Virgibacillus*: a new genus to accommodate *Bacillus pantothenicus* (Proom and Knight 1950). Emended description of *Virgibacillus pantothenicus

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Twelve strains named *Bacillus pantothenicus*, at least 29 *Bacillus* strains representing 16 species belonging to rRNA groups 1 and 2, one *Bacillus dipsosauri* strain, and 38 strains of *Amphibacillus*, *Aneurinibacillus*, *Brevibacillus*, *Halobacillus*, *Paenibacillus*, *Sporosarcina* and *Marinococcus*, were characterized genotypically using amplified rDNA restriction analysis (ARDRA), and phenotypically using routine diagnostic characters comprising 61 biochemical tests in the API System and 15 observations of vegetative cell and sporangial morphology. The *B. pantothenicus* strains were also characterized by fatty acid methyl ester analysis and SDS-PAGE of whole-cell proteins. ARDRA revealed that strains of *B. pantothenicus* formed a cluster quite separate from other species in rRNA group 1, supporting the recognition of the former as a separate genus, for which the name *Virgibacillus* is proposed. The polyphasic data also indicate the presence of an as yet undescribed new species within this genus. The species *Virgibacillus pantothenicus* and related organisms comprising this new genus can be distinguished from members of *Bacillus* rRNA group 1 (*Bacillus sensu stricto*), and from members of *Paenibacillus* and other aerobic endospore-forming bacteria by routine phenotypic tests.

Keywords: amplified rDNA restriction analysis, *Virgibacillus pantothenicus* comb. nov., *Bacillus*, API, fatty acid methyl ester

INTRODUCTION

Bacillus pantothenicus was described by Proom & Knight (21) following a nutritional analysis of over 300 mesophilic soil isolates of *Bacillus* species. Eleven strains requiring pantothenic acid were isolated from eleven different soil samples, taken from widely separated localities in Southern England, by incubating soil suspensions in 4% NaCl broth and plating on nutrient agar at 37 °C. They considered them as members of a species most closely resembling *Bacillus circulans*, but distinct from it, and subsequent studies have confirmed the validity of the species (3, 9, 17). Later isolations have been made from antacids (3), food, water, bile and soil.

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

Comparisons of the 16S rRNA sequences of type strains of various *Bacillus* and *Sporosarcina* species have indicated that *B. pantothenicus* lies at the periphery of rRNA group 1 of Ash *et al.* (1) and shows evolutionary relationships with *Bacillus smithii* and *Bacillus badius*, the recently described *Bacillus dipsosauri* and the non-spore-former *Marinococcus albus* (16), and with the species of the described genus *Halobacillus* (24), which includes *Halobacillus halophilus*, formerly classified as *Sporosarcina halophila* (7).

As a continuation of the collaborative taxonomic study by some members of the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of the Genus *Bacillus* (15), representative strains of *B. pantothenicus* and 22 species from *Bacillus* rRNA group 1 (*Bacillus sensu stricto*), *Bacillus* rRNA group 2 and of the related genera

