Phycococcus dokdonensis sp. nov., isolated from soil

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A Gram-positive, non-motile, coccoid-shaped bacterium, designated strain DS-8^T, was isolated from soil from Dokdo, Korea, and its taxonomic position was investigated by using a polyphasic study. Strain DS-8^T grew optimally at 30 °C and pH 6.5–7.5 in the presence of 0.5–1.0 % (w/v) NaCl. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain DS-8^T joined the type strain of *Phycococcus jejuensis*, with a bootstrap resampling value of 92.5 %, and shared 96.5 % 16S rRNA gene sequence similarity with *P. jejuensis*. The cell-wall peptidoglycan type was based on meso-diaminopimelic acid and the acyl type of the muramic acid was acetyl. The predominant menaquinone was MK-8(H₄). The major fatty acids were iso-C_{15:0} and iso-C_{16:0}. Major polar lipids detected in strain DS-8^T were phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids. The DNA G + C content was 70.7 mol%. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness, revealed that strain DS-8^T differs from *P. jejuensis*. On the basis of the data obtained, strain DS-8^T is considered to represent a novel species of the genus *Phycococcus*, for which the name *Phycococcus dokdonensis* sp. nov. is proposed. The type strain is DS-8^T (=KCTC 19248^T=CCUG 54521^T).

The genus *Phycococcus* was erected by Lee (2006) with the description of *Phycococcus jejuensis* as the sole recognized species. Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Phycococcus* falls within the family *Intrasporangiaceae*, class *Actinobacteria* (Lee, 2006). The genus *Phycococcus* is characterized chemotaxonomically by having the cell-wall peptidoglycan based on meso-diaminopimelic acid, MK-8(H₄) as the predominant menaquinone, muramic acid of the acetyl type, major amounts of phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol, and C_{17:1}ω8c, iso-C_{16:0} and iso-C_{15:0} as major fatty acids. *P. jejuensis* was isolated from a dried seaweed sample on a sandy beach of Jeju in Korea. In the present study, we report on the taxonomic characterization of a *Phycococcus*-like bacterial strain, DS-8^T, which was isolated from soil of Dokdo (37° 14' 12" N 131° 52' 07" E), Korea.

Strain DS-8^T was isolated by means of the standard dilution plating technique at 25 °C on 10 × diluted nutrient agar (Difco). *P. jejuensis* KCCM 42315^T was obtained from the Korean Culture Center of Microorganisms, Seoul, Korea. The morphological, physiological and biochemical characteristics of strain DS-8^T were investigated by using routine cultivation on trypticase soy agar (TSA; Difco) at 30 °C. Cell

morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy. Flagellation was examined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on TSA. Growth in the absence of NaCl and at various NaCl concentrations [0.5 % (w/v) and 1.0–7.0 % (w/v) at intervals of 1.0 %] was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded from the medium formula. The pH range for growth was determined in nutrient broth (Difco) that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units), prior to sterilization, by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber under a nitrogen atmosphere on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v). Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were studied according to Lanyi (1987). Antibiotic susceptibility

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was tested on TSA plates by using discs containing the following antibiotics: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) or carbenicillin (100 µg). Utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 50CH, API ZYM, API 20E and API 20NE systems (bioMérieux); utilization of various substrates was determined by inoculating API 50CH strips with cells suspended in AUX medium (bioMérieux).

Cell biomass of strain DS-8^T for DNA extraction and for analyses of the cell wall, isoprenoid quinones and polar lipids was obtained from cultivation in trypticase soy broth (Difco) at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR by using two universal primers as described by Yoon *et al.* (1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed by using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The isomer type of the diamino acid of the cell-wall peptidoglycan was analysed by the method of Komagata & Suzuki (1987). The cell-wall acyl type was determined as described by Uchida & Aida (1984). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For fatty acid methyl ester analysis, cell mass of strain DS-8^T and *P. jejuensis* KCCM 42315^T was harvested from TSA plates after incubation for 7 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization experiments were performed fluorometrically according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values.

