microbiology research

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Metabacillus schmidteae sp. nov., Cultivated from Planarian *Schmidtea mediterranea* Microbiota

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Abstract: Taxonogenomics combines phenotypic assays and genomic analysis as a means of characterizing novel strains. We used this strategy to study Marseille-P9898^T strain, an aerobic, motile, Gram-negative, spore-forming, and rod-shaped bacterium isolated from planarian *Schmidtea mediterranea*. Marseille-P9898^T is catalase-positive and oxidase-negative. The major fatty acids detected are 12-methyl-tetradecanoic acid, 13-methyl-tetradecanoic acid, and hexadecanoic acid. Marseille-P9898^T strain shared more than 98% sequence similarity with the *Metabacillus niabensis* strain 4T19^T (98.99%), *Metabacillus halosaccharovorans* strain E33^T (98.75%), *Metabacillus malikii* strain NCCP-662^T (98.19%), and *Metabacillus litoralis* strain SW-211^T (97.15%). Marseille-P9898 strain belongs to *Metabacillus* genus. Genomic analysis revealed the highest similarities with Ortho-ANI and dDDH, 85.76% with *Metabacillus halosaccharovorans*, and 34.20% with *Bacillus acidicola*, respectively. These results show that the Marseille-P9898^T strain is a novel bacterial species from *Metabacillus* genus, for which we propose the name of *Metabacillus schmidteae* sp. nov. (Type strain Marseille-P9898^T = CSUR P9898^T = DSM 111480^T).

Keywords: taxonogenomics; *Schmidtea mediterranea*; microbiota; *Metabacillus schmidteae*; planarians

Citation: Kangale, L.J.; Raoult, D.A.; Ghigo, E.; Fournier, P.-E. *Metabacillus schmidteae* sp. nov., Cultivated from Planarian *Schmidtea mediterranea* Microbiota. *Microbiol. Res.* **2021**, *12*, 299–316. https://doi.org/10.3390/microbiolres12020021

Academic Editor: Stefan Schmidt

Received: 7 January 2021
Accepted: 30 March 2021
Published: 2 April 2021

1. Introduction

The birth of genomics, followed by the development of Next Generation Sequencing (NGS) methods, has allowed the characterization, classification, and nomenclature of many prokaryotic species using a taxonogenomics strategy combining phenotypic assays and genome sequencing [1–3]. Phenotypic assays are based on the analysis of morphological, physiological, chemical, and biochemical features of an organism [4]. Genotypic characterization, i.e., the analysis of the genetic material, is essential for species description and sheds light on the evolutionary relationships between various lineages. For the genotypic characterization, first, the gene 16S rRNA sequences are used to determine sequence similarity and for phylogenetic analysis [5]. Then Digital DNA-DNA hybridization values (dDDH) that evaluate the degree of genetic similarity between two genomes have been used for bacterial species demarcation by providing a constant numerical threshold (dDDH value > 70%) for species boundary [6].

Planarian *Schmidtea mediterranea* is an invertebrate living in freshwater and an excellent organism model to investigate the human host-pathogen relationship [7–9]. To understand the role of planarian *S. mediterranea* microbiota in the immune response, we studied its composition and isolated by culturomics [10], a bacterial strain (Marseille-P9898).

Analysis of Marseille-P9898 strain by Matrix-Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) did not allow identifying this strain,

but it revealed that it belongs to the *Bacillus* genus (now *Metabacillus* [11]). *Bacteria* belonging to the *Bacillus* genus [12] are obligate aerobic, endospore-forming bacilli. This genus was first described by Cohn F. in 1872 [13]. The type strain is *Bacillus subtilis* [14]. Recently, phylogenomic and comparative genomic analyses showed six novel genera of *Bacillus* species: *Peribacillus, Cytobacillus, Mesobacillus, Neobacillus, Alkalihalobacillus,* and *Metabacillus* [11]. According to its first characteristics, Marseille-P9898 strain could be clustered among *Metabacillus* (Me.ta.ba.cil'lus. Gr. adv. meta besides; L. masc. n. bacillus a small staff or rod, and *Bacillus*, a bacterial genus; N.L. masc. n. *Metabacillus* a genus besides *Bacillus*), which was approved in the family *Bacillaceae* [15], belonging to the phylum *Firmicutes* [16]. The majority of bacteria from this genus are frequently isolated from hypersaline and saline environments, mainly saline soils, and saline aquatic habitats [17–20]. These bacteria are obligate aerobic or facultative anaerobic [12] and moderately halophilic or halotolerant [21].

In this study, we have characterized the taxonogenomic properties of this strain, designated Marseille-P9898 (=CSUR P9898^{T} = DSM 111480^{T}), which is a novel bacterial species named *Metabacillus schmidteae* sp. nov.

2. Materials and Methods

2.1. Planarian Schmidtea Mediterranea Culture

S. mediterranea flatworms (asexual clonal line ClW4) were kept in tap water filtered at 19 °C. Filtered water was obtained by filtration through a filter containing charcoal and ceramics (Fairey Industrial Ceramics limited, Suffolk, England), and a 0.2 µm membrane (Thermo Scientific Nalgene filtration Products, Mexico City, Mexico). The sterility of filtered water was analyzed before any utilization. Microbiological analysis of filtered water was performed by inoculation of filtered water (25, 50, 75, and 100 µL) in 5 % sheep blood-enriched Columbia agar plate (bioMérieux, Marcy l'étoile, France) and by incubation at various temperatures (5, 10, 19, 28, 37 and 45 °C). After four days, the absence of bacterial colonies was assessed.

2.2. Culture Conditions from Schmidtea mediterranea and Strain Isolation

Worms were starved for 2 weeks, washed in filter-sterilized water. One worm was smashed in a tube with a pestle in 500 µL of PBS, then 100 µL of the crushed mixture was inoculated in 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'étoile, France) and incubated at 28 °C. Individual bacterial colonies were harvested and identified by MALDI-TOF-MS (Microflex spectrometer; Bruker Daltonics, Bremen, Germany) [22]. The MALDI Biotyper RTC software was used to interpret results according to obtained score values. The database used was the MaldiBiotyperDBUpdate_V9. 8468 MSP from the Bruker Daltonics. A bacterial colony is likely identified at the species level for a score $\geq$ 2.0; probably identified for a score between 1.99 and 1.7, but not identified for a score < 1.7. Profile spectrum of the whole cell was obtained after analysis as previously described [22].

2.3. DNA Extraction, Sequencing, Assembly, and Annotation

Genomic DNA extraction (gDNA) from the Marseille-P9898 strain was performed using an EZ1 BioRobot and EZ1 DNA tissue kit (Cat No./ID: 953034, Qiagen, Hilden, Germany). Then gDNA was quantified by a Qubit assay (Life Technologies, Carlsbad, CA, USA). Here, we used two technologies for Marseille-P9898 strain sequencing. First, gDNA was normalized at 0.2 ng/µL, then prepared and sequenced using the Mate-Pair strategy with a Miseq sequencer (Illumina, San Diego, CA, USA), as previously described [23]. Second, gDNA was normalized at 1.5 ng/µl and sequenced using nanopore strategy with a Minion sequencer (Oxford Nanopore MinION™) [24,25]. The reads of Miseq and MinION run were examined using FastQC 0.11.8 to evaluate quality [26]. Two sequencing reads were assembled using Spades [27] genome assembler software for regular and single-cell projects (Galaxy 3.12.0+galaxy1). The "conservative" option was used to reduce mismatches number and short indels. Default parameters were applied for each software. Genomic annotation was obtained using Prokka [28] software (Galaxy version 1.14.5+galaxy1).