Table 1. Strains used and the methods used for their characterization

Name*	LMG no.	Other designations†	Source/comment‡	Method applied‡			
				ARDRA	API	PAGE	FAME
<i>A. aneurinilyticus</i>	15531 ^T	B0205 ^T , NRRL NRS 1589 ^T , ATCC 12856 ^T	Gordon	+	+		
<i>A. aneurinilyticus</i>	15533	B0207, NRRL NRS 1449, Li strain	Gordon	+	+		
<i>Am. xylanus</i>	17667 ^T	DSM 6626 ^T	DSM	+	+		
<i>B. amyloliquefaciens</i>	9814 ^T	B0177 ^T , Campbell F, ATCC 23350 ^T	Gordon	+	+		
<i>B. amyloliquefaciens</i>	12234	B0168, Fukumoto F	Gordon	+	+		
<i>B. azotoformans</i>	9581 ^T	CCM 2849 ^T	CCM	+	+		
<i>B. azotoformans</i>	15444	Pichinoty Ba3	Pichinoty	+	+		
<i>B.adius</i>	7122 ^T	B0180 ^T , DSM 23 ^T	DSM	+	+		
<i>B.adius</i>	12332	B0201, NRS 1407, Appleman	Gordon	+	+		
<i>B. cereus</i>	6923 ^T	B0002 ^T , DSM 31 ^T , ATCC 14579, Ford 13	DSM	+	+		
<i>B. cereus</i>	12334	B0358, F 4810/73 (strain 88)	Vomit, serogroup 1, Melling	+	+		
<i>B. circulans</i>	13261 ^T	B0004 ^T , DSM 11 ^T , ATCC 4513 ^T	DSM	+	+		
<i>B. circulans</i>	12342	B0196, NCTC 5846, Morris	Potted crab, NCTC	+	+		
<i>B. dipsosauri</i>	17413 ^T	NCFB 3027 ^T	NCFB	+	+		
<i>B. firmus</i>	7125 ^T	B0181 ^T , DSM 12 ^T , ATCC 14575 ^T	DSM	+	+		
<i>B. fustiformis</i>	9816 ^T	B0658 ^T , ATCC 7055 ^T , Wellcome 2209	Goodfellow	+	+		
<i>B. fustiformis</i>	17347	B0660, Wellcome 2615	Goodfellow	+	+		
<i>B. insolitus</i>	17757 ^T	B0540 ^T , CCM 2175 ^T , ATCC 2399, B0432 ^T , Stokes W16 B, DSM 5 ^T	Soil, CCM	+	+		
<i>B. insolitus</i>	17758	B0433, Ottow 627	Goodfellow		+		
<i>B. lentus</i>	9579 ^T	B0779 ^T , Gibson urea 165 'type culture'	Gibson	+	+		
<i>B. lentus</i>	12359	B0179, Gibson 238, ATCC 10841	Gibson	+	+		
<i>B. lentus</i>	16798 ^T	NCIMB 8773 ^T	NCIMB	+	+		
<i>B. licheniformis</i>	12360	B0242, Gibson 307	Gibson	+	+		
<i>B. licheniformis</i>	12363 ^T	B0245 ^T , G 46, NCTC 10341	Gibson	+	+		
<i>B. megaterium</i>	7127 ^T	B0010 ^T , DSM 32 ^T , ATCC 14581 ^T	DSM	+	+		
<i>B. megaterium</i>	12409	B0621, Hartman NRRL B-348	Goodfellow	+	+		
<i>B. pantothenicus</i>	7129 ^T	B0018 ^T , DSM 26 ^T , ATCC 14576 ^T	Soil, DSM	+	+	+	+
<i>B. pantothenicus</i>	12366	B0183, DSM 490, NCTC 8122	DSM	+	+	+	+
<i>B. pantothenicus</i>	12367	B0184, DSM 491, NCTC 8124	DSM	+	+	+	+
<i>B. pantothenicus</i>	12368	B0275, NCTC 8123, Wellcome CN 3020	Soil, NCTC	+	+	+	+
<i>B. pantothenicus</i>	12369	B0406, F 2150/77	Canned chicken, Colindale	+	+	+	+
<i>B. pantothenicus</i>	12370	B0413, F 2737/77	Water supply, Colindale	+	+	+	+
<i>B. pantothenicus</i>	17342	B0802, Wellcome CN 3021	Soil, Gibson	+	+	+	+
