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Profundibacter amoris gen. nov., sp. nov., a new member of the *Roseobacter* clade isolated from Loki's Castle Vent Field on the Arctic Mid-Ocean Ridge.

Le Moine Bauer Sven [1, *], Sjøberg Andreas Gilje [1], L'Haridon Stephane [2], Stokke Runar [1], Roalkvam Irene [3], Steen Ida Helene [1], Dahle Håkon [1]

[1] Department of Biological Sciences and K.G. Jebsen Center for Deep Sea Research, University of Bergen, N-5020 Bergen, Norway
[2] Université de Brest (UBO), Institut Universitaire Européen de la Mer (IUEM) - UMR 6197, Laboratoire de Microbiologie des Environnements Extrêmes (LM2E), Plouzané, France
[3] Department of Biological Sciences, University of Bergen, N-5020 Bergen, Norway

* Corresponding author : Sven Le Moine Bauer, email address : sven.lemoinebauer@gmail.com

Abstract :

A bacterial strain, designated BAR1T, was isolated from a microbial mat growing on the surface of a barite chimney at the Loki's Castle Vent Field, at a depth of 2216 m. Cells of strain BAR1T were rod-shaped, Gram-reaction-negative and grew on marine broth 2216 at 10–37 °C (optimum 27–35 °C), pH 5.5–8.0 (optimum pH 6.5–7.5) and 0.5–5.0 % NaCl (optimum 2 %). The DNA G+C content was 57.38 mol%. The membrane-associated major ubiquinone was Q-10, the fatty acid profile was dominated by $C_{18:1}\omega7c$ (91 %), and the polar lipids detected were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid, one unidentified lipid and one unidentified phospholipid. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain BAR1T clustered together with *Rhodobacterales bacterium* PRT1, as well as the genera *Halocynthiibacter* and *Pseudohalocynthiibacter* in a polyphyletic clade within the *Roseobacter* clade. Several characteristics differentiate strain BAR1T from the aforementioned genera, including its motility, its piezophilic behaviour and its ability to grow at 35 °C and under anaerobic conditions. Accordingly, strain BAR1T is considered to represent a novel genus and species within the *Roseobacter* clade, for which the name *Profundibacter amoris* gen. nov., sp. nov. is proposed. The type strain is *Profundibacter amoris* BAR1T (=JCM 31874T=DSM 104147T).

Keywords : isolate, piezophilic, deep-sea, alphaproteobacteria, hydrothermal vent, roseobacter

The *Roseobacter* clade is part of the *Rhodobacteraceae* family within the *Alphaproteobacteria* class (1). The family mainly contains aquatic species, and the clade is a ubiquitous marine group, as isolates and non-cultured cells were obtained from coastal waters, deep waters, marine sediments, and various algae and animals (see references in (2)). The cultured members of the clade are heterotrophic, aerobic, mainly mesophilic, and present a wide range of physiological characteristics (3). Within this clade, the genus *Halocynthiibacter* was proposed in 2014 along with the characterization of *H. namhaensis*, isolated from a sea squirt in South-Korea (4). A second species, *H. arcticus*, was isolated from sediments of the coast of Svalbard in 2015 (5). Members of the *Halocynthiibacter* genus are characterized by rod-shaped cells, aerobic, non-motile, and catalase and oxidase positive (4). The *Pseudohalocynthiibacter* genus was proposed in 2015 along with the characterization of *P. aestuariivivens*, isolated from tidal flat sediments in South-Korea (6). Members of *Halocynthiibacter* and *Pseudohalocynthiibacter* are phylogenetically closely related (>97 % 16S rRNA similarity) and share several characteristics. However, members of *Pseudohalocynthiibacter* can be differentiated from members of *Halocynthiibacter* by their ability to reduce nitrate and the presence of the polar lipid phosphatidylethanolamine. Here, we report the polyphasic characterization of strain BAR1^T, isolated from a deep-sea hydrothermal vent and phylogenetically closely related to the two aforementioned genera. For comparative purposes, *H. namhaensis* RA2-3^T (=KCTC 32362), *H. arcticus* PAMC 20958^T (=KCTC 42129), and *P. aestuariivivens* BS-W9^T (=KCTC 42348) were included in the study.

Strain BAR1^T was isolated from a low-temperature venting site, referred to as the barite field, at the Loki's Castle Vent Field (73°33' N 08°09' E), a well-studied basalt-hosted and sedimentary-influenced hydrothermal system situated at around 2400 m depth on the Arctic Mid-Ocean Ridge system (7). The sample was taken from a microbial mat growing on top of a barite chimney (8) using a hydraulic pump device mounted on a remotely operated vehicle. Primary enrichments were immediately set up shipboard in artificial seawater (as described in (9)), supplemented with 10 mM thiosulfate and 0.02 % yeast extract (YE). Incubation occurred under aerobic conditions, at 23°C and with gentle shaking. The enrichment was then plated on Marine Agar 2216 (Difco) and a pure culture of strain BAR1^T was obtained after several colony transfers. The isolate was thereafter grown on Marine Broth or Agar 2216 (Difco).

A 1448 bp-long sequence of the 16S rRNA gene was obtained using Sanger sequencing technology, and compared against sequences from the Genbank (10) and EzBioCloud (11) databases. Strain BAR1^T showed highest 16S rRNA similarity to *Rhodobacterales bacterium* PRT1 (97.0 %), an obligate piezophilic strain isolated at 8350 m depth in the Puerto Rico trench (12). However, strain PRT1 has not been fully characterized and its taxonomic position on the genus and species levels has not been validated. Strain PRT1 was not included in our study due to the difficulty to generate complete characterization data for obligate piezophilic organisms. The closest relative type strains to strain BAR1^T were *Planktotalea lamellibrachiae* JAM 119^T, *P. aestuariivivens* BS-W9^T (95.8 %), *H. arcticus* PAMC 20958^T (95.8 %) and *H. namhaensis* RA2-3^T (95.7 %). The phylogenetic relationship between these strains and a selection of other *Roseobacter* species was reconstructed using the maximum-likelihood (13) and neighbor-joining (14) algorithms as implemented in the MEGA X software package (15). The robustness of each tree was assessed using 500 bootstrap replications. The two algorithms showed that strain BAR1^T formed a distinct branch with strain PRT1 within a cluster also containing the *Halocynthiibacter* and *Pseudohalocynthiibacter* species

(Fig. 1). *P. lamellibrachiae* JAM 119^T was not included in the comparative study as it did not branch together with strain BAR1^T.

DNA for the sequencing of the BAR1^T genome was extracted using a modified version of the Marmur protocol (16,17). The genome was then sequenced on a Pacific Biosciences Sequel instrument using Sequel Polymerase v2.1, SMRT cells v2 and Sequencing chemistry v2.1. Assembly was performed using CLC Genomics Workbench v11 and resulted in 1 contiguous sequence. By using the contig extension mode in CLC, an overlap was detected resulting in a closed circular genome. Genome polishing and error correction was performed using the Resequencing pipeline on SMRT link (v5.1.0.26412, SMRT Link Analysis Services and GUI v5.1.0.26411) with a consensus concordance of 99.99 %, resulting in a complete genome with a total length of 3 558 757 bp and a mean coverage of 246. The location of the *dnaA* gene was used as start of the circular chromosome. Two 16S rRNA operons, containing identical 16S rRNA genes were detected using Barrnap 0.8 (Torsten Seemann: https//github.com/tseeman/barrnap). The G+C content of the genome is 57.38 mol% which is slightly higher than the range of the known members of the genera *Halocynthiibacter* and *Pseudohalocynthiibacter* (52.9 % to 53.2 % (4–6)). As shown in supplementary table 1, the length of the genomes of strain BAR1^T (3 558 757 bp) and *H. namhaensis* RA2-3^T (3 535 512 bp) are similar while much shorter than the genome of *H. arcticus* PAMC 20958^T (4 329 554 bp). At the time of writing, no genome is available for *Pseudohalocynthiibacter aestuariivivens* BS-W9^T. A detailed comparison of the available genomes from the species present in figure 1 is presented in supplementary table 1, including genome size, gene count, genomic G+C content and ANI and DDH values between strain BAR1^T and each species. The whole genome sequencing project has been registered under the Bioproject PRJNA488700 and the genome deposited in Genbank with accession number CP032125. The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (18). Raw data are available from the SRA database with the accession number SRP159493.

