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Proposal of *Virgibacillus proomii* sp. nov. and emended description of *Virgibacillus pantothenticus* (Proom and Knight 1950) Heyndrickx *et al.* 1998

M. Heyndrickx,[1]† L. Lebbe,[1] K. Kersters,[1] B. Hoste,[1] R. De Wachter,[2] P. De Vos,[1] G. Forsyth[3] and N. A. Logan[3]

Author for correspondence: N. A. Logan. Tel: +44 141 331 3207. Fax: +44 141 331 3208. e-mail: N.A.Logan@gcal.ac.uk

[1] Vakgroep BFM WE10V, Laboratorium voor Microbiologie, Universiteit Gent, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium

[2] Department Biochemie, Universiteit Antwerpen, Universiteitsplein 1, B-2610 Antwerpen, Belgium

[3] School of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA, UK

A polyphasic study of strains originally received as *Bacillus* (now *Virgibacillus*) *pantothenticus*, along with strains representing species belonging to *Bacillus*, *Halobacillus* and *Paenibacillus*, was undertaken using amplified rDNA restriction analysis (ARDRA), fatty acid methyl ester (FAME) analysis, SDS-PAGE of whole-cell proteins and routine diagnostic characters comprising 61 biochemical tests in the API system and 15 observations of vegetative cell and sporangial morphology. It revealed the presence within *Virgibacillus* of an as yet undescribed new species, for which the name *Virgibacillus proomii* is proposed; *V. proomii* can be distinguished from *V. pantothenticus* and members of *Bacillus sensu stricto*, and from members of *Paenibacillus* and other aerobic endospore-forming bacteria, by routine phenotypic tests. The type strain of *Virgibacillus proomii* is LMG 12370^T.

Keywords: *Bacillus*, amplified rDNA restriction analysis, *Virgibacillus pantothenticus*, *Virgibacillus proomii*, polyphasic taxonomy

INTRODUCTION

Bacillus pantothenticus was described by Proom & Knight (1950) following a nutritional analysis of mesophilic soil isolates of *Bacillus* species. Strains requiring pantothenic acid were isolated from different soil samples, taken from widely separated localities in Southern England. They considered them as members of a species most closely resembling *Bacillus circulans* but distinct from it, and subsequent studies confirmed the validity of the species (Claus & Berkeley, 1986; Gordon *et al.*, 1973; Logan & Berkeley, 1984). Later isolations have been made from antacids (Claus & Berkeley, 1986), food, water, bile and soil.

Comparisons of the 16S rRNA sequences of type strains of various *Bacillus* and *Sporosarcina* species have indicated that *B. pantothenticus* lies at the periphery of rRNA group 1 of Ash *et al.* (1991) (*Bacillus sensu stricto*). Following amplified rDNA restriction analysis (ARDRA) and a polyphasic study, the new genus *Virgibacillus* (Heyndrickx *et al.*, 1998) was proposed to accommodate *B. pantothenticus* and two related organisms which appeared to belong to an as yet undescribed new species. Polyphasic taxonomic study of further isolates received as *B. pantothenticus* has confirmed the existence within *Virgibacillus* of a second species which we propose here as *Virgibacillus proomii*.

METHODS

Strains and media. The designations of the strains, their origins and the different methods applied are shown in Table 1. Unless otherwise stated, *V. pantothenticus* strains were grown on Trypticase Soy agar (TSA) and the other *Bacillus* strains on nutrient agar with 1% (w/v) glucose (NAG) at 30 °C for 24–48 h. The *Bacillus dipsosauri* strain was grown at 37 °C on TSA supplemented with 1 M KCl. The strains were checked for purity by plating and phase-contrast microscopy, and were maintained both as lyophilized cultures and as sporulated cultures on slopes of the appropriate above-mentioned medium containing 5 mg $MnSO_4.4H_2O$

†**Present address:** Government Dairy Research Station, Brusselsesteenweg 370, B-9090 Melle, Belgium.

Abbreviations: ARDRA, amplified rDNA restriction analysis; FAME, fatty acid methyl ester; UPGMA, unweighted pair group method with arithmetic averages.

The EMBL accession number for the 16S rRNA gene sequence of *Virgibacillus proomii* strain LMG 12370^T determined in this work is AJ012667.