The morphological, cultural, physiological and biochemical characteristics of strain DS-8^T are given in the species description below or are shown in Table 1. Strain DS-8^T was susceptible to cephalothin, chloramphenicol, gentamicin,

Table 1. Differential phenotypic characteristics between strain DS-8^T and *Phycococcus jejuensis*

Taxa: 1, strain DS-8^T; 2, *P. jejuensis* (data from Lee, 2006). Cells of both taxa are Gram-positive, non-motile cocci. Both taxa are positive for catalase, hydrolysis of aesculin, casein, gelatin and starch, utilization of D-glucose, mannose, mannitol, N-acetylglucosamine, maltose, melibiose, sucrose, trehalose, raffinose, glycogen, gentiobiose, D-arabitol and turanose, and for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase. Both are negative for urease, β-glucuronidase, N-acetyl-β-glucosaminidase and α-fucosidase. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2
Nitrate reduction	–	+
Growth at 4 °C	–	+
Maximum growth temperature (°C)	36	37
Growth in 7.0 % (w/v) NaCl	–	+
Oxidase	+	–
Utilization of:		
Glycerol	–	+
L-Arabinose	–	+
Ribose	–	+
D-Xylose	–	+
D-Galactose	+	w
D-Fructose	+	w
Rhamnose	–	+
Inositol	–	+
Sorbitol	–	+
Cellobiose	+	w
Lactose	w	+
Xylitol	–	+
Salicin	–	+
L-Fucose	–	w
Amygdalin	–	+
Enzyme activity (API ZYM)		
Lipase (C14)	–	+
Trypsin	–	+
α-Chymotrypsin	–	+
α-Mannosidase	w	–
Isolation source	Soil	Seaweed
Major polar lipids*	PI, DPG, PG, PL	PE, PI, DPG
DNA G+C content (mol%)	70.7	74

*DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid.

lincomycin, neomycin, novobiocin, oleandomycin, polymyxin B, streptomycin and tetracycline, but not to ampicillin, carbenicillin, kanamycin or penicillin G (concentrations as indicated above). The almost complete 16S rRNA gene sequence of strain DS-8^T determined in this

study comprised 1481 nt (approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence). Comparative 16S rRNA gene sequence analyses showed that strain DS-8^T was phylogenetically most closely related to members of the family *Intrasporangiaceae*. In the neighbour-joining tree based on 16S rRNA gene sequences, strain DS-8^T joined the type strain of *P. jejuensis* at a bootstrap confidence value of 92.5 % (Fig. 1). This topology was also found in the tree based on the maximum-parsimony algorithm (Fig. 1). Strain DS-8^T exhibited 16S rRNA gene sequence similarity values of 96.5 % to the type strain of *P. jejuensis*, 96.7 % to the type strain of *Terrabacter tumescens* and 93.9–96.4 % to the type strains of other members of the family *Intrasporangiaceae* used in the phylogenetic analysis.

Strain DS-8^T had *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The acyl type of the muramic acid was acetyl. Strain DS-8^T contained menaquinone-8(H₄) as the predominant isoprenoid quinone. The fatty acid profile of strain DS-8^T showed the presence of large amounts of branched, straight-chain and unsaturated fatty acids; the major components were iso-C_{15:0} and iso-C_{16:0}. This fatty acid profile was similar to that of *P. jejuensis*, although there were differences in the proportions of some components (Table 2). Major polar lipids detected in strain DS-8^T were phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids. The DNA G+C content of strain DS-8^T was 70.7 mol%. The cell-wall composition and the predominant menaquinone were similar between strain DS-8^T and *P. jejuensis*, while some differences in the polar lipid and fatty acid profiles were revealed (Lee, 2006; Tables 1 and 2). These differences have been reported for members of some related genera (Hanada *et al.*, 2002; Montero-Barrientos *et al.*, 2005). Accordingly, on the basis of the phylogenetic and chemotaxonomic data, it is appropriate to classify strain DS-8^T within the genus *Phycoccus*.

