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NOTE

Chitinibacter suncheonensis sp. nov., a Chitinolytic Bacterium from a Mud Flat in Suncheon Bay[§]

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A chitinolytic bacterium, designated strain SK16^T, was isolated from a mud flat in Suncheon Bay, Republic of Korea. Strain SK16^T is Gram-negative, strictly aerobic, motile by a polar flagellum, and short rod-shaped. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain belonged to the genus *Chitinibacter* and was most closely related to *Chitinibacter tainanensis* S1^T (98.2% similarity). DNA-DNA hybridization analyses showed a low association value of 20.45±4.08% between them. The major cellular fatty acids, the G+C content of the genomic DNA, and the predominant quinone of the strain were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ *ω7c*; 50.5%) and $C_{12:0}$ (12.5%), 52.26 mol%, and Q-8, respectively. Based on the phylogenetic, chemotaxonomic, and phenotypic properties, strain SK16^T represents a novel species of the genus *Chitinibacter*, for which the name *Chitinibacter suncheonensis* sp. nov. is proposed. The type strain is SK16^T (=KCTC 23839^T =DSM 25421^T).

Keywords: *Chitinibacter suncheonensis* sp. nov., taxonomy, chitinolytic activity, mud flat

The genus *Chitinibacter* belongs to the family *Neisseriaceae*, class Betaproteobacteria. At the time of writing, the genus comprises two recognized species *Chitinibacter tainanensis* and *Chitinibacter alvei* (Chern *et al.*, 2004; Yang *et al.*, 2010). The type species of the genus, *C. tainanensis*, is a Gram-negative, aerobic, oxidase-positive, and catalase-positive bacterium with strong chitinolytic activity, and the closest phylogenetic neighbors of *C. tainanensis* are reported to be *Formivibrio citricus* and *Iodobacter fluvialtilis* (Chern *et al.*, 2004). *C. alvei* is a bacterium with weak chitinolytic activity and has the 16S rRNA gene sequence similarity of 96.0% with *C. tainanensis* (Yang *et al.*, 2010). The present study is concerned with the determination of the taxonomic position of strain SK16^T, a strongly chitinolytic bacterium isolated from mud.

Strain SK16^T was originally isolated from a mud flat in Suncheon Bay (34° 53′ 2.04″ N 127° 30′ 46.80″ E), Republic of Korea. The sample was enriched for chitinolytic microorganisms by incubating for 2 weeks at room temperature in the presence of 5% chitin powder and 1% colloidal chitin. Colloidal chitin was prepared as described by Hsu and Lockwood (1975). The enriched sample was serially diluted with 0.85% NaCl and then spread onto Luria-Bertani (LB) agar plates containing 0.2% colloidal chitin. Strain SK16^T formed colonies with a large clear zone after the incubation of the plates for 3 d at 30°C. The strain was preserved at -80°C in LB broth supplemented with 20% (v/v) glycerol. *C. tainanensis* S1^T was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany, and was used as a reference strain.

Extraction and purification of genomic DNA was carried out as described by Rodriguez and Tait (1983). Amplification of 16S rRNA gene was done by PCR using bacterial universal primers (Ward *et al.*, 1990). The PCR product was cloned using the pGEM T-Easy vector system (Promega, USA), and sequenced by Solgent Co. Ltd. (Korea). Sequence similarities were determined from pairwise 16S rRNA gene sequence comparisons using the EzTaxon-e server (http://eztaxon-e. ezbiocloud.net/; Kim *et al.*, 2012). Sequences were aligned by SINA (v1.2.9) using the SILVA as reference alignment (Pruesse *et al.*, 2012; available at www.arb-silva.de), and the alignment was refined using PHYDIT (Chun, 1995). From a total of 1,465 nucleotides, 1,333 nucleotides were analyzed for tree reconstruction. Phylogenetic analyses were performed by using PAUP* 4.0 (Swofford, 1998). Phylogenetic trees were inferred using neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1993), and maximum-parsimony (Fitch, 1971) algorithms. Distance matrices for the neighbor joining method were generated

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SK16^T is JN981166.

