Bacillus acidicola sp. nov., a novel mesophilic, acidophilic species isolated from acidic *Sphagnum* peat bogs in Wisconsin

Richard A. Albert,^{1†} Julieta Archambault,^{1‡} Ramón Rosselló-Mora,² Brian J. Tindall³ and Mike Matheny^{1§}

Correspondence

Richard A. Albert
richard.albert@marquette.edu

¹Semco BioScience, 630 East Keefe, Milwaukee, WI 53212, USA

²Grup d'Oceanografia Interdisciplinari, Institut Mediterrani d'Estudis Avançats, E-07190 Esporles, Mallorca, Spain

³DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig, Germany

A mesophilic, acidophilic, spore-forming bacterium, strain 105-2^T, was isolated from an acidic *Sphagnum* peat bog in Wisconsin, USA. Strain 105-2^T has 16S rRNA gene sequence similarity to *Bacillus sporothermodurans* DSM 10599^T and *Bacillus oleronius* DSM 9356^T of 97.4 and 97.8 %, respectively. The primary lipoquinone is MK-7 and the major fatty acids are 15 : 0 iso, 15 : 0 anteiso and 17 : 0 anteiso. The predominant polar lipids were found to be diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and a glycolipid. The DNA G + C content was found to be 43.2 mol%. The phenotypic, chemotaxonomic and molecular analyses identified strain 105-2^T as a novel *Bacillus* species, for which the name *Bacillus acidicola* is proposed. The type strain is 105-2^T (= DSM 14745^T = ATCC BAA-366^T = NRRL B-23453^T).

The genus *Bacillus* contains Gram-positive, aerobic or facultatively anaerobic bacteria that are able to produce bacterial endospores. Members are considered ubiquitous because they have been isolated from a wide variety of aquatic and terrestrial environments, ranging from sewage sludge (Demharter & Hensel, 1989), ocean sediments (Rüger *et al.*, 2000) and saline water (Smibert & Krieg, 1994) to desert soils (Roberts *et al.*, 1994).

Gram-positive bacterial endospore formers with the ability to grow at acidic pH values and which possess ω -alicyclic fatty acids are placed in the genus *Alicyclobacillus*. The pH growth range varies with species, from 2–6 to 3.0–5.5 (Wisotzkey *et al.*, 1992). Members of the genus *Bacillus* that

are reported to have the ability to grow at acidic pH values (< 4.6) are *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus subtilis* (Hanlin, 1998).

In this paper, we report the isolation of an aerobic, mesophilic, endospore-forming organism that grows at pH values from 3.5 to 7.0. On the basis of phenotypic characteristics, chemotaxonomic data and genotypic data, the isolate is a previously undescribed species of the genus *Bacillus* and we propose the name *Bacillus acidicola* sp. nov.

Soil and water samples were collected from acidic peat bogs near Necedah, WI, USA. Diluted soil samples and water samples were heat-shocked at 80 °C for 20 min. Each heat-shocked sample (0.5 ml) was added to 10 ml plate-count broth (Difco) adjusted to pH 4.5 by using a citrate/phosphate buffer (0.05 M citrate/0.1 M Na₂HPO₄) (Breznak & Costilow, 1994). Inoculated flasks were incubated at 32 or 37 °C with shaking aeration. After 24 h incubation, 0.1–100 ml culture liquid was transferred to 10 ml plate-count broth at pH 4.5 and incubated at 32 or 37 °C with shaking aeration. After 48 h incubation, samples were streaked onto plate-count agar (PCA; Difco) that had been adjusted to a pH of 4.5 by using citrate/phosphate buffer. Plates were incubated at 37 °C for 48 h. Cultures were purified by subculturing. Three strains, designated 105-2^T, 105-9 and 102-2, were isolated from different acidic *Sphagnum* peat-bog samples.

[†]Present address: Water Quality Center, Marquette University, Civil & Environmental Engineering, PO Box 1881, Milwaukee, WI 53201, USA.

[‡]Present address: Genesis Technologies International, 696 Wimer Industrial Way, Lawrenceville, GA 30045, USA.

