



Review Paper

Pseudomonads: Potential Biocontrol agents of Rice Diseases

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Abstract

Biocontrol mechanism to suppress fungal and bacterial pathogens of rice crop by *Pseudomonas* sp. generally involves the production of antibiotics, siderophores, volatile compounds, hydrocyanic acid (HCN), enzymes and phytohormones. Rice is the staple food of over half of the world population. Diseases are the most important factors that affect rice production causing annual yield losses. Biological control is an eco-friendly, cost effective and sustainable alternative method in disease management.

Keywords: Biocontrol, fungal and bacterial pathogen, *Pseudomonas*.

Introduction

Sustainable agriculture depends on the use of chemical fungicides, pesticides, herbicides and fertilizers. Repeated use of these chemicals is causing severe concern from the health and environmental point of view. In view of these the development of biologically based control method of diseases is now viewed not only as an eco-friendly but also sustainable agriculture. Research on biological control of rice diseases started mainly in the 1980. It is mainly concentrated on the identification, evaluation and formulation of potential biocontrol agents for deployment. Rice (*Oryza sativa* L.) is the most widely cultivated

food crop in the world. Global rice production was 468.1M.T (million tons) during the year 2010 and it rose to a record 480 M.T in 2011. According to Agriculture ministry, India harvested a record 103.41 M.T of rice in the 2011-2012 crop years (July-June) as against 95.98M.T in the previous year¹. More than 90% of rice produced in Asia, where China and India being the lead producer². Diseases are the significant limiting factors that affect rice production causing annual yield losses conservatively estimated 5%³. More than 70 diseases are caused by fungi, bacteria, viruses or nematodes on rice among which rice blast (*Magnaporthe grisea*) is the most serious one⁴.

Table-1
List of rice diseases: (Fungal and Bacterial)^{5,6}

FUNGAL	
Aggregate sheath spot <i>Ceratobasidium oryzae-sativae</i> Gunnell and Webster (anamorph: <i>Rhizoctonia oryzae-sativae</i> (Sawada) Mordue)	Narrow brown leaf spot <i>Cercospora janseana</i> (Racib) O.Const. = <i>C.oryzae</i> Miyake (telemorph: <i>Sphaerulina oryzina</i> K.Hara)
Black kernel <i>Curvularia lunata</i> (Wakk) Boedijn (telemorph: <i>Cochliobolus lunatus</i> R. Nelson & Haasis)	Peckey rice (Kernel spotting) Damage by many fungi including <i>Cochliobolus miyabeanus</i> (Ito and Kuribayashi) Drechs. ex Dastur, <i>Curvularia</i> sp., <i>Fusarium</i> sp. <i>Microdochium oryzae</i> (Hashioka and Yokogi) Samuels and I.C. Halett, <i>Sarocladium oryzae</i> (Sawada) W. Gams and D. Hawksworth
Blast (leaf, neck [rotten neck], nodal and collar <i>Pyricularia grisea</i> Sacc. = <i>P.oryzae</i> Cavara (telemorph: <i>Magnaporthe grisea</i> (Hebert) Barr)	Root rots <i>Fusarium</i> sp. <i>Pythium</i> sp. <i>P.dissotocum</i> Drechs. <i>P.spinosum</i> Sawada
Brown spot <i>Cochliobolus miyabeanus</i> (Ito and Kuribayashi) Drechs. ex Dastur (anamorph: <i>Bipolaris oryzae</i> (Breda de	Seedling blight <i>Cochliobolus miyabeanus</i> (Ito and Kuribayashi) Drechs.ex Dastur, <i>Curvularia</i> spp., <i>Fusarium</i> spp., <i>Rhizoctonia solani</i>

