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Article

Pedobacter ghigonii sp. nov., Isolated from the Microbiota of the Planarian *Schmidtea mediterranea*

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Abstract: The planarian *S. mediterranea* is a platyhelminth with worldwide distribution that can regenerate any part of its body after amputation and has the capacity to eliminate a large spectrum of human bacterial pathogens. Surprisingly, the microbiota of *S. mediterranea* remains poorly investigated. Using the culturomics strategy to study the bacterial component of planarians, we isolated a new bacterial strain, Marseille-Q2390, which we characterized with the taxono-genomic approach that associates phenotypic assays and genome sequencing and analysis. Strain Marseille-Q2390 exhibited a 16S rRNA sequence similarity of 99.36% with *Pedobacter kyungheensis* strain THG-T17^T, the closest phylogenetic neighbor. It is a white-pigmented, Gram-negative, and rod-shaped bacterium. It grows in aerobic conditions and belongs to the family *Sphingobacteriaceae*. The genome of strain Marseille-Q2390 is 5,919,359 bp-long, with a G + C content of 40.3%. By comparing its genome with other closely related strains, the highest Orthologous Average Nucleotide Identity (Ortho-ANI) and digital DNA-DNA hybridization (dDDH) values were 85.71% and 30.50%, respectively, which were found with *Pedobacter soli* strain 15-51^T. We conclude that strain Marseille-Q2390^T is sufficiently different from other nearby species to be classified within a new species for which we propose the name *Pedobacter ghigonii* sp. nov.

Keywords: culturomics; taxono-genomics; *Schmidtea mediterranea*; *Pedobacter ghigonii*



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1. Introduction

The platyhelminth *Schmidtea mediterranea* is an invertebrate living in freshwater such as ponds, lakes, and rivers. It is used as a model of regeneration because of its unique capacity to regenerate after amputation [1]. In addition, planarians have been shown to be among the models useful for the investigation of the host–pathogen relationship in the context of human pathogens [2–4]. The microbiota profile of *S. mediterranea* remains poorly investigated [5,6]. Using a microbial culturomics approach [7], we investigated the *S. mediterranea* microbiota. Culturomics is a strategy in which diversified culture conditions are used to isolate a maximum of bacterial species [8,9]. Through this methodology, we isolated a bacterium [10], Marseille-Q2390, from *S. mediterranea* that could not be identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) [11,12]. We used the taxono-genomics strategy, which combines phenotypic assays and genome sequencing, to characterize this bacterium [13,14]. Regarding genotypic criteria, this was first based on the 16S rRNA gene [15], but the conventional low divergence between two 16S rRNA genes from two organisms resulted in a slight and limited bacterial description [16,17]. However, the use of the genome gave access to complete genetic information and made it possible to evaluate the degrees of genomic similarity using

tools such as the Genome-to-Genome Distance Calculator (GGDC) [18] and Orthologous Average Nucleotide Identity (Ortho-ANI) [19]. This genus *Pedobacter* [20] belongs to the family Sphingobacteriaceae [21] and has mostly been isolated from the environment [21,22] and in animals [23]. The main characteristics of this genus are that it is rod-shaped, aerobic, Gram-negative, and does not involve the formation of endospores; it is catalase-, oxidase-, and phosphatase-positive; the major fatty acids are C15:0 iso, C17:0 iso 3-OH, C15:0 iso 2-OH and C16:1 ω 7c; and the species are phylogenetically closely related at the 16S rRNA gene level (>95%), except *Pedobacter saltans* [20]. Here we describe the bacterium Marseille-Q2390, which exhibited enough genetic and phenotypic differences from closely related species to be classified in a new species for which the name *Pedobacter ghigonii* sp. nov. is proposed.

2. Materials and Methods

2.1. Culture of *Schmidtea mediterranea*

We used the *S. mediterranea* asexual clonal line CIW4 [23], which had been preserved in our laboratory for the previous ten years by cutting animals from tree fragments each month. The *S. mediterranea* were fed once per week with homogenized calf liver. Planarians were grown in filtered tap water at 19 °C. The water used was obtained by a device consisting of two 0.2 μ m filters, one containing charcoal and ceramics (Fairey Industrial Ceramics Limited, Suffolk, England), and the second being a simple 0.22 μ m pore membrane (Thermo Scientific Nalgene Filtration Products, Mexico City, Mexico). Filtered water was checked for sterility prior to being used for *S. mediterranea*, using 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'étoile, France) at different volumes (25, 50, 75, and 100 μ L) and incubated at various temperatures (5, 10, 19, 28, 37, and 45 °C) for four days.

2.2. Isolation and Identification of the Bacteria from *Schmidtea mediterranea*

Before being used for experimentation, *S. mediterranea* worms were starved for two weeks, washed in filter-sterilized water, and then one ground worm was inoculated in Buffered Charcoal Yeast Extract (BCYE) (Oxoid Deutschland GmbH, Wesel, Germany), Luria Bertani (LB), and 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'étoile, France). All inoculated media were incubated at 19, 28, and 37 °C. Each individual bacterial colony was harvested and identified by MALDI-TOF-MS (Microflex spectrometer; Bruker Daltonics, Bremen, Germany). The MALDI Biotyper RTC software was used to interpret the results according to the obtained score values: a colony was judged to be likely to be identified at the species level if it gained a score \geq 2.0; probably identified at the genus level if it gained a score between 1.99 and 1.7; and not identified if it gained a score < 1.7.

2.3. DNA Extraction, Sequencing, Assembly, and Annotation

The genomic DNA of the strain Marseille-Q2390 was extracted using an EZ1 BioRobot and the EZ1 DNA tissue kit (Cat No./ID: 953034, Qiagen, Hilden, Germany). Genomic material was quantified using a Qubit assay (Life Technologies, Carlsbad, CA, USA) at 0.2 ng/ μ L, and then prepared and sequenced using the Mate-Pair strategy with a Miseq sequencer (Illumina, San Diego, CA, USA) [24] using the Nextera XT DNA sample prep kit (Illumina). The Miseq run were checked to evaluate quality using FastQC 0.11.8 [25] then trimmed using Trimmomatic 0.36.6 [26], with default parameters. Sequencing reads were assembled using Spades 3.12 [27], the “conservative” option was used to reduce the number of mismatches and short indels, and default parameters were applied. Genomic annotation of the strain Marseille-Q2390 was made using the Prokaryotic genome annotation 1.14.5 (Prokka) [28] with default parameters.

2.4. Phylogenetic Analysis

The taxonomic assignment was obtained by a BLASTn search in the nr database. A 98.65% sequence similarity threshold was used to delineate a new putative species

by comparison with the phylogenetically closest species found in the nomenclature [29]. Phylogenetic relationships were inferred from the comparison of 16S rRNA sequences using the MEGAX 10.1 software [30,31], using the Maximum Likelihood Phylogenetic model. The sequences were aligned using the MUSCLE algorithm default parameters. Numbers at the nodes were percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree (bootstrap values $\geq 50\%$ were retained). The scale bar indicated a 1% sequence divergence.

2.5. Genomic Comparison

Clusters of Orthologous Groups (COG) [32] functional category comparison was carried out in Blastp. Degrees of genomic similarity were evaluated using the GGDC (<http://ggdc.dsmz.de/ggdc.php>, GGDC Genome-to-Genome Distance Calculator 2.1) [18] and Ortho-ANI (<https://www.ezbiocloud.net/tools/orthoani>, OrthoANI Tool Version 0.93.1) [19] software.

