Paenibacillus (Formerly *Bacillus*) *gordonae* (Pichinoty et al. 1986) Ash et al. 1994 Is a Later Subjective Synonym of *Paenibacillus* (Formerly *Bacillus*) *validus* (Nakamura 1984) Ash et al. 1994: Emended Description of *P. validus*

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A polyphasic study in which we performed an amplified ribosomal DNA restriction analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins, a gas chromatographic analysis of methylated fatty acids, pyrolysis mass spectrometry, a random amplified polymorphic DNA analysis, a phenotypic analysis, and an analysis of the levels of DNA binding of *Paenibacillus gordonae* and *Paenibacillus validus* strains (including both type strains) showed that these organisms form a homogeneous group and that the names *P. gordonae* and *P. validus* are therefore synonyms. *P. validus* has nomenclatural priority, and an emended description of this species is given; the type strain is strain LMG 11161 (= ATCC 43897).

In 1992 the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of the Genus *Bacillus* (15) decided to undertake a collaborative polyphasic taxonomic study of the genus *Bacillus*, with a view toward establishing minimal standards for the description of species and revising the nomenclature of the group, starting with mesophilic and psychrophilic species which form sporangia swollen by ellipsoidal spores. In the following year, Ash et al. (2) proposed that several members of this group should be reclassified in the new genus *Paenibacillus*. Recently, this name was validated (validation list no. 51). This new genus contains *Paenibacillus gordonae* (formerly *Bacillus gordonae*) and *Paenibacillus validus* (formerly *Bacillus validus*). These species are the subject of this study. Ash et al. (2) found a level of sequence similarity of 99.8% between the ribosomal DNAs (rDNAs) of the type strains of *P. gordonae* and *P. validus*.

In 1935 Bredemann and Heigener (3) described the species *B. validus*. Later, Smith et al. (30) and Gordon et al. (10) reclassified *B. validus* strains as *Bacillus circulans* strains, and as the former species was considered to be inadequately described, it was not included on the Approved Lists of Bacterial Names (29). Nakamura and Swezey (24) found that the guanine-plus-cytosine (G+C) content of the DNA of their *B. validus* strain was 54 mol%, and they considered this organism to be unrelated to *B. circulans* sensu stricto strains, whose DNA G+C contents ranged from 37 to 39 mol%. On the basis of the results of new phenotypic and genotypic (DNA-DNA homology) studies, Nakamura (23) revived the name *B. validus* for the same group of organisms to which it was originally applied.

Pichinoty et al. (25) isolated 30 endospore-forming strains from pasteurized soil samples by aerobic enrichment in minimal media containing various aromatic compounds. These authors considered their isolates members of morphological group 2 of Smith et al. (30), as these organisms produced

sporangia which were swollen by ellipsoidal spores. Pichinoty et al. (25) believed that their strains represented a new species (*B. gordonae*) because they were aerobic (unlike most other group 2 strains, which are facultatively anaerobic) and found that they could be clearly differentiated from the two other aerobic members of the group, *Bacillus brevis* and *Bacillus azotoformans*. The reported G+C content of this new *Bacillus* species was 55 ± 0.6 mol% (25).

Our restriction analysis of enzymatically amplified 16S rDNAs from *P. validus* and *P. gordonae* revealed that all 14 strains studied produced similar patterns, and this was considered a strong indication that these organisms are very closely related phylogenetically. These strains were further examined in a polyphasic study to determine the exact taxonomic and nomenclatural implications at the species level.

MATERIALS AND METHODS

For all of the methods used in this study many strains were tested at least twice. In this paper we describe only individual observations, unless indicated otherwise.

Strains used. The designation of each strain which we studied, its origin, its taxonomic status, and the different methods that we used to study it are shown in Table 1. All strains were checked for purity by plating on nutrient agar (pH 7.4) and microscopic examination. The strains were maintained at 4°C on slopes of nutrient agar containing 5 mg of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ per liter to enhance sporulation and as lyophilized cultures.

SDS-PAGE of whole-cell proteins. To obtain material for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), cells were grown in petri dishes on nutrient agar supplemented with 1% (wt/vol) glucose (pH 7.4) for 24 h. We used previously described methods to prepare and electrophorese SDS protein extracts (27). The protein profiles were recorded densitometrically, stored as normalized data in a PC-AT computer, and analyzed numerically with the GelCompar software (Applied Maths, Kortrijk, Belgium) by using the Pearson product moment correlation coefficient. A cluster analysis was performed by the unweighted pair group method using arithmetic averages (UPGMA) clustering technique (32).

