Neptuniibacter pectenicola sp. nov. and *Neptuniibacter marinus* sp. nov., two novel species isolated from a Great scallop (*Pecten maximus*) hatchery in Norway and emended description of the genus *Neptuniibacter.*

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Abstract

Nine isolates obtained from a great scallop hatchery in Norway were characterized using a polyphasic approach. Strains were Gram-negative, aerobic and motile rods with oxidative metabolism. Phylogenetic analysis based on the sequences of 16S rRNA and *rpo*B genes showed that these strains formed two different groups associated with members of the genus *Neptuniibacter*. DNA-DNA hybridization (DDH) and Average Nucleotide Identity (ANI) demonstrated that the isolates constituted two novel species of this genus, which can be phenotypically differentiated from their closest relatives. The names *Neptuniibacter marinus* sp. nov. and *Neptuniibacter pectenicola* sp. nov are proposed, with ATR 1.1^T (=CECT 8938^T=DSM 100783^T) and LFT 1.8^T (=CECT 8936^T=DSM 100781^T) as respective type strains.

Keywords: *Neptuniibacter pectenicola* sp. nov.; *Neptuniibacter marinus* sp. nov.; *Oceanospirillaceae*; *Pecten maximus*

The Great scallop, *Pecten maximus*, is a potential aquaculture species in Norway and other European countries, due to its high price and well established markets [5]. The technology used in Norwegian hatcheries is almost the same as in 1960´s and most of them depend on static or flow-through systems (FTS). The scallops suffer high mortality rates in early life stages, most of them with bacterial ethiology [18]. To improve survival of larvae different methods have been proposed including recirculating systems (RAS). In order to compare the microbiota associated with these two production systems, FTS and RAS, samples of broodstock, larvae, microalgae, water, biofilm, biofilters and water treated with UV light were collected in an experimental hatchery in Norway. A total of 221 isolates were obtained, among which 9 were selected for this study because they appeared to constitute two new species within the genus *Neptuniibacter.*

The genus *Neptuniibacter*, described by Arahal *et al*. [1], belongs to the Family *Oceanospirillaceae* and it is only constituted by two species at the time of writing: *Neptuniibacter caesariensis* and *Neptuniibacter halophilus* [7]. Besides being respectively isolated from seawater and salt pan, bacteria belonging to these species have also been detected forming part of intestinal microbiota of rainbow trout, (*Oncorhynchus mykiss*) [8], being therefore not only related to marine environments. *Neptuniibacter* has been also studied by its capacity of assimilation of taurine-nitrogen [12] and its implication in the degradation of carbazole [17].

In this study, nine isolates obtained from different compartments in the hatchery were phenotypical, chemotaxonomical and genetically characterized to determine their taxonomic position.

Samples were collected from Scalpro AS, an experimental hatchery in Bergen, Norway. Samples of water, biofilm, biofilter, UV treated water and larvae were spread on Marine Agar 2216 (MA; Difco). Different colonies were isolated from plates, purified as pure cultures and stored at -80 ºC in Marine Broth (MB; Pronadisa) supplemented with 20% (v/v) glycerol.

Genomic DNA of pure cultures was obtained using the 'Instagene' matrix (Bio-Rad), following the manufacturer's recommendations. Amplification of 16S rRNA gene of the different strains was carried out using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1510R (5'-GGTTACCTTGTTACGACTT-3') as previously described by Lane [13]. Amplification of *rpo*B gene, codifying for the β-subunit of RNA polymerase, was obtained with primers PasrpoB-L (5'-GCAGTGAAAGARTTCTTTGGTTC-3') and rpoB-R (5'-GTTGCATGTTNGNACCCAT-3') following the protocol described by Korczak *et al.* [11]. Sequences of the reference strains were retrieved from GenBank/EMBL. Sequence data analysis was performed with DNASTAR Lasergene SEQMAN program. Sequences obtained for both genes were subjected to a BLAST search against the latest release of GenBank and compared with avalaible results in EzTaxon-extended database for 16S rRNA gene sequences [10]. Phylogenetic analysis of sequences of both genes were conducted with the program MEGA 6.0 [24], using Neighbour-joining (NJ) and Maximum Likelihood (ML) algorithms with a bootstrap of 1000 replications.

