# **Chapter 6 Beneficial Bacteria for Biological Control** of Fungal Pathogens of Cereals

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# 6.1 Introduction

Fourteen crop plants provide the bulk of human food, of which eight are cereals (Strange and Scott 2005). According to an estimate, 10 % of global food production is lost due to plant disease (Strange and Scott 2005). Reducing disease-associated cereal crop losses is key to both increasing yields and providing a steady and healthy food supply to a burgeoning human population. Common practices for controlling plant disease include plant disease resistance breeding, manipulation of plant culture practices and, to a greater extent, the use of synthetic chemicals (Strange 1993). The persistence and long-term toxicity of fungicides to nontarget organisms, including humans, has generated worldwide concern, both societal and scientific, regarding their future use. This has necessitated the re-evaluation of synthetic chemicals as a final solution to pest disease management (Saxena and Pandey 2001). Many of the synthetic chemicals may lose their usefulness due to revised safety regulations, concern over nontarget effects, or development of resistance in pathogen populations (Emmert and Handelsman 1999). Thus there is a need for new solutions to plant disease problems that provide effective control, while minimizing the negative consequences for human health and the environment (Emmert and Handelsman 1999).

Biological control (i.e., using microorganisms to suppress plant disease) offers a powerful alternative to the use of synthetic chemicals. The rich diversity of the microbial world provides a seemingly endless resource for this purpose. Increasing the abundance of a particular strain in the vicinity of a plant can suppress disease without producing lasting effects on the rest of the microbial community or other organisms in the ecosystem (Gilbert et al. 1993). The basic prerequisite for the

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success of a biological control program is good adaptation of the biocontrol agent to the local environmental conditions in which it is to be used (Romero et al. 2004). Idealistically, biological control would be more robust and durable than chemical disease control in cases where the "biocontrol" agent employs numerous mechanisms of disease suppression (Cook 1993).

Until recently, research on biological control of fungal plant pathogens had been confined within the members of the fungal genera *Trichoderma*. However, growing evidence suggests that bacteria have great potential to control fungal plant pathogens. Growing interest among scientists for bacterial biocontrol agents resulted in identification of a range of bacterial species having great potential against plant pathogens. We present here some highlights of global research activities on bacterial biological control of major fungal cereal diseases, commercial biocontrol products based on bacteria, mode of action of antifungal bacteria, screening methods used for selecting the potential biocontrol bacteria, and future challenges and prospects.

#### 6.2 Antifungal Bacteria Against Major Cereal Diseases

Researches around the world have reported antifungal activity of a range of bacterial species against various cereal diseases (Table 6.1). Although members of various bacterial genera have been found to possess cereal disease suppression capability, it seems that those belonging to fluorescent pseudomonads and *Bacillus* spp. are more effective.

#### 6.2.1 Wheat and Barley Diseases

The major fungal wheat diseases are Fusarium head/seedling blight, Septoria tritici leaf blotch, take-all and net blotch disease caused by *Fusarium* species, *Mycosphaerella graminicola*, *Gaeumannomyces graminis*, and *Pyrenophora teres*, respectively. The bacteria *Pseudomonas fluorescens* (strains MKB 100, MKB 158, and MKB 249), *P. frederiksbergensis* strain MKB 202, and *Chryseobacterium* sp. strain MKB 277 were found to be very effective biocontrol bacteria in reducing Fusarium seedling blight disease symptoms in both wheat and barley seedlings under controlled environmental conditions (Khan et al. 2006). *Pseudomonas fluorescens* strain MKB 100 is also very effective against net blotch disease of barley (Khan et al. 2010). In another study, the bacteria *P. fluorescens* (strains MKB 158 and MKB 249) and *P. frederiksbergensis* strain MKB 202 were found to be effective in reducing Fusarium head blight disease symptoms in both wheat and barley plants under both glasshouse and field conditions (Khan and Doohan 2009). They were also effective in restoration of yield of wheat and barley under field conditions. In the same study *P. fluorescens* MKB 158 and MKB 249 were able to reduce the