Haan)Shoemaker	Kuhn, <i>Sclerotium rolfsii</i> Sacc.(teleomorph: <i>Athelia rolfsii</i> (Curzi) Tu and Kimbrough)
Crown sheath rot <i>Gaeumannomyces graminis</i> (Sacc) Arx and D.Olivier	Sheath blight <i>Thanatephorus cucumeris</i> (A.B.Frank) Donk (anamorph: <i>Rhizoctonia solani</i> Kuhn)
Downy mildew <i>Sclerophthora macrospora</i> (Sacc) Thirumalachar <i>et al.</i>	Sheath spot <i>Rhizoctonia oryzae</i> Ryker and Gooch
Eye spot <i>Drechslera gigantea</i> (Heald and F.A.Wolf) Ito	Sheath rot <i>Sarocladium oryzae</i> (Sawada) W. Gams and D. Hawksworth= <i>Acrocyndrium oryzae</i> Sawada
False smut <i>Ustilaginoidea virens</i> (Cooke) Takah.	Stackburn (Alternaria leaf spot) <i>Alternaria padwickii</i> (Ganguli) M.B.Ellis
Kernel smut <i>Tilletia barclayana</i> (Bref.)Sacc. and Syd. in Sacc. = <i>Neovossia horrida</i> (Takah) Padwick and A. Khan	Stem rot <i>Magnaporthe salvinii</i> (Cattaneo) R. Krause and Webster (synamorphs: <i>Sclerotium oryzae</i> Cattaneo, <i>Nakataea sigmoidae</i> (Cavara) K.Hara)
Leaf smut <i>Entyloma oryzae</i> Syd. and P.Syd.	Water-mold(seed-rot and seedling disease) <i>Achlya conspicua</i> Coker <i>A.klebsiana</i> Pieters <i>Fusarium spp.</i> <i>Pythium spp.</i> <i>P.dissotocum</i> Drechs <i>P.spinosum</i> Sawada
Leaf scald <i>Microdochium oryzae</i> (Hashioka and Yokogi) Samuels and aI.C.Hallett= <i>Rhynchosporium oryzae</i> Hashioka & Yokogi	Black sheath spot <i>Curvularia fallax</i> B.D.Gao et al.
BACTERIAL	
Bacterial blight <i>Xanthomonas oryzae</i> pv. <i>Oryzae</i> (Ishiyama)Swings et al. = <i>X.campestris</i> pv. <i>oryzae</i> (Ishiyama) Dye	Grain rot <i>Pseudomonas glumae</i> Kurita and Tabei
Bacterial leaf streak <i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> (Ishiyama) Swings et al.	Sheath brown rot <i>Pseudomonas fuscovaginae</i> (ex Tanii et al.) Miyajima et al.
Foot rot <i>Erwinia chrysanthemi</i> Burkholder et al.	

Pseudomonas is a genus of gammaproteobacteria belonging to the family Pseudomonadaceae and is gram negative rod shaped having one or more flagella, motile, non-spore forming aerobic strains also have anaerobic respiration with nitrate as the terminal electron acceptor and 58-69% GC content⁷. It is a very large and important family of gram negative bacteria and is chemoheterotrophic with versatile function predominantly present in soil. They have the ability to colonize rhizosphere of a wide variety of crops including cereals, pulses, oilseeds and vegetables^{8,9} and can be used as plant growth promoters and biocontrol agents¹⁰⁻¹². The genus *Pseudomonas* includes both fluorescent and non-fluorescent species. The fluorescent species produce water soluble yellow green pigments and fluorescence under low wave length UV radiation¹³. Although some

Pseudomonas are well known plant pathogen (*Pseudomonas syringae*) but some members of this group are beneficial to plants. They are known to produce secondary metabolites like siderophores, antibiotics, HCN etc¹⁴ and enzymes like proteases and gluconases^{15,16} which made them the most promising group of plant growth promoting rhizobacteria involved in the biocontrol of plant diseases¹⁷⁻¹⁹. Bacterial antagonists have twin advantage of faster multiplication and higher rhizosphere competence hence *Pseudomonas fluorescens* has successfully used for biological control of several plant pathogens²⁰ and its application as biocontrol agents has drawn wide attention because of the production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones^{21,22}.

