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TAXONOMIC DESCRIPTION

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Paracoccus nototheniae sp. nov., isolated from a black rock cod fish (*Notothenia coriiceps*) from the Chilean Antarctic

Peter Kämpfer,[1,*] Rute Irgang,[2,3] Matías Poblete-Morales,[2,3] Guillermo Fernández-Negrete,[4] Stefanie P. Glaeser,[1] Derie Fuentes-Messina[4] and Ruben Avendaño-Herrera[2,3,5,*]

Abstract

An orange-pigmented, oxidase-positive bacterial strain (I-41R45^T), isolated from the kidney of a black rock cod fish sampled in the Chilean Antarctic was studied in a polyphasic taxonomic investigation. Cells of the isolate were coccoid and stained Gram-negative. A comparison of the 16S rRNA gene sequence of strain I-41R45^T with sequences of type strains of most closely related *Paracoccus* species showed highest sequence similarities to *Paracoccus hibiscisoli* (98.4 %), *Paracoccus marcusii* (98.3 %), *Paracoccus haeundaensis* and *Paracoccus carotinifaciens* (both 98.2 %). 16S rRNA gene sequence similarities to all other *Paracoccus* species were below 97 %. The draft genome of strain I-41R45^T had a size of 4.59 Mb with a DNA G+C content of 65.26 mol% and included the prediction and annotation of 4426 coding genes, 1973 protein-coding genes and 46 tRNAs. The fatty acid profile of strain I-41R45^T consisted mainly of the major fatty acids $C_{18:1}\omega7c/\omega9t/\omega12t$ and $C_{18:0}$, typical of the genus *Paracoccus*. DNA–DNA hybridizations between I-41R45^T and type strains of *P. hibiscisoli, P. marcusii*and *P. haeundaensis* resulted in similarity values of 45 % (reciprocal 26 %), 66 % (reciprocal 61 %), and 29 % (reciprocal 36 %), respectively. DNA–DNA hybridization results, together with the differentiating biochemical and chemotaxonomic properties, showed that strain I-41R45^T represents a novel *Paracoccus* species, for which the name *Paracoccus nototheniae* sp. nov. (type strain I-41R45^T=CCM 8875^T=CIP 111632^T), is proposed.

The genus *Paracoccus* was first reported by Davis *et al.* [1]. To date (April 2019, LPSN [2]), the genus is composed of 56 species with validly published names. The Gram-negative, catalase-positive, oxidase-positive, coccus or short-rod members of this genus show a variety of metabolic properties [3–5]. From the *Paracoccus* species published only a few were isolated from marine environments: as sediment [6, 7], sea-sand sample [8], reef coral [9], coastal seawater [10], marine bryozoan [11] and the two only isolates from fish were from Korean food prepared with salted pollock [12] and blues potted cornet fish [13].

An orange-pigmented strain encoded as I-41R45^T was isolated from a black rock cod (*Notothenia coriiceps* Richardson 1844) from a kidney sample on February 2015, during the 51th Expedition to the Chilean Antarctic Naval Base *Arturo Prat* (62° 30′ S 59° 41′ W) on trypticase soy agar supplemented with 1 % NaCl (w/v; TSA-1) plates. The strain revealed typical colony morphological properties of paracocci. Strain I-41R45^T was maintained and sub-cultivated on nutrient agar (NA; Oxoid) at 30 °C for 48 h and subsequently analysed for 16S rRNA gene sequence, fatty acid methyl ester composition of whole cell hydrolysates, further phenotypic features, and DNA–DNA similarities to those *Paracoccus* species most closely related on the basis of 16S rRNA gene sequence similarities. Reference strains for physiological and biochemical tests, DNA–DNA hybridization (DDH) and fatty acid analysis (*Paracoccus hibiscisoli* KACC 18933^T, *Paracoccus marcusii* DSM 11574^T and *Paracoccus haeundaensis* LMG 21903^T) were grown under the same conditions as the new strain.