Sequences of 16S rRNA gene obtained for our isolates showed a range of similarity of 96.09-97.15% with *N. caesariensis* MED92^T (=CECT 7075^T) and between 95.87% and 96.27% with *N. halophilus* antisso-13^T (=LMG 25378^T). The phylogenetic trees obtained by NJ and ML showed that these isolates formed two very robust and independent groups within the genus *Neptuniibacter* and well differentiated from other genera of the Family *Oceanospirillaceae* (Fig. 1). Phylogenetic study based on *rpo*B gene only permited the comparison of our isolates with a few deposited reference sequences, but the results previously obtained with 16S rRNA gene analysis were confirmed, showing the new isolates distributed into two groups separated from other *Neptuniibacter* (Fig. 2). One group comprised 3 isolates (LFT 1.8^T [=CECT 8936^T =DSM 100781^T] LRS 1.6 [=CECT 8937] and LRS 1.10) all of them from scallop larvae, and the second one grouped 6 isolates (ATR 1.1^T [=CECT 8938^T =DSM 100783^T], ATR 1.2, ATR 1.5, BFR 2.2, BFR 3.3 and UV 2.5 [=CECT 8939 =DSM 100784]) obtained from tank and UV-treated water and biofilter in the hatchery.

The isolates were phenotypically characterized by routine biochemical tests following the methodologies described by Romalde *et al.* [21] and MacFaddin [15]. *Neptuniibacter caesariensis* CECT 7075^T and *N. halophilus* LMG 25378^T were included in all the experiments for comparative purposes.

Morphology and motility were observed by light microscopy (ICC50HD; Leica). Catalase activity was determined based on bubble production in H_2O_2. The oxidase activity was determined by oxidation of 1% (w/v) N,N,N',N'-tetramethyl-ρ-phenylenediamine. Gelatinase, lipase, and hydrolysis of aesculin and starch activities were analyzed on MA plates supplemented with 0.4%, 1%, 0.1% and 0.4% (w/v) of substrates, respectively. Growth at various temperatures (4-44 ºC) was determined on MA plates. The optimum pH range for growth was examined in MB using appropriate biological buffers for adjusting the pH to 3, 5, 7, 8, 9, 10 and 11. Tolerance to different salinity concentrations was tested on tubes of Basal Medium (BM) with 4 g l^{-1} neopeptone and 1 g l^{-1} yeast extract supplemented with 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, 6%, 8% and 10% of NaCl and on the other hand, with 0%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 10%, 12% and 15% of Sea Salts (Sigma-Aldrich) incubated at room temperature. Growth was determined by measuring the turbidity at 580nm in a MicroScan TurbidityMeter (Siemens).

Use of different compounds as sole carbon source was tested on Basal Medium Agar (BMA) containing 50% (v/v) of Artificial Sea Water (ASW: 23.38 g l^{-1} NaCl, 24.65 g l^{-1} $MgSO_4x7H_2O$, 1.5 g l^{-1} KCl, 2.2 g l^{-1} $CaCl_2x2H_2O$), 6.1 g l^{-1} Tris-HCl, 1 g l^{-1} NH4Cl, 0.075 g l^{-1} K_2HPO_4, 0.028 g l^{-1} $FeSO_4x7H_2O$ and 1.8% (w/v) American bacteriological Agar (Pronadisa) supplemented with different substrates according Baumann and Baumann [4]. A cell suspension was prepared on MB and 1µl was inoculated using a Multipoint Inoculator (Denley). Plates were incubated during 10 days at 25 ºC obtaining lectures every 24 h.

Additional biochemical test were analyzed using miniaturized systems API ZYM (bioMerieux) according to the manufacturer's instructions but using a Sea Salts solution (3.5‰ w/v) and incubating at 25 ºC during 24 h.

All isolates showed white and translucent colonies, bacilar morphology and great motility by light microscopy. All of them were Gram negative and oxidase positive. Catalase activity was present only in scallop larvae isolates. This characteristic together with the differences in the growth at 37ºC and gelatinase activity allowed us to distinguish the two groups of Norwegian isolates. Unlike previously described species, our isolates could grow at 4ºC but not in a medium containing 6% of NaCl (w/v). They also could be differentiated from other *Neptuniibacter* in the ability of use as sole carbon source cellobiose, L-rhamnose, glycerol or L-histidine whereas were not able to use glutamic acid or putrescine among other substances (Table1).

The Norwegian isolates presented a low enzymatic activity in the API ZYM system. Phosphatase alkaline activity was observed for all strains whereas they showed variable results in esterase, leucine arylamidase and valine arylamidase activities (Fig. S1). Only the isolate LFT 1.8^T was positive for esterase lipase and isolate BFR 3.3 was positive for acid phosphatase. The rest of activities of the API ZYM gallery were negative for all isolates, even for Naphthol-AS-BI-phosphohydrolase activity that is positive in other *Neptuniibacter* species.

