Corynebacterium atrinae sp. nov., isolated from the gastrointestinal tract of a pen shell, *Atrina pectinata*

Pil Soo Kim, Na-Ri Shin, Dong-Wook Hyun, Joon Yong Kim, Tae Woong Whon, Sei Joon Oh and Jin-Woo Bae

Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea

Correspondence
Jin-Woo Bae
baejw@khu.ac.kr

A novel Gram-stain-positive, non-motile, facultatively anaerobic and rod-shaped bacterium, strain PSPT56^T, was isolated from the gastrointestinal tract of a pen shell (*Atrina pectinata*). Optimal growth of strain PSPT56^T was ascertained to occur at 30 °C, pH 8.0 and in the presence of 1–2 % (w/v) NaCl. The strain was catalase-positive and oxidase-negative. The major cellular fatty acids were $C_{18:1}\omega9c$, $C_{16:0}$, $C_{17:1}\omega8c$ and $C_{17:0}$. Tuberculostearic acid was not present. The major cell-wall sugars were ribose, galactose, glucose and arabinose. Peptidoglycan amino acids were *meso*-diaminopimelic acid, alanine and glutamic acid. The predominant isoprenoid quinone was MK-8(H_2). Strain PSPT56^T contained phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid, two unidentified lipids and two unidentified amino-lipids. Mycolic acids were detected as constitutive components of the cell wall. A phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain PSPT56^T was most closely related to *Corynebacterium testudinoris* M935/96/4^T and *Corynebacterium felinum* M714/95/5^T with 98.69 % and 97.01 % similarity, respectively. DNA–DNA hybridization experiments indicated less than 29.9 % relatedness to the phylogenetically closest species. The G+C content of genomic DNA was 67.6 mol%. The phenotypic, phylogenetic and genotypic analyses indicated that strain PSPT56^T represents a novel species within the genus *Corynebacterium*, for which the name *Corynebacterium atrinae* is proposed. The type strain is PSPT56^T (=KACC 17525^T=JCM 19266^T).

The genus *Corynebacterium*, which was created by Lehmann & Neumann (1896), contains more than 100 species of widely known Gram-positive, asporogenous bacteria in the phylum Actinobacteria. The members of the genus *Corynebacterium* are characterized by chemotaxonomic features such as tuberculostearic acid (TBSA) among cellular fatty acids, menaquinone MK-8(H_2) and/or MK-9(H_2) as isoprenoid quinones (Collins *et al.*, 1985; Collins *et al.*, 1982), *meso*-diaminopimelic acid (DAP)-containing A1γ-type cell-wall peptidoglycan (Bernard & Funke, 2012) and arabinose and galactose as major cell-wall sugar components. Many of the constituents of the genus *Corynebacterium* were isolated from human clinical specimens: *Corynebacterium diphtheriae* (Lehmann & Neumann, 1896), *C. falsenii* (Sjödén *et al.*, 1998), *C. lipophiloflavum* (Funke *et al.*, 1997) and *C. tuberculostearicum* (Feurer *et al.*, 2004). Additionally, various animals and food materials have been sources of species of the genus *Corynebacterium*: *Corynebacterium nuruki* (Shin *et al.*, 2011), *C. casei*, *C. mooreparkense* (Brennan *et al.*, 2001), *C. epidermidicanis* (Frischmann *et al.*, 2012), *C. spheniscorum* (Goyache *et al.*, 2003a), *C. sphenisci* (Goyache *et al.*, 2003b), *C. testudinoris* and *C. felinum* (Collins *et al.*, 2001). Here, we described a novel species of the genus *Corynebacterium*, designated strain PSPT56^T, based on physiological, biochemical and genotypic investigations.

Strain PSPT56^T was isolated from the gastrointestinal tract (GIT) of a pen shell (*Atrina pectinata*) collected from offshore of Yeosu, Jeollanam-do, Korea. Homogenized GIT tissue was serially diluted with filtered PBS buffer and spread on trypticase soy agar (TSA; Bacto) plates. These plates were cultured at 25 °C for 1 week. Cultivation on fresh media was performed repeatedly to obtain a pure culture. All tests were conducted in triplicate. For phylogenetic analysis, amplification of the 16S rRNA gene of the isolate was carried out through colony-PCR, as described by Kim *et al.* (2013), with PCR pre-mix (iNtRon Biotechnology) and two universal bacterial-specific primers: forward primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGYTACCTTGTTACGACTT-3′)

Abbreviations: DAP, diaminopimelic acid; DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PSPT56^T is KF135179.

