Collinsella provencensis sp. nov., a new species identified from healthy human gut microbiota.

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Research Article

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Abstract

A Gram-positive and anaerobic bacterium was isolated from the stool sample of a healthy French volunteer. A taxonogenomics approach was adopted to characterize it. Cells are rod shaped non-spore-forming and non-motile. Growth of strain Marseille-P3740 occurs at 37°C in anaerobic atmosphere at pH 7. The 16S ribosomal RNA gene sequence analysis of the novel strain Marseille-P3740 displayed 96.31% similarity in nucleotide sequence with *Collinsella intestinalis* strain JCM 10643, the phylogenetically closest related species with standing in nomenclature. $C_{16:0}$, $C_{18:1n9}$ and $C_{18:2n6}$ are the major cellular fatty acid components. The genomic of strain Marseille-P3740^T is 1.74 Mbp long with 59.1 mol% of G + C content. Based on the taxonogenomic description and the phenotypic and biochemical properties of this bacterium, we propose the strain Marseille-P3740T (= CSUR P3740 = CCUG 70947) as a new species, *Collinsella provencensis* sp. nov.

Introduction

The *Collinsella* genus was first described in 1999 by Kageyama *et al.*, [1] following the transfer of *Eubacterium aerofaciens* to the genus on the basis of deep analysis of 16S rRNA gene sequence variability with other *Eubacterium* sp. At the time of writing, *Collinsella* genus contains five validly described species, including *C. aerofaciens* [1], *C. intestinalis* [2], *C. massiliensis*[3], *C. stercoris* [2] and *C. tanakaei* [4]. Species belonging to the *Collinsella* genus are Gram-positive anaerobic bacilli. They have been isolated from the human intestinal microbiota and can sometimes be associated with diseases [5,6].

Bacterial diversity is believed to play a role in normal physiological functions and in diseases which needs to be better understood [7]. Indeed, the culturomics concept developed in recent years allows, by developing different culture conditions, to expand our knowledge of the human microbiota through the discovery of bacteria never cultivated before [8–11]. Once the bacterium was isolated, we used a taxono-genomic approach including matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and genome sequencing, to establish its description [12,13].

Here, we provide a full description of *Collinsella provencensis* sp. nov., strain Marseille-P3740^T (= CSUR P3740 = CCUG 70947) which discovery was announced by Dione et al. 2018[14].

Materiel And Methods

Strains isolation and identification

As part of a culturomic study investigating the human microbiome, we isolated a bacterial strain Marseille-P3740 from fresh stool of a 32-year-old male volunteer living in France. The patient has endorsed an informed consent, while the study was authorized by the ethics committee of the Institut Federatif de Recherche IFR48 under number 2016-010.

In order to prove the existence of bacteria by culturomics, our stool sample was diluted with phosphate buffer saline and then incubated at 37°C in an anaerobic culture flask (BD BACTEC®, Plus Anaerobic/F Media, Le Pont de Claix, France) which was filled with 5% (V/V) sheep blood and 5% (V/V) sterile-filtered cow rumen. To obtain distinct bacterial colonies, we subcultured onto 5% sheep blood-enriched Columbia agar medium (BioMérieux, Marcy l'Etoile, France) at 37°C after 48 hours.

First identification was attempted with MALDI-TOF Mass Spectrometry (Bruker, Daltonics, Bremen, Germany) as previously reported [15]. The generated spectra from this strain were analyzed using Biotyper 3.0 software, by comparing them to those properly and regularly incremented in the local URMS database (https://www.mediterranee-infection.com/urms-data-base). MALDI-TOF MS could not correctly identify the strain Marseille-P3740, a molecular investigation was therefore carried out by amplifying the 16S rRNA gene using the primer pair fD1 and rP2 (Eurogentec, Angers, France). Then, it was sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), following the protocol previously reported [16]. Using CodonCode Aligner software (http://www.codoncode.com), sequences were assembled and analyzed in order to obtain a consensus sequence as reference sequence of the type strain and submitted it to the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/). A comparative analysis of nucleotides by BLASTn was performed. Thus, only the sequences phylogenetically close to the typical species were recovered to build the phylogenetic tree.

