

***Photobacterium marinum* sp. nov., a marine bacterium isolated from a sediment sample from Palk Bay, India**

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Running title

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains AK15<sup>T</sup> and AK18 are HE573747 and HE614292 respectively.

The novel, cream coloured, Gram-staining-negative, rod-shaped, motile bacteria, designated strains AK15<sup>T</sup> and AK18, were isolated from sediment samples collected from Palk Bay, India. Both strains were positive for arginine dihydrolase, lysine decarboxylase, oxidase, nitrate reduction and methyl red test. The major fatty acids were C<sub>16:0</sub>, C<sub>18:1 ω7c</sub>, C<sub>16:1 ω7c</sub> and/or C<sub>16:1 ω6c</sub> and/or iso-C<sub>15:0</sub> 2-OH (summed feature 3). Polar lipids content of strains AK15<sup>T</sup> and AK18 were found to be phosphatidylethanolamine (PE), two unidentified phospholipids (PL1 and PL2) and three unidentified lipids (L1-L3). The 16S rRNA gene sequence analysis indicated strains AK15<sup>T</sup> and AK18 as the members of the genus *Photobacterium* and closely related to the type strain *Photobacterium jeanii* with pair-wise sequence similarity of 96.7%. DNA-DNA hybridization between strain AK15<sup>T</sup> and AK18 showed a relatedness of 87%. Based on data from the current polyphasic study, strains AK15<sup>T</sup> and AK18 are proposed as novel species of the genus *Photobacterium*, for which the name *Photobacterium marinum* sp. nov. is proposed. The type strain of *Photobacterium marinum* is AK15<sup>T</sup> (= MTCC 11066<sup>T</sup> = DSM 25368<sup>T</sup>).

**Key words:** *Photobacterium marinum*; 16S rRNA gene based phylogeny; chemotaxonomy, *Vibrionaceae*.

The genus *Photobacterium* was established by reclassifying the species "*Micrococcus phosphoreus*" [7] as *Photobacterium phosphoreum* [4]. The genus *Photobacterium* comprises of Gram-negative, motile (with one to three polar flagella) or nonmotile, straight or plump rods, sodium requiring, chemoorganoheterotrophic, with respiratory and fermentative type of metabolism, non spore forming, facultative anaerobes. The DNA G + C content range for the genus *Photobacterium* is between 39-50.6 mol%. Genus *Photobacterium* belongs to the family *Vibrionaceae*. Some members of the genus are bioluminescent. Species of the genus *Photobacterium* are mainly from marine habitat. Their symbiotic association with fishes can be in different ways like, within the body of fishes for the formation of light organs, or as a neutral entity on the surface or within the intestines of fish. They also act as decomposers of dead fishes, or can also act as agent of diseases. During the past five years, a number of novel species were reported, including *Photobacterium atrarenae*, *Photobacterium ganghwense*, *Photobacterium halotolerans*, *Photobacterium kishitanii*, *Photobacterium lutimaris*, *Photobacterium aquimaris*, *Photobacterium gaetbulicola*, *Photobacterium jeanii*, *Photobacterium aphoticum* and *Photobacterium swingsii* [12, 22, 24, 3, 10, 42, 11, 5, 18, 9]. *Photobacterium fischeri* and *Photobacterium logei* were considered as synonyms of *Vibrio fischeri* and *Vibrio logei*, respectively and were transferred to *Aliivibrio* from the genus *Vibrio* [35]. According to Kimura *et al.* [13] *Photobacterium histaminum* [19] is a later heterotypic synonym of *Photobacterium damsela* subsp. *damsela*. The genus *Photobacterium* presently comprises of 20 species and 2 subspecies. In the present study, we focused on the characterization and classification of the strains AK15<sup>T</sup> and AK18, which were isolated from marine sediment by using a polyphasic approach [36]. From the results of phylogenetic and phenotypic analyses, the strains are proposed as the representatives of a novel species of the genus *Photobacterium*.

Strains AK15<sup>T</sup> and AK18 were isolated from the sediment samples collected from Offshore Rameswaram, Palk Bay, India on 18<sup>th</sup> and 17<sup>th</sup> April 2011 (GPS positioning 9°23'-27.00" N 79°10' 25.05" E and 9°23'-13.12"-N 79°13' 36.82" E). The distance between two sampling points and also from the coast was ~6 miles. Strains AK15<sup>T</sup> and AK18 were isolated from the sediment sample collected at depths of 10.5 and 10.9 m respectively. The samples from which the strains AK15<sup>T</sup> and AK18 were isolated had a pH of 8.12 and 6.81 respectively, for both the samples, temperature was 31°C and salinity was 32.62 ppt. Approximately 100 mg of the sediment sample was suspended in 900 ml of sterile saline water (3% NaCl w/v) and subjected to shaking for 2 h. The supernatant was serially diluted and 100 µl was plated on Zobell marine agar (MA) [42] plates and incubated at 30 °C for 15 days. Based

on the colony morphology, two cream coloured colonies were selected and characterized in the present study.

Strains AK15<sup>T</sup> and AK18 were characterized simultaneously with *Photobacterium jeanii* LMG 25436<sup>T</sup> and *Photobacterium rosenbergii* LMG 22223<sup>T</sup> that were procured from LMG culture collection, Belgium. Colony morphology was examined following growth of the strain on MA at 30 °C for 48 h. Cell morphology and motility were observed by using phase contrast microscopy. Motility was also assessed on Motility-Indole-Lysine HiVeg<sup>TM</sup> medium (cat. no. MV847; HIMEDIA) with 0.2% agar (by inoculating the active culture suspension using sterile inoculation needle and checking for spreading of the growth in the medium) and also under phase contrast microscope. Growth at temperatures of 4, 10, 18, 30, 37 and 40°C was ascertained using marine agar medium and salt tolerance [0, 0.5, 1, 2, 3, 4, 5, 6, 8 and 10% (w/v) NaCl] was ascertained using nutrient agar (NA) medium containing peptone (0.5%), beef extract (0.3%) and agar (2.0%). Growth of strains AK15<sup>T</sup> and AK18 at pH 5, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11 and 12 was assessed on MA buffered with citric acid/NaOH (for pH 5 and 6), NaHPO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> (for pH 7 and 8), glycine/NaOH (for pH 9 and 10) or Tris-HCl or NaOH (for pH 11 and 12). Biochemical characteristics such as activity of oxidase, catalase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, nitrate reduction, hydrolysis of aesculin, gelatinase, ONPG, starch, Tween 20/40/60/80, H<sub>2</sub>S production and the sensitivity to 21 different antibiotics using the disc diffusion method with commercially available discs (HIMEDIA) were determined by previously described methods [16, 29]. The zone of inhibition (sensitive or resistant) to the antibiotics was taken from the information given in the HIMEDIA product overleaf (manufacturer instructions). Biochemical and enzymatic characterization of the strains was also performed using Vitek 2 GN (bioMérieux) with incubation at 30 °C, according to the manufacturer's protocol, except that sterile 2.0 % (w/v) NaCl was used to prepare the inoculum.