Gas chromatographic analysis of methylated fatty acids. Cells were grown on Trypticase soy agar containing 3.0% (wt/vol) Trypticase soy broth (BBL) and 1.5% (wt/vol) Bacto Agar (Difco) at 28°C for 24 h. Approximately 60 mg (wet weight) of cells was harvested from each plate from the most diluted quadrant exhibiting confluent growth. We used previously described methods for fatty acid extraction, methyl ester preparation, and methyl ester separation by gas chromatography (33). The fatty acid methyl ester profiles were identified with the Microbial Identification System software (MIS version 3.8; Microbial ID, Inc.,

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TABLE 1. Designations and sources of the *Paenibacillus* strains used and methods used to study them

Strain or species ^a	Other designation(s) ^a	Source ^a	Methods used						
			ARDRA	API tests ^b	PAGE	FAME analysis ^c	DNA homology	Py-MS	RAPD analysis
<i>P. validus</i> LMG 11161 ^T	B1397 ^T , DSM 3037 ^T	DSM	+	+	+	+	+	+	+
<i>P. validus</i> LMG 14018	B1392, NRRL NRS-838A	Nakamura	NR ^d	+	NR	NR	NR	+	+
<i>P. validus</i> LMG 14019	B1393, NRRL NRS-839A	Nakamura	+	+	+	+	+	+	+
<i>P. validus</i> LMG 14020	B1394, NRRL NRS-625	Nakamura	+	+	+	+	+	+	+
<i>P. gordonae</i> LMG 9817 ^T	B1395 ^T , Pichinoty Q 1 ^T , ATCC 29948 ^T	ATCC	+	+	+	+	+	+	+
<i>P. gordonae</i> LMG 14717	B1399, Pichinoty Q 2	Pichinoty	+	NR	+	+	NR	NR	NR
<i>P. gordonae</i> LMG 14663	B1400, Pichinoty Q 3	Pichinoty	+	+	+	+	NR	+	+
<i>P. gordonae</i> LMG 14664	B1401, Pichinoty Q 12	Pichinoty	+	+	+	+	NR	+	+
<i>P. gordonae</i> LMG 14665	B1402, Pichinoty Ipht 1	Pichinoty	+	+	+	+	NR	+	+
<i>P. gordonae</i> LMG 14666	B1403, Pichinoty Pohb 1	Pichinoty	+	+	+	+	+	+	+
<i>P. gordonae</i> LMG 14667	B1404, Pichinoty Pohb 3	Pichinoty	+	+	+	+	+	+	+
<i>P. gordonae</i> LMG 14668	B1405, Pichinoty Tri 5	Pichinoty	+	+	+	+	+	+	+
<i>P. gordonae</i> LMG 14468	Pichinoty Q 13, DSM 6390	DSM	+	NR	+	+	NR	NR	NR
<i>P. gordonae</i> LMG 14469	Pichinoty Pohb 3, DSM 6544	DSM	+	NR	+	+	NR	NR	NR
<i>P. gordonae</i> LMG 14470	Pichinoty Tri 4, DSM 6545	DSM	+	NR	+	+	NR	NR	NR
<i>P. pulvifaciens</i> LMG 13302	B0302, T. Gibson 1301	Gibson	NR	NR	NR	NR	NR	+	NR
<i>P. pulvifaciens</i> LMG 13303	B0307, T. Gibson 11.1301	Gibson	NR	NR	NR	NR	NR	+	NR
<i>P. peoriae</i>	B1412, NRRL NRS 1312	Nakamura	NR	NR	NR	NR	NR	+	NR
<i>P. peoriae</i> LMG 14672	B1413, NRRL NRS 14475	Nakamura	NR	NR	NR	NR	NR	+	NR
<i>P. polymyxa</i> LMG 6319 ^T	DSM 36 ^T , ATCC 842 ^T	DSM	+	NR	+	NR	NR	NR	NR
<i>P. polymyxa</i> LMG 13296	B0264, T. Gibson 43	Gibson	NR	NR	+	NR	NR	NR	NR
<i>P. larvae</i> LMG 9820 ^T	ATCC 9545 ^T	ATCC	NR	NR	+	NR	+	NR	NR
<i>P. larvae</i> LMG 14425	ATCC 25747	ATCC	NR	NR	+	NR	NR	NR	NR
<i>P. larvae</i> LMG 14426	ATCC 25748	ATCC	NR	NR	+	NR	+	NR	NR
<i>P. macquariensis</i> LMG 6935 ^T	DSM 2 ^T , ATCC 23464 ^T	DSM	+	NR	NR	NR	NR	NR	NR

^a ATCC, American Type Culture Collection, Rockville, Md.; B, N. A. Logan *Bacillus* Collection, Glasgow Caledonian University, Glasgow, United Kingdom; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; T. Gibson, T. Gibson collection, University of Bristol, Bristol, United Kingdom; LMG, Laboratorium voor Microbiologie Collection, Universiteit Gent, Ghent, Belgium; Nakamura, L. K. Nakamura, U.S. Department of Agriculture, Peoria, Ill.; NRRL, Northern Regional Research Laboratory Collection, Peoria, Ill.; NRS, N. R. Smith Collection; Pichinoty, F. Pichinoty, UER Scientifique de Luminy, Marseille, France.

