A Numerical Classification of the Genus *Bacillus*

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Three hundred and sixty-eight strains of aerobic, endospore-forming bacteria which included type and reference cultures of *Bacillus* and environmental isolates were studied. Overall similarities of these strains for 118 unit characters were determined by the S_{SM} , S_J and D_P coefficients and clustering achieved using the UPGMA algorithm. Test error was within acceptable limits. Six cluster-groups were defined at 70% S_{SM} , which corresponded to 69% S_P and 48–57% S_J . Groupings obtained with the three coefficients were generally similar but there were some changes in the definition and membership of cluster-groups and clusters, particularly with the S_J coefficient.

The *Bacillus* strains were distributed among 31 major (4 or more strains), 18 minor (2 or 3 strains) and 30 single-member clusters at the 83% S_{SM} level. Most of these clusters can be regarded as taxospecies. The heterogeneity of several species, including *Bacillus brevis*, *B. circulans*, *B. coagulans*, *B. megaterium*, *B. sphaericus* and *B. stearothermophilus*, has been indicated and the species status of several taxa of hitherto uncertain validity confirmed. Thus on the basis of the numerical phenetic and appropriate (published) molecular genetic data, it is proposed that the following names be recognized; *Bacillus flexus* (Batchelor) nom. rev., *Bacillus fusiformis* (Smith *et al.*) comb. nov., *Bacillus kaustophilus* (Prickett) nom. rev., *Bacillus psychrosaccharolyticus* (Larkin & Stokes) nom. rev. and *Bacillus simplex* (Gottheil) nom. rev. Other phenetically well-defined taxospecies included 'B. aneurinolyticus', 'B. apiarius', 'B. cascainensis', 'B. thiaminolyticus' and three clusters of environmental isolates related to *B. firmus* and previously described as 'B. firmus–B. lentus intermediates'. Future developments in the light of the numerical phenetic data are discussed.

INTRODUCTION

Bacteria that produce heat-resistant endospores are classified in several genera in the family *Bacillaceae*. With the exception of the anaerobic, endospore-forming bacteria, the genus *Bacillus* is the largest and best-known member of this family, which also includes the genera *Sporosarcina* and *Sporolactobacillus* (Berkeley & Goodfellow, 1981). Since endospore-formation is a universal feature of these bacteria, spore morphology has traditionally been given considerable weight in their classification and identification.

The earlier taxonomy of the bacilli was very confused, yielding more than 150 named species, often described on the basis of single physiological or ecological features. In a comparative study of over 1000 strains, Smith *et al.* (1952) used spore shape, size and location within the sporangium as a means of differentiating groups within the genus and reduced the number of species to 19. These morphological divisions have remained in general use (Wolf & Barker, 1968; Hobbs & Cross, 1983), despite criticism (Gordon, 1981). Revised and supplemented descriptions of common *Bacillus* species have also been published, together with information on

some unclassified strains (Gordon *et al.*, 1973). However, it was appreciated that the criteria used for this classification were insufficient (Gordon, 1981) and that many strains could not be accommodated within it. Nevertheless, the descriptions of Gordon and her co-workers form the basis of the classification in *Bergey's Manual of Systematic Bacteriology* (Claus & Berkeley, 1986) and, together with strain histories, provide an invaluable framework for *Bacillus* taxonomists.

The inadequacy of *Bacillus* classification has been emphasized by molecular studies. The wide range of base composition in chromosomal DNA indicates genetic diversity (Priest, 1981; Fahmy *et al.*, 1985) and suggests that *Bacillus* species should be reclassified into several genera. Analysis of rRNA by partial oligonucleotide sequencing has indicated a close relationship between the genera *Bacillus*, *Planococcus*, *Sporosarcina*, *Staphylococcus* and *Thermoactinomyces* and revealed *Bacillus* as a fairly coherent taxon (Stackebrandt & Woese, 1981; Stackebrandt *et al.*, 1987) equivalent in phylogenetic depth to the actinobacteria (Goodfellow & Cross, 1984) or the enteric bacteria-vibrio group (Stackebrandt & Woese, 1981), each of which encompasses several genera. Further, DNA homology studies have shown that many accepted *Bacillus* species, notably *B. circulans* (Nakamura & Swezey, 1983a), *B. megaterium* (Hunger & Claus, 1981), *B. sphaericus* (Krych *et al.*, 1980) and *B. stearothermophilus* (Sharp *et al.*, 1980), are markedly heterogeneous and in need of taxonomic revision.

Taxometric studies using a wide range of characters have been shown to be effective for the taxonomic revision of large groups of related bacteria (Goodfellow & Dickinson, 1985; MacDonell & Colwell, 1985). The extensive data bases derived from such studies are increasingly being used for the construction of probabilistic identification matrices (Williams *et al.*, 1985) and for designing media formulations that are selective for the isolation of industrially important bacteria (Goodfellow & Williams, 1986). Numerical taxonomy has been used to classify marine bacilli (Bonde, 1975; Boeyé & Aerts, 1976), and culture collection strains representing the genus *Bacillus* have been analysed for a small number of classical tests (Priest *et al.*, 1981). However, in a more comprehensive study Logan & Berkeley (1981) concluded that further information was needed before *Bacillus* could be subdivided into 'three or more different genera', and 'spectra of strains', notably the *B. firmus*/*B. lentus* and *B. circulans* groups, be unscrambled. Although much remains to be done, these and other studies indicated the value of the numerical taxonomic approach in helping to clarify relationships within the genus *Bacillus*.

The primary aim of the current investigation was to establish the detailed intrageneric relationships of bacilli by examining representative strains for many properties using the numerical taxonomic procedure. It was also anticipated that the resultant data base would be used to construct a frequency matrix for the probabilistic identification of bacilli and for the formulation of media selective for specific bacilli of industrial importance.

METHODS

Strains and culture conditions. Three hundred and sixty-eight test strains were obtained from public and private collections (Table 1); 29 duplicate cultures were also included. Wherever possible type cultures were included. All cultures were stored on nutrient agar (Oxoid CM1) slopes at 4 °C, with the inclusion of 5% (w/v) NaCl for *B. pantothenicus* and adjusted to pH 6.0 with 1.0 M-HCl for *B. coagulans* strains. Suspensions of vegetative cells and endospores were stored in glycerol (20%, v/v) at -20 °C.

Each strain was examined for 118 unit characters (Tables 3-5). Thawed glycerol suspensions were used as inocula wherever possible but for sugar fermentation and organic acid utilization tests 2- to 4-d-old cultures grown on nutrient agar and suspended in physiological saline were used. All tests were done at least once on each strain but were repeated where ambiguous or clearly unexpected results were obtained. Inoculated media were usually incubated at 30 °C but thermophilic and psychrophilic strains were incubated at 50 °C and 15 °C, respectively. Morphological, degradation (with the exception of aesculin, allantoin, arbutin, hippurate and urea, which were done in test tubes), antibiotic sensitivity and physiological tests were done in Petri dishes. Replidishes (Sterilin) were used for 'spreading' organisms such as *B. alvei* and *B. mycoides*. They were also used for sugar fermentation and organic acid utilization tests. Petri and Replidishes were inoculated with a multipoint inoculator (Denley).

Morphology and pigmentation. Colonial morphology was examined on isolated colonies grown on nutrient agar for 2-4 d. Cellular morphology was examined in Gram-stained smears of these cultures, and spores were stained using malachite green (Cowan, 1974). Spore morphology was examined on cultures from soil-extract agar (SxA) (Gordon *et al.*, 1973) in cases where sporulation did not occur on nutrient agar (see Tables 3-5).

Degradative tests. The degradation of adenine and tyrosine (0.5%), elastin (0.3%), casein (1%, w/v, skimmed milk), guanine (0.05%) and testosterone (0.1%) was determined in nutrient agar after 7 and 14 d (2 and 5 d at 50 °C for thermophiles; 14 and 21 d at 15 °C for psychrophiles); clearing of the areas under and around the growth was scored as positive. Gelatin (0.4%) and starch (1%) hydrolysis were detected in the same basal medium after 7 d (2 d for thermophiles; 14 d for psychrophiles) by flooding plates with acidified HgCl₂ (Frazier, 1926) and iodine solution (Gordon *et al.*, 1973) respectively. Hydrolysis of DNA (0.2%) and RNA (0.3%) was observed using Bacto DNase Test agar (Difco) and nutrient agar as nutrient bases, respectively. After incubation for 7 d (2 d for thermophiles; 14 d for psychrophiles) plates were flooded with 1 M-HCl and clear zones recorded as positive. Tweens 20 and 80 (1%, v/v) were incorporated into Sierra's (1957) medium and plates examined for opacity after 7 d (2 d for thermophiles; 14 d for psychrophiles). The hydrolysis of allantoin and urea was detected using the media and methods of Gordon (1966, 1968). Aesculin and arbutin (both 0.1%) degradation was determined by the methods of Williams *et al.* (1983) and examined after 7 d (2 d for thermophiles; 14 d for psychrophiles). Pullulan and pustulan hydrolysis was determined by the methods of Morgan *et al.* (1979) and Martin *et al.* (1980), respectively. Chitinolytic activity was observed after 14 and 21 d (3 and 5 d for thermophiles) as the appearance of zones of clearing in colloidal chitin agar (Hsu & Lockwood, 1975) and hippurate hydrolysis using the method of Gordon *et al.* (1973) after incubation for 14 d (5 d for thermophiles). Lecithinase activity was determined as opalescence in a medium comprising egg-yolk emulsion (5%, v/v; Oxoid) in nutrient agar incubated for 2 d (1 d for thermophiles; 5 d for psychrophiles). Pectin degradation was detected using the modified method of Williams *et al.* (1983); hydrolysis zones were detected after 7 d (2 d for thermophiles; 14 d for psychrophiles).

Antibiotic resistance. Strains were examined for the ability to grow in nutrient agar supplemented with antibiotics (Sigma) at two concentrations (Table 3). The antibiotics used were benzylpenicillin, chloramphenicol, D-cycloserine, erythromycin, gramicidin, nalidixic acid, polymyxin sulphate, rifampicin, streptomycin sulphate and tetracycline. Growth was recorded after 7 d (3 d for thermophiles; 14 d for psychrophiles) and resistance scored as positive.

Acid production from sugars and sugar alcohols. This was detected using the media and methods of Gordon *et al.* (1973). Replidishes were inoculated and examined after 7 d (3 d for thermophiles; 14 d for psychrophiles) for acid production.

Organic acid utilization. The ability of strains to use organic acids was determined using the methods of Gordon *et al.* (1973). Replidishes were examined after 5 d (2 d for thermophiles; 10 d for psychrophiles) for the appropriate colour change.

Tolerance tests. Nutrient agar was used as the basal medium. Growth at 5 °C and 17 °C was recorded after 14 and 21 d, growth at 37 °C after 3 d, and growth at 50 °C and 65 °C after 2 d. Growth at pH 4.5, 6.0, 8.0 and 9.5 was determined in media adjusted to the appropriate pH with HCl or NaOH and recorded after 7 d (3 d at 50 °C). Growth in the presence of NaCl (2, 5 and 10%, w/v) was recorded after 7 d (3 d at 50 °C).

Miscellaneous biochemical tests. Anaerobic growth was determined according to Gordon *et al.* (1973) and gas production from glucose in glucose/peptone water containing Durham tubes. Production of dihydroxyacetone and indole, reduction of nitrate, deamination of phenylalanine, and the Voges-Proskauer test were determined using the standard methods for *Bacillus* strains (Gordon *et al.*, 1973). Hydrolysis of *o*-nitrophenyl β -D-galactoside, the methyl red test, the oxidase reaction and presence of phosphatase were examined using the procedures of Cowan (1974). Ability to grow on MacConkey agar (Oxoid) was recorded after 5 d (2 d at 50 °C; 10 d at 15 °C).

Coding of data. Nearly all the characters existed in one of two mutually exclusive states and were scored plus (1) or minus (0). Qualitative multistate characters were each scored plus (1) for the character state shown and minus (0) for the alternatives. Quantitative multistate characters such as tolerance to NaCl were coded using the additive method of Sneath & Sokal (1973). Characters which did not show any separation value or were poorly reproducible were deleted from the data matrix. The final $n \times t$ table, therefore, contained data for 368 bacteria (t) and 118 unit characters (n ; Tables 3–5).

Computer analysis. Data were analysed using the Clustan 1C package (Wishart, 1978) on a Burroughs B6370 computer using the simple matching (S_{SM}), Jaccard (S_J) and pattern difference (D_P) coefficients (Sneath & Sokal, 1973). Clustering was achieved using the unweighted pair group method with arithmetic averages (UPGMA) algorithm (Sneath & Sokal, 1973).

Test reproducibility. Twenty-nine strains were tested in duplicate and an estimate of test variance calculated (formula 15; Sneath & Johnson, 1972) which was used to calculate the average probability (p) of an erroneous test result (formula 4; Sneath & Johnson, 1972).

RESULTS

Test error

Experimental test error was calculated from the data collected on the 29 duplicate strains. The average probability (p) of an erroneous test result was 3.90% calculated from the pooled

variance ($S^2 = 0.0374$) of all the unit characters for the duplicate cultures. The 29 pairs of duplicate strains showed a mean observed similarity of 93.86% S_{SM} . Some groups of tests were highly reliable, particularly cellular morphology, degradation, acid from sugars, growth, and miscellaneous tests, all of which displayed a variance < 0.03 . The most irreproducible tests were those involving organic acid utilization, in which the indicator change was difficult to read. Nevertheless, these results were included in the study because the variance (0.113) was only slightly greater than the generally accepted level of < 0.1 (Sneath & Johnson, 1972).

Gross taxonomic structure

The data were analysed using the S_{SM} , S_J and D_P coefficients with the UPGMA algorithm. The S_{SM} dendrogram was divided into six aggregate clusters at the 70% similarity (S -) level (Fig. 1; Table 1), which corresponded to 69% S_P . The composition of the cluster-groups was slightly different in the S_{SM} and D_P phenograms (Table 2) but the major and minor clusters were little affected. In the S_J /UPGMA analysis, five cluster-groups were apparent but to delineate them a staggered line from 48 to 57% similarity was required. Given this relaxation of the generally accepted interpretation of dendograms, the composition of the cluster-groups showed good congruence with those obtained in the S_{SM} and D_P analyses. The major variation was observed in the distribution of the clusters of obligate aerobic strains within cluster-groups D and E. The S_{SM} /UPGMA analysis most closely resembled classifications obtained in earlier studies of the genus (Logan & Berkeley, 1981; Priest *et al.*, 1981) and it is presented here in detail.