[§]Present address: Envera, 5026 W. Willow Road, Mequon, WI 53092, USA.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 105-2^T is AF547209.

Phase-contrast micrographs of strain 105-2^T, a photograph showing the colony morphology of strain 102-2 and a thin-layer chromatogram of the type strain's polar lipids are available as supplementary figures in IJSEM Online.

Because the isolates grew best at acidic pH values, standard tests for the characterization of members of the genus *Bacillus* were modified as described below. In addition, it was observed that growth of this organism was improved in the presence of 10–25 % soil extract. Thus, most test media were supplemented with soil extract to enhance growth. The soil extract was prepared as described by Gordon *et al.* (1973). The base medium (plate-count broth) for the determination of the temperature growth range, growth at various NaCl concentrations and the pH growth profile was as follows: tryptone (Difco), 5.0 g l⁻¹; yeast extract (Difco), 2.5 g l⁻¹; D-glucose (Sigma), 1.0 g l⁻¹; and, where used, agar (Difco), 1.5 g l⁻¹. The base formulation was often supplemented with 10–25 % soil extract. A citrate/phosphate buffer (Breznak & Costilow, 1994) was used to control or adjust the pH. All tests were incubated at 37 °C, except for the temperature-profile experiments. For all tests performed in liquid, a visual increase in turbidity was assumed to indicate growth.

For phenotypic characterizations, strains 105-2^T, 105-9 and 102-2 were grown for 24–48 h on acidic PCA or standard PCA, which was used to prepare a heavy cell suspension in citrate/phosphate buffer (pH 4.7–5.0). Tests in liquid media were then inoculated with one to three drops of cell suspension, whereas tests performed on solid media were streaked from the cell suspension. Growth at 2, 5, 7 and 10 % (w/w) NaCl was determined in the base medium supplemented with soil extract (pH 4.5) under aerated conditions for 1–3 days. To determine the pH range for growth, the base medium was prepared in citrate/phosphate buffer for pH values from 4.25 to 6.5. At pH values of 4.0 and below, the base medium was buffered with NaH₂PO₄ (0.1 M) and supplemented with 10 % soil extract. The pH of the test medium was confirmed after autoclaving. Tubes were incubated for 24–72 h. At pH values greater than 6.5, it was observed that phosphate was inhibitory. Therefore, at pH values greater than 6.5, a non-phosphate-buffered agar-based medium supplemented with 10 % soil extract was used. The pH was adjusted with HCl or NaOH prior to autoclaving. After autoclaving, the pH was rechecked and recorded as the pH of the growth medium. Plates were examined visually for growth after 72 h incubation.

Motility was tested in base medium (pH 4.5) containing 0.4 % agar (Holt & Krieg, 1994) and incubated for 24–48 h.

The temperature range for growth was tested in base medium (pH 4.5) supplemented with soil extract to 10 %. Tubes were incubated for up to 10 days.

Utilization of carbohydrates was determined by using procedures described by Gordon *et al.* (1973), with modifications. The modifications included the addition of sterile MgSO₄·7H₂O, after autoclaving, from a 100-fold-concentrated solution, adjusting the initial pH to 6.1–6.3, and adding agar and yeast extract to 1.5 and 0.05 %, respectively. Plates were incubated for up to 7 days. The presence of growth and a pH change (indicated by a colour change, from purple to yellow, of the pH indicator

bromocresol purple) was used to detect acid production from the substrate.

Indole production and gelatin hydrolysis were determined by using the method of Smibert & Krieg (1994), except for the yeast-extract (0.05 %) and soil-extract (25 %) supplementation. The indole test was performed by using Kovács' indole reagent (BBL) after 4 days incubation, whereas the gelatin-hydrolysis test was performed after 7 and 10 days aerated incubation. Casein hydrolysis was determined by the procedure of Smibert & Krieg (1994), except that the medium was acidified by using a citrate/phosphate buffer.

