Fusarium head blight (FHB) caused by the filamentous fungus *Fusarium graminearum* is one of the most severe diseases threatening the production of small grains. Infected grains are often contaminated with mycotoxins such as zearalenone and trichothecences. During survey of contamination by FHB in rice grains, we found a bacterial isolate, designated as BN1, antagonistic to *F. graminearum*. The strain BN1 had branching vegetative hyphae and spores, and its aerial hyphae often had long, straight filaments bearing spores. The 16S rRNA gene of BN1 had 100% sequence identity with those found in several *Streptomyces* species. Phylogenetic analysis of ITS regions showed that BN1 grouped with *S. sambsonii* with 77% bootstrap value, suggesting that BN1 was not a known *Streptomyces* species. In addition, the efficacy of the BN1 strain against *F. graminearum* strains was tested both *in vitro* and *in vivo*. Wheat seedling length was significantly decreased by *F. graminearum* infection. However, this effect was mitigated when wheat seeds were treated with BN1 spore suspension prior to *F. graminearum* infection. BN1 also significantly decreased FHB severity when it was sprayed onto wheat heads, whereas BN1 was not effective when wheat heads were point inoculated. These results suggest that spraying of BN1 spores onto wheat heads during the wheat flowering season can be efficient for plant protection. Mechanistic studies on the antagonistic effect of BN1 against *F. graminearum* remain to be analyzed.

**Keywords**: biocontrol agent, fusarium head blight, *Fusarium graminearum*, *Streptomyces*

Fusarium head blight (FHB) is one of the most severe diseases threatening the production of major cereal crops including wheat, barley, and rice. Infected grains are often contaminated with mycotoxins such as zearalenone and trichothecences that cause mycotoxicosis in humans and animals (Desjardins, 2006; Lee et al., 2009). Although several fungal species of the *Fusarium* genus can cause FHB, the ascomycete fungus *F. graminearum* (telomorph: *Gibberella zeae*) is a major causal agent for FHB in Asia and North America (Leslie and Summerell, 2006). In Korea, *F. graminearum* recently caused severe yield losses in wheat, barley, rice, and maize (Lee et al., 2009, 2010, 2012; Ryu et al., 2011). In addition to yield losses and mycotoxin contamination through disease, planting infected seeds can cause root rot and seedling blight (Leslie and Summerell, 2006).

Despite the toxigenic and pathogenic importance of FHB, there are no FHB resistant cultivars and there is only limited information on biological agents against FHB. Fungicide application has shown partial efficacy for FHB control. Azole-type fungicides, one of the most important classes of systemic site-specific fungicides, reduce grain infection and trichothecene contamination (Pirgozliev et al., 2003). The fungicides inhibit cytochrome P450 sterol 14α-demethylase, resulting in the disturbance of fungal membrane integrity (Buchenauer, 1987). However, resistance to azole fungicides occurs in many plant pathogens including *F. graminearum* (Becher et al., 2010), limiting their widespread application and requiring the development of other approaches including resistant cultivars, cultivation methods, and biological control agents.

One of the most plentiful natural resources for the development of biological agents against plant pathogens are the Gram-positive *Actinobacteria*, which have high guanine and cytosine content in their genome. *Streptomyces*, which has highly differentiated branched mycelia, is the largest genus of *Actinobacteria* (Ventura et al., 2007). Many species belonging to this genus produce diverse secondary metabolites, some of which have antibiotic effects and affect soil microbial communities. In agriculture, the genus *Streptomyces* plays important roles in increasing soil fertility, degrading organic matter, and controlling pathogens. For example, this genus is a major soil decomposer that provides natural fertilizers for plants. Additionally, blasticidin S produced by *S. griseochromogenes* is effective for rice blast disease caused by the filamentous fungus *Magnaporthe*.
oryzea, and polyoxin produced by S. asoensis is used for the control of bacterial rice sheath blight and fungal leaf spot (Watve et al., 2001). The diversity of Streptomyces species in soil provides numerous possibilities to explore potential biological control agents and new secondary metabolites that have antagonistic effects against plant pathogens.