One supplementary figure is available with the online Supplementary Material.

(Lane, 1991). PCR product sequencing was performed using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems), according to the manufacturer's instructions. The reaction mixture was analysed using an automated DNA analyser system (PRISM 3730XL DNA Analyser, Applied Biosystems). The sequence fragments of strain PSPT56^T were assembled using SeqMan (DNASTAR). The assembled near-full-length 16S rRNA gene sequence was compared with other sequences of type strains in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). The 16S rRNA gene comparison revealed that the isolate shared high similarity with *C. testudinoris* M935/96/4^T (98.69 %) and *C. felinum* M714/95/5^T (97.01 %). The 16S rRNA gene sequences of strain PSPT56^T and representatives of species of the genus *Corynebacterium* were aligned using the multiple alignment program CLUSTAL W (Thompson *et al.*, 1994). BioEdit software was used for checking the aligned sequences manually (Hall, 1999). Phylogenetic trees based on the 16S rRNA gene sequences of the isolate and the closely related members of the genus *Corynebacterium* were reconstructed using the MEGA5 software (Tamura *et al.*, 2011). Neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (MP; Kluge & Farris, 1969) and maximum-likelihood (ML; Felsenstein, 1981) methods with 1000 bootstrap replicates were used to confirm phylogenetic correlations (Felsenstein, 1985). The conjoined phylogenetic consensus tree (branch reconstructed by neighbour-joining algorithm with bootstrap value of corresponding branches from MP/ML) based on the 16S rRNA genes of strain PSPT56^T and the type strains of species of the genus *Corynebacterium* is shown in Fig. 1. We obtained *C. testudinoris* CCUG 41823^T (=M935/96/4^T) and *C. felinum* CCUG 39943^T (=M714/95/5^T) from the Culture Collection, University of Göteborg, Sweden (CCUG) to conduct a more comprehensive characterization of strain PSPT56^T.

Temperature and pH ranges for growth and optimal growth conditions of the isolate were examined by cultivation at various temperatures (4, 10, 15, 20, 25, 30, 37, 45, 55 and 65 °C) and at pH 4.0–10.0 (at intervals of 1.0 pH unit) in trypticase soy broth (TSB; Bacto) medium. The pH of the medium was adjusted using 6 M HCl solution (Sigma Aldrich) and 1 M NaOH solution (Sigma Aldrich) with 10 mM MES (for pH 4, 5 and 6), 10 mM TAPS (for pH 7, 8 and 9) or 10 mM Na_2HPO_4 (for pH 10). The NaCl requirement and tolerance were verified in TSB medium with different NaCl concentrations (0, 1, 2, 3, 4, 5, 8, 10, 12 and 15 %, w/v), which was prepared by including all components of TSB except NaCl. The turbidity of each culture was measured at OD_{600} using a spectrophotometer (Synergy MX, BioTek) after 24 h, 48 h, 72 h and 7 days of incubation. Strain PT56^T showed growth at 10–37 °C, pH 6.0–8.0 and in the presence of 0–7 % (w/v) NaCl, and showed optimal growth at 30 °C, pH 8.0 and in the presence of 1–2 % (w/v) NaCl. Unless stated otherwise, all experiments were carried out under optimal growth conditions for 24–48 h.

The Gram staining test was performed using a Gram staining kit (bioMérieux) according to the manufacturer's instructions. Gram staining and cell morphology were observed with a light microscope (ECLIPSE 50*i*, Nikon). Semi-solid agar media were used for checking cellular motility (Tittsler & Sandholzer, 1936). Growth under anaerobic conditions was identified in an anaerobic chamber (Sheldon Manufacturing), filled with an atmosphere composed of $N_2/CO_2/H_2$ (90 : 5 : 5). Cell morphology tests showed that cells were Gram-stain positive and rod-shaped, and formed orange–cream-coloured, circular (0.5–1.0 mm in diameter), smooth and slightly viscous colonies after 48 h on TSA medium at 30 °C.

