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Photobacterium halophilum sp. nov. and a Salt-Loving Bacterium Isolated from Marine Sediment

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Abstract: A Gram-stain-negative, rod-shaped, and facultatively anaerobic bacterium named strain GJ3^T was isolated from coastal sediment of Jeju Island, South Korea. Catalase and oxidase activity were detected in the cell of strain GJ3^T, as well as white pigmented colony and motility with polar flagellum. The cell grew optimally at 30 °C, pH 7.0, in the presence of 4% (*w*/*v*) sodium chloride. Phylogenetic analysis using the 16S rRNA gene sequence indicated that strain GJ3^T was classified to the genus *Photobacterium*, with high sequence similarity to *Photobacterium galatheae* S2753^T (98.30%), *Photobacterium halotolerans* MACL01^T (97.90%), and *Photobacterium panuliri* LBS5^T (96.55%). Strain GJ3^T possessed only ubiquinone-8 (Q-8) as a respiratory quinone and summed feature 8 as the major fatty acid (38.18%). Additionally, the dominant polar lipids phosphatidylglycerol and phosphatidylethanolamine were identified. The complete genome size and G + C content of strain GJ3^T was estimated to be 3,603,274 bp in length and 50.70%, respectively. Polyphasic approach and genomic analyses (e.g., ANI and digital DDH) revealed that strain GJ3^T (=KCTC 72816^T = KMM 6822^T) represented a novel species within the genus *Photobacterium*, and the name *Photobacterium halophilum* sp. nov., is proposed for this novel bacterium.

Keywords: *Photobacterium halophilum*; tidal flat; sediment; genome; polyphasic; novel species

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1. Introduction

The taxonomy study is a definition for nomenclature and classification for (microbial) organisms in nature ecosystem based on their shared properties, including 16S rRNA gene sequence [1,2]. To classify the organisms, the phenotypical properties were considered as a key distinguishing feature; however, it was dramatically included with genotypic characterizations (e.g., DNA–DNA relatedness) [3,4]. Then, the bacterial systematics (i.e., taxonomy study) is supposed as a consensually polyphasic approach [1]. Nonetheless, this polyphasic approach has a critical limitation that this research can apply to the already isolated (i.e., cultivated) microorganisms in the laboratory [5] or technical handling concerns (e.g., DNA–DNA hybridization) [6].

In the sequencing era, the taxonomic assignment for bacterial organisms has been challenged, resulting in reclassification of the incorrectly identified taxa and revisiting their ecological implications [7–11]. At the moment, as a genomic gold standard, genomes are dramatically applied into the definition for prokaryotic species. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) have been most frequently utilized in these sorts of studies [12–14]. Additionally, the value for the average amino acid identity (AAI) is employed for novel species validation [15].

During a culturable-bacterial diversity investigation of marine environments, including coastal sediments on Jeju Island, South Korea, we isolated a novel bacterium belonging to the genus *Photobacterium*, designated as GJ3^T. The genus *Photobacterium* belongs to the family Vibrionaceae and the phylum Proteobacteria. It was first proposed by

Beijerink et al. [16] with the description of *Photobacterium phosphoreum* as the type species. At the time of this writing, the genus *Photobacterium* has 42 species with valid names (https://lpsn.dsmz.de/genus/photobacterium, accessed on 10 January 2022). The species of the genus *Photobacterium* have been mostly isolated from marine and aquatic environments, including samples associated with marine animals [17–25]. Members of this genus share common characteristics of being Gram-stain negative, facultative anaerobic, motile, and rod shaped with cells containing ubiquinone 8 (Q-8) as a major or sole respiratory quinone [16]. Additionally, some *Photobacterium* species are luminous due to the presence of the *lux* gene (*luxCDABEG*), and many marine fish species form bioluminescent through these *Photobacterium* species [26]. Therefore, in this study, we describe its taxonomic properties, including biochemical and genomics traits. Finally, we proposed the strain GJ3^T as a representative novel species of the genus *Photobacterium*.