Lysine decarboxylase and phenylalanine deaminase activities were determined by using the method of Gordon *et al.* (1973), except that the medium pH was adjusted to 5.8 and 5.2 and supplemented with 20 and 25 % soil extract, respectively. Examinations were made periodically during 7 days incubation. Utilization of citrate and propionate was determined according to Gordon *et al.* (1973), with modifications. Yeast extract and soil extract were added (to 0.05 and 20 %, respectively) to the propionate medium, whereas yeast extract only (0.05 %) was added to the citrate medium. The initial pH values of the citrate and propionate media were adjusted to 5.5 and 5.6, respectively. Citrate utilization was observed after 1 and 2 days incubation, whilst propionate utilization was examined after 4 days incubation.

Nitrate reduction was examined according to the method of Gordon *et al.* (1973), with modifications. Yeast extract was added to 0.05 %, soil extract was added to 25 % and the initial pH was adjusted to 5.0. Analysis was performed after 4 days incubation.

Catalase and oxidase tests were performed by using 24 h cultures grown on acidified PCA (pH 4.5) supplemented with soil extract (to 20 %). The catalase test was performed by placing a small amount of the culture on a slide and adding 3 % H₂O₂. The presence of gas bubbles indicated a positive reaction. The oxidase test was performed by using an Oxy-Swab (Remel), according to the manufacturer's recommendations except that the micro-organism was grown on acidified PCA. Gram stains were performed on the same cultures. The Voges–Proskauer reaction was performed by using the procedure described by Gordon *et al.* (1973), except that there was supplementation with yeast extract (0.05 %) and soil extract (20–25 %) at an initial pH of 4.7–4.8. The test was performed in 150 × 18 mm screw-cap test tubes (5 ml per tube) and read after 1, 3 and 7 days.

Respiratory lipoquinones and polar lipids were extracted from freeze-dried cell material (100 mg) by using a two-stage method (Tindall, 1990a, b). Respiratory lipoquinones were separated by TLC as described previously (Tindall, 1990a); UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by reversed-phase HPLC. Polar lipids were separated by two-dimensional silica-gel TLC as described

previously (Tindall, 1990a): total lipid material and specific functional groups were detected by using dodecamolybdo-phosphoric acid (total lipids), Zinzadze reagent (phosphate) (Tindall, 1990a), ninhydrin (free amino groups), the periodic acid–Schiff reaction (α -glycols), Dragendorff's (quaternary nitrogen) (Tindall, 1990a) and anisaldehyde/sulphuric acid (glycolipids), as described previously (Tindall, 1990a, b).

The cellular fatty acid profile was determined by using a modified procedure because strains 105-2^T, 105-9 and 102-2 did not grow in standard growth conditions [tryptic soy broth agar (Difco) at 37 °C for 24 h]. Thus, the strains were grown on tryptic soy broth agar prepared by using KH₂PO₄ (pH 6.2). After growth, bacteria were saponified and the liberated fatty acids were methylated and analysed by capillary GC (Hewlett Packard model 5890A GC) by the Sherlock system (MIDI), according to the manufacturer's instructions.

Genomic DNA was prepared according to the method of Marmur (1961). The G+C content was analysed by hydrolysis of DNA to nucleosides and was quantified by HPLC using the method of Peña *et al.* (2005). The relative G+C contents were calculated by using the following standard DNAs: *Staphylococcus aureus* (strain CECT 239, 34 mol%; Holländer & Pohl, 1980); *Shewanella putrefaciens* (CECT 5346^T, 43.9 mol%; Owen *et al.*, 1978); *Escherichia coli* B (strain CECT 101, 52 mol%; Holländer & Pohl, 1980); *Pseudomonas aeruginosa* (strain DSM 50071^T; 67.2 mol%; Palleroni, 1984); and *Agrococcus jenensis* (strain DSM 9580^T; 74 mol%; Groth *et al.*, 1996). DNA–DNA hybridization experiments were carried out by using a microtitre-plate non-radioactive method (Ziemke *et al.*, 1998).