Catalase and oxidase activities were confirmed by bubble production in 3 % (v/v) hydrogen peroxide and change in colour of a colony by 1 % (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux), respectively. The isolate was catalase-positive and oxidase-negative. API ZYM test strips (bioMérieux) and API Coryne test strips (bioMérieux) with API suspension medium (bioMérieux) were used to confirm the enzyme activities of the isolate and the type strains of reference species, according to the manufacturer's instructions. Acid production from various carbohydrates and metabolism of sole carbon sources were ascertained using API 50 CH test strips (bioMérieux) with 50 CHB/E medium (bioMérieux) and GP2 MicroPlates (Biolog) with GN/GP inoculating fluid (Biolog), respectively. The isolate and *C. testudinoris* CCUG 41823^T differed in their ability to metabolize 3-methyl glucose, succinamic acid and L-asparagine (GN2 MicroPlate), produce acid from amygdalin, cellobiose and gentiobiose (API 50 CH) and their level of enzyme activity for α-glucosidase, pyrazinamidase and β-glucosidase (hydrolysis of aesculin) (API Coryne). The complete results of biochemical tests and the differential characteristics of strain PSPT56^T are presented in Table 1 and the species description.

Chemotaxonomic analyses were performed with the cell biomass of strain PSPT56^T and the type strains of reference species cultured on TSA plates for 24 h. To extract cellular fatty acids, the protocol of the Sherlock Microbial Identification System was applied (MIDI, 1999). The cellular fatty acids were analysed by GC (Agilent 6890 GC, Agilent Technologies). The peaks of the gas chromatogram were identified using the MIDI software package (Sherlock version 6.2B) (Sasser, 1990) with the enclosed TSBA6 library. The dominant cellular fatty acids (>10 %) of strain PSPT56^T were $C_{18:1}\omega9c$ (38.0 %), $C_{16:0}$ (26.3 %), $C_{17:1}\omega8c$ (21.4 %) and $C_{17:0}$ (11.2 %). Neither the novel isolate nor the type strains of reference species contained tuberculostearic acid (10-methyl $C_{18:0}$). The complete cellular fatty acid compositions of strain PSPT56^T and the type strains of reference species are given in Table 2. Isoprenoid quinone extraction was carried out by steeping in chloroform/methanol (2 : 1, v/v) overnight, as described by Collins & Jones (1981). Reverse-phase HPLC with a Thermo ODS HYPERSIL (250 × 4.6 mm) column was employed for analysis of isoprenoid quinone composition. Liquid chromatography (LC) (Ultimate 3000, Dionex) analysis using an ion trap-mass spectrometer equipped

