

## Chapter 9

# Commercial Potential of Microbial Inoculants for Sheath Blight Management and Yield Enhancement of Rice

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## 9.1 Introduction

Rice (*Oryza sativa* L.) is an important staple food crop for a larger part of the world's population and is produced around the globe. Global rice production was approximately 680 million tons in 2009. More than 90% of rice is produced in Asia, with China and India being the lead producers. The other major rice-producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, and Japan (Table 9.1). Rice production in the USA, which started 300 years ago, now has an annual production of 9.2 million tons. Major rice-producing states of the USA are Arkansas, California, Louisiana, Mississippi, Missouri, and Texas. The forecasted increase in global population in the coming years is demanding a need for increase in productivity of rice, although there is only a limited scope for expansion of crop-growing area especially in densely populated countries such as Asia (Meunchang et al. 2006). Use of chemical fertilizers for enhancing rice production is a common practice. However, indiscriminate use of chemical fertilizers to increase grain yields in rice has several concerns such as leaching of fertilizers into ground water, change of microbial balance in soil-root-ecosystem, increased susceptibility of the crop to pests and diseases, and acidification or alkalization of soils.

Rice production is affected by many biotic and abiotic stresses including fungal pathogens that attack the crop from seeding to harvest and cause severe yield losses. Seed-borne pathogens often reduce the germination and inflict qualitative and quantitative yield losses (Haque et al. 2007). Among important fungal diseases, blast (*Magnaporthe oryzae*, formerly *M. grisea* or *Pyricularia oryzae*), sheath blight (*Rhizoctonia solani* AG 1-1A), brown spot (*Bipolaris oryzae*), sheath rot (*Acrocyndrium oryzae*), stem rot (*Sclerotium oryzae*), and bakane (*Gibberella fujikuroi*) cause severe yield losses in rice. Major bacterial diseases include bacterial leaf blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*X. campestris* pv. *oryzicola*) (Bangura and John 1991). Important viral diseases include tungro, grassy stunt, ragged stunt, yellow dwarf, orange leaf, and hoja

**Table 9.1** Production details of major rice-producing countries in the world<sup>a</sup>

Rank	Country	Rice production (million tons)
1	China	187.40
2	India	144.57
3	Indonesia	57.15
4	Bangladesh	43.06
5	Viet Nam	35.94
6	Thailand	32.10
7	Myanmar	31.45
8	Philippines	16.24
9	Brazil	11.06
10	Japan	10.89

<sup>a</sup>FAOSTAT 2007

blanca. Other important ones include diseases caused by nematodes such as white tip (*Aphelenchoides besseyi*) and ufra (*Ditylenchus angustus*) (Datta 1981).

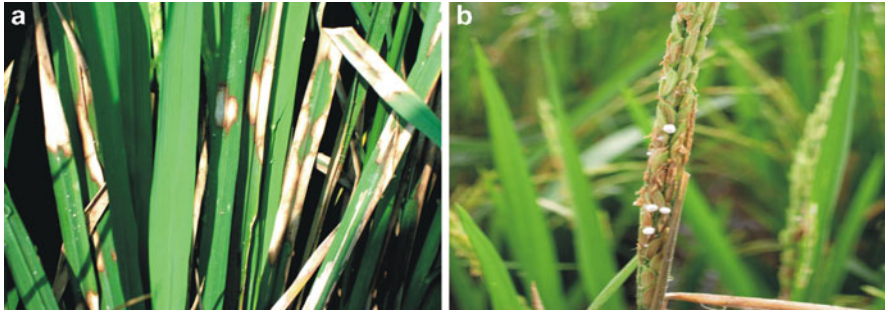
Sheath blight (ShB) is an economically significant disease of rice in all growing areas of the world. Yield losses of up to 50% are reported when susceptible varieties are grown (Prasad and Eizenga 2008). Soil bacteria in rice ecosystems typically exert a significant fungistatic effect on mycelia and sclerotia of the ShB pathogen (Luo et al. 2005). Effective management of ShB with PGPR application has been reported (Mew and Rosales 1986; Vasantha Devi et al. 1989; Kanjanamaneesathian et al. 1998); however, the field results were not consistent due to varying reasons. This review focuses on recent developments in the management of rice ShB with PGPR. The topics covered in the chapter include PGPR application in rice, greenhouse, and field efficacy of PGPR and the scope of applying them in conjunction with chemical fungicides under integrated disease management system (IDM) of ShB. The overall goal of this chapter is to introduce the multistep process that leads to the development of a new microbial inoculant product and its use and to outline the beneficial strategies specifically for ShB disease management of rice. In addition, it attempts to define the major efforts under way to help stimulate the process. Because product development is integrally related to several tasks including intellectual property issues and to regulatory and liability concerns, these topics are also included. Data on product development for rice ShB management are not systematically available. We have, therefore, used information based on our own research efforts and, when possible, made comparisons.

## 9.2 Symptomatology

Initial ShB symptoms appear on lower rice leaf sheaths when the crop is in late tillering or early internode elongation phase. These lesions appear as green–grey water soaked at 0.5–3 cm below the collar region as circular, oblong, or ellipsoid and about 1 cm long. As the disease progresses, the lesions expand with bleached appearance and a brown border. Under favorable conditions (95% relative humidity and temperature of 28–32°C), the disease spreads by runner hyphae to upper parts of plants including leaf blades (Fig. 9.1a). The pathogen also infects the panicle (Fig. 9.1b) and causes chaffiness of lower grains (Lee and Rush 1983).

## 9.3 Disease Cycle

The pathogen survives from one crop season to another as sclerotia and mycelia in plant debris and also through weed hosts in tropical environments (Kobayashi et al. 1997). In temperate regions, the primary source of inoculum is sclerotia produced in previous rice crops (Kozaka 1961). The sclerotia float in water during field preparation and attack newly planted crop. The pathogen produces lesions on



**Fig. 9.1** Sheath blight symptoms on rice leaf blades and panicle (a) leaf blades. (b) Formation of sclerotia on panicle

leaf sheaths and leaf blades. The disease is more aggressive when the crop advances to the reproductive phase, and the pathogen also infects the rice panicles. New sclerotia are produced as the lesions mature and these sclerotia drop into the soil during harvesting, perpetuate, and infect a newly planted crop in the next season (Suparyono et al. 2003).

#### 9.4 Use of Microbial Inoculants

Currently, ShB is managed through cultural and chemical control methods. Mostly, disease management is through use of systemic and non-systemic fungicides. Most widely used fungicides include azoxystrobin, hexaconazole, propiconazole, tebuconazole, carbendazim, trifloxystrobin, validamycin, and jinggangmycin. Use of chemicals in ShB management is creating concerns over environmental pollution, escalated costs, and pathogen resistance to chemicals. Biological control is a viable alternative in ShB disease management. However, the use of biocontrol agents in managing rice diseases is still at its infancy due to varying reasons. A successful bioagent, when applied to rice ecosystem, should be able to survive, establish, proliferate, and control target pathogens. Fungal and bacterial biocontrol agents have been used for control of rice diseases. The popularly used fungal bioagents against ShB include *Trichoderma* spp. and *Gliocladium* spp. These bioagents were applied either as seed treatment, root dip, or foliar spray (Nagaraju et al. 2002). The other effective fungal bioagent is *Helminthosporium gramineum* that produces a toxin called “ophiobolin.” The toxin is effective in reducing ShB incidence under field conditions (Duan et al. 2007). The prevailing anaerobic conditions in rice are unfavorable for the fungal bioagents to survive, establish, and proliferate in the soil.

