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

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Sodium ion requirement and salt tolerance (0, 1, 2, 3, 4, 5, 6,
8 and 10 % NaCl) was assessed in marine minimal medium
[17] supplemented with glucose (5 g l \(^{-1}\)) after 4 days of incubation at 20 °C.

Cell morphology and the presence of a flagellum was deter-
mixed using transmission electron microscopy of negatively
stained cells [14]. The Gram Stain Set 5 kit (BD Difco) and
the Ryu non-staining KOH method [18] were used for test-
ing the Gram reaction. Motility was tested by the hanging-
drop method [19]. The phenotypic characterizations included oxidase activity (BBL Dryslide Oxidase kit; BD)
and catalase activity (H \(_2\)O \(_2\) addition). Other enzymic activi-
ties were determined by cultivation on MA supplemented
with 0.06 % (w/v) azurin cross-linked (AZCL) substrates,
AZCL-amylose, -arabinan, -arabinoxylan, -rhamnogalactur-
onan, -galactomannan, -xylan and -cellulose (Megazymes).
Alginase lyase activity was determined on MA plates con-
taining alginate (0.1 %, w/v; Sigma) and stained with 10 %
(w/v) cetylpyridinium chloride (CPC) after incubation [20].
Carrageenase activity was determined on MB plates solidi-
fied with κ-carrageenan (1 %, w/v; Sigma) and stained with
Lugol solution after incubation [21]. All plates were incu-
bated at 20 °C for 1 to 2 weeks. Susceptibility to tetracycline,
streptomycin, gentamycin, kanamycin, ampicillin, penicillin
G, chloramphenicol and tobramycin was investigated on
MA plates using MIC test strips (Liofichem) according to
the instructions of the manufacturer. Additional growth and
physiological characterizations were performed using API
20 NE and API ZYM strips according to the instructions of
the manufacturer (bioMérieux) except that cell suspensions
were made in 3 % sea salts (Sigma-Alrich) or in API AUX
medium adjusted to 3 % sea salt. For supplementary sugar
utilization experiments, strain S66\(^{\top}\) was cultivated in marine
minimal medium containing 5 g l \(^{-1}\) of the following sugars:
glucose (Sigma-Alrich), galactose (Sigma-Alrich), xylose
(Sigma-Alrich), sucrose (Sigma-Alrich), lactose (Sigma-
Alrich), maltose (Sigma-Alrich), cellulose avicel (Merck),
laminarin (Goëmar), starch (Fluka), amylopectin (Merck),
amylose (Fluka), mannan (Sigma-Alrich), xylan (Sigma-
Alrich), pectin (apple) (Sigma-Alrich), agar (Sigma-
Alrich), agarose (Eurogentec), porphyran (extracted from
Porphyra umbilicalis), alginic acid (Sigma-Alrich), κ-
carrageenan (Danisco), β-carrageenan (Goëmar), λ-carrageenan
(Danisco), fucoidan (extracted from Pelvetia canaliculata)
and ulvan (a 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 modi-
fications of the methods of Miller [22] and Kuykendall et al.,
[23]. The fatty acid methyl ester mixtures were separated
using the Sherlock Microbial Identification System (MIS)
(MIDI, Microbial ID), and peaks were integrated automa-
tically and fatty acid names and percentages calculated by
the MIS Standard Software (Microbial ID). Respiratory
lipoquinones were separated into their different classes (mena-
quinones, ubiquinones, etc.) by TLC on silica gel [24, 25].
Polar lipids were separated by two-dimensional silica gel TLC.
The first direction was developed in chloroform/methanol/water
(65:25:4, by vol.), and the second in chloroform/meth-
anol/acetic acid/water (80:12:15:4, by vol.) [26]. Genomic
DNA was isolated from cells using the Gentra Puregene
Yeast/Bact kit (Qiagen). Whole-genome sequencing of strain
S66\(^{\top}\) was carried out as described by Schultz-Johansen et al.
[27]. The 16S rRNA gene sequence and the deduced GyrB
protein sequence were retrieved from the genome sequence
and compared with partial 16S rRNA gene and GyrB se-
cuences of other species of the genera Paraglaciecola, Glaciecola
and Aliiglaciecola. The nucleotide sequences (16S rRNA gene)
were aligned using the program MAFFT version 7 with the
L-INS-I strategy [28], and phylogenetic trees based on the
neighbor-joining (NJ), maximum-parsimony (MP) and maxi-
mum-likelihood (ML) algorithms were built using MEGA
software (version 5.05) [29] (Fig. 1). Amino acid (GyrB)
sequences were aligned and NJ phylogenetic trees were recon-
structed using the alignment tool in the CLC Main Work-
bench 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 the ML tree) resampled datasets (Fig. S1,
available in the online Supplementary Material). Genomic
relatedness was investigated by comparing the S66\(^{\top}\) genome
sequence with other genomic sequences of type strains of spe-
cies of the genera Paraglaciecola and Glaciecola using the
Genome-Genome Distance Calculator (GGDC) in an online
program at http://ggdc.dsmz.de/distcalc2.php using the align-
ment method Blast+ and the formula 2 for incomplete
sequence with other genomic sequences of type strains of spe-
cies of the genus Paraglaciecola and Glaciecola [30]. The average nucleotide identity (ANI) was similarly calculated using an online server at the Kostas Lab, http://enve-omics.ce.gatech.edu/ani/. These novel
methods for comparing genome sequences in pairwise related-
ness 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 [32–36]. The DNA G+C content of strain
S66\(^{\top}\) was 42.2 mol%.