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Crop	Plant disease	Pathogen	Biocontrol bacteria	References
Wheat	Fusarium seedling blight	Fusarium culmorum	Pseudomonas fluorescens (strains MKB 100, MKB 158, and MKB 249); P. frederiksbergensis strain 202; Chryseobacterium sp. strain MKB 277	Khan et al. (2006)
	Fusarium head blight	Fusarium culmorum	Pseudomonas fluorescens (strains MKB 158 and MKB 249); P. frederiksbergensis strain 202	Khan and Doohan (2009)
	Fusarium head blight	Fusarium graminearum	Lysobacter enzymogenes strain C3	Jochum et al. (2006)
	Septoria tritici leaf blotch	Mycosphaerella graminicola	Bacillus megaterium strain MKB135	Kildea et al. (2008)
Barley	Fusarium seedling blight	Fusarium culmorum	Pseudomonas fluorescens (strains MKB 100, MKB 158, and MKB 249); P. frederiksbergensis strain 202	Khan et al. (2006)
	Fusarium head blight	Fusarium culmorum	Pseudomonas fluorescens (strains MKB 158 and MKB 249); P. frederikshergensis strain 202	Khan and Doohan (2009)
	Net blotch	Pyrenophora teres	Pseudomonas fluorescens strain MKB 100	Khan et al. (2010)
Rice	Rice blast	Pyricularia oryzae	Streptomyces sindeneusis isolate 263	Zarandi et al. (2009)
	Rice blast	Pyricularia oryzae	P. fluorescens strain 7-14	Gnanamanickam and Mew (1992)
	Rice sheath-blight	Rhizoctonia solani	Bacillus megaterium strain 16	Kanjanamaneesathian et al. (2007)
Maize	Banded leaf and sheath blight	Rhizoctonia solani	Bacillus subtilis strain br23	Muis and Quimiob (2006)
	Foot rots and wilting	Fusarium moniliforme	<i>Pseudomonas</i> sp. strain EM85; <i>Bacillus</i> spp. strains MRF and MR-11(2)	Pal et al. (2001)
	Collar rots/stalk rots and root rots and wilting	Fusarium graminearum	<i>Pseudomonas</i> sp. strain EM85; <i>Bacillus</i> spp. strains MRF and MR-11(2)	Pal et al. (2001)
	Charcoal rots	Macrophomina phaseolina	Pseudomonas sp. strain EM85; Bacillus spp. strains MRF and MR-11(2)	Pal et al. (2001)
	Preemergence damping-off	Pythium ultimum	Pseudomonas fluorescens strain AB254	Callan et al. (1990)

mycotoxin contamination of wheat and barley flour. Jochum et al. (2006) observed that treatment of wheat spikelets with the bacterium *Lysobacter enzymogenes* strain C3 significantly reduced Fusarium head blight disease symptoms in greenhouse tests. Kildea et al. (2008) observed that the bacterium *Bacillus megaterium* strain MKB 135 could significantly inhibit the Septoria tritici leaf blotch disease of wheat under field conditions.

# 6.2.2 Rice Diseases

Blast disease of rice caused by the fungus *Pyricularia oryzae* is considered to be one of the major diseases of rice. Zarandi et al. (2009) observed that spraying of rice seedlings with *Streptomyces sindeneusis* isolate 263 resulted in the reduction of blast disease symptoms under glasshouse conditions. Earlier, Gnanamanickam and Mew (1992) observed that spraying of rice seedlings with a cell suspension of the bacterium *P. fluorescens* strain 7-14 reduced the blast disease symptoms under field conditions. Another major rice disease is sheath blight caused by the fungus *Rhizoctonia solani*. Kanjanamaneesathian et al. (2007) reported that spraying of *Bacillus megaterium* strain 16 was as effective as the fungicide "Iprodione" in reducing the percentage of rice seedling with sheath blight disease symptoms.

