

Draft genome sequence of an enterococcus faecalis strain (24FS) that was isolated from healthy infant feces and exhibits high antibacterial activity, multiple-antibiotic resistance, and multiple virulence factors

Article

Published Version

Creative Commons: Attribution 4.0 (CC-BY)

Open Access

El Halfawy, N. M., El-Naggar, Y. and Andrews, S. C. (2019) Draft genome sequence of an enterococcus faecalis strain (24FS) that was isolated from healthy infant feces and exhibits high antibacterial activity, multiple-antibiotic resistance, and multiple virulence factors. Microbiology Resource Announcements. ISSN 2576-098X doi: https://doi.org/10.1128/MRA.00047-19 Available at

http://centaur.reading.ac.uk/84629/

It is advisable to refer to the publisher's version if you intend to cite from the work. See <u>Guidance on citing</u>.

To link to this article DOI: http://dx.doi.org/10.1128/MRA.00047-19

Publisher: American Society for Microbiology



All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online







Draft Genome Sequence of an Enterococcus faecalis Strain (24FS) That Was Isolated from Healthy Infant Feces and **Exhibits High Antibacterial Activity, Multiple-Antibiotic** Resistance, and Multiple Virulence Factors

Nancy M. El Halfawy, a,b Moustafa Y. El-Naggar, a Simon C. Andrewsb

^aBotany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt bSchool of Biological Sciences, University of Reading, Reading, United Kingdom

ABSTRACT Enterococcus faecalis 24FS is a bacteriocin-producing, multiply antibioticresistant, and potentially virulent bacterium isolated from healthy infant feces. The draft 2.9-Mb genome sequence revealed 2,968 protein-encoding genes; 11 antibiotic resistance, 8 virulence, and 3 bacteriocin genes; and 2 plasmids, 4 prophages, 30 insertion sequence (IS) elements, 1 transposon, and 1 integron.

Interococcus faecalis is a common commensal of the human intestine (1, 2) but is also a frequent cause of hospital-acquired enterococcal infections (3). An E. faecalis fecal isolate (24FS) from a healthy 6-month-old infant (Alexandria, Egypt) displayed multipleantibiotic resistance, strong antibacterial properties, and numerous virulence factors and thus provided the opportunity to study the emergence of pathogenic/resistance properties in a nonclinical strain from a developing nation.

Strain 24FS was isolated from infant feces (in 2016) on bile esculin azide agar. Genomic DNA was isolated using a GeneJET purification kit. Genome sequencing was performed by MicrobesNG (Birmingham, UK) on MiSeq and HiSeq 2500 platforms (Illumina) with 30× sequence coverage, giving 924,992 reads with a median insert size of 529 bases and a 136-fold coverage of the genome. The reads were trimmed using Trimmomatic (4) (v 0.38) by identification of adapter sequences, and the quality of trimmed reads was assessed using in-house scripts combined with the BWA-MEM software (5) (v 0.7.9). De novo assembly was performed using SPAdes (6) (v 3.7.0), yielding 46 contigs of more than 1,000 bp. Assembly quality was assessed using QUAST (7). The 2,984,798-bp draft genome has 2,968 protein-encoding genes, 54 tRNA genes, and 111 pseudogenes and a G+C content of 37.38%, as predicted with Rapid Annotations using Subsystem Technology (RAST) (8, 9).

E. faecalis 24FS showed a broad-spectrum antibacterial activity (in spot on the lawn assays [10]), inhibiting the pathogens Listeria monocytogenes 10403S, Salmonella enterica PT4, Staphylococcus aureus, and Pseudomonas aeruginosa PAO1. The PATtyFam algorithm identified three bacteriocin genes encoding a colicin V (20,317 Da), the class lla bacteriocin hiracin-JM79 (7,737 Da), and an enterocin A (13,264 Da). Strain 24FS is α -hemolytic (11), which is consistent with the observed presence of yafA, encoding hemolysin III. It also has high adherence and biofilm-formation capacity (12), correlating with the presence of virulence-related adherence/biofilm genes, namely, gelE (gelatinase), esp (gene encoding a surface protein), ace (collagen adhesin), cvfB (conserved virulence factor B), and ebpABC (pili-encoding genes). The disk diffusion method (13) showed that the strain is resistant to chloramphenicol, tetracycline, and erythromycin but sensitive to penicillin and streptomycin, which is consistent with the presence of corresponding resistance genes (cat, chloramphenicol; tetA, tetC, tetL, tetR, and ykkCD,

Citation El Halfawy NM, El-Naggar MY, Andrews SC. 2019. Draft genome sequence of an Enterococcus faecalis strain (24FS) that was isolated from healthy infant feces and exhibits high antibacterial activity, multiple-antibiotic resistance, and multiple virulence factors. Microbiol Resour Announc 8:e00047-19. https://doi.org/10.1128/MRA.00047-19.

Editor Jason E. Stajich, University of California,

Copyright © 2019 El Halfawy et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Simon C. Andrews, s.c.andrews@reading.ac.uk.