The composition of cluster-group A was largely unaffected by the coefficients used (Table 2). The bacteria encompassed by this taxon all produced acid from a wide range of carbohydrates, were facultative anaerobes with ellipsoidal spores that distended the sporangium, and hydrolysed a variety of polysaccharides including starch and pullulan. Similarly, cluster-group B encompassed bacteria that were aerobic or facultatively anaerobic and produced acid from a variety of sugars. They also formed oval spores which, with the exception of those of *B. laterosporus* and '*B. psychrosaccharolyticus*', did not distend the sporangium. Strains assigned to cluster-group B hydrolysed casein and, with the exception of *B. pumilus*, starch.

Cluster-group C was based on *B. firmus*, *B. pantothenicus*, marine strains and perhaps *B. lentus*, although in the S_{SM} /UPGMA and D_P /UPGMA analyses this species was given cluster-group status. These bacteria were generally weak in their ability to form acid from sugars and grew poorly, if at all, under anaerobic conditions. They produced oval spores and were NaCl tolerant. Considerable affinity was found between cluster-groups C and D, which included '*B. aneurinolyticus*' and *B. sphaericus*, but strains in the latter group were distinguished by lack of acid production from sugars (*B. psychrophilus* was a very weak acid-former). These bacteria displayed a variety of spore morphologies.

Cluster-group E contained *B. lentus* and *B. macquariensis* but the weight of evidence (Table 2) suggests that these taxa might more appropriately be placed in cluster-groups D and A, respectively. Cluster-group F encompassed the two thermophilic taxa *B. coagulans* and *B. stearothermophilus*. These bacteria displayed heterogeneity of spore morphology and fermented a variety of carbohydrates.

The full characteristics of the cluster-groups are given in Table 3.

Composition and characteristics of major and minor clusters

The strains were recovered in 31 major (four or more strains), 18 minor (two or three strains) and 30 single-member clusters at the 83% S_{SM} level (Fig. 1). These clusters have been assigned names according to the distribution of type and reference strains. The characteristics of the major and minor clusters are given in Tables 4 and 5, respectively.

Within cluster-group A, cluster 1 contained 13 strains received as *B. alvei*. They formed a homogeneous phenon at 87% S_{SM} and displayed typical motile micro-colonies (see Parry *et al.*, 1983) and swollen sporangia containing oval, terminal spores. Cluster 3 comprised four strains of '*B. thiaminolyticus*' that were morphologically similar to *B. alvei* but distinguishable by non-motile micro-colonies and positive and negative reactions in the nitrate reduction and Voges-

Proskauer tests, respectively. Of the six strains assigned to cluster 4, four were originally labelled as *B. circulans*, one as *B. alvei* and the other as '*B. sphaericus* var. *rotans*'. These bacteria possessed motile micro-colonies typical of *B. alvei* but differed from the latter in failing to produce dihydroxyacetone and in being negative for nitrate reduction and the Voges–Proskauer reaction. Cluster 7 strains resemble *B. pabuli* (Nakamura, 1984a) and were named accordingly.

The ten strains of *B. macerans* recovered in cluster 5 displayed the typical reactions of this species, in particular the production of gas from sugars, a property shared with *B. polymyxa* (cluster 8). However, the strains in the latter taxon fermented a less extensive range of sugars, hydrolysed casein and produced dihydroxyacetone. Related to *B. polymyxa* at 77.5% S_{SM} were five strains of *B. circulans* including the type strain (cluster 6). These bacteria did not produce gas from glucose. The heterogeneity of strains received as *B. circulans* was evident given their assignment to two major, three minor and four single-member clusters. The sole strain of '*B. filicolonicus*' was recovered as a single member cluster in cluster-group A.

Cluster-group B was numerically the largest in the study. Strains of *B. cereus*, *B. mycoides* and *B. thuringiensis*, assigned to cluster 11 within this cluster-group, were divided at the 89 to 92% S_{SM} level into nine subclusters which approximated to the species and varieties represented. Subclusters 11A and 11B were heterogeneous and contained strains labelled *B. thuringiensis* and *B. cereus*. Subcluster 11C contained seven strains of *B. cereus*, some of which had been associated with food poisoning. Subcluster 11D also contained *B. cereus* strains, some of which were originally designated '*B. cereus* var. *fluorescens*' and '*B. cereus* var. *albolactis*'. *B. thuringiensis* strains were recovered in subcluster 11E and two *B. cereus* strains of serotypes 6 and 8 comprised 11F. Twelve strains of *B. thuringiensis*, including the type strain, formed subcluster 11G. Subcluster 11H was largely composed of *B. cereus* strains, and the final subcluster 11I, contained four strains of *B. mycoides*. *Bacillus cereus* NCIB 8705 and a marine isolate representative of cluster IIC (*B. cereus*) of Bonde (1975) formed single-member subclusters. Although the subclusters largely conformed to the designations *B. cereus*, *B. mycoides* and *B. thuringiensis*, consistent features that distinguished them, with the exception of the rhizoidal colony forms of *B. mycoides*, were not evident. Loosely associated with the *B. cereus* cluster were two marine isolates from group IIC of Bonde (1975), and two strains of '*B. psychrosaccharolyticus*'.

Eight strains of *B. laterosporus*, including the type strain, were recovered in cluster 13. Their close affinity to *B. cereus* (76% S_{SM}) may initially seem surprising, but if the unusual spore morphology is ignored, the taxa have many features in common. Both species contained facultative anaerobes that were largely methyl red positive and reduced nitrate; both degraded a variety of macromolecules and produced acid from a similar range of sugars. A single strain of '*B. pycnoticus*' recovered within the *B. laterosporus* cluster at 86% S_{SM} did not have the characteristic lateral spore position of *B. laterosporus*.

The '*B. subtilis* group', including *B. megaterium*, joined *B. cereus* at 72% S_{SM} . Cluster 14 contained nine strains of which eight were authentic cultures of *B. amyloliquefaciens* or were strains labelled *B. subtilis* from amylase fermentations; one strain was a marine isolate. Although cluster 14 was distinct from *B. subtilis* (cluster 15), consistent differential features were not evident. Fermentation of meso-inositol, lactose and xylose, and hydrolysis of DNA and Tween 80 provide some measure of distinction.

Cluster 15 encompassed strains received as *B. subtilis*, including the type strain. Two strains received as '*B. vulgatus*' and two designated as '*B. atterimus*' were recovered in this cluster. Two marine isolates, representatives of group IVA (*B. subtilis*) and group IIB (*B. megaterium*) of Bonde (1975), were assigned to this cluster as was a second '*B. pycnoticus*' strain. Bacteria in cluster 15 conformed to the typical description of *B. subtilis* since they were obligate aerobes that were positive in the nitrate reduction and Voges–Proskauer tests and produced acid from a variety of sugars.

Strains of *B. pumilus* formed a homogeneous cluster related to *B. subtilis* at 79% S_{SM} . Most of these organisms were received as *B. pumilus*, including two marine isolates, representing Bonde's (1975) group IVB (*B. pumilus*). However, representatives of his group IIB (*B. megaterium*) and

Fig. 1 (continued overleaf). Simplified dendrogram showing the relationships between clusters recovered in the SSM/UPGMA analysis. Asterisks (*) are used to denote clusters containing type strains.

Fig. 1 (continued). Simplified dendrogram showing the relationships between clusters recovered in the SSM/UPGMA analysis.

Table 1. Designation and source of strains assigned to cluster-groups (defined at 70% S_{SM} , UPGMA) and clusters (defined at 83% S_{SM} , UPGMA)

Binomials in inverted commas are not on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and have not been validly published since 1 January 1980. Type strains are marked with an asterisk (*).

Cluster-group A	
Strains assigned to cluster 1 (<i>Bacillus alvei</i>)	
S3–*S5	<i>B. alvei</i> , NCIB 8212, NCIB 8199, NCIB 9371
S6–S8	<i>B. alvei</i> , NCTC 3324, NCTC 3349, NCTC 7583
S9	<i>B. alvei</i> , J. R. Norris, Cadbury Schweppes Ltd, Reading, UK, BO 113
S11–S16	<i>B. alvei</i> , WR 2772, WR 2773, WR 3186 (E. Schreiner, SKG), WR 3187 (E. Schreiner, 4N), WR 3250 (E. Schreiner, A), WR 3251 (E. Schreiner, B)
Strains assigned to cluster 2 (<i>Bacillus apiarius</i>)	
S414, S415	' <i>B. apiarius</i> ', R. E. Gordon, Rutgers University, New Jersey, USA, NRS 1438 (H. Katznelson, BX3), NRS 1439 (H. Katznelson, BX5); bee larvae
Strains assigned to cluster 3 (<i>Bacillus thiaminolyticus</i>)	
S327–S330	' <i>B. thiaminolyticus</i> ', J. R. Norris, BO 286–BO 289 (J. Yamaguchi, M1–M4)
Strains assigned to cluster 4 (<i>Bacillus circulans</i>)	
S10	<i>B. alvei</i> , J. R. Norris, BO 024
S93, S94, S96, S103	<i>B. circulans</i> , J. R. Norris, BO 030, BO 061, BO 197 (T. Gibson, 514), BO 319
S310	' <i>B. sphaericus</i> var. <i>rotans</i> ', H. J. Somerville, Shell, UK, T216
Strains assigned to cluster 5 (<i>Bacillus macerans</i>)	
S185	<i>B. macerans</i> , T. R. G. Gray, University of Essex, UK, NCIB 7588
S186–S189, *S191	<i>B. macerans</i> , NCIB 8160, NCIB 8210, NCIB 8930, NCIB 10443, NCIB 9368
S192	<i>B. macerans</i> , H. J. Somerville, T521
S193–S195	<i>B. macerans</i> , WR 1013, WR 1014, WR 2614 (colonial variant of WR 1014)
Strains assigned to cluster 6 (<i>Bacillus circulans sensu stricto</i>)	
S89, S91	<i>B. circulans</i> , P. A. Hartman, Iowa State University, Ames, USA, NRRL B-381, NRRL B-380
*S92, S95	<i>B. circulans</i> , J. R. Norris, BO 004 (NCTC 2610), BO 196 (NCTC 5849)
S109	<i>B. circulans</i> , NCIB 9555
Strains assigned to cluster 7 (<i>Bacillus pabuli</i>)	
S101, S102	<i>B. circulans</i> , J. R. Norris, BO 317 (T. Gibson, 261), BO 318 (T. Gibson, 287)
Strains assigned to cluster 8 (<i>Bacillus polymyxa</i>)	
S247, S249, S280	<i>B. polymyxa</i> , P. A. Hartman, IA 32 (J. C. Ayres, B-57-3B), ATCC 8523, IA 56
*S251	<i>B. polymyxa</i> , NCIB 8158
S254–S256, S258–S261	<i>B. polymyxa</i> , WR 1417, WR 1756, WR 1966, WR 2161 (banana skin), WR 2179, WR 2186 (garden soil), WR 2494 (potato)
Strains assigned to cluster 9 (<i>Bacillus circulans</i>)	
S88	<i>B. circulans</i> , P. A. Hartman, NRRL B-378
S97, S104	<i>B. circulans</i> , J. R. Norris, BO 266 (NCTC 7578), BO 320 (T. Gibson, 48)
Strains assigned to cluster 10 (<i>Bacillus circulans</i>)	
S99, S100, S105	<i>B. circulans</i> , J. R. Norris, BO 305 (T. Gibson, 137), BO 306 (T. Gibson, 255), BO 321 (T. Gibson, 38)
Single-member clusters	
S90	<i>B. circulans</i> , P. A. Hartman, NRRL B-395
S98	<i>B. circulans</i> , J. R. Norris, BO 267; NCTC 9432
S106	<i>B. circulans</i> , J. R. Norris, BO 322; T. Gibson, 92
S107	<i>B. circulans</i> , J. R. Norris, BO 323; T. Gibson, 279
S110	' <i>B. filicolonicus</i> ', J. R. Norris, BO 322; T. Gibson, 92

Table 1 (*continued*)

Cluster-group B1

Strains assigned to cluster 11 (*Bacillus cereus*/*Bacillus thuringiensis*)Subcluster 11A (*'Bacillus thuringiensis* var. *finitimus*'

- S120 *'B. finitimus'*, J. R. Norris, BO 308; T. Gibson, 1316
 S332 *'B. thuringiensis* var. *alesti'*, P. A. Hartman, BT-3 (serotype 3a)
 S121 *'B. thuringiensis* var. *finitimus'*, P. A. Hartman, BT-2

Subcluster 11B (*Bacillus cereus*)

- S66 *B. cereus*, T. R. G. Gray, B20; NCTC 6474
 S297 *'B. sotto'*, J. R. Norris, BO 021

Subcluster 11C (*Bacillus cereus*)

- S58 *B. cereus*, B. Austin, Heriot-Watt University, Edinburgh, UK, SA 15; swan faeces
 S62, S63 *B. cereus*, P. A. Hartman, IA 36 (Y. L. Quinn; ATCC 11778), NRRL B-344
 S67 *B. cereus*, T. R. G. Gray, B21; NCTC 7464
 S68 *B. cereus*, R. J. Gilbert, Central Public Health Laboratories, Colindale, London, UK, 3502/73; fried rice (serotype 5)
 S72 *B. cereus*, R. J. Gilbert, 4433/73; meat loaf
 S86 *'B. cereus* var. *terminalis'*, WR 7100

Subcluster 11D (*Bacillus cereus*)

- *S60 *B. cereus*, J. R. Norris, BO 002; DSM 31
 S61, S76 *B. cereus*, P. A. Hartman, IA 27, NRS 996
 S74 *B. cereus*, R. J. Gilbert, 4746/77; fried rice (serotype 1)
 S75 *B. cereus*, H. J. Somerville, TI 87; strain T
 S77 *'B. cereus* var. *albolactis'*, NCIB 5097
 S79 *'B. cereus* var. *fluorescens'*, H. J. Somerville, TI 53; NCIB 2600
 S81 *'B. cereus* var. *mycoides'*, NCTC 2603
 S470 *'B. thuringiensis* var. *dendrolinus'*, J. R. Norris, 10; H. Dulmage, 37 (serotype 4ab)

Subcluster 11E (*Bacillus thuringiensis*)