Table 1. Strains used and the methods used for characterization

Name	LMG no.	Other designations*	Source and comment*	Methods applied†			
				ARDRA	API	PAGE	FAME
B. amyloliquefaciens	9814^T	B0177^T, Campbell F, ATCC 23350^T	Gordon	+	+		
B. amyloliquefaciens	12234	B0168, Fukumoto F	Gordon	+	+		
B. azotoformans	9581^T	CCM 2849^T	CCM	+	+		
B. azotoformans	15444	Pichinoty Ba3	Pichinoty	+	+		
B. badius	7122^T	B0180^T, DSM 23^T	DSM	+	+		
B. badius	12332	B0201, NRS 1407, Appleman	Gordon	+	+		
B. cereus	6923^T	B0002^T, DSM 31^T, ATCC 14579, Ford 13	DSM	+	+		
B. cereus	12334	B0358, F 4810/73 (strain 88)	Vomit, serogroup 1, Melling	+	+		
B. circulans	13261^T	B0004^T, DSM 11^T, ATCC 4513^T	DSM	+	+		
B. circulans	12342‡	B0196, NCTC 5846, Morris	Potted crab, NCTC	+	+		
B. dipsosauri	17413^T	NCFB 3027^T	Iguana, NCFB	+	+		
B. firmus	7125^T	B0181^T, DSM 12^T, ATCC 14575^T	DSM	+	+		
B. fusiformis	9816^T	B0658^T, ATCC 7055^T, Wellcome 2209	Goodfellow	+	+		
B. fusiformis	17347	B0660, Wellcome 2615	Goodfellow	+	+		
B. insolitus	17757^T	B0432^T, Stokes W16 B, DSM 5^T	Soil, DSM	+	+		
B. insolitus	17758	B0433, Ottow 627	Goodfellow	+	+		
B. lentus	9579^T	B0779^T, Gibson urea 165 'type culture'	Gibson		+		
B. lentus	12359	B0179, Gibson 238, ATCC 10841	Gibson	+	+		
B. lentus	16798^T	NCIMB 8773^T	NCIMB	+			
B. licheniformis	12360	B0242, Gibson 307	Gibson	+	+		
B. licheniformis	12363^T	B0245^T, G 46, NCTC 10341	Gibson	+	+		
B. megaterium	7127^T	B0010^T, DSM 32^T, ATCC 14581^T	DSM	+	+		
B. megaterium	12409	B0621, Hartman NRRL B-348	Goodfellow	+	+		
B. psychrophilus	6929^T	B0434^T, Stokes W16 A, DSM 3^T	DSM	+	+		
B. psychrophilus	17169	ATCC 23306	ATCC	+	+		
B. pumilus	7132^T	B0019^T, DSM 27^T, ATCC 7061^T	DSM	+	+		
B. pumilus	12259	B0102, G 1036, Lister 2812	Gibson		+		
B. smithii	6327	DSM 459	DSM	+	+		
B. smithii	12526^T	CCUG 27413^T	CCUG	+	+		
B. sphaericus	7134^T	B0012^T, DSM 28^T, G 1013	DSM	+	+		
B. sphaericus	17382	B1144, SS II-1	De Barjac	+	+		
B. subtilis	7135^T	B0014^T, DSM 10^T, ATCC 6051^T	DSM	+	+	+	
B. subtilis	17727	B0905	Cornish pasty, Sandys	+	+		
V. pantothenticus	7129^T	B0018^T, DSM 26^T, ATCC 14576^T, NCDO 1765^T	Soil, DSM	+	+	+	+
V. pantothenticus	12366	B0183, DSM 490, NCTC 8122	DSM	+	+	+	+
V. pantothenticus	12367	B0184, DSM 491, NCTC 8124	DSM	+	+	+	+
V. pantothenticus	12368	B0275, NCTC 8123, Wellcome CN 3020	Soil, NCTC	+	+	+	+
V. pantothenticus	12369	B0406, F 2150/77	Canned chicken, Colindale	+	+	+	+
V. pantothenticus	12370	B0413, F 2737/77	Water supply, Colindale	+	+	+	+
V. pantothenticus	12371	B0417, F 218/78	Infant bile, Colindale	+	+	+	+
V. pantothenticus	17342	B0802, Wellcome CN 3021	Soil, Gibson	+	+	+	+
V. pantothenticus	17343	B0803, Wellcome CN 3022	Soil, Gibson	+	+	+	+
V. pantothenticus	17344	B0805, Wellcome CN 3024	Soil, Gibson	+	+	+	+
V. pantothenticus	17345	B0806, Wellcome CN 3025	Soil, Gibson	+	+	+	+