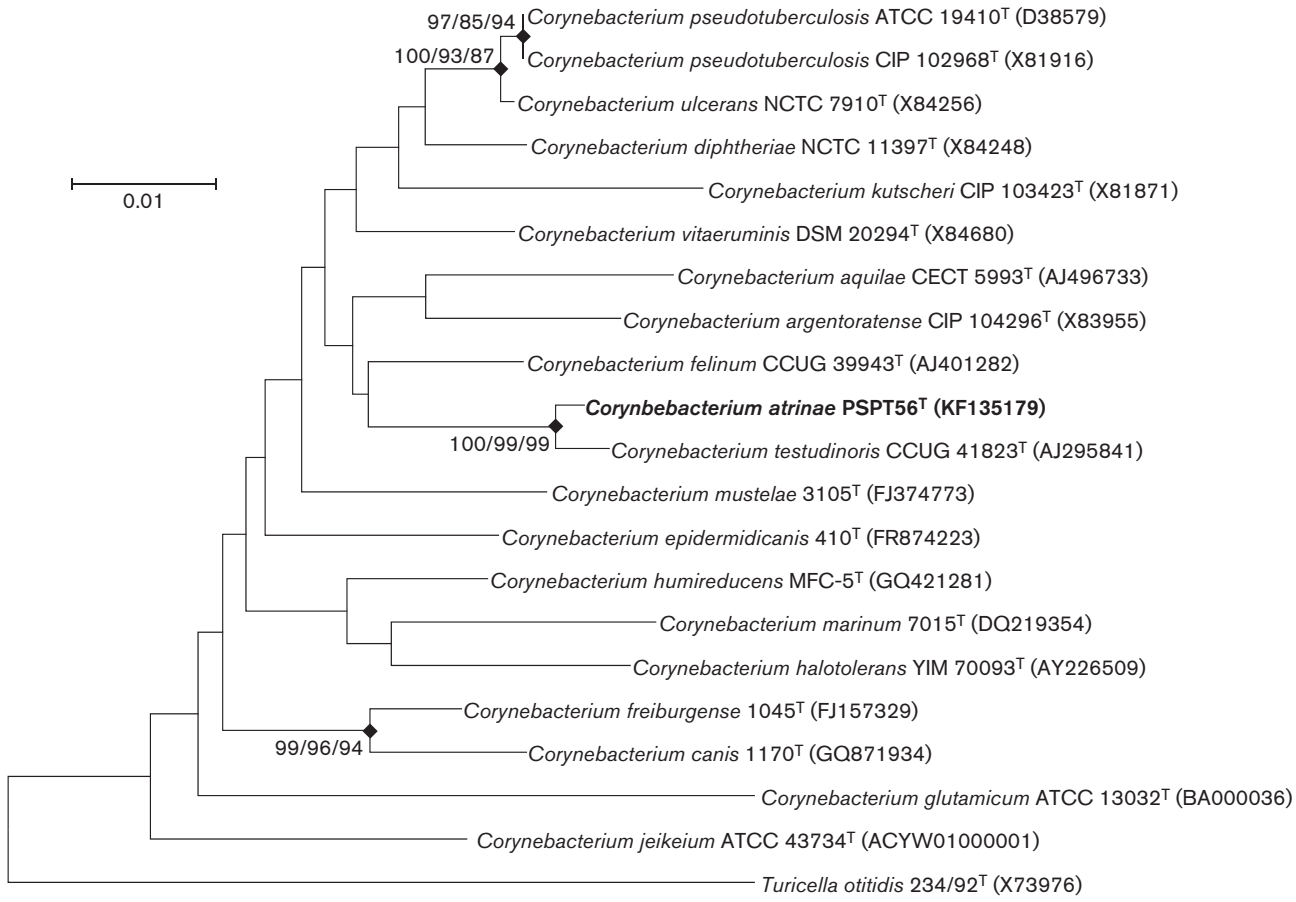

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparisons, mainly reconstructed using the neighbour-joining algorithm (NJ). Maximum-parsimony (MP) and maximum-likelihood (ML) algorithms were applied for additional comparison. Filled diamonds indicate branches present in the phylogenetic trees generated using the three different methods. Numbers on the nodes (>70 %) represent bootstrap values as percentages of 1000 replicates (NJ/MP/ML). *Turicella otitidis* 234/92^T was used as an outgroup. Bar, 0.01 accumulated changes per nucleotide.

with an electrospray ionization probe (HCT, Bruker Daltonics) was performed for intensive discrimination of the quinone constituent, according to the protocol of Taguchi *et al.* (2005). The predominant isoprenoid quinones of strain PSPT56^T were menaquinones MK-8(H_2) (69.6 %), MK-9(H_2) (16.2 %) and MK-7(H_2) (14.2 %), which were similar to those of *C. testudinoris* (analysis performed in this study) and other species in the genus *Corynebacterium* (Collins *et al.*, 1977). The polar lipid composition was identified by extracting polar lipids according to the protocol described by Xin *et al.* (2000) and separating the extracted polar lipids using two-dimensional TLC on a silica gel 60 F_{254} plate (Merck). The reagents used for detecting and identifying the polar lipids were as follows: 10 % ethanolic molybdatophosphoric acid reagent for total lipids, ninhydrin reagent for amino-containing lipids and Zinzadze reagent for phospholipids (Tindall, 1990). The phospholipids were identified using one-dimensional TLC with standard compounds (Sigma Aldrich). The polar lipids of strain PSPT56^T included two unidentified lipids (L1–2), two unidentified aminolipids (AL1–2), an unidentified phospholipid (PL1), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) (Fig. S1a, available in the online Supplementary Material). The isolate shared three phospholipids (PL1, PG, and DPG) with *C. testudinoris*; however, unidentified lipid components such as AL1, AL2, L1 and L2 were different from those of *C. testudinoris* (Fig. S1b). Cell-wall hydrolysates were extracted as described by Schleifer & Kandler (1972). Cell-wall sugars and standard sugars (ribose, galactose, glucose, mannose, arabinose, xylose and rhamnose) (Schön & Groth, 2006) were spotted on a cellulose TLC plate (Merck) and developed twice in ethyl acetate/pyridine/water (100 : 35 : 25, by vol.). To detect sugars on the TLC plate, aniline-phthalate spray reagent was sprayed. Strain PSPT56^T contained ribose, galactose, glucose and arabinose as cell-wall sugars. Cell-wall peptidoglycan amino acids were purified and then hydrolysed according to the method of Bousfield *et al.* (1985). LL-DAP and *meso*-DAP, 2,4-diaminobutyric acid, glutamic acid, glycine, alanine, lysine, threonine and ornithine (Sigma Aldrich) were used as

