



Article Characterization of *Terrihabitans soli* gen. nov., sp. nov., a Novel 0.2 μm-Filterable Soil Bacterium Belonging to a Widely Distributed Lineage of *Hyphomicrobiales* (*Rhizobiales*)

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Abstract: We previously showed that novel filterable bacteria remain in "sterile" (<0.2 µm filtered) terrestrial environmental samples from Japan, China, and Arctic Norway. Here, we characterized the novel filterable strain IZ6^T, a representative strain of a widely distributed lineage. Phylogenetic analysis showed that this strain was affiliated with the Rhizobiales (now proposed as Hyphomicrobiales) of Alphaproteobacteria, but distinct from any other type strains. Strain IZ6^T shared the following chemotaxonomic features with the closest (but distantly) related type strain, Flaviflagellibacter deserti SYSU D60017^T: ubiquinone-10 as the major quinone; phosphatidylethanolamine, phosphatidylcholine, and phosphatidylglycerol as major polar lipids; and slightly high G+C content of 62.2 mol%. However, the cellular fatty acid composition differed between them, and the unsaturated fatty acid $(C_{18:1}\omega7c/C_{18:1}\omega6c)$ was predominantly found in our strain. Moreover, unlike methyrotrophs and nitrogen-fixers of the neighboring genera of *Hyphomicrobiales* (*Rhizobiales*), strain IZ6^T cannot utilize a one-carbon compound (e.g., methanol) and fix atmospheric nitrogen gas. These findings were consistent with the genome-inferred physiological potential. Based on the phylogenetic, physiological, and chemotaxonomic traits, we propose that strain IZ6^T represents a novel genus and species with the name *Terrihabitans soli* gen. nov., sp. nov. (=NBRC 106741^{T} = NCIMB 15058^{T}). The findings will provide deeper insight into the eco-physiology of filterable microorganisms.

Keywords: filter sterilization; filterable bacteria; soil; phylogeny; taxonomy; *Hyphomicrobiales; Rhizo-biales*

1. Introduction

Removal of microorganisms using a micropore filter (e.g., 0.2 μ m pore size) is a common procedure in research laboratories as well as medical and industrial processes [1]. However, some bacteria in nature are known to pass through a 0.2 μ m-pore-size filter and to persist in "sterile" (<0.2 μ m filtered) environmental samples (reviewed in [2–5]). Culturing efforts targeting filterable microorganisms have uncovered novel filterable bacteria, including obligate and facultative ultramicrobacteria as well as slender bacteria [3,5].



Citation: Nakai, R.; Naganuma, T.; Tazato, N.; Kunihiro, T.; Morohoshi, S.; Koide, T.; Kusada, H.; Tamaki, H.; Narihiro, T. Characterization of *Terrihabitans soli* gen. nov., sp. nov., a Novel 0.2 μm-Filterable Soil Bacterium Belonging to a Widely Distributed Lineage of *Hyphomicrobiales* (*Rhizobiales*). *Diversity* **2021**, *13*, 422. https:// doi.org/10.3390/d13090422

Academic Editor: Michael Wink

Received: 24 July 2021 Accepted: 27 August 2021 Published: 1 September 2021

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Such filterable microorganisms have been explored and mined mainly in water samples collected in aquatic systems [5]. On the other hand, culture-independent analyses have also revealed that novel and/or rarely cultivated microorganisms are present in small cell size fractions (<0.8 μ m) of terrestrial soil systems [6]. Nonetheless, there are only a few successes of isolating pure cultures from filtered fractions in soils (e.g., *Verrucomicrobia* isolates [7]).

