Phycococcus aerophilus sp. nov., isolated from air

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A Gram-positive, short rod-shaped, non-spore-forming bacterium, designated 5516T-20^T, was isolated from an air sample. The results of 16S rRNA gene sequence analyses showed that strain 5516T-20^T belonged to the family *Intrasporangiaceae*, having the highest sequence similarities (97.5 and 96.1 %, respectively) with respect to the type strains of *Phycococcus dokdonensis* and *Phycococcus jejuensis*. The value for DNA–DNA hybridization between 5516T-20^T and *P. dokdonensis* DS-8^T was 41 %. Strain 5516T-20^T contained menaquinone MK-8(H₄) as the major isoprenoid quinone, possessed phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol as the polar lipids and contained glucose and ribose as whole-cell sugars. The major fatty acids were C_{17:1}ω8c, iso-C_{16:0}, iso-C_{15:0}, C_{17:0} and iso-C_{14:0}. The G + C content of the DNA from strain 5516T-20^T was 70.5 mol%. On the basis of the data from the polyphasic taxonomic study, strain 5516T-20^T represents a novel species within the genus *Phycococcus*, for which the name *Phycococcus aerophilus* sp. nov. is proposed. The type strain is 5516T-20^T (=KACC 20658^T =DSM 18548^T).

The genus *Phycococcus* was described by Lee (2006) with a single species, *Phycococcus jejuensis*. Recently, the species *Phycococcus dokdonensis* was added to the genus (Yoon *et al.*, 2008). The genus *Phycococcus* includes Gram-positive, coccus-shaped bacteria with *meso*-diaminopimelic acid as the diamino acid of the peptidoglycan, MK-8(H₄) as the predominant menaquinone and C_{17:1}ω8c, iso-C_{16:0}, iso-C_{15:0}, C_{15:0} and C_{17:0} as the major fatty acids. Phylogenetically, the genus belongs to the family *Intrasporangiaceae*, suborder *Micrococcineae* (Stackebrandt *et al.*, 1997).

Strain 5516T-20^T was isolated during the characterization of bacterial strains isolated from air collected with an air sampler (single-stage, multiple-hole impactor, MAS-100; Merck) in the Taean region of the Republic of Korea. The sampler contained Petri dishes with R2A agar (BBL) amended with 0.02 % cycloheximide (Sigma). After sampling, the plates were incubated at 30 °C for 5 days.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5516T-20^T is EF493847.

Cellular fatty acid profiles of strain 5516T-20^T and related strains are available as supplementary material with the online version of this paper.

Strain 5516T-20^T produced white-coloured colonies on R2A medium. The strain was found to be able to grow on nutrient agar and tryptic soy agar, but not on MacConkey agar (all from Difco).

For the phenotypic characterization, R2A medium was used as the basal medium. Gram staining, catalase and oxidase activities and hydrolysis of casein, CM-cellulose, chitin, DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine and xanthine were determined using the methods of Smibert & Krieg (1994). Temperature, salinity and pH for growth were tested at 5, 10, 20, 25, 30, 37, 40 and 45 °C, at 0, 1, 3, 5, 7 and 10 % (w/v) NaCl and at pH 4, 5, 6, 7, 8, 9 and 10. Cell morphology and cell dimensions were examined using phase-contrast microscopy (Axio Imager; Zeiss). Anaerobic growth was checked on R2A agar medium using a BBL anaerobic jar (Becton Dickinson) at >15 % CO₂. Other physiological properties, carbohydrate assimilation and enzyme activities were tested using API 20NE, API ID 32GN and API ZYM test strips according to instructions of the manufacturer (bioMérieux). The results of the physiological and biochemical tests are given in Table 1 and the species description.

