Andreprevotia lacus sp. nov., isolated from a fish-culture pond

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A bacterial strain designated GFC-1^T was isolated from a fish-culture pond in Taiwan and was characterized by using the polyphasic taxonomic approach. Strain GFC-1^T was Gram-negative, aerobic, rod-shaped, motile and non-spore-forming. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain belonged to the genus *Andreprevotia* of the family *Neisseriaceae* and its closest neighbour was *Andreprevotia chitinilytica* JS11-7^T (97.0% sequence similarity). The results of physiological and biochemical tests allowed clear phenotypic differentiation of isolate GFC-1^T from *A. chitinilytica* JS11-7^T. The major fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1}ω7c. The DNA G + C content was 63.0 mol%. On the basis of 16S rRNA gene sequence analysis and chemotaxonomic and physiological data, strain GFC-1^T should be classified as representing a novel species and a second member of the genus *Andreprevotia*, for which the name *Andreprevotia lacus* sp. nov. is proposed. The type strain is GFC-1^T (=BCRC 17832^T=LMG 24502^T).

The genus *Andreprevotia*, a member of the family *Neisseriaceae*, currently encompasses only one species, namely *Andreprevotia chitinilytica* (type strain JS11-7^T), isolated from Halla Mountain forest soil, Jeju Island, Korea (Weon *et al.*, 2007). During our investigations of the biodiversity of bacteria associated with a fish-culture pond located in Taoyuan County, Taiwan, several ivory-coloured bacterial colonies were isolated on R2A agar (BD Difco). Strains showing similar colony morphology were selected and a representative strain, GFC-1^T, was selected for detailed analyses. Strain GFC-1^T was maintained on R2A agar at 25 °C. Subcultivation was performed on R2A agar at 25 °C for between 48 and 72 h. On this medium, strain GFC-1^T was able to grow at 15–37 °C, but not at 10 or 40 °C. The strain was preserved at –80 °C in nutrient broth (NB; Difco) with 20% (v/v) glycerol or by lyophilization.

Cell morphology was observed by phase-contrast microscopy (DM 2000; Leica) at lag, exponential and stationary

phases of growth. Motility was tested by the hanging-drop method. Spot Test Flagella Stain (BD Difco) was used for flagellum staining. A Gram Stain Set S kit (BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were adopted for testing the Gram-staining reaction. Poly-β-hydroxybutyrate granule accumulation was observed under light microscopy after staining the cells with Sudan black. Details of cell morphology are provided in the species description. The pH range for growth was determined by measuring the OD₆₀₀ of the culture grown in NB that had been adjusted prior to sterilization to various pH values (pH 4.0–10.0 at intervals of 0.5 pH units), using appropriate biological buffers (Chung *et al.*, 1995). Verification of the pH values after autoclaving revealed only minor changes. Tolerance to NaCl was tested in NB prepared according to the formula of the BD Difco medium, but adjusted to different NaCl concentrations [0, 0.5 and 1.0–3.0% (w/v) at intervals of 1.0%]. Anaerobic growth was checked by using the Oxoid AnaeroGen system. Strain GFC-1^T was examined for catalase, oxidase, DNase, urease and lipase activities and hydrolysis of starch, casein, chitin,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Andreprevotia lacus* GFC-1^T is EU287926.

CM-cellulose, aesculin, tyrosine and Tweens 20, 40, 60 and 80 by using standard approaches (Gerhardt *et al.*, 1994). Phenotypic characteristics [biochemical tests and carbon-source utilization; API 50CH, API ZYM and API 20NE (all from bioMérieux)] were investigated.

