



HAL
open science

Paraglaciecola hydrolytica sp. nov., a bacterium with hydrolytic activity against multiple seaweed-derived polysaccharides

Pernille Kjersgaard Bech, Mikkel Schultz-Johansen, Mikkel Andreas Glaring, Tristan Barbeyron, Mirjam Czjzek, Peter Stougaard

► To cite this version:

Pernille Kjersgaard Bech, Mikkel Schultz-Johansen, Mikkel Andreas Glaring, Tristan Barbeyron, Mirjam Czjzek, et al.. *Paraglaciecola hydrolytica* sp. nov., a bacterium with hydrolytic activity against multiple seaweed-derived polysaccharides. *International Journal of Systematic and Evolutionary Microbiology*, 2017, 67 (7), pp.2242 - 2247. 10.1099/ijsem.0.001933 . hal-03758577

HAL Id: hal-03758577

<https://hal.science/hal-03758577v1>

Submitted on 6 Sep 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

***Paraglaucicola hydrolytica* sp. nov., a novel bacterium with hydrolytic activity against multiple seaweed-derived polysaccharides.**

Running Title: *Paraglaucicola hydrolytica* sp. nov.

Pernille Kjersgaard Bech^{1†}, Mikkel Schultz-Johansen^{1†}, Mikkel Andreas Glaring¹, Tristan Barbeyron², Mirjam Czjzek², Peter Stougaard¹

¹ Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.

² UPMC University Paris 6, UMR 7139 Marine Plants and Biomolecules, Station Biologique de Roscoff, F-29682 Roscoff, Bretagne, France.

† These authors contributed equally to this work

Corresponding author:

Peter Stougaard,

E-mail: psg@plen.ku.dk. Tel: +45 35 33 26 09.

Subject category: New Taxa: Proteobacteria

The 16S rRNA gene sequence of *Paraglaucicola hydrolytica* S66^T is found in the genome sequence of strain S66^T (GenBank accession number LSNE000000000).

Supplementary material showing a transmission electron micrograph, fatty acid composition, polar lipids, pairwise nucleotide comparison, maximum likelihood and maximum parsimony trees based on 16S rRNA gene sequences, and a neighbour-joining tree based on GyrB amino acid sequences of *P. hydrolytica* S66^T and related species is available in IJSEM Online.

Abstract

A novel bacterial strain, S66^T, was isolated from eelgrass collected on the coastline of Zealand, Denmark. Polyphasic analyses involving phenotypic, phylogenetic and genomic methods were used to characterize strain S66^T. The strain was Gram-negative, rod shaped, aerobic, and displayed optimal growth at 28°C (range from 5 to 33 °C) and at pH 7.5 (range from pH 5 to 9). Furthermore, strain S66^T grew on seaweed polysaccharides agar, agarose, porphyran, κ -carrageenan, alginate and laminarin as sole carbon source. Major fatty acids were C_{16:0}, C_{16:1 ω 7c}, and C_{18:1 ω 7c}. The predominant respiratory quinone was determined to be Q-8 and polar lipids were phosphatidylethanolamine and phosphatidylglycerol. G+C content was determined to be 42.2 mol%. Phylogenetical analyses based on the 16S rRNA gene and GyrB showed that the bacterium was affiliated with the genus *Paraglaciecola* within the *Alteromonadaceae* of the *Gammaproteobacteria*. The identity percentages between 16S rRNA gene and GyrB sequences of strain S66^T and other members of *Paraglaciecola* were 94-95% and 84-85%, respectively. Based on the genome sequence of S66^T the average nucleotide identity (ANI) between strain S66^T and other members of *Paraglaciecola* were 77-80%, and DNA-DNA hybridization (DDH)-prediction showed values less than 24% identity respectively between S66^T and other *Paraglaciecola* spp. The phenotypical, phylogenetical and genomic analyses support the hypothesis that strain S66^T represents a novel species of the genus *Paraglaciecola*, for which the name *Paraglaciecola hydrolytica* is proposed. The type strain is S66^T (LMG 29457^T = NCIMB 15060^T).

Keywords: agarase, carrageenase, psychrotrophic, hydrolytic enzymes

The genus *Glaciecola*, originally described by Bowman et al. (1998), was recently subdivided into *Aliiglaciecola* (Jean et al., 2013), *Paraglaciecola* and *Glaciecola* (Shivaji and Reddy, 2014). The species in the three genera share a number of morphological, biochemical, and physiological features. They are Gram-negative, rod-shaped, non-sporulating and strictly aerobic. The majority of the species contain C_{16:0}, C_{16:1 ω 7c}, and C_{18:1 ω 7c} as major fatty acids and they display G+C contents between 40-46 mol%. All species have been isolated from marine environments such as seawater (*Glaciecola nitratreducens*, (Baik et al., 2006), *Paraglaciecola aquimarina* (Park and Yoon, 2013), *P. polaris* (van Trappen et al., 2004), *P. oceanifecundans* (Shivaji and Reddy, 2014), *Aliiglaciecola lipolytica* (Chen et al. 2009)), sea ice (*G. punicea* and *G. pallidula* (Bowman et al., 1998), *P. psychrophila* (Zhang et al., 2006)), marine sediments (*P. agarilytica*, (Yong et al., 2007), *P. chathamensis* (Matsuyama et al., 2006), *P. arctica* (Zhang et al., 2011), *A. litoralis* (Tanaka et al., 2010), *A. aliphaticivorans* (Jin et al., 2015)), marine invertebrates (*P. mesophila* (Romanenko et al., 2003)) and mangrove water (*A. coringensis* (Gupta et al., 2014)). The species show different growth physiological properties, but all are cold-adapted, a feature confirmed by recent genome analysis (Qin et al., 2014). Two species, *P. agarilytica* and *P. mesophila*, were shown to degrade agar (Yong et al., 2007 and Romanenko et al., 2003).

