Polyphasic identification of *Bacillus* and *Brevibacillus* strains from clinical, dairy and industrial specimens and proposal of *Brevibacillus invocatus* sp. nov.

¹ School of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA, UK

² Laboratorium voor Microbiologie, Universiteit Gent, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium

³ CCUG, Culture Collection, University of Göteborg, Dept of Clinical Bacteriology, Guldhedsgatan 10, S-413 46 Göteborg, Sweden

⁴ Department of Medical Microbiology, Lund University, Sölvegatan 23, S-223 62 Lund, Sweden

⁵ Regional Centre for Communicable Disease Control, University Hospital MAS, Malmö, Sweden

N. A. Logan,¹ G. Forsyth,¹ L. Lebbe,² J. Goris,² M. Heyndrickx,^{2†} A. Balcaen,² A. Verhelst,² E. Falsen,³ Å. Ljungh,⁴ H. B. Hansson⁵ and P. De Vos²

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

Thirty-three clinical, dairy and industrial isolates of aerobic endospore-forming bacteria which were unreactive in routine identification tests were characterized genotypically by using amplified rDNA restriction analysis (ARDRA), 16S rDNA sequencing and DNA–DNA reassociation, and phenotypically by using fatty acid methyl ester (FAME) analysis, SDS-PAGE of whole-cell proteins, API Biotype 100 assimilation tests and 16 other routine phenotypic tests. Three isolates were identified as strains of *Bacillus badius*, 12 as *Brevibacillus agri*, including 3 strains associated with an outbreak of water-borne illness, 4 as *Brevibacillus centrosporus* and 2 as *Brevibacillus parabrevis*; 12 strains contaminating an antibiotic production plant were recognized as members of a new species, for which the name *Brevibacillus invocatus* is proposed, with the type strain LMG 18962^T (= B2156^T = CIP 106911^T = NCIMB 13772^T).

Keywords: *Aneurinibacillus*, *Bacillus badius*, *Brevibacillus*, *Brevibacillus invocatus*, *Brevibacillus agri* waterborne illness

INTRODUCTION

Following DNA relatedness and chemotaxonomic studies, some '*Bacillus brevis*' strains were assigned to the new species '*Bacillus agri*' and '*Bacillus centrosporus*' (Nakamura, 1993), '*Bacillus migulanus*', '*Bacillus choshinensis*', '*Bacillus parabrevis*' and '*Bacillus galactophilus*' (Tagaki *et al.*, 1993), and '*Bacillus reuszeri*', '*Bacillus formosus*' and '*Bacillus borstelensis*' (Shida *et al.*, 1995), but '*Bacillus galactophilus*' was later recognized to be a synonym of '*Bacillus agri*'

(Shida *et al.*, 1994b). Also, '*Bacillus aneurinolyticus*' was revived (Shida *et al.*, 1994a); this organism was already known to resemble '*Bacillus brevis*' and related taxa (Claus & Berkeley, 1986), and *Bacillus badius* phenotypically, but that is to say most such strains are unreactive in many of the biochemical tests upon which the two main identification schemes are based and identifications rely too heavily on negative test results.

Studies on the 16S rDNA sequences of the type strains of '*Bacillus brevis*' and '*Bacillus aneurinolyticus*' suggested that the latter represented a distinct evolutionary line close to that of '*Bacillus brevis*' (Ash *et al.*, 1991) or that it diverged early from the '*Bacillus brevis*' line (Farrow *et al.*, 1992, 1994). On the basis of a 16S rDNA gene sequence analysis of the type strains only, Shida *et al.* (1996) proposed two new genera to accommodate the above-mentioned and allied species: *Aneurinibacillus* contains *Aneurinibacillus aneurinolyticus* and *Aneurinibacillus migulanus*, while *Brevibacillus* contains *Brevibacillus brevis* and the seven species derived from it, *Brevibacillus laterosporus* and the

[†]**Present address:** Government Dairy Research Station, Brussels-esteenweg 370, B-9090 Melle, Belgium.

Abbreviations: ARDRA, amplified rDNA restriction analysis; FAME, fatty acid methyl ester.

The EMBL accession number for the 16S rRNA gene sequence of *Brevibacillus invocatus* LMG 18962^T is AF378232. The accession numbers for the other 16S rRNA gene sequences are: *Brevibacillus agri* LMG 18172, AF378233; *Brevibacillus borstelensis* LMG 15536, AF378230; *Brevibacillus formosus* LMG 16101, AF378234; *Brevibacillus invocatus* LMG 18167, AF378231.

Table 1. Designations and sources of clinical, dairy and pharmaceutical isolates, *Aneurinibacillus*, *Bacillus* and *Brevibacillus* reference strains used and an overview of the different characterization methods applied to them

The following reference strains were studied by ARDRA only: *Bacillus amyloliquefaciens* LMG 9814^T, *Bacillus azotoformans* LMG 9581^T, *Bacillus cereus* LMG 6923^T, *Bacillus circulans* LMG 13261^T, *Bacillus firmus* LMG 7125^T, *Bacillus licheniformis* LMG 6933^T, *Bacillus megaterium* LMG 7127^T, *Bacillus simplex* LMG 11160^T, *Bacillus subtilis* LMG 7135^T, *Bacillus thuringiensis* LMG 7138^T. Abbreviations: ÅL, Å. Ljungh, Department of Medical Microbiology, Lund University, Sweden; ATCC, American Type Culture Collection, Manassas, VA, USA; B, N. A. Logan *Bacillus* Collection, Glasgow Caledonian University, Glasgow, UK; C, antibiotic fermenter contaminant strain designation; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CIP, Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; G, Gibson, T. Gibson Collection, held at Glasgow Caledonian University; Goodfellow, M. Goodfellow, University of Newcastle, Newcastle upon Tyne, UK; Gordon, R. E. Gordon, Rutgers University, New Brunswick, USA; IFO, Institute of Fermentation, Osaka, Japan; LMG, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; NCA, National Canners Association, Washington, DC, USA; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, UK; NRRL, Northern Regional Research Laboratory Collection, Peoria, IL, USA; NRS, N. R. Smith Collection, now held at NRRL; R-, research collection, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; Wellcome, Wellcome Collection of Micro-organisms, Beckenham, Kent, UK.

