Complete genome sequencing of *Pseudomonas parafulva* PpaJBCS1880, a biocontrol and plant growth promoting agent

Swarnalee Dutta¹, Alex Wabyona¹, David Kakembo¹, and Yong Hoon Lee^{1,2*}

식물 병 방제 및 생육촉진 효과를 나타내는 *Pseudomonas parafulva* PpaJBCS1880균주의 유전체 염기서열

더타스와나리¹ · 와비오나알렉스¹ · 카켐보데이비드¹ · 이용훈^{1,2*}[[]

(Received June 27, 2019; Revised August 21, 2019; Accepted August 28, 2019)

Pseudomonas parafulva PpaJBCS1880 (PpaJBCS1880) isolated from rice seeds showed strong antagonistic activity against bacterial plant pathogens by producing lipopeptide. Furthermore, the strain controlled the incidence of bacterial pustule in soybean plants and promoted the growth of rice plants. Here we present complete genome sequence of PpaJBCS1880. The genome comprises of 5,208,480 bp with GC content of 63.4%, which includes 4,487 predicted protein-coding genes, 19 rRNAs, and 74 tRNAs. Genome analysis revealed genes encoding antimicrobial secondary metabolites such as lipopeptide, pyoverdine, phenazine, and hydrogen cyanide, which are known to play essential roles in biocontrol of plant diseases.

Keywords: antagonism, biocontrol, lipopeptide, PGPR

Pseudomonas parafulva (formerly known as Pseudomonas fulva) is a Gram-negative bacterium that is frequently isolated from environment such as rice field (Uchino et al., 2001). In previous reports, P. parafulva CRS01-1 showed antagonism against bacterial and fungal plant pathogens probably by pro-

ducing pyrimidine and benzoate (Liu et al., 2015). P. parafulva PRS09-11288 inhibited Rhizoctonia solani via production of phenazine-1-carboxylic acid (Zhang et al., 2018). In this study, the P. parafulva strain PpaJBCS1880, which was isolated from rice seeds showed strong antibacterial activity against Xanthomonas axonopodis pv. glycines (causal pathogen of bacterial pustule of soybean), Burkholderia glumae (bacterial grain rot of rice), and Xanthomonas oryzae pv. oryzae (bacterial leaf blight of rice) by producing viscosin-like lipopeptide VLIS2 and suppressed incidence of bacterial pustule in soybean plants (Kakembo and Lee, 2019).

Taken together, the results indicate that the *P. parafulva* strains inhibit bacterial or fungal pathogens by producing various secondary metabolites. Therefore, the genomic information of the potential biocontrol agents ultimately increases our understanding of the functions of the strains, which is vital for practical application of biocontrol in the fields. The strain PpaJBCS1880 was deposited in the Korean Agricultural Culture Collection (KACC) with accession number of KACC 92259P.

Total genomic DNA of PpaJBCS1880 was extracted using the commercial kit (GeneAll® ExgeneTM Cell SV Mini Kit)

¹Division of Biotechnology, Chonbuk National University, Iksan 54596, Republic of Korea

²Advanced Institute of Environment and Bioscience, Plant Medical Research Center, and Institute of Bio-industry, Chonbuk National University, Iksan 54596, Republic of Korea

¹전북대학교 생명공학부, ²전북대학교 환경생명신기술연구소

Table 1. The features of Pseudomonas parafulva PpaJBCS1880 genome

| Features | Genome (Chromosome) |
|----------------------------------|---------------------|
| Genome size (bp) | 5,208,480 |
| GC ratio % | 63.4 |
| CDS (total) | 4,544 |
| CDS (coding) | 4,487 |
| Coding % | 98.75 |
| No. of rRNA genes (5S, 16S, 23S) | 19 (7, 6, 6) |
| No. of tRNA genes | 74 |
| No. of pseudogenes | 57 |

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 93,159 reads with an average length of 11,167 bp and genome coverage depth about 157. The complete genome sequence was 5,208,480 bp with the GC content of 63.4%. The chromosome contains 4,487 coding sequences (CDSs), 74 tRNAs and 19 rRNAs (Table 1).