- S64 *B. cereus*, NCIB 6349
 S334 *B. thuringiensis*, WR 4138
 S476, S477 *'B. thuringiensis* var. *aizawai'*, J. R. Norris, 16 (H. Dulmage, 227), 17 (H. Dulmage, 137) (serotype 7)
 S471 *'B. thuringiensis* var. *benyae'*, J. R. Norris, 11; H. Dulmage, 136 (serotype 4ac)
 S472 *'B. thuringiensis* var. *galleriae'*, J. R. Norris, 12; H. Dulmage, 273 (serotype 5ab)
 S466, S467 *'B. thuringiensis* var. *kurstaki'*, J. R. Norris, 6 (H. Dulmage, 187), 7 (H. Dulmage, 89) (serotype 3ab)
 S468 *'B. thuringiensis* var. *sotto'*, J. R. Norris, 8; H. Dulmage 5 (serotype 4ab)
 S478 *'B. thuringiensis* var. *tolworthi'*, J. R. Norris, 18; H. Dulmage, 125 (serotype 9)

Subcluster 11F (*Bacillus cereus*)

- S70, S71 *B. cereus*, R. J. Gilbert, 4370/75 (serotype 6; barbecued chicken), 4431/73 (serotype 8; Indonesian rice dish)

Subcluster 11G (*Bacillus thuringiensis*)

- S335 *B. thuringiensis*, WR 5751
 *S336 *B. thuringiensis*, T. R. G. Gray, B76; NCIB 9134
 S337 *B. thuringiensis*, H. J. Somerville, T 537 (serotype 1)
 S464, S465 *'B. thuringiensis* var. *alesti'*, J. R. Norris, 4 (H. Dulmage, 10), 5 (H. Dulmage, 104)
 S331 *'B. thuringiensis* var. *berliner'*, P. A. Hartman, BT-1; Bonnefoi, BT-1
 S469 *'B. thuringiensis* var. *dendrolinus'*, J. R. Norris, 9; H. Dulmage, 106 (serotype 4b)
 S474 *'B. thuringiensis* var. *entomocidus'*, J. R. Norris, 14; H. Dulmage, 9 (serotype 6)
 S463 *'B. thuringiensis* var. *finitimus'*, J. R. Norris, 3; H. Dulmage, 3
 S473 *'B. thuringiensis* var. *galleriae'*, J. R. Norris, 13; H. Dulmage, 29
 S461, S462 *'B. thuringiensis* var. *thuringiensis'*, J. R. Norris, 1 (H. Dulmage, 39), 2 (H. Dulmage, 17) (serotype 7)

Subcluster 11H (*Bacillus cereus*/*Bacillus thuringiensis*)

- S69, S73 *B. cereus*, R. J. Gilbert, 3605/73 (serotype 3; boiled rice), 4810/72 (serotype 1; vomit)
 S78 *'B. cereus* subsp. *albolactis'*, NCIB 8079
 S475 *B. thuringiensis*, J. R. Norris, 15

Subcluster 11I (*'Bacillus cereus* var. *mycoides'*)

- S80 *'B. cereus* var. *mycoides'*, NCIB 926
 S83-S85 *'B. cereus* var. *mycoides'*, WR 1541, WR 2500, WR 2528
 S482 *'B. cereus* var. *mycoides'*, H. J. Somerville, T193

Table 1 (continued)

Strains assigned to cluster 12 (<i>Bacillus psychrosaccharolyticus</i>)	
S438, S439	' <i>B. psychrosaccharolyticus</i> ', J. L. Stokes, T25B, T27B; soil
Strains assigned to cluster 13 (<i>Bacillus laterosporus</i>)	
S145–S148, S150	<i>B. laterosporus</i> , J. R. Norris, BO 026, BO 115 (T. Gibson, 308), BO 116 (T. Gibson, 1066), BO 262, BO 309 (T. Gibson, 1080)
S151	<i>B. laterosporus</i> , WR 2197
*S152, S154	<i>B. laterosporus</i> , NCIB 8215, NCIB 11046
S289	' <i>B. pycnoticus</i> ', J. R. Norris, BO 311 (T. Gibson, 51)
Single-member clusters	
S65	<i>B. cereus</i> , NCIB 8705
S348	<i>Bacillus</i> sp., G. J. Bonde, 1 (cluster IIC; <i>B. cereus</i>)
S363	<i>Bacillus</i> sp., G. J. Bonde, 354 (cluster IIC; <i>B. cereus</i>)
S366	<i>Bacillus</i> sp., G. J. Bonde, 372 (cluster IIC; <i>B. cereus</i>)
Cluster-group B2	
Strains assigned to cluster 14 (<i>Bacillus amyloliquefaciens</i>)	
*S18–S20	<i>B. amyloliquefaciens</i> , F. E. Young, University of Rochester, NY, USA, F (L. L. Campbell, F), H (L. L. Campbell, H), K (L. L. Campbell, K)
S21	<i>B. amyloliquefaciens</i> , NCIB 10785
S24	<i>B. amyloliquefaciens</i> , J. R. Norris, 30; ATCC 23843
S23	<i>B. subtilis</i> , J. R. Norris, 29, T. Kaneko, N
S234	<i>B. subtilis</i> , ABM Chemical Ltd, Stockport, UK, B20; amylase fermentation
S312	<i>B. subtilis</i> , P. A. Hartman, JR 8; J. Robyt, amylase preparation
S352	<i>Bacillus</i> sp., G. J. Bonde, 50 (cluster 3A)
Strains assigned to cluster 15 (<i>Bacillus subtilis</i>)	
S30	' <i>B. atterimus</i> ', J. R. Norris, BO 096; T. Gibson, 525
S22	<i>B. megaterium</i> , J. R. Norris, 25; T. Kaneko, 203
S290	' <i>B. pycnoticus</i> ', J. R. Norris, BO 322
S31, *S316, S317	<i>B. subtilis</i> , NCIB 2591, NCIB 3610, NCIB 8054
S339, S340	' <i>B. vulgatus</i> ', NCIB 8063, NCIB 8802
S311, S315	<i>B. subtilis</i> , P. A. Hartman, IA 5, W 23
S321, S322	<i>B. subtilis</i> , J. R. Norris, 6 (T. Gibson, 1115), 7 (T. Gibson, 1137)
S32	' <i>B. atterimus</i> ', WR 2192; NCIB 8055
S359, S362	<i>Bacillus</i> sp., G. J. Bonde, 177 (cluster 2B; <i>B. megaterium</i>), 315 (cluster 4A; <i>B. subtilis</i>)
Strains assigned to cluster 16 (' <i>Bacillus subtilis</i> var. <i>niger</i> ')	
S223, S224	' <i>B. niger</i> ', J. R. Norris, BO 099 (T. Gibson, 1208), BO 098 (T. Gibson, 1007)
S225	' <i>B. subtilis</i> var. <i>niger</i> ', E. Hemphill, Syracuse University, NY, USA, 1000
Strains assigned to cluster 17 (<i>Bacillus circulans</i>)	
S87	<i>B. circulans</i> , P. A. Hartman, NRRL B-377
S351	<i>Bacillus</i> sp., G. J. Bonde, 47 (cluster IIIA)
Strains assigned to cluster 18 (<i>Bacillus subtilis</i>)	
S319	<i>B. subtilis</i> , J. R. Norris, 2; T. Gibson, 636
S323	<i>B. subtilis</i> , WR 2745
Strains assigned to cluster 19 (<i>Bacillus pumilus</i>)	
S208, S273, S274	<i>B. megaterium</i> , B. Austin, SA 217, SA 232, SA 218; swan faeces
S209, S275, S276	<i>B. megaterium</i> , B. Austin, CGA 59, G-2-P, CGA 28; Canada goose faeces
S278, *S279	<i>B. pumilus</i> , T. R. G. Gray, B46 (NCTC 7576), B47 (NCTC 8241)
S281, S282	<i>B. pumilus</i> , P. A. Hartman, NRRL B-3275, NRS 630
S283, S284	<i>B. pumilus</i> , NCTC 2595, NCTC 2596
S285–S288	<i>B. pumilus</i> , J. R. Norris, 10 (T. Gibson, 1130), 12 (T. Gibson, 10), 13 (T. Gibson, 47), 14 (T. Gibson, 67)
S354, S355, S358, S361	<i>Bacillus</i> sp., G. J. Bonde, 86 (cluster IV, <i>B. pumilus</i>), 88 (cluster IVB; <i>B. pumilus</i>), 174 (cluster IIB; <i>B. megaterium</i>), 293 (cluster 2C, <i>B. cereus</i>)
Strains assigned to cluster 20 (<i>Bacillus licheniformis</i>)	
S167	<i>B. licheniformis</i> , P. A. Hartman, 9945A; C. B. Thorne, 9945A

Table 1 (continued)

*S168-S173	<i>B. licheniformis</i> , J. R. Norris, 17 (DSM 13), 18 (T. Gibson, 1174), 20 (T. Gibson, 1174), 22 (T. Gibson, 1160), 23 (T. Gibson, 5), 24 (T. Gibson, 1158)
S174-S180	<i>B. licheniformis</i> , NCIB 6816, NCIB 7224, NCIB 8061, NCIB 8537, NCIB 8549, NCIB 8874, NCIB 9668
S181-S183	<i>B. licheniformis</i> , NCTC 962, NCTC 1097, NCTC 2120
S314	<i>B. subtilis</i> , P. A. Hartman, R66-A
S338	<i>B. subtilis</i> , NCIB 9536
Strains assigned to cluster 21 (<i>Bacillus</i> sp.)	
S356, S357	<i>Bacillus</i> sp., G. J. Bonde, 127 (cluster V), 128 (cluster V)
Strains assigned to cluster 22 (<i>Bacillus megaterium</i>)	
S205	<i>B. megaterium</i> , B. Austin, SA 174; swan faeces
S216-S218	<i>B. megaterium</i> , J. R. Norris, BO 075 (T. Gibson, 386), BO 076, BO 077 (T. Gibson, 732)
S215	<i>B. megaterium</i> , P. A. Hartman, NRRL B-348
S219	<i>B. megaterium</i> , J. R. Norris, BO 078, T. Gibson, 186
S220-*S222	<i>B. megaterium</i> , NCIB 7581, NCIB 8291, NCIB 9376
S296	' <i>B. silvaticus</i> ', NCIB 8674
S379	' <i>B. malabarensis</i> ', NCTC 5637
Strains assigned to cluster 23 (<i>Bacillus flexus</i>)	
S211, S212	<i>B. megaterium</i> , R. E. Gordon, NRS 602 (J. R. Porter; G. Brederman; ' <i>B. agrestis</i> '), NRS 665 (B. S. Henry, ' <i>B. flexus</i> ', 131)
Strains assigned to cluster 24 (<i>Bacillus firmus</i>)	
S412	<i>B. firmus</i> , WR 3389; R. E. Gordon, NRS 1147
S347	<i>Bacillus</i> sp., A. Boeyé, Vrije Universiteit, Brussels, Belgium, VUB 231 (group B1); North Sea sediment
Single-member clusters	
S137	' <i>B. globigii</i> ', P. A. Hartman, IA 30
S184	' <i>B. longissimus</i> ', J. R. Norris, BO 339
S202	' <i>B. maroccanus</i> ', NCIB 10500
S343	<i>Bacillus</i> sp., A. Boeyé, VUB 72 (group A2); North Sea sediment
S346	<i>Bacillus</i> sp., A. Boeyé, VUB 211 (group A1); North Sea sediment
Cluster-group C	
Strains assigned to cluster 25 (<i>Bacillus firmus</i>)	
S122, *S123	<i>B. firmus</i> , NCIB 8162, NCIB 9366 (NRS 613)
S126, S127, S129, S130, S131	<i>B. firmus</i> , WR 3317 (R. E. Gordon, NRS 858), WR 3318 (R. E. Gordon, NRS 861), WR 3320 (R. E. Gordon, NRS 860), WR 3384 (R. E. Gordon, NRS 1070), WR 3385 (R. E. Gordon, NRS 1131)
S132	<i>B. firmus</i> , H. J. Somerville, T 544
Strains assigned to cluster 26 (<i>Bacillus</i> sp.)	
S413	<i>B. firmus</i> , WR 3390
S483	<i>Bacillus</i> sp., NRS 1151
Strains assigned to cluster 27 (<i>Bacillus</i> sp.)	
S342, S344	<i>Bacillus</i> sp., A. Boeyé, VUB 33 (cluster B4), VUB 73 (cluster B2); North Sea sediment
S497, S492, S487, S494	<i>Bacillus</i> sp., R. E. Gordon, NRS 1574, NRS 1565, NRS 1570, NRS 1566; M. Turner, SM 34, SM 23, SM 29, SM24; salt marsh
Strains assigned to cluster 28 (<i>Bacillus</i> sp.)	
S482, S495	<i>Bacillus</i> sp., R. E. Gordon, NRS 1147, NRS 1569
Strains assigned to cluster 29 (<i>Bacillus</i> sp.)	
S484, S488-S490	<i>Bacillus</i> sp., R. E. Gordon, NRS 1329, NRS 1572, NRS 1575, NRS 1149 (H. W. Renszer, 1124)
Strains assigned to cluster 30 (<i>Bacillus pantothenicus</i>)	
S227, S228	<i>B. pantothenicus</i> , J. R. Norris, BO 183, BO 184
S230	<i>B. pantothenicus</i> , NCIB 8775
S231, S233-S237	<i>B. pantothenicus</i> , WR 3019, WR 3023, WR 3024, WR 3026, WR 3028, WR 3043; soil

Table 1 (continued)

