Corynebacterium senegalense sp. nov. and *Arthrobacter senegalensis* sp. nov., two new *Actinobacteria* isolated from skin swab from the palm of hand

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Abstract

Corynebacterium senegalense strain Marseille-P4329^T (= CSURP4329) and *Arthrobacter senegalensis* strain Marseille-P4329^T (= CSURP4198) are new species first isolated from human skin. A culturomics approach and taxonogenomics methods were used for these new bacterial species.

Keywords: *Arthrobacter senegalensis* sp. nov., *Corynebacterium senegalense* sp. nov., culturomics, human skin, taxonogenomics
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Introduction

The skin microbiota has great importance for human health. It is involved in many cutaneous diseases and plays a vital role in wound infections [1]. Most bacteria living on the skin belong to three phyla: *Actinobacteria*, *Firmicutes* and *Proteobacteria* [2]. *Actinobacteria* phylum regroup most of bacterial species located in different sites on the skin [3]. The genus *Arthrobacter* is known as a member of the skin flora, moreover *Arthrobacter mysorens* has been implicated in localized skin infection [4]. In contrast, the genus *Corynebacterium* is a commensal of the skin and is one of the most commonly isolated *Actinobacteria* [4,5].

Implications of bacterial diversity in normal physiological functions and susceptibility to diseases has become a crucial topic to explore [6]. In our laboratory we adopt a new approach called culturomics that isolates bacteria under various culture conditions while associating identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and 16S rRNA amplicon sequencing to study the diversity of human bacteria [7–9]. The new species that we present here—*Corynebacterium senegalense* sp. nov. and *Arthrobacter senegalensis* sp. nov.—have been described using a combination of genotypic and phenotypic characteristics, following a taxonogenomic strategy previously described [10,11].

Isolation and growth conditions

In 2017, unidentified bacterial strains were isolated from the palm of hand of two healthy persons living in Ndiop, in rural Senegal. The study was validated by the ethics committee of Senegal (No. 53/MSAS/DPRS/CNERS du 31 mars 2015). Initial growth of *Corynebacterium senegalense* and *Arthrobacter senegalensis* was obtained after 24 h of incubation at 37°C on Columbia Colistin and Nalidixic Acid +5% sheep blood (BioMérieux, Marcy l'Etoile, France) under aerobic conditions at pH 7.3. Samples were screened using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [12]. 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 MEPHI database).

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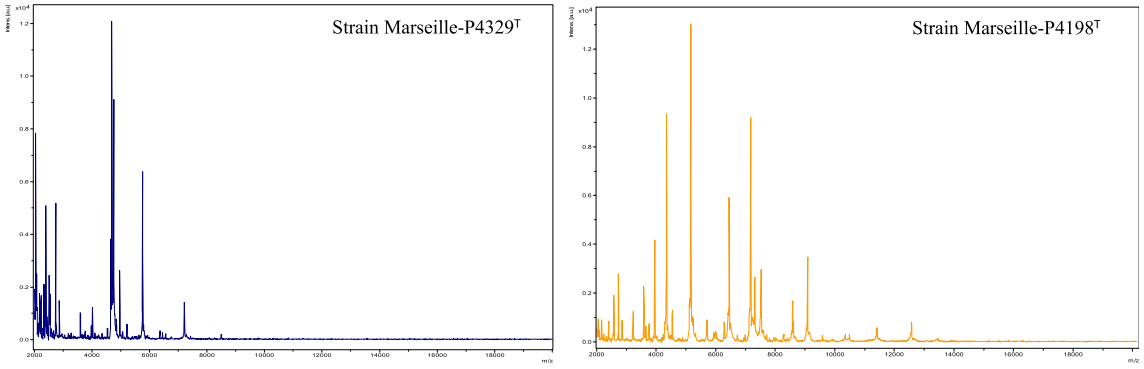

FIG. 1. MALDI-TOF MS reference spectrum of the two new species described. The reference spectra were generated by comparison of spectra from 12 individual colonies for each species.

