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# Description of *Acinetobacter ihumii* sp. nov., *Microbacterium ihumii* sp. nov., and *Gulosibacter massiliensis* sp. nov., three new bacteria isolated from human blood

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## Abstract

Blood is precious tissue that is normally sterile. With the aim of diagnosing the cause of bacteremia, three bacterial strains were isolated from three different individuals. Strains Marseille-P7157$^T$ and Marseille-Q2854$^T$ are Gram-stain positive, non-spore-forming rod-shaped bacteria, while strain Marseille-P8049$^T$ is a Gram-stain negative, motile, non-spore-forming and rod-shaped bacterium. The major fatty acids found (>30%) were hexadecanoic acid for strain Marseille-P8049$^T$ and 12-methyl tetradecanoic acid for both strains Marseille-P7157$^T$ and Marseille-Q2854$^T$. The 16S rRNA gene sequence analysis shows that strains Marseille-P8049 and Marseille-Q2854$^T$ have sequence similarity of 96.8%, 99.04%, and 98.3% with *Acinetobacter ursingii* strain LUH3792 (NR_025392.1), *Gulosibacter faecalis* strain B187 (NR_041812.1), and *Schaalia canis* strain CCUG 41706 (NR_025366.1), respectively. In addition, strains Marseille-Q2854$^T$, Marseille-P8049$^T$ and Marseille-P7157$^T$ shared with their closely related species cited above the following DDH values: 19.5%, 24.4%, and 20.2%, respectively. Based on these phenotypic and genomic findings, we consider that strains Marseille-P8049$^T$ (= CSUR P8049 = CECT 30350), Marseille-P2854$^T$ ( = CSUR Q2854 = CECT 30120) and Marseille-P7157$^T$ ( = CSUR P7157 = CECT 30048) are new bacterial species, for which the names *Acinetobacter ihumii* sp. nov., *Microbacterium ihumii* sp. nov., and *Gulosibacter massiliensis* sp. nov., are proposed.

**Keywords:** *Acinetobacter ihumii*; *Microbacterium ihumii*; *Gulosibacter massiliensis*, new species, human blood, bacteria

## Introduction

Blood is a transport fluid distributed by the heart to all parts of the body, from where it is returned to the heart to repeat the process (Bill 1975, Rosell and Belfrage 1979). Blood is normally sterile. The presence of bacteria in the blood indicates bacteremia. In an effort to establish the etiological diagnosis of bacteremia, three new species belonging to the genera *Acinetobacter*, *Gulosibacter,* and *Microbacterium* were isolated.

Members of the genus *Acinetobacter* are non-fastidious Gram-stain negative coccobacilli belonging to the phylum *Pseudomonadota* (Oren and Garrity 2021). *Acinetobacter* was first described from soil on a calcium acetate-mineral medium as *Micrococcus calcoaceticus* (Beijerinck 1910), before becoming known as *Acinetobacter* in the 1950s (Brisou and Prevot 1954, Baumann *et al.* 1968). The natural habitats of *Acinetobacter* are soil and water. In humans, the genus *Acinetobacter* is described as a serious cause of nosocomial infections (Bergogne-Bérézin *et al.* 1996, Wolff 1996).

The genus *Microbacterium* is non-fastidious Gram-stain positive, rod-shaped, non-spore-forming bacterium belonging to the phylum *Actinomycetota* (Oren and Garrity 2022). The genus *Microbacterium* was proposed in 1919 (Orla-Jensen 1919), and first described in 1983 (Collins *et al.* 1983). In 1998, the genus was reclassified with the unification of the genera *Microbacterium* and *Aureobacterium* (Takeuchi and Hatano 1998). Members of the genus *Microbacterium* have been isolated from a wide range of specimens, including human clinical samples.

The genus *Gulosibacter* belongs to the phylum *Actinomycetota* (Oren and Garrity 2022), the class *Actinomycetia*, the order *Micrococcales* as well as the family *Microbacteriaceae*. Members of this genus are Gram-stain positive, short rod-shaped, non-spore-forming, non-motile, aerobic, catalase-positive, and oxidase-positive bacteria. It was first described in 2004 (Manaia *et al.* 2004). Currently, the genus *Gulosibacter* contains six species validly published (Parte *et al.* 2020).

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Herein, we report three strains: Marseille-Q2854$^T$, Marseille-P8049$^T$, and Marseille-P7157$^T$, isolated from blood samples of patients diagnosed in the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection. We use a taxonogenomics approach (Ramasamy *et al.* 2014), which combines morphological, biochemical, phenotypic, and genomic features of the three novel species described here.

# Materials and methods

## Isolation and growth conditions

Three unidentified strains were isolated from blood culture samples received in our diagnostic laboratory. Strain Marseille-P8049 was isolated from a 57-year-old man with mixed cirrhosis which is aggravated by infection of the right hydrothorax. He is awaiting a liver transplant. Strain Marseille-P7157 was obtained from an external blood culture taken from a 70-year-old man. Finally, strain Marseille-Q2854 is isolated from an 85-year-old woman who came to the emergency room for abdominal pain and urinary tract infection.

