Article

Qipengyuania pacifica sp. nov., a Novel Carotenoid-Producing Marine Bacterium of the Family *Erythrobacteraceae*, Isolated from Sponge (*Demospongiae*), and Antimicrobial Potential of Its Crude Extract

Sanaullah Tareen [1,2], Chandra Risdian [1,3], Mathias Müsken [4] and Joachim Wink [1,*]

1   Microbial Strain Collection (MISG), Helmholtz Centre for Infection Research (HZI),
    38124 Braunschweig, Germany; sanaullahtareen734@gmail.com or sanaullah.tareen@helmholtz-hzi.de (S.T.);
    chandra.risdian@helmholtz-hzi.de (C.R.)
2   Department of Microbiology, Balochistan University of Information Technology Engineering and
    Management Sciences, Quetta 87300, Pakistan
3   Research Unit for Clean Technology, National Research and Innovation Agency (BRIN),
    Bandung 40135, Indonesia
4   Central Facility for Microscopy, Helmholtz Centre for Infection Research (HZI),
    38124 Braunschweig, Germany; mathias.muesken@helmholtz-hzi.de
*   Correspondence: joachim.wink@helmholtz-hzi.de

Abstract: A marine *Alphaproteobacterium* designated as strain NZ-96^T was isolated in February 2021, from a sponge species (*Demospongiae*) collected in muddy sediments with boulders and old chimneys in Otago/Canterbury Slope, Pacific Ocean, New Zealand. The isolate was found to be Gram-negative, rod-shaped, aerobic, motile, and produced yellow-colored colonies. The isolate was positive for alkaline phosphatase, leucine arylamidase, trypsin, catalase, and oxidase and negative for α-galactosidase and urease. It was resistant to many antibiotics including hygromycin, trimethoprim, spectinomycin, ampicillin, oxytetracycline, cephalosporin, bacitracin, and polymyxin. The 16S rRNA gene-based phylogenetic analyses exhibited that strain NZ-96^T belonged to the genus *Qipengyuania* and showed 98.3–98.8% 16S rRNA gene sequence similarity to its closest relatives. The major respiratory quinone was ubiquinone-10 (Q-10). The polar lipid profile consisted of phosphatidylcholine, sphingoglycolipid, phosphatidylglycerol, one unknown polar lipid, and three unknown glycolipids. The major fatty acids were $C_{18:1}\omega12t$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{17:1}\omega6c$, $C_{16:0}2$-OH, and $C_{14:0}$ 2-OH. Carotenoid were produced. The crude extract showed pronounced activity against *Staphylococcus aureus* Newman and *Bacillus subtilis* DSM 10. Pairwise ANI and dDDH values of strain NZ-96^T and closely related phylogenetic hits were below the threshold values of 95% and 70%, respectively. Genes for trehalose biosynthesis, aspartate-semialdehyde dehydrogenase, flagellar biosynthesis, fatty acid biosynthesis, and antibiotics resistance were present, which aids in isolate survival in a sea or ocean environment. The DNA G+C content was 60.8% (by genome). Based on data obtained by the polyphasic approach, strain NZ-96^T (= DSM 112811^T = NCCB 100842^T) represents a novel species of the genus *Qipengyuania*, for which the name *Qipengyuania pacifica* sp. nov. is proposed.

Keywords: carotenoid; *Erythrobacteraceae*; Pacific Ocean; polyphasic analysis; sponge

1. Introduction

Reduction in the discovery of new antimicrobial compounds has led the scientific community to exploit underexplored and extreme ecological niches in search of novel microorganisms. This has led to a particular interest in seas and oceans, which host some of the least explored and most hostile environments on Earth [1]. Sponges (phylum Porifera) in the ocean shelter abundant and diverse symbiotic microbial communities [2]. They are

ecologically productive and a treasure of novel, biotechnologically relevant natural products [3]. The study aimed at the isolation of the novel bacteria from the sea sponge led to the isolation of a strain designated as NZ-96^T. Based on 16S rRNA gene sequences comparative analysis, the isolate was shown to be closely related to *Qipengyuania pelagi* UST081027-248^T, a member of the family *Erythrobacteraceae*. Through phylogenomic core genes reconstruction and analyses of genome similarity, the taxonomy of the family *Erythrobacteraceae* was recently revised, leading to the formation of many novel genera within the family [4]. Taxonomy revision resulted in the transfer of *Erythrobacter pelagi* into a new genus *Qipengyuania* in the family *Erythrobacteraceae*, thus the species is called by its new name, *Qipengyuania pelagi*. Marine organisms [5], marine cyanobacterial mats, seawater, marine and mangrove sediments [6–8], solar saltern [9], ice core [10], and estuarine environments [11] have been found to contain members of the family *Erythrobacteraceae*. Members of the family *Erythrobacteraceae* are Gram-negative, have rod-shaped or pleomorphic coccidal forms and are pink, red, orange, or yellow pigmented and aerobic chemoorganotrophs [12]. The genus *Qipengyuania*, a member of the *Erythrobacteraceae* family [13], belongs to the class *Alphaproteobacteria*, with the representation of *Qipengyuania sediminis* as the type of species [14]. To date, the genus *Qipengyuania* comprises thirteen (13) species with validly published names. https://lpsn.dsmz.de/genus/qipengyuania (accessed on 29 February 2022) [15].

The present work was carried out to determine the taxonomic status of NZ-96^T using a polyphasic study involving the phenotypic and chemotaxonomic characterization, a detailed phylogenetic investigation based on 16S rRNA gene sequences, and genetic analyses of genome data.

2. Materials and Methods

2.1. Isolation, Cultivation, and Maintenance of Bacteria

A sponge species sample (Demospongiae) was collected in February 2017 during a collection expedition using a remotely operated vehicle (ROV), located at a depth of 596 m below the ocean surface at muddy sediments with boulders and old chimneys in the Otago/Canterbury Slope (45°02' N, 171°90' W), Pacific Ocean, New Zealand. An artificial seawater wash was used to remove loosely bound bacterial cells and debris from the sponge for bacterial isolation. A sponge tissue sample ($\pm$1 cm^3) was ground and added to 9 mL of sterile artificial seawater. The serial dilutions technique (10^{-2}–10^{-6}) was carried out and 0.1 mL was spread on seawater glutamate (SWG) agar medium (artificial seawater (3.9% (*w/v*) of sea salt from ATI Coral Ocean), 0.1% sodium glutamate, and 1.6% agar (Difco) [16] with antifungal agent cycloheximide (50 mg/L). Incubation was processed at 30 °C for 2–4 weeks and growth was checked under the stereomicroscope. The strain NZ-96^T was isolated by repeated streaking on MA (Marine agar, Difco). The strain was preserved in marine broth 2216 (MB, Difco) with 50% (*v/v*) glycerol at −80 °C.

All characterization of the strain, unless otherwise specified, are based on cultures incubated at 30 °C for 3 days on MA or MB medium.

2.2. Morphological, Cultural, and Physiological Characterization

Over the course of 1 week, morphological observations were performed on MA 2216 medium incubated at 30 °C. Phase-contrast microscopy and electron microscopy (SEM and negative staining TEM) were used to analyze the cell morphology. A scanning electron microscopy (SEM) was carried out following the previous method with a few modifications [17]. In a nutshell, 1-day-old bacterial broth culture were fixed in aldehydes solution (2% glutaraldehyde for 30 min, followed by 5% formaldehyde for 30 min). After twice washing with TE buffer (10 mM TRIS, 1 mM EDTA, and pH 6.9), it was dehydrated in a graded series of acetone (10, 30, 50, 70, 90, and 100%). After drying the samples with liquid CO$_2$, they were sputter-coated with gold-palladium and subsequently examined with a Zeiss Merlin field emission scanning electron microscope applying the HE-SE detector and the inlens-SE detector in a 75:25 ratio. For transmission electron microscopy (TEM), a sample drop was placed on with a thin carbon film, taken off with a grid, twice washed

before being negatively stained with 4% uranyl acetate, and subsequently examined with a Zeiss Libra 120 at different magnifications.