<i>B. pantothenicus</i>	17343	B0803, Wellcome CN 3022	Soil, Gibson	+	+	+	+
<i>B. pantothenicus</i>	17344	B0805, Wellcome CN 3024	Soil, Gibson	+	+	+	+
<i>B. pantothenicus</i>	17345	B0806, Wellcome CN 3025	Soil, Gibson	+	+	+	+
<i>B. pantothenicus</i>	17367	B0807, Wellcome CN 3026	Soil, Gibson	+	+	+	+
<i>B. pantothenicus</i>	17369	B0809, Wellcome CN 3043	Soil, Gibson	+	+	+	+
<i>B. psychrophilus</i>	6929 ^T	B0434 ^T , Stokes W16 A, DSM 3 ^T	DSM	+	+		
<i>B. psychrophilus</i>	17169	ATCC 23306	ATCC	+	+		
<i>B. pumilus</i>	7132 ^T	B0019 ^T , DSM 27 ^T , ATCC 7061 ^T	DSM	+	+		
<i>B. pumilus</i>	12259	B0102, G 1036, Lister 2812	Gibson		+		
<i>B. smithii</i>	6327	DSM 459	DSM	+	+		
<i>B. smithii</i>	12526 ^T	CCUG 27413 ^T	CCUG	+	+		
<i>B. sphaericus</i>	7134 ^T	B0012 ^T , DSM 28 ^T , G 1013	DSM	+	+		
<i>B. sphaericus</i>	17382	B1144, SS II-1	de Barjac	+	+		
<i>B. subtilis</i>	7135 ^T	B0014 ^T , DSM 10 ^T , ATCC 6051 ^T	DSM	+	+		
<i>B. subtilis</i>	17727	B0905	Cornish pasty, Sandys	+	+		
<i>Br. agri</i>	15103 ^T	B4008 ^T , CCUG 31345 ^T , NRRL NRS 1219 ^T	CCUG	+	+		
<i>Br. agri</i>	15592	B4001, NRRL B-1157	Clinical, Nakamura	+	+		
<i>Br. borstelensis</i>	16009 ^T	B4029 ^T , IFO 15714 ^T , NRRL NRS 818 ^T	IFO	+	+		
<i>Br. borstelensis</i>	15599	B4009, NRRL NRS 1373, 'B. agri'	Hot spring, Nakamura	+	+		
<i>Br. brevis</i>	16703 ^T	ATCC 8246 ^T	ATCC	+	+		
<i>Br. brevis</i>	17054	ATCC 11301, NRS 799	ATCC	+	+		
<i>Br. formosus</i>	16010 ^T	B4039 ^T , NRRL NRS 863 ^T	Nakamura	+	+		
<i>Br. formosus</i>	16101	B4040, NRRL NRS 910	Nakamura	+	+		
<i>Br. laterosporus</i>	16000 ^T	B4035 ^T , DSM 25 ^T , ATCC 64 ^T , Ford 29	DSM	+	+		
<i>Br. laterosporus</i>	15436	B0116, G 1066, 'B. orpheus'	Gibson	+	+		
<i>Br. parabrevis</i>	15971 ^T	B4047 ^T , ATCC 10027 ^T , NRRL NRS 605 ^T	ATCC	+	+		
<i>Br. parabrevis</i>	15428	B0271, G 108, NCTC 7577	NCTC	+	+		
<i>H. halophilus</i>	17431 ^T	DSM 2266 ^T	DSM	+	+		
	17432	DSM 2500	DSM	+	+	+	
	17435	DSM 5938	DSM	+	+	+	
<i>H. trueperi</i>	17437 ^T	DSM 10404 ^T	DSM	+	+		
<i>H. litoralis</i>	17438 ^T	DSM 10405 ^T	DSM	+	+	+	
<i>M. albus</i>	17430 ^T	DSM 20748 ^T	DSM	+	+		
<i>M. halophilus</i>	17439 ^T	DSM 20408 ^T	DSM	+	+		
<i>P. alvei</i>	13253 ^T	B0016 ^T , DSM 29 ^T	DSM	+	+		
<i>P. alvei</i>	13254	B0610, CN 2772	Goodfellow	+	+		
<i>P. azotofixans</i>	14658 ^T	B1406 ^T , Seldin P3L5	Seldin	+	+		
<i>P. azotofixans</i>	14659	B1407, Seldin F-102	Seldin	+	+		
<i>P. larvae</i> subsp. <i>larvae</i>	9820 ^T	B1415 ^T , ATCC 9545 ^T	ATCC	+	+		
<i>P. larvae</i> subsp. <i>larvae</i>	14425	B1416, ATCC 25747	ATCC	+	+		