The isolation of DNA and generation of PCR products for 16S rRNA were performed by using the MicroSeq full-gene protocol, which uses dRhodamine-labelled dye terminators yielding double-stranded sequence data. MicroSeq (Applied Biosystems) contains a PCR and sequencing module, bacterial identification and analysis software and a 16S rRNA gene sequence library. Bacterial genomic DNA isolation and PCR amplification of the 1548 bp of the 16S rRNA gene were performed according to the manufacturer's instructions. Double-stranded sequence analysis of the first 1548 bp was completed by using an ABI Prism 377 or 3100 Avant genetic analyser (Applied Biosystems). Sequencing reactions were run on a 4.5 % Long Ranger (FMC BioProducts) and a 33 % urea electrophoresis gel by using an ABI Prism 377 sequencer (Applied Biosystems). DNA sequence data were analysed and assembled with AutoAssembler software (Applied Biosystems). Bacterial identification based on 16S rRNA gene sequence data were assigned by using MicroSeq microbial identification and analysis software (Applied Biosystems).

Complete 16S rRNA gene sequences were compared initially with reference sequences in GenBank (<http://www.ncbi.nlm.nih.gov>) by using BLAST (Altschul *et al.*, 1997).

New 16S rRNA gene sequences were then added to an alignment of about 50 000 homologous bacterial 16S rRNA primary structures as implemented in the ARB software package, and corresponding to the released database available at <http://www.arb-home.de> (Ludwig *et al.*, 2004). Phylogenetic analyses were performed by the use of different datasets and by the use of the three different algorithms – neighbour joining, maximum parsimony and maximum likelihood – as implemented in the ARB software package. Finally, a consensus tree was achieved after evaluation of the resulting trees when using different algorithms and datasets as recommended by Ludwig *et al.* (1998).

Three strains, designated 105-2^T, 105-9 and 102-2, were isolated from different acidic *Sphagnum* peat bog samples collected near Necedah, WI, USA. All strains were catalase-positive, oxidase-negative, Gram-positive, endospore-forming rods.

The cell shape of the type strain, 105-2^T, was affected significantly by culture conditions. On solid medium (acidified PCA supplemented with soil extract) after 24 h incubation, cells were rod-shaped [see Supplementary Fig. S1(a), available in IJSEM Online]. In liquid medium (acidified plate-count broth supplemented with soil extract) under aerated conditions, cells displayed filamentous cellular morphology after 24 h growth [see Supplementary Fig. S1(b)]. Ellipsoidal endospores were located centrally in swollen sporangia [see Supplementary Fig. S1(c)]. Colony size was highly variable. After 48 h growth on PCA at 37 °C, the diameters of colonies of strain 105-2^T ranged between 0.6 and 2.1 mm. The colony diameter of strain 102-2, grown under the same conditions, ranged from 0.6 to 5.2 mm (see Supplementary Fig. S2, available in IJSEM Online).

The results based on the full 16S rRNA gene sequence showed that strain 105-2^T is related to *Bacillus oleronius* DSM 9356^T and *Bacillus sporothermodurans* DSM 10599^T (Fig. 1). The full gene sequence similarity between strain 105-2^T and *B. oleronius* DSM 9356^T was 97.8 %, whilst that between strain 105-2^T and *B. sporothermodurans* DSM 10599^T was 97.4 %. All other values with respect to *Bacillus* species with validly published names were lower (<96 % 16S rRNA gene sequence similarity).

The phenotypic characteristics and DNA G+C contents of strains 105-2^T, 105-9 and 102-2 and related species (*B. oleronius* DSM 9356^T and *B. sporothermodurans* DSM 10599^T) are summarized in Table 1. Examination of the information in Table 1 indicates that there are significant phenotypic differences between strain 105-2^T and *B. oleronius* DSM 9356^T and *B. sporothermodurans* DSM 10599^T.

