Hymenobacter erythromyxa sp. nov., and *Hymenobacter radioresistens* sp. nov., Gamma and UV radiation resistant bacteria isolated from a Gamma ray irradiated soil

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Abstract

Two Gram-negative, gamma and UV tolerant bacterial strains, DG25A^T and DG25B^T, were isolated from soil samples exposed to 3kGy gamma radiation collected in Seoul, Korea. The cells grown on R2A agar at 25 °C were pink to red in colour. The phylogenetic analysis based on 16S rRNA gene sequence revealed that the strains belong to the genus *Hymenobacter.* The closest degrees of 16S rRNA gene sequence similarities to these strains, DG25A^T and DG25B^T, were found with *Hymenobacter tibetensis* NRRL B-51271^T (96.5% and 96.6%, respectively). The multilocus sequence analysis (MLSA) tree based on the 14 housekeeping genes suggested that these two strains are distinct from other *Hymenobacter* species and represent novel candidates. The G+C contents of genomic DNA of DG25A^T and DG25B^T are 57.2 and 56.8 mol%, respectively. Chemotaxonomic data revealed that the strains contain iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ *ω5c*, summed feature3 ($C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*), and summed feature4 (anteiso B-$C_{17:1}$ / iso I- $C_{17:1}$) as the major fatty acids, and major polar lipid for both strains was phosphatidylethanolamine (PE). In addition, the strains showed resistance to gamma and UV radiation. Based on these data, each of strains DG25A^T and DG25B^T should be classified as a novel species, for which the names *Hymenobacter erythromyxa* sp. nov. (DG25A^T =KCTC 32451^T= JCM 19445^T) *and Hymenobacter radioresistens* sp. nov., are proposed (DG25B^T = KCTC 32452^T = JCM 19444^T).

Introduction

The genus *Hymenobacter* was first described by Hirsch et al. (Hirsch et al., 1998) belonging to the family *Cytophagaceae* of the phylum *Bacteriodetes*. The *Hymenobacter* species contain predominant amounts of branched fatty acids (iso and anteiso-types) in the cell membranes, phosphatidylethanolamine as major polar lipid, and high G + C contents of genomic DNA (55–65 mol%) (Buczolits et al., 2006). At the time of writing, the genus *Hymenobacter* contained 94 species with validated names (http://www.bacterio.net/hymenobacter.html). In addition, several *Hymenobacter* species, *H. actinosclerus* (Collins et al., 200), *H. tibetensis* (Dai et al., 2009) and *H. xinjiangensis* (Zhang et al., 2007) were reported to show resistance to gamma and UV radiation. In this study, two novel gamma and UV radiation resistant strains were characterised by a polyphasic taxonomic approach, including phenotypic, genotypic, and chemotaxonomic methods. Based on the results, the strains were proposed as novel species in the genus of *Hymenobacter*.

Materials And Methods

Organism and culture conditions

Two novel radiation-resistant strains, DG25A^T and DG25B^T, were isolated from a soil sample irradiated with 3 kGy gamma radiation via plating onto R2A agar (Difco) and incubated at 25°C for 7 days. The soil sample was collected in Seoul (GPS; N 37° 33' 30" E 127° 00' 01"), 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. Colonies were purified by sub-culturing and preserved in a glycerol solution (20%, w/v) at -70°C.

Morphology, Physiology and Biochemical analysis

Cells were grown on R2A agar at 25°C for 3 days for morphological observation. The cell morphology and motility were examined with light microscopy and transmission electron microscopy (TEM, Call Zeiss LIBRA 120). The Gram reaction was performed using the non-staining method as described by Buck (Buck, 1982). Pigments were extracted using 95% ethanol, and then the absorption spectrum between 300 and 700 nm was measured with a UV spectrophotometer (UV-2450, Shimazu) (Gosink et al., 1998). Flexirubin-type pigments were analysed based their color shift after exposure to a KOH solution. Oxidase and catalase activity was determined by the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine and by assessing bubble formation with 3% (v/v) hydrogen peroxide solution, respectively. API 20NE and API 50CH test panels (bioMérieux) were employed for other physiological and biochemical analysis. API ZYM test system was employed according to the recommendations of the manufacturer (bioMérieux) for studying enzyme activities of the strains. Growth on various standard bacteriological media was assessed using trypticase soy agar (TSA, Difco), Luria-Bertani agar (LB, Difco), nutrient agar (NA, Difco), and R2A agar (Difco). Growth at different temperatures (4, 15, 20, 25, 30, 37, and 42 ˚C) and NaCl concentration (0, 1, 2, 3, 4, 5, and 10%; w/v) were determined on R2A agar (Difco) for 3 days. Growth at various pHs (4, 5, 6, 7, 8, 9, 10, and 11) was assessed in R2A broth (MB Cell) at 25 °C.