Table 1 (cont.)

Name*	LMG no.	Other designations†	Source/comment‡	Method applied‡			
				ARDRA	API	PAGE	FAME
<i>P. lautus</i>	11157 [†]	B1396 [†] , DSM 3035 [†] , B. Henry 160	DSM	+	+		
<i>P. lautus</i>	14015	B1388, NRRL NRS-1171	Nakamura	+	+		
<i>P. macerans</i>	13281 [†]	B0497 [†] , NCTC 6335 [†]	NCTC	+	+		
<i>P. macerans</i>	13283	B0110, Gibson 557	Gibson	+	+		
<i>P. polymyxa</i>	13294 [†]	B0011 [†] , DSM 36 [†]	DSM	+	+		
<i>P. polymyxa</i>	13296	B0264 [†] , Gibson 43, NCTC 7575	Gibson	+	+		
<i>P. validus</i>	11161 [†]	B1397 [†] , DSM 3037 [†]	DSM	+	+		
<i>P. validus</i>	14018	B1392, NRRL NRS-838A	Nakamura	+	+		
<i>S. ureae</i>	17366 [†]	B0819 [†] , G 181, NRS 42, CCM 684 [†]	CCM	+	+		
<i>S. ureae</i>	17363	B0727, G urea 155	Gibson	+	+		

* Genus abbreviations: *A.*, *Aneurinibacillus*; *Am.*, *Amphibacillus*; *B.*, *Bacillus*; *Br.*, *Brevibacillus*; *H.*, *Halobacillus*; *M.*, *Marinococcus*; *P.*, *Paenibacillus*; *S.*, *Sporosarcina*.

† ATCC, American Type Culture Collection, Rockville, MD, USA; B, N. A. Logan *Bacillus* collection, Glasgow Caledonian University, Glasgow, UK; CCUG, Culture Collection University of Göteborg, Göteborg, Sweden; CN, Wellcome Collection of Microorganisms, Beckenham, Kent, UK; 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; Gordon, Rutgers University, New Brunswick, USA; Goodfellow, University of Newcastle, Newcastle upon Tyne, UK; LMG, BCCM/LMG Bacteria collection Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; Nakamura, US Department of Agriculture, Peoria, IL, USA; 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; Pichinoty, UER Scientifique de Luminy, Marseille, France; Seldin, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

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

Aneurinibacillus, *Brevibacillus* and *Paenibacillus* were initially characterized by amplified rDNA restriction analysis (ARDRA) (14). This method revealed that *B. pantothenicus* represents a phylogenetic group sufficiently different from other *Bacillus* species to warrant the status of a separate genus. We therefore carried out a polyphasic taxonomic study, which also included strains of *Amphibacillus xylanus*, *Halobacillus halophilus*, *Halobacillus trueperi*, *Halobacillus litoralis*, *Marinococcus halophilus* and *Marinococcus albus*, to clarify the positions of these strains at different taxonomic levels. We report here on the findings of that study, which support the recognition of a new genus, which we name *Virgibacillus*, to accommodate *B. pantothenicus* and related organisms which appear to belong to an as-yet-undescribed new species.

METHODS

Strains and media. The designations of the strains, their origins and the different methods applied are shown in Table 1. Unless otherwise stated, *Bacillus pantothenicus* 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 *Halobacillus* strains were grown on Bacto Marine agar (Difco) at 30 °C and *Bacillus dipsosauri* on TSA supplemented with 1 M KCl at 37 °C. *Marinococcus albus* and *Marinococcus halophilus* were grown on the *M. albus* medium at 37 and 30 °C, respectively (6). *Amphibacillus xylanus* was grown at 37 °C aerobically

and anaerobically (Anaerobic System, Forma Scientific model 1024) on the medium described by Niimura *et al.* (18). The strains were checked for purity by plating and phase-contrast microscopy, and were maintained both as lyophilized cultures and (except for *Marinococcus* strains) as sporulated cultures on slopes of the appropriate above-mentioned medium containing 5 mg MnSO₄·4H₂O 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 using a slight modification of the method of Pitcher *et al.* (19), as described previously (14). DNA was prepared from *A. xylanus* grown aerobically as well as anaerobically. The DNA preparations were visually inspected by 1% (w/v) agarose gel electrophoresis, quantified spectrophotometrically at 260 nm, and stored at –20 °C.

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 (14). The restriction patterns for each strain were normalized and combined. The combined patterns were subjected to numerical analysis by the Gelcompar 4.0 software (Applied Maths) using the Dice coefficient and the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm.