Strain BAR1^T was shown to be Gram-reaction negative, as revealed by the KOH test described by Ryu (19). The cells are 1-2 µm long rods. On MA plates, according to the criteria described in Tindall *et al.* (20), colonies formed after 1 week were less than 1 mm in diameter, beige, circular, convex, smooth, had an entire margin and were viscous if touched with a needle. In young Marine Broth 2216 cultures, some motile cells could be observed. The protocol described by Heimbrook *et al.* (21), which uses the Ryu stain (22) to observe the presence of flagella under light microscopy, revealed the presence of monotrichous flagella in some cells in young cultures (Supplementary figure 1). In all the following experiments, growth was assessed using spectrophotometry at a wavelength of 600 nm. The temperature growth range was tested in Marine Broth 2216 at 4, 10, 15, 20, 25, 27, 30, 32, 35, 37 and 40 °C, but growth was observed only at 10-37 °C (optimum 27-35 °C). The pH growth range was tested in Marine Broth 2216 with 10 mM MES buffer (pH 5.0, 5.5 and 6.0), 10 mM PIPES buffer (pH 6.0, 6.5 and 7.0), 10 mM HEPES buffer (pH 7.0, 7.5 and 8.0), and 10 mM Tris-HCl buffer (pH 7.5, 8.0 and 9.0), but growth was observed only at pH 5.5-8.0 (optimum pH 6.5-7.5). Tolerance to various ionic strengths was tested in Marine Broth 2216 with NaCl concentrations of 0, 0.5, 1, 2, 3, 4, 5, 7 and 10 %, but growth was only observed at 0.5-5 % NaCl (optimum 2 % NaCl). All the following growth, enzymatic and antibiotic resistance tests were performed on strain BAR1^T as well as on type strains of *H. arcticus* PAMC 20958^T, *H. namhaensis* RA2-3^T and *P. aestuariivivens* BS-W9^T for comparison purposes. Tolerance to high hydrostatic

pressure was tested in sterile plastic syringes of 5 ml filled with 3 ml Marine Broth 2216 and 1 ml of tetradecafluorohexane as oxygen provider. The syringes were incubated at 10, 20, 25, 30, 35, 40 and 50 MPa in stainless steel pressure vessel-incubators custom-built by Top Industrie (Industrial zone 'Le Plateau de Biere', Dammarie-les-Lys, France). Tested at 30°C, strain BAR1^T could grow at all pressures with an optimum between 30-35 Mpa, with a decrease in doubling time from 15 h at 0.1 MPa to 9.8 h at 32 MPa (Supplementary figure 2). Strain BAR1^T should therefore be considered as piezophilic. Tested at 20°C, *P. aestuariivivens* BS-W9^T , *H. namhaensis* RA2-3^T and *H. arcticus* PAMC 20958^T showed a decreasing growth rate with increasing pressure, and no or nearly no growth at 40 and 50 MPa (Supplementary figure 2). Strain BAR1^T could grow in Marine Broth 2216 without magnesium salts ($MgCl_2$ and $MgSO_4$). Growth on MA plates was tested under anaerobic conditions (80:20 N_2:CO_2 in the gas phase), microaerobic conditions (8-9 % oxygen) using a Campygen atmosphere generator system (Oxoid), and aerobic conditions. Nitrate reduction was tested using Marine Broth 2216 supplemented with 0.1% $NaNO_3$ and 0.17% agar to create suboxic conditions. The ability of strain BAR1^T to reduced nitrate to nitrite was demonstrated by colorimetry with the Tetra test NO_2 (Tetra) and measured by spectrophotometry at a wavelength of 539 nm. Catalase activity was tested by mixing a colony with 3 % hydrogen peroxide as described by Tindall *et al.* (20), and oxidase activity was tested using Diatabs (Rosco Diagnostica). Methods described in Tindall *et al. w*ere used to assess the hydrolysis of urea, esculin, casein, agar, lecithin and starch (20). Indol production from tryptophan and hydrolysis of Tween 20 and gelatin were assessed according to the methods described by Hansen and Sørheim, with the exception of using saturated $(NH_4)_2SO_4$ instead of $HgCl_2$ to reveal gelatinase activity (23). The hydrolysis of hypoxanthine was assessed by adding 4 g L^{-1} hypoxanthine to MA plates. Furthermore, an enzymatic fingerprint was produced using an API ZYM strip (Biomérieux). The susceptibility to various antibiotics was tested on MA plates using the disc diffusion method (24). Growth in the presence of tetracyclin (30 µg), ampicillin (10 µg), neomycin (30 µg), strepomycin (50 µg), penicillin G (10 µg), rifampycin (30 µg), kanamycin (30 µg), or chloramphenicol (50 µg) was evaluated after 7 days of incubation. The ability to use various carbon sources was tested in a modified Marine Broth (0.1 g $C_6H_5FeO_7$, 19.45 g NaCl, 5.9 g $MgCl_2$•$6H_2O$, 3.24 g $MgSO_4$•$7H_2O$, 1.8 g $CaCl_2$•$2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.08 g KBr, 34 mg $SrCl_2$•$6H_2O$, 22 mg H_3BO_3, 4 mg Na_2SiO_3, 2.4 mg NaF, 1.6 mg NH_4NO_3, 8 mg Na_2HPO_4•$2H_2O$) supplemented with 0.02 % yeast extract and 0.2 % of either D-(+)-sucrose, D-(+)-galactose, D-(+)-glucose, D-(+)-xylose, D-(+)-maltose, D-(+)-cellobiose, D-(+)-mannose, D-(-)-ribose, D-(-)-fructose, L-(-)-alanine, L-(-)-serine, L-(-)-lysine, L-(-)-arabinose, α-cellulose, lactose, mannitol, tryptone, peptone, or 20 mM of either acetate, citrate, formate or pyruvate. Strain BAR1^T could only grow with peptone or tryptone, even when the medium was supplemented with 0.02 % tryptone, 0.02 % peptone and 0.5 % Wolfe's vitamin solution. The results remained unchanged. When tested on other basal media (the marine basal minimal medium described by Geng *et al.* (25), the basal medium described by Baumann and Baumann (26) and the M1 medium described by Le Moine Bauer *et al. (27)*) supplemented with 0.02 % yeast extract and 0.5 % Wolfe's vitamin solution, strain BAR1^T did not even grow on 0.2 % peptone or tryptone. The results of the comparative analysis between strain BAR1^T and members of the *Halocynthiibacter* and *Pseudohalocynthiibacter* genera are presented in Table 1. A complete phenotypic description of strain BAR1^T can be found in the description of the novel species.

Biomass for the analysis of fatty acids, respiratory quinones and polar lipids was produced from colonies grown on MA plates at 23 °C. Cells were harvested and frozen after 3 days of incubation. All analyses were performed by the Identification Service of the Leibniz-Institut DSMZ. Respiratory lipoquinones were extracted using the two stage method described by Tindall (28), separated by thin layer chromatography on silica gel and further analyzed by high-performance liquid chromatography. Polar lipids were extracted using a chloroform/methanol mixture (29) and separated by two-dimensional silica gel thin layer chromatography. Lipids were detected using functional group specific reagents (20). Saponification, methylation, and extraction of the fatty acid methyl esters were done following a protocol modified from Miller (30) and Kuykendall *et al.* (31) and separated using a Sherlock Microbial Identification System (MIDI, Microbial ID). The sole respiratory quinone detected in strain BAR1^T was Q10, a characteristic shared with the close relatives. The polar lipids detected were phosphatidylcholine, phosphatidylglycerol, one unindentified aminolipid, one unidentified lipid (all shared with members of the *Halocynthiibacter* and *Pseudohalocynthiibacter* genera), phosphatidylethanolamine (shared with *P. aestuariivivens*) and one unidentified phospholipid not found in the close relatives (Supplementary figure 3). The fatty acid profile of strain BAR1^T was dominated by the monounsaturated $C_{18:1}\omega7c$ (91 %), a feature also found in the fatty acid profiles of *Halocynthiibacter* and *Pseudohalocynthiibacter* members (67.7-84.5 %, table 2).