Rice ecosystems are rich in bacteria (Yin and Mew unpublished data; Mew et al. 2004). They also have greater adaptability to rice ecosystems compared to fungal antagonists. Of them, plant growth-promoting rhizobacteria (PGPR) have been

used in controlling rice diseases. Besides, these PGPR also contribute to enhanced growth of the seedlings, induction of systemic resistance against diseases and thereby increases yields (Pathak et al. 2004). Bacterial strains of the genera such as *Aeromonas*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Klebsiella*, *Pseudomonas*, and *Serratia* were identified as PGPR (Tripathi et al. 2005; Raj et al. 2004; Dey et al. 2004; Jaizme-vega et al. 2004; Joo et al. 2004; Bonaterra et al. 2003; Cezon et al. 2003; Esitken et al. 2003; Garica et al. 2003; Munir et al. 2003; Kokalis-Burelle et al. 2002; Khalid et al. 2003; Murphy et al. 2003; Preeti et al. 2002; Gupta et al. 1995; Bertand et al. 2001; Hamaoui et al. 2001; NandaKumar et al. 2001b; Pan et al. 1999; Arndt et al. 1998; De Freitas et al. 1997; Shishido et al. 1996; Babalola et al. 2003; Mirza et al. 2001; Podile and Kishore 2006). In addition to enhancement in plant growth, PGPR were also contributed to increase N uptake, phytohormone synthesis, phosphate solubilization, and acquisition of ferric iron through production of siderophores (Lalande et al. 1989; Glick 1995; Bowen and Rovira 1999).

Use of PGPR in rice to control major diseases and to enhance yields was earlier reported (Lucas et al. 2009). A variety of beneficial bacteria were found to colonize the rhizosphere and aerial parts of rice. Nitrogen-fixing activity and indoleacetic acid (IAA) production was detected in roots and submerged shoots of field-grown rice due to these beneficial bacteria (Mehnaz et al. 2001). Rhizosphere bacterial isolates of rice have an excellent potential of producing biofertilizers. Inoculation of PGPR in rice increased total dry weight of plants, total N and P uptake through N fixation, P solubilization capacity, and IAA production (Meunchang et al. 2006). Use of biofertilizers in cereals was found to significantly increase plant growth and yields (Boddey et al. 1986; Fages 1994; Kapulnik et al. 1981; Kennedy and Tchan 1992; Pereira et al. 1988). Frequent rhizosphere colonizers of cereal crops and grasses include N-fixing bacteria such as *Azospirillum*, *Acetobacter*, *Azoarcus*, *Herbaspirillum* spp. (Baldani et al. 1986; Bally et al. 1983; Bilal et al. 1990; Dobreiner and Day 1976; Gillis et al. 1989; Reinhold-Hurek et al. 1993), *Aeromonas*, and *Enterobacter* spp. (Mehnaz et al. 2001).

### 9.4.1 Mode of Delivery

Field efficacy of a PGPR strain partly depends on the method of delivery. PGPR and their formulations are generally delivered as seed treatment, soil amendment, or root dip in bacterial suspensions prior to transplanting. Other important methods also include foliar spray or through drip irrigation in different crops (Podile and Kishore 2006). Success of the PGPR strain is dependent on understanding the use of specific delivery system and its advantages over other methods. In rice, PGPR is delivered through seed, as soil amendment, seedling dip, and foliar spray, and through combinations of these methods. Against rice ShB, the popular delivery systems are through seed, soil, and foliar applications (Nakkeeran et al. 2005).

#### 9.4.1.1 Seed Treatment

An ideal bacterial antagonist when treated to seed should colonize the rhizosphere during seed germination (Weller 1983), and several application methods can be used to accomplish this. Treating seeds with different PGPR was found to be highly effective in managing rice ShB disease. Seed coating of *P. fluorescens* (B41) was found to be comparatively more effective than soil drenching and foliar sprays against ShB under greenhouse conditions (Kazempour 2004). Seed bacterization of *Pseudomonas* strain GRP3 followed by root dipping resulted in ShB reduction in rice up to 46% (Pathak et al. 2004). Seed treatment with PGPR mixtures also resulted in effective ShB management. Soaking rice seeds in *P. fluorescens* mixture of strains PF1 and PF2 at  $10^8$  cfu  $g^{-1}$  for 24 h were effective in reducing ShB incidence under field conditions (Nandakumar et al. 2001a). Seed bacterization with fluorescent *Pseudomonads* such as *P. fluorescens* and *P. putida* V14i was highly effective in reducing ShB severities by 68 and 52%, respectively, in seed bed and field experiments (Malarvizhi 1987) due to protection of the plants from infection. Subsequent planting in the same field after planting the first crop, in which the seeds were treated with bacteria, also showed reduced ShB severity (Mew and Rosales 1986). Seed treatment with peat-based formulation of *P. fluorescens* (PfALR2) at the rate of 20 g  $kg^{-1}$  resulted in ShB disease control effectively under greenhouse and field conditions (Rabindran and Vidhyasekaran 1996). Induced systemic resistance, plant growth promotion, and sheath blight control was observed by treating rice seeds with three isolates of *Pseudomonas aeruginosa*. The biocontrol agents were also found effective in reducing blast and brown spot diseases in rice due to increased accumulation of salicylic acid and pathogenesis-related peroxidases (Saikia et al. 2006).

#### 9.4.1.2 Seedling Dip

The ShB pathogen is soil-borne, attacks the rice seedlings, and establishes host-pathogen relationship by root entry (Nakkeeran et al. 2005). Seedling root dip treatment of rice prior to transplanting for a period of 2 h in talc-based formulations of PGPR mixtures at 20 g/L reduced ShB incidence effectively (Nandakumar et al. 2001a). Earlier, seedling root dip in a talc-based formulation of *P. fluorescens* before transplantation into main field suppressed ShB disease and improved grain yields (Rabindran and Vidhyasekaran 1996). A novel application method of *B. megaterium* multiplied in empty fruit bunches (EFB) as carrier was reported. The rice seedlings when treated with bacterial inoculum multiplied in EFB carrier had significantly enhanced plant height, number of roots, and dry matter of root and shoot. The method offered a scope of developing new delivery system and granulation of bioinoculants for effective control of diseases as well as for enhancing grain yields (Al-Taweil et al. 2009).

