Variovorax soli sp. nov., isolated from greenhouse soil

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A Gram-negative, rod-shaped, non-spore-forming bacterium, strain GH9-3^T, isolated from greenhouse soil, was investigated in a polyphasic study. The novel organism grew at 10–35 °C, 0–3 % NaCl and pH 5–9. It had ubiquinone 8 (Q-8) as the predominant isoprenoid quinone and possessed C_{16:0}, summed feature 3, C_{17:0} cyclo and C_{18:1} ω 7c as the major fatty acids (together representing 87.4 % of the total). The DNA G+C content was 67.1 mol%. 16S rRNA gene sequence analysis of strain GH9-3^T showed that it grouped within the *Variovorax* cluster, with highest sequence similarities to *Variovorax paradoxus* IAM 12373^T (98.3 %) and *Variovorax dokdonensis* DS-43^T (98.0 %). DNA–DNA hybridization values between strain GH9-3^T and *V. paradoxus* DSM 30034^T and *V. dokdonensis* DS-43^T were 38 and 29 %, respectively. Based on phenotypic, chemotaxonomic and phylogenetic features, it is proposed that strain GH9-3^T represents a novel species of the genus *Variovorax* with the name *Variovorax soli* sp. nov. The type strain is GH9-3^T (=KACC 11579^T = DSM 18216^T).

The genus *Variovorax*, which was created to accommodate *Alcaligenes paradoxus* (Willems *et al.*, 1991), is affiliated to the family *Comamonadaceae* of the *Betaproteobacteria* (Anzai *et al.*, 2000). The type species of this genus is *Variovorax paradoxus*, and another species in the genus, *Variovorax dokdonensis*, was recently proposed from a soil isolate (Yoon *et al.*, 2006).

Strain GH9-3^T was isolated from greenhouse soil cultivated with lettuce (*Lactuca sativa* L.) in Wanju region, Korea. The soil sample was serially diluted with 0.85 % NaCl (w/v), spread on R2A (Reasoner & Geldreich, 1985) and incubated at 30 °C for 3 days. The isolate was maintained on R2A and stored at –80 °C with 15 % glycerol (v/v).

The morphological, physiological and biochemical characteristics of strain GH9-3^T were investigated using routine cultivation on R2A medium at 30 °C. Gram reaction and cell morphology were observed by light microscopy and negatively stained whole cells were viewed by transmission electron microscopy. Catalase and oxidase activity and hydrolysis of casein, carboxymethylcellulose, DNA, gelatin,

tyrosine and starch were determined as described by Smibert & Krieg (1994). Growth was assessed at 5, 10, 20, 25, 30, 35, 40 and 45 °C, at pH 4, 5, 6, 7, 8, 9 and 10, and at 0, 1, 3, 5 and 7 % NaCl. Growth and biochemical tests were also carried out using API 20NE, API ID 32 GN and API ZYM commercial kits (bioMérieux).

PCR amplification of the 16S rRNA gene was carried out as described previously (Kwon *et al.*, 2003). Sequencing was performed using an automated DNA sequencer model 3100 (Applied Biosystems). The 16S rRNA gene sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). A phylogenetic analysis was performed using MEGA version 3.1 (Kumar *et al.*, 2004). Distances (using distance options according to the Kimura two-parameter model) and clustering using the neighbour-joining method and maximum-parsimony (Kumar *et al.*, 2004) were determined using bootstrap values based on 1000 replicates.

Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). Whole-cell fatty acids were analysed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990) after cultivation on R2A for 2 days at 30 °C. Determination of DNA G+C content was according to

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GH9-3^T is DQ432053.

the method of Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18-S; Supelco).

DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted using a non-radioactive DIG High Prime DNA Labelling and Detection Starter kit II (Roche Molecular Biochemicals). Hybridized DNA was visualized using a DIG luminescent detection kit (Roche).

Table 1. Phenotypic comparison of strain GH9-3^T and the type strains of *Variovorax* species

Strains: 1, GH9-3^T (*V. soli* sp. nov.); 2, *V. paradoxus* DSM 30034^T; 3, *V. dokdonensis* DS-43^T. Data were obtained in this study unless indicated. All three strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase and negative for starch hydrolysis, anaerobic growth with nitrate, indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis, β -galactosidase, lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. All three strains assimilated D-glucose, L-arabinose, potassium gluconate, adipic acid, malic acid, suberic acid, lactic acid, 3-hydroxybenzoic acid, L-histidine, 3-hydroxybutyric acid and 4-hydroxybenzoic acid and did not assimilate *N*-acetylglucosamine, D-maltose, capric acid, trisodium citrate, L-rhamnose, inositol, sucrose, itaconic acid, glycogen, L-serine, salicin, D-melibiose or L-fucose. +, Positive; –, negative; W, weakly positive.