The separation of the *Pseudohalocynthiibacter* genus from the *Halocynthiibacter* genus was proposed based on differences in phylogeny, polar lipid and fatty acid profiles and some phenotypic characteristics (6). Similarly, our results showed that strain BAR1^T possesses several features segregating it from these two genera. Strain BAR1^T was phylogenetically most closely related to the uncharacterized *Rhodobacterales bacterium* PRT1, and the two algorithms used to build the phylogenetic trees separated these two strains from the *Halocyntiibacter* and *Pseudohalocynthiibacter* genera (Fig. 1). The 16S rRNA similarity between strain BAR1^T and any member of the the *Halocyntiibacter* and *Pseudohalocynthiibacter* genera is lower than 96%. As well, the DDH and ANI values between strain BAR1^T and *H. arcticus* PAMC 20958^T and *H. namhaensis* RA2-3^T are very low (respectively 19.9 and 70.0 for the former, and 18.3 and 70.1 for the later, supplementary table 1). The ability to reduce nitrate, the API ZYM profile, the antibiotic resistance profile and the membrane polar lipid composition suggested a closer relationship of BAR1^T to *P. aestuariivivens* BS-W9^T than to *H. arcticus* PAMC 20958^T and *H. namhaensis* RA2-3^T (Table 1 and 2). However, strain BAR1^T also possessed an unidentified phospholipid in the membrane and its percentage of the $C_{18:1}\omega7c$ fatty acid was the highest among the aforementioned strains (Table 2). Also, it had a higher DNA G+C content than the other strains. Phenotypically, it was the only strain tested able to grow at 35 °C and under anaerobic conditions with nitrate as an electron acceptor. However, its carbon substrate range was very limited compared to *P. aestuariivivens* BS-W9^T and *H. arcticus* PAMC 20958^T (Supplementary table 2). It was also the only strain to show a piezophilic behavior, motility, the ability to hydrolyze urea and inability to hydrolyze Tween 20 (Table 1). Accordingly, we suggest that strain BAR1^T should be classified as a novel species of a novel genus within the *Roseobacter* clade for which the name *Profundibacter amoris* gen. nov., sp. nov., is proposed. A comparison between the characteristics of strain BAR1T and the closely related genera is shown in supplementary table 3.

Description of *Profundibacter* gen. nov.

Profundibacter (Pro.fun.di.bac'ter. L. neut. n. *profundum* the depths of the sea; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Profundibacter*, rod-shaped bacterium living in the deep-sea).

Cells are Gram-stain-negative and rod-shaped. They grow under aerobic and/or anaerobic conditions and exhibit motility ability at some stages of the growth. Catalase and oxidase tests are positive. Nitrate is used as an electron acceptor under anaerobic conditions. The predominant ubiquinone is Q-10. The main fatty acid is $C_{18:1}\omega 7c$. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, one unidentified lipid, one unidentified aminolipid and one unidentified phospholipid. The type species is *Profundibacter amoris* sp.nov.. According to 16S rRNA gene sequence analysis, it is a member of the family *Rhodobacteraceae* and the class *Alphaproteobacteria.*

Description of *Profundibacter amoris* sp.nov.

Profundibacter amoris (a.mo'ris. N.L. gen. masc. n. *amoris*, of AMOR, arbitrary name (homonymous with L. *amor*, love) derived from AMOR (Arctic Mid-Ocean Ridge System), where the isolate was found).

In addition to the characters described for the genus, the species is characterized by the following properties. Cells are 1-2 µm long and grow in Marine Broth 2216 and on Marine Agar. Young cultures exhibited some motile cells. Growth occurs at 10-37 °C (optimum 27-35 °C), pH 5.5-8 (optimum pH 6.5-7.5) and NaCl concentration of 0.5-5 % (optimum 2 %). The cells show a piezophilic behavior, with shortest doubling times measured at 30-35 MPa (ca. 2/3 of doubling time at 0.1 MPa). Growth can occur without the presence of magnesium salts and under anaerobic, microaerobic and aerobic conditions. The strain can reduce nitrate to nitrite and hydrolyze urea but cannot hydrolyze Tween 20, gelatin, hypoxanthine, esculin, casein, agar, lecithin and starch, nor produce indol from tryptophan. In the API ZYM strip, esterase (C4) and leucine arylamidase are positive while alkaline phosphatase, esterase lipase (C8) and acid phosphatase are weakly positive. In a modified Marine Broth 2216 with different carbon sources, cells could grow on tryptone and peptone, but not on D-(+)-sucrose, D-(+)-galactose, D-(+)-glucose, D-(+)-xylose, D-(+)-maltose, D-(+)-cellobiose, (D-(+)-mannose, D-(-)-ribose, D-(-)-fructose, L-(-)-alanine, L-(-)-serine, L-(-)-lysine, L-(-)-arabinose, α-cellulose, lactose, mannitol, acetate, citrate, formate or pyruvate. They are resistant to tetracyclin, but susceptible to neomycin, kanamycin, ampicillin, streptomycin, penicillin G, rifampicin and chloramphenicol. The DNA G+C content is 57.38 mol%

The type strain BAR1^T (=JCM 31874^T =DSM 104147^T) was isolated from a microbial mat growing on a barite chimney at ca. 2400 m depth, in a low-temperature venting area at the deep-sea hydrothermal system Loki’s Castle (73°33’ N 08°09’ E), on the Arctic Mid-Oceanic Ridge System. The GenBank accession number of the 16S rRNA gene sequence is MH883801. The GenBank accession number for the complete genome is CP032125.

Author statements

For this article, SLMB has been involved in the investigation, the validation, the writing (original draft preparation) the visualization and the supervision, AGS has been involved in the investigation, SLH has been involved in the investigation and the writing (review and editing), RS has been involved in the investigation, formal analysis and the writing (review and editing), IR has been involved in the supervision and the writing (review and editing), HD has been involved in the resources, the supervision and the writing (review and editing), and IHS has been involved in the supervision and the writing (review and editing). The authors declare no conflict of interest.

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Figure 1. Maximum-likelihood tree showing the phylogenetic relationship between strain BAR1^{T}, *Rhodobacterales bacterium* PRT1, and a selection of close relatives belonging to the *Roseobacter* clade. Numbers at nodes are bootstrap values based on 500 resamplings; only values ≥70 are shown. Black points represent branches supported by neighbor-joining algorithms. Bar, 0.020 nucleotide substitutions per site.