### 9.4.1.3 Soil Application

Soil application of PGPR has also been reported to be an effective method of controlling soil-borne diseases of rice (Rabindran and Vidhyasekaran 1996). For effective suppression of ShB, the population thresholds of the antagonist in soil should be higher than  $1 \times 10^6$  cfu g<sup>-1</sup> during early stages of infection by *R. solani* (Li et al. 2003). Effective management of ShB is feasible only when the applied bioagents survive, establish, proliferate, and control pathogen populations in soils. A strain of *B. licheniformis* (CHM1) isolated from rice fields was found to be highly effective in protecting rice seedlings from ShB disease as well as in plant-growth promotion when applied as soil drenching around root zone (Wang et al. 2009). Soil application of peat formulation of *P. fluorescens* (PfALR2) effectively controlled ShB disease under greenhouse and field conditions (Rabindran and Vidhyasekaran 1996). Broadcasting of talc-based formulation mixtures of Pf1 and Pf7 at 30 days of transplanting of rice seedlings reduced ShB and increased grain yields significantly under field conditions (Nandakumar et al. 2001a). The population levels of PGPR in rice fields are an important factor for effective control of ShB disease. Mixing the potting soil with bacterial suspensions of different *P. aeruginosa* mutants coupled with a soil drench at a concentration of  $5 \times 10^7$  cfu g<sup>-1</sup> elicited ISR in rice seedlings to blast and ShB diseases under greenhouse conditions (Vleeschauwer and Hofte 2005).

### 9.4.1.4 Foliar Application

Survival rates and application efficiencies of PGPR as foliar sprays against plant diseases is generally affected by variations in microclimate. Nutrient concentrations of amino acids, organic acids, and sugars that exude through lenticels, stomata, and hydathodes vary in the phyllosphere (Nakkeeran et al. 2005). The efficacy of PGPR against ShB under greenhouse and field conditions is dependent on time of application. Spraying of *P. fluorescens* at 7 days before pathogen inoculation resulted in effective ShB reduction (59–64%) over simultaneous application at 7 days after inoculation. Further, grain yields and 1,000 grain weight were also enhanced with the prophylactic sprays (Rajbir Singh and Sinha 2005). Commercial formulations of *P. fluorescens* (Ecomonas and Florezen P) when sprayed three times at 10-day interval after disease initiation under field conditions resulted in ShB control by 14–38% besides significant increase in grain yields (Vijay Krishna Kumar et al. 2009). Foliar sprays with floating pellet formulation of *B. megaterium* were effective in rice ShB suppression under greenhouse conditions (Wiwattanapatapee et al. 2007). Spraying of antifungal metabolites of *Streptomyces* spp. (SPM5C-2) at the rate of 500 µg ml<sup>-1</sup> significantly decreased ShB and blast disease development by 82 and 76%, respectively, under greenhouse conditions (Prabavathy et al. 2006). Broadcasting of floating pellets formulation and spraying of water-soluble formulation of *B. megaterium* resulted in effective control of rice ShB disease under greenhouse and field conditions (Kanjnamaneesathian et al. 2007).

#### 9.4.1.5 Multiple Delivery Systems

Protection of spermosphere, rhizosphere, and phylloplane from infection courts of plant pathogens through multiple delivery systems of PGPR offers a comprehensive means of plant disease management (Nakkeeran et al. 2005). Talc-based formulations of two *P. fluorescens* strains (PF1 and PF7) when applied through seed, root, soil, and foliar sprays significantly reduced ShB and pest (leaf-folder) incidence in rice under greenhouse and field conditions. The bacterial mixture performed better than individual strains with a reduction of 62% of ShB and 47–56% of leaf folder incidence (Radja Commare et al. 2002). Combined applications of *P. fluorescens* strains (PF1, FP7, and PB2) as bacterial suspensions or as talc-based formulations through seed, root, foliar, and soil application significantly reduced the ShB incidence (45%) under greenhouse and field conditions over their individual applications. Further, a significant increase in yield was obtained with application of mixtures over their individual applications. Fluorescent *Pseudomonas* application (PF1 and FP7) either as a suspension or talc-based formulation through seed, root, soil, and foliar means effectively reduced rice ShB incidence and promoted plant growth and grain yields (Nandakumar et al. 2001a). Similar results on ShB disease suppression and enhanced yields were reported with peat-based formulations of *P. fluorescens* (PfALR2) as seed treatment, root treatment, soil application, and foliar spraying. Further, the efficacy of combined application methods was comparable with fungicide treatments (Rabindran and Vidhyasekaran 1996).

#### 9.4.2 Formulations

A formulated PGPR should ideally possess high rhizosphere competence, plant growth promotion, ease for large-scale multiplication, wide range of plant disease control, consistent in disease control, and compatible with environment and other rhizobacteria (Nakkeeran et al. 2005). Besides, the bacterial inoculants should be able to tolerate desiccation, heat, oxidizing agents, and UV radiations (Jeyarajan and Nakkeeran 2000). The formulated product should meet the important criteria such as satisfactory shelf life, non-phytotoxic nature, water solubility, ability to withstand environmental fluctuations and compatibility with other agrochemicals. Besides, it should be cost-effective with ready availability of carriers at a cheaper rate and should not impart mammalian toxicity (Nakkeeran et al. 2005; Jeyarajan and Nakkeeran 2000).

The carrier materials used in PGPR formulations are broadly categorized into organic and inorganic ones. The commonly used organic carriers are peat, turf, talc, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, press mud, sawdust, and vermiculite (Nakkeeran et al. 2005). In general, PGPR survive longer in carriers with smaller particle sizes than in those with larger particle sizes. Carriers with smaller size will have more surface area that enables increased resistance to desiccation of PGPR through increased coverage of bacterial cells (Dandurand et al. 1994).



Commonly available PGPR formulations are talc formulations, peat formulations, press mud formulations, vermiculite formulations (Nakkeeran et al. 2005), water-soluble granular formulations, liquid formulations, floating pellet formulations, and formulations with EFB as carriers. Details of different PGPR formulations that exhibited effective control of rice ShB disease under greenhouse or field conditions are given in Table 9.2.

### 9.4.3 Shelf life

Effective disease control by PGPR is possible only when the formulated product delivers a sufficient number of viable cells. So, determining the shelf life and viability of a commercial bio-product is a crucial step. The shelf life of PGPR in the formulated product is dependent on the type of carrier material used. Talc is an excellent carrier material for PGPR with low moisture equilibrium, relative hydrophobicity, reduced moisture absorption, and chemical inertness (Nakkeeran et al. 2005). The population levels of PGPR (fluorescent Pseudomonads) did not decline in talc powder with 20% xanthan gum after storage for 2 months at 4°C (Kloepper and Scroth 1981).

Vermicompost is comparatively a better carrier material than lignite for bioinoculants with high nitrogen, phosphorus, potassium, copper, manganese, and iron besides possessing an ideal pH. The shelf life of vermicompost-based formulations is greater than that of lignite-based ones. The population levels of *B. megaterium* and *P. fluorescens* were very high ( $7.6 \times 10^8$  and  $1 \times 10^8$  cfu g<sup>-1</sup> of dry weight, respectively) at the end of 360 days when vermicompost was used as carrier (Gandhi and Saravanakumar 2009).

The shelf life of peat-based formulations depends on the availability of good quality peat. Heat sterilization of peat results in release of toxic substances that are detrimental to bacteria thus affecting their population levels in the formulation (Bashan 1998). The population levels of *P. fluorescens* ( $2.8 \times 10^6$  cfu g<sup>-1</sup>) in peat-based formulation was maintained up to 8 months (Vidhyasekaran and Muthamilan 1995), whereas the shelf life of *P. chlororaphis* and *B. subtilis* were more than 6 months (Kavitha et al. 2003; and Nakkeeran et al. 2004). The use of press mud and vermiculite-based PGPR formulations are also in practice. The viability of *Azospirillum* spp. in press mud formulation is higher than in lignite (Muthukumarasamy et al. 1997) whereas in vermiculite, its viability is retained up to 10 months (Saleh et al. 2001).

