



RESEARCH ARTICLE

Taxono-genomics description of *Olsenella lakotia* SW165^T sp. nov., a new anaerobic bacterium isolated from cecum of feral chicken [version 1; peer review: 2 approved]

Supapit Wongkuna^{1,2}, Sudeep Ghimire^{2,3}, Tavan Janvilisri⁴, Kinchel Doerner⁵, Surang Chankhamhaengdecha ⁶, Joy Scaria ^{2,3}

¹Doctor of Philosophy Program in Biochemistry (International Program), Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand

²Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, South Dakota, 57007, USA

³South Dakota Center for Biologics Research and Commercialization, Brookings, South Dakota, 57007, USA

⁴Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand

⁵Department of Biology and Microbiology, South Dakota State University, Brookings, South Dakota, 57007, USA

⁶Department of Biology, Faculty of Science, Mahidol University, Bangkok, 10400, Thailand

V1 First published: 08 Sep 2020, 9:1103
<https://doi.org/10.12688/f1000research.25823.1>
 Latest published: 08 Sep 2020, 9:1103
<https://doi.org/10.12688/f1000research.25823.1>

Abstract

Background: The microbial community residing in the animal gastrointestinal tract play a crucial role in host health. Because of the high complexity of gut microbes, many microbes remain unclassified. Deciphering the role of each bacteria in health and diseases is only possible after its culture, identification, and characterization. During the culturomics study of feral chicken cecal sample, we cultured a possible novel strain SW165^T.

Methods: For the possible novel strain SW165^T, phenotypic characterization was performed using colony morphology, Gram staining, growth in different temperature and pH and motility. Biochemical assays included carbon source utilization, enzymatic activity, cellular fatty acids and short chain fatty acid production. 16S rRNA sequencing and whole genome sequencing and comparison was performed for genetic analysis.

Results: This strain was isolated from cecal content of feral chickens in Brookings, South Dakota, USA. Phylogenetic analyses based on 16S rRNA gene sequence revealed that the closest valid neighbor was *Olsenella profusa* DSM 13989^T (96.33% similarity) within the family *Atopobiaceae*. Cells were Gram-stain-positive and obligately anaerobic bacilli in chains. The optimum temperature and pH for the growth of the microorganism were 37-45°C and pH 6.0-7.5 respectively. This strain produced acetic acid as the primary fermentation product. Major fatty acids were C_{12:0}, C_{14:0}, C_{14:0} DMA and summed feature 1 (C_{13:1} at 12-13 and C_{14:0} aldehyde). Strain SW165^T exhibited a genome size of 2.43 Mbp with a G+C content of 67.59 mol%, which is the second highest G+C content among members of the genus *Olsenella*.

Open Peer Review

Reviewer Status

| | Invited Reviewers | |
|---|-------------------|--------|
| | 1 | 2 |
| version 1 | | |
| 08 Sep 2020 | report | report |
| 1. Yung-Fu Chang , Cornell University, Ithaca, USA | | |
| 2. Mohamed Seleem , Virginia Polytechnic Institute and State University, Blacksburg, USA | | |
| Any reports and responses or comments on the article can be found at the end of the article. | | |

The digital DNA-DNA hybridization and OrthoANI values between SW165^T and DSM 13989^T were only 17.6 ± 5.3 and 74.35%, respectively.

Conclusion: Based on the phenotypic, biochemical, and genomic analyses, we propose the new species of the genus *Olsenella*, and name it *Olsenella lakotia* SW165^T sp. nov., (=DSM 107283 =CCOS 1887) as the type strain.

Keywords

Olsenella lakotia SW165^T sp. nov., culturomics, chicken gut microbiota, taxono-genomics

Corresponding authors: Surang Chankhamhaengdecha (surang.cha@mahidol.ac.th), Joy Scaria (joy.scaria@sdstate.edu)

Author roles: **Wongkuna S:** Data Curation, Formal Analysis, Methodology, Validation, Visualization, Writing – Original Draft Preparation; **Ghimire S:** Methodology; **Janvilisri T:** Funding Acquisition, Writing – Review & Editing; **Doerner K:** Writing – Review & Editing; **Chankhamhaengdecha S:** Funding Acquisition, Supervision, Writing – Review & Editing; **Scaria J:** Funding Acquisition, Investigation, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This research project was supported by Mahidol University to SC and TJ, and was funded in part by the USDA National Institute of Food and Agriculture, Hatch projects SD00H532-14 and SD00R540-15, and a grant from the South Dakota Governor's Office of Economic Development to JS.

Copyright: © 2020 Wongkuna S *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Wongkuna S, Ghimire S, Janvilisri T *et al.* **Taxono-genomics description of *Olsenella lakotia* SW165^T sp. nov., a new anaerobic bacterium isolated from cecum of feral chicken [version 1; peer review: 2 approved]** F1000Research 2020, 9:1103 <https://doi.org/10.12688/f1000research.25823.1>

First published: 08 Sep 2020, 9:1103 <https://doi.org/10.12688/f1000research.25823.1>

Introduction

The chicken gut harbors highly diverse microbes¹. The gut microbes are known for their nutritional benefits by producing short chain fatty acids, enzymes, amino acids along with their ability to resist pathogens, immunity development and maintain homeostasis². Even though culture independent methods have highlighted the functional capability of gut microbes, validation of these functions requires their cultivation, identification, and characterization. Most of the intestinal bacteria have been never isolated in the laboratory^{3,4} thus hindering the understanding of their ecological and functional roles in the gut. Recently, “culturomics” strategy drives discovery of previously uncultured species based on modified culture conditions, such as media, temperature, pH and atmosphere and rapid identifying methods; matrix-assisted desorption ionization- time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing⁵⁻⁷. We employed culturomics to isolate bacteria from cecum of feral chickens. Based on bacterial isolation and identification, strain SW165^T was found as a new species within the genus *Olsenella*.

The members of the genus *Olsenella* are strictly anaerobic, Gram-positive, non-motile, non-spore-forming bacilli or cocci. This genus was first named by Dewhirst *et al.* 2001⁸ and amended by Zhi *et al.* 2009⁹ and Kraatz *et al.* 2011¹⁰. This genus has recently been reclassified as a member of the family *Atopobiaceae* under order Coriobacteriales, class Coriobacteria, and phylum Actinobacteria¹¹. The genus *Olsenella* consists of nine published species; *O. uli*¹², *O. profusa*⁸, *O. umbonata*¹⁰, *O. scatoligenes*¹³, *O. urinifantia*¹⁴, *O. congonesis*¹⁵, *O. proveniensis*¹⁶, *O. phocaensis*¹⁶, and *O. mediterranea*¹⁶. The members of this genus are strictly anaerobic, Gram positive, non-motile, non-spore forming rod shaped with G+C content of DNA 62–64%^{8,13,17}. The main habitats of the *Olsenella* are the oral cavity and gastrointestinal tract of humans¹⁸⁻²¹, animals and various anaerobic environmental sites²²⁻²⁴. In chicken cecum, many members of genus *Olsenella* have been reported in the chicken microbiome in metagenomic-based studies^{15,25}. However, only *O. uli* was isolated from chicken gut²⁶.