Phylogenetic analysis and genome sequencing

The 16S rRNA genes of the strains were amplified using a PCR with universal bacterial primer set, 9F, 785F, 800R, and 1492R (Weisburg et al., 1991), and sequenced by SolGent. Inc. (Daejeon, Korea). The complete 16S rRNA sequences were compiled with SeqMan software (DNASTAR Inc.) and compared with those of related taxa using the EzTaxon-e (http://eztaxon-e.ezbiocloud.net). The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) and maximum-parsimony method (Fitch, 1971) in MEGA 5 program (Tamura et al., 2011) after editing with the BioEdit program (Hall, 1991) and multiple alignments of the data by the CLUSTAL_X program (Thompson et al., 1997). Furthermore, the Kimura two-parameter model was used to calculate pairwise distances (Kimura and Suzuki, 1987) and bootstrap values were calculated with 1,000 replicates to obtain confidence levels for the branches (Felsenstein, 1985).

Multilocus sequence analysis (MLSA) tree was constructed using the 14-housekeeping gene (*gyr*A, *gyr*B, *rec*A, *rpo*A, *rpo*B, *atp*A, *atp*B, *atp*C, *atp*D, *atp*E, *gap*A, *eno*, *pfk*A, and *pyk*A) sequences in this study and those retrieved from NCBI GenBank. The ML phylogenetic tree based on the concatenated sequence of 14 housekeeping genes (20,346bp) was constructed as described above. Average nucleotide identity (ANI) was used to calculate the genomic similarities between the strains (Richter and Rossello-Mora, 2009). The complete genome sequence of strains DG25A^T (GenBank accession no. CP012623) and DG25B^T

(GenBank accession no. CP010054), which were published previously (Kim et al., 2015ª; Kim et al., 2015b), were used for ANI-MUMmer (ANIm) and ANI-Blast (ANIb) calculations with the JSpecies Web Service (JSpecies WS; http://jspecies.ribohost.com/jspeciesws/) (Richter and Rossello-Mora, 2009).

Genomic DNA was extracted and purified with the Genomic-tip system 100/G (QIAGEN, Japan). DNA-DNA hybridisation was performed following the method prescribed in Ezaki et al. (Ezaki et al., 1989). For determination of the G+C content of DNA, genomic DNA was degraded enzymatically and analysed using reverse-phase HPLC as previously described (Mesbah et al., 1989; Tamaoka and Komagata, 1984).

Chemotaxonomic characterisation

For cellular fatty acid methyl ester analysis, cells were allowed to grow on R2A agar at 25 °C for 3 days. Analysis was performed according to the Sherlock Microbial Identification System (MIS) (Sasser, 1990). Cellular polar lipids were extracted according to the procedures described by Minnikin*et al*. (Minnikin et al., 1984) and were identified by two-dimensional thin-layer chromatogram. To produce the TLC chromatograms, we used chloroform/methanol/water (65:25:4, v/v/v) for the first mobile dimension, and chloroform/methanol/acetic acid/water (80:12:15:4, v/v/v/v) for the second mobile dimension. The four different type polar lipids were detected and compared by spraying the appropriate four detection reagents (Minnikin et al., 1984; Komagata and Suzuki, 1987); namely: molybdophosphoric acid solution (to show total lipids), ninhydrinsolution (to appear aminolipids), α-naphthol (to show glycolipids), and molybdenum blue (to show phospholipids).