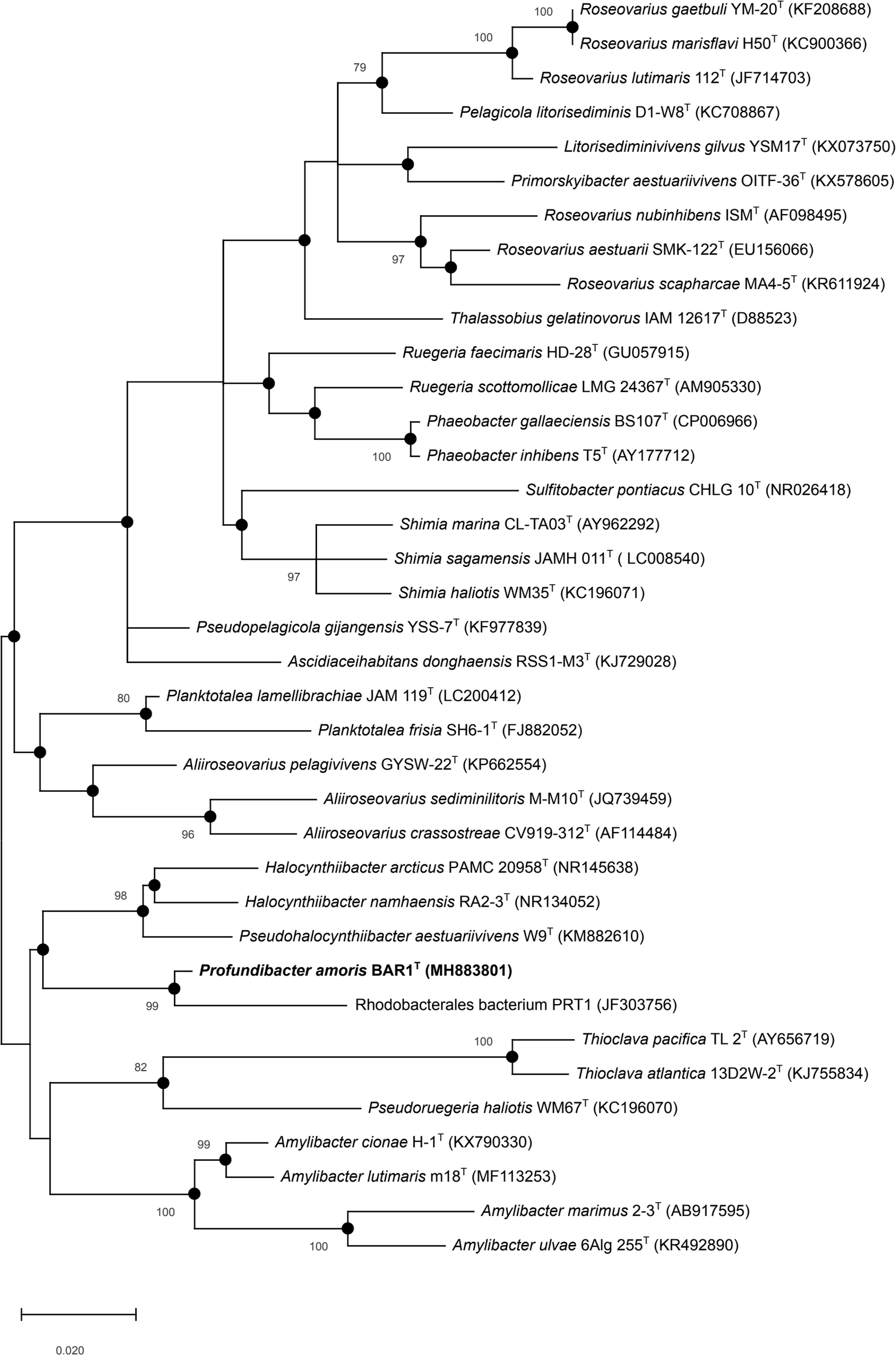
100
Roseovarius gaetbuli YM-20T (KF208688)
100
Roseovarius marisflavi H50T (KC900366)
79
Roseovarius lutimaris 112T (JF714703)
Pelagicola litorisediminis D1-W8T (KC708867)
Litorisediminivivens gilvus YSM17T (KX073750)
Primorskyibacter aestuariivivens OITF-36T (KX578605)
Roseovarius nubinhibens ISMT (AF098495)
97
Roseovarius aestuarii SMK-122T (EU156066)
Roseovarius scapharcae MA4-5T (KR611924)
Thalassobius gelatinovorus IAM 12617T (D88523)
Ruegeria faecimaris HD-28T (GU057915)
Ruegeria scottomollicae LMG 24367T (AM905330)
Phaeobacter gallaeciensis BS107T (CP006966)
100
Phaeobacter inhibens T5T (AY177712)
Sulfitobacter pontiacus CHLG 10T (NR026418)
Shimia marina CL-TA03T (AY962292)
Shimia sagamensis JAMH 011T (LC008540)
97
Shimia haliotis WM35T (KC196071)
Pseudopelagicola gijangensis YSS-7T (KF977839)
Ascidiaceihabitans donghaensis RSS1-M3T (KJ729028)
80
Planktotalea lamellibrachiae JAM 119T (LC200412)
Planktotalea frisia SH6-1T (FJ882052)
Aliiroseovarius pelagivivens GYSW-22T (KP662554)
Aliiroseovarius sediminilitoris M-M10T (JQ739459)
96
Aliiroseovarius crassostreae CV919-312T (AF114484)
Halocynthiibacter arcticus PAMC 20958T (NR145638)
98
Halocynthiibacter namhaensis RA2-3T (NR134052)
Pseudohalocynthiibacter aestuariivivens W9T (KM882610)
Profundibacter amoris BAR1T (MH883801)
99
Rhodobacterales bacterium PRT1 (JF303756)
100
Thioclava pacifica TL 2T (AY656719)
82
Thioclava atlantica 13D2W-2T (KJ755834)
Pseudoruegeria haliotis WM67T (KC196070)
99
Amylibacter cionae H-1T (KX790330)
Amylibacter lutimaris m18T (MF113253)
100
Amylibacter marimus 2-3T (AB917595)
100
Amylibacter ulvae 6Alg 255T (KR492890)
0.020

Table 1. Comparison of a selection of phenotypic characteristics of strain BAR1^T and the relative *Halocynthiibacter* and *Pseudohalocynthiibacter* members.

Strains: 1, BAR1^T; 2, *P. aestuariivivens* BS-W9^T; 3, *H. arcticus* PAMC 20958^T, 4, *H. namhaensis* RA2-3^T. All data are from this study unless otherwise stated. In the API ZYM, all strains were negative for lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, N-acetyl- β-glucosaminidase, α-mannosidase and α-fucosidase. All strains were oxidase and catalase positive and were sensible to ampicillin, streptomycin, penicilin G, rifampicin and chloramphenicol. No strain could hydrolyse starch, agar, casein, lecithin and gelatine, nor produce indol from tryptophan. Note that several of the features observed in our study are contradictory to the results reported in the respective original publication. +, positive; -, negative; NO, not observed; w, weak.

Characteristic	**1**	**2**	**3**	**4**
Temperature growth range (optimum) in °C	10-37 (27-35)	10-30 (25-30)	10-27 (21)	4-30 (25)
pH growth range (optimum)	5.5-8 (6.5-7.5)	5.5-8 (7-8)	5.5-9.5 (7-7.5)	6-7.5 (7-7.5)
NaCl growth range (optimum) in %	0.5-5 (2)	1-7 (2)	0.5-7.5 (2)	0.5-4 (2)
Growth under anaerobic conditions	+	-	-	-
Motility	+	NO	NO	NO
Pressure optimum in MPa	30-35	0.1	0.1	0.1
Growth without Mg^{2+}	+	+	+	-
Tween 20 hydrolysis	-	+	+*	+
NO_3^- reduction	+	+	-	-
Urea hydrolysis	+	-	-	-
Esculin hydrolysis	-	-	+	-
API ZYM tests				
Alkaline phosphatase	w	w	+	w
Esterase (C4)	+	+	w	+*
Esterase lipase (C8)	w	-*	-*	w
Leucine arylamidase	+	+	+	+
Acid phosphatase	w	+	+	+*
Naphthol-AS-BI-phosphohydrolase	-	-	-*	-*
α-galactosidase	-	-	w*	-
β-galactosidase	-	-	+*	-
α-glucosidase	-	-	+	-
Antibiotic resistance				
Tetracyclin	+	+	-	+
Neomycin	-	-	-	+*
Kanamycin	-	-	-	+*
DNA G+C content in mol%	57.3	53.2†	53.2†	52.9†

†, data taken from original publication.
*, result contradictory to the one reported in the original publication.

