





Draft Genome Sequences of Two Commensal *Enterococcus faecalis* Strains Isolated from American Black Vultures (*Coragyps atratus*) in Brazil

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ABSTRACT We report the draft genome sequences of two commensal *Enterococcus faecalis* strains (designated Ca-2 and Ca-18) recovered from the cloacae of two healthy American black vultures (*Coragyps atratus*) in Rio de Janeiro, Brazil. The strains were found to carry a variety of antimicrobial resistance and virulence-associated genes.

Enterococcus faecalis is an opportunistic bacterium found in the intestinal microbiome of humans and animals (1, 2). The emergence of this species as a major nosocomial pathogen has been associated with its ability to acquire antimicrobial resistance and virulence genes (1, 3) and constitutes a significant concern for both human and veterinary medicine (1, 4, 5).

We present the draft genome sequences of two *E. faecalis* strains (Ca-2 and Ca-18) isolated from two American black vultures (*Coragyps atratus*) admitted to a wildlife rehabilitation center in Rio de Janeiro, Brazil. Cloacal content samples were inoculated into Enterococcosel broth (BD Microbiology Systems, USA). After 24 h at 37°C, an aliquot of each culture was streaked onto an Enterococcosel agar plate and incubated under the same conditions.

Genomic DNA was obtained from 1.5 mL of each culture grown in tryptic soy broth overnight at 37°C, using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA); it was prepped using the Nextera XT kit and sequenced on a HiSeq 2500 sequencer (Illumina Inc., San Diego, CA, USA) with 125-bp paired-end reads. The read quality metrics were evaluated using FastQC v0.11.18 (6). The reads were trimmed (Trim Galore v0.6.5) (7), assembled (Unicycler v0.4.8) (8), and annotated (RASTtk v1.073) (9) using the resources of the Pathosystems Resource Integration Center (PATRIC) v3.6.9 (10). Default parameters were used for all software tools. Sequencing and assembly data for the two genomes are summarized in Table 1.

VirulenceFinder v2.0 (11) was used to identify genes playing important roles in the pathogenesis of enterococcal infections. Both strains harbored genes coding for gelatinase production (*gelE*), endocarditis antigen (*efaA*), aggregation substance (*agg*), and sortase (*srtA*), which are associated with adherence, aggregation, and invasion of host tissues (2, 4). Other virulence-associated genes identified in both strains included *cad*, *cOB1*, *CCF10*, and *camE* (coding for sex pheromones) (4, 12), *tpx* (protection against oxidative stress) (4, 13), and *elrA* (the enterococcal Rgg-like regulator gene associated with macrophage evasion) (4, 14). Genes associated with cytolysin production (*cylA*, *cylB*, *cylL*, and *cylM*) (2, 4) and collagen adhesion (*ace*) (4) were identified only in strain Ca-18.

ResFinder v4.1 (15) was used to identify the following major antimicrobial resistance genes in strain Ca-2: *ant(6)-Ia*, *aph(3')-III*, *sat-4*, and *str* (aminoglycoside); *erm(B)* (macrolide,

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TABLE 1 Basic characteristics of the whole-genome assemblies of two *Enterococcus faecalis* strains isolated from American black vultures in Rio de Janeiro, Brazil

Parameter	Data for <i>Enterococcus faecalis</i> strain:	
	Ca-2	Ca-18
Genome size (bp)	3,059,826	3,072,615
No. of raw reads	490,885	624,609
GC content (%)	37.30	37.37
No. of contigs	59	92
Mean coverage (×)	46	58
No. of CDSs ^a	3,058	3,069
<i>N</i> ₅₀ (bp)	173,562	134,282
No. of tRNAs	52	53
No. of rRNAs	3	3
SRA accession no.	SRR8163627	SRR8163626
GenBank assembly no.	RJJO0000000.2	RJJP0000000.2

^a CDSs, coding DNA sequences.

lincosamide and streptogramin B); *lnu(G)* (lincosamide); *tet(L)* (tetracycline); *catA* (chloramphenicol); and *dfp* (trimethoprim). Both strains carried the *tet(M)* (tetracycline resistance) and *IsaA* (streptogramin resistance) genes. Mutations in *GyrA* (Glu88Gly) and *ParC* (Ser85Ile), implicated in fluoroquinolone resistance (16, 17), were identified in strain Ca-2 by alignment with reference sequences (GenPept accession numbers [BAB69479.1](https://www.ncbi.nlm.nih.gov/GenPept/BAB69479.1) and [BAB69481.1](https://www.ncbi.nlm.nih.gov/GenPept/BAB69481.1), respectively). Both strains contained the plasmid repUS43, and strain Ca-2 also carried rep_{9c} (18). One confirmed CRISPR region was identified in Ca-18 (19). Moreover, using PHASTER (20), one intact prophage was predicted in the Ca-2 genome and two intact prophages in the Ca-18 genome. Multilocus sequence typing (21) identified the strains as sequence type 33 (ST330) (Ca-2) and ST82 (Ca-18).