Table-2

Pseudomonas as a biocontrol agent for major rice diseases

Disease	Pathogen (Causal agent)	Biological control agent developed	Reference
Blast (Bl)	<i>Pyricularia oryzae</i> (Telimorph: <i>Magnaporthe grisea</i>)	<i>Pseudomonas fluorescens</i>	23, 24,25
Sheath blight(Sh B)	<i>Rhizoctonia solani</i> (Telimorph: <i>Thanetophorus cucumeris</i>)	<i>Pseudomonas fluorescens Pseudomonasputida</i>	26,27,28, 29
Sheath-rot(Sh-R)	<i>Sarocladium oryzae</i>	<i>Pseudomonas fluorescens</i>	30,31,32
Bacterial Blight (BB)	<i>Xanthomonas oryzae pv.oryzae</i>	<i>Pseudomonas fluorescens</i>	33,34,35

Mechanism of Pathogen Suppression

Antibiotic mediated suppression: *Pseudomonas fluorescens* has a gene cluster that produces antibiotics including compounds such as 2, 4-diacetylphloroglucinol (DAPG), phenazine, pyrrolnitrin, pyoluteorin and biosurfactant antibiotics³⁶. *Pseudomonas fluorescens* is uniquely capable of synthesizing many of these antibiotics not only to enhance its own fitness but also to help in the maintenance of soil health and bioprotection of crop from pathogens^{37,38}. Strains of plant associated *Pseudomonas fluorescens* produce DAPG and was detected by PCR-based screening method that used primers Phl2a and Phl2b and amplified 745-bp fragment characteristics of DAPG. HPLC, 1H NMR and IR analysis provided further evidence for its production and showed that compound (DAPG) suppress the growth of *Xanthomonas oryzae*pv. *Oryzaea* causal agent of the bacterial blight of rice in vitro assay suppress up to 59%-64% in net house and in vivo.e.in field experiments^{39,40}. *P.aeruginosa* (PUPa3) isolated from rhizospheric soil of rice produce Phenazine-1-Carboxamide (PCN) control rice disease caused by *Sarocladium oryzae* and *Rhizoctonia solani*⁴¹. Phenazine-1-Carboxylic acid (PCA) produced by *P.fluorescens* suppress the leaf and neck blast of rice⁴². *Sarocladium oryzae* (Sawada) the causative agent of rice sheath rot is highly sensitive to *P.fluorescens*due to the production of antibiotics⁴³⁻⁴⁸.

HCN mediated suppression: Hydrocyanic acid production plays a major role in suppression of the growth of phytopathogen. HCN inhibits the electron transport thereby the energy supply to the cell is disrupted leading to the death of the organism. It inhibits the proper functioning of enzymes and natural receptors by reversible mechanism of inhibition⁴⁹. It is also known to inhibit the action of cytochrome oxidase⁵⁰. HCN is a volatile compound which on interaction with fungi can easily degrade its cell wall^{51,52} presented evidence that HCN is

involved in biological control by *Pseudomonas fluorescens* strain CHAO. HCN production by *Pseudomonas* isolated from Potato and wheat rhizosphere⁵³. Plant associated *Pseudomonas fluorescens* produce HCN, a broad-spectrum antimicrobial compound involved in the biological control of root diseases where HCN synthase is encoded by three biosynthetic genes hcnA, hcnB and hcnC⁵⁴.