Strain S66^T was isolated from eelgrass (*Zostera* sp.) collected on the coast of northwest Zealand, Denmark. Bacteria from leaves of eelgrass were cultivated on Marine Agar (MA; Difco) and strain S66^T was selected due to its ability to produce pits in the solid agar medium. Temperature optimum was determined by growth in Marine Broth 2216 (MB; Difco) at pH 7.5 and pH optimum was similarly determined in MB at 20°C. Cultivation was for 1 to 6 days. Cell morphology was determined using transmission electron microscopy of negatively stained cells (Romanenko et al., 2003). Phenotypic characterizations (Gram-staining and catalase, oxidase, DNase, caseinase,

gelatinase and lipase (Tween 80)) activities were carried out according to standard procedures. Other enzymatic activities were determined by cultivation on MA supplemented with 0.06% (w/v) azurin cross-linked (AZCL) substrates, AZCL-amylose, -arabinan, -arabinoxylan, -rhamnogalacturonan, -galactomannan, -xylan, and -cellulose (Megazymes). Ability to hydrolyse alginate was determined on plates solidified with alginate (2%, w/v; Sigma) and stained for alginate lyase activity with 10% (w/v) cetylpyridinium chloride (CPC) after incubation (Gacesa & Wusteman, 1990). All plates were incubated at 20°C for one to two weeks. Susceptibility to antibiotics was investigated on MA plates using droplets of antibiotic containing the following: Apramycin (50 µg), chloramphenicol (50 µg), gentamicin (10 µg), kanamycin (50 µg), penicillin G (2 µg), streptomycin (50 µg), tobramycin (1.6 µg) and tetracycline (30 µg). Additional physiological characterizations were performed using API 20 E and API ZYM strips according to the instructions of the manufacturer (bioMérieux). For sugar utilization experiments strain S66^T was cultivated in Marine Minimal Medium (Thomas et al., 2011) containing 5 g/L of the following sugars: glucose (Sigma-Aldrich G8270), galactose (Sigma-Aldrich G0750), xylose (Sigma-Aldrich X3877), sucrose (Sigma-Aldrich S0389), lactose (Sigma-Aldrich L2643), maltose (Sigma-Aldrich M-5885), cellulose avicel (Merck 1.02330), laminarin (Goëmar HII2H52/01 A), starch (Fluka 03967), amylopectin (Merck 1252), amylose (Fluka 10130), mannan (Sigma-Aldrich M7504), xylan (Sigma-Aldrich X-0627), pectin (apple) (Sigma-Aldrich P-8471), agar (Sigma-Aldrich A7002), agarose (Eurogentec EP-0010-05), porphyran (extracted from *Porphyra umbilicalis*), alginic acid (Sigma-Aldrich A7128), *ι*-carrageenan (Danisco 2544-97-01), *κ*-carrageenan (Goëmar X-6913), *λ*-carrageenan (Danisco 2544-89-01), fucoidan (extracted from *Pelvetia canaliculata*) and ulvan (gift from Münster University).

Whole fatty acids were extracted from cells cultivated on MA at 20°C. Analyses of respiratory quinones, polar lipids, and fatty acids were carried out by the Identification Service, DSMZ,

Braunschweig, Germany. Fatty acids were subjected to saponification, methylation and extraction using minor modifications of the method of Miller (1982) and Kuykendall et al., (1988). Genomic DNA was isolated from cells using the Genra Puregene Yeast/Bact kit (Qiagen). Whole genome sequencing of strain S66^T was carried out as described (Schultz-Johansen et al., 2016). The 16S rRNA gene sequence and the deduced GyrB protein sequence were retrieved from the genome sequence and compared to partial 16S rRNA gene and GyrB sequences of other *Paraglaciecola*, *Glaciecocola*, and *Aliiglaciecocola* species. The nucleotide sequences (16S rRNA gene) were aligned using the program MAFFT version 7 with the L-INS-I strategy (Kato et al. 2002) and phylogenetic trees based on the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) algorithms were built using MEGA software (version 5.05; Tamura et al, 2011) (Fig. 1; Supplementary Fig. S4, S5). Amino acid (GyrB) sequences were aligned and neighbor-joining phylogenetic trees were constructed using the alignment tool in the CLC Main Workbench 7.6.3 (CLC bio, Qiagen Aarhus). The tree topologies were evaluated using bootstrap analyses of 1000 (for NJ and MP trees) or 100 (for ML tree) resampled datasets (Supplementary Fig. S3). Genomic relatedness was investigated by comparing the S66^T genome sequence with other genomic sequences of type strains of *Paraglaciecola* and *Glaciecocola* using the Genome-Genome Distance Calculator (GGDC) (Meier-Kolthoff et al., 2013) and average nucleotide identity (ANI) (Kostas Lab: <http://enve-omics.ce.gatech.edu/ani/> and EzGenome: <http://www.ezbiocloud.net/ezgenome/ani>). These novel methods for comparing genome sequences in pairwise relatedness analyses have been shown to be as good as or better than the traditional DNA-DNA hybridization method because they are reproducible, easier to perform, and compatible with genome sequences (Figueras et al., 2014; Goris et al., 2007; Stropko et al., 2014; McGinnis et al. 2015; Jung et al., 2015). G+C content was 42.2 mol%.

Strain S66^T grew aerobically with optimal temperature and pH for growth at 28°C and pH 7.5, respectively (Table 1). Other growth physiological features are summarized in Table 1. The major fatty acids of strain S66^T were C_{16:1}ω7c (41.6%), C_{16:0} (14.8%), and C_{18:1}ω7c (12.3), which were similar to fatty acid composition of other members of *Paraglaiecola*. (Supplementary Table S1). The predominant respiratory quinone of strain S66^T was ubiquinone-8 (Q-8) which is in accordance with the quinone composition of *G. aquimarina*. The only other *Paraglaiecola* species that has been investigated for quinone composition is *P. oceanifecundans* which contains the menaquinone MK-7 (Shivaji and Reddy 2014). The major polar lipids of strain S66^T were phosphatidylethanolamine and phosphatidylglycerol (Supplementary Fig. S2), which is also the case for other *Paraglaiecola*, *Glaciecola*, and *Aliiglaciecola* species. Cells of strain S66^T were Gram negative, straight or irregular shaped rods with a polar flagellum (Supplementary Fig S1). Colonies on MA were yellowish-brown, circular and convex with a mucoid consistency. The presence of holes under and around colonies cultivated on media solidified with agar or carrageenan revealed the expression of agarase activity, and the presence of a clear halo around colonies grown on alginate after CPC staining indicated alginate lyase activity. In addition, strain S66^T hydrolyzed AZCL-amylose, -galactomannan, -xylan and -cellulose. Growth was observed on glucose, maltose, xylose, lactose and sucrose whereas growth on galactose was weak (Table 1 and Supplementary Table S3). Of the plant-derived polysaccharides tested, growth was observed on starch, amylopectin, xylan, and apple pectin with limited growth recorded on amylose and no growth observed on cellulose or mannan. Strain S66 was able to utilize the complex algal polysaccharides agar, agarose, porphyran, alginate, and laminarin as a sole carbon and energy source, and to a limited extent also κ-carrageenan, but not λ- and ι-carrageenan, fucoidan and ulvan (Supplementary Table S3).