During the course of mining ultra-small microorganisms, we isolated and cultivated a novel 0.2 µm-filterable *Rhizobiales* bacterium, strain IZ6^T, from a filtered suspension of forest soil [8]. We also obtained its phylogenetically closely-related isolates from other filtered samples (i.e., bark soil, sand, and travertine) collected in geographically remote areas such as Japan, China, and Arctic Norway [8]. These isolates formed a novel genus-level cluster in the 16S rRNA gene sequence-based phylogenetic tree [9]. Previous investigations also showed that strain IZ6^T was not a typical ultramicrobacterium (cell volume, <0.1 μ m³ [3,5]) under the optimal culture condition, but it does exhibit cell plasticity resulting in some filterable cells under a colder temperature condition [9]. This study characterized a cultivated representative strain IZ6^T of a widely distributed terrestrial lineage by using polyphasic approaches including morphological, physiological, biochemical, and chemotaxonomic analyses in addition to phylogenomic analysis. Based on the characterizations, we propose the novel genus and species, Terrihabitans soli gen. nov., sp. nov., for this strain. Note that the order *Hyphomicrobiales* of the class *Alphaproteobacteria* was recently proposed as a replacement for *Rhizobiales* [10] and is herein tentatively described as *Hyphomicrobiales* (*Rhizobiales*) in reference to previous research [11].

2. Materials and Methods

2.1. Phylogenetic and Phylogenomic Analysis

The full-length 16S rRNA gene sequence of strain IZ6^T was retrieved from the genome sequence (AP023361 [9]). The sequence was BLASTn-searched against the NCBI nt/nr database (accessed, June 2021) for the identification of the closest type strains, taxonomically undescribed strains, and uncultivated clones/phylotypes. To evaluate genome-scale similarities, the whole-genome-based average nucleotide identity (gANI) of strain $IZ6^{T}$ against the closest type strains identified using BLASTn was calculated using the ANI calculator [12]. The two-way averaged amino acid identity (AAI) and alignment fractions (AF) values were also calculated by using the AAI and AF calculators [13,14]. For the whole genome-based taxonomic analysis, the genome sequence data were analyzed by the Type (Strain) Genome Server (TYGS [15]) (accessed, June 2021). For the phylogenomic inference, all pairwise comparisons among the set of genomes were conducted using the Genome BLAST Distance Phylogeny approach (GBDP), and accurate intergenomic distances were inferred under the algorithm "trimming" and distance formula d_5 [16]. A total of 100 distance replicates were calculated for each genome. Digital DNA-DNA hybridization (DDH) values and confidence intervals were calculated using the recommended settings of the GGDC 2.1 [16]. The resulting intergenomic distances were used to infer a balanced minimum-evolution tree with branch support via FASTME 2.1.6.1, including SPR postprocessing [17]. Branch support was inferred from 100 pseudo-bootstrap replicates. The trees were rooted at the midpoint [18] and visualized with PhyD3 [19].

2.2. Metagenomic Database Search for Potential Habitat Prediction

The potential distribution and habitability of strain IZ6^T and its close relatives were predicted by the IMNGS platform [20], which is a database search against metagenomederived 16S rRNA gene amplicon datasets, as well as the ProkAtlas search, which contains multiple 16S rRNA gene sequences labeled by one environmental category [21]. Both tools were performed with the sequence similarity threshold of 99%, using the sequence of IZ6^T as the query.

2.3. Morphological, Physiological, and Biochemical Characterization

Strain IZ6^T was originally isolated from a 0.2 μ m filtrate of a suspension of forest soil collected in March 2009 from western Japan, as previously described [8]. $IZ6^{T}$ is routinely cultivated and maintained in R2A agar "DAIGO" (Nihon Pharmaceutical, Tokyo, Japan) at 25 °C. For colony observation, strain IZ6^T was grown on R2A agar at 25 °C for 7 days. The cell morphology was observed in a previous study [9]. The cell motility was examined by microscopy (Olympus BX-50F4; Olympus Optical, Tokyo, Japan) after cultivation at 25 °C in R2A liquid medium (R2A broth "DAIGO"; Nihon Pharmaceutical). Gram staining was carried out using a Favor G Nissui kit (Nissui Pharmaceutical, Tokyo, Japan). Growth at a range of temperatures (4 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 37 °C) was assessed on R2A agar. Growth at various pH levels (pH 4–12) and salt concentrations (0-2% [w/v])NaCl) was also determined on R2A agar; the pH was adjusted by adding 1M or 6M HCl or NaOH prior to sterilization. Anaerobic growth was tested using a chamber with an Anaero Pack system (Mitsubishi Gas Chemical, Tokyo, Japan). Catalase and oxidase activities were assessed following the previously reported method [22]. Physiological and biochemical characteristics and enzyme activities were tested at 25 °C using the API 20NE and APIZYM test strips (bioMérieux, Tokyo, Japan) according to the manufacturer's instructions. Growth on methanol was tested with a minimal medium K containing methanol (0.5%, v/v) as reported in a previous study [23]. Nitrogen fixation potential was assessed based on the growth at 25 °C for 3-4 weeks on a nitrogen-free MS2N medium following the method of Bao et al. [24]. To account for the effect of nitrogen carryover derived from the pre-cultivation medium (R2A medium) before inoculation, cells grown in MS2N were inoculated into a fresh MS2N medium, and further growth was confirmed.