#### 6.2.3 Maize Diseases

Biocontrol activity of bacteria has been found against several maize diseases. Muis and Quimio (2006) observed that treatment of maize seeds with *Bacillus subtilis* strain br23 could significantly restore grain yield of maize in banded leaf and sheath blight infested field plots and its effect was better than the fungicide "captan" used for seed treatment. Pal et al. (2001) reported that bacterial genera, namely, *Pseudomonas* sp. EM85 and *Bacillus* spp. MRF and MR-11(2) could significantly reduce foot rots and wilting, collar rots/stalk rots and root rots and wilting, and charcoal rots of maize under field conditions. Callan et al. (1990) found that *P. fluorescens* AB254 could provide protection against preemergence damping-off in naturally infested soil.

# 6.3 Mode of Action

For a successful biological control program against fungal cereal diseases, it is very important to understand the mode of action of potential biocontrol bacteria. A biocontrol bacterium may employ different mechanism(s) to antagonize a pathogen such as by competition, antibiosis, and elicitation of induced systemic resistance (ISR) in the host plant. A biocontrol bacterium may compete against a pathogen for space and/or nutrients. A fast growing bacterium may outgrow the pathogen and thus restrict the growth of a pathogen on or around the plant. Moreover, bacteria may compete for essential nutrients with the pathogen. Members of *Pseudomonas* species and *Bacillus* species produce a range of different antibiotics against plant pathogens which might contribute to their disease suppression effect (reviewed by Shoda 2000; Haas and Defago 2005). Members of *Pseudomonas* species produce several types of antibiotics including phloroglucinols, pyrolnitrin, pyoluteorin, cyclic peptides, phenazines besides siderophore and production of volatile antibiotic hydrogen cyanide, which may contribute to disease suppression effect as well. Members of Bacillus species produce several types of antibiotics including siderophores, lipopeptides, bacillomycin, iturin, mycosubtilin, bacilysin, fengymycin, and mycobacillin. A biocontrol bacterium may suppress the plant disease by eliciting ISR mechanism in the plant, in which due to colonization of the biocontrol bacteria the plant develops resistance against invading pathogens. Members of *Pseudomonas* species and *Bacillus* species have been shown to elicit ISR mechanism in different plant species against various fungal pathogens.

#### 6.4 Screening Methods

Research on biological control starts with the generation of a bacterial culture collection and screening of the isolates against the pathogen. Conventionally, the bacterial isolates are co-cultivated on agar plates along with the pathogen (Fig. 6.1). If the growth of the pathogen is inhibited due to presence of any bacterium, then it is thought to have antagonistic activity and further tested in planta. However, this method has severe drawback as it can detect only direct antagonism mediated by secretion of antibiotic(s) in the agar plate. Moreover, these in vitro tests cannot mimic exactly the environmental conditions on the plants in the field where the biocontrol agent will encounter the pathogen. On the contrary, a potential biocontrol bacterium may not show in vitro inhibitory activity against a pathogen. Furthermore, in vitro dual culture tests cannot detect the other two modes of bacterial antagonism, i.e., competition for space and nutrients and elicitation of ISR. Ideally any screening tests for potential biocontrol bacteria should include the four elements, viz. plant, pathogen, bacterial isolate, and the proper environment where the disease occurs (i.e., field tests). This is practically impossible while screening a large collection of bacteria. The next option will be glasshouse tests in pots including all the four elements. If the second option is also not possible, then at least an in vitro test should include at least plant (tissue such as seed or leaf segment), pathogen, and bacterial isolate (Fig. 6.2). This test will still exclude the "environment" element. It will save time and space for screening a large bacterial collection. However, the bacteria which show disease suppressing effect in such test should then be tested in the glasshouse and field. For example, in our in vitro studies P. fluorescens MKB 156



**Fig. 6.1** Growth inhibition of *Fusarium* spp. by *Pseudomonas flourescens* (strain MKB 156) in dual cultures tests. *F. graminearum* (strain HUGR9) (**a**) and *F. poae* (strain HUPO3) (**b**) grown in the absence (**I**) and presence (**II**) of *Pseudomonas fluorescens* (strain MKB 156) for 7 days on potato dextrose agar plates