2.6. Phenotypic Characteristics of Strain Marseille-Q2390

Culturing of strain Marseille-Q2390 was attempted at various growth temperatures (4, 19, 28, 30, 37, and 45 °C) in 5% sheep blood-enriched Columbia agar (bioMérieux) under aerobic and anaerobic atmospheres (using GasPak™ EZ generators (Becton-Dickinson, Maryland, MD, USA)). A sporulation assay was undertaken by thermal shock. Bacteria were exposed to a temperature of 80 °C for 30 min. Then, bacterial growth was monitored for four days. The bacterial growth was also tested in various salinity (0, 20, 40, 50, 60, 80, and 100 g/L) and pH (5, 5.5, 6, 6.5, 7.5, 8.5, 9, and 10) conditions. Gram staining and motility from fresh colonies were observed using a DM1000 photonic microscope (Leica Microsystems, Nanterre, France) with a 40× objective lens and 10× ocular lens. Bacterial structure was evaluated by scanning electron microscope (Hitachi SUV5000) (Hitachi High-Technologies Corporation, Science & Medical Systems Business Group, Tokyo, Japan). Catalase and oxidase activities were investigated using BBL DrySlide, in accordance with the manufacturer's instructions (Becton Dickinson, Le Pont de Claix, France). The biochemical characteristics were identified using API strips (API ZYM [33–35], API 20NE [36,37], API 20E [38,39], and API 50CH [40–43], bioMérieux).

2.7. Antibiotic Susceptibility

After 48 h of growth, the colonies of the strain Marseille-Q2390 were suspended in saline to match the McFarland 0.5 turbidity standard. Columbia agar enriched with 5% sheep blood (bioMérieux) was inoculated with a suspension of the bacterial isolate. E-test strips (bioMérieux) were put on the surface of the 5% sheep blood-enriched Columbia agar, and the agar was incubated in an aerobic atmosphere at 28 °C for 48 h. The susceptibility of the strain Marseille-Q2390 was assessed for the benzylpenicillin, amoxicillin, ampicillin, ceftriaxone, imipenem, ciprofloxacin, amikacin, gentamicin, streptomycin, daptomycin, doxycycline, metronidazole, rifampicin, fosfomycin, vancomycin, and tigecycline. 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 [44].

2.8. Analysis of Cellular Fatty Acids of the Strain Marseille-Q2390

Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS. Two samples were prepared with 120 mg of bacterial biomass per tube harvested from several culture plates. Fatty acid methyl esters were prepared as described by Sasser [45] and GC/MS analysis was carried out as previously described [46]. Briefly, 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). A spectral database search was performed using MS Search 2.0, operated with the Standard Reference Database 1A (NIST, Gaithersburg, MA, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK).

3. Results and Discussions

3.1. MALDI-TOF-MS

MALDI-TOF-MS analysis showed that the spectrum of the strain Marseille-Q2390 corresponds to the spectrum of *Pedobacter soli* with a score of 1.8. This spectrum similarity score of 1.8 does not allow the classification of the strain Marseille-Q2390 as *Pedobacter soli*, because this value is less than 2. However, it was probably a strain belonging to the genus of *Pedobacter* at an earlier time since this score was between 1.7 and 1.99.

3.2. Phylogenetic Analysis

The gene 16S rRNA sequence from strain Marseille-Q2390 was 1519 bp. A sequence similarity calculation using the BLASTn search in the nr database indicated that the closest relatives of the strain Marseille-Q2390 were *Pedobacter kyungheensis* strain THG-T17^T (99.36%) [47], *Pedobacter roseus* strain CL-GP80^T [48], *P. soli* strain 15-51^T [49], *P. borealis* strain G-1^T [50], *P. alluvionis* strain NWER-II11^T [50], *Pedobacter miscanthi* strain RS10^T [51], *P. ginsenosidimutans* strain THG-45^T [52], *P. suwonensis* strain 15-52^T [53], *P. jejuensis* strain THG-DR3^T [54], *P. kyonggii* strain K-4-11-1^T [55], *P. nototherniae* strain 36B243^T [56], *P. psychrotolerant* strain V5RD^T [55], *P. zaeae* strain 22^T [57], *P. agri* PB92^T [58], *P. terrae* strain DS-57^T [59], *P. rhizosphaerae* strain 01-96^T [49], *P. jeongneungensis* strain BH45^T [60], *P. vanadiisoli* strain XNV015^T [61], *P. humicola* strain R135^T [62], *P. lithocola* strain CCM 8691^T [63], *P. sandarakinus* strain DS-27^T [64], *P. jamesrossensis* strain CCM 8689^T [63], *P. petrophilus* strain CCM 8687^T [63], *P. ginsengiterrae* strain DCY49^T [65], *P. heparinus* strain DSM 2366^T [21], *P. changchengzhani* strain E01020^T [66], *P. seoulensis* strain THG-G12^T [67], and *P. schmidtea* strain EGT [23], for which the similarity values and accession numbers are presented in Table 1. Although the species name *Pedobacter wanjuense* strain PL247-sym is not taxonomically correct, it is important to point out that there is a closely related 16S rRNA sequence in the genebank repository (KP277503.1) [68]. The 16S rRNA-based phylogenetic tree showed that strain Marseille-Q2390, *P. soli* strain 15-51^T, and *P. kyungheensis* strain THG-T17^T formed a monophyletic group with a high bootstrap value (54%), which was supported by both tree-making analyses (Figure 1). Strain Marseille-Q2390 is a member of the family Sphingobacteriaceae [21] within the phylum Bacteroidetes [69], from the class of Sphingobacteriia [70] and the order of Sphingobacteriales [71], and of the genus *Pedobacter* [20] (Table 2). This result confirmed the data from the MALDI-TOF-MS analysis, showing that it is of the genus *Pedobacter*. Without this, the use of one gene (16S rRNA) would not have been sufficient to confirm such a result, so it would have been necessary to use the complete genome.

Table 1. Taxonomic assignment obtained by a BLASTn search of 16S rRNA genes of the strain Marseille-Q2390.

Names	Cover	Marseille-Q2390	
		Identification Percentage	Accession
<i>Pedobacter kyungheensis</i> strain THG-T17 ^T	82%	99.36%	NR_132668.1
<i>Pedobacter roseus</i> strain CL-GP80 ^T	89%	98.68%	NR_043555.1
<i>Pedobacter soli</i> strain 15-51 ^T	97%	98.59%	NR_115008.1
<i>Pedobacter borealis</i> strain G-1 ^T	92%	98.23%	NR_044381.1
<i>Pedobacter alluvionis</i> strain NWER-III1 ^T	92%	98.21%	NR_044382.1
<i>Pedobacter miscanthi</i> strain RS10 ^T	95%	97.99%	NR_164958.1
<i>Pedobacter ginsenosidimutans</i> strain THG-45 ^T	95%	97.93%	NR_108685.1
<i>Pedobacter suwonensis</i> strain 15-52 ^T	94%	97.78%	NR_043543.1
<i>Pedobacter jejuensis</i> strain THG-DR3 ^T	93%	97.68%	NR_133810.1
<i>Pedobacter kyonggii</i> strain K-4-11-1 ^T	95%	97.65%	NR_159165.1
<i>Pedobacter nototherniae</i> strain 36B243 ^T	91%	97.62%	NR_164976.1
<i>Pedobacter psychrotolerant</i> strain V5RD ^T	89%	97.57%	NR_152669.1
<i>Pedobacter zeae</i> strain 22 ^T	97%	97.57%	NR_156064.1
<i>Pedobacter agri</i> PB92 ^T	95%	97.45%	NR_044339.1
<i>Pedobacter terrae</i> strain DS-57 ^T	97%	97.43%	NR_044005.1
<i>Pedobacter rhizosphaerae</i> strain 01-96 ^T	97%	97.31%	NR_122096.1
<i>Pedobacter jeongneungensis</i> strain BH45 ^T	95%	97.30%	NR_132685.1
<i>Pedobacter vanadiisoli</i> strain XNV015 ^T	95%	97.30%	NR_153693.1
<i>Pedobacter humicola</i> strain R135 ^T	95%	97.03%	NR_149278.1
<i>Pedobacter lithocola</i> strain CCM 8691 ^T	97%	96.90%	NR_156883.1
<i>Pedobacter sandarakinus</i> strain DS-27 ^T	97%	96.82%	NR_043665.1
<i>Pedobacter jamesrossensis</i> strain CCM 8689 ^T	97%	96.68%	NR_156882.1
<i>Pedobacter petrophilus</i> strain CCM 8687 ^T	97%	96.61%	NR_156885.1
<i>Pedobacter ginsengiterrae</i> strain DCY49 ^T	91%	96.55%	NR_109023.1
<i>Pedobacter heparinus</i> strain DSM 2366 ^T	99%	96.49%	NR_074519.1
<i>Pedobacter changchengzhani</i> strain E01020 ^T	99%	96.09%	NR_164993.1
<i>Pedobacter seoulensis</i> strain THG-G12 ^T	92%	96.08%	NR_145561.1
<i>Pedobacter schmidtea</i> EG ^T	100%	96.12%	LS453293.1