Radiation resistance test

To determine the resistant survival rate to gamma radiation, cells grown in TGY (1% tryptone, 0.1% glucose and 0.5% yeast extract) broth or agar. Cultures grown to the early stationary phase were divided into 1 ml aliquots without change of broth and were exposed to a cobal-60 gamma irradiator on ice. The source strength was approximately 100 kCi at 70 Gy min^{-1} dose rate, and the actual doses were within 2% of the target dose. Irradiated cells were diluted, plated in triplicate on TGY agar plates and incubated for 3 days before survivors were scored*. Deinococcus radiodurans* $R1^T$ (=DSM 20539^T) (Brooks and Murray, 1981; White et al., 1999) and *Escherichia coli* $K12^T$ (=KCTC 1116^T) (Kämpfer et al., 2008) cells used as positive and negative control strains for the experiment (Lim et al., 2006; Im et al., 2008; Lim et al., 2012).

To measure ultraviolet radiation resistance, the early stationary phase cells grown (~10^8 CFU/ml) on TGY medium was exposed to different doses of ultraviolet radiation (Im et al., 2013; Selvam et al., 2013). The cells treated as described below, serially diluted with 0.85 % NaCl, spotted on TGY agar plates, and incubated at 25 °C for 3 days prior to the enumeration of colonies. Cells were placed on TGY agar plate (with the lids off) and irradiated at room temperature using a UVC ultraviolet cross-linker (UVP, CX-2000, CA, USA) at 254 nm. The applied dose rate was 20 $J/m^2/s$, and the different radiation doses were achieved by adjusting the total exposure times.

Results

Morphology, Physiology and Biochemical analysis

Cells of strains DG25A^T and DG25B^T were Gram-negative, aerobic, non-flagella rods (Fig. 3), and pink to red colored when cultured on R2A agar at 25°C. Strain DG25B^T produced red pigments with absorbance spectral peaks at 318.0, 482.5 and 504.0 nm, and the strain DG25B^T produced peaks at 304.0, 480.5 and 496.0 nm; these spectra were similar to those of *H. tibetensis* NRRL B-51271^T (Fig. S1). Cells were able to grow at a temperature range of 10-30°C, but not at 4C°, 37C°, and 42C°. The optimum growth temperature was 25 °C. The strain DG25B^T tolerated up to 1% NaCl, but the strain DG25A^T did not grow in 1% NaCl. Cells grew weakly on NA but not on LB and TSA. The results regarding the physiological characteristics of strains DG25A^T and DG25B^T are summarised in the species description below, and a comparison of differential characteristics with type strain of closely related species is shown in Table 1.

Phylogenetic and genome sequence analysis

The complete 16S rRNA gene sequences of the strains were stretches of 1457 and 1455 nucleotides, and showed a high level of similarity (99.5%) to each other, but the morphological and phenotypical characterisation showed several distinguishable characters. Strain DG25A^T produced reddish and mucoid colonies, but strain DG25B^T pink-red with dry colonies. The phylogenetic trees based on the 16S rRNA gene and MLSA genes with concatenated sequence of the housekeeping gens showed that, the strains are placed within the *Hymenobacter* genus with high bootstrap support (Figs. 1 and 2). The calculated ANI value between strains DG25A^T and DG25B^T were 88.95% (ANIb) and 90.23% (ANIm), which were below the threshold values for species delineation (Minnikin et al., 1984), which also confirmed by the low levels of DNA-DNA hybridisation values between the stains DG25A^T and DG25B^T is 52±2% (reciprocal 56±3%). Based on the low booststrap value (79%) of 16S rRNA phylogenetic tree (Fig. 2), ANI values the strains evidently belong to two different novel species in the genus *Hymenobacter* (Table S1). The highest degrees of sequence similarities of the strains DG25A^T and DG25B^T were found to be *Hymenobacter tibetensis* NRRL B-51271^T (96.5% and 96.6% 16S rRNA sequence similarity, respectively), and the closest degrees of sequence similarity to another genus found to be *Adhaeribacter terreus* DNG6^T (Zhang et al., 2009) (90.3% and 90.4%, respectively).