Phylogenetic analyses of 16S rRNA gene and GyrB sequences showed that strain S66^T was distantly related to other *Paraglaiececola* species (Fig. 1 and Supplementary Fig. S3, S4 and S5) with approximately 95% identity to other 16S rRNA genes and 84-85% identity to GyrB amino acid sequences (Supplementary Table S2). ANI and GGDC analyses confirmed the phylogenetic results determined from 16S rRNA gene and GyrB sequences. The ANI and GGDC values for strain S66^T when compared to other *Paraglaiececola* species were less than 81 % and less than 24 %, respectively (Table 2). A pairwise genome comparison of other *Paraglaiececola* species showed that *P. agarilytica* and *P. chathamensis* may represent the same species since ANI and GGDC values between were 98.5 % and 87 %, respectively. For comparison, the ANI and GGDC values between genomes from *P. agarilytica* NO2^T and *P. agarilytica* 4H-3-7+YE-5 are 98.5 % and 86 %, respectively. For species delineation, ANI values of 95-96 % and GGDC values of 70 %, respectively, are normally accepted (Goris et al., 2007; Richter & Rosselló-Móra, 2009; Auch et al., 2010). In conclusion, phylogenetic characterizations using 16S rRNA gene and GyrB sequences together with whole genome pairwise comparisons show that strain S66^T represents a novel species of the genus *Paraglaiececola*, for which the name *Paraglaiececola hydrolytica* sp. nov. is proposed.

Description of *Paraglaiececola hydrolytica* sp. nov.

Paraglaiececola hydrolytica (hy.dro.ly'ti.ca. Gr. n. *hydor*, water; Gr. adj. *lytikos*, dissolving, splitting; N. L. adj. *hydrolytica*, splitting with water, referring to the hydrolytic activity of the bacterium).

Cells are Gram negative, aerobic, motile (0.4-0.6 µm wide and 0.8-1.0 µm long) with a polar flagellum Colonies on MA are yellowish-brown, convex, circular and mucoid in consistency.

Growth is observed between 5 and 33 °C with an optimum at 28°C, between pH 5 and 9 with an optimum at pH 7.5, and between 2% and 5% NaCl with an optimum at 3% NaCl. Catalase- and

oxidase-positive. In the API ZYM test, alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, *N*-acetyl- β -glucosaminidase, and α -fucosidase activities are positive and valine arylamidase is weakly positive, whereas cystine arylamidase and trypsin activities are negative. Starch, agar, furcelleran, κ -carrageenan and alginate are hydrolysed. In the API 20 E test, tryptophan deaminase is positive but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production, urea hydrolysis, indole production, acetoin production and gelatinase activity are negative. D-glucose, L-rhamnose, D-sucrose, D-melibiose, L-arabinose, D-xylose, L-lactose, maltose and galactose (weakly) are used, but not D-mannitol. Resistant to penicillin (2 μ g) and tetracycline (30 μ g); sensitive to chloramphenicol (50 μ g), gentamicin (10 μ g), kanamycin (50 μ g), streptomycin (50 μ g), tobramycin (1.6 μ g) and apramycin (weak; 50 μ g). The major cellular fatty acids are C_{16:1} ω 7c, C_{16:0}, and C_{18:1} ω 7c. G+C content was 42.2 mol%. The predominant respiratory quinone is Q-8 and polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The type strain is S66^T (LMG 29457^T = NCIMB 15060^T), isolated from leaves of eelgrass (*Zostera* sp.) collected on the coast of northwest Zealand, Denmark.

Acknowledgements

We thank Helle J. Martens, University of Copenhagen for performing the transmission electron micrographs of strain S66^T. This work was supported by a grant from the Novo Nordisk Foundation grant no. NNF12OC0000797 and from The Danish Council for Independent Research, Technology and Production Sciences (0602-02399B) to Peter Stougaard.

References

- Baik KS, Park YD, Seong CN, Kim EM, Bae KS, & Chun J. (2006).** *Glaciecola nitratreducens* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **56**, 2185-2188.
- Bowman JP, McCammon SA, Brown JL, & McMeekin TA (1998).** *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. *Int J Syst Bacteriol* **48**, 1213–1222.
- Chen LP, Xu HY, Fu S-Z, Fan HX, Liu YH, Liu SJ & Liu ZP (2009).** *Glaciecola lipolytica* sp. nov., isolated from seawater near Tianjin city, China. *Int J Syst Evol Microbiol* **59**, 73–76.
- Figueras MJ, Beaz-Hidalgo R, Hossain MJ, & Liles MR (2014).** Taxonomic affiliation of new genomes should be verified using average nucleotide identity and multilocus phylogenetic analysis. *Genome Announc* **4** e00927-14.
- Gacesa P & Wusteman FS (1990).** Plate assay for simultaneous detection of alginate lyases and determination of substrate specificity. *Appl Environ Microbiol* **56**, 2265-2267.
- Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, & Tiedje JM (2007).** DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* **57**, 81-91.
- Jean WD, Hsu CY, Huang SP, Chen JS, Lin S, Su MH, & Shieh WY (2013).** Reclassification of [*Glaciecola*] *lipolytica* and [*Aestuariibacter*] *litoralis* in *Aliiglaciecola* gen. nov., as *Aliiglaciecola lipolytica* comb. nov. and *Aliiglaciecola litoralis* comb. nov., respectively. *Int J Syst Evol Microbiol* **63**, 2859–2864.
- Jung YJ, Lee YM, Baek K, Hwang CY, Cho Y, Hong SG, Kim JH, & Lee HK (2015).** *Algibacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from marine sediment. *Int J Syst Evol Microbiol* **65**, 1735-1740.

Kuykendall LD, Roy MA, O'Neill JJ, & Devine TE (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358-361.

Matsuyama H, Hirabayashi T, Kasahara H, Minami H, Hoshino T & Yumoto I (2006). *Glaciecola chathamensis* sp. nov., a novel marine polysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **56**, 2883–2886.

McGinnis JM, Cole JA, Dickinson MC, Mingle LA, Lapierre P, Musser KA, & Wolfgang WJ. (2015). *Paracoccus sanguinis* sp. nov., isolated from clinical specimens of New York State patients. *Int J Syst Evol Microbiol* **65**, 1877-1882.

Miller LT (1982). A single derivatization method for bacterial fatty acid methyl esters including hydroxy acids. *J Clin Microbiol* **16**: 584-586.