Table 1 (*cont.*)

Name	LMG no.	Other designations*	Source and comment*	Methods applied†			
				ARDRA	API	PAGE	FAME
V. pantothenticus	17367	B0807, Wellcome CN 3026	Soil, Gibson	+	+	+	+
V. pantothenticus	17368	B0808, Wellcome CN 3027	Soil, Gibson	+	+	+	+
V. pantothenticus	17369	B0809, Wellcome CN 3043	Soil, Gibson	+	+	+	+

* ATCC, American Collection of Type Cultures, Manassas, VA, USA; B, N. A. Logan *Bacillus* collection, Glasgow Caledonian University, Glasgow, UK; CCM, Czechoslovakian Collection of Microorganisms, Brno, CSSR; CCUG, Culture Collection University of Göteborg, Göteborg, Sweden; CN, Wellcome Collection of Microorganisms, Beckenham, Kent, UK; Colindale, Central Public Health Laboratory, London, UK; De Barjac, H. De Barjac, Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; Gibson, T. Gibson collection, held at University of Bristol, Bristol, UK; Goodfellow, M. Goodfellow, University of Newcastle, Newcastle upon Tyne, UK; Gordon, R. E. Gordon, Rutgers University, New Brunswick, USA; LMG, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; Melling, J. Melling, CAMR, Porton Down, Salisbury, UK; NCFB, National Collection of Food Bacteria, AFRC Institute of Food Research, Reading, UK; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, UK; NRRL, Northern Regional Research Laboratory collection, Peoria, IL, USA; NRS, N. R. Smith collection, now held at NRRL; Pichinoty, F. Pichinoty, UER Scientifique de Luminy, Marseille, France; Sandys, G. H. Sandys, Public Health Laboratory, Plymouth, UK.

† API, tests in API system, other biochemical tests and morphological observations; ARDRA, amplified rDNA restriction analysis; FAME, fatty acid methyl ester analysis; PAGE, polyacrylamide gel electrophoresis of whole-cell proteins.

‡ Accession number for this strain is now LMG 18014.

l^{-1} (to enhance sporulation). Slopes were incubated for 48 h or longer, until spores could be observed by microscopy, then stored in the dark at 4 °C.

DNA preparation. Total genomic DNA was purified for ARDRA using a slight modification of the method of Pitcher *et al.* (1989), as described previously (Heyndrickx *et al.*, 1996b, 1998). For DNA–DNA binding experiments and DNA base composition studies, DNA was prepared as described previously (Heyndrickx *et al.*, 1996a).

ARDRA. Enzymically amplified 16S rDNA was obtained by PCR and analysed by restriction digestion with five restriction enzymes (*Hae*III, *Dpn*II, *Rsa*I, *Bfa*I and *Tru*9I) as described previously (Heyndrickx *et al.*, 1996b). The restriction patterns were analysed as described by Heyndrickx *et al.* (1998).

Percentage DNA–DNA binding. DNA–DNA binding reactions of representative strains of each species were determined spectrophotometrically by using the initial renaturation method of De Ley *et al.* (1970) and expressed as percentages, as described previously (Willems *et al.*, 1989).

DNA base composition. Mean G+C contents were determined using the thermal denaturation method (De Ley & Van Muylem, 1963) and the equation of Marmur & Doty (1962) as modified by De Ley (1970). The G+C content of *Virgibacillus* sp. LMG 17368 was measured using the method of Tamaoka & Komagata (1984).

Gas chromatographic analysis of fatty acid methyl esters (FAMEs). Cells were grown and analysed as described by Heyndrickx *et al.* (1998), using the methods of Vauterin *et al.* (1991).

SDS-PAGE of whole-cell proteins. Cells were obtained as described by Heyndrickx *et al.* (1998), the SDS protein extracts prepared and electrophoresed according to Pot *et al.* (1994), and the data collected and interpreted as described by Vauterin & Vauterin (1992).