The 16S rRNA gene sequence was analysed as described by Chen *et al.* (2001). Analysis of the sequence data was performed by using the software packages BioEdit (Hall, 1999) and MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). A distance-matrix method (distance options according to the Kimura two-parameter model; Kimura, 1983) including clustering by the neighbour-joining (Saitou & Nei, 1987) (Fig. 1), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods was used. In each case, bootstrap values were calculated based on 1000 replications. The 16S rRNA gene sequence of strain GFC-1^T was a partial stretch of 1398 bp (GenBank accession no. EU287926). 16S rRNA gene sequence analysis indicated that strain GFC-1^T belongs to the family *Neisseriaceae* of the class *Betaproteobacteria*. Strain GFC-1^T formed a distinct subline within the genus *Andreprevotia*. Sequence-similarity calculations (over 1350 bp) indicated that strain GFC-1^T was related closely to *A. chitinilytica* JS11-7^T (97.0% 16S rRNA gene sequence similarity), *Deefgea rivuli* WB 3.4-79^T (95.6%) and *Silvimonas terrae* KM-45^T (94.9%). Lower sequence similarities (<94.5%) were found with representative members of all other genera listed in Fig. 1. The overall topologies of the phylogenetic trees obtained with neighbour-joining, maximum-likelihood or maximum-parsimony methods were similar.

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification system (Microbial ID; Sasser, 1990). The predominant fatty acid constituents of strain GFC-1^T were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1}ω7c. The fatty acid profile of strain GFC-1^T was

consistent with that reported for *A. chitinilytica* JS11-7^T, but their fatty acid proportions differed (Table 1). The DNA G+C content of strain GFC-1^T was estimated as described by Mesbah *et al.* (1989) to be 63.0±0.1 mol% (n=3). DNA–DNA hybridization experiments were carried out with *A. chitinilytica* JS11-7^T by the method outlined by Ezaki *et al.* (1989). The results of DNA–DNA hybridization indicated low levels (27.5±6.8%) of relatedness between strain GFC-1^T and *A. chitinilytica* JS11-7^T (Wayne *et al.*, 1987).

Sensitivity of strain GFC-1^T to antibiotics was tested by spreading cells (0.5 on the McFarland scale) on Mueller–Hinton agar (BD Difco) plates. The antibiotic discs (Oxoid) contained the following antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), penicillin G (10 µg), streptomycin (10 µg), sulfamethoxazole plus trimethoprim (23.75+1.25 µg) or tetracycline (30 µg). The effect of antibiotics on cell growth was assessed after 3 days incubation. Detailed results of the phenotypic and biochemical characterization of strain GFC-1^T are provided in Table 2 and in the species description. Carbon substrate-utilization tests with various sugars and organic acids as substrates showed few positive results.

Strain GFC-1^T is clearly different from *A. chitinilytica* and occupies a separate position in the genus *Andreprevotia*. This genotypic insight was supported by a unique combination of chemotaxonomic and biochemical characteristics (Tables 1 and 2). It is clear from the genotypic and phenotypic data that strain GFC-1^T represents a second species of the genus *Andreprevotia*. The name *Andreprevotia lacus* sp. nov. is proposed for this taxon.

Description of *Andreprevotia lacus* sp. nov.

Andreprevotia lacus (la'cus. L. gen. n. *lacus* of a lake, basin or tank, referring to the place of isolation of the type strain).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain GFC-1^T (*Andreprevotia lacus* sp. nov.) and related taxa in the class *Betaproteobacteria*. The neighbour-joining method was employed with the distance model of Jukes & Cantor (1969). Numbers at nodes are percentage bootstrap values (>50%) based on both the neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered by using the maximum-likelihood and maximum-parsimony tree-making algorithms. Bar, 0.01 substitutions per nucleotide position.

Table 1. Fatty acid compositions of strain GFC-1^T (*Andreprevotia lacus* sp. nov.) and *A. chitinilytica* JS11-7^T

Strains: 1, GFC-1^T; 2, *A. chitinilytica* JS11-7^T. Strain GFC-1^T was cultured on tryptic soy agar (BD Difco) at 28 °C for 48 h as described for *A. chitinilytica* JS11-7^T (Weon *et al.*, 2007). Values are percentages of total fatty acid content. —, <0.5% or 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.

Fatty acid	1	2
C _{10:0} 3-OH	1.8	1.6
C _{12:0}	—	2.7
C _{12:0} 3-OH	2.1	2.0
C _{14:0}	5.7	0.6
C _{15:1} ω 6c	0.7	1.0
C _{16:0}	25.9	31.1
C _{17:0}	0.7	1.0
C _{17:0} cyclo	—	2.3
C _{17:1} ω 6c	0.6	—
C _{18:0}	—	0.7
C _{18:1} ω 7c	22.2	15.8
Summed feature 3*	38.5	39.5

*Summed features are groups of fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH.

