Pontibacter rubellus sp. nov., and *Pontibacter situs* sp. nov. bacteria isolated from soil

Soohyun Maeng
Seoul Women's University
Yuna Park
Seoul Women's University
Tuvshinzaya Damdintogtokh
Seoul Women's University
Hyejin Oh
Seoul Women's University
Minji Bang
Seoul Women's University
Sathiyaraj Srinivasan
Seoul Women's University
Myungkyum Kim (biotech@swu.ac.kr)
Seoul Women's University

Research Article

Keywords: Hymenobacteraceae, Pontibacter, Taxonomy

Posted Date: September 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-582338/v1

Abstract

Gram-stain-negative, aerobic, non-flagellated strains 172403-2^T and BT310^T were isolated from the soil collected in Pyeongchang city and Uijeongbu city, Korea. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strains 172403-2^T and BT310^T formed a distinct lineage within the family *Hymenobacteraceae* (order *Chitinophagales*, class *Chitinophagia*) and were most closely related to members of the genus *Pontibacter*, *Pontibacter chitinilyticus* 17gy-14^T (95.7%), and *Pontibacter populi* HLY7-15^T (97.1% 16S rRNA gene sequence similarity) respectively. The optimal growth of strains 172403-2^T and BT310^T occurred at pH 7.0, in the absence of NaCl, and 25°C and 30°C, respectively. The predominant cellular fatty acids were iso-$C_{15:0}$ and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B). The major respiratory quinone of the two strains was MK-7. The major polar lipid of the two strains was phosphatidylethanolamine. Biochemical, chemotaxonomic and phylogenetic analyses indicated that strains 172403-2^T and BT310^T represent novel bacterial species within the genus *Pontibacter*, for which the names *Pontibacter rubellus* and *Pontibacter situs* are proposed. The type strains of *Pontibacter rubellus* and *Pontibacter situs* are 172403-2^T and BT310^T, respectively.

Introduction

The genus *Pontibacter* is a member of the family *Hymenobacteraceae* in the phylum *Bacteroidetes.* The family *Hymenobacteraceae* contains six genera (http://www.bacterio.net) and *Pontibacter* is one of the largest genera. The genus *Pontibacter* was first proposed by Nedashkovskaya et al. (2005) with *P. actiniarum* as a type species. At the time of writing, the genus *Pontibacter* is comprised of 38 species (http://www.bacterio.net/Pontibacter.html).

The species of *Pontibacter* have been isolated mainly from soil samples (Srinivasan et al. 2014; Chhetri et al. 2019), seawater (Kang et al. 2013), tidal flat (Park et al. 2016), plant rhizospheres (Xu et al. 2014), pond sediments (Singh et al. 2015), actinians (Nedashkovskaya et al. 2005) and desert (Zhang et al. 2008). The members of genus *Pontibacter* are Gram-stain-negative, aerobic, rod-shaped, and motile or non-motile. The common chemotaxonomic features are phosphatidylethanolamine (PE) as the main polar lipid and MK-7 as the main respiratory quinone. The branched-chain fatty acids such as iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, summed feature 3 (iso-$C_{15:0}$ 2OH / $C_{16:1}$ *ω7c*) and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B) are the main cellular fatty acids. (Nedashkovskaya et al. 2005).

Materials And Methods

Organism and culture conditions

Strains 172403-2^T and BT310^T were isolated from a soil sample collected in Uijeongbu city, South Korea. Soil (1 g) was added to 10 mL if sterile normal saline and shaken at 37 °C for 1h, and then serially diluted. 100 µL of the diluent was spread on Reasoner's 2A (R2A, Difco) agar and incubated at 25°C; after

3 days, various colonies were selected and purified. Strains 172403-2^T and BT310^T were stored at -80°C in 20% (v/v) glycerol with R2A broth. The 16S rRNA gene sequences of the strains were compared with the closely related sequences including *Pontibacter populi* HLY7-15^T and *Pontibacter amylolyticus* 9-2^T using the EzBioCloud (https://www.ezbiocloud.net).