Phenotypic characterization and numerical analysis. Strains were grown and maintained on TSA plus $MnSO_4$, and characterization and numerical analysis followed the methods of Logan & Berkeley (1984), as described by Heyndrickx *et al.* (1998).

16S rDNA sequencing. A fragment of the 16S rRNA gene (corresponding to positions 8–541 in the *Escherichia coli* numbering system) of *V. proomii* LMG 12370^T was amplified by PCR using conserved primers (5′-AGAGTTTGATCCTGGCTGAG-3′ and 5′-AAGGAGGTGATCCAGCCGCA-3′). The PCR products were purified using a QIAquick PCR Purification kit (Qiagen) according to the manufacturer's instructions. Sequencing was performed using an Applied Biosystems 377 DNA sequencer and the protocols of the manufacturer (Perkin-Elmer) using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit. The sequencing primers used were as described by Coenye *et al.* (1999). A dissimilarity matrix was constructed. The sequences of strains belonging to the same phylogenetic group were retrieved from the EMBL database and aligned with the consensus sequence via the TREECON software (Van de Peer & De Wachter, 1997). A FASTA search (Pearson & Lipman, 1988) was also completed via the EMBL sequence database.

RESULTS AND DISCUSSION

The 14 *Virgibacillus* strains were separated from all the other organisms studied in the numerical analysis of their combined ARDRA patterns, and were subdivided into two groups (Fig. 1). The largest group, formed at a similarity level of 92%, comprised 10 strains and included the type strain of *V. pantothenticus*, LMG 7129^T. Nearest neighbours of this group, joined at a similarity level of 80%, were the

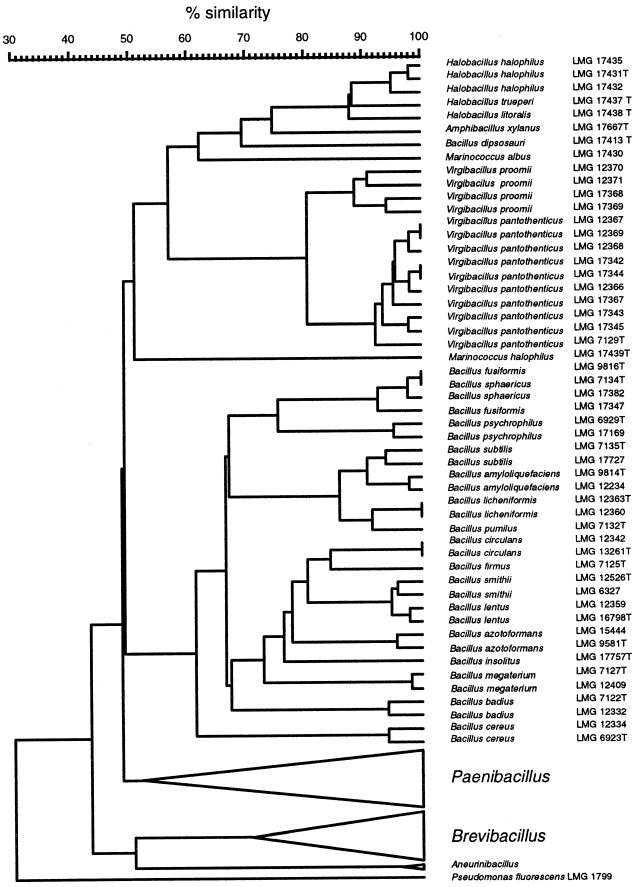

Fig. 1. Dendrogram based on UPGMA (unweighted pair group method with arithmetic averages) clustering of correlation coefficients of normalized 16S rDNA restriction profiles (ARDRA) of *V. pantothenticus*, *V. proomii* and a selection of *Bacillus* (*sensu lato*) strains belonging to different rRNA groups.

four strains LMG 12370, 12371, 17368 and 17369; these showed 89% similarity to each other and form the group being proposed as the new species *V. proomii*.

From Table 2 it can be seen that the lowest dissimilarity in rDNA sequence was found between *V. pantothenticus* and *V. proomii*. The FASTA searches (not shown) did not reveal sequence similarities with other taxa of higher than 97%, supporting the separate species status of *Virgibacillus proomii*.

