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

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**Abstract**

*Selenomonas felix* strain Marseille-P3560T (=CSURP3560) is a new species isolated from human sputum. © 2019 The Author(s). Published by Elsevier Ltd.

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**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 Phylogeny and Infections (MEPHI) database; http://www.mediterraneoinfection.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.

**Strain identification**

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done by using the primer pair fD1 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 dianae* 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 1. Phenotypic characterization of Selenomonas felix based on biochemical tests

| Test                              | Result |
|-----------------------------------|--------|
| API 50 CH Control                 | −      |
| Control                           | −      |
| Glycerol                          | −      |
| Erythrol                          | −      |
| α-Arabinose                       | +      |
| β-Arabinose                       | −      |
| d-Ribose                          | +      |
| L-Ribose                          | +      |
| d-Xylose                          | +      |
| L-Xylose                          | +      |
| d-Adonitol                        | +      |
| Methyl-β-D-xylopyranoside         | +      |
| d-Galactose                       | +      |
| d-Glucose                         | +      |
| d-Fructose                        | +      |
| d-Manose                          | +      |
| L-Manose                          | +      |
| Dulcitol                          | +      |
| Inositol                          | +      |
| d-Mannitol                        | +      |
| Methyl-α-D-mannopyranoside        | −      |
| Methyl-α-D-glucopyranoside        | −      |
| N-Acetylglucosamine               | −      |
| Amygdaline                        | −      |
| Arginine                          | −      |
| Esculine                          | −      |
| Salicin                           | +      |
| d-Cellobiose                      | −      |
| d-Maltose                         | +      |
| d-Lactose                         | +      |
| d-Melibiose                       | +      |
| d-Saccharose                      | +      |
| d-Trehalose                       | +      |
| Inuline                           | +      |
| d-Melezitose                      | −      |
| d-Raffinose                       | +      |
| Aminoxydes                        | +      |
| Glycogene                         | +      |
| Xylool                            | +      |
| Gentiobiose                       | +      |
| d-Turanose                        | +      |
| d-Lyxoase                         | +      |
| α-Lyxose                          | +      |
| d-Fucose                          | +      |
| L-Fucose                          | +      |
| d-Arabinol                        | +      |
| L-Arabinol                        | +      |
| Potassium gluconate               | +      |
| Potassium 2-cetoglucurate         | −      |
| Potassium 5-cetoglucurate         | +      |
| API ZYM Control                   | −      |
| Control                           | −      |
| Alkaline phosphatase              | −      |
| Esterase (C4)                     | −      |
| Esterase lipase (C8)              | −      |
| Lipase (C14)                      | −      |
| Leucine arylationide              | −      |
| Valine arylationide               | −      |
| Tryptase                          | −      |
| α-Chymotrypsine                   | −      |
| Acid phosphatase                  | +      |
| Naphthol-AS-BI-phosphohydrolase   | +      |
| d-Galactosidase                   | +      |
| β-Galactosidase                   | +      |
| β-Glucuronidase                   | −      |
| Glucosidase                       | +      |
| β-Glucosidase                     | −      |
| N-Acetyl-β-glucosaminidase        | −      |
| d-Mannosidase                     | +      |

+, positive result; −, negative result.

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and reference spectrum generated.
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 flagella (Fig. 3). Strain Marseille-P3560T showed catalase-negative and oxidase-negative activities. API 50C and API ZYM tests were performed at 37°C under anaerobic conditions (Table 2).

![Phylogenetic tree showing position of Selenomonas felix strain Marseille-P3560T relative to other phylogenetically close neighbours.](image1)

![Electron micrograph of Selenomonas felix strain Marseille-P3560T was acquired with Hitachi TM4000Plus tabletop scanning electron microscope. Note flagella around bacteria. Scale bar and acquisition settings are detailed on micrograph.](image2)

**TABLE 2. Description of Selenomonas felix according to digitalized protologue TA00880 ([www.imedea.uib.es/dprotologue](http://www.imedea.uib.es/dprotologue))**

| Characteristic                        | Value                                                                 |
|---------------------------------------|-----------------------------------------------------------------------|
| Taxonumber                            | TA00880                                                              |
| Date of entry                         | 2019-04-19                                                           |
| First submission date                 | 2019-04-19                                                           |
| Draft number/date                     | 003                                                                  |
| Version                               | Submitted                                                            |
| Species name                          | Selenomonas felix                                                    |
| Genus name                            | Selenomonas                                                           |
| Specific epithet                      | Selenomonas felix                                                    |
| Species status                        | sp. nov.                                                             |
| Species etymology                     | Selenomonas felix sp. nov. felix’ [pronounced fay’lix]               |
| L. adj. felix, ‘lucky’ [referring to its presence in sputum sample of healthy person] |                                                       |
| E-mail of corresponding author        | edmondkuete@yahoo.fr                                                 |
| E-mail of submitter                   | Kuete Yimagou Edmond                                                 |
| Designation of type strain            | Marseilles-P3560T                                                    |
| Strain collection numbers             | C5LRP3560                                                           |
| 16S rRNA gene accession number        | LT725659                                                             |
| Genome accession number [EMBL]        | FYC000000000                                                        |
| 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| Guts                                                                |
| Gram stain                            | Negative                                                             |
| Cell shape                            | Rod                                                                  |
| If motile                             | Flagellar                                                            |
| Sporulation (resting cells)           | None                                                                 |
| Highest temperature for growth        | 45                                                                  |
| Temperature optimum                   | 37                                                                  |
| Habitat                               | Human                                                               |

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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 Termanaerovibrio 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 Termanaerovibrio acidaminovorans to 90.97% with Selenomonas flueggei.

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).

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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 FYCJ00000000 respectively.

Deposit in culture collections

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

Conflict of interest

None declared.

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