

Selenomonas felix sp. nov., a new bacterium isolated from human sputum

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Abstract

Selenomonas felix strain Marseille-P3560^T (=CSURP3560) is a new species isolated from human sputum.

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Phylogeny and Infections (MEPHI) database; <http://www.mediterraneeinfection.com/article.php?larub=280&titre=urms-database>). The initial growth was obtained after 48 hours' culture on Columbia agar with 5% sheep's blood in anaerobic conditions at 37°C at pH 7.5.

Introduction

Culturomics is the concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once a bacterium is isolated, a taxonogenomic approach is used, including MALDI-TOF MS, phylogenetic analysis, main phenotypic description (Table 1) and genome sequencing, to describe it [5,6].

Isolation and growth conditions

In 2017 we isolated from a human sputum sample an unidentified bacterial strain. The study was validated by the ethics committee of IHU Méditerranée Infection under number 2016-011. A screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with Microbes Evolution

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done by using the primer pair fDI and rP2 (Eurogentec, Angers, France), and sequencing by the Big Dye Terminator v1.1 Cycle Sequencing Kit and the ABI Prism 3130xl Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (<http://www.codoncode.com>). Strain *Selenomonas felix* exhibited a 96% sequence identity with *Selenomonas diana* strain ATCC 43527 (GenBank accession no. NR_041805.1), the phylogenetically closest species with standing in nomenclature (Fig. 2). We consequently classified this strain as a member of a new species within the genus *Selenomonas*, family *Selenomonadaceae*, phylum *Firmicutes*.

Phenotypic characteristics

Colonies were pink in colour and circular in shape, with a mean diameter of 1 mm. Bacterial cells were Gram negative

TABLE I. Phenotypic characterization of *Selenomonas felix* based on biochemical tests

Test	Result
API 50 CH	–
Control	–
Glycerol	–
Erythrol	–
D-Arabinose	–
L-Arabinose	+
D-Ribose	–
D-Xylose	+
L-Xylose	+
D-Adonitol	+
Methyl- β -D-xylopyranoside	+
D-Galactose	–
D-Glucose	+
D-Fructose	+
D-Mannose	+
L-Sorbose	+
L-Rhamnose	–
Dulcitol	+
Inositol	–
D-Mannitol	–
D-Sorbitol	–
Methyl- α -D-mannopyranoside	–
Methyl- α -D-glucopyranoside	–
N-Acetylglucosamine	–
Amygdaline	–
Arbutine	–
Esculine	–
Salicine	+
D-Cellobiose	–
D-Maltose	+
D-Lactose	+
D-Melibiose	+
D-Saccharose	+
D-Trehalose	+
Inuline	+
D-Melezitose	–

TABLE I. Continued

Test	Result
D-Raffinose	+
Amidon	+
Glycogene	+
Xylitol	–
Gentibiose	+
D-Turanose	+
D-Lyxose	+
D-Tagatose	+
D-Fucose	+
L-Fucose	+
D-Arabitol	+
L-Arabitol	+
Potassium gluconate	+
Potassium 2-cetogluconate	–
Potassium 5-cetogluconate	+
API ZYM	
Control	–
Alkaline phosphatase	–
Esterase (C4)	–
Esterase lipase (C8)	–
Lipase (C14)	–
Leucine arylamidase	–
Valine arylamidase	–
Cystine arylamidase	–
Trypsine	–
α -Chymotrypsine	–
Acid phosphatase	+
Naphthol-AS-BI-phosphohydrolase	+
α -Galactosidase	+
β -Galactosidase	+
β -Glucuronidase	–
α -Glucosidase	+
β -Glucosidase	–
N-Acetyl- β -glucosaminidase	–
α -Mannosidase	–

+, positive result; –, negative result.