Numerical analysis of SDS-PAGE patterns of whole-cell proteins (Fig. 2) showed a similar arrangement of the *Virgibacillus* strains (except for strain LMG 12366) to that seen with ARDRA (Fig. 1): a large group (nine strains), including *V. pantothenticus* LMG 7129^T, showed identical protein patterns (similarity level of at least 89%), and the four strains LMG 12370, 12371, 17368 and 17369 showed 88% similarity and linked with the larger *Virgibacillus* group at 76% similarity. The one exception was *V. pantothenticus* LMG 12366, which was the nearest neighbour of the larger group of nine strains at 78% similarity. The aberrant clustering of this strain with respect to the ARDRA picture was merely caused by a dense protein band with a molecular mass of $5{\cdot}6 \times 10^4$ Da. Indeed, by exclusion of this band from numerical analysis (Costas, 1992), this strain clustered within the group of nine strains containing the *V. pantothenticus* type strain (not shown).

Fatty acid profiles of the *Virgibacillus* strains are shown in Table 3. Although the profiles of both groups of strains show the major fatty acids to be iso-$C_{15:0}$ and anteiso-$C_{15:0}$, the ratios of these major components are quite different in each group; in *V. pantothenticus* the ratio is about 1:3, and in *V. proomii* it is 1:1. Furthermore, iso-$C_{13:0}$ is always lower than 0·5% of total fatty acids in *V. pantothenticus*, whilst it is at least 1% in *V. proomii*.

Table 2. 16S rDNA sequence dissimilarities (fraction of the observed difference per total number of compared nucleotides) for the type strains of *V. pantothenticus* and *V. proomii*, and representative strains of seven other related taxa

Key: 1, *Amphibacillus xylanus* JCM 7361; 2, *Virgibacillus pantothenticus* NCDO 1765^T; 3, *Virgibacillus proomii* LMG 12370^T; 4, *Bacillus salexigens* C20-Mo; 5, *Bacillus subtilis* NCDO 1769; 6, *Brevibacillus brevis* NCIMB 9372; 7, *Halobacillus litoralis* SL-4; 8, *Paenibacillus polymyxa* NCDO 1774; 9, *Halobacillus halophilus* NCIMB 9251.

	1	2	3	4	5	6	7	8	9
2	0·07164	0·000							
3	0·07163	0·02861	0·000						
4	0·09314	0·05015	0·04545	0·000					
5	0·09765	0·07554	0·07631	0·08267	0·000				
6	0·09663	0·11180	0·11037	0·12221	0·10036	0·000			
7	0·07838	0·05391	0·05871	0·06759	0·07616	0·09928	0·000		
8	0·11176	0·11706	0·11533	0·13014	0·12472	0·11386	0·12227	0·000	
9	0·07433	0·05621	0·06211	0·07847	0·08834	0·10813	0·02019	0·12473	0·000

Table 3. Comparison of the mean fatty acid profiles of 4 *V. proomii* and 10 *V. pantothenticus* strains as measured by GC analysis of FAMEs

The data given are mean values with the minimal and maximal values within the particular species given below. Only the fatty acids accounting for at least 0·5% of the total fatty acid content are listed.

Fatty acid	13:0 iso	13:0 anteiso	14:0 iso	14:0	15:0 iso	15:0 anteiso	16:0 iso	16:0	17:0 iso	17:0 anteiso
V. proomii	1·9	0·64	6·0	2·3	33·5	33·0	4·9	7·6	4·3	5·8
	0·96–3·9	0·00–1·1	2·9–11·4	1·4–3·8	23·8–39·9	24·0–40·0	3·9–7·8	5·9–11·0	2·5–6·2	1·6–10·6
*V. pantothenticus**	—	0·5	4·3	1·2	15·8	47·4	8·3	5·2	2·8	13·5
	—	0·0–0·87	2·7–6·4	0·7–1·6	11·9–19·2	37·8–52·0	6·4–11·2	3·2–8·1	2·1–3·3	10·3–18·9

* Data from Heyndrickx *et al*. (1998).

Fig. 2. Normalized computer profiles of protein patterns (PAGE) of *V. pantothenticus* and *V. proomii* strains, as well as representatives of *Halobacillus*, *Bacillus* and *Paenibacillus* as outgroups.

