Virgibacillus soli sp. nov., isolated from mountain soil

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A Gram-positive bacterium (strain CC-YMP-6^T) was isolated from soil samples collected from Yang-Ming Mountain, Taiwan. On the basis of 16S rRNA gene sequence analysis, strain CC-YMP-6^T clearly belonged to the genus *Virgibacillus* and was most closely related to the type strains of *Virgibacillus halophilus* (96.2 % similarity) and *Virgibacillus kekensis* (96.3 %). The predominant isoprenoid quinone was menaquinone MK-7 and the polar lipid profile was composed of the major components diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and one unidentified phospholipid plus moderate amounts of two unidentified aminophospholipids and a phospholipid. The polyamine pattern comprised spermidine as the single major component with spermine and putrescine present in minor amounts. The major fatty acids of strain CC-YMP-6^T were iso-C_{15:0} and anteiso-C_{15:0}. The results of physiological and biochemical tests allowed the clear phenotypic differentiation of strain CC-YMP-6^T from all recognized species of the genus *Virgibacillus*. Strain CC-YMP-6^T is therefore considered to represent a novel species of the genus *Virgibacillus*, for which the name *Virgibacillus soli* sp. nov. is proposed. The type strain is CC-YMP-6^T (=DSM 22952^T=CCM 7714^T).

The genus *Virgibacillus* was proposed by Heyndrickx *et al.* (1998) to accommodate endospore-forming organisms previously assigned to *Bacillus pantothenicus*. At the time of writing, the genus comprised 20 recognized species, namely *V. pantothenicus* (Heyndrickx *et al.*, 1998), *V. proomii* (Heyndrickx *et al.*, 1999), *V. carmonensis*, *V. marismortui*, *V. necropolis*, *V. picturae* and *V. salexigens* (Heyrman *et al.*, 2003), *V. halodenitrificans* (Yoon *et al.*, 2004), *V. dokdonensis* (Yoon *et al.*, 2005), *V. koreensis* (Lee *et al.*, 2006), *V. halophilus* (An *et al.*, 2007), *V. olivae* (Quesada *et al.*, 2007), *V. salarius* (Hua *et al.*, 2008), *V. chiguensis* (Wang *et al.*, 2008), *V. kekensis* (Chen *et al.*, 2008), *V. salarius* (Hua *et al.*, 2008), *V. salinus* (Carrasco

et al., 2009), *V. sedimenti* (Chen *et al.*, 2009), *V. arcticus* (Niederberger *et al.*, 2009) and *V. byusanensis* (Yoon *et al.*, 2010).

Strain CC-YMP-6^T was isolated from soil samples collected from Yang-Ming Mountain (Taiwan) on nutrient agar (NA; Oxoid). The isolate was maintained on NA after incubation at 30 °C for 48 h, and was preserved at –80 °C in nutrient broth (NB; Oxoid) with 20 % (v/v) glycerol or by lyophilization. Orange to brownish colonies with a diameter of 2–4 mm were observed after 3 days of incubation on Caso agar (Merck 105458). Morphological properties and Gram-staining were investigated by phase-contrast microscopy as described previously (Kämpfer *et al.*, 2009, 2010). Strain CC-YMP-6^T formed highly motile rod-shaped cells of about 0.5 × 5 µm (some longer cells were observed). A Gram-positive to Gram-variable reaction was observed after incubation for 24 h at 28 °C in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-YMP-6^T is EU213011.

Supplementary figures are available with the online version of this paper.

NB. Endospore formation was observed after 3 days of cultivation at 30 °C on Caso agar supplemented with 10 mg MnSO₄·H₂O. Strain CC-YMP-6^T produced spherical to ellipsoidal spores in a terminal position (see Supplementary Fig. S1 in IJSEM Online). Extracellular deposits were observed (Supplementary Fig. S1), but no parasporal bodies. Anaerobic growth was detected in tryptone soy broth in tubes with an overlay of paraffin oil after 3 days of incubation.