Cultural and morphological characteristics were determined from cultures grown on NA at 28 °C. The Gram reaction was tested on the basis of a modified method of Gerhardt *et al.* [14]. Motility was tested under a light microscope on cells grown for 3 days in nutrient broth (Oxoid) at 30 °C. Oxidase activity was tested using the Oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Catalase activity was tested by gas formation after saturation

Author affiliations: [1]Institut für Angewandte Mikrobiologie, Universität Giessen, Giessen, Germany; [2]Universidad Andrés Bello, Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Viña del Mar, Chile; [3] Centro FONDAP, Interdisciplinary Center for Aquaculture Research (INCAR), Viña del Mar, Chile; [4]Fraunhofer Chile Research Foundation, Center for Systems Biotechnology, Santiago, Chile; [5]Universidad Andrés Bello, Centro de Investigación Marina Quintay (CIMARQ), Quintay, Chile.

***Correspondence:** Peter Kämpfer, peter.kaempfer@umwelt.uni-giessen.de; Ruben Avendaño-Herrera, reavendano@yahoo.com *or* ravendano@unab.cl

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I-41R45^T is MH065728. This Whole Genome Sequence project has been deposited as a genome assembly at the DDBJ/ENA/GenBank under the accession RQRT00000000.

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with hydrogen peroxide of fresh biomass grown for 48 h on NA (Oxoid). Growth was investigated at different temperatures (4, 8, 10, 30, 37, 45 and 50 °C) on NA. NaCl tolerance was evaluated in tryptic soy broth (TSB; Oxoid) supplemented with 0.5–10.0 % (w/v) NaCl. Growth on different culture media was also tested such as brain hearth infusion agar (Oxoid), MacConkey's agar (Oxoid) and Reasoner's 2A (R2A) agar.

Strain I-41R45^T showed a Gram-negative staining behaviour and formed visible (diameter about 2 mm) orange colonies within 48 h at 30 °C. Strain I-41R45^T was negative for oxidase and catalase activity. Cells of the strain were non-motile, non-spore forming cocci (approx. 1.1–1.3 µm wide and approx. 1.1–1.5 µm long). Strain I-41R45^T grew well on NA, brain heart infusion agar, R2A agar, TSA and MacConkey agar (Oxoid). No growth was observed below 10 °C and above 36 °C. Strain I-41R45^T also grew with 0–9 % (w/v) NaCl, but not at 10 % NaCl and in R2A broth adjusted to pH 5.0–7.5 with a pH meter (Mettler Toledo, FiveEasy FE20).

Detailed physiological characterization and biochemical tests were performed to assess the carbon source utilization pattern and hydrolysis of chromogenic substrates as described by Kämpfer *et al.* [15]. In addition, other carbon-source utilization test kits (API ZYM, API20NE and API 32GN) were employed according to the manufacturer's instructions. The strain utilized many carbon sources, similar to all *Paracoccus* species, and was able to hydrolyse many chromogenic substrates. The biochemical/physiological data are given in Table 1 and in the species descriptions and a clear differentiation from the most closely related *Paracoccus* species was possible.

The 16S rRNA gene of strain I-41R45^T was PCR-amplified with universal 16S rRNA gene targeting primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′, [16]) and sequenced with primers 8F and E786F (5′-GATTAGATACCCTGGTAG-3′) [17]) by LGC Genomics (Berlin, Germany). First phylogenetic identification including sequence similarity analysis was performed in the EzTaxon type strain 16S rRNA gene sequence database [18]. The phylogenetic relationship of strain I-41R45^T was investigated in detail using the All-Species Living Tree Project (LTP; [19]) database release LTPs128 (February 2017) and ARB release 5.2 [20]. Sequences not included in the LTP database were implemented after alignment by SINA (version 1.2.11; [21]). The alignment of all *Paracoccus* species and selected outgroup sequences was proofed manually before analysis. Phylogenetic trees were reconstructed with the maximum-likelihood (ML) method using RAxML version 7.04 [22] with GTR-GAMMA and rapid bootstrap and the maximum-parsimony (MP) method using DNAPARS version 3.6 [23], and the neighbour-joining (NJ) method using ARB NJ and the Jukes–Cantor correction [24]. The ML and MP trees were calculated with 100, the NJ tree with 1000 resamplings (bootstrap-analysis; [25]) and sequence information between gene termini 58–1446 (according to the numbering of the *rrnB* of *Escherichia coli*; [26]) were considered. The 16S rRNA gene sequence of *Paracoccus acridae* SCU−M53^T (KT634253) was shorter and added according to the Pasimony criteria to the phylogenetic trees after tree reconstruction without changing the overall tree topology using the ARB Parsimony quick add mode. Type strains of the next related genera within the family *Rhodobacteriaceae*, *Methylarcula*, *Gemmobacter*, *Cereibacter* and *Pseudorhodobacter* were included in the phylogenetic analysis to stabilize the resolution of the phylogenetic tree. The type strain of *Hyphomonas polymorpha* (*Hyphomonadaceae*; [27]) was used as an outgroup. The trees were compared and conserved nodes were marked in the ML tree which is depicted in Fig. 1.