Strains assigned to cluster 31 (<i>'Bacillus carotarum'</i> sensu Gibson)	
S51–S55	<i>'B. carotarum'</i> , J. R. Norris, BO 079, BO 080, BO 081, BO 272, BO 303; T. Gibson, 148, 242, 511, 21 (NCIB 4821), 122
Strains assigned to cluster 32 (<i>Bacillus simplex</i>)	
S210	<i>'B. simplex'</i> , R. E. Gordon, NRS 335
S213	<i>'B. teres'</i> , R. E. Gordon, NRS 986
Strains assigned to cluster 33 (<i>Bacillus</i> sp.)	
S498, S499	<i>B. megaterium</i> , R. E. Gordon, NRS 608, NRS 828
S485, S486	<i>Bacillus</i> sp., NRS 1369, NRS 1370
Strains assigned to cluster 34 (<i>Bacillus pulvifaciens</i>)	
S264, S265	<i>B. pulvifaciens</i> , WR 3622 (W. C. Haynes, NRS 1283), WR 3623 (W. C. Haynes, NRS 1285)
S266	<i>B. pulvifaciens</i> , H. de Barjac, Institut Pasteur, Paris, strain LES; human origin
Strains assigned to cluster 35 (<i>'Bacillus cascainensis'</i>)	
S416, S418–S422	<i>'B. cascainensis'</i> , ATCC 11968; R. E. Gordon, NRS 1471, NRS 1473, NRS 1474a, NRS 1475, NRS 1474b
S417	<i>'B. cascainensis'</i> , R. E. Gordon, NRS 1470
Single-member clusters	
S56	<i>'B. carotarum'</i> , J. R. Norris, BO 314
S114	<i>'B. epiphytus'</i> , J. R. Norris, BO 293
S341	<i>'B. loehnisii'</i> , NCTC 4825
S197	<i>'B. macroides'</i> , J. R. Norris, BO 204; ATCC 12905
S226	<i>'B. pacificus'</i> , J. R. Norris, BO 291
S378	<i>Sporolactobacillus inulinus</i> , NCIB 9743
S493	<i>Bacillus</i> sp., R. E. Gordon, NRS 1562
S496	<i>Bacillus</i> sp., R. E. Gordon, NRS 1573
Cluster-group D	
Strains assigned to cluster 36 (<i>'Bacillus aneurinolyticus'</i>)	
S25–S29	<i>'B. aneurinolyticus'</i> , J. R. Norris, BO 205 (ATCC 12866), BO 206 (NRS 1448), BO 207 (NRS 1450), BO 208 (NRS 1450), BO 209 (NRS 1451)
Strains assigned to cluster 37 (<i>Bacillus brevis</i>)	
S35–S37	<i>B. brevis</i> , J. R. Norris, BO 118 (T. Gibson, 539), BO 117 (T. Gibson, 442), BO 270 (NCTC 7096)
S38, *S39	<i>B. brevis</i> , NCIB 8803, NCIB 9372
S40	<i>B. brevis</i> , T. R. G. Gray, B54; NCTC 7577
S42, S44–S47	<i>B. brevis</i> , WR 2904, WR 2922, WR 2932, WR 2934, WR 3005; soil
Strains assigned to cluster 38 (<i>Bacillus azotoformans</i>)	
S423–S428	<i>B. azotoformans</i> , F. Pichinoty, UER Scientifique de Huming, Marseilles, France, 1, 2, 9, 32, 34, 36; garden soil
Strains assigned to cluster 39 (<i>Bacillus badius</i> / <i>'Bacillus freudenreichii'</i>)	
S33, S34	<i>B. badius</i> , J. R. Norris, BO 180 (NCTC 10333), BO 201 (M. D. Appleman, NRS 1407)
S133, S380	<i>'B. freudenreichii'</i> , J. R. Norris, BO 200 (ATCC 7053), BO 199 (T. Gibson, 68)
Strains assigned to cluster 40 (<i>Bacillus psychrophilus</i>)	
*S433–S436	<i>B. psychrophilus</i> , J. L. Stokes, Washington State University, USA, W16A (soil), W3 (river water), W5 (soil), W70A
S441	<i>Bacillus</i> sp., J. L. Stokes, T75
Strains assigned to cluster 41 (<i>Bacillus brevis</i>)	
S48, S49	<i>B. brevis</i> , WR 3006, WR 3010
Strains assigned to cluster 42 (<i>Bacillus fusiformis</i>)	
S134–S136	<i>'B. fusiformis'</i> , J. R. Norris, BO 297 (T. Gibson, 1014), WR 2009, WR 2520
S301	<i>B. sphaericus</i> , T. R. G. Gray, B22; NCTC 7582

Table 1 (*continued*)

Strains assigned to cluster 43 (<i>Bacillus sphaericus</i>)	
S298, *S300, S350	<i>B. sphaericus</i> , NCIB 8216, NCIB 9370, G. J. Bonde, 13
S302	<i>B. sphaericus</i> , P. A. Hartman, NRS 348
S303–S307	<i>B. sphaericus</i> , WR 1652, WR 2105, WR 2205, WR 2518, WR 2594
Single-member clusters	
S430	<i>B. globisporus</i> , T. L. Stokes, W8
S432	<i>B. insolitus</i> , T. L. Stokes, W16B
S295	' <i>B. repens</i> ', J. R. Norris, BO 301
S299	' <i>B. sphaericus</i> var. <i>rotans</i> ', NCIB 8867
S349	<i>Bacillus</i> sp., G. J. Bonde, 6 (cluster IIA)
S353	<i>Bacillus</i> sp., G. J. Bonde, 52 (cluster I)
S365	<i>Bacillus</i> sp., G. J. Bonde, 453 (cluster IIAT)
Cluster-group E	
Strains assigned to cluster 44 (<i>Bacillus lentus</i>)	
S156	<i>B. firmus</i> , R. E. Gordon, NRS 769
S164	<i>B. firmus</i> , WR 3321, R. E. Gordon, NRS 769
*S155	<i>B. lentus</i> , NCIB 8773; NRS 670; T. Gibson, 165
S158, S159	<i>B. lentus</i> , R. E. Gordon, NRS 883 (T. Gibson, 165), NRS 1262 (T. Gibson, 258)
S160	<i>B. lentus</i> , J. R. Norris, BO 179; T. Gibson, 238
S165, S166	<i>B. lentus</i> , WR 3322 (R. E. Gordon, NRS 749), WR 3323
Strains assigned to cluster 45 (<i>Bacillus macquariensis</i>)	
*S199, S201	<i>B. macquariensis</i> , J. R. Norris, BO 188 (NCTC 10419), BO 190 (NCTC 10421)
Single-member cluster	
S163	<i>B. lentus</i> , WR 2789
Cluster-group F	
Strains assigned to cluster 46 (<i>Bacillus coagulans sensu stricto</i>)	
S443, S447, S448	<i>B. coagulans</i> , J. Wolf, University of Leeds, UK, C77, C12, C88
S445, S449	<i>B. coagulans</i> , WR 2972, WR 2822; soil
Strains assigned to cluster 47 (<i>Bacillus coagulans</i>)	
S444	<i>B. coagulans</i> , J. Wolf, C32
S446	<i>B. coagulans</i> , unknown origin
S450	<i>B. coagulans</i> , WR 2974; mud
Strains assigned to cluster 48 (<i>Bacillus stearothermophilus sensu stricto</i>)	
S454–S456	<i>B. stearothermophilus</i> , J. Wolf, T128, T168, T210
S459	<i>B. stearothermophilus</i> , WR 4592
Strains assigned to cluster 49 (<i>Bacillus kaustophilus</i>)	
S451, S452, S457	<i>B. stearothermophilus</i> , T1, T39, T349
S458, S460	<i>B. stearothermophilus</i> , WR 4591, WR 4288
Single-member clusters	
S113	' <i>B. cirroflagellosus</i> ', J. R. Norris, BO 290
S453	<i>B. stearothermophilus</i> , J. Wolf, T76

IIC (*B. cereus*) were also included in this cluster. *B. pumilus* strains are readily distinguished from others in the 'subtilis group' by being unable to hydrolyse starch or reduce nitrate.

Several minor clusters contained organisms that shared a high overall similarity with both the *B. subtilis* and *B. pumilus* strains. Three strains of '*B. subtilis* var. *niger*' formed a homogeneous cluster in both the S_{SM} and D_P analyses. These organisms were not pigmented on nutrient agar and showed no consistent single features that allowed them to be distinguished from typical strains of *B. subtilis*. Similarly, a single strain of '*B. globigii*', often considered to be closely related to either *B. subtilis* or *B. licheniformis*, was recovered as a single-member cluster in this area. Cluster 18 contained two strains received as *B. subtilis*; these organisms were unusual in being

unable to produce acid from xylose, salicin and mannose. A single marine isolate, a representative of cluster A2 of Boeyé & Aerts (1976), was recovered between the *B. pumilus* and *B. licheniformis* clusters. Cluster A2 strains were described as '*B. pumilus*/*B. licheniformis* intermediates' in the original publication.

Sixteen strains of *B. licheniformis*, which formed a tight group at 91% S_{SM} , fused with three additional strains to form cluster 20. These strains on the periphery of cluster 20 were *B. subtilis* NCIB 9536, originally deposited as '*B. tinakiensis*', *B. licheniformis* NCIB 9668 and a strain received as *B. subtilis* R66-A. Cluster 20 conformed to the standard description of *B. licheniformis*.

Eleven strains of *B. megaterium* formed a fairly diffuse taxon (cluster 22) that showed a relatively close affinity with the '*B. subtilis* group'. This cluster included the type strain of *B. megaterium*, and strains labelled '*B. malabarensis*' and '*B. silvaticus*'. The cluster 22 strains formed large cells and conformed to the current description of *B. megaterium sensu stricto*, i.e. they were strictly aerobic, degraded a variety of polysaccharides, were predominantly urease positive and mainly Voges-Proskauer negative. Several minor clusters were associated with the *B. megaterium* taxon. Cluster 21 contained two marine isolates representing Bonde's (1975) group V (*B. licheniformis*); '*B. longissimus*' S184 and '*B. maroccanus*' S202 were recovered as single-member clusters and may represent new centres of variation. Two clusters which fused at 83% S_{SM} were peripherally associated with the *B. megaterium* cluster. Cluster 23 contained strains originally labelled '*B. agrestis*' and '*B. flexus*'. Strains in these taxa have been considered to belong to the species *B. megaterium* (Gordon *et al.*, 1973). They can be distinguished from *B. megaterium sensu stricto* as they do not hydrolyse aesculin or form acid from arabinose or xylose. A strain of *B. firmus* and a marine isolate from group B1 of Boeyé & Aerts (1976), a cluster thought to be related to *B. firmus*, comprised cluster 24.

Cluster-group C contained *B. firmus*, *B. pantothenicus* and a number of unnamed or poorly described strains. Eight strains of *B. firmus* were assigned to a tight taxon (cluster 25) which had the recognized characteristics of this species. These bacteria formed oval, central spores that did not distend the sporangium, were obligately aerobic, produced acid from a restricted range of sugars, and reduced nitrate. '*B. epiphytus*' S114 was recovered on the periphery of the *B. firmus* cluster in the S_{SM} and D_P analyses but seemed sufficiently dissimilar not to be included. Clusters 26 to 29 contained organisms described by Gordon *et al.* (1977) as '*B. firmus*-*B. lentus* intermediates'. It is presently difficult to identify features that will distinguish these clusters, although acid production from sugars might be useful. Single-member clusters representing salt-marsh isolates of the so-called '*B. firmus*-*B. lentus* spectrum' were also recovered in this area of the dendrogram, as was '*B. pacificus*' S226. Cluster 27 included two marine isolates that were assigned to clusters B2 and B4, both equated with *B. firmus*, by Boeyé & Aerts (1976).

Nine strains of *B. pantothenicus* comprised cluster 30. These NaCl-tolerant bacteria had a variable spore morphology but oval spores predominated. They grew anaerobically and produced acid from a restricted range of sugars. Strains labelled '*Bacillus loehnisii*' are generally considered to belong to the species *B. sphaericus* but the single strain bearing this name in the present study was recovered as a single-member cluster near the *B. pantothenicus* taxon. Five strains received as '*B. carotarum*' constituted cluster 31, with the sixth strain on the periphery of this cluster. All six strains contained oval central spores with some swelling of the sporangium and produced acid from a limited range of sugars; some were urease positive. '*B. simplex*' and '*B. teres*' are often considered to be closely related to *B. megaterium*. Strains bearing these names were assigned to cluster 32; they were distinguished from *B. megaterium* by reducing nitrate and failing to hydrolyse aesculin, pullulan or urea. A single isolate of '*B. macroides*' was recovered adjacent to cluster 33; the latter contained two strains received as *B. megaterium* and two '*B. firmus*-*B. lentus* intermediates'.

Cluster 34 encompassed three strains of *B. pulvifaciens* which showed 77% similarity (S_{SM}) with the sole isolate of *Sporolactobacillus inulinus* examined. The *B. pulvifaciens* strains produced oval, central spores that distended the sporangium, and produced acid from a restricted range of sugars.

Table 2. A comparison of the composition of cluster-groups from several taxometric analyses of the genus *Bacillus*

For clarity, only principal taxa in cluster-groups are given. For full composition of cluster-groups from the S_{SM} /UPGMA analysis, see Table 1. The cluster-group designations used by Priest *et al.* (1981) and Logan & Berkeley (1981) are shown in parentheses. NI, Not included.