Isolation of DNA was performed by using the commercial DNA extraction kit (GenElute Plant Genomic DNA kit; Sigma). The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Multiple sequence alignment and analysis of the data were performed with the MEGA software package, version 4 (Tamura *et al.* 2007), as well as with the ARB software package (version of December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURF 95 database (release of July 2008; Pruesse *et al.*, 2007). Genetic distances were calculated (distance options according to the Kimura two-parameter model) and clustering was performed with the neighbour-joining method and the maximum-parsimony method (results not shown) by using MEGA version 4, and bootstrap values based on 1000 replications. Tree reconstruction via the maximum-likelihood method with fastDNAmI (Olsen *et al.*, 1994) and a 30 % conservatory filter (only alignment

columns in which the frequency of the most abundant nucleotide is equal to or above 30 % are included in the calculation) was performed with the ARB software package (Fig. 1). Tree topology was further tested without filters. No differences were detected between these trees.

The 16S rRNA gene sequence of strain CC-YMP-6^T was a continuous stretch of 1526 bp. Sequence similarity calculations showed that the closest relatives of strain CC-YMP-6^T were *V. halophilus* 5B73C^T (96.2 %) and *V. kekensis* YIM kkny16^T (96.3 %).

For polyamine, quinone and polar lipid analyses, cells were grown in PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C. Analyses were carried out as described by Busse & Auling (1988), Altenburger *et al.* (1996, 1997) and Tindall (1990a, b). HPLC analyses of the quinone system and polyamines were performed by using the instruments described by Stolz *et al.* (2007). The polyamine pattern consisted of (per g dry weight) 3.2 µmol spermidine, 0.1 µmol spermine and 0.03 µmol putrescine. Similar polyamine patterns have been reported for other mesophilic bacilli in the genera *Bacillus*, *Paenibacillus*, *Brevibacillus* and *Aneurinibacillus*. Thermophilic bacilli such as species of the genera *Geobacillus* and *Alicyclobacillus* have spermine as the major polyamine (Hamana *et al.*, 1989, 1993; Meier-Stauffner *et al.*, 1996). The quinone system of strain CC-YMP-6^T was composed of menaquinone

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences showing the position of strain CC-YMP-6^T among its closest relatives (available from the EMBL database; accession numbers in parentheses). The phylogenetic tree was constructed by using the ARB software package (version of December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURF 95 database (release of July 2008; Pruesse *et al.*, 2007). Tree building was performed by using the maximum-likelihood method with fastDNAmI (Olsen *et al.*, 1994). For better clarity, only a subset of the sequences used for treeing is shown. Bar, 0.1 nt substitutions per nucleotide position.

MK-7 (93 %) and MK-6 (7 %). In the polar lipid profile (see Supplementary Fig. S2), the major components were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and one unidentified phospholipid (PL1). Moderate amounts of two unidentified aminophospholipids (APL1, APL2) and one phospholipid (PL2) and traces of an unidentified phospholipid (PL3), an aminolipid, a third aminophospholipid (APL3) and two lipids (L1, L2), not stainable with any of the applied specific spray reagents, were found. The three aminophospholipids showed a highly hydrophilic chromatographic behaviour. The presence of phosphatidylethanolamine has been reported for several species of the genus *Virgibacillus*, including *V. pantothenicus*, but not for others (e.g. *V. carmonensis*) and only as a trace component in *V. salexigens* (Wainø *et al.*, 1999; Heyrman *et al.*, 2003). On the other hand, two phospholipids with a chromatographic behaviour similar to PL1 and PL2 (Supplementary Fig. S2) were reported for *V. salexigens*, but not for *V. pantothenicus*. However, this polar lipid profile supported the assignment of strain CC-YMP-6^T to the genus *Virgibacillus*.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain CC-YMP-6^T was similar to those of recognized species of the genus *Virgibacillus*, and closely matched those of *V. kekensis* and *V. halophilus* (Table 1).

Results of comparative physiological characterization are given in the species description below and in Table 2, with methods as described previously (Kämpfer *et al.*, 1991, 2009). The observed differences in fatty acid profiles and physiological test results together with the phylogenetic data presented suggest that strain CC-YMP-6^T represents a novel species of the genus *Virgibacillus*, for which the name *Virgibacillus soli* sp. nov. is proposed.

Description of *Virgibacillus soli* sp. nov.