Park S. & Yoon JH (2013). *Glaciecola aquimarina* sp. nov., a gammaproteobacterium isolated from coastal seawater. *Antonie van Leeuwenhoek* **103**, 1141–1148.

Qin QL, Xie BB, Yu Y, Shu YL, Rong JC, Zhang YJ, Zhao DL, Chen XL, Zhang XY, Chen B, Zhou BC, & Zhang YZ (2014). Comparative genomics of the marine bacterial genus *Glaciecola* reveals the high degree of genomic diversity and genomic characteristic for cold adaptation. *Environ Microbiol* **16**, 1642-1653.

Richter M & Rosselló-Móra R (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* **106**, 19126-19131.

Romanenko LA, Zhukova NV, Rohde M, Lysenko AM, Mikhailov VV & Stackebrandt E (2003). *Glaciecola mesophila* sp. nov., a novel marine agar-digesting bacterium. *Int J Syst Evol Microbiol* **53**, 647–651.

Schultz-Johansen M, Glaring MA, Bech PK, & Stougaard P (2016). Draft genome sequence of a novel marine bacterium, *Paraglaciecola* sp. strain S66, with hydrolytic activity against seaweed polysaccharides. *Genome Announc* **4** e00304-16.

Shivaji S & Reddy GS (2014). Phylogenetic analyses of the genus *Glaciecola*: emended description of the genus *Glaciecola*, transfer of *Glaciecola mesophila*, *G. agarilytica*, *G. aquimarina*, *G. arctica*, *G. chathamensis*, *G. polaris* and *G. psychrophila* to the genus *Paraglaciecola* gen. nov. as *Paraglaciecola mesophila* comb. nov., *P. agarilytica* comb. nov., *P. aquimarina* comb. nov., *P. arctica* comb. nov., *P. chathamensis* comb. nov., *P. polaris* comb. nov. and *P. psychrophila* comb. nov., and description of *Paraglaciecola oceanifecundans* sp. nov., isolated from the Southern Ocean. *Int J Syst Evol Microbiol* **64**, 3264-3275.

Stropko SJ, Pipes SE, & Newman JD (2014). Genome-based reclassification of *Bacillus cibi* as a later heterotypic synonym of *Bacillus indicus* and emended description of *Bacillus indicus*. *Int J Syst Evol Microbiol* **64**, 3804-3809.

Thomas F, Barbeyron T, & Michel G (2011). Evaluation of reference genes for real-time quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. *J Microbiol Methods* **84**, 61-66.

Van Trappen, S., Tan, T. L., Yang, J., Mergaert, J. & Swings, J. (2004). *Glaciecola polaris* sp. nov., a novel budding and prosthecate bacterium from the Arctic Ocean, and emended description of the genus *Glaciecola*. *Int J Syst Evol Microbiol* **54**, 1765–1771.

Yong, J. J., Park, S. J., Kim, H. J. & Rhee, S. K. (2007). *Glaciecola agarilytica* sp. nov., an agar-digesting marine bacterium from the East Sea, Korea. *Int J Syst Evol Microbiol* **57**, 951–953.

Zhang, D. C., Yu, Y., Chen, B., Wang, H. X., Liu, H. C., Dong, X. Z. & Zhou, P. J. (2006). *Glaciacola psychrophila* sp. nov., a novel psychrophilic bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **56**, 2867–2869.

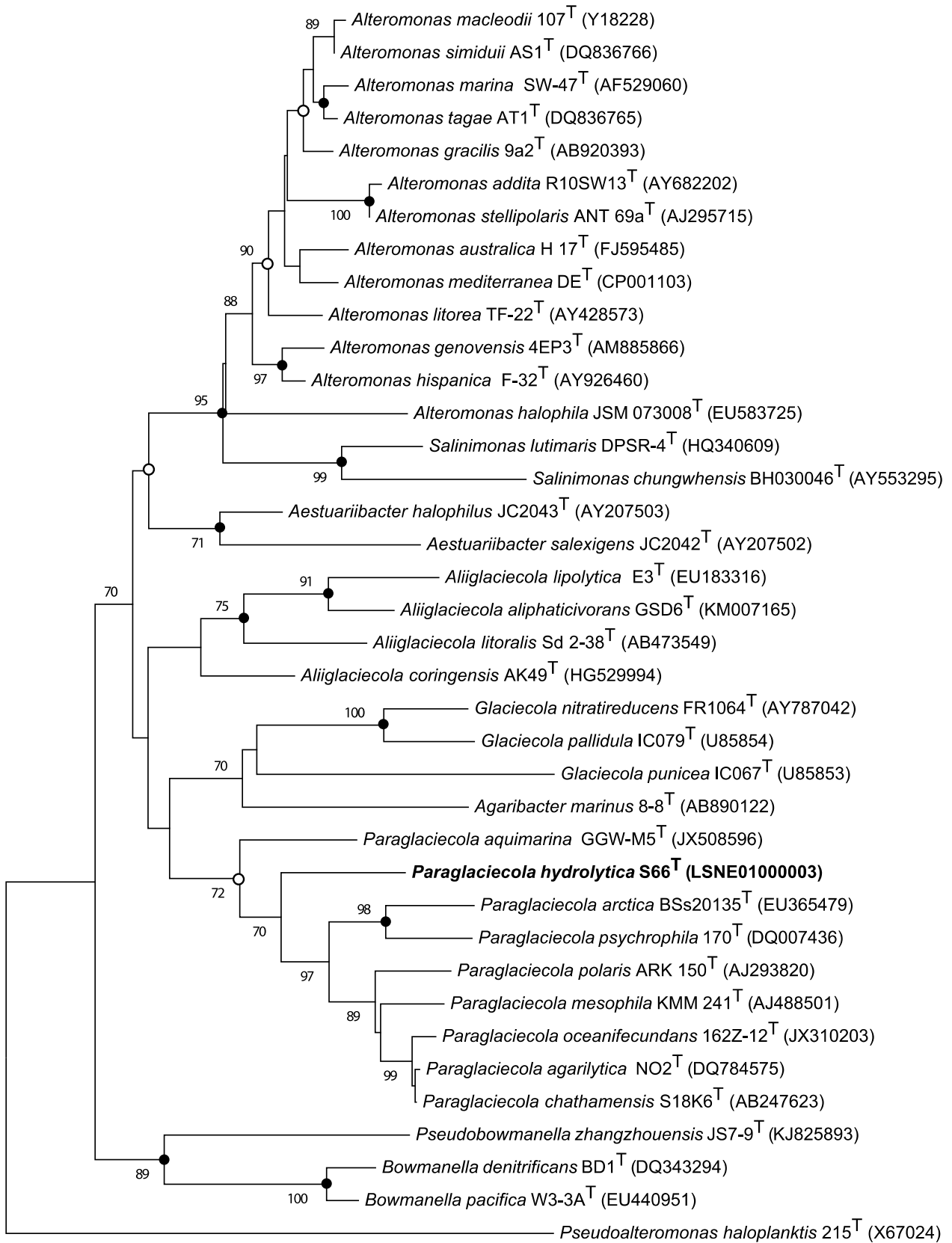
Zhang, Y. J., Zhang, X. Y., Mi, Z. H., Chen, C. X., Gao, Z. M., Chen, X. L., Yu, Y., Chen, B. & Zhang, Y. Z. (2011). *Glaciacola arctica* sp. nov., isolated from Arctic marine sediment. *Int J Syst Evol Microbiol* **61**, 2338–2341.