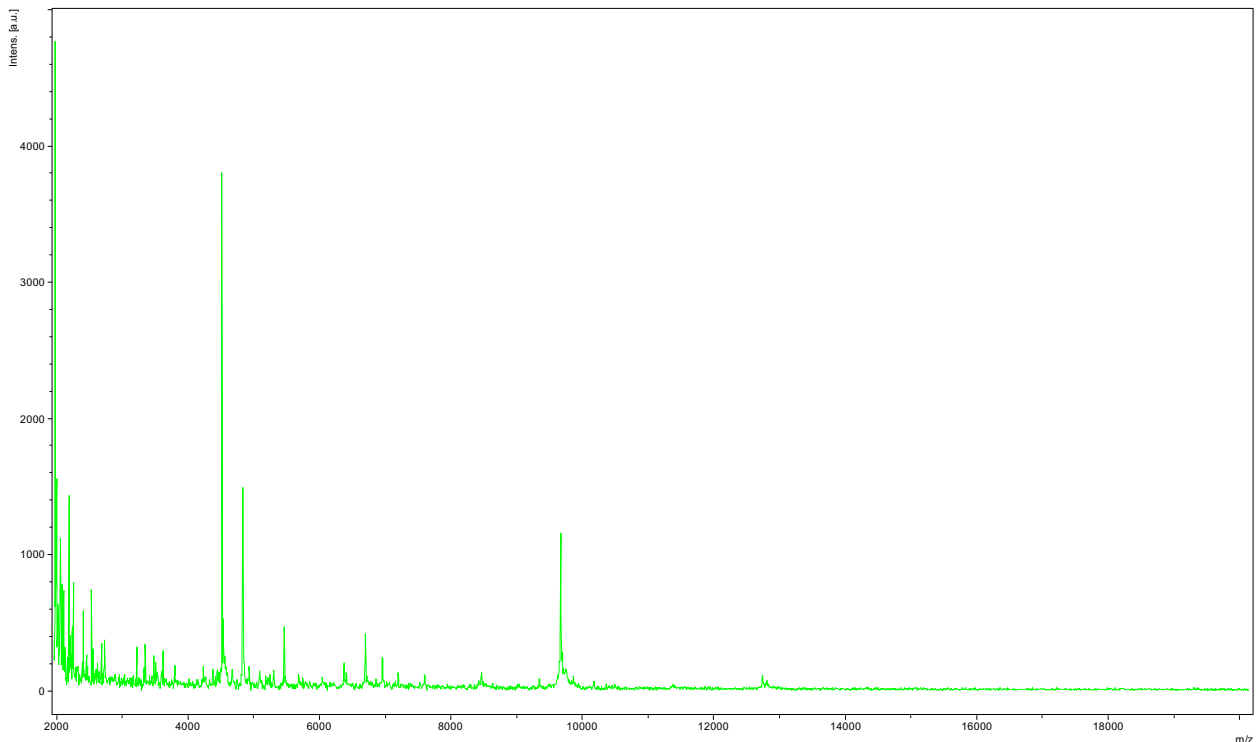
**FIG. I.** MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and reference spectrum generated.

FIG. 2. Phylogenetic tree showing position of *Selenomonas felix* strain Marseille-P3560^T relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using Muscle v3.8.31 with default parameters, and phylogenetic inferences were obtained using maximum likelihood method within MEGA 7 software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 100 times to generate majority consensus tree. Scale bar indicates 5% nucleotide sequence divergence.

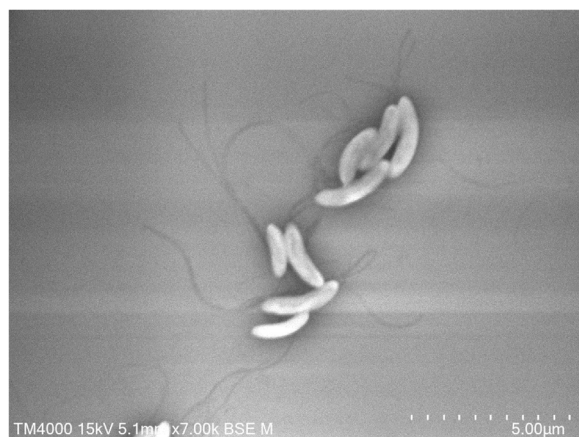
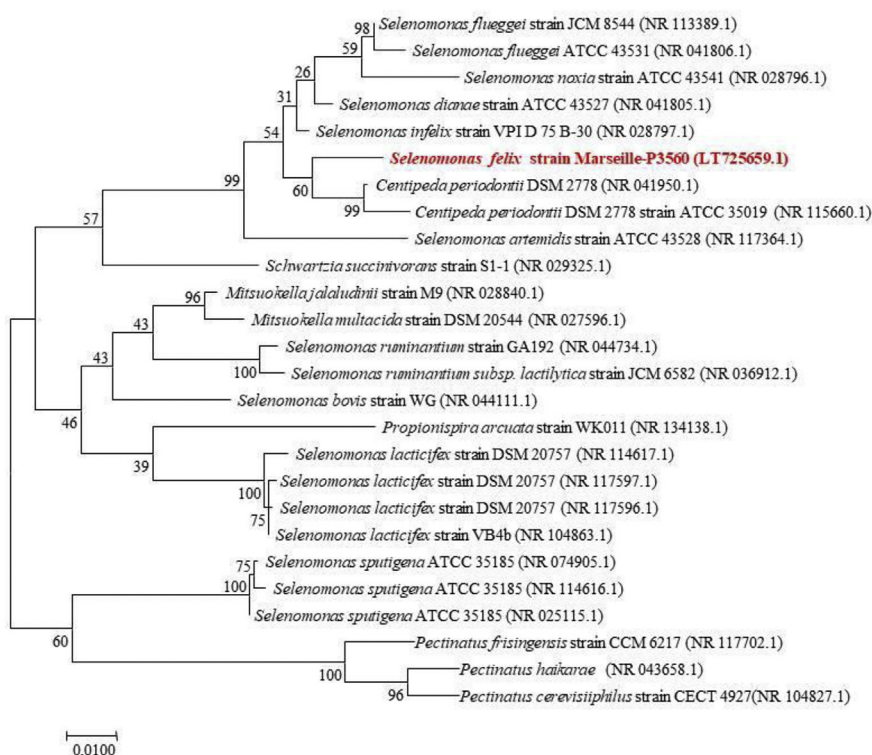