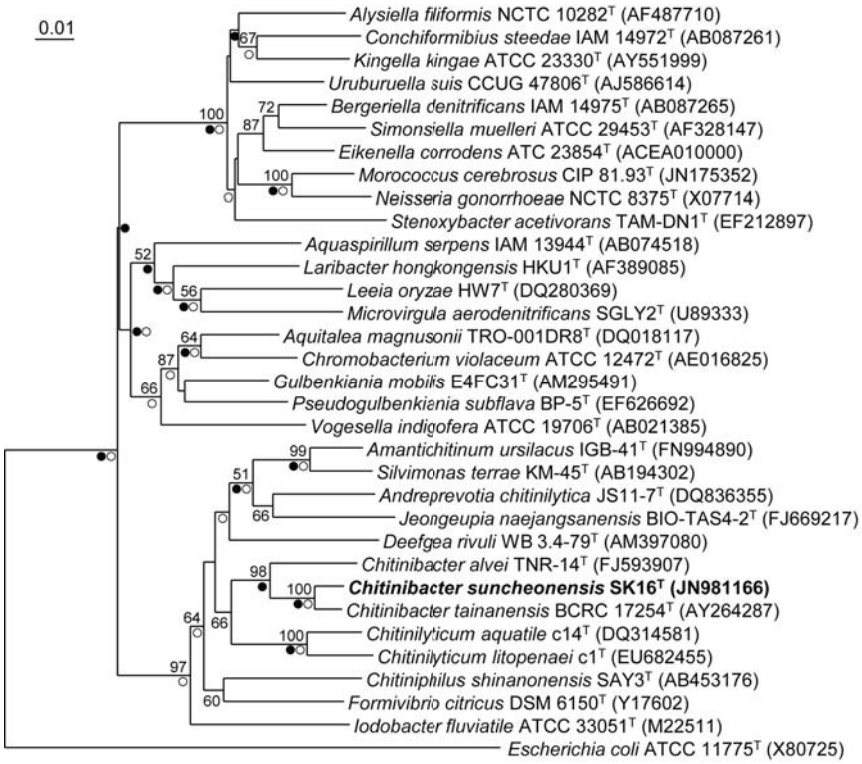

Fig. 1. Neighbor-joining tree based on 1,333 unambiguously aligned 16S rRNA gene sequences showing the position of strain SK16^T among members of the family *Neisseriaceae*. Bootstrap values (>50%) based on 1,000 resamplings are shown as percentages at branch nodes. Circles indicate that corresponding nodes were recovered in trees generated with the maximum-likelihood method (filled circles) and the maximum-parsimony method (open circles). *E. coli* ATCC 11775^T (X80725) was used as an outgroup. Bar, 0.01 nucleotide substitution per position.

according to the model of Jukes and Cantor (1969). The robustness of the topology in the neighbor-joining phylogenetic tree was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings.

The nearly complete 16S rRNA gene sequence of strain SK16^T consisting of 1,465 nucleotides showed the highest similarity of 98.2% to the type species of the genus *Chitinibacter*, *C. tainanensis* S1^T, and 96.2% similarity to the second species of the genus, *C. alvei* TNR-14^T, suggesting that the strain is a novel species belonging to the genus *Chitinibacter* (Fig. 1). The similarities of the 16S rRNA gene sequence of strain SK16^T to those of the members of the other genera in the family *Neisseriaceae* were lower than 95.3%: *Deefgea chitinilytica* Nsw-4^T (95.2%) (Chen *et al.*, 2010), *Deefgea rivuli* WB 3.4-79^T (94.0%) (Stackebrandt *et al.*, 2007), *Iodobacter fluviatile* ATCC 33051^T (93.2%) (Logan, 1989), and *Formivibrio citricus* DSM 6150^T (93.0%) (Hippe *et al.*, 1999). Overall topologies of the phylogenetic trees obtained with the neighbor-joining, maximum-likelihood and maximum-parsimony methods were similar to each other.