Genomic DNA for DNA-DNA hybridization (DDH) and determination of the G+C content was extracted using Wizard® Genomic DNA Purification Kit (Promega). The G+C content was determined from triplicate samples of strains ATR 1.1^T and LFT 1.8^T following the method described by González and Saiz-Jimenez [9], using EvaGreen and 30% formamide. *Lactococcus lactis* subsp. *lactis* CECT 4433^T, *Escherichia coli* CECT 433^T, *Neptuniibacter caesariensis* CECT 7075^T and *Pseudomonas aeruginosa* CECT 4122^T were used as controls. DDH between strains ATR 1.1^T, LFT 1.8^T and *N. caesariensis* CECT 7075^T were performed by the hydroxyapatite/microtitre method [28] with a hybridization temperature (Tm) of 60 ºC.

In addition, DNA of isolates ATR 1.1^T and LFT 1.8^T was extracted with High Pure PCR Template Preparation kit (Roche) and sequenced at Sistemas Genómicos (Valencia, Spain) using Illumina paired-end sequencing technology. The quality of reads was analyzed using Trimmomatic 0.32 [6]. Genome assembly was performed using SPAdes 3.6.1 [19]. Accesion numbers of whole genome sequences of LFT 1.8^T and ATR 1.1^T are LUTR00000000.1 and LUTS00000000.1, respectively. The versions described in this paper are version LUTR01000000.1 and LUTS01000000.1, respectively. The Average Nucleotide Identity (ANI), using BLAST (ANIb) and MUMmer (ANIm) algorithms, was calculated using the software J-species (V1.2.1) as described by Richter and Roselló-Móra [20]. OrthoANI percentages were calculated as described Lee et al. [10]. *In silico* DDH value was estimated between strains ATR 1.1^T, LFT 1.8^T and *N. caesariensis* MED92^T (Accession number AAOW00000000.1) using the genome-to-genome distance calculator (GGDC2.0) [2, 3, 16].

DDH reassociation values obtained for strains ATR 1.1^{T} and LFT 1.8^{T} are shown in Table 2. For ATR 1.1^{T} DDH values of 56.46%, 43.73 and 51.07% were obtained with strains LFT 1.8^{T}, and respectively. For strain LFT 1.8^{T}, DDH values of 32.1% with *N. caesariensis* CECT 7075^{T} and 42.0% with *N. halophilus* LMG 25378^{T} were observed. Results obtained using *in silico* DDH for ATR 1.1^{T} were 24.50% ± 2.39 with LFT 1.8^{T} and 18.00% ± 2.25 with *N. caesariensis* MED 92^{T} (Table 2). The strain LFT 1.8^{T} showed a percentage of similarity of 18.10% ± 2.25 with *N. caesariensis* MED92^{T}. In any case, the values were lower than the 70% established as threshold for species definition [28].

ANIb, ANIm and OrthoANI values between ATR 1.1^{T}, LFT 1.8^{T} and *N. caesariensis* MED92^{T} are listed in Table 2. Values ranged from 71.25% to 81.59% for ANIb, from 83.28% to 86.06% for ANIm, and from 72.12 to 81.67 for Ortho ANI. Values obtained from the three indexes were consistent and below the proposed cut-off for species boundary (95~96%) [20].

The DNA G+C mol% content of strains ATR 1.1^{T} and LFT 1.8^{T} was determined by whole genome sequencing data and also was estimated by thermal denaturation method. G+C contents of ATR 1.1^{T} and LFT 1.8^{T} were of 42.8 mol% and 45.7 mol% when calculated from the whole genome sequences, and of 47.5 mol% and 49.3 mol% by the fluorimetric method. Thus, variations of 4.7% and 3.6% were observed for the G+C content of these strains depending on the procedure utilized. In the original description of *N. halophilus*, Chen *et al.* [7] reported that the type strain presents a G+C content of 54.2 ± 1.0 mol%. This value could mean a conflict when compared to that of strain ATR 1.1^{T} (obtained from genome sequence) due to the fact that these type strains differ in more than 10 mol%, and therefore they would belong theoretically to different genera [23, 27, 28]. Such incongruence does not exist when compared with the value obtained by the fluorimetric method. However, it is important to note that the result by Chen et al. [7] was obtained by using HPLC method, that although seems to be more accurate the the fluorimetric technique, is still an indirect method for of G+C content measurement.

Whole-cell fatty acid analysis was concurrently performed on cells of the isolates ATR 1.1^T, LFT 1.8^T, *N. caesariensis* CECT 7075^T and *N. halophilus* LMG 25378^T grown for 2 days at 25º C on MA (Difco). Extraction and analysis of whole-cell fatty acids was carried out using the MIDI system in accordance with the protocols and instrument specifications recommended by the manufacturer [22] using Phospholipid Fatty Acids (PFLAD1) and Environmental Aerobes (TSBA) databases. Extraction and analysis of respiratory quinones and polar lipids were carried out by the Identification Service of the DSMZ using thin layer chromatography following the methodology described by Tindall [25, 26].