GC analysis of fatty acid methyl esters (FAMES). Cells were grown on TSA at 28 °C for 24 h as recommended by the Microbial Identification System (MIS; Microbial ID). Methods for fatty acid extraction, methyl ester preparation and methyl ester separation by GC were described previously

(25). The FAME profiles were identified and clustered using the MIS software version 4.0.

SDS-PAGE of whole-cell proteins. Cells for SDS-PAGE were obtained after a 24 h growth on nutrient agar with 1% (w/v) glucose (NAG) at 30 °C. Methods for preparation and electrophoresis of SDS protein extracts and for UPGMA cluster analysis of the protein profiles were described previously (20, 26).

Phenotypic characterization and numerical analysis. Strains were grown on TSA or, in the case of *B. dipsosauri*, *Amphibacillus*, *Halobacillus* and *Marinococcus* strains, on the special media mentioned above, and were characterized by the methods of Logan & Berkeley (16), using API tests and microscopic characters: 12 miscellaneous biochemical tests from the API 20E strip and 49 carbohydrate tests in the API 50CH gallery (using the API CHB suspension medium) were accompanied by 15 observations on vegetative cells and sporangia by phase-contrast microscopy (presence of motile cells, chains of cells, curved rods, rods with tapered ends, vacuoles, spores, swollen sporangia, parasporal crystals, parasporal bodies; shape of spores ellipsoidal, cylindrical or spherical, position of spores terminal, subterminal or central/paracentral). Biochemical tests were scored on a range of 0 (negative) to 5 (strong positive); microscopic observations were scored as 0 (absent/negative) or 1 (present/positive). Redundant tests (i.e. positive or negative for all strains) were excluded, and the data were subjected to numerical taxonomic analysis using the Genstat 5 program. Similarities were calculated using the general similarity coefficient of Gower (S_G) (10). Where two organisms were both negative for an API test, such a matching was given a weighting of zero in the calculation to avoid negative results making undue contributions to similarity. The results were then examined by UPGMA cluster analysis.

RESULTS AND DISCUSSION

The 12 *B. pantothenicus* strains clustered together, but separate from all the other organisms studied, in the numerical analysis of their combined ARDRA patterns, and are subdivided into two groups (Fig. 1). The largest group, forming at a similarity level of 92%, consisted of 10 strains and included the type strain of *B. pantothenicus*, LMG 7129^T. Nearest neighbours of this group, joining at a similarity level of 81%, were *B. pantothenicus* LMG 12370 and *B. pantothenicus* LMG 17369, which showed an ARDRA similarity of 91% to each other. This cluster of 12 strains, henceforth referred to as the *B. pantothenicus* group, was as a whole somewhat more closely related (similarity level 58%) to the spore-forming halophilic organisms *B. dipsosauri*, *H. halophilus*, *H. litoralis* and *H. trueperi*, to the halophilic non-spore-forming organism *M. albus* and to the spore-forming alkaliphilic organism *A. xylanus*, than to *Bacillus* rRNA groups 1 and 2 (1), *Paenibacillus* (formerly *Bacillus* rRNA group 3) (2), and *Aneurinibacillus* and *Brevibacillus* (formerly *Bacillus* rRNA group 4) (23). This ARDRA clustering of the *B. pantothenicus* group is consistent with the most recent phylogenetic trees based upon 16S rRNA (rDNA) sequences of type strains alone (16, 23, 24).

It is noteworthy that according to the ARDRA patterns, *M. halophilus* (type species of the genus)

belongs to a separate phylogenetic lineage which agrees well with the sequence analysis of Farrow *et al.* (8). *M. albus* did not show any appreciable relationship by ARDRA to the former species, confirming the sequence analysis of Lawson *et al.* (16) and suggesting that it should be removed from the genus *Marinococcus*.