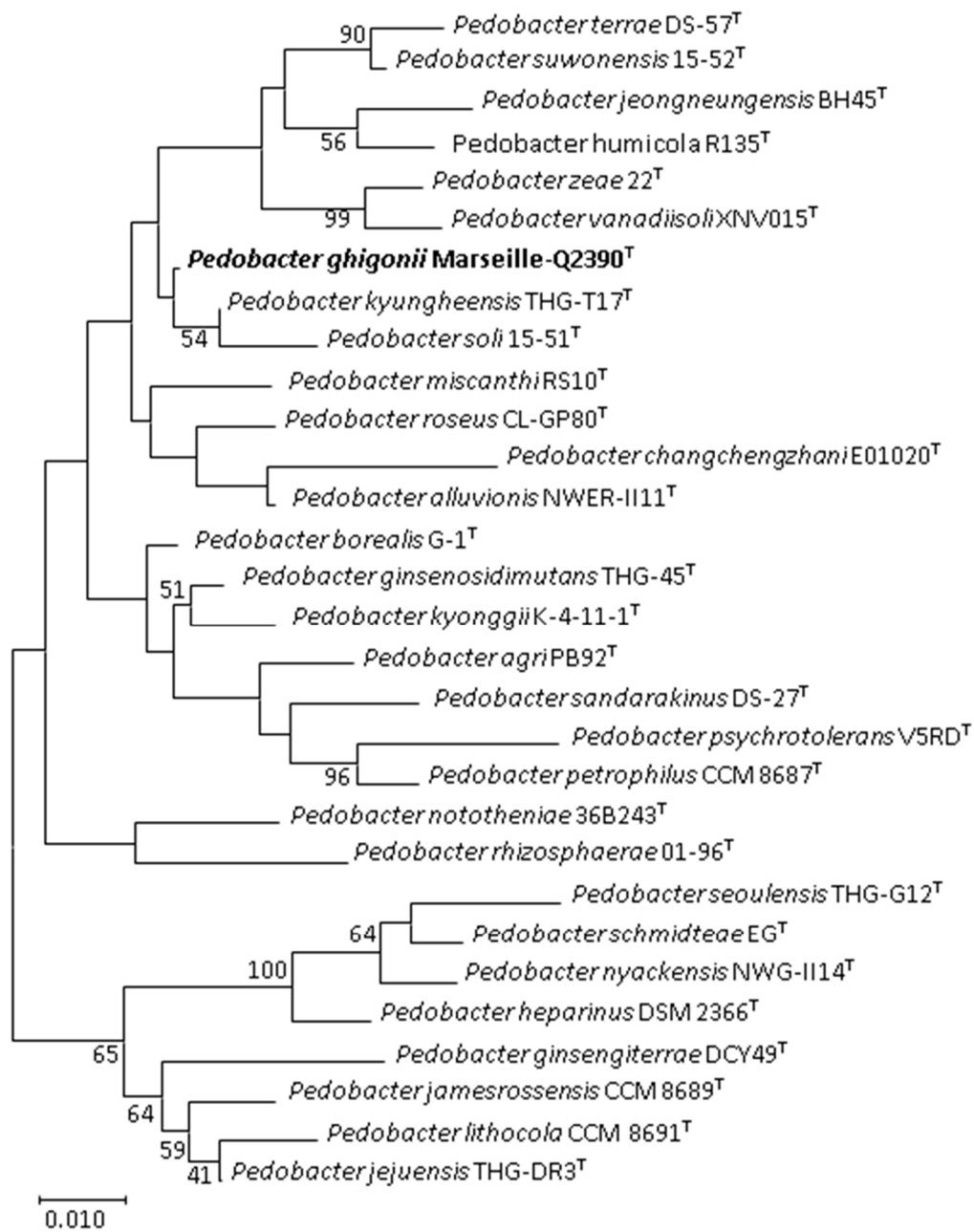


Figure 1. Phylogenetic tree based on 16S rRNA sequence comparison highlighting the position of strain Marseille-Q2390 relative to other closely related species.

Table 2. Classification and general features of the strain Marseille-Q2390.

Property	Term
Current classification	Domain: Bacteria [10]
	Phylum: <i>Bacteroidetes</i> [69]
	Class: <i>Sphingobacteriia</i> [70]
	Order: <i>Sphingobacteriales</i> [71]
	Family: <i>Sphingobacteriaceae</i> [21]
	Genus name: <i>Pedobacter</i> [20]
	Species name: <i>ghigonii</i>
	Specific epithet: <i>Pedobacter ghigonii</i>
	Type strain: Marseille-Q2390
	Species status
Gram stain	Negative
Cell shape	Rod-shaped
Motility	Motile
Sporulation	Non-spore-forming
Temperature range for growth	4–30
Temperature optimum	28
pH range for growth	5.5–10
pH optimum	7.5
pH category	Neutro-alkalophilic
Lowest NaCl concentration for growth	0
Highest NaCl concentration for growth	20 g/L
Salinity optimum	9 g/L
O ₂ conditions for strain testing	Aerobiosis
Catalase	Positive
Oxydase	Positive
Habitat	Gut microbiota of <i>Schmidtea mediterranea</i>
Biotic relationship	Symbiotic