Fig. 6.2 Bacterial inhibition of in vitro coleoptile retardation of germinating wheat (cv. GK-Othalom) seeds caused by *Fusarium culmorum* (strain FCF 200). Effect of seed treatment with *Pseudomonas flourescens* (strain MKB 158) on *F. culmorum*-induced coleoptile growth retardation. Nontreated (I) and bacterial-treated (II) seeds were germinated on *F. culmorum*-inoculated potato dextrose agar plates and photographed 4 days postincubation

has shown growth inhibitory activity against *Fusarium* species, the pathogens of Fusarium seedling blight disease (Fig. 6.1) (Khan et al. 2006). However, this bacterium is unable to control Fusarium seedling blight disease (Khan et al. 2006) in glasshouse conditions. On the other hand, *P. fluorescens* MKB 158 can inhibit both *Fusarium* seedling and head blight diseases of cereals, but did not show any inhibitory activity in dual culture tests against the same pathogens (Khan et al. 2006; Khan and Doohan 2009).

# 6.5 Commercial Biocontrol Products

Research on biological control of plant diseases is relatively new compared to pest control. Despite this, a few commercial products are already available in the market against cereal diseases. We have presented some examples of the commercially available bacterial biocontrol products which can be used against fungal cereal diseases in Table 6.2. The bacterium Burkholderia cepacia is in the market under the trade name "Deny" (Helena Chemical Company, TN, USA) which can be used to control wheat and barley diseases caused by the fungal genera *Rhizoctonia*, *Pythium*, Fusarium. Pratibha Biotech (Hyderabad, India) has been marketing a P. fluorescens under the trade name "Flick" which can be used to control blast, sheath blight, sheath rot, brown spot, and seedling rot diseases of rice. Scientific Agriculture Laboratory (Madurai, India) has a product based on a *P. fluorescens* under the trade name "Fluroissal" which can be used to control root rot, stem rot, and wilt diseases of rice, wheat, and maize. Biotech International Ltd. (Greater Noida, India) has a product based on a *P. fluorescens* under the trade name "Biomonas" which can be used to control cereal diseases caused by Rhizoctonia, Pythium, Fusarium. The same company markets another bacterium Bacillus subtilis under the trade name "Biosubtilin" which can be used to control general fungal diseases of cereals. Jay Bio Tech (Pune, India) has a product based on a P. fluorescens under the trade name "Jay-Pseudo" which can be used to control blast, sheath blight of rice. BioAgri AB (Stockholm, Sweden) markets a *Pseudomonas chlororaphis* under the trade names "Cedomon" and "Cerall" which can be used to control diseases caused by *Fusarium* spp. in wheat, rye, and triticale.

# 6.6 A Case Study: Biological Control of Fusarium Diseases of Cereals

#### 6.6.1 Diseases Caused by Fusarium species

*Fusarium* fungi can cause diseases on most cultivated plants, including all members of the Gramineae (Parry et al. 1995). *Fusarium* spp. cause seedling blight, foot rot, and head blight [Fusarium head blight (FHB)] diseases of cereals (Parry et al. 1995). Seedling blight and foot rot diseases cause extensive damage to growing seedlings (Wiese 1977) and lead to a reduction in plant establishment, number of heads, and grain yield (Wong et al. 1992; Humphreys et al. 1998). FHB is a major cereal disease worldwide (McMullen et al. 1997). *Fusarium* infection of cereal heads leads to a reduction in the yield and quality of the cereal grains (Pirgozliev et al. 2003). The infected grains carry over inoculum that can cause Fusarium seedling blight when such seeds are sown (Winson et al. 2001). *Fusarium* spp. can produce a wide range of toxins [i.e., deoxynivalenol (DON)] in the infected heads

