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Draft genome sequence of *Cronobacter sakazakii* strain MEZCS99 sequence type 3 isolated from chicken in South Africa

Mohamed E. El Zowalaty[a,b,*], Linda Falgenhauer[c], Stephen Forsythe[d]

[a] *Veterinary Medicine and Food Security Research Group, Medical Laboratory Sciences Program, Faculty of Health Sciences, Abu Dhabi Women's Campus, Higher Colleges of Technology, Abu Dhabi 41012, United Arab Emirates*
[b] *Zoonosis Science Center, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden*
[c] *Institute of Hygiene and Environmental Medicine, Justus Liebig University Giessen, Giessen, Germany*
[d] *Foodmicrobe.com, Adams Hill, Keyworth, Nottingham NG12 5GY, United Kingdom*

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ABSTRACT

Objectives: *Cronobacter sakazakii* is an emerging opportunistic foodborne pathogen that is frequently associated with life-threatening infections such as infantile septicemia, meningitis, and necrotizing enterocolitis. The emergence of antimicrobial-resistant, livestock-associated *C. sakazakii* is a great public health concern. Here, we report on the first draft genome sequence of *C. sakazakii* strain MEZCS99 sequence type 3 (ST3) isolated from feces from a healthy chicken in KwaZulu-Natal Province, South Africa.
Methods: The genomic DNA of *C. sakazakii* was sequenced using an Illumina MiSeq platform (Illumina Inc., San Diego, CA). Generated reads were trimmed and de novo assembled. The assembled contigs were analyzed for virulence and antimicrobial resistance genes, extra-chromosomal plasmids, and multilocus sequence type (MLST). To compare the sequenced strains to other previously sequenced *C. sakazakii* strains, available raw read sequences of *C. sakazakii* were downloaded and all sequence files were treated identically to generate a core genome phylogenetic tree.
Results: Intrinsic beta-lactam resistance gene *blaCSA-1* was detected in MEZCS99. No colistin or other antibiotic resistance genes were detected. MEZCS99 belonged to ST3 and harbored an extra-chromosomal plasmid (IncFIB (pCTU3)). The genome of MEZCS99 strain showed two CRISPR/Cas cluster arrays of I-E (n = 1) and I-F (n = 1) type.
Conclusion: The genome sequence of strain MEZCS99 will serve as a reference point for molecular epidemiological studies of livestock-associated *C. sakazakii* in Africa. In addition, this study allows in-depth analysis of the genomic structure and will provide valuable information that helps understand the pathogenesis and antimicrobial resistance of livestock-associated *C. sakazakii*.

1. Introduction

Cronobacter sakazakii (*C. sakazakii*) is a rare opportunistic foodborne pathogen that is associated with life-threatening and severe infections such as infantile septicemia, meningitis, necrotizing enterocolitis [1], and nosocomial infections such as urinary tract infections, pneumonia, and wound infections in adults [2,3]. *Cronobacter* species (formerly known as *Enterobacter sakazakii*) are motile by peritrichous flagella, non-spore forming, oxidase-negative, catalase-positive, facultative anaerobic, rod-shaped Gram-negative bacteria in the *Enterobacteriaceae* family [1]. *Cronobacter sakazakii* resists osmotic stress and desiccation, enabling the bacterium to be detected in stored powdered infant formula (PIF) even after 2.5 y [4], where infections can occur through the ingestion of contaminated rehydrated PIF. Nevertheless, it has been recovered from a wide range of plants, plant-based food ingredients, different environments including households, livestock facilities, and food manufacturing operations (specifically PIF production facilities) [1]. *Cronobacter sakazakii* has infrequently been reported from animals or meat products yet was recently reported from buffalo feces [5]. *Cronobacter sakazakii* can harbor a range of antimicrobial resistance genes, including colistin resistance *mcr-9* and *mcr-10* genes [6].

Here, we report the draft genome sequence of *Cronobacter sakazakii* strain MEZCS99 isolated from fecal material of a

* Corresponding author at: Division of Health Sciences, Abu Dhabi Women's Campus, Higher Colleges of Technology, Abu Dhabi 41012, United Arab Emirates.
E-mail address: elzow001@gmail.com (M.E. El Zowalaty).