^b API System tests and morphological observations.

^c FAME, fatty acid methyl ester.

^d NR, not reported.

Newark, Del.) and were clustered by using the statistical program CLUS (Microbial ID, Inc.).

Py-MS. Cells were grown on nutrient agar for 24 to 36 h at 30°C. Bacterial sample preparation and pyrolysis mass spectrometry (Py-MS) of the samples were performed as described previously (9). Three samples of each strain were analyzed. The raw data were first normalized with the resident PYSTAT program (13), and this was followed by a principal-component analysis to reduce the data for a canonical variates analysis, both by the GENSTAT program (13), which produced a generalized distance matrix. This matrix was transformed to a similarity level matrix by using the general similarity coefficient of Gower (11) to make the data amenable to a UPGMA analysis.

Phenotypic characterization and numerical analysis. Strains were characterized by the methods of Logan and Berkeley (18) by using the following API tests and other phenotypic tests and observations: 16 biochemical tests (gas production from carbohydrates, casein digestion, growth and hemolysis on horse blood agar, and 12 of the API 20E strip tests) and 49 carbohydrate tests in API 50CH galleries (using the API CHB suspension medium), nine growth temperature tests (with temperatures ranging from 4 to 55°C), and 12 characteristics determined by phase-contrast microscopy, including motility, vegetative cell curvature, shape of the cell ends, chain formation, the presence of spores, spore shape (ellipsoidal, cylindrical, or spherical) and position (central and paracentral, subterminal, or terminal), and swelling of the sporangia. The results of biochemical tests were scored on a scale of 0 (negative) to 5 (strongly positive); and the results of growth temperature tests and microscopic observations were scored as 0 (absent or negative) or 1 (present or positive). The data were subjected to a numerical taxonomic analysis by using the GENSTAT 5 program. Levels of similarity, with negative matches in the API tests both ignored and included, were calculated by using the similarity coefficient of Gower, and the results were examined by performing an UPGMA analysis. Anaerobic growth at 30°C was tested for in tubes containing thioglycolate medium (Brewer) (Oxoid) and tubes containing the anaerobic agar of Gordon et al. (10) as described by Claus and Berkeley (4), with growth at the medium-air interfaces acting as aerobic controls in both cases, as well as in nutrient agar containing 1% (wt/vol) glucose and incubated anaerobically (anaerobic system BR 38; Oxoid) in parallel with aerobic control plates containing the same medium. Results were recorded after 2, 5, and 7 days.

DNA preparation. For DNA-DNA binding experiments (see below), DNA was

extracted from 5 to 10 g (wet weight) of cells grown in Roux flasks containing 130 ml of nutrient agar for 48 h at 30°C by using a slight modification of the method of Marmur (21), as described previously (36). For the amplified rDNA restriction analysis (ARDRA) (see below), total genomic DNA was purified by a method slightly modified from the method of Pitcher et al. (26); cells grown in petri dishes containing nutrient agar for 24 to 48 h at 30°C were lysed in an additional step by incubating them with lysozyme at a final concentration of 50 mg/ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) for 30 min at 37°C. After the DNA was purified, it was treated with RNase at a final concentration of 250 µg/ml of TE buffer for 1 h at 37°C. The quality of the purified DNA was monitored by 1% (wt/vol) agarose gel electrophoresis, and the amount of DNA was determined on the basis of the A_{260} .

Levels of DNA-DNA binding. DNAs from representative strains of each species were used to determine the levels of DNA-DNA binding (expressed as percentages) spectrophotometrically by using the initial renaturation method of De Ley et al. (7), as described previously (35).

DNA base composition. The mean G+C contents were determined by the thermal denaturation method (8) and were calculated by using the equation of Marmur and Doty (22) as modified by De Ley (6).