Phenotypic characterization

Strain Marseille-P3740 was cultured in aerobic, microaerophilic and anaerobic atmospheres (Thermo Scientific, Dardilly, France) and at different temperatures (25, 28, 37, 45 and 56°C) and varied pH (5 to 8.5) to evaluate its growth under these conditions on 5% sheep blood-enriched Columbia agar medium (bioMérieux). The Gram staining, catalase and oxidase tests, as well as spore-forming were realized as previously described [17]. In addition, to study the biochemical characteristics of the Marseille-P3740 strain, API ZYM and API 50 CH (bioMérieux) strips were used according to the manufacturer's instructions. To reveal the shape of the bacterial cells, a negative staining of the strain Marseille-P3740 was carried out and observed under a scanning electron microscope (Hitachi High-Technologies, Tokyo, Japan) following the protocol described by Belkacemi *et al*., 2019 [18].

Genome characteristics

Genomic DNA extraction was realized using the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) adapted to EZ1 biorobot. It was sequenced by the MiSeq instrument (Illumina Inc, San Diego, CA, USA) using the Nextera Mate Pair and Nextera XT Paired End (Illumina) sample preparation kit, following the same protocol previously used [19]. Three known softwares were used to correctly assemble this genome, including Spades [20], Velvet [21] and Soap Denovo [22]. To manage trimmed or untrimmed sequences, MiSeq and Trimmomatic softwares were used, respectively [23]. In addition, we used the GGDC (Genome-to-Genome Distance Calculator) web server available online (http://ggdc.dsmz.de) to calculate the genomic

similarities [24]. This allowed us to obtain DNA-DNA hybridization (DDH) values of these compared genomes. The average nucleotide identity (OrthoANI) was also accessed using the OAT software [25].

Ethical approval

The volunteer has given freely his authorization by signed and informed consent for advanced studies to be done on the collected sample. In addition, all the methods used in this study were performed in accordance with relevant guidelines and regulations conformed to Declaration of Helsinki.

Results

Strain identification and phylogenetic analysis

MALDI-TOF mass spectrometry could not correctly identify the strain Marseille-P3740. Thus, the obtained reference spectrum was added to the local database. Similarity analysis based on 16S rRNA of strain Marseille-P3740 against GenBank showed the highest nucleotide sequence similarities of 96.31 % sequence identity with *Collinsella intestinalis* strain JCM 10643 (Genbank accession number: NR_113165.1), which was the phylogenetically closest species. This value obtained is below the threshold of 98.65% recommended to delimit the species barrier in bacteria [24,26]. Therefore, the strain Marseille-P3740 was considered as a potentially new species belonging to the genus *Collinsella* within the *Coriobacteriaceae* family in the phylum *Actinobacteria* (Figure 1).

Phenotypicproperties of the strain

After the isolation step, the strain Marseille-P3740 was cultured in order to obtain pure and isolated colonies on 5% sheep blood enriched Columbia agar. The colonies were small and transparent. Bacterial cells were Gram-positive. No spore forming after thermal shock (10 minutes at 80°C) for strain Marseille-P3740. In addition, the shape of the bacterium was demonstrated by a photomicrograph obtained with the Hitachi TM4000 instrument (Figure 2). Bacterial cells appeared to be rod-shaped with a mean length of 1 µm and a mean diameter of 0.5 µm. This bacterium is fastidious but grows under anaerobic conditions at pH 7 on 5% sheep blood enriched Columbia agar after 48 hours. No growth in aerobic or microaerophilic atmosphere was observed. However, it is also possible to obtain bacterial colonies at temperatures ranging from 28 to 45°C, knowing that optimal growth occurs at 37° C in an anaerobic atmosphere. The use of API ZYM and 50 CH (bioMérieux) galleries revealed that strain Marseille-P3740^T presented enzymatic activities, such as acid phosphatase, alkaline phosphatase and naphthol-AS-BI-phosphohydrolase, while D-mannose, D-raffinose and D-trehalose were positive. However, negative reactions were observed with lipase, trypsin, achymotrypsin, α-mannosidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase and β-glucosidase, erythritol, arabinose, D-ribose, D-adonitol, xylose, methyl-β-D-xylopranoside, D-galactose, glucose, arbutin, fructose, L-sorbose, L-rhamnose, D-lyxose, dulcitol, inositol, D-mannitol, esculin ferric citrate, methyl-αD-glucopranoside, N-acetyl-β-glucosaminidase, glycogen, methyl-αD-xylopranoside, amygdalin, salicin, D-cellobiose, D-