In the numerical analysis of API tests and other phenotypic characters (Fig. 3), the *Virgibacillus* strains were divided into two distinct clusters which merged at 77·5% S_G. The larger of the two *Virgibacillus* clusters formed at 85% S_G, and contained the same 10 strains that clustered together in the ARDRA (Fig. 1) and the revised PAGE dendrograms. The four remaining strains of *Virgibacillus* (LMG 12370, 12371, 17368 and 17369) clustered loosely at 82·5% S_G, a result also consistent with the findings of the other analyses. This separation reflects distinct biochemical and morphological profiles for the two sub-groups of *Virgibacillus* which allow their ready separation using routine tests (Table 4).

The DNA–DNA relatedness of the type strain of *V. pantothenticus* with *V. proomii* strain LMG 12370 was 23%, whereas *V. proomii* strains LMG 12370 and 17368 showed 93% relatedness. These data confirm the distinction of the two *Virgibacillus* groups at the species level and support the recognition of the LMG 12370, 12371, 17368 and 17369 group as a new species of *Virgibacillus*.

In conclusion, our polyphasic data show that the *V. pantothenticus* cluster contains two distinct taxa. The larger group of 10 strains contains the type strain of *V. pantothenticus*, whilst the smaller cluster, comprising four strains from different environments, is proposed as the new species *Virgibacillus proomii*, with LMG 12370 as the type strain. The description of the new species and an emended description of *V. pantothenticus* follow, and Table 4 shows characteristics for separating the two species and for their distinction from some other aerobic endospore-forming bacteria.

Description of *Virgibacillus proomii*

Virgibacillus proomii (proom.i.i. M.L. adj. *proomii* of Proom, referring to Harold Proom, the person who, with B. C. J. G. Knight, first isolated a member of this species and who described *Bacillus pantothenticus*).

Cells are motile, Gram-positive, usually long, rods (0·5–0·7 by 2–8 mm) which sometimes, especially in older cultures, form chains and/or filaments. They bear spherical to ellipsoidal endospores which lie in

Table 4. Characteristics for distinguishing between *Virgibacillus* species and some species of *Bacillus*, *Halobacillus* and *Paenibacillus*

+, >85% positive; (+), 75–84% positive; v, variable (26–74% positive); (−), 16–25% positive; −, 0–15% positive; w, weak positive reaction.

Characteristic*	*V. pantothenticus*	*V. proomii*	*B. circulans*	*B. subtilis*	*B. licheniformis*	*B. dipsosauri*	*P. polymyxa*	*P. validus*	*H. litoralis*
Chains of cells and/or filaments	+	+	(−)	(−)	(+)	+	−	−	+
Sporangia†									
Spore shape	ES	ES	E	E	E	S	E	E	E(S)
Spore position	T(S)	T(S)	ST	PS	PS	T	PS	ST	PS
Sporangia swollen	+	+	+	−	−	+	+	+	+
Anaerobic growth	+	+	+	−	+	−	+	−	−
Gelatin hydrolysis	+	v	−	+	+	−	v	−	+
Casein hydrolysis	+	+	−	+	+	−	+	−	−
Gas from carbohydrates	−	−	−	−	−	−	+	−	−
Acid from:									
Amygdalin	+	−	+	+	+	+	+	−	−
D-Arabinose	+	−	+	−	−	+	−	−	−
L-Fucose	+	−	−	−	−	+	−	−	−
Glycerol	+	−	v	+	+	+	+	+	w
Glycogen	−	v	+	+	+	+	+	v	−
meso-Inositol	−	+	+	+	(+)	−	−	+	−
Methylxyloside	−	−	+	−	−	+	+	−	−
L-Rhamnose	+	(−)	(−)	−	+	+	v	−	−
D-Turanose	+	−	+	+	+	−	+	+	w

* With the exception of microscopic observations, anaerobic growth and casein hydrolysis, all characteristics were determined using tests in API 20E and 50 CHB systems.

† Spore shape: E, ellipsoidal; S, spherical. Spore position: P, central/paracentral; S, subterminal; T, terminal. Shapes or positions infrequently observed are shown in parentheses.