### 9.4.4 Root Colonization

Of different soil microbial populations, bacteria residing in the rhizosphere are the most beneficial. Bacterial communities in the rhizosphere vary in different root

**Table 9.2** Currently used PGPR formulations against rice sheath blight disease

PGPR strain	Type of formulation	Method of application	References
<i>Bacillus megaterium</i>	EFB (empty fruit bunches) powder	Seedling dip	Al-Taweil et al. (2009)
<i>Bacillus licheniformis</i> CHM1	Bacterial cell suspension	Soil application	Wang et al. (2009)
<i>Bacillus megaterium</i> (No 16)	Floating pellets	Broadcasting	Kanjanamaneesathian et al. (2007)
<i>Bacillus megaterium</i> (No 16)	Water soluble granules	Foliar spray	Kanjanamaneesathian et al. (2007)
<i>Pseudomonas fluorescens</i> (PF1 and FP7)	Bacterial suspension/talc-based	Seed + root + soil + foliar	Nandakumar et al. (2001a)
<i>Pseudomonas fluorescens</i> and <i>Pseudomonas aeruginosa</i>	Bacterial cell suspension	Seed + root + soil	Vleeschauwer and Hofte (2005)
<i>Pseudomonas fluorescens</i> (PfALR2)	Peat based	Seed + root + soil + foliar	Rabindran and Vidhyasekaran (1996)
<i>Pseudomonas fluorescens</i> (PF1 and FP7)	Talc-based	Seed+root+soil+foliar	Radja Commare et al. (2002)
Beneficial bacteria (NF1, NF3, NF52, NF49, CT6-37, and W23)	Bacterial cell suspension	Foliar spray	Lai Van E et al. (2001)
Fluorescent and non- fluorescent <i>Pseudomonads</i>	Bacterial cell suspension	Seed treatment	Mew and Rosales (1986); Vasantha Devi et al. (1989)
<i>Bacillus megaterium</i>	Floating pellets	Broadcast + spray	Wiwattanapatee et al. (2007)
<i>Pseudomonas fluorescens</i>	Talc-based	Foliar spray	Vijay Krishna Kumar et al. (2009)
<i>Pseudomonas aeruginosa</i>	Bacterial cell suspension	Foliar spray	Saikia et al. (2006)
Antagonistic bacteria	Bacterial cell suspension	Seed + foliar	Chen et al. (1996)

zones and their composition can be altered by changes in root exudate composition (Yang and Crowley 2000). Root exudates of rice plants were found to exert a positive influence on the motility of these bacteria toward plant roots (Bacilio-Jimenez 2003). Earlier studies indicated that the rhizosphere isolates of rice were able to induce IAA production and have phosphate solubilization capacity. Further, these PGPR isolates were found to promote seed germination, root length, plant height, and dry matter production of shoot and roots in rice (Ashrafuzzaman et al. 2009). Application of bio-inoculants was found to enhance rice growth through production of total sugars, reducing sugars, amino nitrogen content, PGP substances in the root exudates, and biological nitrogen fixation. The microbial consortium viz., *Azospirillum lipoferum*-Az204, *B. megaterium* var. *phosphaticum*, and *P. fluorescens* Pf1 when applied to rice improved the colonization potential, sustainability within the inoculants, and enhanced plant growth when compared to their application individually (Raja et al. 2006).

Mirza et al. (2006) reported a nitrogen-fixing, phytohormone-producing *Pseudomonas* isolate (strain K1) that had a capacity of fixing nitrogen in inoculated rice plants and its efficacy was comparable to non-*Pseudomonas* nitrogen-fixing PGPR. Use of PGPR also alleviates zinc deficiency in rice plants. Zinc deficiency is a serious problem in rice production (Anon 1993). Inoculation of rice fields with PGPR had a significant positive impact on root length (54% increase), root weight (74%), root volume (62%), root area (75%), shoot weight (23%), panicle emergence index (96%), and Zn mobilization efficiency, thereby reducing the cost incurred in the application of chemical Zn fertilizers (Muhammad et al. 2007). Application of diazotrophs such as *Rhizobium leguminosarum* bv. *trifolii* (E11), *Rhizobium* spp. (IRBG74), and *Bradyrhizobium* sp. IRBG271 in lowland rice fields enhanced N, P, and K uptake by 10–28% due to rhizobial inoculation. In addition, the uptake of Fe was enhanced by 15–64%. Further, the growth promotion in rice was due to changes in growth physiology or root morphology rather than biological nitrogen fixation (BNF) (Biswas et al. 2000).

## 9.5 Sheath Blight Management

In rice, PGPR offer a promising means of controlling plant diseases besides contributing to the plant resistance, growth, and grain yields (Mew and Rosales 1992). Of different PGPR, fluorescent *Pseudomonads* and *Bacillus* spp. group of bacteria are widely used against ShB. Their application promotes plant growth by direct and indirect mechanisms. Direct growth promotion is due to production of phytohormones, solubilization of phosphates, increased uptake of iron through production of siderophores, and volatile metabolites. Indirect way of plant growth promotion is due to mechanisms of antibiosis, competition for space and nutrients, parasitism or lysis of pathogen hyphae, inhibition of pathogen-produced enzymes or toxins, and through induced systemic resistance (ISR). The ISR in rice against ShB is either due to enhanced chitinase or peroxidase activity (Nandakumar et al.

2001b). However, no correlation was observed between chitinase production and ShB suppression (Thara and Gnanamanickam 1994). Strains of *P. fluorescens* were found to produce siderophores, volatile metabolites, extracellular secretions, and antibiotics that were inhibitory to ShB pathogen. Further, the strains reduced germination and caused lysis of sclerotia (Kazempour 2004). Rhizosphere isolates of *P. fluorescens* produced  $\beta$ -1,3-glucanase, salicylic acid, and HCN, and a significant relationship was observed between antagonism of the bacterium and the production of these substances (Nagarajkumar et al. 2004).

*Bacillus* spp. are endospore-producing gram-positive bacteria, and some strains have been used in biocontrol of rice diseases. Strains of *B. subtilis* and *B. megaterium* exhibit inhibition of *Rhizoctonia solani* (Luo et al. 2005). The fermented product of *Bacillus* (Drt-11) is highly inhibitory to the sclerotial germination, hyphal growth, and colony diameter besides enhancing rice seedling growth (Chen and Hui 2006). Strains of *Bacillus* produce a thermo and proteinase – stable antagonistic substance (P1) that is effective against rice ShB and blast pathogens (He et al. 2002). The *B. subtilis* (AUBS1) strain produces phenylalanine ammonia-lyase (PAL), peroxidase (PO), and certain pathogenesis-related (PR) proteins in rice leaves when applied against ShB disease. Accumulation of thaumatin-like proteins, glucanases, and chitinases are the other important substances in plants against ShB by these bioagents (Jayaraj et al. 2004). The other promising bacteria against rice ShB include *Streptomyces* spp. and *Serratia marcescens*. The antifungal metabolites of *Streptomyces* spp. (PM5, SPM5C-1, and SPM5C-2) were highly effective against mycelial growth of rice ShB and blast pathogens under in vitro conditions. Greenhouse studies revealed that spraying of the strain SPM5C-2 at 500  $\mu\text{g ml}^{-1}$  significantly reduced ShB and blast diseases by 82 and 76%, respectively (Prabavathy et al. 2006). Culture filtrates of *S. marcescens* exhibited enhanced reduction of sclerotial viability of ShB pathogen, when applied with reduced doses of fungicides such as flutolanil, pencycuron, and validamycin (Someya et al. 2005).