Several filamentous fungi, yeast, and bacteria have antagonistic effects against *F. graminearum* (Luz et al., 2003; Pirgozliev et al., 2003) such as *in vitro* antifungal activity (Chan et al., 2003; Xue et al., 2009), the reduction of disease severity (Nourozian et al., 2006; Stockwell et al., 2001; Xue et al., 2009), systemic movement within infected spikes (Yuen et al., 2003), mycotoxin accumulation (Dawson et al., 2004), and pathogen survival (Bujold et al., 2001). Although many studies have focused on the development of biological agents against this disease, only a few biological agents including *Bacillus subtilis* and *Cryptococcus laurentii* strains have been evaluated under diverse growing conditions and developed as commercial biological agents (Pryor et al., 2007; Zhang et al., 2005). Therefore, more biological agents from natural environmental conditions need to be found and applied as alternatives to chemical approaches for control of FHB.

We found a bacterial colony that inhibited the mycelial growth of *F. graminearum*, suggesting that the bacterial strain can be developed as a biological agent against *F. graminearum*. In the present study, we identified the strain based on the sequences of the 16S rRNA gene and internal transcribed space (ITS) region as well as its morphological characteristics, and tested the efficacy of the strain against *F. graminearum* both *in vitro* and *in vivo*. Wheat heads treated with the BN1 strain spore suspension had significantly reduced FHB disease severity caused by *F. graminearum*, showing that the BN1 strain was able to control FHB.

### Materials and Methods

#### Isolation and identification of the bacterial strain BN1.

A bacterial colony was isolated from rice kernels that were placed on potato dextrose agar (PDA; Leslie and Summerell, 2006) to purify *F. graminearum* isolates. The bacterial colony was grown on lysogeny broth (LB; Sambrook and Russell, 2001) agar plate. To obtain chromosomal DNA, the strain, designated as BN1, was incubated in 3 ml of LB broth for 48 h at 28°C in an orbital shaker (200 rpm). Chromosomal DNA was isolated using the NucleoSpin columns (Macherey-Nagel, Duren, Germany) following manufacturer’s instruction. The primers ITS-38R (5'-CGGCCATCCTG-3') and ITS-72F (5'-TGCGGCTG GATCTCCTT-3') (Normand et al., 1996) were used for amplification of the ITS region, and 16S-530F (5'-GACTGAGTGCCAGCMGGCCGG-3') and 16S-1494R (5'-TGACTGACTGAGGTTACCTTGTACGACTT-3') primers were used for amplification of the 16S rRNA gene (Borneeman and Triplett, 1997). The amplified 16S rRNA gene was directly purified using MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology, Sungnam, Korea) and directly sequenced. In the case of ITS region, the purifiedPCR product was cloned in pGEM-T Easy vector (Promega, Madison, WI), and the clone were sequenced. All the amplified DNA fragments and plasmid clones were sequenced using an automated 3730XL DNA sequencer (ABI, Tokyo, Japan). The sequences were used for a BlastN search in the NCBI GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

#### Phylogenetic analysis.

ITS sequences from the 13 *Streptomyces* spp. were retrieved from GenBank database. The sequences were aligned using the MEGA4.1 software with default parameters (Tamura et al., 2007), and trimmed manually at the same position that only includes ITS region. The ITS sequence of *Actinomyces* sp. strain F0330 (GenBank accession number NZ_ACTB01000202) was used as the outgroup for the phylogenetic analysis. A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) in the MEGA4.1 software with the following parameters: complete deletion of gaps, Kimura-2, both transitions and transversions substitutions included, and 2,000 bootstrap replicates.

#### Preparation of inoculum.

The bacterial strain BN1 was incubated in 30 ml of LB for 72 h at 28°C in an orbital shaker (200 rpm) to induce spore production. To harvest BN1 spores, the culture was filtered with two layers of miracloth and spores were harvested by centrifugation at 10,000 g. Spores were washed twice with distilled water and resuspended with 0.1% (v/v) Tween 20 solution (10⁶ spores/ml). To induce spore production of *F. graminearum* strain JL001, the strain was incubated in 30 ml of carboxymethylcellulose (CMC) medium as previously described (Capellini and Peterson, 1965). The cultures were also filtered with two layers of miracloth and spores were harvested by centrifugation at 10,000 g. Spores were washed twice with distilled water and diluted to a final 10⁵/ml concentration in 0.1% (v/v) Tween 20 solution.

#### Antifungal activity assay of the bacterial strain BN1 on diverse media.

The biological efficacy of the BN1 strain against *F. graminearum* was tested on four different media including PDA, yeast malt agar (YMA; Harris, 2005), complete medium (CM), and minimal medium (MM; Leslie