Morphology, physiology and biochemical analysis

Cell morphology was observed by transmission electron microscopy (JEOL, JEM1010) after 3 days of incubation on R2A at 30 °C. Gram-staining reaction was performed according to the standard Gram reaction kit (bioMérieux). The growth temperature range was tested at 4, 10, 15, 25, 30, 35, 37, 42 and 45 °C. The salt tolerance was measured in R2A supplemented with various concentrations of NaCl (1–10% at intervals of 1%, w/v). The pH range was measured in R2A from pH 4.0 to 10.0 with an interval of 0.5 units using R2A broth at 25°C. Oxidase activities of strains 172403-2^T and BT310^T were tested using 1% (w/v) tetramethyl- *p*-phenylene diamine diamine (Smibert and Krieg 1981) and catalase activities were tested by measuring bubble production after applying 3% (v/v) hydrogen peroxide solution (Cappuccino and Sherman 2002). Growth on the different mediums was observed on R2A agar, nutrient agar (NA, BD Difco), tryptic soy agar (TSA, BD Difco), MacConkey agar (BD Difco) and lysogeny broth (LB, BD Difco). API 20NE (bioMérieux) was used to determine the utilization and fermentation of various carbon sources and API ZYM (bioMérieux) was used to determine the enzymic activities of the strains according to the manufacturer's instructions.

Phylogenetic analysis and Genome sequencing

The genomic DNA of strains 172403-2^T and BT310^T were extracted using a genomic DNA extraction kit (Qiagen). The 16S rRNA gene was Amplified using a standard PCR method with a universal bacterial primer set 27F and 1492R (Weisburg et al. 1991). The amplified 16S rRNA gene was sequenced by Macrogen (Korea) with the 518F, 785F, 800R and 926R universal primers. To determine the taxonomic positions of strains 172403-2^T and BT310^T, 16S rRNA gene sequences of closely related taxa were obtained from EzBioCloud (http://ezbiocloud.net) and EzEditor2 program was used for the alignment of the sequences. The phylogenetic tree was constructed using the MEGAX program (Kumar et al. 2018) and neighbor-joining (NJ) algorithm (Saitou and Nei 1987). A bootstrap analysis with 1,000 replicates was conducted (Felsenstein 1985).

For genome sequencing, genomic DNA of strains 172403-2^T and BT310^T were extracted using a MagAttract HMW DNA kit (Qiagen, Germany) according to the manufacturer's instructions. The SMRT sequencing was performed on the Pacific Biosciences RSII sequencer (PacBio) according to standard protocols (MagBead Standard Seq version 2 loading, 1180 min movie) using the P4-C2 chemistry at the DNALink (www.dnalink.com), Korea. The complete genome sequences of the strains were deposited to Genbank (www.ncbi.nlm.nih.gov/) and annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016) and Rapid Annotations using Subsystems Technology (RAST version 2.0, with annotation scheme "Classic RAST,"

added automatically fix errors, fix frameshifts, and backfill gaps (Overbeek et al. 2014). The genome-based phylogenetic tree was reconstructed using the UBCG set pipeline (Na et al. 2018), with which we used a concatenated sequence dataset of 92 single-copy bacterial core genes (www.ezbiocloud.net/tools/ubcg).

Chemotaxonomic characteristics

The polar lipids of strains 172403-2^T and BT310^T were extracted (Minnikin et al. 1984) and examined using two-dimensional thin-layer chromatography (TLC). The separated polar lipids were identified by spraying several reagents as described by Komagata and Suzuki (1987). Lipoquinones were extracted with Sep-Pak Vac cartridges (Waters) and analyzed by high-performance lipid chromatography (HPLC) method (Hiraishi et al. 1996). For cellular fatty acids analysis, strains 172403-2^T and BT310^T were incubated on R2A agar for 3 days at 25°C. The cellular fatty acids were purified by saponification, methylation and extraction procedures as previously described (Sasser 1990). The fatty acid methyl esters (FAME) were identified using the Sherlock Microbial Identification System V6.01 (MIS, database TSBA6, MIDI Inc., Newark, DE, USA).