Fatty acid methyl esters were prepared and analyzed using the Sherlock Microbial Identification System (MIDI-6890 with database TSBA6) according to the standard protocol. For this purpose, strains AK15<sup>T</sup>, AK18 and type strain *Photobacterium jeanii* LMG 25436<sup>T</sup> and *Photobacterium rosenbergii* LMG 22223<sup>T</sup> were grown on MA plates at 30°C for one to two days after growth standardization (All strains were at same physiological age). Polar lipids were extracted and analyzed according to the method described by Komagata and Suzuki [14]. Polar lipids were determined in freeze-dried cells. PHB accumulation was determined by Nile blue A staining method according to Ostle and Holt [20]. The

DNA of strains AK15<sup>T</sup> and AK18 were isolated according to the procedure of Marmur [18] and the G + C content was determined from melting point (*T<sub>m</sub>*) curves [28] obtained by using a Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). *Escherichia coli* strain DH5- $\alpha$  DNA was used as a standard in determining the DNA G + C content.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories Inc.) and sequenced as described previously [15]. The resultant sequences of the 16S rRNA gene (1492 and 1502 nt of strains AK15<sup>T</sup> and AK18 respectively) were subjected to BLAST sequence similarity searches [1] and the EzTaxon server [6] to identify the nearest taxa. All 16S rRNA gene sequences of closely related members of the genera *Photobacterium*, *Enterovibrio*, *Grimontia* and *Salinivibrio* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL\_X program [32] and the alignment was then corrected manually. Evolutionary analyses were conducted in MEGA5 [31]. The evolutionary history was inferred using the maximum-likelihood [30] and neighbor-joining [24] methods. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [8]. The evolutionary distances were computed using the Kimura 2-parameter method [12] and are in the units of the number of base substitutions per site. Sequences of different house-keeping genes like recombination repair protein (*recA*), topoisomerase I (*topA*), a cell-division protein (*ftsZ*) and an actin-like cytoskeleton protein (*mreB*) were obtained as described previously [5]. All positions containing gaps and missing data were eliminated. There were a total of 2936 positions in the final dataset (including concatenated sequences of 16S rRNA gene, *ftsZ*, *mreB*, *recA* and *topA* genes). DNA-DNA hybridization was performed by the membrane filter method [34] as described previously [22, 27]. REP, ERIC and BOX-PCR were performed according to the protocol described by Versalovic et al. [37]. RAPD-PCR was carried out with two random primers [5'-CTTGAGTGGA-3' and 5'-GAGATGACA-3'] with 10 pm concentration. Initial denaturation at 95°C for 15 sec, and subsequent denaturation at 95°C for 5 sec. Primer annealing was performed at 35-40°C for 10 sec, and primer extension was performed at 72°C for 30 sec. The final extension step was 5 min at 72°C. Total 35 cycles were performed. Nearly equal concentration of the template DNA was taken for all the strain studied (98-105 ng/ $\mu$ l). The final volume of the PCR reaction is 25  $\mu$ l and 1  $\mu$ l template was added.

The cellular fatty acid composition of strains AK15<sup>T</sup> and AK18 showed a spectrum of 23 and 24 fatty acids with a pronounced dominance of (>5%) of C<sub>16:0</sub>, C<sub>18:1  $\omega$ 7c</sub>, C<sub>16:1  $\omega$ 7c</sub> and/or C<sub>16:1  $\omega$ 6c</sub> and/or iso-

C<sub>15:0</sub> 2-OH (summed feature 3) (Supplementary Table 1). Strain AK18 also contained iso-C<sub>16:1</sub> I and/or C<sub>14:0</sub> 3-OH (summed feature 2) as dominant (>5%). Compared with *Photobacterium jeanii* LMG 25436<sup>T</sup> and *Photobacterium rosenbergii* LMG 22223<sup>T</sup>, the composition of fatty acids differed considerably in strains AK15<sup>T</sup> and AK18 (Supplementary Table 1). Compared with other members of the genus *Photobacterium* the major fatty acid composition differed considerably, but C<sub>16:0</sub> is present in all species tested and C<sub>18:1 ω7c</sub> in majority of species tested (Table 1). Major polar lipid composition (phosphatidylethanolamine [PE] and unidentified phospholipids [PL2]) was similar in all studied strains. The other common polar lipids in all strains studied include two unidentified lipids (L1, L3) (Supplementary Fig. S1). The strains AK15<sup>T</sup>, AK18 and *Photobacterium jeanii* LMG 25436<sup>T</sup> differed from the strain *Photobacterium rosenbergii* LMG 22223<sup>T</sup> by the presence of one unidentified phospholipid (PL1), one unidentified lipid (L2) and the absence of one unidentified aminophospholipid (APL), three unidentified phospholipids (PL3-PL5) and one unidentified lipid (L4) (Supplementary Fig. S1)(Table 2). The G + C content of DNA of the strains AK15<sup>T</sup> and AK18 were 46.8 and 46.5 mol% respectively.

The 16S rRNA gene analysis placed strains AK15<sup>T</sup> and AK18 within the genus *Photobacterium*. The results indicated that at the 16S rRNA gene sequence level, strains AK15<sup>T</sup> and AK18 were close to each other (with 99.1% pair-wise 16S rRNA gene sequence similarity) and to the single phylogenetic neighbor, *Photobacterium jeanii* the sole member of the genus, with a pair-wise sequence similarity of 96.7%. The pair-wise sequence similarity with the other members of the genus *Photobacterium* was below 96.4 to 94.6%. Phylogenetic tree (based on the 16S rRNA gene sequences) obtained using the maximum-likelihood (Fig. 1) method revealed clustering of the strains AK15<sup>T</sup> and AK18 together and with *Photobacterium jeanii* with a sequence dissimilarity of 4.0% (96.0% similarity) all together clustered with six *Photobacterium* species that include *Photobacterium ganghwense*, *Photobacterium gaetbulicola*, *Photobacterium lutimaris*, *Photobacterium rossenbergii*, *Photobacterium aphoticus* and *Photobacterium halotolerans* (Fig. 1). Phylogenetic tree (based on the concatenated sequences of 16S rRNA gene, *ftsZ*, *mreB*, *recA* and *topA* genes) obtained using the maximum-likelihood (Fig. 2) method revealed clustering of the strains AK15<sup>T</sup> and AK18 together and with *Photobacterium rossenbergii* and all together clustered with three strains of *Photobacterium jeanii* (Fig. 2). The phylogenetic distance/sequence dissimilarity between AK15<sup>T</sup> and the two related strains *Photobacterium rossenbergii* and *Photobacterium jeanii* is nearly 16.9 and 17.2 % respectively (83.1 and 82.8 % similarity). DNA-

DNA hybridization between strains AK15<sup>T</sup> and AK18 showed a reassociation of 87%. This value was above 70%, confirming that the novel strains are members of the same species [38]. A number of differences were observed between the two strains AK15<sup>T</sup> and AK18 and also with type strain in all three Rep-PCR genomic fingerprint patterns (REP-, ERIC-, BOX-PCR) (Supplementary Fig. S2). In case of REP-PCR, amplification was not obtained for strains AK15<sup>T</sup> and *Photobacterium jeanii* LMG 25436<sup>T</sup>. In case of RAPD-PCR, there was no amplification with all three strains.

The characteristics that differentiated strains AK15<sup>T</sup> and AK18 from the type strains of species *Photobacterium*, are given in Table 1. Although the strains AK15<sup>T</sup> and AK18 are in line with the genus *Photobacterium*, they differed with the members of the genus *Photobacterium* in many characteristics (Table 1). Based on the blast and phylogenetic analysis, a detailed comparison was made between the characteristics of strains AK15<sup>T</sup> and AK18 with the type strains *Photobacterium jeanii* LMG 25436<sup>T</sup> and *Photobacterium rosenbergii* LMG 22223<sup>T</sup> of the family *Vibrionaceae* (Tables 1, 2, Supplementary Table 1) and a number of differences were observed. For instance, strains AK15<sup>T</sup> and AK18 differed from *Photobacterium jeanii* with respect to cell size, salinity growth range, temperature growth range, catalase activity, hydrolysis of complex substrates, susceptibility to different antibiotics and fatty acid composition (Tables 1, 2, Supplementary Table 1). Thus, the cumulative differences that strain AK15<sup>T</sup> exhibits with the above closely related type strains unambiguously supports the creation of a new species of the genus *Photobacterium* for which the name *Photobacterium marinum* sp. nov. is proposed.

