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Lysinibacillus timonensis sp. nov., *Microbacterium timonense* sp. nov., and *Erwinia mediterraneensis* sp. nov., three new species isolated from the human skin

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Abstract

Lysinibacillus timonensis strain Marseille-P5727^T (=CSURP5727), *Microbacterium timonense* strain Marseille-P5731^T (=CSURP5731) and *Erwinia mediterraneensis* strain Marseille-P5165^T (=CSURP5165) are three new species isolated from the human skin.

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Introduction

The skin is the biggest protective organ in humans. However, the cutaneous microbiota is involved in many skin diseases and plays a role in wound infections [1]. The majority of bacteria colonizing human skin belong to three phyla: *Firmicutes*, *Actinobacteria* and *Proteobacteria* [2].

As part of a culturomics study of the human microbiota [3,4], we isolated from skin samples of three different healthy Senegalese individuals, three new bacteria that could not be identified by our systematic matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany). Each of the three species has been described according to their following main phenotypic description, phylogenetic analysis and genome sequencing [5,6].

Isolation and growth conditions

In 2017, we isolated three unidentified bacterial strains from the skin swabs of an 18-year-old man, a 35-year-old man living in Ndiop and a 15-year-old girl living in Dielmo. The study was validated by the ethics committee of Senegal (No. 53/MSAS/DPRS/CNERS on 31 March 2015). A screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics) as previously described [7]. The spectra obtained (Fig. 1) were imported into MALDI BIOTYPYER 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with MEPHI database). The initial growth of *Lysinibacillus timonensis* sp. nov., strain Marseille-P5727, was obtained after 24 h of culture on a Chapman–mannitol salt agar (BioMérieux, Marcy l'Etoile, France) in aerobic conditions at 37°C and pH 7.4. *Microbacterium timonense* sp. nov., strain Marseille-P5731 and *Erwinia mediterraneensis* sp. nov., strain Marseille-P5165 both grew on Columbia Colistin and Nalidixic Acid agar supplemented with 5% sheep blood (BioMérieux) under aerobic conditions at 37°C with pH 7.3.

Strain identification

The 16S rRNA gene was sequenced to classify these bacteria. Amplified by using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer

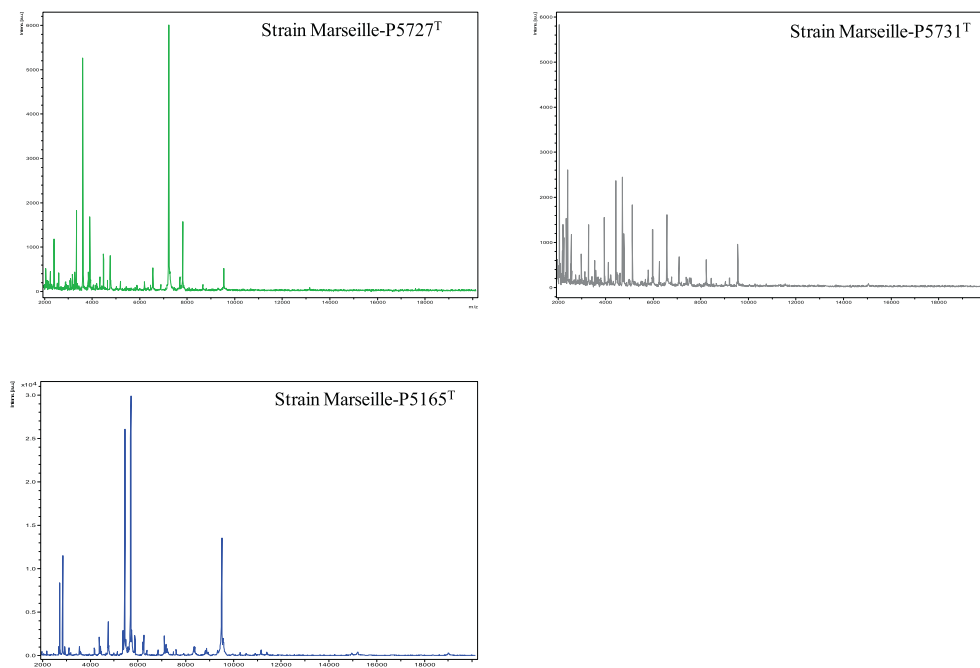


FIG. 1. MALDI-TOF MS reference spectrum of the three new species described above. The reference spectra were generated by comparison of spectra from 12 individual colonies for each species.