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Table 1
Number of single nucleotide polymorphism (SNP) differences between the *Cronobacter sakazakii* sequence type 3 (ST3) isolates

	978	C_1	ZV_6148_17	1915003	984	MOD1_Jor146	C_5	MEZCS99	ZV_132_17	C_10	MOD1_16MP002185
978		2055	2061	2044	51	2056	2057	2052	2163	2055	2091
C_1	2055		266	251	2040	127	2	255	368	0	294
ZV_6148_17	2061	266		229	2046	267	268	263	322	266	302
1915003	2044	251	229		2029	252	253	244	327	251	287
984	51	2040	2046	2029		2041	2042	2037	2148	2040	2076
MOD1_Jor146	2056	127	267	252	2041		129	256	369	127	293
C_5	2057	2	268	253	2042	129		257	370	2	296
MEZCS99	2052	255	263	244	2037	256	257		361	255	291
ZV_132_17	2163	368	322	327	2148	369	370	361		368	404
C_10	2055	0	266	251	2040	127	2	255	368		294
MOD1_16MP002185	2091	294	302	287	2076	293	296	291	404	294	

healthy chicken (*Gallus gallus domesticus*) in Verelum, KwaZulu-Natal Province in August 2018. The sample was collected in 10 mL of 0.1% buffered peptone water (Merck, South Africa), incubated for 24 h, enriched in peptone broth, and streaked onto nutrient agar (Thermo Scientific, England). A slant of the bacterial culture was shipped to the University of Minnesota Genomic Center (St. Paul, MN) for whole-genome sequencing, as previously reported [7]. The isolate was cultured on sheep blood agar for 18 to 24 h at 37 °C in the presence of 5% CO_2, and a single colony-forming unit was cultured in tryptic soy broth (Merck KGaA, Germany) to the inoculum density of 0.5 McFarland turbidity standard using Thermo Scientific™ Sensititre™ Nephelometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA isolation was performed using a Qiagen DNeasy Blood & Tissue kit (Lucigen, WI, USA) according to the manufacturer's protocol.

Sequencing libraries were prepared using a Nextera XT library sample preparation kit (Illumina Inc., San Diego, CA). Sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA) using the v3 reagent kit with 2 × 300nt paired-end (PE) read length.

Raw sequencing reads were quality-controlled and assembled using the ASA^{3}P tool [8]. An average read length of 206 nt and coverage of 39 × was obtained. Contigs smaller than 200 nucleotides were discarded from further analysis. The genome size was 4,339,652 bp with a G+C (guanine-cytosine content) content of 56.99%, an N_{50} value of 192,224 bp, and 53 contigs. The genome was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) from the National Center for Biotechnology Information (NCBI), which identified 4004 coding DNA sequences and 74 tRNAs, 11 ncRNA, and 5 rRNA genes.

The genome sequence of *C. sakazakii* MEZCS99 is the first report of *C. sakazakii* isolated from chicken feces in South Africa, indicating their potential role in environmental contamination and food animals. The only two other *C. sakazakii* strains of African origin in the PubMLST *Cronobacter* database (IDs 846 and 847; https://pubmlst.org/organisms/cronobacter-spp) were reported from Nigeria. They differ from MEZCS99 in that they were isolated from infant formula and are different sequence types (ST304 and ST296, compared with ST3).

The MEZCS99 strain belongs to sequence type 3, which is in clonal complex 3 (CC3). Strains from this clonal complex are frequently isolated from infant formula [1]. TblastN using the reference gene catalogue for antimicrobial resistance genes (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#) against MEZCS99 was performed, and it was revealed that MEZCS99 carried only the intrinsic beta-lactam resistance gene, *blaCSA-1*, which confers resistance to cephalothin. No other antibiotic resistance genes were detected. PlasmidFinder v2.1 [9] and CRISPRCasFinder v2.0.3 [10] were used to determine plasmid and CRISPR arrays, respectively. MEZCS99 harbored a IncFIB (pCTU3) plasmid. The genome of MEZCS99 strain showed two CRISPR/Cas cluster arrays of I-E (n = 1) and I-F (n = 1) type (Table 1). The presence of *C. sakazakii*-specific virulence genes was determined using the BLAST tool of PubMLST (https://pubmlst.org/organisms/cronobacter-spp) [11] (Supplementary Table S1). MEZCS99 harbored 29 of 33 of the virulence genes analyzed, including genes involved in motility, flagella production, sialic acid utilization, and hemolysis. It is worth mentioning that MEZCS99 lacks genes *galF* and *galS*; however, their relevance is still unknown.

A maximum-likelihood phylogeny was inferred from the core genome alignment of ten *C. sakazakii* ST3 publicly available genomes (Supplementary Table S2) downloaded in PubMLST (https://pubmlst.org/organisms/cronobacter-spp) using ParSNP, implemented in Harvest Suite, v.1.1.2 [12], and visualized with ITOL v.5 [13]. As shown in Fig. 1, *C. sakazakii* ST3 isolates are divided in two clades with two and nine isolates.