Gram staining was carried out following the manufacturer's instructions using a Gram stain kit (Sigma-Aldrich CA, USA). The RAL color code (https://www.ralfarben.de accessed on 29 February 2022) was used to determine the culture's color [18]. N, N-dimethyl-p-phenylenediamine dihydrochloride, and 3% (*v/v*) hydrogen peroxide solutions were employed to determine the oxidase and catalase activities, respectively. The strain growth under anaerobic conditions at 30 °C was evaluated on MA in an anaerobic chamber by employing the Anaerocult A, as per the manufacturer's instructions. The hanging drop method was used for motility testing [6]. Varying temperatures (4, 15, 20, 25, 30, 37, and 44 °C) were used to determine the growth on MA medium. Growth was also observed on CY medium (Casitone 3.00 g, $CaCl_2 \times 2.H_2O$ 1.36 g, Yeast extract 1.00 g, Agar 16.00 g, Distilled water 1000 mL, pH 7.2) augmented with 39 g of artificial sea salts (3.9% (*w/v*) of sea salt from ATI Coral Ocean). The pH range 3-11 with one unit difference of pH was used to examine the growth in MB. The pH validation was performed after autoclaving. Resistance towards sodium chloride was evaluated with varying concentrations of (2.5, 5, 7.5, and 10% (*w/v*)) NaCl on agar medium containing (per liter) 5.9 g $MgCl_2$, 3.24 g Na_2SO_4, 1.8 g $CaCl_2$, 0.55 g KCl, 5.0 g peptone, 0.1 g ferric citrate, and 1.0 g yeast extract (pH 7.6) [19]. Biochemical and physiological properties were examined using API ZYM, API 20E, and API 20NE strips (Biomerieux, Marcy l'Etoile, France), and the Biolog Gen III Microplate system (Biolog, CA, USA) as per the manufacturer's instructions.

A 24-well plate was used with 1% filter sterilized carbon sources (arabinose, cellulose, fructose, inositol, mannitol, raffinose, rhamnose, sucrose, and xylose) added to MA medium for carbon sources utilization. An antibiotic resistance test was performed on agar plates containing filter-sterilized antibiotics with the final concentration of 50 μg/mL^{-1} except for oxytetracycline ampicillin, chloramphenicol, and hygromycin. The antibiotics used were bacitracin (50 μg/mL), polymyxin (50 μg/mL), gentamicin (50 μg/mL), oxytetracycline (10 μg/mL), trimethoprim (50 μg/mL), ampicillin (100 μg/mL), chloramphenicol (30 μg/mL), spectinomycin (50 μg/mL), kanamycin (50 μg/mL), cephalosporin (50 μg/mL), fusidic acid (50 μg/mL), thiostrepton (50 μg/mL), and hygromycin (150 μg/mL) [20,21].

2.3. 16S rRNA Gene Sequence and Phylogenetic Analysis

The phylogenetic analysis of the isolate was based on a 16S rRNA gene sequence. Strain NZ-96^T was cultivated in MB medium for 3 days at 30 °C. A Nucleospin microbial DNA kit (Macherey-Nagel, Düren, Germany) was used to extract the genomic DNA. Primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGA CTT-3') were used for the amplification of the 16S rRNA gene. Following analysis by the gel electrophoresis, the PCR product was purified using the Nucleospin gel and PCR clean-up kit (Macherey Nagel) as per the manufacturer's instructions. Primers F1100 (5'-CAACGAGCGCAACCC-3'), R1100 (5'-GGGTTGCGCTCGTTG-3'), and R518 (5'-CGTATTACCGCGGCTGCTGG-3') were applied for sequencing in addition to the primers used for primary PCR, to assure that both nucleotide directions were covered. The program BioEdit (version 7.0.5.3) was used for the assembly of acquired sequences. The cap contig function of the BioEdit sequence editor (version 7.0.5.3) was used to build the consensus sequences and compared with the NCBI-BLAST nucleotide database using the FASTA search tool [20]. Phylogenies were deduced by the web server of GGDC (Meier-Kolthoff et al., 2021) available at http://ggdc.dsmz.de/ accessed on 29 February 2022 using the DSMZ phylogenomics pipeline [22] adapted to single genes. MUSCLE was used to create multiple sequence alignment [23]. Maximum likelihood (ML) and maximum parsimony (MP) trees were deduced from the RAxML [24] and TNT [25] alignments, respectively. For ML, rapid bootstrapping in connection with the autoMRE bootstopping criterion [26] and subsequent search for the best tree was used. For MP, 1000 bootstrapping replicates were carried out in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence addition replicates. The sequences were checked for a compositional bias

using the X^2 test as implemented in PAUP* [27]. The 16S rRNA gene sequence of strain NZ-96^T was deposited at Genbank under the accession no. MZ569436.

2.4. Chemotaxonomy

To study chemotaxonomic fingerprints of respiratory quinones, fatty acids, and polar lipids, freeze-dried cells were used. Cells of strain NZ-96^T, *Qipengyuania pelagi* UST081027-248^T, *Qipengyuania citreus* RE35F/1^T, and *Erythrobacter aureus* YH-07^T, were incubated for 3 days in MB at 30 °C and harvested and washed three times with sterile phosphate buffer saline prior to freeze-drying.

The polar lipids extraction was carried out by adding 6.75 mL of chloroform-methanol-0.3% aqueous sodium chloride (50-100-40 *v/v*) solution to 50 mg of lyophilized biomass and run overnight on a rotator. The extraction was based on the method by Minnikin et al. [28]. Separation of polar lipids was performed by a two-dimensional thin-layer chromatography (2D-TLC) on silica gel, using five TLC plates (10 × 10) per isolate. Each plate was spotted with 10 µL lipid extracts, followed by treatment with chloroform/methanol/water (65:25:4) and chloroform/methanol/acetic acid/water (80:12:15:4) by volume for the first and second dimensions, respectively. A range of reagents were used for the detection of polar lipids with varying functional groups [29]. Phosphomolybdic acid was applied on the first plate to detect all polar lipids. Aminolipids detection was performed by staining the second plate with ninhydrin, followed by the application of molybdenum blue to the same plate for the detection of phospholipids. To detect glycolipids, the third plate was stained with α-naphthol [28]. Anisaldehyde and Dragendorff's reagents were applied to the fourth and fifth plates, respectively. Polar lipids were visualized by heating all the stained plates at 100–120 °C, except the molybdenum blue and Dragendorff's stained plates, which were developed at room temperature.

Extraction using a ternary solvent system of hexane/methanol/water mixture in a ratio of (2:1:1), respectively, was carried out for the presence or absence of bacteriochlorophyll *a* and carotenoids [30], and subsequent analysis by reverse-phase high-performance liquid chromatography (Agilent 1260 series) equipped with diode-array detection and mass spectrometry (RP-HPLC-DAD-MS). High-resolution electron spray ionization mass spectrometry (HR-ESI-MS) data were generated by Maxis ESI-TOF-MS spectrometer (Bruker). The RP-HPLC system used Acquity C18 column 2.1 × 50 mm, 1.7 µm with gradient elution employing two mobile phases (solvent A: water + 0.1 formic acid and solvent B: acetonitrile + 0.1 formic acid) with the flow rate of 0.6 mL/min. The gradient system was 5% B in the first 0.5 min, increasing gradually to 100% B in 19.5 min, and finally withholding for 5 min at 100% B. Isoprenoid quinones were extracted according to the method of MinniKin et al., (1984) [28] and were analyzed by HPLC following Risdian et al. [31] with slight modifications using isocratic condition acetonitrile/isopropanol 65:35 (*v/v*) and flow rate 0.3 mL/min. Cellular fatty acids were extracted and methylated to yield fatty acid methyl esters (FAMEs) following the method of Sasser [32]. The FAME analysis was carried out using 6890N gas chromatography with a Macherey Nagel Optima 5 column (5% phenyl, 95% dimethylpolysiloxane; 50 m length; 0.32 mm inner diameter; and 0.25 µm film thickness) and equipped with a flame ionization detector (FID). Identification of FAMEs were performed by comparing them with the in-house reference standard.