Evaluation of fatty acid composition revealed that the two groups of Norwegian isolates, *N. caesariensis* and *N. halophilus* shared the major fatty acids, including $C_{10:0}$ 3OH, $C_{16:0}$, Summed Feature 3 ($C_{16:1}\omega 7c$ /$C_{16:1}$ $\omega 6c$) and Summed Feature 8 ($C_{18:1}\omega 7c$ or $C_{18:1}$ $\omega 6c$) although differences in their percentages were observed (Table 3). Strain ATR 1.1^T could be differentiated from the other species by a high percentage of $C_{18:0}$ (Table 3).

Regarding to polar lipids and respiratory quinones analysis, ATR 1.1^T presented diphosphatidylglycerol, phosphatidylethanolamine, phosphatydylglycerol and an unidentificated phosphoaminolipid as the major compounds of polar lipids profile. A weak presence of unknown phospholipid, glycolipid and aminolipid was also detected (Fig. 3A). Isolate LFT 1.8^T showed a polar lipid profile formed by phosphatidylethanolamine and an unknown phosphoaminolipid as major compounds. A moderate amount of phosphatidylglycerol and diphosphatidylglycerol and weak peaks of unidentified phosphoaminolipid, phospholipid and lipid were detected (Fig.3B). Ubiquinone Q8 was the principal respiratory quinone in both groups of isolates.

According to the results obtained in the phenotypic characterization, genomic and phylogenetic analysis, and chemotaxonomic studies, we conclude that these groups of isolates constitute two novel species within the genus *Neptuniibacter* for which the names *Neptuniibacter marinus* sp. nov. and *Neptuniibacter pectenicola* sp. nov are proposed, with strains ATR 1.1^T (=CECT 8938^T= DSM 100783^T) and LFT 1.8^T (=CECT 8936^T= DSM 100781^T) as the type strains.

Description of *Neptuniibacter marinus* sp. nov.

Neptuniibacter marinus (ma. ri' nus. L. masc. adj. *marinus*, from the seawater.)

Cells are rods, motile, Gram-negative, oxidase positive and catalase negative. Colonies are white-traslucent, with entire margens and 1-2 mm of diameter on MA. The temperature range for growth is 4ºC – 30ºC (optimum: 25ºC), whereas the pH range is pH 7.0-10.0, except for the strains ATR 1.1^T and BFR 2.2, which can grow at pH 5. Growth in medium without NaCl or sea salts was not observed. The range of NaCl to grow is 1-8% (optimum: 2.5-3%), except for isolate UV 2.5 which only can grow at 2.5-3% NaCl, and for Sea Salts the range is 2-6% (optimum of 5%). All strains are negative for indol, citrate and reduction of nitrates to nitrites except the type strain, ATR 1.1^T. No hydrolysis of gelatin, starch, tween 80 and aesculine is observed. According to API ZYM, all strains present alkaline phosphatase and leucine arylamidase activity except isolate BFR 3.3. Isolates ATR 1.1^T, ATR 1.2 and ATR 1.5 show esterase (C4) activity. Valine arylamidase is present in ATR 1.1^T, ATR 1.2, BFR 2.2 and BFR 3.3, and only strain BFR 3.3 show acid phosphatase activity. All strains can use as sole carbon source β-hydroxybutyric acid, fumaric acid, citric acid, lactic acid, propionic acid, *trans*-aconitic acid, malic acid, succinic acid, L-rhamnose, D-alanine, piruvate, sodium acetate, cellobiose and glycerol. Type strain ATR 1.1^T can use L-serine. Major fatty acids detected are Summed feature 3 which contains $C_{16:1}$ ω7c/ $C_{16:1}$ ω6c (36.8%), $C_{16:0}$ (25.7%), Summed feature 8 composed by $C_{18:1}$ ω7c or $C_{18:1}$ ω6c (23.7%), $C_{10:0}$ 3OH (5.1%) and $C_{18:0}$ (4.1%).

Polar lipids profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unidentified phosphoaminolipid as major compounds and to a lesser extent unknown phospholipid, aminolipid and glycolipid. Ubiquinone Q8 is major respiratory quinone.

The type strain ATR 1.1^T (= CECT 8938^T=DSM 100783^T) was isolate from water of a tank in a hatchery of Great scallop (*Pecten maximus*) in Bergen, Norway (60º 30' 53.77" N 4º 54' 14.75" W). The DNA G+C content of type strain is 42.8 mol%.

Description of *Neptuniibacter pectenicola* sp. nov.

Neptuniibacter pectenicola (pec. te. ni' co. la., L. n. *pecten* scallop; L. suff *–cola* dweller; N. L. n. *pectenicola* scallop dweller).

