

The Complete Genome of *Bacillus amyloliquefaciens* subsp. *plantarum* CAU B946 Contains a Gene Cluster for Nonribosomal Synthesis of Iturin A

Jochen Blom,^a Christian Rueckert,^a Ben Niu,^{b,c} Qi Wang,^b and Rainer Borriss^{c,d}

Computational Genomics, Center for Biotechnology (CeBiTec), Universität Bielefeld, Bielefeld, Germany^a; MOA Key Laboratory of Plant Pathology, Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing, People's Republic of China^b; Bakteriengenetik, Institut für Biologie, Humboldt-Universität Berlin, Berlin, Germany^c; and ABITEP GmbH, Berlin, Germany^d

The genome of the rhizobacterium *Bacillus amyloliquefaciens* subsp. *plantarum* CAU B946 was 4.02 Mb in size and harbored 3,823 genes (coding sequences [CDS]). Nine giant gene clusters were dedicated to nonribosomal synthesis of antimicrobial compounds. Remarkably, strain CAU B946 possessed a gene cluster involved in synthesis of iturin A.

Plant growth-promoting rhizobacteria (PGPR) have been applied as environmentally friendly alternatives of agrochemicals to improve crop yield and quality (10). The rhizobacterial strains belonging to *Bacillus amyloliquefaciens* subsp. *plantarum* (2) are known for their ability to promote plant growth by producing indole-3-acetic acid (IAA) (8) and volatile compounds (1). *B. amyloliquefaciens* FZB42 was shown to produce an array of secondary metabolites (3, 5, 11, 15, 16) which are important in controlling plant pathogens. Due to these properties, *B. amyloliquefaciens* subsp. *plantarum* is increasingly used as biofertilizer and as a biocontrol agent in agriculture (1). Recently, several representatives of industrially important *B. amyloliquefaciens* subsp. *amyloliquefaciens*, including the strain DSM7^T, have been completely sequenced (7, 14, 17, 18). However, from the plant-associated *B. amyloliquefaciens* subsp. *plantarum* group, only FZB42^T has been completely sequenced (4). Here, we report the genome sequence of the plant-associated strain CAU B946.

Strain CAU B946, isolated from the rice rhizosphere, was identified by 16S rRNA gene and *gyrA* gene sequencing and by physiological and biochemical analysis as being *Bacillus amyloliquefaciens* subsp. *plantarum* (2). Due to its capability to produce antibiotics, some products developed from strain CAU B946 have already been applied as biofungicides to control several plant diseases, such as tobacco black shank, rice sheath blight, cotton fusarium wilt, cotton verticillium wilt, and wheat scab (Q. Wang, unpublished data).

Genomic DNA prepared from strain CAU B946 was used for construction of a 3-kb-long paired-end library with a GS FLX library preparation kit in combination with GS FLX paired-end adaptors (both Roche, Mannheim, Germany) according to the manufacturer's protocol. The reads were assembled using the GS *de novo* assembler, and the resulting scaffolds were oriented based on the occurrence of unique single nucleotide polymorphisms (SNPs) in the repetitive rRNA gene clusters. Utilization of the paired-end information allowed scaffolding of the contigs larger than 500 bp. Gap closure was done by long-range PCR (using Phusion polymerase; New England BioLabs, Frankfurt am Main, Germany) and subsequent Sanger sequencing (IIT Biotech, Bielefeld, Germany). Prediction of protein-encoding sequences was initially accomplished with REGANOR (12). Manual and automatic annotations were done using the annotation software GenDB 2.4 (13).

The complete genome sequence of strain CAU B946 consisted of a circular 4,019,861-bp chromosome with a G+C value of 46.51%. The genome was slightly larger than that of strain FZB42 and was characterized by many phage-derived genes not present in the FZB42 genome. The chromosome consisted of 3,823 genes (coding sequences [CDS]), 10 rRNA operons, and 95 tRNAs.

