**Chryseobacterium schmidteae** sp. nov. a novel bacterial species isolated from planarian *Schmidtea mediterranea*

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Marseille-P9602T is a *Chryseobacterium*-like strain that we isolated from planarian *Schmidtea mediterranea* and characterized by taxono-genomic approach. We found that Marseille-P9602T strain exhibits a 16S rRNA gene sequence similarity of 98.76% with *Chryseobacterium scophthalmum* LMG 13028T strain, the closest phylogenetic neighbor. Marseille-P9602T strain was observed to be a yellowish-pigmented, Gram-negative, rod-shaped bacterium, growing in aerobic conditions and belonging to the *Flavobacteriaceae* family. The major fatty acids detected are 13-methyl-tetradecanoic acid (57%), 15-methylhexadecenoic acid (18%) and 12-methyl-tetradecanoic acid (8%). Marseille-P9602 strain size was found from genome assembly to be of 4,271,905 bp, with a 35.5% G+C content. The highest values obtained for Ortho-ANI and dDDH were 91.67% and 44.60%, respectively. Thus, hereby we unravel that Marseille-P9602 strain is sufficiently different from other closed related species and can be classified as a novel bacterial species, for which we propose the name of *Chryseobacterium schmidteae* sp. nov. Type strain is Marseille-P9602T (= CSUR P9602T = CECT 30295T).

Using genotypic, chemotaxonomic and phenotypic characteristics of members of *Flavobacterium* and *weeksella* genus allowed revising the classification of the novel *Chryseobacterium* genus1 with *Chryseobacterium gleum* type strain2. Several genus members were isolated from soil, plant, waste water, fish, sewage, sludge, lactic acid beverage, oil, contaminated soil, and clinical samples3–12. Some species of this genus such as *Chryseobacterium indolgenes*, *Chryseobacterium oranimense* and *Chryseobacterium gleum* are responsible for human pathologies13,14; others are involved in the production of natural bioactive substances such as prebiotics, antioxidants, and proteases15–17. *Chryseobacterium* cells were observed to be gram-negative, non-motile, non-spore-forming rods, with parallel sides and rounded ends. Typically, these cells are 0.5 mm wide and 1 to 3 mm long1. All strains grow at 30 °C; most strains grow at 37 °C. Growth on solid media is typically pigmented (yellow to orange). Colonies were observed to be translucent (occasionally opaque), circular, convex, or low convex, smooth, and shiny, with entire edges1. In this study, we used the genomic and taxonomy strategy that combines phenotypic assays and genome sequencing18–21 to further characterize a *Chryseobacterium*-like bacterial strain isolated from planarian *Schmidtea mediterranea* species. *S. mediterranea* platyhelminth is a zoophage invertebrate living in freshwater like ponds, lakes, and rivers22. This flatworm is a model organism for regeneration, because of its unique capacity to regenerate after amputation23, as well as to investigate host–pathogen interaction24–26.

**Materials and methods**

**Culture of Schmidtea mediterranea.** *S. mediterranea* animals are asexual (clonal line ClW4), kept in laboratory for 10 years and fed with calf liver, maintained in filtered tap water at 19 °C as previously described27.

**Isolation and identification of bacteria from Schmidtea mediterranea.** Before experiments, animals were starved for two weeks, washed in sterile water and then one worm was inoculated in Buffered Charcoal Yeast Extract (BCYE) (Oxoid Deutschland GmbH, Wesel, Germany), Luria Bertani (LB) and 5% sheep

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blood-enriched Columbia agar (bioMérieux, Marcy l’étoile, France) and incubated at 19, 28 and 37 °C. Bacterial colonies were identified by MALDI-TOF-MS (Microflex spectrometer; Bruker Daltonics, Bremen, Germany) 29. As previously described 27. Briefly, a colony was likely identified at the species level for a score ≥ 2.0; probably identified for a score between 1.99 and 1.7, but not identified for a score < 1.7.