Name as received	Present taxonomic status (if changed)	Study no.	Other designations	Source
<i>Aneurinibacillus aneurinilyticus</i>		LMG 15531 ^T	B0205 ^T , ATCC 12856 ^T	Gordon
<i>Aneurinibacillus aneurinilyticus</i>		LMG 15533	B0207, NRRL NRS-1449, Li strain	Gordon
<i>Aneurinibacillus migulanus</i>		LMG 15427 ^T	B0270 ^T , NCTC 7096 ^T , ATCC 9999 ^T	NCTC
<i>Aneurinibacillus migulanus</i>		LMG 16098	B4036, NRRL NRS-606	NRRL
<i>Aneurinibacillus thermoaerophilus</i>		LMG 17165 ^T *	DSM 10154 ^T	DSM
<i>Aneurinibacillus thermoaerophilus</i>		LMG 17166 ^T *	DSM 10155	DSM
<i>Bacillus badius</i>		LMG 7122 ^T	B1903 ^T , B0180 ^T DSM 23 ^T	DSM
<i>Bacillus badius</i>		LMG 12332	B0201, NRS 1407	Gordon
<i>Bacillus</i> sp.	<i>Bacillus badius</i>	LMG 18004	B2083	Gelatin processing plant
<i>Bacillus</i> sp.	<i>Bacillus badius</i>	LMG 18005	B2084	Gelatin processing plant
<i>Bacillus</i> sp.	<i>Bacillus badius</i>	LMG 18006	B2085	Gelatin processing plant
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18007	B2086	Gelatin processing plant
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18008	B2378	Gelatin processing plant
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18071	B1577, ÅL 1	Water-borne illness
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18072	B1578, ÅL 2	Water-borne illness
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18073	B1579, ÅL 3	Water-borne illness
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18960*	B2142, C2392	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18965	B2143, C2543	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18162	B2159, C3793	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18171	B2144, C2714	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus agri</i>	LMG 18172†	B2146, C2922	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18161*	B2153, C3739	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18164	B2150, C3735	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18165	B2141, C0006	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18166	B2163, C4309	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18167†	B2161, C3950	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18169	B2158, C3776	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18170	B2162, C3994	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18959‡	B2151, C3736	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18961§	B2164, C4014	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18962††	B2156 ^T , NCIMB 13772 ^T , CIP106911 ^T , C3774 ^T	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18963	B2152, C3738	Antibiotic fermenter
<i>Bacillus</i> sp.	<i>Brevibacillus invocatus</i>	LMG 18966‡	B2155, C3749	Antibiotic fermenter
<i>Brevibacillus agri</i>	<i>Brevibacillus parabrevis</i>	LMG 15101	B4000, CCUG 27608	Blood isolate
<i>Brevibacillus brevis</i>	<i>Brevibacillus parabrevis</i>	LMG 15104	B1915, CCUG 29760	Breast abscess
<i>Brevibacillus brevis</i>	<i>Brevibacillus parabrevis</i>	LMG 15426	B0118, Gibson 539	Gibson
<i>Brevibacillus brevis</i>	<i>Brevibacillus agri</i>	LMG 15431	B0629, Wellcome 2934	Goodfellow
<i>Brevibacillus brevis</i>	<i>Brevibacillus borstelensis</i>	LMG 15536	B0627, Wellcome 2918	Goodfellow
<i>Brevibacillus brevis</i>	<i>Brevibacillus agri</i>	LMG 19651*	B2097, R-2139	Sterilized milk
<i>Brevibacillus brevis</i>	<i>Brevibacillus agri</i>	LMG 19652*	B2098, R-2140	Sterilized milk
<i>Brevibacillus laterosporus</i>	<i>Brevibacillus centrosporus</i>	LMG 15107	B4018, CCUG 28806	Human blood
<i>Brevibacillus laterosporus</i>	<i>Brevibacillus centrosporus</i>	LMG 15108	B4019, CCUG 28967	Human blood
<i>Brevibacillus laterosporus</i>	<i>Brevibacillus</i> sp.	LMG 15111	B4022, CCUG 29854	Industrial isolate
<i>Brevibacillus laterosporus</i>	<i>Brevibacillus centrosporus</i>	LMG 15112	B4023, CCUG 29969	Human blood
<i>Brevibacillus laterosporus</i>	<i>Brevibacillus centrosporus</i>	LMG 15113	B4024, CCUG 31314	Bronchio-alveolar lavage
<i>Brevibacillus agri</i>		LMG 15103 ^T	B4008 ^T , NRRL NRS-1219 ^T , CCUG 31345 ^T	CCUG
<i>Brevibacillus agri</i>		LMG 15592	B4001, NRRL B-1157	NRRL, clinical isolate
<i>Brevibacillus agri</i>		LMG 15593	B4003, NRRL B-1158	NRRL, clinical isolate
<i>Brevibacillus agri</i>		LMG 15594	B4004, NRRL NRS- 873, 'Bacillus hollandicus'	NRRL
<i>Brevibacillus agri</i>		LMG 15595	B4005, NRRL NRS- 874, 'Bacillus hollandicus'	NRRL
<i>Brevibacillus agri</i>		LMG 15596	B4006, NRRL NRS-1002, 'Bacillus ventricosus'	NRRL
<i>Brevibacillus agri</i>		LMG 15597	B4007, NRRL NRS-1028	NRRL
<i>Brevibacillus agri</i>		LMG 16099	B4025, NRRL NRS-616,	NRRL
<i>Brevibacillus borstelensis</i>		LMG 16009 ^T	B4029 ^T , NRRL NRS-818 ^T , IFO15714 ^T	IFO
<i>Brevibacillus borstelensis</i>		LMG 15429	B0626, Wellcome 2904	Goodfellow

Table 1 (cont.)