Virgibacillus soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are Gram-positive to Gram-variable rods, about 0.5 × 5 µm (some longer cells may occur), showing facultatively anaerobic respiratory metabolism, but not denitrification. Endospores are spherical to ellipsoidal in a terminal position. Extracellular deposits are detected but not parasporal bodies. Good growth occurs after 3 days of incubation on NA at 25–30 °C, and also on tryptone soy agar. Moderate growth occurs after 3–7 days of incubation in NB under anaerobic conditions. On both agar media, orange to brownish colonies are produced. Optimal temperature for growth is 25–30 °C; growth occurs at 15–40 °C, but not at 5 or 45 °C. Optimal pH for growth is 7.5–8.5; growth occurs at

Table 1. Whole-cell fatty acid profiles of strain CC-YMP-6^T and related species of the genus *Virgibacillus*

Strains: 1, CC-YMP-6^T (data from the present study); 2, *V. kekensis* YIM kkn16^T; 3, *V. halophilus* KCTC 13935^T (An *et al.*, 2007); 4, *V. marismortui* KCTC 3845^T; 5, *V. halodenitrificans* KCTC 3790^T; 6, *V. olivae* DSM 18098^T (Quesada *et al.*, 2007); 7, *V. carmonensis* KCTC 3819^T; 8, *V. proomii* KCTC 3822^T; 9, *V. pantothenicus* KCTC 3539^T; 10, *V. dokdonensis* KCTC 3933^T (An *et al.*, 2007); 11, *V. necropolis* KCTC 3820^T; 12, *V. salexigens* KCTC 3844^T; 13, *V. koreensis* KCTC 3823^T. Other reference data are from Lee *et al.* (2006). The fatty acid profiles of *V. halophilus* DSM 12325^T and *V. kekensis* DSM 10037^T were confirmed in parallel tests with strain CC-YMP-6^T under identical conditions. Data are percentages of the total fatty acids; components representing <1.0 % of the total are not shown.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
C _{14:0}	8.4	1.7	2.1			1.3				2.1			1.8
C _{15:0}			1.4			1.1							5.1
C _{16:0}	6.9	2.7	5.3	1.8	1.1	3.1	1.0	4.0	1.3	12.7	1.6	5.1	5.9
C _{18:0}		2.1				1.2							
C _{16:1} ω7c alcohol	1.2	8.1		1.1	3.1	1.3	6.5				2.4		3.4
C _{16:1} ω11c	3.3					1.7					1.0		2.2
C _{18:1} ω9c		1.1				1.2							
iso-C _{13:0}	2.1									1.0			
anteiso-C _{13:0}	1.2									1.8			
iso-C _{14:0}	6.4	13.3	5.6	2.9	7.4	2.1	3.3	2.4	2.3	2.1	2.2	4.2	8.3
iso-C _{15:0}	37.0	2.6	21.2	39.0	2.4	33.7	5.7	15.1	7.1	15.5	3.8	34.0	6.1
anteiso-C _{15:0}	28.2	50.4	35.8	33.9	51.8	28.4	58.29	38.5	55.3	47.0	67.9	30.5	43.4
iso-C _{16:0}	2.0	6.1	10.3	4.3	11.8	3.9	6.4	7.3	7.2	3.7	4.2	8.4	14.4
iso-C _{17:0}	1.0		1.8	5.6		10.1		6.3	2.0	2.4		5.6	
anteiso-C _{17:0}	2.3	7.0	16.1	8.6	19.5	9.2	11.1	24.9	24.0	11.5	12.2	9.3	7.0
Summed features*													
3							5.0				2.1		
4					1.6								

*Summed features are groups of two or three fatty acids that could not be separated by GC using the MIDI system: summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7t.

Table 2. Differential characteristics between strain CC-YMP-6^T and related species of the genus *Virgibacillus*