Result And Discussion

Morphology, physiology and biochemical analysis

Strains 172403-2^T and BT310^T were isolated from soil collected in the Pyeongchang and Uijeongbu city, respectively. The colonies of strain 172403-2^T on R2A agar medium were observed to be red, convex and circular after 72-hour incubation at 25°C, whereas strain BT310^T formed orange color colonies. Both strains were Gram-staining-negative, non-flagellated and short rods (Fig. 1). Strain 172403-2^T could grow at 10-30°C, pH 5.0-9.0 without NaCl. Strain BT310^T could grow at 10-30°C, pH 5.0-9.0 and with 3% of NaCl. The differences between the novel isolates and the reference strains were provided in Table 1. Strain 172403-2^T could be differentiated from strain BT310^T based on several characteristics, such as growth at 3% of NaCl. Strain 172403-2^T could be differentiated from *P. chitinilyticus* 17gy-14 ^{T} on the basis of arginine dihydrolase, *α*-chymotrypsin, *α*-fucosidase, *β*-galactosidase (ONPG), *α*-glucosidase (starch hydrolysis), *β*-glucosidase, *α*-mannosidase enzyme activities and assimilation of D-glucose, D-maltose, D-mannose, D-mannitol and *N*-acetyl-D-glucosamine. Strain BT310^T could be differentiated from *P. populi* HLY7-15 ^{T} on the basis of acid phosphatase, *α*-chymotrypsin, cystine arylamidase, esterase (C8), naphtol-AS-BI-phosphohydrolase enzyme activities and assimilation of D-glucose and D-mannose. (Table 1).

Genome sequencing and Phylogenetic analysis

The genome size of strain 172403-2^T was 5,025,066 bp (genome coverage of 29.7X) and consisted of 4,192 coding sequences (CDSs) and 38 tRNA genes. DNA G+C content was 48.6 mol%. The obtained genome of strain 172403-2^T was submitted to the GenBank/EMBL/DDBJ under the accession

number NZ_JADQDR000000000. The genome size of strain BT310^T was 4,294,440 bp (genome coverage of 45.8X) and consisted of 3,618 coding sequences (CDSs), and 38 tRNA genes. DNA G+C content was 45.2 mol%. The obtained genome of strain BT310^T was submitted to the GenBank/EMBL/DDBJ under the accession number NZ_JAELXU010000000.

Based on 16S rRNA gene sequence similarity, strains 172403-2^T and BT310^T were affiliated with the family *Hymenobacteraceae* and showed high sequence similarities with the genus *Pontibacter*. Strain 172403-2^T was most closely related to *P. chitinilyticus* 17gy-14 ^{T} (95.7% 16S rRNA gene sequence similarity) and strain BT310^T was most closely related to *P. populi* HLY7-15 ^{T} (97.0% 16S rRNA gene sequence similarity). After the construction of neighbor-joining phylogenetic tree (Fig. 2), the strains each formed an independent cluster, which clearly showed that strains 172403-2^T and BT310^T belong to the genus *Pontibacter* and represent two novel species. The phylogenomic genomic tree (Fig. S1) based on UBCGs supported the 16S rRNA genes based phylogenetic tree.

The genome of the strains 172403-2^T and BT310^T contain genes associated with nitrogen metabolism, including denitrification. Both the strains 172403-2^T and BT310^T contain the key enzymes such as cytochrome nitrite reductase (EC:1.7.2.1) and Nitric-oxide reductase (EC 1.7.99.7) involved in the denitrification process (Xu at al. 2014, Philippon et al. 2021). Furthermore, genes associated with menaquinone biosynthesis are also identified in the genome, which is an essential component of the electron transfer pathway in prokaryotes (Dairi 2012).

