

Genome Sequence of the Leaf-Colonizing Bacterium *Bacillus* sp. Strain 5B6, Isolated from a Cherry Tree

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Plant growth-promoting bacteria colonize various habitats, including the phyllosphere. Here, we present the high-quality draft genome sequence of *Bacillus* sp. strain 5B6, which was isolated from the leaf of a cherry tree. The 3.9-Mb genome uncovers its potential for understanding the nature of leaf colonization as well as antibiosis against plant pathogens.

Plant growth-promoting bacteria (PGPB) colonize plant surfaces, including the phylloplane and the rhizoplane, and exert beneficial effects on plant health (5, 11). PGPB have been utilized as biofertilizers and biological control agents against a wide range of phytopathogens, including bacteria and fungi (6). Some species of the genus *Bacillus* were reported as members of PGPB contributing to the supply of mineral nutrients or phytohormones to plants or suppression of plant pathogens by producing antibiotics (12). Studies on plant growth-promoting bacilli have been focused on strains isolated from the rhizosphere with the purpose of application to the root system (4, 8a, 12), while leaf-associated bacilli are much less studied in spite of their potential (9, 14). *Bacillus* sp. strain 5B6, isolated from the leaf of *Prunus avium* L., exhibited a leaf-colonizing capacity and promoted plant growth. The 16S rRNA gene sequences of 5B6 are closely related to those of *Bacillus methylotrophicus* CBMB205 (100%) and *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 (99.9%), well-known plant growth-promoting rhizobacteria.

The genome sequence of 5B6 was determined by next-generation sequencing technology. From a 350-bp paired-end library, 101-bp reads of 3.913 Gb (~1,016-fold coverage) were produced with Illumina/Solexa HiSeq 2000 (NICEM, Republic of Korea). Total paired reads were *de novo* assembled with CLC Genomics Workbench (CLC bio, Inc.). Twenty-five contigs were rearranged in an orderly manner into 17 scaffolds with SSPACE (1). Gap closing was performed using IMAGE (16), perl scripts developed in-house, and primer walking. tRNA and rRNA genes were predicted by tRNAscan-SE and RNAmmer. A total of 3,898 coding sequences were predicted by Glimmer and annotated using information from RAST, GenBank, PFAM, and KEGG (8). The average nucleotide index (ANI) value was calculated with Jspecies (13), and a phage locus occupying a 33.9-kb region was detected by PHAST (17).

Final assembly consists of a single contig of 3,899,657 bp with 46.64% G+C content. Two thousand eight hundred seventy-nine protein-coding sequences were assigned predicted functions. The genome has 10 rRNA operons and 86 tRNAs. The ANI value between 5B6 and FZB42 is 98.7%, whereas the value between 5B6 and DSM7, the type strain of *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens*, is 93.6% (2, 3, 10). Thus, 5B6 is most likely a strain of *B. amyloliquefaciens* based on *in silico* DNA-DNA hybrid-

ization. The genome contains gene clusters possibly involved in biosynthesis of antibiosis-related surfactants such as *srf*, *bmy*, and *fen* (3, 15). Besides these three sets, four gene clusters for biosynthesis of nonribosomal peptides and polyketides that may function as antibiotics similar to bacilysin, difficidin, bacillaene, and macrolactin and two for synthesis and transport of bacillibactin, a high-affinity siderophore that may inhibit the growth of fungal pathogens (12), are present. The genome information suggests that 5B6 has the potential to colonize in the phyllosphere and to act as a biocontrol agent. Comparative genome analysis among strains from various habitats would further help us to study plant-PGPB interactions in each ecological niche.

Nucleotide sequence accession numbers. The draft genome sequence has been deposited in GenBank under accession no. AJST00000000 (first version, AJST01000000). The sequence is also available from the Genome Encyclopedia of Microbes (GEM; <http://www.gem.re.kr>) (7).

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REFERENCES

1. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* 27: 578–579.
2. Borriss R, et al. 2011. Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM 7T and FZB42T: a proposal for *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* subsp. nov. and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on complete genome sequence comparisons. *Int. J. Syst. Evol. Microbiol.* 61:1786–1801.
3. Chen XH, et al. 2007. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nat. Biotechnol.* 25:1007–1014.
4. Compant S, Duffy B, Nowak J, Clement C, Barka EA. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* 71:4951–4959.
5. Esitkena A, Pirlakb L, Turanc M, Sahin F. 2006. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. *Sci. Hortic.* 110:324–327.
6. Heil M, Bostock RM. 2002. Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Ann. Bot.* 89:503–512.
7. Jeong H, Yoon SH, Yu DS, Oh TK, Kim JF. 2008. Recent progress of microbial genome projects in Korea. *Biotechnol. J.* 3:601–611.
8. Kim BK, et al. 2011. Genome sequence of an ammonia-oxidizing soil archaeon, “*Candidatus Nitrosoarchaeum koreensis*” MY1. *J. Bacteriol.* 193:5539–5540.
- 8a. Kim JF, et al. 2010. Genome sequence of the polymyxin-producing plant-probiotic rhizobacterium *Paenibacillus polymyxa* E681. *J. Bacteriol.* 192: 6103–6104.
9. Kishore GK, Pande S, Podile AR. 2005. Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut (*Arachis hypogaea* L.). *Lett. Appl. Microbiol.* 40:260–268.
10. Konstantinidis KT, Tiedje JM. 2005. Genomic insights that advance the species definition for prokaryotes. *Proc. Natl. Acad. Sci. U. S. A.* 102:2567–2572.
11. Lindow SE, Brandl MT. 2003. Microbiology of the phyllosphere. *Appl. Environ. Microbiol.* 69:1875–1883.
12. Lugtenberg B, Kamilova F. 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63:541–556.
13. Richter M, Rossello-Mora R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. U. S. A.* 106: 19126–19131.
14. Ryu C, et al. 2011. Potential for augmentation of fruit quality by foliar application of bacilli spores on apple tree. *Plant Pathol. J.* 27:164–169.
15. Stanley NR, Lazazzera BA. 2004. Environmental signals and regulatory pathways that influence biofilm formation. *Mol. Microbiol.* 52:917–924.
16. Tsai IJ, Otto TD, Berriman M. 2010. Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps. *Genome Biol.* 11:R41. doi:10.1186/gb-2010-11-4-r41.
17. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res.* 39:W347–W352.