maltose, glycerol, D-lactose, D-melibiose, inulin, D-melezitose, amidon, D-turanose, D-tagatose, gentiobiose, L-fucose, L-arabitol, potassium 2-ketogluconate and potassium gluconate. Strain Marseille-P3740^T showed catalase-negative and oxidase-negative activities. The Table 1 comparing the main phenotypic criteria shows that the strain Marseille-P3740 differs from other *Collinsella* by not consuming glucose. Fatty acid methyl esther analysis was performed as previously described [13].The major fatty acid were hexadecanoic acid (41 %) and 9-octadecenoic acid (23.6 %). Very few other structures were described. Minor amounts of other unsaturated and saturated fatty acids were also detected, including unsaturated structures (Table 2).

Genome sequencing and comparison

The genome of *Collinsella provencensis* strain Marseille-P3740 is 1,737,922 bp long with a 58.2 mol% G+C content.

The genome size, the number of proteins and the number of genes of strain Marseille-P3740 were lower than those of the other genomes studied here (Table 3). On the other hand, it has a genome with a GC percentage higher than that of *C. bouchesdurhonensis,* but lower than that of *C. intestinalis*, *C. vaginalis*, *C. stercoris* and *C. tanakaei* (62.4, 64.4, 62.7 and 60.2 mol% respectively). Calculation of the degree of genomic similarity of strain Marseille-P3740 with closely related species showed that values ranged from 72.57% between *C. bouchesdurhonensis* and *C. vaginalis* to 82.09% between *C. intestinalis* and *C. stercoris*. On the other hand, *C. provencensis* compared to the other *Collinsella* species, showed values ranging from 78.01% with *C. stercoris* to 72.90% with *C. bouchesdurhonensis*. Therefore, we found that the OrthoANI values among closely related species (Figure 3) were below the value at the 95% threshold recommended for delineating species barrier in prokaryotes. However, the analysis of the DDH values calculated between the genomes of the *Collinsella* species studied here, showed that no value is close to the threshold value (70%) making it possible to delimit a new bacterial species. Indeed, 27.3% was the highest DDH value obtained between *C. stercoris* and *C. phocaeensis*, while the lowest DDH value obtained (21.6%) was shared between *C. vaginalis* and *C. aerofaciens* (Table 4).

Conclusion

The results obtained from phenotypic, phylogenetic and genomic analysis, such as 16S rRNA sequence similarity, OrthoANI values lower than 95%, strongly confirmed that strain Marseille-P3740^T is a new bacterial species called *Collinsella provencensis* sp. nov.

Description of *Collinsella provencensis* sp. nov.

Collinsella provencensis (pro.ven.cen'sis, N.L. fem. adj. *provencensis*, pertaining to Provence, the region of France where the strain was isolated). Optimum growth of colonies was obtained at 37°C on 5% sheep blood enriched Columbia agar during after 48 hours. They appear small and transparent. *C. provencensis* is a Gram-positive rod-shaped bacterium with a mean length of 1 µm and a mean diameter of 0.5 µm. Strain Marseille-P3740^T produced alkaline phosphatase, acid phosphatase, naphthol-AS-BI-

phosphohydrolase and D-trehalose. However, negative reactions were observed for trypsin, β-galactosidase, α-glucosidase, glycerol, D-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-lactose, D-saccharose, glycogen, D-fucose and D-arabitol. Strain Marseille-P3740^T is catalase-negative and oxidase-negative. The genome size of *Collinsella provencensis* strain Marseille-P3740^T is about 1.74 Mbp long with 58.1 mol% G+C content. The Genbank accession number for the 16S rRNA gene sequence of strain Marseille-P3740^T is LT722680 and for the whole genome shotgun project is FZRI00000000.This strain was isolated from fresh stool of a healthy French man.

Declarations

Conflict of interest:

None to declare

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Tables

Table 1: Differential phenotypic features of **1**, *Collinsella provencensis* sp. nov. strain Marseille-P3740; **2**, *Collinsella intestinalis* strain JCM 10643 [2]; **3**, *Collinsella stercoris* strain DSM 13279 [2]; **4**, *Collinsella vaginalis* strain Marseille-P2666 [27]; **5**, *Collinsella tanakaei* strain YIT 1206 [4].

