Koreibacter algae gen. nov., sp. nov., isolated from seaweed

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A Gram-positive, aerobic, non-motile, rod-shaped actinomycete, designated strain DSW-2^T, was isolated from a seaweed sample collected around Mara Island, Jeju, Republic of Korea.

Comparative 16S rRNA gene sequence analysis showed that strain DSW-2^T belongs to the suborder *Micrococcineae* and forms a distinct clade separated from representatives of the several families of this order. Levels of 16S rRNA gene sequence similarity between the novel strain and members of this suborder were lower than 96.4 %. The peptidoglycan type is A3 α with Lys–Ser as the interpeptide bridge. Whole-cell sugars are glucose and galactose. The major menaquinone is MK-9(H₄). The predominant fatty acid is ai-C_{15:0}. The polar lipids are phosphatidylglycerol and phosphatidylinositol. The DNA G + C content was 68.3 mol%. On the basis of the chemotaxonomic markers and phylogenetic distinctiveness presented here, it is evident that the isolate represents a novel taxon within the suborder *Micrococcineae*. The name *Koreibacter algae* gen. nov., sp. nov. is proposed, with the type strain DSW-2^T (=KCTC 13436^T =DSM 22126^T).

The suborder *Micrococcineae* (Stackebrandt *et al.*, 1997) is one of the largest and most diverse taxa in the class *Actinobacteria* and originally contained nine families: *Micrococcaceae*, *Brevibacteriaceae*, *Cellulomonadaceae*, *Dermabacteraceae*, *Dermatophilaceae*, *Intrasporangiaceae*, *Jonesiaceae*, *Microbacteriaceae* and *Promicromonosporaceae*. Five novel families have been subsequently added to the suborder on the basis of phylogenetic analysis: *Bogoriellaceae*, *Dermacoccaceae*, *Rarobacteraceae*, *Sanguibacteraceae* (Stackebrandt & Schumann, 2000) and *Yaniellaceae* (Li *et al.*, 2008). To include these novel taxa, the descriptions of these families were recently emended on the basis of phylogenetic analyses and the signature patterns of their 16S rRNA gene sequences, and, based on this revision, the new family *Beutenbergiaceae* was created in the suborder *Micrococcineae* (Zhi *et al.*, 2009). This paper describes the classification of a *Micrococcineae* strain that was isolated from seaweed.

Strain DSW-2^T was isolated from a sample of an unknown seaweed collected around Mara Island in Jeju, Republic of Korea. The seaweed was directly spread onto SC-SW agar (1 % soluble starch, 0.03 % casein, 0.2 % KNO₃, 0.2 % NaCl, 0.2 % KH₂PO₄, 0.002 % CaCO₃, 0.005 % MgSO₄·7H₂O, 0.001 % FeSO₄·7H₂O, 1.8 % agar; 60 % natural seawater and 40 % distilled water) and the plate was incubated at 30 °C for 2 weeks. The isolate was maintained

on marine agar (MA; Difco) and in 20 % (v/v) glycerol suspensions containing 60 % natural seawater and 20 % distilled water at –20 °C and –80 °C, respectively.

Cell morphology and motility were observed by using phase-contrast and transmission electron microscopy, with cells grown on MA at 30 °C for 5 days. Colony characteristics were observed on MA at 30 °C for 5 days. Conditions for growth were examined on MA at 4, 10, 20, 25, 30, 37, 40 and 45 °C and pH 3.5, 4.1–12.1 (in intervals of one pH unit), 13.0 and 14.0 and on tryptic soy agar (TSA; Difco) supplemented with 1–12 % (w/v) NaCl (in intervals of 1 %). Gram stain, oxidase and catalase activities and degradation and utilization of carbohydrates were tested by using previously described methods (Lee & Lee, 2008). Enzyme activities were determined by using the API ZYM kit (bioMérieux), according to the manufacturer's directions.