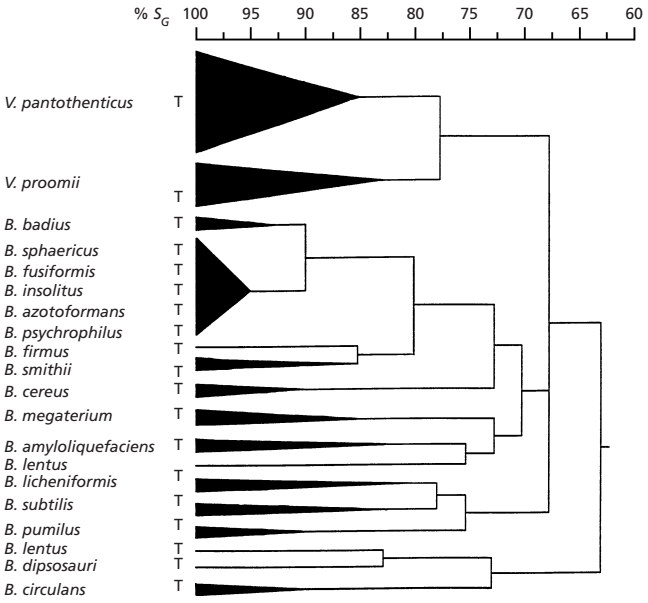

Fig. 3. Simplified phenogram based on the UPGMA clustering of similarity coefficients (S_G) of 76 phenotypic characters of representatives of 14 *Virgibacillus* strains and of 32 representatives of 18 *Bacillus* species. T indicates position of type strain.

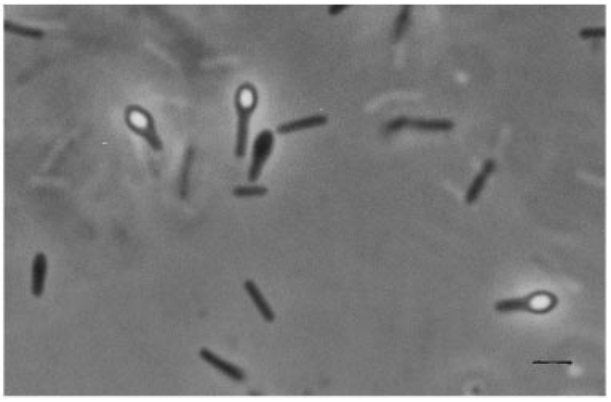

Fig. 4. Photomicrograph of sporangia and vegetative cells of the type strain of *V. proomii* viewed by phase-contrast microscopy; sporangia are swollen with subterminal to terminal ellipsoidal spores. Bar, 2 μm.

terminal, sometimes subterminal, positions in swollen sporangia (Fig. 4). After 2 d on TSA colonies are 1–4 mm in diameter, low convex, circular and slightly irregular, butyrous (sometimes slightly tenacious when cells form filaments), creamy-grey and almost opaque with an eggshell or matt appearance; the appearance under ×10 magnification is reminiscent of white soapflakes in a greyish matrix. After 4 d colonies smell of ammonia and are 5–10 mm in diameter with lobed and/or fimbriate margins. Organisms are facultatively anaerobic and catalase-positive, and they may have a nutritional requirement for pantothenic acid, thiamin, biotin and amino acids. The Voges–Proskauer reaction is negative. Hydrogen sulphide is not produced, but a few strains may give positive reactions for arginine

dihydrolase and citrate utilization in the API 20E strip. Indole is not produced. Nitrate is not reduced to nitrite. Hydrolysis of aesculin and of casein positive; hydrolysis of gelatin usually positive. Growth may occur between 15 and 50 °C, with an optimum of about 37 °C. Acid without gas is produced from the following carbohydrates: *N*-acetylglucosamine, arbutin, D-fructose, galactose, D-glucose, inositol, maltose, D-mannose, ribose, salicin, D-tagatose and trehalose. Acid production from the following carbohydrates is variable: amygdalin, cellobiose and gluconate (these three usually negative), glycogen, methyl α-D-glucoside, rhamnose, starch and sucrose. Acid is not produced from the following carbohydrates: adonitol, D- or L-arabinose, D- or L-arabitol, dulcitol, erythritol, D- or L-fucose, β-gentiobiose, glycerol, inulin, 2-keto-D-gluconate, 5-keto-D-gluconate, lactose, D-lyxose, mannitol, D-melibiose, D-melezitose, methyl α-D-mannoside, methylxyloside, D-raffinose, sorbitol, L-sorbose, D-turanose, xylitol or D- or L-xylose. The major cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$, present in about equal amounts. iso-$C_{13:0}$ is always present at a level of more than 0·5% of the total fatty acids. The G+C content is 37 mol% for the type strain, LMG 12370^T, and 36·8 mol% for strain LMG 17368; the 16S rRNA sequence of the type strain is deposited at EMBL under accession number AJ012667. In the variable characters listed above, the type strain is weak for hydrolysis of gelatin; and acid without gas is produced from methyl α-D-glucoside, starch and sucrose, but not from amygdalin, cellobiose, gluconate, glycogen and rhamnose.