The sequenced 16S rRNA gene fragment of strain I-41R45^T (accession number MH065728) was a continuous stretch of 1377 unambiguous nucleotides (termini 58–1500; *E. coli* numbering [26]).

Strain I-41R45^T shared highest 16S rRNA gene sequence similarities to species of the genus *Paracoccus* as indicated by EzTaxon BLAST analysis. Pairwise 16S rRNA gene sequence similarities (calculated on the basis of EzTaxon) above 97 % were obtained between strain I-41R45^T and the type strains of four *Paracoccus* species, *Paracoccus hibiscisoli* (98.4 %), *Paracoccus marcusii* (98.3 %), *Paracoccus haeundaensis* and *Paracoccus carotinifaciens* (both 98.2 %). In the 16S rRNA gene sequence based phylogenetic trees, strain I-41R45^T was placed within the genus *Paracoccus* in a cluster with the next related species listed above (Fig. 1).

The Fraunhofer Chile Research Foundation carried out draft genome sequencing of strain I-41R45^T. Libraries were reconstructed using the NEXTERA XT library preparation kit, and sequenced on a MiSeq instrument using the 2×300 cycles, paired-end V3 chemistry (Illumina), leading to 1 176 475 reads pairs (35–301 bp long), which were assembled using SPAdes version 3.11.1 [28] software. The draft genome sequence obtained had a total size of 4 597 015 bp, with 235 contigs (>500 bp), an N50 value of 51 739 bp and a G+C content of 65.26 mol%. These groups of contig sequences were processed with the Tychus pipeline [29] in order to get the prediction and annotation of 4426 coding genes, 46 tRNA and 1 tmRNA. Of the 4426 predicted genes, 1973 were hypothetical proteins. The 16S rRNA gene sequence located in contig RQRT01000123 was identical to the 16S rRNA gene sequence obtained by the Sanger sequencing approach (accession number MH065728).

In order to perform the average nucleotide identity (ANI) calculations, the genomic sequence information from the five *Paracoccus* strains available in the RSAT prokaryotes database [30] was downloaded. The three next related type strains were so far not genome sequenced. Classical DNA–DNA hybridization (DDH) was therefore applied for species delineation (see below). With the available phylogenetically close genomes, we performed a pairwise ANI calculation

Table 1. Physiological characteristics of strain I-41R45^T and related type strains of species of the genus *Paracoccus*

Strains: 1, I-41R45^T; 2, *Paracoccus hibiscisoli* KACC 18933^T (THG-T2.31^T); 3, *Paracoccus marcusii* DSM 11574^T; 4, *Paracoccus haeundaensis* LMG P-21903^T (BC74171^T); 5, *Paracoccus carotinifaciens* E-396^T; 6, *Paracoccus denitrificans* KCTC 2530^T. Data for strains 1–3 were generated in this study and confirmed and adjusted by data given by Lin *et al.* [23]. Data for strains 4–6 were obtained from Lin *et al.* [23]. All strains are Gram-reaction-negative and rod-shaped. +, Positive; w, weakly positive; —, negative.