Table 2. Comparison of the fatty acid profiles of strain BAR1^T and the relative *Halocynthiibacter* and *Pseudohalocynthiibacter* members.

Strains: 1, BAR1^T; 2, *P. aestuariivivens* BS-W9^T; 3, *H. arcticus* PAMC 20958^T, 4, *H. namhaensis* RA2-3^T. The data for the close relatives was obtained from the respective original publications. In all analyzes the cells were harvested after 3 days on MA. Tr, traces (<0,5%); ECL, equivalent chain length.

Characteristic	**1**	**2**	**3**	**4**
Straight-chain saturated				
$C_{16:0}$	Tr	0.7	-	1.0
$C_{17:0}$	-	-	1.1	-
$C_{18:0}$	1.2	3.5	4.3	7.4
$C_{10:0}$ 3-OH	1.9	3.4	1.9	5.2
$C_{18:0}$ 3-OH	1.3	7.5	-	8.2
$C_{19:0}$ 10-methyl	0.7	Tr	-	0.6
Monounsaturated				
$C_{15:1}\omega 8c$	0.6	-	-	-
$C_{17:1}\omega 7c$	1.1	-	-	-
$C_{18:1}\omega 7c$	91.9	67.7	84.5†	74.5
$C_{18:1}\omega 7c$ 11-methyl	0.9	3.9	4.0	-
$C_{18:1}\omega 9c$	-	0.7	-	1.8
$C_{19:0}\omega 8c$ cyclo	-	7.9	-	-
Unknown fatty acid ECL 11,799	-	-	4.2	-
Summed feature				
2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH)	-	2.7	-	-
3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$)	Tr	Tr	-	0.6
7 (unknown fatty acid ECL 18,846)	-	Tr	-	-

† In the original publication corresponds to summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$)

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Article title: *Profundibacter amoris* gen. nov., sp. nov., a new member of the *Roseobacter* clade isolated at Loki's Castle Vent Field on the Arctic Mid-Oceanic Ridge.

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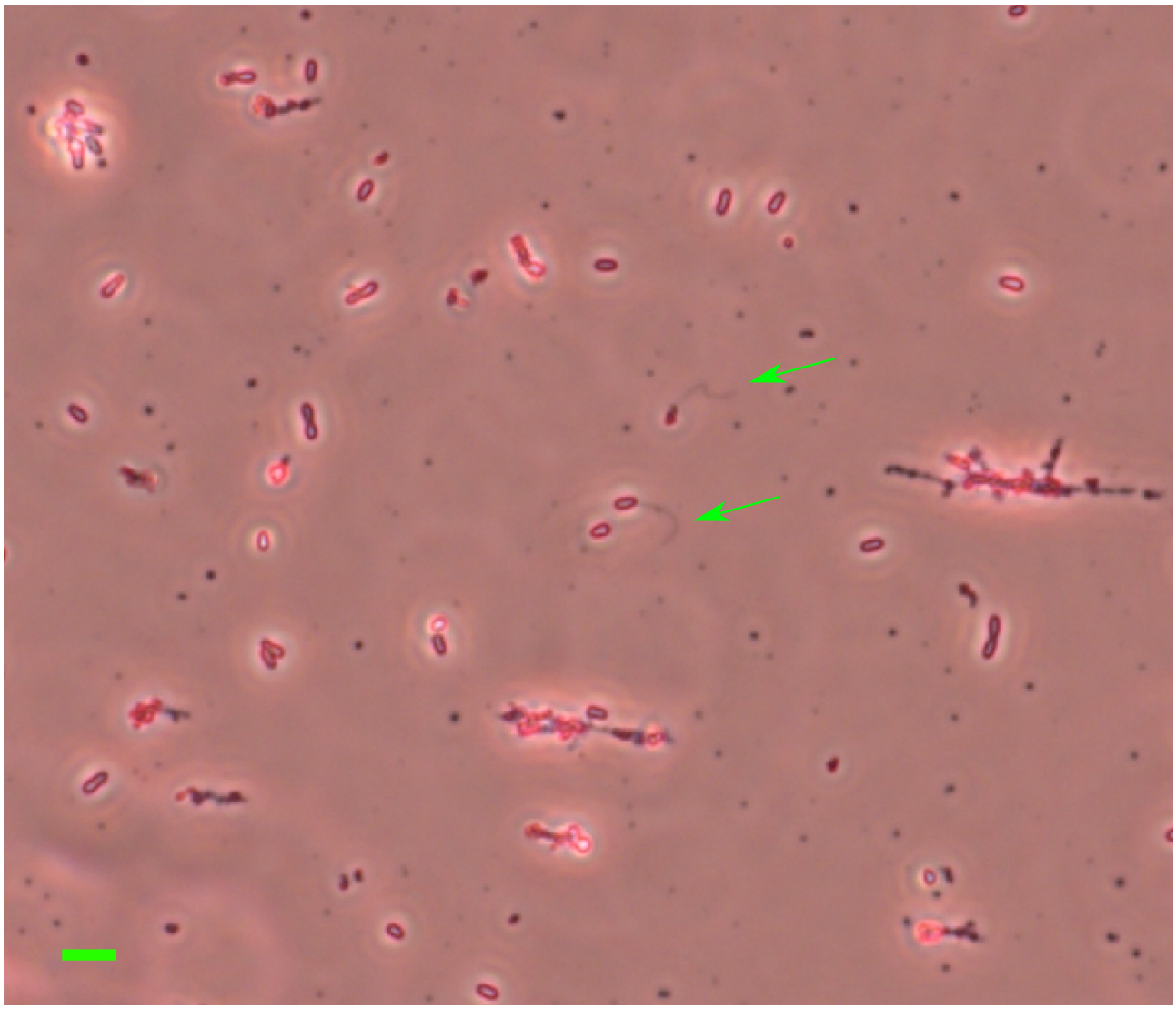
Journal: International Journal of Systematic and Evolutionary Microbiology.

Corresponding author: Sven Le Moine Bauer, University of Bergen, Department of Biological Sciences and K.G. Jebsen Center for Deep Sea Research, N-5020 Bergen, Norway.
sven.lemoinebauer@gmail.com

Supplementary figure 1 : Light microscopy picture of strain BAR1^T cells exhibiting monotrichous flagella after staining using the protocol described in Heimbrook *et al.* (1989).

Magnigfication : x100 with immersion oil ; green bar, 2 µm ; green arrows, flagella



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Supplementary figure 2 : Growth of the tested strains under different hydrostatic pressures.

Growth of *Halocynthiibacter namhaensis* (Figure 1), *Halocynthiibacter arcticus* (Figure 2), *Pseudohalocynthiibacter aestuariivivens* (Figure 3) and strain BAR1^T (Figure 4) under various hydrostatic pressures. Growth curve of strain BAR1^T at 32 MPa (Figure 5). The figures were made in R using the « ggplot2 » package.

Figure 1 : Growth of *Halocynthiibacter namhaensis* at 0.1-50 MPa. The cultures were inoculated at 2,75e+07 cells per mL (black points). The growth was measured after 5 days of incubation (colorized points). Black dash, average growth of the replicates.