The genome length of strain DG25A^T was 3,777,136 bp (136.0×) and consisted of 2,991 protein-coding genes and 49 RNA genes including 6 rRNA genes and 43 tRNA genes. The genome length of strain DG25B^T was 3,874,646 bp (50.0×) and consisted of 3,153 protein-coding genes and 49 RNA genes including 6 rRNA genes and 43 tRNA genes. The G+C content of genomic DNA from strains DG25A^T and DG25B^T were 57.2 and 56.8 mol%, within the range given for the genus *Hymenobacter*.

Chemotaxonomic characterisation

The major cellular fatty acids of the strains DG25A^T and DG25B^T were iso-$C_{15:0}$ (14.0% and 12.9%, respectively), anteiso-$C_{15:0}$ (11.7% and 12.1%), $C_{16:1}$ *ω5c* (15.3% and 13.7%), summed feature 3 ($C_{16:1}$ *ω6c/ ω7c*) (12.0% and 16.4%), and summed feature 4 (iso I -$C_{17:1}$ /anteiso B) (13.4% and 14.2%), which are predominant in most *Hymenobacter* species (Chunga et al., 2010). The minor cellular fatty acids of the strains DG25A^T and DG25B^T were iso-$C_{14:0}$, iso-$C_{15:0}$ 3OH, $C_{16:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$, $C_{17:0}$ 2OH, iso-$C_{17:0}$ 3OH, iso G-$C_{15:1}$, iso H -$C_{16:1}$, and $C_{17:1}$ *ω6c*. However, some qualitative and quantitative differences in the fatty acid composition were observed between the novel strains and *H. tibetensis* NRRL B-51271^T. For example, in contrast to *H. tibetensis* NRRL B-51271^T, the strains DG25A^T and DG25B^T had larger amounts of iso-$C_{16:0}$, iso H-$C_{16:1}$, and summed feature 4 (iso I-$C_{17:1}$ /anteiso B) and contained smaller amounts of iso-$C_{15:0}$, iso G -$C_{15:1}$, and $C_{16:1}$ *ω5c* (Table. 2). The major polar lipids of the strain DG25B^T were phosphatidylethanolamine (PE), unidentified aminophospholipids, unidentified aminolipids, and unidentified polar lipids (Fig. S2), similar to those of other *Hymenobacter* species (Dai et al., 2009; Chunga et al., 2010). The major polar lipids of strains DG25A^T and DG25B^T are identical but some minor unidentified polar lipids are absent in the strain DG25A.

Radiation resistance test

The strains DG25A^T and DG25B^T also appeared to be tolerant to gamma radiation and UV radiation. At the lowest Gamma radiation dose tested (3 kGy), *E. coli* did not survive, whereas the strains DG25A^T, DG25B^T and *D. radiodurans* R1^T showed high resistance (83%, 80% and 88% survival, respectively). At 9 kGy gamma radiation, the strains DG25A^T and DG25B^T showed 30% and 43% survival; comparable to 68% survival of *D. radiodurans* R1^T (Fig. S3a) and formed the characteristic sigmoidal survival (shoulder) curves for resistance to gamma-radiation. At the lowest UV dose tested (400 Jm^{-2}), *E. coli* did not survive, whereas 43% and 39% survival were observed in the strains DG25A^T and DG25B^T (50% for *D. radiodurans* R1^T); and after exposure to 800 Jm^{-2} UV doses, 0.8% and 0.4% of the strains DG25A^T and DG25B^T survived (Fig. S3b).

Conclusion

The polyphonic taxonomic analysis results indicate that strains DG25A^T and DG25B^T are members of the genus *Hymenobacter*, as they have the typical features of genus *Hymenobacter* with the presence of major fatty acids, polar lipids, and genomic DNA G + C mol% range. The strains DG25A^T and DG25B^T could be distinguished from the most closely related type strain of *H. tibetensis* NRRL B-51271^T by carbon-source fermentation, enzyme production, and fatty acid composition. In addition, the strains have resistance to gamma radiation-resistant most similar with *D. radiodurans* R1^T, the first species showed such a higher degree of resistance in the genus *Hymenobacter.* Based on the phenotypic, phylogenetic, genomic, and chemotaxonomy properties, strains DG25A^T and DG25B^T are considered to represent two new species within the genus *Hymenobacter*, for which the names *Hymenobacter erythromyxa* sp. nov., and *Hymenobacter radioresistens* sp. nov., was proposed.