ARDRA. Enzymatically amplified 16S rDNA was obtained by performing a PCR in 50 µl of PCR buffer (10 mM Tris-HCl [pH 8.4], 1.5 mM MgCl₂, 50 mM KCl) containing 10 nmol of each deoxynucleoside triphosphate (Pharmacia Biotech Benelux, Roosendaal, The Netherlands), 20 pmol of each primer (forward primer 5'-AGTTTGTATCCTGGCTCAG-3' and reverse primer 5'-TACCTTGT TACGACTTACCCCA-3'; *Escherichia coli* nucleotide positions 10 to 27 and 1507 to 1485, respectively; Pharmacia Biotech Benelux), 1.25 U of *EuroTaq* DNA polymerase (Eurogentec, Seraing, Belgium), and 250 ng of template DNA. A Perkin-Elmer model 9600 thermocycler was used with the following program: an initial denaturation step at 95°C for 5 min was followed by 40 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min and a final extension step at 72°C for 10 min. The presence and yield of the specific PCR product (length, about 1,500 bp) were monitored by 1% (wt/vol) agarose gel electrophoresis. Restriction digestion of the amplified 16S rDNA was carried out for 2 h at 37°C in 20-µl reaction mixtures containing 10 µl of the PCR product solution, 2 µl of a commercially supplied incubation buffer, and 5 U of one of the following restriction enzymes: *Hae*III (Boehringer, Mannheim, Germany), *Rsa*I (Pharmacia Biotech Benelux), *Dpn*II (New England Bio-

FIG. 1. (A) Schematic representation of normalized composite 16S rDNA restriction profiles from an ARDRA of *P. gordonae* (g), *P. validus* (v), *P. polymyxa* (p), and *P. macquariensis* (m) strains obtained with restriction enzymes *HaeIII*, *DpnII*, *RsaI*, *BfaI*, and *MseI*. Lane M contained a molecular weight marker. (B) Normalized computer profiles of the *RsaI* restriction patterns of the 16S rDNAs of *P. gordonae* LMG 9817^T, LMG 14468, LMG 14469, LMG 14470, LMG 14663, LMG 14664, LMG 14665, LMG 14666, LMG 14667, LMG 14668, and LMG 14717, *P. validus* LMG 11161^T, LMG 14019, and LMG 14020, *P. polymyxa* LMG 6319^T, and *P. macquariensis* LMG 6935^T (strains LMG 6319^T and LMG 6935^T were used as outgroup organisms). Lane M contained a molecular weight marker.

labs), *BfaI* (New England Biolabs), or *MseI* (New England Biolabs). After 4 μ l of sample buffer (4 g of sucrose and 2.5 mg of bromophenol blue in 10 ml of TE buffer) was added, 20- μ l portions of the restriction digests were analyzed by 2% (wt/vol) Metaphor agarose (FMC BioProducts, Rockland, Maine) gel electrophoresis at 3V/cm for 130 min in 89 mM Tris–89 mM boric acid–2 mM EDTA (TBE) buffer (pH 8.0) containing 0.5 μ g of ethidium bromide per ml. After an

additional 20 min of staining with 1 μ g of ethidium bromide per ml in TBE buffer and 5 min of destaining with distilled water, the gels were photographed under UV illumination by using Polaroid type 665 positive/negative film. The negatives were scanned with a ScanJet IIp scanner (Hewlett-Packard) for normalization of the restriction patterns with the GelCompar software by using the profile of plasmid pBR322 digested with *AluI* (Pharmacia Biotech Benelux) as a reference

FIG. 2. Normalized computer profiles of PAGE protein patterns of *P. gordonae* and *P. validus* strains. The scale on the upper left indicates percent similarity. *P. polymyxa* and *P. larvae* were used as outgroup organisms. The irregular high-molecular-weight bands to the right of the arrow were omitted from the numerical analysis presented in a dendrogram based on the UPGMA linkage of correlation coefficients. Abbreviations: G, *P. gordonae*; V, *P. validus*; P, *P. polymyxa*; L, *P. larvae*.

profile. The five normalized restriction patterns obtained for each strain were assembled into a composite profile and analyzed by using the Dice similarity coefficient and the UPGMA clustering algorithm. Bands whose intensities were definitely lower than the intensities of immediately adjacent bands (probably because of microheterogeneity in the 16S rRNA alleles) were not included in this numerical analysis.