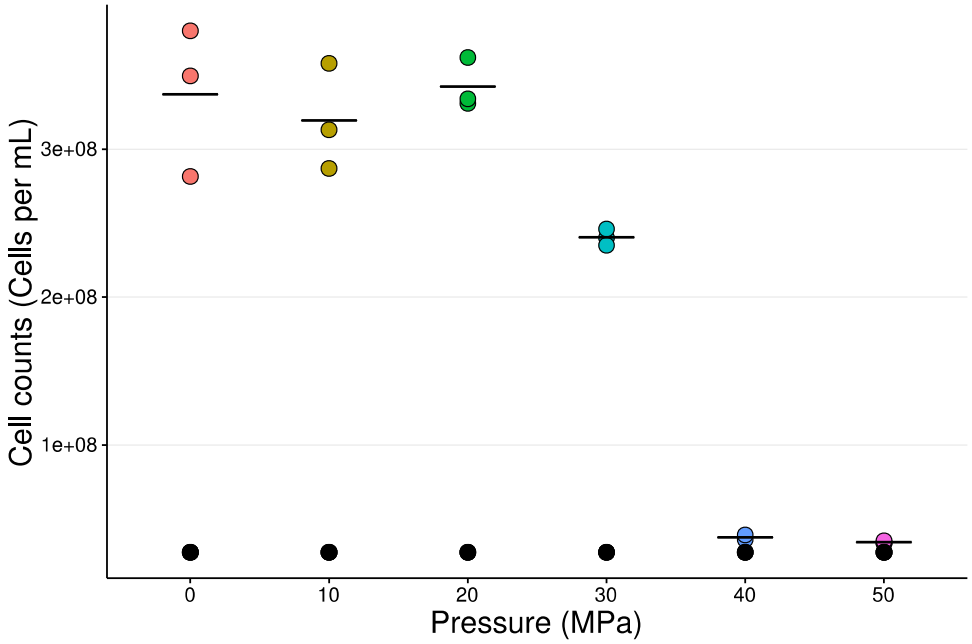


Figure 2: Growth of *Halocynthiibacter arcticus* at 0.1-50 MPa. The cultures were inoculated at 8,07e+06 cells per mL (black points). The growth was measured after 5 days of incubation (colorized points). Black dash, average growth of the replicates.

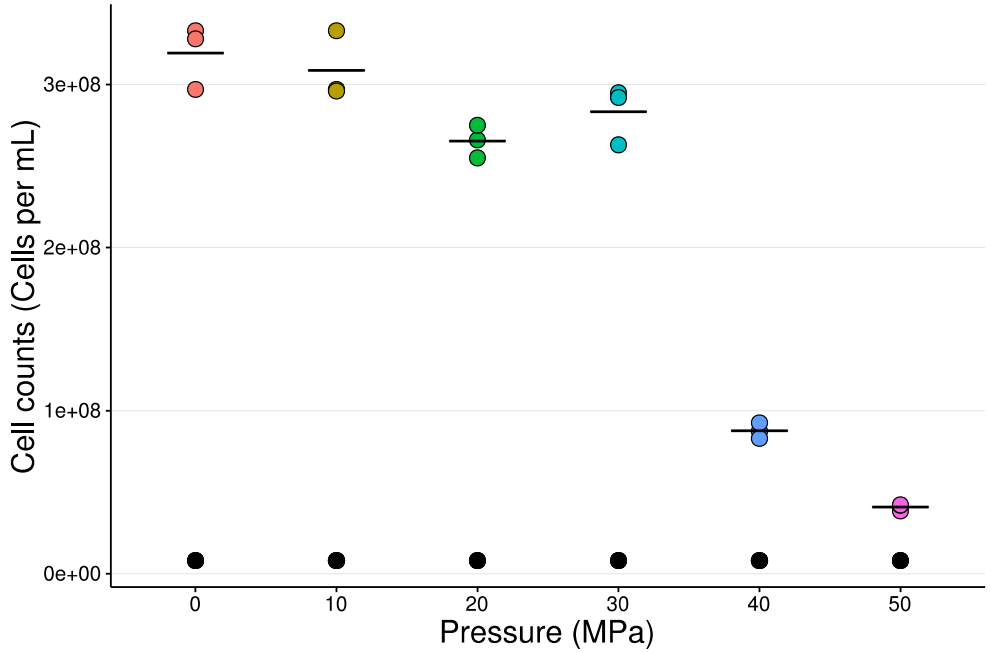


Figure 3: Growth of *Pseudohalocynthiibacter aestuariivivens* at 0.1-50 MPa. The cultures were inoculated at 7,17e+06 cells per mL (black points). The growth was measured after 5 days of incubation (colorized points). Black dash, average growth of the replicates.

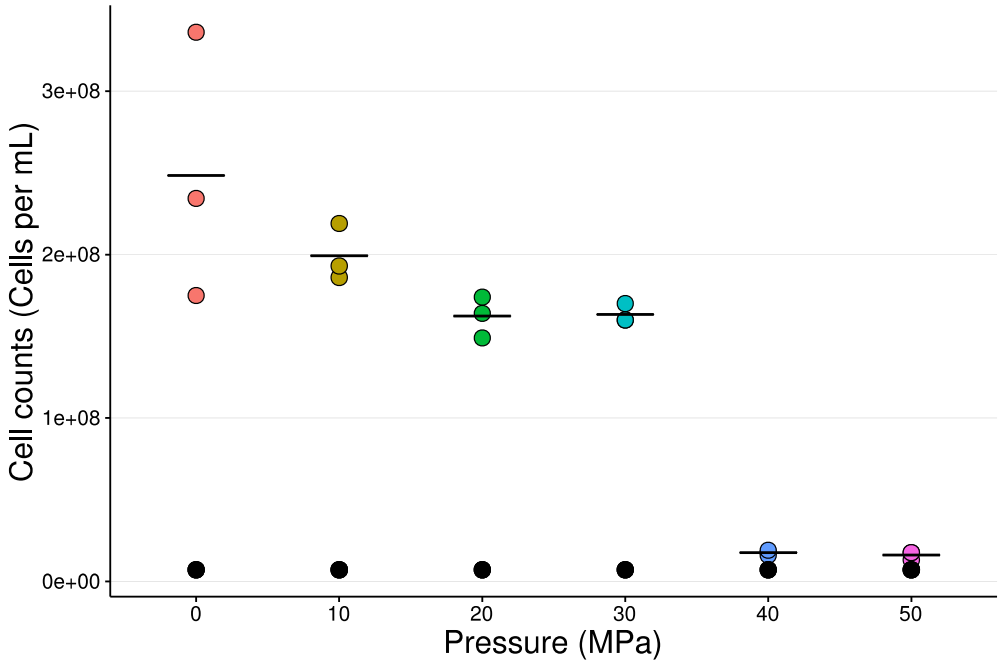
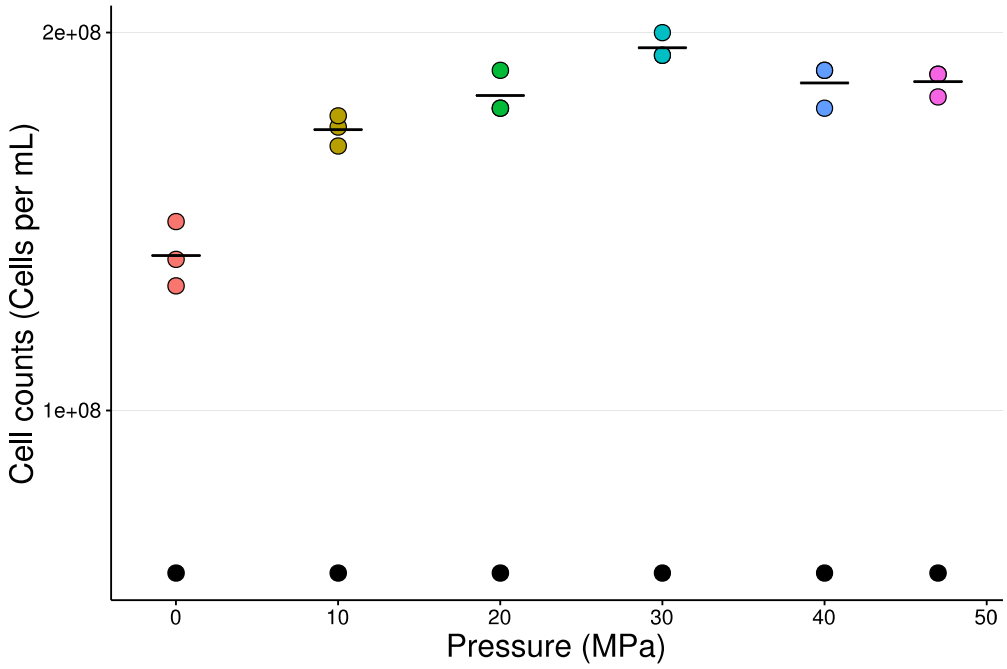


Figure 4: Growth of strain BAR1^{T} at 0.1-50 MPa. Two experiments were run to determine the pressure optimum. In the first experiment (first plot), the cultures were inoculated at 5,7e+07 cells per mL (black points) and growth was measured after 2 days (colorized points). In the second experiment (second plot), the cultures were inoculated at 3,8e+06 cells per mL (black points) and growth was measured after 4 days (colorized points). Black dash, average growth of the replicates.



