


# Complete genome sequence of *Serratia rubidaea* AO4-P6, a biocontrol bacterium against fire blight of apple tree

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## 사과 과수화상병을 억제하는 세균, *Serratia rubidaea* AO4-P6 균주의 전장 유전체 분석

전제승 · 김서현 · 오성숙 · 정혜주 · 송재경\* 

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Fire blight is a fatal plant disease caused by the pathogen *Erwinia amylovora* on rosaceous plant, especially apple. *Serratia rubidaea* strain AO4-P6, a biocontrol bacterium against fire blight is isolated from the organic farming soil in Asan-si, Korea. Here, we report the whole genome sequence of *S. rubidaea* strain AO4-P6. The sequence analysis revealed that *S. rubidaea* strain AO4-P6 has a single 4,907,279 bp circular chromosome with a DNA G + C-content of 59.2%. This chromosome contains 4,508 protein-coding sequences together with 22 rRNA and 84 tRNA genes. Further genome analysis revealed that strain AO4-P6 possessed the genes related to the production of disease suppressive secondary metabolites, such as safracin, pyrrolnitrin, and siderophore, as well as the genes associated with bacterial competitions such as chitinase, and multi-drug resistance protein.

**Keywords:** *Serratia rubidaea*, AO4-P6, biocontrol, fire blight, genome

Fire blight caused by the pathogen *Erwinia amylovora* is one of the most destructive plant disease on rosaceous plants including apple and pear. Since fire blight was discovered, it has caused enormous economic losses in the orchards due to

reduction in crop yield but also loss of entire trees (Zhao *et al.*, 2019). Chemical, agrotechnical, biological and quarantine methods have been developed and used to protect plant against fire blight worldwide (Sharifazizi *et al.*, 2017). Due to the increasing rate in chemical pesticide resistance and the raising awareness in sustainable agriculture, biological fire blight management is considered as a promising alternative (Paulin, 2000). Via *in vitro* antibacterial activity test or *in planta* bio-screening assay on apple seedlings, the strain AO4-P6 was selected to use as biocontrol agent against fire blight.

*Serratia rubidaea* strain AO4-P6 was isolated from the organic farming soil in Asan, Korea and grown in tryptic soy broth (TSB) at 28°C for 40 h under aerobic condition at pH between 4.0 and 9.0 (optimum 7.0). To evaluate the antagonistic effect of the strain AO4-P6, its *in planta* disease suppressive activity against *E. amylovora* on M9 apple seedlings was examined. As shown in Fig. 1A, *S. rubidaea* strain AO4-P6 showed to significantly reduce disease severity by 57% when compared with non-treated control. To examine its genomic characteristics, genomic DNA was extracted from the culture of *S. rubidaea* strain AO4-P6 using a MGTM genomic DNA purification kit (MGmed), according to the manufacturer's protocols. The whole genome of *S. rubidaea* strain AO4-P6

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**Table 1.** Genome features of *Paenibacillus tritici* PH55-1

Genome features	Chromosome
Genome size (bp)	4,904,279
G + C content (%)	59.2
Protein-coding genes (CDSs)	4,508
Number of rRNAs	8, 7, 7 (5S, 16S, 23S)
Number of tRNAs	84
ncRNAs	13
Number of pseudogenes	32
Plasmids	0
Accession number (GenBank)	CP110227

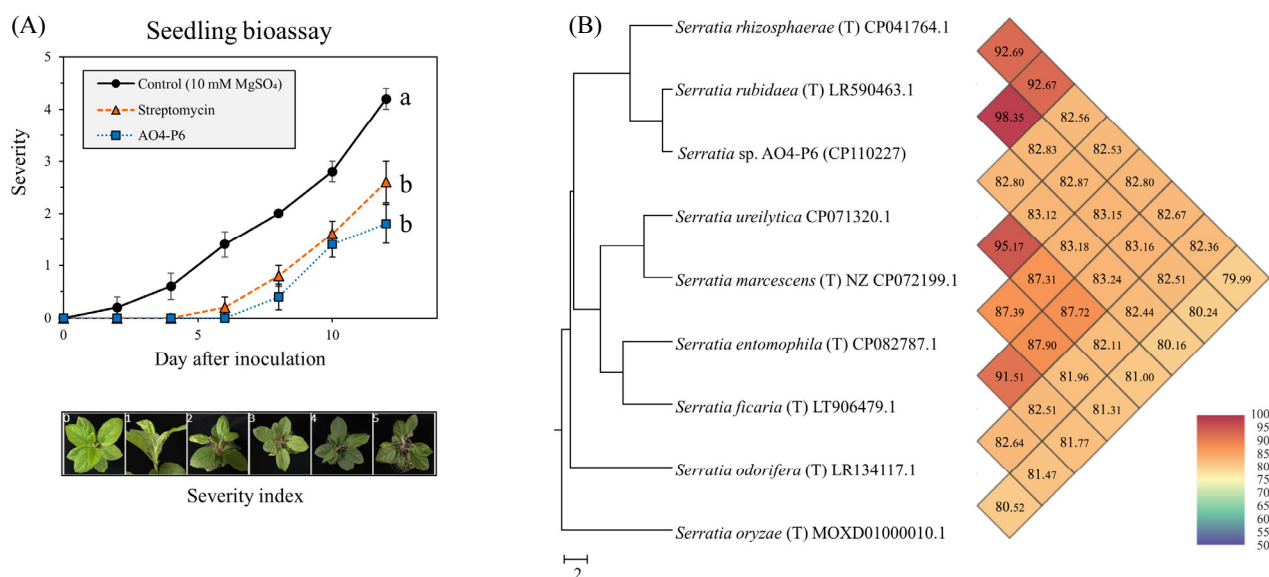
was sequenced by using the PacBio Sequel sequencing platform (Pacific Biosciences) and NovaSeq6000 platform (Illumina) at Macrogen. The genome was assembled using Microbial Assembly Application by Macrogen, and annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al.*, 2016). The complete genome sequence of *S. rubidaea* strain AO4-P6 was found to comprise 4,907,279 bp with an average DNA GC-content of 59.2% and absence of plasmids. The *S. rubidaea* strain AO4-P6 chromosome contains 4,508 protein-coding sequences (CDSs) and 22 rRNA and 84 tRNA genes (Table 1). The OrthoANI values between *S.*