Fig. 1.

Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain S66^T and related taxa. Numbers at nodes are bootstrap values shown as percentages of 1000 replicates; only values >70% are shown. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the ML and MP algorithms, while open circles indicate that the nodes were only recovered in the tree generated with the ML algorithm.

Pseudoalteromonas haloplanktis 215^T (GenBank accession no. X67024) was used as an outgroup.

Scale bar, 0.01 changes per nucleotide position.



0.01

Table 1. Phenotypic characteristics of strain S66^T and species of *Paraglaciecola*.

1, Strain S66^T (*P. hydrolytica* sp. nov.); 2, *P. agarilytica* NO2^T (data from Yong et al., 2007); 3, *P. aquimarina* GGW-M5^T (Park and Yoon, 2013); 4, *P. arctica* BSs20 135^T (Zhang et al., 2011); 5, *P. chathamensis* S18K6^T (Matsuyama et al., 2006); 6, *P. mesophila* KMM 241^T (Romanenko, 2003); 7, *P. oceanifecundans* 162Z-12^T (Shivaji and Reddy 2014); 8, *P. polaris* LMG 21857^T (van Trappen et al., 2004); 9, *P. psychrophila* 170^T (Zhang et al. 2006). All strains were positive for motility, oxidase and catalase activities and negative for indole reaction. +, positive; -, negative; w, weakly positive or delayed; nd, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Growth									
Temp optimum, °C	28		30	25		25-28	25		12
Temp range, °C	5-33	7-30	4-37	4-28	4-30	7-35	4-30	5-30	4-15
pH optimum	7.5		7-8			8.0-8.5			5-10
pH range	5-9		5.5-8		5-9	5.5-9.5	6-10		6-9
NaCl optimum, %	3		2	2		2-5			
NaCl, range %	2-5	2-8	0.5-5	1-5	1-10	1-8	2-9	1-10	1-6
Hydrolysis of									
Starch	+	+	-	-	nd	+	-	+	+
Agar	+	+	nd	-	nd	+	nd	-	-
Carrageenan	+	nd	nd	-	nd	+	nd	nd	nd
Utilization of									
D-Glucose	+	-	+	+	+	+	-	+	+
D-Mannitol	-	+	+	-	+	+	-	+	+
L-Rhamnose	+	-	+	-	+	-	-	-	-
D-Sucrose	+	-	+	-	+	+	-	+	+
D-Melibiose	+	-	-	-	nd	+	-	+	+
L-Arabinose	+	-	-	-	-	-	+	-	-
D-Xylose	+	nd	+	nd	+	-	-	+	nd
Lactose	+	nd	+	nd	-	-	-	-	+
Galactose	w	+	+	nd	+	-	-	+	+
Maltose	+	-	+	-	+	+	-	+	+
Enzymes (API-ZYM)									
Alkaline phosphatase	+	nd	+	+	nd	nd	+	+	+
Leucine arylamidase	+	nd	+	+	+	-	nd	+	+
Valine arylamidase	w	+	w	+	-	w	nd	w	+
Cystine arylamidase	-	-	-	w	-	-	nd	-	w
α-Chymotrypsin	+	w	-	-	w	-	nd	-	+
Trypsin	-	w	-	w	+	-	nd	w	w
N-Acetyl-β-glucosaminidase	+	-	+	-	-	-	nd	-	-
DNA G+C mol %	42.2	45	42.4	40.3	44.8	44.6-44.8	nd	44.0	42.9

Table 2. Whole genome relatedness analysis of strain S66^T and related species of *Paraglaciicola* and *Glaciicola*.

Lower left shows the average nucleotide identity (ANI) and upper right shows the genome-to-genome distance calculation (GGDC).

GenBank accession numbers for the genome sequences are given in the top row.

		GGDC									
		<i>P. hydrolytica</i>	<i>P. agarilytica</i>	<i>P. agarilytica</i>	<i>P. arctica</i>	<i>P. chathamensis</i>	<i>P. mesophila</i>	<i>P. polaris</i>	<i>P. psychrophila</i>	<i>G. nitratireducens</i>	<i>G. punicea</i>
		S66 ^T	NO2 ^T	4H-3-7	BSs20 135 ^T	S18K6 ^T	KMM 241	LMG 21857 ^T	170 ^T	FR1064 ^T	ACAM 611 ^T
Accession nos.		LSNE000000000	BAEK000000000	NC_015497	BAEO010000000	BAEM000000000	BAEP000000000	BAER000000000	NC_020514	NC_016041	NZ_BAET000000000
ANI	<i>P. hydrolytica</i> S66 ^T		19.9	20.7	18.5	20.2	22.0	20.9	20.2	23.5	22.1
	<i>P. agarilytica</i> NO2 ^T	77.9		86.0	20.1	87.4	24.2	20.9	20.2	25.6	21.3
	<i>P. agarilytica</i> 4H-3-7	78.8	98.5		21.4	86.7	23.8	20.4	21.1	27.7	24.1
	<i>P. arctica</i> BSs20 135 ^T	77.0	77.8	78.5		19.9	20.1	21.1	24.7	23.5	21.3
	<i>P. chathamensis</i> S18K6 ^T	78.6	98.6	98.5	77.5		23.6	20.3	20.6	24.1	21.3
	<i>P. mesophila</i> KMM 241	80.6	83.4	82.8	79.3	82.8		21.0	23.0	24.5	23.6
	<i>P. polaris</i> LMG 21857 ^T	78.4	80.8	79.8	79.4	79.5	80.6		21.4	24.1	22.7
	<i>P. psychrophila</i> 170 ^T	77.6	77.1	78.2	81.9	77.0	79.2	78.4		27.0	24.1
	<i>G. nitratireducens</i> FR1064 ^T	77.6	78.5	79.8	77.3	78.4	78.7	78.0	77.4		21.2
	<i>G. punicea</i> ACAM 611 ^T	77.5	77.7	78.1	77.5	77.2	77.1	76.2	78.3	77.7	

Supplementary Table S1

Cellular fatty acid composition of strain *Paraglaciecola hydrolytica* S66^T and type strains of selected members of the genera *Paraglaciecola*, *Glaciecola*, and *Aliiglaciecola*.

