AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Whole-Genome Sequencing of *Alcaligenes faecalis* HZ01, with Potential to Inhibit Nontuberculous Mycobacterial Growth

Henry Marcel Zalona Fernandes,[a] Emilyn Costa Conceição,[b,c] Sandro Patroca da Silva,[d] Edson Machado,[e] Maria Carolina Sisco,[a] Abhinav Sharma,[f] Karla Valéria Batista Lima,[d,g] Marília Lima da Conceição,[d,g] Ana Carolina da Silva Carvalho,[h] Karla Rodrigues Miranda,[a] Rafael Silva Duarte,[a] Daniela Sales Alviano,[a] Rubens Clayton da Silva Dias[i]

[a]Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil
[b]Department of Science and Innovation, National Research Foundation Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
[c]Programa de Pós-graduação em Pesquisa Clínica e Doenças Infecciosas, Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil
[d]Instituto Evandro Chagas, Ananindeua, Pará, Brazil
[e]Laboratório de Genética Molecular de Microrganismos, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil
[f]Faculty of Engineering and Technology, Liverpool John Moores University, Liverpool, United Kingdom
[g]Pós-Graduação Biologia Parasitária na Amazônia, Instituto de Ciências Biológicas e da Saúde, Universidade do Estado do Pará, Belém, Pará, Brazil
[h]Instituto de Química, Universidade Federal do Rio de Janeiro, Campus Macaé, Macaé, Rio de Janeiro, Brazil
[i]Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

Henry Marcel Zalona Fernandes and Emilyn Costa Conceição contributed equally to this work. Author order was determined in order of increasing seniority.

ABSTRACT *Alcaligenes faecalis* is a Gram-negative rod that is ubiquitous in the environment and is an opportunistic human pathogen. Here, we report the whole-genome sequencing analysis of *A. faecalis* HZ01, which presents mycobacterial growth inhibitory activity and was isolated from a contaminated culture of *Mycobacterium chubuense* ATCC 27278.

Alcaligenes faecalis is a Gram-negative rod, nonfermenting, aerobic, mobile, and peritrichous bacterium (1). This opportunistic pathogen is widely distributed in the environment and is related to nosocomial diseases (2, 3), with biotechnological potential in the pharmaceutical industry and in bioremediation of contaminated environments (4), such as the production of antibacterial substances (5–7). Although *A. faecalis* represents a promising source for new bioactive substances, there is limited literature on genomic approaches (8).

During the development of previous studies, we observed a contaminant microorganism that had grown on a *Mycobacterium chubuense* ATCC 27278 culture at 37°C on Middlebrook 7H10 medium and exhibited mycobacterial growth inhibitory activity (Fig. 1A to C). In a similar study, it was verified that the antibacterial activity of *A. faecalis* is via a live-cell and contact-dependent mechanism (9). The ATCC strain was obtained from our mycobacterial collection. To isolate the contaminant microorganism, we selected three colonies showing a halo of mycobacterial growth inhibition, and then they were individually streaked on another Middlebrook 7H10 medium plate and incubated at 37°C for 48 h. The contaminant microorganism was identified as *A. faecalis* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) of pure cultures of the three isolates obtained originally and was stored at −80°C in nutrient broth supplemented with glycerol (final concentration of 15% [vol/vol]) (10).

Following the bacterial culture in MacConkey agar in a 37°C incubator for 48 h, we performed genomic DNA extraction using the QIAamp DNA minikit (Qiagen, Hilden, Germany) and library preparation using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA). Whole-genome sequencing (WGS) was conducted on the Illumina NextSeq 500 platform with 2 × 150-bp paired-end reads.

Citation Fernandes HMZ, Conceição EC, da Silva SP, Machado E, Sisco MC, Sharma A, Lima KVB, da Conceição ML, da Silva Carvalho AC, Miranda KR, Silva Duarte R, Alviano DS, da Silva Dias RC. 2021. Whole-genome sequencing of *Alcaligenes faecalis* HZ01, with potential to inhibit nontuberculous mycobacterial growth. Microbiol Resour Announc 10:e00521-21. https://doi.org/10.1128/MRA.00521-21.

Editor Catherine Putonti, Loyola University Chicago

Address correspondence to Henry Marcel Zalona Fernandes, henry_zalona@hotmail.com.