The DNA G+C contents were determined as described by Shin *et al.* (1996). The nucleoside mixture was separated by HPLC with an Agilent Eclipse 5 μm C_{18} column (250×4.6 mm^2). The DNA G+C contents of strain SK16^T and *C. tainanensis* S1^T were 52.26 and 56.14 mol%, respectively. The DNA G+C content of *C. alvei* TNR-14^T was reported to be 57.5 mol% (Yang *et al.*, 2010). DNA-DNA hybridization experiments were carried out in triplicate using reagents from Roche Diagnostics (Switzerland) according to the method described by Seldin and Dubnau (1985). The level of DNA-DNA relatedness between strain SK16^T and *C. tainanensis* S1^T was 20.45±4.08%, far below the 70% cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987).

Strain SK16^T and *C. tainanensis* S1^T were tested under the same conditions for various phenotypic properties described for *C. tainanensis* (Chern *et al.*, 2004). Gram stain was performed using the Gram stain kit (Difco, USA). Cell morphology and motility of exponentially growing cells in liquid culture were observed by transmission electron microscopy (Tecnai 12; Philips, Netherlands) using freshly prepared wet mounts according to the previously described procedure (Bruns *et al.*, 2001). The effects of temperature, pH, and NaCl concentration on growth were determined by measuring the turbidities (OD_{600}) of the cultures grown for 5 d under shaking. For the determination of the temperature range for growth, cells were grown in LB broth at 4, 10, 15, 20, 25, 30, 35, 40, 42, and 45°C. The pH range for growth was determined by growing cells at 37°C in LB broth adjusted to various pHs (pH 2.0 to 11.0 at intervals of 1.0 pH unit) with 1 N HCl or 1 N NaOH. Tolerance to NaCl was monitored by growing cells at 37°C in LB broth containing various concentrations of NaCl (0, 0.5, 1–10%, w/v, at intervals of 1.0%). Anaerobic growth on LB agar was tested at 37°C for 14 d using the GasPak anaerobic system (Anaerocult® A; Merck, Germany). DNase activity was determined with DNase test agar (Becton Dickinson, USA). Hydrolysis of Tween 20 (1%, w/v) was tested using LB agar as the basal medium as described by Tindall *et al.* (2007). Biochemical and physiological properties were tested using the API 20NE (bioMérieux, France), the API ZYM (bioMérieux), and the GN2 MicroPlate (Biolog, USA) according to the manu-

facturer's instructions.

Strain SK16^T formed yellowish, circular, slightly convex and semitranslucent colonies on an LB agar medium within 48 h of growth at 37°C. Colonies on an LB agar medium containing 0.2% colloidal chitin were surrounded by a large clear zone. Strain SK16^T was a Gram-negative, strictly aerobic, motile by a single polar flagellum, and short rod-shaped bacterium of 1.9 μm×0.9 μm in mean cell dimension (Supplementary data Fig. S1). Strain SK16^T grew well at 15–37°C (optimum 30–37°C) and at pH 7.0–10.0 (optimum pH 7.0–8.0) in the presence of 0–2% (w/v) NaCl. Unlike *C. tainanensis* S1^T which grew at 42°C, strain SK16^T did not grow at 42°C, though both the strain had the same optimum temperature, 30–37°C. Strain SK16^T grew at pH 10.0 but not at pH 6.0, while *C. tainanensis* S1^T and *C. alvei* TNR-14^T showed growth at pH 6.0 but not at pH 10.0. Strain SK16^T showed growth in the presence of 2% NaCl, while *C. tainanensis* S1^T and *C. alvei* TNR-14^T did not show growth. Phenotypic characteristics that serve to differentiate strain SK16^T from the type strains of *C. tainanensis* and *C. alvei* are presented in Table 1. Detailed results of the phenotypic and biochemical analyses are provided in the species description. Strain SK16^T was susceptible to antibiotics ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, minocycline, colistin, and trimethoprim/ sulfamethoxazole.