Description of Hymenobacter erythromyxa **sp. nov.**

Hymenobacter erythromyxa (Gr. adj. *eruthros*, red; Gr. n. *muxa*, mucus, slime; N.L. n. *erythomyxa*, the red slime).

Cells are Gram-negative, aerobic, non-motile, and rod-shaped. Growth after 3days on R2A agar at 10–30°C (optimum 25–30°C). Unable to grow at 5, or 42°C. Colonies on R2A agar are red-pigmented and mucoid. Growth occurs at pH 6–7. Cells grew weakly on NA but not on LB and TSA. Oxidase and catalase activity are positive. Glucose fermentation and indole production are negative. Nitrate reduction is positive without the production of N_2. Resistant to UV irradiation and gamma radiation.

In the API ZYM system, positive for acid phosphatase, alkaline phosphatase, *α*-chymotrypsin, cysteine arylamidase, esterase C4, esterase C8, *α*-glucosidase, leucine arylamidase, naphtol-AS-BI-phosphohydrolase, trypsin, *N*–acetyl-*β*-glucosaminidase, and valine arylamidase; but trypsin showed week reaction. Negative activies were recored for *α*-fucosidase, *α*-galactosidase, *β*-galactosidase (ONPG), *β*-glucosidase, *β*-glucuronidase, lipase C14, and *α*-mannosidase. In the API 20NE and 50CH systems, esculin and 5-ketogluconate was assimilated and the remaining were negative. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ *ω5c*, summed feature 3 ($C_{16:1}$ *ω6c*/*ω7c*), and summed feature 4 (iso I-$C_{17:1}$ /anteiso B). The major polar lipid is phosphatidylethanolamine. The G + C content of genomic DNA of the type strain is 57.2 mol%.

The type strain DG25A^T (KCTC 32451^T = JCM 19445^T) was isolated from a soil sample collected in Seoul, Korea.

Description of Hymenobacter radioresistens **sp. nov.**

Hymenobacter radioresistens (N.L. pref. radio- (from L. n. radius, ray, beam), pertaining to radiation; L. part. adj. resistens, resisting; N.L. masc. part. adj. *radioresistens*, ray-resisting, because of high resistance to gamma-ray irradiation).

Cells are Gram-negative, aerobic, non-motile, and rod-shaped. Growth after 3 days on R2A agar at 10–30°C (optimum 25–30°C). Unable to grow at 5, or 37, °C. Colonies on R2A agar are pink pigmented, smooth, and mucoid. Growth occurs at pH 6–7. Cells grew weakly on NA but not on LB and TSA. Oxidase and catalase activity are positive. Glucose fermentation and indole production are negative. Nitrate reduction is positive without production of N_2. Resistant to UV irradiation and gamma radiation.

In the API ZYM system, positive for acid phosphatase, alkaline phosphatase, cysteine arylamidase, esterase C4, esterase C8, *α*-glucosidase (starch hydrolysis), leucine arylamidase, naphtol-AS-BI-phosphohydrolase, trypsin, *N*–acetyl-*β*-glucosaminidase, and valine arylamidase; but negative for *α*-chymotrypsin, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase (ONPG), *β*-glucosidase, *β*-glucuronidase, lipase C14, and *α*-mannosidase. In the API 20NE and 50CH systems, positive for fermentation of D-adonitol, amidon, L-arabinose, D-cellobiose, esculin ferric citrate, D-galactose, gentiobiose, D-glucose,

glycogen, 5-ketogluconate (potassium), D-mannose, D-raffinose, D-saccharose (sucrose), and D-trehalose; negative for *N*-acetyl-glucosamine, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, gluconate (potassium), glycerol, L-histidine, inositol, inulin, 2-ketogluconate (potassium), D-lactose, D-lyxose, maltose, D-mannitol, mehtyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, D-melezitose, methyl-β-D-xylose, D-melobiose, L-rhamnose, D-ribose, D-sorbitol, salicin, L-sorbose, D-tagatose, D-turanose, xylitol, D-xylose, and L-xylose. The major polar lipid is phosphatidylethanolamine. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ $\omega 5c$, summed feature 3 ($C_{16:1}\omega 6c/\omega 7c$), and summed feature 4 (iso I-$C_{17:1}$ /anteiso B). The G + C content of genomic DNA of the type strain is 56.8 mol%.