Cells are rods, motile, Gram-negative, oxidase and catalase positive. Colonies are white-traslucent, with entire margens and 1-2 mm of diameter on MA. All strains can grow from 4ºC to 37ºC with an optimum of 25ºC. All isolates require at least 1% of NaCl or 2% of Sea Salts in the medium to grow. pH tolerance range from 7 to 10 except for type strain who can grow at pH 5. Negative to indol, citrate, reduction of nitrates and hydrolysis of starch, tween 80 and aesculine but isolates can hydrolyze gelatin. According to API ZYM, all strains present alkaline phosphatase and valine arylamidase but not lipase (C14), cystine arylamidase, tripsine, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α and β- galactosidase, β-glucuronidase, α and β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Strains LFT 1.8^T and LRS 1.6 also present esterase (C4) and leucine arylamidase activities. Type strain also produces esterase lipase (C8).

All isolates can use as sole carbon source the following compounds: γ- aminobutyric acid, β-hydroxybutyric acid, fumaric acid, citric acid, lactic acid, propionic acid, *trans*-aconitic acid, malic acid, succinic acid, cellobiose, L- histidine, D-alanine, L-rhamnose, pyruvate and sodium acetate but they can not use *myo*-inositol, N-acetyl-glucosamine, D-sorbitol, glycine, aspartic acid, arabinose, L-leucine, L-threonine, glutamic acid, L-arginine, sucrose, trehalose, sarcosine, putrescine, lysine, D-manitol, salicine, glycerol, D-saccaric acid, gluconic acid, amigdalin, melibiose,lactose, maltose, D-mannose, D-galactose, tyrosine, L-citruline, ornithine, D-ribose, D-xylose, glucose and D-fructose. Only strain LRS 1.10 can use L- serine as unique carbon source. Major fatty acids present on type strain are Summed feature 8 containing $C_{18:1}$ ω7c or $C_{18:1}$ ω6c (37.00%), Summed feature 3 composed by $C_{16:1}$ ω7c/ $C_{16:1}$ ω6c (23.0%), $C_{16:0}$ (22.8%), $C_{10:0}$ 3OH (8.7%) and $C_{12:1}$ 3OH (2.0%).

Polar lipids profile is formed by phosphatidylethanolamine and unknown phosphoaminolipid as major components, phosphatidylglycerol and diphosphatidylglycerol in a moderate quantity and weak amount of unidentified lipid and phospholipid. The major respiratory quinone is ubiquinone Q-8.

The type strain LFT 1.8^T (=CECT 8936^T=DSM 100781^T) was isolate from Great scallop (*Pecten maximus*) larvae in a hatchery of Bergen, Norway (60º 30’ 53.77” N 4º 54’ 14.75” W). The DNA G+C content of type strain is 45.7 mol%.

Emended description of the genus *Neptuniibacter* Arahal *et al.* 2007 emended by Chen *et al.* 2012.

The description of the genus *Neptuniibacter* is based on that given previously by Arahal *et al*. [1] with the following modifications. All species of the genus are catalase positive except *Neptuniibacter marinus* and the minimum G+C content of the genus is 42.8 mol%.

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Table 1: Differential characteristics among strains of *N. marinus* sp. nov., *N. pectenicola* sp. nov., *N. caesariensis* CECT 7075^T and *N. halophilus* LMG 25378^T. Data obtained in this work.

Phenotypic trait	***N. marinus*** **sp. nov. (n=6)**	***N. pectenicola*** **sp. nov. (n=3)**	***N. caesariensis*** **CECT 7075^T**	***N. halophilus*** **LMG 25378^T**
Enzymatic activity:				
Catalase	–	+	+	+
Gelatinase	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	–	–	+	+
Growth at:				
4ºC	+	+	–	–
37ºC	–	+	+	+
6% NaCl	–	–	+	+
Use as sole carbon source:				
L-rhamnose	+	+	–	–
Glutamic acid	–	–	+	+
Putrescine	–	–	+	+
Cellobiose	+	+	–	–
Glycerol	+	–	–	+
L-histidine	–	+	–	+

Table 2. Results of wet DDH, together with ANI and GGDC calculations (%) from the genome comparisons of *Neptuniibacter marinus sp. nov and Neptunibacter pectenicola sp. nov.* and the other members of the genus *Neptuniibacter*.