The compositions of cellular fatty acids of strain SK16^T and *C. tainanensis* S1^T were determined using the standard Microbial Identification System (MIDI) (Yang *et al.*, 1993; Lee *et al.*, 1996). Cells were grown in Tryptic soy broth (Difco) for 48 h at 37°C. Fatty acids were extracted, methylated, and analyzed by gas chromatography (6890; Agilent Technologies, USA). The major fatty acids of strain SK16^T were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$) (50.5%), $C_{12:0}$ (12.5%), $C_{16:0}$ (10.9%), and $C_{12:0}$ 3-OH (7.8%), and those of *C. tainanensis* S1^T were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$) (52.8%), $C_{12:0}$ 3-OH (13.1%), $C_{16:0}$ (7.9%), and $C_{12:0}$ (6.6%) (Table 2). Isoprenoid quinones

Table 1. Differential phenotypic characteristics of strain SK16^T and closely related *Chitinibacter* species

The data for strain SK16^T and *C. tainanensis* S1^T are from this study, and the data for *C. alvei* TNR-14^T were taken from Yang *et al.* (2010). +, Positive; -, negative; w, weakly positive.

Characteristic	SK16^T	*C. tainanensis* S1^T	*C. alvei* TNR-14^T
Growth at/with:			
37°C	+	+	-
42°C	-	+	-
pH 6.0	-	+	+
pH 9.0	+	+	-
pH 10.0	+	-	-
2% NaCl	+	-	-
Hydrolysis of:			
Chitin	+	+	w
DNA	-	-	+
Tween 20	-	-	+
API ZYM results			
Alkaline phosphatase	-	-	+
Esterase lipase (C8)	-	-	+
Valine arylamidase	-	+	-
Cystine arylamidase	-	+	-
Acid phosphatase	+	w	-
α-Glucosidase	-	-	+
Esterase (C4)	w	+	+
API 20NE results			
Nitrate reduction	+	-	+
β-Glucosidase	-	-	w
Protease	-	-	+
Glucose assimilation	+	+	-
Oxidation of (Biolog GN2):			
Glycogen	-	-	w
Tween 40	-	-	+
Tween 80	-	-	w
Maltose	w	-	+
Citric acid	-	-	+
Succinic acid	-	-	+
L-Alaninamide	-	-	+
L-Alanine	-	-	+
L-Alanyl glycine	-	-	+
L-Asparagine	-	-	+
L-Aspartic acid	-	-	+
L-Glutamic acid	-	-	w
Glycyl L-aspartic acid	-	-	+
Glycyl L-glutamic acid	-	-	+
L-Histidine	-	-	+
L-Phenylalanine	-	-	w
L-Serine	-	-	+
Glycerol	-	-	+
D-Mannose	-	-	+
Turanose	w	w	-
D-Gluconic acid	+	-	+
α-Ketobutyric acid	w	-	-
DNA G+C content (mol%)	52.46	56.14	57.5

Table 2. Cellular fatty acid compositions of strain SK16^T and closely related *Chitinibacter* species

Values for SK16^T and *C. tainanensis* S1^T are from this study. Values for *C. alvei* TNR-14^T (R2A agar, 20°C, 3 d) were from Yang *et al.* (2010). Only those fatty acids representing >1% of the total cellular fatty acids of either of the strains are shown. -, Less than 1.0% or not detected. Summed features represent groups of two or three fatty acids that could not be separated by GLC with the Microbial Identification (MIDI). Summed feature 3 comprised iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$.