2.4. Phylogenetic Analysis

For the taxonomic assignment, we used the nr database (Standard databases) for the BLASTn search. 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 [29]. Phylogenetic relationships were inferred from the comparison of 16S rRNA sequences using MEGAX 10.1 software [30,31]. The method for estimating phylogenetic trees was maximum likelihood. Sequences were aligned using the MUSCLE algorithm with default parameters. Numbers shown at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. Only bootstrap values $\geq$ 50% were retained. Scale bare indicates a 0.01% sequence divergence.

2.5. Genomic Comparison

The degree of genomic similarity was evaluated using GGDC [32] (http://ggdc.dsmz.de/ggdc.php, GGDC Genome-to-Genome Distance Calculator 2.1 and Orthologous Average Nucleotide Identity [33] (https://www.ezbiocloud.net/tools/orthoani, accessed on 1 January 2021, OrthoANI Tool version 0.93.1) software. Comparison of COG functional categories was carried out using BLASTP (E-value 10^{-3}, coverage 0.7 and identity percent 30%) against the clusters of Metabacillus orthologous groups (COG) database.

2.6. Phenotypic and Biochemical Features

The growth of the Marseille-P9898 strain was analyzed at different temperatures (4, 19, 28, 30, 37, and 45 °C) in 5% sheep blood-enriched Columbia agar (bioMérieux) under anaerobic and aerobic atmospheres using GasPak™ EZ generators (Becton-Dickinson, Marynland, MD, USA). The capacity to grow in distinct salinity (0, 20, 40, 50, 60, 80 and 100 g of NaCl/l) and pH (5, 5.5, 6, 6.5, 7.5, 8.5, 9, and 10) conditions were tested. Sporulation capacity was tested by thermal shock. For this purpose, bacteria were exposed to 80 °C temperature for 30 min, and then bacterial growth was monitored for 4 days. Gram staining and motility of fresh colonies were observed using a DM1000 light microscope (Leica Microsystems, Nanterre, France) with 100× lens and oil immersion. To evaluate bacterial structures, we used scanning electron microscopy (Hitachi SU5000) (Hitachi High-Technologies Corporation, Tokyo, Japan). Catalase and oxidase activities were tested using a BBL DrySlide according to the manufacturer's instructions (Becton Dickinson, Le Pont de Claix, France). API strips (API ZYM [34–36], API 20NE [37,38], API 20E [39,40] and API 50CH [41–44], bioMérieux) were used to study the biochemical characteristics for Marseille-P9898 strain and *Metabacillus niabensis* strain 4T19^{T} [45].

2.7. Antibiotic Susceptibility

Bacterial susceptibility to benzylpenicillin, amoxicillin, ampicillin, ceftriaxone, imipenem, ciprofloxacin, amikacin, gentamicin, streptomycin, daptomycin, doxycyclin, metronidazole, rifampicin, fosfomycin, vancomycin, and tigecycline was assessed using *E*-tests. For this purpose, we used a 0.5 McFarland concentration of Marseille-P9898 strain and *Metabacillus niabensis* strain 4T19^{T}.

2.8. Chemotaxonomic Analysis

Cellular fatty acid methyl ester (FAME) analysis of Marseille-P9898 strain and *Metabacillus niabensis* strain 4T19^{T} was performed by GC/MS. Fatty acid methyl esters were prepared as published by Sasser [46] and GC/MS analysis was realized as previously described [47]. 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). 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).

3. Results

3.1. Phenotypic and Biochemical Characteristics

The Marseille-P9898 strain was isolated on 5% Columbia agar enriched with sheep blood (bioMérieux) after 2 days of growth at 28 °C in an aerobic atmosphere. This strain grows at a temperature ranging from 19 to 50 °C, and under pH from 7.5 to 10 (alkaline). Marseille-P9898 strain can grow at NaCl concentration lower than 10 g/L (Table 1). After 4 days of incubation at 28 °C on blood-enriched Columbia agar, Marseille-P9898 strain colonies were white, round, and smooth, with a 3–5 mm diameter and a convex shape (Supplementary data Figure S1). Electron microscopy revealed that Marseille-P9898 strain cells are rod-shaped and have a 2.70 µm mean length, a 0.42 µm mean width, and a polar flagellum (Figure 1). Bacterial cells are Gram-negative (Figure 2), motile, and spore-forming bacilli (Supplementary data Figure S2). The endospore formation occurs in the terminal position. Marseille-P9898 strain is catalase positive and oxidase negative. Bacterial metabolism was assessed using API 50CHB/E, API 20NE, API Zym, and API 20E strips (Table 2). Marseille-P9898 strain differs from *Metab. halosaccharovorans*, *Metab. niabensis*, *Metab. malikii* and *Metab. litoralis* compared to α-glucosidase, L-rhamnose, methyl-αD-glucopyranoside, glycogen, potassium gluconate, natriumpyruvat, glucose, trisodium citrate, because Marseille-P9898 strain needs these substrates to grow. Interestingly, identification by MALDI-TOF-MS of Marseille-P9898 strain showed a score of 1.77, matching with *Metabacillus niabensis* (spectrum in supplementary data Figure S3). Because this value was less than 2, the Marseille-P9898 strain cannot be identified as *Metabacillus niabensis*. This score only indicates that the Marseille-P9898 strain belongs to the *Metabacillus* genus. To define the species type, we performed a genomic approach.