The optimal growth conditions were determined for each strain. Thus, the temperature (room temperature, 28, 37, 45, and 56°C), the atmosphere (aerobic, anaerobic, and microaerophilic) and the incubation time (24, 48, and 72 h) were varied. About 12 bacterial colonies of each strain were tested using MALDI-TOF MS, as previously described (Seng *et al.* 2009). The specific generated spectra were analyzed and implemented in the local database (https://www.mediterranee-infection.com/urms-data-base/).

## Phenotypic and morphological characteristics

Carbohydrate fermentation and the presence of enzymatic activities for these three strains were studied using the API 50CH and ZYM strips respectively, following the manufacturer's indications. Spore-formation was determined using a suspension of the pure species heated at 80°C for 20 min and then 50 µL of the suspension was inoculated on COS plates, and incubated in the optimal growth condition. Growth indicates spore-forming ability. Additionally 10 µL of the suspension was observed by scanning electron microscope SU5000 (Hitachi group, Tokyo, Japan) for spore formation.

The optical microscope DM1000 (Leica Microsystems) was used to determine the Gram stain and the motility or not of each strain. Bacterial cell morphology was determined by the scanning electron microscope SU5000. Oxidase and catalase tests were done following standard procedures (Mayrer 1974). Salt tolerance was evaluated at varied concentrations of NaCl (50, 75, 100, and 150 g/L) on Columbia agar (COS, bioMérieux, Marcy l'Etoile, France). The growth of these strains was also tested at different pH (6, 6.5, 7, 7.5, and 8). Cellular fatty acid methyl ester (FAME) analysis was performed using gas chromatography/mass spectrometry as previously described (Dione *et al.* 2016). ETEST® strips (bioMérieux) were used to determine the minimal inhibitory concentration to selected antibiotics in optimal growth conditions according to the recommendations of the European committee on antimicrobial susceptibility testing (EUCAST) (https://www.eucast.org/).

## Genome characterization

Genomic DNA (gDNA) extraction of the strains Marseille-Q2854$^T$, Marseille-P8049$^T$ and Marseille-P7157$^T$ was performed as previously described (Lo *et al.* 2022). The assembly was done with a pipeline grouping different software (Velvet (Zerbino and Birney 2008), Soap Denovo (Luo *et al.* 2012) and Spades (Bankevich *et al.* 2012)) and different criteria (number of scaffolds, N50, number of N) were selected in order to create the best assembly. GapCloser software (Xu *et al.* 2019) was used to close gaps within genomes. Annotation of genomes was done with Prokka version 1.14.6 (Seemann 2014). To assess the genomic characteristics of the studied bacteria, average nucleotide identity (ANI) and digital DNA-DNA Hybridization (dDDH) were calculated using OrthoANI software (Lee *et al.* 2016) and the GGDC website (http://ggdc.dsmz.de), respectively.

# Results

## Strain identification and phylogenetic analysis

Strains Marseille-Q2854$^T$, Marseille-P8049$^T$ and Marseille-P7157$^T$ were isolated on Columbia agar with 5% sheep blood at 37°C after 48 h of incubation. The identification attempts by MALDI-TOF MS did not give a reliable result (score under 1.7) because there were no spectra corresponding to the existence of these bacterial strains in the database. Their reference spectra were added in our local database for future correct identification.

The 16S rRNA gene phylogenetic analysis showed that the strain Marseille-P8049$^T$ exhibited 96.88% sequence identity with *Acinetobacter ursingii* strain LUH3792 (GenBank accession number: NR_025392.1). Conversely, the strains Marseille-P7157$^T$ and Marseille-P2854$^T$ exhibited 99.04% and 99.48% with *Gulosibacter faecalis* strain B187 (Accession No: NR 041812.1) and *Microbacterium aerolatum* strain V-73 (Accession No: NR 028944.1), respectively, the phylogenetically closest species. In addition, phylogenetic trees of each strain comparing them to their closest species show that the strains Marseille-Q2854$^T$, Marseille-P8049$^T$, and Marseille-P7157$^T$ have a distinct position (Fig. 1).

## Phenotypic and biochemical characteristics

Strains Marseille-P7157$^T$ and Marseille-Q2854$^T$ are Gram-stain positive and non-spore-forming rod-shaped bacteria (Fig. 2), with catalase-positive and oxidase-negative activities. Strain Marseille-P8049$^T$ is a Gram-stain negative, motile, non-spore-forming and rod-shaped bacterium (Fig. 2). Strain Marseille-P8049$^T$ has catalase-positive and oxidase-negative activities. The mean length and width of the strain Marseille-P7157$^T$ were 1.3 $\mu$m and 0.5 $\mu$m, respectively. Bacterial cells of strains Marseille-Q2854$^T$ and Marseille-P8049$^T$ had a mean length of 1.06 $\mu$m and 1.11 $\mu$m and a mean width of 0.32 $\mu$m and 0.56 $\mu$m, respectively. The growth of strains Marseille-P7157$^T$, Marseille-Q2854$^T$, and Marseille-P8049$^T$ was observed at temperatures ranging from 25 to 37°C after 24–48 h of incubation under aerobic, anaerobic, and microaerophilic atmospheres. Their optimal growth conditions were observed at 37°C after 48 h of incubation under aerobic atmosphere.