Physiological growth features of strain S66\(^{\top}\) and representa-
tives of other species of the genus Paraglaciecola are listed in
Table 1. The major fatty acids of strain S66\(^{\top}\) were C\(_{16:1}\)
ω7c (41.6 %), C\(_{16:0}\) (14.8 %) and C\(_{18:1}\) ω7c (12.3 %), which
is similar to the fatty acid compositions of other members of
the genus Paraglaciecola (Table S1). The respiratory quino-
one of strain S66\(^{\top}\) was determined to be ubiquinone-8 (Q-
8), which is in accordance with the quinone composition of
Glaciecola aquimarina. The only other species of the genus
Paraglaciecola that has been investigated for quinone com-
position is Paraglaciecola oceanificundans, which contains
the menaquinone MK-7 [3]. The major polar lipids of strain
S66\(^{\top}\) were phosphatidylethanolamine, phosphatidylglycerol
and an unknown aminolipid (Fig. S2), which is also the case
for other species of the genus Paraglaciecola, Glaciecola
and Aliiglaciecola [2, 3, 5, 7, 9, 13, 15]. Cells of strain S66\(^{\top}\)
were Gram-reaction-negative, straight or irregular-shaped rods.
Fig. 1. NJ tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain S66 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\textsuperscript{T} (GenBank accession no. X67024) was used as an outgroup. Bar, 0.01 changes per nucleotide position.
with a polar flagellum (Fig. S3). Cell aggregates were formed when grown in liquid medium. In contrast to other members of the genus *Paraglaciecola*, colonies of S66<sup>T</sup> were beige to pale orange on MA, and they were circular and convex with a mucoid consistency. The presence of holes under and around colonies cultivated on media solidified with agar 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. Carrageenase activity was observed for colonies growing on plates solidified with a mucoid consistency. The presence of holes under and around colonies of S66<sup>T</sup> hydrolysed AZCL-amylose, -galactomannan, -xylan and -cellulose. Growth was observed on glucose, maltose, xylose, lactose and sucrose whereas growth on starch, amylopectin, xylan and apple pectin with limited growth recorded on amylose and no growth observed on cellulose or mannan. Strain S66<sup>T</sup> was able to utilize the complex algal polysaccharides agar, agarose, porphyran, alginate, and laminarin as sole carbon and energy sources, and to a limited extent also κ-carrageenan, but not λ- or τ-carrageenan, fucoidan or ulvan (Table S2).

Phylogenetic analyses of 16S rRNA gene and GyrB sequences showed that strain S66<sup>T</sup> was distantly related to other species of the genus *Paraglaciecola* (Figs 1 and S1) with approximately 95% identity to other 16S rRNA gene sequences and 84–85% identity to GyrB amino acid sequences (Table S3). ANI and GGDC analyses confirmed the phylogenetic results determined from 16S rRNA gene and GyrB sequences. The ANI and GGDC values for strain S66<sup>T</sup> when compared with other species of the genus *Paraglaciecola* were less than 81% and less than 24%, respectively (Table S4). A pairwise genome comparison of other species of the genus *Paraglaciecola* showed that *Paraglaciecola agarlytica* and *Paraglaciecola chathamensis* may represent the same species since ANI and GGDC (formula 2) values between them were 98.5 and 87%, respectively (also