3.3. Genomic Comparison

The genome sequence from strain Marseille-Q2390 was assembled into 41 contigs for a total size of 5,921,534 bp (N50, 292.871; L50, 7; coverage, 20×) with a G + C content of 40.3%. A total of 4.870 predicted protein-coding genes were identified, along with 7 rRNA, 49 tRNAs, and 1tmRNA (Table 3). The genome of strain Marseille-Q2390 was compared with those of *P. kyungheensis*, *P. zeae*, *P. alluvionis*, *P. borealis*, *P. ginsenosidimutans*, *P. kyonggii*, *P. soli*, *P. suwonensis*, *P. terrae*, and *P. suwonensis*. With regard to contigs, size, CDSs, GC%, tRNAs, and rRNAs, all strains were shown to have different characteristics. Digital DNA-DNA hybridization (dDDH) values obtained using the GGDC software for the strain Marseille-Q2390 ranged from 23.60% with *P. suwonensis* and *P. terrae* to 30.50% with *Pedobacter soli* and *P. kyungheensis* (Table 4 and Table S1). As strain Marseille-Q2390 was mostly clustered with *Pedobacter soli* and *P. kyungheensis* (30.50%), such values were lower than the 70% threshold recognized to delineate bacterial species [18]. Accordingly, Marseille-Q2390 is a new species of *Pedobacter*. Similarly, the Ortho-ANI values (Figure 2 and Table S1) ranged from 79.48% with *P. suwonensis* to 85.71% with *P. soli*, which was lower than the 95% threshold used to discriminate species [19]. The strain Marseille-Q2390 was grouped with the genus *Pedobacter soli* with a lower identity percentage, and thus

Marseille-Q2390 was found to be a new species of *Pedobacter*. The distribution of genes in COG functional categories is presented in Figure 3 and Table S2. We can note that the proteins [A], [Y], [Z], [W], and [X], which are typically involved in several functions in bacteria, was not produced in the genus *Pedobacter*. Moreover, the other proteins were produced in all the species studied, which means that the quality of the proteins was similar in all compared species; but the quantity produced was different from one species to another species. Thus, with the genomic data we could confirm that strain Marseille-Q2390 belongs to a separate *Pedobacter* species.

Table 3. Main genomic characteristics of the strain Marseille-Q2390 and other closely related *Pedobacter* species.

Name	Contigs	Size (bp)	CDSs	GC%	tRNAs	rRNAs	Refseq
<i>P. soli</i>	38	6,006,420	4,923	40.5	49	6	NZ_FMZH00000000.1
<i>P. ghigonii</i>	41	5,921,534	4,870	40.3	49	7	CAESCM00000000.1
<i>P. kyungheensis</i>	67	6,358,642	5,270	40.5	52	6	NZ_JSYN00000000.1
<i>P. borealis</i>	216	5,544,917	4,610	38.4	50	3	NZ_JAUG00000000.1
<i>P. zeae</i>	15	5,444,802	4,567	40.3	49	3	NZ_JACIEF00000000.1
<i>P. alluvionis</i>	20	6,037,645	5,006	38.4	46	4	NZ_RCCCK00000000.1
<i>P. kyonggii</i>	73	6,186,183	5,107	38.8	50	8	NZ_SIXF00000000.1
<i>P. ginsenosidimutans</i>	86	6,517,553	5,301	38.7	52	5	NZ_LMZQ00000000.1
<i>P. suwonensis</i>	40	5,803,831	4,738	39.5	47	3	NZ_FOJM00000000.1
<i>P. terrae</i>	63	5,755,101	4,783	38.8	46	3	NZ_FNCH00000000.1

Table 4. Digital DNA-DNA hybridization (dDDH) values obtained through a comparison of all studied genomes using the Genome-to-Genome Distance Calculator (GGDC), formula 2 (DDH estimates based on identities/HSP length).

Digital DNA-DNA Hybridization	1	2	3	4	5	6	7	8	9
<i>P. borealis</i>	23.90								
<i>P. ginsenosidimutans</i>	23.30	45.70							
<i>P. zeae</i>	24.50	26.10	25.70						
<i>P. kyonggii</i>	23.60	45.80	44.40	25.50					
<i>P. ghigonii</i>	30.50	24.50	23.80	24.00	23.80				
<i>P. alluvionis</i>	23.50	31.70	31.10	26.30	31.10	23.90			
<i>P. soli</i>	56.40	23.70	23.50	24.40	23.50	30.50	23.40		
<i>P. suwonensis</i>	24.20	26.70	26.30	25.20	26.00	23.60	27.50	23.90	
<i>P. terrae</i>	23.60	28.40	27.60	25.30	27.50	23.60	30.30	23.70	31.70

Taxa: 1, *P. kyungheensis*; 2, *P. borealis*; 3, *P. ginsenosidimutans*; 4, *P. zeae*; 5, *P. kyonggii*; 6, *P. ghigonii*; 7, *P. alluvionis*; 8, *P. soli*; 9, *P. suwonensis*; 10, *P. terrae*. The strain Marseille-Q2390 had a higher percentage of hybridization with *Pedobacter soli* and *P. kyungheensis* compared to other species, but these hybridization values were below the threshold of 70% recognized for the delimitation of bacterial species [18].

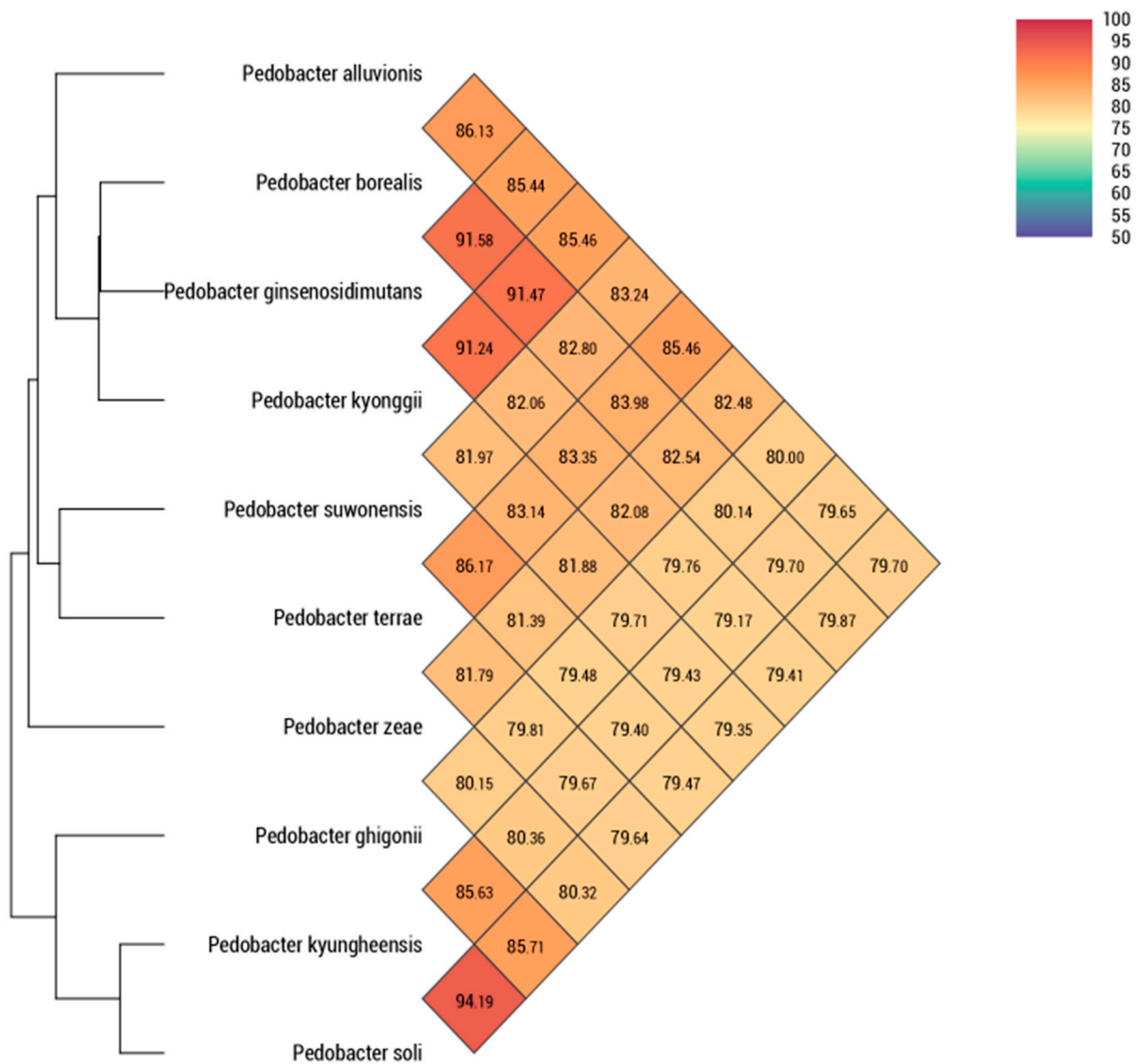


Figure 2. Heatmap generated with Orthologous Average Nucleotide Identity (Ortho-ANI) values calculated using the OAT software, comparing *Pedobacter ghigonii* and other closely related species with standing in the nomenclature. The color code indicates the closest species with green and the farthest with red. The strain Marseille-Q2390 is mainly grouped with the genus *Pedobacter solis* and, with less similarity, *Pedobacter kyungheensis* [19].