In order to identify a potential biocontrol agent, researchers have been spending their time on several microbes in areas of isolation, identification, and purification which is routine. This is a laborious process demanding efforts of time and man-hours. Here, we have provided our own selection of a potential microbial inoculant against rice ShB.

### **9.5.1 Screening of Different PGPR Against ShB Pathogen and Seedling Growth Promotion Under Laboratory Conditions**

Seventy PGPR strains that belong to *Bacillus*, *Paenibacillus*, *Brevibacillus*, and *Arthrobacter* were selected from the bacterial culture collection of the Phytopathology Laboratory of Auburn University. These PGPR strains were found to be highly effective in inducing growth-promoting effects in various crops. These strains were screened against rice ShB pathogen, ShB lesion spread and in promoting rice

**Table 9.3** Benefits and use rates of Integral in different crops

Crop	Method of application	Rates of application	Target pathogens
Peanut	In-furrow	0.1–1.2 fl oz/acre	<i>Rhizoctonia</i> , <i>Fusarium</i> , <i>Aspergillus</i>
Cotton, vegetables, soybean corn	In-furrow	0.1–1.2 fl oz/acre	<i>Rhizoctonia</i> , <i>Fusarium</i>
Non-bearing plants (Cherry) in greenhouses	Soil mix	1.3–13 fl oz/acre	<i>Fusarium</i> , <i>Rhizoctonia</i>
Cotton	Seed	0.6–2.4 fl oz/100 lb seed	<i>Fusarium Rhizoctonia</i>
Soybeans	Seed	0.13 fl oz/100 lb seed	<i>Fusarium Rhizoctonia</i>
Green beans, snap beans, lima beans, kidney beans, navy beans, pinto beans, wax beans, pole beans, garden beans, peas, field beans	Seed	0.6–2.4 fl oz/100 lb seed	<i>Fusarium Rhizoctonia</i>
Alfalfa, forage, turf	Seed	0.2–12 fl oz/100 lb seed	<i>Fusarium Rhizoctonia</i>
Wheat, barley	Seed	0.1–0.6 fl oz/100 lb seed	<i>Fusarium Rhizoctonia</i>
Field corn, sweet corn	Seed	0.6–2.4 fl oz/100 lb seed	<i>Fusarium</i>
Canola	Seed	1.6–3.8 fl oz/100 lb seed	<i>Fusarium Rhizoctonia</i>

seedling growth under laboratory conditions. The mycelial growth inhibition of *R. solani* was as high as 83% with *B. subtilis* MBI 600, compared to the control. Only four strains completely inhibited the germination of sclerotia. The ShB lesion spread was determined by highest relative lesion height method (HRLH) and for effective strain (*B. subtilis* MBI 600) was found to be only 2.9 as against control (100). Highest seedling vigor of 13,600 was recorded in comparison to that of control (4,867) on 10-day-old seedlings. The PGPR strain, *B. subtilis* MBI 600 was found to be highly effective in all the screening assays and was selected for further studies.

To further test the efficacy of *B. subtilis* MBI 600, the strain was produced in a commercial proprietary liquid formulation by Becker Underwood, Ames, Iowa, USA. The formulated strain MBI 600 has a proprietary trade name as Integral<sup>®</sup>. The product is stored at room temperatures prior to use. The minimum concentration of Integral in liquid formulation is  $2.2 \times 10^{10}$  cfu ml<sup>-1</sup>. The details of different application methods of Integral are shown in Table 9.3.

### 9.5.2 Efficacy of Integral

To assess the biocontrol suppression of ShB by using antagonistic bacteria and their combination with fungicide under field conditions for a long time, antagonistic bacteria and fungicide used to control ShB must be evaluated for durability effect.

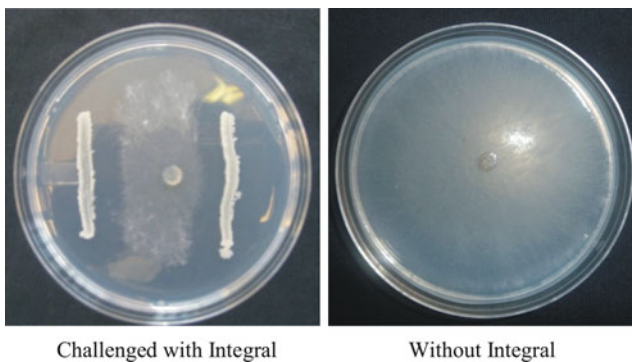
Improved plant growth and health by PGPR is either due to direct mechanisms such as improvement in plant uptake through solubilization of mineral phosphates

and other nutrients (De Freitas et al. 1997; Gaur 1990), nitrogen fixation (Boddey and Dobereiner 1995), and phytohormone production such as indole 3-acetic acid, gibberellic acid, cytokinins, and ethylene (Arshad and Frankenberger 1993; Glick 1995). Indirect growth promotion is through biological control of plant pathogens by producing siderophores (Scher and Baker 1982), antibiotics (Shanahan et al. 1992), hydrogen cyanide (Flaishman et al. 1996), lytic enzymes, and competition for nutrients and space.

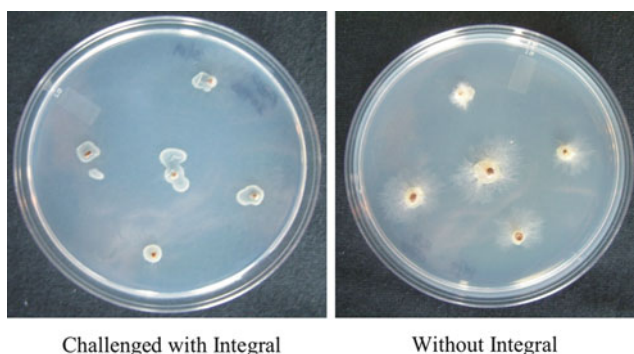
### 9.5.2.1 In-Vitro Inhibition of ShB Pathogen

The *B. subtilis* MBI 600 strain of Integral was further characterized for determining its mode of action against ShB pathogen. The PGPR strain was isolated from the formulation on TSA and confirmation of its purity was carried out using 16s rDNA sequence homology and by measuring the 16s rDNA sequence with 1,409 base pairs of the isolate. The BLAST analysis of the sequencing results confirmed 100% similarity with *B. subtilis*. The MBI 600 strain was highly effective against the ShB pathogen, *R. solani* and repeatedly shown significant results in inhibiting mycelial growth (Fig. 9.2) and germination of sclerotia (Fig. 9.3) under in-vitro conditions. A strong zone of inhibition (3 mm) between mycelial growth of pathogen and bacterium was observed. Inhibition of sclerotial germination was about 98% at a concentration of  $2.2 \times 10^9$  cfu ml<sup>-1</sup> whereas at a concentration of  $2.2 \times 10^8$  cfu ml<sup>-1</sup>, the inhibition was 37%. Integral was not effective in inhibiting sclerotial germination at concentrations of  $2.2 \times 10^6$  and  $2.2 \times 10^7$  cfu ml<sup>-1</sup>. Highest inhibition of sclerotial growth was obtained at a concentration of  $2.2 \times 10^9$  cfu ml<sup>-1</sup> (79%), followed by at  $2.2 \times 10^8$  cfu ml<sup>-1</sup> (72%). Integral was also effective at lower concentrations with sclerotial growth inhibitions ranging from 29 to 60% (Table 9.4).

