Sellimonas caecigallum sp. nov., description and genome sequence of a new member of the *Sellimonas* genus isolated from the cecum of feral chicken

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Abstract

An obligately anaerobic, non-motile, Gram-positive coccobacillus strain SW451 was isolated from pooled caecum contents of feral chickens. Based on taxono-genomic, and biochemical analyses, the strain SW451 represents a new species of the genus *Sellimonas*, for which the name *Sellimonas caecigallum* sp. nov. is proposed. The type strain of *Sellimonas caecigallum* is SW451 (=DSM 109473^T = CCOS 1879^T).

Keywords: Culturomics, feral chicken, new species, *Sellimonas caecigallum*, taxono-genomics
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Introduction

The chicken gut contains a diverse microbial community which is dominated by the phyla Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes [1–3]. Gut commensal bacteria play an important role in host metabolism, immune system and pathogen protection [4,5]. Recently, the gut microbiota has been studied using culture-independent and culture-dependent techniques. Sequencing-based analysis of the gut microbiome is useful for identifying dynamic changes in microbiota composition, whereas culturing is used to access biological functions of individual microbiota members and their interactions [6–8]. However, the cultivation and isolation of several bacterial species from gut microbiota remains a daunting problem. Here, we report the cultivation and characterization of a new bacterial species from the caecum of feral chicken. The proposed *Sellimonas caecigallum* sp. nov. strain SW451 (=DSM 109473^T = CCOS 1879^T) is a new member of genus *Sellimonas*.

Methods

Isolation, growth conditions and strain identification

Strain SW451 was isolated from the caecum of feral chicken by culturing in strict anaerobic conditions in a Coy Lab anaerobic chamber containing 85% nitrogen, 10% hydrogen and 5 % carbon dioxide. Modified brainheart infusion (BHI-M) medium, which contained 37 g/L of BHI, 5 g/L of yeast extract, 1 mL of 1 mg/mL menadione, 0.3 g of L-cysteine, 1 mL of 0.25 mg/L of resazurin, 1 mL of 0.5 mg/mL hemin, 10 mL of vitamin and mineral mixture, 1.7 mL of 30 mM acetic acid, 2 mL of 8 mM propionic acid, 2 mL of 4 mM butyric acid, 100 µL of 1 mM isovaleric acid and 1% pectin and inulin, was used for strain culturing and maintenance. Genomic DNA of the strain SW451 was extracted using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. 16S rRNA gene sequences were amplified using universal primer set 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-ACCTTGTTACGACTT-3′) [9,10]. The PCR amplicon was sequenced using the Sanger dideoxy method (ABI 3730XL; Applied Biosystems, Foster City, CA, USA). The 16S rRNA gene sequence of SW451 was compared with closely related strains obtained from GenBank (www.ncbi.nlm.nih.gov/genbank/) and EZtaxon databases (www.ezbiocloud.net/eztaxon) [11].