Numerical analysis of SDS-PAGE patterns of whole cell proteins (data not shown) showed a similar arrangement of the *B. pantothenicus* group (except for *B. pantothenicus* LMG 12366) as that seen with ARDRA (Fig. 1): a large group (nine strains), including *B. pantothenicus* LMG 7129^T, showing identical protein patterns (similarity level of at least 90%), and the two strains *B. pantothenicus* LMG 12370 and *B. pantothenicus* LMG 17369 having a mutual similarity of 95%, and linking with the larger group at 80% similarity. The exception, *B. pantothenicus* LMG 12366, was found as the nearest neighbour of the larger group of nine strains at 80% similarity. The slightly aberrant clustering of this strain with respect to the ARDRA picture was caused by a dense protein band with a molecular mass of 56 kDa. Indeed, by exclusion of this band (5) from numerical analysis, this strain joined the group of nine strains containing the *B. pantothenicus* type strain. *H. halophilus* strains LMG 17432, 17435 and 17438, which could be cultivated on NAG after a preculture on Bacto Marine agar, showed completely different protein patterns and are thus clearly distinct from the *B. pantothenicus* group.

In a numerical analysis of the FAME data of the *B. pantothenicus* group (not shown), the same 10 *B. pantothenicus* strains as in the ARDRA analysis (Fig. 1) formed a cluster at a Euclidian distance of 12.7. *B. pantothenicus* LMG 12367 was the only strain linked at the lower border of this cluster. All of the nine other strains grouped within a Euclidian distance of about 10, a level which has recently been suggested for species delineation (22). The mean fatty acid profile (Table 2) of this group probably represents the authentic *B. pantothenicus* species, and consists of major amounts of anteiso-C_{15:0}, iso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}, with an iso-C_{15:0}/anteiso-C_{15:0} ratio of 0.33. *B. pantothenicus* LMG 12370 and *B. pantothenicus* LMG 17369, clustering together at a Euclidian distance of 7.0, again have a distinct position, lying at 24.6 Euclidian distance from the main *B. pantothenicus* group. The main difference in their mean fatty acid profile (Table 2, group 2) was a higher iso-C_{15:0}/anteiso-C_{15:0} ratio of around 1.0.

In the numerical analysis of API tests and other phenotypic characters (data not shown), the *B. pantothenicus* group was divided into two distinct clusters which merged at 67% S_G and which then joined with the clusters containing most of the other *Bacillus* species at 46% S_G . This separation reflects distinct biochemical and morphological profiles for *B. pantothenicus* which allow its ready separation from other aerobic endospore-forming bacteria and from *Marinococcus*. The larger of the two *B. pantothenicus* clusters

Fig. 1. Dendrogram based on the UPGMA clustering of similarity coefficients (S_D) of normalized combined 16S rDNA restriction profiles (ARDRA) of 12 *Bacillus pantothenicus* (here reclassified as *Virgibacillus pantothenicus*) strains, the type strain of *B. dipsosauri*, five *Halobacillus* strains and two *Marinococcus* strains. To show the localization of the *V. pantothenicus* strains, we included 29 representatives of *Bacillus* rRNA groups 1 and 2, 14 *Paenibacillus* strains, 12 *Brevibacillus* strains, two strains of *Aneurinibacillus* and two strains of *Sporosarcina ureae*. Restriction enzymes used were *Hae*III, *Dpn*II, *Rsa*I, *Bfa*I and *Tru*9I. *Pseudomonas fluorescens* LMG 1799 was used as outgroup reference. T indicates position of type strain.

Table 2. Comparison of the mean fatty acid profile of 12 named *B. pantothenicus* strains as measured by GC analysis of their methyl esters

The data given are mean values with the minimal and maximal values within the particular group of strains given below. Only 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
<i>B. pantothenicus</i> group 1*		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
<i>B. pantothenicus</i> group 2†	1.4	0.7	3.8	2.0	34.9	34.0	4.1	8.8	5.1	5.7
	1.1–1.6	0.0–0.7	2.9–4.6	1.6–2.4	34.8–35.1	31.9–36.1	3.9–4.2	6.7–10.9	4.0–6.2	4.9–6.4

* Group 1 consists of strains LMG 7129^T, 17342, 17367, 17344, 12366, 17345, 12368, 17343, 12369 and 12367.