Regarding salt tolerance, strains Marseille-P7157$^T$, Marseille-Q2854$^T$ and Marseille-P8049$^T$ were unable to grow on media with salt concentrations superior to 10% NaCl. Optimal growth is obtained with a pH ranging from 6.5 to 8.5, with an optimal growth pH of 7.5.

Using API ZYM strips, strain Marseille-P7157$^T$ was positive for esterase, esterase lipase, leucine arylamidase, and acid phosphatase. Strain Marseille-P8049$^T$ was positive for esterase, esterase lipase, leucine arylamidase, and naphthol-AS-BI-phosphohydrolase. Strain Marseille-P2854$^T$ was positive for leucine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, and $\alpha$-glucosidase. The use of API 50 CH strips revealed that es-

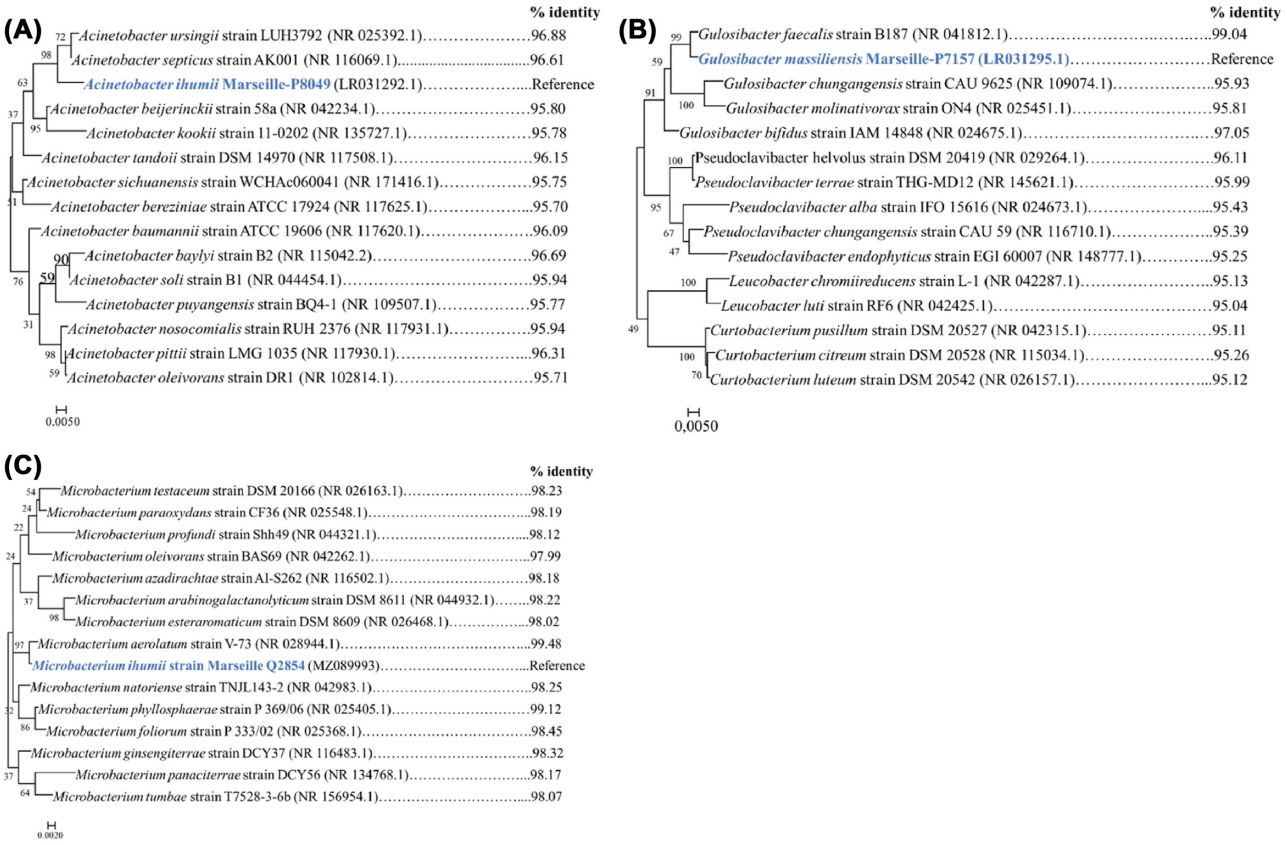


**Figure 1.** Phylogenetic tree showing the position of the strains Marseille-Q2854$^T$, Marseille-P8049$^T$, and Marseille-P7157$^T$ relative to other phylogenetic neighbors, based on the *16S rRNA* gene sequences. The respective GenBank accession numbers for *16S rRNA* genes are indicated in parenthesis. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic inferences obtained using the maximum likelihood method within MEGA 7 software (Kumar *et al.* 2008). Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree.

culin ferric citrate and D-trehalose (only for Marseille-P7157$^T$) were the only carbohydrates consumed by these three species. All negative reactions obtained with API strips are assembled in Supplementary Table S1. A comparison of the main phenotypic criteria of these three species with their phylogenetically closest species is reported in Table 1.