The type strain DG25B^T (= KCTC 32452^T = JCM 19444^T) was isolated from a soil sample collected in Seoul, Korea.

Declarations

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Tables

Table 1. Differential characteristics between strains DG25A^T, DG25B^{T}andclosely related species.

Characteristics	1	2	3
Growth at/with			
10 ˚C	+	+	-
1% NaCl	-	+	w
on Nutritent agar	w	w	+
Enzyme activities (API ZYM)			
α-Chymotrypsin	+	-	w
Esterase (C4)	+	+	-
Esterase (C8)	+	+	-
α-Galactosidase	-	-	+
β-Galactosidase (ONPG)	-	-	+
β-Glucosidase	-	-	+
Leucinearylamidase	+	+	-
Trypsin	w	+	+
Production of acid (API 50CH)			
L-Arabinose	-	+	-
D-Cellobiose	-	+	-
Esculin	+	+	-
D-Galactose	-	+	-
Gentiobiose	-	+	-
Glycogen	-	+	-
D-Lactose	-	-	+
Maltose	-	-	+
D-Mannose	-	+	-
D-Raffinose	-	+	-
Starch	-	+	-
D-Sucrose	-	+	-
D-Trehalose	-	+	-
5-Ketogluconate	+	+	-

D-Adonitol (Ribitol)	-	+	-
Xylitol	-	-	+
L-Histidine	-	-	+
DNA G+C content (mol%)	57.2	56.8	55.8

Strains: (1) DG25A^T, (2) DG25B^T, (3) *Hymenobacter tibetensis* NRRL B-51271^T

All data were from this study except for the DNA G+C contents of *H. tibetensis* NRRL B-51271^T (Dai et al., 2006). All strains grew at10-30˚C on R2A agar, but did not grow on 4˚C, and 42˚C.

All strains produced *N*–acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, cystinearylamidase, naphtol-AS-BI-phosphohydrolase, and valinearylamidase. They did not produce *α*-fucosidase, *β*-glucuronidase, lipase (C14), and *α*-mannosidase. +, positive; w, weak positive; -, negative.

Table 2. Cellular fatty acid profiles of strains DG25A^T, DG25B^T and a closely related reference strain *H. tibetensis* NRRL B-51271^T.

Fatty acids	1	2	3
Saturated			
15:0 iso	14.0	12.9	25.3
15:0 anteiso	11.7	12.1	11.7
15:0 iso 3OH	1.4	1.7	Tr
16:0	1.5	1.0	Tr
16:0 iso	5.1	5.8	1.8
17:0 iso	5.0	2.2	4.6
17:0 anteiso	4.6	2.4	Tr
17:0 2OH	tr	1.1	ND
17:0 iso 3OH	3.4	2.8	1.1
Unsaturated			
15:1 iso G	tr	tr	2.9
16:1 iso H	5.4	7.3	Tr
16:1 *ω* 5*c*	15.3	13.7	22.4
17:1 *ω 6c*	1.1	1.3	ND
†Summed Feature 3 (16:1 *ω*7*c* / 16:1 *ω*6*c*)	12.0	16.4	14.6
†Summed Feature 4 (17:1 iso I / 17:1 anteiso B)	13.4	14.2	8.2

Strains: (1) DG25A^T, (2) DG25B^T, (3) *H. tibetensis* NRRL B-51271^T

All strains were grown on R2A at 25 °C for 3 days. Values are percentages and only fatty acids accounting for more than 1% from one of the strains are indicated. For unsaturated fatty acids, the position of the double bond was located by counting from the methyl(*ω*) end of the carbon chain. The *cis* isomers were indicated by the suffixes *c*.

†Summed feature contained two fatty acids, which could not separated by GLC with the MIDI system.