2.5. Secondary Metabolites Production and Minimum Inhibitory Concentration Assay

Strain NZ-96^T was incubated in a 250 mL Erlenmeyer flask carrying 100 mL MB medium with 2% (*v/v*) of purified adsorbent resin XAD-16N at 30 °C with 160 rpm agitation for 4 days. The adsorbent was filtered through a sieve and collected in a flask after incubation. A total of 50 mL of acetone was added and placed at room temperature for 1 h in darkness. To remove the XAD resin the eluate was filtered (ROTH, membrane 240 mm, folded filter) after incubation. Utilizing a rotary evaporator, the acetone was removed to yield a solid residue (40 °C, 1 mb). The obtained crude extract was stored at −20 °C after being dissolved in (100:1) methanol. The minimum inhibitory concentrations (MICs) assay

was conducted against a panel of microorganisms following the previous method [33]. The indicator strains used were *Escherichia coli* acrB JW25113, *Staphylococcus aureus* Newman, *Citrobacter freundii* DSM 30039, *Candida albicans* DSM 1665, *Bacillus subtilis* DSM 10, *Escherichia coli* wild type BW25113, *Mycobacterium smegmatis* ATCC 700084, *Pseudomonas aeruginosa* DSM 19882, *Mucor hiemalis* DSM 2656, *Pichia anomala* DSM 6766, and *Acinetobacter baumannii* DSM 30008. The strains were added to 40 mL of their respective growth medium (Mueller–Hinton Broth medium containing 0.5% casein peptone, 0.5% protease peptone, 0.1% meat extract, 0.1% yeast extract, pH 7.0 for bacteria, MYC medium containing 1.0% glucose, 1.0% phytone peptones, and 50-mM HEPES 11.9 g/L, pH 7.0 for fungi and yeasts [34]) and mixed well. Diluted cultures in a volume of 150 μL were transferred into each well of a 96-well plate (initial OD_{600} for the bacteria was 0.01; and for fungi and yeasts, 0.05). The first row was augmented by an additional 130 μL of indicator culture. Crude extracts (20 μL) of the strain NZ-96^T were added to the first row. The extract was serially diluted (1:1) by transferring 150 μL from one well to the next in a 96-well plate. Methanol and antibiotics were used as negative and positive controls, respectively. Thereafter, the microtiter plates were incubated on a microplate shaker incubator at 160 rpm at 30 or 37 °C for 24 h. Visual inspection of the indicator strain was performed after incubation. Turbidity absence in a well showed strain inhibition.
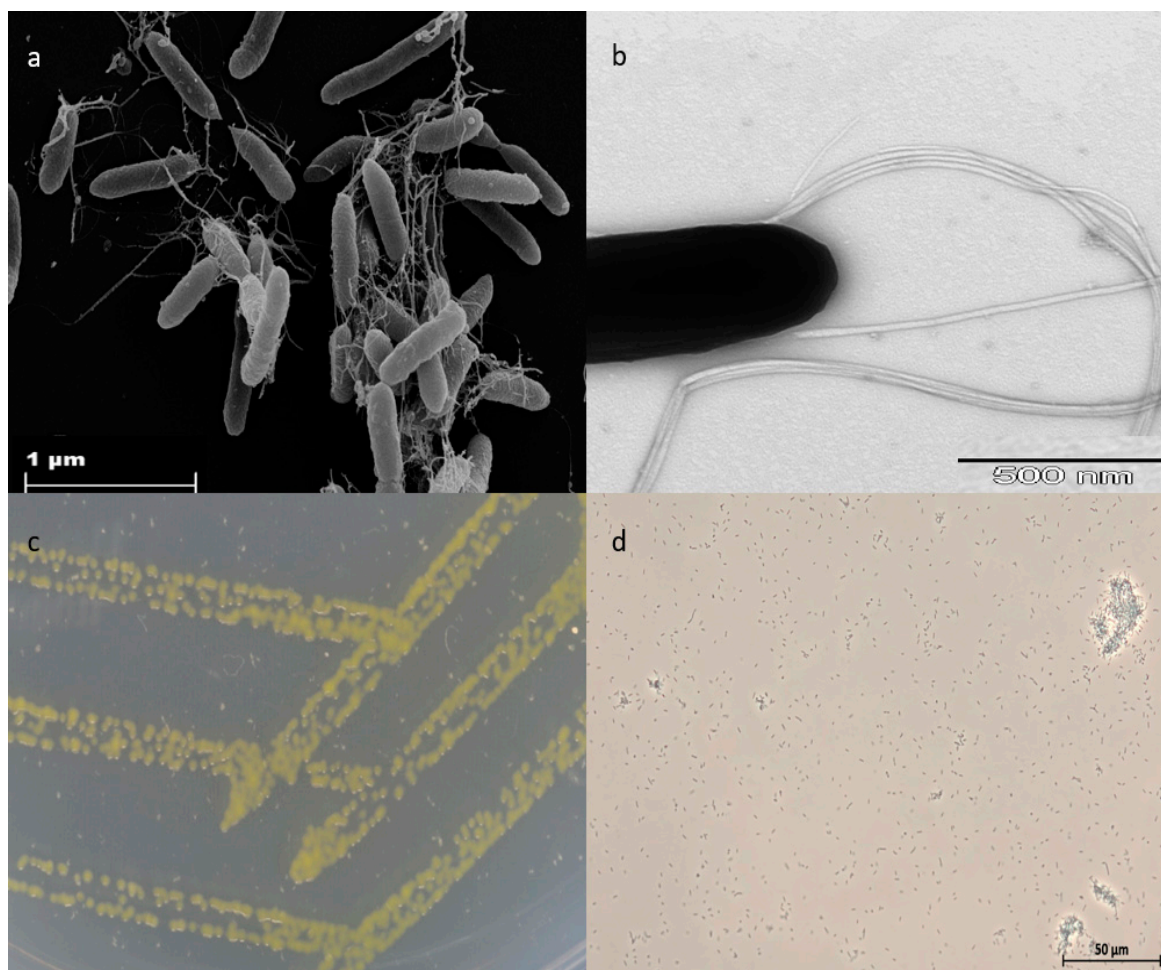
2.6. Genome Analysis

Genomic DNA was extracted from a pure culture grown for 3 days on MA at 30 °C using a Nucleospin Microbial DNA kit (Macherey-Nagel, Düren, Germany) following the protocol from the manufacturer. Genome sequencing was conducted at the sequencing facility (HZI Braunschweig, Germany) using the Illumina MiSeq (300 bp, paired-end reads) with NexteraXT protocol for library preparation. Unicycler [35] and Prokka [36] were employed for de novo assembly and genome annotation, respectively. Draft genome sequences were submitted to NCBI Genbank and analyzed using ContEst16S (www.ezbiocloud.net/tools/contest16s, accessed on 29 August 2021) [37] to verify the purity of the genome. The whole-genome sequence of strain NZ-96^T was deposited at DDBJ/ENA/GenBank under the accession JAHWXO000000000. The guanine and cytosine (G+C) content of the genomic DNA (mol%) was calculated using genome assembly annotation 1.14.0 (https://github.com/tseemann/prokka, accessed on 1 September 2021). Rapid Annotation using Subsystem Technology (RAST) (https://rast.nmpdr.org/, accessed on 2 September 2021) [38] and antibiotics and secondary metabolite analysis shell (antiSMASH) (https://antismash.secondarymetabolites.org/, accessed on 2 September 2021) [39] were used for the analysis of metabolic reconstruction and secondary metabolite gene clusters prediction, respectively. Responsible genes for strain NZ-96^T survival in the sea environment were deciphered using the RAST server. Based on the genome sequence of NZ-96^T and its closely related phylogenetic neighbours, a phylogenomic tree was produced using the type (strain) genome server (TYGS) (https://tygs.dsmz.de/, accessed on 6 September 2021). For the phylogenomic inference, Genome Blast Distance Phylogeny (GBDP) was employed for all pairwise comparisons of genomes and under the algorithm 'trimming' as well as distance formula d5 [22]. The accurate intergenomic distances were inferred with 100 distance replicates. Using the amino acid sequences of the complete proteome, a second GBDP phylogenomic analysis was deduced, promising a better-resolved phylogeny. Pairwise average nucleotide identity (ANI) values between strain NZ-96^T and its closest phylogenetic relatives were determined using the EzBiocloud server (www.ezbiocloud.net/tools/ani, accessed on 15 September 2021). Digital DNA–DNA hybridization (dDDH) values between the genome data of strain NZ-96^T and its closest type strains were estimated using the Genome to Genome Distance Calculator (GGDC; version 2.1) [40].

3. Results and Discussions

3.1. Morphological and Physiological Characteristics

In February 2017, Strain NZ-96[T] was isolated from a sponge sample obtained at a depth of 596 m below the ocean surface in Otago/Canterbury slope (45° 02/N, 171° 90/W), Pacific Ocean, New Zealand. Cells of strain NZ-96[T] were catalase and oxidase-positive, Gram-negative, non-spore-forming, and straight rods that occur singly and in pairs. Colonies were circular, zinc yellow-colored, and convex with entire margins (Figure 1). A scanning electron microscopy image of strain NZ-96[T] depicts distinctly the rod-shaped morphology (2.5–3.0 um in length and 0.5–0.6 um wide) and cell-connecting filaments that represent the bundle-forming flagella-like structure (Figure 1). Strain NZ-96[T] grew at 15–37 °C (optimal growth was shown at 20–30 °C). No growth was observed at 4 °C and 44 °C. Growth was displayed at pH 6–9 (optimum growth shown at pH 6). Growth was observed in the presence of 0–7.5% (*w/v*) NaCl with optimum growth at 2.5% (*w/v*) NaCl. The strain also grew on CY medium, apart from marine agar 2216 (Difco). An antibiotic susceptibility test showed that the strain was sensitive to thiostrepton (50 μg/mL), fusidic acid (50 μg/mL), kanamycin (50 μg/mL), and gentamycin (50 μg/mL). However, resistance was shown against, spectinomycin (50 μg/mL), ampicillin (100 μg/mL), oxytetracycline (10 μg/mL), trimethoprim (50 μg/mL), cephalosporin (50 μg/mL), hygromycin (150 μg/mL), bacitracin (50 μg/mL), chloramphenicol (30 μg/mL), and polymyxin (50 μg/mL).