2. Materials and Methods

2.1. Isolation and Culture Conditions

Strain GJ3^T was isolated from a coastal sediment sample on Jeju Island (March 2019), South Korea (GPS; 33°27′15 N, 126°18′26 E). The sample was collected into a falcon tube, stored in a container maintained at 4 °C by icepack, and transported to the laboratory. To isolate bacteria, a sediment sample was placed in a sterile 50-mL conical tube and slurried with artificial seawater (ASW) [27]. After serial dilution to 10^{-5}, the diluted slurry was spread onto marine agar 2216 (MA; BD Difco) plates. For two weeks, these plates were incubated at 30 °C. Single colonies were transferred to new MA plates. Each colony was subcultured on MA plates at least five times to obtain a pure colony. Cells were subcultured and grown on MA at 30 °C for 3 days before being stored at −80 °C as a suspension in marine broth (MB; BD Difco) supplemented with 30% (*w*/*v*) glycerol for subsequent analyses. Based on 16S rRNA gene sequence similarity and phylogenetic position, *Photobacterium galatheae* S2753^T (LMG 28894^T) and *Photobacterium halotolerans* MACL01^T (LMG 22194^T) were selected and obtained from Belgian Coordinated Collections of Microorganisms (BCCM/LMG) as experimental reference strains for comparison with strain GJ3^T. Strain GJ3^T was deposited in the Korean Collection for Type Cultures (KCTC) and Collection of Marine Microorganisms (KMM) as KCTC 72816^T and KMM 6822^T, respectively. Unless otherwise stated, all strains were grown on MA medium under their optimal culture conditions. However, during paper writing after polyphasic experiments, *Photobacterium salinisoil* LAM9072^T has been validly published by Li et al. [28]. We conducted whole-genome-based taxonomic analyses (such as digital DNA–DNA hybridization (dDDH), average nucleotide identity (ANI), and average amino acid identity (AAI)) for genomes of *P. salinisoli* and the isolated strain GJ3^T (see DDH results and discussion on genomics relatedness).

2.2. Phylogenetic Analysis

To determine the phylogenetic position of strain GJ3^T, genomic DNA (gDNA) was extracted using a commercial gDNA extraction kit (GeneAll Biotechnology Co., Ltd., Seoul, Korea) according to the manufacturer's instructions. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using gDNA as template and universal bacterial primer set 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) as previously described [29]. For sequencing the amplified 16S rRNA gene, the PCR product was purified using a PCR purification kit (GeneAll Biotechnology Co., Ltd.) according to the manufacturer's instructions. Sequencing of the 16S rRNA gene was performed using primers 27F, 518F, 800R, and 1492R as described previously [29,30]. Finally, the 16S rRNA gene sequence of strain GJ3^T (1474bp) was obtained using SeqMan software (DNASTAR) and compared with sequences of related taxa obtained from the GenBank database (www.ncbi.nlm.nih.gov, accessed on 12 January 2022) and the EzBioCloud server (https://www.ezbiocloud.net, accessed on 12 January 2022). Sequences were edited and aligned using Clustal_X of BioEdit program [31]. Evolutionary distances were calculated using the Kimura two-parameter model [32]. The neighbor-joining [33], maximum parsi-

mony [34], and maximum likelihood methods [35] were used to reconstruct phylogenetic trees in MEGA X software [36], with bootstrap values based on 1000 replications.