Cells of strain DSW-2^T were aerobic, Gram-positive, non-motile rods (0.3–0.5 × 1.5–2.3 µm) (Fig. 1). Colonies of the cells were light yellow, circular and convex with entire margins, approximately 0.5–0.8 mm in diameter after incubation on MA at 30 °C for 5 days. Physiological and biochemical properties are given in the genus and species descriptions.

Genomic DNA was isolated according to the method of Hopwood *et al.* (1985). Amplification of the 16S rRNA gene and sequencing were performed as described previously (Lee & Lee, 2008). The partial 16S rRNA gene sequence of strain DSW-2^T determined in this study (1401 nt) was subjected to a preliminary BLAST search against

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSW-2^T is FM995611.

Details of additional sequences used in the construction of Fig. 2 and 16S rRNA gene sequence similarities are available as supplementary material with the online version of this paper.

Fig. 1. Transmission electron micrograph of a cell of strain DSW-2^T grown on MA at 30 °C for 5 days. Bar, 0.5 µm.

GenBank entries, which showed maximum similarities of less than 96% to members of the families *Cellulomonadaceae*, *Sanguibacteraceae* and *Promicromonosporaceae* in the suborder *Micrococcineae*. The CLUSTAL X program (Thompson *et al.*, 1997) was used to create a multiple alignment of the sequences of strain DSW-2^T and representatives of the suborder *Micrococcineae*. The alignment was optimized manually according to the secondary structure of the *Escherichia coli* 16S rRNA molecule (Brosius *et al.*, 1978). A total of 1201 unambiguous nucleotides present in all strains (*E. coli* positions 45 and 1464) was used for tree construction. Phylogenetic analyses were performed with several treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). An evolutionary distance matrix was calculated using the model of Jukes & Cantor (1969) and a phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (Felsenstein, 1985) was performed with 1000 resamplings.

The 16S rRNA gene sequence tree (Fig. 2 and Supplementary Table S1, available in IJSEM Online) shows that strain DSW-2^T belongs to the suborder *Micrococcineae* and forms a lineage distinct from those of several *Micrococcineae* representatives. 16S rRNA gene sequence similarity values between strain DSW-2^T and representatives of *Micrococcineae* genera were as follows: in the family *Cellulomonadaceae*, 96.1–96.4% (*Oerskovia*), 93.8–95.6% (*Cellulomonas*) and 94.8% (*Actinotalea*); in the family *Sanguibacteraceae*, 95.3–96.1% (*Sanguibacter*); in the family *Promicromonosporaceae*, 95.3–96.1% (*Cellulosimicrobium*), 94.7–95.3% (*Isophtericola*) and 94.2–95.8% (*Promicromonospora*); and in the family *Beutenbergiaceae*, 95.2% (*Beutenbergia*). Levels of 16S rRNA gene sequence similarity between the novel strain and other representatives of this suborder were 94.6% or less (Supplementary Table S2, available in IJSEM Online). In other analyses

with a total of 1339 nt present in all strains, 16S rRNA gene sequence similarity values between strain DSW-2^T and representatives of this suborder were slightly lower ($0.4 \pm 0.3\%$) than those described above (data not shown).

The amino acid composition of the cell-wall peptidoglycan was determined by reversed-phase HPLC (Waters 2690) as described previously (Lee, 2007). Purified cell-wall material was prepared according to the method of Hancock (1994). Cell-wall sugars were analysed by GC as described by Saddler *et al.* (1991). Menaquinones were analysed by HPLC as described by Kroppenstedt (1985). Polar lipids were determined by TLC as described by Minnikin *et al.* (1975). Cellular fatty acids of strain DSW-2^T were determined by using cells grown on TSA for 3 days at 30 °C and analysis of the methyl esters was performed according to the instructions of the Microbial Identification System (version 6; MIDI). The G + C content of the DNA was determined by HPLC according to Mesbah *et al.* (1989).