Figure 1. (**a**) Scanning electron micrograph (SEM) of cells grown on marine agar with the formation of cell-connecting fibers, (**b**) TEM of a single cell with cell-connecting fibers, (**c**) growth of strain NZ-96[T] on marine agar after 72 h, (**d**) phase contrast microscopy of strain NZ-96[T] showing rod-shaped morphology.

Biochemical and physiological properties of strain NZ-96^T at 30 °C according to API ZYM (Table S1), API 20E (Table S2), API 20NE (Table S3) strips, and the Biolog Gen III Microplate system (Table S4) showed a pronounced activity for acid phosphatase, leucine arylamidase, valine arylamidase, alkaline phosphatase, trypsin, naphthol-AS-BI-phosphohydrolase, cystine arylamidase, α-glucosidase, inositol and rhamnose fermentation, arginine dihydrolase, and esculin hydrolysis. Whereas no activity was seen for β-galactosidase, β-glucuronidase, α-galactosidase, β-glucosidase, ornithine decarboxylase, β-galactosidase (ONPG), N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, urease, melibiose fermentation, and mannose assimilation. Strain NZ-96^T showed activity for lysine decarboxylase and citrate utilization in comparison to *Qipengyuania pelagi* JCM 14468^T and *Qipengyuania citreus* DSM 14432^T which showed no activity. In contrast to *Qipengyuania pelagi* UST081027-248^T and *Qipengyuania citreus* RE35F/1^T (which reduces nitrate, strain NZ-96^T was negative for the said property), cells of strain NZ-96^T are motile rods; whereas *Qipengyuania pelagi* JCM 14468^T and *Qipengyuania citreus* RE35F/1^T cells are non-motile. Strain NZ-96^T was positive for α-glucosidase, whereas *Qipengyuania citreus* RE35F/1^T was negative for the said property. A detailed comparison of phenotypic properties and enzymatic activities among strain NZ-96^T and comparative analyses strains are shown in Tables S1–S3 of Supplementary Materials.
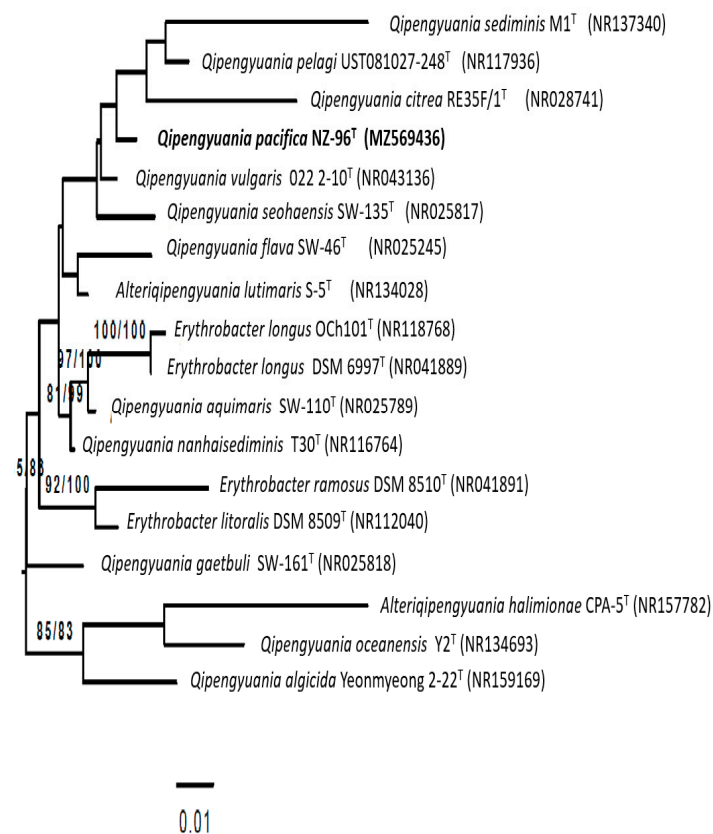
3.2. 16S rRNA Gene-Based Phylogenetic Analysis

Sequence similarity comparisons of the 16S rRNA gene using NCBI- BLAST [41] depicted that strain NZ-96^T was closely related to *Qipengyuania citreus* RE35F/1^T (98.8%), *Erythrobacter aureus* YH-07^T (98.3%), and *Qipengyuania pelagi* UST081027-248^T (98.3%). Inference of the tree (Figure 2 maximum likelihood tree rooted by midpoint rooting) based on an almost complete 16S rRNA gene sequence (1425 nucleotides) depicted that strain NZ-96^T forms a well-defined tight cluster with *Qipengyuania pelagi* UST081027-248^T coupled with high bootstrap value. Based upon the 16S rRNA gene sequence similarities and phylogenetic analysis, *Qipengyuania pelagi* UST081027-248^T, *Qipengyuania citreus* RE35F/1^T, and *Erythrobacter aureus* YH-07^T were designated as the reference strains for comparative analysis.
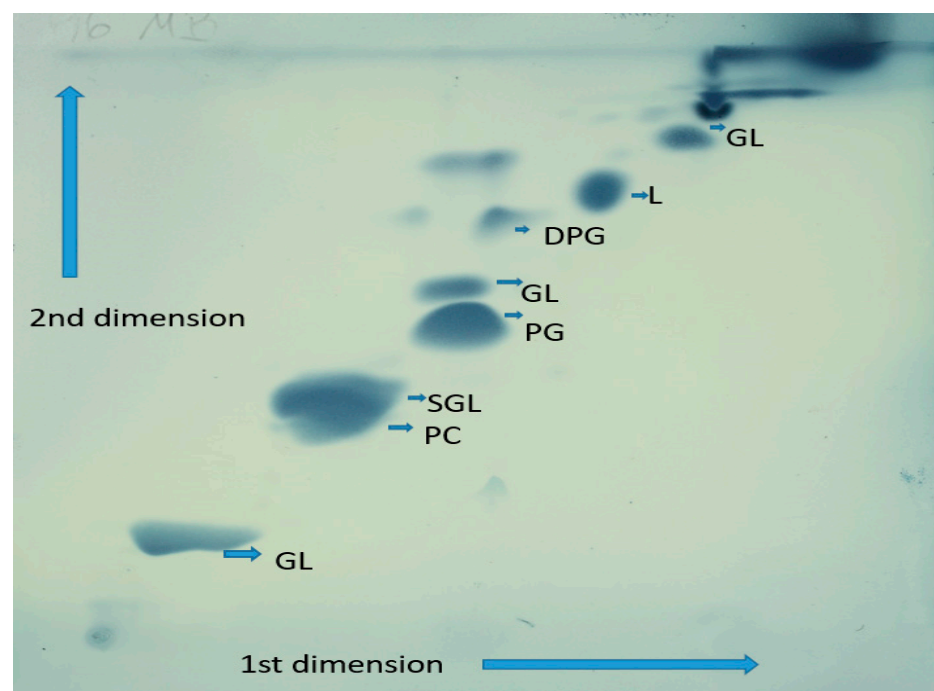
3.3. Chemotaxonomy

Analyses of the chemical composition of cell constituents of NZ-96^T showed that it carries chemotaxonomic features that are in agreement with those prescribed for members of the family *Erythrobacteraceae* [42]. The respiratory quinone profiles consisted exclusively of ubiquinone-10 (Q-10), which is in agreement with the description of genus *Qipengyuania* [12]. The polar lipid profile of NZ-96^T is composed of phosphatidylcholine, sphingoglycolipid, phosphatidylglycerol, one unknown polar lipid, and three unknown glycolipids (Figure 3). The major fatty acids of strain NZ-96^T are $C_{18:1}\omega12t$ (40.3%), $C_{16:0}$ (11.9%), $C_{16:1}\omega7c$ (9.9%), $C_{17:1}\omega6c$ (9.5%), $C_{16:0}$2-OH (5.9%), and $C_{14:0}$2-OH (5.1%). The fatty acid profiles of strain NZ-96^T and its closely related members were presented in Table 1. Differences in the percentages of major fatty acids $C_{18:1}\omega12t$, $C_{16:0}$, and $C_{16:1}\omega7c$ distinguish the strain from its close relatives. The absence of $C_{19:0}$ and $C_{17:1}\omega8c$, and the presence of $C_{18:0}$ and some other quantitative differences between the novel strain NZ-96^T and closely related strains can be considered as a distinguishing characteristic for the novel species.