2.3. Phenotypic and Biochemical Characteristics

Unless otherwise specified, strain GJ3^T and selected reference strains (*P. galatheae* LMG 28894^T and *P. halotolerans* LMG 22194^T) were cultivated on MA at 30 °C until the mid-exponential phase to obtain the best results for physiological and biochemical characterization. Cell morphology and size were examined by transmission electron microscopy (JEOLJEM-1010, JEOL Ltd., Tokyo, Japan) using negative staining with 1% (*w*/*v*) phosphotungstic acid. Cell motility was tested with the hanging-drop method [37]. The temperature range and optimum temperature for growth were determined by incubation on MA at 4, 10, 15, 18, 25, 30, 37, 42, and 45 °C. The pH range and optimal pH for growth were determined on MB to pH 4.0–10.0 at increments of 0.5 pH units adjusted by the following buffering system: homopiperazine-1,4-bis-2-ethanesulfonic acid (pH 4.0–5.0), 2-(*N*-morpholino) ethanesulfonic acid (pH 5.5–6.5), 1,3-bis[tris(hydroxymethyl)methylamino]propane (pH 7.0–8.5), and 3-(cyclohexylamino)-1-propanesulfonic acid (pH 9.0–10.0). To estimate salinity tolerance, the novel strain was grown in artificial seawater (ASW) [27] medium containing 0 to 10% (*w*/*v*) NaCl (at intervals of 1%) supplemented with peptone (1%, *w*/*v*) and yeast extract (0.5%, *w*/*v*). Gram staining was performed using a BD Gram-staining kit. Anaerobic growth was examined over two weeks on MA plates using a BD GasPak^{TM} EZ Anaerobe pouch system (BD Diagnostics). Catalase activity was determined based on the production of oxygen bubbles after mixing cells with 3% (*v*/*v*) H_2O_2. An oxidase test was performed using 1% (*w*/*v*) tetramethyl-*p*-phenylenediamine (Merck). The hydrolysis of skim milk (0.2%, *w*/*v*), CM-cellulose (0.2%, *w*/*v*), starch (0.2%, *w*/*v*), and tween 60 (1.0%, *w*/*v*) was determined based on the formation of clear zones around colonies after applying suitable solutions based on the method described by Smibert and Krieg [38]. DNA degradation was tested using DNase test agar (BD Difco) containing up to 3.0% NaCl. Biochemical tests were carried out using API 20NE, API 32GN, and API ZYM test strips (bioMérieux) with bacterial suspensions in ASW medium. For antimicrobial susceptibility test, strain GJ3^T was grown on MA in the presence of disks containing various antimicrobial compounds (penicillin G, 10 IU; tetracycline, 30 μg; gentamicin, 10 μg; oxacillin, 1 μg; clindamycin, 2 μg; kanamycin, 30 μg; streptomycin, 10 μg; ampicillin, 10 μg; sulfamethoxazole/trimethoprim, 23.75/1.25 μg; neomycin, 30 μg) for two weeks at 30 °C.

2.4. Chemotaxonomic Analyses

Whole-cell fatty acid methyl ester compositions of GJ3^T and two reference strains were determined using a 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). Cultivation of strains, harvesting of cells, fatty acid extraction, and analysis of fatty acid methyl esters were performed according to recommendations of the Sherlock Microbial Identification System (MIDI). For fatty acid analysis, all strains were grown on MA for 3 days at 30 °C under aerobic conditions. They were then identified using the TSBA identification library version 5.0. For quinone and polar lipid analyses, GJ3^T, *P. galatheae* LMG 28894^T, and *P. halotolerans* LMG 22194^T cultures first underwent lyophilization. Quinones were extracted as described by Hu et al. [39]. Quinone component was separated and identified by reversed-phase high-performance liquid chromatography as previously described [30]. Polar lipids were extracted according to procedures described by Minnikin et al. [40] and separated by two-dimensional thin-layer chromatography as described by Minnikin et al. [41].

2.5. Whole-genome Sequencing and Genome Analyses

For whole-genome sequencing, 20-kb SMRTbell^{TM} template libraries were constructed using the extracted gDNA. Whole-genome sequencing of GJ3^T was performed using a PacBio RSII platform (Pacific Biosciences, Menlo Park, CA, USA) at DNA Link, Inc. (Seoul, Korea). Sequences were obtained from high-quality reads with about 244-fold coverage

and assembled using a hierarchical genome assembly process (HGAP, v3.0) with assembly polishing using Quiver [42]. To examine species boundaries, ANI [43] values, AAI [44], and dDDH were calculated using an ANI calculator (http://enve-omics.ce.gatech.edu/ani/, accessed on 13 January 2022), AAI (http://enveomics.ce.gatech.edu/aai/, accessed on 13 January 2022), and a genome-to-genome comparison (GGDC) [45], respectively. Before genomic trait analysis, the genome of GJ3^T was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) [46]. There was no difference between the 16S rRNA gene sequence identified from the whole-genome sequence and that obtained by the PCR-Sanger sequencing method (see phylogenetic analysis).