Characteristic	1	2	3	4	5	6
Orange to red pigment	+	—	+	+	+	—
Motility	—	—	—	—	+	—
Temperature range for growth (°C)	4–36	10–35	25–30	10–40	20–35	10–40
pH range for growth	6–10	5–8	6–8	7–8	6–10	5–8
NaCl tolerance (%)	0–9	0–4	0–6	1–6	0–6	0–5
Activity and assimilation of (API 20NE and 32GN):						
Nitrate reduction	—	—	—	+	—	+
Glucose acidification	+	+	—	—	—	—
Arginine dihydrolase	—	+	—	+	—	—
Urease	—	+	—	—	—	+
Protease	—	+	w	—	—	—
D-Mannose	+	+	+	—	+	+
Gluconate	+	+	+	+	—	+
Adipate	—	+	+	—	—	—
Citrate	+	+	+	+	+	+
Phenylacetate	w	+	+	—	—	—
D-Mannitol	+	+	+	—	+	+
Melibiose	+	+	+	—	—	—
D-Sorbitol	+	+	+	—	—	—
L-Arabinose	+	+	+	+	—	—
Propionate	—	+	+	+	+	+
L-Histidine	—	+	—	—	+	+
L-Proline	+	+	—	—	—	+
D-Ribose	+	+	+	—	—	+
Inositol	+	+	+	—	—	+
Sucrose	+	+	+	—	—	—
Maltose	+	+	+	—	+	+
Suberate	—	+	+	+	—	—
Sodium malonate	—	+	+	—	—	—
DL-Lactate	+	+	+	+	—	+
L-Alanine	+	+	+	+	—	+
L-Serine	+	+	—	+	—	+
Salicin	+	+	+	—	+	—
API ZYM:						
Leucine arylamidase	w	w	+	+	+	+
Cystine arylamidase	—	+	+	+	+	+
Trypsin	—	+	—	—	—	+
Naphthol-AS-BI-phosphohydrolase	—	w	+	—	—	+
β-Glucuronidase	—	w	—	—	—	—
α-Mannosidase	—	+	—	—	—	+

using the JSpecies web server [31], and the following parameters to establish a significantly different species: <94 % identity for BLAST analysis (ANIb), <96 % MUMer analysis (ANIm), and <0.99 for tetranucleotides frequency analysis (TETRA; [32]). Regarding ANIb and ANIm analysis, we found values of identity over 95 % for two non-type strain genome sequences, *Paracoccus yeei* TT13 and strain FDAARGOS_252 (accession number GCA_002749495.1 and GCA_002073635.2) which were included in the analysis. The results expected that the two strains belonging the same species, and also ratified by the 0.99 value for TETRA analysis. However, none of the two strains were further