Properties	1	2	3	4	5
Cell diameter (µm)	0.3-0.5	0.3-0.5	0.3-0.4	0.3-0.5	0.5-1
Oxygen requirement	OA	OA	OA	OA	OA
Gram staining	+	+	+	+	+
Spore formation	−	−	−	−	−
Motility	−	−	−	−	−
Production of:					
Acid phosphatase	+	+	+	+	+
Catalase	−	na	na	−	−
Lipase (C14)	−	−	−	−	−
Trypsin	−	−	−	−	−
α-galactosidase	−	−	−	−	−
N-acetyl-β-glucosaminidase	−	+	+	+	−
Acid from:					
Mannose	+	+	+	+	+
Glucose	−	+	+	+	+
Mannitol	−	−	−	−	−
Lactose	−	−	+	+	+
Fructose	−	+	+	na	na
Ribose	−	+	−	na	na
G+C content (mol%)	58.2	64.4	61.2	64.6	60.8
Source	Human feces	Human feces	Human feces	Human vagina	Human feces

OA, obligately anaerobic; +, positive result; −, negative result; na, data not available

Table 2: Fatty acid profiles (%) of *Collinsella provencensis* strain Marseille-P3740^{T}.

Fatty acids	Name	*C. provencensis**	*C. vaginalis**
$C_{16:00}$	Hexadecanoic acid	41.0 ± 1.9	23.5±0.5
$C_{18:1n9}$	9-Octadecenoic acid	23.6 ± 0.5	37.5±1.0
$C_{18:2n6}$	9,12-Octadecadienoic acid	15.8 ± 0.8	11.3±0.3
$C_{18:00}$	Octadecanoic acid	11.9 ± 0.8	18.5±0.4
$C_{20:4n6}$	5,8,11,14-Eicosatetraenoic acid	1.9 ± 0.7	ND
$C_{14:00}$	Tetradecanoic acid	1.6 ± 0.1	3.5±0.3
$C_{17:00}$	Heptadecanoic acid	1.4 ± 0.3	ND
$C_{16:1n7}$	9-Hexadecenoic acid	1.2 ± 0.2	ND
$C_{12:00}$	Dodecanoic acid	TR	TR
$C_{15:00}$	Pentadecanoic acid	TR	TR
$C_{17:1n8}$	9-Heptadecenoic acid	TR	ND

*Mean peak area percentage; TR = trace amounts < 1; ND, not found.

Table 3: Genome comparison of closely related species with *Collinsella provencensis* strain Marseille-P3740T

Species	Size (Mbp)	G+C (mol%)	Protein	rRNA	tRNA	Genes
C. provencensis	1.74	58.2	1,428	5	49	1,517
C. intestinalis	1.78	62.4	1,515	11	52	1,594
C. bouchesdurhonensis	1.88	57.9	1,630	4	48	1,703
C. vaginalis	2.16	64.4	1,761	3	47	1,919
C. stercoris	2.48	62.7	1,932	3	52	2,111
C. tanakaei	2.49	60.2	2,120	12	55	2,225

Table 4: Comparison of DDH values calculated from the server of Genome-to-Genome Distance Calculator 2.1 (http://ggdc.dsmz.de/).

	Cpro	*Cvag*	*Ctan*	*Cste*	*Cpho*	*Cmas*	*Cbou*	*Caer*
Cpro	100%							
Cvag	22.6 ±4.7%	100%						
Ctan	24.4 ±4.8%	22.5 ±4.7%	100%					
Cste	25.7 ±4.8%	23.2 ±4.7%	26.1 ±4.9%	100%				
Cpho	24.4 ±4.8%	23.4 ±4.7%	26.3 ±4.8%	27.3 ±4.8%	100%			
Cmas	22.7 ±4.7%	22.1 ±4.7%	22.0 ±4.7%	22.7 ±4.8%	22.0 ±4.7%	100%		
Cbou	23.0 ±4.7%	22.5 ±4.7%	23.1 ±4.8%	22.8 ±4.7%	22.2 ±4.7%	21.9 ±4.7%	100%	
Caer	22.8 ±4.8%	21.6 ±4.7%	22.9 ±4.7%	23.7 ±4.8%	22.3 ±4.7%	21.8 ±4.7%	24.5 ±4.8%	100%

Cpro, *Collinsella provencensis* strain Marseille-P3740; ***Cvag***, *Collinsella vaginalis* strain Marseille-P2666; ***Ctan***, *Collinsella tanakaei* strain YIT 1206; ***Cste***, *Collinsella stercoris* strain DSM 13279; ***Cpho***, *Collinsella phocaeensis* strain Marseille-P3245; ***Cmas***, *Collinsella massiliensis* strain CSURP902; ***Cbou***, *Collinsella bouchesdurhonensis* strain Marseille-P3296; ***Caer***, *Collinsella aerofaciens* strain CCUG 28087.