Taxa: 1, strain CC-YMP-6^T; 2, *V. kekensis* YIM kkny16^T; 3, *V. halophilus* (An *et al.*, 2007); 4, *V. marismortui* (Arahal *et al.*, 1999, 2000; Heyrman *et al.*, 2003); 5, *V. halodenitrificans* (Yoon *et al.*, 2004); 6, *V. olivae* (Quesada *et al.*, 2007); 7, *V. carmonensis* (Heyrman *et al.*, 2003); 8, *V. proomii* (Heyndrickx *et al.*, 1999; Heyrman *et al.* 2003); 9, *V. pantothenicus* (Heyndrickx *et al.*, 1999; Heyrman *et al.* 2003); 10, *V. dokdonensis* (Yoon *et al.*, 2005); 11, *V. necropolis* (Heyrman *et al.* 2003); 12, *V. salexigens* (Garabito *et al.*, 1997; Wainø *et al.*, 1999; Heyrman *et al.*, 2003); 13, *V. koreensis* (Lee *et al.*, 2006). +, Positive; –, negative; v, variable; w, weakly positive; pr, predominant; tr, trace; ND, no data available. Data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Spore shape*	E, S	E, S	E	E	E	S, E	E, S	S, E	S, E	S, E	E	E	E
Spore position†	T	T	ST	T, ST	T, ST	T, ST	ST	T, ST	T, ST	T, ST	C, ST, T	C, ST, T	T
Anaerobic growth	+	–	–	–	+	–	–	+	+	+	–	–	+
Temperature range (°C)	15–40	10–50	5–45	15–50	10–45	20–45	10–40	15–50	15–50	15–50	10–40	15–45	10–45
Growth in/at:													
0 % (w/v) NaCl	+	+	+	–	–	+	–	ND	ND	+	W	–	–
20 % (w/v) NaCl	–	+	–	–	+	V(+)	–	–	–	+	–	+	–
pH 10	+	+	+	–	–	–	ND	–	–	ND	ND	+	–
Hydrolysis of:‡													
Aesculin	–	–	ND	+	V(–)	V(+)	W	+	+	+	–	+	+
Casein	–	–	ND	+	–	V(+)	+	+	+	+	+	+	ND
Gelatin	+	–	ND	+	+	+	–	V	+	+	W	+	–
Starch	+	+	ND	–	–	+	ND	ND	ND	+	ND	–	ND
H ₂ S production‡	–	–	–	+	–	ND	–	–	V	–	–	+	–
Nitrate reduction‡	+	+	+	+	+	+	+	–	V	–	+	–	–
Acid production from:													
N-Acetylglucosamine	–	–	+	+	+	ND	–	+	+	+	W	W	–
D-Galactose	–	–	W	–	+	–	–	+	–	+	–	W	–
D-Glucose	W	+	+	+	+	–	–	+	–	+	W	W	W
D-Fructose	–	+	+	–	V(+)	+	–	+	–	+	W	W	+
D-Mannitol	–	W	+	–	+	–	–	+	–	–	–	–	–
D-Mannose	–	–	+	+	+	–	–	+	–	+	W	W	–
Trehalose	–	W	+	–	+	ND	–	+	+	–	W	–	W
Menaquinones (%)													
MK-6	7	6.1	ND	1.8	2.0	ND	2	1	1	Minor	2	2	ND
MK-7	93	93.2	pr	97.6	97.7	ND	98	99	98	pr	97	98	pr
MK-8	ND	0.7	ND	0.6	0.3	ND	1	1	1	ND	tr	tr	ND

*E, ellipsoidal; s, spherical.

†C, central; ST, subterminal; T, terminal.

‡Unless otherwise indicated, results of these tests for *V. halophilus* DSM 12325^T and *V. kekensis* DSM 10037^T were confirmed in parallel tests with strain CC-YMP-6^T under identical conditions.

pH 7.0–10.0. NaCl is not required for growth; tolerates up to 6 % (w/v) NaCl. Catalase- and oxidase-positive. Gelatin and starch are hydrolysed. H₂S, indole and acetoin are not produced. Nitrate is reduced to nitrite under aerobic conditions. Positive for gelatinase activity, but negative for urease, β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and citrate utilization. Acid production from different sugars is detailed in Table 2. Glucose and sucrose are utilized as sole source of carbon (weak results after 7 days of incubation). The quinone system comprises menaquinone MK-7 with minor amounts of MK-6. The polar lipid profile is composed of the major components diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and one unidentified phospholipid and moderate amounts of two unidentified

aminophospholipids and one phospholipid. Trace amounts of two polar lipids, an aminolipid, a third aminophospholipid and a third phospholipid are detectable. The polyamine pattern consists of the major component spermidine; spermine and putrescine are present in minor amounts. The major fatty acids are iso-C_{15:0} and anteiso-C_{15:0}.

The type strain, CC-YMP-6^T (=DSM 22952^T=CCM 7714^T), was isolated from soil samples collected from Yang-Ming Mountain, Taiwan.

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