



Whole-Genome Sequence of *Bacillus subtilis* WS1A, a Promising Fish Probiotic Strain Isolated from Marine Sponge of the Bay of Bengal

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ABSTRACT This study reports the draft genome sequence of a promising fish probiotic, *Bacillus subtilis* strain WS1A, that possesses antimicrobial activity against *Aeromonas veronii* and suppressed motile *Aeromonas* septicemia in *Labeo rohita*. The *de novo* assembly resulted in an estimated chromosome size of 4,148,460 bp, with 4,288 open reading frames.

The *Bacillus subtilis* strain WS1A was isolated from a marine sponge from the Saint Martin's Island area of the Bay of Bengal, Bangladesh. WS1A was cultured on Zobell agar plates (1, 2). It can grow in both marine water and freshwater media. It demonstrated *in vitro* antimicrobial activity against *Aeromonas veronii*, prevented motile *Aeromonas* septicemia in an Indian major carp species (*Labeo rohita*) (3, 4), and is considered a promising probiotic candidate. Prior permission was obtained from the Institute of Biotechnology and Genetic Engineering ethical review committee for the animal experiments (approval number IBGE-ERC-005).

WS1A was grown in Zobell broth at 28°C for 24 h, and then the genomic DNA was extracted using the GeneJET genomic DNA purification kit (Thermo Fisher Scientific, USA). The DNA was processed according to the Illumina XT protocol (5). In brief, 1 ng of normalized DNA was fragmented and “tagged” via tagmentation (6). The fragmented DNA was indexed accordingly and subjected to a 600-cycle sequencing protocol using the MiSeq benchtop sequencer (Illumina, Inc.) at 50.0× coverage. Initial identification of bacteria was performed by using the Bacterial Analysis Pipeline v.1.0.4 (7). Removal of sequence adaptors and quality filtering were performed by using Trimmomatic v0.38 and PRINSEQ v0.20.3, respectively (8, 9). The *de novo* assembly and quality evaluation of the assembled draft genome were completed using SPAdes v3.9.0 (10) and QUAST v5.0.2 (11), respectively. Annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (12). Gene prediction was carried out using Prodigal v1.20 and the predicted proteins were searched for similarity against the UniProt protein database using BLASTp v2.10.0 (13), following pathway identification by the KEGG Automatic Annotation Server (KAAS) v2.1. The genome was screened to determine putative antibiotic resistance genes (ResFinder v4.0) (14), plasmids (PlasmidFinder v2.0) (15), virulence factors (VirulenceFinder v2.0) (16), and pathogenicity toward the human host (PathogenFinder v1.1) (17).

The annotated chromosome length, GC content, and N_{50} value of the assembled genome were 4,148,460 bp (151 contigs), 43.6%, and 199,148 bp, respectively. The largest and smallest contigs were 433,806 bp and 257 bp, respectively. The open

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reading frames of the genome were predicted and annotated using the Rapid Annotations using Subsystems Technology (RAST) server (classic RAST FIGfams v70) (18), which showed 333 subsystems and 96 RNA genes.

Genome analyses identified several orthologs of intrinsic genes of a potential probiotic bacterium, such as those encoding proteins involved in the biosynthesis of riboflavin, vitamin B₆, and amino acids (*ilvD*) and in carbon utilization (*pta*). The genome also codes for antimicrobial peptides such as bacillaene, subtilin, bacillibactin, surfactin, fengycin, bacilysin, and subtilosin A (antiSMASH v5.1.2) (19). No genes coding for putative virulence factors, no plasmids, and no antibiotic resistance genes were identified in the genome using VirulenceFinder v2.0 (16), PlasmidFinder v2.0 (15), and ResFinder v4.0 (14), respectively, with default parameters. The genome sequence information will help to exploit the probiotic potential of this strain.

Data availability. The whole-genome shotgun project for *B. subtilis* strain WS1A has been deposited at GenBank under the assembly accession number [JABFHE000000000.1](https://www.ncbi.nlm.nih.gov/assembly/JABFHE000000000.1). Raw reads and raw sequencing data are available under the accession number [SRR11868367](https://www.ncbi.nlm.nih.gov/srr/SRR11868367), BioProject accession number [PRJNA630208](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA630208), and BioSample accession number [SAMN14828537](https://www.ncbi.nlm.nih.gov/biosample/SAMN14828537).

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There are no conflicts of interest regarding this paper.

REFERENCES

- Anand TP, Bhat AW, Shouche YS, Roy U, Siddharth J, Sarma SP. 2006. Antimicrobial activity of marine bacteria associated with sponges from the waters off the coast of South East India. *Microbiol Res* 161:252–262. <https://doi.org/10.1016/j.micres.2005.09.002>.
- Lu Z, Guo W, Liu C. 2018. Isolation, identification, and characterization of novel *Bacillus subtilis*. *J Vet Med Sci* 80:427–433. <https://doi.org/10.1292/jvms.16-0572>.
- Paul SI. 2018. Marine sponge and sponge associated bacteria inhibiting fish pathogen. MS thesis. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.
- Rahman MM, Kawser AQMR, Islam MT. 2019. Probiotic bacilli in sustainable aquaculture, p 305–334. *In* Islam MT, Rahman M, Pandey P, Boehme MH, Haesaert G (ed), *Bacilli and agrobiotechnology: phytostimulation and biocontrol*. Springer, London, United Kingdom.
- Illumina. 2019. Nextera XT DNA library prep reference guide. Illumina, San Diego, CA. https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-05.pdf.
- Akter T, Rahman MM, Tay ACY, Ehsan R, Islam MT. 2020. Whole-genome sequence of fish pathogen *Enterococcus faecalis* strain BFFF11. *Microbiol Resour Announc* 9:e01447-19. <https://doi.org/10.1128/MRA.01447-19>.
- Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, Sicheritz-Pontén T, Aarestrup FM, Ussery DW, Lund O. 2014. Benchmarking of methods for genomic taxonomy. *J Clin Microbiol* 52:1529–1539. <https://doi.org/10.1128/JCM.02981-13>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27:863–864. <https://doi.org/10.1093/bioinformatics/btr026>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.
- Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
- Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52:1501–1510. <https://doi.org/10.1128/JCM.03617-13>.
- Cosentino S, Larsen MV, Aarestrup FM, Lund O. 2013. PathogenFinder: distinguishing friend from foe using bacterial whole genome se-

- quence data. PLoS One 8:e77302. <https://doi.org/10.1371/journal.pone.0077302>.
18. Overbeek R, Olson R, Push 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:D204–D214. <https://doi.org/10.1093/nar/gkt1226>.
19. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de los Santos ELC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0: improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res 45:W36–W41. <https://doi.org/10.1093/nar/gkx319>.