	N. marinus **sp. nov. ATR 1.1^{T}**	***N. pectenicola*** **sp. nov. LFT 1.8^{T}**	***N. caesariensis*** **MED92^{T}**	***N. halophilus*** **LMG 25378^{T}**
Wet DDH				
ATR 1.1^{T}	100			
LFT 1.8^{T}	56.46	100		
MED92^{T}	43.76	32.1	ND*	ND
LMG 25378^{T}	51.07	42.0	ND	ND
ANIb				
ATR 1.1^{T}	100	81.58	71.51	ND
LFT 1.8^{T}	81.59	100	71.51	ND
MED92^{T}	71.25	71.35	100	ND
ANIm				
ATR 1.1^{T}	100	86.05	83.28	ND
LFT 1.8^{T}	86.06	100	83.34	ND
MED92^{T}	83.30	83.36	100	ND
OrthoANI				
ATR 1.1^{T}	100			ND
LFT 1.8^{T}	81.67	100		ND
MED92^{T}	72.15	72.12	100	ND
GGDC				
ATR 1.1^{T}	100			ND
LFT 1.8^{T}	24.5 ± 2.4	100		ND
MED92^{T}	18.0 ± 2.25	18.1 ± 2.25	100	ND

*ND, not determined

Genomes used in the *in silico* analyses: *N. marinus* sp. nov. ATR 1.1^{T} (LUTR00000000.1); *N. pectenicola* LFT 1.8^{T} (LUTS00000000.1); *N. caesariensis* MED92^{T} (AAOW00000000.1).

Table 3: Fatty acid composition obtained from type strains of *N. marinus* sp. nov., *N. pectenicola* sp. nov., *N. caesariensis* and *N. halophilus.*

Major fatty Acids	***N. marinus* sp. nov. ATR 1.1^T**	***N. pectenicola* sp. nov. LFT 1.8^T**	***N. caesariensis* CECT 7075^T**	***N. halophilus* LMG 25378^T**
$C_{10:0}$ 3OH	5.1	8.7	5.0	5.2
$C_{12:1}$ 3OH	1.4	2.0	ND	ND
$C_{14:0}$	1.3	*tr*	*tr*	*tr*
Summed feature 3	36.8	23.0	39.0	42.2
$C_{16:0}$	25.7	22.8	15.3	15.1
$C_{18:1}$ ω9c	1.0	ND	ND	*tr*
Summed feature 8	23.7	37.0	37.3	33.5
$C_{18:0}$	4.1	1.9	1.2	*tr*
$C_{19:0}$ 10- methyl	ND	1.9	ND	*tr*

Values are percentages of the total fatty acids; fatty acids that make up <1 % of the total are indicated by *tr*. ND: Not detected. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* isomer is indicated by the suffixes *c*. Summed features are groups of two fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains $C_{16:1}$ω7*c*/$C_{16:1}$ω6*c* and Summed feature 8 contains $C_{18:1}$ω7*c* and/or $C_{18:1}$ω6*c*. All data obtained in the present study.

Figure legends

Fig. 1. Phylogenetic reconstruction based on the nucleotide sequences of 16S rRNA gene by the ML algorithm, showing the position of the novel *Neptuniibacter* species. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the nodes. Bold circles indicate that corresponding nodes were coincident in the tree generated with NJ algorithm. Bar, 0.02 substitutions per nucleotide position.

Fig. 2. Phylogenetic reconstruction based on the nucleotide sequences of *rpo*B gene by the NJ algorithm, showing the position of the novel *Neptuniibacter* species. Bootstrap values (expressed as percentages of 1000 replications) are shown at the nodes. Bar, 0.05 substitutions per nucleotide position.

Fig. 3. Polar lipid profiles of type strains of *N. marinus* sp. nov. ATR 1.1^{T} (A) and *N. pectenicola* sp. nov. LFT 1.8^{T} (B). DPG: Diphosphatidylglycerol; PE: Phosphatidylethanolamine; PG: Phosphatidylglycerol; PL: Phospholipid; PN: Phosphoaminolipid; GL: Glycolipid; AL: Aminolipid; L: lipid.