Chemotaxonomic characterization

The total cellular fatty acids of strains 172403-2^T and BT310^T and their most related species were shown in Table 2. The predominant fatty acids of strain 172403-2^T were iso-$C_{15:0}$ (28.7%) and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B) (22.1%). The fatty acid profile of strain 172403-2^T was similar to those of the most closely related type strain but can be differentiated from closely related specie based on relatively high amounts of iso-$C_{15:0}$, iso-$C_{16:0}$3OH, iso-$C_{17:0}$ 3OH and small amount of $C_{14:1}$ ω5*c*, iso-$C_{15:1}$F (Table 2). The predominant fatty acids of strain BT310^T were iso-$C_{15:0}$ (29.5%) and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B) (20.9%). The fatty acid profile of strain BT310^T was similar to those of the most closely related type strain but can be differentiated from closely related species based on relatively high amounts of iso-$C_{15:1}$ G, iso-$C_{16:1}$ H, $C_{16:1}$ ω5*c*, $C_{17:1}$ ω6*c* and small amount of iso-$C_{17:0}$ 3OH (Table 3). The major polar lipid of strains 172403-2^T and BT310^T was phosphatidylethanolamine (PE). The total polar lipids profile of strain 172403-2^T showed phosphatidylethanolamine (PE), one aminolipid, one aminophospholipid, two glycolipids and two unknown polar lipids (Fig. S2). The total polar lipids profile of strain BT310^T showed phosphatidylethanolamine (PE), one aminolipid, one aminophospholipid, one glycolipid, two phospholipids, and six unknown polar lipids (Fig. S3). The major respiratory quinone of strains 172403-2^T and BT310^T was MK-7, which is common in the species of the genus *Pontibacter.*

Based on phenotypic, phylogenetic and biochemical characteristics, we concluded that strain 172403-2^T and strain BT310^T represent two novel species in the genus *Pontibacter*, for which the name *Pontibacter rubellus* and *Pontibacter situs* are proposed. The NCBI accession numbers for 16S rRNA sequences of the strains 172403-2^T and BT310^T are MW237669 and MT795756, respectively.

Description of *Pontibacter rubellus* sp. nov.

Pontibacter rubellus (ru.bel'lus. L. masc. adj. *rubellus* reddish).

The cells are Gram-stain-negative, non-flagellated and short rod-shaped. Colonies on R2A agar are convex, circular and red colored after 72 hours of growth at 25°C. Cells are around 0.4 µm wide and 0.5 µm long. Growth occurs at 10-30°C and pH 5.0-9.0 (optimum 7.0). Cells grow well on R2A agar, TSA, LB and NA but not on MAC agar. Oxidase and catalase activities are positive. In API 20NE test, strain 172403-2^T was positive for *β*-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), and *β*-galactosidase (PNPG). But negative for nitrate reduction, arginine dihydrolase, urease, D-glucose, L-arabinose, D-mannitol, D-maltose, gluconate, D-mannose, production of indole, production of acid from glucose, *N*-acetyl-D-glucosamine, caprate, adipate L-malate, citrate, and phenyl acetate. In API ZYM test, strain 172403-2^T was positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, and *N*–acetyl-*β*-glucosaminidase. weakly positive for esterase (C4), esterase (C8), cystine arylamidase, and *α*-galactosidase. But negative for lipase (C14), trypsin, *α*-chymotrypsin, *β*-galactosidase (ONPG) and *β*-glucuronidase, *α*-glucosidase (starch hydrolysis), *β*-glucosidase, *α*-mannosidase. and *α*-fucosidase. The major respiratory quinone is MK-7. The dominant cellular fatty acids are iso-$C_{15:0}$ and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B). The major polar lipid is phosphatidylethanolamine (PE).

The type strain for *Pontibacter rubellus*, 172403-2^T (=KCTC 62072^T = NBRC XXXX^T) was isolated from soil in Korea. The GenBank accession number for 16S rRNA gene sequence of strain 172403-2^T is MW237669.

Description of *Pontibacter situs* sp. nov.

Pontibacter situs (si.tus. L. masc. adj. *situs* soil).