Marseille-Q2854$^T$ and Marseille-P7157$^T$ had the same major fatty acids, which respectively are 12-methyl-tetradecanoic acid (43.7% and 55.5%), 14-methyl-hexadecanoic acid (27.7% and 32.7%), and 14-methyl-pentadecanoic acid (18.9% and 9.8%), as indicated in Table 2. For strain Marseille-P8049 $^T$, the major fatty acids were hexadecanoic acid (30.6%), 9-octadecenoic acid (25.8%) and dodecanoic acid (18.2%). The antibiotic susceptibility of the three strains was tested and their respective minimum inhibitory concentrations (MIC) (mg/L) are shown in Supplementary Table S2.

## Genomic analysis

The genome size of strain Marseille-P8049$^T$ was 3.1 Mbp with 40.5 mol% of G + C content and 2915 protein-coding genes. Its assembly was achieved on 28 contigs, with the longest contig, contig7, being 0.6 Mb (Fig. 3). The genome size of strain Marseille-P7157$^T$ was 2.7 Mbp, with 67.2 mol% of G + C content and 2570 protein-coding genes. Its assembly was achieved on 2 contigs, with contig1 being 2.1 Mb (Fig. 3). Strain Marseille-P7157$^T$ has a genome size of 2.9 Mbp, with 68.5 mol% of G + C content and 2940 protein-coding genes. Its assembly was achieved on 24 contigs, with the longest contig, contig1, being 0.7 Mb (Fig. 3). The number of genes associated with general cluster orthologous groups (COGs) for the strains Marseille-Q2854$^T$, Marseille-P8049$^T$, Marseille-P7157$^T$ is shown in Supplementary Table S3. For these strains, the major proportion of genes was assigned to a general function prediction only.

The genomic analysis with the TYGS server (https://tygs.dsmz.de/) confirms that the three studied strains cluster perfectly with their respective phylogenetically closest species, with very strong bootstrap values (100%), as shown in Supplementary Figure S1.

The degree of genomic similarity of strains Marseille-Q2854$^T$, Marseille-P8049$^T$, and Marseille-P7157$^T$ with their closely related species was estimated using the OrthoANI software (Lee *et al.* 2016) (Table 3). For strain Marseille-P8049$^T$, values ranged from 75.2% with *Acinetobacter pittii* to 83.5% with *Acinetobacter ursingii*. The values from genomic analysis concerning strain Marseille-Q7157$^T$ are between 71.9% with *Pseudoclavibacter helvolus* to 82.0% with *Gulosibacter faecalis* (Table 3). For strain Marseille-Q2854$^T$, OrthoANI values ranged from 78.8% with *Microbacterium foliorum* and *Microbacterium phyllosphaerae* to 83.7% with *Microbacterium aerolatum* (Table 3). In addition, the digital DNA-DNA hybridization (dDDH) calculation showed that strains Marseille-P8049$^T$, Marseille-P7157$^T$ and Marseille-Q2854$^T$ shared genomic similarity of 27 ± 4.9%, 24.4 ± 4.8%, and 22 ± 4.7%, respectively with closely related species, such as *Acinetobacter ursingii*, *Gulosibacter faecalis*, and *Microbacterium aerolatum*, respectively (Table 3).