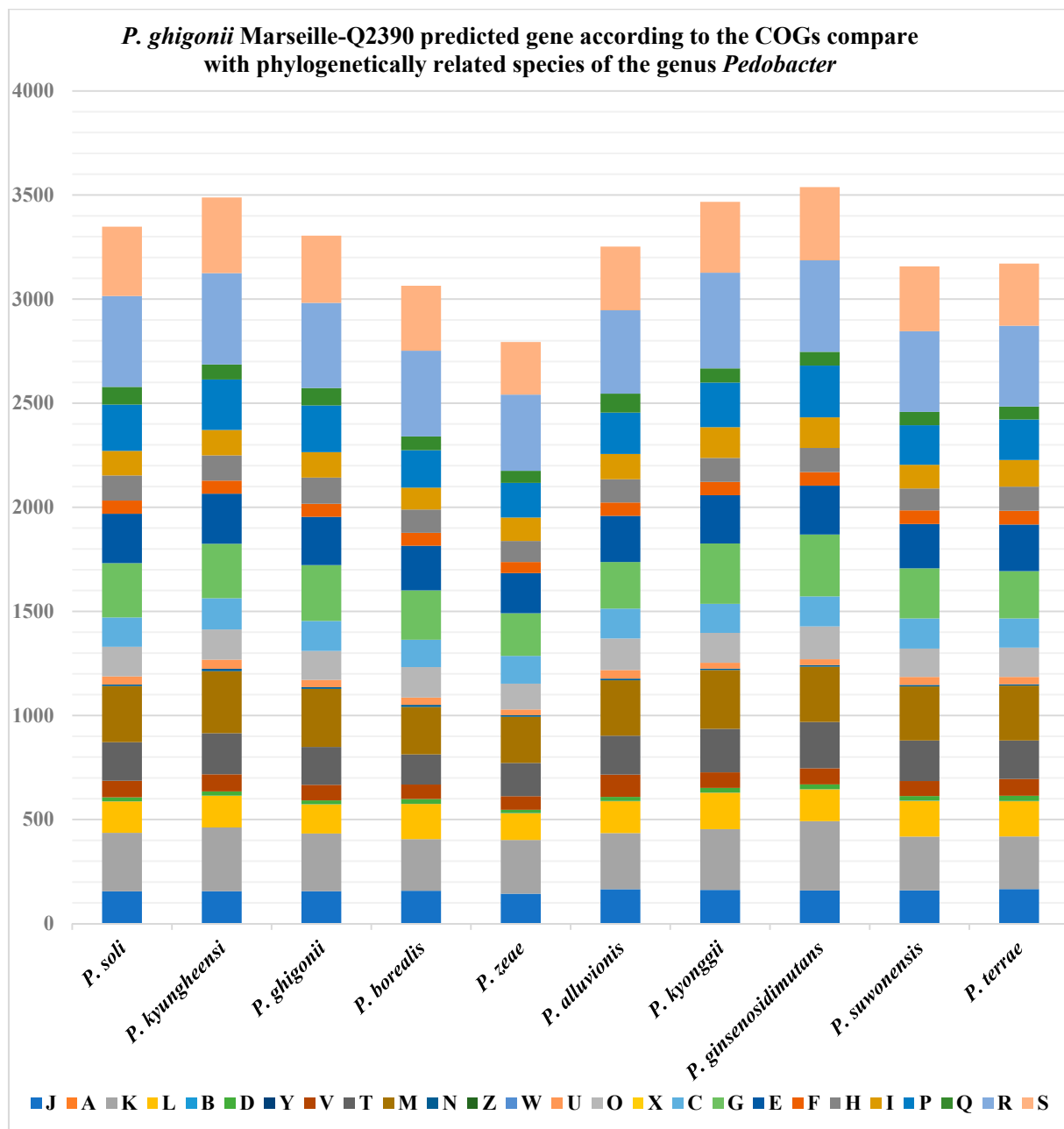


Figure 3. Comparison of gene functional categories of the strain Marseille-Q2390 and other *Pedobacter* species.

3.4. Phenotypic Characteristics of Strain Marseille-Q2390

Strain Marseille-Q2390 was isolated on Columbia agar after two days at 28 °C in an aerobic atmosphere at pH 7. Strain Marseille-Q2390 grew at temperatures ranging from 4 to 30 °C in an aerobic atmosphere and at pH values ranging from 6 to 10 (neutro-alkalophilic bacterium). It also grew at salinity concentrations lower than 9 g/L. After four days of culture on Columbia agar, colonies of strain Marseille-Q2390 were white, small (0.3 mm median diameter), circular with a convex shape, and smooth. Bacterial cells were Gram-negative (Figure 4), rod-shaped, non-spore-forming, and motile bacilli, but without any flagellum. Their mean length and width were 2.25 µm and 0.86 µm, respectively (Figure 5). Strain Marseille-Q2390 exhibited positive oxidase and catalase activities. Positive and negative reactions obtained using API 50CHB/E, API 20NE, API Zym, and API 20E strips are show in Table 5. These data were compared to those of closely related species data, including *P. soli* 15–51^T and *P. borealis* G1^T, as previously described [49,50].

Strain Marseille-Q2390 differed from all other compared species of *P. soli* in the use of α -mannosidase, L-arabinose, D-xylose, D-galactose, D-fructose, L-rhamnose, methyl- α D-mannopyranoside, methyl- α D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, starch, glycogen, gentiobiose, and D-turanose.