Strains: 1, Strain S66^T; 2, *P. mesophila* KMM241^T (data from Romanenko et al. 2003), 3, *G. nitratreducens* FR1064^T (Baik et al. 2006); 4, *A. lipolytica* E3^T (Chen et al. 2009). Data are percentages of total fatty acids. -, Fatty acids not detected or below 1%.

Fatty acid	1	2	3	4
Straight-chain				
C _{12:0}	1.5	2.6	1.6	3.5
C _{14:0}	3.7	1.2	7.0	4.1
C _{15:0}	2.9	-	1.0	1.8
C_{16:0}	14.8	33.0	20.5	17.0
C _{17:0}	1.0	2.1	1.5	3.4
C _{18:0}	-	1.9	1.9	1.5
Unsaturated				
C _{15:1} <i>ω</i> 8 <i>c</i>	1.4	-	-	-
C_{16:1} <i>ω</i>7<i>c</i>	41.6	43.0	38.2	34.5
C _{17:1} <i>ω</i> 8 <i>c</i>	7.9	3.2	2.6	5.0
C_{18:1} <i>ω</i>7<i>c</i>	12.3	5.2	7.7	17.3
Hydroxy				
C _{10:0} 3-OH	1.9	-	-	-
C _{11:0} 3-OH	1.7	-	-	-
C _{12:0} 3-OH	2.6	-	2.8	2.9
C _{12:1} 3-OH	3.2	-	-	2.1

Supplementary Table S2

Pairwise nucleotide comparison between *Aliiglaciecola*, *Galciecola*, and *Paraglaciecola* species' 16S rRNA gene sequences (lower left), and between deduced GyrB amino acid sequences (upper right). 1, *P. hydrolytica* S66^T; 2, *P. psychrophila* 170^T; 3, *P. arctica* BSs20 135^T; 4, *P. agarilytica* NO2^T; 5, *P. oceanifecundans* 162Z-12^T; 6, *P. chathamensis* S18K6^T; 7, *P. mesophila* KMM 241^T; 8, *P. polaris* LMG 21857^T; 9, *P. aquimarina* GGW-M5^T; 10, *A. lipolytica* E3^T; 11, *G. nitratireducens* FR1064^T; 12, *G. pallidula* ACAM 615^T; 13, *G. punicea* ACAM 611^T; 14, *Alteromonas macleodii* ATCC 27126^T.

		GyrB													
		1	2	3	4	5*	6	7	8	9*	10	11	12	13	14
16S rRNA	1		85.5	85.0	84.7		84.5	83.9	83.5		81.8	75.6	75.3	73.6	78.6
	2	94.9		94.8	83.5		83.4	83.4	83.0		82.2	74.7	75.1	73.6	80.3
	3	95.9	97.3		82.8		82.7	82.5	82.2		80.9	74.7	74.7	73.9	79.4
	4	94.0	94.8	95.7			99.9	96.4	94.8		80.4	75.9	75.6	73.9	80.3
	5	95.3	95.1	96.0	99.1										
	6	95.5	95.3	96.3	99.3	99.5		96.5	94.9		80.3	75.8	75.4	73.8	80.2
	7	95.9	96.5	97.3	98.1	98.3	98.7		95.5		80.8	75.9	75.7	74.3	79.9
	8	95.6	96.0	97.1	97.8	98.0	98.4	97.9			80.2	75.6	75.1	73.6	79.3
	9	95.5	95.5	96.0	96.3	96.5	96.7	96.5	96.5						
	10	94.2	93.6	93.8	94.0	94.2	94.4	94.3	94.0	94.6		75.4	75.1	74.6	80.6
	11	92.6	93.0	93.6	94.3	94.5	94.8	95.0	95.3	94.2	92.1		94.4	81.2	76.5
	12	92.2	93.3	93.5	93.6	93.9	94.2	94.9	94.1	94.2	91.1	97.5		81.0	76.3
	13	91.4	92.7	93.1	92.3	92.6	92.9	93.6	92.5	92.4	91.6	92.9	92.5		75.3
	14	93.5	92.4	92.7	93.3	93.8	93.9	93.2	93.2	94.5	92.6	93.2	93.3	91.2	

* The genome sequence of *P. oceanifecundans* 162Z-12^T and *P. aquimarina* GGW-M5^T has not been determined and thus, no GyrB protein sequence has been found.

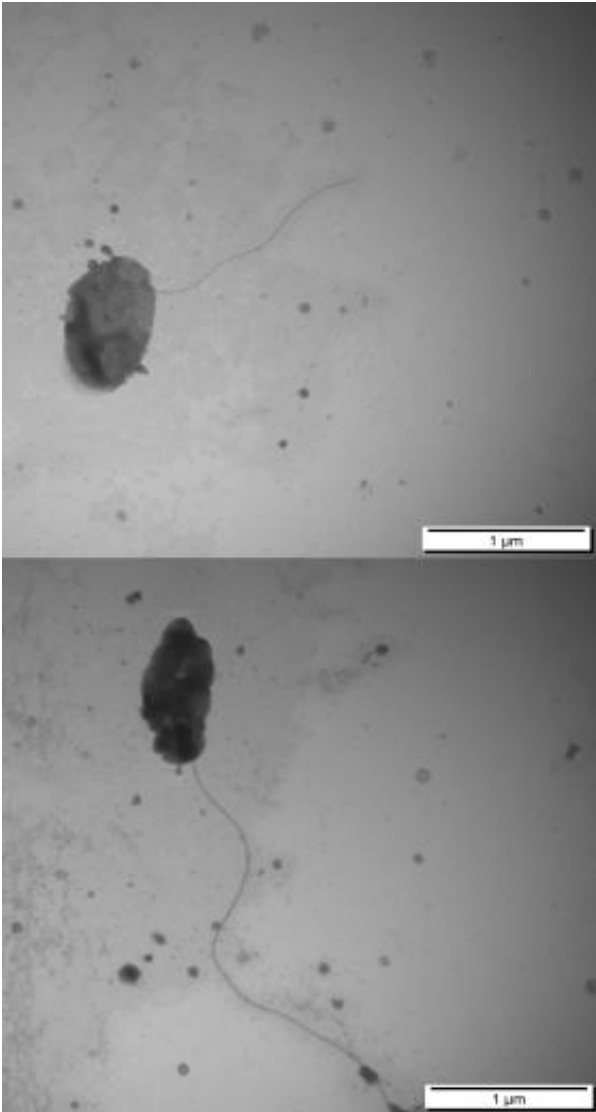
Supplementary Table S3.Utilization of carbohydrates by strain S66^T.