BChl *a* was absent. The ternary solvent extract spectra exhibited peak retention time at 22.16 min with UV absorption at 425 nm (the characteristic spectra of carotenoid-like pigments).

Figure 2. Maximum-likelihood phylogenetic tree based on almost complete 16S rRNA gene sequence of strain NZ-96^T and its most closely related species, inferred under the GTR+GAMMA model. The numbers above the branches are support values when larger than 60% from ML (**left**) and MP (**right**) bootstrapping. Bar 0.01 substitution per nucleotide position.

Figure 3. Polar lipids observed in strain NZ-96^T are DPG: diphosphatidylglycerol; GL1-3: unknown glycolipid; PGL: unknown phosphoglycolipid; L: Unknown polar lipid; PG: Phosphatidylglycerol; SGL: Sphingoglycolipid; PC: Phosphatidylcholine.

Table 1. Fatty acids profiles of strain NZ-96[T] and the phylogenetically closest relatives. Strains 1: NZ-96[T]; 2: *Qipengyuania citreus* RE35F/1[T]; 3: *Erythrobacter aureus* YH-07[T]; and 4: *Qipengyuania pelagi* UST081027-248[T]. Values are percentages of the total fatty acids. The bold type represents the major fatty acids. -: Not detected.

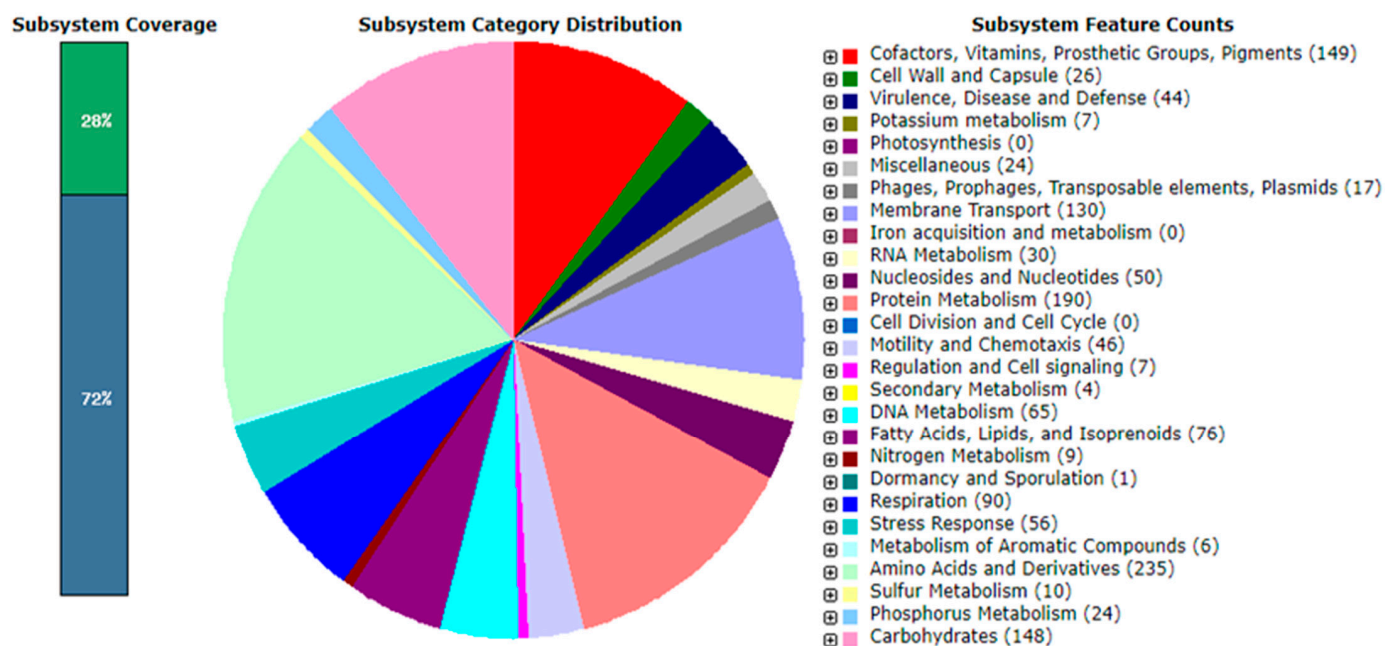| Fatty Acids	1	2	3	4
Saturated				
$C_{15:0}$	1.57	—	—	5.76
$C_{16:0}$	**11.98**	3.32	4.21	5.11
$C_{17:0}$	1.91	1.93	—	4.63
$C_{18:0}$	1.21	—	—	—
$C_{19:0}$	—	1.63	—	—
Branched				
$C_{17:0}$a	0.77	0.78	—	3.97
Unsaturated				
$C_{17:1}\omega6c$	**9.54**	**17.76**	7.66	**34.69**
$C_{18:1}\omega12t$	**40.29**	—	**55.06**	—
$C_{16:1}\omega7c$	**9.93**	6.75	**10.31**	3.04
$C_{16:1}\omega7t$	0.72	1.12	1.14	—
$C_{17:1}\omega8c$	—	1.69	—	—
$C_{18:1}\omega7c$	—	—	—	**20.92**
Hydroxy				
$C_{14:0}2$-OH	5.09	3.23	5.89	4.25
$C_{16:0}2$-OH	5.89	3.77	6.36	—
$C18:1\omega9t$ and/or $C_{18:1}\omega7c$	—	**40.52**	—	—

3.4. Antimicrobial Potential

The extract of strain NZ-96[T] exhibited a varying degree of inhibition activity against the tested strains. A minimal inhibitory concentration of crude extracts from NZ-96[T] showed high activity against Gram-positive bacteria, *Bacillus subtilis* DSM 10, and *Staphylococcus aureus* Newman. Whereas only the highest concentration of NZ-96[T] XAD extract in the first well showed activity against tested Gram-negative bacteria, *Pseudomonas aeruginosa* PA14 DSM 19882, and *Escherichia coli* DSM 1116. No antimicrobial activity was observed against fungal and yeast organisms such as, *Pichia anomala* DSM 6766, *Candida albicans* DSM 1665, and *Mucor himalis* DSM 2656 in this approach. The strain NZ-96[T] crude extract was fractionated through a high-performance liquid chromatography (HPLC) and subsequently tested in bioassays against the susceptible strains, i.e., *Staphylococcus* aureus Newman and *Bacillus subtilis* DSM 10 in order to determine the peak-related activities (Figure S1). The micro-fractionation was performed in 96-well plates to narrow down the active compounds of the crude extract.