Efficacy of Integral was evaluated in reducing ShB lesions on rice leaves under in vitro conditions by detached leaf piece assay. Integral concentrations of



**Fig. 9.2** Inhibition of mycelial growth of *Rhizoctonia solani* challenged with Integral



**Fig. 9.3** Inhibition of sclerotial germination of *Rhizoctonia solani* by Integral

**Table 9.4** Efficacy of Integral on sclerotial germination and sheath blight lesion symptoms of rice

Concentration <sup>1</sup>	% Inhibition of sclerotial germination <sup>2</sup>	% Inhibition of sclerotial growth compared to control <sup>3</sup>	ShB lesion spread <sup>4</sup>
$2.2 \times 10^6$ CFU/ml	0 <sup>c</sup>	28.5 <sup>d</sup>	92.6 <sup>b</sup>
$2.2 \times 10^7$ CFU/ml	0 <sup>c</sup>	59.5 <sup>c</sup>	71.6 <sup>c</sup>
$2.2 \times 10^8$ CFU/ml	36.7 <sup>b</sup>	71.8 <sup>b</sup>	22.7 <sup>d</sup>
$2.2 \times 10^9$ CFU/ml	97.7 <sup>a</sup>	78.8 <sup>a</sup>	4.7 <sup>e</sup>
Control	0 <sup>c</sup>	—	99.2 <sup>a</sup>

Means followed by a common letter in the columns are not significantly different at  $p \leq 0.05$

<sup>1</sup>Integral applied at these concentrations to test on sclerotial germination, growth of mycelia, and suppression of ShB lesions

<sup>2</sup>Sclerotial germination was recorded at 3 days after incubation

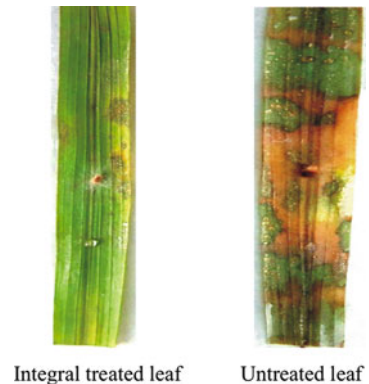
<sup>3</sup>Sclerotial growth was recorded at 5 days after incubation and

<sup>4</sup>ShB lesion spread was recorded by Highest Relative Lesion Height method at 7 days after inoculation

$2.2 \times 10^6$  through  $2.2 \times 10^9$  cfu ml<sup>-1</sup> were sprayed onto rice leaf pieces (8 cm) separately. Later the leaves were inoculated with 1-week-old sclerotia of *R. solani* at the centre and leaves were incubated in Petri dishes containing moistened filter papers. ShB lesion length around sclerotium was recorded after 5 days and disease severity was assessed by highest relative lesion height (HRLH) method. As shown in Fig. 9.4, Integral at  $2.2 \times 10^9$  cfu ml<sup>-1</sup> significantly reduced ShB lesion spread on detached rice leaves (4.7) (Table 9.4). At other concentrations, the lesion spread ranged from 23 to 93 as against untreated control (100).

Integral was tested positive for production of siderophores. However, production of IAA, HCN, cellulase, chitinase, and phosphate solubilizing capacity when tested were negative. Siderophores are low-molecular-weight iron-chelating agents produced by PGPR that can create iron nutrient competition in soils to plant pathogens. Since the element iron is present in low quantities in soils, siderophore production is a strategy by the PGPR to compete with soil-borne plant pathogens.

**Fig. 9.4** Suppression of sheath blight lesions in a detached leaf assay with Integral



### 9.5.2.2 Rice Plant Growth Promotion

Seed treatment with Integral was highly effective in promoting rice seedling development both under laboratory and greenhouse conditions. Under *in vitro* conditions, significantly higher root and shoot lengths were observed with Integral over untreated control. Increase in root and shoot lengths was noticed with an increase in Integral concentration from  $2.2 \times 10^6$  cfu ml<sup>-1</sup> to  $2.2 \times 10^9$  cfu ml<sup>-1</sup>. As shown in Fig. 9.5, highest root and shoot lengths (47.5 and 39.1 mm, respectively) were recorded at a concentration of  $2.2 \times 10^9$  cfu ml<sup>-1</sup> as against control (14.3 and 7.6 mm of root and shoot lengths respectively).

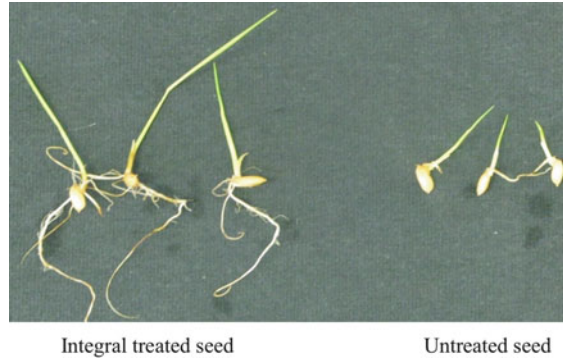
Under greenhouse conditions, seed treatment with Integral significantly improved rice seed germination, seedling emergence, and plant growth. The percent germination of seeds sown in 15 cm pots filled with field soil was highest at a concentration of  $2.2 \times 10^9$  cfu ml<sup>-1</sup> (88.9%) as against untreated control (61.1%) at 7 days after sowing (DAS). Integral application significantly improved root and shoot lengths at 15 DAS. Highest root length and shoot length were recorded at a concentration of  $2.2 \times 10^9$  cfu ml<sup>-1</sup> (166 and 335 mm respectively) as against untreated control (73 and 222 mm respectively) (Fig. 9.6).

### 9.5.2.3 Chemical Compatibility

Currently, ShB disease management strategy is through use of systemic fungicides and also with certain non-systemic fungicides (Pal et al. 2005). Pathogen resistance to these systemic fungicides is of concern, thus demanding integration of PGPR in IDM. Since, host plant resistance to ShB range only from very susceptible to moderately susceptible levels in rice (Groth and Bond 2007), use of chemical fungicides has become a necessary component for an effective ShB management. For effective functioning of PGPR under the ambit of IDM, their compatibility with commonly used fungicides and insecticides in rice is also mandatory (Mew et al. 2004).