S_{SM}	D_P	S_I	Priest <i>et al.</i> (1981)	Logan & Berkeley (1981)
Cluster-group A				
<i>B. alvei</i>	<i>B. alvei</i>	<i>B. alvei</i>	<i>B. alvei</i> (6)	<i>B. circulans</i> (V)
<i>B. circulans</i>	<i>B. circulans</i>	<i>B. circulans</i>	<i>B. circulans</i> (6)	NI
' <i>B. apiarius</i> '	' <i>B. apiarius</i> '	' <i>B. apiarius</i> '		
' <i>B. thiaminolyticus</i> '	' <i>B. thiaminolyticus</i> '	' <i>B. thiaminolyticus</i> '		
<i>B. macerans</i>	<i>B. macerans</i>	<i>B. macerans</i>	<i>B. macerans</i> (6)	<i>B. macerans</i> (V)
<i>B. polymyxa</i>	<i>B. macquariensis</i>		<i>B. polymyxa</i> (6)	<i>B. polymyxa</i> (V)
			<i>B. coagulans</i> (6)	<i>B. macquariensis</i> (V)
Cluster-group B (I and II)				
<i>B. cereus</i>	<i>B. cereus</i>	<i>B. cereus</i>	<i>B. cereus</i> (5A)	<i>B. cereus</i> (I)
<i>B. laterosporus</i>	<i>B. laterosporus</i>	<i>B. laterosporus</i>		
<i>B. amyloliquefaciens</i>	<i>B. amyloliquefaciens</i>	<i>B. amyloliquefaciens</i>	<i>B. amyloliquefaciens</i> (5B)	<i>B. amyloliquefaciens</i> (IV)
<i>B. subtilis</i>	<i>B. subtilis</i>	<i>B. subtilis</i>	<i>B. subtilis</i> (5B)	<i>B. subtilis</i> (IV)
<i>B. pumilus</i>	<i>B. pumilus</i>	<i>B. pumilus</i>	<i>B. pumilus</i> (5B)	<i>B. pumilus</i> (IV)
<i>B. licheniformis</i>	<i>B. licheniformis</i>	<i>B. licheniformis</i>	<i>B. licheniformis</i> (5B)	<i>B. licheniformis</i> (IV)
<i>B. megaterium</i>	<i>B. megaterium</i>	<i>B. megaterium</i>	<i>B. megaterium</i> (5B)	<i>B. megaterium</i> (IV)
<i>B. psychrosaccharolyticus</i>	<i>B. psychrosaccharolyticus</i>	<i>B. psychrosaccharolyticus</i>		NI
	' <i>B. cascainensis</i> '			
	<i>B. badius</i>			
	' <i>B. freudenreichii</i> '			
	<i>B. pulvifaciens</i>	<i>B. pulvifaciens</i>		NI
	<i>B. polymyxa</i>	<i>B. polymyxa</i>		
Cluster-group C				
<i>B. firmus</i>	<i>B. firmus</i>	<i>B. firmus</i>	<i>B. firmus</i> (I)	<i>B. firmus</i> (II)
<i>B. pantothenicus</i>			<i>B. pantothenicus</i> (I)	
' <i>B. carotarum</i> '			NI	' <i>B. carotarum</i> ' (II)
<i>B. pulvifaciens</i>				NI
' <i>B. cascainensis</i> '			' <i>B. cascainensis</i> ' (I)	NI
			<i>B. lentus</i> (I)	<i>B. lentus</i> (II)
			<i>B. psychrophilus</i> (I)	
				<i>B. brevis</i> (II)
				<i>B. badius</i> (II)
				' <i>B. aneurinolyticus</i> ' (II)
				<i>B. alvei</i> (II)
				<i>B. laterosporus</i> (II)
				' <i>B. thiaminolyticus</i> ' (II)

Table 3. *Percentage distribution of positive characters to cluster-groups defined at the 70% level (S_{SM})*

Cluster-group* . . . Number of strains . . .	A 59	B 154	C 52	D 46	E 10	F 17
<i>Colonial morphology</i>						
1. Flat/raised	90	74	61	76	60	94
2. Smooth	97	66	100	100	100	94
3. Rhizoidal	0	5	0	0	20	0
4. Entire	36	32	86	74	80	59
5. Opaque	61	97	96	89	0	35
6. Pigmented	3	4	27	0	0	0
7. Motile colonies	30	0	0	0	0	0
<i>Cellular morphology</i>						
8. Length > 3 μ m	75	45	21	67	10	47
9. Diameter > 0.9 μ m	0	42	8	15	0	0
10. Ends round	90	100	98	100	90	100
11. Single	76	33	33	76	30	71
12. Vacuoles present	0	41	4	0	0	0
13. Gram-variable	7	95	73	33	80	59
14. Gram-positive	0	58	29	6	40	6
15. Spores oval	100	99	94	80	100	100
16. Spores round	0	0	11	28	0	0
17. Spores central	24	99	65	59	100	35
18. Spores terminal	75	3	21	50	0	59
19. Spores bulging	47	92	25	87	20	76
20. Sporulation 24 h	10	17	0	35	0	100
21. Sporulation 72 h	86	88	70	87	60	100
22. Sporulation 120 h	100	99	81	96	80	100
23. Sporulation SxA†	100	99	94	98	100	100
<i>Degradation of:</i>						
24. Adenine	0	15	2	28	0	18
25. Aesculin	100	99	65	7	100	94
26. Allantoin	7	16	10	15	0	0
27. Arbutin	100	100	38	15	100	100
28. Casein	68	100	100	61	0	41
29. Chitin	28	38	0	0	0	0
30. DNA	68	95	94	100	0	100
31. Elastin	10	53	13	17	0	0
32. Gelatin	90	100	100	72	0	71
33. Guanine	0	1	13	0	0	0
34. Hippurate	57	21	52	39	80	100
35. Lecithin	52	88	71	28	0	0
36. Pectin	15	28	4	0	0	0
37. Pullulan	83	64	60	0	100	53
38. Pustulan	12	1	0	0	0	0
39. RNA	80	100	81	67	0	88
40. Starch	98	75	46	0	100	100
41. Testosterone	85	1	2	50	30	29
42. Tween 20	100	100	98	72	80	100
43. Tween 80	73	80	86	65	80	47
44. Tyrosine	12	32	27	28	0	0
45. Urea	20	21	4	26	80	0
<i>Resistance to (μg ml⁻¹):</i>						
46. Benzylpenicillin (8)	3	42	11	0	20	0
47. Benzylpenicillin (4)	20	52	11	6	30	0
48. Chloramphenicol (8)	15	25	21	13	0	0
49. Chloramphenicol (4)	19	35	29	24	0	0
50. Cycloserine (128)	73	35	13	65	10	0
51. Cycloserine (64)	86	56	50	85	30	0
52. Erythromycin (1)	37	30	35	37	80	6
53. Erythromycin (0.5)	66	34	23	30	80	6

Table 3 (continued)

Cluster-group* . . . Number of strains . . .	A 59	B 154	C 52	D 46	E 10	F 17
54. Gramicidin (64)	73	61	27	78	10	0
55. Gramicidin (32)	83	73	35	83	30	0
56. Nalidixic acid (32)	22	7	54	72	90	100
57. Nalidixic acid (16)	17	22	65	83	100	100
58. Polymyxin (16)	80	89	6	50	0	6
59. Polymyxin (8)	88	90	27	72	10	6
60. Rifampicin (0.25)	22	14	25	4	0	0
61. Rifampicin (0.125)	34	43	31	11	0	0
62. Streptomycin (16)	49	35	0	59	100	0
63. Streptomycin (8)	66	52	4	61	100	0
64. Tetracycline (2)	46	43	0	20	0	0
65. Tetracycline (1)	49	72	0	37	0	0
<i>Acid from:</i>						
66. Adonitol	36	0	0	0	0	6
67. Arabinose	49	35	0	0	0	0
68. Cellobiose	97	96	40	2	60	35
69. Dulcitol	0	0	2	0	0	0
70. Erythritol	2	0	11	0	0	18
71. Fructose	75	94	40	24	30	71
72. Galactose	85	40	25	0	60	23
73. Glucose	98	99	79	15	80	100
74. Glycerol	90	94	71	59	20	41
75. <i>meso</i> -Inositol	30	35	2	0	0	29
76. Lactose	73	23	19	0	80	0
77. Maltose	100	93	60	9	50	88
78. Mannitol	60	42	63	2	70	47
79. Mannose	63	59	17	0	70	71
80. Raffinose	97	48	21	2	60	23
81. Rhamnose	7	4	11	0	10	0
82. Salicin	76	94	19	0	60	18
83. Sorbitol	25	26	13	0	10	0
84. Sucrose	100	80	75	2	40	71
85. Trehalose	93	98	75	2	10	88
86. Xylose	63	43	0	0	50	35
<i>Utilization of:</i>						
87. Acetate	19	55	48	63	40	12
88. Citrate	12	74	44	30	10	59
89. Formate	34	77	44	50	20	18
90. Gluconate	49	47	27	4	30	6
91. Lactate	24	62	31	37	20	35
92. Malonate	5	10	21	4	0	6
93. Succinate	32	41	60	50	10	29
<i>Growth at:</i>						
94. pH 4.5	0	15	23	0	10	12
95. pH 6.0	78	96	98	87	100	94
96. pH 7.2	100	100	100	100	100	82
97. pH 8.0	100	100	100	100	100	35
98. pH 9.5	95	99	100	80	100	0
99. 5 °C	12	3	6	9	20	0
100. 17 °C	78	97	94	72	100	6
101. 37 °C	100	100	100	89	90	94
102. 50 °C	27	34	25	43	0	100
103. 65 °C	0	0	0	0	0	53
<i>Growth in (% w/v):</i>						
104. NaCl (2)	83	100	100	87	100	47
105. NaCl (5)	58	99	96	70	80	29
106. NaCl (10)	2	52	79	0	20	6

Table 3 (continued)

Cluster-group* . . .	A	B	C	D	E	F
Number of strains . . .	59	154	52	46	10	17
<i>Miscellaneous tests:</i>						
107. Anaerobic growth	98	52	23	0	20	57
108. Gas from glucose	36	0	0	0	0	0
109. Dihydroxyacetone production	41	40	0	4	0	0
110. Indole production	27	2	0	2	0	0
111. Growth on MacConkey agar	41	68	77	63	80	35
112. Methyl red test	78	76	6	0	20	47
113. Nitrate reduction	75	75	52	67	30	100
114. ONPG†	100	57	48	13	100	47
115. Oxidase	72	29	6	41	80	0
116. Phenylalanine deamination	0	29	32	6	0	0
117. Phosphatase	37	71	13	20	20	65
118. Voges-Proskauer reaction	36	80	0	0	0	18

* For ease of computation, these cluster-groups do not take into account data for single-member clusters.

† SxA, soil extract agar.

‡ ONPG, *o*-nitrophenyl β -D-galactoside.

The final phenon in cluster-group C comprised seven strains originally described as '*Krusella cascaianensis*' (Castellani, 1954) but subsequently transferred to *Bacillus* as '*B. cascaianensis*' (Castellani, 1955). Most of these bacteria formed endospores that were oval and central but did not swell the sporangium; their other characteristics are given in Table 4.

Cluster-group D contained the alkali-forming strains that have limited, if any, reaction in sugar-containing media. Five strains of '*B. aneurinolyticus*' formed a homogeneous group (cluster 36) that was closely related to *B. brevis* (cluster 37). Both of these taxa accommodated strains with oval central spores that distended the sporangium. They were obligate aerobes, reduced nitrate and with the occasional exception did not produce acid from carbohydrates. The two species were distinguished by the failure of '*B. aneurinolyticus*' strains to grow in 5% (w/v) NaCl or to hydrolyse casein, gelatin or hippurate. Six strains of *B. azotoformans* were recovered close to *B. brevis* in cluster 38. These species had many features in common, but *B. azotoformans* strains can be distinguished as they are unable to grow at 50 °C and fail to hydrolyse casein, gelatin, hippurate or RNA. In the S_{SM} /UPGMA analysis, cluster 39 contained strains of *B. badius* and '*B. freudenreichii*' but these taxa were separated in the analyses based on S_j and D_p coefficients. The *B. badius* strains were negative for nitrate reduction and urease production, but hydrolysed casein and gelatin; the '*B. freudenreichii*' strains gave the opposite reactions.

Five psychrophilic isolates were assigned to cluster 40, *B. psychrophilus*. These bacteria produced spherical spores that distended the sporangium and most grew at 5 °C but not at 37 °C. Cluster 41, which was recovered in all three analyses, contained two *B. brevis* strains. Morphologically similar to *B. brevis sensu stricto*, these strains differed by degrading adenine, allantoin and elastin, and were also urease positive and did not reduce nitrate.

The *B. sphaericus* and '*B. sphaericus* var. *fusiformis*' strains were recovered in two discrete clusters in all three analyses, suggesting that the latter should be given species status as *B. fusiformis*. The remaining strains in cluster-group D were recovered as single-member clusters and included '*B. sphaericus* var. *rotans*' S299, *B. globisporus* S430, *B. insolitus* S432, '*B. repens*' S295 and three marine isolates.

Cluster-group E contained a single major cluster, *B. lentus*. The bacteria in this taxon had limited action on macromolecules, formed acid from few sugars other than glucose and produced oval central spores that did not swell the sporangium. Two strains of *B. macquariensis* were recovered in a minor cluster adjacent to *B. lentus*.

The thermophilic bacilli were recovered in cluster-group F. Eight strains of *B. coagulans* were recovered in two clusters, one of which, cluster 46, conformed to *B. coagulans sensu stricto* (Wolf

Type B; Wolf & Sharp, 1981). Similarly, the *B. stearothermophilus* strains were assigned to two major phena. Cluster 48 contained strains belonging to Groups 2 and 3 (*B. stearothermophilus* Donk) of Walker & Wolf (1971). These two groups of bacteria fused at 84% S_{SM} but were assigned to separate clusters in the S_j analysis. Thus, the characteristics shown in Table 4 may not be typical for *B. stearothermophilus sensu stricto*. Cluster 49 equated with Group 1 of Walker & Wolf (1971) (*B. kaustophilus*).

DISCUSSION

It is encouraging that the three analyses presented here and the two previous comprehensive taxometric studies of bacilli (Logan & Berkeley, 1981; Priest *et al.*, 1981) are essentially congruous despite the use of widely different data bases. Indeed, the assignment of species to cluster-groups seems to reflect a natural classification that is largely consistent with DNA base composition (Priest, 1981). The cluster-groups can be equated with genera in some groups of bacteria, but additional data derived from 16S rRNA sequencing or hybridization studies are needed before any dismemberment of the genus *Bacillus* can be proposed with confidence. For the present, the cluster-groups should be used as a framework for further taxonomic studies, and to this end their characteristics have been considered above. The ensuing discussion concentrates on species of *Bacillus* that currently present taxonomic problems.

Cluster-group A. This study confirms the heterogeneity of strains currently classified as *B. circulans*. Gibson & Topping (1938) described *B. circulans* as a 'complex' rather than a species, a view that persisted for some time (Proom & Knight, 1955; Wolf & Chowdbury, 1971; Gibson & Gordon, 1974). It is now apparent that the description of *B. circulans* encompasses a variety of genotypically unrelated bacteria. The mol% G + C of 123 strains identified as *B. circulans* varied between 37 and 61 (Nakamura & Swezey, 1983*a*), and in DNA reassociation experiments nearly half of these bacteria were assigned to 10 homology groups, while the remaining 61 strains were unclassified (Nakamura & Swezey, 1983*b*). From these studies, four species names previously considered as synonyms of *B. circulans*, namely *B. amylolyticus*, *B. lautus*, *B. pabuli* and *B. validus*, were reintroduced (Nakamura, 1984*a*). Our numerical classification included few of the strains examined by Nakamura & Swezey (1983*a, b*) but it is possible to equate the two studies. Cluster 6 contained the type strain and has properties in accord with those of *B. circulans sensu stricto* (Nakamura, 1984*a*). Further evidence for homogeneity of this cluster is indicated by the inclusion of strain S109 (NCIB 9555), which originally bore the name '*B. aporrhoeus*' but is now considered to be a synonym of *B. circulans* (Gordon *et al.*, 1973) and shares 50 to 60% DNA sequence homology with the type strain of *B. circulans* (Nakamura & Swezey, 1983*b*). Cluster 7 is similar to *B. pabuli* in most respects. Similarly, strains assigned to cluster 9 have much in common with *B. amylolyticus* and cluster 10 can perhaps be equated with *B. lautus*, although it contains strains that hydrolyse Tween 80 and do not produce acid from rhamnose. The numerical classification also underpins the taxonomic integrity of *B. alvei*, *B. macerans* and *B. polymyxa*. It is also evident that strains of '*B. apiarius*' and '*B. thiaminolyticus*' form well-circumscribed taxa which may merit species status when DNA base composition data become available.