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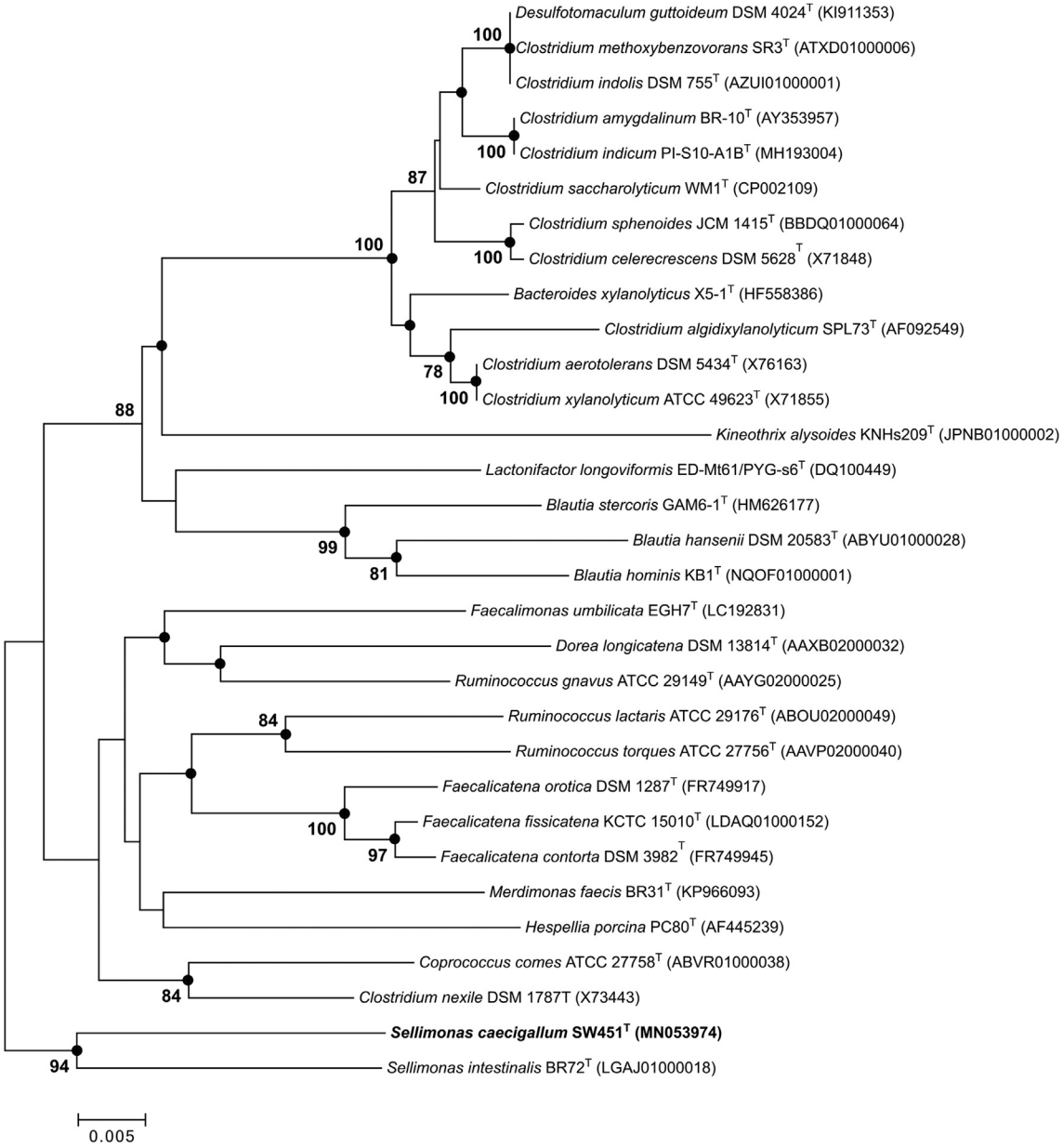

FIG. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of *Sellimonas caecigallum* within closely related taxa in the genus *Sellimonas* of *Clostridium* cluster XIVa. GenBank accession numbers of the 16S rRNA gene sequences are given in parentheses. Black circles indicate that the corresponding branches were also recovered both by maximum-likelihood and maximum parsimony methods. Numbers at nodes are shown as percentages of bootstrap >70%. Bar, 0.005 substitutions per nucleotide position.

Phylogenetic analysis

A phylogenetic tree was constructed with MEGA7 software [12] using 16S rRNA gene sequences of the strain SW451 and the closely related valid species. Multiple alignments were generated using the CLUSTAL-W algorithm [13]. Reconstruction of phylogenetic trees was carried out using the maximum-likelihood [14], maximum-parsimony [15] and neighbour-joining [16] methods. The distance matrices were generated using Kimura's two-parameter model. Bootstrap resampling analysis of 1000 replicates was performed to estimate the confidence of tree topologies [17,18].