The taxono-genomics approach uses combination of phenotypic and genotypic characterization to describe new bacteria^{27,28}. Phenotypic investigation includes morphological, physiological, and biochemical assays. Genome-based and 16S-based analysis are used in genotypic characterization. In this study, strain SW165^T was described using taxono-genomics and compared to its closely related phylogenetic neighbors. Following analysis, we found that strain SW165^T belongs to a novel species for which the name *Olsenella lakotia* SW165^T sp. nov. is proposed.

Methods

Strain isolation

The cecal content of feral chickens was collected in Brookings, South Dakota, USA. For cultivation, the samples were transferred into an anaerobic workstation (Coy Laboratory) containing 85% nitrogen, 10% hydrogen and 5 % carbon dioxide and plated on a modified Brain Heart Infusion (BHI-M) agar containing 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% of pectin and inulin. After 3 days of incubation at 37°C under anaerobic conditions, single colony of strain SW165 was identified by MALDI-TOF mass spectrometry using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany). The strain was maintained in BHI-M medium and stored with 10% (v/v) Dimethyl Sulfoxide (DMSO) at -80°C.

Phenotypic and biochemical tests

For morphological characterization, the strain SW165^T was anaerobically cultivated in BHI-M medium, pH 6.8-7.2, at 37°C. Colony morphologies were examined after 2–3 days of incubation on BHI-M agar plates. Gram-staining was performed using a Gram-Staining kit set (Difco), according to the manufacturer's instructions. Cell morphologies were examined by scanning electron microscopy (SEM) of cultures during exponential growth. Aerotolerance was examined by incubating cultures for 2 days separately under aerobic and anaerobic conditions. Growth of strain SW165^T at 4, 20, 30, 37, 40 and 55°C was determined. For determining the range of pH for growth of SW165^T, the pH of the medium was adjusted to pH 4.0–9.0 with sterile anaerobic stock solutions of 0.1 M HCl and 0.1 M NaOH. Motility of this microorganism was determined using motility medium with triphenyltetrazolium chloride (TTC)²⁹. The growth was indicated by the presence of red color, reduced form of TTC after it is absorbed into bacterial cell wall.

Biochemical tests to determine standard taxonomic characteristics for strain SW165^T were performed in triplicate. The utilization of various substrates as sole carbon and energy sources and enzyme activities were performed using the AN MicroPlate (BIOLOG) and API ZYM (bioMérieux) according to the manufacturer's instructions. Reference strain, DSM 13989^T purchased from the DSMZ culture collection and isolated strains SW165^T were simultaneously cultured in BHI-M medium at 37°C for 24 h under anaerobic condition before cell biomass were harvested for cellular fatty acid analysis. The fatty acids were extracted, purified, methylated and analyzed using gas chromatography (Agilent 7890A) based on the instruction from Microbial Identification System (MIDI)³⁰. Metabolic end-products such as short-chain fatty acids of strain SW165^T and DSM 13989^T grown in BHI-M were determined using a gas chromatography. The cultures were deproteinized with 25% metaphosphoric acid before supernatant collection. The supernatant was analyzed for the presence of acetic acid, butyric acid, isovaleric acid and propionic acid using GC (Thermo Scientific™ TRACE™ 1310 GC equipped with a TraceGOLD™ TG-WaxMS A GC column.).

16S RNA phylogenetical analysis

Genomic DNA of the strain SW165^T was extracted using a DNeasy Blood & Tissue kit (Qiagen), according to the manufacturer's instructions. 16S rRNA gene sequence was amplified using universal primer set 27F (5'- AGAGTTTGTATCMTGG CTCAG-3'; Lane *et al.*, 1991) and 1492R (5'- ACCTTGTTA

CGACTT-3'; Stackebrandt et al., 1993)^{31,32}, and sequenced using a Sanger DNA sequencer (ABI 3730XL; Applied Biosystems). The 16S rRNA gene sequence of SW165 was then compared with closely related strains from the GenBank (www.ncbi.nlm.nih.gov/genbank/) and EZBioCloud databases (www.ezbiocloud.net/eztaxon)³³. Alignment and phylogenetic analysis were conducted using MEGA7 software³⁴. Multiple sequence alignments were generated using the CLUSTAL-W³⁵. Reconstruction of phylogenetic trees was carried out using the maximum-likelihood (ML)³⁶, maximum-parsimony (MP)³⁷, and neighbor-joining (NJ)³⁸ methods. The distance matrices were generated according to Kimura's two-parameter model. Bootstrap resampling analysis of 1000 replicates was performed to estimate the confidence of tree topologies.

Genome sequencing and analysis

The whole genome sequencing of strain SW165^T was performed using Illumina MiSeq sequencer using 2x 300 paired end V3 chemistry. The reads were assembled using Unicycler that builds an initial assembly graph from short reads using the de novo assembler SPAdes 3.11.1³⁹. The quality assessment for the assemblies was performed using QUAST5.0.2⁴⁰. Genome annotation was performed using Rapid Annotation using Subsystem Technology (RAST) server⁴¹. The digital DNA-DNA hybridization (dDDH) was performed using Genome-to-Genome Distance Calculator (GGDC) web server (<http://ggdc.dsmz.de>) to estimate the genomic similarity between strain SW165^T and the closest phylogenetic neighbor. Average nucleotide identity (ANI) between strain SW165^T and the closely related strains was also calculated using the OrthoANI software⁴². Distribution of functional categories of strain SW165^T was compared to *Olsenella* species and was presented in a heatmap generated using Explicet version 2.10.5⁴³.

Results

Strain SW165^T was isolated from cecal contents of feral chicken in anaerobic chamber (Coy Laboratory Product, MI, USA). Colonies of SW165^T on BHI-M agar were 0.2–0.5 cm in diameter, appeared white, smooth, and umbonate with entire circular edges when grown at 37°C anaerobically after 48 hours of incubation. After cultivation, the colonies of this strain were subjected to identification by MALDI-TOF using a Microflex spectrometry (Bruker Daltonics, Bremen, Germany). MALDI-TOF did not identify the strain as the scores obtained were < 1.70. Thus, full length 16S rRNA gene was sequenced using Sanger sequencing method. The 16S rRNA of the strain SW165^T showed 96.33% identity with *O. profusa* DSM 13989^T (GenBank accession no. [AF292374](https://www.ncbi.nlm.nih.gov/nuccore/AF292374)), the validly closest species within phylogenetical nomenclature (Figure 1). The current cut off for species delineation from its nearest neighbor based on 16S rRNA is 98.7%⁴⁴. As the identity of 16S rRNA of strain SW165^T was lower than threshold, it was considered as a representative of putatively novel species within the genus *Olsenella* in the family *Atopobiaceae*. Phylogenetically, the strain was found to cluster together with other members of genus *Olsenella*, as shown in Figure 1, validating that SW165^T belongs to genus *Olsenella* taxonomically.

Phenotypic growth of strain SW165^T was observed on modified BHI-M agar after 2–3 days of incubation at temperature between 37°C and 45°C and pH between 6.0–7.0. The optimum temperature and pH for the growth were at 45°C and pH 7.0, respectively. Strain SW165^T grew only under anaerobic conditions, suggesting obligate anaerobic nature. Bacterial cells were Gram-stain-positive bacilli (0.5–2.0 µm), growing in pairs or as short chains and were non-motile (Figure 2).