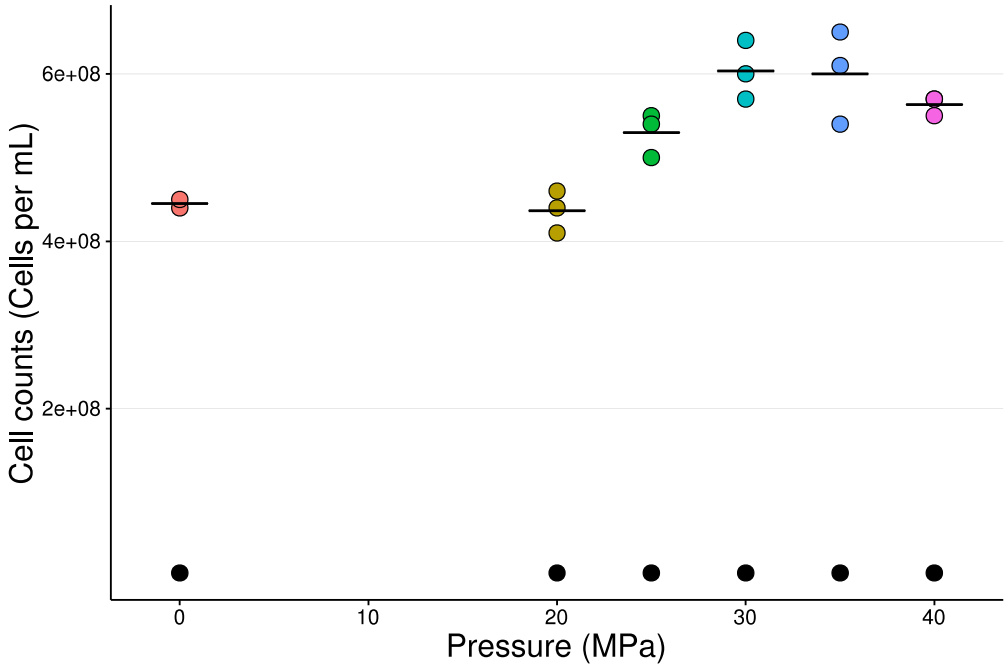
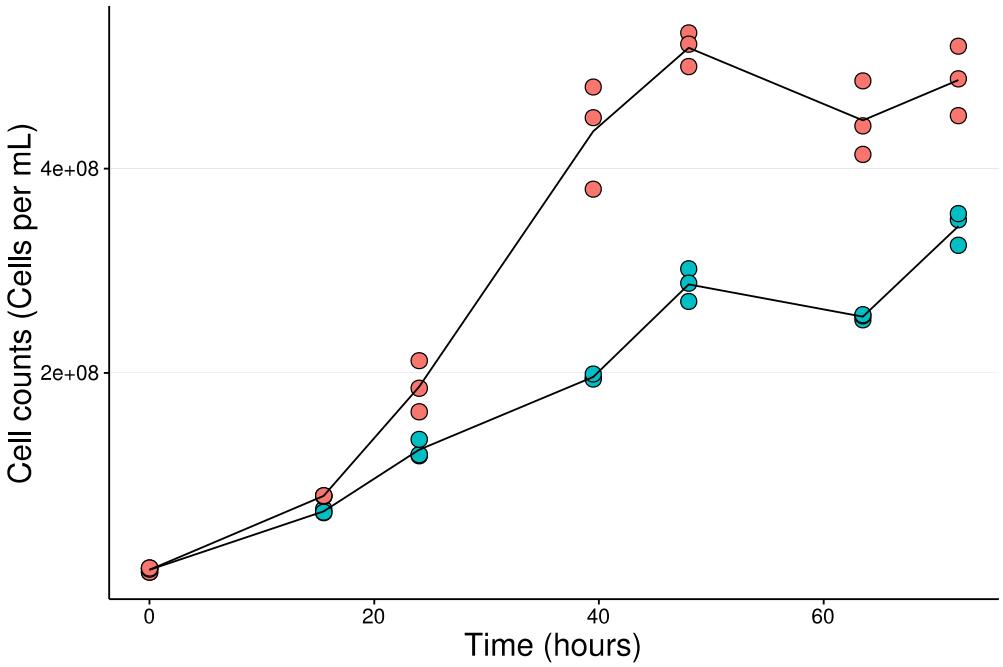
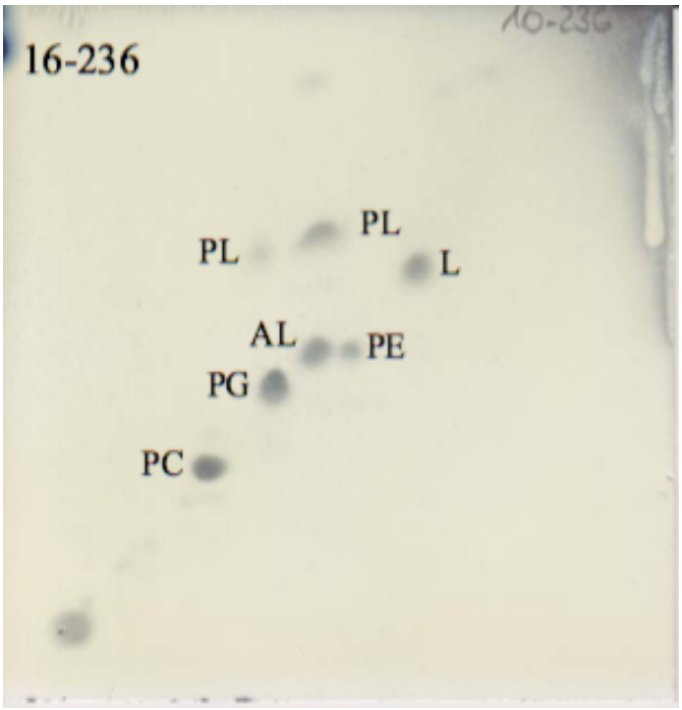


Figure 5 : Growth curve of strain BAR1^{T} at 0,1 MPa (blue points) and 32 MPa (red points). The line connects the averages at each measure point. The growth rates presented in the main body of the article were calculated using the growth measured between 15,5 and 39,5 hours.



Supplementary figure 3 : Polar lipid composition

DSMZ identification service report



AL, Aminolipid; L, Lipid ; PC, Phosphatidylcholine ; PE, Phosphatidylethanolamine ; PG, Phosphatidylglycerol ; PL, Phospholipid.

Supplementary table 1: Genome statistics for BAR1^T and the closest related species as depicted by Figure 1.