RAPD analysis. Random amplified polymorphic DNA (RAPD) analysis PCR were carried out with whole-cell lysates by using a single arbitrary primer. Approximately 5 µg of cells was obtained from colonies grown on nutrient agar for 24 to 36 h at 30°C. After the cells were suspended in 15 µl of sterile distilled water, the preparation was boiled for 4 to 8 min and centrifuged for 20 s at 10,000

× g. Then 10 µl of the resulting supernatant was added to 50 µl of a PCR mixture containing 1× Super Taq reaction buffer (10 mM Tris-HCl [pH 9.0], 50 mM KCl, 0.01% [wt/vol] gelatin, 1.5 mM MgCl₂, 0.1% Triton X-100), 10 nmol of each deoxynucleoside triphosphate (Pharmacia Biotech, St. Albans, United Kingdom), 1.5 U of Super Taq DNA polymerase (HT Biotechnology, Cambridge, United Kingdom), and 25 pmol of primer 42423 (5'-GGGCGTTGTCGGTGTTCATG-3'; Department of Biochemistry, University of Bristol, Bristol, United Kingdom) (20). The PCR were carried out with an OmniGene temperature cycler (model CM 220; Hybaid, Ltd., Teddington, United Kingdom) by using the following program: initial denaturation at 94°C for 3 min was followed by four cycles of intermediate product formation consisting of denaturation at 94°C for

FIG. 3. Dendrogram based on UPGMA linkage of Gower correlation coefficients for Py-MS data for *P. gordonae*, *P. validus*, *P. pulvificiens*, and *P. peoriae* strains.

45 s, annealing at 26°C for 2 min, and extension at 72°C for 2 min, and then the main amplification step (30 cycles consisting of 94°C for 45 s, 36°C for 1 min, and 72°C for 2 min) was followed by the final extension step at 72°C for 5 min. A 20- μ l portion of the PCR products and a 1-kb DNA ladder (Gibco BRL) which was used as a molecular weight marker were analyzed by 1% (wt/vol) agarose gel electrophoresis in TBE buffer containing 0.5 μ g of ethidium bromide per ml at 100 V for 1.5 to 3 h. The band patterns were photographed under UV illumination with Polaroid type 677 positive-image film. The photos were scanned with a ScanJet IIP scanner (Hewlett-Packard), and the scanned images were analyzed with the Gelmanager program (Biosystematica, Tavistock, United Kingdom) by using the Pearson product moment correlation coefficient and the UPGMA clustering algorithm.

Nucleotide sequence accession number. The 16S rDNA sequence of strain DSM 3037^T (T = type strain) has been deposited in the EMBL data library under accession number M77489.

RESULTS AND DISCUSSION

ARDRA. Bands whose intensities were definitely lower than the intensities of adjacent bands appeared in some restriction patterns (for example, the band at about 340 bp in the *P. gordonae* and *P. validus* patterns in Fig. 1B). These less pronounced bands were probably caused by additional restriction sites in a minority of the 16S rRNA alleles (12, 31). In a numerical analysis (data not shown) of the ARDRA patterns of *Bacillus* rRNA group 3 (1) strains, we found that all of the *P. gordonae* and *P. validus* strains clustered at levels of similarity of more than 86% and that the type strains of the two taxa were linked at a level of similarity of 95%. The amplified 16S rDNA restriction patterns of all 11 *P. gordonae* strains examined and the 3 *P. validus* strains examined (Fig. 1A and B) exhibited high levels of internal similarity compared with the patterns of two outgroup organisms, *Paenibacillus polymyxa* and *Paenibacillus macquariensis*.

Although with all methods that rely on multivariate analysis levels of similarity may vary with the kind and number of strains included, our ARDRA data strongly indicate that *P. gordonae* and *P. validus* should be united at the species level. The high level of rDNA restriction pattern similarity is supported by a high level of rDNA sequence similarity (99.8%) between the *P. gordonae* and *P. validus* type strains (2). Finally, restriction analysis of enzymatically amplified rDNAs, as applied in this study, has been used previously to delineate species (12, 14, 17, 28, 31).

In order to confirm the phylogenetic homogeneity indicated by the rDNA data, the strains were also examined in a polyphasic study in which we performed SDS-PAGE of whole-cell proteins, a gas chromatographic analysis of methylated fatty acids, a Py-MS analysis, a RAPD analysis, a biochemical and morphological analysis, and DNA-DNA binding studies.

SDS-PAGE. The PAGE (Fig. 2) data revealed that all of the *P. gordonae* and *P. validus* strains clustered at a similarity level of about 75% (data not shown). This rather low level of similarity was due to the irregular pattern of one or two dense protein bands in a zone at the top of the gel. It has been shown previously that the characteristics of such zones often mask the real levels of similarity between overall profiles (5). If the data for this zone (all bands with molecular weights of more than 1.4×10^5 in the protein profile) (Fig. 2) were not included in the numerical analysis, all of the *P. gordonae* and *P. validus* strains except *P. gordonae* LMG 9817^T clustered at levels of similarity of more than 90%, and *P. gordonae* LMG 9817^T was linked at a level of similarity of 84%. The latter value is still very high and definitely more than the levels of linkage to the nearest neighbors, *P. polymyxa* and *Paenibacillus larvae* (Fig. 2). Therefore, these results support the results based on rDNA data.