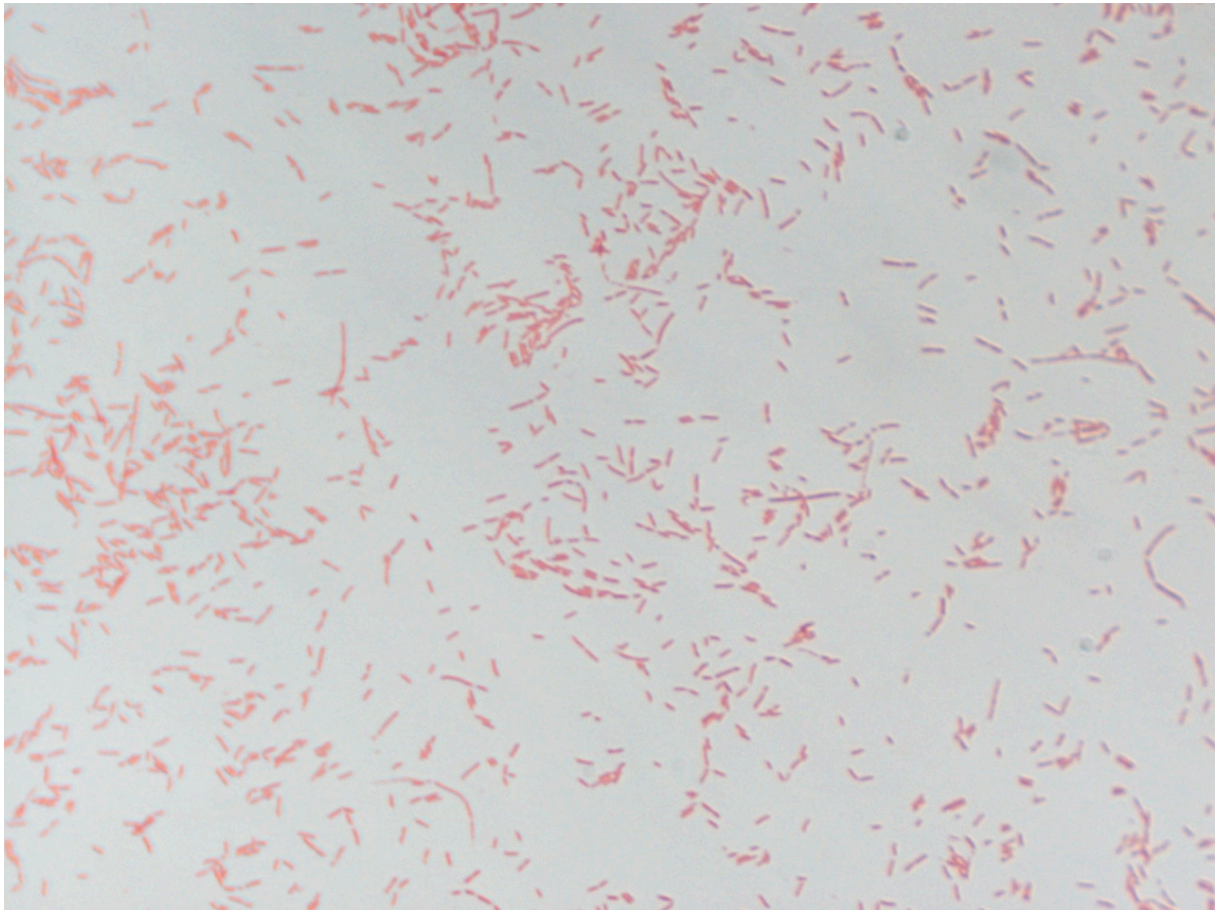


Figure 4. Gram staining of the strain Marseille-Q2390 at 100 \times magnification.

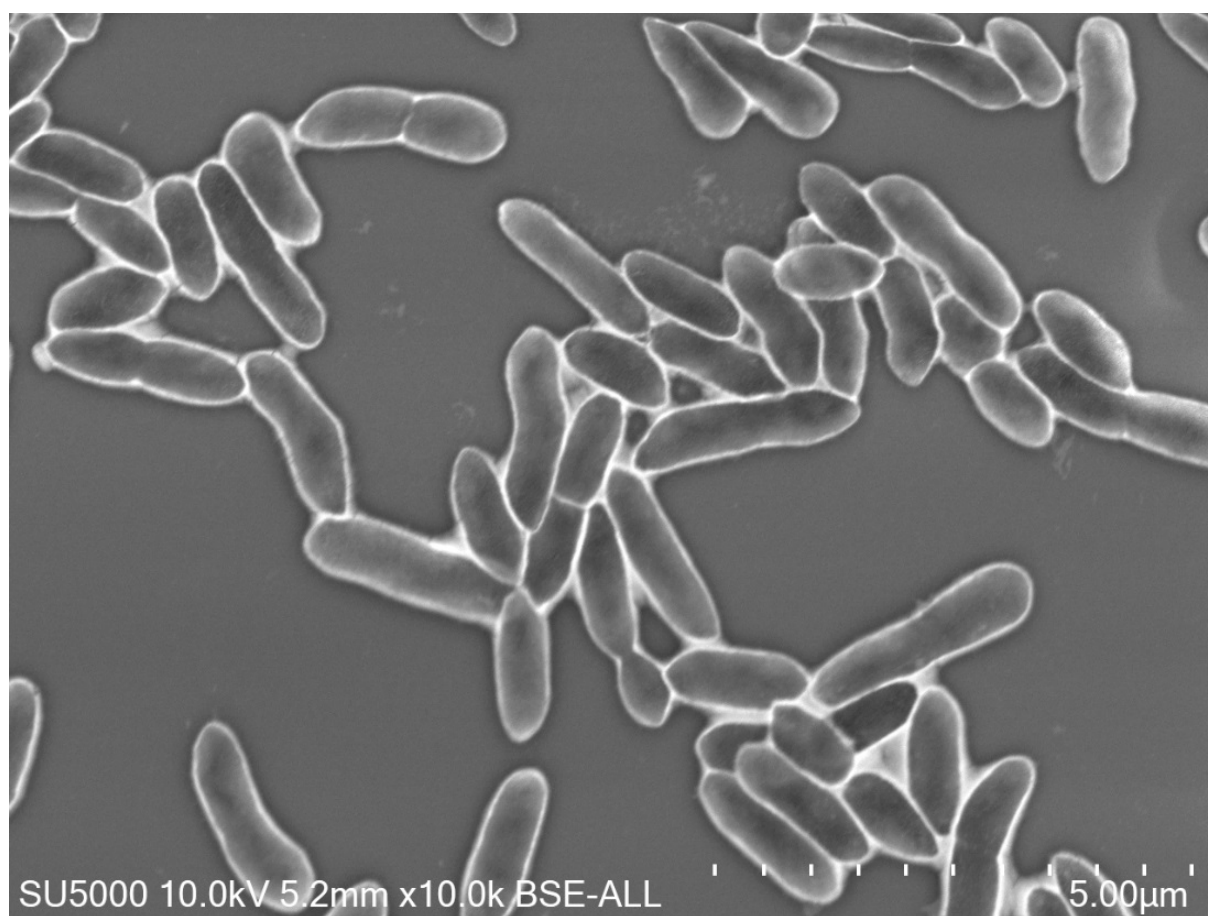


Figure 5. Transmission electron microscopy of the strain Marseille-Q2390. Bacterium was rod-shaped and without flagellum. Scale bar = 5 μ m.

Table 5. Physiological characteristics of strain Marseille-Q2390 and phylogenetically related species of the genus *Pedobacter*.

Characteristics	1	2	3	4	5	6	7	8	9	10
Gram-staining	-	-	-	-	-	-	-	-	-	-
Sporulation	-	-	-	-	-	-	-	-	-	-
Growth temperature range ($^{\circ}$ C)	4–30	4–35	4–30	4–40	4–35	4–30	1–37	0–32	15–30	4–30
Aerobic growth	+	+	+	+	+	+	+	+	+	+
Source	Planarian	Rhizosphere	Soil	Soil	Soil	Soil	Rhizosphere	Soil	Maize root	Floodplain
Colony color	White	Pinkish yellow	Reddish pink	Pink	Orange	Pink	Pinkish yellow	Light salmon	Pinkish yellow	Reddish pink
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+
Enzyme activity (API ZYM):										
Alkaline phosphatase	+	+	+	+	+	+	+	+	+	+
Esterase (C4)	+	+	+	-	+	+	+	+	+	+
Esterase lipase (C8)	+	+	+	+	+	+	+	+	+	+
Lipase (C14)	-	-	-	-	-	-	-	-	-	-
Leucine arylamidase	+	+	+	+	+	+	+	+	+	+
Valine arylamidase	+	+	+	+	+	+	+	+	+	+
Cystine arylamidase	+	+	+	-	-	-	-	+	+	+
Trypsin	+	+	+	-	-	+	+	+	+	+
α -chymotrypsin	-	-	-	-	-	+	-	NA	-	-

Table 5. Cont.