The cells are Gram-stain-negative, non-flagellated and short rod-shaped. Colonies on R2A agar are convex, circular and red colored after 72 hours of growth at 25°C. Cells are around 0.9-1.1 µm wide and 2.2-2.4 µm long. Growth occurs at 10-30°C and pH 5.0-9.0. Cells grow well on R2A agar, TSA, LB and NA but not on MAC agar. Oxidase activity is negative and catalase activity is positive. In API 20NE test, strain BT310^T was positive for *β*-glucosidase (esculin hydrolysis). But negative for nitrate reduction, production of indole, production of acid from glucose, arginine dihydrolase, urease, *β*-galactosidase (PNPG), D-maltose, D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, adipate and L-malate. protease (gelatin hydrolysis), D-mannitol, gluconate, caprate, citrate and phenyl acetate. In API ZYM test, strain

BT310^T was positive for alkaline phosphatase, esterase (C4), esterase (C8), leucine arylamidase, valine arylamidase, and trypsin. But negative for lipase (C14), cystine arylamidase, *α*-chymotrypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase (ONPG), *β*-glucuronidase, *α*-glucosidase (starch hydrolysis), *β*-glucosidase, *N*–acetyl-*β*-glucosaminidase and *α*-mannosidase and *α*-fucosidase. The major respiratory quinone is MK-7. The dominant cellular fatty acids are summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B) and iso-$C_{15:0}$. The major polar lipid is phosphatidylethanolamine (PE).

The type strain for *Pontibacter situs*, BT310^T (=KCTC 72363^T = NBRC 114378^T) was isolated from soil in Korea. The GenBank accession number for 16S rRNA gene sequence of strain BT310^T is MT795756.

Declarations

Acknowledgments

This research was supported by a research grant from Seoul Women's University (2021), the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (No. 2021R1F1A1061389) and by the grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202002203).

Author's contributions

All authors equally contributed in this work.

Compliance with ethical standards

Conflict of interest

All authors certify that there is no conflict of interest.

References

1. Cappuccino JG, Sherman N (2002) Microbiology A laboratory manual, 6th edn. Pearson Education, Inc. Benjamin Cummings, California
2. Dairi T (2012) Menaquinone biosyntheses in microorganisms. Methods Enzymol 515:107–122
3. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
4. Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high performance liquid chromatography and photodiode array detection. J Gen Appl Microbiol 42:457–469
5. Kang JY, Joung Y, Chun J, Kim H, Joh K, Jahng KY (2013) *Pontibacter saemangeumensis* sp. nov., isolated from seawater. Int J Syst Evol Microbiol 63:565–569

6. Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207
7. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549
8. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
9. Na SI, Kim YO, Yoon SH, Ha SM, Baek I et al (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280–285
10. Nedashkovskaya OI, Kim SB, Suzuki M et al (2005) *Pontibacter actiniarum* gen. nov., sp. nov., a novel member of the phylum '*Bacteroidetes*', and proposal of *Reichenbachiell*a gen. nov. as a replacement for the illegitimate prokaryotic generic name *Reichenbachia Nedashkovskaya* et al. 2003. Int J Syst Evol Microbiol 55:2583–2588
11. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R (2014) The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. D206-14
12. Park S, Park J, Lee KH, Yoon J (2016) *Pontibacter litorisediminis* sp. nov., isolated from a tidal flat. Int J Syst Evol Microbiol 66:4172–4178
13. Philippon T, Tian J, Bureau C, Chaumont C, Midoux C et al (2021) Denitrifying bio-cathodes developed from constructed wetland sediments exhibit electroactive nitrate reducing biofilms dominated by the genera *Azoarcus* and *Pontibacter*. Bioelectrochemistry 140:107819
14. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Bio Evol 4:406–425
15. Sasser M (1990) Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. In: MIDI Technical Note 101. MIDI Inc, Newark
16. Singh AK, Garg N, Lal R (2015) *Pontibacter chinhatensis* sp. nov., isolated from pond sediment containing discarded hexachlorocyclohexane isomer waste. Int J Syst Evol Microbiol 65:2248–2254
17. Smibert RM, Krieg NR (1981) General characterization. Manual of methods for general bacteriology. American Society for Microbiology, Washington DC, pp 409–442
18. Srinivasan S, Lee JJ, Lee S, Kim MK (2014) *Pontibacter humi* sp. nov., isolated from mountain soil. Current Microbiol 69:263–269
19. Tatusova T, DiCuccio M, Badretdin A et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624
20. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44(14):6614–6624