Cluster-group B. DNA reassociation studies support the view that *B. cereus*, *B. mycoides* and *B. thuringiensis* comprise a single species (Somerville & Jones, 1972; Seki *et al.*, 1978). In this respect, it is interesting that crystal toxin synthesis is often plasmid-encoded and transmissible from *B. thuringiensis* to *B. cereus* by 'conjugation' (Gonzalez *et al.*, 1982). The numerical phenetic data underline the close relationship between *B. cereus* and *B. thuringiensis*, although strains bearing these names were largely allocated to separate subclusters within cluster 11. Some strains of *B. cereus* are responsible for diarrhoeal and emetic types of food poisoning (Gilbert, 1979) and others for quite severe medical and veterinary pathogenic conditions (Turnbull *et al.*, 1979); a serotyping scheme has been developed for the identification of these strains (Kramer *et al.*, 1982). It has been claimed that strains of *B. cereus* responsible for the emetic form of food-poisoning can be distinguished from diarrhoeal and non-food-poisoning strains by numerical

Table 4. *Percentage distribution of positive characters to major clusters defined at the 83% level (S_{SM})*

	<i>B. albei</i>	<i>'B. thiaminolyticus'</i>	<i>B. circulans</i>	<i>B. macerans</i>	<i>B. circulans sensu stricto</i>	<i>B. polymyxa</i>	<i>B. cereus/B. thuringiensis</i>	<i>B. laterosporus</i>	<i>B. anyoliquefaciens</i>	<i>B. subtilis</i>	<i>B. pumilus</i>	<i>B. licheniformis</i>
Cluster number. . .	1	3	4	5	6	8	11	13	14	15	19	20
Number of strains. . .	13	4	6	10	5	11	56	9	9	15	20	19
<i>Colonial morphology</i>												
1. Flat/smooth	100	75	100	100	100	82	98	44	56	67	75	32
2. Smooth	100	75	100	100	100	91	76	100	11	73	55	26
3. Rhizoidal	0	0	0	0	0	0	9	0	0	0	0	0
4. Entire	0	100	0	40	20	46	0	89	22	47	65	21
5. Opaque	69	75	83	0	60	73	100	89	100	93	95	100
6. Pigmented	0	0	0	0	0	0	0	0	0	0	0	5
7. Motile colonies	92	0	100	0	0	0	0	0	0	0	0	0
<i>Cellular morphology</i>												
8. Length > 3 μ m	85	0	67	70	40	82	98	67	0	0	0	0
9. Diameter > 0.9 μ m	0	0	0	0	0	0	98	0	0	0	0	0
10. Ends round	100	0	100	100	100	100	100	100	100	100	100	100
11. Single	100	100	100	60	100	0	17	89	100	80	25	16
12. Vacuoles present	0	0	0	0	0	0	100	0	0	0	0	0
13. Gram-variable	39	0	0	0	20	0	100	100	100	100	90	90
14. Gram-positive	15	0	0	0	0	0	72	100	89	80	15	42
15. Spores oval	100	100	100	100	100	100	100	100	100	100	100	100
16. Spores round	0	0	0	0	0	0	0	0	0	0	0	0
17. Spores central	23	0	0	0	20	64	100	100	100	100	100	100
18. Spores terminal	77	100	100	100	80	36	0	0	11	0	0	0
19. Spores bulging	92	100	100	100	100	91	0	89	0	0	0	0
20. Sporulation 24 h	0	0	0	20	20	9	15	0	0	7	10	0
21. Sporulation 72 h	62	100	100	90	100	100	100	89	0	87	80	100
22. Sporulation 120 h	92	100	100	100	100	100	100	89	100	100	95	100
23. Sporulation SxA	100	100	100	100	100	100	100	100	100	100	100	100
<i>Degradation of:</i>												
24. Adenine	0	0	0	0	0	0	0	0	0	60	55	5
25. Aesculin	100	100	100	100	100	100	100	67	100	100	100	100
26. Allantoin	0	100	0	0	0	0	0	83	0	0	0	63
27. Arbutin	100	100	100	100	100	100	100	100	100	100	100	100
28. Casein	100	100	83	0	0	100	100	100	100	100	100	100
29. Chitin	0	100	0	100	0	0	47	100	0	0	0	95
30. DNA	100	100	100	0	100	55	100	100	33	93	100	100
31. Elastin	0	100	0	0	20	0	9	67	100	100	100	79
32. Gelatin	100	100	67	100	100	100	100	100	100	100	100	100
33. Guanine	0	0	0	0	0	0	0	0	0	0	0	0
34. Hippurate	100	100	100	50	0	0	2	67	11	27	100	0
35. Lecithin	0	0	0	0	0	0	100	55	0	0	0	0
36. Pectin	0	0	0	0	0	64	0	0	0	27	85	95
37. Pullulan	100	100	100	50	100	100	95	0	33	60	30	47
38. Pustulan	0	0	67	0	0	0	2	0	0	0	0	0
39. RNA	100	100	100	0	100	100	100	100	100	100	100	100
40. Starch	100	100	100	100	100	100	86	0	100	100	0	100
41. Testosterone	0	0	67	0	20	0	0	0	0	0	0	0
42. Tween 20	100	100	100	100	100	100	100	100	100	100	100	100
43. Tween 80	46	0	50	100	80	100	98	100	0	87	100	100

Table 4 (continued)

Character	<i>B. megaterium</i>	<i>B. firmus</i>	<i>Bacillus</i> sp.	<i>Bacillus</i> sp.	<i>B. pantothenicus</i>	' <i>B. carotarum</i> '	<i>Bacillus</i> sp.	' <i>B. cascadenensis</i> '	' <i>B. aneurinolyticus</i> '	<i>B. brevis</i>	<i>B. azotoformans</i>	<i>B.adius</i> /' <i>B. freudenreichii</i> '	<i>B. psychrophilus</i>	<i>B. fusiformis</i>	<i>B. sphaericus</i>	<i>B. lentus</i>	<i>B. coagulans sensu stricto</i>	<i>B. stearothermophilus sensu stricto</i>	<i>B. kaustophilus</i>
	22 11	25 8	27 6	29 4	30 9	31 5	33 4	35 7	36 5	37 11	38 6	39 4	40 5	42 4	43 9	44 8	46 5	48 4	49 5
1.	73	88	17	75	100	0	0	100	100	91	83	50	100	25	56	63	100	75	100
2.	73	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	80
3.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	82	37	100	100	100	100	100	100	60	82	100	50	100	25	67	75	80	100	0
5.	100	100	100	100	100	100	100	43	100	100	83	100	60	100	100	0	80	25	0
6.	27	37	67	100	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0
7.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.	54	0	0	25	0	80	75	0	100	27	100	100	0	100	89	0	100	0	0
9.	54	0	0	0	0	60	0	0	60	0	0	50	40	0	0	0	0	0	0
10.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
11.	45	25	83	0	0	0	75	100	100	100	0	75	100	0	100	50	20	100	100
12.	54	0	0	0	0	20	25	0	0	0	0	0	0	0	0	0	0	0	0
13.	100	25	100	50	78	100	75	71	20	0	0	100	100	25	33	100	60	50	60
14.	45	12	50	25	22	20	25	29	0	0	0	25	40	0	0	63	0	25	0
15.	100	100	100	100	100	100	100	57	100	91	100	100	0	0	0	100	100	100	100
16.	0	0	17	0	11	0	0	0	0	9	0	0	100	100	100	0	0	0	0
17.	100	88	83	100	0	100	0	57	80	82	0	100	100	50	11	100	80	0	0
18.	0	12	17	0	100	0	0	0	20	27	100	25	0	50	89	0	100	100	100
19.	0	0	17	0	100	0	0	0	80	100	100	0	100	100	89	0	60	100	100
20.	82	0	0	0	0	0	0	0	20	45	0	0	0	50	67	0	100	100	100
21.	100	88	67	75	67	60	100	43	40	91	100	100	60	100	100	88	100	100	100
22.	100	88	67	75	89	60	100	57	60	100	100	100	100	100	100	100	100	100	100
23.	100	100	100	100	100	100	100	57	80	100	100	100	100	100	100	100	100	100	100
24.	0	0	0	0	0	0	0	14	0	0	0	0	0	100	78	0	0	0	0
25.	100	0	100	100	100	40	100	100	0	27	0	0	20	0	0	100	100	100	100
26.	0	0	0	0	0	20	100	0	0	0	0	0	100	0	0	0	0	0	0
27.	100	0	100	100	100	60	100	100	0	27	0	0	80	0	0	100	100	100	100
28.	100	100	100	100	100	100	100	100	0	100	0	50	100	100	89	0	0	75	20
29.	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.	100	100	100	100	89	100	100	100	100	100	100	100	100	100	100	0	100	100	100
31.	0	50	33	25	0	0	0	0	0	0	0	0	0	75	33	0	0	0	0
32.	100	100	100	100	100	100	100	100	0	100	0	50	100	100	100	0	0	100	100
33.	9	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34.	9	88	0	50	0	100	100	86	0	91	0	50	100	0	11	100	100	100	100
35.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37.	91	100	83	100	78	0	25	14	0	0	0	0	0	0	0	100	0	100	100
38.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39.	100	100	83	100	11	100	100	100	100	100	0	50	0	50	100	0	80	75	100
40.	100	100	83	100	11	40	0	0	0	0	0	0	0	0	0	100	100	100	100
41.	0	0	0	0	11	0	0	0	0	55	0	0	80	100	78	37	0	0	100
42.	100	100	100	75	100	100	100	100	0	82	0	100	100	100	100	100	100	100	100
43.	45	100	83	50	100	100	100	86	0	55	50	50	100	100	89	100	0	0	100

Table 4 (*continued*)

Cluster number. . .	1	3	4	5	6	8	11	13	14	15	19	20
Number of strains. . .	13	4	6	10	5	11	56	9	9	15	20	19
44. Tyrosine	8	100	0	0	0	0	76	55	0	0	0	0
45. Urea	31	100	0	0	0	0	21	0	0	0	0	32
<i>Resistance to ($\mu\text{g ml}^{-1}$):</i>												
46. Benzylpenicillin (8)	15	0	0	0	0	0	95	44	11	0	0	0
47. Benzylpenicillin (4)	31	0	67	10	60	0	98	89	11	7	0	21
48. Chloramphenicol (8)	0	0	100	10	0	0	3	11	0	0	80	84
49. Chloramphenicol (4)	0	0	100	10	0	9	15	22	11	0	100	90
50. Cycloserine (128)	69	100	100	100	0	91	64	11	33	47	15	0
51. Cycloserine (64)	100	100	100	100	20	91	98	33	0	67	55	0
52. Erythromycin (1)	39	100	100	100	100	0	20	0	89	0	45	68
53. Erythromycin (0.5)	69	100	100	100	100	64	23	33	100	0	60	100
54. Gramicidin (64)	100	100	83	100	20	73	32	0	78	93	100	95
55. Gramicidin (32)	100	100	100	100	40	100	48	0	89	100	100	100
56. Nalidixic acid (32)	0	100	0	0	100	0	2	0	0	0	0	42
57. Nalidixic acid (16)	0	100	17	0	100	0	7	67	0	13	5	100
58. Polymyxin (16)	100	100	17	100	80	100	100	100	100	100	100	100
59. Polymyxin (8)	100	100	83	100	80	100	100	100	100	100	100	100
60. Rifampicin (0.25)	0	0	83	30	20	27	21	0	11	27	0	5
61. Rifampicin (0.125)	0	0	83	70	20	55	60	0	22	60	20	32
62. Streptomycin (16)	54	0	100	100	0	18	26	67	89	73	0	42
63. Streptomycin (8)	92	0	100	100	0	64	60	89	11	73	0	95
64. Tetracycline (2)	15	100	100	100	0	36	81	11	0	53	0	37
65. Tetracycline (1)	23	100	100	100	20	36	97	67	100	67	40	58
<i>Acid from:</i>												
66. Adonitol	92	0	17	10	100	0	2	0	0	0	0	0
67. Arabinose	0	75	67	100	20	100	0	0	22	7	100	100
68. Cellobiose	92	100	100	100	100	100	84	22	100	100	100	100
69. Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0
70. Erythritol	0	0	0	10	0	0	0	0	0	0	0	0
71. Fructose	0	100	100	100	100	100	86	67	100	100	100	100
72. Galactose	54	100	100	90	100	91	3	0	0	33	90	95
73. Glucose	92	100	100	100	100	100	100	100	100	100	100	100
74. Glycerol	92	100	83	100	100	91	91	100	100	100	100	100
75. <i>meso</i> -Inositol	39	50	17	20	100	0	2	0	33	100	10	95
76. Lactose	0	100	100	100	100	91	2	0	100	13	45	68
77. Maltose	100	100	100	100	100	100	100	100	100	100	50	100
78. Mannitol	8	0	83	90	100	91	0	100	100	100	100	100
79. Mannose	15	100	100	90	100	82	21	0	89	100	100	100
80. Raffinose	100	100	100	90	80	100	3	0	100	100	75	95
81. Rhamnose	0	0	17	20	20	0	0	0	0	0	0	25
82. Salicin	0	100	100	100	100	100	91	44	100	100	100	100
83. Sorbitol	0	0	0	70	100	18	3	0	89	93	0	95
84. Sucrose	100	100	100	100	100	100	65	0	100	100	100	100
85. Trehalose	69	100	100	100	100	100	98	100	89	100	100	100
86. Xylose	0	0	100	100	100	91	0	0	11	80	95	95
<i>Utilization of:</i>												
87. Acetate	15	0	33	30	0	18	76	89	0	20	0	58
88. Citrate	0	50	50	0	0	18	80	11	22	87	90	95
89. Formate	0	50	33	60	20	82	91	67	0	93	40	95
90. Gluconate	23	0	67	100	60	36	43	0	11	100	0	100
91. Lactate	0	0	33	50	40	18	79	0	0	60	10	85
92. Malonate	0	0	0	10	0	18	5	0	0	0	0	10
93. Succinate	0	0	33	80	0	46	14	99	78	87	100	100
<i>Growth at:</i>												
94. pH 4.5	0	50	0	0	0	0	0	0	11	47	20	0
95. pH 6.0	0	100	100	100	100	100	100	44	100	100	100	100
96. pH 7.2	100	100	100	100	100	100	100	100	100	100	100	100
97. pH 8.0	100	100	100	100	100	100	100	100	100	100	100	100
98. pH 9.5	92	100	100	100	100	100	98	100	100	100	100	100
99. 5 °C	0	0	17	0	20	0	0	0	0	0	0	0
100. 17 °C	54	50	100	100	100	100	98	100	78	100	100	95