Characteristics	1	2	3	4	5	6	7	8	9	10
D-Saccharose	-	+	+	+	+	+	+	+	+	+
D-Trehalose	-	+	+	NA	+	NA	+	NA	+	+
Inulin	-	-	-	NA	+	NA	-	NA	-	+
D-Melezitose	-	+	-	NA	-	NA	+	NA	-	-
D-Raffinose	-	+	+	NA	+	NA	+	NA	+	+
Starch	-	+	+	-	+	+	+	NA	-	+
Glycogen	-	+	-	+	-	-	+	-	+	-
Xylitol	-	-	-	NA	-	NA	-	NA	-	-
Gentiobiose	-	+	+	NA	+	NA	+	NA	-	+
D-Turanose	-	+	+	NA	+	NA	+	NA	+	+
D-lyxose	-	-	-	NA	-	NA	-	-	+	-
D-Tagatose	-	-	-	NA	-	NA	-	-	+	-
D-Fucose	-	-	-	-	-	NA	-	NA	-	-
L-Fucose	-	-	-	-	-	-	-	-	+	-
D-Arabitol	-	-	-	NA	-	NA	-	NA	-	-
L-Arabitol	-	-	-	-	-	-	-	-	-	-
Potassium Gluconate	-	-	-	-	-	-	-	-	-	-
Potassium 2-ketoGluconate	-	-	-	-	-	-	-	-	-	-
Potassium 5-ketogluconate	-	-	+	-	-	-	-	-	-	-
API 20E										
Natriumthiosulfat	-	NA	NA	NA	-	NA	NA	-	NA	NA
L-tryptophan	+	NA	NA	-	-	NA	NA	-	NA	NA
Indole production	-	NA	-	-	-	-	-	-	-	-
API 20NE										
Potassium nitrate	-	NA	-	-	-	-	-	-	-	-
L-arginine	-	NA	+	-	-	-	-	-	-	+
Urea	-	NA	-	-	-	-	-	-	-	-
Gelatin	-	+	+	NA	+	+	+	+	+	+
Capric acid	+	NA	NA	+	-	-	-	-	-	NA
Adipic acid	-	NA	NA	-	-	-	-	-	-	NA
Malic acid	+	NA	NA	-	-	+	-	-	-	-
Trisodium citrate	+	NA	NA	-	-	-	-	-	+	-
Phenylacetic acid	-	NA	NA	-	-	-	-	-	-	NA

Taxa: 1, Marseille-Q2390; 2, *Pedobacter soli* 15-51^T [49]; 3, *P. borealis* G1^T [50]; 4, *P. kyungheensis* strain THG-T17^T [47]; 5, *P. terrae* strain DS-57^T [59]; 6, *P. ginsenosidimutans* strain THG-45^T [52]; 7, *P. suwonensis* strain 15-52^T [53]; 8, *P. kyonggii* strain K-4-11-1^T [55]; 9, *Pedobacter zeae* strain 22^T [57]; 10, *Pedobacter alluvionis* strain NWER-III1^T [50]. The data were completed using previously described [47,49,50,52,53,55,57,59] characteristics and those obtained in the present study. positive (+);negative (-); NA, data not available.

3.5. Antibiotic Susceptibility

Strain Marseille-Q2390 was susceptible to ceftriaxone, imipenem, ciprofloxacin, amikacin, gentamicin, streptomycin, doxycyclin, rifampicin, fosfomycin, and tigecycline, but it was resistant to benzylpenicillin, amoxicillin, ampicillin, daptomycin, metronidazole, and vancomycin (Table 6). Accordingly, strain Marseille-Q2390 was found to be resistant to antibiotics of the β -lactamin family.

Table 6. Antimicrobial susceptibility and MIC values of strain Marseille-Q2390.

Drug (Antibiotics)	CC µg/mL	<i>P. ghigonii</i> MIC µg/mL
Benzylpenicilin	0.016–256	>256
Amoxicillin	0.016–256	>256
Ampicillin	0.016–256	>256
Ceftriaxone	0.016–256	128
Imipenem	0.002–32	0.047
Ciprofloxacin	0.002–32	0.25
Amikacin	0.016–256	1
Gentamicin	0.64–1024	0.5
Streptomycin	0.064–1024	6
Daptomycin	0.016–256	>256
Doxycyclin	0.016–256	1.5
Metronidazole	0.016–256	>256
Rifampicin	0.002–32	16
Fosfomycin	0.064–1024	192
Vancomycin	0.016–256	>256
Tigecyclin	0.016–256	4

CC: tested range of drug concentration in µg/mL, MIC: minimum inhibition of concentration in µg/mL.

3.6. Cellular Fatty Acids Analysis

The fatty acids were 13-methyl-tetradecanoic acid (54.5%), 9-hexadecenoic acid (11.1%), 11-hexadecenoic acid (8.6%), 3-hydroxy-15-methyl-hexadecenoic acid (5.5%), 15-methyl-hexadecenoic acid (3.5%), 3-hydroxy-13-methyl-tetradecanoic acid (3.5%), and 3-methyl-butanoic acid (3.0%). Minor amounts of other fatty acids included hexadecanoic acid (1.1%), 8-methyl-decanoic acid (1.7%), 14-methyl-hexadecenoic acid (1.1%), 9-methyl-decanoic acid (1.8%), and 8-pentadecenoic acid (1.1%) (Table 7). Comparing the fatty acid profile of strain Marseille-Q2390 with *P. soli* 15–51^T, Marseille-Q2390 can be seen to differ from *P. soli* 15–51^T due to the presence of 11:0 anteiso, 5:0 iso, and 15:1ω7. 13-methyl-tetradecanoic acid (15:0 iso) was found in all strains; thus, 15:0 iso could be used as a *Pedobacter* signature marker.

Table 7. Cellular fatty acid composition of strain Marseille-Q2390 and related species of the genus *Pedobacter*.