Strain identification

To classify these bacteria, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [13]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P4329^T exhibited a 97.56% sequence identity with *Corynebacterium lipophiloflavum* strain DSM 44291 (GenBank Accession number: NR_026370.1), the phylogenetically closest species with standing in nomenclature (Fig. 2a). However, the strain Marseille-P4198^T exhibited a 98.10% sequence identity with *Arthrobacter crystallopoietes* strain DSM 20117 (GenBank Accession number: NR_026189.1), the phylogenetically closest species with standing in nomenclature (Fig. 2b). We consequently classify these strains as members of new species within the phylum *Actinobacteria*.

Phenotypic characteristics

Corynebacterium senegalense strain Marseille-P4329^T colonies were yellow, circular and shiny with a smooth surface. Bacterial cells were Gram-positive, non-motile, non-spore-forming, club-shaped rods, 0.9 µm long and 0.6 µm wide. Strain Marseille-P4329^T showed catalase-positive and oxidase-negative activities. *Arthrobacter senegalensis* strain Marseille-P4198^T colonies were grey with irregular edges. Bacterial cells were Gram-negative, motile, 1.2 µm long and 0.5 µm wide. Strain Marseille-P4198^T showed catalase-positive and oxidase-negative activities (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 [14]. The assembly was performed with a pipeline incorporating different softwares (Velvet [15], Spades [16] and Soap Denovo [17], on trimmed data (MiSeq and Trimmomatic [18] 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 by using different criteria (number of scaffolds, N50, number of N). The degree of genomic similarity of these two strains with their closely related species was estimated using OrthoANI software [19]. The genome strain Marseille-P4329^T is 2 311 841 bp long with 52.8% G+C content. OrthoANI values among closely related species ranged from 68.48% between *Corynebacterium terpenotabidum* and *Corynebacterium casei* to 82.08% between *Corynebacterium senegalense* and *Corynebacterium timonense*. When *Corynebacterium senegalense* was compared with these closely related species, values ranged from 69.61% with *Corynebacterium casei* to 82.08% with *Corynebacterium timonense* .

The genome of strain Marseille-P4198^T had a length of 4 027 190 bp with 66.1% G+C content. OrthoANI values among closely related species ranged from 64.80% between