Fatty acid methyl esters. The fatty acid compositions of all strains (Table 2) were very similar. A comparison of the mean

TABLE 2. Fatty acid profiles of 11 *P. gordonae* strains and 3 *P. validus* strains as determined by a gas chromatographic analysis of their methyl esters

Taxon	Mean % (range)													Summed feature S ^a
	10:0	14:0 iso	14:0	15:0 iso	15:0 anteiso	15:0	16:1 ω 7C	16:0 iso	16:1 ω 11	16:0	Iso 17:1 ω 10C	17:0 iso	17:0 anteiso	
<i>P. gordonae</i>	0.9 (0.8-1.2)	3.0 (2.7-3.6)	1.8 (1.4-2.1)	13.9 (11.0-15.8)	51.5 (49.5-52.9)	0.7 (0.5-1.1)	2.5 (2.2-3.1)	6.1 (5.2-7.4)	5.8 (4.1-6.9)	4.9 (3.7-6.0)	1.0 (0.7-1.4)	3.3 (2.8-4.1)	3.9 (3.1-4.5)	0.6 (0.5-0.7)
<i>P. validus</i>	0.9 (0.7-1.3)	3.4 (3.1-3.9)	2.1 (1.8-2.5)	10.3 (9.7-11.3)	51.9 (50.6-52.7)	0.9 (0.8-1.1)	2.6 (2.5-2.7)	7.2 (6.9-7.5)	7.0 (6.7-7.6)	5.6 (5.4-5.6)	0.5 (0.0-0.8)	2.7 (2.2-3.5)	4.5 (3.9-5.0)	0.5 (0.0-0.8)

^a Summed feature 5 is 17:1 iso I and/or 17:1 anteiso B.

FIG. 4. RAPD patterns of *P. gordonae* and *P. validus* strains obtained with primer 42423. Lane 1 contained the 1-kb molecular weight ladder; lanes 2 to 5 contained *P. validus* LMG 14018, LMG 14019, LMG 14020, and LMG 11161^T, respectively; and lanes 6 to 13 contained *P. gordonae* LMG 9817^T, LMG 14717, LMG 14663, LMG 14664, LMG 14665, LMG 14666, LMG 14667, and LMG 14668, respectively.

compositions of the different strains of each taxon revealed that the qualitative patterns were the same and that there were only minor quantitative differences, as indicated by the minimum and maximum levels of each fatty acid. Furthermore, in

a numerical analysis (data not shown) all of the strains clustered below a Euclidian distance of 5, which confirmed that this group of strains was homogeneous and supported the hypothesis that these organisms are members of a single species.

Py-MS. The dendrogram in Fig. 3 shows the results of a numerical analysis of the Py-MS data for eight strains of *P. gordonae*, four strains of *P. validus*, two strains of *Paenibacillus pulvificiens*, and two strains of *Paenibacillus peoriae* (members of the latter two species were used as outgroups). All of the *P. gordonae* and *P. validus* strains clustered at high levels of similarity (range, 89 to 99.4%), while the level of similarity between the type strains was 92%. Similarly, the two-dimensional canonical variates analysis plots of the first and second vectors and the first and third vectors in the Py-MS analysis (data not shown) clearly revealed the close affinities of the members of the two species, as indicated by the overlapping confidence limits around group means determined by using the chi-square procedure (16, 19).

RAPD patterns. The band patterns obtained in the RAPD PCR analysis of *P. gordonae* and *P. validus* strains were very similar (Fig. 4). When we performed a numerical analysis of the PCR data, the strains formed two mixed clusters linked at similarity levels of more than 90%. Within one of the clusters the type strains were linked at a similarity level of 97%.

Biochemical, physiological, and morphological characteristics. In our numerical analysis of API data and morphological observations, the *P. validus* and *P. gordonae* strains were recovered in one diffuse cluster with levels of similarity ranging from 67.5 to 95% when negative matches in API data were ignored and from 80 to 97.5% when negative matches were included. Some of the levels of similarity between strains assigned to the two different species were greater than the levels

FIG. 5. Photomicrograph of sporangia and vegetative cells of *P. validus* LMG 11161^T viewed by phase-contrast microscopy. The sporangia are irregular in shape and greatly swollen with subterminal to terminal ellipsoidal spores. Bar = 2 μ m.