The purified cell wall of strain DSW-2^T contained lysine as the diagnostic diamino acid. The molar ratio of Ala/Ser/Glu/Lys was estimated to be 1.5:0.6:1.0:1.0. Asp and Gly were not detected. These results suggested that the structure of the linkage is Lys–Ser and that the peptidoglycan type is A3 α (Schleifer & Kandler, 1972). The cell-wall sugar was galactose. The predominant menaquinone was MK-9(H₄). The polar lipids were phosphatidylglycerol and phosphatidylinositol. The cellular fatty acid profile of strain DSW-2^T contained ai-C_{15:0} (80.7%), ai-C_{17:0} (3.9%), C_{16:0} (3.5%), i-C_{14:0} (2.5%), i-C_{16:0} (2.1%), C_{18:0} (1.8%), C_{14:0} (1.1%) and i-C_{15:0} (1.1%). The G + C content of the DNA was 68.3 mol%.

The 16S rRNA gene sequence analysis clearly showed that strain DSW-2^T belongs to the suborder *Micrococcineae*. This is also supported by the possession of 16S rRNA gene sequence signature nucleotides of the *Micrococcineae* lineage (Zhi *et al.*, 2009). However, the novel strain can be readily differentiated from phylogenetically closely related genera of this suborder by its chemotaxonomic properties and, in particular, the combination of the diamino acid type and the composition of interpeptide bridges in the cell-wall peptidoglycan (Table 1). For example, the genera *Cellulosimicrobium*, *Oerskovia* and *Sanguibacter* contain lysine as the diamino acid in the cell-wall peptidoglycan and have MK-9(H₄) as the major menaquinone, as does strain DSW-2^T, but they clearly differ from strain DSW-2^T in the composition of the interpeptide bridges in the cell-wall peptidoglycan, the major cellular fatty acids and requirement for oxygen for growth.

On the basis of its phenotypic features and distinct phylogenetic position, strain DSW-2^T is considered to be well separated from all genera of the suborder *Micrococcineae*. The name *Koreibacter algae* gen. nov., sp. nov. is proposed.

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain DSW-2^T amongst representatives of the suborder *Micrococccineae*, constructed from an evolutionary distance matrix with the neighbour-joining method. The taxa and sequences used for the genus or family groups and not shown are detailed in Supplementary Table S1. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Asterisks indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. *Glycomyces harbinensis* IMSNU 20070 was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

Table 1. Differential characteristics of strain DSW-2^T from the phylogenetically closely related genera of the suborder Micrococcineae

Taxa: 1, *Koreibacter algae* gen. nov., sp. nov., DSW-2^T; 2, *Actinotalea* (Bagnara *et al.*, 1985; Yi *et al.*, 2007); 3, *Beutenbergia* (Groth *et al.*, 1999); 4, *Cellulosimicrobium* (Schumann *et al.*, 2001; Brown *et al.*, 2006; Yoon *et al.*, 2007); 5, *Cellulomonas* (An *et al.*, 2005; Brown *et al.*, 2005; Elberson *et al.*, 2000; Jones *et al.*, 2005; Rivas *et al.*, 2004); 6, *Isoptericola* (Stackebrandt *et al.*, 2004; Zhang *et al.*, 2005; Groth *et al.*, 2005); 7, *Oerskovia* (Stackebrandt *et al.*, 2002); 8, *Promicromonospora* (Alonso-Vega *et al.*, 2008; Busse *et al.*, 2003; Kalakoutskii *et al.*, 1989; Takahashi *et al.*, 1987; Jiang *et al.*, 2009); 9, *Sanguibacter* (Huang *et al.*, 2005; Hong *et al.*, 2008). A, Aerobic; F, facultatively anaerobic.