21. Weisburg WG, Barns SM, Pellerier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703
22. Xu L, Zeng XC, Nie Y, Luo X, Zhou E, Zhou L, Pan Y, Li W (2014) *Pontibacter diazotrophicus* sp. nov., a novel nitrogen-fixing bacterium of the family *Cytophagaceae*. PLoS One 9:e92294
23. Zhang L, Zhang Q, Luo X, Tang Y, Dai J, Li Y, Wang Y, Chen G, Fang C (2008) *Pontibacter korlensis* sp. nov., isolated from the desert of Xinjiang, China. Int J Syst Evol Microbiol 58:1210–1214

Tables

Table 1. Differential characteristics of strains 172403-2^{T}and BT310^T and closely related species

Taxa: 1, strain 172403-2^T; 2, strain BT310^T; 3, *P. chitinilyticus* 17gy-14 ^{T}; 4, *P. populi* HLY7-15^T. Data of reference strain was obtained from previous studies (Chhetri et al.2019 and Xu et al. 2012).

+, positive; -, negative; w, weak positive.

Characteristic	1	2	3	4
Growth at 1% NaCl	-	+	+	+
Enzyme activity				
N–Acetyl-*β*-glucosaminidase	+	-	+	-
Acid phosphatase	+	-	+	+
Arginine dihydrolase	-	-	+	-
α-Chymotrypsin	-	-	+	+
Cystine arylamidase	w	-	+	+
Esterase (C4)	w	+	+	+
Esterase (C8)	w	+	+	-
α-Fucosidase	-	-	+	-
α-Galactosidase	w	-	+	-
β-Galactosidase (ONPG)	-	-	+	-
α-Glucosidase (starch hydrolysis)	-	-	+	-
β-Glucosidase (Esculin hydrolysis)	+	w	+	-
β-Glucosidase	-	-	+	-
α-Mannosidase	-	-	+	-
Naphtol-AS-BI-phosphohydrolase	+	-	+	+
Trypsin	-	+	+	+
Urease	-	-	+	-
Assimilation				
D-Glucose	-	-	+	+
D-Maltose	-	-	+	-
D-Mannose	-	-	+	+
D-Mannitol	-	-	+	-
N-Acetyl-D-glucosamine	-	-	+	-

Table 2. Cellular fatty acid profiles of strains 172403-2^{T}and BT310^T and closely related species Taxa: 1, strain 172403-2^T; 2, strain BT310^T; 3, *P. chitinilyticus* 17gy-14 ^{T}; 4, *P. populi* HLY7-15^T. Data of reference

strain was obtained from previous studies (Chhetri et al. 2019 and Xu et al. 2012). TR, trace (<1%); ND, not detected.

Fatty acids

	1	2	3	4	
Saturated					
13:0 iso 3OH	ND	ND	ND		1.0
14:0	TR	TR	1.0	ND	
15:0 iso	28.7	29.5	20.9		16.5
15:0 anteiso	4.5	2.4	11.1	ND	
15:0 iso 3OH	3.1	5.5	ND	1.8	
16:0	TR	TR	TR	9.1	
16:0 iso	2.4	ND	ND	4.3	
16:0 3OH	TR	1.0	ND	ND	
17:0	1.2	TR	1.5	2.0	
17:0 iso	5.6	TR	4.5	2.6	
17:0 anteiso	2.3	ND	3.6	ND	
17:0 iso 3OH	9.6	6.8	ND	11.0	
18:0	ND	ND	ND	7.9	
Unsaturated					
14:1 ω 5c	ND	ND	5.0	ND	
15:1 iso F	ND	ND	1.8		1.1
15:1 iso G	TR	1.2	ND	ND	
15:1 *ω 6c*	TR	1.7	ND		1.4
16:1 iso H	2.0	4.5	2.6		1.7
16:1 *ω* 5*c*	4.3	6.3	2.2		2.2
17:1 ω6c	6.3	6.2	2.1		3.5
18:1 ω 9c	ND	ND	ND		1.7
19:0 cyclo ω 8c	ND	ND	1.7	ND	