Table 4 (continued)

Character	22 11	25 8	27 6	29 4	30 9	31 5	33 4	35 7	36 5	37 11	38 6	39 4	40 5	42 4	43 9	44 8	46 5	48 4	49 5
44.	0	0	0	0	56	100	50	0	100	36	0	100	0	0	0	0	0	0	0
45.	82	0	0	0	0	60	0	0	0	0	0	50	100	75	0	100	0	0	0
46.	9	0	0	0	0	0	100	0	0	0	0	0	0	0	0	25	0	0	0
47.	45	0	0	0	0	0	100	0	0	0	0	0	0	25	22	37	0	0	0
48.	0	0	0	0	78	0	50	0	0	0	0	0	0	75	11	0	0	0	0
49.	9	0	0	0	67	40	50	100	0	18	0	0	0	100	33	0	0	0	0
50.	0	0	0	0	0	40	100	0	100	82	0	0	40	100	89	0	0	0	0
51.	9	0	0	0	100	60	100	71	100	100	67	0	100	100	89	0	0	0	0
52.	9	100	0	0	67	0	50	0	0	18	100	0	0	100	33	100	20	0	0
53.	27	100	0	0	0	0	50	0	0	18	100	0	0	25	33	100	20	0	0
54.	27	0	0	25	0	20	50	100	100	100	100	25	60	25	78	0	0	0	0
55.	73	0	17	50	11	60	50	100	100	100	100	25	80	25	89	25	0	0	0
56.	0	12	0	0	100	100	100	100	100	73	100	0	0	100	100	100	100	100	100
57.	0	50	0	0	100	100	100	100	100	100	100	0	20	100	100	100	100	100	100
58.	0	0	0	0	0	0	0	0	100	100	0	0	0	25	44	0	20	0	0
59.	9	12	0	0	33	0	0	100	100	100	33	0	0	100	100	0	20	0	0
60.	0	75	0	25	0	20	50	0	0	0	0	0	0	11	0	0	0	0	0
61.	0	88	0	25	0	100	0	0	0	0	0	0	0	25	33	0	0	0	0
62.	0	0	0	0	0	0	0	0	100	91	0	0	0	25	100	100	0	0	0
63.	0	0	0	0	0	0	0	0	100	100	0	0	0	25	100	100	0	0	0
64.	0	0	0	0	0	0	0	0	0	27	0	0	0	0	67	0	0	0	0
65.	9	0	0	0	0	0	0	14	0	64	0	0	20	0	100	0	0	0	0
66.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0
67.	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68.	100	0	67	0	11	100	50	100	0	0	0	0	20	0	0	50	20	0	100
69.	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71.	100	12	67	0	67	80	0	100	0	73	0	0	60	0	0	0	60	100	100
72.	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	50	0	0	20
73.	100	100	100	0	78	100	0	100	40	9	17	0	60	0	0	100	100	100	100
74.	100	88	83	50	11	100	75	100	100	82	50	50	60	0	33	12	0	25	80
75.	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
76.	27	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
77.	100	100	100	50	22	80	50	0	20	0	0	0	60	0	0	37	60	100	100
78.	100	88	100	50	0	100	75	100	0	0	0	0	0	25	0	88	0	75	100
79.	45	0	17	0	0	0	0	100	0	0	0	0	0	0	0	24	20	75	100
80.	0	0	0	0	0	60	0	100	0	9	0	0	0	0	0	0	0	75	20
81.	0	0	0	0	0	0	0	86	0	0	0	0	0	0	0	0	0	0	0
82.	100	12	0	0	11	0	0	100	0	0	0	0	0	0	0	50	0	25	40
83.	54	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
84.	100	100	100	0	100	80	50	100	0	0	0	0	0	25	0	50	60	100	20
85.	100	12	67	75	100	100	50	100	0	0	0	0	20	0	0	12	60	100	100
86.	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	20	0	100
87.	91	100	17	25	11	100	100	0	40	82	0	75	0	100	100	50	0	50	0
88.	100	0	50	100	11	80	100	0	0	45	33	0	0	100	33	12	0	50	100
89.	100	100	0	0	11	100	100	0	0	45	0	75	0	100	100	12	0	0	40
90.	100	0	0	25	0	100	100	0	0	0	0	0	0	50	0	12	0	0	0
91.	100	50	0	25	11	100	100	0	60	0	33	25	0	100	78	0	80	0	20
92.	91	100	0	0	0	0	0	0	20	0	0	0	0	25	0	0	0	0	20
93.	100	100	50	100	0	100	100	0	0	45	17	100	0	100	100	12	0	50	60
94.	100	75	17	0	0	100	0	0	0	0	0	0	0	0	0	12	0	25	0
95.	100	100	100	100	100	100	75	100	100	100	100	100	60	100	55	100	100	75	100
96.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
97.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	50	80
98.	100	100	100	100	100	100	100	100	0	100	33	100	100	100	100	100	0	0	0
99.	0	0	0	0	0	40	0	0	0	0	0	0	80	0	0	0	0	0	0
100.	100	75	83	100	100	100	100	100	20	64	33	100	100	100	100	100	0	0	0

Table 4 (continued)

Cluster number. . .	1	3	4	5	6	8	11	13	14	15	19	20
Number of strains. . .	13	4	6	10	5	11	56	9	9	15	20	19
101. 37 °C	100	100	100	100	100	100	100	100	100	100	100	100
102. 50 °C	0	0	17	100	60	0	0	0	89	87	60	95
103. 65 °C	0	0	0	0	0	0	0	0	0	0	0	0
<i>Growth in (% w/v):</i>												
104. NaCl (2)	100	100	100	30	100	73	100	100	100	100	100	100
105. NaCl (5)	92	50	100	0	100	9	100	100	100	100	100	100
106. NaCl (10)	0	0	0	0	0	9	40	0	89	80	100	5
<i>Miscellaneous tests:</i>												
107. Anaerobic growth	100	100	100	100	100	100	100	100	0	0	0	100
108. Gas from glucose	0	0	0	100	0	100	0	0	0	0	0	0
109. Dihydroxyacetone production	100	0	0	0	0	100	2	11	100	60	100	95
110. Indole production	92	100	0	0	0	0	0	33	0	0	0	0
111. Growth on MacConkey agar	39	25	100	20	100	73	98	0	67	0	95	0
112. Methyl red test	69	100	67	100	100	62	100	67	11	33	95	95
113. Nitrate reduction	8	100	17	100	20	100	97	100	78	100	0	100
114. ONPG	100	100	100	100	100	100	12	0	89	100	100	100
115. Oxidase	100	100	100	100	0	0	69	11	0	0	10	10
116. Phenylalanine deamination	0	0	0	0	0	0	15	0	89	0	0	95
117. Phosphatase	100	100	17	0	20	9	100	0	100	67	15	100
118. Voges-Proskauer reaction	77	0	0	0	0	100	91	0	100	100	100	100

analysis of phenotypic features (Logan *et al.*, 1979), although there are no clear diagnostic features. The results of the present study indicate that strains associated with incidents of food poisoning cannot be separated easily from other strains using phenotypic tests. Strains of '*B. cereus* var. *mycoides*' were recovered in a separate subcluster (11I), and were distinguished by their characteristic colonial morphology.

B. psychrosaccharolyticus was recovered as a well-defined cluster in this study, a result in line with earlier work (Laine, 1970; Gyllenberg & Laine, 1971). This species was not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), but is listed as *species incertae cedis* in *Bergey's Manual of Systematic Bacteriology* (Claus & Berkeley, 1986). It is evident from the present and earlier studies that the epithet *B. psychrosaccharolyticus* should be reintroduced (see below).

The '*B. subtilis* group', defined at 78% S_{SM} , contained clusters identified as *B. amyloliquefaciens*, *B. licheniformis*, *B. pumilus* and *B. subtilis*. *B. amyloliquefaciens* and *B. subtilis* strains share little DNA sequence homology (Welker & Campbell, 1967; Seki *et al.*, 1975; Priest, 1981), can be separated by pyrolysis gas-liquid chromatography (O'Donnell *et al.*, 1980) and can be distinguished by a few phenotypic properties (Priest *et al.*, 1987). Our results support the recent demonstration that strains of *B. amyloliquefaciens*, unlike those of *B. subtilis*, produce acid from lactose (Nakamura, 1987), which is a useful distinguishing character.

The clear separation of *B. licheniformis*, *B. pumilis* and *B. subtilis* has been noted in other taxometric studies (Bonde, 1975; Durand *et al.*, 1979; O'Donnell *et al.*, 1980; Logan & Berkeley, 1981). These species comprise discrete DNA homology groups (reviewed by Priest, 1981) and can be readily distinguished by a number of presumptively diagnostic features (Table 4). It was also encouraging that strains labelled as '*B. atterimus*' and '*B. vulgatus*' fell within the *B. subtilis* cluster, as such strains were recovered in the *B. subtilis* DNA homology group by Seki *et al.* (1975). Three strains of '*B. subtilis* var. *niger*', were assigned to a separate cluster that was closely related to *B. subtilis*. However further studies are required to determine the taxonomic status of '*B. subtilis* var. *niger*', as a strain of this taxon showed 95% DNA homology with the type strain of *B. subtilis* (Seki *et al.*, 1975).

Strains labelled as *B. megaterium* were assigned to three DNA homology groups by Hunger & Claus (1981). The homology group corresponding to *B. megaterium sensu stricto* is represented by cluster 22. A second DNA homology group contains strains originally labelled as '*B. simplex*'

Table 4 (continued)

Character	22	25	27	29	30	31	33	35	36	37	38	39	40	42	43	44	46	48	49
	11	8	6	4	9	5	4	7	5	11	6	4	5	4	9	8	5	4	5
101.	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100	100	100	75	100
102.	0	0	33	0	100	40	0	0	100	100	0	50	0	0	0	0	100	100	100
103.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
104.	100	100	100	100	100	100	100	100	100	100	67	100	80	25	100	100	20	50	80
105.	100	100	100	100	100	100	100	100	0	100	33	100	80	25	89	100	0	25	60
106.	0	100	83	100	100	100	0	100	0	0	0	0	0	0	0	12	0	0	0
107.	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	50	0
108.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
110.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111.	100	63	100	75	100	100	100	14	0	36	33	100	100	100	100	100	20	25	60
112.	73	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	100	75	0
113.	0	100	0	0	89	100	0	0	100	91	100	50	80	0	44	37	100	100	100
114.	100	0	83	100	89	0	0	100	0	18	0	0	80	0	0	100	80	25	20
115.	0	0	17	0	22	0	0	0	60	0	83	25	20	50	89	100	0	0	0
116.	100	75	67	50	56	0	0	0	0	0	0	25	0	25	11	0	0	0	0
117.	54	0	17	0	0	20	0	0	80	0	0	0	0	50	33	0	80	25	100
118.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60	0	0

and '*B. teres*'. These strains are phenetically different from *B. megaterium sensu stricto*, and in the taxometric study of Priest *et al.* (1981) they were recovered in a different aggregate cluster from *B. megaterium*. In the present study, '*B. simplex*' S210 and '*B. teres*' S213 formed cluster 32 (cluster-group C), thereby supporting the distinction between these organisms and strains belonging to *B. megaterium sensu stricto*.

The third DNA homology group described by Hunger & Claus (1981) encompassed strains originally labelled as '*B. agrestis*' and '*B. flexus*'. These organisms were recovered in a separate phenon (cluster 23) in both this and a previous study (Priest *et al.*, 1981) and clearly represent a distinct species. Since the name '*B. flexus*' (Batchelor, 1919) has priority over '*B. agrestis*' (Werner, 1933) the former epithet is used for the reintroduced taxon (see below). Single-member clusters recovered in cluster-group B included strains of '*B. longissimus*' (Mishustin & Tepper, 1948) and '*B. maroccanus*' (Delaporte & Sasson, 1967). More strains of these taxa must be isolated and studied before their taxonomic status can be clarified.

Cluster-group C. Two groups of strains labelled as '*B. carotarum*' and a third group associated with this name were recovered in cluster-group C. Strains S51 to S55 (cluster 31) were isolated by Gibson (1935) and identified by him as '*B. carotarum sensu* Koch 1888. Two other strains originally labelled as '*B. carotarum*' (NRS 608 and NRS 828) were donated by Gordon and thought to be original isolates from G. Bredemann and C. Stapp & N. H. Claussen, respectively (R. E. Gordon, personal communication). These were recovered in cluster 33. The third group comprised strains originally labelled as '*B. simplex*' and '*B. teres*'. Such strains were assigned to DNA homology group B by Hunger & Claus (1981) and were considered to belong to '*B. carotarum*' by Gibson & Gordon (1974). These were recovered as cluster 32. The fact that strains assigned to clusters 31, 32, and 33 have many properties in common helps to explain the confusion that has arisen with respect to the taxonomy of '*B. carotarum*'. It is, however, clear from the present study that these taxa are distinct. The integrity of cluster 32 is supported by DNA base composition and reassociation data (Hunger & Claus, 1981) and merits species status. Since these strains were not original isolates of Koch (1888), and to avoid confusion, they cannot be given the name '*B. carotarum*'. The name '*B. simplex*' (Gottheil, 1901) should be reintroduced for the taxon represented by cluster 32 as this epithet has priority over '*B. teres*' (Neide, 1904). Further DNA studies are needed to confirm the taxonomic status of clusters 31 and 33.