**Fig. 9.5** Effect of Integral on rice seed development



**Fig. 9.6** Efficacy of Integral on rice seedling growth



Combined applications of PGPR with chemical fungicides are an important IDM package against ShB. Of different PGPR, Pseudomonads and *Bacillus* spp. were found to be very effective as a supplement in IDM. Greenhouse and field studies against rice ShB with different PGPR isolated from farmyard manure, rice seed, phyllosphere, and rhizosphere proved that three bacteria, *P. fluorescens* (PF-9), *Bacillus* sp. (B-44), and a chitinolytic bacterium (Chb-1) are compatible with carbendazim at 500 and 1,000 ppm concentrations. Of these, PF-9 was most effective in reducing ShB severity either alone or in combination with one spray of 0.1% carbendazim, followed by combination of PF-9 and B-44 (Laha and

Venkataraman 2001). The *B. subtilis* (Bs-916) when applied along with jinggangmycin was found to colonize the root system effectively. Further, the population density of BS-916 was maintained in its presence without any further decline (Chen et al. 2003).

In order to use Integral in ShB management, it has to be compatible with existing agronomic practices and commonly used chemical fungicides in rice production systems. In our ongoing research, we have attempted to study our classical product Integral according to the assays described by Shanmugam and Narayanasamy (2009) under in vitro conditions. Briefly, in this assay, a loop full of MBI 600 strain onto Nutrient Agar (NA) plates amended with various concentrations (100–1,000 ppm) of fungicides such as propiconazole, validamycin, benomyl, carbendazim, tricyclazole, mancozeb, azoxystrobin, and hexaconazole. Plates were later incubated at room temperature for 48 h and growth of bacterium was monitored. Further compatibility studies with azoxystrobin and carbendazim were carried out according to Omar et al. (2006) wherein 100 µL of bacterial inoculum was added to 250 ml yeast peptone glucose (YPG) liquid medium amended with fungicides at concentrations at 200 and 400 ppm and incubated on a shaker, growth of bacterium was enumerated on NA after serial dilution.

Integral exhibited good tolerance to hexaconazole, propiconazole, and validamycin, moderately to tricyclazole and slightly to benomyl and mancozeb at 1,000 ppm. It was highly compatible with carbendazim and azoxystrobin up to 400 ppm whereas complete inhibition was obtained with these fungicides at 800 ppm (Table 9.5). Compatibility to azoxystrobin and carbendazim showed up to 400 ppm, and good growth of Integral was in carbendazim- and azoxystrobin-amended medium (Figs. 9.7 and 9.8).

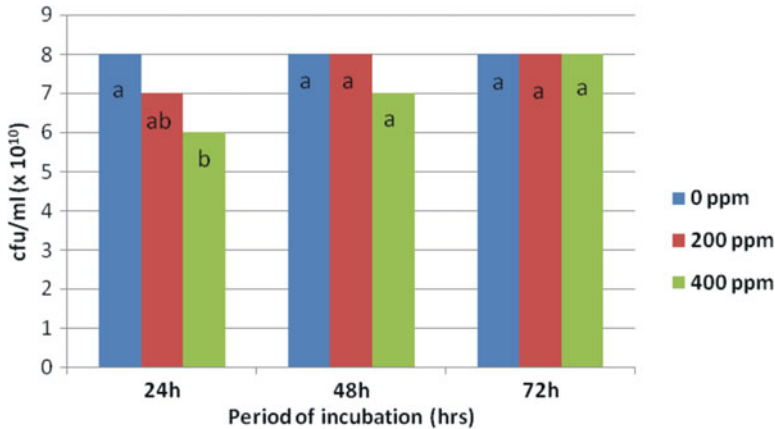
#### 9.5.2.4 Efficacy of PGPR Against ShB Under Greenhouse and Field Conditions

The time of application of PGPR has significant influence in the management of ShB disease. Ren et al. (2006) reported that the optimum time of application

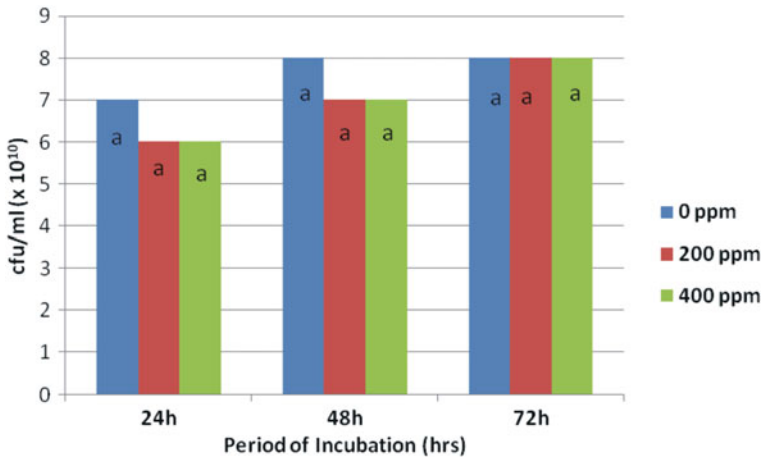
**Table 9.5** Compatibility of Integral with commonly used fungicides

Fungicides	Fungicide concentrations (ppm) <sup>a</sup>					
	100	200	400	600	800	1,000
Propiconazole	+++	+++	+++	+++	+++	+++
Validamycin	+++	+++	+++	+++	+++	+++
Benomyl	+++	+++	+++	+++	++	+
Carbendazim	+++	+++	+++	+	--	--
Tricyclazole	+++	+++	+++	+++	+++	++
Mancozeb	+++	+++	+++	++	+	+
Azoxystrobin	+++	+++	+++	+	--	--
Hexaconazole	+++	+++	+++	+++	+++	+++

<sup>a</sup>Growth of Integral in NA amended with fungicides: +++ = Good; ++ = Moderate; + = Slight; -- = No growth



**Fig. 9.7** Compatibility of Integral with Carbendazim. Values are means of five replications. Means followed by a common letter are not significantly different at  $p \leq 0.05$



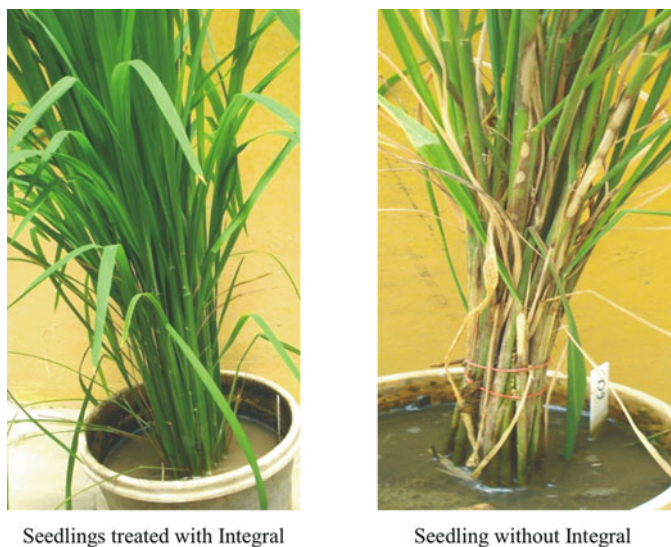
**Fig. 9.8** Compatibility of Integral with Azoxystrobin. Values are means of five replications. Means followed by a common letter are not significantly different at  $p \leq 0.05$

of PGPR against ShB under field conditions was during the first day of inoculation of *R. solani*. Reduction in ShB severity under field conditions by PGPR is also dependent on the bacterial concentration. It is interesting to note that the PGPR when applied as consortia and in conjunction with other fungal antagonists offered synergistic effect over their individual applications in ShB disease reduction. Talc-based formulations of two *P. fluorescens* strains (PF1 and PF7), when applied as seed, soil, and root dip treatments and foliar sprays, significantly reduced ShB and leaf-folder incidence under greenhouse and field conditions. The PGPR mixture proved to be more effective over their individual applications (Radja Commare et al. 2002). Combined use of *P. fluorescens* and *Trichoderma viride* was effective