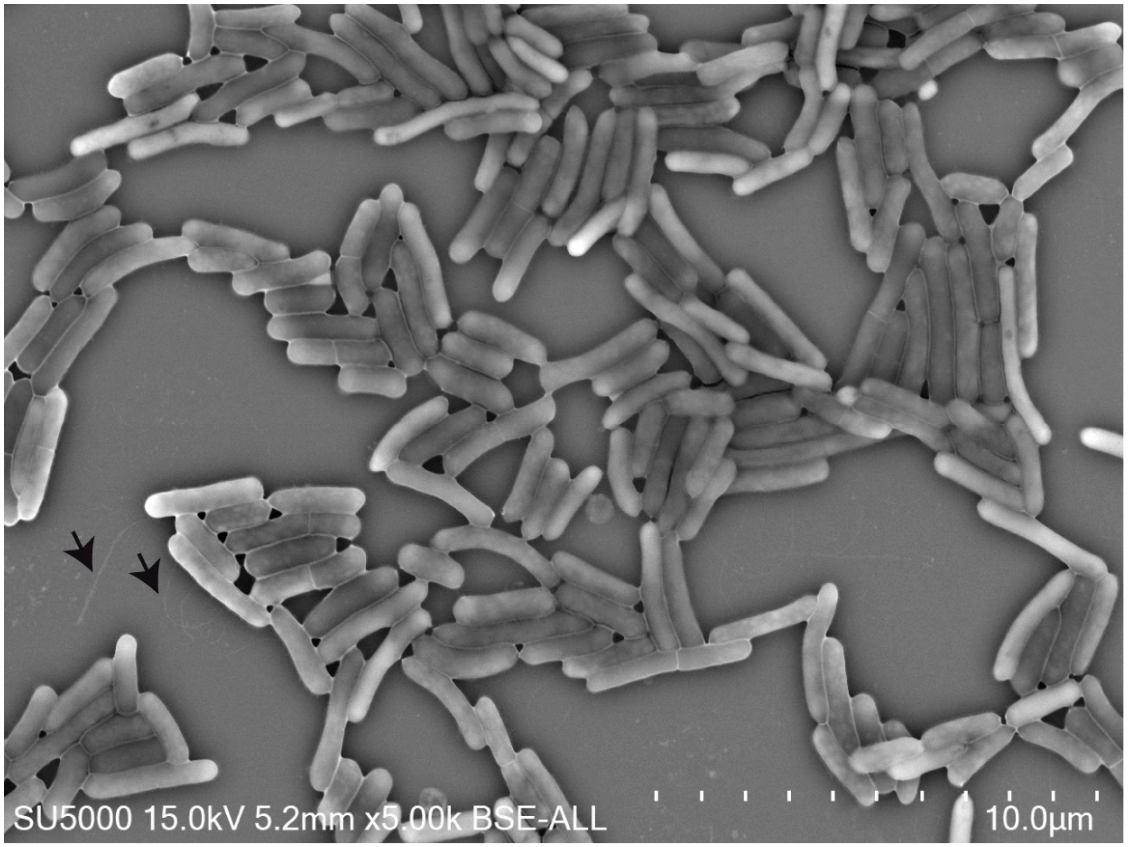

Figure 1. Transmission electron microscopy of Marseille-P9898 strain. Bacteria are rod-shaped and exhibit a polar flagellum (black arrow). Scale bar = 10.0 µm.

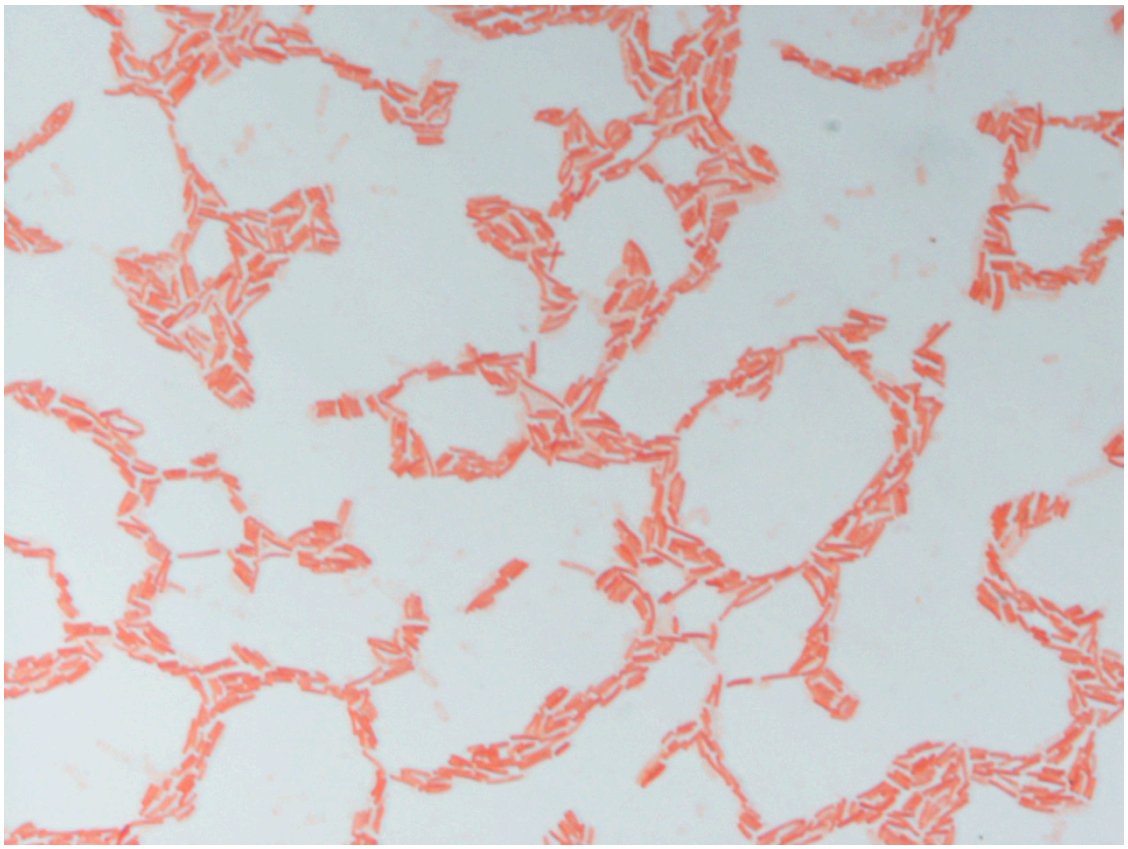

Figure 2. Gram staining of Marseille-P9898 strain.

3.2. Phylogenetic Analysis

The gene 16S rRNA sequence from the Marseille-P9898 strain had a size of 1548 bp. Using phylogenetic comparison with the GenBank database, we found that Marseille-P9898 strain had similarity at the level of an 16S rRNA gene sequence with *Metabacillus niabensis* strain 4T19^T [45] (98.99%), *Metab. halosaccharovorans* strain E33^T [48] (98.75%), *Metab. malikii* strain NCCP-662^T [49] (98.19%), *Metab. litoralis* strain SW-211^T [50] (97.15%), *Bacillus frigoritolerans* strain DSM 8801^T [51,52] (95.99%), *Mesob. foraminis* strain CV53^T [53] (95.97%), *Mesob. subterraneus* strain COOI3B^T [54] (95.85%), *Metab. herbersteinensis* strain D-15a^T [55] (97.21%), *Neob. niacini* strain IFO15566^T [56] (95.95%), *B. acidicola* strain 105-2^T [57] (95.36%), *B. oryzisoli* strain 1DS3-10^T [58] (96.10%), *B. flexus* strain IFO15715^T [59] (95.69%), *Metab. indicus* strain Sd/3^T [60] (96.21%), *Cytob. gottheilii* strain WCC 4585^T [61] (95.91%), *B. horikoshii* strain DSM 8719^T [62] (95.55%), *Perib. simplex* DSM 1321^T [59] (95.68%), *B. tianshenii* strain YIM M13235^T [63] (95.27%), *Metab. fastidiosus* strain DSM91^T (96.48%), *B. aryabhattai* B8W22^T [64] (95.50%), *Metab. endolithicus* strain JC267^T [65] (98.21%), *Neob. bataviensis* strain NBRC 102449^T [66] (95.94%), *Metab. galliciensis* strain BFLP-1^T [67] (96.18%), *Neob.. novalis* strain IDA3307^T [66] (95.89%), *B. licheniformis* DSM 13^T (94.45%), and *Metab. crassostreae* JSM 100118^T [68] (98.07%). The most closely related species to the Marseille-P9898 strain was the *Metab. niabensis* strain 4T19^T, *Metab. halosaccharovorans* strain E33^T, *Metab. malikii* strain NCCP-662^T and *Metab. litoralis* strain SW-211^T. A phylogenetic tree based on the maximum-likelihood algorithm revealed that the Marseille-P9898 strain is shared in a cluster of members of the *Metabacillus* genus (Figure 3), belonging from *Bacillaceae* family and *Firmicutes* phylum. On the phylogenetic tree, it was clear that the Marseille-P9898 strain presented a distinct taxon separated from other species of genus *Bacillus*. We selected nine closely related species to make a comparison genomic (Table 3 and Supplementary Data Table S1).