**Sequencing, assembly, and annotation.** First, using EZ1 automate and DNA tissue kit (Qiagen, Hilden, Germany), bacterial genomic DNA was extracted and then quantified using a Qubit assay (Life Technologies, Carlsbad, CA, USA) at 0.2 ng/µl. Second, bacterial genomic DNA was prepared and sequenced using Mate-Pair strategy with a Miseq sequencer (Illumina, San Diego, CA, USA) 39. Next, sequencing reads were assembled using Spades software (Galaxy version 3.12.0 + galaxy1) 30 and genomic annotation was obtained using Prokka (Rapid Prokaryotic Genome Annotation) 31. Finally, taxonomic assignation was done by BLASTn search performed against nr database. A sequence similarity threshold of 98.65% by comparison with the phylogenetically closest species with standing in nomenclature was used to delineate a putative novel species 32.

**Phylogenetic analysis, and genomic comparison.** Phylogenetic relationships were inferred from comparison of 16S rRNA gene sequences using MEGAX (version 10.1) software 33, 34. Sequences were aligned using MUSCLE algorithm setup with default parameters, and numbers at the nodes were percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. Only bootstrap values ≥ 50% were retained. For the Phylogenetic tree based on the core genes, we generated a core-genome alignment using Roary 3.13.0 35 with 70% identity. We obtained an alignment of 1535 core genes from which we inferred a phylogenetic tree using FastTree 2.1.10 36. Degrees of genomic similarity were evaluated using the GGDC 37 (http://ggdc.dsmz.de/ggdc.php#) and Orthologous Average Nucleotide Identity 38 (https://www.ezbiocloud.net/tools/orthoani, OrthoANI Tool version 0.93.1) softwares. Comparison COG functional categories were carried out using Blast P (E-value 10-3, coverage 0.7 and identity percent 30%) against clusters of orthologous groups (COG) database.

**Phenotypic characteristics.** Growth of Marseille-P9602 strain and Chryseobacterium scophthalmum LMG 13028 T strain (purchased to DSMZ) (ATCC 700,039 = CCM 4109 = CCUG 33,454 = CIP 104,199 = DSM 16,779 = MM1) 39 was attempted at various temperatures such as 4, 19, 28, 30, 37 and 45 °C in 5% sheep blood-enriched Columbia agar (bioMérieux) under anaerobic atmosphere using GasPak EZ generators (Becton–Dickinson, Maryland, USA), as well under aerobic atmosphere. Strain ability to sporulate was investigated by thermal shock. Briefly, bacteria were exposed at 80 °C temperature for 30 min and then bacterial growth was assessed for 4 days. The capacity to grow under various salinity (0, 20, 40, 50, 60, 80 and 100 g of NaCl/l) and pH conditions (5, 5.5, 6, 6.5, 7.5, 8.5, 9 and 10) was also investigated. Gram staining and motility of fresh colonies were observed using a DSM1000 photonic microscopy (Leica Microsystems, Nanterre, France) with an ocular of 10 x and 40 x objective lens. Bacterial structure was defined using a scanning electron microscopy (Hitachi SUV5000) (Hitachi High-Technologies Corporation, Tokyo, Japan). Enzymatic activities such as catalase and oxidase activities were analysed with a BBL DrySlide following manufacturer's instructions (Becton Dickinson, Le Pont de Clai, France). API strips (API ZYM 40-42, API 20NE 43, 44, API 20E 45, 46 and API 50CH 47-50, bioMérieux) were used to study strains biochemical characteristics.

**Antibiotic susceptibility of Marseille-P9602 strain.** Bacterial susceptibility to benzylpenicillin, amoxicillin, ampicillin, ceftriaxone, imipenem, ciprofloxacin, amikacin, gentamicin, streptomycin, daptomycin, doxycycline, metronidazole, rifampicin, fosfomycin, vancomycin and tigecycline was assessed using E-tests and a 0.5 McFarland concentration of Marseille-P9602 and LMG 13028 T strains. MICs were read at the point of intersection between the developed elliptical zone of inhibition and the test strip. Interpretation of the MICs was carried out according to NCCLS recommendations for bacterial isolates grown aerobically 31.