Data availability. The raw sequence reads have been deposited at the NCBI Sequence Read Archive (SRA) under the BioProject accession number [PRJNA503970](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA503970). The whole-genome shotgun project versions described in this paper are listed in Table 1.

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REFERENCES

- Torres C, Alonso CA, Ruiz-Ripa L, León-Sampedro R, Del Campo R, Coque TM. 2018. Antimicrobial resistance in *Enterococcus* spp. of animal origin. *Microbiol Spectr* 6. <https://doi.org/10.1128/microbiolspec.ARBA-0032-2018>.
- He Q, Hou Q, Wang Y, Li J, Li W, Kwok LY, Sun Z, Zhang H, Zhong Z. 2018. Comparative genomic analysis of *Enterococcus faecalis*: insights into their environmental adaptations. *BMC Genomics* 19:527. <https://doi.org/10.1186/s12864-018-4887-3>.
- Mikalsen T, Pedersen T, Willems R, Coque TM, Werner G, Sadowy E, van Schaik W, Jensen LB, Sundsfjord A, Hegstad K. 2015. Investigating the mobilome in clinically important lineages of *Enterococcus faecium* and *Enterococcus faecalis*. *BMC Genomics* 16:282. <https://doi.org/10.1186/s12864-015-1407-6>.
- Woods SE, Lieberman MT, Lebreton F, Trowel E, de la Fuente-Núñez C, Dzink-Fox J, Gilmore MS, Fox JG. 2017. Characterization of multi-drug resistant *Enterococcus faecalis* isolated from cephalic recording chambers in research macaques (*Macaca* spp.). *PLoS One* 12:e0169293. <https://doi.org/10.1371/journal.pone.0169293>.
- Gawryszewska I, Malinowska K, Kuch A, Chrobak-Chmiel D, Trokheim LL, Hryniewicz W, Sadowy E. 2017. Distribution of antimicrobial resistance determinants, virulence-associated factors and clustered regularly interspaced palindromic repeats loci in isolates of *Enterococcus faecalis* from various settings and genetic lineages. *Pathog Dis* 75:ftx021. <https://doi.org/10.1093/femspd/ftx021>.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Krueger F. 2012. Trim Galore: a wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, with some extra functionality for MspI-digested RRBS-type (reduced representation bisulfite-seq) libraries. http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, 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, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich

- EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45:D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
11. 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>.
 12. Beukers AG, Zaheer R, Goji N, Amoako KK, Chaves AV, Ward MP, McAllister TA. 2017. Comparative genomics of *Enterococcus* spp. isolated from bovine feces. *BMC Microbiol* 17:52. <https://doi.org/10.1186/s12866-017-0962-1>.
 13. La Carbona S, Sauvageot N, Giard JC, Benachour A, Posteraro B, Auffray Y, Sanguinetti M, Hartke A. 2007. Comparative study of the physiological roles of three peroxidases (NADH peroxidase, alkylhydroperoxide reductase and thiol peroxidase) in oxidative stress response, survival inside macrophages and virulence of *Enterococcus faecalis*. *Mol Microbiol* 66: 1148–1163. <https://doi.org/10.1111/j.1365-2958.2007.05987.x>.
 14. Dumoulin R, Cortes-Perez N, Gaubert S, Duhutrel P, Brinster S, Torelli R, Sanguinetti M, Posteraro B, Repoila F, Serron P. 2013. Enterococcal Rgg-like regulator ElrR activates expression of the *elrA* operon. *J Bacteriol* 195: 3073–3083. <https://doi.org/10.1128/JB.00121-13>.
 15. Bortolaia V, Kaas RF, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AR, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 75:3491–3500. <https://doi.org/10.1093/jac/dkaa345>.
 16. Tankovic J, Mahjoubi F, Courvalin P, Duval J, Leclerc R. 1996. Development of fluoroquinolone resistance in *Enterococcus faecalis* and role of mutations in the DNA gyrase *gyrA* gene. *Antimicrob Agents Chemother* 40:2558–2561. <https://doi.org/10.1128/AAC.40.11.2558>.
 17. Sun Y, Lu H, Zhang X, Wu Q, Bi W, Liu H, Cao J, Zhou T. 2018. Phenotype and genotype alteration during adaptive evolution of *Enterococcus faecalis* to antimicrobials. *Infect Genet Evol* 62:80–85. <https://doi.org/10.1016/j.meegid.2018.03.029>.
 18. 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>.
 19. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C. 2018. CRISPRCasFinder, an update of CRISPRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res* 46: W246–W251. <https://doi.org/10.1093/nar/gky425>.
 20. Arndt D, Jason R, 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>.
 21. Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 3:124. <https://doi.org/10.12688/wellcomeopenres.14826.1>.