To further analyze the biochemical properties of the strain, we performed the carbon source utilization assay using BIOLOG AN microplate and compared it to closely related taxa. Strain SW165^T consumed various carbon sources for the growth, which differed from related strains in the utilization of D-fructose, L-fucose, D-galactose, maltose, D-melibiose and D-raffinose, and in the non-utilization of dulcitol. Based on enzymatic activity test, the strain produced several enzymes, including alkaline phosphatase, leucine arylamidase, cysteine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, and β-glucosidase. Interestingly, alkaline phosphatase α-galactosidase, β-galactosidase and β-glucuronidase are not reported from its closest neighbors (Table 1). Furthermore, the dominant cellular fatty acids of the strain SW165^T were saturated, including C_{12:0} (25.5%) and C_{14:0} (22.83%). Moreover, other dominant fatty acids were C_{14:0} DMA (15.61%) and summed feature 1 [C_{13:1} and/or C_{14:0} aldehyde; 13.94%]. However, there were distinct quantities of some fatty acids between SW165^T and the relative strains (Table 2). The major short chain fatty acids produced by SW165 when cultivated in BHI-M were acetic acid (3.74 mM) followed by propionic acid (0.53 mM).

We examined the genome of the strain SW165^T to investigate its differentiation from the neighbors. The genome size of strain SW165^T was 2,427,227 bp with 67.59mol% G+C content. The draft genome was assembled into 33 contigs with 2,228 protein-coding sequences and 52 RNAs (Table 3) and is visualized as Figure 3. The genomes sizes for the *Olsenella* species were comparable to one another except for *O. urinifantis* whose was only 1.75 Mbps. However, the G+C contents strain SW165^T and *O. mediterranea* were the highest but comparable to other neighboring *Olsenella* species (Table 3). The genome of SW165^T possessed a total of 1,230 genes with putative function and 998 genes as hypothetical proteins. Among 1,230 genes, 823 were classified as features in subsystem, following functional categories (COGs). Majority of categories included amino acid and derivatives (172 genes), carbohydrates (163 genes), and protein metabolism (132 genes) (Extended data: Supplemental Table 1⁴⁵).

Furthermore, we compared the genome of SW165^T to its neighbor using OrthoANI as shown in Figure 4. The genome of SW165 was only 73.41% identical to its nearest neighbor *O. profusa* DSM 13989^T. Also, the OrthoANI values for SW165^T and closely related strains ranged from 65.40 to 74.18% (Figure 4) indicating that the genome of SW165^T is unique compared to its neighbors. The proposed cut off for OrthoANI

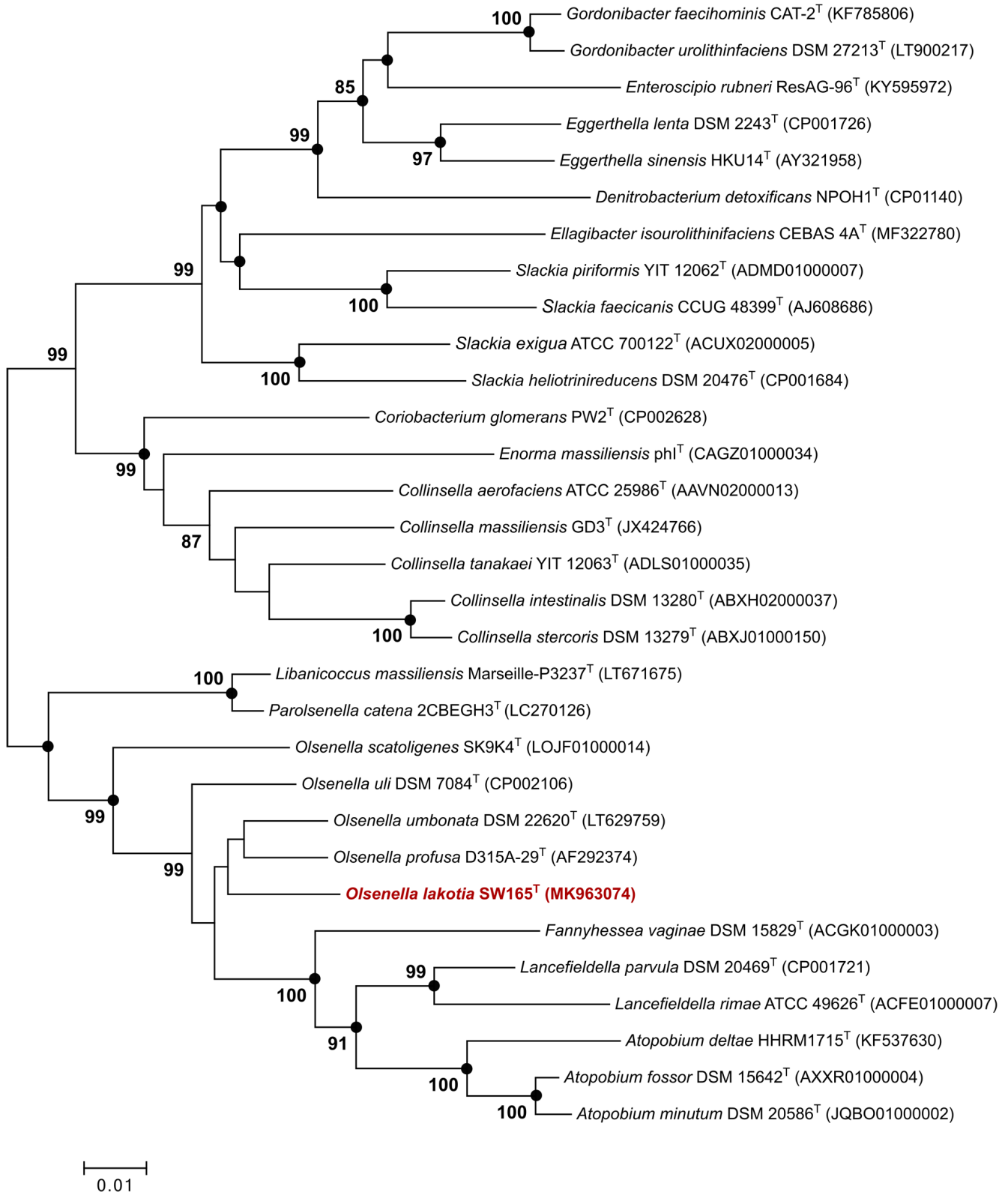


Figure 1. 16S rRNA based neighbor-joining tree of SW165^T and its neighbors. Tree shows phylogenetic position of *Olsenella lakotia* DSM 107283^T and closely related species in the family *Atopobiaceae*. GenBank accession numbers of the 16S rRNA gene sequences are given in parentheses. Black circles indicate that the corresponding branches were also recovered both by maximum-likelihood and maximum parsimony methods. Bootstrap values (based on 1000 replications) greater than or equal to 70% are shown in percentages at each node. Bar, 0.01 substitutions per nucleotide position.