**Analysis of cellular fatty acids of strain Marseille-P9602.** Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS for both Marseille-P9602 and LMG 13028 T strain. Fatty acid methyl esters were prepared as described by Sasser 32 and GC/MS analysis was realized as previously described 32. Briefly, Marseille-P9602 and LMG 13028 T strains were inoculated in 5% sheep blood-enriched Columbia agar and incubated at 28 °C. Fatty acid methyl esters were separated using an Elite 5-MS column and monitored by mass spectrometry (Clarus 500—SQ 8 S, Perkin Elmer, Courtaboeuf, France). Spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (NIST, Gaithersburg, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK).
Results and discussion

Phylogenetic analysis and genomic comparison. The gene 16S rRNA sequence from Marseille-P9602 strain was observed to be 1513 bp-long. A sequence similarity calculation using BLASTn search in the nr database indicated that the closest relatives of Marseille-P9602 strain are *Chryseobacterium scophthalmum* strain LMG 13028T, *Chryseobacterium piscium* strain LMG 23089T, *Chryseobacterium balustinum* strain NBRC 15053T and *Chryseobacterium indoltheticum* strain LMG 4025T. Therefore, Marseille-P9602 strain belongs to *Chryseobacterium* genus within the *Flavobacteriaceae* family and the *Bacteroidetes* phylum.

Table 1. The taxonomic assignment obtained by a BLASTn search in the nr database. Marseille-P9602 strain has a high sequence similarity, but a sequence cover lower\(^3\), with *Chryseobacterium scophthalmum* strain LMG 13028T, *Chryseobacterium piscium* strain LMG 23089T, *Chryseobacterium balustinum* strain NBRC 15053T and *Chryseobacterium indoltheticum* strain LMG 4025T.

| Name                                                      | Cover (%) | Identity (%) | Accession          |
|-----------------------------------------------------------|-----------|--------------|--------------------|
| *Chryseobacterium scophthalmum* strain LMG 13028T         | 97        | 98.76        | NR_025386.1        |
| *Chryseobacterium piscium* strain LMG 23089T              | 98        | 98.36        | NR_042410.1        |
| *Chryseobacterium balustinum* strain NBRC 15053T          | 97        | 98.75        | NR_113721.1        |
| *Chryseobacterium indoltheticum* strain LMG 4025T         | 96        | 97.98        | NR_042926.1        |
| *Chryseobacterium taihusense* strain THMBM1\(^7\)         | 99        | 96.62        | NR_109542.1        |
| *Chryseobacterium arelyticum* strain F-Fue-04III\(^a\)    | 99        | 96.53        | NR_042503.1        |
| *Chryseobacterium aquaticum* strain 10-46\(^c\)           | 96        | 97.34        | NR_042642.1        |
| *Chryseobacterium lactis* strain KC1864\(^4\)             | 97        | 97.01        | NR_126256.1        |
| *Chryseobacterium sinjiangense* strain TSBY-67\(^I\)      | 100       | 96.09        | NR_131771.1        |
| *Chryseobacterium sordelllicola* strain NBRC 100864\(^T\) | 97        | 98.89        | NR_113952.1        |
| *Chryseobacterium forskose* strain CC-H3-2\(^T\)          | 98        | 96.57        | NR_036872.1        |
| *Chryseobacterium aureum* strain 17S1E7\(^T\)             | 99        | 96.02        | NR_170500.1        |
| *Chryseobacterium hominis* strain NF802\(^a\)             | 99        | 96.08        | NR_042517.2        |
| *Chryseobacterium taimonianum* strain G972\(^T\)          | 99        | 96.02        | NR_164881.1        |
| *Chryseobacterium polytrichastri* strain YG4-6\(^T\)      | 99        | 96.07        | NR_134710.1        |
| *Chryseobacterium echinoidorum* strain CC-CZW010\(^T\)    | 99        | 95.95        | NR_145657.1        |
| *Chryseobacterium endophyticum* strain CC-YTH209\(^T\)    | 98        | 96.30        | NR_156142.1        |
| *Chryseobacterium taiwanense* strain BCRC 17412\(^T\)     | 99        | 95.61        | NR_043715.1        |
| *Chryseobacterium vystaatense* strain R-23566\(^T\)       | 98        | 96.17        | NR_042370.1        |
| *Chryseobacterium joostei* strain LM18212\(^T\)           | 98        | 96.17        | NR_025387.1        |
| *Chryseobacterium geocarposphaerae* strain 91A-561\(^T\)  | 97        | 96.54        | NR_133727.1        |
| *Chryseobacterium gleum* strain NBRC 15054\(^T\)          | 97        | 96.40        | NR_113722.1        |