Given the relatively high 16S rRNA gene sequence similarity values, DNA–DNA hybridization was carried out between the three novel isolates and *B. oleronius* DSM 9356^T and *B. sporothermodurans* DSM 10599^T. The results are

Fig. 1. 16S rRNA gene sequence phylogenetic reconstruction, based on a neighbour-joining algorithm, showing the affiliation of *B. acidicola* to its closest relatives. The tree topology was reconstructed by comparing the results of several treeing approaches based on neighbour-joining, maximum-parsimony and maximum-likelihood algorithms with different datasets, as implemented in the ARB software package (Ludwig *et al.*, 2004). The tree topology has also been examined by using alignment masks that allow only conserved homologous positions for its calculation. In all cases, topologies were identical, so neither multifurcation (Ludwig *et al.*, 1998) nor bootstrapping was necessary. Bar, 2% sequence dissimilarity.

summarized in Table 1. DNA–DNA hybridization values indicated that strains 105-2^T, 105-9 and 102-2 formed an independent group with values that were always higher than 63%. In contrast, DNA–DNA hybridization values between the novel isolates and *B. oleronius* DSM 9356^T and *B. sporothermodurans* DSM 10599^T were always below 42%. These values, along with the similarity in polar lipids and physiological characteristics (Table 1), are consistent with placement of strains 105-2^T, 105-9 and 102-2 in a single species (Rosselló-Mora & Amann, 2001) distinct from *B. oleronius* DSM 9356^T and *B. sporothermodurans* DSM 10599^T.

The DNA G + C contents of strains 105-2^T, 105-9 and 102-2 were 43.2, 43.2 and 42.3 mol%, respectively.

The polar lipids of strains 105-2^T, 105-9 and 102-2 were essentially similar, showing only slight quantitative differences; the polar lipid pattern for strain 105-2^T is available as Supplementary Fig. S3 in IJSEM Online. Three major phospholipids and a single glycolipid were present. A number of minor components were also detected, but they could not be identified unambiguously. Very little work has been published in the past few decades on the polar lipid composition of bacilli, which makes it difficult to put the present work into perspective. The most comprehensive review of the literature is by O'Leary & Wilkinson (1988).

The cellular fatty acid profiles of the three strains are presented in Table 2. Three fatty acids, 15:0 iso, 15:0 anteiso and 17:0 anteiso, were the most common cellular fatty

acids, comprising approximately 90% of the cellular fatty acids extracted. Branched fatty acids, 14- to 17-carbon iso and anteiso series, are typically the major fatty acids found in *Bacillus* cell membranes (Kämpfer, 1994; Kaneda, 1977). Whilst the fatty acids alone do not give good differentiation within the bacilli, some major groups may be discerned. However, taken alone, these do not always provide unambiguous differentiation to genus level.

The major lipoquinone present in strains 105-2^T and 105-9 was MK-7. Strain 102-2 was unusual in that it produced almost equal amounts of MK-7, MK-6, MK-5, MK-4 and MK-3, indicating that the regulation of the biosynthesis of the isoprenoid side chain may be defective.

Given the sparse amount of data on the chemical composition of bacilli, the significance of the present data cannot be evaluated fully. Comparison of the lipid patterns with those of members of the genera *Gracilibacillus* and *Salibacillus* published by Wainø *et al.* (1999) reveals clear differences. In other cases, the *R_f* values of polar lipids reported in the literature cannot be compared directly, although it is evident that there is a significant degree of chemical diversity within this group of organisms, which were previously classified predominantly on the basis of morphological criteria.

On the basis of all the data obtained, we conclude that strains 105-2^T, 105-9 and 102-2 belong to a novel mesophilic, acidophilic *Bacillus* species, for which the name *Bacillus acidicola* sp. nov. is proposed.

Table 1. Differentiating characteristics of *B. acidicola*, *B. oleronius* and *B. sporothermodurans*

Strains: 1, *B. acidicola* strains 105-2^T, 105-9 and 102-2; 2, *B. oleronius* DSM 9356^T (data from Kuhnigk *et al.*, 1995); 3, *B. sporothermodurans* DSM 10599^T (data from Pettersson *et al.*, 1996). Symbols: +, positive; −, negative; (+), weakly positive; NK, information not known; v, variable reaction; v*, strains 105-2^T and 102-2 reacted positively; +*, only strains 105-2^T and 102-2 were tested; 43·2*, G+C content (mol%) for strains 105-2^T and 105-9 (that for strain 102-2 was 42·3 mol%). All of the species are motile, catalase-positive and negative for anaerobic growth, growth in 10% NaCl and nitrate reduction.