*rubidaea* type strain and AO4-P6 genome sequences were 98.35% which were calculated using the OrthoANI algorithm version v0.93.1 (Lee *et al.*, 2016) (Fig. 1B). The strain AO4-P6 genome has a gene associated with the synthesis of chitinase (*chi*), which is known to chitin degradation related enzyme, several genes (*mtdA*, *mtdB*, *mtdC*, *mtdK*, *mtdL*, *mtdN*, and *mtdO*) which code the multidrug resistance related proteins, and genes (*acrA*, *acrB*, and *acrZ*) which code multidrug efflux pump related proteins. In addition, the AntiSMASH 6.0 (Blin *et al.*, 2021) analysis showed that the strain AO4-P6 codes the genes related to disease suppressive secondary metabolites, such as safracin, pyrrolnitrin, and siderophores.

Overall, the sequence analysis of the strain AO4-P6 genome revealed that it possesses several genes that could elicit antagonistic effect on pathogen. Thus, *S. rubidaea* strain AO4-P6 can be a useful candidate for bio-controlling of fire blight on apple trees.

#### Nucleotide sequence accession number

The whole genome sequence of *S. rubidaea* strain AO4-P6 described in this study was deposited to the National Center for Biotechnology Information (NCBI) with the accession number



**Fig. 1.** Disease severity curve (A) and genetic similarity test of strain AO4-P6 (B). Disease severity curve was examined after *E. amylovora* treatment on AO4-P6 pre-treated M9 apple seedlings. Streptomycin (500 mg/L), an antibiotic, and 10 mM MgSO<sub>4</sub> were utilized as a positive and a negative control, respectively. Different letters show significant difference between the treatments (Non-parametric Kruskal-Wallis-test, followed by Tukey's post-hoc test,  $P < 0.05$ ). Data are presented as mean  $\pm$  standard error,  $n = 5$ . UPGMA and Heatmap were generated with OrthoANI values calculated from the OAT software. The letter (T) means type strain of the species. The scale bar means 2% sequence divergence.

CP110227. Additionally, the strain was deposited to the Korean Agricultural Culture Collection (KACC) (accession number KACC 81224BP).

## 적 요

과수화상병은 병원균인 *Erwinia amylovora*에 의해 발생하는 식물병으로 장미과 식물, 특히 사과나무에 치명적이다. 아산시의 유기농 토양으로부터 분리된 *Serratia rubidaea* AO4-P6 균주는 과수화상병을 억제하는 세균이다. 우리는 *S. rubidaea* AO4-P6 균주의 전장 유전체를 분석을 통해 AO4-P6 균주의 유전체가 4,907,290 bp와 59.2%의 G + C 함량을 가진 하나의 환형 염색체로 구성되었음을 밝혔다. AO4-P6 균주의 유전체는 4,508개의 단백질을 암호화하는 염기서열을 가졌으며, 22개의 rRNA와 84개의 tRNA 유전자를 포함하였다. 유전자 분석결과는, AO4-P6 균주가 safracin, pyrrolnitrin, siderophores 등의 병원균 억제와 관련된 이차대사산물을 암호화하는 유전자를 가졌으며, chitinase, 약제저항성 단백질과 같은 미생물의 경쟁과 관련된 유전자를 가졌음을 밝혔다.

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## Conflict of Interest

The authors have no conflict of interest to report.

## References

- Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Weezel GP, Medema MH, and Weber T.** 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res.* **49**, W29–W35.
- Lee I, Kim YO, Park SC, and Chun J.** 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int. J. Syst. Evol. Microbiol.* **66**, 1100–1103.
- Paulin JP.** 2000. *Erwinia amylovora*: general characteristics, biochemistry and serology, pp. 87–115. In Vanneste JL. (ed.), Fire blight: the disease and its causative agent, *Erwinia amylovora*. CABI Books, CAB international, Wallingford, United Kingdom.
- Sharifazizi M, Harighi B, and Sadeghi A.** 2017. Evaluation of biological control of *Erwinia amylovora*, causal agent of fire blight disease of pear by antagonistic bacteria. *Biol. Control* **104**, 28–34.
- Tatusova T, DiCuccio M, Badretdin A, Chetvermin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, and Ostell J.** 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **44**, 6614–6624.
- Zhao Y, Tian Y, Wang L, Geng L, Zhao W, Hu B, and Zhao Y.** 2019. Fire blight disease, a fast-approaching threat to apple and pear production in China. *J. Integr. Agric.* **18**, 815–820.