The 16S rRNA sequence from Marseille-P9602 strain was assembled into 56 contigs for a total size of 4,276,845 bp (Cover, 56x; \(N\)\(_{50}\), 151,068; \(L\)\(_{50}\), 9) with a 33.5% G + C content. A total of 3881 predicted protein-coding genes were identified, along with 9 rRNAs, 67 tRNAs, 1 tmRNA and 1 repeat region; and this genome was compared with other closely related *Chryseobacterium* genomes (Table 3). Based on the Digital DNA-DNA hybridization values (dDDH) obtained using GGDC software, Marseille-P9602 strain values ranged from 21.40% with *C. aureum* and *C. lactis* to 44.60% with *C. scophthalmum* (Table 3). These values were below the 70% threshold recognized for the delimitation of bacterial species. Ortho-ANI values of Marseille-P9602 strain ranged from 76.65% with *C. aureum* to 91.67% with *C. scoph-

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Table 1. The taxonomic assignment obtained by a BLASTn search in the nr database. Marseille-P9602 strain has a high sequence similarity, but a sequence cover lower\(^3\), with *Chryseobacterium scophthalmum* strain LMG 13028T, *Chryseobacterium piscium* strain LMG 23089T, *Chryseobacterium balustinum* strain NBRC 15053T and *Chryseobacterium indoltheticum* strain LMG 4025T.
thalmum, which is lower than the 95% threshold used to distinguish species (Table 3). These values of genomic comparison showed that Marseille-P9602 strain is probably a novel species in the *Chryseobacterium* genus. The distribution of genes in COG functional categories is presented in Fig. 2 and Table 4. Few differences were observed between these species. In addition, by comparison of the genomes of Marseille-P9602 strain and of the 11 closest species, we highlighted 100 specific and unique genes to the Marseille-P9602 strain (Supplementary data S1). Taken together, these results confirm that Marseille-P9602 strain belongs to a separate *Chryseobacterium* species.

**Phenotypic analysis and biochemical characteristics.** Marseille-P9602 strain was isolated on COS agar after 2 days at 28 °C in aerobic atmosphere at pH 7.5. We observed that Marseille-P9602 strain grows at temperatures ranging from 4 to 30 °C in aerobic atmosphere and at pH values ranging from 6.5 to 9 (Neutro-alkalophilic bacterium). In contrast, LMG 13028^T^ strain grows at pH 6. Marseille-P9602 strain grows at salinity concentrations lower than 12 g of NaCl/l; however, in contrast, LMG 13028^T^ strain needs a NaCl concentration lower than 25 g/l. After 4 days culture on COS agar, Marseille-P9602 strain colonies were observed to be yellowish, small (0.4 mm median diameter), circular with a convex shape and smooth. Bacterial cells (Fig. 3) are Gram-negative (Fig. 3A), rod-shaped, non-spore-forming bacilli and non-motile, but without any flagellum. Their mean length and width are 3.15 μm and 0.66 μm, respectively (Fig. 3B). Marseille-P9602 strain was found to be oxidase positive and catalase negative. Bacterial metabolism was characterized using API 50CHB/E, API 20NE, API Zym and API 20E strips (Table 5). Marseille-P9602 strain differs from *C. scophthalmum*, *C. indoltheticum*, *C. piscium*, and *C. balustinum* regarding catalase, α-glucosidase, inositol and urea.

