

Draft Genome Sequence of the Halophilic Bacterium *Halobacillus* sp. Strain BAB-2008

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The *Halobacillus* sp. strain BAB-2008 is a moderately halophilic, rod-shaped, Gram-positive, orange-pigmented, carotenoid-producing bacterium isolated from saline soil near Zazam-Solar Park Road, Gujarat, India. Here we present the 3.7-Mb genome sequence to provide insights into its functional genomics and potential applications for carotenoid and enzyme production.

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The genus *Halobacillus* was identified by Spring et al. (1) and comprises 18 species with validly published names: *H. halophilus*, *H. litoralis*, and *H. trueperi* (1), *H. salinus* (2), *H. karajensis* (3), *H. locialis* (4), *H. aidingensis* and *H. dabanensis* (5), *H. yeomjeoni* (6), *H. campisalis* (7), *H. profundi* and *H. kuroshimensis* (8), *H. faecis* (9), *H. mangrovei* (10), *H. alkaliphilus* (11), *H. naozhouensis* (12), *H. salsuginis* (13), and *H. seohaensis* (14). The *Halobacillus* genus comprises moderate halophiles which can grow and produce enzymes over a very wide range of salinities, making them very attractive for research and for screening of novel enzymes with unusual properties (15). Many moderate halophiles produce carotenoids as a protective mechanism against photooxidation processes. Carotenoids have major applications in the food industry as food-coloring agents and as additives in health food products (16). The halophilic aspect of these bacteria has exciting potential, for instance, in their possible application in agriculture to construct salt-resistant plants carrying prokaryotic genes encoding enzymes for the synthesis of osmoprotective compounds (17).

The halophilic bacterium *Halobacillus* sp. strain BAB-2008 was isolated from a soil sample near Zazam-Solar Park Road, District Patan (23°55'782"N, 71°18'480"E), Gujarat, India, by using the traditional dilution-plating method. Preliminary characterization revealed that *Halobacillus* sp. BAB-2008 is Gram positive, orange pigmented, and halophilic (can grow at up to 15% NaCl).

Whole-genome sequencing of the strain was done with a high-throughput Ion Torrent Personal Genome Machine with an Ion Torrent Server (Torrent suite version 3.2). Following the manufacturer's protocol, 22.11× coverage data and a total of 1,078,490 mate-paired reads (shortest read with 91 bp and longest read with 176 bp) were obtained. *De novo* assembly was performed using the MIRA-3 assembler (version 3.1.0). The automatic annotation of the genome was performed using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>), which utilizes GeneMark (18), Glimmer (19), and tRNAscan-SE searches (20), and using the RAST server (21) with the SEED database (22). Functional

annotation of genes was performed using the KEGG Automatic Annotation Server (23).

The total length of the genome was found to be 3,784,751 bp, which was distributed in 137 contigs (scaffold N_{50} = 51,319 bp). The G+C content was 46.83%. The *Halobacillus* sp. BAB-2008 harbored 410 subsystems, having 3,935 protein-coding genes, 60 tRNAs, and 9 rRNAs. Carotenoid biosynthesis genes (*crtN*, *crtP*, *crtB*, and *crtM*) present on the chromosome of *Halobacillus* sp. BAB-2008 indicate that this species might have possible applications in carotenoid production. Genes encoding serine proteinases, amylases, nucleases, esterases, and lipases were also identified on the chromosome.

Thus, the ability to grow under halophilic conditions, the presence of a clearly defined carotenoid biosynthetic pathway, and the probable potential toward enzyme production make *Halobacillus* sp. BAB-2008 an organism of industrial importance. Moreover, this bacterium may be used as a model organism for molecular study of the osmoregulatory mechanisms which help moderate halophiles grow over a wide range of salt concentrations.

Nucleotide sequence accession number. The draft genome sequence of *Halobacillus* sp. BAB-2008 has been deposited at GenBank under the accession number [ANPF00000000](https://www.ncbi.nlm.nih.gov/nuccore/ANPF00000000).

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REFERENCES

1. Spring S, Ludwig W, Marquez MC, Ventosa A, Schleifer KH. 1996. *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov. and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. Int. J. Syst. Bacteriol. 46:492–496.
2. Yoon JH, Kang KH, Park YH. 2003. *Halobacillus salinus* sp. nov., Isolated from a salt lake on the coast of the East Sea in Korea. Int. J. Syst. Evol. Microbiol. 53:687–693.
3. Amoozegar MA, Malekzadeh F, Malik KA, Schumann P, Spröer C. 2003. *Halobacillus karajensis* sp. nov., A novel moderate halophile. Int. J. Syst. Evol. Microbiol. 53:1059–1063.

