





Complete Genome Sequence of *Bacillus* subtilis J-5, a Potential Biocontrol Agent

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ABSTRACT *Bacillus subtilis* J-5 was isolated from tomato rhizosphere soil and exhibited strong inhibitory activity against *Botrytis cinerea*. To shed light on the molecular mechanism underlying the biological control on phytopathogens, the whole genome of this strain was sequenced. Genes encoding antimicrobial compounds and the regulatory systems were identified in the genome.

B acillus subtilis has been widely described as an important biocontrol strain. It has the ability to suppress phytopathogens by producing antimicrobial compounds, such as antibiotics, lantibiotics, and bacillibactin, competing for nutrients and niches with pathogens, and inducing systemic resistance to pathogens (1–3). In addition, *B. subtilis* is approved to promote plant growth (4). Recently, we isolated a strain from tomato rhizosphere soil. This strain has strong inhibitory effect on the growth of *Botrytis cinerea*, a fungal pathogen causing gray mold in tomato. Biochemical and molecular biological analyses assigned this strain to *B. subtilis* J-5. The lipopeptides were identified from the culture broth of *B. subtilis* J-5. Moreover, the volatile substances of *B. subtilis* J-5 were demonstrated to strongly inhibit the growth of *B. cinerea in vitro*. To gain more knowledge on the genetic equipment of this bacterium and provide more insight into the mechanism by which this bacterium plays its biocontrol roles, we sequenced and annotated the complete genome sequence of this strain.

Whole-genome sequencing of strain J-5 was performed on the PacBio RSII sequencing platform at Novogene (Beijing, China). The genomic DNA was randomly sheared to 10-kb target size using PacBio RSII (5). It gives 87 million bases with approximately 211-fold genome sequence coverage. The reads were assembled with SMRT Portal 2.03 software (6). We used GeneMarkS (7) (http://topaz.gatech.edu/) to predict bacterial coding genes. Genomic islands was predicted using software IslandPath-DIOMB. The rRNAmmer software was used to predict rRNAs, the tRNAscan software was employed to predict tRNA regions and tRNA secondary structure, and the Rfam software was used to predict small RNAs (sRNAs). The secondary metabolite gene cluster was identified using the antiSMASH program (8). Gene annotation was added using the NCBI Prokaryotic Genome Annotation Pipeline (9).

The complete genome sequence of *B. subtilis* J-5 is characterized by a circular chromosome of 4,117,900 bp, with a mean G+C content of 46.11%. The chromosome contains 4,312 coding genes, 87 tRNAs, 27 rRNAs, and 9 sRNAs. No plasmid was found in this strain. Genome analysis revealed that the genome of J-5 contains 9 gene clusters devoted to the synthesis of antimicrobial compounds, including polyketide synthase (PKS) antibiotics (type 2 PKS [T2PKS], TransPKS, type 3 PKS [T3PKS], transAT PKS-nonribosomal peptide synthetase [TATPKS-NRPS]), NRPS antibiotics (NRPS-bacteriocin, NRPS), lantibiotics, bacillibactin, and terpene. The two-component signal transduction systems function in response to prokaryotes under a variety of external conditions. It was uncovered that a total of 48 two-component systems (TCS) exist in the genome of strain J-5, including ComP/ComA (10) DegS/DegV (11), QseC/QseB (12), PhoR/PhoP (13),

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and DesK/DesR (14). The genes encoding RpoS and RpoN (15), which play roles in bacterial adaptation to environmental stress, were also identified in the genome of this strain.

Accession number(s). The complete genome sequence of *B. subtilis* J-5 has been deposited at GenBank under the accession number CP018295. The version described in this paper is the first version. This strain has been deposited at the China General Microbiological Culture Collection Center (CGMCC no. 11750).

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REFERENCES

- Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Paré PW, Bais HP. 2010. The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 3:130–138. https://doi.org/10.4161/cib.3.2.10584.
- 2. Desoignies N, Schramme F, Ongena M, Legrève A. 2013. Systemic resistance induced by *Bacillus* lipopeptides in *Beta vulgaris* reduces infection by the rhizomania disease vector *Polymyxa betae*. Mol Plant Pathol 14:416–421. https://doi.org/10.1111/mpp.12008.
- Lakshmanan V, Selvaraj G, Bais HP. 2014. Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol 166: 689–700. https://doi.org/10.1104/pp.114.245811.
- Rudrappa T, Czymmek KJ, Paré PW, Bais HP. 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556. https://doi.org/10.1104/pp.108.127613.
- Ee R, Lim YL, Yin WF, Chan KG. 2014. De novo assembly of the quorum sensing Pandoraea sp. strain RB-44 complete genome sequence using PacBio single-molecule real-time sequencing technology. Genome Announc 2(2):e00245-14. https://doi.org/10.1128/genomeA.00245-14.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/ nmeth.2474.
- Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes: implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. https://doi.org/10.1093/nar/29.12.2607.
- 8. Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T. 2013. antiSMASH 2.0 a versatile platform for genome mining ofsecondary metabolite producers. Nucleic Acids Res 41:W204–W212. https://doi.org/10.1093/nar/gkt449.

- Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta) genomic annotation. OMICS 12:137–141. https://doi.org/10.1089/omi.2008.0017.
- Wang X, Luo C, Liu Y, Nie Y, Liu Y, Zhang R, Chen Z. 2010. Three nonaspartate amino acid mutations in the ComA response regulator receiver motif severely decrease surfactin production, competence development and spore formation in *Bacillus subtilis*. J Microbiol Biotechnol 20:301–310.
- Mariappan A, Makarewicz O, Chen XH, Borriss R. 2012. Two-component response regulator DegU controls the expression of bacilysin in plantgrowth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. J Mol Microbiol Biotechnol 22:114–125. https://doi.org/10.1159/000338804.
- Liu J, Hu L, Xu Z, Tan C, Yuan F, Fu S, Cheng H, Chen H, Bei W. 2015. Actinobacillus pleuropneumoniae two-component system QseB/QseC regulates the transcription of PilM, an important determinant of bacterial adherence and virulence. Vet Microbiol 177:184–192. https://doi .org/10.1016/j.vetmic.2015.02.033.
- Guo Q, Li S, Lu X, Li B, Ma P. 2010. PhoR/PhoP two-component regulatory system affects biocontrol capability of *Bacillus subtilis* NCD-2. Genet Mol Biol 33:333–340. https://doi.org/10.1590/S1415-47572010005000032.
- Beckering CL, Steil L, Weber MH, Völker U, Marahiel MA. 2002. Genomewide transcriptional analysis of the cold shock response in *Bacillus* subtilis. J Bacteriol 184:6395–6402. https://doi.org/10.1128/JB.184.22 6395-6402 2002
- Sapi E, Theophilus PA, Pham TV, Burugu D, Luecke DF. 2016. Effect of RpoN, RpoS and LuxS pathways on the biofilm formation and antibiotic sensitivity of *Borrelia burgdorferi*. Eur J Microbiol Immunol (Bp) 6:272–286. https://doi.org/10.1556/1886.2016.00026.

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