Name as received	Present taxonomic status (if changed)	Study no.	Other designations	Source
<i>Brevibacillus borstelensis</i>		LMG 15599‡	B4009, NRRL NRS-1373, 'Bacillus agri'	NRRL, hot spring
<i>Brevibacillus borstelensis</i>		LMG 16103‡	B4030, NRRL NRS-1029b, 'Bacillus schuylikillensis'	NRRL
<i>Brevibacillus borstelensis</i>		LMG 16208‡	B4028, NCIMB 8803, Knaysi 80	NCIMB
<i>Brevibacillus brevis</i>		LMG 16703†	B1881†, ATCC 8246†	ATCC
<i>Brevibacillus brevis</i>		LMG 17054‡	B2025, ATCC 11031	ATCC
<i>Brevibacillus brevis</i>		LMG 17055‡	ATCC 35690	ATCC
<i>Brevibacillus centrosporus</i>		LMG 15106†	B4016†, CCUG 31347†, NRS 664†	CCUG
<i>Brevibacillus centrosporus</i>		LMG 15600‡	B4012, NRRL NRS-135, NCA 11773-2	NRRL, from spinach
<i>Brevibacillus centrosporus</i>		LMG 15601	B4013, NRRL NRS-136, NCA 11773-8	NRRL
<i>Brevibacillus centrosporus</i>		LMG 15602	B4015, NRRL NRS-632, 'Bacillus rarus'	NRRL
<i>Brevibacillus centrosporus</i>		LMG 15604	B4017, NRRL NRS-925, 'Bacillus pallidus'	NRRL
<i>Brevibacillus centrosporus</i>		LMG 15713	B4014, NRRL NRS-628	NRRL
<i>Brevibacillus choshinensis</i>		LMG 15968†	B4046†, ATCC 51359†	ATCC
<i>Brevibacillus choshinensis</i>		LMG 16095*	B4032, NRRL NRS-376	NRRL, soil
<i>Brevibacillus choshinensis</i>		LMG 16096	B4033, NRRL NRS-378	NRRL, soil
<i>Brevibacillus choshinensis</i>		LMG 16097	B4034, NRRL NRS-380	NRRL, soil
<i>Brevibacillus formosus</i>		LMG 16010†	B4039†, NRRL NRS-863†	NRRL
<i>Brevibacillus formosus</i>		LMG 16101	B4040, NRRL NRS-910	NRRL
<i>Brevibacillus laterosporus</i>		LMG 16000†‡	B4035†, DSM 25†, ATCC 64†, Ford 29†	DSM
<i>Brevibacillus laterosporus</i>		LMG 15109‡	B4020, CCUG 29453, NRRL B-4190	CCUG
<i>Brevibacillus laterosporus</i>		LMG 15110‡	B4021, CCUG 29454, NRRL B-14295	CCUG
<i>Brevibacillus laterosporus</i>		LMG 15433‡	B0026, 8/1/15/1	Bristol
<i>Brevibacillus laterosporus</i>		LMG 15434‡	B0043, 8/1/15/1 colonial variant	Bristol
<i>Brevibacillus laterosporus</i>		LMG 15435‡	B0115, G 308, NCTC 7579	Gibson
<i>Brevibacillus laterosporus</i>		LMG 15436‡	B0116, G 1066, 'Bacillus orpheus'	Gibson
<i>Brevibacillus laterosporus</i>		LMG 15437‡	B0262	Laboratory contaminant
<i>Brevibacillus laterosporus</i>		LMG 15439‡	B0309, G 1080, 'Bacillus orpheus'	Gibson
<i>Brevibacillus laterosporus</i>		LMG 15440‡	B0311, G 5, 'Bacillus pyenoticus'	Gibson
<i>Brevibacillus laterosporus</i>		LMG 15441‡	B0616, NRS 682, 'Bacillus orpheus'	Goodfellow
<i>Brevibacillus parabrevis</i>		LMG 15971†	B4047†, NRS 605†, ATCC 10027†	ATCC
<i>Brevibacillus parabrevis</i>		LMG 15428	B0271, NCTC 7577, G 108	Gibson
<i>Brevibacillus parabrevis</i>		LMG 15973	B4048, ATCC 8185, NRS 751,	ATCC, soil, gramicidin strain
<i>Brevibacillus parabrevis</i>		LMG 16011	B4037, NRS 779, ATCC 8186, IFO 12333	IFO
<i>Brevibacillus parabrevis</i>		LMG 16100*‡	B4038, NRRL NRS-815	NRRL
<i>Brevibacillus reuszeri</i>		LMG 16012†	B4042†, IFO 15719†, NRRL NRS-1206†, Reuszer 39†	IFO
<i>Brevibacillus reuszeri</i>		LMG 16105	B4043, NRRL NRS-1207, Reuszer 723	NRRL
<i>Brevibacillus reuszeri</i>		LMG 16106*	B4044, NRRL NRS-1208, Reuszer 842	NRRL
<i>Brevibacillus thermoruber</i>		LMG 16910†‡	B1858†, DSM 7064†	DSM

* Strain not included in FAME analysis.

† Strain subjected to 16S rDNA sequencing.

‡ Strain giving unclear PAGE profile and so not shown in Figs 2 and 3; or in the case of strains LMG 15533, LMG 16100, LMG 16910†, LMG 17165 and LMG 17166, not included in analysis.

§ Strain not included in ARDRA.

|| Strain not characterized by tests in API system, other biochemical tests and morphological observations.

thermophile *Brevibacillus thermoruber*. Heyndrickx *et al.* (1997) transferred another thermophilic species, 'Bacillus thermoaerophilus', to *Aneurinibacillus* following a polyphasic taxonomic study.

Thus, strains which might previously have been assigned to 'Bacillus brevis' now represent some 10 mesophilic species in two genera. Their distinctions are based mainly upon DNA relatedness studies, molecular probing and chemotaxonomic analyses of the relatively few available isolates, using databases which are largely restricted to reference laboratories and unsuitable for organisms only occasionally encountered in routine laboratories. Distinction of most *Aneurinibacillus* and *Brevibacillus* species is not possible using the currently available *Bacillus* identification schemes and separation remains difficult even with much wider selections of phenotypic tests (Heyndrickx *et al.*, 1997). Also, another unreactive species, *Bacillus*

badius, may easily be misidentified as a member of these genera. It is unfortunate that the extensive splitting proposed by the various recent taxonomic studies has not revealed characteristic phenotypic profiles which would be of value in the routine laboratory.