The genome and biosynthetic gene cluster analysis revealed for the first time that PpaJBCS1880 produces novel viscosinlike lipopeptide VLIS2, which predicted to have D-Ser at the second position (Kakembo and Lee, 2019). It also contains genes for the production of pyoverdines, phenazine, and hydrogen cyanide, which contribute to biocontrol activity of the strain (Couillerot et al., 2009). Furthermore, in our hands, PpaJBCS1880 also promoted growth of rice plant. The production of siderophore such as pyoverdine and enzyme such as alkaline phosphatase might contribute to the growth promotion, which requires further exploration (Thakur et al., 2014; Rosier et al., 2018). Overall, the genome analysis indicated that PpaJBCS1880 promises to be a potential candidate for biocontrol as well as plant growth promotion. The complete genome will provide new insight to increase our understanding of the functions of the genes that remain underexplored for biocontrol performance of bacterial biocontrol agents.

Nucleotide sequence accession number

The genome of *P. parafulva* PpaJBCS1880 was made publicly available at NCBI GenBank (Acc. No. CP031641.1).

적 요

벼 종자에서 분리한 Pseudomonas parafulva PpaJBCS1880 (PpaJBCS1880) 균주는 lipopeptide를 분비하여 식물의 세균 병원균에 대해 강력한 항균력을 나타냈다. 또한, PpaJBCS1880 는 콩불마름병의 발생을 억제하였을 뿐만 아니라, 벼의 생육을 촉진하였다. 이에 따라, 본 연구에서는 PpaJBCS1880 균주의 전체염기서열을 해독하고 분석하였는데, 총 염기서열은 5,208,480 bp였고, GC 함량은 63.4%였다. 염색체는 4,487개의 단백질을 암호화하였고, 19개의 rRNA와 74개의 tRNA로구성되어 있었다. 유전체의 분석을 통해 2차 대사산물인 lipopeptide, pyoverdine, phenazine 및 hydrogen cyanide 등을 생산하는 것을 확인하였는데, 이들 대사산물에 의해 항균력, 생물방제 및 생육촉진 효과를 나타내는 것으로 판단된다.

Acknowledgements

We gratefully acknowledge a grant from Next-Generation BioGreen 21 Program (Project No. PJ01316301), Rural Development Administration. This research was also supported by National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (Project No. 2017R1A2B2002221) funded by the Korean government (MSIT), Republic of Korea.

References

Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, et al. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat. Methods 10, 563–569.

Couillerot O, Prigent-Combaret C, Caballero-Mellado J, and Moënne-Loccoz Y. 2009. *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Lett. Appl. Microbiol.* **48**, 505–512.

Kakembo D and Lee YH. 2019. Analysis of traits for biocontrol performance of *Pseudomonas parafulva* PpaJBCS1880 against

- bacterial pustule in soybean plants. Biol. Cont. 134, 72-81.
- Liu Q, Zhang Y, Yu N, Bi Z, Zhu A, Zhan X, Wu W, Yu P, Chen D, Cheng S, et al. 2015. Genome sequence of Pseudomonas parafulva CRS01-1, an antagonistic bacterium isolated from rice field. J. Biotechnol. 206, 89–90.
- Rosier A, Medeiros FHV, and Bais HP. 2018. Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. *Plant Soil* 428, 35–55.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin 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.
- **Thakur D, Kaushal R, and Shyam V.** 2014. Phosphate solubilising microorganisms: role in phosphorus nutrition of crop plants-a review. *Agricul. Rev.* **35**, 159–171.
- Uchino M, Shida O, Uchimura T, and Komagata K. 2001. Recharacterization of *Pseudomonas fulva* lizuka and Komagata 1963, and proposals of *Pseudomonas parafulva* sp. nov. and *Pseudomonas cremoricolorata* sp. nov. *J. Gen. Appl. Microbiol.* 47, 247–261.
- Zhang Y, Chen P, Ye G, Lin H, Ren D, Guo L, Zhu B, and Wang Z. 2018. Complete genome sequence of *Pseudomonas parafulva* PRS09-11288, a biocontrol strain produces the antibiotic phenazine-1-carboxylic acid. *Curr. Microbiol.* **76**, 1087–1091.