Abbreviations: ND, not detected; tr, trace (<1.0%)

Figures

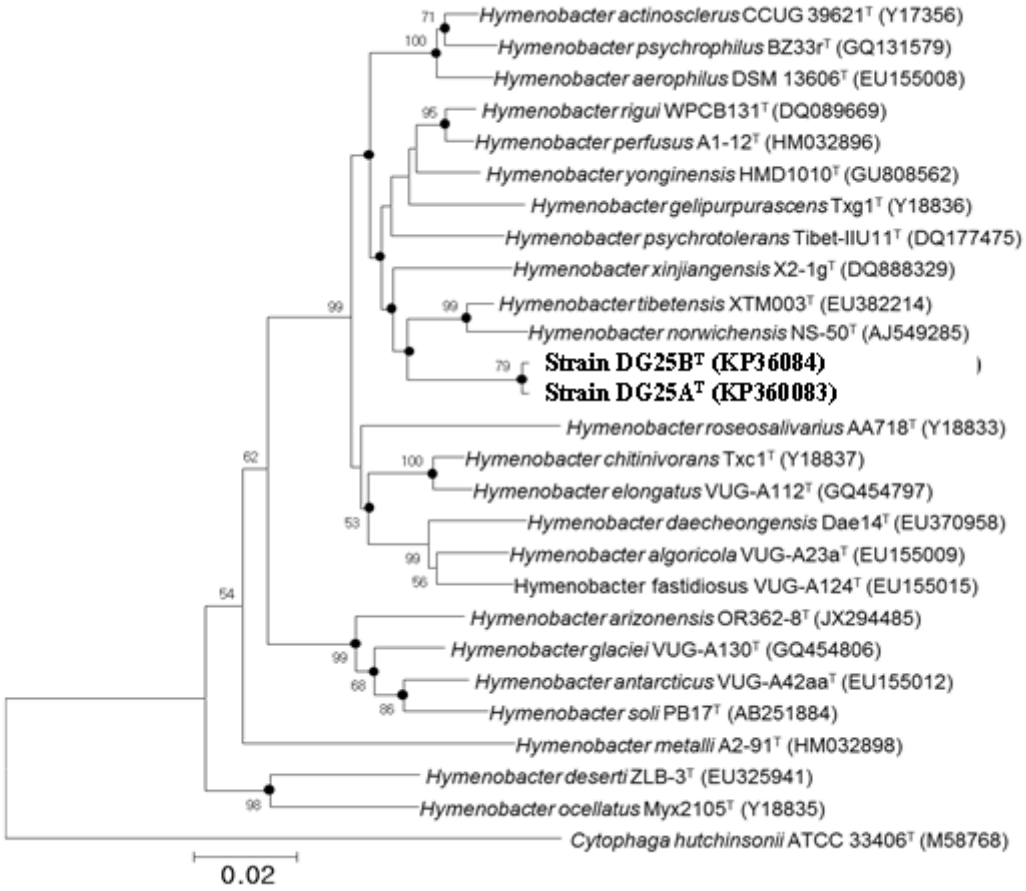

Figure 1

Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences of strain DG25A^T, DG25B^T and related taxa. The scale bar represents 0.02 substitutions per nucleotide position. Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are shown at the branch points. Filled circles indicate the common nodes recovered from the maximum-parsimony algorithm.

