genomeA
Journals.ASM.org

Draft Genome Sequence of *Alicyclobacillus acidoterrestris* Strain ATCC 49025

Moshe Shemesh,[a] Ronit Pasvolsky,[a] Noa Sela,[b] Stefan J. Green,[c,d] Varda Zakin[a]

Department of Food Quality and Safety, Agricultural Research Organization The Volcani Center, Bet-Dagan, Israel[a]; Department of Plant Pathology and Weed Research, Agricultural Research Organization The Volcani Center, Bet-Dagan, Israel[b]; DNA Services Facility, University of Illinois at Chicago, Chicago, Illinois, USA[c]; Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois, USA[d]

***Alicyclobacillus acidoterrestris* is a spore-forming Gram-positive, thermo-acidophilic, nonpathogenic bacterium which contaminates commercial pasteurized fruit juices. The draft genome sequence for *A. acidoterrestris* strain ATCC 49025 is reported here, providing genetic data relevant to the successful adaptation and survival of this strain in its ecological niche.**

Received 20 July 2013 **Accepted** 7 August 2013 **Published** 5 September 2013

Citation Shemesh M, Pasvolsky R, Sela N, Green SJ, Zakin V. 2013. Draft genome sequence of *Alicyclobacillus acidoterrestris* strain ATCC 49025. Genome Announc. 1(5):e00638-13. doi:10.1128/genomeA.00638-13.

Address correspondence to Moshe Shemesh, moshesh@agri.gov.il.

Alicyclobacillus acidoterrestris is capable of surviving extremely harsh conditions, for instance during industrial food processing (1–3). *A. acidoterrestris* is a spore-forming Gram-positive bacterium, which is widespread in soil and frequently isolated from a wide variety of commodities as a contaminant (3, 4). Since it can lead to food spoilage, *A. acidoterrestris* contamination can cause enormous economic losses mainly in the fruit juice industries; therefore, this microorganism is considered a major challenge in the food industry (3, 5). *A. acidoterrestris* survives across a broad range of temperatures (25 to 60°C) and pH conditions (pH 2 to 6); it can also survive pasteurization and is able to grow during food storage (3, 5). Thus, *A. acidoterrestris* is the predominant spoilage species within the *Alicyclobacillus* genus (5). To develop our understanding of the survival strategies used by *A. acidoterrestris* in natural environments, a draft genome sequence was generated for strain ATCC 49025. The wild-type isolate of *A. acidoterrestris* ATCC 49025 was purchased from American Type Culture Collection (ATCC) and was kindly provided by Ronit Ben Avraham from Milouda Laboratories (Israel). Genomic DNA was isolated from liquid culture using a genomic DNA purification kit (Sigma-Aldrich) and prepared for shotgun sequencing using the PrepX ILM DNA library kit (IntegenX, Pleasanton, CA). DNA was initially sheared using a Covaris S2 acoustic shearing device, and subsequent to sequencing, adapter-ligated fragments were size selected (400–800 bp) using the Pippin prep automated electrophoresis instrument (Sage Scientific, Beverly, MA). Sequencing was performed on an Illumina HiSeq2000 instrument, employing paired-end 100-base reads. Approximately 13 M reads were generated in pairs and assembled by the *de novo* assembler within the software package CLC Genomics Workbench v 6.0 (CLCbio, Cambridge, MA). A total of 207 contigs of length ≥500 bases were generated, with a sum of 4,063,548 bp, an N_{50} of 44,524 bases, and an average coverage of >100×. More than 96% of the reads mapped to the draft genome contigs. The contigs were successfully used for annotation and gene prediction by Rapid Annotations using Subsystems Technology (RAST) (6). The overall GC content of 52.2% encompasses 4,145 predicted protein-encoding genes.

In response to stressful conditions, bacteria can initiate a developmental pathway leading to the formation of dormant endospores (7). Sporulation transcriptional activator (Spo0A) is a critical regulator for the entrance of bacteria to the sporulation pathway (8). A BLAST analysis was performed to identify sequences in the draft genome sharing high sequence similarity to Spo0A. The putative Spo0A gene in strain ATCC 49025 shows 71% similarity to the sequence encoding Spo0A in two sequenced strains of *Alicyclobacillus acidocaldarius*, Tc-4-1 and DSM 446. The *A. acidoterrestris* Spo0A protein is also similar to that of *Bacillus subtilis* 168 (61% similarity), *Bacillus licheniformis* ATCC 14580 (60% similarity), and *B. halodurans* (57% similarity). The sporulation kinase A (KinA), which activates Spo0A by phosphorylation, was also found to be conserved in *A. acidoterrestris*. Thus, KinA protein shows 32% similarity to the PAS/PAC sensor signal transduction histidine kinase of the *A. acidocaldarius* strains as well as to KinA of *B. subtilis* 168.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited at GenBank under the accession no. AURB00000000.

ACKNOWLEDGMENTS

We thank Ronit Ben Avraham from Milouda Laboratories (Israel) for kindly providing the *A. acidoterrestris* wild-type strain. We thank Shlomo Sela and Eddie Cytryn from ARO for the helpful discussions.

This work was not supported by any external funding.

REFERENCES

1. **Connor CJ, Luo H, Gardener BB, Wang HH.** 2005. Development of a real-time PCR-based system targeting the 16S rRNA gene sequence for rapid detection of *Alicyclobacillus* spp. in juice products. Int. J. Food Microbiol. **99:**229–235.
2. **Minamikawa M, Kawai Y, Inoue N, Yamazaki K.** 2005. Purification and characterization of Warnericin RB4, anti-*Alicyclobacillus* bacteriocin, produced by *Staphylococcus warneri* RB4. Curr. Microbiol. **51:**22–26.

3. **Steyn CE, Cameron M, Witthuhn RC.** 2011. Occurrence of *Alicyclobacillus* in the fruit processing environment—a review. Int. J. Food Microbiol. **147:**1–11.
4. **Groenewald WH, Gouws PA, Witthuhn RC.** 2008. Isolation and identification of species of *Alicyclobacillus* from orchard soil in the Western Cape, South Africa. Extremophiles **12:**159–163.
5. **Spinelli AC, Sant'Ana AS, Rodrigues-Junior S, Massaguer PR.** 2009. Influence of different filling, cooling, and storage conditions on the growth of *Alicyclobacillus acidoterrestris* CRA7152 in orange juice. Appl. Environ. Microbiol. **75:**7409–7416.
6. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9:**75. doi:10.1186/1471-2164-9-75.
7. **Stragier P, Losick R.** 1996. Molecular genetics of sporulation in *Bacillus subtilis*. Annu. Rev. Genet. **30:**297–241.
8. **Molle V, Fujita M, Jensen ST, Eichenberger P, Gonzalez-Pastor JE, Liu JS, Losick R.** 2003. The Spo0A regulon of *Bacillus subtilis*. Mol. Microbiol. **50:**1683–1701.