	Carbon source	Growth
Simple sugars	Glucose	++
	Galactose	+
	Sucrose	++
	Lactose	++
	Xylose	++
	Maltose	++
Plant polysaccharides	Amylose	+
	Amylopectin	++
	Cellulose	-
	Mannan	-
	Pectin (Apple)	++
	Starch	++
	Xylan	++
Algal Polysaccharides	Agar	++
	Agarose	++
	Alginate	++
	Fuoidan	-
	<i>ι</i> -carrageenan	-
	<i>κ</i> -carrageenan	+
	<i>λ</i> -carrageenan	-
	Laminarin	++
	Porphyran	++
	Ulvan	-

The bacterium was grown in Minimal Marine Medium with the listed carbohydrates as the sole carbon source. Growth is described as normal (++), limited or stalled (+), or no growth (-).

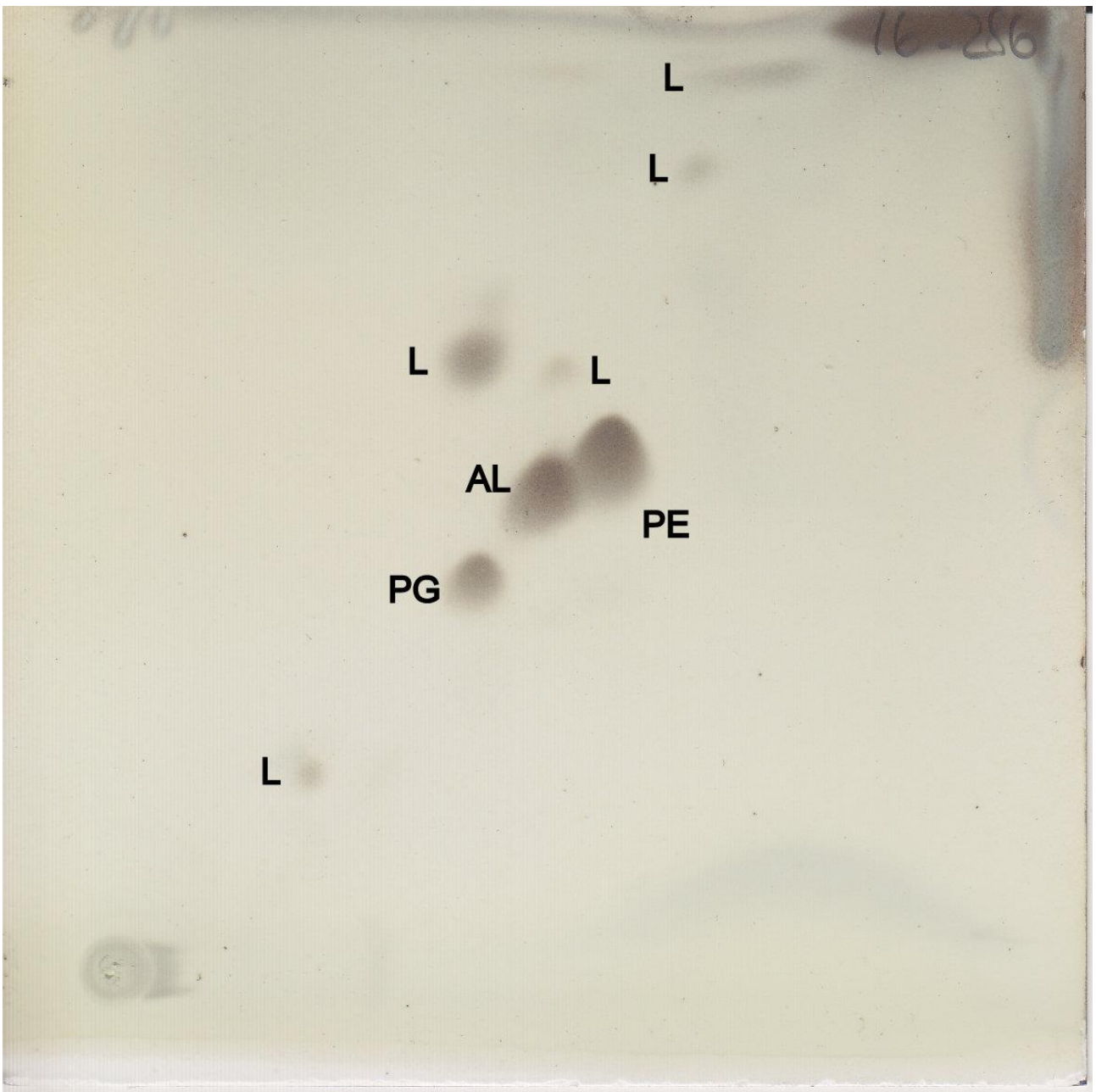
Supplementary Fig. S1

Transmission electron micrographs of negatively stained cells of strain S66^T showing a single polar flagellum.