Unreactive, aerobic, endospore-forming isolates from an outbreak of water-borne illness, from other clinical specimens, from 'sterilized' milk and from pharmaceutical manufacturing plants have been submitted to our laboratories for identification over the last few years. These referrals reflect the difficulties such organisms present to routine laboratories, where it is important to know that the isolate is neither *Bacillus anthracis* nor *Bacillus cereus*, nor yet a member of another species with a record of opportunistic pathogenicity. This prompted us to carry out a polyphasic taxonomic study of unreactive, aerobic endospore-

formers to identify these organisms, and in so doing we recognized a new species which we propose as *Brevibacillus invocatus*.

METHODS

Strains. The designations of the clinical and pharmaceutical isolates and reference strains, their origins and the different methods applied to them are shown in Table 1. Strains LMG 18071–18073 were isolated in connection with an outbreak of water-borne illness in four Swedish towns (total population 84 500) in November 1995, following the accidental overload of a purification plant with raw water. Responses to a questionnaire distributed in two affected towns and one unaffected control town revealed that 64–86% of affected households complained about water odour and taste, and that about 15–17% of persons in the affected towns reported symptoms of (in order of decreasing frequency) abdominal pain, diarrhoea, myalgia, vomiting and fever. Symptoms appeared in slightly less than 24 h and most patients recovered in 1–4 days. Strains LMG 18161, 18162, 18164–18167, 18169–18172, 18959–18963 and 18965 were contaminants isolated from a pharmaceutical fermenter plant and its antibiotic raw product over a period of several months from 1996 to 1997. Strains LMG 18004–18008 were isolated from an industrial plant which produces gelatin from animal skin and bone for pharmaceutical capsule manufacture. Strains LMG 19651 and 19652 were isolated from sterilized milk. Strains LMG 15101, 15104, 15107, 15108, 15112 and 15113 were isolated from clinical specimens in a Swedish medical laboratory; their clinical sources are indicated in Table 1. Strains of *Aneurinibacillus thermoaerophilus* were only analysed by amplified rDNA restriction analysis (ARDRA) in the present study as their high growth temperature (55 °C) requirement made direct phenotypic comparison with the other species untenable; the relationship of this species to the other species of *Aneurinibacillus* and to *Brevibacillus* species was discussed by Heyndrickx *et al.* (1997).

Cultivation and maintenance of strains. Unless otherwise stated, all strains were grown for the different analyses on nutrient agar with 1% (w/v) glucose (pH 7.4) (NAG) at 30 °C for 24–48 h. The strains were checked for purity by plating and phase-contrast microscopic examination and were maintained both as lyophilized cultures and as sporulated cultures on NAG slopes 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 at 4 °C.

DNA preparation. DNA was prepared and stored as described by Logan *et al.* (2000).

ARDRA. Enzymically amplified 16S rDNA was obtained by PCR and analysed by restriction digestion with five restriction enzymes (*Hae*III, *Dpn*II, *Rsa*I, *Bfa*I and *Tru*9I) as described previously (Heyndrickx *et al.*, 1996b). Identification of isolates was attempted by comparing ARDRA profiles with a database of over 1000 authentic strains of aerobic endospore-formers.

DNA base composition. DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters Symmetry Shield C8 column thermostabilized at 37 °C. The solvent was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5% (v/v) acetonitrile. Non-methylated λ phage DNA (Sigma) was used as the calibration reference.

Percentage DNA–DNA binding. DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements (excitation filter 360 nm, emission filter 465 nm). The optimal renaturation temperature was determined according to the equation of De Ley *et al.* (1970).

16S rDNA sequencing. This was carried out as described by Logan *et al.* (2000).

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

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

Phenotypic characterization and numerical analysis. Organisms were cultivated and characterized and the results subjected to numerical analysis and expressed as percentage *S*_G, following the methods described by Heyndrickx *et al.* (1997). Five strains were characterized blind, in duplicate, to give an indication of test error.

RESULTS AND DISCUSSION

The ARDRA cluster containing the type strain of *Brevibacillus agri* (Fig. 1) also contained *Brevibacillus brevis* LMG 16703^T and so it is evident that this technique was unable to discriminate between these two species. The type strains of *Brevibacillus centrosporus*, *Brevibacillus choshinensis* and *Brevibacillus formosus*, LMG 15106^T, 15968^T and 16010^T, and other authentic strains of these species were also not recovered in separate clusters by ARDRA, and all of these five species appeared as members of what we may refer to as the ‘*Brevibacillus brevis* ARDRA complex’, which formed at 88% similarity. This lack of discrimination between some *Brevibacillus* species in ARDRA was observed to a lesser extent by Heyndrickx *et al.* (1997) when the *Brevibacillus brevis* and *Brevibacillus agri* strains were not separated in ARDRA, but were separable by PAGE and API and other phenotypic characters; in that study, however, most *Brevibacillus* species were represented by only two strains. In the present work, some members of the *Brevibacillus brevis* ARDRA complex were separated by PAGE (Figs 2 and 3), with most strains of *Brevibacillus agri* being recovered in a distinct cluster, but API and other phenotypic tests were unable to separate all the members of the complex satisfactorily (Fig. 4). However, in the latter analysis the estimated probability of test inconsistency was 4%, indicating that separations of much less than 6–8% *S*_G were probably of little significance. In the 16S rDNA sequence comparison tree (Fig. 5), most species of the genus *Brevibacillus* were represented by more than one strain and it can be seen that several of the species were not well separated. This is perhaps not surprising, as such species were delineated primarily upon the basis of DNA related-

Fig. 1. Dendrogram of UPGMA clustering of correlation coefficients (Dice) of normalized 16S rDNA restriction analysis data (ARDRA) of representatives of *Aneurinibacillus migulanus* (2 strains), *Aneurinibacillus aneurinilyticus* (2 strains) and *Aneurinibacillus thermoaerophilus* (2 strains), and 5 *Bacillus badius* strains and 81 *Brevibacillus* strains. For details of strains see Table 1. The molecular mass marker contained, from right to left, 10 bands of 910, 659, 521, 403, 281, 257, 226, 136, 100 and 63 bp. The marker was prepared as described by Heyndrickx et al. (1996b).

Fig. 2. Normalized computer profiles of from SDS-PAGE analyses of whole-cell proteins of *Brevibacillus* members and *Bacillus badius*. The dendrogram is based on UPGMA clustering of the correlation coefficient (r) of total protein profiles. The zone used for clustering analysis was between points 30 and 330 (a complete lane contained 400 points).