Received 8 June 2021
Accepted 6 September 2021
Published 30 September 2021

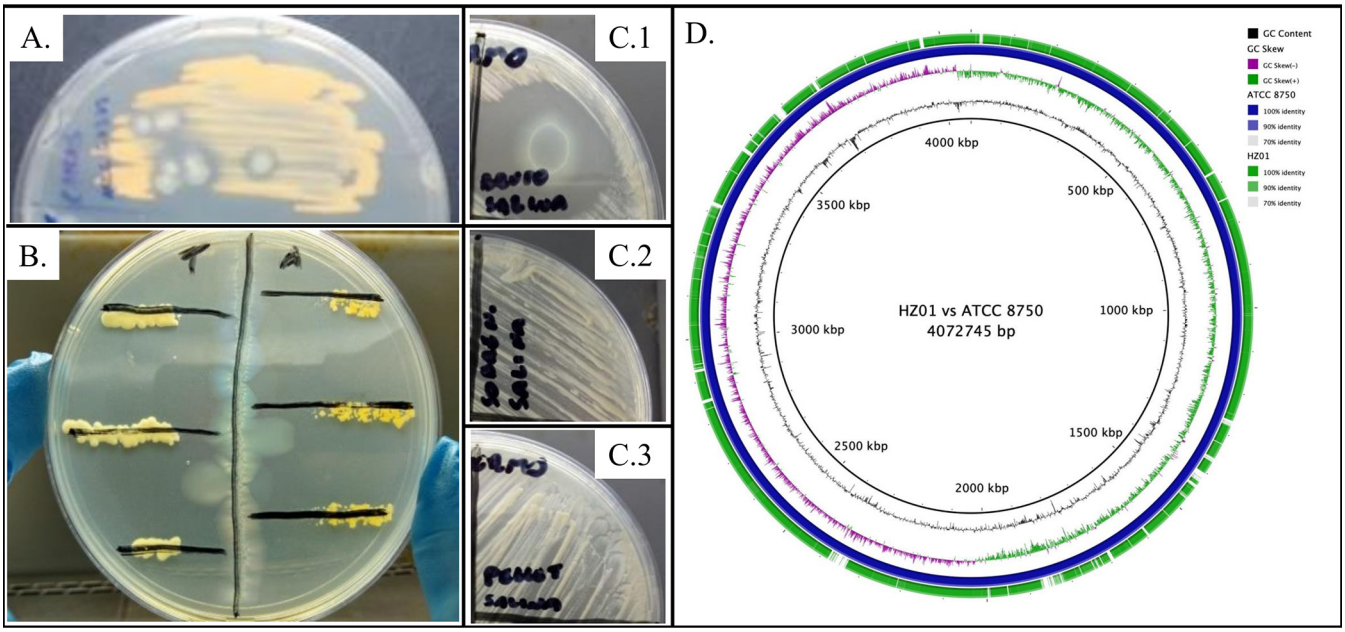

FIG 1 Antimycobacterial activity of *Alcaligenes faecalis* HZ01. (A) *Mycobacterium chubuense* ATCC 27278 culture from which *A. faecalis* HZ01 was originally isolated. (B) Antibiosis test exhibiting *A. faecalis* HZ01 (central line) antimycobacterial activity on *Mycobacterium thermoresistibile* ATCC 19527 (lines on the left) and *Mycobacterium aichiense* ATCC 27280 (lines on the right), using the cross-streak method. (C) Antimycobacterial activity analysis of *A. faecalis* HZ01 cell suspension (C.1), cell-free supernatant (C.2), and lysed pellet (C.3) on a *M. thermoresistibile* ATCC 19527 culture. (D) Genomic comparison of the *A. faecalis* HZ01 isolate against the reference genome of *A. faecalis* ATCC 8750.

The sequencing quality was evaluated using FastQC v0.11.9 (11), before and after the reads were trimmed with Trimmomatic v0.39 (12). *De novo* assembly was performed with SPAdes v3.14.0 (13), assembly quality was evaluated with QUAST v5.0.2 (14), and annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.2 (15). For genome comparison, the Artemis Comparison Tool and BLAST Ring Image Generator (BRIG) v3.0 were used (16, 17). For variant calling, we used Snippy v4.6.0 (https://github.com/tseemann/snippy). We used PlasmidSeeker v1.3 and PlasmidFinder v2.1 to investigate the presence of plasmids (18, 19). We used default parameters for all software.

A total of 8,369,218 reads were obtained, and the genome coverage was 606×. We obtained a total of 7,854,398 reads after quality trimming. By mapping the reads obtained against *A. faecalis* subsp. *faecalis* (ATCC 8750) (https://genomes.atcc.org/genomes/a6829cff570e4f50) using the Burrows-Wheeler aligner (20), we observed that 86.68% of the reads were properly paired against the reference genome. After *de novo* assembly, we obtained 57 contigs; the largest contig had 848,880 bp. The draft genome obtained had a total length of 4,141,412 bp, with a GC content of 56.79% (Fig. 1D). The N_{50} and N_{75} values were 669,949 bp and 410,060 bp, respectively. There was no presence of plasmids. We found 7,873 complex variants, 191 deletions, 188 insertions, 993 multiple-nucleotide polymorphisms (MNPs), 4,7401 single-nucleotide polymorphisms (SNPs), and a total of 5,6647 variants.