		Active against cereal	
Product name	Active ingredient	diseases	Company
Deny	Burkholderia cepacia	Barley and wheat diseases caused by <i>Rhizoctonia</i> , <i>Pythium</i> , <i>Fusarium</i>	Helena Chemical Company 225 Schilling Blvd., Collierville, TN 38017 USA
Flick	Pseudomonas fluorescens	Blast, sheath blight, sheath rot, brown spot, and seedling rot diseases of rice	Prathibha Biotech 5-5-35/75, Kukat Pally, Hyderabad, India
Fluroissal	Pseudomonas fluorescens	Root rot, stem rot, wilt diseases of rice, wheat, and maize	Scientific Agriculture Laboratory 3/321, Kavimani Street, Indian Bank Colony, Narayanapuram, Madurai- 625014, Tamil Nadu, India
Biomonas	Pseudomonas fluorescens	Cereal diseases caused by Rhizoctonia, Pythium, Fusarium	Biotech International Ltd. No. B-2160-C, Surajpur, Greater Noida-301306, Uttar Pradesh, India
Biosubtilin	Bacillus subtilis	Fungal diseases of cereals	Biotech International Ltd. No. B-2160-C, Surajpur, Greater Noida-301306, Uttar Pradesh, India
Jay-Pseudo	Pseudomonas fluorescens	Blast, sheath blight of rice	Jay Bio Tech 32, Market Yard, Gultekdi, Pune-411037, Maharashtra, India
Cedomon and Cerall	Pseudomonas chlororaphis	Diseases caused by <i>Fusarium</i> spp. in wheat, rye, and triticale	BioAgri AB Uppsala Stockholm, SE-75109, Sweden

Table 6.2 Examples of commercial bacterial biocontrol agents available against cereal diseases

which are hazardous to animal and human health (Placinta et al. 1999). There has been limited success in controlling *Fusarium* diseases of cereals by cultural, genetical, and chemical measures (Pirgozliev et al. 2003). Recently, biological control has shown some promise against *Fusarium* diseases of cereals (Johansson et al. 2003; Khan et al. 2006; Khan and Doohan 2009).

## 6.6.2 Global Scenario

During 1942, a severe FHB outbreak in Ireland decreased wheat yield by up to 55 % (McKay 1957). A second outbreak during 1954 was responsible for wheat and oat yield reductions up to 50 % (McKay 1957). Severe infestations were widespread in wheat crops in England in 1982, 1992, and 1993 (Parry et al. 1984; Jennings and Turner 1996). In Romania, Tusa et al. (1981) and Munteanu et al. (1972) reported

that, in epidemic years, FHB of wheat caused losses of approximately 40 % in some regions of the country, with up to 70% yield loss recorded in some fields. In Hungary, according to Kukedi (1972), wheat yields were depressed by 40–50 % in some areas following a severe attack of FHB in 1970. A survey carried out between 1951 and 1985 in Yangtze river valley of China recorded 19 FHB outbreaks, with grain yields of wheat reduced by 5-15 % in years when moderate epidemics of FHB were recorded and up to 40 % in years when disease epidemics were severe (Zhuping 1994). During 1980, in the Atlantic Provinces of Canada, FHB was responsible for between 30 % and 70 % wheat yield loss (Martin and Johnston 1982). FHB epidemics in wheat and barley occurred in southern Idaho in 1982 and 1984 and resulted in estimated yield losses as high as 50 % (Michuta-Grimm and Foster 1989). In the USA, FHB has reached epidemic levels in several years during the last decade, causing yield losses and discounted prices were paid for the reduced quality seed (Windels 2000). From 1998 to 2000 direct and secondary economic losses due to FHB for all crops in the Northern Great plains and Central USA were estimated to be worth \$2.7 billion (Nganje et al. 2002).

# 6.6.3 Biological Control

It is surprising that there are so few reports of biocontrol of FHB, given the importance of the disease. It could be presumed that the short time period during which cereal heads are sensitive to the disease could offer an ideal opportunity for a biological solution to the FHB problem and would avoid the hazards associated with late fungicide application (Parry et al. 1995). Although biocontrol using either microorganisms or biochemicals offers a positive alternative to chemical pesticides, the overall contribution of biocontrol represents about 1 % of agricultural chemical sales, whereas fungicides represent approximately 15 % of pesticide sales (Lidert 2001; Fravel 2005). No commercial biocontrol product has yet been released for the control of FHB disease of cereals, but there is experimental evidence that indicates that this is a feasible disease control strategy.