Hydroxy

13:0 iso 3OH	ND	ND	ND		1.0

15:0 iso 3OH	ND	ND		2.5	ND	
Summed Feature 1 (15:1 iso H / 13:0 3OH)	TR	1.6	ND			1.5
Summed Feature 3 (16:1 *ω 6c* / 16:1 ω 7c)	2.2	3.6		5.3		2.4
Summed Feature 4 (17:1 iso I / 17:1 anteiso B)	22.1	20.9		26.2		18.5
Summed Feature 8 (18:1 *ω 7c* / 18:1 ω 6c)	ND	TR	ND			3.3

Table 3 is not available with this version

Figures

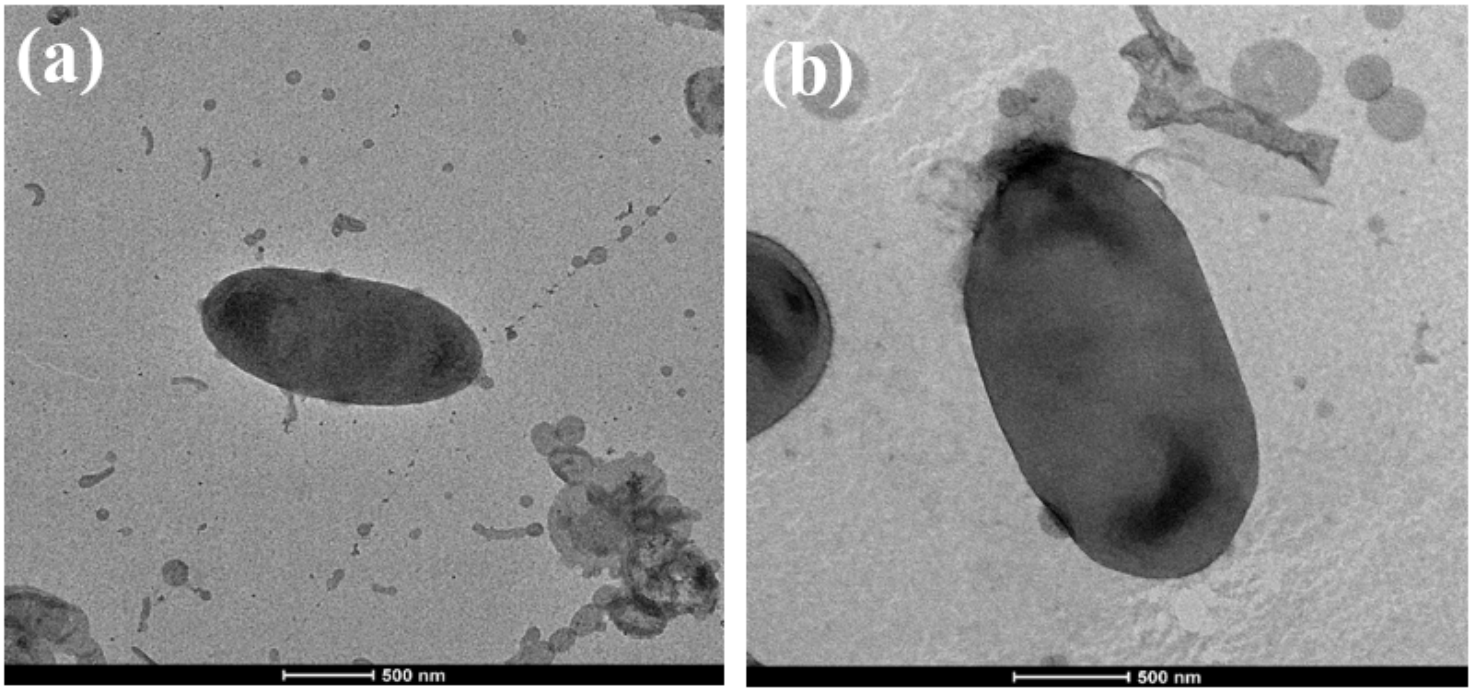

Figure 1

Transmission electron micrographs of strains 172403-2T (a) and BT310T (b).