**Antibiotic susceptibility.** Marseille-P9602 strain growth is inhibited by benzylpenicillin, amikacin, amoxicillin, ampicillin, gentamicin, ciprofloxacin ceftriaxone, streptomycin, doxycycline, tigecycline, rifampicin, and vancomycin; but not by daptomycin, fosfomycin, and metronidazole (Table 6). We noticed that amikacin inhibits the growth of Marseille-P9602, but not LMG 13028^T^ strain.
Chryseobacterium ureilyticum strain F-Fue-04IIIIaa\textsuperscript{T}
Chryseobacterium lactis strain KC1864\textsuperscript{T}
Chryseobacterium aureum strain 17S1E7\textsuperscript{T}
Chryseobacterium gleum strain NBRC 15054\textsuperscript{T}
Chryseobacterium timonianum strain G972\textsuperscript{T}
Chryseobacterium vrystaatense strain R-23566\textsuperscript{T}
Chryseobacterium joostei strain LMG 18212\textsuperscript{T}
Chryseobacterium formosense strain CC-H3-2\textsuperscript{T}
Chryseobacterium hominis strain NF802\textsuperscript{T}
Chryseobacterium taiwanense strain BCRC 17412\textsuperscript{T}
Chryseobacterium indoltheticum strain LMG 4025\textsuperscript{T}
Chryseobacterium geocarposphaerae strain 91A-561\textsuperscript{T}
Chryseobacterium taihuense strain THBMB1\textsuperscript{T}
Chryseobacterium polyrichastri strain YG4-6\textsuperscript{T}
Chryseobacterium piscium strain LMG 23089\textsuperscript{T}
Chryseobacterium balustinum strain NBRC 15053\textsuperscript{T}
Chryseobacterium scophthalmum strain LMG 13028\textsuperscript{T}
\textbf{Chryseobacterium schmidtiae Marseille-P9602\textsuperscript{T}}
Chryseobacterium endophyticum strain CC-YTH209\textsuperscript{T}
Chryseobacterium aquaticum strain 10-46\textsuperscript{T}
Chryseobacterium xinjiangense strain TSBY-67\textsuperscript{T}
Chryseobacterium soldanellicola strain NBRC 100864\textsuperscript{T}

**Figure 1.** Phylogenetic tree and Core-genome. (A) Phylogenetic tree based on 16S rRNA sequence comparison highlighting the position of Marseille-P9602 strain relative to other closely related species. Only bootstrap values ≥ 50\% were shown. (B) Core-genome-based phylogenetic relationships of Marseille-P9602 strain relative to other closely related species.
Cellular fatty acids analysis. The fatty acids 13-methyl-tetradecanoic acid (56.7%), 15-Methylhexadecanoic acid (18.1%), 12-methyl-tetradecanoic acid (7.5%), 3-methyl-butanolic acid (4.9%), 3-hydroxy-15-methyl-Hexadecanoic acid (3.3%), Hexadecanoic acid (1.2%) and 11-methyl-Dodecanoic acid (1.2%) were detected in Marseille-P9602 strain. Trace (< 1%) of unsaturated and saturated fatty acids such as 15-methyl-Hexadecanoic acid, 9,12-Octadecadienoic acid, 12-methyl-Tridecanoic acid, Pentadecanoic acid, 9-Octadecenoic acid, 3-hydroxy-Hexadecanoic acid, Tetradecanoic acid, 9-Hexadecenoic acid, and Octadecenoic acid were detected. The fatty acid 3-hydroxy-13-methyl-Tetradecanoic was not detected in Marseille-P9602 strain, in contrast to C. auresens.

Species description. Chryseobacterium schmidteae is a novel genus. We propose the name of Chryseobacterium schmidteae Marseille-P9602 strain. To date, this novel strain has never been identified in any other environment.

Conclusion

Based on the results obtained by the taxono-genomic approach, we confirm that Marseille-P9602 strain belongs to a novel species from Chryseobacterium genus. We propose the name of Chryseobacterium schmidteae Marseille-P9602 strain. To date, this novel strain has never been identified in any other environment.

Table 3. Main genomic characteristics of Marseille-P9602 and other closely related Chryseobacterium species. OrthoANI values calculated using OAT software38. dDDH values obtained by comparison of all studied genomes using GGDC, formula 2 (DDH Estimates Based on Identities/HSP length).
Nucleotide sequence accession number. 16S rRNA gene sequence and genome sequence were deposited in GenBank under the accession numbers LR797929 and CAESCJ000000000.1, respectively. The raw data for the assembly were deposited in EMBL-EBI under the run accession ERR4143501 and the experiment accession ERX4110774.
Table 4. Functional annotation of predicted genes according to the COGs database.