### **Description of *Photobacterium marinum* sp. nov.**

*Photobacterium marinum* (ma.ri'num. L. neut. adj. *marinum* of the sea, marine).

Cells are Gram-negative, facultative anaerobic, oval to slightly curved rod shaped, motile, 1.0-1.2 µm wide and 1.5-2.0 µm long. Cells of strain AK18 are longer (2.0-3.0 µm) than AK15<sup>T</sup>. Colonies on marine agar are circular, 1 mm in diameter, smooth, shiny, translucent and raised with entire margins. Grows at 10 to 37 °C with an optimum temperature of 30-37°C and tolerates up to 8% (w/v) NaCl and requires NaCl for growth. Grows at pH 5 to 10 with an optimum pH of 7-8. Oxidase, arginine dihydrolase, lysine decarboxylase, activity is present but catalase and ornithine decarboxylase activities are absent. The methyl red reaction and nitrate reduction to nitrite are positive. H<sub>2</sub>S and indole are not produced. Voges-Proskauer's reaction is negative. PHB granules produced. Bioluminescence is not observed. Tween 20/40/60 were hydrolyzed. Gelatin and Tween 80 hydrolysis are variable (strain

AK15<sup>T</sup> is negative for gelatin and positive for Tween 80). Aesculin, agar, casein and starch are not hydrolyzed. Positive for Ala-Phe-Pro-arylamidase and urease activities but negative for glutamyl arylamidase pNA,  $\gamma$ -glutamyl-transferase, lipase, tyrosine arylamidase, phosphatase, glycine arylamidase, glu-gly-arg-arylamidase, ELLMAN, L-pyrrolydonyl-arylamidase,  $\beta$ -*N*-acetylglucosaminidase,  $\beta$ -glucosidase,  $\beta$ -xylosidase,  $\beta$ -alanine arylamidase pNA, L-proline arylamidase,  $\alpha$ -glucosidase,  $\beta$ -*N*-acetylgalactosaminidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase and  $\beta$ -glucuronidase activities. Fermentation of glucose is positive. Gas is produced from D-glucose. Do not utilize adonitol, L-arabitol, D-cellobiose, citrate, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, malonate, 5-keto-D-gluconate, L-histidine, courmarate, L-malate and L-lactate. Negative for L-lactate and succinate alkalisation. Susceptible to ( $\mu$ g per disc unless indicated) amoxicillin (30), ampicillin (10), azithromycin (15), gentamicin (10), nitrofurantoin (300), norfloxacin (10), tobramycin (10) and trimethoprim (5), moderately susceptible to neomycin (30) and resistant to bacitracin (10), kanamycin (30), methicillin (5), pencillin-G (10 units), vancomycin (30). Strain AK15<sup>T</sup> is susceptible to nalidixic acid (30), novobiocin (30), streptomycin (10) and moderately susceptible to cephalothin (30), erythromycin (15), rifampicin (5), tetracycline (30). Strain AK18 is susceptible to cephalothin (30), erythromycin (15), moderately susceptible to nalidixic acid (30), novobiocin (30), streptomycin (10) and resistant to rifampicin (5) and tetracycline (30). Polar lipid consisted of phosphatidylethanolamine (PE), two unidentified phospholipids (PL1 and PL2) and three unidentified lipids (L1-L3). The G + C content of DNA of the strains AK15<sup>T</sup> and AK18 were 46.8 and 46.5 mol%.

The type strain AK15<sup>T</sup> (= MTCC 11066<sup>T</sup> = DSM 25368<sup>T</sup>), and the reference strain AK18 were isolated from a sediment sample collected from Offshore Rameswaram, Palk Bay, India.

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comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. Int. J. Syst. Evol. Microbiol. 57, 2823-2829.

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## Legends to Figures

**Fig. 1.** Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains AK15<sup>T</sup> and AK18 and closely related members of the family *Vibrionaceae*. Filled circles indicate branching topologies that are common in the trees obtained using the neighbor-joining and maximum-likelihood methods. Numbers at the nodes are bootstrap values >70%. *Vibrio anguillarum* NCMB 6<sup>T</sup> (AM235737) was used as an out group. Bar, 0.01 substitution per nucleotide position.

**Fig. 2.** Maximum-likelihood phylogenetic tree, on concatenated 16S rRNA, *ftsZ*, *mreB*, *recA* and *topA* gene sequences showing the position of the novel strains (*P. marinum* sp. nov.). Filled circles indicate branching topologies that are common in the trees obtained using the neighbor-joining and maximum-likelihood methods. Numbers at the nodes are bootstrap values >70%. *Aeromonas hydrophila* subsp. *hydrophila* ATCC 7966<sup>T</sup> was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

**Supplementary Fig. S1.** Two-dimensional thin-layer chromatogram of polar lipids of the strain AK15<sup>T</sup> (a), *Photobacterium jeanii* LMG 25436<sup>T</sup> (b) and *Photobacterium rosenbergii* LMG 22223<sup>T</sup> (c). Detection of total lipids by molybdato-phosphoric acid. Abbreviations: PE, phosphatidylethanolamine; APL, unidentified aminophospholipid; L1-4, unidentified lipids; PL1-5, unidentified phospholipids.

**Supplementary Fig. S2.** Characteristic Rep-PCR genomic fingerprint patterns of strains AK15<sup>T</sup>, AK18 and *Photobacterium jeanii* LMG 25436<sup>T</sup>. Lane 1 corresponds to 100 bp DNA Marker. Lane 2, 5 & 8 corresponds to fingerprint patterns of strain *Photobacterium jeanii* LMG 25436<sup>T</sup>, lanes 3, 6 & 9 corresponds to fingerprint patterns of strain AK18 and lanes 4, 7 & 10 corresponds to fingerprint patterns of strain AK15<sup>T</sup>. Lanes 2, 3 & 4 corresponds to BOX element genomic fingerprints; lanes 5, 6 & 7 corresponds to ERIC (enterobacterial repetitive intergenic consensus) and lanes 8, 9 & 10 corresponds to REP (repetitive extragenic palindromic).

**Supplementary Fig. S3.** Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains AK15<sup>T</sup> and AK18 and closely related members of the family *Vibrionaceae*. Numbers at the nodes are bootstrap values >70%. *Vibrio anguillarum* NCMB 6<sup>T</sup> (AM235737) was used as an out group. Bar, 0.01 substitution per nucleotide position.

**Supplementary Fig. S4.** Neighbor-joining phylogenetic tree, based on concatenated 16S rRNA, *ftsZ*, *mreB*, *recA* and *topA* gene sequences showing the position of the novel strains (*P. marinum* sp. nov.). Numbers at the nodes are bootstrap values >70%. *Aeromonas hydrophila* subsp. *hydrophila* ATCC 7966<sup>T</sup> was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

**Table 1.** Phenotypic features that distinguish strains AK15<sup>T</sup> and AK18 from closely related species of the genus *Photobacterium*

Data from the present study. All strains were negative for L-pyrrolydonyl-arylamidase,  $\beta$ -galactosidase, glutamyl arylamidase,  $\gamma$ -glutamyl-transferase,  $\beta$ -glucosidase,  $\beta$ -xylosidase,  $\beta$ -alanine arylamidase, tyrosine arylamidase,  $\alpha$ -glucosidase, glycine arylamidase,  $\beta$ -glucuronidase, glu-gly-arg-arylamidase activities, L-lactate and succinate alkalisation, H<sub>2</sub>S production, courmarate, adonitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, citrate, malonate, 5-keto-D-gluconate, L-histidine, L-malate, L-lactate utilization and ELLMAN reaction under Vitek 2 GN (bioMérieux) assay.