FIG. 3. Electron micrograph of *Selenomonas felix* strain Marseille-P3560^T was acquired with Hitachi TM4000Plus tabletop scanning electron microscope. Note flagella around bacteria. Scale bar and acquisition settings are detailed on micrograph.

and rod shaped; ranged in length from 2.42 to 2.49 μm and in width from 0.48 to 0.57 μm ; and were motile with multiple flagellae (Fig. 3). Strain Marseille-P3560^T showed catalase-negative and oxidase-negative activities. API 50CH and API ZYM tests were performed at 37°C under anaerobic conditions (Table 2).

TABLE 2. Description of *Selenomonas felix* according to digitalized protologue TA00880 (www.imedeaiuib.es/dprotologue)

Characteristic	Value
Taxonnumber	TA00880
Date of entry	2019-04-19
First submission date	2019-04-19
Draft number/date	003
Version	Submitted
Species name	<i>Selenomonas felix</i>
Genus name	<i>Selenomonas</i>
Specific epithet	<i>Selenomonas felix</i>
Species status	sp. nov.
Species etymology	<i>Selenomonas felix</i> sp. nov. fe'lix [pronounced fay'lix]. L. adj. <i>felix</i> , 'lucky' [referring to its presence in sputum sample of healthy person]
E-mail of corresponding author	edmondkuete@yahoo.fr
Submitter	Kuete Yimagou Edmond
E-mail of submitter	edmondkuete@yahoo.fr
Designation of type strain	Marseille-P3560 ^T
Strain collection numbers	CSURP3560
16S rRNA gene accession number	LT725659
Genome accession number [EMBL]	FYJ00000000
Data on origin of sample from which strain had been isolated	
Country of origin	France
Region of origin	Paca
Source of isolation	Sputum
Sampling date	2016-08-12
Temperature of sample	37°C
pH of sample	7.5
Source of isolation of nontype strains	Gut
Gram stain	Negative
Cell shape	Rod
If motile	Flagellar
Sporulation (resting cells)	None
Highest temperature for growth	45
Temperature optimum	37
Habitat	Human



Heatmap generated with OrthoANI values calculated from the OAT software.
Please cite Lee et al. 2015.