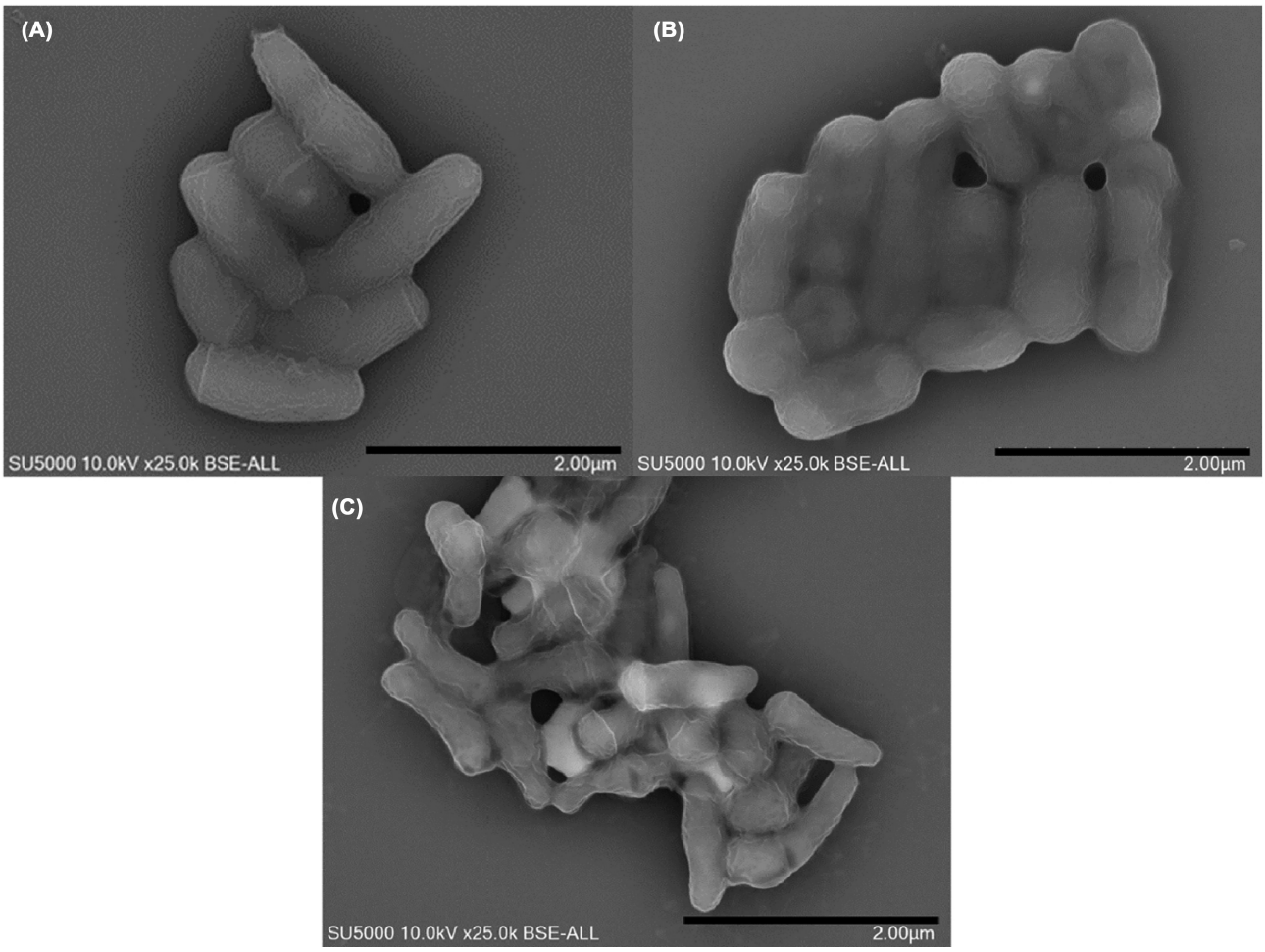


**Figure 2.** Electron micrographs of the strains captured using the SU5000® scanning electron microscope. **(A)** Strain Marseille-P7157$^T$; **(B)** Strain Marseille-P8049$^T$; **(C)** Strain Marseille-Q2854$^T$.

**Table 1.** Different characteristics of *Acinetobacter ihumii* sp. nov., strain Marseille-P8049$^T$, *Gulosibacter massiliensis* sp. nov., strain Marseille-P7157$^T$ and *Microbacterium ihumii* sp. nov., strain Marseille-Q2854$^T$ with their phylogenetically closest related species.

| | Acinetobacter species | | | Gulosibacter species | | | Microbacterium species | | |
|---|---|---|---|---|---|---|---|---|---|
| **Characteristics** | **1** | **2** | **3** | **a** | **b** | **c** | **i** | **ii** | **iii** |
| Cell size (µm) | 1.1–0.5 | NR | NR | 1.3–0.5 | 0.3–1.1 | 0.3–1.1 | 1.0–0.3 | 0.5–1.9 | NR |
| Optimum pH | 7.5 | NR | NR | 7.5 | 8.0 | 8.0 | 7.5 | NR | NR |
| Optimum temperature | 37°C | 37°C | 37°C | 37°C | 30°C | 30°C | 37°C | 37°C | 25°C |
| D-arabitol | - | - | - | - | - | - | - | NR | - |
| Arbutin | - | NR | NR | - | - | - | - | - | + |
| D-glucose | - | - | - | - | - | - | - | + | + |
| D-lactose | - | + | + | - | - | - | - | - | - |
| D-mannose | - | - | - | - | - | - | - | + | + |
| D-melezitose | - | NR | NR | - | - | - | - | NR | + |
| D-melibiose | - | NR | NR | - | - | - | - | NR | + |
| N-acetylglucosamine | - | - | - | - | NR | NR | - | NR | + |
| D-raffinose | NR | - | - | - | - | - | - | NR | + |
| L-rhamnose | - | - | - | - | - | - | - | + | + |
| Sucrose | - | - | - | - | - | - | - | + | + |
| D-trehalose | - | - | - | + | - | - | - | - | + |
| D-xylose | - | - | - | - | - | - | - | + | - |
| DNA G + C content (mol %) | 40.5 | 40.1 | 38.2 | 67.2 | 67.0 | 62.0 | 68.5 | 69.3 | 64.0 |

**+**: positive reaction; **-**: negative reaction; NR: Not reported; **1**: *Acinetobacter ihumii* Marseille-P8049$^T$; **2**: *Acinetobacter ursingii*; **3**: *Acinetobacter septicus*; **a**: *Gulosibacter massiliensis* Marseille-P7157$^T$; **b**: *Gulosibacter faecalis*; **c**: *Gulosibacter bifidus*; **i**: *Microbacterium ihumii* Marseille-Q2854$^T$; **ii**: *Microbacterium aerolatum*; **iii**: *Microbacterium phyllosphaerae*.