Fig. 3. (a) Clustering of SDS-PAGE analysis of *Brevibacillus centrosporus* strains also shown in Fig. 2, but using only the zone between points 52 and 330 and thus omitting the high molecular mass bands above 85 kDa (all bands to the left of the dotted line) that are present in some, but not all strains of this species and which disturbed the clustering in Fig. 2. (b) Clustering of SDS-PAGE analysis of *Brevibacillus choshinensis* strains also shown in Fig. 2, but using only the zone between points 52 and 330 and thus omitting the high molecular mass bands above 85 kDa (all bands to the left of the dotted line) that are present in some, but not all strains of this species and which disturbed the clustering in Fig. 2. (c) Clustering of SDS-PAGE analysis of *Brevibacillus agri* strains also shown in Fig. 2, but using only the zone between points 52 and 330 and thus omitting the high molecular mass bands above 85 kDa (all bands to the left of the dotted line) that are present in some, but not all strains of this species and which disturbed the clustering in Fig. 2. (d) Clustering of SDS-PAGE analysis of *Bacillus badius* strains also shown in Fig. 2, but using only the zone between points 70 and 330 and thus omitting the high molecular mass bands above 70 kDa (all bands to the left of the dotted line) that are present in some, but not all strains of this species and which disturbed the clustering in Fig. 2.

Fig. 4. Simplified phenogram based upon the UPGMA clustering of similarity coefficients (S_G) of 115 phenotypic characters of 4 *Aneurinibacillus* strains, 5 *Bacillus* strains and 81 *Brevibacillus* strains. T indicates the position of the type strain.

ness (Nakamura, 1993; Takagi *et al.*, 1993; Shida *et al.*, 1995).

The three strains LMG 18071–18073, isolated in association with an outbreak of water-borne illness in

Sweden, showed 100 % similarity in ARDRA (Fig. 1) and lay close (91 %) to the cluster containing *Brevibacillus agri* LMG 15103^T. Although the strains were inseparable by ARDRA, their profiles in PAGE and in API and other phenotypic tests showed some variation (Figs 2, 3 and 4), indicating that they did not represent three isolations of the same strain. In PAGE, strain LMG 18072 was recovered as an outlier of the main *Brevibacillus agri* group (Fig. 2), but this was largely owing to a poor profile with weak bands. For reasons unknown, some *Brevibacillus* strains (especially those belonging to *Brevibacillus laterosporus*, but occasionally representatives of other species too) showed many fuzzy protein bands in the SDS-PAGE analysis. In a few cases the PAGE pattern could be improved under more stringent conditions of the timing of harvesting the cells, lysis of the cells, etc. In all other cases we did not include the PAGE patterns in the numerical analysis. The generation of fuzzy protein banding has also been reported for other taxa (e.g. Doignon-Bourcier *et al.*, 1999). In the API and other phenotypic test analyses (Fig. 4), strains LMG 18071–18073 were recovered in a loose cluster which formed at 80 % S_G and which merged with clusters containing the type strains of *Brevibacillus brevis*, *Brevibacillus agri*, *Brevibacillus formosus* and *Brevibacillus parabrevis* at only 74 % S_G . The cluster containing LMG 18071–18073 also included strains isolated from the antibiotic fermenter, gelatin, milk and clinical specimens (Fig. 4); all of these other strains were identified as *Brevibacillus agri* (see below). As DNA–DNA binding experiments showed LMG 18071 to have relatedness of 86.3–92.2 % to *Brevibacillus agri* LMG 15103^T in reciprocal experiments (Table 2), it can be concluded that the water-borne illness isolates are members of *Brevibacillus agri*. Although this is the first report of *Brevibacillus agri* being associated with illness, there have been occasional reports of '*Bacillus brevis*' (the species from which *Brevibacillus agri* was derived) being implicated in corneal infection and food poisoning (Logan, 1988), and *Brevibacillus agri* strains LMG 15592 and 15593 were also clinical isolates (Table 1).

The 12 strains LMG 18161, 18164–18167, 18169, 18170, 18959, 18961–18963 and 18966 were all isolated from the same antibiotic fermenter plant over a period of several months and they clustered as distinct groups in numerical analyses of ARDRA, PAGE, API and other phenotypic test data (Figs 1–4, where they are labelled as *Brevibacillus invocatus*). In ARDRA (Fig. 1), 10 of the strains clustered at 90.5 % (± 2 %) similarity, while strain LMG 18963 was an outlier joining at 87.5 % similarity. Nine strains of this group clustered by 92.5 % similarity in the PAGE analysis (Fig. 2), with LMG 18963 joining at 84 % similarity. All 12 strains characterized by API and other phenotypic characters also clustered together; nine strains grouped at 91 % S_G , with LMG 18961 and LMG 18966 joining at 86 % and LMG 18963 joining at 80 %; thus, LMG 18963 was found to lie at the border

Fig. 5. 16S rDNA bootstrap analysis (500 calculations) of a neighbour-joining (Bionumerics software; Applied Maths) clustering of the type strains of *Brevibacillus* species and other relevant references. Unknown bases were discarded and the gapcost was 0%. Possible variation of deposited sequences is demonstrated by the inclusion of repeated sequence determination for the type strain of *Brevibacillus laterosporus*. The figure also shows the low discrimination potential of this methodology for *Brevibacillus parabrevis*, *Brevibacillus reuszeri*, *Brevibacillus formosus*, *Brevibacillus brevis* and *Brevibacillus choshinensis*.

of the main group according to ARDRA, PAGE, API and other phenotypic characters. The results indicate that this group of antibiotic fermenter isolates could be distinguished from other *Brevibacillus* species using routine phenotypic tests. Strains 18167 and 18962 showed 70% DNA relatedness (mean value of reciprocal experiments; Table 2). In the 16S rDNA sequence comparison tree (Fig. 5), these two strains were close to each other (above 99% similarity in 16S rDNA sequence) and well separated (less than 97% similarity in sequence) from their nearest relatives, *Brevibacillus agri*, *Brevibacillus borstelensis*, *Brevibacillus centrosporus*, *Brevibacillus laterosporus* and *Brevibacillus thermoruber*. We therefore propose this group of isolates as a new species of *Brevibacillus*, with the name *Brevibacillus invocatus*. A description of the organism is given below and Table 3 shows the discriminating characters at the species level.