Table 1. Characteristics and classification of strain Marseille-P9898.

Property	Term
Current classification	Kingdom: Bacteria [69] Phylum: *Firmicutes* [16] Class: *Bacilli* [70] Order: *Bacillales* [71] Family: *Bacillaceae* [15] Genus: *Metabacillus* [11] Species name: *Schmidteae* Specific epithet: *Metabacillus schmidteae* Type strain: Marseille-P9898
Species status	sp. nov.
Gram stain	Negative
Cell shape	Rod-shape
Motility	Motile
Sporulation	Spore-forming
Temperature range for growth	19 to 50
Temperature optimum	28
pH range for growth	7.5 to 10
pH optimum	8
pH category	alkaline
Lowest NaCl concentration for growth	0
Highest NaCl concentration for growth	12 g/L
Salinity optimum	10 g/L
O2 conditions for strain testing	Strict aerobic
Catalase	Positive
Oxidase	Negative
Habitat	Planarians
Biotic relationship	Microbiota planarian

Table 2. Biochemical characteristics of Marseille-P9898 and phylogenomically related species. Taxa: 1, Marseille-P9898; 2, *Metabacillus niabensis* 4T19^T; 3, *Metabacillus halosaccharovorans* E33^T; 4, *Metabacillus litoralis* SW-211^T. Data of E33^T and SW-211^T strains were obtained using previously published work [48,50]. Data of Marseille-P9898 and 4T19^T strains were obtained in the present study. +, positive; −, negative; NA, data not available.

Property	**1**	**2**	**3**	**4**
Gram-staining	−	+	+	−
Mobility	Motile	Motile	Motile	Motile
Sporulation	+	+	+	+
Growth temperature range (°C)	19–50	15–40	20–45	4–45
Aerobic growth	Strict aerobic	Aerobic	Strict aerobic	Strict aerobic
Source	Planarians	Cotton waste	Lake	Tidal flat
Colony color	White	Yellowish white	Cream	Yellowish white
Catalase	+	+	+	+
Oxidase	−	−	+	+
ENZYMATIC ACTIVITIES				
2-nitrophenyl-βD-galactopyranoside (ONPG)	−	+	NA	NA
4-Nitrophenyl-βD-Galactopyranoside	+	+	NA	NA
Acid phosphatase	−	−	NA	+
Alkaline phosphatase	+	−	NA	+
Cystine arylamidase	−	−	−	−
Esculin ferric citrate (hydolyse)	+	+	+	NA

Table 2. *Cont.*

Property	**1**	**2**	**3**	**4**
Esterase (C4)	+	+	NA	+
Esterase lipase (C8)	+	+	NA	+
Gelatin	–	–	+	+
Indole production	–	–	–	–
L-arginin	–	–	–	–
Leucine arylamidase	–	–	–	–
Lipase (C14)	–	–	NA	–
L-lysin	–	–	–	–
L-ormithin	–	–	–	–
N-acetyl-β-glucosaminidase	–	–	NA	–
Naphtol-AS-BI-phosphohydrolase	+	+	NA	+
Natriumpyruvat	+	–	NA	–
Natriumthiosulfat	–	–	–	–
Trinatriumcitrat	–	–	NA	NA
Trypsin	–	–	NA	–
Urea	–	–	–	–
Valine arylamidase	–	–	–	–
α-chymotrypsin	–	–	NA	–
α-fucosidase	–	–	NA	–
α-galactosidase	–	–	NA	–
α-glucosidase	+	–	NA	–
α-mannosidase	–	–	NA	–
β-glucosidase	–	+	NA	–
β-glucuronidase	+	–	NA	–
CARBOHYDRATE ASSIMILATION				
Adipic acid	–	–	NA	NA
Amygdalin	+	+	NA	NA
Arbutin	–	+	NA	NA
Capric acid	–	–	NA	NA
D-adonitol	–	–	NA	–
D-arabinose	–	–	+	NA
D-arabitol	+	–	NA	NA
D-cellobiose	+	+	+	+
D-fructose	+	+	+	+
D-fucose	–	–	NA	NA
D-galactose	+	+	+	+
D-glucose	+	+	+	+
D-lactose	+	+	+	+
D-lyxose	+	–	NA	NA
D-maltose	+	–	+	+
D-mannitol	+	–	+	–

Table 2. *Cont.*

Property	**1**	**2**	**3**	**4**
D-mannose	+	+	+	–
D-melezitose	+	–	NA	+
D-melibiose	+	+	+	+
D-raffinose	+	+	+	+
D-ribose	–	–	+	+
D-saccharose	+	+	+	+
D-sorbitol	–	+	NA	–
D-tagatose	–	–	NA	NA
D-trehalose	+	+	+	+
D-turanose	–	–	NA	NA
Dulcitol	–	–	NA	NA
D-xylose	+	+	+	+
Erythritol	–	–	NA	NA
Gentiobiose	+	+	NA	NA
Glycerol	+	+	+	NA
Glycogen	–	+	NA	NA
Inositol	–	+	NA	–
Inulin	+	+	NA	NA
L-arabinose	+	-	+	+
L-arabitol	–	–	NA	NA
L-fucose	–	–	NA	NA
L-rhamnose	–	+	NA	+
L-sorbose	–	–	NA	NA
L-xylose	–	–	+	NA
Malic acid	+	–	NA	+
Methyl-αD-glucopyranoside	+	–	–	NA
Methyl-αD-mannopyranoside	–	–	–	NA
Methyl-βD-xylopyranoside	+	+	NA	NA
N-Acetyl-glucosamine	+	–	NA	NA
Phenylacetic acid	–	–	NA	NA
Potassium 2-ketoGluconate	–	–	NA	NA
Potassium 5-ketogluconate	–	–	NA	NA
Potassium gluconate	+	–	NA	NA
Salicin	+	+	+	NA
Starch	+	+	+	+
Trisodium citrate	+	–	NA	–
Xylitol	–	–	NA	NA
SUBSTRAT REDUCTION				
L-tryptophan	+	+	NA	–
Potassium nitrate	–	+	–	NA