3.5. Genome Analysis

The draft assembled genome of strain NZ-96[T] yielded 54 contigs with a total sequence length of 3,497,702 bp. Annotation resulted in 3400 coding sequences, 48 tRNA genes, 6 rRNA operons, and a single tmRNA gene. The DNA G+C content of strain NZ-96[T] was 60.8 mol%, a value in line with the range reported for members of genus *Qipengyuania*, i.e., 60.6–66.7% [4].
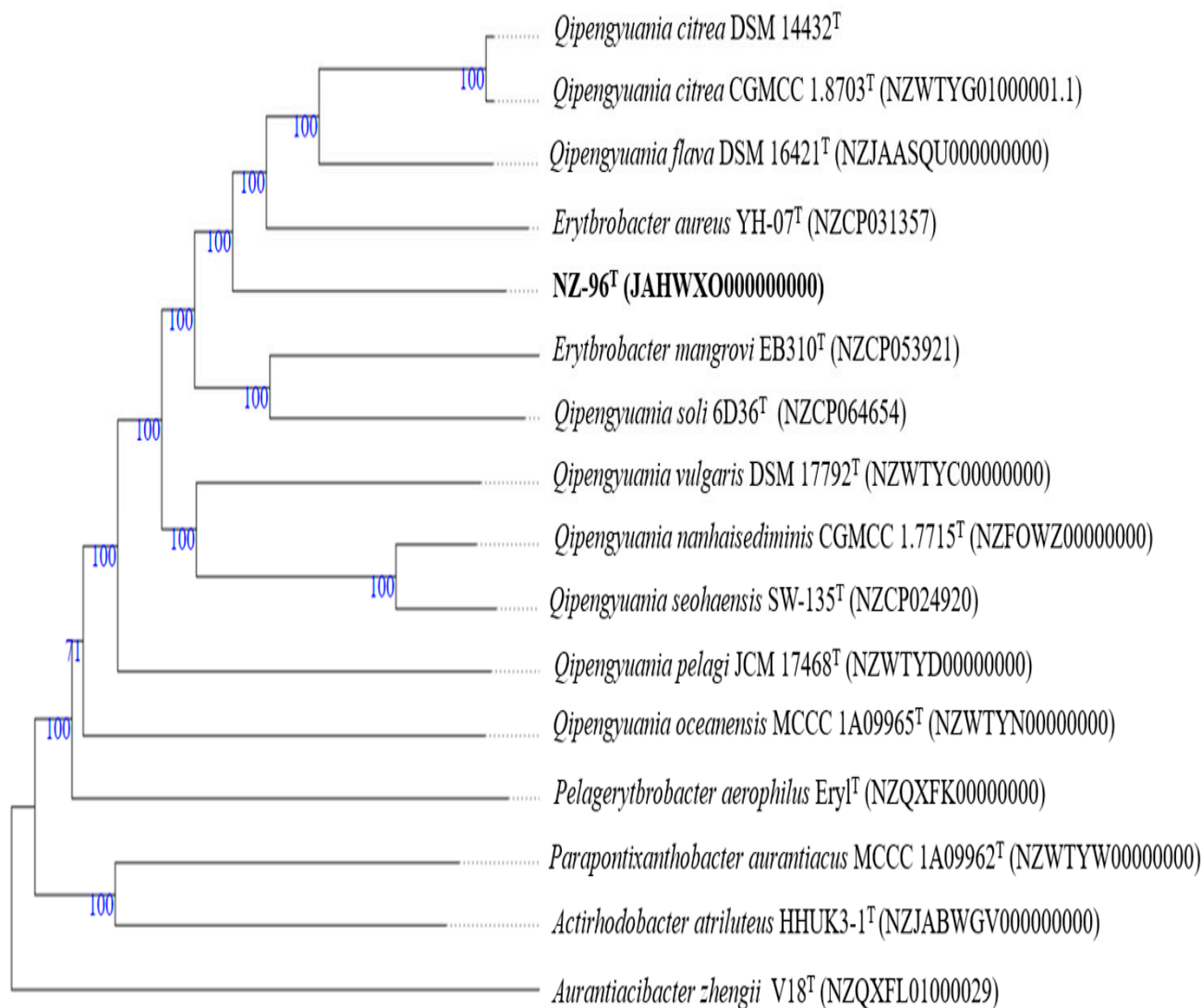
A RAST analysis revealed that only 28% of the annotated genes in the genome of strain NZ-96[T] were assigned to the subsystems category (Figure 4). Among the subsystem categories, amino acids and derivate metabolism had the highest feature counts (235), followed by protein metabolism with 190 feature counts. Then, 32 feature counts were detected for resistance to antibiotics and toxic compounds; whereas, genomes of *Qipengyuania citreus* RE35F/1[T], *Qipengyuania pelagi* UST081027248[T], and *Erythrobacter aureus* YH-07[T] contained 40, 29, and 18 feature counts, respectively. The characteristic genes and gene products present in strain NZ-96[T] were determined via the RAST analysis (Figure 4). Strain NZ-96[T] contained the proteins involved in the metabolism of carbohydrates, biosynthesis of amino acids and derivatives, metabolism of fatty acids, lipids, isoprenoids, motility, and chemotaxis. Concerning ecology, gene products related to the resistance of antibiotics and toxic compounds such as copper, zinc, and chromium compounds were present. The anti-SMASH server predicted three secondary metabolite biosynthesis gene clusters displaying similarities to terpene (66%), beta lactone, lasso peptide, and hserlactone biosynthetic gene clusters (Figure S2). A genome analysis indicated the absence of the photosynthetic gene cluster (PGC) involved in the BChl *a* biosynthetic pathway (*bch* genes, *puf* genes, *puh* genes, and regulatory genes). However, strain NZ-96[T] harbored *crt* genes responsible for the biosynthesis of carotenoids.

Figure 4. Subsystem category distribution of strain NZ-96[T] based on RAST annotation server (https://rast.nmpdr.org/, accessed on 2 September 2021).

A phylogenomic tree (Figure S3) with low average branch support of 61.8% generated with TYGS server using genome sequence showed that the strain NZ-96[T] was separated from the closest phylogenetic neighbors *Qipengyuania pelagi* UST081027248[T] and *Qipengyuania citrea* RE35F/1[T] based on the 16S rRNA gene sequence analyses. A second whole-proteome-based tree was well-supported with high average branch support of 97.8%, depicting the reliable placement of strain NZ-96[T] in the tree. Strain NZ-96[T] was placed in a group that contains members of *Qipengyuania* and a couple strains that are put in single quotes (Figure 5). Pairwise ANI values between genome sequence of strain NZ-96[T] and the closest phylogenetic hits *Qipengyuania citreus* RE35F/1[T], *Erthrobacter aureus* YH-07[T], *Qipengyuania pelagi* UST081027248[T], and *Qipengyuania seohaensis* SW-135[T] were 79.38%, 78.93%, 75.43%, and 75.81%, respectively, values clearly below the threshold value of 95–96% that corresponds to the species boundary. The dDDH values between strain NZ-96[T] and strains *Qipengyuania citreus* RE35F/1[T], *Erythrobacter aureus* YH-07[T], *Qipengyuania pelagi*

UST081027248^T, and *Qipengyuania seohaensis* SW135^T were 22.1%, 21.4%, 21.4%, and 19.4%, respectively (Table 2); values well-below the cut-off value of 70% (dDDH) generally used for species delineation. The ANI and DNA relatedness values warrant that strain NZ-96^T represents a new species of the genus *Qipengyuania*. Detailed contrasting characteristics that distinguish strain NZ-96^T from its closely related phylogenetic relatives are provided in Table 3.

Figure 5. Tree deduced with FastME 2.1.6.1 [43] from whole-proteome-based GBDP distances. The branch lengths are scaled via GBDP distance formula d5. Branch values are GBDP pseudo-bootstrap support values >60% from 100 replications, with average branch support of 97.8%.

Table 2. 16S, ANI, and dDDH values of NZ-96^T and its closely related phylogenetic species.

Reference Type Strains	16S	ANI	dDDH
Qipengyuania citreus RE35F/1^T	98.80	79.38	22.10
Erythrobacter aureus YH-07^T	98.38	78.93	21.40

Table 2. *Cont.*

Reference Type Strains	16S	ANI	dDDH
Qipengyuania pelagi UST081027-248[T]	98.31	75.43	21.40
Qipengyuania seohaensis SW-135[T]	98.02	75.81	19.40
Qipengyuania vulgaris 022 2-10[T]	98.15	77.31	23.50
Sphingomonas phyllosphaerae FA2[T]	97.75	70.00	20.20
Qipengyuania aquimaris SW-110[T]	97.74	75.68	19.60
Qipengyuania gaetbuli SW-161[T]	97.66	76.43	19.70
Erythrobacter litoralis DSM 8509[T]	97.22	72.61	18.80

Table 3. Differential properties of strain NZ-96[T] and closely related type strains.

Properties	1	2	3	4
Motility	+	−	−	+
Esterase (C 4)	w+	w+	+	+
Esterase lipase (C 8)	w+	+	+	+
Cystine arylamidase	+	w+	w+	w+
Trypsin	+	w+	+	+
α-chymotrypsin	w+	+	−	+
Naphthol-AS-BI-phosphohydrolase	+	w+	+	+
α-glucosidase	+	+	−	−
lysine decarboxylase	w+	−	−	−
Citrate utilization	w+	−	−	−
Tryptophan deaminase	w+	+	+	+
Gelatinase	−	w+	−	−
Nitrate reduction	−	+	+	+
Arginine dihydrolase	+	−	−	−
Inositol fermentation	+	−	−	−
L-Alanine	−	+	+	w+
L-Galactonic acid lactone	+	w+	+	w+
L-Lactic acid	+	−	w+	−
D-Salicin	+	−	−	w+
Citric acid	−	+	+	−
L-Histidine	−	−	w+	+
L-pyroglutamic acid	+	w+	+	+
Stachyose	−	−	w+	+
N-acetyl Neuraminic acid	+	−	w+	+

Table 3. *Cont.*

Properties	1	2	3	4
D-serine	−	+	−	+
Polar lipids	PC, SGL, PG, DPG, 3GL, 1L.	* DPG, PC, PE, PG, 2L, 1P.	** PE, PG, DPG, PC, SGL.	*** DPG, PC, PE, PG, SGL, 2L.
Fatty acids				
$C_{16:0}$	11.98%	5.11%	3.32%	4.21%
$C_{18:1}\omega12t$	40.29%	ND	ND	55.06
$C_{16:0}2$-OH	5.89	ND	3.77%	6.36%

Strains **1**: NZ-96[T]; **2**: *Qipengyuania pelagi* JCM 14468[T]; **3**: *Qipengyuania citreus* DSM 14432[T]; **4**: *Erythrobacter aureus* DSM 107319[T]; +: positive; − negative; w+: weakly positive; ND: not determined; DPG: diphosphatidylglycerol; PE: phosphatidylethanolamine; PC: phosphatidylcholine; SGL: sphingoglycolipid; PG: phosphatidylglycerol; L: Unknown polar lipid; PL: unknown phospholipid; GL: unknown glycolipid; PG: phosphatidylglycerol, and P: unknown pigmented lipid. *All data are from this study except polar lipids type strains data marked with *, **, and *** are from previous studies. *: Data from [19]; **: data from [7]; ***: data from [44].