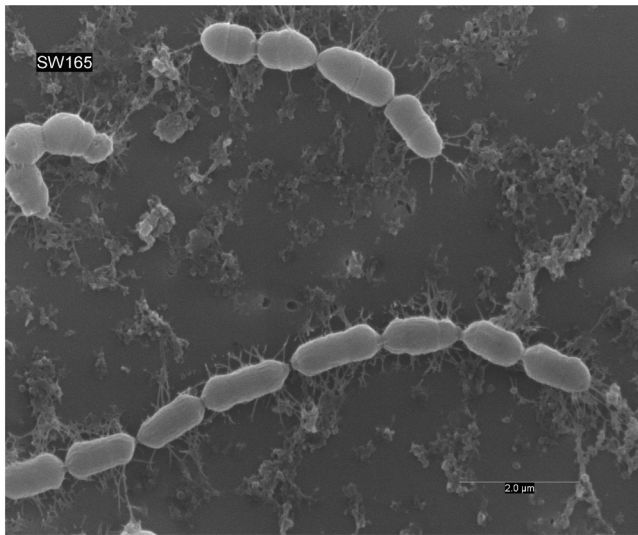


Figure 2. Scanning electron micrograph of *O. lakotia* SW165^T. Cells were anaerobically cultured for 24 hours at 37° C in BHI-M medium. Bar, 2 μm; uncropped/unedited image.

for the species delineation is 95-96% identity^{46,47}. In addition, dDDH between SW165^T and the closest neighbor, *O. profusa* DSM 13989^T was only 17.6 ± 5.3. These values were lower than threshold of ANI and dDDH for delineating prokaryotic species, suggesting that these strains are distinct species. Also, the gene distribution into COGs was comparable in all eight compared *Olsenella* genomes (Figure 5). Hence, the phenotypic and genetic discrepancy of the SW165^T with its close neighbor apparently supports that strain SW165^T represents a new species of the genus *Olsenella*.

Discussion

Culturomics of the gut microbiota has evolved as a strong tool to increase the isolation of diverse previously uncultured bacteria from the gut^{5,48}. The cultivation of the gut microbiota enables to improve the health through an enhanced understanding of their roles in the gut ecosystem and finally to the host. Thus, using the culturomics strategy, we were able to isolate previously uncultured bacterium SW165^T from cecal content of feral chicken and finally characterize and describe it using taxonogenomics as a novel microorganism.

Table 1. Characteristics of *O. lakotia* SW165^T and closely related strains. Column headers show Strains designated in the following numbers: 1 - SW165^T; 2 - *O. profusa* DSM 13989^T; 3 - *O. uli* DSM 7084^T; 4 - *O. umbonata* DSM 22620^T; 5 - *O. scatoligenes* DSM 28304^T. 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; ND, not determined.

| Characteristic | 1 | 2 | 3 † | 4 † | 5 ‡ |
|---------------------------|---|---|-----|-----|-----|
| Gram stain | + | + | + | + | + |
| Growth at 37o C | + | + | + | + | + |
| Motility | - | - | - | - | - |
| Carbon source (BIOLOG AN) | | | | | |
| Arbutin | + | + | ND | ND | ND |
| D-Cellulose | + | + | - | - | + |
| Dextrin | + | + | ND | ND | ND |
| D-Fructose | + | - | ND | ND | ND |
| L-Fucose | + | - | ND | ND | ND |
| D-Galactose | + | - | ND | ND | ND |
| α-D-Glucose | + | + | ND | ND | ND |
| Dulcitol | - | + | ND | ND | ND |
| Maltose | + | - | - | + | ND |
| D-Mannose | + | + | - | + | ND |
| D-Melibiose | + | - | - | - | ND |
| D-Raffinose | + | - | ND | ND | ND |

| Characteristic | 1 | 2 | 3 † | 4 † | 5 ‡ |
|-----------------------------------|-------|---------|---------|---------|---------|
| Salicin | + | + | - | - | + |
| Sucrose | + | + | - | + | ND |
| Turanose | + | + | ND | ND | ND |
| Enzyme activity (API ZYM) | | | | | |
| Alkaline phosphatase | + | - | + | - | ND |
| Esterase (C 4) | - | - | + | + | - |
| Leucine arylamidase | + | + | + | + | ND |
| Cystine arylamidase | + | + | - | - | ND |
| α-chymotrypsin | w | - | ND | ND | ND |
| α-galactosidase | + | - | ND | ND | ND |
| β-galactosidase | + | - | - | - | + |
| β-glucuronidase | + | - | ND | ND | ND |
| α-glucosidase | + | + | - | + | + |
| β-glucosidase | + | + | + | - | + |
| Short-chain fatty acid production | A | L, a, f | L, a, f | L, a, f | L, a, f |
| DNA G+C content (mol%) | 67.59 | 64 | 64.7 | 64.9 | 62.1 |

†Data from Kraatz *et al.* (2011)¹⁰

‡Data from *et al.* (2015)¹³

A/a, acetic acid; L, lactic acid; f, formic acid. Capital letters indicate major end products.

Table 2. Cellular fatty acid compositions of *O. lakotia* SW165^T and related neighbors. Strains: 1 - SW165^T; 2 - *O. profusa* DSM 13989^T; 3 - *O. uli* DSM 7084^T; 4 - *O. umbonata* DSM 22620^T; 5 - *O. scatoligenes* DSM 28304^T. Values are percentages of total fatty acids detected. Fatty acids with contents of less than 1% in all strains are not shown; ND, Not detected.

| Fatty acid composition | 1 | 2 | 3 ‡ | 4 ‡ | 5 ‡ |
|------------------------|-------|-------|------|------|------|
| Straight chain | | | | | |
| C10:00 | 3.34 | ND | ND | ND | ND |
| C12:00 | 25.5 | ND | 2.8 | ND | ND |
| C14:00 | 22.83 | 9.94 | 1.3 | 31.6 | 25.9 |
| C16:00 | 2.69 | 5.82 | 4.3 | 6.2 | 2.7 |
| C16:0 aldehyde | 0.92 | 3.48 | ND | ND | ND |
| Demethylacetal (DMA) | | | | | |
| C12:0 DMA | 8.44 | ND | ND | ND | ND |
| C14:0 DMA | 15.61 | 4.33 | ND | ND | ND |
| C16:0 DMA | 1.21 | 13.97 | ND | ND | ND |
| C18:0 DMA | 0.65 | 1.18 | ND | ND | ND |
| Branched | | | | | |
| C14:0 iso | ND | 22.34 | ND | ND | ND |
| C13:0 anteiso | ND | 1.79 | ND | ND | ND |
| C15:0 anteiso | ND | 14.48 | ND | ND | ND |
| C15:0 anteiso DMA | ND | 5.17 | ND | ND | ND |
| Unsaturated | | | | | |
| C18:1 ω 9c | 1.82 | 3.9 | 69.8 | 20.7 | 25.7 |
| C18:2 ω 6,9c | 0.89 | 2.03 | ND | ND | ND |
| Summed Feature 1 | 13.94 | 2.15 | ND | 11.3 | 20.7 |
| Summed Feature 13 | ND | 5.17 | ND | ND | ND |

‡Data from *et al.* (2015)¹³

*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} anteiso and/or C_{14:0} 2-OH.