Characteristic	<i>Photobacterium marinum</i> AK-15 <sup>T</sup>	<i>Photobacterium jeanii</i> LMG 25436 <sup>T</sup>	<i>Photobacterium rosenbergii</i> LMG 22223 <sup>T</sup>
Temperature growth range (°C)	10-37	15-37	15-35
Optimum temperature (°C)	30-37	20-37	20-30
Salinity growth range (%)	2-8	0.5-4	1-7
Optimum NaCl concentration (% w/v)	2-4	0.5-2	2-6
pH growth range	5-10	5-9	6-10
Optimum pH for growth	7-8	7-8	7-8.5
<b>Hydrolysis of:</b>			
Casein	-	-	w
Starch	-	-	+
Tween 80	+	-	+
<b>Vitek 2 GN (bioMérieux):</b>			
Ala-Phe-Pro-arylamidase	+	-	+
$\beta$ -N-acetylglucosaminidase	-	-	+
L-proline arylamidase	-	-	+
Lipase	-	+	-
Urease	+	+	-
$\beta$ -N-acetylgalactosaminidase	-	-	+
$\alpha$ -galactosidase	-	-	+
Phosphatase	-	+	-
Polar lipids	PE, PL1, PL2, L1-L3	PE, PL1, PL2, L1-L3	PE, APL, PL2- PL5, L1, L3, L4

Strains AK15<sup>T</sup> and AK18 were differed with respect to the Tween 80 hydrolysis and Ala-Phe-Pro-arylamidase (positive and negative respectively).

Symbols: +, positive; -, negative; w, weak; PE, phosphatidylethanolamine; APL, unidentified aminophospholipid; L1-4, unidentified lipids; PL1-5, unidentified phospholipids.

**Supplementary Table 1.** Fatty acid profiles of strains AK15<sup>T</sup>, AK18 and closely related species of the genus *Photobacterium*

Data from the present study. Strains AK15<sup>T</sup>, AK18, *Photobacterium jeanii* LMG 25436<sup>T</sup> and *Photobacterium rosenbergii* LMG 22223<sup>T</sup> were grown on MA plates at 30 °C for one to two days. Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 5% or more of the total fatty acids are in bold. ND, not detected. Fatty acids that are less than 1% for all three species were not shown.

Fatty acid	<i>Photobacterium marinum</i> AK-15 <sup>T</sup>	<i>Photobacterium marinum</i> AK-18	<i>Photobacterium jeanii</i> LMG 25436 <sup>T</sup>	<i>Photobacterium rosenbergii</i> LMG 22223 <sup>T</sup>
C <sub>11:0</sub> 3-OH	1.1	0.9	0.4	nd
C <sub>12:0</sub>	1.3	1.4	<b>5.7</b>	4.1
C <sub>12:0</sub> 3-OH	4.9	4.8	4.8	1.8
iso-C <sub>13:0</sub>	nd	0.2	1.4	nd
C <sub>14:0</sub>	4.7	4.9	<b>7.3</b>	<b>5.3</b>
C <sub>15:0</sub>	nd	nd	nd	nd
iso-C <sub>15:0</sub>	0.8	1.4	2.5	nd
anteiso-C <sub>15:0</sub>	1.1	0.9	0.5	nd
iso-C <sub>15:0</sub> 3-OH	0.8	1.0	0.8	nd
C <sub>16:0</sub>	<b>13.7</b>	<b>13.9</b>	<b>18.0</b>	<b>17.9</b>
iso-C <sub>16:0</sub>	1.1	1.2	0.3	nd
C <sub>16:0</sub> N alcohol	1.2	1.6	nd	<b>10.1</b>
C <sub>16:1</sub> ω <sub>7</sub> C alcohol	1.1	1.6	nd	<b>11.9</b>
C <sub>17:0</sub>	1.5	2.0	1.6	nd
C <sub>17:1</sub> ω <sub>8</sub> C	1.1	0.9	0.7	nd
iso-C <sub>17:0</sub>	1.1	1.6	1.0	nd
anteiso-C <sub>17:0</sub>	1.9	1.4	0.3	nd
isoC <sub>17:1</sub> ω <sub>9</sub> C	nd	nd	nd	nd
C <sub>18:0</sub>	1.1	1.5	1.0	nd
C <sub>18:1</sub> 2-OH	nd	1.3	nd	nd
C <sub>18:1</sub> ω <sub>7</sub> C	<b>21.2</b>	<b>20.0</b>	<b>14.0</b>	<b>7.0</b>
C <sub>18:1</sub> ω <sub>9</sub> C	1.3	0.4	0.3	nd
C <sub>19:0</sub> 10-methyl	1.4	2.0	nd	nd
C <sub>20:2</sub> ω <sub>6,9</sub> C	2.6	nd	nd	nd
Summed feature 1	1.0	1.1	0.9	nd
Summed feature 2	4.8	<b>5.5</b>	<b>7.5</b>	2.3
Summed feature 3	<b>21.1</b>	<b>21.3</b>	<b>25.7</b>	<b>39.6</b>

#Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed Feature 1 comprises iso-C<sub>15:1</sub> H/I and/or C<sub>13:0</sub> 3-OH; Summed Feature 2 comprises iso-C<sub>16:1</sub> I and/or C<sub>14:0</sub> 3-OH; Summed Feature 3 comprises C<sub>16:1</sub> ω<sub>7</sub>C and/or iso-C<sub>15:0</sub> 2-OH.

**Supplementary Table 2.** Phenotypic features that distinguish strains AK15<sup>T</sup> and AK18 from the species of the genus *Photobacterium*

Taxa: 1, strain AK15<sup>T</sup> (data from this study); 2, strain AK18 (data from this study); 3, \**P. jeanii* LMG 25436<sup>T</sup> (data from this study and from Chimentto et al. [5]); 4, \**P. rosenbergii* (data from this study and from Thompson et al. [33]); 5. *P. gaetbulicola* (data taken from Kim et al. [11]); 6. *P. lutimaris* (data taken from Jung et al. [10]); 7. *P. ganghwense* (data taken from Park et al. [21]); 8. *P. aphoticum* (data taken from Lucena et al. [17]); 9. *P. halotolerans* (data taken from Rivas et al. [23]); 10. *P. phosphoreum*; 11. *P. angustum*; 12. *P. damselae* subsp. *damselae*; 13. *P. damselae* subsp. *piscicida*; 14. *P. iliopiscarium*; 15. *P. leiognathi*; 16. *P. profundum* (data for taxa 10-16 were taken from An Thyssen and Frans Ollevier [2]; Ast et al. [3]); 17. *P. aquimaris* (data taken from Yoshizawa et al. [41]); 18. *P. kishitanii* (data taken from Ast et al. [3]); 19. *P. lipolyticum* (data taken from Yoon et al. [40]); 20. *P. swingsii* (data taken from Gomez-Gil et al. [9]); 21. *P. frigidophilum* (data taken from Seo et al. [25]); 22. *P. aplysiae* (data taken from Seo et al. [26]); 23. *P. indicum* (data taken from Xie and Yokota [39]).

