



Genome Sequence of a Gram-Positive Diazotroph, *Paenibacillus durus* Type Strain ATCC 35681

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Here, we report the complete genome sequence of *Paenibacillus durus* type strain ATCC 35681, which can fix atmospheric nitrogen even in the presence of nitrate.

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Paenibacillus durus ATCC 35681^T is a nitrogen-fixing bacterium and can be found as free-living or in a symbiotic relationship with plant roots. Here, we report the complete genome sequence of *P. durus* ATCC 35681^T, a unique strain which can fix atmospheric nitrogen even in the presence of nitrate (1). Members of this species are normally found within the maize, sugarcane, wheat, and sorghum rhizospheres (2). It was previously known as *Bacillus azotofixans, Paenibacillus azotofixans*, and *Paenibacillus durum* before its taxonomic status was revised to its current name of *P. durus* (3, 4).

In a previous study, three *nifH*-homologues were reported in *P. durus* (5). The occurrence of multiple *nifH* genes within a single organism suggested that they serve to modulate nitrogenase activity under certain environmental conditions (6). Such traits make the study of the regulation of the multiple *nifH* genes of *P. durus* under varying environmental conditions and its adaptation to different ecological niches interesting.

The genome was sequenced using the Pacific Biosciences RS (PacBio) and Illumina HiSeq2000 platforms. A 10-Kb single-pass library was performed for PacBio while for the Illumina platform, a 500 bp paired-end library was used. These resulted in 14-fold and 110-fold coverage for the PacBio and Illumina platforms, respectively. PacBio genome data was assembled using the "RS_HGAP_Assembly.2" protocol included in SMRT Portal version 2.3.0. The Illumina reads were used for error correction. CONTIGuator version 2.7, a bacterial genome finishing software tool, was used to re-arrange and join all the contigs into one scaffold (7, 8). PCR primers were subsequently developed to close any remaining gaps between the scaffolds by DNA amplification and sequencing.

The genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline to predict protein coding genes, structural RNAs, small noncoding RNAs, and tRNAs (9). Additional annotations were performed using the RAST server (SEED and rapid annotation tools) (10) and BASys (11).

The genome of *P. durus* ATCC 35681^{T} consists of a circular chromosome of 5,575,484 bp in length and a G+C content of 51%. No plasmid was detected. Among the 5,233 predicted genes,

4,808 were identified as protein coding genes and 125 were RNAs. From the assigned protein coding genes, 2,904 has putative function while the remaining 1,904 genes were annotated as hypothetical proteins.

Spore formation and nitrogen-fixation genes were detected in the genome of *P. durus* ATCC 35681^T. The genome harbors six copies of *nifH* homologs and one of these encoded an alternative dinitrogenase reductase (5, 12). This phenomenon of having multiple copies of *nifH* has been reported in various diazotrophs such as *Clostridium pasteurianum*, which likewise has six copies of *nifH*, one of which codes for the alternative vanadium-containing nitrogenase (6, 13, 14). The *nifH1* homolog lies within a nitrogen fixation gene cluster consisting of *nifB1*, *nifH1*, *nifD1*, *nifK*, *nifE*, *nifN*, *nifX*, *hesA*, *nifV* resembling the arrangement in *Paenibacillus* sp. WLY78 (15).

Nucleotide sequence accession number. The genome sequence of *P. durus* ATCC 35681^{T} has been deposited in GenBank under the accession number CP011114.

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REFERENCES

- Seldin L, Van Elsas JD, Penido EGC. 1984. Bacillus azotofixans sp. nov., a nitrogen-fixing species from Brazilian soils and grass roots. Int J Syst Bacteriol 34:451–456. http://dx.doi.org/10.1099/00207713-34-4-451.
- Seldin L, Rosado AS, da Cruz DW, Nobrega A, van Elsas JD, Paiva E. 1998. Comparison of *Paenibacillus azotofixans* strains isolated from rhizo-

plane, rhizosphere, and non-root-associated soil from maize planted in two different Brazilian soils. Appl Environ Microbiol **64:**3860–3868.