**Table 2.** Cellular fatty acid composition (%) of **1**, *Acinetobacter ihumii* sp. nov., strain Marseille-P8049$^T$, **2**, *Gulosibacter massiliensis* sp. nov., strain Marseille-P7157$^T$ and **3**, *Microbacterium ihumii* sp. nov., strain Marseille-Q2854$^T$.

| | | Mean relative %[a] | | |
|---|---|---|---|---|
| **Fatty acids** | **Name** | **1** | **2** | **3** |
| $C_{16:0}$ | Hexadecanoic acid | 30.6 | TR | 1.8 |
| $C_{12:0}$ | Dodecanoic acid | 18.2 | - | - |
| $C_{12:03\text{-}OH}$ | 3-Hydroxydodecanoicacid | 4.6 | - | - |
| $C_{14:03\text{-}OH}$ | 3-hydroxy-Tetradecanoicacid | 3.8 | - | - |
| $C_{15:0\ anteiso}$ | 12-methyl-tetradecanoicacid | - | 55.5 | 43.7 |
| $C_{15:0\ iso}$ | 13-methyl-tetradecanoicacid | - | TR | 4.8 |
| $C_{16:0\ iso}$ | 14-methyl-Pentadecanoicacid | - | 9.8 | 18.9 |
| $C_{16:1n7}$ | 9-hexadecenoic acid | 16.1 | - | - |
| $C_{17:0\ anteiso}$ | 14-methyl-Hexadecanoicacid | - | 32.7 | 27.7 |
| $C_{17:0\ iso}$ | 15-methyl-Hexadecanoicacid | - | TR | 1.8 |
| $C_{18:1n9}$ | 9-Octadecenoic acid | 25.8 | TR | TR |

[a]Mean peak area percentage; TR = trace amounts < 1%.

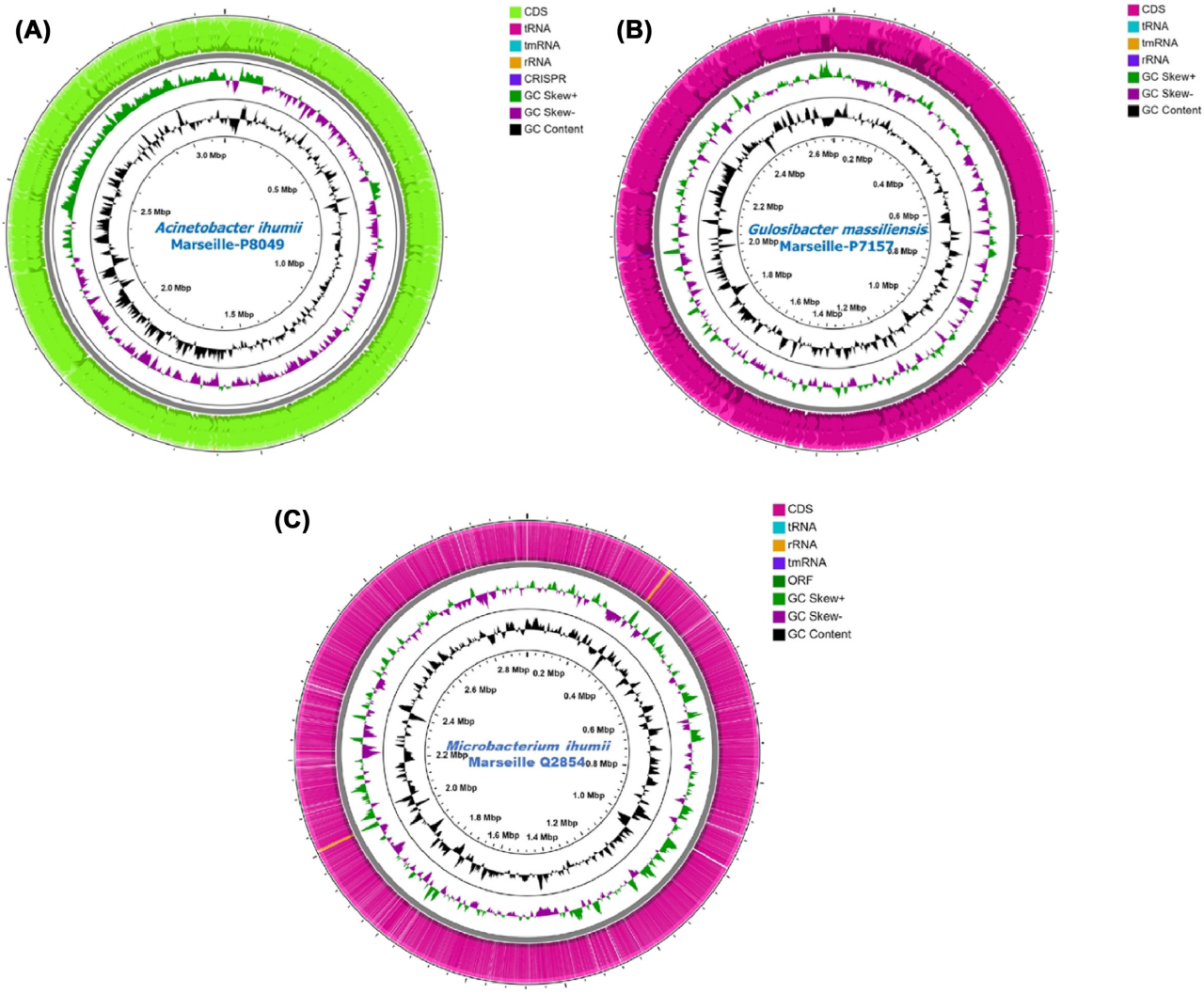


**Figure 3.** Circular map of the genome of strains Marseille-P7157$^T$ **(A)** Marseille-P8049$^T$ **(B)**, and Marseille-Q2854$^T$ **(C)** using the CGView server (Grin and Linke 2011). Different components were highlighted with colored bands.