Symbols: o, oval; cv, curved; r, rod; cb, coccobacilli; pl, pleomorphic; co, coccoid; +, positive; -, negative; w, weak; v, variable; S, susceptible; R, resistant; SF, summed feature; nd, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Cell shape	o, cv-r	o, cv-r	cb	R	o-r	o	o-r	r	r	r	r	r	r	pl	cb	r	co-r	co-r	pl	cb	r	r	pl	
Motility	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	nd	+	+	+
Growth, 4°C	-	-	-	-	-	-	+	-	+	+	v	-	-	+	-	+	-	+	+	+	-	-	-	
Growth, 37°C	+	+	+	+	+	+	+	+	+	-	+	v	v	-	+	-	-	-	-	-	-	-	-	
Salinity tolerance (% w/v)	8	8	4	4-6	2-5	2-3	5-6	7	8	nd	8	6	3	2	6	nd	3.5	nd	6	6	1-3.5 %	5	nd	
Catalase	-	-	+	not given	+	w	+	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+	-	
Oxidase	+	+	+	+	+	+	+	+	+	-	v	v	+	nd	v	+	-	w	+	+	+	+	-	
Arginine dihydrolase	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	nd	
Lysine decarboxylase	+	+	-	-	-	-	-	nd	-	+	-	v	-	+	-	-	-	+	-	-	nd	nd	-	
Indole production	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	nd	-	+	-	+	-	+	
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	
Glucose fermentation	+	+	+	+	ng	+nd	+	+	+	nd	nd	nd	nd	nd	nd	nd	-	+	nd	nd	+	nd	+	
Gas production from D-glucose	+	+	- not given	-not given		nd	-	-	nd	+	-	+	-	+	-	-	nd	nd	nd	nd	nd	nd	+	

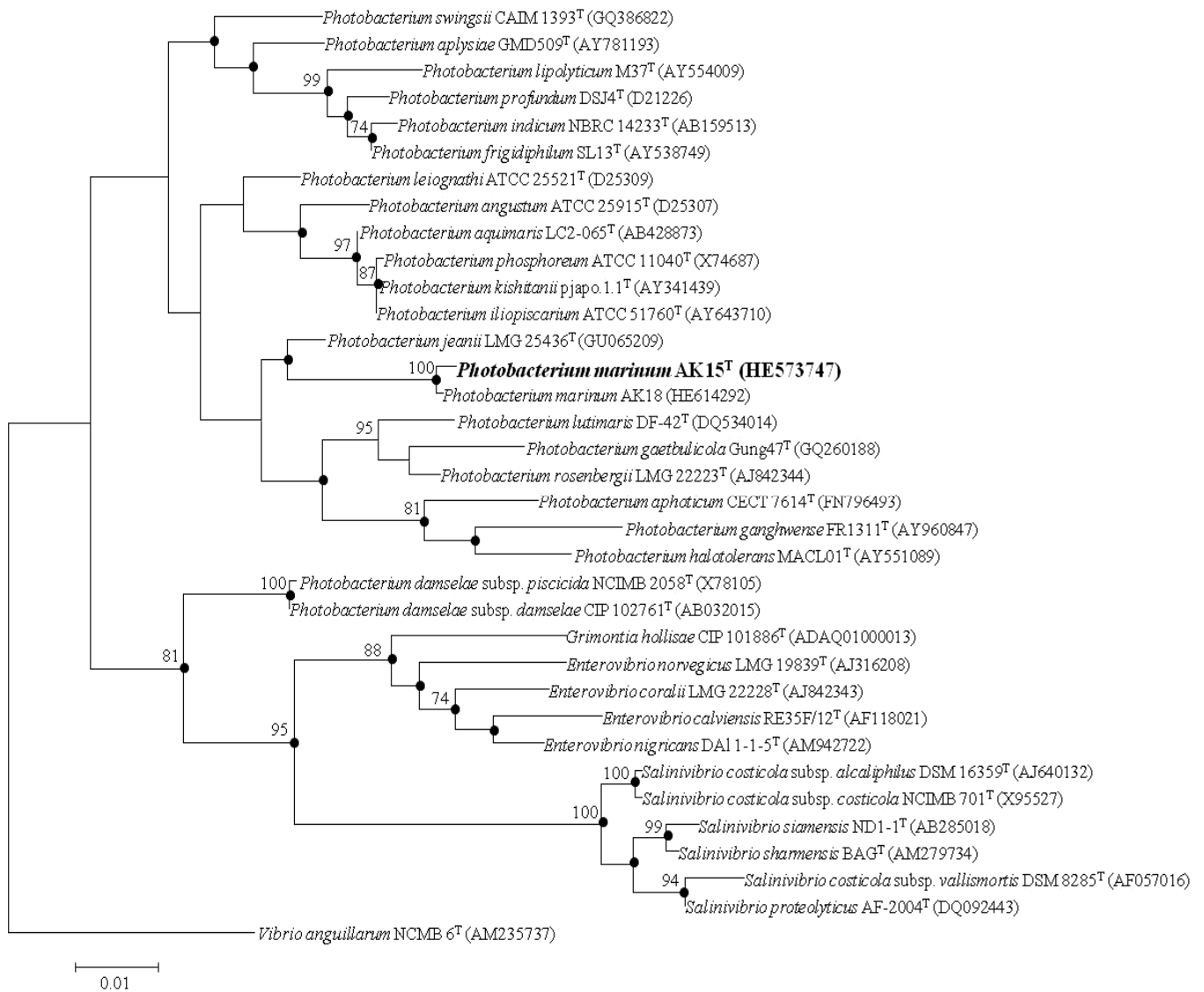


Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Susceptibility to vibriostatic agent O/129	S	S	S	R	nd	nd	nd	nd	R	nd	S	S	nd	S	S	R	nd	nd	nd	S	S	S	nd	
Gelatinase	-	w	+	-	-	-	+	-	+	-	v	v	-	-	-	nd	-	-	-	+	+	+	-	
Lipase	-	-	+	+	+	-	-	-	-	-	v	v	+	nd	+	nd	-	w	-	-	+	+	nd	
Luminescence	-	-	-	-	+	nd	-	-	-	+	-	-	-	-	+	-	+	+	nd	-	-	nd	nd	
PHB accumulation	+	+	+	+	nd	nd	-	nd	nd	+	nd	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Major fatty acids	C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 3	C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 2, SF 3	C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 2, SF 3	C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>16:1 ω7C</sub> , alco hol, C <sub>18:1 ω7c</sub> , SF 3	C <sub>18:1 ω7c</sub> , SF 3, C <sub>16:0</sub>	SF 3, C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 3	C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 3	C <sub>16:1 ω7c</sub> , C <sub>16:0 ω6c</sub> , C <sub>18:1 ω7c</sub> , C <sub>18:1 ω7c</sub> , C <sub>12:0</sub> , C <sub>16:0</sub>	SF 3, C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 3	C <sub>12:0</sub> , C <sub>12:0</sub> , 3-OH, C <sub>14:0</sub> , C <sub>16:0</sub>	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:1</sub>	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:1</sub>	nd	nd	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:1</sub>	C <sub>16:1</sub> , C <sub>16:0</sub> , C <sub>18:1</sub> , SF3	C <sub>12:0</sub> , C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>18:0</sub> , SF3	C <sub>12:0</sub> , C <sub>12:0</sub> , 0 3-OH, C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>18:1 ω7c</sub> , SF 2, SF 3	C <sub>16:0</sub> , SF 3	nd	C <sub>12:0</sub> , C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>18:1</sub> , C <sub>20:5 ω3c</sub>	C <sub>16:0</sub> , C <sub>14:0</sub> , C <sub>16:0</sub> , C <sub>18:1</sub> , C <sub>20:5 ω3c</sub>	C <sub>16:0</sub> , C <sub>16:0</sub> , C <sub>18:1</sub>	16 : 0, 16 : 1 and 18 : 1, 12 : 0 3-OH and 14 : 0 3-OH
DNA G+C content (mol%)	46.8	46.5	*45.5	*47.6-47.9	50.6 %	48.3 %	44.0	nd	49.8	40.8	41.0	40.6-41.4	41.1-41.3	39.0	41.6	42.0	42.2	40.2	47.0	46.7-48.7	43.8	45.0	40.0	