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 Marseille-P5727^T exhibited a 95.47% sequence identity with *Lysinibacillus endophyticus* strain C9 (GenBank Accession number NR_146821.1), the phylogenetically closest species with standing in nomenclature. Strain Marseille-P5731^T exhibited a 98.08% sequence identity with *Microbacterium hominis* strain DSM 12509 (GenBank Accession number: NR_042480.1), the phylogenetically closest species with standing in nomenclature. Strain Marseille-P5165^T exhibited a 97.46% sequence identity with *Erwinia iniecta* strain B120 (GenBank Accession number NR_137333.1), the phylogenetically closest species with standing in nomenclature. Based on the 16S rRNA sequences, we consequently rank the strains as members of new species within the following three phyla: *Proteobacteria*, *Actinobacteria* and *Firmicutes* (Fig. 2).

Phenotypic characteristics

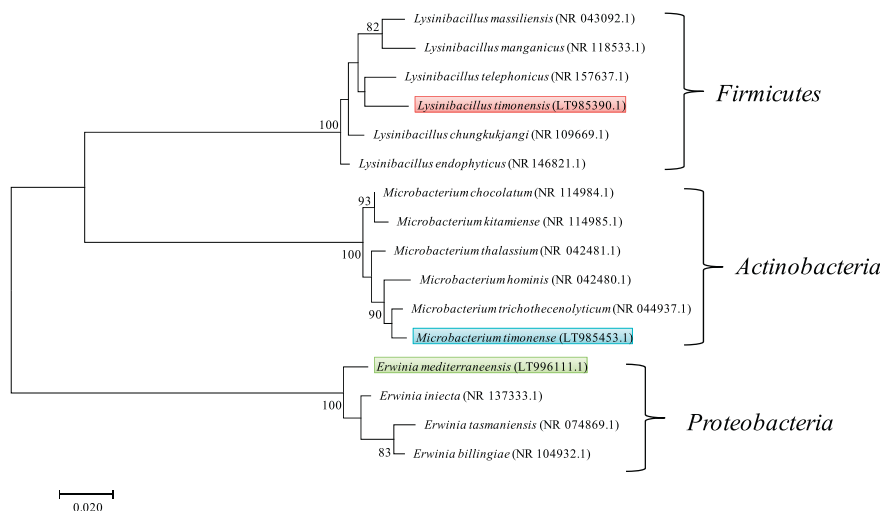
Lysinibacillus timonensis strain Marseille-P5727^T presents opaque colonies with a greyish colour. Bacterial cells were Gram-negative, non-motile, spore-forming, club-shaped rods, 3.74 μm long and 0.61 μm wide. Strain Marseille-P5727^T showed catalase-positive and oxidase-negative activities. *Microbacterium timonense* strain Marseille-P5731^T has grey colonies with irregular edges, creamy. Bacterial cells were Gram-negative,

motile and measured 1.25 μm in length and 0.53 μm in width. Strain Marseille-P5731^T showed catalase activity, but not oxidase activity. *Erwinia mediterraneensis* strain Marseille-P5165^T presented colonies with a smooth surface and regular edges. Cells stained as Gram-negative. They are motile and measure means of 1.98 μm long and 0.62 μm wide. Strain Marseille-P5165^T is catalase-positive and oxidase-positive (Fig. 3).

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA Tissue Kit and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired end (Illumina), as previously described [9]. Assembly was performed with a pipeline incorporating different softwares (VELVET [10], SPADES [11] and SOAP DENOVO [12]), and trimmed data (MISEQ and TRIMMOMATIC [13] softwares) or untrimmed data (only MISEQ software). GAPCLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value <25% of the mean depth were removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N). The degree of genomic similarity of these three strains with closely related species was estimated using ORTHOANI software [14]. The genome of strain Marseille-P5727^T was 4 220 229 bp long with a 35.7 mol% G+C content. ORTHOANI values among closely related species ranged from 70.55% between *Lysinibacillus manganicus* and *Lysinibacillus*