Characteristic	1	2	3	4	5	6	7	8	9
Oxygen requirement	A	F	A	F	A or F	F	F	A	F
Major menaquinone	MK-9(H ₄)	MK-10(H ₄)	MK-8(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄), MK-9(H ₂), MK-9	MK-9(H ₄)	MK-9(H ₄) or MK-9	MK-9(H ₄)
Diamino acid	Lys	L-Orn	Lys	L-Lys	L-Orn	L-Lys	L-Lys	L-Lys	L-Lys
Interpeptide bridge	Lys-Ser	D-Asp	L-Lys-L-Glu	D-Ser-D-Asp or L-Thr-D-Asp	D-Asp or D-Glu	D-Asp or D-Glu	L-Thr-D-Glu or L-Thr-D-Asp	Gly-Ala	D-Ser-D-Glu
Predominant fatty acid(s)	ai-C _{15:0}	C _{14:0} , ai-C _{15:0} , C _{16:0}	ai-C _{15:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{16:0} , C _{16:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{15:0} , C _{16:0} , ai-C _{17:0} , C _{18:0}	ai-C _{15:0} , i-C _{15:0} , C _{14:0} , C _{16:0} , i-C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , i-C _{15:0} , C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , i-C _{15:0} , C _{15:0}	ai-C _{15:0} , C _{16:0} , C _{18:0}
DNA G + C content (mol%)	68.3	76	71	72.9–74.5	71–76	70–73.8	71	70–70.8	69–73

Description of *Koreibacter* gen. nov.

Koreibacter (Ko.re.i.bac'ter. N.L. n. *Korea*, Korea; N.L. masc. n. *bacter* rod; N.L. masc. n. *Koreibacter* a Korean rod, a rod isolated from Korea, referring to the site from which the type strain of the type species was isolated).

Cells are Gram-positive, aerobic, oxidase-negative, catalase-positive, non-motile rods (0.3–0.5 × 1.5–2.3 µm). The peptidoglycan type is A3α with Lys–Ser as the interpeptide bridge. The whole-cell sugar is galactose. The major menaquinone is MK-9(H₄). The predominant fatty acid is ai-C_{15:0}. The polar lipids are phosphatidylglycerol and phosphatidylinositol. The DNA G + C content is 68.3 mol%. Phylogenetically, the genus is a member of the suborder Micrococcineae, order Actinomycetales. The type species is *Koreibacter algae*.

Description of *Koreibacter algae* sp. nov.

Koreibacter algae (al'gae. L. gen. n. *algae* of alga, seaweed).

The morphological and chemotaxonomic characteristics are the same as those given in the genus description. Colonies are light yellow, circular, convex with entire margins and approximately 0.5–0.8 mm in diameter after incubation on MA at 30 °C for 5 days. Growth occurs at 10–37 °C (optimum 25–30 °C) and at pH 4.1–12.1 (optimum pH 7.1), but not at pH 3.5 and 13.0, and with 0–10 % NaCl (optimum 1–4 %). DNA is degraded, but casein, cellulose, chitin, elastin, hypoxanthine, starch, L-tyrosine and xanthine are not. Enzyme tests with the API ZYM system are positive for α-glucosidase and N-acetyl-β-

glucosaminidase, weakly positive for esterase (C4) and esterase lipase (C8) and negative for alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. Acid is produced from maltose, L-rhamnose, trehalose and glycerol, but not from D-arabinose, L-arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-glucose, inulin, lactose, D-mannose, melezitose, α-methyl D-glucoside, α-methyl D-mannoside, raffinose, salicin, L-sorbose, sucrose, D-xylose, D-dulcitol, meso-erythritol, myo-inositol, D-mannitol, D-sorbitol or D-xylitol. The predominant cellular fatty acid is ai-C_{15:0}. The DNA G + C content of the type strain is 68.3 mol%.

The type strain, DSW-2^T (=KCTC 13436^T =DSM 22126^T), was isolated from a seaweed sample taken on the coast of Mara Island, Jeju, Republic of Korea.

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