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GENOME SEQUENCES

Whole-Genome Sequence of *Aneurinibacillus migulanus* TP115, a Potential Fish Probiotic Isolated from *Oreochromis niloticus* Gut

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ABSTRACT We report the whole-genome sequence of a promising fish probiotic, *Aneurinibacillus migulanus* TP115, which was isolated from the gut of Nile tilapia (*Oreochromis niloticus*). The *de novo* assembly resulted in an estimated chromosome size of 5,556,554 bp, with 5,576 open reading frames.

Fish probiotics are particularly important for growth promotion and disease management in aquaculture (1). *Aneurinibacillus migulanus* is a prospective biocontrol agent against bacterial pathogens because of its ability to produce various antimicrobial compounds (2). The presented genome information for *A. migulanus* TP115 will facilitate the understanding of its safety and probiotic potential for safe use in aquaculture.

We isolated *A. migulanus* TP115 in 2018 from a healthy Nile tilapia in Bangladesh. The abdomen of the fish was cut aseptically, and the gut was removed for the isolation of probiotic bacteria. One-gram homogenates of the intestinal segments were serially diluted and spread on de Man, Rogosa, and Sharpe (MRS) agar plates, followed by incubation at 28°C for 48 h. A single whitish-gray colony growing on the MRS plate after 48 h of incubation was identified as *Aneurinibacillus migulanus* (GenBank accession number MW512511.1) based on 16S rRNA gene sequence homology. The primers used for amplification were 8F (5′-AGAGT TTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). TP115 exhibits potent *in vivo* growth promotion and suppresses motile *Aeromonas* septicemia in *Oreochromis niloticus* (3). Prior permission was obtained from the Institute of Biotechnology and Genetic Engineering (IBGE) ethical review committee for the animal experiments (approval number IBGE-ERC-008).

The genomic DNA of TP115 was extracted from the original stock in 2019. The same single colony as used for 16S rRNA sequencing was inoculated in MRS broth and incubated at 28°C for 48 h. High-quality genomic DNA was extracted using a GeneJET genomic DNA purification kit (Thermo Fisher Scientific, USA), and the quality and quantity of the DNA were checked using a NanoDrop spectrophotometer (Thermo Fisher Scientific). A paired-end DNA library was prepared using the Nextera XT library preparation kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions (4). Sequencing (600 cycles) was performed using the MiSeq benchtop sequencer (Illumina) (5, 6) and yielded a total of 3,528,592 reads (1,764,296 paired-end reads) and 762,217,704 bases. Trimmomatic v.0.38 (7) was used to remove the sequence adaptors, and quality filtering was performed using PRINSEQ v.0.20.3 (8). *De novo* assembly was performed using SPAdes v.3.9.0 (9), and QUAST v.5.0.2 (10) was used for quality assessment of the assembled genome. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok) (11). Using SpeciesFinder v.2.0 (https://cge.food.dtu.dk/services/SpeciesFinder), the bacterium was identified as *Aneurinibacillus migulanus* (12). Default parameters were used for all software unless otherwise specified.

The chromosome size of TP115 is 5,556,554 bp distributed in 113 contigs, with a G+C content of 43.7%, genome coverage of 137×, and no plasmids. The genome contains 5,576 total coding sequences (CDSs), with 5,405 CDSs encoding putative proteins. The N_{50} and L_{50} values of the assembly were 126,253 bp and 12, respectively. The largest and smallest

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contigs were 567,280 bp and 505 bp, respectively. PGAP predicted 134 RNA genes (108 tRNA genes, 20 rRNA operons, and 6 noncoding RNA [ncRNA] genes) and 171 pseudogenes. RAST v.2.0 (13) predicted 316 subsystems and 2,090 protein-coding genes putatively assigned to functional categories. Using antiSMASH v.6.1.1, a total of 13 gene clusters for secondary metabolites were predicted for the genome of the *A. migulanus* TP115 (14). β-Lactones, cyclic lactone autoinducers, terpenes, siderophores, ranthipeptides, proteusins, nonribosomal peptides, polyketides, arylpolyenes, sactipeptides, and lasso peptides are among the predicted secondary metabolites.

Data availability. The whole-genome shotgun project for *Aneurinibacillus migulanus* TP115 has been deposited in GenBank under the accession number JAMZFU000000000. The version described in this paper is version JAMZFU010000000. The raw reads and raw sequencing data are available under SRA accession number SRX15894335, BioProject accession number PRJNA851951, and BioSample accession number SAMN29257231.

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REFERENCES

1. El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MA, Dhama K, Abdel-Latif HM. 2021. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol 117:36–52. https://doi.org/10.1016/j.fsi.2021.07.007.
2. Alenezi FN, Weitz HJ, Belbahri L, Nidhal J, Luptakova L, Jaspars M, Woodward S. 2015. Draft genome sequence of *Aneurinibacillus migulanus* NCTC 7096. Genome Announc 3:e00234-15. https://doi.org/10.1128/genomeA.00234-15.
3. Rahman A. 2019. Isolation and identification of indigenous fish gut bacteria and their disease suppression effects in tilapia. MS thesis. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.
4. 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.
5. Rahman MM, Paul SI, Akter T, Tay AC, Foysal MJ, Islam MT. 2020. Whole-genome sequence of *Bacillus subtilis* WS1A, a promising fish probiotic strain isolated from marine sponge of the Bay of Bengal. Microbiol Resour Announc 9:e00641-20. https://doi.org/10.1128/MRA.00641-20.
6. Akter T, Rahman MM, Tay ACY, Ehsan R, Islam MT. 2020. Whole-genome sequence of fish pathogenic *Enterococcus faecalis* strain BFFF11. Microbiol Resour Announc 9:e01447-19. https://doi.org/10.1128/MRA.01447-19.
7. 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.
8. Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10.1093/bioinformatics/btr026.
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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.
10. 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.
11. 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.
12. 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.
13. 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.
14. 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.