The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a public bioinformatics platform available at https://tygs.dsmz.de (accessed on 13 January 2022) for whole genome-based taxonomic analysis [47]. Information on nomenclature, synonymy, and associated taxonomic literature was obtained from TYGS's sister database, the List of Prokaryotic names with Standing in Nomenclature (LPSN, available at https://lpsn.dsmz.de, accessed on 13 January 2022) [48]. For phylogenomic inference, all pairwise comparisons among the set of genomes were conducted using GBDP and accurate intergenomic distances inferred under the algorithm "trimming" and distance formula d5 [45]. One hundred distance replicates each were calculated. Digital DDH values and confidence intervals were calculated using recommended settings of the GGDC 3.0 [45,48]. Resulting intergenomic distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1, including SPR postprocessing [49]. Branch support was inferred from 100 pseudo-bootstrap replicates each. Trees were rooted at the midpoint [50] and visualized with PhyD3 [51].

Prophage, clustered regularly interspaced short palindromic repeats (CRISPRs), and secondary metabolite clusters were identified and annotated by the online tools phage search tool enhanced release (PHASTER), CRISPRFinder, and antiSMASH bacterial version (v. 6.0), respectively [52–54].

3. Results and Discussion

3.1. Phylogenetic, Phylogenomic, and Genome Features

The 16S rRNA gene sequence revealed that strain GJ3^T was clearly clustered to the genus Photobacterium (Figure 1). Based on the sequence similarity in EzBioCloud server, GJ3^T was most closely related to *Photobacterium galatheae* S2753^T (98.30% 16S rRNA gene sequence similarity), followed by *Photobacterium halotolerans* MACL01^T (97.90%), and *Photobacterium panuliri* LBS5^T (96.55%). The sequence similarity between GJ3^T and the additional-validated strain *P. salinisoil* LAM 9072^T was 98.55%. Additionally, the resultant phylogenomics tree provided strong evidence that strain GJ3^T belonged to the genus *Photobacterium* (Figure 2).

From genome, the branch lengths are scaled in terms of GBDP distance formula d5. Numbers above branches are GBDP pseudo-bootstrap support values > 60 assemble, a complete circular genome 3,603,274 bp length with a G+C content of 50.70% and two plasmids. The values for ANI, AAI, and dDDH between GJ3^T and two selected reference strains were calculated by 79.24%, 81.94%, and 23.50% (*P. galatheae* LMG 28894^T), and 77.90%, 81.83%, and 22.30% (*P. halotolerans* LMG 22194^T), respectively. In addition, those values between GJ3^T and *P. salinisoli* LAM 9072^T (GenBank access. No. GCA_003614885) were 81.11%, 81.99%, and 22.30%, respectively. Despite its 16S rRNA gene sequence similarity with *P. salinisoli* (98.55%) was slightly higher than *P. galatheae* (98.30%), values of ANI, AAI, and dDDH from whole-genome-based genomic analyses indicated that GJ3^T was clearly distinguished from *P. salinisoli* as a novel species. Strain GJ3^T clearly represented a novel species in the genus *Photobacterium* based on ANI and dDDH criterion values [43,47].