Strain DS-8^T exhibited a mean DNA–DNA relatedness value of 12 % to *P. jejuensis* KCCM 42315^T. Strain DS-8^T

was distinguishable from *P. jejuensis* based on differences in several phenotypic properties, as shown in Table 1. This phylogenetic distinctiveness, together with the DNA–DNA relatedness data and differential phenotypic properties, was sufficient to categorize strain DS-8^T as representing a novel species of the genus *Phycoccus* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), for which the name *Phycoccus dokdonensis* sp. nov. is proposed.

Description of *Phycoccus dokdonensis* sp. nov.

Phycoccus dokdonensis (dok.do.nen'sis. N.L. masc. adj. *dokdonensis* of Dokdo, Korea, from where the type strain was isolated).

Cells are Gram-positive, non-spore-forming, non-motile cocci (0.3–0.7 µm). Colonies on TSA are circular, convex, smooth, glistening, greyish yellow in colour and 0.6–1.2 mm in diameter after incubation for 7 days at 30 °C. Optimal growth occurs at 30 °C; growth occurs at 10 and 36 °C, but not at 4 or 37 °C. Optimal pH for growth is between 6.5 and 7.5; growth occurs at pH 5.0 and 8.5, but not at pH 4.5 or 9.0. Growth occurs in the presence of 0–5.0 % (w/v) NaCl, with optimum growth in the presence of 0.5–1.0 % (w/v) NaCl. Growth does not occur under anaerobic conditions on TSA or on TSA supplemented with potassium nitrate. Hypoxanthine and Tweens 20, 40, 60 and 80 are hydrolysed, but xanthine and L-tyrosine are not. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H₂S and indole are not produced. Aesculin, melezitose, starch, D-lyxose, gluconate and malate are utilized, but erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, arbutin, inulin, D-tagatose, D-fucose, L-arabitol, 2-ketogluconate, 5-ketogluconate, adipate, caprate, citrate and phenylacetate are not. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid. The acyl type of the muramic acid is acetyl. The predominant menaquinone is MK-8(H₄). The major fatty acids (>10 % of the total fatty acids) are iso-C_{15:0} and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DS-8^T and some other related taxa. Bootstrap values (based on 1000 replications) are shown as percentages at each node; only values ≥50 % are shown. Dots indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Brevibacterium linens* DSM 20425^T was used as outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid content (% of total) of strain DS-8^T and *Phycoccus jejuensis* KCCM 42315^T

Strain: 1, DS-8^T; 2, *P. jejuensis* KCCM 42315^T. Fatty acids that represented <0.5% in both strains were omitted. Data are from the present study. —, Not detected.

Fatty acid	1	2
Straight-chain fatty acid		
C _{14:0}	0.2	0.7
C _{15:0}	2.3	8.2
C _{16:0}	0.9	2.3
C _{17:0}	4.5	9.7
C _{18:0}	0.7	0.4
Unsaturated fatty acid		
C _{15:1ω6c}	1.1	0.9
C _{17:1ω6c}	2.5	—
C _{17:1ω8c}	4.8	23.6
C _{18:1ω9c}	0.9	4.1
Branched fatty acid		
iso-C _{14:0}	8.0	3.8
iso-C _{15:0}	30.1	17.1
anteiso-C _{15:0}	8.4	4.0
iso-C _{16:1}	2.0	—
iso-C _{16:0}	20.8	13.5
iso-C _{17:0}	0.9	0.6
anteiso-C _{17:0}	2.0	1.6
iso-C _{18:0}	0.4	0.7
10-Methyl fatty acid		
C _{16:0}	1.2	0.2
C _{17:0}	4.1	1.9
Hydroxy fatty acid		
C _{17:0} 3-OH	0.9	2.8
Summed feature*		
3	1.3	1.9
6	0.4	0.9

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 comprised C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. Summed feature 6 comprised C_{19:1ω9c} and/or C_{19:1ω11c}.

iso-C_{16:0}. Major polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids. The DNA G+C content is 70.7 mol% (HPLC). Other phenotypic properties are given in Table 1.

The type strain, DS-8^T (=KCTC 19248^T=CCUG 54521^T), was isolated from soil from Dokdo, Korea.

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