Figure 1

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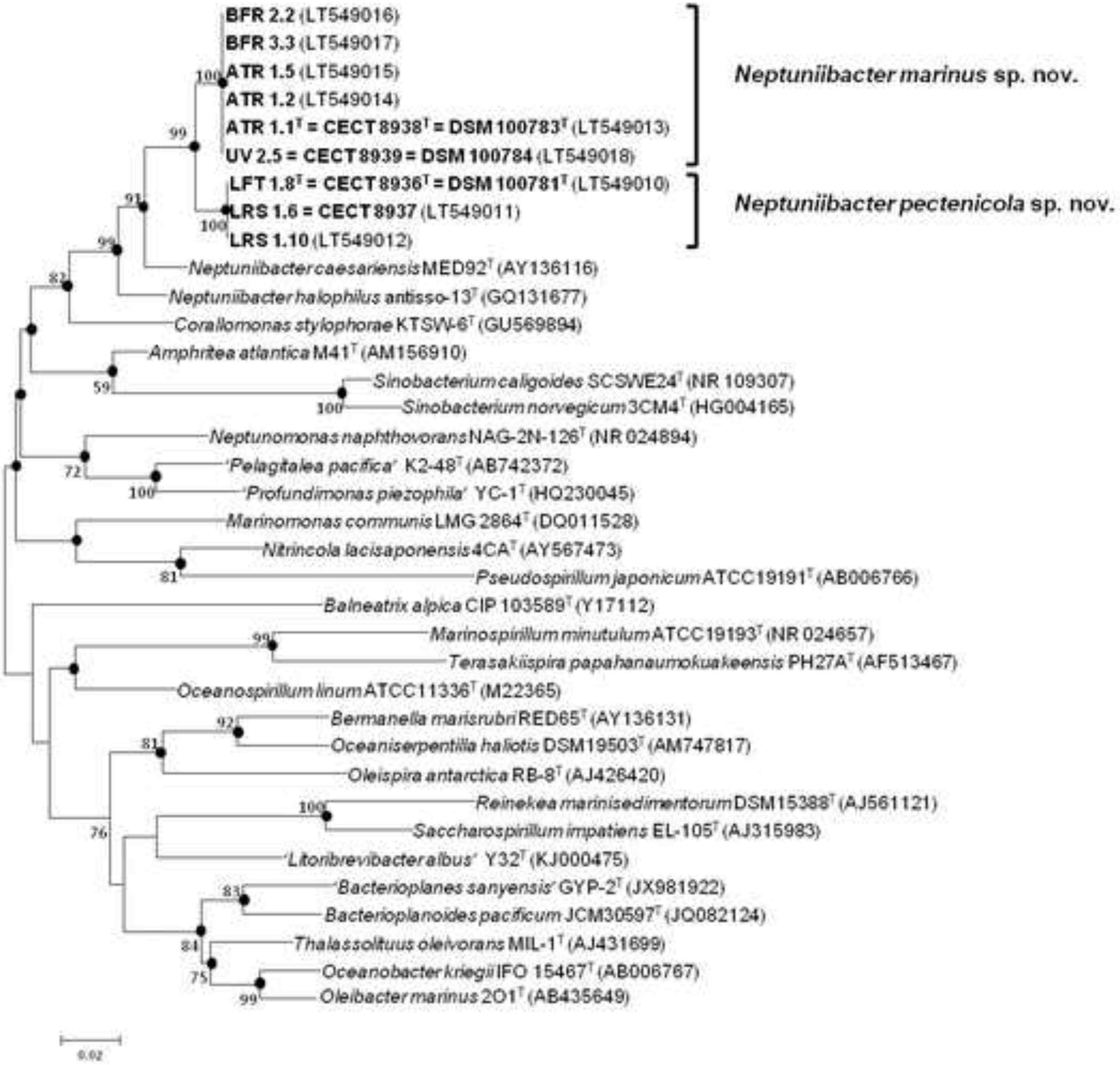

Figure 2

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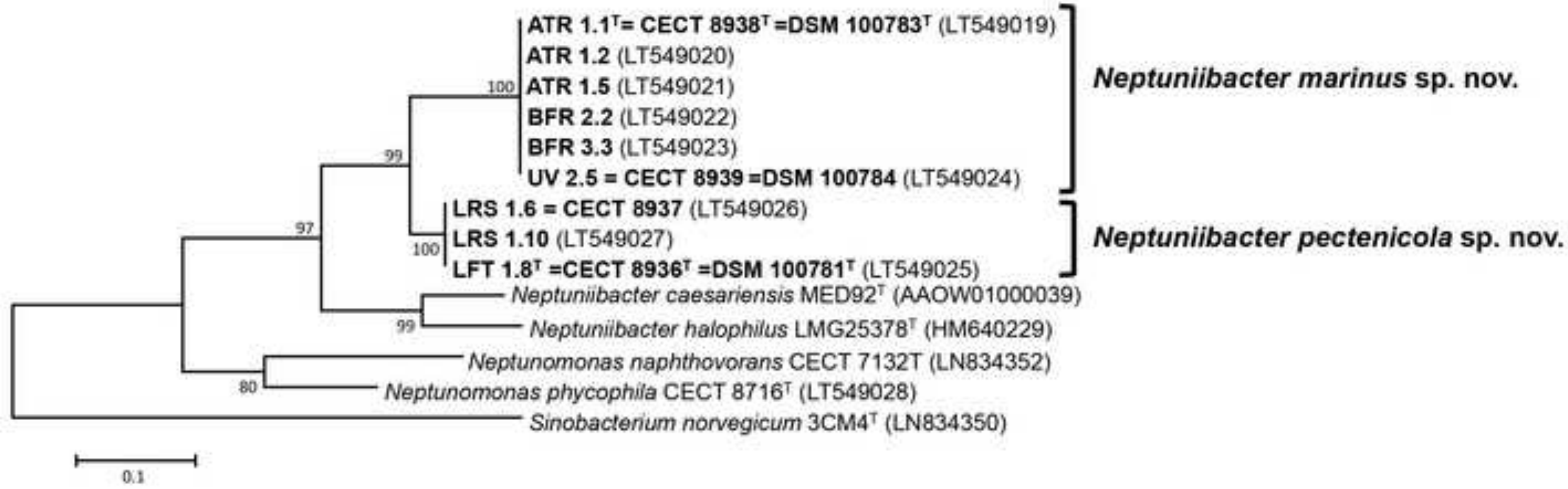