- 3. Trüper HG. 2003*Paenibacillus durus* (Collins et al. 1994, formerly *Clostridium durum* Smith and Cato 1974) has priority over *Paenibacillus azotofixans* (Seldin et al. 1984). Opinion 73. Int J Syst Evol Microbiol 53:931.
- Rosado AS, van Elsas JD, Seldin L. 1997. Reclassification of *Paenibacillus durum* (formerly *Clostridium durum* Smith and Cato 1974) Collins et al. 1994 as a member of the species *P. azotofixans* (formerly *Bacillus azotofixans* Seldin et al. 1984) Ash et al. 1994. Int J Syst Bacteriol 47:569–572. http://dx.doi.org/10.1099/00207713-47-2-569.
- Choo Q-C, Samian M-R, Najimudin N. 2003. Phylogeny and characterization of three *nifH*-homologous genes from *Paenibacillus azotofixans*. Appl Environ Microbiol 69:3658–3662. http://dx.doi.org/10.1128/ AEM.69.6.3658-3662.2003.
- Wang SZ, Chen JS, Johnson JL. 1988. The presence of five *nifH*-like sequences in *Clostridium pasteurianum*: sequence divergence and transcription properties. Nucleic Acids Res 16:439–454. http://dx.doi.org/ 10.1093/nar/16.2.439.
- Galardini M, Biondi EG, Bazzicalupo M, Mengoni A. 2011. CONTIGuator: a bacterial genomes finishing tool for structural insights on draft genomes. Source Code Biol Med 6:11. http://dx.doi.org/10.1186/ 1751-0473-6-11.
- Galardini M, Mengoni A, Bazzicalupo M. 2015. Mapping contigs using CONTIGuator, p. 163–176. *In* Bacterial Pangenomics. Springer.
- 9. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity GM, 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. http://dx.doi.org/10.1089/omi.2008.0017.

- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42: D206-D214. http://dx.doi.org/10.1093/nar/gkt1226.
- Van Domselaar GH, Stothard P, Shrivastava S, Cruz JA, Guo A, Dong X, Lu P, Szafron D, Greiner R, Wishart DS. 2005. BASys: a web server for automated bacterial genome annotation. Nucleic Acids Res 33: W455–W459. http://dx.doi.org/10.1093/nar/gki593.
- Xie J-B, Du Z, Bai L, Tian C, Zhang Y, Xie J-Y, Wang T, Liu X, Chen X, Cheng Q, Chen S, Li J. 2014. Comparative genomic analysis of N2-fixing and non-N2-fixing *Paenibacillus* spp.: organization, evolution and expression of the nitrogen fixation genes. PLoS Genet 10:e1004231. http://dx.doi.org/10.1371/journal.pgen.1004231.
- Kentemich T, Danneberg G, Hundeshagen B, Bothe H. 1988. Evidence for the occurrence of the alternative, vanadium-containing nitrogenase in the cyanobacterium *Anabaena variabilis*. FEMS Microbiol Lett 51:19–24. http://dx.doi.org/10.1111/j.1574-6968.1988.tb02960.x.
- Mulligan ME, Haselkorn R. 1989. Nitrogen fixation (nif) genes of the cyanobacterium Anabaena species strain pcc 7120. The nifB-fdxN-nifSnifU operon. J Biol Chem 264:19200–19207.
- Wang L, Zhang L, Liu Z, Zhao D, Liu X, Zhang B, Xie J, Hong Y, Li P, Chen S, Dixon R, Li J. 2013. A minimal nitrogen fixation gene cluster from *Paenibacillus* sp. WLY78 enables expression of active nitrogenase in *Escherichia coli*. PLoS Genet 9:e1003865. http://dx.doi.org/10.1371/ journal.pgen.1003865.