2.4. Chemotaxonomic Analyses

The chemotaxonomic properties of strain IZ6^T were characterized by data on the cellular fatty acid composition, major respiratory quinone, polar lipids, and genomic GC content. For the fatty acid analysis, cells grown on R2A agar at 25 °C for 5 days were harvested and used. Fatty acids methyl esters were prepared and analyzed using the protocol of the Sherlock Microbial Identification system version 6.0 (Microbial ID; MIDI Inc., Newark, DE, USA). The fatty acid profile was compared and determined with the TSBA library (TSBA6 6.20). For the quinone determination, cells were grown on R2A agar at 25 °C for 5 days and were harvested and freeze-dried. The total lipids of the cells were extracted by a modification of the previously reported method [25], and the quinones in the crude extract were purified using a Sep-Pak plus silica column (Waters, Milford, MA, USA). The molecular type and concentration of each quinone extracted were analyzed by an ultra-performance liquid chromatography (UPLC) system (Acquity UPLC system; Waters) equipped with a photodiode array detector (UPPDA-E; Waters), an Eclipse plus C18 column (3.0×150 mm; pore size, 3.5μ m; Agilent Technologies, Tokyo, Japan), and Empower 2 software (Waters). The quinone species was finally determined based on the linear relationship between the logarithm of the UPLC retention time and the number of isoprene units according to the equivalent number of isoprene units of quinone components as reported by Tamaoka et al. [26]. For the characterization of polar lipids, extracted lipids were also separated by two-dimensional HPTLC silica gel 60 (Merck, Tokyo, Japan) following the previously reported experimental method and condition [27]. The genomic G + C content was calculated from the complete genome sequence of this strain (AP023361 [9]).

3. Results and Discussion

3.1. Phylogenetic Affiliation and Phylogenomic Placement of Strain IZ6^T

The full-length 16S rRNA gene sequence of strain IZ6^T was moderately related to *Hyphomicrobiales* (*Rhizobiales*) members of *Alphaproteobacteria*, but showed no more than 97% sequence identity to those of known type strains as of June 2021. The closest type strain was *Flaviflagellibacter deserti* SYSU D60017^T, which was isolated from desert soil [28], and

shared 96.9% sequence identity with strain IZ6^T. Type strain SYSU D60017^T is assigned to the family *Rhizobiaceae* in the NCBI taxonomy and to Ca. Methylopilaceae in the Genome Taxonomy Database (GTDB), although the family-level classification is not assigned in the List of Prokaryotic names with Standing in Nomenclature (LPSN). Note that this taxonomic issue regarding the placement of the family of strains IZ6^T and SYSU D60017^T will be discussed later. As of June 2021, the taxonomically undescribed isolates most closely related (16S rRNA gene sequence identities, 98.7–100%) to strain IZ6^T were five filterable strains (DDBJ/ENA/NCBI accession nos. AB539978, AB539979, AB539981, AB539982, and AB540022) isolated from 0.2 µm-filtered fractions of terrestrial samples collected in Japan, China, and Arctic Norway, as reported in our previous study [8]. These data indicate that strain IZ6^T and its close relatives are taxonomically novel candidates belonging to the *Hyphomicrobiales* (*Rhizobiales*).

The whole-genome-based average nucleotide identity (gANI) and the alignment fractions (AF) value of strain $IZ6^{T}$ against the closest type strain *F. deserti* were 75.4% and 0.50–0.59, respectively. These values were somewhat higher than the threshold for genus-level differentiation [29], while the value (69.9%) of the averaged amino acid identity (AAI) falls within the genus-level threshold (60–80% [30]). Moreover, a phylogenomic tree based on the Genome BLAST Distance Phylogeny (GBDP) approach indicated that although this strain was relatively close to *F. deserti*, this represents a novel genus-level lineage (Figure 1). This result was consistent with the phylogenetic novelty inferred from the previous 16S rRNA gene-based phylogenetic tree [9].



