Draft Genome Sequence of *Salmonella enterica* subsp. *enterica* Serovar Gallinarum Biovar Pullorum Strain FCAV198, a Brazilian Chicken Pathogen

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Salmonella enterica subsp. enterica serovar Gallinarum biovar Pullorum is a bird-restricted pathogen which causes pullorum disease. The strain FCAV198 was isolated from a pool of chicken ovaries in Brazil, and its genome may be helpful for studies involving molecular mechanisms related to pathogenesis and other related applications.

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Pullorum disease (PD), which is caused by *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Pullorum, was reported worldwide in 2012, but its occurrence was especially high in Asian countries (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail). PD is known as a systemic disease responsible for high mortality in young birds (1). Some of the birds that recover from infection become persistently infected and transmit *Salmonella* biovar Pullorum vertically (2). The bird restriction and aspects of PD pathogenesis, mainly the persistence of infection, are thought to be associated with gene losses (O. C. Freitas Neto, personal communication). The genome decay in *Salmonella* biovar Pullorum, caused by pseudogene accumulation, has already been observed in other *Salmonella enterica* subsp. *enterica* genomes (3–5).

Genetic variability among *Salmonella* biovar Pullorum strains has been demonstrated by use of several different molecular approaches over the past few years (6–8), highlighting the need for more genome sequencing to reveal the core genes. In addition, genomic comparisons among the closely related pathogens *Salmonella* biovar Pullorum and *Salmonella* serovar Gallinarum biovar Gallinarum could be helpful to clarify the genome evolution and molecular basis of their distinct pathogenicity and epidemiology. The *Salmonella* biovar Pullorum strain FCAV198 was isolated in Brazil from a pool of chicken ovaries in 2008. This strain was serotyped and granted by the National Agriculture and Livestock Laboratory (LANAGRO), which is the official laboratory of the Brazilian Ministry of Agriculture, Livestock and Supply and gives support to the surveillance of animal diseases.

The whole genome was sequenced using the SOLiD 4.0 sequencing system, which produced 65 million mate-paired reads of 50 bp, with insert sizes from 1,000 to 2,000 bp. *De novo* assembly was carried out using the CLC Genomics workbench 6.5.1 (CLC bio, Aarhus, Denmark) and generated 128 contigs. The draft genome sequence has a G+C content of 52.2% and a total size of 4,783,588 bp, as expected for *Salmonella* spp. (4, 5). The annotation and metabolic reconstruction were performed using the Prokka pipeline version 1.7 (Prokaryotic Genome Annotation System) and Rapid Annotations using Subsystems Technology (RAST) server (9), respectively. With these methods, 4,652 coding sequences (CDSs; with an average length of 847 bp), 65 tRNAencoding genes, and 27 rRNA-encoding genes were predicted. It is noteworthy that the last 182,819 bp (from contigs 48 to 127) corresponds to mobile genetic elements, plasmids, and other genomic sequences which had no matches to the closely related *Salmonella* biovar Pullorum strain CDC 1983-67 (NC_02221). Some of these regions may be acquired by lateral gene transfer and encoding of potential biovar-specific traits, such as were observed in other *Enterobacteriaceae* (10, 11).

Experimental studies by *in vivo* infection indicate that the strain FCAV198 is pathogenic for chickens and shows the typical biochemical profile observed in other *Salmonella* biovar Pullorum strains (12). Furthermore, the genetic variability observed among the strains of *Salmonella* biovar Pullorum (6–8) suggests that their genomes are under different evolutive forces. Indeed, the genome sequencing approach of several different biovars could shed light on the biology of *Salmonella* biovar Pullorum and on PD disease, which is still responsible for economic losses to the poultry industry worldwide.

Nucleotide sequence accession number. The annotated draft genome sequence was deposited in DDBJ/EMBL/GenBank under accession number AZRG00000000. The version described in this paper is the first version.