Due to increasing challenges in treating multidrug-resistant infections, such as mycobacterial diseases, and the global shortage of successful drug therapy options, the discovery of new antimicrobial agents is necessary to improve patient outcomes.

Data availability. The *A. faecalis* HZ01 WGS data were deposited in DDBJ/ENA/GenBank under accession number JAFMOE000000000 (the version described in this paper is JAFMOE010000000), BioSample accession number SAMN17762316, BioProject accession number PRJNA698913, and SRA accession number SRR13612681.

ACKNOWLEDGMENTS

We are grateful to Marlei Gomes da Silva for technical assistance during the bacterial isolation.

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (finance code 001).

All authors report no conflicts of interest.

REFERENCES

1. Bizet J, Bizet C. 1997. Strains of *Alcaligenes faecalis* from clinical material. J Infect 35:167–169. https://doi.org/10.1016/S0163-4453(97)91710-2.
2. Tena D, Fernández C, Lago MR. 2015. *Alcaligenes faecalis*: an unusual cause of skin and soft tissue infection. Jpn J Infect Dis 68:128–130. https://doi.org/10.7883/yoken.JJID.2014.164.
3. Al-Zakhari R, Suhail M, Ataallah B, Aljammali S, Grigos A. 2020. Rare but fatal case of cavitary pneumonia caused by *Alcaligenes faecalis*. Cureus 12:e8934. https://doi.org/10.7759/cureus.8934.
4. Ju S, Lin J, Zheng J, Wang S, Zhou H, Sun M. 2016. *Alcaligenes faecalis* ZD02, a novel nematicidal bacterium with an extracellular serine protease virulence factor. Appl Environ Microbiol 82:2112–2120. https://doi.org/10.1128/AEM.03444-15.
5. Quiroz-Castañeda RE, Mendoza-Mejía A, Obregón-Barboza V, Martínez-Ocampo F, Hernández-Mendoza A, Martínez-Garduño F, Guillén-Solís G, Sánchez-Rodríguez F, Peña-Chora G, Ortíz-Hernández L, Gaytán-Colín P, Dantán-González E. 2015. Identification of a new *Alcaligenes faecalis* strain MOR02 and assessment of its toxicity and pathogenicity to insects. Biomed Res Int 2015:570243. https://doi.org/10.1155/2015/570243.
6. Abdsharad A, Usup G, Sahrani FK, Ahmad A. 2016. Antimicrobial activity and determination of bioactive components from marine *Alcaligenes faecalis* extract against a sulfate-reducing bacteria. AIP Conf Proc 1784:e020010.
7. Kapley A, Tanksale H, Sagarkar S, Prasad AR, Kumar RA, Sharma N, Qureshi A, Purohit HJ. 2016. Antimicrobial activity of *Alcaligenes* sp. HPC 1271 against multidrug resistant bacteria. Funct Integr Genomics 16:57–65. https://doi.org/10.1007/s10142-015-0466-8.
8. Basharat Z, Yasmin A, He T, Tong Y. 2018. Genome sequencing and analysis of *Alcaligenes faecalis* subsp. *phenolicus* MB207. Sci Rep 8:3616. https://doi.org/10.1038/s41598-018-21919-4.
9. Fuqua A. 2020. Characterization of the broad-spectrum inhibitory capability of Alcaligenes faecalis and A. viscolactis against potential pathogenic microorganisms. Undergraduate thesis. East Tennessee State University, Johnson City, TN.
10. Martin MS, Santos IC, Carlton DD, Jr, Stigler-Granados P, Hildenbrand ZL, Schug KA. 2018. Characterization of bacterial diversity in contaminated groundwater using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Sci Total Environ 622:1562–1571. https://doi.org/10.1016/j.scitotenv.2017.10.027.
11. Andrews S. 2015. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc.
12. 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.
13. 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.
14. 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.
15. 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.
16. Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. 2005. ACT: The Artemis Comparison Tool. Bioinformatics 21:3422–3423. https://doi.org/10.1093/bioinformatics/bti553.
17. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402.
18. Roosaare M, Puustusmaa M, Möls M, Vaher M, Remm M. 2018. PlasmidSeeker: identification of known plasmids from bacterial whole genome sequencing reads. PeerJ 6:e4588. https://doi.org/10.7717/peerj.4588.
19. Carattoli A, Zankari E, Garcìa-Fernandez A, Larsen M, Lund O, Villa L, Aarestrup F, 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.
20. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.