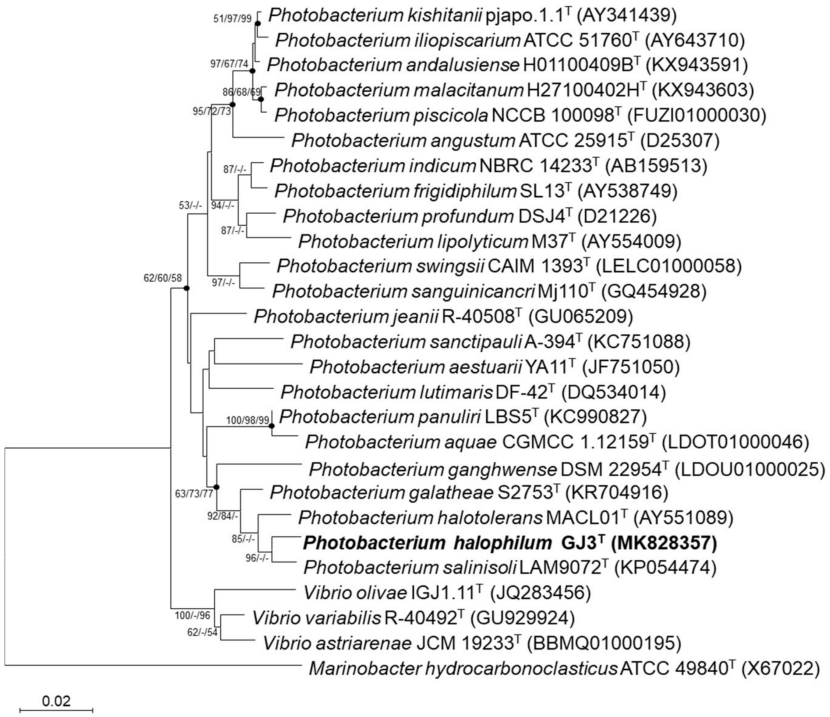

Figure 1. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain GJ3^T and related strains, including validated members of the genus *Photobacterium*. The sequence of *Marinobacter hydrocarbonoclasticus* ATCC 49840^T (X67022) was used as an outgroup in this study. GenBank accession numbers are given in parentheses. Bootstrap values (>50%) based on 1000 replications are shown at nodes based on neighbor-joining, maximum likelihood, and maximum parsimony methods. Filled circles indicated that the corresponding nodes were also obtained in the trees generated with the neighbor-joining, maximum likelihood, and maximum parsimony methods. Bars, 0.02 substitutions per nucleotide position.