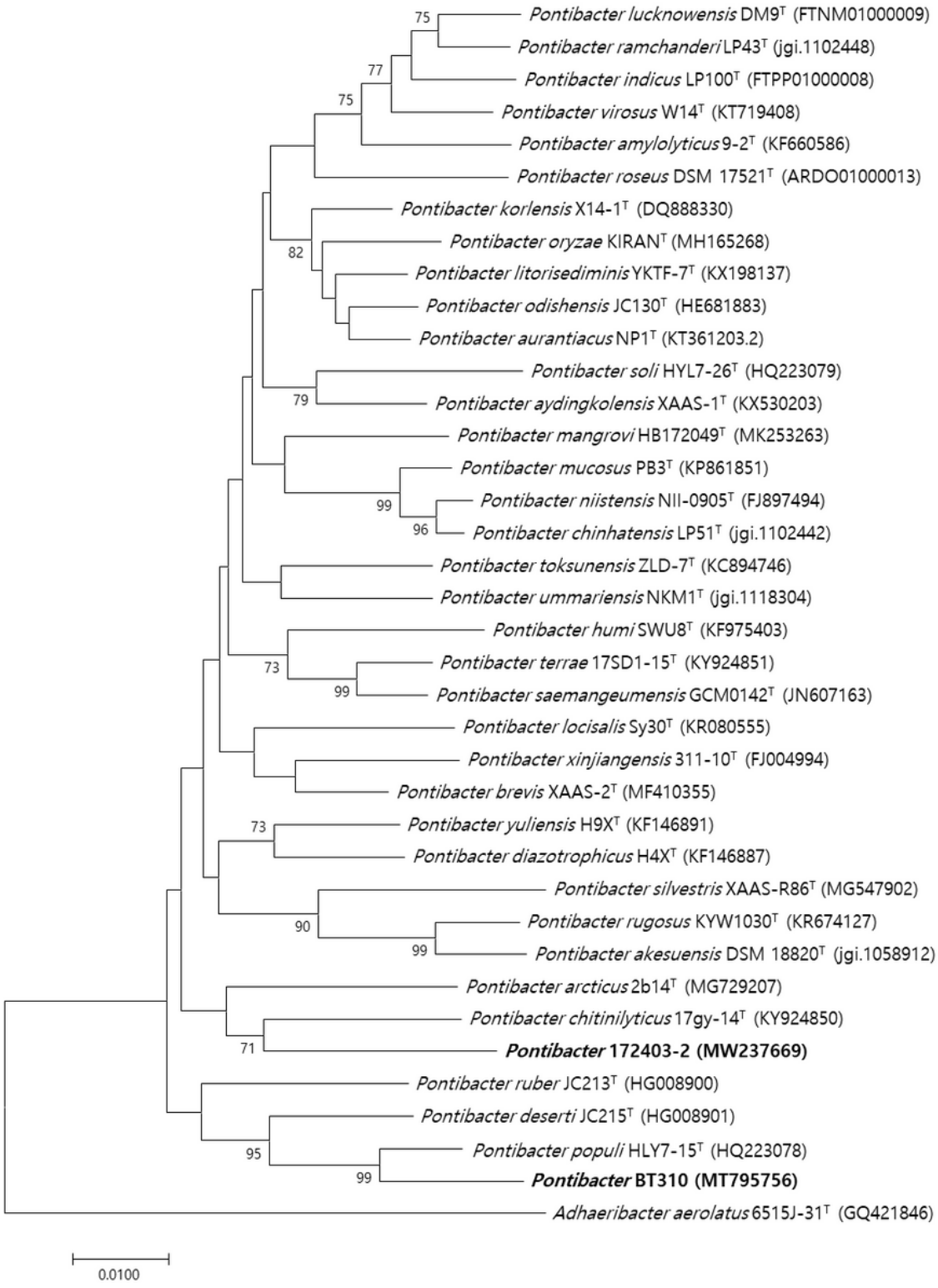

Figure 2

Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strains 172403-2T and BT310 T and other species of the genus Pontibacter. Numbers at nodes are bootstrap percentages (>70 %) based on the Neighbour-joining algorithms. Adhaeribacter aerolatus 6515J-31T was used as outgroup. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.docx