Table 1. Differential characteristics of strain PSPT56^T and closely related species of the genus *Corynebacterium*

Strains: 1, PSPT56^T; 2, *C. testudinoris* CCUG 41823^T; 3, *C. felinum* CCUG 39943^T. Data for sole-carbon-source metabolism, acid production from carbohydrates and enzyme activity were obtained using GP2 MicroPlates, API 50CH, and API Coryne and API ZYM, respectively. All data were derived from the current study, except those indicated. All strains metabolized dextrin, D-fructose, α-D-glucose, maltotriose, D-mannose, acetic acid, α-hydroxybutyric acid and L-lactic acid (GP2 Microplates). All strains produced acids from D-ribose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, maltose and trehalose (API 50 CH). All strains were positive for leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase (API ZYM). +, Positive; −, negative; V, variable.

Characteristic	1	2	3
Metabolism of:			
3-Methyl glucose, succinamic acid	−	+	−
L-Asparagine	+	−	+
Arbutin, salicin, maltose, D-psicose, *N*-acetyl-D-glucosamine, trehalose, sucrose, *N*-acetyl-β-D-mannosamine, methyl β-D-glucoside, D-ribose, α-ketoglutaric acid, β-hydroxybutyric acid, D-lactic acid methyl ester, L-malic acid, pyruvatic acid methyl ester, succinic acid monomethyl ester, pyruvic acid, succinic acid, L-glutamic acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine	+	+	−
Acid production from:			
Amygdalin, cellobiose, gentiobiose	−	+	−
Arbutin, aesculin, salicin, sucrose, turanose	+	+	−
Starch	−	−	+
Enzyme activity and reaction			
Reduction of nitrate to nitrite	+	+	−
Esterase (C4), esterase lipase (C8)	+	+	−
α-Glucosidase	+	V*	+
Pyrazinamidase	+	−*	+*
Pyrrolidonyl arylamidase	−	−*	+*
β-Glucosidase (hydrolysis of aesculin)	V	−*	−*

*Data from Collins *et al.* (2001).

standard compounds. Peptidoglycan hydrolysates of the novel isolate and standard compounds were separated on a cellulose TLC plate by developing in methanol/H_2O/6 M HCl/pyridine (80 : 26 : 4 : 10, by vol.). The isolate possessed *meso*-DAP, alanine and glutamic acid in the peptidoglycan. Mycolic acids were extracted and purified according to Yano & Saito (1972). Mycolic acids detection was performed by developing the compound extracted from the novel isolate with that from *C. testudinoris* as a positive control, on a silica TLC aluminium sheet, as described by Goodfellow *et al.* (1976). Strain PSPT56^T contained mycolic acid.