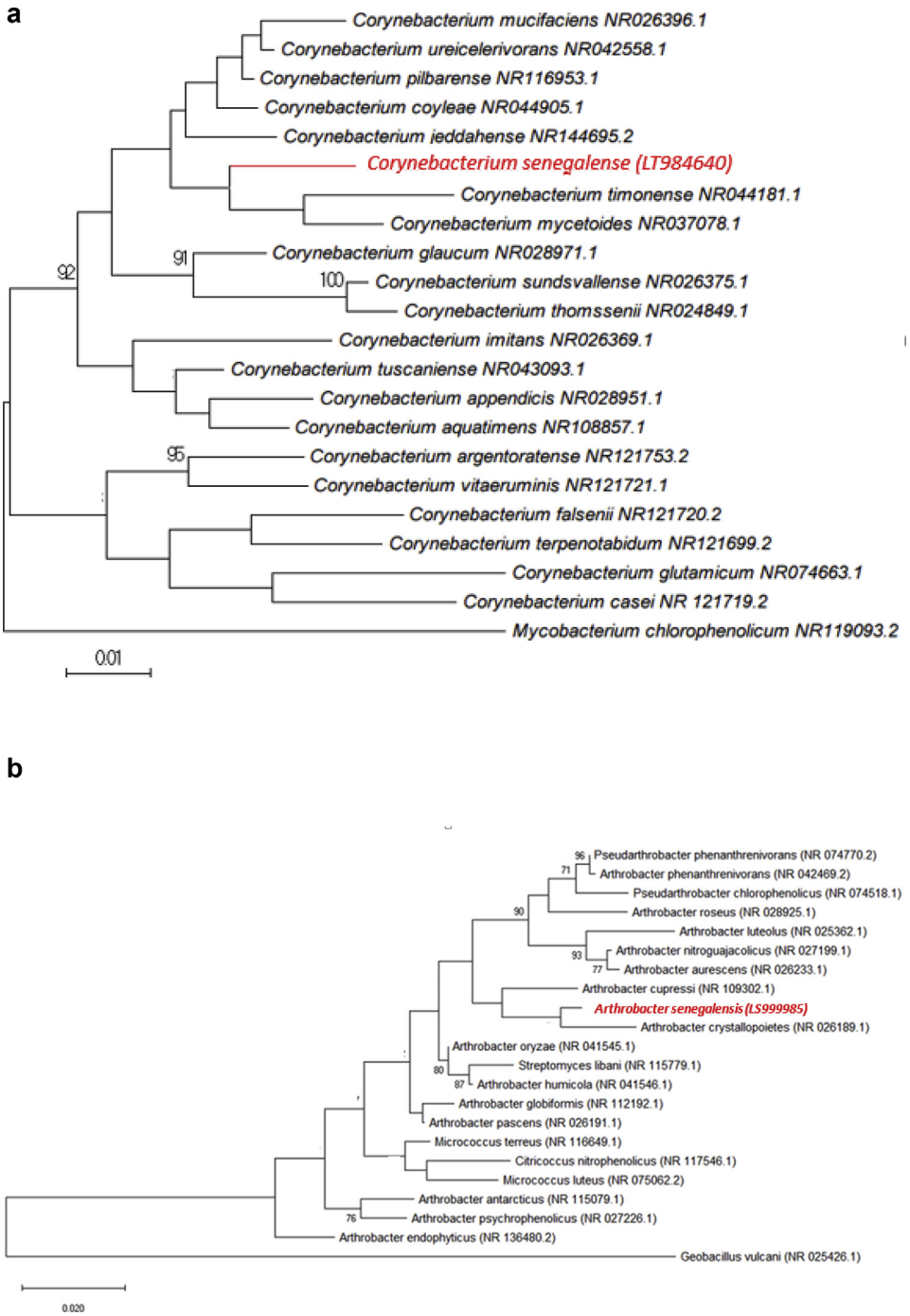

FIG. 2. (a) Phylogenetic tree showing the position of *Corynebacterium senegalense* strain Marseille-CSURP4329^T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with 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 500 times to generate a majority consensus tree. The scale bar indicates a 1% nucleotide sequence divergence. (b) Phylogenetic tree showing the position of *Arthrobacter senegalensis* strain Marseille-CSURP4198^T relative to other phylogenetically close neighbours. 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.

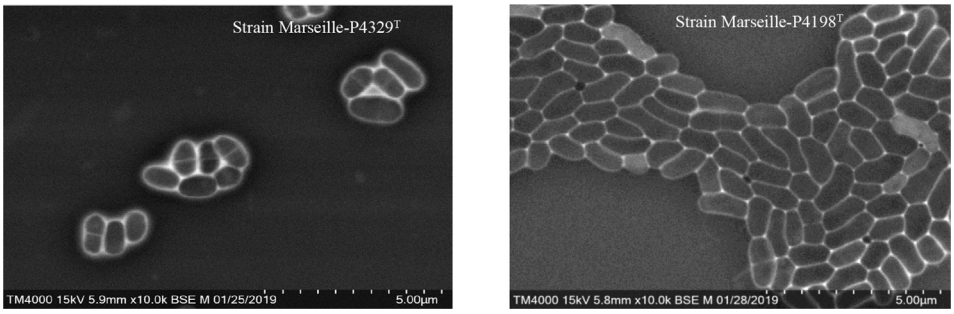

FIG. 3. Electron micrographs of *Corynebacterium senegalense* Strain Marseille-P4329^T *Arthrobacter senegalensis* strain Marseille-P4198^T were acquired with a Hitachi TM4000Plus tabletop scanning electron microscope (SEM). A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes 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) approximately 60 cm in height and 33 cm in width to evaluate bacterial structure. Scales and acquisition settings are shown in the figure.