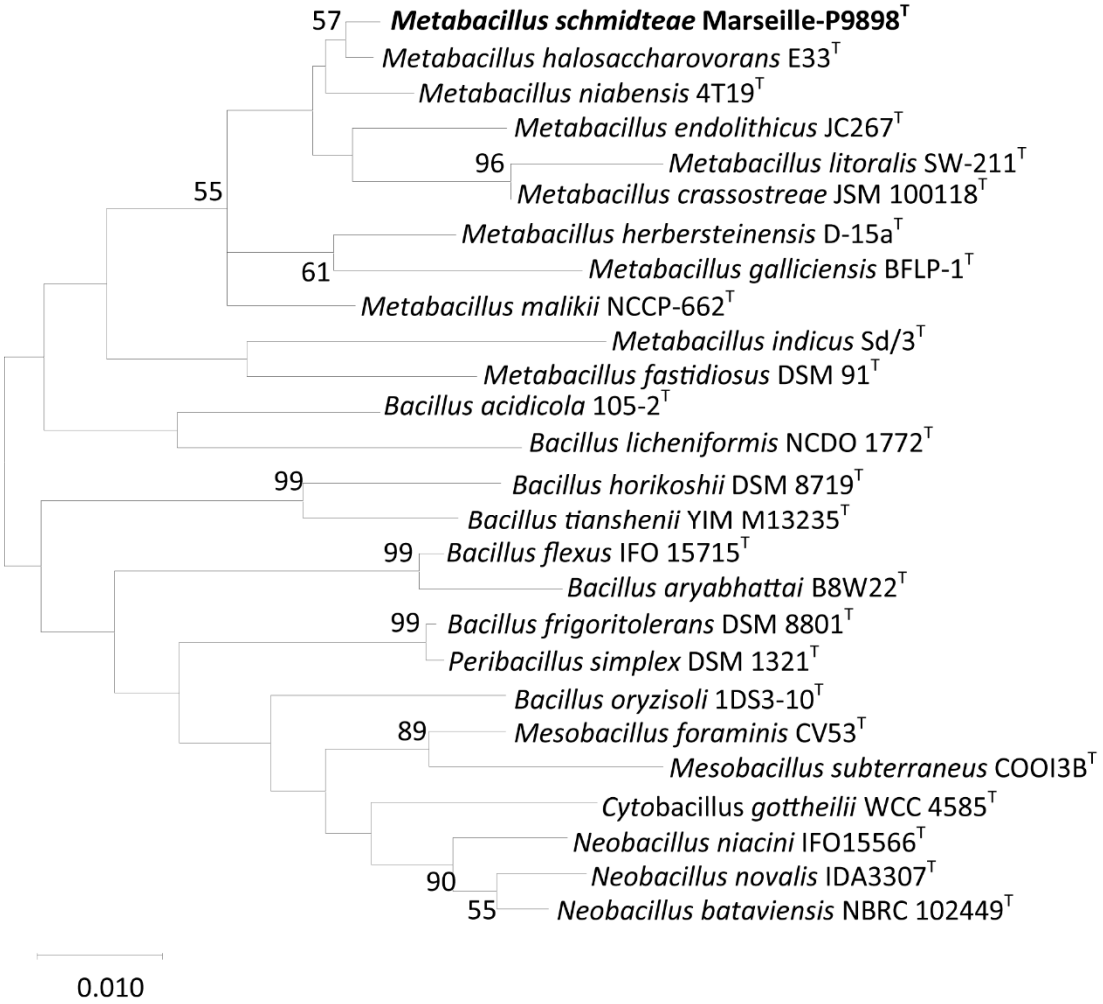

Figure 3. Phylogenetic tree carried out from a comparative analysis of 16S rRNA gene sequences indicating the relationships between Marseille-P9898 strain and related species. Sequences are aligned using the MUSCLE algorithm with default parameters and phylogenies are inferred by the MEGAX software (version 10.1). Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. Only bootstrap values $\geq$ 50% are retained. Scale bare indicates a 0.01% sequence divergence.

Table 3. Main genomic characteristics of strain Marseille-P9898 and closely related species.

Name	Size (bp)	GC%	Contigs	Refseq
Bacillus flexus	3,906,163	37.6	259	BCVD01000001.1
Bacillus acidicola	5,137,992	39.4	10	LWJG01000001.1
Mesobacillus foraminis	5,730,823	43.0	35	SLVV01000001.1
Metabacillus halosaccharovorans	5,399,327	36.1	8	MTIR01000001.1
Metabacillus litoralis	5,230,624	35.9	1	NZ_CP033043.1
Metabacillus niabensis	4,987,608	35.5	462	NZ_CADEPK010000000
Neobacillus niacini	2,201,253	38.3	143	JRYQ01000001.1
Metabacillus schmidteae	5,499,502	35.8	3	CAESCH000000000.1
Mesobacillus subterraneus	4,571,170	43.9	42	RSFW01000001.1
Bacillus frigoritolerans	5,475,560	43.9	1	NZ_CP030063.1

3.3. Genomic Comparison

Marseille-P9898 strain genome was assembled from three contiguous sequences (N_{50}, 4,279,115; L_{50}, 1; coverage 12x) of 5,499,502 bp with a G+C content of 35.8% (Table 3).

Marseille-P9898 strain presents a total of 5318 predicted protein-coding genes, 48 complete rRNAs, 114 tRNAs, and 1 tmRNA.

dDDH hybridization values were obtained using GGDC software and were reported in Table 3. Marseille-P9898 strain shows values ranged from 22.30% with *Metab. niabensis* to 34.20% with *B. acidicola*. These values were below the 70% threshold recognized for delineation of distinct species [32]. Ortho-ANI values ranged from 67.64% with *Mesob. subterraneus* to 85.76 % with *Metab. halosaccharovorans*, which was lower than the 95% threshold used to discriminate bacterial species [33] (Figure 4 and Table 4). These results were sufficient to separate the Marseille-P9898 strain from *Metab. halosaccharovorans* strain E33^T, *Metab. niabensis* strain 4T19^T, *Metab. malikii* strain NCCP-662^T, *Metab. litoralis* strain SW-211^T, and other most closely related species of genus *Metabacillus*. The distribution of genes into COG categories was similar among all compared genomes (Figure 5 and Table 5). Taken together, these results confirmed that the Marseille-P9898 strain belongs to a separate *Bacillus* species.