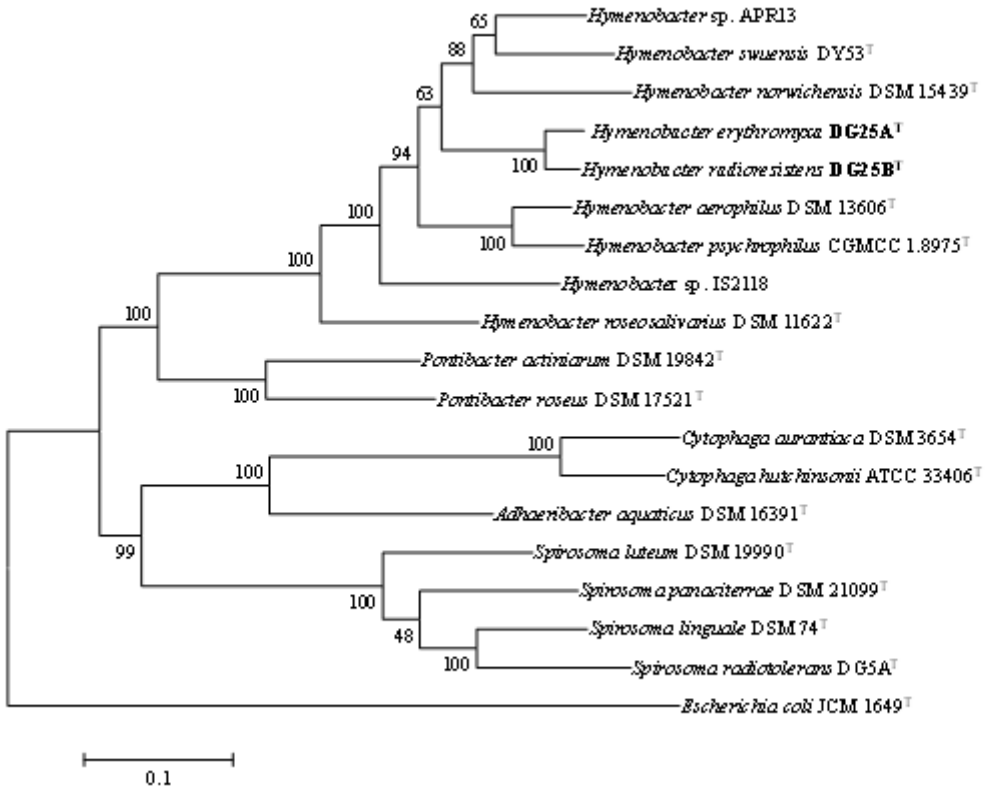

Figure 2

Maximum-likelihood phylogenetic tree based on concatenated sequence of 14 housekeeping genes (20,346bp) indicates the phylogenetic position of the isolates DG25A^T, DG25B^T and other related taxa.

The scale bar represents 0.01 substitutions per nucleotide position. Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are shown at the branch points. Filled circles indicate the common nodes recovered from the maximum-parsimony algorithm.

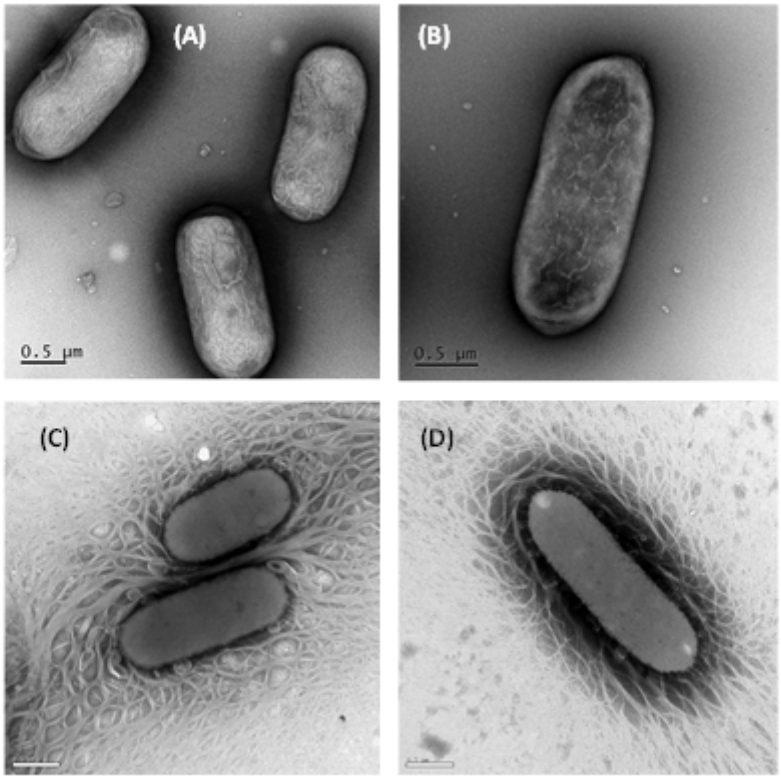

Figure 3

Transmission electron microscopy of strains DG25A^T (A and B) and DG25B^T (C and D) grown on R2A agar for 3 days, negatively stained with phosphotunstic acid (PTA). Bar = 0.5 m.

Supplementary Files

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- SupplementryMaterial.docx