Other strains isolated from the antibiotic fermenter plant and its product, during the same period as the isolations we have allocated to *Brevibacillus invocatus*, were also clearly members of the genus *Brevibacillus*. Strain LMG 18965 showed the same ARDRA pattern

as two authentic strains of *Brevibacillus brevis* (Fig. 1) and it might have been provisionally identified as a strain of *Brevibacillus brevis*. However, the type strain of that species was recovered within the *Brevibacillus agri* part of the *Brevibacillus brevis* ARDRA complex (at above 92% similarity) and hence these two species cannot be separated by this technique. Indeed, PAGE analysis allocated LMG 18965 to the same cluster as the type strain of *Brevibacillus agri*. Four other antibiotic fermenter isolates, LMG 18162, 18171, 18172 and 18960, showed 100% similarity in ARDRA and clustered with the two strains LMG 18007 and 18008 which were isolated from the gelatin processing plant (Fig. 1). The loose clustering (82% S_G) of these seven strains in API and other phenotypic test analyses (Fig. 4) is consistent with the main *Brevibacillus agri* cluster forming at only 81% similarity in PAGE analysis (see also Fig. 3c). Strains LMG 19651 and 19652, from 'commercially sterilized' milk, are also found in the *Brevibacillus brevis* ARDRA complex; they could not be separated from each other, nor from the above-mentioned seven strains, on the basis of their ARDRA patterns. In PAGE the two milk strains showed 97% similarity and lay within the *Brevibacillus*

Table 2. Percentage DNA-DNA relatedness values between *Brevibacillus* strains

Mean values are given for reciprocal measurements which were nearly always within a $\pm 5\%$ relatedness difference. The exception was *Brevibacillus agri* LMG 18960 versus *Brevibacillus agri* LMG 15103^T, but as both values in this case were clearly above 70% relatedness, the taxonomic conclusion was not affected.

Strain	G+C (mol %)	LMG 15106 ^T	LMG 16012 ^T	LMG 15103 ^T	LMG 15971 ^T	LMG 16009 ^T	LMG 7123 ^T	LMG 15968 ^T	LMG 15113	LMG 18962 ^T	LMG 18167	LMG 18071	LMG 18007	LMG 18960	LMG 19651
<i>Br. centrosporus</i> LMG 15106 ^T	49.8	100													
<i>Br. reuszeri</i> LMG 16012 ^T	46.4	8	100												
<i>Br. agri</i> LMG 15103 ^T	53.5	26	7	100											
<i>Br. parabrevis</i> LMG 15971 ^T	52.2	24	8	55	100										
<i>Br. borstelensis</i> LMG 16009 ^T	51.9	21	9	28	27	100									
<i>Br. brevis</i> LMG 7123 ^T	47.2	21	8	31	34	22	100								
<i>Br. choshinensis</i> LMG 15968 ^T	49.8	38	8	27	24	23	25	100							
<i>Br. centrosporus</i> LMG 15113	49.7	70	9	28	24	30	25	38	100						
<i>Br. invocatus</i> LMG 18962 ^T	49.8	25		19		17			100						
<i>Br. invocatus</i> LMG 18167	49.1								101	100					
<i>Br. agri</i> LMG 18071	53.7			92							100				
<i>Br. agri</i> LMG 18007	53.6	26		98		19			20			100			
<i>Br. agri</i> LMG 18960	53.6	24		96		17			16				89	100	
<i>Br. agri</i> LMG 19651	53.5	23		88		20			19				87	88	100

agri cluster. They also clustered with the five antibiotic fermenter strains and the two gelatin strains in the API and other phenotypic test analyses. The PAGE, API and other phenotypic test results of these nine strains thus support their identification as *Brevibacillus agri* and the rDNA sequence comparison placed strain LMG 18172 in a cluster with four other strains of this species (Fig. 5). Strain LMG 15431 was received as '*Bacillus brevis*' in 1977, and our ARDRA, PAGE, API and other phenotypic test data all support the transfer of this strain to *Brevibacillus agri*.

Strain LMG 15111 lay at the periphery of the *Brevibacillus brevis* complex in ARDRA (Fig. 1) and in the phenotypic analysis (Fig. 4), while in PAGE it joined with the *Brevibacillus parabrevis* and *Brevibacillus reuszeri* clusters at low similarity. This Swedish industrial isolate is therefore regarded here as an unaffiliated member of *Brevibacillus*.

Three further isolates from the gelatin processing plant, LMG 18004, 18005 and 18006, clustered in ARDRA (Fig. 1) with the type and one other strain of *Bacillus badius* at above 92% similarity, and so can be regarded as members of this species. These isolates were also identified, according to their fatty acid pattern in the MIDI commercial system, as *Bacillus badius* at around 84.5% similarity. Furthermore, they also clustered with the *Bacillus badius* type strain in PAGE analysis (if particular zones containing heavy protein bands were omitted; Figs 2 and 3d) and in the analysis of API and other phenotypic test data (Fig. 4). In the latter analysis, *Bacillus badius* strains were not separable from *Aneurinibacillus aneurinilyticus* and *Aneurinibacillus migulanus*, a problem also observed by Heyndrickx *et al.* (1997). In previous PAGE interpretations (Heyndrickx *et al.*, 1996a) it was shown that heavy protein bands in a particular zone can greatly disturb cluster analyses of PAGE patterns which appear very similar on visual examination. The polyphasic approach is clearly decisive and allocates the three gelatin isolates to *Bacillus badius*.