Genome sequencing and comparison

The whole genome sequencing of strain SW451 was performed using an Illumina MiSeq sequencer with Illumina V3 2 × 250 chemistry. The reads were assembled using UNICYCLER, which builds an initial assembly graph from short reads using the *de novo* assembler SPADES 3.11.1 [19]. The quality assessment for

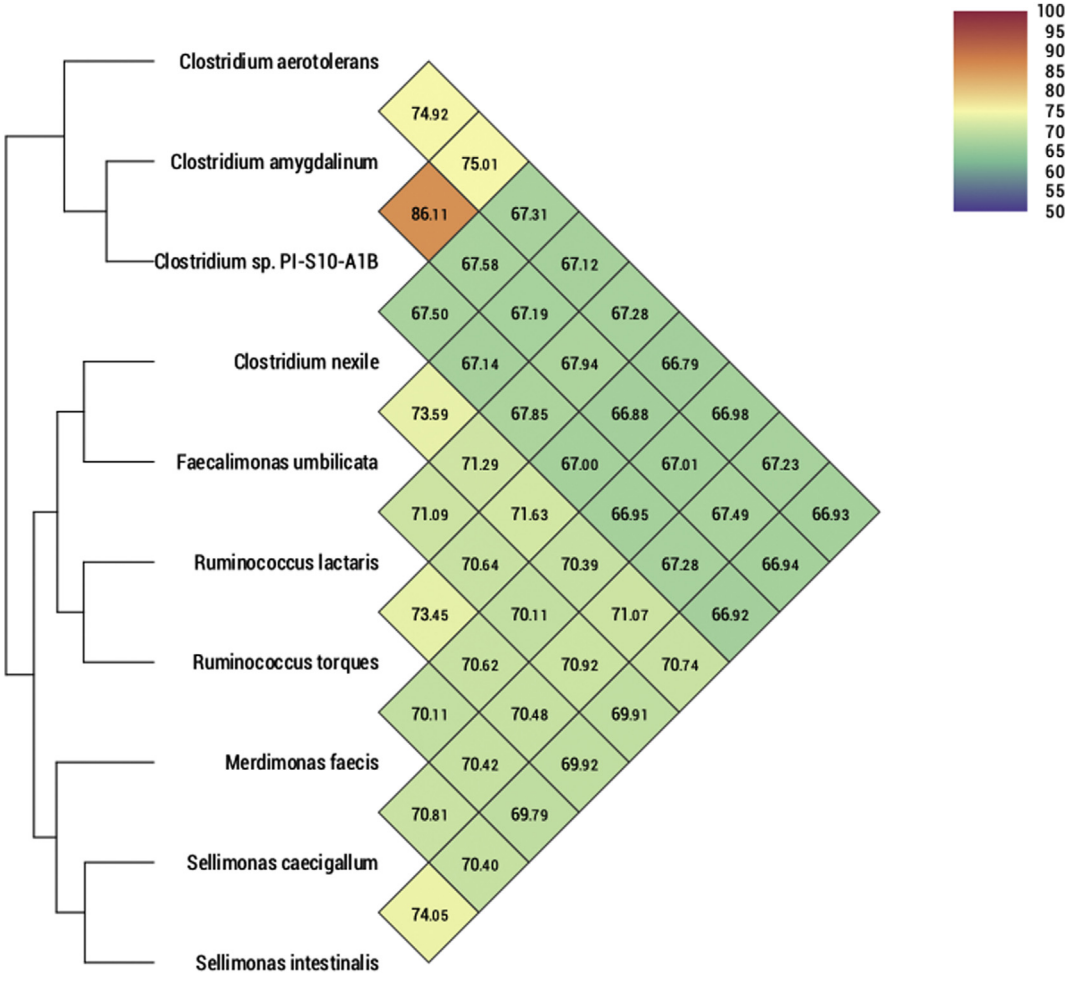

FIG. 2. Average nucleotide identity comparison of Strain SW451 and related species with a valid taxonomy. Heatmap was generated with OrthoANI values calculated using the OAT software between *Sellimonas caecigallum* and other closely related species with standing in nomenclature.

the assembly was performed using QUAST [20]. The average nucleotide identity was calculated between SW451 and the closest related strains using OrthoANI software [21]. Digital DNA–DNA hybridization between SW451 and related species was estimated *in silico* by automated calculation on Genome-to-Genome Distance Calculation web server version 2.1 (https://ggdc.dsmz.de/) [22].