The genomic DNA of strain PSPT56^T, *C. testudinoris* and *C. felinum* was extracted as described by Rochelle *et al.* (1992) for genotypic analyses. A fluorimetric method with SYBR Gold I (Invitrogen) and a real-time PCR thermocycler (BioRad) was used to estimate the G+C content of the genomic DNA (Gonzalez & Saiz-Jimenez, 2002). Genomic DNA of *Escherichia coli* K-12 (50.8 mol%), *Bacteroides fragilis* JCM 11019^T (43.1 mol%), *Bacteroides thetaiotaomicron* JCM 5827^T (42.9 mol%) and *Ruegeria pomeroyi* DSS-3^T (64.1 mol%) was used for calibration. The DNA G+C content of the isolate was 67.6 mol%. This value fell within the DNA G+C content range of the genus *Corynebacterium* (51–74 mol%) (Bernard & Funke, 2012). To elucidate the genetic relativeness between strain PSPT56^T and the most closely related species, DNA–DNA hybridization (DDH) was performed using the genome-spotted microarray method (Bae *et al.*, 2005; Chang *et al.*, 2008). The DDH values were calculated from the signal-to-noise ratio of the genomic probes (Loy *et al.*, 2005). The values of DDH between the isolate and the type strains of reference species were as follows: 29.9±3.4 % (49.4±17.6 % reciprocal) to *C. testudinoris* CCUG 41823^T and 6.9±4.2 % (26.4±15.2 % reciprocal) to *C. felinum* CCUG 39943^T. The DDH values were below the novel genotypic species threshold of 70 % (Wayne *et al.*, 1987).

The results of phylogenetic, phenotypic and genotypic analyses indicate that strain PSPT56^T represents a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium atrinae* sp. nov. is proposed.

Description of *Corynebacterium atrinae* sp. nov.

Corynebacterium atrinae (a.tri′nae. N.L. gen. n. *atrinae* of *Atrina*, isolated from the gastrointestinal tract of a pen shell, *Atrina pectinata*).

Cells are Gram-stain positive, rod-shaped, non-motile, catalase-positive and oxidase-negative. Colonies are circular, orange–cream-coloured, smooth and slightly viscous on TSA medium after 48 h at 30 °C. Growth occurs at temperatures between 10 and 37 °C, at pH 6.0–8.0 and the presence of 0–7 % (w/v) NaCl. Optimal growth conditions

Table 2. Cellular fatty acid composition of strain PSPT56^T and the type strains of reference species

Strains: 1, PSPT56^T; 2, *C. testudinoris* CCUG 41823^T; 3, *C. felinum* CCUG 39943^T. All data were obtained from the current study. Values are percentages of total fatty acids; those that comprised <0.5 % for all strains were omitted. tr, Trace (<0.5 %); –, not detected.

Fatty acid	1	2	3
Saturated acids			
$C_{9:0}$	–	1.3	tr
$C_{14:0}$	tr	0.6	3.2
$C_{16:0}$	26.3	27.4	31.7
$C_{17:0}$	11.2	–	6.0
$C_{18:0}$	0.8	1.0	1.4
$C_{19:0}$	0.6	0.7	tr
Unsaturated acids			
$C_{15:1}\omega8c$	–	1.3	–
$C_{16:1}\omega9c$	–	0.9	–
$C_{17:1}\omega8c$	21.4	26.3	13.8
$C_{18:3}\omega6c$ (6, 9, 12)	–	0.8	–
$C_{18:1}\omega9c$	38.0	39.7	8.8
Summed feature 3*	0.6	tr	33.0
Branched acids			
$C_{16:0}$ N alcohol	–	0.7	0.6
iso-$C_{18:0}$ H	tr	0.8	–

*Summed features were used when two or three fatty acids could not be separated using the Microbial Identification System. Summed features 3 comprised $C_{16:1}\omega7c$/$C_{16:1}\omega6c$.

are 30 °C, pH 8.0 and in the presence of 1–2 % (w/v) NaCl. Acid is produced from D-ribose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, arbutin, aesculin, salicin, maltose, sucrose, trehalose and turanose (API 50 CH). Metabolizes dextrin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, arbutin, D-fructose, α-D-glucose, maltose, maltotriose, D-mannose, methyl β-D-glucoside, D-psicose, D-ribose, salicin, sucrose, trehalose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketoglutaric acid, D-lactic acid methyl ester, L-lactic acid, L-malic acid, pyruvatic acid methyl ester, succinic acid monomethyl ester, pyruvic acid, succinic acid, L-glutamic acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine and L-asparagine (Biolog GP2 Microplates). The following enzyme activities and reactions are positive based on the API ZYM and API Coryne test strip: esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase (hydrolysis of aesculin) (API ZYM), reduction of nitrate to nitrite, pyrazinamidase and α-glucosidase (API Coryne). The following enzyme activities and reactions are negative based on the API ZYM and API Coryne test strip: alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase (API ZYM), pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, β-glucosidase (hydrolysis of aesculin), urease and gelatin hydrolysis (API Coryne). The major cell-wall sugars are ribose, glucose, arabinose and galactose. The predominant quinones are menaquinones MK-8(H_2), MK-7(H_2) and MK-9(H_2). The major cellular fatty acids (>10 %) are $C_{18:1}\omega9c$, $C_{16:0}$, $C_{17:1}\omega8c$ and $C_{17:0}$. Tuberculostearic acid is absent. Mycolic acids are contained. Polar lipids comprise two unidentified lipids, an unidentified phospholipid, two unidentified aminolipids, phosphatidylglycerol and diphosphatidylglycerol. Alanine, glutamic acid and *meso*-DAP are contained in the peptidoglycan. Mycolic acids are present.