**Table 3.** dDDH and average nucleotide identity (ANI) values obtained by comparison of strains Marseille-Q2854$^T$, Marseille-P8049$^T$, Marseille-P7157$^T$ and the closest species. The table is filled diagonally. The upper right part for dDDH values and the lower part represent ANI values. 100%, assumed to be in the middle, is removed to show a better separation between the two calculations.

| | Acinetobacter species | | | | |
|---|---|---|---|---|---|
| | ***A. baylyi*** | ***A. ihumii*** | ***A. pittii*** | ***A. septicus*** | ***A. ursingii*** |
| *Acinetobacter baylyi* | | 21.2 ± 4.7% | 21.2 ± 4.7% | 21.4 ± 4.7% | 21.5 ± 4.7% |
| *Acinetobacter ihumii* | 77.5% | | 20.2 ± 4.6% | 26.6 ± 4.9% | 27.0 ± 4.9% |
| *Acinetobacter pittii* | 75.3% | 75.2% | | 20.2 ± 4.7% | 20.5 ± 4.7% |
| *Acinetobacter septicus* | 77.5% | 83.1% | 75.5% | | 73.0 ± 5.8% |
| *Acinetobacter ursingii* | 77.7% | 83.5% | 75.4% | 96.8% | |
| Gulosibacter species | | | | | |
| | *G. bifidus* | *G. faecalis* | *G. massiliensis* | *P. helvolus* | *P. terrae* |
| *Gulosibacter bifidus* | | 20.8 ± 4.7 | 20.5 ± 4.6% | 20.2 ± 4.6% | 18.6 ± 4.5% |
| *Gulosibacter faecalis* | 73.8% | | 24.4 ± 4.8% | 19.2 ± 4.6% | 19.0 ± 4.5% |
| *Gulosibacter massiliensis* | 74.0% | 82.0% | | 19.7 ± 4.6% | 18.9 ± 4.5% |
| *Pseudoclavibacter helvolus* | 70.3% | 72.0% | 71.9% | | 30.9 ± 4.9% |
| *Pseudoclavibacter terrae* | 70.3% | 72.1% | 72.1% | 86.1% | |
| Microbacterium species | | | | | |
| | *M. aerolatum* | *M. foliorum* | *M. ginsengiterrae* | *M. ihumii* | *M. phyllosphaerae* |
| *Microbacterium aerolatum* | | 26.9 ± 4.8 | 25.6 ± 4.9% | 22.0 ± 4.7% | 21.8 ± 4.7% |
| *Microbacterium foliorum* | 78.6% | | 28.8 ± 4.9% | 22.1 ± 4.7% | 21.2 ± 4.7% |
| *Microbacterium ginsengiterrae* | 82.4% | 78.0% | | 25.2 ± 4.8% | 21.2 ± 4.7% |
| *Microbacterium ihumii* | 83.7% | 78.8% | 82.3% | | 21.9 ± 4.7% |
| *Microbacterium phyllosphaerae* | 78.7% | 85.1% | 78.1% | 78.8% | |

## Discussion

The use of MALDI-TOF in clinical microbiology has allowed rapid and accurate identification of several microorganisms and consequently the discovery of new bacterial species (Fall *et al.* 2015). Therefore, it is considered a first-line tool in routine diagnosis in several clinical laboratories (Lo *et al.* 2015).

The three bacterial species studied all had a percentage of sequence similarity of the 16S rRNA gene above the recommended threshold value (Kim *et al.* 2019). Indeed, the 16S similarity value is not only the gold standard to prove the belonging of a bacterial strain to a taxon. Therefore, a polyphasic approach is integrated by modern taxonomists in the description of new species (Vandamme *et al.* 1996, Ramasamy *et al.* 2014). Thus, DDH and ANI calculations are made with the genomics of each species compared to those that are phylogenetically closer. In this study, all DDH values obtained were well below the 70% threshold value recommended for delineating the species barrier in prokaryotes (Auch *et al.* 2010). In addition, the calculated ANI values were all below to 95%–96%, the threshold necessary for a strain to be considered as member belonging to a bacterial species (Meier-Kolthoff *et al.* 2013).

## Conclusion

The unique phenotypic, biochemical, and genomic characteristics possessed by the strains Marseille-P8049$^T$, Marseille-Q2854$^T$, and Marseille-P7157$^T$ allowed us to conclude that they are new bacterial species compared to others with a valid published name. They were classified as new species and named *Acinetobacter ihumii* sp. nov., *Microbacterium ihumii* sp. nov., and *Gulosibacter massiliensis* sp. nov., respectively.