The novelty of a prokaryotic organism is universally determined by the comparison of 16S rRNA gene sequence homology⁴⁹. The threshold values are used at distinct taxonomic levels⁴⁶. In this context, the newly discovered bacterium was initially validated using the full-length 16S rRNA gene sequences, which were thereafter used for taxonomic classification. Phylogenetic analysis of 16S rRNA gene showed that the novel strain SW165^T clustered with closely related taxa in the genus *Olsenella* within the family *Atopobiaceae* (Figure 1). This genus composes of nine species, most of which are members of gut microbiota of humans and animals. However, *O. uli* is only

a species that have been isolated from chicken gut²⁶. Remarkable, this study revealed a new member of *Olsenella* from gut microbiota of chicken.

Phenotypic analyses are performed to differentiate closely valid bacteria. Based on phenotypic tests, strains SW165^T appeared several distinct properties compared to other members of the genus *Olsenella* (Table 1 and Table 2). The obvious distinct phenotypic features were observed in biochemical tests including enzymatic activity and carbon source utilization, thereby they might be important parameters for discriminating closely related species. These differences suggested the novelty of this microorganism belonging to *Olsenella*.

In addition to 16S based comparison, whole genome can be used for distinguishing, distinct bacteria. Recently, digital DNA-DNA hybridizations (dddH) becomes a key measurement in delineation of prokaryotic species. It is an *in-silico* genome-to-genome comparison inferring whole genome distance to mimic DDH⁵⁰. Besides, Average Nucleotides Identification (ANI) is another particular tool that confirm the taxonomic delineation. It measures the overall similarity between two genome sequences⁵¹. Recent publication of novel bacteria trend to perform genome-based analysis to support the results of 16S rRNA gene-based analysis. The strengths of genome-based analyses include comparison of all nucleotides in prokaryotic taxonomy and functional prediction⁵². Based on genomic evidence, strain SW165^T showed low similarity in terms of OrthoANI with *Olsenella* species of the family *Atopobiaceae* (Figure 4). Further, genome features and distribution of predicted functional categories of strain SW165^T was corresponded to all other *Olsenella* species (Figure 5 and Table 3). Thus, we proposed the strain SW165^T as a new species *Olsenella lakotia* SW165^T sp. nov., within the family *Atopobiaceae*.

Description of *Olsenella lakotia* SW165^T sp. nov.

O. lakotia sp. nov. (la.ko'tia N.L n. referring to native American tribe). Cells are strictly anaerobic, Gram-positive streptobacillus and non-motile. The average size of each cell is 0.5–2.0 μ m. Colonies are visible on BHI-M agar after 2 days and are approximately 0.2–0.5 cm in diameter, cream-white, smooth, slightly umbonate with entire circular margin. The microorganism exhibits optimal growth in BHI-M medium at 45°C and pH 7.0. The strain utilizes arbutin, cellobiose, dextrin, D-fructose, L-fucose, D-galactose, α -D-glucose, maltose, D-mannose, D-melibiose, D-raffinose, salicin, sucrose and turanose as a carbon source. Positive enzymatic reactions are obtained for alkaline phosphatase, leucine arylamidase, cysteine arylamidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase and β -glucosidase. The volatile fatty acid produced by this strain is acetic acid. The primary cellular fatty acids are C_{12:0}, C_{14:0}, C_{14:0} DMA and summed feature 1. The genome is 2,427,227 bp and its G+C is 67.59 mol%.

The type strain SW165^T (=DSM 107283 =CCOS 1887) was isolated from the cecum of feral chicken was deposited in the DSMZ and CCOS collections under accession numbers DSM 107283 and CCOS 1887 (*Extended data*: Supplemental

Table 3. General genome characteristic of *O. lakotia* SW165^T and its neighbors.

| Species | Strain | Size (Mbp) | % G+C | CDSs | rRNA | tRNA | GenBank accession number |
|--------------------------------------|-----------------|------------|-------|------|------|------|--------------------------|
| <i>O. lakotia</i> SW165 ^T | DSM 107283 | 2.43 | 67.6 | 2228 | 3 | 49 | PRJNA545153 |
| <i>O. profusa</i> | F0195 | 2.72 | 64.2 | 2610 | 3 | 48 | GCA_000468755.1 |
| <i>O. umbonata</i> | DSM 22620 | 2.35 | 64.9 | 2060 | 12 | 57 | GCA_900105025.1 |
| <i>O. uli</i> | DSM 7084 | 2.05 | 64.7 | 1772 | 3 | 49 | GCA_000143845.1 |
| <i>O. scatoligenes</i> | SK9K4 | 2.47 | 62.4 | 2110 | 3 | 47 | GCA_001494635.1 |
| <i>O. urinifantis</i> | Marseille-P3197 | 1.75 | 64.3 | 1558 | 7 | 48 | GCA_900155635.1 |
| <i>O. phocaensis</i> | Marseille-P2936 | 2.24 | 66.3 | 2190 | 3 | 50 | GCF_900120385.1 |
| <i>O. mediterranea</i> | Marseille-P3256 | 2.37 | 67.6 | 2045 | 6 | 52 | GCA_900119385.1 |

