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Draft genome sequences of *Pseudomonas syringae* pv. *syringae* strain WSPS007 causing bacterial shoot blight on apple

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사과가지마름병원세균 *Pseudomonas syringae* pv. *syringae* WSPS007 균주의 유전체 해독

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Pseudomonas syringae pv. *syringae* strain WSPS007 was isolated from infected twigs (*Malus pumila*) in 2013 in Yeongju, Gyeongbuk Province, Republic of Korea. Here, we report the draft genome sequence of WSPS007 with a chromosome size of 6,238,498 bp (59.04% G+C content). The genome comprises 5,379 CDS, 16 rRNA genes, and 65 tRNA genes. The *P. syringae* pv. *syringae* strain WSPS007 genome possesses an ice-nucleating activation (INA) gene and an antifreeze operon that may be related to frost damage by this pathogen. Thus, the genome sequence determined in this study will be useful in understanding the relationship between the outbreak of bacterial shoot blight disease and frost damage in northern Gyeongbuk Province.

Keywords: *P. syringae* pv. *syringae*, apple, bacterial shoot blight, ice-nucleation

Previously, *Pseudomonas syringae* pv. *syringae* had been reported as a causal agent of bacterial shoot blight on apple (*Malus pumila*) in Korea (Lee *et al*., 2015). In this paper, the authors also described disease incidence of greater than 20% in 20 apple orchards of northern Gyeongbuk Province. Therefore, bacterial shoot blight disease is likely emerging as a new pathogen, creating a problem that is reducing apple yields in those areas. Cultivators from these regions frequently suggest frost damage as the reason for death of trees from this disease. In fact, *P. syringae* pv. *syringae* is well known to cause severe symptoms on apple trees when cold periods are lengthened in early spring and the symptoms are accelerated by ice-nucleation activity of the pathogen (Kennelly *et al*., 2007). Diseases caused by *P. syringae* pv. *syringae* in apple trees were recently reported from Norway (Perminow *et al*., 2018) and the United States (Gašić *et al*., 2018). In these two reports, the diseases were stated to be caused by the ice-nucleating strains of *P. syringae* pv. *syringae*, and the symptoms were significantly aggravated during long periods of cold in the spring. Therefore, the aforementioned concerns of cultivators from northern Gyeongbuk Province are plausible, especially with prolonged cold during the spring, based on the environmental conditions of Korea, Norway, and New York State. Thus, we analyzed the complete genome sequence of the representative *P. syringae* pv. *syringae* strain WSPS007, which was isolated from infected twigs in 2013 in Yeongju, Gyeongbuk Province, Republic of

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Draft genome sequencing of *P. syringae* pv. *syringae* WSPS007 was performed using Pacific Biosciences RSII sequencing platform (Pacific Biosciences) with a 20 kb SMRTbell™ templates library at ChunLab, Inc. Sequences were assembled using the HGAP2 protocol (Pacific Biosciences) and the sequencing depth was 181.74 × coverage of the genome. Genes encoding tRNAs and rRNA operons were searched using the tRNA-scan-SE 1.3.1 database and the Rfam 12.0 database, respectively. The draft genome size of strain WSPS007 was 6,238,498 bp on a single chromosome with 59.04% G+C content. A total of 5,397 CDS, 16 rRNA genes, and 65 tRNA genes were identified (Table 1). Based on the opinions of farmers, we hypothesized that *P. syringae* pv. *syringae* WSPS007 must have ice-nucleating activity expressed by an ice-nucleation gene. *P. syringae* pv. *syringae* WSPS007 showed ice-nucleation activity that could freeze water when the bacterial suspension ($O.D._{600nm}$ = 0.1) was placed on aluminum foil and kept at -5°C for 5 min. The ice-nucleation activity (INA) gene was found as single copy (3,891 bp in size) by the Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) of the National Center for Biotechnology Information (NCBI). The size of the INA gene is larger than in normal prokaryotic microorganisms. Its genome also has an antifreeze gene that is homologous to that in *P. syringae* pv. *syringae* B728a (Feil *et al*., 2005). Thus, *P. syringae* pv. *syringae* WSPS007 can secrete ice-nucleation and antifreeze proteins that simultaneously activate the formation of ice crystals outside the cell and inhibit growth of external ice into the cytoplasm for improved survival of the bacteria, respectively. In addition, the antifreeze gene was organized as an operon composed of a glycosyltransferase, a type I secretion system, and an ABC transporter (periplasmic substrate-binding protein), which may participate in glycosylation and secretion of the antifreeze protein (Feil *et al*., 2005). Conclusively, *P. syringae* pv. *syringae* WSPS007 has the ability to induce ice-nucleating activity by the INA gene and antifreeze operon, thus, we believe that damage in the apple orchards of northern Gyeongbuk Province might be caused by *P. syringae* pv. *syringae* during extended cold periods. We hope to further understand the distinct characterization of frost damage with ice-nucleation activity in Korea.

Table 1. Genome features of *P. syringae* pv. *syringae* strain WSPS007

Genome features	Value
Genome size (bp)	6,238,498
G+C content (%)	59.04
tRNA	65
rRNA	16
CDS	5,379
No. of contigs	3
Sequencing depth of coverage	181.74 ×

Nucleotide sequence accession number

The draft genome sequence of the chromosome of *Pseudomonas syringae* pv. *syringae* WSPS007 has been deposited in the GenBank database under accession number RZUC 00000000. The strain was deposited in the Korean Agricultural Culture Collection (KACC) under the number of KACC 21200.

적 요

Pseudomonas syringae pv. *syringae* WSPS007 균주는 대한민국 경상북도 영주시 사과 과원에서 나타난 가지마름병 병징으로부터 2013년 분리되었다. 본 논문에서는 6,238,498 bp (59.04% G+C 함량)인 WSPS007 균주의 전체 염기서열을 보고한다. 전체 지놈은 5,379개의 코딩서열, 65개의 tRNA, 16개의 rRNA 유전자를 가지고 있다. 특히 WSPS007 균주의 전체 염기서열 분석은 냉해와 관련된 빙핵 활성 유전자 클러스터를 중심으로 분석을 수행하였으며, *P. syringae* pv. *syringae* 국외 대표 균주인 PssB728a와 유사한 빙핵활성 유전자 구성을 가지고 있는 것으로 나타났다. 따라서 본 논문에서의 염기서열 분석 결과를 바탕으로 경상북도 일원 사과 과원에서 동해로 추정되는 병원균의 원인을 규명하기 위한 기본 자료를 제공하는데 있어서 의의가 있다고 사료된다.

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