Detailed differential characteristics that distinguish strain NZ-96[T] from its closely related phylogenetic relatives are provided in Table 3. Given these combined chemotaxonomic, morphological, phylogenetic, and phylogenomic analyses, strain NZ-96[T] guarantees classification in the genus *Qipengyuania*. The level of DNA–DNA association and phenotypic characteristics confirmed that the strain constitutes a separate species. Therefore, based on the data presented, we propose to include strain NZ-96[T] in the genus *Qipengyuania* as *Qipengyuania pacifica* sp. nov.

4. Description of *Qipengyuania pacifica* sp. nov.

Qipengyuania pacifica (pa.ci′fi.ca. L. fem. adj. pacifica) pertains to the Pacific Ocean from where the type strain was isolated. The cells are Gram-stain-negative, motile, rod-shaped, and aerobic. Colonies on marine agar (MA) were circular, smooth, opaque, convex with entire margins, zinc-yellow colored, and 0.7–1.0 mm in diameter at 30 °C for 3 days. Flagellum was present. BChl *a* was not produced. Growth occurred at 15–37 °C (optimum growth at 20–30 °C), at pH 6–9 (optimum growth at pH 6), and in 0–7.5% NaCl (optimum growth at 2.5% (*w/v*) NaCl). Positive activity was observed for catalase and oxidase tests. No reduction in nitrate was observed. The cells were negative for the production of H_2S, indole, and acetoin. The enzyme reactions for valine arylamidase, cystine arylamidase, alkaline phosphatase, leucine arylamidase, trypsin, acid phosphatase, esculin hydrolysis, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and arginine dihydrolase were positive. The enzyme reactions for α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, β-galactosidase (ONPG), ornithine decarboxylase, urease, gelatinase were negative. Dextrin, D-trehalose, cellobiose, sucrose, turanose, raffinose, α-D-lactose, β-methyl-D-glucoside, D-salicin, N-acetyl-β-d-mannosamine, N-acetyl-D-galactosamine, N-acetylneuraminic acid, D-fucose, L-fucose, L-rhamnose, D-arabitol, Myo-inositol, glycerol, D-fructose-6-PO4, L-arginine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, lincomycin, D-galacturonic acid, L-galactonic acid lactone, D-glucose-6-PO4, D-gluconic acid, D-galactose, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, L-lactic acid, α-keto-glutaric acid, L-malic acid, Tween 40, β-hydroxy-d, L-butyric acid, propionic acid, and acetic acid can be used as sole carbon sources. The principal fatty acids are $C_{18:1}\omega12t$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{17:1}\omega 6c$. The polar lipids profile includes phosphatidylcholine, sphingoglycolipid, phosphatidylglycerol, one unknown polar lipid, and three unknown glycolipids. The respiratory quinone is ubiquinone-10 (Q-10). The G+C content is 60.8 mol%.

The type strain NZ-96[T] (=DSM 112811[T] = NCCB 100842[T]) was isolated from a sponge sample. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and draft genome sequence of strain NZ-96[T] is MZ569436 and JAHWXO000000000, respectively.

5. Conclusions

Morphological, cultural, and chemotaxonomic markers, in addition to a phylogenomic tree analysis, revealed that strain NZ-96[T] belongs to the genus *Qipengyuania*. The strain can be distinguished from the closely related type strains by several striking characteristics. Crude extracts from NZ-96[T] showed high activity against Gram-positive bacteria, *Staphylococcus aureus* Newman, and *Bacillus subtilis* DSM 10. Whereas weak activity was displayed against tested Gram-negative bacteria, *Pseudomonas aeruginosa* PA14 DSM 19882, and *Escherichia coli* DSM 1116. No antimicrobial activity was observed against fungal and yeast organisms such as, *Pichia anomala* DSM 6766, *Candida albicans* DSM 1665, and *Mucor himalis* DSM 2656 in this approach. On the basis of the polyphasic approach, we propose to include strain NZ-96[T] in the genus *Qipengyuania*. as *Qipengyuania pacifica* sp. nov.

References

1. Back, C.R.; Stennett, H.L.; Williams, S.E.; Wang, L.; Ojeda Gomez, J.; Abdulle, O.M.; Duffy, T.; Neal, C.; Mantell, J.; Jepson, M.A.; et al. A New *Micromonospora* Strain with Antibiotic Activity Isolated from the Microbiome of a Mid-Atlantic Deep-Sea Sponge. *Mar. Drugs* **2021**, *19*, 105. [CrossRef]
2. Webster, N.S.; Taylor, M.W. Marine Sponges and Their Microbial Symbionts: Love and Other Relationships. *Environ. Microbiol.* **2012**, *14*, 335–346. [CrossRef]
3. Schmitt, S.; Tsai, P.; Bell, J.; Fromont, J.; Ilan, M.; Lindquist, N.; Perez, T.; Rodrigo, A.; Schupp, P.J.; Vacelet, J.; et al. Assessing the Complex Sponge Microbiota: Core, Variable and Species-Specific Bacterial Communities in Marine Sponges. *ISME J.* **2012**, *6*, 564–576. [CrossRef]
4. Xu, L.; Sun, C.; Fang, C.; Oren, A.; Xu, X.W. Genomic-Based Taxonomic Classification of the Family *Erythrobacteraceae*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 4470–4495. [CrossRef]
5. Zhuang, L.; Lin, B.; Xu, L.; Li, G.; Wu, C.J.; Luo, L. *Erythrobacter spongiae* sp. nov., isolated from marine sponge. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 1111–1116. [CrossRef]
6. Fang, C.; Wu, Y.H.; Sun, C.; Wang, H.; Cheng, H.; Meng, F.X.; Wang, C.S.; Xu, X.W. *Erythrobacter zhengii* sp. nov., a bacterium isolated from deep-sea sediment. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 241–248. [CrossRef]

7. Denner, E.B.M.; Vybiral, D.; Koblízek, M.; Kämpfer, P.; Busse, H.-J.; Velimirov, B. *Erythrobacter citreus* sp. nov., a yellow-pigmented bacterium that lacks Bacteriochlorophyll *a*, isolated from the western mediterranean sea. *Int. J. Syst. Evol. Microbiol.* **2002**, *52*, 1655–1661. [CrossRef]

8. Lei, X.; Zhang, H.; Chen, Y.; Li, Y.; Chen, Z.; Lai, Q.; Zhang, J.; Zheng, W.; Xu, H.; Zheng, T. *Erythrobacter luteus* sp. nov., isolated from mangrove sediment. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 2472–2478. [CrossRef]

9. Subhash, Y.; Tushar, L.; Sasikala, C.; Ramana, C.V.; Ch Ramana, C.V. *Erythrobacter odishensis* sp. nov. and *Pontibacter odishensis* sp. nov. isolated from dry soil of a solar saltern. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 4524–4532. [CrossRef]

10. Xing, T.; Liu, Y.; Wang, N.; Xu, B.; Liu, K.; Shen, L.; Gu, Z.; Guo, B.; Zhou, Y.; Liu, H. *Erythrobacter arachoides* sp. nov., isolated from ice core. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 4235–4239. [CrossRef]

11. Park, S.; Won, S.M.; Yoon, J.H. *Erythrobacter marisflavi* sp. nov., isolated from estuary water. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 2696–2702. [CrossRef]

12. Tonon, L.A.C.; Moreira, A.P.B.; Thompson, F. The Family *Erythrobacteraceae*. In *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*; Springer: Berlin/Heidelberg, Germany, 2014; Volume 9783642301, pp. 213–235. [CrossRef]

13. Shiba, T.; Simidu, U. *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains Bacteriochlorophyll *a*. *Int. J. Syst. Bacteriol.* **1982**, *32*, 211–217. [CrossRef]

14. Feng, X.M.; Mo, Y.X.; Han, L.; Nogi, Y.; Zhu, Y.H.; Lv, J. *Qipengyuania sediminis* gen. nov., sp. nov., a Member of the Family *Erythrobacteraceae* Isolated from Subterrestrial Sediment. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 3658–3665. [CrossRef]