There has been very limited research on biological control Fusarium seedling blight disease of cereals. In a screen for potential disease control organisms and agents, the bacteria *P. fluorescens* (strains MKB 100, MKB 158, and MKB 249), *P. frederiksbergensis* strain MKB 202, and *Chryseobacterium* sp. strain MKB 277 significantly reduced the extent of wheat and barley seedling blight disease symptoms caused by *F. culmorum* (up to 91 % reduction) (Khan et al. 2006). Strains of *Bacillus cereus* and *Stenotrophomonas maltophilia* have also been shown to reduce Fusarium seedling blight disease caused by *F. graminearum* under glasshouse conditions (Bello et al. 2002). In Sweden, Johansson et al. (2003) reported that treatment of winter and spring wheat with selected isolates of fluorescent pseudomonads and *Pantoea* sp. suppressed seedling blight of wheat caused by *F. culmorum* and *M. nivale* as effectively as did the fungicide guazatine in repeated glasshouse and field trials (by >85 %, relative to control treatments).

The bacteria, *P. fluorescens* strains MKB 158 and MKB 249 and *P. frederiksbergensis* strain 202 were capable of reduced both the severity of FHB disease symptoms caused by *F. culmorum* on wheat and barley ( $\geq$ 23 %; *P*  $\leq$  0.050) and the disease-associated loss in 1,000-grain weight ( $\geq$ 16 %; *P*  $\leq$  0.050) under both glasshouse and field conditions when applied 24 h prepathogen inoculation (Khan and Doohan 2009). Glasshouse studies showed that these bacteria were more effective in controlling disease when applied 24 h pre- as opposed to 24 h postpathogen inoculation. The most striking finding was that, in the *F. culmorum*inoculated field trials, treatment with either of the two *P. fluorescens* strains (MKB 158 or MKB 249) also significantly reduced the DON levels in wheat and barley grain (74–78 %; *P*  $\leq$  0.050). This was the first report detailing the ability of fluorescent pseudomonad bacteria to control FHB disease and simultaneously reduce mycotoxin contamination of wheat and barley under field conditions.

Interestingly, the bacterium *P. fluorescens* strain MKB 158 caused a suppression of expression of key Fusarium gene (Trichodiene synthase) involved in trichothecene mycotoxin biosynthesis in the infected stem base tissue of wheat and augmented expression of a wheat class III plant peroxidase gene (a pathogenesis-related plant defense gene). A soil inoculation test showed that this bacterium can control wheat and barley seedling blight disease symptoms when spatially separated from the pathogen which indicated that it can elicit ISR mechanism in the seedling against the disease. Subsequent functional genomics analysis in our laboratory revealed that MKB 158-mediated ISR against Fusarium in the barley seedling takes place involving novel plant hormone-mediated pathways (Khan et al. unpublished data). Further research is underway in this line to understand the exact role played by these hormones in barley defense against Fusarium. We have not confirmed yet whether the strain MKB 158 can elicit similar ISR against FHB disease as well. Our preliminary functional genomic studies indicate that at least it can elicit a local resistance mechanism with upregulation of many wheat genes in the heads (Petti et al. 2010). The bacterium significantly affected the accumulation of 1,203 barley transcripts associated with diverse functions, including detoxification, cell wall biosynthesis, and the amplification of host defense responses. The transcriptome studies also revealed new insights into bacterium-mediated priming of host defenses against necrotrophs, including the positive effects on grain filling, lignin deposition, oxidative stress responses, and the inhibition of protease inhibitors and proteins that play a key role in programmed cell death.

## 6.7 Future Challenges and Prospects

Intensification of crop cultivation to feed the burgeoning human population demands use of chemicals for controlling cereal diseases. Growing concern about the effects of chemicals has led to increase in demands for organic products throughout the globe. Although research on biological control of plant diseases involving fungi is quite old, there is no commercial biocontrol product available that can substitute chemicals. Growing evidence suggests the potential of bacteria to control fungal cereal diseases. However, more research is necessary to find suitable candidate for each fungal disease which should be able to control diseases in variable environmental conditions as per with chemical agents. Research should target the diverse bacterial populations throughout the globe to find suitable agents, acknowledging the fact that conventional techniques have targeted only the culturable bacteria which constitute only less than 1 % in any given habitat. Therefore, advance molecular tools such as metagenomic research should be employed to explore for potential antifungal genes among the unculturable bacteria.

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