| Code | Value | Description                                      |
|------|-------|--------------------------------------------------|
| [I]  | 154   | Translation, ribosomal structure and biogenesis   |
| [A]  | 0     | RNA processing and modification                   |
| [K]  | 170   | Transcription                                    |
| [L]  | 163   | Replication, recombination and repair             |
| [B]  | 0     | Chromatin structure and dynamics                  |
| [J]  | 20    | Cell cycle control, cell division, chromosome partitioning |
| [Y]  | 0     | Nuclear structure                                 |
| [V]  | 77    | Defense mechanisms                                |
| [T]  | 116   | Signal transduction mechanisms                    |
| [M]  | 222   | Cell wall/membrane/envelope biogenesis            |
| [N]  | 6     | Cell motility                                    |
| [Z]  | 0     | Cytoskeleton                                     |
| [W]  | 0     | Extracellular structures                          |
| [U]  | 23    | Intracellular trafficking, secretion, and vesicular transport |
| [O]  | 105   | Posttranslational modification, protein turnover, chaperones |
| [X]  | 0     | Mobilome: prophages, transposons                  |
| [C]  | 119   | Energy production and conversion                  |
| [G]  | 114   | Carbohydrate transport and metabolism             |
| [E]  | 173   | Amino acid transport and metabolism               |
| [F]  | 55    | Nucleotide transport and metabolism               |
| [H]  | 91    | Coenzyme transport and metabolism                 |
| [I]  | 101   | Lipid transport and metabolism                    |
| [P]  | 136   | Inorganic ion transport and metabolism            |
| [Q]  | 39    | Secondary metabolites biosynthesis, transport and catabolism |
| [R]  | 342   | General function prediction only                  |
| [S]  | 237   | Function unknown                                  |

Table 4. Functional annotation of predicted genes according to the COGs. Functional annotation of Marseille-P9602 predicted genes according to the COGs database.

Figure 3. Micrograph of Marseille-P9602 strain. (A) Micrograph of Marseille-P9602 strain after Gram staining, (B) Transmission electron microscopy micrograph of Marseille-P9602 strain.
| Properties                        | 1 | 2 | 3 | 4 | 5 |
|----------------------------------|---|---|---|---|---|
| Gram-staining                    | − | − | − | − | − |
| Sporulation                      | − | − | − | − | − |
| Growth temperature range (°C)    | 4–30 | 4–30 | 5–30 | 5–35 | 5–37 |
| Aerobic growth                   | + | + | + | + | + |
| Source                           | Planarian | S. maximus | sea | fish | fish |
| Colony colour                    | Yellowish | Yellowish | Yellow | Yellow | Yellow |
| Catalase                         | − | + | + | + | + |
| Oxidase                          | + | + | + | + | + |

**Enzyme activity (API ZYM):**

| Enzyme activity (API ZYM)              | 1 | 2 | 3 | 4 | 5 |
|----------------------------------------|---|---|---|---|---|
| Alkaline phosphatase                   | + | + | + | + | NA |
| Esterase (C4)                         | + | + | − | NA | − |
| Esterase lipase (C8)                  | − | − | + | NA | NA |
| Lipase (C14)                          | + | + | − | NA | − |
| Leucine aminopeptidase                | + | + | + | NA | NA |
| Valine aminopeptidase                 | − | − | + | NA | NA |
| Cystine aminopeptidase                | + | + | + | NA | − |
| Trypsin                               | − | − | − | NA | − |
| α-chymotrypsin                        | + | + | + | NA | − |
| Acid phosphatase                      | + | + | + | + | NA |
| Naphthol-AS-BI-phosphohydrolase       | − | − | + | NA | NA |
| α-galactosidase                       | − | − | − | NA | NA |
| β-galactosidase                       | − | − | − | NA | − |
| β-glucuronidase                       | − | + | − | NA | NA |
| α-glucosidase                        | − | + | + | NA | NA |
| β-glucosidase                        | + | + | − | NA | + |
| N-acetyl-β-glucosaminidase            | − | − | + | NA | NA |
| α-mannosidase                        | − | − | − | NA | NA |
| α-fucosidase                         | + | + | − | NA | NA |