Figure 1. Phylogenomic tree of *Terrihabitans soli* IZ6^T and its relatives as inferred by FastME 2.1.6.1 [17]

from the Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5 . The numbers above branches are GBDP pseudo-bootstrap support values of >60% from 100 replications, with average branch support of 71.8%. The tree was rooted at the midpoint [18]. The distribution of the genome size is shown as a bar graph on the right side; the genomes ranged from 3.1 Mbp to 9.8 Mbp. The data for strain IZ6^T are colored orange, and all other data are colored blue. The scale bar indicates the branch length value.

It should be noted that the genome size (3.11 Mb) of $IZ6^{T}$ is smaller than that (3.72 Mb) of *F. deserti* and was one of the smallest sizes among the free-living members in *Hyphomicrobiales* (*Rhizobiales*) (Figure 2). The smaller genomes all belong to host-associated (e.g., parasitic) members of the genera *Bartonella*, Ca. Tokpelaia, Ca. Liberibacter, Ca. Hodgkinia. In general, planktonic and filterable ultramicrobacteria in aquatic systems tend to have small genomes, known as so-called "genome streamlining" (1–2 Mb [5,31,32]). Although strain $IZ6^{T}$ does not exhibit the typical cell size of ultramicrobacteria, as described below, its genome size is at the boundary between those of free-living and host-associated bacteria. This suggests that genome reduction and/or streamlining of $IZ6^{T}$ is currently underway, but this will require further validation, including genome analysis of other filterable relatives.



Figure 2. Relationship between the genome size and the number of protein-coding genes in *Hyphomicrobiales* (*Rhizobiales*) genomes. A total of 373 genomic datasets registered with the genome completion status "finished" and the alphaproteobacterial orders "*Hyphomicrobiales*" and "*Rhizobiales*" were downloaded from the Integrated Microbial Genomes and Microbiomes (IMG/M) [33,34] (accessed on June 2021). The scientific names of some representative members, including our strain indicated by a yellow-filled circle, are shown; the host-associated (e.g., parasitic) members are circled by a gray dashed line.

3.2. Morphological, Physiological, and Biochemical Characteristics of Strain IZ6^T

Cells of strain IZ6^T were rods with a size of $1.5-2.0 \times 0.4-0.5 \mu m$ (cell volume, ~0.36 μm^3) under the 25 °C culture condition exhibiting good growth. This is not the size of typical ultramicrobacteria, which generally have a cell volume of <0.1 μm^3 [3,5]. However, our previous study showed that smaller cells, as well as some ultra-small cells and cell-like particles (cell/particle volume, ~0.06 μm^3), were observed when the temperature condition was changed from 25 °C to 15 °C [9]. This cell plasticity may be related to the ability to pass through 0.2 μm -pore-sized filters. The strain was Gram-stain negative, catalase-positive, and oxidase-positive. Growth was observed at 10–30 °C, but not at 4 °C or 37 °C, and at pH 6–10 with weak growth at pH 5 or 11; it should be noted that potential changes in pH during cultivation can affect the pH growth range data. Growth was also observed at 0–1.0% NaCl, with no growth at 1.5% NaCl or above. In the API 20NE and API ZYM tests, alkaline phosphatase, esterase, esterase lipase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase were positive, and all others were negative.

The *Hyphomicrobiales* (*Rhizobiales*) group is phenotypically diverse and contains methanotrophs, methylotrophs, and nitrogen fixers [10,35]. In contrast, strain IZ6^T exhibited no growth on methanol-amended medium and nitrogen-free medium. This result is consistent with the absence of the gene sequences encoding the key enzymes (i.e., methanol dehydrogenase and methane monooxygenase for one-carbon compound metabolism as well as nitrogenase for nitrogen fixation) in the IZ6^T genome [9]. The previous genomic annotation also identified the presence of a nitrate reductase gene (*nasA*) in the genome [9], but the test for nitrate reduction by using API 20NE was negative under the condition tested. Further verification of this aspect of the characterization will be needed.