Fatty Acids	Name	1	2	3	4	5	6	7	8	9	10
Straight-Chain Saturated											
10:0	Decanoic acid	tr	-	-	1.92	-	-	-	-	-	-
14:0	Tetradecanoic acid	tr	tr	1.0	-	-	-	tr	1.2	2.6	0.7
15:0	Pentadecanoic acid	tr	-	2.0	-	-	-	-	-	-	1.1
16:0	Hexadecanoic acid	1.1	4.1	-	2.54	1.9	4.7	1.6	2.2	tr	0.7
14:0 2-OH	2-hydroxy-tetradecanoic acid	-	tr	tr	-	-	-	-	tr	tr	-
16:0 3-OH	3-hydroxy-hexadecanoic acid	tr	2.1	2.2	-	1.6	1.4	tr	1.4	tr	-
17:0 2-OH	2-hydroxy-hexadecenoic acid	-	-	-	-	-	1.7	-	tr	-	-
Branched Saturated											
11:0 anteiso	8-methyl-decanoic acid	1.1	-	-	-	-	-	-	-	-	-
13:0 anteiso	10-methyl-dodecanoic acid	tr	-	-	-	-	-	-	-	-	-
17:1 anteiso	14-methyl-hexadecenoic acid	1.1	1.0	2.3	-	1.2	-	-	1.5	tr	-
15:0 anteiso		-	tr	2.1	1.76	2.1	3.3	tr	tr	tr	1.7
5:0 iso	3-methyl-butanoic acid	3.0	-	-	-	-	-	-	-	-	-
8:0 iso	6-methyl-octanoic acid	tr	-	-	-	-	-	-	-	-	-
11:0 iso	9-methyl-decanoic acid	1.8	tr	-	-	-	-	-	-	tr	-
14:0 iso	12-methyl-tridecanoic acid	tr	-	-	-	-	-	-	-	-	-
15:0 iso	13-methyl-tetradecanoic acid	54.5	29.6	25.4	24.8	28.3	30.1	35.4	27.0	37.0	36.6
17:1 iso	15-methyl-hexadecenoic acid	3.5	tr	tr	-	1.2	-	-	tr	tr	-
15:0 3-OH iso	3-hydroxy-13-methyl-tetradecanoic acid	3.5	2.0	3.3	4.41	3.5	-	2.4	2.6	3.0	-
16:0 3-OH iso	3-hydroxy-13-methyl-hexadecanoic acid	-	tr	tr	-	tr	-	-	tr	tr	-
17:0 3-OH iso	3-hydroxy-15-methyl-hexadecenoic acid	5.5	12.3	14.6	20.1	20.4	18.2	-	12.8	7.9	13.7

Table 7. Cont.

Fatty Acids	Name	1	2	3	4	5	6	7	8	9	10
Mono-Unsaturated											
14:1 ω 5	9-tetradecenoic acid	tr	-	-	1.33	-	-	-	-	tr	-
15:1 ω 7	8-pentadecenoic acid	1.7	-	-	-	-	-	-	-	-	-
16:1 ω 7	9-hexadecenoic acid	11.1	33.1	30.6	30.0	27.7	24.5	27.2	29.3	31.4	20.2
16:1 ω 5	11-hexadecenoic acid	8.6	1.4	2.7	-	1.7	tr	1.4	1.4	1.4	21.1
17:1 ω 9	9-hexadecanoic acid	-	6.9	6.6	6.51	6.1	3.4	7.4	4.7	4.5	3.4
18:1 ω 9	9-octadecenoic acid	tr	-	-	-	-	-	-	-	-	-
18:2 ω 6	9,12-octadecadienoic acid	tr	-	-	-	-	-	-	-	-	-
18:1 ω 5	11-octadecenoic acid	-	tr	1.3	-	tr	-	-	tr	tr	-

Taxa: 1, Marseille-Q2390; 2, *Pedobacter soli* 15–51^T [49]; 3, *P. borealis* G1^T [50]; 4, *P. kyungheensis* strain THG-T17^T [47]; 5, *P. terrae* strain DS-57^T [59]; 6, *P. ginsenosidimutans* strain THG-45^T [52]; 7, *P. suwonensis* strain 15–52^T [53]; 8, *P. kyonggii* strain K-4-11-1^T [55]; 9, *Pedobacter zeae* strain 22^T [57]; 10, *Pedobacter alluvionis* strain NWER-III1^T [50]. tr, trace (<1%); -, not detected.

4. Conclusions

On the basis of phenotypic and genomic data, we confirmed that strain Marseille-Q2390 belongs to a new species within the *Pedobacter* genus for which we propose the name *Pedobacter ghigonii* sp. nov. strain Marseille-Q2390^T. This strain is abundant among the *Schmidtea mediterranea* planarians but has not yet been identified in any other environment. Identification of the strain Marseille-Q2390 makes it possible to study its involvement in the gut microbiota of planarians.

4.1. Protologue

Pedobacter ghigonii (ghi.go.ni'i/N.L. masc. Gen. from Ghigo, family name of Eric Ghigo a French researcher who works on planarians) is a bacterium belonging to the family *Sphingobacteriaceae* within the phylum *Bacteroidetes*. Type strain Marseille-Q2390^T was isolated, on 5% sheep blood-enriched Columbia agar after 2 days at 28 °C in aerobic atmosphere at pH 7, from the microbiota of the planarian *Schmidtea mediterranea*. Colonies were small, circular, smooth, white, and convex. Cells were Gram-negative, rod-shaped, motile, and non-spore-forming bacilli showing positive catalase and oxidase activities. The fatty acids were 3-methyl-Butanoic acid, 13-methyl-tetradecanoic acid, 15-methyl-Hexadecenoic acid, 3-hydroxy-13-methyl-Tetradecanoic acid, 3-hydroxy-15-methyl-Hexadecenoic acid, 9-Hexadecenoic acid, and 11-Hexadecenoic acid. Positive activities were detected for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase. Lipase (C14), α -chymotrypsin, and β -glucuronidase were the negative activities. Glucose, mannose, esculin ferric citrate, and maltose were assimilated, but not glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl- β D-xylopyranoside, D-galactose, D-fructose, L-sorbose, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl- α D-mannopyranoside, methyl- α D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-lactose, D-melibiose, D-saccharose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium, or 5-ketogluconate. Positives reactions were observed for L-tryptophan, natrium pyruvate, N-acetyl-glucosamine, capric acid, malic acid, and trisodium citrate. No reaction was observed for L-lysine, L-ornithine, trisodium citrate, natrium thiosulfate, indole production, gelatin, potassium nitrate, L-arginine, urea, adipic acid, or phenylacetic acid. The genome of strain Marseille-Q2390^T is 5,919,359 bp-long with a G+C content of 40.3%. The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LR797942 and CAESCM000000000.1, respectively. Type strain Marseille-Q2390^T was deposited in the Collection de Souche de l'Unité des Rickettsies (CSUR).

4.2. Nucleotide Sequence Accession Numbers

The 16S rRNA gene sequence (BioProject: PRJEB37821) was deposited in GenBank under accession numbers LR797942.1. BioProject PRJNA224116, BioSample SAMEA6828267, and Assembly GCF_903166585.1 were deposited in GenBank under accession number NZ_CAESCM010000000, and consist of sequences CAESCM010000001–CAESCM010000041. The raw data from Illumina MiSeq paired-end sequencing (BioProject: PRJEB37821, Experiment: ERX4110733 and BioSample: SAMEA6830360 (ERS4557981)) were deposited in the sequence read archive (SRA) under run accession numbers ERR4143460.

4.3. Deposit in Culture Collections

Strain Marseille-Q2390^T was deposited in the CSUR strain collections under number Q2390.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/microbiolres12020019/s1>, Table S1: dDDH and OrthoANI analysis of phylogenetically related species of the strain Marseille-Q2390, Table S2: Functional annotation of strain Marseille-Q2390 predicted gene according to the COGs database.

Author Contributions: L.J.K. conceived and realized the experiments, analyzed the data, prepared figures, and drafted the manuscript. D.R. and F.P.-E. designed the experiments, conceived the experiments, analyzed the data, and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The 16S rRNA gene sequence (BioProject: PRJEB37821) was deposited in GenBank under accession numbers LR797942.1. BioProject PRJNA224116, BioSample SAMEA6828267, and Assembly GCF_903166585.1 were deposited in GenBank under accession number NZ_CAESCM010000000, and consist of sequences CAESCM010000001–CAESCM010000041. The raw data from Illumina MiSeq paired-end sequencing (BioProject: PRJEB37821, Experiment: ERX4110733 and BioSample: SAMEA6830360 (ERS4557981)) were deposited in the sequence read archive (SRA) under run accession numbers ERR4143460.

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