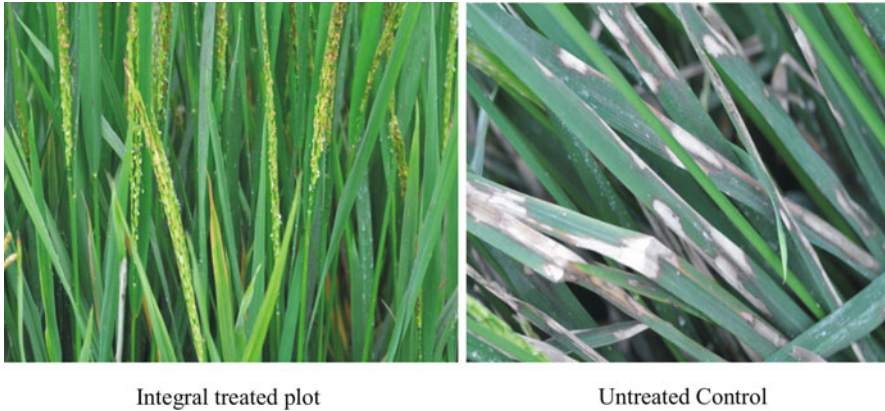
in rice ShB reduction and enhanced seedling growth (Mathivanan et al. 2006). *Bacillus* spp. exhibited synergistic effect when used in conjunction with *T. viride* (Das et al. 1998) and *Gliocladium virens* (Sarmah 1999) against ShB. The fermented product of *Bacillus* strain Drt-11 when applied in combination with biofungicide, Jinggaameisu WP (20%) proved to be more effective against ShB over their individual applications (Chen and Hui 2006).

Our own studies with Integral under greenhouse and field studies effectively reduced ShB incidence in rice. In a typical greenhouse assay, Integral was evaluated at concentrations of  $2.2 \times 10^6$  to  $2.2 \times 10^9$  cfu ml<sup>-1</sup> as seed treatment (ST), seedling root dip (SD), and foliar sprays (FS). Seed treatment with Integral at concentrations of  $2.2 \times 10^8$  and  $2.2 \times 10^9$  cfu ml<sup>-1</sup> significantly improved percent germination over untreated control. Further, the root and shoot lengths were significantly improved (12.2 and 40.7 cm respectively) at  $2.2 \times 10^9$  cfu ml<sup>-1</sup> as against untreated control (7.9 and 33.8 cm respectively) at 25 DAS. Significant reduction in ShB severity (9.2 vs. 24.1 in untreated control) (Fig. 9.9), increase in plant height (73.2 vs. 62.7 cm in untreated control) and number of tillers/plant (11.9 vs. 8.0 in untreated control) was obtained when Integral was applied at  $2.2 \times 10^9$  cfu ml<sup>-1</sup> as seed treatment (ST) + seedling root dip (SD) + foliar sprays (FS).

Our field studies at Andhra Pradesh Rice Research Institute (APRRI), India during 2009 indicated significant improvement in root and shoot lengths or rice seedlings in nursery with Integral seed treatment at  $2.2 \times 10^8$  and  $2.2 \times 10^9$  cfu ml<sup>-1</sup> over untreated control. On a transplanted crop, Integral application as ST + SD + FS at  $2.2 \times 10^9$  cfu ml<sup>-1</sup> significantly reduced sheath blight severity (19.2 vs. 69.7 in control) and percent diseased tillers (25.1 vs. 99.4 in control)



**Fig. 9.9** Suppression of sheath blight severity with Integral under greenhouse conditions



**Fig. 9.10** Sheath blight severity under field conditions

(Fig. 9.10). Plant height (98.1 vs. 78.5 cm in control), tillers/plant (12.8 vs. 10.0 in control), and grain yields were maximum with Integral application at  $2.2 \times 10^9$  cfu ml<sup>-1</sup> as ST + SD + FS. Integral was also effective in reducing ShB severity and promoting growth and grain yields at a concentration of  $2.2 \times 10^8$  cfu ml<sup>-1</sup>.

## 9.6 Conclusions

As shown by the examples discussed in this chapter, PGPR have good potential in the management of rice ShB. It is generally believed that the field efficacy of a particular PGPR strain is dependent on its root colonization capacity. According to this reasoning, rhizosphere competence of a PGPR strain is a desirable trait for its effective root colonization and subsequent disease control. Earlier reports indicated that diversity of PGPR in rice rhizosphere is changing according to soil salinity. With increase in soil salinity, the population levels of *Pseudomonas* spp. decreased. In non-saline sites of rice rhizosphere, fluorescent *Pseudomonads* are the dominant species whereas in saline sites, these were replaced by salt tolerant species such as *P. alcaligenes* and *P. pseudoalcaligenes*. Further, organic farming was found to significantly enhance the diversity of PGPR populations in saline soils (Rangarajan et al. 2002).

It should be noted that although rhizosphere competence is considered important for effective PGPR biocontrol agents, there could be exceptions. For example, if a particular PGPR-based product is applied as a foliar spray, rhizosphere colonization would not be strictly required. For example, with the product Integral, which was highly effective in biocontrol of ShB in field trials in India, one application method was a foliar spray. To date, there are no published studies examining possible rhizosphere competence or root colonization on rice by the PGPR strain contained in Integral (*B. subtilis* strain MBI600).

Several biotic and abiotic factors also have significant impact on the field consistency of a formulated PGPR strain in rice ShB management. Since gram-positive bacilli produce endospores that can withstand desiccation and have a long shelf life, they are considered to be ideal candidates for commercial use against ShB. Fungicidal compatibility of selected PGPR strain is another important factor that determines the efficacy of PGPR under IDM. Consistent efforts are therefore needed to select PGPR strain with all the desirable traits that contribute to effective rice ShB management.

Although several advantages have been reported with the use of microbial inoculants in rice, variability in effectiveness of field performance remains a constraint. To overcome this, comprehensive basic research is essential in the areas of selection of microbial agents that focus on identifying strains that occupy the same ecological niche with that of pathogens such as roots, the phylloplane, and vascular systems. Application of novel techniques such as PCR, RFLP, and RAPD for rapid identification of bacterial strains with desirable traits like biocontrol and growth-promoting mechanisms are therefore necessary. Integrating these basic research concepts with studies on greenhouse and field studies are therefore essential before devising IDM approaches for ShB management in rice. The PGPR that are identified in these respects should be maintained as important genetic resource, which will be useful for future studies that form an alternate to the presently available chemical control of ShB.

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