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REFERENCES

- 1. Barrow PA, Freitas Neto OC. 2011. Pullorum disease and fowl typhoid new thoughts on old diseases: a review. Avian Pathol. 40:1–13. http://dx .doi.org/10.1080/03079457.2010.542575.
- Berchieri Junior A, Murphy CK, Marston K, Barrow PA. 2001. Observations on the persistence and vertical transmission of *Salmonella enterica* serovars pullorum and gallinarum in chickens: effect of bacterial and host genetic background. Avian Pathol. 30:221–231. http://dx.doi.org/10.1080/03079450120054631.
- Feng Y, Xu HF, Li QH, Zhang SY, Wang CX, Zhu DL, Caor FL, Li YG, Johnston RN, Zhou J, Liu GR, Liu SL. 2012. Complete genome sequence of *Salmonella enterica* serovar Pullorum RKS5078. J. Bacteriol. 194:744. http://dx.doi.org/10.1128/JB.06507-11.
- 4. Thomson NR, Clayton DJ, Windhorst D, Vernikos G, Davidson S, Churcher C, Quail MA, Stevens M, Jones MA, Watson M, Barron A, Layton A, Pickard D, Kingsley RA, Bignell A, Clark L, Harris B, Ormond D, Abdellah Z, Brooks K, Cherevach I, Chillingworth T, Woodward J, Norberczak H, Lord A, Arrowsmith C, Jagels K, Moule S, Mungall K, Sanders M, Whitehead S, Chabalgoity JA, Maskell D, Humphrey T, Roberts M, Barrow PA, Dougan G, Parkhill J. 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res. 18:1624–1637. http://dx.doi.org/10.110 1/gr.077404.108.
- Holt KE, Thomson NR, Wain J, Langridge GC, Hasan R, Bhutta ZA, Quail MA, Norbertczak H, Walker D, Simmonds M, White B, Bason N, Mungall K, Dougan G, Parkhill J. 2009. Pseudogene accumulation in the evolutionary histories of *Salmonella enterica* serovars Paratyphi A and Typhi. BMC Genomics 10:36. http://dx.doi.org/10.1186/1471-2 164-10-36.

- Li J, Smith NH, Nelson K, Crichton PB, Old DC, Whittam TS, Selander RK. 1993. Evolutionary origin and radiation of the avian-adapted nonmotile salmonellae. J. Med. Microbiol. 38:129–139. http://dx.doi.org/10. 1099/00222615-38-2-129.
- Olsen JE, Skov MN, Christensen JP, Bisgaard M. 1996. Genomic lineage of *Salmonella enterica* serotype Gallinarum. J. Med. Microbiol. 45: 413–418. http://dx.doi.org/10.1099/00222615-45-6-413.
- Porwollik S, Santiviago CA, Cheng P, Florea L, Jackson S, McClelland M. 2005. Differences in gene content between *Salmonella enterica* serovar Enteritidis isolates and comparison to closely related serovars Gallinarum and Dublin. J. Bacteriol. 187:6545–6555. http://dx.doi.org/10.1128/JB.18 7.18.6545-6555.2005.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/ 1471-2164-9-75.
- Kingsley RA, Msefula CL, Thomson NR, Kariuki S, Holt KE, Gordon MA, Harris D, Clarke L, Whitehead S, Sangal V, Marsh K, Achtman M, Molyneux ME, Cormican M, Parkhill J, MacLennan CA, Heyderman RS, Dougan G. 2009. Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. Genome Res. 19:2279–2287. http://dx.doi.org/10.1101/gr.0910 17.109.
- Nie H, Yang F, Zhang X, Yang J, Chen L, Wang J, Xiong Z, Peng J, Sun L, Dong J, Xue Y, Xu X, Chen S, Yao Z, Shen Y, Jin Q. 2006. Complete genome sequence of *Shigella flexneri* 5b and comparison with *Shigella flexneri* 2a. BMC Genomics 7:173. http://dx.doi.org/10.1186/1471-2164-7 -173.
- Crichton PB, Old DC. 1990. Salmonellae of serotypes gallinarum and pullorum grouped by biotyping and fimbrial-gene probing. J. Med. Microbiol. 32:145–152. http://dx.doi.org/10.1099/00222615-32-3-145.