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Complete genome sequencing of *Pseudomonas fluorescens* NBC275, a biocontrol agent against fungal pathogens of plants and insects

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식물 및 곤충의 곰팡이 병원균에 항균력을 가진 *Pseudomonas fluorescens* NBC275 균주의 유전체 염기서열

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Pseudomonas fluorescens NBC275 (Pf275) isolated from soil sample collected at riverside of Nakdonggang showed antagonistic activity against fungal pathogens of plants and insects. Here we present complete genome sequence of Pf275. The genome comprises of 6,610,362 bp with GC content of 60.9%, which includes 5,869 predicted protein-coding genes, 16 rRNAs, and 65 tRNAs. Genome analysis revealed gene clusters encoding antimicrobial secondary metabolites such as pyoverdine, 2, 4-diacetylphloroglucinol, and phenazine, which are known to play essential roles in biocontrol of diseases.

Keywords: *Pseudomonas fluorescens*, antagonism, biocontrol, genome sequence, secondary metabolite

Pseudomonas fluorescens strains have been extensively studied to use as biocontrol agents against plant diseases because they antagonize plant pathogens by producing diverse secondary metabolites (Haas and Keel, 2003; Gliese *et al.*, 2010). The comparison of three *P. fluorescens* genomes revealed that only 61% of genes are shared, which indicating considerable divergence of the genomes (Silby *et al.*, 2009). Recently, the whole genome analysis of plant-associated *P. fluorescens* strains also revealed diversity in genome size and genes related to chemotaxis and motility as well as secondary metabolite biosynthesis (Hernández-Salmerón *et al.*, 2017). Therefore, the genomic information of a potential biocontrol strain can improve understanding for the biocontrol performance of the strain.

In this study, the *P. fluorescens* strain Pf275 isolated from soil sample collected at riverside of Nakdonggang, South Korea, exhibited broad antifungal activity against plant pathogens, *Botrytis cinerea*, *Rhizoctonia solani*, and *Alternaria solani* as well as insect pathogens, *Metarhizium anisopliae* and *Beauveria bassiana.* The strain was deposited in the Korean Collection for Type Cultures (KCTC) with accession number of KCTC 13360BP.

Total genomic DNA of Pf275 was extracted using the

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commercial kit (GeneAll® ExgeneTM Cell SV mini kit) and whole-genome sequencing was performed by Macrogen Inc. using PacBio single molecule real-time (SMRT) technology. The sequencing reads were assembled using the RS Hierarchical Genome Assembly Process 3.0 (HGAP) within the SMRT Portal 2.3 (Chin *et al*., 2013). Annotation of the genome was performed using the National Center for Biotechnology Information (NCBI)'s Prokaryotic Genome Annotation Pipeline 2.0 (PGAP) (Tatusova *et al*., 2016). Whole genome sequencing by the PacBio platform produced a total of 94,864 reads with an average length of 10,722 bp and genome coverage depth, about 123. The complete genome sequence was 6,610,362 bp with the GC content of 60.9% and no functional plasmid was detected. The chromosome contains 5,869 coding sequences (CDSs), 65 tRNAs, 16 rRNAs, and 156 pseudogenes (Table 1). Among the CDSs, 4,351 CDS (74.13%) have known functions, while 1,518 CDS (25.86%) have unknown functions.

The genome analysis indicated that Pf275 produces secondary metabolites such as 2, 4-diacetylphloroglucinol, pyoverdines, phenazines, and hydrogen cyanide, which contribute to biocontrol activity of the strain (Couillerot *et al.*, 2009, 2011). The presence of alkaline phosphatase and 1-aminocyclopropane-1-carboxylic acid deaminase genes indicated plant growth-promoting traits (Redondo-Nieto *et al.*, 2013). This strain also possesses around 25 genes related to denitrification which is associated with rhizosphere competence of fluorescent pseudomonads (Ghirardi *et al*., 2012). Overall, the genome analysis indicated that Pf275 promises to be a potential candidate for biocontrol as well as plant growth promotion. The complete genome will allow better understanding of genomic traits involved in antagonism and biocontrol activity of *P. fluorescens*.

Table 1. The features of *Pseudomonas fluorescens* Pf275 genome

Features	Genome (Chromosome)
Genome size (bp)	6,610,362
GC ratio %	60.9
CDS (total)	6,025
CDS (coding)	5,869
Coding %	97.41
Average CDS length (nt)	335.0
No. of rRNA genes (5S, 16S, 23S)	16 (6, 5, 5)
No. of tRNA genes	65
No. of pseudogenes	156
Number of genes with assigned function	4,351
Number of genes without assigned function	1,518

Nucleotide sequence accession number

The genome of *P. fluorescens* NBC275 was made publicly available at NCBI GenBank (Acc. No. CP031648.1).

적요

낙동강 주변에서 채취한 토양으로부터 분리한 *Pseudomonas fluorescens* NBC275 (Pf275) 균주는 식물과 곤충에 병을 일으키는 곰팡이류에 우수한 항균력을 보였다. 본 연구에서는 Pf275 균주의 전체염기서열을 해독하고 분석하였는데, 총 염기서열은 6,610,362 bp였고, GC 함량은 60.9%였다. 염색체는 5,869개의 단백질을 암호화하였고, 16개의 rRNA와 65개의 tRNA로 구성되어 있었다. 유전체의 분석을 통해 항균력을 나타내는 2차 대사산물을 암호화하는 유전자를 확인할 수 있었는데, Pf275 균주는 pyoverdine, 2, 4-diacetylphloroglucinol 및 phenazine 등의 항균물질을 생산하였고, 이들 대사산물에 의해 항균력 및 생물방제효과를 나타내는 것으로 판단된다.

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