Characteristic	1	2	3
Temp. growth range (°C)	15–45	NK	20–45/50
pH growth range	3·5–7·0	NK	NK
Spore shape	Ellipsoidal	Oval	Ellipsoidal
Spore location	Central	Subterminal	Terminal
Sporangia swollen	+	+	−
Oxidase	−	NK	+
Growth in NaCl at:			
2%	+	NK	+
5%	−	+	v
7%	−	+	NK
Gelatin hydrolysis	−	+	−
Casein hydrolysis	−	−	(+)
Indole production	−	−	NK
Voges–Proskauer	−	+	−
Lysine decarboxylase	+	−	NK
Phenylalanine deaminase	−	−	NK
Assimilation of:			
Propionate	−	−	NK
Citrate	+	−	+
Acid production from:			
D-Glucose	+	+	−
D-Mannitol	+	−	−
D-Xylose	+	−	−
L-Sorbose	−	−	NK
D-Sorbitol	−	−	NK
D-Lactose	v*	−	−
L-Rhamnose	−	−	NK
D-Glycerol	+	(+)	NK
Sucrose	+	−	NK
D-Cellobiose	+	+	−
D-Ribose	+	(+)	NK
D-Mannose	+	−	−
D-Maltose	+	(+)	NK
D-Fructose	+	+	−
D-Arabinose	−	−	NK
L-Arabinose	−	−	NK
D-Galactose	+*	−	−
G+C content (mol%)	43·2*	35	36
DNA–DNA hybridization (%):			
DSM 14745 ^T labelled†	100–63‡	32	35
Strain 105-9 labelled†	100–84§	39	42

Table 1. cont.

†Standard DNAs double-labelled with biotin and digoxigenin to hybridize against non-labelled DNA.

‡Hybridization was 100, 79 and 63% with strains 105-2^T, 105-9 and 102-2, respectively.

§Hybridization was 100, 100 and 84% with strains 105-2^T, 105-9 and 102-2, respectively.

Description of *Bacillus acidicola* sp. nov.

Bacillus acidicola (a.ci.di'co.la. N.L. n. *acidum* an acid; L. suff. *-cola* an inhabitant of a place, a resident; N.L. masc. n. *acidicola* an inhabitant of acidic environments).

Cells are rod-shaped, 3·1–5·9 µm in length and 1·0–1·6 µm wide and occur singly or in chains. In liquid culture, cells can form filamentous rods that are 1·0–1·3 µm wide. Colonies on PCA after 48 h incubation are 0·6–2·1 mm in diameter. Colonies are smooth, shiny, circular and entire with a slight yellow tint. The temperature and pH ranges for growth are 15–45 °C and 3·5–7·0, respectively. Cells are Gram-positive. Other characteristics are listed in Table 1. The cellular fatty acids are listed in Table 2. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. A single glycolipid is also detected. MK-7 is the primary lipoquinone of strains 105-2^T and 105-9. Strain 102-2 produces almost equal amounts of MK-7, MK-6, MK-5, MK-4 and MK-3. The DNA G+C content for strains 105-2^T and 105-9 is 43·2 mol%, whilst that for strain 102-2 is 42·3 mol%.

The organism was isolated from acidic *Sphagnum* peat bogs in Wisconsin, USA. The type strain is 105-2^T (=DSM 14745^T = ATCC BAA-366^T = NRRL B-23453^T).

Table 2. Major cellular fatty acids of *B. acidicola* strains 105-2^T, 105-9 and 102-2

Strains were grown on tryptic soy broth agar (pH 6·2), prepared by using KH₂PO₄, for 24 h at 37 °C.

Fatty acid	Content (%) in strain:		
	105-2 ^T	105-9	102-2
14:0	0·91	1·46	0·87
15:0 iso	57·86	59·09	53·92
15:0 anteiso	24·30	21·19	28·58
16:1ω11c	0·69	—	—
16:0 iso	—	0·79	0·83
16:0	0·83	0·97	—
17:1 iso I/17:1 anteiso B	1·95	1·51	1·95
iso 17:1ω10c	1·67	1·46	1·27
17:0 iso	3·33	4·71	3·28
17:0 anteiso	8·47	8·83	9·31

Acknowledgements

We would like to acknowledge the excellent technical assistance of Amanda Hansen and Billie Samson. In addition, Maria Valens is acknowledged for her assistance with DNA G+C content and DNA–DNA hybridization analyses. Cellular fatty acid profiling and 16S rRNA gene sequencing were performed by Accugenix (Newark, DE, USA). Micrographs were taken with the assistance of the Microscopy Center at the University of Wisconsin at Milwaukee.