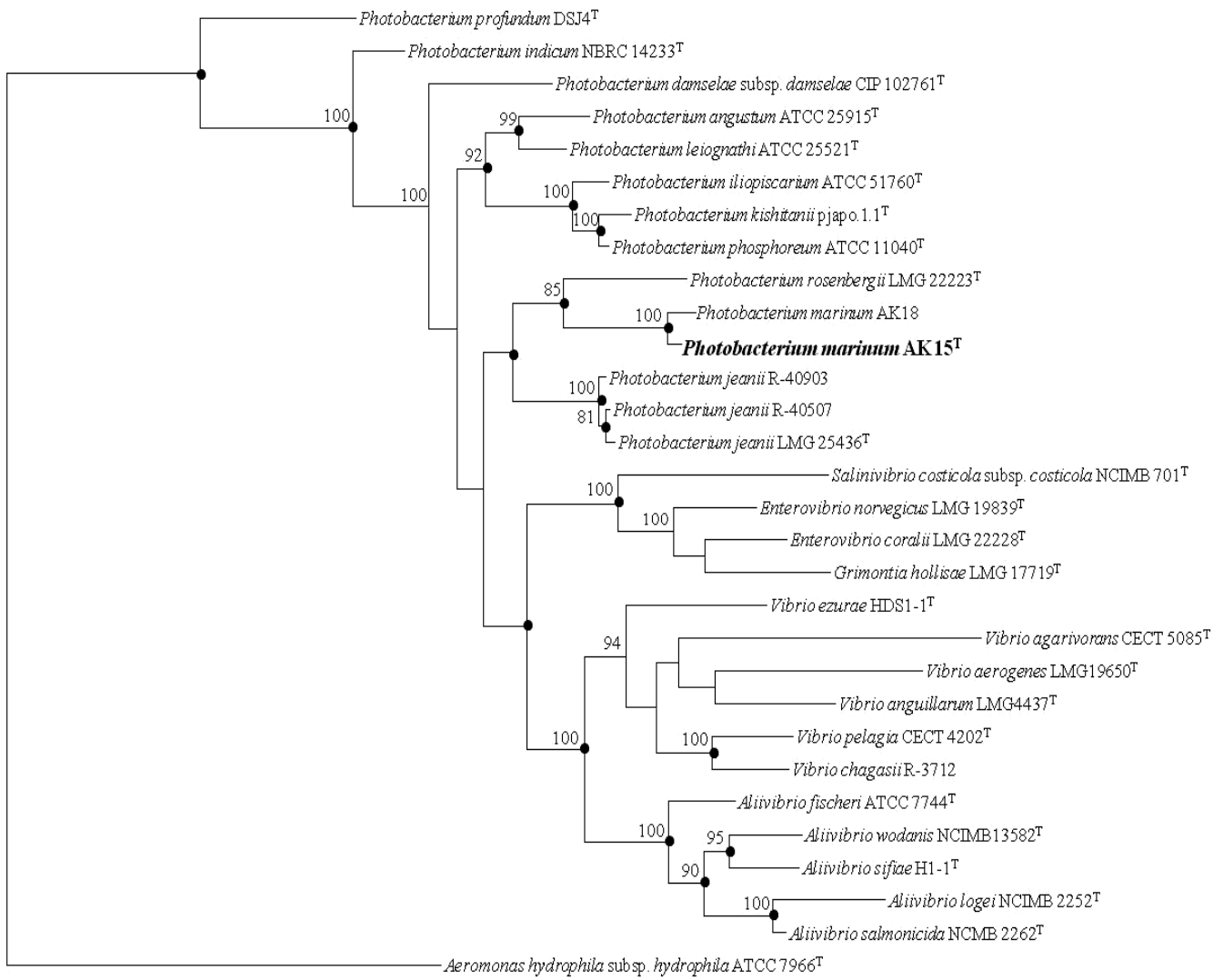
\*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed Feature 2 comprises iso-C<sub>16:1</sub> I and/or C<sub>14:0</sub> 3-OH; Summed Feature 3 comprises C<sub>16:1 ω7C</sub> and/or iso-C<sub>15:0</sub> 2-OH

**Supplementary Table 3.** Sequence accession numbers used in the multilocus sequence typing (MLST) phylogenetic analysis

