderma* produce antibiotics at low pH. *T. hamatum* and *T. harzianum* produce lytic enzymes (chitinases and glucanases) that attack the hyphae and sclerotia of the pathogen.
- Extent of pathogen suppression by antagonistic fungi is influenced by several parameters, the most important is pH. Neutral and alkaline soil affect biocontrol agents.

2.4.2. Mechanism of blast pathogen suppression

- Strains of *Pseudomonas fluorescens* (*Pf*7-14) produce antifungal antibiotics that inhibit germination of conidia of the blast pathogen. The exact chemical nature of the antibiotics is not known.
- Many fluorescent pseudomonads and other plant growth-promoting rhizobacteria cause induced systemic resistance (ISR) in rice in response to treatments with *P. fluorescens* strains *Pf*7-14, and *Pp*V14i, which is an important mechanism of biological suppression of blast. Treatment increases

the level of salicylic acid that increases ISR, which in turn suppresses rice blast by up to 25%.

2.5. Formulations of Biocontrol Agents

The success of a biocontrol agent depends largely on the ability of the introduced agent to establish itself in the new environment and maintain a threshold population on the planting material or rhizosphere. Biological control agents are living materials. Commercial production and application at farm level demands a few prerequisites, such as:

i. they have to be viable for longer period (longer shelf life), and
ii. they need to be tolerant to variable weather conditions and physiological stresses associated with transportation, storage, and application.

Cost-effective formulations that are easy to handle and have no adverse effects on seed germination or plant growth are essential.

2.5.1. Formulation of fungal antagonists

Fungal antagonists can be formulated by fluid-bed granulation using dextrin as a binder and a reduced content of alginate. Alginate gel has also been used to prepare formulations of biocontrol bacteria as well as fungi. Fungal antagonists can also be formulated as wettable powder, granular or powder.

2.5.2. Formulation of bacterial antagonists

Bacterial antagonists have been formulated in a variety of ways to control plant pathogens. The sporulating, gram-positive bacteria offer a biological solution to the problem of biocontrol agent formulation. Gram-positive microorganism offer heat- and desiccation-resistant spores that can be formulated into stable, dry-powder products. The non-spore-forming organisms are more difficult to formulate because they do not have the survival mechanisms of spores. The gram negative microorganisms have a short life and are readily killed by desiccation. These are traditionally formulated into various solid carriers such as wettable powder. Liquid formulations with either aqueous or mineral oil are user friendly. These media allow slow, continual growth of the organism or suspend growth to a starved level.

2.5.3. Formulation of rice pathogen biocontrol agents

A number of commercial, bacterial biofungicides and mycofungicides for disease control of different crops are available. Biocontrol agents for rice disease control are not yet commercially available. However, tests revealed that formulations of antagonistic bacterial strains *Pp*V14I and *Pf*7-14 with combination of methyl cellulose and talc at 1:4 and $CaCO_3$ are most satisfactory. In this formulation, bacteria can survive up to 10 months. Formulated *Pp*V14I applied as seed treatment, root dip or foliar sprays can suppress sheath blight up to 60%. While formulation of *Pf*7-14 applied as seed and multiple foliar sprays can suppress 60% and 72% of leaf and neck blast, respectively. The Plant Protection Department of the Jiangsu Academy of Agricultural Sciences isolated ,developed and formulated a strain of *B. subtilis* (*B*-916) for rice sheath blight. It is now being used in farmers' fields on a limited scale.

3. Evaluation of Biocontrol Agents

3.1. Steps for Screening of Biocontrol Agents

The following steps should be followed for the evaluation, development and deployment of biocontrol agents:

1. Survey and collection of plant and soil samples
2. Isolation of microorganisms in pure culture
3. Primary and secondary screening in the laboratory and greenhouse against the target pathogens
4. Field test or trials
5. Formulation of the product
6. Pilot tests of the product on a semi-commercial scale in larger farms
7. Market development

3.2. Protocol for Screening and Bioassay of Bacterial Agents

Step I. Collection of samples - Collect healthy and infected rice leaf, leaf sheath, roots, healthy and diseased seeds, rhizosphere soils and water from the field.

Step II. Isolation and purification of bacterial isolates - Perform serial dilution of collected plant, soil and water samples. Select candidate isolates and purify in plated PPM agar and streak in PPM agar slants.

Step III. Pathogenicity test - Inject suspensions of different isolates into 21-day-old rice seedlings. Identify and remove the isolates that are pathogenic to rice seedlings. Exclude isolates that exhibit yellowing, browning, spotting, lesion formation or death of 21-day-old test plants.

Step IV. *In vitro* test - Perform *in vitro* test against major fungal pathogens of rice or against target pathogens.

Step V. Selection of antagonistic isolates - Select bacterial isolates that exhibit antagonism towards one or more target fungal pathogens by *in vitro* antagonism test.

Step VI. Seedling test/Plant growth promotion test/ Seedling vigor test - Soak rice seeds in a suspension of the antagonistic bacteria for 24 hours. Plant the soaked seeds in sterilized soil and maintain in the greenhouse for 14 days. Measure shoot and root length at 7 and 14 day and compare with control seedlings (from water soaked seeds). Determine seed germination level. Differentiate antagonists into (i) promoter of plant growth, (ii) deleterious to seedling germination and growth, and (iii) no effect on seedling germination and growth.

Step VII. Greenhouse and field screening - Screen the bacterial isolates in the greenhouse and also under field conditions. Screening should aim to determine (i) effect on disease incidence, (ii) effect on focal expansion of the disease, and (iii) carry-over effect on the disease.

3.3. Attributes of Successful Biocontrol Agents

An ideal biocontrol agent should satisfy most, if not all, of the following attributes:

- Must not be pathogenic to plants and animals
- Level of pathogen control must be high
- Should live longer in soil or host tissues
- Should have rapid reproductive capacity
- Should be a good competitor
- Should have high survival rate in soil or host tissues
- Should be capable of controlling more than one pathogen
- Should be suitable for long-term storage
- Should be compatible to use with agro-chemicals viz. fertilizers, pesticides etc.
- Its application should result in higher rice yields

3.4. Potential Advantages of Biological Control

- Decrease disease intensity leading to higher production
- Reduce the use of chemical fungicides and nematicides
- Reduce likelihood of undesirable effects (environment pollution, effects on non-target organisms, resistance development against pesticides) from chemical pesticide
- Provide greater flexibility in rice disease management
- Can play a key role in integrated management of rice diseases
- Safe for the users and the farming community

3.5. Potential Disadvantages of Biological

- May have deleterious effects on non-target micro-organisms
- Pathogens may develop resistance to the biocontrol agent
- Pathogen replacement may follow control of target disease pathogen
- Seasonal/weather phenomena can make biocontrol agent ineffective

3.6. Critical Steps for Commercialization of Biofungicides

- Isolation of active strains against specific/non-specific pests
- Development of bioassays
- Laboratory evaluations
- Laboratory scale up
- Cost-effective formulation process
- Development of user-friendly formulations
- Field evaluations
- Product registration
- Chemical production
- Market acceptance of new technology

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