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Actinomyces bouchesdurhonensis sp. nov. and *Actinomyces mediterranea* sp. nov., isolated from human stomach and duodenum

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ABSTRACT

We propose here the main characteristics of the new bacteria *Actinomyces bouchesdurhonensis* strain Marseille-P2825 (CSUR P2825) isolated from a gastric liquid sample of a 60-year-old man and *Actinomyces mediterranea* strain Marseille-P3257 (CSUR P3257) isolated from a duodenal liquid sample of a 76-year-old woman.

Culturomics is a complementary approach to metagenomics for the study of the human microbiome [1]. Since 2012, culturomics enabled to extend the known repertoire of the gut microbiota with the characterization of more than 100 new species [2]. By applying this technique to staggered samples of the digestive tract, we isolated two bacterial strains that could not be identified using our routine identification by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [3]. Subsequently, we sequenced their 16S rRNA gene using the fD1-rP2 primers as previously described [4] and a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France).

The first of these two strains, strain Marseille-P2825, was isolated in March 2016 from a stomach lavage sample of a 60-year-old man who underwent an upper endoscopy for the etiological investigation of an iron deficiency anemia. The second strain, strain Marseille-P3257, was cultivated in May 2016, from a duodenum lavage sample of a 76-year-old woman who underwent an upper endoscopy for assessment of an esophagitis. The two patients gave signed informed consents and the ethics committee of the Institut Fédératif de Recherche IFR48 validated the study under the number 2016-010.

Initial growth of strain Marseille-P2825 was obtained after inoculation of the specimen on Columbia agar enriched with 5% of sheep blood (COS, bioMérieux, Marcy l'Etoile, France). The strain grew after three days of incubation in anaerobic atmosphere (AnaeroGen™ Compact, OXOID Ltd, Thermo Scientific®, Dardilly, France) at 37 °C. In contrast, strain Marseille-P3257 was obtained after a 7-day pre-incubation in an anaerobic blood culture bottle (BD BACTEC®, Plus Anaerobic/F Media, Le Pont de Claix, France) supplemented with 5 ml of a 0.2 µm filtered-sterilized rumen and 5 ml of sheep blood. The strain grew after subculture for two days on 5% sheep blood-enriched Columbia agar in the same conditions as described above (bioMérieux). Growth of both strains was also obtained in microaerophilic atmosphere (CampyGenTM Compact, OXOID Ltd, Thermo Scientific®, Dardilly, France) at 37 °C. Agar-grown colonies from both strains were round and translucent, with a mean diameter of 0.2 mm. Bacterial cells were Gram-positive bacilli, ranging in length and width from 500 to 800 nm for both, and from 1300 to 1700 nm for strain Marseille-P2825 and from 1300 to 2700 nm for strain Marseille-P3257, respectively. The two strains were neither motile nor endospore forming. They exhibited no catalase and no oxidase activities.

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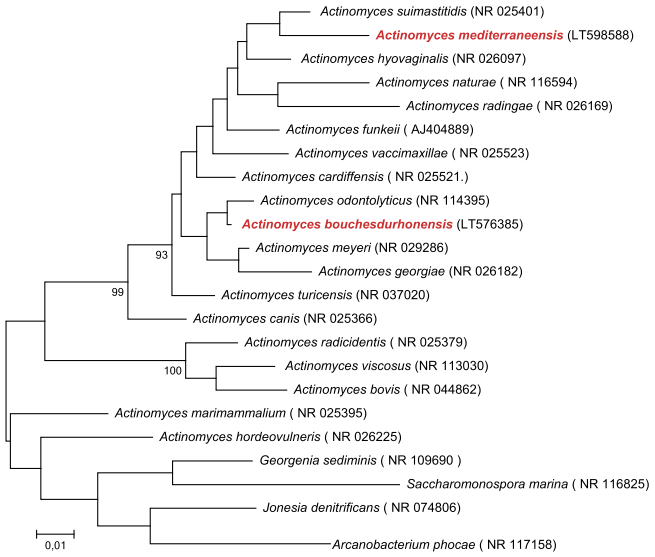

Fig. 1. Phylogenetic tree showing the position of *A. bouchesdurhonensis* sp. nov., strain Marseille-P2825^T and *A. mediterranea* sp. nov., strain Marseille-P3257^T relatives to other phylogenetically-close neighbours. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic inferences were obtained using the neighbor-joining method with 500 bootstrap replicates, within the MEGA6 software. Only bootstrap values greater than 95% are displayed. The scale bar represents a 1% nucleotide sequence divergence.

Strain Marseille-P2825 exhibited a 98.3% sequence similarity with *Actinomyces odontolyticus* strain ATCC 17929^T (Genbank accession number AJ234040), the phylogenetically closest species with standing in nomenclature [5]. Strain Marseille-P3257 exhibited a 93.9% sequence identity with *Actinomyces hyovaginalis* strain ATCC 51367^T (X69616). As the minimum 16S rRNA sequence similarity observed among *Actinomyces* species genera is 85.0% [6], the similarity values that we obtained putatively classify them as new species within this genus (Fig. 1). The genus *Actinomyces* was discovered in 1877 [7] and currently contains 47 species with validly published names.

We thus propose the creation of two new species: *Actinomyces bouchesdurhonensis* sp. nov. (bou.ches.du.rho.nen'sis, N.L. masc. adj. *Bouchesdurhonensis* pertaining to Bouches–du-Rhône, the department in Southern France) and *Actinomyces mediterranea* sp. nov. (me.di.ter.ra'ne.a, L. fem. adj. *Mediterranea* of Mediterranea, the Latin name of the Mediterranean sea). Strain Marseille-P2825^T is the type strain of *A. bouchesdurhonensis* sp. nov. and strain Marseille-P3257^T is the type strain of *A. mediterranea* sp. nov.

The MALDI-TOF Spectrum of *A. bouchesdurhonensis* strain Marseille-P2825^T and *A. mediterranea* strain Marseille-P3257^T are available at http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database.

Nucleotide sequence accession number

The 16S r RNA gene sequences of strains Marseille-P2825^T and Marseille-P3257^T were deposited in Genbank under Accession numbers LT576385 and LT598588, respectively.

Deposit in a culture collection

Strain Marseille-P2825^T and strain Marseille-P3257^T were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under numbers P2825 and P3257, respectively.

Conflict of interest

None to declare.

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