4. Yoon JH, Kang KH, Oh TK, Park YH. 2004. *Halobacillus localis* sp. nov., A halophilic bacterium isolated from a marine solar saltern of the Yellow Sea in Korea. *Extremophiles* 8:23–28.
5. Liu WY, Zeng J, Wang L, Dou YT, Yang SS. 2005. *Halobacillus dabanensis* sp. nov. and *Halobacillus aidingensis* sp. nov., isolated from salt lakes in Xinjiang, China. *Int. J. Syst. Evol. Microbiol.* 55:1991–1996.
6. Yoon JH, Kang SJ, Lee CH, Oh HW, Oh TK. 2005. *Halobacillus yeomjeoni* sp. nov., Isolated from a marine solar saltern in Korea. *Int. J. Syst. Evol. Microbiol.* 55:2413–2417.
7. Yoon JH, Kang SJ, Jung YT, Oh TK. 2007. *Halobacillus campisalis* sp. nov., Containing meso-diaminopimelic acid in the cell-wall peptidoglycan, and emended description of the genus *Halobacillus*. *Int. J. Syst. Evol. Microbiol.* 57:2021–2025.
8. Hua NP, Kanekiyo A, Fujikura K, Yasuda H, Naganuma T. 2007. *Halobacillus profundi* sp. nov. and *Halobacillus kuroshimensis* sp. nov., moderately halophilic bacteria isolated from a deep-sea methane cold seep. *Int. J. Syst. Evol. Microbiol.* 57:1243–1249.
9. An SY, Kanoh K, Kasai H, Goto K, Yokota A. 2007. *Halobacillus faecis* sp. nov. in a spore-forming bacterium isolated from a mangrove area on Ishigaki Island, Japan. *Int. J. Syst. Evol. Microbiol.* 57:2476–2479.
10. Soto-Ramírez N, Sánchez-Porro C, Rosas-Padilla S, Almodóvar K, Jiménez G, Machado-Rodríguez M, Zapata M, Ventosa A, Montalvo-Rodríguez R. 2008. *Halobacillus mangrovi* sp. nov., A moderately halophilic bacterium isolated from the black mangrove *Avicennia germinans*. *Int. J. Syst. Evol. Microbiol.* 58:125–130.
11. Romano I, Finore I, Nicolaus G, Huertas FJ, Lama L, Nicolaus B, Poli A. 2008. *Halobacillus alkaliphilus* sp. nov., A halophilic bacterium isolated from a salt lake in Fuente de Piedra, southern Spain. *Int. J. Syst. Evol. Microbiol.* 58:886–890.
12. Chen YG, Liu ZX, Zhang YQ, Zhang YX, Tang SK, Borrathybay E, Li WJ, Cui XL. 2012. *Halobacillus naozhouensis* sp. nov., a moderately halophilic bacterium isolated from a sea anemone. *Antonie Van Leeuwenhoek* 96:99–107.
13. Chen YG, Zhang YQ, Liu ZX, Zhuang DC, Klenk HP, Tang SK, Cui XL, Li WJ. 2009. *Halobacillus salsuginis* sp. nov., a moderately halophilic bacterium from a subterranean brine. *Int. J. Syst. Evol. Microbiol.* 59:2505–2509.
14. Yoon JH, Kang SJ, Oh TK. 2008. *Halobacillus seohaensis* sp. nov., isolated from a marine solar saltern in Korea. *Int. J. Syst. Evol. Microbiol.* 58:622–627.
15. Kushner DJ. 1986. Molecular adaptation of enzymes, metabolic systems and transport systems in halophilic bacteria. *FEMS Microbiol. Rev.* 39:121–127.
16. Köcher S, Jurgen B, Müller V, Sandmann G. 2009. Structure, function and biosynthesis of carotenoids in the moderately halophilic bacterium *Halobacillus halophilus*. *Arch. Microbiol.* 191:95–104.
17. Ventosa A, Nieto JJ, Oren A. 1998. Biology of moderately halophilic bacteria. *Microbiol. Mol. Biol. Rev.* 62:504–544.
18. Borodovsky M, McIninch J. 1993. GeneMark: parallel gene recognition for both DNA strands. *Comput. Chem.* 17:123–133.
19. Delcher AL, Hormon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* 27:4636–4641.
20. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
21. 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.
22. Disz T, Akhter S, Cuevas D, Olson R, Overbeek R, Vonstein V, Stevens R, Edwards RA. 2010. Accessing the SEED genome databases via Web services API: tools for programmers. *BMC Bioinformatics* 11:319.
23. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.* 35:W182–W185.