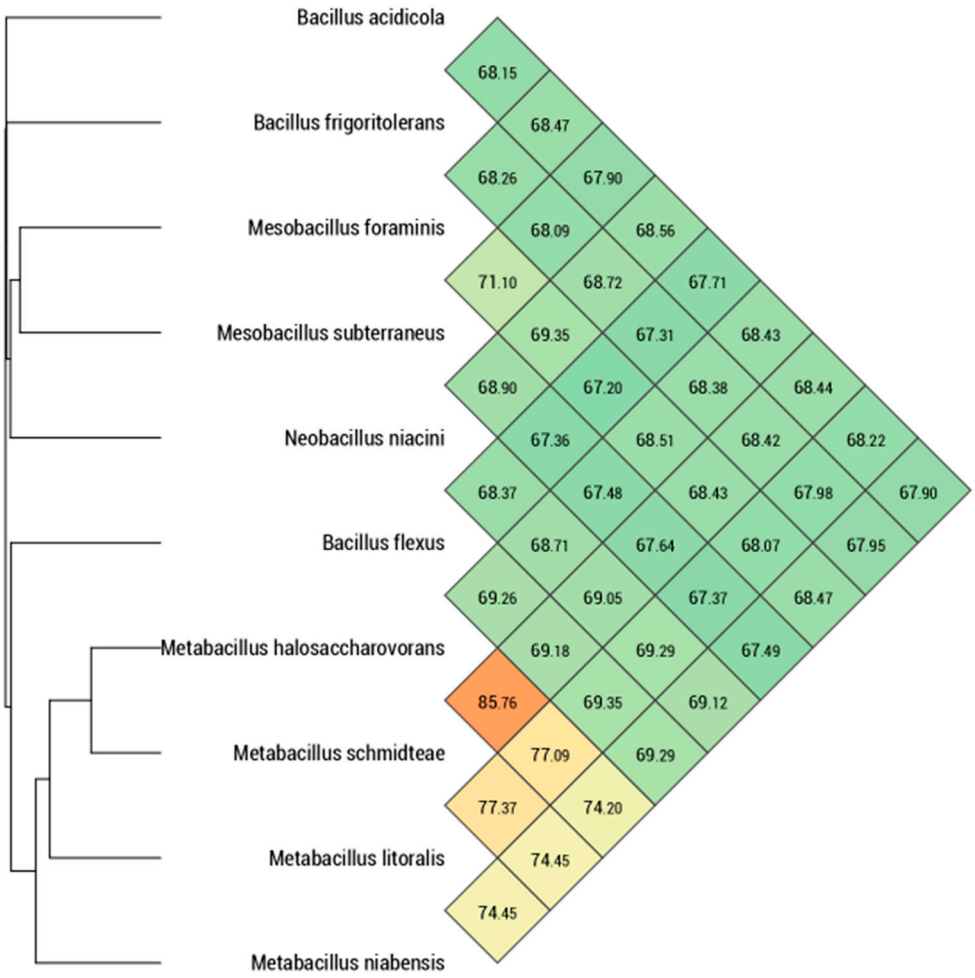

Figure 4. The numeric map is generated with Ortho-ANI values calculated using OAT software between the Marseille-P9898 strain and related species with nomenclature classification. Color code shows closely (in red) to the farthest (in green) related species. *Metabacillus halosaccharovorans*, *Metabacillus litoralis*, and *Metabacillus niabensis* are closely related to *Metabacillus schmidteae*.

Table 4. dDDH values obtained by comparison of related genomes studied using GGDC, formula 2 (DDH Estimates Based on Identities/HSP length). Taxa: 1, *Bacillus acidicola*; 2, *Bacillus flexus* 3, *Mesobacillus foraminis*; 4, *Metabacillus halosaccharovorans*; 5, *Metabacillus litoralis*; 6, *Metabacillus niabensis*; 7, *Neobacillus niacini*; 8, *Metabacillus schmidteae*; 9, *Mesobacillus subterraneus*.

	1	2	3	4	5	6	7	8	9
Bacillus flexus	28.20								
Mesobacillus foraminis	27.80	19.80							
Metabacillus halosaccharovorans	31.80	23.70	27.90						
Metabacillus litoralis	39.10	23.70	27.00	22.70					
Metabacillus niabensis	27.00	20.10	22.00	22.00	22.60				
Neobacillus niacini	25.90	19.90	21.50	25.50	29.70	23.10			
Metabacillus schmidteae	34.20	24.40	27.10	31.10	23.00	22.30	27.70		
Mesobacillus subterraneus	25.30	21.70	18.80	29.90	31.30	20.70	21.30	30.10	
Bacillus frigoritolerans	32.50	26.70	25.60	31.30	34.10	28.70	24.70	32.80	28.20

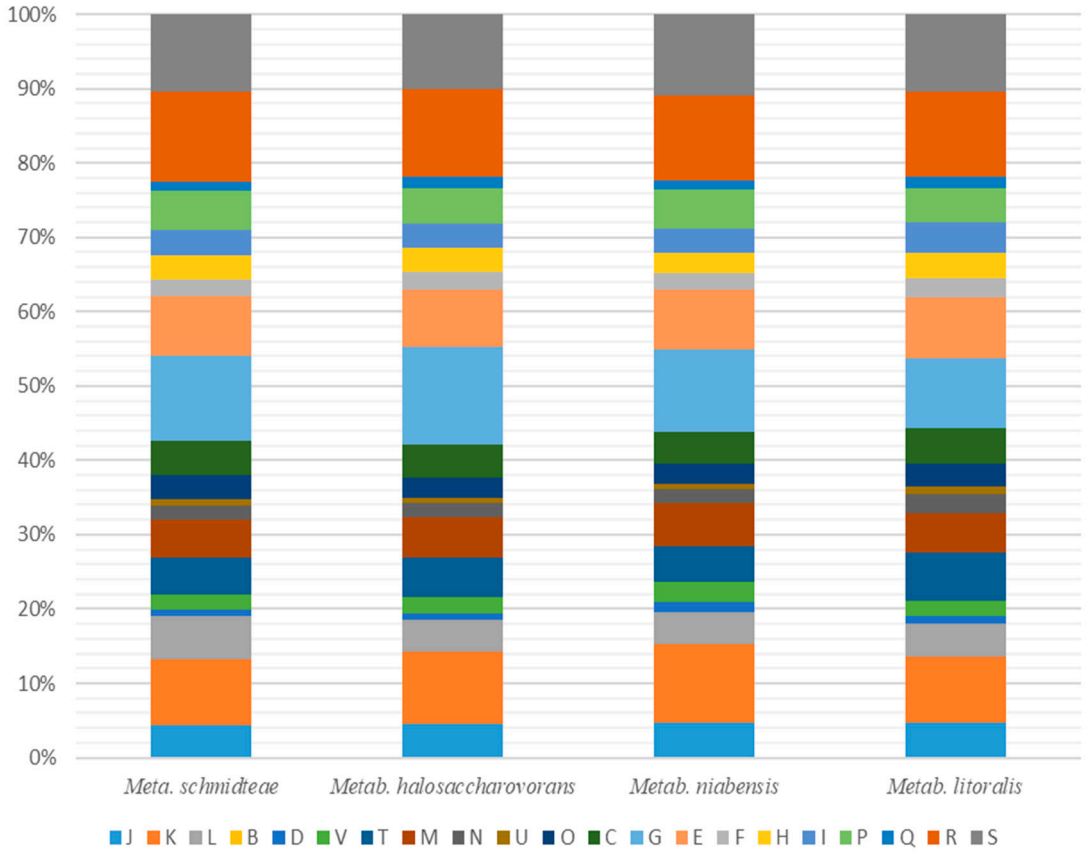

Figure 5. Comparison of predicted genes according to the COGs of Marseille-P9898 strain and related species. *Metabacillus halosaccharovorans*, *Metabacillus litoralis*, and *Metabacillus niabensis* are closely related to *Metabacillus schmidteae*. **[B]** Chromatin structure and dynamics; **[C]** Energy production and conversion; **[D]** Cell cycle control, cell division, chromosome partitioning; **[E]** Amino acid transport and metabolism; **[F]** Nucleotide transport and metabolism; **[G]** Carbohydrate transport and metabolism; **[H]** Coenzyme transport and metabolism; **[I]** Lipid transport and metabolism; **[J]** Translation, ribosomal structure, and biogenesis; **[K]** Transcription; **[L]** Replication, recombination, and repair; **[M]** Cell wall/membrane/envelope biogenesis; **[N]** Cell motility; **[O]** Posttranslational modification, protein turnover, chaperones; **[P]** Inorganic ion transport and metabolism; **[Q]** Secondary metabolites biosynthesis, transport, and catabolism; **[R]** General function prediction only; **[S]** Function unknown; **[T]** Signal transduction mechanisms; **[U]** Intracellular trafficking, secretion, and vesicular transport; **[V]** Defense mechanisms; **[W]** Extracellular structures; **[X]** Mobilome: prophages, transposons; **[Z]** Cytoskeleton.