† Group 2 consists of strains LMG 12370 and 17369.

formed at 76% S_G , and contained the same 10 strains that clustered together in the ARDRA (Fig. 1), PAGE and FAME dendrograms. The two remaining strains of *B. pantothenicus* (LMG 12370 and 17369) clustered loosely at 70% S_G , a result also consistent with the findings of the ARDRA (Fig. 1), SDS-PAGE and FAME analyses. Given that, in ARDRA, *M. albus* LMG 17430 clustered with *Halobacillus* strains, and yet *M. halophilus* showed no appreciable relatedness to *M. albus*, it is of interest that the strains of *Halobacillus* and *M. albus* also clustered together in the API and other phenotypic test analysis, while *M. halophilus* was recovered as an outlier of the *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus* cluster, joining at 55% S_G , finally joining with the *Halobacillus* and *M. albus* cluster at only 29.5% S_G .

In conclusion, our polyphasic data show that the *B. pantothenicus* group is distinct from *Bacillus* rRNA groups 1 and 2, as well as from the related genera *Paenibacillus*, *Brevibacillus* and *Aneurinibacillus*. Although it shows a distant phylogenetic relationship to the alkaliphilic spore-forming genus *Amphibacillus*, to the moderately halophilic spore-forming genus *Halobacillus*, and to the moderately halophilic non-spore-forming genus *Marinococcus*, it differs from the last two genera in its facultative anaerobic metabolism and from each of the three mentioned genera in the following respective characteristics (Table 3): *Amphibacillus* is an obligate alkalophile and lacks isoprenoid quinones (18), *Halobacillus* contains an uncommon cell wall murein of the Orn-D-Asp type (24), whereas *B. pantothenicus* contains a cell wall murein based on meso-diaminopimelic acid like most *Bacillus* spp. (3), and *Marinococcus* differs by its inability to grow on media without NaCl, its coccoid morphology and its absence of endospores (11). Therefore we propose to elevate the *B. pantothenicus* group to the rank of genus, for which we propose the name *Virgibacillus* gen. nov. At present this genus contains only one validly described species, the type species *V. pantothenicus*. However our polyphasic data indicate that

strains LMG 12370 and 17369 represent a new *Virgibacillus* species; more strains must be obtained and characterized to describe the phenotypic and genotypic variation of this species.

The description of the new genus and an emended description of its species follow, and a comparison of some of the salient features of other genera of aerobic spore-forming bacteria and *Marinococcus*, is shown in Table 3. Cultural, microscopic and API characters, and the fatty acid profile were obtained in the present study. Other characters are taken from the description of *B. pantothenicus* given by Claus & Berkeley (3).

Description of *Virgibacillus* gen. nov.

Virgibacillus (Vir.gi.ba.cil'lus. L. n. *virga* a green twig, transf., a branch in a family tree; L. dim. n. *bacillus* from *Bacillus*, a genus of aerobic endospore-forming bacteria; *Virgibacillus* a branch of the genus *Bacillus*).

Cells are motile, Gram-positive rods (0.5–0.7 by 2–5 µm) which often form chains. They bear spherical to ellipsoidal endospores which lie in terminal, sometimes subterminal, positions in swollen sporangia. After 2 d on Trypticase Soy agar colonies are small (0.5–2 mm in diameter), circular and slightly irregular, smooth, glossy or sometimes matt, flat, butyrous, creamy to yellowish white and almost opaque. Members of the genus are facultatively anaerobic and catalase positive. They have a nutritional requirement for pantothenic acid, thiamin, biotin and amino acids. In the API 20E strip and in conventional tests the Voges–Proskauer reaction is negative, indole is not produced, nitrate reduction to nitrite is variable. Hydrolysis of casein and of aesculin positive, hydrolysis of gelatin usually positive, and 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 ONPG in the API 20E strip. Growth is stimulated by 4% NaCl and not inhibited by 10% NaCl. Growth may occur between

Table 3. Salient features of some genera of aerobic, endospore-forming bacteria and *Marinococcus*

Characters are taken from the following references: *Bacillus* (3), *Paenibacillus* (13), *Aneurinibacillus* (12, 23), *Brevibacillus* (23), *Halobacillus* (24), *Alicyclobacillus* (28), *Amphibacillus* (18), *Sporosarcina* (4), and *Marinococcus* (11). Abbreviations: DAP, direct-linked *meso*-diaminopimelic acid; E, ellipsoidal; S, spherical; E-S, ellipsoidal to spherical; C, cylindrical; B, ellipsoidal or cylindrical spores bent into kidney or banana shape; V, character varies according to species.