The characteristics of strain $IZ6^{T}$ lacking methanol utilization and nitrogen fixation capacity were shared with the closest type strain *F. deserti* [28]. However, in addition to differences in motility and growth temperature, pH, and salinity, several enzymatic activities also differed between $IZ6^{T}$ and *F. deserti*, indicating clear phenotype differences (Table 1). Moreover, strain $IZ6^{T}$ also showed a different trend from the phenotypic characteristics of strains of two other relatively related genera (Table 1).

Characteristic	1	2	3	4
Isolation source	Forest soil	Desert soil	Forest soil	Wheat soil
Motility	Nonmotile	Motile	Nonmotile	Motile
Growth at/in the				
presence of:				
4 °C	_	+	—	_
37 °C	—	+	+	+
pH 9	+	—	+	—
1.5% (w/v) NaCl	—	+	—	—
Assimilation of glucose	_	+	+	+
Enzymatic activities:				
Lipase (C14)	—	+	—	—
Leucine arylamidase	—	+	+	—
Valine arylamidase	—	+	+	—
Cystine arylamidase	—	+	+	—
Trypsin	-	+	+	-

Table 1. Differential phenotypic characteristics of strain IZ6^T, its closest type strain (*Flaviflagellibacter deserti* SYSU D60017^T), and two other related strains of the *Hyphomicrobiales* (*Rhizobiales*).

Strains: 1, IZ6^T (data from this study); 2, *Flaviflagellibacter* (data from [28]). Both strains were Gram-stain negative, aerobic, catalase-positive, and oxidase-positive. In the API 20NE kit, both strains were negative for all tests except for glucose assimilation, as shown below. In the API ZYM, both strains were positive for alkaline phosphatase, esterase, esterase lipase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, and were negative for all tests except for the enzymatic activities, where differences were observed as shown below. Data from two other strains of the *Hyphomicrobiales* (*Rhizobiales*) were used for comparison purposes; 3, *Pinisolibacter* [36]; 4, *Methylopila* [37]. Note that these two relatively related genera were selected from the previous 16S rRNA gene-based phylogenetic tree [9].

3.3. Chemotaxonomic Characteristics of Strain IZ6^T

The dominant cellular fatty acids (>5% of the total cellular fatty acids) were $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$ (71.6%), $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ (5.9%), $C_{19:0}$ cyclo $\omega8c$ (5.48%), and $C_{16:0}$ (5.26%). Other minor fatty acids (0.5%~5%) were $C_{17:0}$ (2.94%), $C_{18:0}$ (2.38%), $C_{16:0}$ 3-OH (1.99%), $C_{17:1}\omega6c$ (0.86), $C_{12:0}$ 3-OH (0.72%), $C_{17:0}$ cyclo (0.62), and $C_{14:0}$ (0.5%). The respiratory quinone of strain IZ6^T was determined to be ubiquinone-10 (Q-10) that is a typical trait of *Hyphomicrobiales* (*Rhizobiales*) members [36]. The polar lipids detected were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and two unidentified polar lipids (Figure S1). The genomic G + C content was 62.2 mol%, as calculated from the IZ6^T genome sequence (accession no. AP023361). The results of comparing these chemotaxonomic features with those of other relatives in the *Hyphomicrobiales* (*Rhizobiales*) are shown in Table 2. In particular, the unsaturated fatty acid(s) ($C_{18:1}\omega7c/C_{18:1}\omega6c$) was predominantly (>70% of the total cellular fatty acids) found in our strain, compared to the proportions (18–52%) of members of two related genera (*Flaviflagellibacter*). This suggests that the unsaturated fatty acid-rich characteristic is one of the key features of strain IZ6^T.

Table 2. Differential chemotaxonomic characteristics of strain $IZ6^T$ and its three related genera of the *Hyphomicrobiales* (*Rhizobiales*).