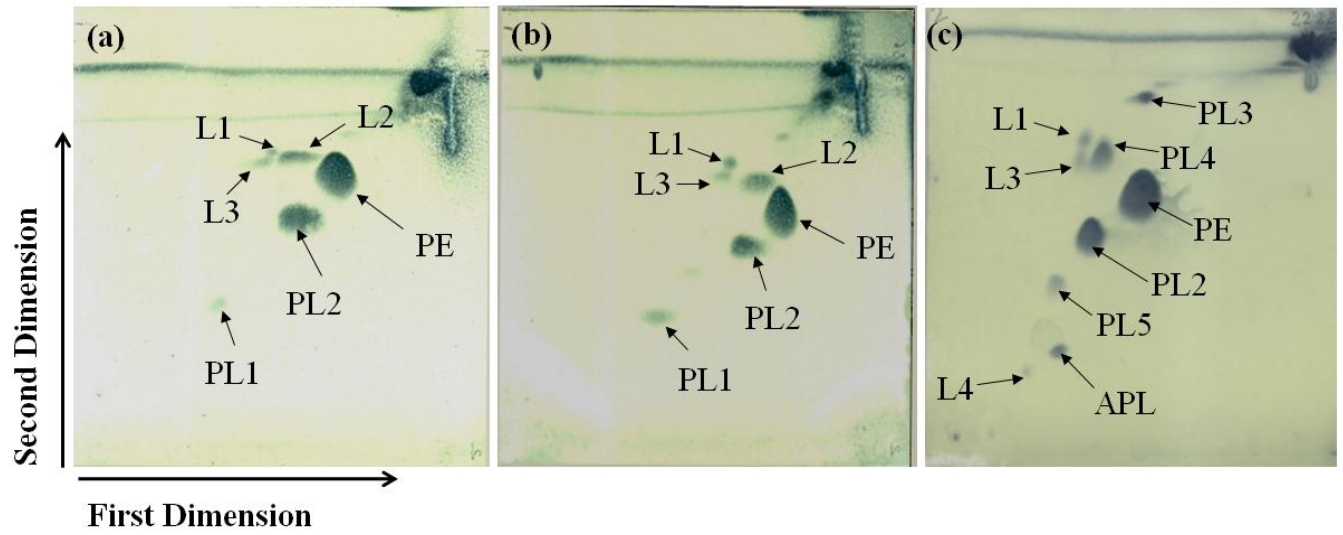
S. No.	Taxon	Sequence accession number of the genes used				
		16S rRNA	<i>ftsZ</i>	<i>mreB</i>	<i>recA</i>	<i>topA</i>
1.	<i>Photobacterium marinum</i> AK15 <sup>T</sup>					
2.	<i>Photobacterium marinum</i> AK18					
3.	<i>Photobacterium jeanii</i> R-40903	GU065211	GU065218	GU065221	GU065224	GU065215
4.	<i>Photobacterium jeanii</i> R-40507	GU065209	GU065216	GU065219	GU065222	GU065213
5.	<i>Photobacterium jeanii</i> R-40508 <sup>T</sup>	GU065210	GU065217	GU065220	GU065223	GU065214
6.	<i>Photobacterium rosenbergii</i> LMG 22223 <sup>T</sup>	AJ842344	DQ907328	DQ907395	AJ842360	DQ907467
7.	<i>Photobacterium angustum</i> ATCC 25915 <sup>T</sup>	D25307	DQ907318	DQ907385	AJ842354	DQ907457
8.	<i>Photobacterium leiognathi</i> ATCC 25521 <sup>T</sup>	D25309	DQ907324	DQ907391	AJ842364	DQ907463
9.	<i>Photobacterium iliopiscarium</i> ATCC 51760 <sup>T</sup>	AY643710	DQ907322	DQ907389	AJ842362	DQ907461
10.	<i>Photobacterium phosphoreum</i> ATCC 11040 <sup>T</sup>	X74687	DQ907327	DQ907394	AJ842365	DQ907466
11.	<i>Photobacterium damsela</i> subsp. <i>damsela</i> CIP 102761 <sup>T</sup>	AB032015	DQ907319	DQ907386	AJ842357	DQ907458
12.	<i>Photobacterium kishitanii</i> pjapo.1.1 <sup>T</sup>	AY341439	AB453688	AB453696	EF415552	AB453692
13.	<i>Photobacterium indicum</i> NBRC 14233 <sup>T</sup>	AB159513	DQ907323	DQ907390	EF415543	DQ907462
14.	<i>Photobacterium profundum</i> DSJ4 <sup>T</sup>	D21226				
15.	<i>Aliivibrio fischeri</i> ATCC 7744 <sup>T</sup>	AY341436	DQ907344	JF815027	AJ842417	DQ907482
16.	<i>Aliivibrio logei</i> NCIMB 2252 <sup>T</sup>	AJ437616	AB464981	AB464991	EU257776	AB464986
17.	<i>Aliivibrio salmonicida</i> NCMB 2262 <sup>T</sup>	X70643	DQ907375	DQ907447	EU257778	DQ907517
18.	<i>Aliivibrio sifiae</i> H1-1 <sup>T</sup>	AB464964	AB464982	AB464992	AB464996	AB464987
19.	<i>Aliivibrio wodanis</i> NCIMB13582 <sup>T</sup>	AJ132227	DQ907383	DQ907455	EF380244	DQ907523
20.	<i>Enterovibrio corali</i> LMG 22228 <sup>T</sup>	AJ842343	DQ907329	DQ907396	AJ842347	EF114217
21.	<i>Enterovibrio norvegicus</i> LMG 19839 <sup>T</sup>	AJ316208	EF027349	DQ907397	AJ842348	EF114216
22.	<i>Grimontia hollisae</i> LMG 17719 <sup>T</sup>		EF027348	DQ907398	AJ842351	EF114215
23.	<i>Listonella anguillarum</i> LMG4437 <sup>T</sup>	AM235737	DQ907334	DQ907406	AJ842375	DQ907471
24.	<i>Listonella pelagia</i> CECT 4202 <sup>T</sup>	AJ293802	DQ907369	DQ907442	AJ580872	DQ907511
25.	<i>Salinivibrio costicola</i> subsp. <i>costicola</i> NCIMB 701 <sup>T</sup>	X74699	EF027351	DQ907399	AJ842367	DQ90746
26.	<i>Vibrio aerogenes</i> LMG19650 <sup>T</sup>	AF124055	DQ907330	DQ907401	AJ842368	EF114212
27.	<i>Vibrio agarivorans</i> CECT 5085 <sup>T</sup>	AJ310647	DQ907332	DQ907403	FJ178184	DQ907470
28.	<i>Vibrio chagasii</i> R-3712	AJ316199	DQ996590	DQ481637	AJ580874	DQ481649
29.	<i>Vibrio ezur</i> ae HDS1-1 <sup>T</sup>	AY426980	DQ907343	DQ907414	AJ842413	DQ907481
30.	<i>Aeromonas hydrophila</i> subsp. <i>hydrophila</i> ATCC 7966 <sup>T</sup>	CP000462	CP000462	CP000462	CP000462	CP000462