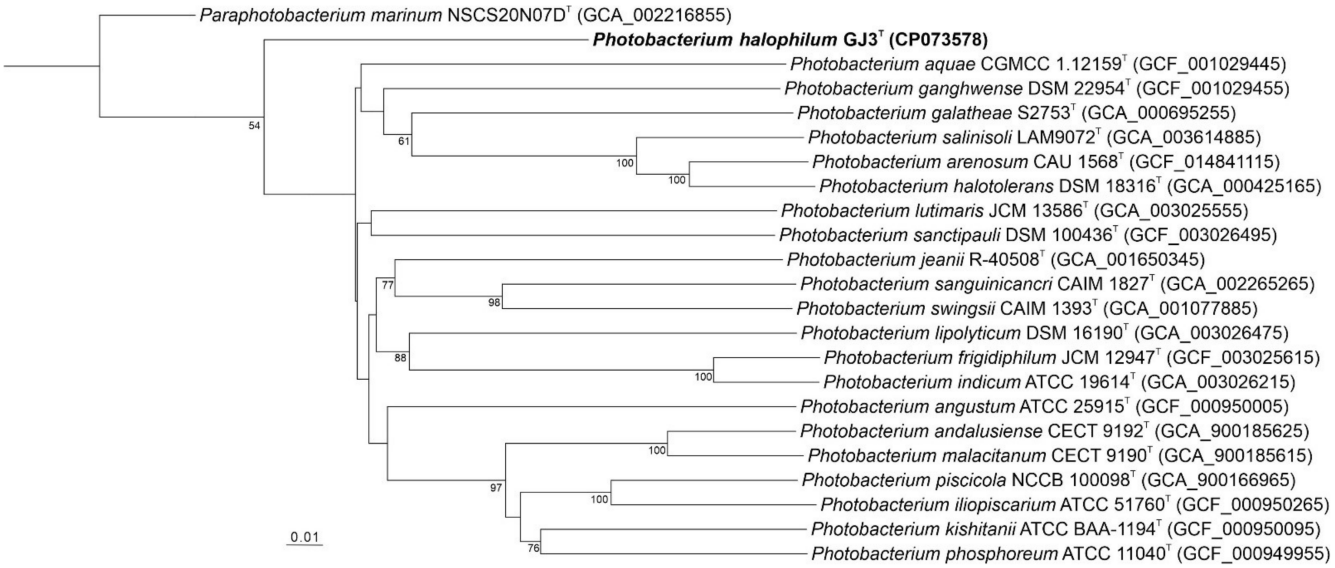

Figure 2. Whole-genome sequence-based tree inferred with FastME 2.1.6.1 [49] from GBDP distances calculated from strain GJ3^T and relative genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. Numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with average branch support of 55.6%. The tree was rooted at the midpoint [51].

Strain GJ3^T genome was annotated by PGAP predicted 4719 coding sequences (CDSs) and 164 RNAs, including 125 transfer RNAs and 6 non-coding RNA genes. A total of 2259 CDSs were matched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (47.87% of total CDSs). Based on KEGG results, quinone electron carrier (ArcBA) enzymes for anaerobic respiration and low nitrogen availability (GlnDBLGA) were found in the genome. Anaerobic respiration enzymes (FrdABCD) for converting fumarate to succinate and regulating the expression of anaerobic respiration gene (NarQL) and dissimilatory nitrate reduction (NapAB and NirBD) were also found in the genome, indicating that strain GJ3^T can grow under a low oxygen environment [55,56].

Strain GJ3^T genome encoded enzymes for phosphate limitation (PhoRB), misfolded proteins (CpxAR, HtrA), and Mg^{2+} starvation and antimicrobial peptide (PhoQP). In addition, the genome contained gene contents entirely for bacterial chemotaxis, including flagella assembly (*cheWYARYVB*). The genome of strain GJ3^T included entire genes for the biosynthesis of ectoine and glycine betaine as a compatible solute. These have been implicated in the maintenance of osmotic equilibrium in hypersaline environments [57]. The resultant comparative genomic analysis for the genus *Photobacterium* showed that biosynthetic gene cluster for ectoine has been well presented in a number of species of the genus [58]. These results indicate that strain GJ3^T might have an ecological advantage for surviving in various environments, including nutrient starvation. Additionally, using antiSMASH analysis, the genome encoded the genes for the biosynthesis for non-ribosomal peptide synthetase cluster, althiomycin, and type I PKS (polyketide synthase) with antagonistic properties, including antibiotics [59]. Unexpectedly, the gene cluster for butyrolactone synthesis has firstly been identified in the *Photobacterium* spp. genomes. The butyrolactone is related to a quorum sensing molecule, regulating for antibiotic production and cell differentiation in streptomyces [60]. Taken together, although natural secondary-metabolic compounds are commonly produced from various organisms, including plant, this result indicates that strain GJ3^T may have a competitive benefit against other organisms [61].

Interestingly, the gene encoding the biosynthesis of siderophores aerobactin (*iuc*) was found in the genome of strain GJ3^T. This gene has been widely reported in members of pathogenic genera, including *Vibrio* [62]. The gene involved in siderophores synthesis is known as related to virulence (e.g., *Klebseilla* [63]). However, for non-pathogenic species, the gene might have been acquired via horizontal gene transfer (HGT) [64]. It was deduced that this gene might have a strategic advantage for iron uptake from the environment. To the best of our knowledge, in the genus *Photobacterium*, except strain GJ3^T, the *iuc* gene encoded in the *P. halotolerans* MELD1 genome was reported [65]. Also, we only identified three incomplete prophage regions (score < 70 estimated by PHASTER) in the strain GJ3^T genome with slightly lower GC range (47.09–47.86%) than its genome GC (50.70%). In these prophage sequences, genes involved in transposase, integrase, hypothetical protein, exonuclease, recombination, and tail assembly were identified. However, we found no CRISPRs in the genome. Taken together, these results indicate that strain GJ3^T might have a little stress for genetic exchange of foreign DNA (i.e., HGT via phage), and there might be no driving force for evolution opportunity (e.g., genome conservation) in their habits [66,67].