TABLE 3. Phenotypic characteristics of *P. validus* and *P. gordonae* strains

Characteristic ^a	<i>P. validus</i>	<i>P. gordonae</i>
Motility	+	+
Ellipsoidal spores	+	+
Subterminal spores	+	+
Swollen sporangia	+	+
Esculin hydrolysis	+	+
Anaerobic growth	—	—
Arginine dihydrolase activity	—	—
Citrate utilization	—	—
Indole production	—	—
Gelatin liquefaction	—	—
Nitrate reduction	v	v
ONPG hydrolysis ^c	—	—
Voges-Proskauer reaction	v	v
Acid produced from ^d :		
D-Fructose, galactose, D-glucose, glycerol, <i>meso</i> -inositol, maltose, mannitol, methyl-D-glucoside, ribose, sucrose, D-trehalose, D-turanose, and D-xylose	+	+
Glycogen, D-mannose, D-melibiose, D-raffinose, and starch	v	v
D-Arabitol	—	v
D-Cellobiose	—	v
Dulcitol	v	+
Inulin	—	v
Lactose	—	v
D-Sorbitol	—	v
Xylitol	—	v

^a All characteristics except anaerobic growth and microscopic properties were determined by using API 20E and API 50 CHB System tests; anaerobic growth was tested for as described in the text.

^b +, 90% or more of the strains react positively; —, 90% or more of the strains react negatively; v, less than 90% of the strains react positively or negatively for this characteristic.

^c ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

^d Acid was not produced from adonitol, amygdalin, D- and L-arabinose, L-arabitol, arbutin, erythritol, D- and L-fucose, gentiobiose, gluconate, 2-keto-D-gluconate, 5-keto-D-gluconate, D-lyxose, D-melezitose, methyl-D-mannoside, methyl-xyloside, *N*-acetylglucosamine, L-rhamnose, salicin, L-sorbose, and L-xylose.

of similarity between strains assigned to the same species. Although the type strains of the two species could be distinguished by API tests, overall the two species exhibited similar patterns of reactions (Table 3), had similar microscopic morphologies (Fig. 5), and could be distinguished from related *Paenibacillus* species (Table 4). None of the strains exhibited anaerobic growth with any of the three methods used to test this characteristic. Thus, in our hands *P. gordonae* and *P. validus* behaved as strict aerobes.

DNA-DNA binding studies. A level of DNA binding (Table 5) of 89% between the type strains of *P. gordonae* and *P. validus* and levels of binding of at least 89 and 92% within the *P. gordonae* group and the *P. validus* group, respectively, confirmed that all of the strains of these two taxa should be assigned to the same species (34).

Conclusion. When Smith et al. (30) and Gordon et al. (10) reclassified *B. validus* as *B. circulans*, they recognized the marked heterogeneity of the latter species, but found that division of the group was impossible as the many combinations of variant properties did not allow recognition of discrete groups, and they wished to emphasize the similarities of their strains rather than the differences between them. Nakamura and Swezey (24) considered *B. validus* unrelated to *B. circulans* sensu stricto on the basis of the different G+C contents of the DNAs of these taxa. Nakamura (23) revived the name *B. va-*

TABLE 4. Characteristics that differentiate *P. validus* from related *Paenibacillus* species

Characteristic ^a	<i>P. validus</i>	<i>P. alvei</i>	<i>P. larvae</i>	<i>P. pulvifaciens</i>
Anaerobic growth	—	+	+	+
Arginine dihydrolase activity	—	—	—	+
Indole production	—	+	—	—
Gelatin liquefaction	—	+	+	+
ONPG hydrolysis ^b	—	+	—	—
Acid produced from:				
D-Xylose	+	—	—	—
Adonitol	—	+	—	—
D-Fructose	+	—	—	—
Mannitol	+	—	—	+
<i>N</i> -Acetylglucosamine	—	+	+	+
Maltose	+	+	—	—

^a All characteristics except anaerobic growth were determined by using API 20E and API 50 CHB System tests; *P. validus* strains were tested for anaerobic growth as described in the text, and data for other species were obtained from references 4 and 10.

^b ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

lidus on the basis of DNA-DNA homology data and found a pattern of phenotypic characteristics which correlated with the characteristics of the *B. validus* DNA relatedness group. In our polyphasic analysis, we were not able to separate the strains classified by Pichinoty et al. as *B. gordonae* (25) and the *B. validus* strains by using morphological and biochemical characteristics and a range of chemotaxonomic and genomic characteristics. We concluded that the name *P. gordonae* is a synonym of *P. validus*, and an emended description of *P. validus* based on our data and on data from Pichinoty et al. (25), Nakamura (23), and Ash et al. (2) is given below.