The type strain is PSPT56^T (=KACC 17525^T=JCM 19266^T), isolated from the gastrointestinal tract of a pen shell (*Atrina pectinata*) at Yeosu, Jeollanam-do, Korea. The DNA G+C content of the type strain is 67.6 mol%.

Acknowledgements

We thank Dr J. P. Euzéby (École Nationale Vétérinaire, France) for etymological advice, and Hae-Won Lee and Dr Seong Woon Roh (Jeju Center, Korea Basic Science Institute, Korea) for isoprenoid quinone analyses. This work was supported by grants from the Mid-career Researcher Program of the National Research Foundation (2012-0008806) and the National Institute of Biological Resources (NIBR, no. 2013-02-001).

References

Bae, J. W., Rhee, S. K., Park, J. R., Chung, W. H., Nam, Y. D., Lee, I., Kim, H. & Park, Y. H. (2005). Development and evaluation of genome-probing microarrays for monitoring lactic acid bacteria. *Appl Environ Microbiol* **71**, 8825–8835.

Bernard, K. A. & Funke, G. (2012). Genus *Corynebacterium*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 5, The *Actinobacteria* pp. 245–289. Edited by M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, K. Suzuki, W. Ludwig & W. B. Whitman. New York: Springer.

Bousfield, G. R., Sugino, H. & Ward, D. N. (1985). Demonstration of a COOH-terminal extension on equine lutropin by means of a common acid-labile bond in equine lutropin and equine chorionic gonadotropin. *J Biol Chem* **260**, 9531–9533.

Brennan, N. M., Brown, R., Goodfellow, M., Ward, A. C., Beresford, T. P., Simpson, P. J., Fox, P. F. & Cogan, T. M. (2001). *Corynebacterium mooreparkense* sp. nov. and *Corynebacterium casei* sp. nov., isolated from the surface of a smear-ripened cheese. *Int J Syst Evol Microbiol* **51**, 843–852.

Chang, H. W., Nam, Y. D., Jung, M. Y., Kim, K. H., Roh, S. W., Kim, M. S., Jeon, C. O., Yoon, J. H. & Bae, J. W. (2008). Statistical superiority of genome-probing microarrays as genomic DNA-DNA hybridization in revealing the bacterial phylogenetic relationship compared to conventional methods. *J Microbiol Methods* **75**, 523–530.

Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Collins, M. D., Goodfellow, M. & Minnikin, D. E. (1982). A survey of the structures of mycolic acids in *Corynebacterium* and related taxa. *J Gen Microbiol* **128**, 129–149.

Collins, M. D., Goodfellow, M., Minnikin, D. E. & Alderson, G. (1985). Menaquinone composition of mycolic acid-containing actinomycetes and some sporoactinomycetes. *J Appl Bacteriol* **58**, 77–86.

Collins, M. D., Hoyles, L., Hutson, R. A., Foster, G. & Falsen, E. (2001). *Corynebacterium testudinoris* sp. nov., from a tortoise, and *Corynebacterium felinum* sp. nov., from a Scottish wild cat. *Int J Syst Evol Microbiol* **51**, 1349–1352.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Feurer, C., Clermont, D., Bimet, F., Candréa, A., Jackson, M., Glaser, P., Bizet, C. & Dauga, C. (2004). Taxonomic characterization of nine strains isolated from clinical and environmental specimens, and proposal of *Corynebacterium tuberculostearicum* sp. nov. *Int J Syst Evol Microbiol* **54**, 1055–1061.