Figures

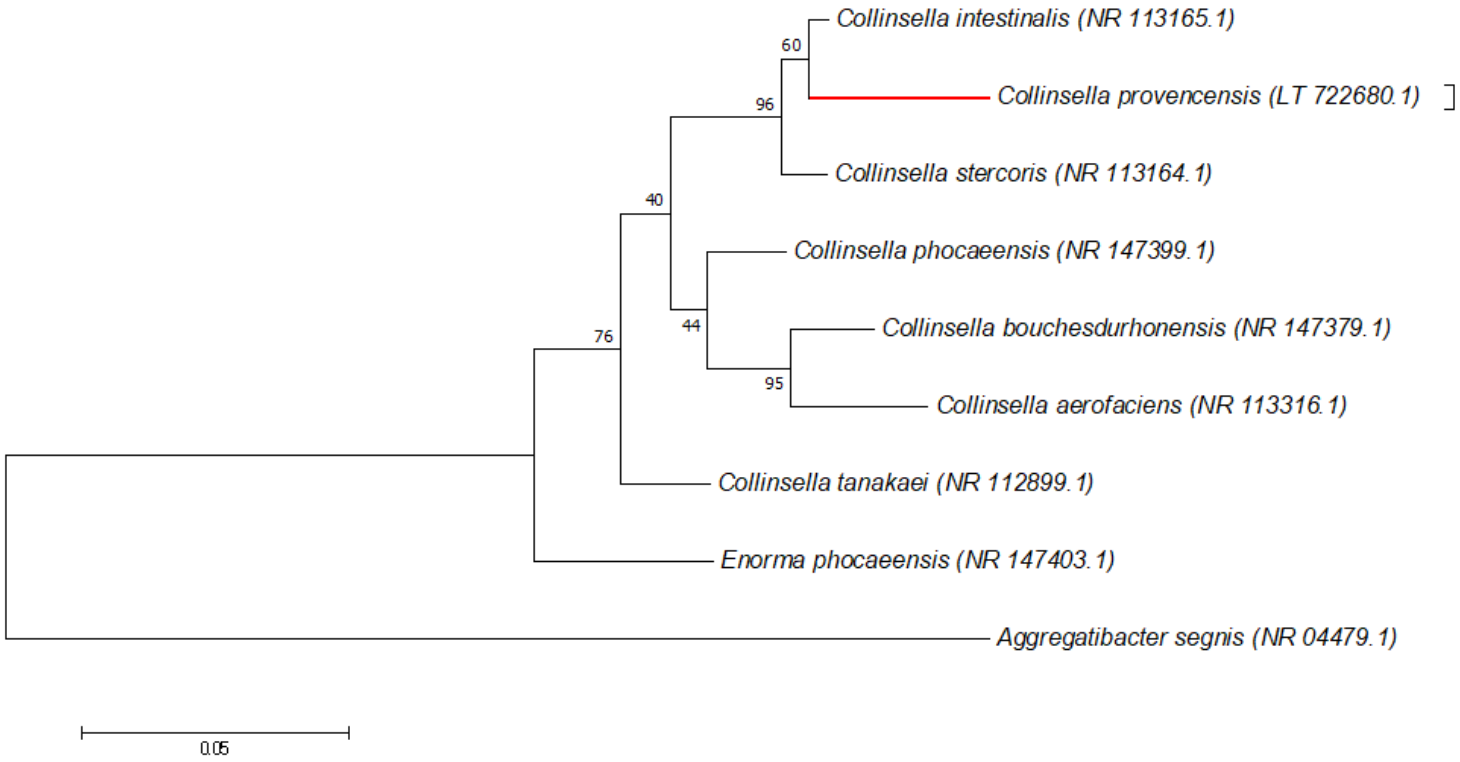

Figure 1

Phylogenetic tree highlighting the position of Collinsella provencensis sp. nov., based on the 16S rRNA gene sequences relative to the most closely related type species within the genus Collinsella. Genbank accession numbers are putted in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference were obtained using the Maximum likelihood method and the MEGA X software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 5% nucleotide sequence divergence.