Character	<i>Virgibacillus</i>	<i>Bacillus</i>	<i>Paenibacillus</i>	<i>Aneurinibacillus</i>	<i>Brevibacillus</i>	<i>Halobacillus</i>	<i>Alicyclobacillus</i>	<i>Amphibacillus</i>	<i>Sporosarcina</i>	<i>Marinococcus</i>
No. of species	1	>60	13	2	10	3	3	1	1	3
Murein	DAP	V*	DAP	DAP	DAP	Orn-D-Asp	ND	DAP	L-Lys-Gly-D-Glu	DAP
Cell shape	Rods	Rods	Rods	Rods	Rods	Rods and cocci	Rods	Rods	Cocci	Cocci
Spores	+	+	+	+	+	+	+	+	+	—
Spore shape	E-S	E, C, S, B	E	E	E	E, S	E	E	S	NA
Sporangia swollen	+	V	+	+	+	+ / NA	+ / —	ND	NA	NA
Anaerobic growth	+	V	V	—	+ / —	—	V	+	—	—
Optimum temperature (°C)	37	15–55	30	37	30–48	35	48–65	37	30	30
Optimum pH	7	7–9.5	7	7	7	7.5	3	9–10	8	7.5
Growth in 10% NaCl	+	V	—	—	—	+	—	—	—	+
Major isoprenoid quinone	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	None	MK-7	MK-7 (MK-8)
Major cellular fatty acids	Iso + anteiso-C _{15:0}	V	Anteiso-C _{15:0}	iso-C _{15:0} , C _{16:0}	Anteiso-C _{15:0}	ND	ω -Alicyclic acids	Anteiso-C _{15:0} , C _{16:0} , iso-C _{16:0}	ND	ND
G + C (T _m)	36.9†–38.3‡	32–69	40–54	41.1–43.4	42.8–57.4	40–43	51.6–60.3	36–38	40–41.5	44.9–49.3

NA, Not applicable.

ND, No data available.

* Predominant type is DAP.

† According to Fahmy *et al.* (7).

‡ Mean of two measurements (T_m method).

15 and 50 °C, with an optimum of about 37 °C. Acid without gas is produced from the following carbohydrates in the API 50CHB gallery: *N*-acetylglucosamine, arbutin, D-fructose, galactose, D-glucose, maltose, D-mannose, ribose, salicin, D-tagatose, trehalose; acid production from the following carbohydrates is variable: amygdalin, D-arabinose, cellobiose, L-fucose, β -gentiobiose, gluconate, glycerol, glycogen, inositol, lactose, methyl α -D-glucoside, methyl α -D-mannoside, rhamnose, sorbitol, starch, sucrose and D-turanose. The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. The major quinone is menaquinone 7 (27). Murein of the type strain of the type species has been reported as *meso*-DAP direct type (3). The G + C content has been reported (7) as 36.9 (T_m) while we found 38.3 mol% to be the mean of two determinations for the type strain of the only species, *Virgibacillus pantothenticus*.

Description of *Virgibacillus pantothenticus* (Proom and Knight 1950, 539^{AL}) comb. nov.

The description is identical to that given by Claus & Berkeley (3) and to the description of the genus given above. The type strain is strain ATCC 14576 (= LMG 7129, NCIMB 8775, NCTC 8162). In the variable API characters listed above, its reactions are positive for H₂S production (weak), citrate utilization, gelatin hydrolysis, and production of acid without gas from: amygdalin, methyl α -D-glucoside, methyl α -D-mannoside, rhamnose, starch, sucrose and D-turanose; its

reactions are negative for arginine dihydrolase, nitrate reduction, ONPG, and for production of acid without gas from D-arabinose, cellobiose, L-fucose, β -gentiobiose, gluconate, glycerol, glycogen, inositol, lactose and sorbitol.

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