Fatty acid	SK16^T	*C. tainanensis* S1^T	*C. alvei* TNR-14^T
Saturated fatty acids			
$C_{12:0}$	12.50	6.60	3.29
$C_{12:0}$ 3-OH	7.78	13.06	4.06
$C_{14:0}$	1.54	-	2.46
$C_{15:0}$	2.40	1.50	
$C_{16:0}$	10.92	7.86	27.22
$C_{16:0}$ 3-OH	2.38	2.91	1.87
$C_{19:0}$ 10-methyl	1.81	1.32	
Unsaturated fatty acids			
$C_{15:1}$ $\omega6c$	1.39	2.24	
$C_{15:1}$ $\omega8c$	1.22	-	
$C_{18:1}$ $\omega7c$	1.22	4.17	
Summed In Feature:*			
3	50.54	52.82	54.03

were purified and analyzed by HPLC (SP930D; Young Lin, Korea) as described by Shin *et al.* (1995). Strain SK16^T contained Q-8 as the major quinone (79.2%).

The phylogenetic, chemotaxonomic, and phenotypic properties suggest that strain SK16^T represents a novel species of the genus *Chitinibacter*, for which the name *Chitinibacter suncheonensis* sp. nov. is proposed.

Description of *Chitinibacter suncheonensis* sp. nov.

Chitinibacter suncheonensis (sun.cheon.en'sis. N.L. adj. *suncheonensis* pertaining to Suncheon Bay, Republic of Korea, where the type strain was isolated).

Cells are Gram-negative rods (1.9 μm × 0.9 μm in mean cell dimension) that are motile by means of a single flagellum. Colonies on LB agar are yellowish, circular, slightly convex and semitranslucent. Colonies are approximately 1.0–2.0 mm in diameter after growth on LB agar for 72 h at 37°C. Colonies on LB/ 0.2% colloidal chitin medium are surrounded by a large clear zone. Cells grow well at 15–37°C (optimum 30–37°C) and at pH 7.0–10.0 (optimum pH 7.0–8.0) in the presence of 0–2% (w/v) NaCl. Cells are strictly aerobic. Positive for hydrolysis of chitin and negative for hydrolysis of DNA and Tween 20. In API 20NE strip, cells are positive for glucose acidification and glucose assimilation; negative for β-glucosidase (esculin hydrolysis), protease (gelatine hydrolysis), indole production, arginine dihydrolase, urease, and β-galactosidase; negative for assimilation of arabinose, mannose, caprate, adipate, and phenyl acetate. Nitrate is reduced to nitrite; nitrite is reduced. In the API ZYM system, cells are positive for acid phosphatase, leucine arylamidase, N-acetyl-β-glucosaminidase; weakly positive for esterase (C4); negative for alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase. In the Biolog GN2 Microplate system, it utilizes the following carbon substrates: dextrin, D-gluconic acid, N-acetyl-D-glucosamine, D-fructose, and α-D-glucose; grows weakly on maltose, pyruvic acid methyl ester, turanose, and α-ketobutyric acid; do not utilize glycogen, Tween 40, Tween 80, citric acid, malonic acid, succinic acid, L-alaninamide, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-phenylalanine, L-serine, glycerol, D-mannose, α-cyclodextrin, N-acetyl-D-galactosamine, adonitol, D-arabitol, cellobiose, *i*-erythritol, L-fucose, D-galactose, gentiobiose, *myo*-inositol, α-D-lactose, lactulose, D-mannitol, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, xylitol, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, α-ketovaleric acid, D,L-lactic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, hydroxyl-L-proline, L-leucine, L-ornithine, L-proline, L-pyroglutamic acid, D-serine, D,L-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, D,L-α-glycerol phosphate, glucose 1-phosphate, and glucose 6-phosphate. Susceptible to ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, minocycline, colistin, and trimethoprim/sulfamethoxazole. The major cellular fatty acids (>5% of total fatty acid) are summed feature 3 (iso-$C_{15:0}$ 2OH and/or $C_{16:1}$ $\omega7c$), $C_{12:0}$, $C_{16:0}$, and $C_{12:0}$ 3OH. The predominant isoprenoid quinone is Q-8. The G+C content of genomic DNA is 52.26 mol%.

The type strain, SK16^T (=KCTC 23839^T =DSM 25421^T), was isolated from the mud and seawater obtained from a mud flat in Suncheon Bay, Republic of Korea.

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