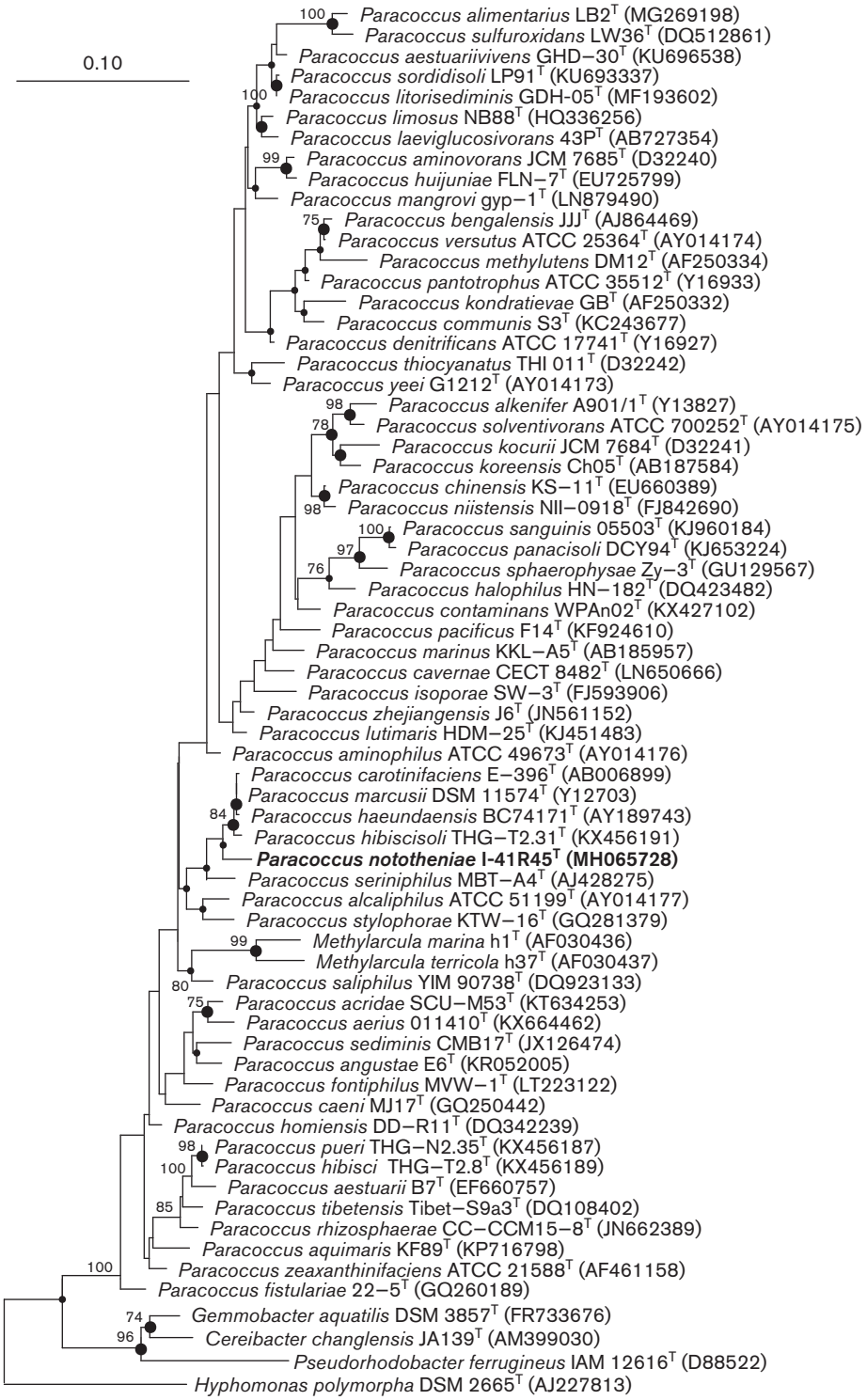


Fig. 1. Phylogenetic placement of strain I-41R45^T within the genus *Paracoccus*. The maximum-likelihood tree was generated in ARB using the LTP database version 128 (February 2017) and based on 16S rRNA gene sequence positions 58–1446 (according to *E. coli* numbering, [17]). Numbers at nodes represent bootstrap values (≥70 %) based on 100 replications. The type strain of the species *Hyphomonas polymorpha* was used as out-group. Sequences of *Gemmobacter* and *Pseudorhodobacter* type strains were removed after tree reconstruction without changing the overall tree topology. Nodes marked with black circles were also present in the maximum-parsimony and neighbour-joining trees. Lager circles represent nodes with ≥70 % bootstrap support in at least one of the other trees. Bar: 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles (%) of strain I-41R45^T and related type strains of *Paracoccus* species

Strains: 1, I-41R45^T; 2, *Paracoccus marcusii* DSM 11574^T; 3, *Paracoccus haeundaensis* LMG P-21903^T (BC74171^T). All data from this study, unless otherwise indicated. Strains were grown on R2A agar for 48 h at 28 °C prior to extraction.

Fatty acid	1	2	3
Saturated:			
$C_{18:0}$	5.3	6.6	6.9
Hydroxy:			
$C_{10:0}$3-OH	3.9	3.1	4.0
$C_{17:1}\omega 8c$	1.5	–	1.5
Summed features:*			
2	–	–	2.1
7	86.1	85.5	82.2
Unknown:†			
ECL 11.799	–	3.1	3.4

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 contains $C_{14:0}$ 3-OH, / $C_{16:1}$ ISO I. Summed feature 7 contains $C_{18:1}\omega 7c$/ω9t/ω12t.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. Equivalent chain length (ECL) is given.

studied in a taxonomic purpose. On the other hand, the average values obtained for both ANIb and ANIm analysis, with the I-41R45^T and available *Paracoccus* type strain genomes did not exceed the 80 %, result ratified by an average value for TETRA near 0.9, which represents the best value obtained with *Paracoccus contaminans* RKI 16-01929^T (accession number GCA_002105555.1; data not shown).

For further genotypic analysis, genomic DNA of strain I-41R45^T and reference strains genomic DNA was extracted by the method of Pitcher *et al.* [33].

DDH experiments were performed between strain I-41R45^T and type strains of the three species with pairwise 16S rRNA gene sequence similarities above 97 % according to the method of Ziemke *et al.* [34] (except that for nick translation 2 µg DNA were labelled during 3 h of incubation at 15 °C). Strain I-41R45^T showed low DDH values to *P. hibiscisoli* KACC 18933^T (45 %, reciprocal 26 %), *P. marcusii* DSM 11574^T (66 %, reciprocal 61 %) and *P. haeundaensis* LMG 21903^T (29 %, reciprocal 36 %), respectively.