Characteristic	1	2	3	4
Major fatty acids	C _{18:1} <i>w</i> 7 <i>c</i> /C _{18:1} <i>w</i> 6 <i>c</i>	$C_{19:0}\omega 8c$ cyclo, $C_{18:1}\omega 7c/C_{18:1}\omega 6c$	$\begin{array}{c} {\rm C}_{18:1}\omega7c/{\rm C}_{18:1}\omega6c,\\ {\rm C}_{16:1}\omega7c/{\rm C}_{16:1}\omega6c,\\ {\rm C}_{16:0}\end{array}$	C _{18:1} <i>w</i> 7
Main quinone	Q-10	Q-10	Q-10	Q-10
Polar lipids	DPG, PC, PE, PG, UL	DPG, PC, PE, PG, UL	DPG, PC, PE, PG, PME, UL	PC, PE
G + C content (mol%)	62.2	63.8	68.4	66–70

Taxa: 1, strain IZ6^T (data from this study); 2, *Flaviflagellibacter* (data from [28]); 3, *Pinisolibacter* [36]; 4, *Methylopila* [38]. Note that the most closely related genus to IZ6^T and two relatively related genera were selected from the BLASTn-search result in this study and the previous 16S rRNA gene-based phylogenetic tree [9]. DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidyl-*N*-methylethanolamine; UL, unidentified polar lipids.

3.4. Potential Distribution and Habitability of Close Relatives of Strain IZ6^T

Comparative 16S rRNA gene sequence analysis revealed that strain $IZ6^{T}$ showed high sequence similarities to uncultured environmental sequences, as shown below. Environmentally derived sequences closely related (98.5–99.2%) to strain $IZ6^{T}$ were detected in a low-level-radioactive-waste site (accession no. GQ264001 [39]) and loamy soil (HQ119195 [40]) in the United States. Further, based on a search using the IMNGS platform [20], which is a database search against metagenome-derived 16S rRNA gene amplicon datasets, we also found that the $IZ6^{T}$ 16S rRNA gene sequence matched 3718 datasets, comprising mainly 2078 soil, 443 rhizosphere, and 219 plant metagenome datasets with a sequence similarity threshold of 99%. In agreement with this, the top three potential habitats inferred by using the ProkAtlas (which contains multiple 16S rRNA gene sequences labeled by one environmental category [21]) with a threshold of 99% were soil (habitat preference score, 45.4%), rhizosphere (31.0%), and plant (15.9%). These results suggested that this filterable bacterium and its close relatives inhabit different habitats in the terrestrial environment.

3.5. Proposal of a Novel Genus and Species for Strain IZ6^T

Based on the clear phenotypic differences as well as the relatively low 16S rRNA gene sequence identity (<97%) and low AAI (<70%), strain IZ6^T can be distinguished from the closest genus and species of *F. deserti*. *F. deserti* was first described as a novel bacterium closely clustered with *Methylocystaceae* clade II on the phylogenetic tree but with low sequence identity (<94%) to known neighbor genera [28]. Members of the *Methylocystaceae* lineage are known not to be a monophyletic group in the phylogenetic

tree and are separated into four different clades [28,35]: clade I (type II methanotrophs: e.g., *Methylocystis* and *Methylosinus*), clade II (methylotrophs: e.g., *Methylosulfonomonas*, and *Methylopila*), clade III (non-methanotrophs: e.g., *Chthonobacter* and *Pleomorphomonas*), and clade IV (non-methanotrophs such as *Terasakiella*). In this context, it has been pointed out that the classification of members of the class *Alphaproteobacteria* is difficult because it relies on the interpretation of insufficiently resolved 16S rRNA gene-based trees for diverse members [10]. On the other hand, recent genome-scale phylogenetic analysis has improved the taxonomic classification of the alphaproteobacterial members, including the aforementioned genera related to *Methylocystaceae* [10]. Future studies will need to include strain IZ6^T and *F. deserti*, as well as their relatives (e.g., *Pinisolibacter*; note that, as of June 2021, no genome information is available in public databases), in such genome-based taxonomic analyses to revisit their phylogenetic systematics.

On the basis of the phylogenetic, morphological, physiological, and chemotaxonomic traits, we propose the novel name *Terrihabitans soli* gen. nov., sp. nov. for strain IZ6^T.

Description of Terrihabitans gen. nov.