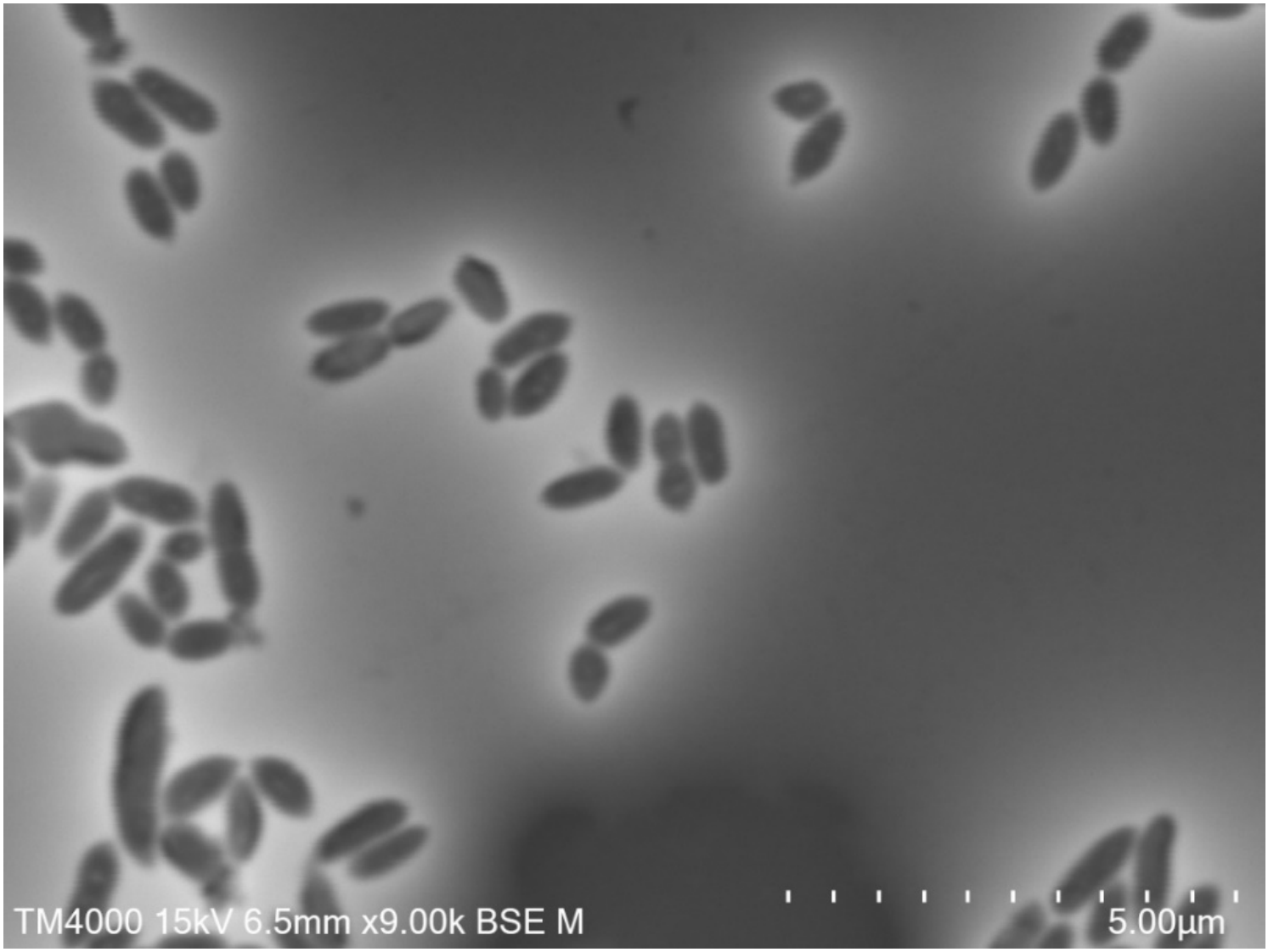

Figure 2

Scanning electron microscopy of stained Collinsella provencensis sp. nov., (Hitachi TM4000). Scales and acquisition settings are shown on figure.