### Description of Gulosibacter massiliensis sp. nov.

*Gulosibacter massiliensis* (mas.si.lien'sis, L. fem. *massiliensis* from Massilia, the Latin name of Marseille, France, where the type strain was isolated). It is Gram-positive, motile, non-spore-forming, rod-shaped and aerobic bacterium, catalase-positive, and oxidase-negative. The cells have a mean length of 1.3 $\mu$m and a mean width of 0.5 $\mu$m. Positive reactions were observed with esterase, esterase lipase, leucine arylamidase and acid phosphatase, while negative reactions were observed with alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, $\alpha$-chymotrypsin, naphthol-AS-BI-phosphohydrolase, $\alpha$-galactosidase, $\beta$-galactosidase, $\beta$-glucuronidase, $\alpha$-and $\beta$-glucosidase, N-acetyl-$\beta$-glucosaminidase, $\alpha$-mannosidase, and $\alpha$-fucosidase. It ferments esculin ferric citrate and D-trehalose. The most abundant fatty acids (>9%) were 12-methyl-tetradecanoic acid, 14-methyl-hexadecanoic acid and 14-methyl-pentadecanoic acid, while minor amounts of unsaturated and linear fatty acids were also detected.

The genome is 2075 628 bp long, with G + C content of 67.2 mol%. The 16S rRNA and genome sequences are available in GenBank under accession numbers LR031295 and UYZX01000001, respectively.

The type strain Marseille-P7157$^T$ (= CSUR P7157 = CECT 30048) was isolated from a human blood sample.

### Description of *Acinetobacter ihumii* sp. nov.

*Acinetobacter ihumii* (i.hu.mi'i. N.L. gen. masc. n. *ihumii*, based on the acronym IHUMI, meaning Institut Hospitalo-Universitaire Méditerranée-Infection, the clinical lab where the type strain was isolated). It is an aerobic Gram-negative bacterium. Bacterial cells are motile, non-spore-forming and rod-shaped, with a mean length of 1.1 $\mu$m and width of 0.5 $\mu$m, catalase positive and oxidase negative. Enzymes such as esterase, esterase lipase, leucine arylamidase, and naphthol-AS-BI-phosphohydrolase are present, while those such as alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, $\alpha$-chymotrypsin, acid phosphatase, $\alpha$-galactosidase, $\beta$-galactosidase, $\beta$-glucuronidase, $\alpha$-glucosidase, $\beta$-glucosidase, N-acetyl-$\beta$-glucosaminidase, $\alpha$-mannosidase, and $\alpha$-fucosidase are

absent. Only esculin ferric citrate was positive among the carbohydrates tested. The most abundant fatty acids (>18%) were hexadecanoic acid, 9-octadecenoic acid, and dodecanoic acid. Two specific 3-hydroxy structures were also described.

The genome is 3 299 805 bp long with G + C content of 40.5 mol%. The 16S rRNA and genome sequences are deposited in the GenBank database under accession numbers LR031292 and UYYC00000000, respectively.

The strain Marseille-P8049$^T$ (= CSUR P8049 = CECT 30350) was the type strain of *Acinetobacter ihumii* sp. nov., which was isolated from a human blood sample.

## Description Microbacterium ihumii sp. nov.

*Microbacterium ihumii* (i.hu.mi'i. N.L. gen. masc. n. *ihumii*, based on the acronym IHUMI, meaning Institut Hospitalo-Universitaire Méditerranée-Infection, the clinical lab where the type strain was isolated). It is an aerobic Gram-positive bacterium. The cells are non-motile, non-spore-forming with a short rod-shape (mean length = 1.06 $\mu$m and mean width = 0.32 $\mu$m), catalase-positive, and oxidase negative. It possesses leucine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase and $\alpha$-glucosidase. A positive reaction was obtained with esculin ferric citrate, but no reaction was observed with other carbohydrates and its derivatives (heterosides, polyalcohols, and uronic acids). In addition, the major fatty acids were branched $C_{15:0\ anteiso}$, $C_{17:0\ anteiso}$, and $C_{16:0\ iso}$. Minor amounts of unsaturated and linear fatty acids were also detected.

The genome is 2954541 bp long and the G + C content is 68.5 mol%. The 16S rRNA and genome sequences are available in GenBank under accession numbers MZ089993 and CAHJXQ000000000, respectively.

The type strain Marseille-Q2854$^T$ (= CSUR Q2854 = CECT 30120) was isolated from a human blood sample.

## Supplementary data

Supplementary data are available at *FEMSLE* online.

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## Author contributions

Conceptualization, JCL, DR; methodology, CIL and MTA; validation, PEF and FF; formal analysis, AY; investigation, SA, FG; Strains culture, AY and SS; writing-original draft preparation, AY, SS, and OLFTS; writing-review and editing, CIL, MTA, GD; supervision, CIL, FF, MM; funding acquisition, DR. All authors have read and agreed to the published version of the manuscript.

## Ethical approval

The clinical samples were obtained in the context of diagnostic screening. Patients were informed of the possible use of their samples for research purposes and retained their right to refuse approval at any time. Indeed, according to the French Jardé Law (Loi no 2012–300 du 5 mars 2012 and Décret no 2016–1537 du 16 Novembre 2016 published in the 'Journal Officiel de la République Française), as this study did not involve specific collection of samples or use medical/personal data from patients, neither institutional ethical approval nor individual patient consent was required for this non-invasive study. As a result, this study was approved by the Ethics committee of the IHU under number No. 2022–004.

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***Conflicts of interest statement.*** Didier Raoult is a consultant in microbiology for the Hitachi High-Tech Corporation from March 2018 until March 2020. The rest of the authors declare that they have no relevant conflicts of interest.

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