Table 5. Functional annotation of predicted genes according to the COGs database.

CODE	VALUE	DESCRIPTION
		Information storage and processing
[J]	169	Translation, ribosomal structure, and biogenesis
[A]	0	RNA processing and modification
[K]	347	Transcription
[L]	221	Replication, recombination, and repair
[B]	1	Chromatin structure and dynamics
		Cellular processes and signaling
[D]	36	Cell cycle control, cell division, chromosome partitioning
[V]	0	Defense mechanisms
[T]	81	Signal transduction mechanisms
[M]	190	Cell wall/membrane/envelope biogenesis
[N]	197	Cell motility
[Z]	77	Cytoskeleton
[W]	0	Extracellular structures
[U]	0	Intracellular trafficking, secretion, and vesicular transport
[O]	30	Posttranslational modification, protein turnover, chaperones
[X]	125	Mobilome: prophages, transposons
		Metabolism
[C]	181	Energy production and conversion
[G]	444	Carbohydrate transport and metabolism
[E]	308	Amino acid transport and metabolism
[F]	92	Nucleotide transport and metabolism
[H]	120	Coenzyme transport and metabolism
[I]	137	Lipid transport and metabolism
[P]	205	Inorganic ion transport and metabolism
[Q]	47	Secondary metabolites biosynthesis, transport, and catabolism
		Poorly characterized
[R]	469	General function prediction only
[S]	403	Function unknown

3.4. Chemotaxonomic Analysis

The major fatty acids present in the Marseille-P9898 strain were 12-methyl-tetradecanoic acid (62.6%), 13-methyl-tetradecanoic acid (12.5%), Hexadecanoic acid (7.1%), 15-methyl-Hexadecanoic acid (3.8%), 12-methyl-Tridecanoic acid (3.3%), Pentadecanoic acid (2.7%), 12-methyl-Hexadecanoic acid (2.3%), 7-hexadecenoic acid (3.1%), 11-methyl-dodecanoic acid (1.1%), and 15-methyl-hexadecanoic acid (1.6%) (Table 6). 12-methyl-tetradecanoic acid was the most present in the Marseille-P9898 strain, *Metab. halosaccharovorans*, *Metab. niabensis*, *Metab. malikii,* and *Metab. litoralis*. Marseille-P9898 differed from *Metab. halosaccharovorans*, *Metab. niabensis*, *Metab. malikii*, and *Metab. litoralis* in Pentadecanoic acid and 11-methyl-Dodecanoic acid.

3.5. Antibiotic Susceptibility

Drugs such as amikacin, fosfomycin, benzylpenicillin, ciprofloxacin, amoxicillin, ceftriaxone, daptomycin, doxycycline, rifampicin, vancomycin, and ampicillin do not inhibit Marseille-P9898 strain growth (Table 7). Comparing Marseille-P9898 strain and *Metab. niabensis*, we found that fosfomycin inhibited the Marseille-P9898 strain, but not *Metab. Niabensis* growth.

Table 6. Cellular fatty acid composition of Marseille-P9898 compared with related species. Taxa: 1, Marseille-P9898; 2, *Metabacillus niabensis* 4T19^T; 3, *Metabacillus halosaccharovorans* E33^T; 4, *Metabacillus litoralis* SW-211^T. The data of strain E33^T and strain SW-211^T are obtained using previously published work [48,50]. A of the fatty acid methyl esters was performed by gas-liquid chromatography according to the instructions for the Microbial Identification System (MIDI). Data of Marseille-P9898 and 4T19^T strains were obtained in the present study. tr, Trace (<1 %); −, not detected; +, present; NA, data not available.

Fatty Acids	Name	1	2	3	4
	Straight-chain saturated				
15:0	Pentadecanoic acid	2.7	tr	–	–
16:0	Hexadecanoic acid	7.1	15.7	–	5.5
	Branched saturated				
13:0 iso	11-methyl-Dodecanoic acid	1.1	tr	–	–
14:0 iso	12-methyl-Tridecanoic acid	3.3	5.8	–	10.0
15:0 iso	13-methyl-tetradecanoic acid	12.5	7.2	21.0	15.6
16:0 iso	12-methyl-Hexadecanoic acid	2.3	6.0	–	12.5
17:0 iso	15-methyl-Hexadecanoic acid	1.6	1.7	–	–
15:0 anteiso	12-methyl-tetradecanoic acid	62.6	45.0	43.5	34.8
17:0 anteiso	15-methyl-Hexadecanoic acid	3.8	11.4	9.5	6.4
	Monounsaturated				
16:1ω9	7-Hexadecenoic acid	3.1	2.1	–	–
18:1n9	9-Octadecenoic acid	–	1.1	–	–

Table 7. Antimicrobial susceptibility and MIC values of Marseille-P9898 strain. 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-P9898 MIC	4T19^T MIC
amikacin	0.016–256	50	1.5
fosfomycin	0.064–1024	16	>1024
benzylpenicelin	0.016–256	0.016	0.016
ciprofloxacin	0.002–32	19	0.38
amoxicillin	0.016–256	0.016	0.016
ceftriaxone	0.016–256	0.064	0.50
daptomycin	0.016–256	25	1
doxycyclin	0.016–256	0.016	0.016
vancomycin	0.016–256	0.094	0.50
ampicillin	0.016–256	0.016	0.016

4. Discussion

Planarian *S mediterranea* is an organism model to investigate the regeneration [72] and host-pathogen relationship [7–9]. To understand the implication of microbiota in the *S. mediterranea* antimicrobial response, we investigated its composition. While, recently, we have already published the identification of *Pedobacter schmidteae* sp. nov., as a novel bacterium isolated from this microbiota [73], here, we report the identification of a second novel bacteria species, which belongs to Metabacillus genus called *Metabacillus schmidteae* sp. nov.

Description of novel bacterial species requires several approaches, the most recurrent one being the taxonogenomic approach [1–3]. Using this approach, we describe the main phenotypic and genotypic features of the Marseille-P9898 strain. The use of MALDI-TOF-MS showed a protein profile, but it did not allow the discrimination between the Marseille-P9898 strain and *Metabacillus niabensis*. 16S rRNA gene sequence presented a

value of more than 98.65% [29], a cut-off to demarcate new species. We know that the conventional low divergence between two 16S rRNA genes from two organisms results in a slight and limited bacterial description [74,75]. Nonetheless, gene 16S rRNA sequence gives us the information concerning genomic sequence analysis by tools such as Genome-to-Genome Distance Calculator (GGDC) [32] and Orthologous Average Nucleotide Identity (Ortho-ANI). Genomics allows the evaluation of the degrees of genomic similarity among species [33]. Taken together, genomic data showed that Marseille-P9898 is a novel bacterial species of genus *Metabacillus* called *Metabacillus schmidteae* sp. nov.