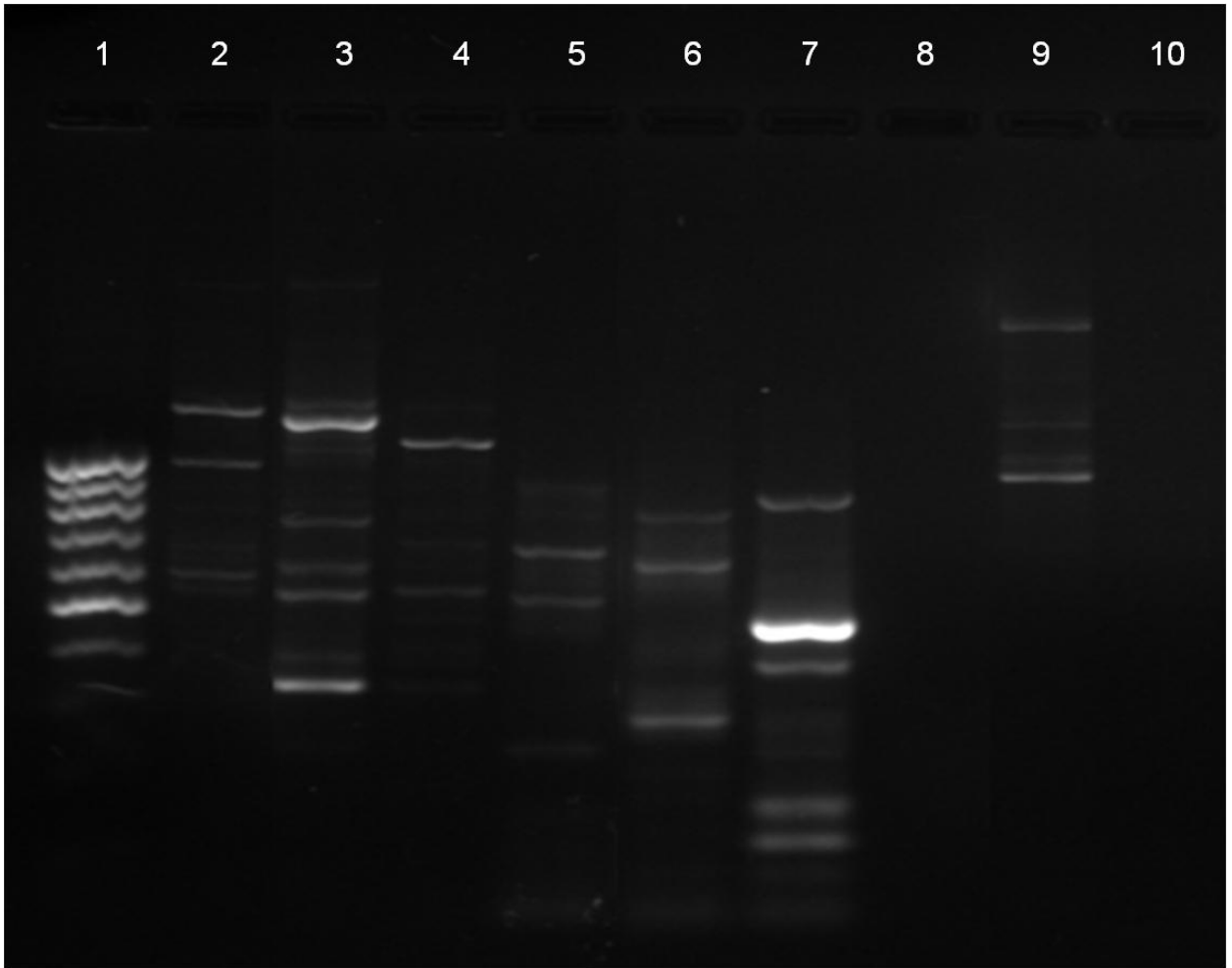
**Fig.1**



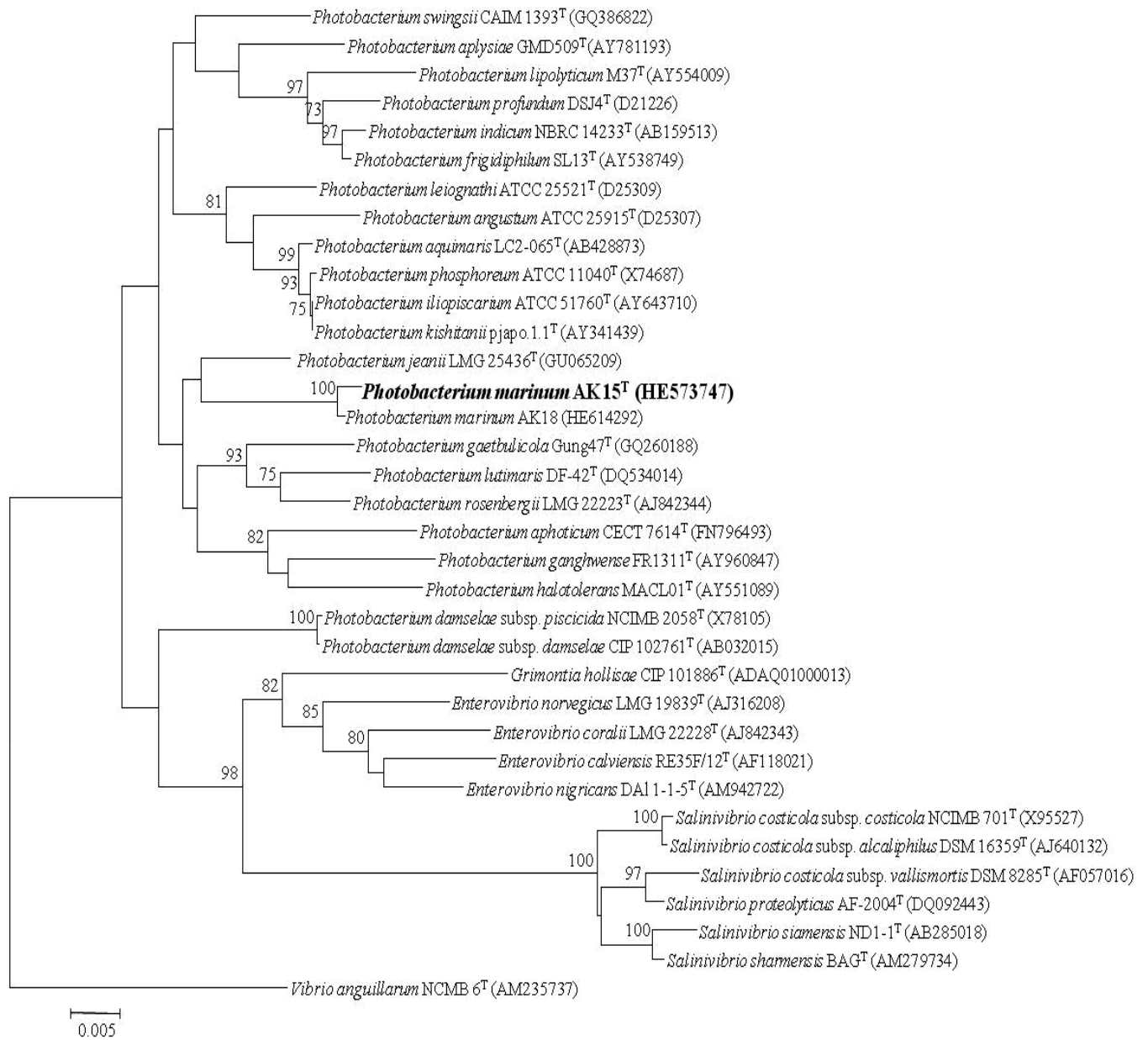
**Fig.2**



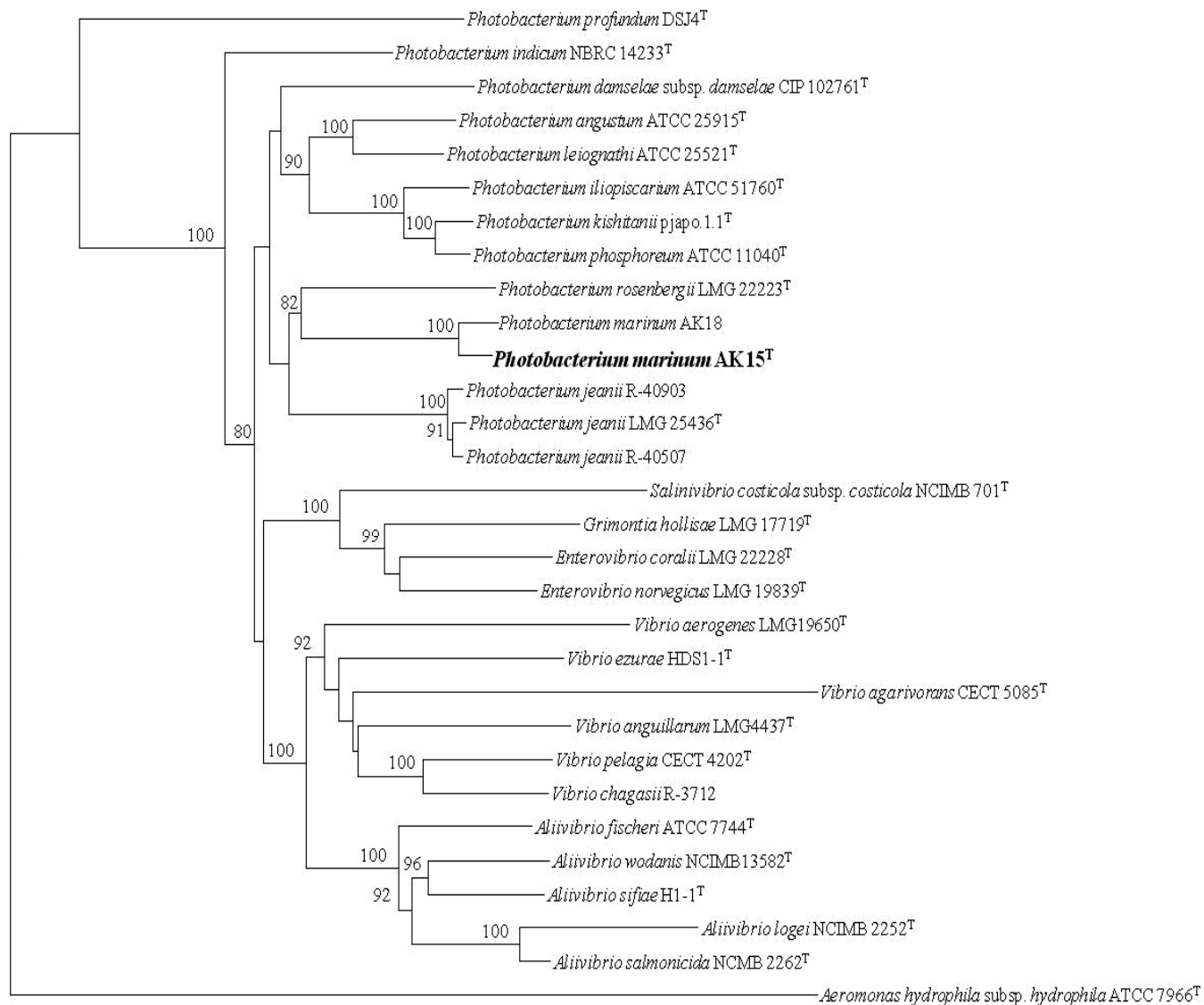
Supplementary Fig. S1



**Supplementary Fig. S2**



**Supplementary Fig. S3**



**Supplementary Fig. S4**