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First Report of Sheath Rot of Rice Caused by *Pantoea ananatis* in Korea

Okhee Choi¹, Hyunyoung Kim¹, Yongsang Lee¹, Jinwoo Kim^{1*}, Jae Sun Moon^{2*} and Ingyu Hwang³

¹Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju 660-701, Korea

²Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-633, Korea

³Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

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Sheath rot symptoms in rice (*Oryza sativa*) were commonly observed during rice growing season in southern part of Korea in 2009 and 2010. Symptoms on glumes and stems of rice ranged from necrotic spot to brown discoloration. Lesion appearance was not uniform, and the margins were often diffused with an indistinct chlorosis. No water-soaking symptom was observed (Fig. 1A). We isolated bacteria from small pieces (3–5 mm) of infected tissue of stem or glume. Tissues were surface-sterilized for 30 s in 1% hypochlorite solution followed by rinsing in sterile distilled water. Tissues were macerated using a pipette tip in 100- μ l of water in a 1.5 ml Eppendorf tube. The macerated tissue was left to soak for 10 min, after which 5 μ l of liquid suspension were streaked onto Tryptic Soy Broth agar medium (TSA). Representative colonies were purified by repeated sub-cultures. Bacterial isolates were stored at –80°C in 25% glycerol.

A representative isolate was grown for 16 h on TSA and suspended at a concentration of 1×10^8 colony forming units/ml in sterile distilled water. Five 4-week-old rice plants were hand-infiltrated with bacterial suspensions or water as a negative control. Inoculated rice plants were kept in greenhouse at 28°C and examined daily to monitor disease symptom development. After 7 days of incubation, sheath rot symptoms were reproduced as observed in paddy fields (Fig. 1B), and the bacterium was re-isolated from the lesions to satisfy Koch's postulates. The control plants injected with water were asymptomatic (Fig. 1C).

To identify isolated bacterium, 16SrDNA, DNA gyrase subunit B (*gyrB*), and RNA polymerase beta subunit (*rpoB*) genes were amplified with proper primers (Navarrete et al., 2010; Yamamoto and Harayama 1995) and all DNA sequences were determined. BLAST search of 16S rDNA (1,506 bp), *gyrB* (1,256 bp), and *rpoB* (359 bp) sequences against the database of type strains of valid prokaryotic names found 99% similarity to those of *Pantoea ananatis* (Brady et al., 2009). In the phylogenetic tree (Fig. 2), the representative isolate was placed within a clade comprising a reference strain of *P. ananatis*.

Fig. 1. Naturally occurred symptoms of rice sheath rot caused by *Pantoea ananatis* (A), and reproduced disease symptoms by artificial inoculation (B). Water treatment was used as a negative control (C). Pictures were taken 10 days after inoculation.

Fig. 2. Phylogenetic tree based on 16S rDNA sequences of *Pantoea* strains. DNA sequences from the NCBI nucleotide database were aligned using ClustalW and a phylogenetic tree was constructed using the neighbor-joining method and visualized with TreeView. Numbers above the branches indicate the bootstrap values. Bars indicate number of nucleotide substitutions per site. The identified isolate PA13 in this study is in bold.

Based on symptoms, pathogenicity, and molecular analysis, we concluded the bacterium isolated from sheath rot is *P. ananatis*. To our knowledge, this is the first report of rice sheath rot caused by *P. ananatis* in Korea. It appears that recent environmental conditions during rice growing season in Korea are favorable for development of sheath rot caused by *P. ananatis*. Recent appearance of rice sheath rot indicates that the disease can be wide spread under favorable weather conditions for infection of *P. ananatis* and symptom development in Korea and other national and international rice growing regions (Cother et al., 2010).

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*Corresponding authors (Jinwoo Kim: E-mail: jinwoo@gnu.ac.kr
 Jae Sun Moon: E-mail: jsmoon@kribb.re.kr)