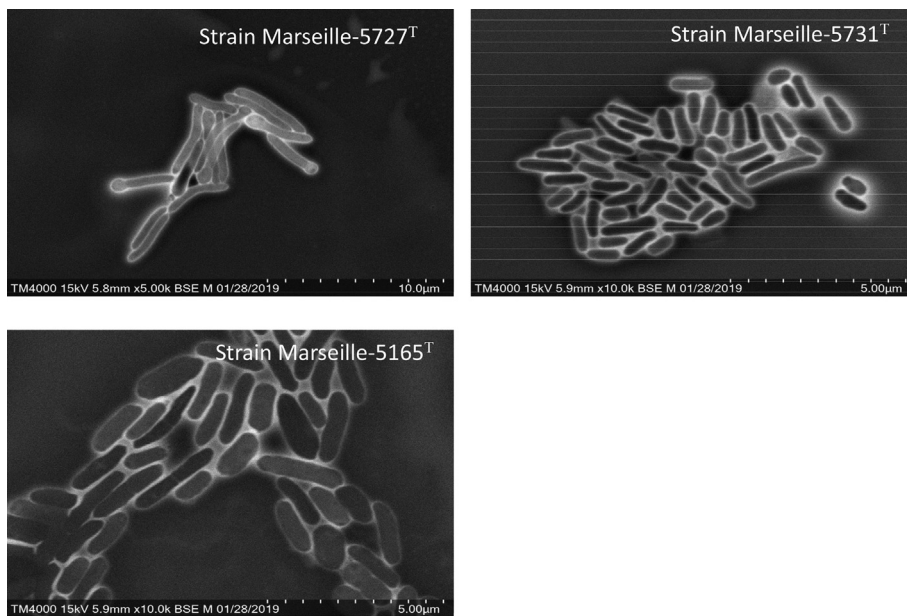
FIG. 2. Phylogenetic tree showing the position of *Lysinibacillus timonensis* strain Marseille-CSURP5727^T, *Microbacterium timonense* strain Marseille-CSURP5731^T and *Erwinia mediterraneensis* strain Marseille-CSURP5165^T relative to other phylogenetically closest species. The respective GenBank Accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 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 2% nucleotide sequence divergence.



odysseyi to 84.36% between *Lysinibacillus boronitolerans* and *Lysinibacillus fusiformis*. When *Lysinibacillus timonensis* was compared with these closely related species, values ranged from 71.21% with *Microbacterium boronitolerans* to 77.78% with *Lysinibacillus massiliensis*.

The genome of strain Marseille-P5731^T was 3 961 226 bp long with a 70.1 mol% G+C content. ORTHOANI values among closely related species ranged from 65.81% between *Microbacterium hominis* and *Microbacterium oleivorans* to 83.27% between *Microbacterium timonense* and *Microbacterium*

FIG. 3. Electron micrograph of *Lysinibacillus timonense* Strain Marseille-P5727^T, *Microbacterium timonense* Strain Marseille-P5731^T, *Erwinia mediterraneensis* Strain Marseille-P5165^T was acquired with a Hitachi TM4000Plus tabletop scanning electron microscope (SEM). A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000). Scales and acquisition settings are shown in the figure.



trichothecenolyticum. When *Microbacterium timonense* was compared with these closely related species, values ranged from 73.74% with *Microbacterium oleivorans* to 75.31% with *Microbacterium hydrocarbonoxydans*.

The genome of strain Marseille-P5165^T was 4 275 595 bp long with a 54.9% G+C content. ORTHOANI values among closely related species ranged from 62.76% between *Erwinia insecta* and *Erwinia oleae* to 79.34% between *Pantoea agglomerans* and *Pantoea cyripedii*. When *Erwinia mediterraneensis* was compared with these closely related species, values ranged from 74.94% with *Erwinia oleae* to 79.02% with *Pantoea agglomerans* (Fig. 4).

Conclusion

Based on the results for unique phenotypic characteristics, including MALDI-TOF spectrum, a 16S rRNA sequence divergence >1.3% and an ORTHOANI value <95% with the phylogenetically closest species with standing in nomenclature, we formally proposed strains Marseille-P5727^T, Marseille-P5731^T and Marseille-P5165^T, respectively, as being the type strains of *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., and *Erwinia mediterraneensis* sp. nov., which are new species in the Bacteria domain.

Description of *Lysinibacillus timonensis* sp. nov.

Lysinibacillus timonensis (ti.mon.en'sis. N.L. masc. adj. *timonensis* the name of quarter La Timone where the strain was isolated). Isolated from the palm of the hand of a healthy person living in rural Senegal. *Lysinibacillus timonensis* is Gram-negative, aerobic and spore-forming; it is a club-shaped rod and showed catalase-positive and oxidase-negative activities. The strain develops readily on Chapman–mannitol salt agar with aerobic and non-mobile cells with a mean length of 3.74 µm and a mean width of 0.61 µm. The G+C content of the genome is 70.1mol%. The 16S rRNA and genome sequences of *L. timonensis* strain Marseille-P5727^T (CSURP5727) are deposited in GenBank under Accession numbers LT985390 and OLMT00000000, respectively.

Description of *Microbacterium timonense* sp. nov.