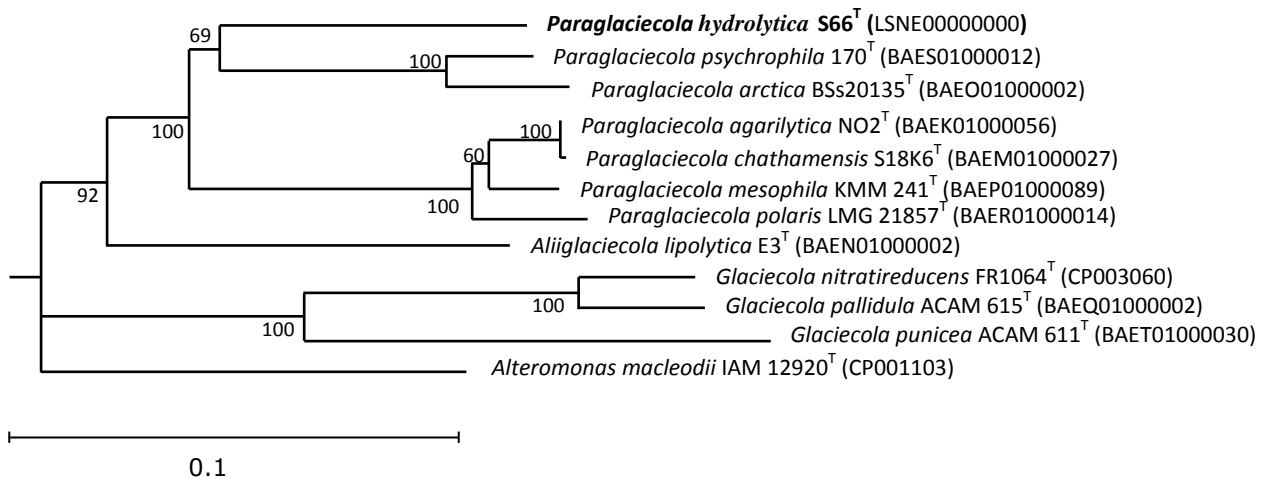
Supplementary Fig. S2

Two-dimensional thin layer chromatography (TLC) of polar lipids from *Paraglaciecola hydrolytica* S66^T. AL, aminolipid; L, lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.



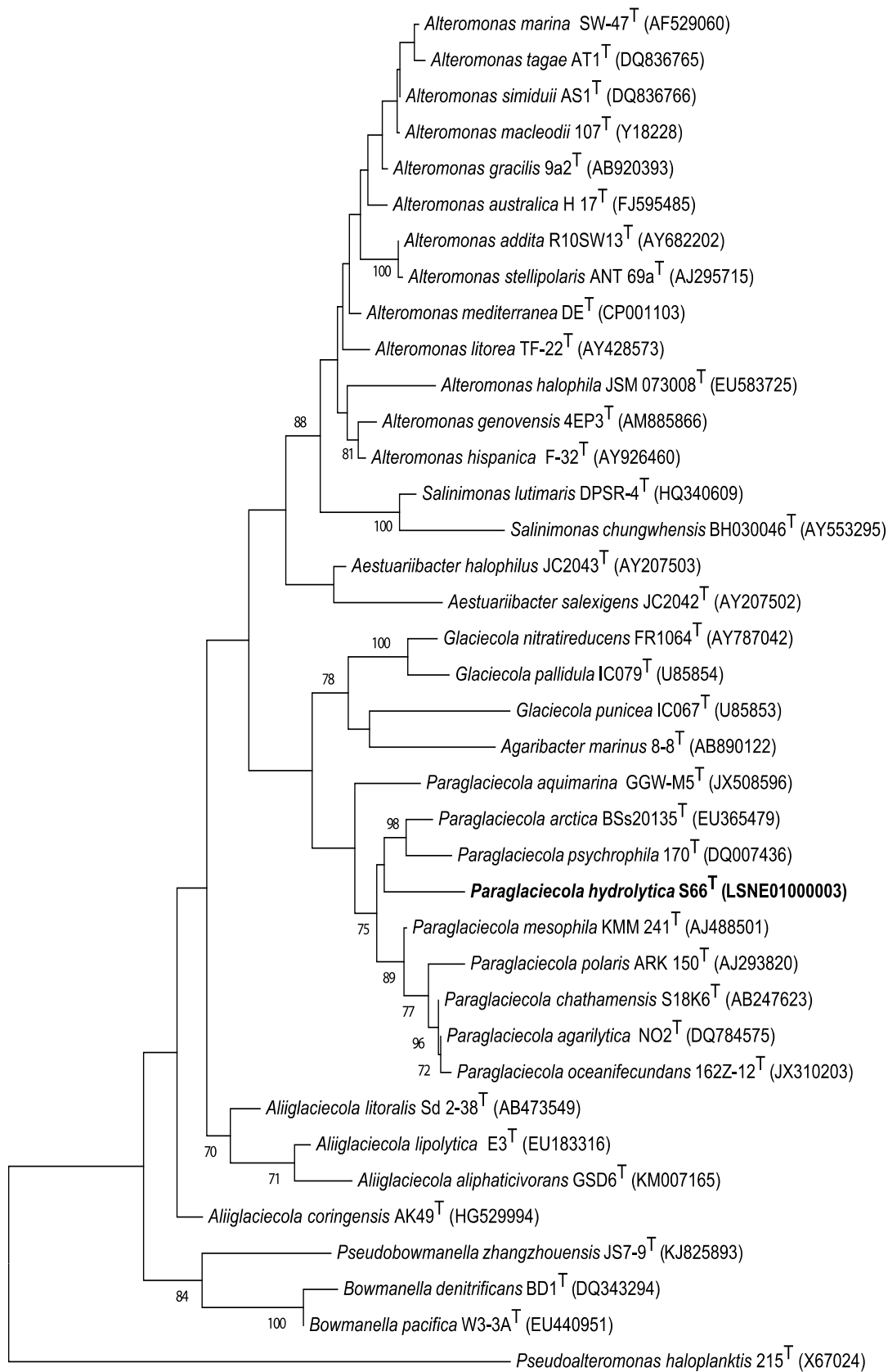
Supplementary Fig. S3

Phylogenetic tree showing the position of strain S66^T in relation to other species within the genera *Paraglaciecola*, *Glaciecola*, and *Aliiglaciecola*. The tree was constructed based on GyrB amino acid sequences deduced from the genome sequence and using the neighbour-joining method. Bootstrap values greater than 50% are shown at the nodes. The scale bar shows 10 % amino acid substitutions.



Supplementary Fig. S4

Maximum likelihood tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain S66^T and related taxa. Numbers at nodes are bootstrap values shown as percentages of 100 replicates; only values >70% are shown. *Pseudoalteromonas haloplanktis* 215^T (GenBank accession no. X67024) was used as an outgroup. Bar, 0.01 changes per nucleotide position.



0.05

Supplementary Fig. S5

Maximum parsimony tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain S66^T and related taxa. Numbers at nodes are bootstrap values shown as percentages of 1000 replicates; only values >70% are shown. *Pseudoalteromonas haloplanktis* 215^T (GenBank accession no. X67024) was used as an outgroup. Bar, 0.01 changes per nucleotide position.