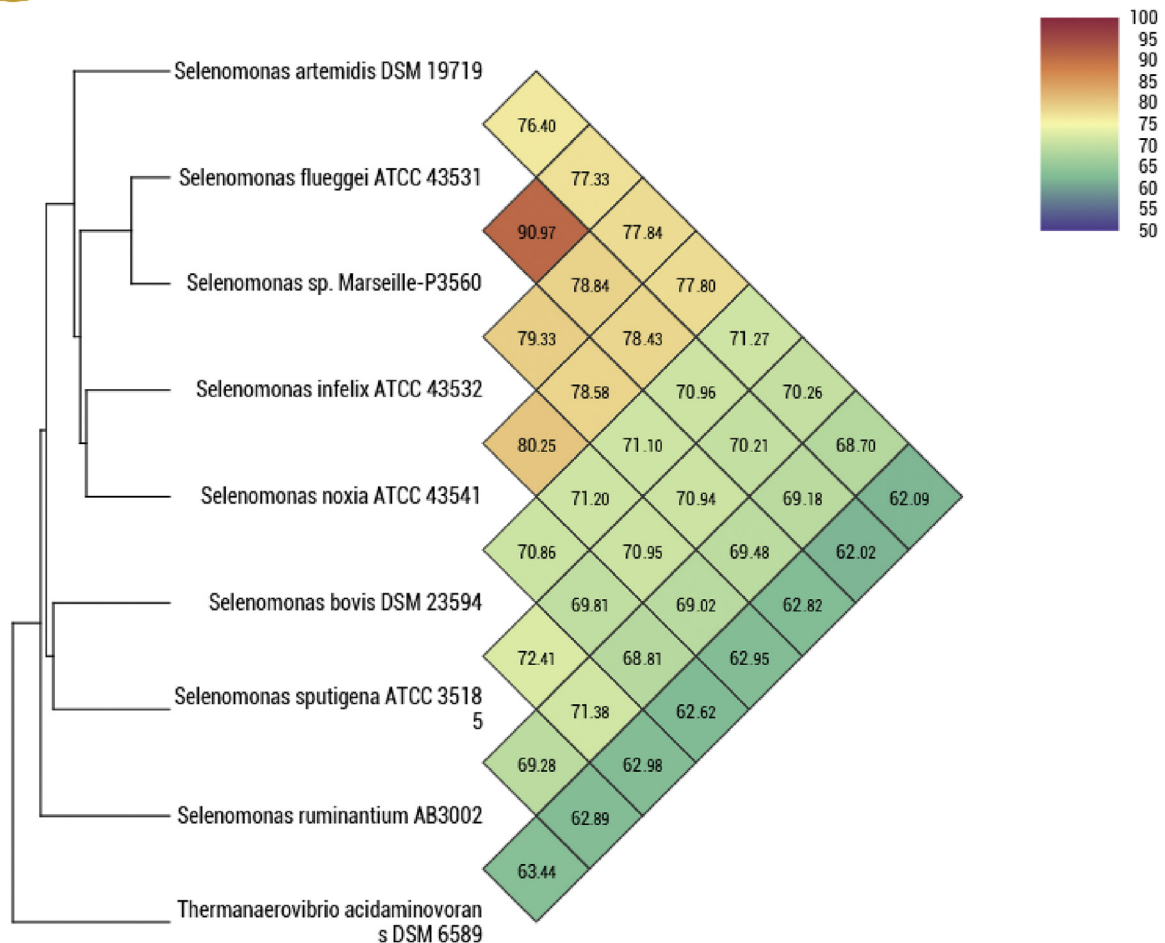


FIG. 4. Heat map generated with OrthoANI values calculated using OAT software between genus and species, and other closely related species with standing in nomenclature.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced by MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different software (Velvet [10], Spades [11] and Soap Denovo [12]) on trimmed (Trimmomatic [13]) or raw data. GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value lower than 25% of the

mean depth were removed. The best assembly was selected by using different criteria (17 scaffolds, 19 contigs).

The genome of strain Marseille-P3560^T is 2 402 833 bp long with a 56.8 mol% G+C content and contains 2393 predicted genes. The degree of genomic similarity of strain Marseille-P3560^T with closely related species was estimated by OrthoANI software [14]. Values among closely related species (Fig. 4) ranged from 62.02% between *Thermanaerovibrio acidaminovorans* and *Selenomonas flueggei* to 90.97% between Marseille-P3560^T and *Selenomonas flueggei*. When the isolate was compared to these closely species, values ranged from 62.82% with *Thermanaerovibrio acidaminovorans* to 90.97% with *Selenomonas flueggei*.

Conclusion

Strain *Selenomonas felix* exhibited a 16S rRNA sequence divergence <98.65% and an OrthoANI value < 95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. It is consequently proposed as the type strain of the new species *Selenomonas felix* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT725659 and FYCJ000000000 respectively.

Deposit in culture collections

Strain Marseille-P3560^T was deposited in the collections under number CSURP3560.

Conflict of interest

None declared.

Acknowledgements

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