Determination of phenotypic characteristics

Colony morphology of strain SW451 was determined after 2–3 days of incubation on BHI-M agar plates. Gram-staining was performed using a Gram-Staining kit set (BD-Difco, Franklin Lakes, NJ, USA), according to the manufacturer's instructions. Cell morphologies of cultures during exponential growth were examined by scanning electron microscopy. Aerotolerance was examined by incubating cultures for 2 days under aerobic and anaerobic conditions. Growth of strain SW451 at 4, 20, 30, 37, 40 and 55°C was determined. For pH range, the pH of the medium was adjusted to pH 4–9 with sterile anaerobic solutions of 0.1 M HCl and 0.1 M NaOH. The motility of this microorganism was determined using motility medium with triphenyl tetrazolium chloride (TTC) [23]. The growth was indicated by the presence of red coloration—the reduced form of TTC after it is absorbed into the bacterial cell wall.

Determination of biochemical characteristics

Other biochemical tests, including utilization of various substrates and enzyme activities, were determined using the AN MicroPlate (Biolog, Hayward, CA, USA) and API ZYM (bioMérieux, Marcy l'Étoile, France), respectively, according to the manufacturer's instructions. For cellular fatty acid analysis, strain SW451 was cultured in BHI-M medium at 37°C for 24 h under anaerobic conditions. Cellular fatty acids were obtained from cell biomass and analysed by gas chromatography (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instruction of Microbial Identification System (MIDI) [24].

Results

Based on the results of 16S rRNA gene sequencing, the closest taxa of SW451 were *Sellimonas intestinalis* DSM 103502^T (95.24 % similarity) and *Clostridium nexile* KCTC 5578^T (94.47%), followed by *Merdimonas faecis* KCTC 15482^T (94.34%) and *Faecalimonas umbilicata* DSM 103426T (94.08%), respectively. The results of phylogenetic analysis showed that SW451 was clustered with the *Sellimonas* clade and it represents a new member of the genus *Sellimonas* (Fig. 1). The draft genome of strain SW451 has a total length of 2.67 Mbp. The genomic G + C content of SW451 was 45.13 mol%. The ANI values between SW451 and its valid neighbour species were a range of 67.23%–71.07%, which were significantly less than the proposed ANI cut-off of the same species (95%–96 %) [25] (Fig. 2). Furthermore, estimation of digital DNA-DNA hybridization between SW451 and the closest relative, *Sellimonas intestinalis* DSM 103502^T, showed a relatively low percentage (17.1%–21.6%), demonstrating that the strain SW451 is a novel species [26].

Cells of the strain SW451 were Gram-positive coccobacilli with the size of 1.0–1.5 μm (Fig. 3). Colonies on BHI-M agar were ivory yellow, raised with entire edge, 0.2–0.5 cm in diameter. Strain SW451 grew between 37°C and 45°C with optimum growth at 45°C. The optimum pH for growth was 7, and growth was observed at pH 6–7.5. The strain grew strictly under anaerobic condition, indicating that it is an obligate anaerobe. Strain SW451 used D-arabitol, D-fructose, L-fucose, D-galacturonic, palatinose and rhamnose, and exhibited positive detection of alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase and α-glucosidase. Based on the results obtained in the Biolog AN microplate and API ZYM, the carbon utilization and enzyme activity of strain SW451 were different from those of the closely related strains (Table 1). The predominant cellular fatty acids of strain SW451 included C16:0 (20.80%), C14:0