Characteristic	1	2	3
Flagella	1–3	Peritrichous ^{a*}	Peritrichous ^b
DNA G + C content (mol%)	67.1	67.0 ^a	66.0 ^b
Nitrate reduction	+	+	–
Urease	–	–	+
Valine arylamidase	–	W	–
Acid phosphatase	+	–	+
α -Glucosidase	–	+	W
Assimilation of:			
D-Mannose	–	+	–
D-Mannitol	+	+	–
Phenylacetic acid	+	–	–
D-Ribose	–	+	–
Sodium malonate	–	+	–
Sodium acetate	–	+	–
L-Alanine	–	–	+
Potassium 5-ketogluconate	–	+	–
D-Sorbitol	+	+	–
Propionic acid	+	–	+
Valeric acid	+	–	+
Potassium 2-ketogluconate	+	–	+
L-Proline	–	+	+

*Data taken from: a, Willems *et al.* (1991); b, Yoon *et al.* (2006).

Colonies of strain GH9-3^T were irregular and yellow in colour on R2A medium. Cells were non-spore-forming, motile, short rods, approximately 0.5–0.7 $\times$ 1.0–1.5 μ m. The strain grew on R2A, nutrient agar (Difco), trypticase soy agar (Difco) and MacConkey agar (Difco). A phenotypic comparison of strain GH9-3^T and the other *Variovorax* species is shown in Table 1.

The major isoprenoid quinone of GH9-3^T was ubiquinone 8 (Q-8). Strain GH9-3^T contained high proportions of C_{16:0} (33.2%), summed feature 3 (29.3%, comprising C_{15:0} iso 2-OH and/or C_{16:1} ω 7c), C_{17:0} cyclo (15.9%) and C_{18:1} ω 7c (9.0%) (Table 2). No significant differences in fatty acid profiles were found from other *Variovorax* species. The DNA G + C content was 67.1 mol%.

The almost complete 16S rRNA gene sequence of strain GH9-3^T (1457 nt) was used in the phylogenetic analysis. Isolate GH9-3^T showed highest sequence similarities to *V. paradoxus* IAM 12373^T (98.3%) and *V. dokdonensis* DS-43^T (98.0%). Sequence similarities to the other species shown in Fig. 1 were below 97%. According to the phylogenetic tree, strain GH9-3^T formed a robust cluster (90% bootstrap values) with *V. paradoxus* and *V. dokdonensis*, indicating that this strain is a member of the genus *Variovorax*. DNA–DNA hybridizations between strain GH9-3^T and *V. paradoxus* DSM 30034^T and *V. dokdonensis* DS-43^T yielded relatedness values of 38 and 29%, respectively. These values are well below the threshold of 70% suggested for species delineation (Stackebrandt & Goebel, 1994), indicating that strain GH9-3^T represents a genomic species separate from *V. paradoxus* and *V. dokdonensis*. On the basis of these results, strain GH9-3^T represents a novel species of the genus *Variovorax*, for which the name *Variovorax soli* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of strain GH9-3^T and the type strains of *Variovorax* species

Strains: 1, GH9-3^T (*V. soli* sp. nov.); 2, *V. paradoxus* DSM 30034^T; 3, *V. dokdonensis* DS-43^T. –, Less than 1% or not detected.

Fatty acid	1	2	3
C _{10:0} 3-OH	5.1	3.7	3.2
C _{12:0}	3.2	3.7	4.0
C _{12:0} 3-OH	–	–	3.3
C _{14:0}	–	–	1.0
C _{14:0} 2-OH	–	2.7	–
C _{15:0}	–	–	1.8
C _{16:0}	33.2	29.8	25.2
C _{17:0} cyclo	15.9	17.1	23.6
C _{17:0}	1.0	1.2	1.6
C _{18:1} ω 7c	9.0	13.1	13.1
Summed feature 3*	29.3	23.2	21.0

*Summed feature 3 comprised C_{15:0} iso 2-OH and/or C_{16:1} ω 7c.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain GH9-3^T. Bootstrap values are shown as percentages of 1000 replicates. Only bootstrap values above 50 % are shown. GenBank accession numbers are shown in parentheses. Bar, 0.5 nucleotide substitutions per 100 nt.

Description of *Variovorax soli* sp. nov.

Variovorax soli (so'li. L. neut. gen. n. *soli* of soil, the source of the organism).

Cells are Gram-negative, motile, non-spore-forming, short and rod-shaped (0.5–0.7 × 1.0–1.5 µm). Colonies are irregular and light yellow in colour after 2 days on R2A. Able to grow between 10 and 35 °C (optimal growth at 30 °C) and at pH 5.0–9.0 (optimal growth at pH 7.0). Tolerates up to 3 % NaCl. Catalase- and oxidase-positive. Hydrolyses tyrosine and urea (prolonged incubation of over 2 weeks), but does not hydrolyse casein, carboxymethylcellulose or DNA. The major cellular fatty acids are C_{16:0}, summed feature 3, C_{17:0} cyclo and C_{18:1ω7c}. Q-8 is the predominant isoprenoid quinone. The G + C content is 67.1 mol%.

The type strain, GH9-3^T (=KACC 11579^T = DSM 18216^T), was isolated from greenhouse soil cultivated with lettuce in Wanju region, Republic of Korea.

Acknowledgements

This study was supported by a programme of international co-research work between the Rural Development Administration (RDA), South Korea, and DSMZ, Germany.

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