

The Genome of Plant Growth-Promoting *Bacillus amyloliquefaciens* subsp. *plantarum* Strain YAU B9601-Y2 Contains a Gene Cluster for Mersacidin Synthesis

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The genome of rhizobacterium *Bacillus amyloliquefaciens* subsp. *plantarum* YAU B9601-Y2 was 4.24 Mb in size and harbored 3,991 coding sequences (CDS). Giant gene clusters were dedicated to nonribosomal synthesis of antimicrobial lipopeptides and polyketides. Remarkably, CAU B946 possessed a gene cluster involved in synthesis of mersacidin.

The aerobic, endospore-forming rhizobacteria belonging to *Bacillus amyloliquefaciens* subsp. *plantarum* are known for enhancing the yield of crop plants and to suppress microbial plant pathogens (2, 3). The type strain, *B. amyloliquefaciens* FZB42^T, was shown to synthesize an array of antimicrobial secondary metabolites (5, 7, 10, 14) and to produce plant growth-promoting compounds, such as indole-3-acetic acid (IAA) (9), and volatile compounds (2). Recently, several representatives of industrially important *B. amyloliquefaciens* subsp. *amyloliquefaciens*, including type strain DSM7^T, have been sequenced (8, 13, 15, 16). However, from the plant-associated *B. amyloliquefaciens* subsp. *plantarum* group, only FZB42^T and CAU B946 have been completely sequenced (1, 6). Here, we report the genome sequence of the plant-associated strain YAU B9601-Y2.

Strain YAU B9601-Y2, isolated from the wheat rhizosphere in North China, was identified as being *B. amyloliquefaciens* subsp. *plantarum* (3). The strain suppresses a broad spectrum of pathogenic fungi; promotes growth and rooting of crops and vegetables; improves the drought tolerance of wheat, corn, and broad bean; and reduces the number of nematodes at tomato and tobacco roots (Y. He, unpublished data).

Genomic DNA prepared from YAU B9601-Y2 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 RRN contigs. 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, Germany) and subsequent Sanger sequencing (IIT Biotech, Bielefeld, Germany). Prediction of protein-encoding sequences was initially accomplished with REGANOR (11). Manual and automatic annotations were done using the annotation software GenDB 2.4 (12).

The complete genome sequence of YAU B9601-Y2 consisted of a circular 4,242,774-bp chromosome with a G+C value of 45.85%. The genome was larger than FZB42 due to many phase insertions not present in FZB42. The chromosome consisted of 3,991 genes (CDS), 10 rRNA operons, and 91 tRNAs.

Nine gene clusters, covering 8.5% of the whole genome, were

involved in nonribosomal synthesis of lipopeptides, such as bacilomycin D and fengycin; the polyketides bacillaene, difficidin, and macrolactin; and the dipeptide bacilysin. The complete gene cluster for synthesis and modification of mersacidin, a type B lantibiotic, was detected in YAU-B9601. Notably, the mersacidin operon detected in YAU B946 did mirror perfectly the gene cluster of the mersacidin producer strain HIL Y-85 (4).

Nucleotide sequence accession number. The complete sequence of YAU B9601-Y2 has been deposited in EMBL (accession number [HE774679.1](https://www.ebi.ac.uk/EMBL/nuclseq/HE774679.1)).

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REFERENCES

- Blom J, Rueckert C, Niu B, Borriss R. 2012. The complete genome of *Bacillus amyloliquefaciens* subsp. *plantarum* CAU B946 contains a gene cluster for nonribosomal synthesis of iturin A. *J. Bacteriol.* 194:1845–1846.
- 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.
- 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.
- Chatterjee S, et al. 1992. Mersacidin, a new antibiotic from *Bacillus*: fermentation, isolation, purification and chemical characterization. *J. Antibiot.* 45:832–838.
- Chen XH, et al. 2006. Structural and functional characterization of three

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- polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB42. *J. Bacteriol.* **188**:4024–4036.
6. 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.
 7. 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.
 8. 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.
 9. Idris ES, 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.
 10. 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.
 11. Linke B, McHardy AC, Neuweber 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.
 12. Meyer F, et al. 2003. GenDB—an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res.* **31**:2187.
 13. Ruckert C, Blom J, Chen XH, 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.
 14. Schneider K, et al. 2007. Macrolactin is the polyketide synthesis product of the *pkS2* cluster of *Bacillus amyloliquefaciens* FZB42. *J. Nat. Prod.* **70**:1417–1423.
 15. 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.
 16. 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.