Until now, *Metabacillus* have been isolated from diverse environments including soil, hyper-saline aquatic, and marine coastal region [11]. We revealed that *Metabacillus schmidteae* is also hosted by aquatic animals such as planarians, precisely in their microbiota. We have observed that *Metabacillus schmidteae* is a Gram-negative bacteria (fresh and older cultures) and it is the first Gram-negative one from *Metabacillus* genus, which is usually Gram-positive or Gram-variable stained [11]. It possesses a polar motile flagellum, and it is strictly aerobic as other species of the genus *Metabacillus*. *Metabacillus schmidteae* can make spores under harsh conditions as reported for other species, except for *Metabacillus weihaiensis* [11]. In addition, biochemical analysis shows that *Metabacillus* (*Metabacillus schmidteae* Marseille-P9898^T, *Metabacillus niabensis* 4T19^T, *Metabacillus halosaccharovorans* E33^{T,} and *Metabacillus litoralis* SW-211^T) use as substrates for their growth, D-galactose, D-glucose, D-fructose, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, and D-raffinose. Chemotaxonomic analysis indicates that *Metabacillus* (*Metabacillus schmidteae* Marseille-P9898^T, *Metabacillus niabensis* 4T19^T, *Metabacillus halosaccharovorans* E33^{T,} and *Metabacillus litoralis* SW-211^T) produce membrane proteins such 15:0 iso (13-methyl-tetradecanoic acid), 15:0 anteiso (12-methyl-tetradecanoic acid), 17:0 anteiso (15-methyl-Hexadecanoic acid). The data of chemotaxonomic and biochemical analysis could be used as a protein or biochemical marker to identify the genus *metabacillus. Metabacillus schmidteae* is now the second bacteria newly identified in planarian microbiota [73]. Its contribution in regeneration, as well as the antimicrobial capacity of the planarian, remains to be investigated.

5. Conclusions

Using taxonogenomic analysis, we described and characterized the *Marseille-P9898* strain, hereby for the first time isolated from planarian *S. schmidteae* microbiota. Marseille-P9898 is phylogenetically related to *Metabacillus* genus. Chemotaxonomic and biochemical analysis and genomics comparison allowed considering Marseille-P9898 as a novel species of *Metabacillus* genus. For this strain *Marseille-P9898*, we propose the name of *Metabacillus schmidteae* sp. nov. (Marseille-P9898^T = CSUR P9898^T = DSM 111480^T).

5.1. Protologue

Metabacillus schmidteae (schmid.te'ae. N.L. gen. n. schmidteae of the planarian genus *Schmidtea*, from which Marseille-P9898 strain was isolated) is a bacterium belonging to the *Bacillaceae* family within *Firmicutes* phylum. Marseille-P9898^T type strain is strictly aerobic, alkaliphilic, Gram-negative, spore-forming, rod-shaped, and motile. After 2 days at 28 °C on Columbia agar at pH 7.5, colonies were small, circular, smooth, white, and convex. Growth occurs at 19–50 °C with 0–12 g/L NaCl and pH 7.5–10. Optimal growth occurs at 28 °C, pH 8, and 10 g/L NaCl. Catalase positive and oxidase negative. Reactions were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), Naphthol-AS-BI-phosphohydrolase, β-glucuronidase, and α-glucosidase; assimilate to glycerol, L-arabinose, D-xylose, methyl-βD-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, methyl-αD-glucopyranoside, N-acetylglucosamine, amygdalin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, gentiobiose, D-lyxose, D-raffinose, starch, D-arabitol, 2-nitrophenyl-βD-galactopyranoside, L-tryptophan, natriumpyruvat, 4-Nitrophenyl-βD-Galactopyranoside, malic acid, and trisodium citrate. Negative reactions were observed

for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucosidase, and N-acetyl-β-glucosaminidase; no assimilate to α-mannosidase, α-fucosidase, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, Dulcitol, Inositol, D-sorbitol, methyl-αD-mannopyranoside, arbutin, glycogen, xylitol, D-turanose, D-tagatose, D-fucose, L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-arginin, L-lysin, L-ormithin, trinatriumcitrat, natriumthiosulfat, urea, indole production, gelatin, potassium nitrate, capric acid, adipic acid, and phenylacetic acid. Marseille-P9898^{T} strain is sensitive to amikacin, fosfomycin, benzylpenicillin, ciprofloxacin, amoxicillin, ceftriaxone, daptomycin, doxycycline, rifampicin, vancomycin, and ampicillin. Fatty acids were 12-methyl-tetradecanoic acid (62.6%), 13-methyl-tetradecanoic acid (12.5%), Hexadecanoic acid (7.1%), 15-methyl-Hexadecanoic acid (3.8%), and 12-methyl-Tridecanoic acid (3.3%). Marseille-P9898^{T} type strain (CSUR P9898^{T} = DSM 111480) was isolated from planarian microbiota. The 16S rRNA gene sequence and genome sequence were deposited in GenBank under accession numbers LR797940 and CAESCH000000000.1, respectively.

5.2. Nucleotide Sequence Accession Number

The 16S rRNA gene sequence and genome sequence were deposited in GenBank under accession numbers LR797940 and CAESCH000000000.1, respectively. Raw data of Illumina MiSeq paired-end and MinION sequencing were deposited in EMBL-EBI under run accession ERR4143811 and ERR4143810; and experiment Accession ERX4111084 and ERX4111083, respectively.

5.3. Deposit in Culture Collections

Marseille-P9898^{T} strain was deposited in the CSUR and DSMZ strain collections under numbers CSUR P9898 and DSM 111480, respectively.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/microbiolres12020021/s1, Figure S1: Colonies color Colonies color of *Metabacillus schmidteae*, Figure S2: Electron Micrograph of *Metabacillus schmidteae* Spore, Figure S3: MALDI-TOF spectrum of *Metabacillus schmidteae*, Table S1: GenBank accession numbers of the type strains.

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

Funding: The study was funded by the Méditerranée-Infection foundation, the National Research Agency under the program "Investissements d'avenir", reference ANR-10-IAHU-03 and by Région Provence Alpes Côte d'Azur and European funding FEDER IHUBIOTK.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The 16S rRNA gene sequence and genome sequence were deposited in GenBank under accession numbers LR797940 and CAESCH000000000.1, respectively. Raw data of Illumina MiSeq paired-end and MinION sequencing were deposited in EMBL-EBI under run accession ERR4143811 and ERR4143810; and experiment Accession ERX4111084 and ERX4111083, respectively.

Acknowledgments: LJK is a student at the Aix-Marseille University and funded by the Méditerranée-Infection foundation. The study was funded by the Méditerranée-Infection foundation, the National Research Agency under the program "Investissements d'avenir", reference ANR-10-IAHU-03 and by Région Provence Alpes Côte d'Azur and European funding FEDER IHUBIOTK. We also thank Aurelia Caputo (IHU Méditerranée-Infection, France) for submitting the raw data assembly, 16S rRNA and genomic sequences to GenBank and Nicholas Armstrong (IHU Méditerranée-Infection, France) for cellular fatty acid methyl ester analysis. We thank Giovanna Mottola (C2VN-AMU-INSERM 1263-INRAE 1260 and AP-HM, Marseille, France) for her help in English editing.

Conflicts of Interest: The authors have no conflicts of interest to declare. The funding sources had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Abbreviations

DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
CSUR	Collection de Souches de l'Unité des Rickettsies
dDDH	digital DNA-DNA hybridization
GGDC	Genome-to-Genome Distance Calculator
MALDI-TOF-MS	Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry
MEGA	Molecular Evolutionary Genetics Analysis
FAME	Fatty Acid Methyl Ester

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