3.2. Physiology and Chemotaxonomy

The isolated strain GJ3^T was Gram-stain-negative, catalase- and oxidase-positive, facultatively anaerobic, white pigmented, motile, and rod shaped (Figure S1). Cells grew at 15–37 °C (optimum: 30 °C), pH 5.5–8.5 (optimum: 7.0), and with 0.5–9.0% (*w*/*v*) NaCl (optimum: 4%) under aerobic conditions. Cells were sensitive to ampicillin, kanamycin, tetracycline, gentamicin, neomycin, and sulfamethoxazole/trimethoprim, but resistant to penicillin, oxacillin, clindamycin, and streptomycin. Degradation activity for starch, skim milk, DNase, cellulose, and Tween 60 was not observed. In addition, strain GJ3^T was negative for reduction of nitrates to nitrite, reduction of nitrates to nitrogen, indole production, and glucose acidification. Numerous additional phenotypic characteristics, as

listed in Table 1, can be used to differentiate strain GJ3^T from the experimental reference strains used in this study.

Table 1. Differential physiological characteristics of strain GJ3^T and reference strains of the genus *Photobacterium*.

Characteristics	1	2	3	4 *
Temperature range (°C)	15–37	15–40	4–37	10–40
NaCl range (%, *w*/*v*)	0.5–9.0	0.5–9.0	0–8.0	0–9.0
Oxidase	+	+	+	−
Nitrate reduction ($NO_3^- \rightarrow NO_2^-$)	−	+	+	−
Glucose acidification	−	+	+	ND
Utilization of (as sole carbon and energy source):				
D-Mannose	+	−	+	−
Gluconate	−	−	+	+
Adipate	−	−	+	ND
Malate	−	+	+	ND
D-Mannitol	−	−	−	+
L-Arabinose	+	−	+	ND
D-Maltose	−	+	+	ND
Citrate	−	−	−	+
Glycogen	−	+	+	+
Enzyme activity:				
Protease (gelatin hydrolysis)	+	−	+	−
Esterase (C4)	−	+	+	+
Esterase lipase (C8)	−	+	+	+
α-glucosidase	−	−	−	+
N-Acetyl-D-glucosamine	−	+	+	ND
DNA G + C content (%, mol/mol)	50.7	49.5	49.8	50.2

Strains: 1. GJ3^T; 2. *P. galatheae* LMG 28894^T; 3. *P. halotolerans* LMG 22194^T; 4. *P. salinisoli* LAM 9072^T. All strains were Gram-stain-negative and catalase positive. All strains were positive for activities of valine arylamidase and acid phosphatase, but negative for arginine dihydrolase and β-glucosidase (esculin hydrolysis) as a sole source of carbon and energy. +, Positive; −, Negative; ND, not determined. 1, 2, 3 data from in this study; and *, data from Li et al. [28].

The major fatty acid found in strain GJ3^T was summed feature 8 ($C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*, 38.18%), which is consistent with the fatty acid compositions of other type strains of the genus *Photobacterium* (Table 2). Individual polar lipids were identified using molybdophosphoric acid, molybdenum blue, ninhydrin, α-naphtol reagents [40,41], and Dragendorff's reagent (Sigma). Phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), and phospholipid (PL) were identified from the strain GJ3^T (Figure S2). Ubiquinone 8 (Q-8) was the sole ubiquinone expressed in the GJ3^T strain, which was consistent with the results obtained with other validated strains.

Based on morphological, physiological, and chemotaxonomic properties, as per the results of phylogenetic and genomics analysis (i.e., phylogenomics, dDDH relatedness, ANI, and AAI), GJ3^T belongs to the genus *Photobacterium*, and represents a novel species.