Siderophores mediated suppression: Siderophores are iron binding extracellular compound with low molecular weight and high affinity for ferric iron that are secreted by microorganisms to take up iron from the environment⁵⁵. The ability of sequester iron provides a competitive advantage to microorganisms. Siderophore chelate ferric ions with a high specific activity and serves as vehicles for the transport of ferric iron in to the microbial cell⁵⁶. Transport of iron in to the cell is mediated by a membrane receptor that specifically recognizes ferric-siderophore complex⁵⁷. Since iron is an essential element and a cofactor in various oxidoreductive enzymatic reaction binding of this element to siderophore create an artificial deficiency in the soil and their mode of action in suppression of disease were thought to be solely based on competition for iron with the pathogen^{58,59}. Fluorescent Pseudomonads are characterized by the production of yellow-green pigments termed pyoverdines which fluoresce under UV light and function as siderophores⁶⁰. The role of siderophores produced by fluorescent *Pseudomonas* in plant growth promotion was first reported by Kloepper J.W. and Schroth M.N.⁶¹ and was later reported to be implicated in the suppression of plant pathogens^{62,63}. Competition for iron between pathogens and siderophores of fluorescent *Pseudomonas* has been implicated in the biocontrol of wilt diseases caused by *Fusarium oxysporum*^{64,65}. Pyoverdines chelate iron in the rhizosphere and deprive pathogens of iron which is required for (Pseudobactin and ferrooxamine B) that chelate the scarcely available iron and thereby prevent pathogens from acquiring iron⁶⁶. Siderophore production by plant growth promoting fluorescent *Pseudomonas* sp.RB13 was effective against several fungal and bacterial pathogens⁶⁷. Siderophore mutant of fluorescent *Pseudomonas* have been used to elucidate the role of siderophores in biocontrol. Mutants were obtained by exposure to UV⁶⁸. NTG and Tn5 transposon mutagenesis⁶⁹⁻⁷¹ were compared with their wild types with respect to the suppression of diseases. The wild type strains were more effective in suppressing the diseases than Sid-mutants. Similarly the wild strain of *Pseudomonas putida* significantly suppressed chlamyospore of *Fusarium oxysporum*⁷² whereas the sid mutant fails to do so. Several strains of siderophore producing *P.fluorescens* have been shown to inhibit *Fusarium oxysporum*, *Rhizoctonia solani* and *Acrocladium oryzae*⁷³. Siderophores produced by *Pseudomonas* showed good antifungal activity against plant deleterious fungi, viz. *Aspergillus niger*, *A.flavus*, *A.oryzae*, *F.oxysporum* and *Sclerotium rolfisii*⁷⁴. The iron concentration in soil was lowered by the addition of an iron chelator⁷⁵. Fluorescent pseudomonas when grown on casamino acid medium under iron deficiency trihydroxamate type pyoviridines forming hexadentate ligands with Fe³⁺ ions were

found and these siderophores were antagonistic to fungal pathogens like *Fusarium oxysporum*, *Alternaria* and *Colletotrichum capsici*⁷⁶. Recently, the pseudobactin siderophore of *P.fluorescens* WCS374r was found to be an important determinant of ISR against blast disease of rice⁷⁷.

Enzymes and phytohormones mediated suppression: Chitinase, P-1,3 gluconase and cellulase are especially important fungus controlling enzymes due to their ability to degrade the fungal cell wall components such as chitin, P1,3 glucan and glucosidic bonds⁷⁸⁻⁸⁰. *Pseudomonas fluorescens* produce chitinase which involved in lysis and fragmentation of fungal cell wall and suppression of phytopathogenic fungi⁸¹. Chitinase excreting microorganisms have been reported as efficient biocontrol agents⁸²⁻⁸⁴. Fluorescent *Pseudomonas* produce plant growth promoting hormones and enzymes which suppress the growth of phytopathogenic fungi^{85,86}. *Pseudomonas fluorescens* (AUPF25) produce protease, IAA and siderophore showed inhibition of mycellial growth of *Pyricularia oryzae* a causal organisms of blast disease of rice⁸⁷.

Conclusion

Chemical pesticides harm the environment and host-plant resistant, which is based on a single gene, may not be durable in the field leading to frequent resistance breakdowns. It is imperative to develop environment-friendly and sustainable control strategies. Biological control is an ecology-conscious, cost effective and sustainable alternative method in disease management. As reported by many researchers that *Pseudomonas* produce different antibiotics which suppress the rice disease causing pathogens it is important to find out these antibiotics are active against other diseases of rice or not. There is need to investigate the function of HCN, Siderophores, enzymes and phytohormones produced by *Pseudomonads* in rhizospheric soil of rice field and their biocontrol against rice diseases as these are the most important compounds which inhibits the rice disease causing pathogens.

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