Strain CAU B946 possessed a unique type I restriction modification system. Nine gene clusters involved in nonribosomal synthesis of lipopeptides, polyketides, and bacilysin were identified. Similar to the genome of FZB42 (6), about 8.5% of the whole CAU B-946 genome was involved in nonribosomal synthesis of antimicrobial compounds and siderophores. In addition, the complete gene cluster for synthesis and modification of the highly modified microcin plantazolicin (9), recently detected in strain FZB42 (16), was present in the genome of CAU B946.

Nucleotide sequence accession number. The complete sequence of the CAU B-946 genome has been deposited in EMBL (accession number [HE617159](https://www.ebi.ac.uk/EMBL/nuclseq/HE617159)).

ACKNOWLEDGMENTS

We thank Hai Sun for his help in annotating the B946 genome.

Financial support for R.B. from the competence network Genome Research on Bacteria (GenoMikPlus) and the Chinese-German collaboration program by the German Ministry for Education and Research (BMBF) is gratefully acknowledged.

REFERENCES

1. Borriss R. 2011. Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents, p 41–76. In Maheshwari DK (ed), *Bacteria in agrobiology: plant growth responses*. Springer Heidelberg, Heidelberg, Germany.
2. Borriss R, et al. 2011. Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM7^T and FZB42^T: 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 X-H, et al. 2006. Structural and functional characterization of three

Received 18 December 2011 Accepted 19 January 2012

Address correspondence to Rainer Borriss, rborriss@abitep.de.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.06762-11

- polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB42. *J. Bacteriol.* 188:4024–4036.
4. 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.
 5. Chen XH, et al. 2009. Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *J. Biotechnol.* 140:38–44.
 6. Chen XH, Koumoutsis A, Scholz R, Borriss R. 2009. More than anticipated—production of antibiotics and other secondary metabolites by *Bacillus amyloliquefaciens* FZB42. *J. Mol. Microbiol. Biotechnol.* 16:14–24.
 7. Geng W, et al. 2011. Complete genome sequence of *Bacillus amyloliquefaciens* LL3, which exhibits glutamic acid-independent production of poly- γ -glutamic acid. *J. Bacteriol.* 193:3393–3394.
 8. Idris EE, Iglesias DJ, Talon M, Borriss R. 2007. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Mol. Plant Microbe Interact.* 20:619–626.
 9. Kalyon B, et al. 2011. Plantazolicin A and B: structure elucidation of ribosomally synthesized thiazole/oxazole peptides from *Bacillus amyloliquefaciens* FZB42. *Org. Lett.* 13:2996–2999.
 10. Kloepper JW, Leong J, Teintze M, Schroth MN. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* 286:885–886.
 11. Koumoutsis A, et al. 2004. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *J. Bacteriol.* 186:1084–1096.
 12. Linke B, McHardy AC, Neuweger H, Krause L, Meyer F. 2006. REGANOR: a gene prediction server for prokaryotic genomes and a database of high quality gene predictions for prokaryotes. *Appl. Bioinformatics* 5:193–198.
 13. Meyer F, et al. 2003. GenDB—an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res.* 31:2187.
 14. Rueckert C, Blom J, Chen X-H, Reva O, Borriss R. 2011. Genome sequence of *Bacillus amyloliquefaciens* type strain DSM7^T reveals differences to plant-associated *Bacillus amyloliquefaciens* FZB42. *J. Biotechnol.* 155:78–85.
 15. Schneider K, et al. 2007. Macrolactin is the polyketide synthesis product of the *pkc2* cluster of *Bacillus amyloliquefaciens* FZB42. *J. Nat. Prod.* 70:1417–1423.
 16. Scholz R, et al. 2011. Plantazolicin, a novel microcin B17/streptolysin S-like natural product from *Bacillus amyloliquefaciens* FZB42. *J. Bacteriol.* 193:215–224.
 17. Yang H, Liao Y, Wang B, Lin Y, Pan L. 2011. Complete genome sequence of *Bacillus amyloliquefaciens* XH7, which exhibits production of purine nucleosides. *J. Bacteriol.* 193:5593–5594.
 18. Zhang G, et al. 2011. Complete genome sequence of *Bacillus amyloliquefaciens* TA208, a strain for industrial production of guanosine and ribavirin. *J. Bacteriol.* 193:3142–3143.