References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Breznak, J. A. & Costilow, R. N. (1994). Physicochemical factors in growth. In *Methods for General and Molecular Bacteriology*, pp. 137–154. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Demharter, W. & Hensel, R. (1989). *Bacillus thermocloacae* sp. nov., a new thermophilic species from sewage sludge. *Syst Appl Microbiol* **11**, 272–276.
- Gordon, R. E., Haynes, W. & Pang, C. H.-N. (1973). *The Genus Bacillus* (Agriculture Handbook no. 427). Washington, DC: United States Department of Agriculture.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Hanlin, J. H. (1998). Spoilage of acidic products by *Bacillus* species. *Dairy Food Environ Sanit* **18**, 655–659.
- Hölländer, R. & Pohl, S. (1980). Deoxyribonucleic acid base composition of bacteria. *Zentbl Bakteriol A* **246**, 236–273.
- Holt, J. G. & Krieg, N. R. (1994). Enrichment and isolation. In *Methods for General and Molecular Bacteriology*, pp. 179–215. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Kämpfer, P. (1994). Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* **17**, 86–98.
- Kaneda, T. (1977). Fatty acids of the genus *Bacillus*: an example of branched-chain preference. *Bacteriol Rev* **41**, 391–418.
- Kuhnigk, T., Borst, E.-M., Breunig, A., König, H., Collins, M. D., Hutson, R. A. & Kämpfer, P. (1995). *Bacillus oleronius* sp. nov., a member of the hindgut flora of the termite *Reticulitermes santonensis* (Feytaud). *Can J Microbiol* **41**, 699–706.
- Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M. & Schleifer, K.-H. (1998). Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* **19**, 554–568.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- O'Leary, W. M. & Wilkinson, S. G. (1988). Gram-positive bacteria. In *Microbial Lipids*, pp. 117–201. Edited by C. Ratledge & S. G. Wilkinson. London: Academic Press.
- Owen, R. J., Legros, R. M. & Lapage, S. P. (1978). Base composition, size and sequence similarities of genome deoxyribonucleic acids from clinical isolates of *Pseudomonas putrefaciens*. *J Gen Microbiol* **104**, 127–138.
- Palleroni, N. J. (1984). Family *Pseudomonadaceae*. In *Bergey's Manual of Systematic Bacteriology*, pp. 141–219. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Peña, A., Valens, M., Santos, F., Buczolits, S., Antón, J., Kämpfer, P., Busse, H.-J., Amann, R. & Rosselló-Mora, R. (2005). Intraspecific comparative analysis of the species *Salinibacter ruber*. *Extremophiles* **9**, 151–161.
- Pettersson, B., Lembke, F., Hammer, P., Stackebrandt, E. & Priest, F. G. (1996). *Bacillus sporothermodurans*, a new species producing highly heat-resistant endospores. *Int J Syst Bacteriol* **46**, 759–764.
- Roberts, M. S., Nakamura, L. K. & Cohan, F. M. (1994). *Bacillus mojavensis* sp. nov., distinguishable from *Bacillus subtilis* by sexual isolation, divergence in DNA sequence, and differences in fatty acid composition. *Int J Syst Bacteriol* **44**, 256–264.
- Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.
- Rüger, H.-J., Fritze, D. & Spröer, C. (2000). New psychrophilic and psychrotolerant *Bacillus marinus* strains from tropical and polar deep-sea sediments and emended description of the species. *Int J Syst Evol Microbiol* **50**, 1305–1313.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 617, 620, 622. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Wainø, M., Tindall, B. J., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.
- Wisotzkey, J. D., Jurtshuk, P., Jr, Fox, G. E., Deinhard, G. & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* **42**, 263–269.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.