Cells are Gram-negative, aerobic, rod-shaped, non-spore-forming and motile by means of a single polar flagellum. Poly- β -hydroxybutyrate granules are accumulated. After 48 h growth on R2A agar at 25 °C, the mean cell size is 0.5 μ m in width and 1.0–2.0 μ m in length. Visible, round, entire, convex and ivory-coloured colonies are formed on R2A agar. Colony size is approximately 1.8–2.2 mm in diameter after 48 h at 25 °C. Growth occurs at 15–37 °C, 0–1% (w/v) NaCl and pH 7.0–9.0 [optimum at 30 °C, 0–0.5% (w/v) NaCl and pH 7.0]. Positive for oxidase and catalase activities and chitin hydrolysis. Negative for hydrolysis of DNase, lipase (corn oil), Tweens 20, 40, 60 and 80, starch, CM-cellulose, aesculin, tyrosine and casein. Positive (API 20NE) for nitrate reduction, glucose acidification, arginine dihydrolase, urease, β -glucosidase, protease, β -galactosidase and assimilation of glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, adipate, malate and citrate; negative for indole production and assimilation of phenylacetate, gluconate and caprate. Positive (API ZYM) for alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and *N*-acetyl- β -glucosaminidase; negative for C14 lipase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase. Major fatty acids are summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1} ω 7c. Additional phenotypic properties are listed in Table 2. The following

Table 2. Characteristics that distinguish strain GFC-1^T (*Andreprevotia lacus* sp. nov.) from *A. chitinilytica* JS11-7^T

Strains: 1, GFC-1^T; 2, *A. chitinilytica* JS11-7^T. Data for isolation source and DNA G+C content for *A. chitinilytica* JS11-7^T were taken from Weon *et al.* (2007); all other data listed were obtained in this study. Both strains were positive for utilization of D-ribose, D-galactose, D-fructose, *N*-acetylglucosamine, D-lyxose and potassium 5-ketogluconate. The following characteristics were negative for both strains: utilization of glycerol, erythritol, L-xylose, adonitol, methyl β -D-xylopyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, amygdalin, arbutin, salicin, D-lactose, α -melibiose, sucrose, trehalose, inulin, α -melezitose, α -raffinose, xylitol, turanose, D-tagatose, L-fucose, L-arabitol and potassium 2-ketogluconate. +, Positive; —, negative; R, resistant; S, sensitive.

Characteristic	1	2
Isolation source	Fish-culture pond	Forest soil
Arginine dihydrolase	+	—
Urease	+	—
β -Galactosidase	+	—
β -Glucosidase	—	+
Alkaline phosphatase	+	—
C8 esterase lipase	+	—
Assimilation of (API 20NE):		
Glucose	+	—
Arabinose	+	—
Mannose	+	—
Maltose	+	—
Gluconate	—	+
Adipate	+	—
Malate	+	—
Citrate	+	—
Hydrolysis of:		
Starch	—	+
Corn oil	—	+
Tween 20	—	+
Tween 40	—	+
Tween 60	—	+
Tween 80	—	+
Susceptibility to nalidixic acid	S	R
DNA G+C content (mol%)	63	62

compounds are utilized in the API 50CH test system: D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, D-lyxose and potassium 5-ketogluconate. Negative for assimilation of glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, methyl β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucoside, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, D-lactose, α -melibiose, sucrose, trehalose, inulin, α -melezitose, α -raffinose, starch, glycogen, xylitol, β -gentiobiose, turanose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate. Sensitive to tetracycline, kanamycin, rifampicin, gentamicin,

chloramphenicol, nalidixic acid, novobiocin and streptomycin, but resistant to ampicillin, penicillin G and sulfamethoxazole plus trimethoprim. The DNA G+C content of the type strain is 63.0 mol%.

The type strain is GFC-1^T (=BCRC 17832^T=LMG 24502^T), isolated from a fish-culture pond located in Taoyuan County, Taiwan.

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