Table 1. Differential phenotypic characteristics of strain 5516T-20^T, *P. dokdonensis* DS-8^T and *P. jejuensis* KSW2-15^T

Unless indicated, data are from this study except those for *P. dokdonensis* DS-8^T, which were taken from Yoon *et al.* (2008). All strains are positive for catalase, for hydrolysis of aesculin, casein, gelatin and starch and for β -galactosidase, but all are negative for indole production and urease. In API 20NE and API ID 32GN test strips, all strains assimilate D-glucose, D-mannose, D-mannitol, maltose, potassium gluconate, *N*-acetylglucosamine, malic acid, sucrose, glycogen and melibiose, but do not assimilate L-arabinose, capric acid, trisodium citrate, phenylacetic acid, potassium 5-ketogluconate or potassium 2-ketogluconate. In API ZYM test strips, all strains show positive results for esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and β -glucosidase, but show negative results for lipase (C14), cystine arylamidase, trypsin, β -glucuronidase, *N*-acetyl- β -glucosaminidase and α -fucosidase. +, Positive; –, negative; (+), weakly positive; ND, no data available.

Characteristic	Strain 5516T-20 ^T	<i>P. jejuensis</i> KSW2-15 ^T	<i>P. dokdonensis</i> DS-8 ^T
Isolation source	Air sample	Seaweed	Soil
Cell shape	Short rod	Coccus*	Coccus
Oxidase	–	–*	+
Nitrate reduction	–	+	–
Hydrolysis of DNA	–	+	ND
Assimilation of:			
Adipic acid	–	(+)	–
D-Ribose	–	+	–
Inositol	–	+	–
Sodium acetate	+	+	ND
Lactic acid	–	+	ND
L-Alanine	(+)	–	ND
Salicin	+	+	–
L-Fucose	+	–	–
D-Sorbitol	–	+	–
Propionic acid	+	+	ND
Valeric acid	+	+	ND
L-Histidine	–	+	ND
3-Hydroxybutyric acid	+	+	ND
L-Proline	–	+	ND
Enzyme activities			
Alkaline phosphatase	–	+	+
Esterase (C4)	–	+	+
Valine arylamidase	–	–	+
Cystine arylamidase	–	–	+
α -Galactosidase	–	+	+
α -Chymotrypsin	+	–	–
α -Mannosidase	–	+	(+)
DNA G + C content (mol%)	70.5	74*	70.7

*Data taken from Lee (2006).

Sequencing of the 16S rRNA gene was performed as described previously (Kwon *et al.*, 2003). Phylogenetic analysis was performed using the software package MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data by CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic dendrograms were constructed using the neighbour-joining and maximum-parsimony methods with bootstrap percentages based on 1000 replications. A continuous stretch of approximately 1400 bp of strain 5516T-20^T was used in the analysis. According to the neighbour-joining tree (Fig. 1), strains 5516T-20^T, *P. dokdonensis* DS-8^T and *P. jejuensis* KSW2-15^T formed a separate clade within the family *Intrasporangiaceae* with high bootstrap support (94%). This branch was also supported by the maximum-parsimony tree. The highest levels of sequence similarity

between strain 5516T-20^T and recognized species were found with respect to the type strains of *P. dokdonensis* and *P. jejuensis* (97.5 and 96.1 %, respectively).

DNA–DNA hybridization was carried out using the filter hybridization method, as described by Seldin & Dubnau (1985). Probe labelling was conducted using the non-radioactive DIG-High prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). The DNA–DNA hybridization value between 5516T-20^T and *P. dokdonensis* DS-8^T was 41 %.