Species	Strain	BioProject	BioSample	GenBank assembly acc.	DDH*	ANI**	Genome coverage	# contigs	Contig N50	DNA G+C (%)	Genome size (bp)	Gene count
Profundibacter amoris	BAR1^T	PRJNA488700	SAMN09939831	GCA_003544895.1	-	-	246	1	3 558 757	57.3	3 558 757	3565
Aliiroseovarius crassostreae	CV919-312^T	PRJNA291771	SAMN03952659	GCA_001307765.1	18.7	71.6	100	25	625 830	58.4	3 723 455	3759
Aliiroseovarius sediminilitoris	M-M10^T	PRJEB16889	SAMN05444851	GCA_900109955.1	18.3	71.1	310	6	3 043 064	58.7	3 413 458	3391
Aliiroseovarius pelagivivens	GYSW-22^T	PRJEB25094	SAMEA104628693	GCA_900302485.1	18.5	71.3	181	4	2 500 797	58.1	3 331 553	3280
Pseudoruegeria haliotis	WM67^T	PRJNA402500	SAMN07621391	GCA_003003255.1	18.7	70.6	178	33	360 437	63	5 044 743	4773
Pseudoruegeria sabulilitoris	GJMS-35^T	PRJNA303057	SAMN04297160	GCA_001558155.1	18.6	71.2	100	148	205 481	62	5 324 675	5014
Pseudoruegeria marinistellae	SF-16^T	PRJNA299081	SAMN04193328	GCA_001509585.1	20.4	70.5	100	41	470 381	63	5 421 999	5045
Pseudoruegeria lutimaris	HD-43^T	PRJEB15972	SAMN04488026	GCA_900099935.1	18.9	71.0	230	193	71 923	62	5 811 514	5757
Pseudoruegeria aquimaris	SW-255^T	PRJEB19730	SAMEA102067918	GCA_900172235.1	18.6	72.8	42	60	114 539	66.6	3 691 014	3655
***Pseudoruegeria* sp.**	SK021	PRJNA360595	SAMN06212649	GCA_002119405.1	19.3	71.0	245	189	94 596	60.1	3 966 811	3858
Halocynthiibacter arcticus	PAMC 20958^T	PRJNA269208	SAMN03252591	GCA_000812665.2	19.9	70.0	23	1	4 329 554	53	4 329 554	4292
Halocynthiibacter namhaensis	RA2-3^T	PRJNA269555	SAMN03254441	GCA_000812685.1	18.3	70.1	12	74	110 746	53	3 535 512	3596
Thioclava atlantica	13D2W-2^T	PRJNA196741	SAMN02945012	GCA_000737065.1	18.3	70.7	195	47	235 161	65	3 928 443	3813
Thioclava pacifica	TL 2^T	PRJNA210304	SAMN02893935	GCA_000714535.1	17.7	70.7	100	42	567 911	64	3 728 293	3615
Planktotalea frisia	SH6-1^T	PRJNA262299	SAMN05660220	GCA_003254185.1	20.2	70.7	284	200	41 424	54	4 159 922	4356
Amylibacter cionae	m18	PRJNA427757	SAMN08272062	GCA_002860325.1	17.5	70.3	1739	17	397 164	56.7	4 286 347	4014

* DDH – Genome-to-Genome Distance Calculator at http://ggdc.dsmz.de/ggdc.php# (1). Same species DDH >= 70%
** ANI – Average Nucleotide Identity Calculator at https://www.ezbiocloud.net/tools/ani (2). Same species ANI >= 94%

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Supplementary table 2 : Growth of the tested strains on different carbon sources.

Strains: 1, BAR1^T; 2, *P. aestuariivivens* BS-W9^T; 3, *H. arcticus* PAMC 20958^T, 4, *H. namhaensis* RA2-3^T. All data are from this study. The growth was assessed in MB medium without peptone, 0,02 % yeast extract, and supplemented with 0,2 % of the carbon source tested. The growth was measured using spectrophotometry at a wevelength of 600 nm. Positive results were validated if the strain still grew more than a negative control (without the carbon source tested) after 3 consecutive transfers. Negative results were validated after two failed attempt to grow the strain on carbon source. For strain BAR1^T, different conditions were tested without any change in the carbon utilisation profile (see main text). The results obtained for *P. aestuariivivens* BS-W9^T are in concordance with the ones published in its characterization. The results obtained for *H. arcticus* PAMC 20958^T cannot be compared to the ones published in its characterization due to the use of different methods. The abscence of growth of *H. namhaensis* RA2-3^T on tryptone is contradictory to the results published in its characterization. +, positive; -, negative.

Carbon source	**1**	**2**	**3**	**4**
D(+) sucrose	-	-	+	-
D(+) galactose	-	-	+	-
D(+) glucose	-	-	+	-
D(+) xylose	-	-	+	-
D(+) maltose	-	-	+	-
D(+) cellobiose	-	-	+	-
D(+) mannose	-	-	+	-
D(-) ribose	-	-	+	-
D(-) fructose	-	-	+	-
D(-) arabinose	-	-	-	-
α-cellulose	-	-	-	-
Lactose	-	-	-	-
Acetate	-	+	-	-
Citrate	-	-	+	-
Pyruvate	-	+	+	-
Formate	-	-	-	-
Mannitol	-	-	+	-
Peptone	+	+	+	+
Tryptone	+	+	+	-
L(-) alanine	-	+	-	-
L(-) serine	-	+	-	-
L(-) lysine	-	+	-	-

Supplementary table 3 : Characteristic comparison between strain BAR1^T and the most closely related genera from figure 1.

The table lists the characteristics of the validely published members of each genus at the time of writing. The data was collected from the original published characterizations. Some of the features originate from the species description instead of the genus description. The characteristics listed are present in at least one species of each genus, and do not imply that each species possess all of them. Note that in some publications $C_{18:1}\omega7c$ was replaced by Summed Feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}\omega6c$). Taxa : 1, strain BAR1^T ; 2, *Halocynthiibacter* (1,2) ; 3, *Pseudohalocynthiibacter* (3) ; 4, *Thioclava* (4–11) ; 5, *Amylibacter (12*–15) ; 6, *Pseudoruegeria (16*–21) ; 7, *Planktotalea (22*–24) ; 8, *Ascidiaceihabitans* (25) ; 9, *Pseudopelagicola* (26) . +, positive ; -, negative ; coc, coccoid ; ovo, ovoid ; FAnaer, facultatively anaerophilic ; Aer, aerophilic ; PC, phospholipid ; PE, phosphatidylethanolamine ; PG, phosphatidylglycerol ; L, lipid ; AL, aminolipid ; PL, phospholipid ; APL, aminophospholipid ; PGL, phosphoglycolipid ; PS, phosphatidylserine ; dPG, diphosphatidylglycerol ; GL, glycolipid.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Motility	+	-	-	+/-	-	-	+/-	-	-
Cell shape	Rod	Rod	Rod/coc/ovo	Rod	Rod	Rod	Rod/ovo	Rod/coc/ovo	Rod
Oxygen regime	FAnaer	Aer	Aer	Aer	Aer	Aer/FAnaer	Aer	Aer	Aer
Nitrate reduction	+	-	+	+/-	-	+/-	+/-	-	+
Catalase, oxidase	+, +	+, +	+, +	+, +	+, +	+, +	+/-, +	+, +	+, +
Major ubiquinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10
Major polar lipids	PC, PE, PG, L, AL, PL	PC, PG, L, AL	PC, PE, PG, L, AL	PE, PG, GL, PL, APL, PGL	PC, PE, PG, L, AL, PL	PC, PE, PG, PS, L, AL, dPG, PL, GL	PC, PG, L, AL, PL	PC, PE, PG, L, AL, dPG	PC, PG, L, AL
Major fatty acids (>20%)	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$
DNA G+C (mol%)	57.38	52.9-53.2	53.2	60.3-65.3	50.4-56.7	62-73.5	53.8-57.1	55.8	55.5

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2. Baek K, Lee YM, Shin SC, Hwang K, Hwang CY, Hong SG, et al. *Halocynthiibacter arcticus* sp. nov., isolated from Arctic marine sediment. Int J Syst Evol Microbiol. 2015 Nov;65(11):3861–5.

3. Won S-M, Park S, Park J-M, Kim B-C, Yoon J-H. *Pseudohalocynthiibacter aestuariivivens* gen. nov., sp. nov., isolated from a tidal flat. Int J Syst Evol Microbiol. 2015 May;65(Pt 5):1509–14.
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