**Assimilation of (API 50 CH/B)**

| Assimilation of (API 50 CH/B)         | 1 | 2 | 3 | 4 | 5 |
|---------------------------------------|---|---|---|---|---|
| Glycérol                             | − | − | NA | NA | NA |
| Erythritol                           | − | − | NA | NA | − |
| d-arabinose                           | − | − | − | NA | − |
| l-arabinose                           | − | − | − | NA | − |
| d-ribose                             | − | − | NA | NA | − |
| d-xylene                             | − | − | − | − | NA |
| l-xylene                             | − | − | − | − | NA |
| d-adonitol                           | − | − | − | − | NA |
| Methyl-d-xylopyranoside               | − | − | NA | − | NA |
| d-galactose                           | − | − | − | − | NA |
| d-glucose                             | + | + | + | + | − |
| d-fructose                            | + | + | + | NA | NA |
| d-mannose                             | + | + | + | + | NA |
| l-sorbose                             | + | − | NA | NA | NA |
| l-rhamnose                            | − | + | − | − | NA |
| Dulcitol                              | − | + | − | − | NA |
| Inositol                              | + | − | − | − | + |
| d-mannitol                            | + | + | − | − | + |
| Methyl-α-d-mannopyranoside            | − | − | NA | NA | − |
| Methyl-β-d-glucosaminide              | − | − | NA | NA | NA |
| Amygdalin                             | + | + | NA | NA | NA |
| Arbutin                               | − | − | NA | NA | NA |
| Esculin ferric citrate                | + | + | + | + | NA |
| Salicin                               | − | − | − | NA | NA |
| α-cellobiose                          | − | − | − | − | − |

Continued
| Properties          | 1     | 2     | 3     | 4     | 5     |
|---------------------|-------|-------|-------|-------|-------|
| d-maltose           | −     | −     | +     | −     | −     |
| d-lactose           | −     | −     | −     | −     | +     |
| d-melibiose         | −     | −     | NA    | −     | NA    |
| d-saccharose        | −     | −     | NA    | −     | NA    |
| d-trehalose         | +     | +     | −     | −     | NA    |
| Inulin              | −     | −     | −     | −     | NA    |
| d-melezitose        | −     | −     | −     | −     | NA    |
| d-raffinose         | −     | −     | −     | −     | NA    |
| Starch              | −     | −     | −     | −     | −     |
| Glycogen            | −     | −     | −     | NA    | NA    |
| Xylitol             | −     | −     | NA    | −     | NA    |
| Gentiofribose       | +     | +     | −     | +     | NA    |
| d-turanose          | −     | −     | NA    | −     | NA    |
| d-lyxose            | −     | −     | NA    | −     | NA    |
| d-tagatose          | −     | −     | NA    | −     | NA    |
| d-lucose            | −     | −     | NA    | −     | NA    |
| l-lucose            | −     | −     | NA    | −     | −     |
| l-arabitol          | −     | −     | NA    | −     | NA    |
| Potassium gluconate | −     | −     | −     | −     | NA    |
| Potassium 2-ketoGluconate | − | − | NA | − | NA |
| Potassium 5-ketogluconate | − | − | NA | − | NA |
| API 20E             |       |       |       |       |       |
| l-lysin             | −     | −     | NA    | NA    | NA    |
| l-orumithin         | −     | −     | NA    | NA    | +     |
| Trinatriumcitrat    | −     | −     | NA    | NA    | NA    |
| Natriumthiosulfat   | −     | −     | +     | NA    | −     |
| l-tryptophan        | +     | +     | NA    | NA    | NA    |
| Indole production   | +     | +     | +     | NA    | +     |
| Natriumpyruvat      | +     | −     | NA    | NA    | NA    |
| API 20NE            |       |       |       |       |       |
| Potassium nitrate   | +     | +     | −     | +     | +     |
| l-arginine          | −     | −     | +     | NA    | +     |
| Urea                | −     | +     | +     | −     | +     |
| Gelatin             | +     | +     | +     | −     | NA    |
| N-acetyl glucosamine| −     | −     | NA    | −     | NA    |
| Capric acid         | −     | −     | −     | −     | −     |
| Adipic acid         | −     | −     | −     | −     | −     |
| Malic acid          | −     | −     | −     | −     | −     |
| Trisodium citrate   | −     | −     | −     | −     | −     |
| Phenylacetic acid   | −     | −     | −     | −     | −     |

Table 5. Biochemical characteristics of Marseille-P9602 and phylogenomically related species. Taxa: 1, Marseille-P9602; 2, C. scophthalmum; 3, C. indolthecicum; 4, C. piscium; 5, C. balustinum. The results presented for 1 and 2 are those obtained in the present study. The results presented for 3, 4 and 5 were completed using previously published studies. The results presented for 3, 4 and 5 were completed using previously published studies. Positive (+); negative (−); NA, non-available. Marseille-P9602 strain differs from C. scophthalmum, C. indolthecicum, C. piscium, and C. balustinum regarding catalase, α-glucosidase, inositol and urea.
Table 6. Antimicrobial susceptibility and MIC values of Marseille-P9602 and *Chryseobacterium scophthalmum* LMG 13028T strains. CC Tested range of drug concentration in µg/ml (microgram/milliliter). MIC Minimum inhibition of concentration in µg/ml (microgram/milliliter).