Received 11 January 2019 Accepted 27 February 2019 Published 28 March 2019

tetracycline; and *ermB*, erythromycin), as revealed using the Comprehensive Antibiotic Resistance Database (CARD) (14). Genes conferring resistance to aminoglycosides [ANT(6)-la and APH(3')-Illa] and fluoroquinolones (*gyrA* and *gyrB*) were also detected, although the corresponding resistance was not tested. In addition, genes mediating iron transport were identified, including *feuABC* (ferric iron uptake), *hmuUV* (haem uptake), *feoAB* (ferrous iron uptake), and *fetAB* (iron export).

The CRISPR finder tool (15) identified two CRISPR elements with five or nine direct repeats of 36 bp, with one associated with *cas*-family proteins. A conjugative Tn916 transposon, associated with tetracycline resistance *tet*(M), was also identified. In addition, a class II integron with three antibiotic-resistance gene cassettes (*dfrA1*, dihydroflorate reductase; *sat2*, streptothricin acetyltransferase; and *aad1*, aminoglycoside adenyltransferase) was found and predicted to confer resistance to trimethoprim, streptothricin, and streptomycin, respectively. Two plasmids (16) were identified, namely, pS194 and pAD1 (4.3 and 60 kb, respectively). The PHASTER tool (17) identified one intact (PHAGE_Entero_vB_IME197, 57.1 kb), one incomplete (PHAGE_Entero_vB_IME197, 14.6 kb), and two questionable (PHAGE_Entero_phiFL1A, 38.8 kb; and PHAGE_Brocho_NF5, 14.2 kb) prophage regions. One putative integrative conjugative element (ICE) (T4SS) of 431,826 bp was identified by ICEfinder (18) containing a type IV secretion gene cluster. Thirty insertion sequence (IS) elements were predicted with IS finder (19).

These genomic data assist understanding of the emergence of antibiotic resistance and virulence in nonclinical *E. faecalis* isolates.

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number PGCH00000000. The version described in this paper is the first version, PGCH01000000. The raw sequencing data are available in the Sequence Read Archive (SRA) database under the accession number SRR8433214.

ACKNOWLEDGMENTS

We thank the Newton-Mosharafa program, cofunded by the British Council and Ministry of Higher Education (Egypt), for financial support of Nancy M. El Halfawy for her research at the University of Reading.

We thank MicrobesNG for performing the next-generation sequencing (NGS). We declare no conflict of interest.

REFERENCES

- Corr SC, Hill C, Gahan CGM. 2009. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. Adv Food Nutr Res 56:1–15. https://doi.org/10.1016/S1043-4526(08)00601-3.
- Guzman Prieto AM, van Schaik W, Rogers MRC, Coque TM, Baquero F, Corander J, Willems RJL. 2016. Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? Front Microbiol 7:788. https://doi.org/10.3389/fmicb.2016.00788.
- Agudelo Higuita NI, Huycke MM. 2014. Enterococcal disease, epidemiology, and implications for treatment. In Gilmore MS, Clewell DB, Ike Y, and Shankar N (ed), Enterococci: from commensals to leading causes of drug resistant infection. Massachusetts Eye and Ear Infirmary, Boston, MA.
- 4. Bolger A, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
- 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.
- 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.
- 7. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assess-

- ment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Ill, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https:// doi.org/10.1038/srep08365.
- Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Seung Yoo H, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC: the bacterial bioinformatics database and analysis resource. Nucleic Acids Res 42:D581–D591. https://doi.org/10 .1093/nar/gkt1099.
- Schillinger U, Lücke FK. 1989. Antibacterial activity of Lactobacillus sake isolated from meat. Appl Environ Microbiol 55:1901–1906.
- Foulquié Moreno MR, Callewaert R, Devreese B, Van Beeumen J, De Vuyst L. 2003. Isolation and biochemical characterisation of enterocins produced by enterococci from different sources. J Appl Microbiol 94: 214–229. https://doi.org/10.1046/j.1365-2672.2003.01823.x.
- Heikens E, Bonten MJM, Willems RJL. 2007. Enterococcal surface protein Esp is important for biofilm formation of *Enterococcus faecium* E1162. J Bacteriol 189:8233–8240. https://doi.org/10.1128/JB.01205-07.
- 13. Baeur AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility

Volume 8 Issue 13 e00047-19

- testing by standardized single disc method. Am J Clinl Pathol 45: 493–496. https://doi.org/10.1093/ajcp/45.4 ts.493.
- 14. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45:D566–D573. https://doi.org/10.1093/nar/gkw1004.
- Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a Web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:W52–W57. https://doi.org/10.1093/nar/gkm360.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of

- plasmids using Plasmid Finder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016.
 PHASTER: a better, faster version of the PHAST phage search tool.
 Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.
- Liu M, Li X, Xie Y, Bi D, Sun J, Li J, Tai C, Deng Z, Ou H-Y. 2019. ICEberg 2.0: an updated database of bacterial integrative and conjugative elements. Nucleic Acids Res 47:D660–D665. https://doi.org/10.1093/nar/gky1123.
- Varani AM, Siguier P, Gourbeyre E, Charneau V, Chandler M. 2011. ISsaga is an ensemble of web-based methods for high throughput identification and semi-automatic annotation of insertion sequences in prokaryotic genomes. Genome Biol 12:R30. https://doi.org/10.1186/gb -2011-12-3-r30.

Volume 8 Issue 13 e00047-19