Using MEGA5 [14], the core SNPs determined using ParSNP were transferred in a homology matrix (Table 1). Based on this matrix, the closest relative to MEZCS99 is the isolate 1915003 (PubMLST ID 3225) isolated from a food ingredient in China; however, the high number of SNPs (n = 244) infers there is only a very distant relationship.

The present study represents important findings on the detection of *C. sakazakii*, a desiccation-resistant coliform of increased concern to infant health which may have a zoonotic potential and further public health risk requiring extensive investigation. The transmission of *Cronobacter* amongst farm animals is unknown and may act as a previously unrecognized reservoir leading to bacterially contaminated food. Monitoring and genomic characterization of *C. sakazakii* in livestock, food chain animals, and food production environments will help us understand its pathogenesis, prevent transmission, and improve food safety and quality by reducing public health risks associated with the presence of *C. sakazakii* in food products.

Data availability

The whole-genome shotgun sequences have been deposited at DDBJ/ENA/GenBank under the BioProject number PRJNA716986 (BioSample accession number SAMN23419544 and GenBank accession number JAJNNJ000000000). The version described in this paper is the first version, JAJNNJ000000000.1. The sequences have been submitted to the Sequence Read Archive (SRA) under the accession numbers SRR17485924 and SRX13656415. The genome was deposited to the *Cronobacter* PubMLST database under ID number 3395.

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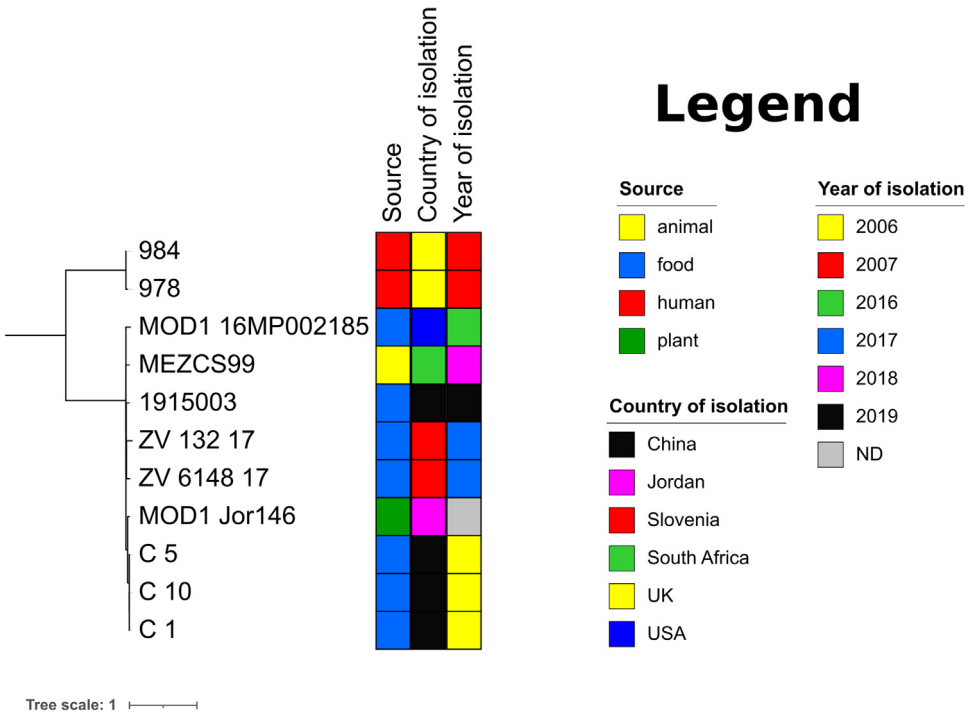

Fig. 1. Single nucleotide polymorphism (SNP)-based phylogenetic tree of MEZCS99 and all *Cronobacter sakazakii* ST3 isolates available at PubMLST (as of 11th February 2022, https://pubmlst.org/organisms/cronobacter-spp,).

Germany. The project was also supported by discretionary funds from Professor Doctor M.E. El Zowalaty.

Conflicts of interest

None declared

Ethical approval

This study was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Durban, South Africa) [reference numbers. AREC 071/017 and AREC 014/018; and South Africa Department of Agriculture, Forestry and Fishery Act No. 35 of 1984 Section 20 approval reference no. 12/11/1/5].

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.08.016.

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