Terrihabitans (Ter.ri.ha'bi.tans. L. fem. n. *terra*, earth; L. pres. part. *habitans*, inhabiting; N.L. part. adj. used as a masc. n. *Terrihabitans*, earth [soil] dweller, referring to the type of ecosystem inhabited by the bacteria)

Cells are rod-shaped and grow chemoorganotrophically and aerobically. Gram-stain negative, non-motile, and non-spore-forming. The cellular fatty acids are $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, $C_{19:0}$ cyclo $\omega8c$, and $C_{16:0}$. The respiratory quinone was ubiquinone-10 (Q-10). The polar lipids contain diphosphatidylglycerol, phosphatidyl-choline, phosphatidylethanolamine, phosphatidylglycerol, and unknown polar lipids. The DNA G + C content is circa 62 mol%. The genome size is circa 3.1 Mbp.

The genus is a member of the class *Alphaproteobacteria* [41] of the phylum *Proteobacteria* [42]. The type species is *Terrihabitans soli*.

Description of *Terrihabitans soli* sp. nov.

Terrihabitans soli (so'li. L. gen. n. *soli*, of soil, the source of the type strain)

General descriptions of morphological and chemotaxonomic features are as given in the genus description. Cells grown in R2A liquid medium are rod-shaped with a size of about $1.5-2.0 \times 0.4-0.5 \mu m$ (cell volume, ~0.36 μm^3). Colonies on R2A agar medium are circular, light white, smooth, <1.0 mm in diameter, and slightly raised. Grows on R2A medium aerobically but not under anaerobic conditions. The temperature range for growth on R2A is 10 °C to 30 °C. Cells of reduced size, as well as some ultra-small cells and cell-like particles (cell/particle volume, ~0.06 μm^3), are observed when the temperature condition is changed from 25 °C to 15 °C. The pH range for growth on R2A is pH 6–10 (note that potential changes in pH during cultivation can affect the pH growth range data). NaCl concentrations of 0–0.5% in R2A medium are tolerated, and weak growth occurs at 1%. Catalase-positive and oxidase-positive. Enzymatic activities are positive for alkaline phosphatase, esterase, esterase lipase, acid phosphatase, and naphthol-AS-BIphosphohydrolase.

The type strain, strain $IZ6^T$ (= NBRC 106741^T = NCIMB 15058^T), was isolated from the 0.2 µm-filtered filtrates of a suspension of forest soil from Okuizumo, Shimane, Japan. The G + C content of the genomic DNA is 62.2 mol%, and the genome size is 3.11Mb. The genome sequence has been deposited in DDBJ/ENA/NCBI under accession no. AP023361.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/ 10.3390/d13090422/s1. Figure S1: Polar lipids of strain IZ6^T after separation by two-dimensional HPTLC. DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; UL1 and UL2, unidentified polar lipids.

Author Contributions: Conceptualization, R.N.; methodology, experiments, and data analysis, R.N., N.T., T.K. (Tadao Kunihiro), S.M. and T.K. (Tomomi Koide); writing and visualization, R.N.;

discussion, review, and editing, all authors; project supervision, R.N.; and funding acquisition, R.N., T.N. (Takeshi Naganuma), H.K. and H.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a Grant-in-Aid for JSPS Fellows (JSPS KAKENHI no. JP13J03441 to R.N.), a Grant-in-Aid for Challenging Exploratory Research (no. JP15K14424 to R.N.), a Grant-in-Aid for Young Scientists (A) (no. JP15H05620 to R.N.), and a Grant-in-Aid for Scientific Research (C) (no. JP23570117 to T. Naganuma). This study was also partially funded by a Grant-in-Aid for Challenging Exploratory Research (JSPS KAKENHI no. JP21K19394 to R.N.) and a Grant-in-Aid for Scientific Research on the Innovative Area "Post-Koch Ecology" (MEXT KAKENHI no. JP19H05683 to R.N., H.K., H.T., and JP19H05679 to R.N.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Strain IZ6^T is deposited at the Biological Resource Center, NITE (NBRC) in Japan and the National Collection of Industrial, Food, and Marine Bacteria (NCIMB) in the United Kingdom under accession nos. NBRC 106741^T and NCIMB 15058^T, respectively.

Acknowledgments: We thank Aharon Oren of the Hebrew University of Jerusalem (Jerusalem, Israel) for helpful comments and suggestions on nomenclature.

Conflicts of Interest: The authors declare no conflict of interest.

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