Microbacterium timonense (ti.mo.nen'se N.L. neut. adj. *timonense*, pertaining to La Timone the name of the hospital in Marseille, France, where the first strains were isolated). Isolated from the palm of the hand of healthy person living in rural Senegal, *M. timonense* strain Marseille-P5731^T is Gram-negative, non-spore-forming and showed catalase activity, but not oxidase activity. The strain develops readily on 5% Columbia agar enriched with sheep blood with aerobic and mobile cells with a mean length of 1.25 µm and a mean width of 0.53 µm. The G+C content of the genome is 70.1%. The 16S rRNA and genome

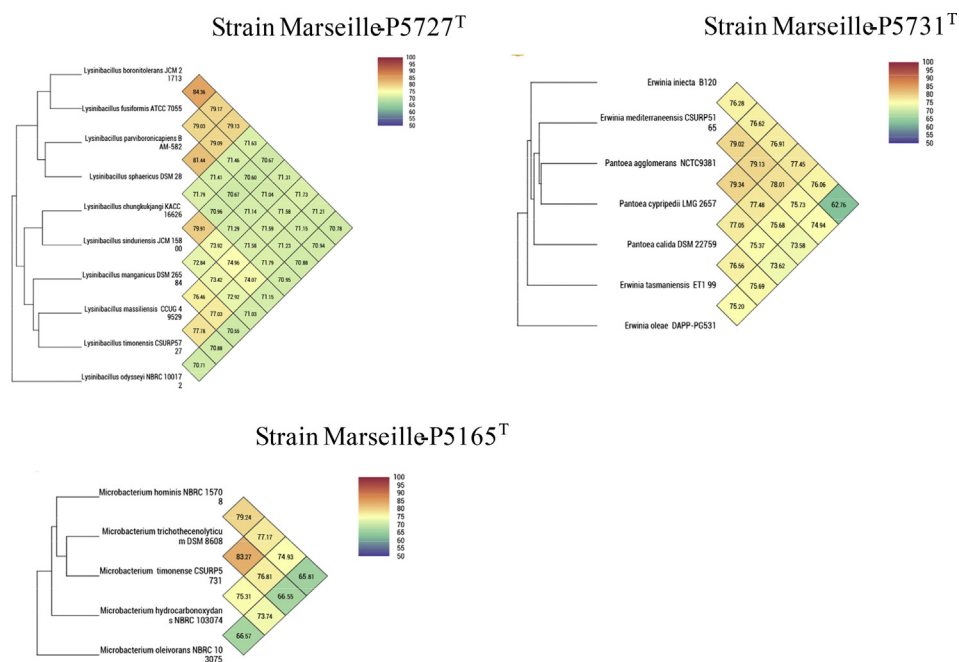


FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., *Erwinia mediterraneensis* sp. nov. and other closely related species with standing in nomenclature.

sequences of *M. timonense* strain Marseille-P5731^T (CSURP5731) are deposited in GenBank under Accession numbers LT985453 and OLMV00000000, respectively.

Description of *Erwinia mediterraneensis* sp. nov.

Erwinia mediterraneensis (me.di.ter.ra.ne.en' sis, L. masc. adj., *mediterraneensis* of Medi-terraneum, the Latin name of the Mediterranean Sea by which Marseille is located, and where strain P5165 was isolated). They are Gram-negative, motile and have mean length of 1.98 µm and mean width of 0.62 µm. Strain Marseille-P5165^T shows catalase and oxidase activities. The strain develops readily on 5% Columbia agar enriched with sheep blood. The G+C content of the genome is 54.9%. The 16S rRNA and genome sequences of *E. mediterraneensis* strain Marseille-P5165^T (CSURP5165) are deposited in GenBank under accession numbers LT996111 and UWOB00000000, respectively.

Nucleotide sequence accession number. The 16S rRNA gene and genome sequences of *Lysinibacillus timonensis* were deposited in GenBank under Accession numbers LT985390 and OLMT00000000, respectively. The 16S rRNA gene and genome sequences of *Microbacterium timonense* were deposited in GenBank under Accession numbers LT985453 and OLMV00000000, respectively. The 16S rRNA gene and genome sequences of *Erwinia mediterraneensis* were deposited in GenBank under Accession numbers LT996111 and UWOB00000000, respectively.

Deposit in culture collection. Strain Marseille-P5727^T, strain Marseille-P5731^T and strain Marseille-P5165^T were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) under the following numbers, respectively: CSURP5727, CSURP5731 and CSURP5165.

Conflict of interest

None to declare.

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Ethics and consent

The study and consent procedures were approved by the Senegalese Comité National d'Ethique pour la Recherche en Santé, ethics committee in accordance with the SEN protocol No. 53/MSAS/DPRS/CNERS on 31 March 2015 as well as by the ethics committee of the Institut Hospitalo-Universitaire Méditerranée Infection. The volunteers gave written consent.

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