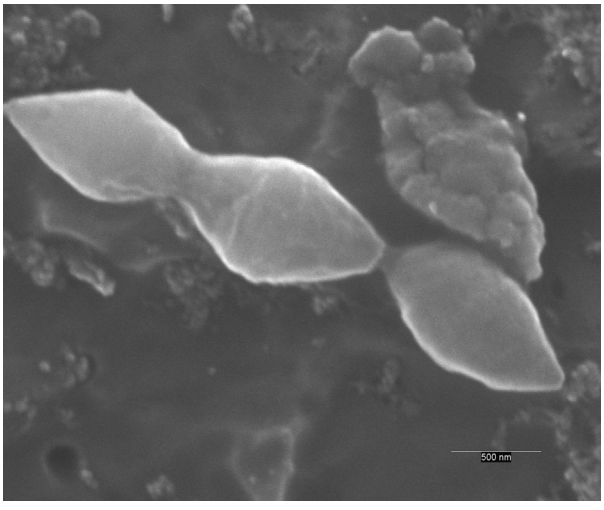

FIG. 3. Scanning electron micrograph of cells of SW451. Cells were anaerobically cultured for 1 day at 37°C in BHI-M medium. Bar, 2 μm.

TABLE 1. **Characteristics of SW451 and closely related strains**

Characteristic	1	2	3 [a]	4 [b]
Gram stain	+	+	+	
Growth at 45°C	+	+	+	
Motility	–	–	ND	–
Carbon source (BIOLOG AN)				
D-Arabitol	+	–	ND	ND
D-Fructose	+	+	ND	ND
L-Fucose	+	+	ND	ND
D-Galactose	–	+	ND	ND
D-Galacturonic	+	+	ND	ND
D-Glucose	–	+	+	–
D-Lactose	–	+	+	–
Maltose	–	+	–	–
Palatinose	+	+	ND	ND
Raffinose	–	+	–	ND
Rhamnose	+	+	ND	ND
Sucrose	–	–	+	ND
Enzyme activity (API ZYM)				
Alkaline phosphatase	w	–	ND	+
Esterase (C4)	–	–	+	+
Leucine arylamidase	+	+	–	ND
Proline arylamidase	–	–	+	+
Valine arylamidase	w	+	–	ND
α-Chymotrypsin	–	w	–	ND
Acid phosphatase	w	+	ND	ND
α-Galactosidase	w	+	ND	+
β-Galactosidase	+	+	ND	+
α-Glucosidase	+	+	ND	+
N-Acetyl-β-glucosaminidase	–	+	ND	ND
DNA G + C content (mol%)	45.13	45.3	40.1	47

Strains: 1, SW451; 2, *Sellimonas intestinalis* DSM 103502T; 3, *Clostridium nexile* KCTC 5578T; 4, *Merdimonas faecis* KCTC 15482T. Results for metabolic end products of SW165 are from this study with cells that were cultured for 3 days at 37°C in BHI-M.
+, positive; –, negative; w, weak; ±, variable; ND, not determined.
[a]Data from Seo *et al.* (2016).
[b]Data from Seo *et al.* (2017).

TABLE 2. **Cellular fatty acid compositions of strains SW451 and related strains**

	1	2	3 [a]	4 [b]
Straight chain				
$C_{12:0}$	3.62	2.45	5.6	3.4
$C_{14:0}$	17.98	21.75	8.3	25
$C_{16:0}$	20.80	27.45	36	18.6
$C_{16:0}$ aldehyde	7.03	1.94	–	–
$C_{18:0}$ aldehyde	1.50	0.6	–	–
Demethylacetal (DMA)				
$C_{14:0}$ DMA	6.10	1.97	–	–
$C_{16:0}$ DMA	15.83	9.28	–	–
$C_{18:0}$ DMA	5.93	3.14	–	–
Unsaturated				
$C_{13:1}$ at 12–13	4.28	0.8	–	2.6
$C_{16:1}$ *ω7c*	2.35	4.41	–	–
$C_{18:1}$ *ω7c*	2.07	7.25	–	–
$C_{18:1}$ *ω7c* DMA	2.97	8.56	–	–
Summed feature 1 [c]	4.28	0.8	2.9	7.4
Summed feature 8 [c]	1.19	1.59	5.7	0.9
Summed feature 10 [c]	2.07	7.25	–	–