15. Parte, A.C. LPSN—List of Prokaryotic Names with Standing in Nomenclature (Bacterio.Net), 20 Years On. *Int. J. Syst. Evol. Microbiol.* **2018**, *68*, 1825–1829. [CrossRef]

16. Hosoya, S.; Arunpairojana, V.; Suwannachart, C.; Kanjana-Opas, A.; Yokota, A. *Aureispira marina* gen. nov., sp. nov., a gliding, Arachidonic Acid-Containing Bacterium Isolated from the Southern Coastline of Thailand. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2931–2935. [CrossRef]

17. Landwehr, W.; Kämpfer, P.; Glaeser, S.P.; Rückert, C.; Kalinowski, J.; Blom, J.; Goesmann, A.; Mack, M.; Schumann, P.; Atasayar, E.; et al. Taxonomic Analyses of Members of the *Streptomyces cinnabarinus* Cluster, Description of *Streptomyces cinnabarigriseus* sp. nov. and *Streptomyces davaonensis* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2018**, *68*, 382–393. [CrossRef]

18. Safaei, N.; Nouioui, I.; Mast, Y.; Zaburannyi, N.; Rohde, M.; Schumann, P.; Müller, R.; Wink, J. *Kibdelosporangium persicum* sp. nov., a new member of the *Actinomycetes* from a Hot Desert in Iran. *Int. J. Syst. Evol. Microbiol.* **2021**, *71*, 004625. [CrossRef]

19. Wu, H.X.; Lai, P.Y.; Lee, O.O.; Zhou, X.J.; Miao, L.; Wang, H.; Qian, P.Y. *Erythrobacter Pelagi* sp. nov., a member of the Family *Erythrobacteraceae* isolated from the Red Sea. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 1348–1353. [CrossRef]

20. Tareen, S.; Risdian, C.; Müsken, M.; Wink, J. *Alteriqipengyuania Abyssalis* sp. nov., a Novel Member of the Class *Alphaproteobacteria* Isolated from Sponge, and Emended Description of the Genus *Alteriqipengyuania*. *Diversity* **2021**, *13*, 670. [CrossRef]

21. Mohr, K.I.; Garcia, R.O.; Gerth, K.; Irschik, H.; Müller, R. *Sandaracinus Amylolyticus* gen. nov., sp. nov., a starch-degrading soil myxobacterium, and Description of *Sandaracinaceae* fam. nov. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 1191–1198. [CrossRef]

22. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.P.; Göker, M. Genome Sequence-Based Species Delimitation with Confidence Intervals and Improved Distance Functions. *BMC Bioinform.* **2013**, *14*, 60. [CrossRef]

23. Edgar, R.C. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [CrossRef]

24. Stamatakis, A. RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef]

25. Goloboff, P.A.; Farris, J.S.; Nixon, K.C. TNT, a Free Program for Phylogenetic Analysis. *Cladistics* **2008**, *24*, 774–786. [CrossRef]

26. Pattengale, N.D.; Alipour, M.; Bininda-Emonds, O.R.P.; Moret, B.M.E.; Stamatakis, A. How Many Bootstrap Replicates Are Necessary? *J. Comput. Biol.* **2010**, *17*, 337–354. [CrossRef]

27. Swofford, D.L. PAUP Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0 Beta 10. Sinauer Associates, Sunderland—References—Scientific Research Publishing. 2002. Available online: https://www.scirp.org/(S(lz5mqp453edsnp5 5rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=1958232 (accessed on 14 June 2021).

28. Minnikin, D.E.; O'Donnell, A.G.; Goodfellow, M.; Alderson, G.; Athalye, M.; Schaal, A.; Parlett, J.H. An Integrated Procedure for the Extraction of Bacterial Isoprenoid Quinones and Polar Lipids. *J. Microbiol. Methods* **1984**, *2*, 233–241. [CrossRef]

29. Goldberg, S.R.; Correa, H.; Haltli, B.A.; Kerr, R.G. *Fulvivirga Aurantia* sp. nov. and *Xanthovirga Aplysinae* gen. nov., sp. nov., Marine Bacteria Isolated from the Sponge *Aplysina Fistularis*, and Emended Description of the Genus *Fulvivirga*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 2766–2781. [CrossRef]

30. Bóna-Lovász, J.; Bóna, A.; Ederer, M.; Sawodny, O.; Ghosh, R. A Rapid Method for the Extraction and Analysis of Carotenoids and Other Hydrophobic Substances Suitable for Systems Biology Studies with Photosynthetic Bacteria. *Metabolites* **2013**, *3*, 912–930. [CrossRef]

31. Risdian, C.; Landwehr, W.; Rohde, M.; Schumann, P.; Hahnke, R.L.; Spröer, C.; Bunk, B.; Kämpfer, P.; Schupp, P.J.; Wink, J. *Streptomyces Bathyalis* sp. nov., an Actinobacterium Isolated from the Sponge in a Deep Sea. *Antonie Van Leeuwenhoek* **2021**, *114*, 425–435. [CrossRef]

32. Sasser, M. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. Technical Note # 101. 2001. Available online: https://www.researchgate.net/publication/284025789_Identification_of_bacteria_by_gas_chromatography_of_cellular_ fatty_acids (accessed on 29 February 2022).

33. Khosravi Babadi, Z.; Ebrahimipour, G.; Wink, J.; Narmani, A.; Risdian, C. Isolation and Identification of *Streptomyces* sp. Act4Zk, a Good Producer of Staurosporine and Some Derivatives. *Lett. Appl. Microbiol.* **2021**, *72*, 206–218. [CrossRef]

34. Khodamoradi, S.; Hahnke, R.L.; Mast, Y.; Schumann, P.; Kämpfer, P.; Steinert, M.; Rückert, C.; Surup, F.; Rohde, M.; Wink, J. *Streptomonospora litoralis* sp. nov., a halophilic thiopeptides producer isolated from Sand Collected at Cuxhaven Beach. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2021**, *114*, 1483–1496. [CrossRef]

35. Wick, R.R.; Judd, L.M.; Gorrie, C.L.; Holt, K.E. Unicycler: Resolving Bacterial Genome Assemblies from Short and Long Sequencing Reads. *PLoS Comput. Biol.* **2017**, *13*, e1005595. [CrossRef]

36. Seemann, T. Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef]

37. Lee, I.; Chalita, M.; Ha, S.M.; Na, S.I.; Yoon, S.H.; Chun, J. ContEst16S: An Algorithm That Identifies Contaminated Prokaryotic Genomes Using 16S RNA Gene Sequences. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 2053–2057. [CrossRef]

38. Aziz, R.K.; Bartels, D.; Best, A.; DeJongh, M.; Disz, T.; Edwards, R.A.; Formsma, K.; Gerdes, S.; Glass, E.M.; Kubal, M.; et al. The RAST Server: Rapid Annotations Using Subsystems Technology. *BMC Genom.* **2008**, *9*, 1–15. [CrossRef]

39. Blin, K.; Shaw, S.; Steinke, K.; Villebro, R.; Ziemert, N.; Lee, S.Y.; Medema, M.H.; Weber, T. AntiSMASH 5.0: Updates to the Secondary Metabolite Genome Mining Pipeline. *Nucleic Acids Res.* **2019**, *47*, W81–W87. [CrossRef]

40. Meier-Kolthoff, J.P.; Göker, M.; Spröer, C.; Klenk, H.P. When Should a DDH Experiment Be Mandatory in Microbial Taxonomy? *Arch. Microbiol.* **2013**, *195*, 413–418. [CrossRef]

41. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic Local Alignment Search Tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]

42. Kosako, Y.; Yabuuchi, E.; Naka, T.; Fujiwara, N.; Kobayashi, K. Proposal of *Sphingomonadaceae* fam. Nov., Consisting of *Sphingomonas* Yabuuchi et Al. 1990, *Erythrobacter* Shiba and Shimidu 1982, *Erythromicrobium* Yurkov et Al. 1994, *Porphyrobacter* Fuerst et Al. 1993, Zymomonas Kluyver and van Niel 1936, and Sandaracinobac. *Microbiol. Immunol.* **2000**, *44*, 563–575. [CrossRef]

43. Lefort, V.; Desper, R.; Gascuel, O. FastME 2.0: A Comprehensive, Accurate, and Fast Distance-Based Phylogeny Inference Program. *Mol. Biol. Evol.* **2015**, *32*, 2798. [CrossRef]

44. Tang, T.; Sun, X.; Dong, Y.; Liu, Q. *Erythrobacter aureus* sp. nov., a plant growth-promoting bacterium isolated from sediment in the Yellow Sea, China. *3 Biotech* **2019**, *9*, 430. [CrossRef] [PubMed]