Chemotaxonomic investigations, i.e. analysis of the peptidoglycan (Schleifer & Kandler, 1972), polar lipids and

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the position of strain 5516T-20^T. Numbers at nodes indicate bootstrap percentages (based on 1000 resamplings); only values >50% are given. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.01 substitutions per nucleotide position.

menaquinones (Minnikin *et al.*, 1984), whole-cell sugars (Staneck & Roberts, 1974), mycolic acids (Minnikin *et al.*, 1975) and fatty acids (standard protocol of the Sherlock Microbial Identification System; Sasser, 1990), were performed as described previously. DNA G+C contents were determined using an HPLC method (Mesbah *et al.*, 1989). The peptidoglycan of strain 5516T-20^T contained meso-diaminopimelic acid, alanine and glutamic acid, i.e. it was of the A1 γ (A31) type according to the catalogue of strains of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) (DSMZ, 2001). Phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol were present in the phospholipid profiles. Strain 5516T-20^T contained MK-8(H₄) as the predominant menaquinone. Glucose and ribose were detected as whole-cell sugars. No mycolic acids were detected in the cell wall. The major fatty acids of strain 5516T-20^T were C_{17:1} ω 8c (18.6%), iso-C_{15:0} (15.7%), iso-C_{16:0} (14.6%) and C_{17:0} (9.4%). More detailed fatty acid profiles of strains tested in this study are given in Supplementary Table S1 (available in IJSEM Online). The G+C content of the DNA from strain 5516T-20^T was 70.5 mol%.

Phylogenetically, strain 5516T-20^T formed a robust cluster with *P. dokdonensis* DS-8^T and *P. jejuensis* KSW2-15^T that was separable from recognized members of the family *Intrasporangiaceae* (Fig. 1). The menaquinone content and the diagnostic amino acid of the cell-wall peptidoglycan are in agreement with the description of the genus *Phycoccus*. The major fatty acid profiles were similar, although there was variation with respect to some components among strains (Supplementary Table S1). In terms of species demarcation, strain 5516T-20^T showed a relatively low level

of DNA–DNA hybridization (41%) with respect to *P. dokdonensis* DS-8^T, confirming that the isolate represented a separate genomic species within the genus *Phycoccus* (Wayne *et al.*, 1987). In addition, strain 5516T-20^T, *P. jejuensis* KSW2-15^T and *P. dokdonensis* DS-8^T differed from each other in terms of several phenotypic characteristics (Table 1). On the basis of the data presented, therefore, strain 5516T-20^T represents a novel species of the genus *Phycoccus*, for which the name *Phycoccus aerophilus* sp. nov. is proposed.

Description of *Phycoccus aerophilus* sp. nov.

Phycoccus aerophilus (ae.ro.phi'lus. Gr. n. *aer* air; Gr. adj. *philos* loving; N.L. masc. adj. *aerophilus* air-loving).

Cells are strictly aerobic, Gram-positive, non-spore-forming, short rods (0.8–0.9 $\times$ 1.1–1.5 μ m). Colonies are white, round and convex with clear margins on R2A medium. Growth is observed at 5–37 $^{\circ}$ C (optimally at 30 $^{\circ}$ C), pH 5–9 (optimally at pH 6–7) and 0–7% NaCl (w/v). Hydrolyses casein, starch, Tween 80, tyrosine and xanthine, but does not hydrolyse chitin, CM-cellulose, hypoxanthine or pectin. Positive for hydrolysis of aesculin and gelatin and for β -galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease (API 20NE test strip). Assimilates D-glucose, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, malic acid, sucrose, sodium acetate, glycogen, salicin, melibiose, L-fucose, propionic acid, valeric acid and 3-hydroxybutyric acid. Weakly assimilates L-alanine. Does not assimilate L-arabinose, capric acid, adipic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium

malonate, lactic acid, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-sorbitol, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID 32GN test strips). The diagnostic cell-wall diamino acid is *meso*-diaminopimelic acid and the peptidoglycan type is A1 γ . The whole-cell sugars are glucose and ribose. The polar lipids comprise phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol. The predominant fatty acids are C_{17:1} ω 8c, iso-C_{15:0}, iso-C_{16:0} and C_{17:0}. The major menaquinone is MK-8(H₄). The DNA G+C content of the type strain is 70.5 mol%.

The type strain, 5516T-20^T (=KACC 20658^T =DSM 18548^T), was isolated from air sampled in Taean, Republic of Korea.

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