| Drug (Antibiotics) | CC µg/ml | Marseille-P9602 MIC | LMG 13028T MIC |
|--------------------|----------|---------------------|----------------|
| Benzylpenicillin   | 0.016–256| 12                  | 8              |
| Amikacin           | 0.016–256| 8                   | >256           |
| Ampicillin         | 0.016–256| 128                 | 48             |
| Gentamicin         | 0.64–1024| 6                   | 64             |
| Ciprofloxacin      | 0.002–32 | 0.25                | 0.38           |
| Ceftriazone        | 0.016–256| 12                  | 12             |
| Streptomycin       | 0.064–1024| 2                  | 32             |
| Daptomycin         | 0.016–256| >256                | >256           |
| Doxycyclin         | 0.016–256| 1                   | 2              |
| Tetracycline       | 0.016–256| 3                   | 4              |
| Gentamicin         | 0.064–1024| >256               | >256           |
| Metronidazole      | 0.016–256| >256                | >256           |
| Rifampicin         | 0.002–32 | 0.004               | 0.38           |
| Vancomycin         | 0.016–256| 48                  | 24             |

Table 7. Cellular fatty acid composition of Marseille-P9602 strain compared with related species. Taxa: 1, Marseille-P9602; 2, *C. scophthalmum*; 3, *C. piscium*; 4, *C. indoltheticum*; 5, *C. balustinum*. The results presented for 1 and 2 were obtained in the present study. The results presented for 3, 4 and 5 were completed using previously published studies. Analysis of the fatty acid methyl esters was performed by Gas liquid chromatography according to the instructions for the Microbial Identification System (MIDI). tr, Trace (<1%); not detected (−); present (+); NA, data not available.

| Fatty acids Name       | 1     | 2     | 3     | 4     | 5     |
|------------------------|-------|-------|-------|-------|-------|
| 14:0                   | tr    | tr    | –     | –     | –     |
| 15:0                   | tr    | –     | –     | –     | –     |
| 16:0                   | 1.2   | 1.0   | 1.1   | 1.2   | 2     |
| 18:0                   | tr    | –     | –     | –     | –     |
| 16:0 3-OH              | tr    | –     | 1.3   | 1.5   | 1     |
| 17:0 2-OH              | –     | –     | –     | 1.8   | –     |
| 5:0 iso                | 4.9   | 9.6   | 4.8   | –     | –     |
| 13:0 iso               | 1.2   | tr    | 0.9   | tr    | tr    |
| 14:0 iso               | tr    | tr    | –     | –     | –     |
| 15:0 iso               | 56.7  | 50.6  | 38.3  | 32.3  | 33    |
| 17:0 iso               | tr    | 1.1   | 1.2   | tr    | 1     |
| 15:0 3-OH iso          | 3.8   | 2.4   | 5.1   | 3     | –     |
| 16:0 3-OH iso          | 10.8  | 22.1  | 9     | –     | –     |
| 17:0 3-OH iso          | tr    | 2.0   | tr    | –     | –     |
| 15:0 anteiso           | 7.5   | tr    | 2.7   | 5.3   | tr    |
| 16:1ω7                 | 9      | 10.8  | 22.1  | 9     | –     |
| 17:1ω9                 | 18.1  | 20.9  | 18.7  | 4.7   | 27    |
| 18:1ω6                 | tr    | tr    | –     | –     | –     |
| 18:1ω9                 | tr    | tr    | –     | –     | –     |
| Unknown                | 4.5   | 6.6   | 1.2   | –     | 2     |
Deposit in culture collections. Marseille-P9602T strain was deposited in the Collection of Souches de l’Unité des Rickettsies (CSUR) and Colección Española De Cultivos Tipo (CECT) strain collections under the numbers CSUR P9602 and CECT 30295, respectively.

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Author contributions

L.J.K., isolated the bacterium, conceived, and realised the experiments, analysed the data, prepared figures, and drafted the manuscript. D.R., E.G. and P.-E.F. designed the experiments, conceived the experiments, analysed the data, and drafted the manuscript, and finalized the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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