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Complete Genome Sequence of *Bacillus subtilis* BSn5, an Endophytic Bacterium of *Amorphophallus konjac* with Antimicrobial Activity for the Plant Pathogen *Erwinia carotovora* subsp. *carotovora*[▽]

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Here, we present the complete genome sequence of *Bacillus subtilis* strain BSn5, isolated from *Amorphophallus konjac* calli tissue and showing strong inhibitory activity to *Erwinia carotovora* subsp. *carotovora*, which causes *Amorphophallus* soft rot disease and affects the industry development of this organism.

Strain BSn5 was isolated from *Amorphophallus konjac* calli tissue during the course of *Amorphophallus konjac* gene transformation. Its 16S rRNA sequence shares 99.0% similarity with that of *Bacillus subtilis*, which has been widely used in the agricultural biocontrol field (1, 2). Its crude protein extract, with 30% ammonium sulfate precipitation from the culture supernatant, mainly consisting of a 31.6-kDa protein as detected by SDS-PAGE, showed strong inhibitory activity toward *Erwinia carotovora* subsp. *carotovora* (9), which causes soft rot disease in a wide variety of plants. However, the exact active component has not been identified yet (3, 5, 11, 14).

Whole-genome sequencing of BSn5 was performed with a strategy involving Solexa paired-end sequencing technology. A total of 4,751,320 pair end reads were generated to reach a depth of 169-fold coverage with an Illumina Solexa GA IIx (Beijing Genomics Institute at Shenzhen, China), and about 97.2% of the reads were assembled into 30 scaffolds using the SOAPdenovo alignment tool (http://soap.genomics.org.cn/index.html#intro2). Both the gaps within the scaffolds and those between the scaffolds were filled through sequencing of PCR products by primer walking through the use of an ABI 3730 capillary sequencer.

Its complete genome sequence is composed of a circular 4,093,599-bp chromosome with a mean GC content of 43.85%. There are 4,177 coding genes, 10 rRNA operons, and 83 tRNAs in the chromosome as annotated by PGAAP (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). Comparative genome analysis was performed with Mauve and Artemis software (7, 13).

Based on the phylogenetic trees inferred from the whole genomes by PTreeRec (8), strain BSn5 was found (in order from near to far) close to *B. subtilis* 168, *Bacillus subtilis natto* BEST 195, *Bacillus subtilis* subsp. *spizizenii* W23, and *Bacillus amyloliquefaciens* FZB42.

Compared with *B. subtilis* strain 168 (4, 12), 9 DNA fragments (>5 kb) were found inserted and 8 DNA fragments (>5 kb) were lost in strain BSn5. These changes were involved in prophage, cell wall synthesis, antibiotic synthesis, sporulation regulation, mobile elements, a restriction modification system, and the major facilitator superfamily MFS, which may lead to strain BSn5, an endophytic bacterium.

More than 4.8% of the BSn5 genome may be devoted to synthesizing antimicrobial products, including NRPS, PKS, hybrid NRPS/PKS antibiotics, and lantibiotics. Among these products, the genes related to the synthesis of a potential NRPS/PKS antibiotic (BSn5_04295 to BSn5_04355) and a paenibacillin-like lantibiotic (BSn5_12550) were absent in *B. subtilis* 168. Also, some lipopeptides and polyketides, including surfactin, fengycin, bacillibactin, and bacillaene, might be produced by strain BSn5 due to its complete *sfp* gene but were not expressed in *B. subtilis* 168, due to a mutation of *sfp* (6). Meanwhile, a sublancin-specifying gene cluster and a spore-killing factor synthesis cluster in *B. subtilis* 168 were lost in stain BSn5. All the differences between these strains likely endowed increased fitness on strain BSn5 in *Amorphophallus konjac*, while strain 168 tends toward fitness under laboratory conditions (10).

In summary, the genome sequence of the strain BSn5 provides the opportunity to further understand the property of an endophytic strain and reveals a potential to produce secondary metabolites.

Nucleotide sequence accession number. The complete genome sequence of strain BSn5 is available in GenBank under accession number CP002468.

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