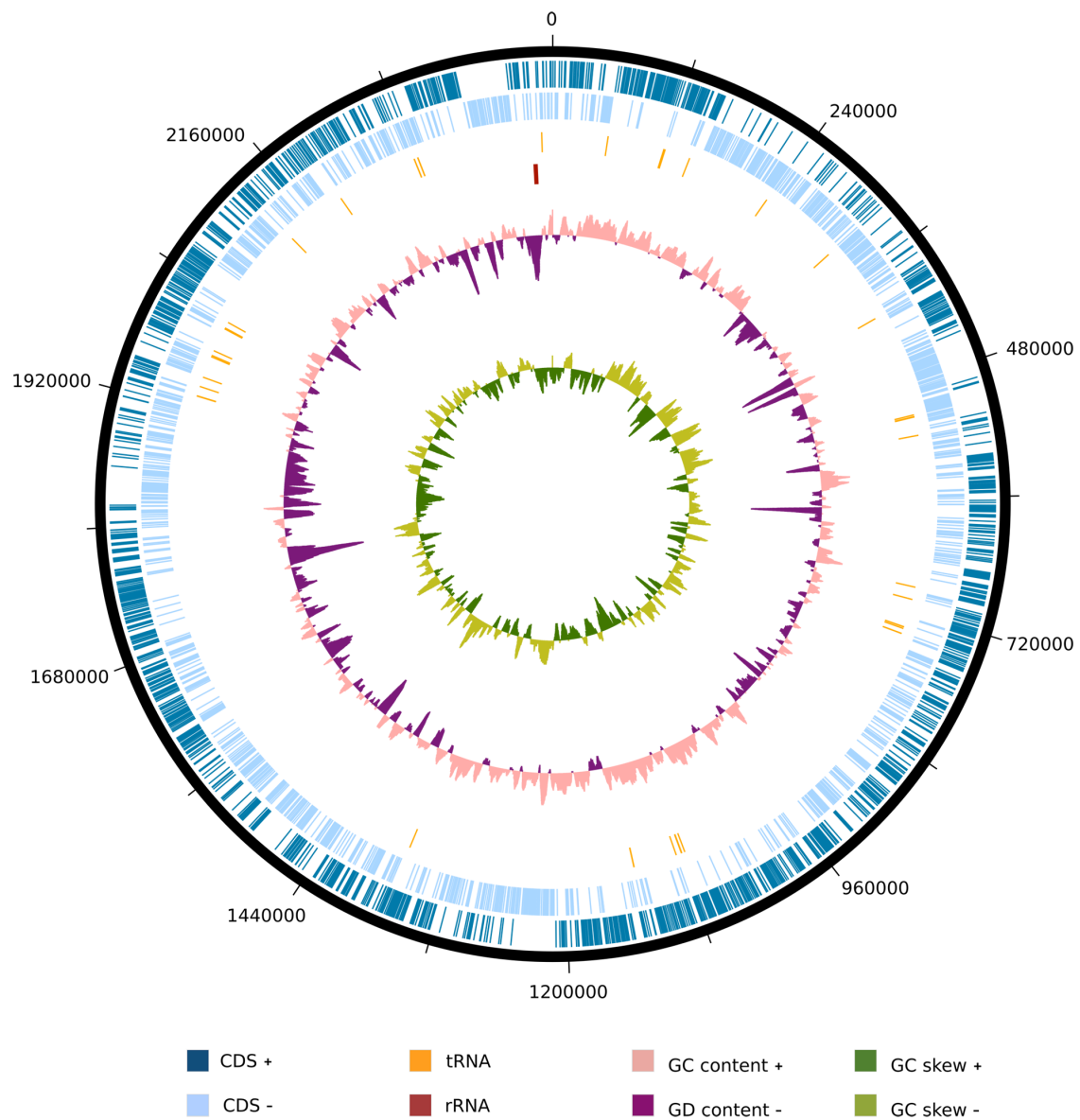


Figure 3. Graphical circular map of *O. lakotia* SW165^T genome. From outside to the center: coding sequences on the forward strand (CDS +), coding sequences on the reverse strand (CDS -), tRNAs, rRNAs, GC content, and GD skew.

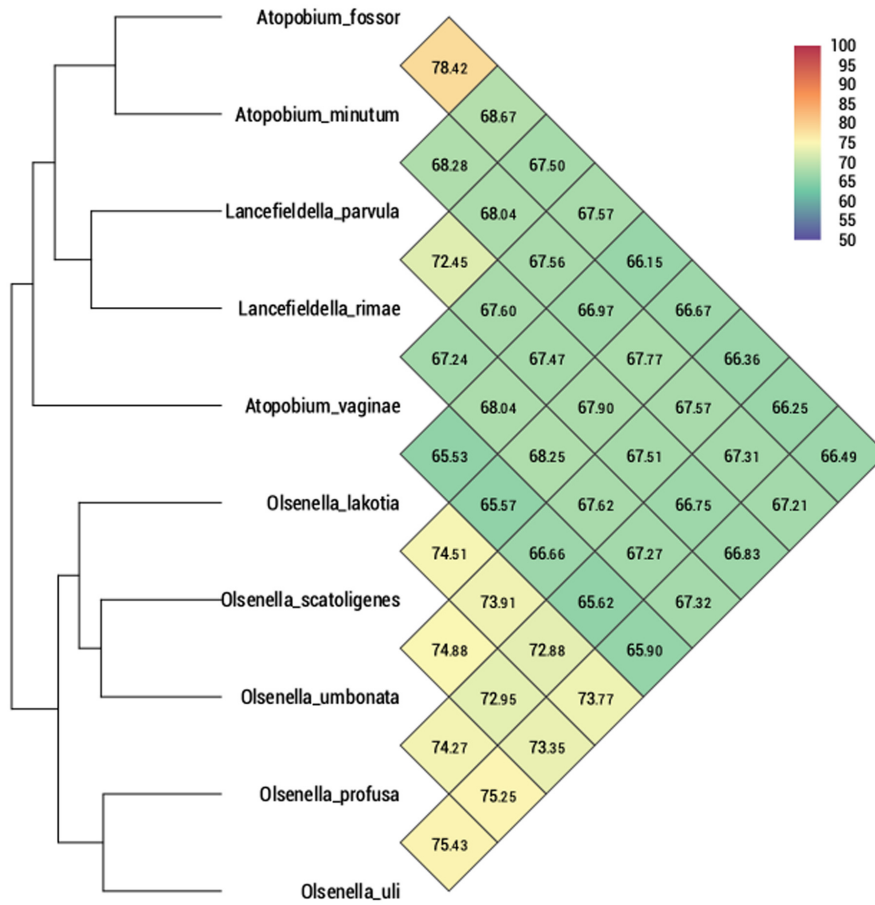


Figure 4. Average nucleotide Identity comparison of *O. lakotia* SW165^T and closely related strains. Heatmap represents OrthoANI values generated using OAT software between *O. lakotia* and related taxa with valid taxonomy.

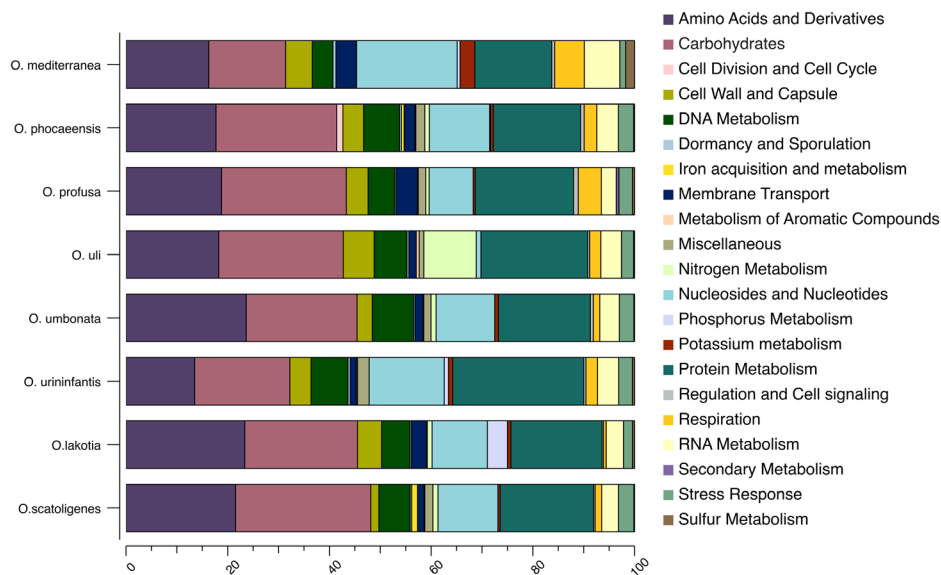


Figure 5. Distribution of functional features of predicted coding sequences of *O. lakotia* SW165^T and its neighbors. The functional features were predicted based on the clusters of orthologous groups. Heatmap was generated from genome annotation of individual species by RAST using Explicit software.

Data 1⁵³), respectively. The 16S rRNA and genome sequence are available in GenBank under accession numbers MK963074 and BioProject PRJNA545153, respectively.

Data availability

Underlying data

Olsenella sp. strain SW165 16S ribosomal RNA gene, partial sequence, Accession number MK963074: <https://www.ncbi.nlm.nih.gov/nuccore/MK963074>

Olsenella lakotia SW165 Genome sequencing and assembly, Accession number PRJNA545153: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA545153>

Extended data

Figshare: Extended data; Supplemental Table 1 (Functional categories (COGs) from genome of strain SW165^T), <https://doi.org/10.6084/m9.figshare.12793544.v145>.