3.3. Description of *Photobacterium halophilum* sp. nov.

Photobacterium halophilum (ha.lo′phi.lum. Gr. masc. n. hals, halos salt; Gr. masc. adj. philos loving; N.L. neut. adj. halophilum salt-loving).

Gram-stain-negative, facultatively anaerobic, white pigmented, rod-shaped (1.0 × 2.5 μm), and motile with flagellum. Colonies are circular, convex, and entire, developed after incubation for 3 days at 30 °C on MA. Cell growth occurs between 15 and 37 °C (optimal temperature 30 °C), pH 5.5–8.5 (optimal pH 7.0), and 0.5–9.0% (*w*/*v*) NaCl (optimum of 4.0% (*w*/*v*) NaCl). Cells are catalase and oxidase positive, but are negative for reduction of nitrates to nitrite, reduction of nitrates to nitrogen, indole production, and glucose acidification. Moreover, cells cannot hydrolyze starch, skim milk, DNA, or Tween 60.

Table 2. Cellular fatty acid profiles (% of totals) of GJ3^T and reference strains of the genus *Photobacterium*.

	1	2	3
Saturated			
$C_{12:0}$	6.37	6.14	5.17
$C_{14:0}$	0.87	1.06	1.43
$C_{16:0}$	15.75	17.67	17.05
$C_{17:0}$	0.88	1.47	1.29
$C_{18:0}$	0.60	TR	TR
Unsaturated			
$C_{16:1}$ *ω5c*	TR	TR	0.79
$C_{16:1}$ *ω9c*	1.79	2.00	2.62
$C_{17:1}$ *ω6c*	TR	0.54	0.78
$C_{17:1}$ *ω8c*	1.77	2.08	1.96
$C_{18:1}$ *ω9c*	0.87	TR	0.56
Branched-chain fatty acid			
$C_{16:0}$ iso	0.79	1.53	2.34
$C_{17:0}$ iso	TR	TR	0.68
Hydroxy fatty acids			
$C_{18:0}$ 2OH	0.81	TR	TR
$C_{12:0}$ 3OH	TR	4.16	4.56
Summed feature			
2; $C_{12:0}$ aldehyde	3.89	3.66	3.88
3; $C_{16:1}$ *ω6c*/$C_{16:1}$ *ω7c*	23.71	28.98	28.36
8; $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*	38.18	26.29	23.99

Strains: 1. GJ3^T; 2. *P. galatheae* LMG 28894^T; 3. *P. halotolerans* LMG 22194^T. All strains were aerobically grown on marine agar plates at 30 °C for 3 days. These values indicate total fatty acid percentages. TR, trace amount (<0.5%). All data presented are those obtained from the present study.

Cells are positive for utilization of D-mannose, D-glucose, and L-arabinose, but negative for utilization of gluconate, adipate, malate, phenyl-acetate, D-mannitol, salicin, D-melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, N-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine as sole carbon and energy sources. Its cells are positive for activity of protease (gelatin hydrolysis), alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase, but negative for arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), β-galactosidase (PNPG), esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, a-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuroniase, α-glucosidase, β-glucosidase, a-mannosidase, and a-fucosidase activities. Q-8 is the only isoprenoid quinone. Its polar lipids include phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, and phospholipid. The major fatty acid is summed feature 8. The G + C content of the type strain was 50.70% as estimated by genome sequence.

The type strain GJ3^T (= KCTC 72816^T = KMM 6822^T) was isolated from the marine sediment collected from Jeju Island, South Korea.

The GenBank accession number for the 16S rRNA gene sequence is MK828357. The whole genomes, including two plasmid sequences, have been deposited in GenBank under the accession number CP073578, CP073579, and CP073580.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d14030188/s1, Figure S1: Cell morphology, Figure S2: Polar lipids profile.

Author Contributions: M.K. and S.-J.P. designed the experiments. M.K., K.-E.L., I.-T.C. and S.-J.P. performed the experiments. I.-T.C. provided support for the experiments. M.K. and S.-J.P. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization.

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