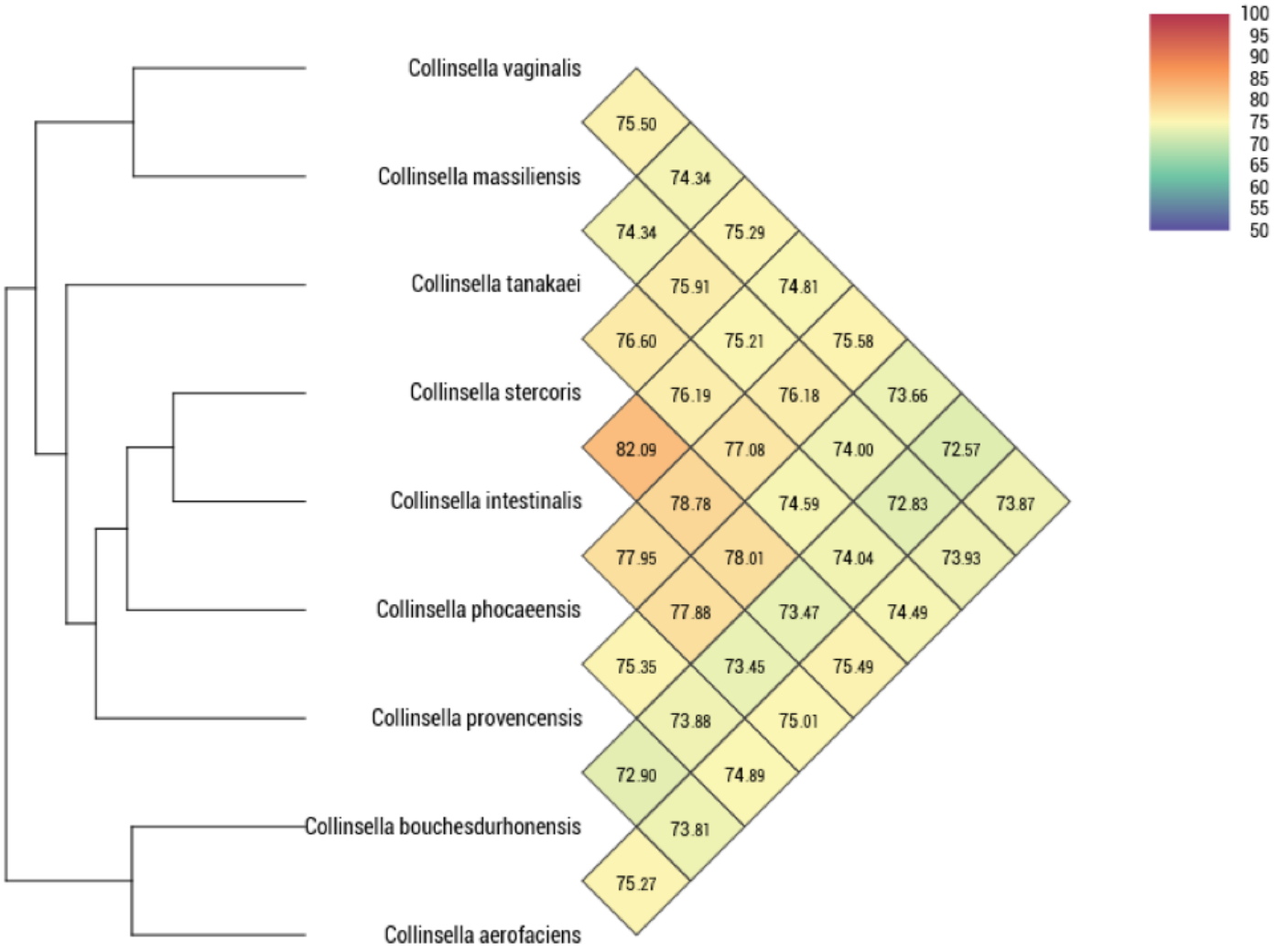

Figure 3

Heatmap generated with OrthoANI values calculated using the OAT software between Collinsella provencensis sp. nov., and other closely related species with standing in nomenclature.