Turning to some further clinical isolates, three strains isolated from human blood specimens in Sweden, LMG 15107, 15108 and 15112, and strain LMG 15113 from a bronchio-alveolar lavage specimen, clustered together at 89% similarity in ARDRA (Fig. 1), within a cluster containing the type and other strains of *Brevibacillus centrosporus*. If the molecular mass zone above 85 kDa was omitted from the numerical analysis of the PAGE patterns of LMG 15107, 15108, 15112 and 15113, a grouping of at least 77% similarity was obtained (Fig. 3a; see also comment on the *Bacillus badius* PAGE data above). The *Brevibacillus centrosporus* reference strain LMG 15604, together with strain LMG 15113 were far removed from the reference strains of *Brevibacillus centrosporus* in the PAGE analysis (Fig. 3a). In the analysis of API and other phenotypic test data (Fig. 4) strains LMG 15107, 15108 and 15112 clustered together at 82% S_G , but LMG 15113 showed less than 72% S_G to the others and none of them were recovered close to strains

Table 3. Phenotypic characters for differentiating *Brevibacillus invocatus* from other *Brevibacillus* species, *Aneurinibacillus* species and *Bacillus badius*

v, Between strain variation.

Character*	<i>Br. invocatus</i>	<i>Br. agri</i>	<i>Br. borstelensis</i>	<i>Br. brevis</i>	<i>Br. centrosporus</i>	<i>Br. choshinensis</i>	<i>Br. formosus</i>	<i>Br. laterosporus</i>	<i>Br. parabrevis</i>	<i>Br. reuszeri</i>	<i>Br. thermoruber</i>	<i>A. aneurinilyticus</i>	<i>A. migulanus</i>	<i>A. thermoaerophilus</i>	<i>Bacillus badius</i>
Hydrolysis of:															
Casein	—	+	+	+	—	—	+	+	+	—	+	—	—	+	+
Gelatin	—	+	+	+	—	—	+	+	+	—	+	—	—	+	+
Growth at:															
55 °C	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—
20 °C	+	v	—	—	+	+	+	v	+	+	—	—	+	—	+
Assimilation of:															
D-Alanine	—	v	+	+	—	—	+	—	+	—	—	+	+	+	+
L-Alanine	+ †	+	+	+	—	—	+	+	+	+	—	+	+	+	+
L-Aspartate	—	+	—	+	+	+	+	+	+	+	—	+	+	+	+
D-Fructose	—	+	+	+	—	—	+	+	—	v	—	—	+	v	—
Fumarate	—	+	+	v	+	v	+	+	—	—	—	+	+	+	+
D-Gluconate	—	+	+	+	+	—	+	—	+	+	—	v	—	—	—
Glutamate	+	+	+	+	+	—	+	+	+	+	—	+	+	+	+
DL-Glycerate	—	—	+	—	—	—	—	+	—	+	—	—	—	v	—
Glycerol	—	+	+	+	—	—	+	+	+	—	—	+	+	+	+
2-Ketoglutarate	+	+	—	v	+	—	+	v	+	+	—	+ †	v	+	+
DL-Lactate	+	+	—	—	+	—	+	—	—	+	—	+	+	+	+
Lactulose	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—
Maltose	—	+	—	+	—	—	+	+	+	—	—	—	—	—	—
D-Mannitol	+	+	—	+	+	v	+	+	+	+	—	—	—	v	—
N-Acetyl-D-glucosamine	—	+	—	+	+	+	+	+	+	+	—	— †	—	+	—
Phenylacetate	+	+	+	+	—	—	+	—	+	+	—	+ †	+	+	+
Quinate	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—
Sucrose	—	+	—	+	—	—	+	—	+	—	—	—	—	—	—
D-Trehalose	—	+	—	+	—	—	+	+	+	—	—	—	—	—	—
D-Turanose	—	+	—	+	—	—	+	—	+	—	—	—	—	v	—

* With the exceptions of hydrolysis of casein and gelatin, and growth at 55 and 20 °C, all characters were determined using tests in the API Biotype 100 System.

† Reaction for > 85 % strains tested.

received as *Brevibacillus centrosporus*; they were outliers of the *Brevibacillus brevis*-*Brevibacillus agri*-*Brevibacillus formosus*-*Brevibacillus parabrevis* group. However, in DNA-DNA binding experiments (Table 2) strain LMG 15113 showed 70% DNA relatedness (reciprocal values of 63 and 77%) to the type strain of *Brevibacillus centrosporus* (LMG 15106^T). Although this mean DNA relatedness value might be interpreted as the borderline for a new species, we regard the four clinical isolates as members of *Brevibacillus centrosporus*.

Two other Swedish clinical isolates, LMG 15101 and 15104, joined at 96% similarity in ARDRA and clustered with the type strain of *Brevibacillus parabrevis*, LMG 15971, at 87% (Fig. 1). Although this rather heterogeneous clustering based on rRNA suggests a taxonomic heterogeneity at the species level, the above three strains, together with LMG 15426, 15973, 16011, 15428 and 16100, were well separated from the other *Brevibacillus* species in the ARDRA clustering. Furthermore, LMG 15101 clustered well above 80% similarity with the type strain of *Brevibacillus parabrevis* in ARDRA, and at 83% with this type strain in PAGE, although LMG 15104 lies closer to *Brevibacillus agri* in PAGE (Fig. 2). We therefore tentatively regard this group of isolates as members of *Brevibacillus parabrevis*. The heterogeneity of the group was also seen in the API and other phenotypic test data (Fig. 4). Indeed, some strains (LMG 15101 and 15104) were recovered as outliers of the phenotypically unresolved *Brevibacillus brevis*-*Brevibacillus agri*-*Brevibacillus formosus*-*Brevibacillus parabrevis* group.

While members of the species *Brevibacillus agri*, *Brevibacillus brevis*, *Brevibacillus centrosporus*, *Brevibacillus choshinensis* and *Brevibacillus formosus* were not readily separated in ARDRA, the other members of this genus, *Brevibacillus borstelensis*, *Brevibacillus laterosporus*, *Brevibacillus parabrevis* and *Brevibacillus reuszeri*, were more easily distinguished by this technique (Fig. 1). The clusters of *Brevibacillus borstelensis* and *Brevibacillus reuszeri*, strains, however, lay close and merged at 89% similarity in ARDRA, with one *Brevibacillus borstelensis* strain (LMG 16103) being recovered as an outlier of this group. Strain LMG 15536 was received as a strain of '*Bacillus brevis*' in 1977, but in PAGE it clustered with the type strain of *Brevibacillus borstelensis* at 90% similarity, and by API and other phenotypic tests, where *Brevibacillus borstelensis*, *Brevibacillus laterosporus* and *Brevibacillus invocatus* were convincingly separated from the other members of this genus (Fig. 4), LMG 15536 joined with the *Brevibacillus borstelensis* cluster at 95% S_G . In ARDRA it was not recovered in any of the species clusters and, when taken with the data from the other methods, this suggests that the ARDRA pattern was disturbed, perhaps by an incomplete restriction digest. Moreover, in a FASTA search the 16S rDNA sequence of LMG 15536 showed more than 99% similarity with the type strain of *Brevibacillus borstelensis*.