Figshare: Supplemental Data 1 (DSMZ and CCOS accession numbers of strain SW165^T), <https://doi.org/10.6084/m9.figshare.12793610.v153>.

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgements

SW gratefully acknowledges Science Achievement Scholarship of Thailand (SAST) for providing fellowship. The authors would like to thank Electron Microscopy Core Facility at the Bowling Green State University, Ohio, USA for assistance with scanning electron microscopy.

A previous version of this article was published on bioRxiv: <https://doi.org/10.1101/670927>

References

- Huang P, Zhang Y, Xiao K, et al.: **The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids.** *Microbiome*. 2018; **6**(1): 211. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shang Y, Kumar S, Oakley B, et al.: **Chicken gut microbiota: importance and detection technology.** *Front Vet Sci*. 2018; **5**: 254. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Walker AW, Duncan SH, Louis P, et al.: **Phylogeny, culturing, and metagenomics of the human gut microbiota.** *Trends Microbiol*. 2014; **22**(5): 267–74. [PubMed Abstract](#) | [Publisher Full Text](#)
- Stewart EJ: **Growing unculturable bacteria.** *J Bacteriol*. 2012; **194**(16): 4151–60. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lagier JC, Khelaifia S, Alou MT, et al.: **Culture of previously uncultured members of the human gut microbiota by culturomics.** *Nat Microbiol*. 2016; **1**: 16203. [PubMed Abstract](#) | [Publisher Full Text](#)
- Rettedal EA, Gumpert H, Sommer MO: **Cultivation-based multiplex phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria.** *Nat Commun*. 2014; **5**: 4714. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zou Y, Xue W, Luo G, et al.: **1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses.** *Nat Biotechnol*. 2019; **37**(2): 179–85. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dewhirst FE, Paster BJ, Tzellas N, et al.: **Characterization of novel human oral isolates and cloned 16S rDNA sequences that fall in the family Coriobacteriaceae: description of *olsenella* gen. nov., reclassification of *Lactobacillus uli* as *Olsenella uli* comb. nov. and description of *Olsenella profusa* sp. nov.** *Int J Syst Evol Microbiol*. 2001; **51**(Pt 5): 1797–804. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zhi XY, Li WJ, Stackebrandt E: **An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa.** *Int J Syst Evol Microbiol*. 2009; **59**(Pt 3): 589–608. [PubMed Abstract](#) | [Publisher Full Text](#)
- Kraatz M, Wallace RJ, Svensson L: ***Olsenella umbonata* sp. nov., a microaerotolerant anaerobic lactic acid bacterium from the sheep rumen and pig jejunum, and emended descriptions of *Olsenella*, *Olsenella uli* and *Olsenella profusa*.** *Int J Syst Evol Microbiol*. 2011; **61**(Pt 4): 795–803. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gupta RS, Chen WJ, Adeolu M, et al.: **Molecular signatures for the class Coriobacteriia and its different clades; proposal for division of the class Coriobacteriia into the emended order Coriobacteriales, containing the emended family Coriobacteriaceae and Atopobiaceae fam. nov., and Eggerthellales ord. nov., containing the family Eggerthellaceae fam. nov.** *Int J Syst Evol Microbiol*. 2013; **63**(Pt 9): 3379–97. [PubMed Abstract](#) | [Publisher Full Text](#)
- Olsen I, Johnson JL, Moore LV, et al.: ***Lactobacillus uli* sp. nov. and *Lactobacillus rimae* sp. nov. from the human gingival crevice and emended descriptions of *Lactobacillus minutus* and *Streptococcus parvulus*.** *Int J Syst Bacteriol*. 1991; **41**(2): 261–6. [PubMed Abstract](#) | [Publisher Full Text](#)
- Li X, Jensen RL, Hojberg O, et al.: ***Olsenella scatoligenes* sp. nov., a 3-methylindole- (skatole) and 4-methylphenol- (*p*-cresol) producing bacterium isolated from pig faeces.** *Int J Syst Evol Microbiol*. 2015; **65**(Pt 4): 1227–33. [PubMed Abstract](#) | [Publisher Full Text](#)
- Morand A, Chabrol B, Fournier PE: **“*Olsenella urinifantis*”, a new bacterial species isolated from a urine sample of a 26-day-old boy suffering from gastroesophageal reflux.** *Human Microbiome Journal*. 2016; **2**: 17–8. [Publisher Full Text](#)
- Bilen M, Cadoret F, Dubourg G, et al.: **‘*Olsenella congonensis*’ sp. nov., identified in human stool sample.** *New Microbes New Infect*. 2017; **19**: 132–3. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ricaboni D, Mailhe M, Vitton V, et al.: ***Olsenella provencensis* sp. nov., *Olsenella phocaeensis* sp. nov., and *Olsenella mediterranea* sp. nov. isolated from the human colon.** *Human Microbiome Journal*. 2017; **4**: 22–3. [Publisher Full Text](#)
- Goker M, Held B, Lucas S, et al.: **Complete genome sequence of *Olsenella uli* type strain (VPI D76D-27C).** *Stand Genomic Sci*. 2010; **3**(1): 76–84. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Munson MA, Banerjee A, Watson TF, et al.: **Molecular analysis of the microflora associated with dental caries.** *J Clin Microbiol*. 2004; **42**(7): 3023–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bahrani-Mougeot FK, Paster BJ, Coleman S, et al.: **Diverse and novel oral bacterial species in blood following dental procedures.** *J Clin Microbiol*. 2008; **46**(6): 2129–32. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Krogus-Kurikka L, Kassinen A, Paulin L, et al.: **Sequence analysis of percent G+C fraction libraries of human faecal bacterial DNA reveals a high number of Actinobacteria.** *BMC Microbiol*. 2009; **9**: 68. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Khachatryan ZA, Ktsoyan ZA, Manukyan GP, et al.: **Predominant role of host genetics in controlling the composition of gut microbiota.** *PLoS One*. 2008; **3**(8): e3064. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hernandez JD, Scott PT, Shephard RW, et al.: **The characterization of lactic acid producing bacteria from the rumen of dairy cattle grazing on improved pasture supplemented with wheat and barley grain.** *J Appl*