Emended description of *Virgibacillus pantothenticus* (Proom and Knight 1950, 539^{AL}) Heyndrickx *et al.* 1998

Virgibacillus pantothenticus (pan.to.then′tic.us. M.L. *acidum pantothenticum* pantothenic acid; M.L. adj. *pantothenticus* relating to pantothenic acid).

Cells are motile, Gram-positive, usually long, rods (0·5–0·7 by 2–8 mm) which sometimes, especially in older cultures, form chains and/or filaments. They bear spherical to ellipsoidal endospores which lie in terminal, sometimes subterminal, positions in swollen sporangia. After 2 d on TSA colonies are 1–4 mm in diameter, low convex, circular and slightly irregular, butyrous (sometimes slightly tenacious when cells form filaments), creamy-grey and almost opaque with an eggshell or glossy appearance; the appearance under ×10 magnification is reminiscent of white soapflakes in a greyish matrix. After 4 d colonies smell of ammonia and are 5–10 mm in diameter with lobed and/or fimbriate margins. Organisms are facultatively anaerobic and catalase-positive. They have a nutritional requirement for pantothenic acid, thiamin, biotin and amino acids. The Voges–Proskauer reaction is negative. Hydrogen sulphide is usually not produced, but a few strains give weak positive reactions in the API 20E strip; a few strains also give positive reactions for arginine dihydrolase, citrate utilization, and *o*-nitrophenyl β-D-galactoside in the API 20E strip. Indole is not produced. Nitrate reduction to nitrite is variable. Hydrolysis of aesculin and of casein positive; hydrolysis of gelatin usually positive. Growth is stimulated by 4% NaCl and not inhibited by 10% NaCl. Growth may occur between 15 and 50 °C, with an optimum of about 37 °C. Acid without gas is produced from the following carbohydrates: *N*-acetylglucosamine, D-arabinose, arbutin, D-fructose, galactose, D-glucose, glycerol, maltose, D-mannose, methyl α-D-glucoside, rhamnose, ribose, salicin, starch, sucrose, D-tagatose, trehalose and D-turanose. Acid production from the following carbohydrates is variable: amygdalin, cellobiose, L-fucose, β-gentiobiose, gluconate, lactose, methyl α-D-mannoside and sorbitol. Acid is not produced from the following carbohydrates: adonitol, L-arabinose, D- or L-arabitol, dulcitol, erythritol, D-fucose, glycogen, inositol, inulin, 2-keto-D-gluconate, 5-keto-D-gluconate, D-lyxose, mannitol, D-melibiose, D-melezitose, methylxyloside, D-raffinose, L-sorbose, xylitol or D- or L-xylose. The major cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$ in a ratio of 1:3; iso-$C_{13:0}$ is present at a level of less than 0·5% of the total fatty acid content. The major quinone is menaquinone 7 (Watanuki & Aida, 1972). Murein of the type strain has been reported as *meso*-DAP direct type (Claus & Berkeley, 1986). The G+C content is 36·9 mol% for the type strain, LMG 7129^T. In the variable characters listed above, its reactions are positive for H_2S production (weak), citrate utilization, gelatin hydrolysis, and production of acid without gas from amygdalin, cellobiose and methyl α-D-mannoside; its reactions are negative for arginine dihydrolase, nitrate reduction, ONPG, and production of acid without gas from L-fucose, β-gentiobiose, gluconate, lactose and sorbitol.

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