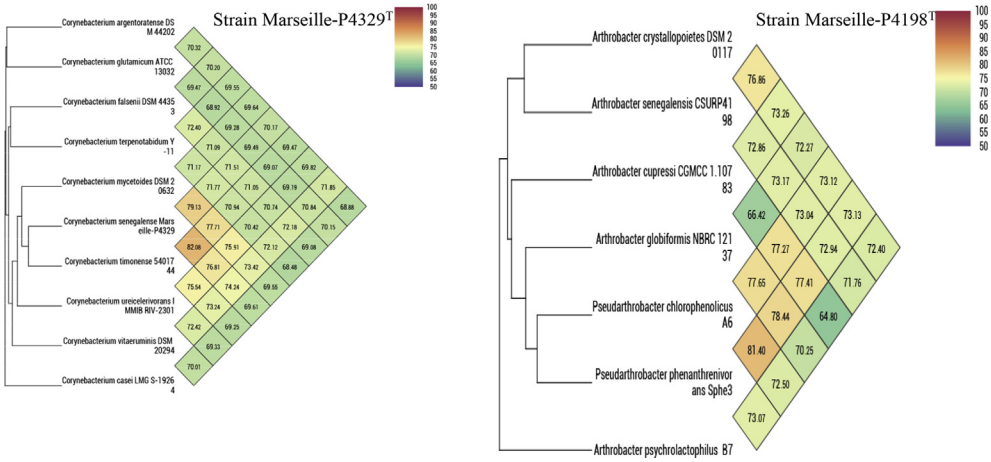

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Corynebacterium senegalense* sp. nov., *Arthrobacter senegalensis* sp. nov. and other closely related species with standing in nomenclature.

Arthrobacter cupressi and *Arthrobacter psychrolactophilus* to 81.40% between *Pseudoarthrobacter chlorophenolicus* and *Pseudoarthrobacter phenanthrenivorans*. When *Arthrobacter senegalensis* was compared with these closely related species, values ranged from 71.76% with *Arthrobacter psychrolactophilus* to 76.86% with *Arthrobacter crystallopoietes* (Fig. 4).

Conclusion

Strain Marseille-P4329^T and strain Marseille-P4198^T exhibited 16S rRNA sequence divergence >1.3% and OrthoANI values < 95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. Based on these results, we consequently proposed the strains Marseille-P4329^T and Marseille-P4198^T as, respectively, the type strains of *Corynebacterium senegalense* sp. nov. and *Arthrobacter senegalensis* sp. nov.

Description of *Corynebacterium senegalense* sp. nov.

Corynebacterium senegalense (se.ne.ga.len'se, L. fem. adj. *senegalense* related to Senegal, the name of the country where the sample was collected). Isolated from the palm of the hand of a healthy person living in rural Senegal. *Corynebacterium senegalense* is a Gram-positive, aerobic, non-spore-forming, club-shaped rod and showed catalase-positive and oxidase-negative activities.

The strain develops readily on Columbia agar enriched with 5% sheep blood with aerobic and non-mobile cells with a mean length of 0.965 µm and a mean width of 0.654 µm. The G+C content of the genome is 52.8%. The 16S rRNA and genome sequences of *C. senegalense* strain Marseille-P4329^T (CSURP4329) are deposited in GenBank under Accession numbers LT984640 and OVSI00000000, respectively.

Description of *Arthrobacter senegalensis* sp. nov.

Arthrobacter senegalensis (se.ne.ga.len'sis, L. masc. adj. *senegalensis* related to Senegal, the name of the country where the sample was collected). Isolated from the palm of the hand of a healthy person living in rural Senegal. *Arthrobacter senegalensis* is Gram-negative, aerobic, non-spore-forming, with irregular edges, and showed catalase-positive and oxidase-negative activities.

The strain develops readily on Columbia agar enriched with 5% sheep blood with aerobic and non-mobile cells with a mean length of 1.250 µm and a mean width of 0.530 µm. The G+C content of the genome is 66.1%. The 16S rRNA and genome sequences of *A. senegalensis* strain Marseille-P4198^T (CSURP4198) are deposited in GenBank under Accession numbers LS999985 and PRJEB28787, respectively.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences of *Corynebacterium senegalense* sp. nov. were deposited in GenBank under Accession numbers LT984640 and OVSI00000000, respectively.

The 16S rRNA gene and genome sequences of *Arthrobacter senegalensis* sp. nov. were deposited in Genbank under accession number LS999985 and PRJEB28787 respectively.

Deposit in culture collection

Strain Marseille-P4329^T and Strain Marseille-P4198^T were deposited in the CSUR collection under the numbers (= CSURP4329) and (= CSURP4198), respectively.

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Conflicts 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 14/30 under number 00053. All the individuals includes in this study and living in Dielmo and Ndiop gave a written consent.

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