Figure 3

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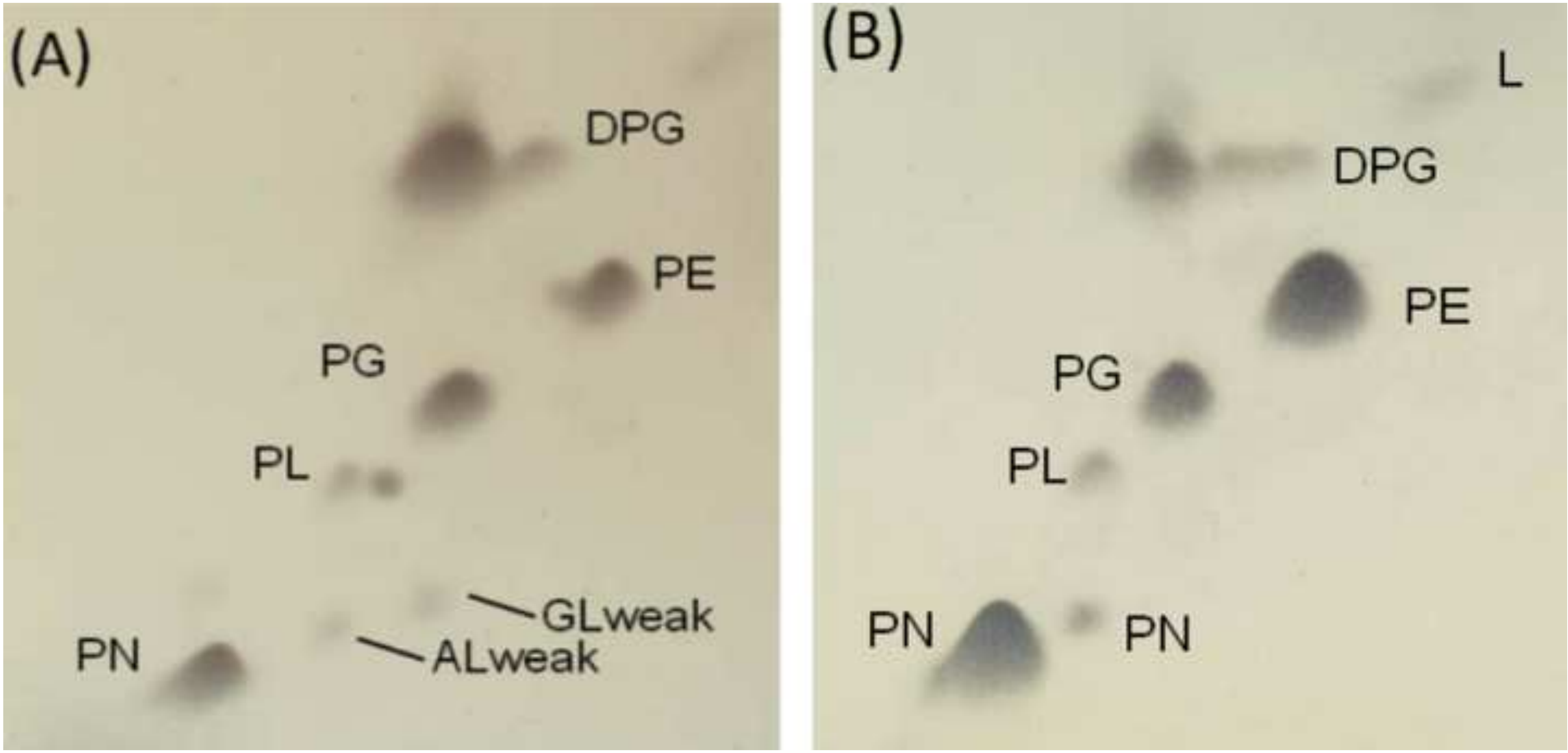

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