Emended description of *Paenibacillus validus*. *Paenibacillus validus* (va'li.dus. L. adj. *validus*, strong, vigorous). Motile, round-ended rods that are 0.5 to 1.0 μm wide by 5.0 to 7.0 μm long occur singly and in pairs. Sporangia are irregular in shape and often greatly swollen by subterminal to terminal ellipsoidal spores which may lie laterally and/or at an angle in the swollen part and which may sometimes be in the unswollen part of the sporangium.

Isolated from soil. Strictly aerobic and catalase positive. The organism grows on routine media such as nutrient agar, producing greyish white, translucent, smooth, circular, entire colonies that are 1 to 2 mm in diameter after 3 days at 30°C; colonies on nutrient agar have a loose butyrous consistency and become tenacious with age. The optimum growth temperature range is 28 to 35°C, the maximum temperature is 42 to 50°C, and the minimum temperature is 5 to 10°C.

Gelatin and *o*-nitrophenyl-β-D-galactopyranoside are not hydrolyzed, citrate is not utilized, and arginine dihydrolase, hydrogen sulfide, urease, and indole are not produced as determined by API 20E strip tests; weak Voges-Proskauer and nitrate reduction reactions are detected in some strains.

In API CH 50 galleries when API CHB suspension medium is used, acid is produced from the following substrates: D-fructose, D-galactose, D-glucose, glycerol, *meso*-inositol, maltose, D-mannitol, methyl-D-glucoside, ribose, sucrose, D-trehalose, D-turanose, and D-xylose. Acid is not produced from adonitol, amygdalin, D- and L-arabinose, L-arabitol, arbutin, erythritol, D- and L-fucose, gentiobiose, D-gluconate, 2-keto-D-gluconate, 5-keto-D-gluconate, D-lyxose, D-melezitose, methyl-D-mannoside, methyl-D-xyloside, *N*-acetylglucosamine, L-rhamnose, salicin, L-sorbose, and L-xylose. Different strains give different results with D-cellobiose, dulcitol, glycogen, inulin,

TABLE 5. G+C contents of and DNA binding data for *Paenibacillus* strains

Strain	G+C content (mol%)	% DNA binding ^a							
		<i>P. gordonae</i> LMG 9817 ^T	<i>P. gordonae</i> LMG 14666	<i>P. gordonae</i> LMG 14667	<i>P. gordonae</i> LMG 14668	<i>P. validus</i> LMG 11161 ^T	<i>P. validus</i> LMG 14019	<i>P. validus</i> LMG 14020	<i>P. larvae</i> LMG 14426
<i>P. gordonae</i> LMG 9817 ^T	52.3	100							
<i>P. gordonae</i> LMG 14666		94	100						
<i>P. gordonae</i> LMG 14667		94	97	100					
<i>P. gordonae</i> LMG 14668		89		96	100				
<i>P. validus</i> LMG 11161 ^T	50.9	89				100			
<i>P. validus</i> LMG 14019						96	100		
<i>P. validus</i> LMG 14020							92	100	
<i>P. larvae</i> LMG 14426	42.2		17						100
<i>P. larvae</i> LMG 9820 ^T	42.3						4		100

^a Data are the means of the values from at least two experiments. The *P. larvae* strains were used as outgroup organisms.

lactose, D-mannose, D-arabitol, D-melibiose, D-raffinose, D-sorbitol, starch, and xylitol.

The following fatty acids are present: 14:0 iso, 14:0, 15:0 iso, 15:0 anteiso, 16:1 ω7C, 16:0 iso, 16:1 ω11, 16:0, 17:0 iso, and 17:0 anteiso.

The average G+C content is 51 to 52 mol% (as determined by the renaturation method) or 54 to 55 mol% (as determined by the buoyant density method). The level of internal DNA relatedness of the strains is more than 70%. ARDRA tests in which *Hae*III, *Rsa*I, *Dpn*II, *Bfa*I, and *Mse*I are used produce similar patterns. See Materials and Methods for 16S rDNA sequence data.

The type strain is strain LMG 11161 (= ATCC 43897 = B1397 = DSM 3037 = NRRL NRS-1000), and this strain has the characteristics of the species described above. This strain is positive for the Voges-Proskauer test, nitrate reduction, and acid production from glycogen, D-mannose, D-melibiose, D-raffinose, and starch and negative for acid production from D-cellobiose, dulcitol, inulin, lactose, D-sorbitol, and xylitol.

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