The genomic DNA G+C content of strain I-41R45^T obtained from the WGS of 65.26 mol%; was in the range of the G+C content values reported for the genus *Paracoccus*, i.g. 66.3 mol% for the type species *P. denitrificans* [1], 69.1 % for *P. hibiscisoli* [35], 66 % for *P. marcusii* [36], and 66.9 % for *P. haeudaensis* [37].

The analysis of cellular fatty acid profiles of whole cell hydrolysates was done as described previously [38] and revealed a typical *Paracoccus* profile with the following most abundant fatty acids $C_{18:1}\omega 7c/\omega$9t/ω12t and $C_{18:0}$. The complete fatty acid pattern of strain I-41R45^T is shown in Table 2 in comparison with those of the type strains of the most closely related *Paracoccus* species.

On the basis of the results of this polyphasic study, it is obvious that strain I-41R45^T represents a novel species of the genus *Paracoccus* for which the name *Paracoccus nototheniae* sp. nov. is proposed.

DESCRIPTION OF *PARACOCCUS NOTOTHENIAE* SP. NOV.

Paracoccus nototheniae (no.to.the′ni.ae. N.L. gen. n. *nototheniae* of the black rock cod fish *Notothenia coriiceps*, the isolation source).

Orange colonies (diameter about 2 mm) form on NA at 30 °C. Cells show Gram-negative staining. Cells are non-motile, non-spore forming cocci, approx. 1.1–1.3 µm wide and 1.1–1.5 µm long. Aerobic. Oxidase- and catalase-positive. Good growth occurs after 48 h on NA, brain heart infusion agar, TSA and R2A agar. Weak growth on MacConkey agar at 28 °C. Growth occurs on NA at 10–36 °C, but not at 4 and 50 °C. Cells grow at 28 °C in the presence of 0–9.0 % NaCl not at 10 % (w/v) as an additional ingredient of TSB.

The following compounds are utilized as a sole source of carbon: acetate, L-arabinose, D-galactose, D-glucose, maltose, D-mannose, D-fructose, D-ribose, D-sorbitol, L-arbutin, cellobiose, gluconate, trehalose, D-mannitol, sucrose, D-xylose, salicin, *i*-inositol, melibiose, L-rhamnose, adonitol, maltitol, fumarate, DL-lactate, citrate, *cis*-aconitate, 4-aminobutyrate, glutarate, 2-oxoglutarate, pyruvate, L-alanine, L-aspartate, L-serine and L-proline. The following compounds are not utilized as a sole source of carbon: *N*-acetylgalactosamine, *N*-acetylglucosamine, propionate, adipate, azelate, DL-3-hydroxybutyrate, *trans*-aconitate, mesaconate, itaconate, suberate, ß-alanine, L-leucine, L-histidine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

The chromogenic substrates *p*-nitrophenyl-ß-D-xylopyranoside bis-*p*-nitrophenyl-phosphate, bis-*p*-nitrophenyl-phenyl-phosphonate, 2-deoxythymidine-2′-*p*-nitrophenyl-phosphate, *p*-nitrophenyl-α-D-glucopyranoside, p-nitrophenyl-ß-D-glucuronide, bis-*p*-nitrophenyl-phosphoryl-choline, γ-L-glutamate-*p*-nitroanilide, and L-proline-*p*-nitroanilide are not hydrolysed; *p*-nitrophenyl-ß-D-glucopyranoside, *p*-nitrophenyl-ß-D-galactopyranoside and L-alanine-*p*-nitroanilide are hydrolysed. The major cellular fatty acids are $C_{18:1}\omega 7c/\omega$9t/ω12t and $C_{18:0}$.

The type strain, I-41R45^T (=CCM 8875^T=CIP 111632^T), was isolated from the kidney of a black rock cod (*Notothenia coriiceps*) on February 2015, during the 51th Expedition to the Chilean Antarctic Naval Base *Arturo Prat* (62° 30′ S 59° 41′ W).

The genomic G+C content of the type strain is 65.26 mol% (genome sequence). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I-41R45^T is MH065728; the Whole Genome Sequencing (WGS) project has been deposited under the accession RQRT00000000.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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