### Table 1. Phenotypic characteristics of strain S66<sup>T</sup> and species of the genus *Paraglaciecola*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>Growth</td>
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<tr>
<td>Temp. optimum (°C)</td>
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<td>Temp. range (°C)</td>
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<td>10–30</td>
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<td>NA</td>
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<tr>
<td>pH range</td>
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<td>6–10</td>
<td>6–10</td>
<td>5–9</td>
<td>6–10</td>
<td>NA</td>
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<tr>
<td>NaCl optimum (%)</td>
<td>3–4</td>
<td>3–4</td>
<td>4–5</td>
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<tr>
<td>NaCl range (%)</td>
<td>1–6</td>
<td>1–8</td>
<td>1–8</td>
<td>1–10</td>
<td>2–9</td>
<td>1–10</td>
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<td>Aggregates in liquid medium</td>
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<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Pigmentation</td>
<td>Beige/pale orange</td>
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<td>No</td>
<td>No</td>
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<td>Hydrolysis of:</td>
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<tr>
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<td>+</td>
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<td>+</td>
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<td>Agar</td>
<td>+</td>
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<td>+</td>
<td>ND</td>
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<td>–</td>
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<td>Carrageenan</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Alginate</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
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<td>ND</td>
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<td>Utilization of:</td>
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<tr>
<td>Glucose</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Arabinose</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Enzymes</td>
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<td>Alkaline phosphatase</td>
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<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>w</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>w</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>Trypsin</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>w</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>42.2</td>
<td>44.6–44.8</td>
<td>45</td>
<td>44.8</td>
<td>ND</td>
<td>44.0</td>
</tr>
</tbody>
</table>
reported by [16]). For comparison, the ANI and GGDC values between genomes of Paraglaciecola agarilytica NO2T and Paraglaciecola agarilytica 4H-3-7+YE-5 were 98.5 and 86 %, respectively. For species delineation, threshold ANI values of 95–96 % and GGDC (formula 2) values of 70 %, respectively, are normally accepted [33, 37, 38]. In conclusion, phylogenetic characterizations using 16S rRNA gene and GyrB sequences together with whole-genome pairwise comparisons show that strain S66T represents a novel species of the genus Paraglaciecola, for which the name Paraglaciecola hydrolytica sp. nov. is proposed.

**DESCRIPTION OF PARAGLACIECOLA HYDROLYTICA SP. NOV.**

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

Cells are Gram-reaction-negative, aerobic and motile (0.4–0.6 µm wide and 0.8–1.0 µm long) with a polar flagellum. Colonies on MA are beige to pale orange, convex, circular and 1–2 mm in diameter. Growth is observed at temperatures between 10 and 25 °C with an optimum at 20–25 °C, between pH 7 and 9 with an optimum at pH 7.5, and with between 1 and 6 % NaCl with an optimum at 3–4 % NaCl when determined in marine minimal medium. Catalase- and oxidase-positive. Positive enzyme reactions are observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and N-acetyl-β-glucosaminidase. A weakly positive reaction is seen for trypsin and β-glucosidase whereas negative reactions are found for nitrate reduction, indole production, arginine dihydrolase, urea hydrolysis and gelatinase. Aesculin, starch, agar, α-carrageenan and alginate are degraded. Utilizes D-glucose, sucrose, D-xyllose, lactose, maltose, D-mannitol, L-arabinose (weakly), D-mannose (weakly) and galactose (weakly), but not N-acetyl-glucosamine, capric acid, citrate or phenylacetic acid. Resistant to penicillin (2 µg ml-1) and sensitive to (minimum inhibition concentration) tetracycline (6 µg ml-1), chloramphenicol (0.5 µg ml-1), gentamicin (1 µg ml-1), kanamycin (3–4 µg ml-1), streptomycin (3–4 µg ml-1), tobramycin (1.5 µg ml-1) and ampicillin (0.5 µg ml-1). The major cellular fatty acids are C16:0,ω7c, C16:1ω7c, C18:0 and C18:1ω7c. The respiratory quinone is Q-8, and polar lipids are phosphatidylethanolamine, phosphatidylglycerol, an unknown aminolipid, and five unknown lipids. The type strain is S66T (=LMG 29457T=NCIMB 15060T =DSM 102834T), isolated from leaves of eelgrass (Zostera sp.) collected on the coast of northwest Zealand, Denmark. The DNA G+C content of the type strain is 42.2 mol %.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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**Ethical statement**

This work was carried out without the use of animals or humans.

**References**


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