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 109473T = CCOS 1879T).

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

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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].
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
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 10350^T^ (95.24 % similarity) and Clostridium nexilis KCTC 5578^T^ (94.47%), followed by Merdimonas faecis KCTC 15482^T^ (94.34%) and Fae-calimonas umbilicata DSM 103426^T^ (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 closest valid neighbour species were a range of 67.23% – 95% [25] (Fig. 2). Furthermore, estimation of digital DNA-DNA hybridization between SW451 and the closest relative, Sellimonas intestinalis DSM 10350^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, 0.2–1.0 mm 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 acid, 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 (17.98%), C15:0, C17:0 (6.3), C16:1ω7c (4.28), C18:1ω9c (5.93), C19:0 DMA (3.14) and C20:0 DMA (2.07). The fatty acid content of strain SW451 was different from those of the closely related strains, and these differences may contribute to the taxonomic position of the new species SW451.

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

| Characteristic                      | 1   | 2   | 3   | 4   |
|------------------------------------|-----|-----|-----|-----|
| Gram stain                         | +   | +   | +   | +   |
| Growth at 45°C                     | +   | +   | ND  | ND  |
| Motility                           | –   | –   | ND  | –   |
| Carbon source (API ZYM)            |     |     |     |     |
| Alkaline phosphatase               | w   | –   | ND  | +   |
| Esterase (C4)                      | –   | +   | ND  | +   |
| Leucine arylamidase                | +   | –   | ND  | +   |
| Proline arylamidase                | –   | +   | ND  | –   |
| Valine arylamidase                 | w   | +   | ND  | +   |
| α-Chymotrypsin                     | –   | w   | –   | ND  |
| Acid phosphatase                   | w   | +   | ND  | ND  |
| α-Galactosidase                    | w   | +   | ND  | +   |
| β-Galactosidase                    | +   | +   | ND  | +   |
| β-Glucosidase                      | +   | +   | ND  | +   |
| N-Acetyl-(β-glucoaminidase)        | –   | +   | ND  | +   |
| DNA G + C content (mol%)           | 45.13 | 45.3 | 40.1 | 47 |

Strains: 1, SW451; 2, Sellimonas intestinalis DSM 10350^T^; 3, Clostridium nexilis KCTC 5578^T^; 4, Merdimonas faecis KCTC 15482^T^. Values are percentages of total fatty acids detected.

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

| Fatty Acid                | 1  | 2  | 3   | 4   |
|---------------------------|----|----|-----|-----|
| Straight chain            |    |    |     |     |
| C12:0                     | 3.62 | 2.45 | 5.6 | 3.4 |
| C14:0                     | 17.98 | 21.75 | 8.3 | 25  |
| C16:0                     | 20.80 | 27.45 | 36  | 18.6|
| C16:0 Aldehyde            | 7.03 | 1.94 | –   | –   |
| C18:0 Aldehyde            | 1.50 | 0.6  | –   | –   |
| Demethylacetal (DMA)      |    |    |     |     |
| C14:0 DMA                 | 6.10 | 1.97 | –   | –   |
| C16:0 DMA                 | 15.83 | 9.28 | –   | –   |
| C18:0 DMA                 | 5.93 | 3.14 | –   | –   |
| Unsaturated               |    |    |     |     |
| C12:0 at 12-13            | 4.28 | 0.8  | –   | 2.6 |
| C14:0 ω7c                 | 2.35 | 4.41 | –   | –   |
| C18:0 ω7c                 | 2.07 | 7.25 | –   | –   |
| C19:0 ω7c DMA             | 2.97 | 8.56 | –   | –   |
| Summed feature 1^a         | 4.28 | 0.8  | 2.9 | 7.4 |
| Summed feature 8           | 1.19 | 1.59 | 5.7 | 0.9 |
| Summed feature 10          | 2.07 | 7.25 | –   | –   |

Strains: 1, SW451; 2, Sellimonas intestinalis DSM 10350^T^; 3, Clostridium nexilis KCTC 5578^T^; 4, Merdimonas faecis KCTC 15482^T^. Values are percentages of total fatty acids detected.

^a Data from Seo et al. (2017).
^b Data from Seo et al. (2018).
**TABLE 3. Description of strain SW451, according to the digitized protologue (www.imedea.uib.es/dprotologue) website**

| Taxonumber | TA01013 |
|------------|---------|
| 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. caecum*, 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 cocclobacillus-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, α-phatase, 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 dimethylacetate. 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 a chicken where the type strain was isolated. The overall characteristics of the strain are summarized in Table 3. 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, α-phatase, 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 dimethylacetate. 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 a chicken where the type strain was isolated. The overall characteristics of the strain are summarized in Table 3. |

**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.

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[13] Thompson JD, Higgins DG, Gibson TJ. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–80.

[14] Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 1981;17:368–76.

[15] Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 1971;20:406–16.

[16] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–25.

[17] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783–91.

[18] Amrane S, Hocquart M, Afouda P, Kuete E, Pham TP, Dione N, et al. Metagenomic and culturomic analysis of gut microbiota dysbiosis during Clostridium difficile infection. Sci Rep 2019;9:12807.

[19] Wick RR, Judd LM, Gorris CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595.

[20] Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics 2013;29:1072–5.

[21] Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.

[22] Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 2013;14:60.

[23] Shields P, Cathcart L. Motility test medium protocol. Washington, DC: American Society for Microbiology; 2011.

[24] Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. In: MIDI Technical Note 101 Newark, DE: MIDI Inc.; 1990.

[25] Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 2014;64:346–51.

[26] Meier-Kolthoff JP, Klenk HP, Goker M. Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. Int J Syst Evol Microbiol 2014;64:352–6.