Careful examination of the FAME data showed that this technique is of limited value for the discrimination of most *Brevibacillus* species (detailed FAME data are given in a table available in the online version of this paper at <http://ijs.sgmjournals.org>). However, the FAME data have some diagnostic value for a few species. We will consider here only the mean data for those species for which at least five strains have been investigated: *Brevibacillus agri*, *Brevibacillus borstelensis*, *Brevibacillus laterosporus*, *Brevibacillus parabrevis*, *Brevibacillus choshinensis* and *Brevibacillus invocatus*. For the last two species, the mean of the ratio anteiso-C15:0 versus iso-C15:0 was above 3.0, while for the other four species it was, at most, 1.0. Furthermore, *Brevibacillus borstelensis* and *Brevibacillus parabrevis* could be discriminated from these other species by having much lower amounts of iso-C14:0 (below 4%). Finally *Brevibacillus laterosporus* showed less than 1% of 16:1 ω 7c alcohol while all others had at least 2% or more of this component.

The difficulties that may attend the identification of these unreactive, aerobic endospore-forming bacteria in the routine laboratory are clear. Useful routine phenotypic characters are few (Table 3) and do not always give clear distinctions between the currently recognized species. Even when a polyphasic approach is taken, identifications are not always clear-cut, as we found with some of our clinical isolates. Aside from the new species *Brevibacillus invocatus*, which is phenotypically distinct, it may be questioned whether the current taxonomy of *Brevibacillus* best serves the needs of the diagnostic bacteriologist, and it might be argued that certain species might better be merged to give a more practically useful classification of this genus.

Description of *Brevibacillus invocatus* sp. nov.

Brevibacillus invocatus (in.vo.ca'tus; M.L. adj. *invocatus*, uninvited, referring to the isolation of strains of this organism as contaminants of an industrial fermentation).

Gram-negative, motile, rod-shaped cells, 0.5–1.0 μ m by 2.0–6.0 μ m. Strictly aerobic. The ellipsoidal spores are borne subterminally, occasionally terminally, and swell the sporangia (Fig. 6). Grows on routine media such as nutrient agar and trypticase soy agar. Growth at 30 °C is initially slow, with more rapid growth following 24 h incubation; after 3–4 days the slightly umbonate colonies are 1–8 mm in diameter, with slightly irregular margins. Colonies are brownish-yellow, some with a single whitish concentric zone at the margin, and they are butyrous and have silky surfaces; the centres are opaque and the edges translucent. Catalase-positive. Nitrate reduction-negative. Casein, gelatin, starch and urea are not hydrolysed and indole is not produced. Growth temperatures range from 15 to 35 °C. Growth occurs between pH 6.0 and 8.5. Few carbohydrates are assimilated and acid is produced weakly, if at all, from them; amino acids and some organic acids are used as carbon sources. The

Fig. 6. Photomicrograph of sporangia and vegetative cells of the type strain of *Brevibacillus invocatus* viewed by phase-contrast microscopy; ellipsoidal spores lie subterminally in slightly swollen sporangia. Bar, 2 μ m.

following carbon sources are assimilated in the API Biotype 100 System: L-glutamate, DL-3-hydroxybutyrate, 2-ketoglutarate, DL-lactate, D-mannitol, phenylacetate, L-proline, quinate, D-ribose and L-tyrosine and L-alanine. Propionate and L-serine are usually assimilated. Assimilation varies between strains for 2-keto-D-gluconate, L-rhamnose and succinate. Aesculin hydrolysis varies between strains. The following carbon sources are occasionally assimilated: adonitol, 4-aminobutyrate, L-aspartate, D-fructose, fumarate, D-galacturonate, DL-glycerate, L-malate, malonate, palatinose, D-trehalose and D-xylose. The following substrates are not assimilated: *N*-acetyl-D-glucosamine, *cis*- and *trans*-aconitate, D-alanine, 5-aminovalerate, L-arabinose, D- and L-arabitol, benzoate, betain, caprate, caprylate, D-cellobiose, citrate, *m*-coumarate, dulcitol, *i*-erythritol, ethanolamine, L-fucose, D-galactose, β -gentiobiose, gentisate, D-glucose, D-glucosamine, D-gluconate, D-glucuronate, glutarate, glycerol, histamine, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, hydroxyquinoline- β -glucuronide, *myo*-inositol, itaconate, 5-keto-D-gluconate, α -lactose, lactulose, D-lyxose, D-mannose, D-melezitose, 1-*O*-methyl- α -galactopyranoside, 1-*O*-methyl- β -galactopyranoside, 1-*O*-methyl- α -D-glucopyranoside, 1-*O*-methyl- β -D-glucopyranoside, 3-*O*-methyl-D-glucopyranose, D-malate, maltitol, maltose, maltotriose, D-melibiose, mucate, 3-phenylpropionate, protocatechuate, putrescine, D-raffinose, D-saccharate, D-sorbitol, L-sorbose, sucrose, D-tagatose, D- and L-tartrate, *meso*-tartrate, tricarballoylate, trigonelline, tryptamine, L-tryptophan, D-turanose and xylitol. The major cellular fatty acid components (accounting for more than 1% of the total fatty acid content) are, in descending order of content: anteiso-C15:0, iso-C15:0, iso-C14:0, C16:1 ω 7*c* alcohol, iso-C16:0 and iso-C17:1 ω 10*c*. The G + C content ranges from 49.7 mol% for the type strain to 49.1 for LMG 18167. The type strain is LMG 18962^T (= B2156^T = CIP 106911^T = NCIMB 13772^T) and in the variable assimilation characters listed above it is

positive for L-alanine, 2-keto-D-gluconate, propionate and L-serine, and negative for L-rhamnose, succinate and aesculin.

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