Strains: 1, SW451; 2, *Serromonas intestinalis* DSM 103502T; 3, *Clostridium nexile* KCTC 5578T; 4, *Merdimonas faecis* KCTC 15482T. Values are percentages of total fatty acids detected.
Fatty acids with contents of <1% in all strains are not shown; –, not detected.
[a]Data from Seo *et al.* (2016).
[b]Data from Seo *et al.* (2017).
[c]Summed features are fatty acids that could not be separated using the MIDI System. Summed feature 1 contains $C_{13:1}$ and/or $C_{14:0}$ aldehyde. Summed feature 13 contains $C_{15:0}$ ante-iso and/or $C_{14:0}$ 2-OH; summed feature 8 comprises $C_{17:1}$ *ω8c* and/or $C_{17:2}$ at 16; summed feature 10 contains $C_{18:1}$ *ω7c* and/or unknown.

TABLE 3. Description of strain SW451, according to the digitized protologue (www.imedea.uib.es/dprotologue) website

Taxonumber	TA01013
Species name	*Sellimonas caecigallum*
Genus name	*Sellimonas*
Species epithet	*caecigallum*
Species status	sp. nov.
Species etymology	ce.ci.gal'lum N.L. gen. n. referring to the caecum of a chicken where the type strain was isolated.
Designation of the type strain	SW451
Strain collection numbers	DSM 109473
16s rRNA gene accession number	MN053974
Genome accession number	PRJNA551641
Genome size	2.67 Mbp
GC mol%	45.13
Country of origin	United State
Region of origin	Brookings, South Dakota
Source of isolation	caecum of feral chickens
Gram stain	positive
Cell shape	small-chain coccobacilli
Cell size	1.0–1.5 µm
Motility	non-motile
Colony morphology	0.2–0.5 cm in diameter, ivory yellow, raised with entire edge
Temperature range	37–45°C
Temperature optimum	45°C
ph range for growth	6–7.5
pH optimum	7
Relationship to O_2	anaerobe

(17.98%) and C16:0 dimethylacetal (15.83%), which differed from the reference strains (Table 2). The overall characteristics of the strain are summarized in Table 3.

Description of *Sellimonas caecigallum* SW451 sp. nov.

Sellimonas caecigallum sp. nov. (referring to L. n. *caecum*, caecum; L. n. *gallus*, of a chicken; N.L. neut. n. *caecigallum*, from the caecum of a chicken). Cells are strictly anaerobic, Gram-strain-positive and non-motile. Average size of each cell is 1.0–1.5 µm and they are coccobacillus-shaped. Colonies are visible on BHI-M agar after 2 days and are approximately 0.2–0.5 cm in diameter, ivory yellow, raised with entire edge. The strain exhibits optimal growth in BHI-M medium at 45°C and pH 7. The strain uses D-arabitol, D-fructose, L-fucose, D-galacturonic, palatinose and rhamnose as a carbon source. Positive enzymatic reactions are obtained for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase and α-glucosidase. The primary cellular fatty acids are C16:0, C14 :0 and C16:0 dimethylacetal. The genome of this strain is 2.67 Mbp in length with 45.13 mol% of G + C content. It was isolated from the caecum of feral chicken. The type strain is SW451 (=DSM 109473^T = CCOS 1879^T).

Nucleotide sequence accession number

The 16S rRNA gene and genome sequence were deposited in GenBank and SRA under accession numbers MN053974 and PRJNA551641, respectively.

Deposition of the strain in culture collections

Strain SW451 was deposited in The Leibniz Institute DSMZ—German Collection of Microorganisms and Cell Cultures GmbH and The National Culture Collection of Switzerland under numbers DSM 109473 and CCOS 1879, respectively.

Conflict of interest

None to declare.

Acknowledgements

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