Frischmann, A., Knoll, A., Hilbert, F., Zasada, A. A., Kämpfer, P. & Busse, H. J. (2012). *Corynebacterium epidermidicanis* sp. nov., isolated from skin of a dog. *Int J Syst Evol Microbiol* **62**, 2194–2200.

Funke, G., Hutson, R. A., Hilleringmann, M., Heizmann, W. R. & Collins, M. D. (1997). *Corynebacterium lipophiloflavum* sp. nov. isolated from a patient with bacterial vaginosis. *FEMS Microbiol Lett* **150**, 219–224.

Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

Goodfellow, M., Collins, M. D. & Minnikin, D. E. (1976). Thin-layer chromatographic analysis of mycolic acid and other long-chain components in whole-organism methanolysates of coryneform and related taxa. *J Gen Microbiol* **96**, 351–358.

Goyache, J., Vela, A. I., Collins, M. D., Ballesteros, C., Briones, V., Moreno, J., Yorio, P., Domínguez, L., Hutson, R. & Fernández-Garayzábal, J. F. (2003a). *Corynebacterium spheniscorum* sp. nov., isolated from the cloacae of wild penguins. *Int J Syst Evol Microbiol* **53**, 43–46.

Goyache, J., Ballesteros, C., Vela, A. I., Collins, M. D., Briones, V., Hutson, R. A., Potti, J., García-Borboroglu, P., Domínguez, L. & Fernández-Garayzábal, J. F. (2003b). *Corynebacterium sphenisci* sp. nov., isolated from wild penguins. *Int J Syst Evol Microbiol* **53**, 1009–1012.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kim, P. S., Shin, N. R., Kim, J. Y., Yun, J. H., Hyun, D. W. & Bae, J. W. (2013). *Gibbsiella papilionis* sp. nov., isolated from the intestinal tract of the butterfly *Mycalesis gotama*, and emended description of the genus *Gibbsiella*. *Int J Syst Evol Microbiol* **63**, 2607–2611.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. New York: Wiley.

Lehmann, K. B. & Neumann, R. (1896). *Atlas und Grundriss der Bakteriologie und Lehrbuch der Speziellen Bakteriologischen Diagnostik*. Munich: J. F. Lehmann.

Loy, A., Schulz, C., Lücker, S., Schöpfer-Wendels, A., Stoecker, K., Baranyi, C., Lehner, A. & Wagner, M. (2005). 16S rRNA gene-based oligonucleotide microarray for environmental monitoring of the betaproteobacterial order "*Rhodocyclales*". *Appl Environ Microbiol* **71**, 1373–1386.

MIDI (1999). *Sherlock Microbial Identification System Operating Manual, version 3.0*. Newark, DE: MIDI, Inc.

Rochelle, P. A., Fry, J. C., Parkes, R. J. & Weightman, A. J. (1992). DNA extraction for 16S rRNA gene analysis to determine genetic diversity in deep sediment communities. *FEMS Microbiol Lett* **100**, 59–65.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Schön, R. & Groth, I. (2006). Practical thin layer chromatography techniques for diaminopimelic acid and whole cell sugar analyses in the classification of environmental actinomycetes. *J Basic Microbiol* **46**, 243–249.

Shin, N. R., Jung, M. J., Kim, M. S., Roh, S. W., Nam, Y. D. & Bae, J. W. (2011). *Corynebacterium nuruki* sp. nov., isolated from an alcohol fermentation starter. *Int J Syst Evol Microbiol* **61**, 2430–2434.

Sjödén, B., Funke, G., Izquierdo, A., Akervall, E. & Collins, M. D. (1998). Description of some coryneform bacteria isolated from human clinical specimens as *Corynebacterium falsenii* sp. nov. *Int J Syst Bacteriol* **48**, 69–74.

Taguchi, R., Houjou, T., Nakanishi, H., Yamazaki, T., Ishida, M., Imagawa, M. & Shimizu, T. (2005). Focused lipidomics by tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **823**, 26–36.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tittsler, R. P. & Sandholzer, L. A. (1936). The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* **31**, 575–580.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.

Yano, I. & Saito, K. (1972). Gas chromatographic and mass spectrometric analysis of molecular species of corynomycolic acids from *Corynebacterium ulcerans*. *FEBS Lett* **23**, 352–356.