- Microbiol.* 2008; **104**(6): 1754–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
23. Leser TD, Amenuvor JZ, Jensen TK, et al.: **Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited.** *Appl Environ Microbiol.* 2002; **68**(2): 673–90.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Weiss A, Jerome V, Freitag R, et al.: **Diversity of the resident microbiota in a thermophilic municipal biogas plant.** *Appl Microbiol Biotechnol.* 2008; **81**(1): 163–73.
[PubMed Abstract](#) | [Publisher Full Text](#)
25. Ferrario C, Alessandri G, Mancabelli L, et al.: **Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses.** *Environ Microbiol.* 2017; **19**(11): 4771–83.
[PubMed Abstract](#) | [Publisher Full Text](#)
26. Medvecky M, Cejkova D, Polansky O, et al.: **Whole genome sequencing and function prediction of 133 gut anaerobes isolated from chicken caecum in pure cultures.** *BMC Genomics.* 2018; **19**(1): 561.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Fournier PE, Drancourt M: **New Microbes New Infections promotes modern prokaryotic taxonomy: a new section "TaxonoGenomics: new genomes of microorganisms in humans".** *New Microbes New Infect.* 2015; **7**: 48–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Patil PP, Kumar S, Midha S, et al.: **Taxonomics reveal multiple novel genospecies associated with clinical isolates of *Stenotrophomonas maltophilia*.** *Microb Genom.* 2018; **4**(8): e000207.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Shields P, Cathcart L: **Motility test medium protocol.** *American Society for Microbiology.* 2011.
[Reference Source](#)
30. Sasser M: **Identification of bacteria by gas chromatography of cellular fatty acids.** MIDI Technical Note 101 Newark, DE: MIDI Inc. 1990.
[Reference Source](#)
31. Lane DJ: **16S/23S rRNA. Sequencing.** In: Stackebrandt E and Goodfellow M, *Nucleic Acid Techniques in Bacterial Systematics.* John Wiley and Sons, New York. 1991.
[Reference Source](#)
32. Stackebrandt E, Liesack W: **Nucleic acids and classification.** In: Goodfellow M and O'Donnell AG (ed.), *Handbook of new bacterial systematics.* Academic Press, London, England. 1993.
[Reference Source](#)
33. Kim OS, Cho YJ, Lee K, et al.: **Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species.** *Int J Syst Evol Microbiol.* 2012; **62**(Pt 3): 716–21.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Kumar S, Stecher G, Tamura K: **MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets.** *Mol Biol Evol.* 2016; **33**(7): 1870–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
35. 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**(22): 4673–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Felsenstein J: **Evolutionary trees from DNA sequences: a maximum likelihood approach.** *J Mol Evol.* 1981; **17**(6): 368–76.
[PubMed Abstract](#) | [Publisher Full Text](#)
37. Fitch WM: **Toward defining the course of evolution: minimum change for a specific tree topology.** *Systematic Zoology.* 1971; **20**(4): 406–16.
[Publisher Full Text](#)
38. Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Mol Biol Evol.* 1987; **4**(4): 406–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Wick RR, Judd LM, Gorrie CL, et al.: **Unicycler: resolving bacterial genome assemblies from short and long sequencing reads.** *PLoS Comput Biol.* 2017; **13**(6): e1005595.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Gurevich A, Saveliev V, Vyahhi N, et al.: **QUAST: quality assessment tool for genome assemblies.** *Bioinformatics.* 2013; **29**(8): 1072–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Aziz RK, Bartels D, Best AA, et al.: **The RAST Server: rapid annotations using subsystems technology.** *BMC Genomics.* 2008; **9**: 75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Goris J, Konstantinidis KT, Klappenbach JA, et al.: **DNA-DNA hybridization values and their relationship to whole-genome sequence similarities.** *Int J Syst Evol Microbiol.* 2007; **57**(Pt 1): 81–91.
[PubMed Abstract](#) | [Publisher Full Text](#)
43. Robertson CE, Harris JK, Wagner BD, et al.: **Explicit: graphical user interface software for metadata-driven management, analysis and visualization of microbiome data.** *Bioinformatics.* 2013; **29**(23): 3100–1.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Stackebrandt E, Ebers J: **Taxonomic parameters revisited: tarnished gold standards.** *Microbiology Today.* 2006; **33**: 152–5.
[Reference Source](#)
45. Wongkuna S, Ghimire S, Janvilisri T, et al.: **Extended data. figshare.** Dataset. 2020.
<http://www.doi.org/10.6084/m9.figshare.12793544.v1>
46. Kim M, Oh HS, Park SC, et al.: **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**(Pt 2): 346–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
47. Lee I, Ouk Kim Y, Park SC, et al.: **OrthoANI: An improved algorithm and software for calculating average nucleotide identity.** *Int J Syst Evol Microbiol.* 2016; **66**(2): 1100–3.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Browne HP, Forster SC, Anonye BO, et al.: **Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation.** *Nature.* 2016; **533**(7604): 543–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
49. Clarridge JE 3rd: **Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases.** *Clin Microbiol Rev.* 2004; **17**(4): 840–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
50. Auch AF, von Jan M, Klenk HP, et al.: **Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison.** *Stand Genomic Sci.* 2010; **2**(1): 117–34.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
51. Figueras MJ, Beaz-Hidalgo R, Hossain MJ, et al.: **Taxonomic affiliation of new genomes should be verified using average nucleotide identity and multilocus phylogenetic analysis.** *Genome Announc.* 2014; **2**(6): e00927–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
52. Konstantinidis KT, Tiedje JM: **Genomic insights that advance the species definition for prokaryotes.** *Proc Natl Acad Sci U S A.* 2005; **102**(7): 2567–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
53. Wongkuna S, Ghimire S, Janvilisri T, et al.: **Supplementary Data 1. figshare.** Dataset. 2020.
<http://www.doi.org/10.6084/m9.figshare.12793610.v1>

Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 24 September 2020

<https://doi.org/10.5256/f1000research.28500.r71021>

© 2020 Seleem M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Mohamed Seleem 

Virginia Polytechnic Institute and State University, Blacksburg, USA

This manuscript describes the isolation and characterization of a proposed new species “lakotia” within the genus *Olsonella*. The isolate has been characterized using the combination of genome sequencing and phenotypic assays. The phenotypic characterization includes nutrient utilization using Biolog plates, enzyme profiling using API zyme, and cellular membrane fatty acid profiling using FAME. At the phenotypic level, the proposed new species show clear differences with *Olsonella umbonata*, the closest taxonomic neighbor. At the genomic level, the proposed new species is only 74% similar to other species in the genus *Olsonella*. Therefore, the proposed species meets the genomic and phenotypic requirements of being designated as a new species. However, some minor changes could improve the clarity of the manuscript:

- Table 1. Tittle of the table reads “ Characteristics of *O. lakotia* and closely related strains” Authors should instead give the phenotype reported here. Therefore, please revise the title to improve the clarity; eg. Phenotypic properties of *O. Lakota*... Likewise, in the table, column header should be “Phenotpic properties” instead of “characteristic”. Please give species names in the column headers instead of numbers.
- Table 3. Authors have given bioproject number instead of genbank accession number for *O. lakotia* SW165. The genbank accession number was anot accessible from the Bioproect page in NCBI. Please remove the bioproject number and instead give Genbank SRA accession number here.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 September 2020

<https://doi.org/10.5256/f1000research.28500.r71020>

© 2020 Chang Y. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Yung-Fu Chang 

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

This manuscript "[Taxono-genomics description of *Olsenella lakotia* SW165T sp. nov., a new anaerobic bacterium isolated from the cecum of feral chicken](#)" was submitted by Scaria *et al.* The authors have characterized a new bacterium strain isolated from chicken. It is a well-written manuscript and the data is very solid to support their conclusion. I have enclosed the manuscript with a minor revision which you can see [here](#).

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbial pathogenesis, molecular diagnosis, vaccine development, genomics, and proteomics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 14 Sep 2020

Surang Chankhamhaengdecha, Faculty of Science, Mahidol University, Bangkok, Thailand

Thank you for reading and correcting our manuscript. We really appreciate you approval.

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research