Table 5. *Distribution of positive characters to minor clusters defined at the 83% level (S_{SM})*

	<i>'B. aparius'</i>	<i>B. pabuli</i>	<i>B. circulans</i>	<i>B. circulans</i>	<i>B. psychrosaccharolyticus</i>	<i>'B. subtilis</i> var. <i>niger'</i>	<i>B. circulans</i>	<i>B. subtilis</i>	<i>Bacillus</i> sp.	<i>B. flexus</i>	<i>B. firmus</i>	<i>Bacillus</i> sp.	<i>Bacillus</i> sp.	<i>B. simplex</i>	<i>B. pulvificiens</i>	<i>B. brevis</i>	<i>B. macquariensis</i>	<i>B. coagulans</i>
Cluster number. . .	2	7	9	10	12	16	17	18	21	23	24	26	28	32	34	41	45	47
Number of strains. . .	2	2	3	3	2	3	2	2	2	2	2	2	2	2	3	2	2	3
<i>Colonial morphology</i>																		
1. Flat/raised	2	2	1	2	2	2	0	2	1	2	2	2	1	2	0	2	2	3
2. Smooth	2	2	3	3	2	2	0	1	2	2	2	2	2	2	3	2	2	0
3. Rhizoidal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
4. Entire	2	0	3	2	1	1	1	1	1	0	2	0	2	2	3	2	2	2
5. Opaque	0	2	3	3	2	3	2	1	2	2	2	2	2	2	3	2	0	1
6. Pigmented	2	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0
7. Motile colonies	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cellular morphology</i>																		
8. Length >3 µm	2	3	3	3	0	0	1	0	0	1	0	2	2	0	0	1	1	3
9. Diameter >0.9 µm	0	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	0	0
10. Ends round	0	2	3	3	2	3	2	2	2	2	2	2	2	2	2	2	2	3
11. Single	2	2	3	3	2	0	2	2	2	0	2	0	0	0	0	2	0	2
12. Vacuoles present	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
13. Gram-variable	0	0	0	0	2	3	1	2	2	2	2	2	2	1	2	2	0	2
14. Gram-positive	0	0	0	0	0	1	0	1	2	0	0	2	2	0	0	0	0	0
15. Spores oval	2	2	3	3	2	3	2	2	1	2	2	2	2	2	3	2	2	3
16. Spores round	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
17. Spores central	2	1	0	1	2	3	2	2	1	2	2	2	2	2	3	0	2	2
18. Spores terminal	0	1	3	2	0	2	0	0	1	0	0	0	0	0	0	2	0	1
19. Spores bulging	2	2	3	3	2	0	1	0	2	0	0	0	0	0	3	2	2	1
20. Sporulation 24 h	0	1	0	1	0	2	2	0	1	2	1	0	0	0	0	2	0	3
21. Sporulation 72 h	1	2	2	3	1	3	2	0	2	2	2	2	2	2	1	2	0	3
22. Sporulation 120 h	2	2	3	3	2	3	2	2	2	2	2	2	2	2	3	2	0	3
23. Sporulation SxA	2	2	3	3	2	3	2	2	2	2	2	2	2	2	3	2	2	3
<i>Degradation of:</i>																		
24. Adenine	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2	0	3
25. Aesculin	2	2	3	3	2	3	2	2	2	0	2	0	2	0	0	0	2	2
26. Allantoin	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	2	0	0
27. Arbutin	2	2	3	3	2	3	2	2	2	2	2	1	2	2	0	0	2	3
28. Casein	2	2	2	1	2	3	2	2	2	2	2	2	2	2	3	2	0	3
29. Chitin	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0
30. DNA	2	2	0	2	2	3	2	2	2	2	2	2	2	1	0	2	0	3
31. Elastin	0	0	0	0	2	1	2	2	1	2	2	0	0	0	0	2	0	0
32. Gelatin	2	2	0	2	2	3	2	2	2	2	2	2	2	2	3	2	0	3
33. Guanine	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
34. Hippurate	2	0	1	3	0	0	1	0	0	0	0	1	0	2	1	0	0	3
35. Lecithin	2	0	0	0	2	3	2	1	2	0	1	2	1	0	0	0	0	0
36. Pectin	0	2	0	0	0	1	0	2	2	0	0	0	0	0	2	0	0	0
37. Pullulan	2	0	3	0	2	2	0	1	0	2	1	2	2	0	1	0	2	0
38. Pustulan	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39. RNA	2	2	3	1	2	3	2	2	2	2	2	2	2	1	3	2	0	3
40. Starch	2	2	3	2	2	3	2	2	2	2	1	2	1	2	0	0	2	3
41. Testosterone	0	0	3	1	0	0	0	0	0	0	1	0	0	0	0	2	0	0
42. Tween 20	2	2	3	3	2	3	2	2	2	2	0	2	2	2	3	2	0	3
43. Tween 80	2	2	3	3	2	3	2	1	2	1	1	2	1	1	2	2	0	3
44. Tyrosine	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
45. Urea	2	0	0	2	2	0	0	0	2	2	0	0	0	0	0	2	0	0

Cluster number. ...	2	7	9	10	12	16	17	18	21	23	24	26	28	32	34	41	45	47
Number of strains. ...	2	2	3	3	2	3	2	2	2	2	2	2	2	2	3	2	2	3
<i>Resistance to ($\mu\text{g ml}^{-1}$):</i>																		
46. Benzylpenicillin (8)	0	0	0	0	1	3	0	0	0	0	0	2	0	0	0	0	0	0
47. Benzylpenicillin (4)	0	0	0	0	1	3	1	0	0	0	0	2	0	0	0	0	0	0
48. Chloramphenicol (8)	0	2	0	0	1	1	0	0	2	0	0	2	0	0	0	2	0	0
49. Chloramphenicol (4)	0	2	1	0	1	2	0	0	2	0	0	2	0	2	0	2	0	0
50. Cycloserine (128)	0	2	3	0	1	2	1	0	0	0	0	0	0	0	1	2	2	0
51. Cycloserine (64)	2	2	3	0	1	3	2	2	0	0	0	1	1	1	3	2	2	0
52. Erythromycin (1)	0	2	0	0	0	0	0	0	2	1	0	2	0	0	0	2	0	0
53. Erythromycin (0.5)	0	2	0	0	0	0	0	0	2	2	0	2	0	0	0	2	0	0
54. Gramicidin (64)	2	0	0	0	1	3	2	2	2	1	2	2	0	0	1	2	0	0
55. Gramicidin (32)	2	0	0	1	2	3	2	2	2	1	2	2	0	0	1	2	0	0
56. Nalidixic acid (32)	0	0	3	1	0	0	0	0	2	0	0	0	0	2	0	2	1	3
57. Nalidixic acid (16)	0	0	3	1	0	0	0	0	2	0	0	0	0	2	3	2	2	3
58. Polymyxin (16)	2	2	0	0	0	3	2	2	2	0	0	0	0	0	3	2	0	0
59. Polymyxin (8)	2	2	1	0	0	3	2	2	2	0	0	0	0	1	3	2	1	0
60. Rifampicin (0.25)	0	1	0	0	0	0	2	1	0	0	0	2	0	1	0	1	0	0
61. Rifampicin (0.125)	0	1	0	0	1	1	2	2	2	1	2	2	0	1	0	1	0	0
62. Streptomycin (16)	2	2	0	0	0	1	0	2	2	0	0	0	0	0	0	2	2	0
63. Streptomycin (8)	2	2	0	0	1	2	0	2	2	0	0	0	0	0	2	2	2	0
64. Tetracycline (2)	0	0	0	0	1	0	2	2	0	0	0	0	0	0	0	0	0	0
65. Tetracycline (1)	2	0	0	0	1	3	2	2	2	0	1	0	0	0	0	0	0	0
<i>Acid from:</i>																		
66. Adonitol	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67. Arabinose	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
68. Cellobiose	2	2	3	2	1	3	2	0	2	0	1	0	0	2	0	0	2	0
69. Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70. Erythritol	0	0	0	0	0	0	0	0	0	0	2	1	0	2	3	0	0	3
71. Fructose	0	2	3	3	2	3	2	2	2	2	2	0	0	0	0	0	2	0
72. Galactose	2	1	3	3	0	3	0	0	2	2	2	1	0	2	3	0	2	3
73. Glucose	2	2	3	3	2	3	2	2	2	2	2	1	0	2	3	0	2	3
74. Glycerol	2	2	2	1	2	3	2	0	2	2	2	2	2	2	2	2	0	2
75. meso-Inositol	2	1	0	0	0	3	2											

Table 5 (continued)

Cluster number. . .	2	7	9	10	12	16	17	18	21	23	24	26	28	32	34	41	45	47
Number of strains. . .	2	2	3	3	2	3	2	2	2	2	2	2	2	2	3	2	2	3
100. 17 °C	0	2	0	3	2	3	2	2	0	2	2	2	2	2	3	2	2	1
101. 37 °C	2	2	3	3	0	3	2	2	2	2	2	2	2	2	3	2	1	3
102. 50 °C	0	1	1	0	0	2	1	0	0	0	0	0	0	0	0	2	0	3
103. 65 °C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Growth in (% w/v):</i>																		
104. NaCl (2)	2	2	3	3	2	3	2	2	2	2	2	2	2	2	3	2	2	1
105. NaCl (5)	0	2	3	3	1	3	2	2	2	2	2	2	2	2	1	2	0	1
106. NaCl (10)	0	0	0	0	0	2	0	2	0	2	2	1	2	0	0	0	0	1
<i>Miscellaneous tests:</i>																		
107. Anaerobic growth	2	2	2	2	0	2	2	0	2	0	0	0	0	0	3	0	2	3
108. Gas from glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109. Dihydroxyacetone production	0	0	0	0	0	3	1	0	2	0	0	0	0	0	0	1	0	0
110. Indole production	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111. Growth on MacConkey agar	0	2	2	0	2	3	2	2	1	2	2	2	2	2	1	1	2	1
112. Methyl red test	0	2	3	2	1	0	0	0	2	0	0	0	0	0	2	0	0	0
113. Nitrate reduction	2	0	3	3	2	3	2	2	2	0	0	2	0	2	2	0	0	3
114. ONPG	2	2	3	3	0	2	2	2	2	2	2	0	1	0	0	0	2	2
115. Oxidase	2	2	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
116. Phenylalanine deamination	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
117. Phosphatase	2	0	0	0	1	1	0	1	2	2	1	0	1	2	2	0	2	1
118. Voges-Proskauer reaction	0	0	0	0	1	3	2	2	2	0	0	0	0	0	0	0	0	0

Cluster-group C also contained isolates from marine or saline environments. In a numerical taxonomic study of 138 bacilli isolated from the North Sea, Boeyé & Aerts (1976) recognized two major clusters representing *B. firmus* and the *B. subtilis* group. Similarly, Bonde (1975, 1976) examined several hundred bacilli from marine sources and found that *B. firmus*, species of the '*B. subtilis* group', and *B. sphaericus* were common. In the present investigation, most of the representatives from the studies of Bonde (1975, 1976) and Boeyé & Aerts (1976) were assigned to cluster-groups B and C.

B. firmus has been a problematical taxon (Gordon *et al.*, 1977). In the present study, the *B. firmus* strains formed a compact phenon, a result in agreement with earlier work (Priest *et al.*, 1981; Logan & Berkeley, 1981). *B. firmus* was also distinguished from *B. lentus* (cluster 44) in DNA pairing experiments (Priest, 1981; Seki *et al.*, 1983) which showed that the sequence homology between representatives of these species was very low. Many bacilli isolated from saline environments have been described as intermediate between *B. firmus* and *B. lentus* and, as a result, strains in these taxa have been considered to form a 'spectrum' (Gordon *et al.*, 1977). In the present numerical classification, however, most of the *B. firmus/B. lentus* intermediates were recovered in three related but distinct phenons, clusters 27, 28 and 29, within cluster-group C. These findings are supported by DNA reassociation data which indicate that very little homology exists between 'intermediate strains' and *B. firmus* (Priest, 1981; Seki *et al.*, 1983). Strains NRS 1575 and NRS 1570, for example, not only belong to different clusters but have been assigned to distinct DNA homology groups (Seki *et al.*, 1983). Similarly, strain NRS 1151 was assigned to an individual homology group and was recovered in cluster 26. Further nucleic acid reassociation data are needed to resolve the taxonomic status of clusters 27, 28 and 29.

Other phenons that comprised distinct taxa within cluster-group C include *B. pantothenicus* and *B. pulvificiens*. Strains received as '*Krusella cascaianensis*' (Castellani, 1954) produce ellipsoidal spores (Castellani, 1955; Gordon *et al.*, 1973) and have been transferred to the genus *Bacillus* as '*B. cascaianensis*' (Castellani, 1955). The present study indicates that this epithet should be reintroduced, but DNA base composition data on representative strains are needed to complement the present description of this taxon. A single strain of '*B. epiphytus*' was recovered on the periphery of the *B. firmus* cluster; this relationship has been noted by others (Gibson &

Gordon, 1974; Bonde, 1976; Logan & Berkeley, 1981). DNA data are required to clarify the status of '*B. epiphytus*'. Similarly, '*B. loehnisii*', '*B. pacificus*' and '*B. macroides*' formed single-member clusters in cluster-group C. '*Bacillus loehnisii*' is generally regarded to be similar to *B. pasteurii* and '*B. freudenreichii*' (Gibson, 1934) but in the present study, *B. pasteurii* strains were not included and '*B. freundenreichii*' was recovered in cluster group D. '*Bacillus macroides*', on the other hand, was assigned to the *B. firmus* aggregate group by Logan & Berkeley (1981); its placement in cluster-group C is consistent with this.

Cluster-group D. Most of the *B. brevis* strains were recovered in cluster 37, but the allocation of two strains to cluster 41 was in good agreement with an earlier taxometric study where *B. brevis* was shown to be heterogeneous (Priest *et al.*, 1981). Five strains of '*B. aneurinolyticus*' formed a homogeneous phenon closely related to *B. brevis*. Previously, casein hydrolysis was considered the only feature available to distinguish between these taxa (Claus & Berkeley, 1986), but additional differential characteristics have been highlighted in this study. The name '*B. aneurinolyticus*' should be reintroduced when confirmatory DNA base composition data become available.

B. sphaericus strains have been assigned to at least five DNA homology groups (Seki *et al.*, 1978; Krych *et al.*, 1980), but still appear to be phenotypically uniform. Previous taxometric studies have placed *B. sphaericus* in a single phenon (Logan & Berkeley, 1981; Priest *et al.*, 1981) but in the present analysis four strains labelled '*B. sphaericus* var. *fusiformis*' were assigned to a separate cluster. This cluster corresponds to DNA homology group IIB of Krych *et al.* (1980). It is evident that the strains of cluster 41 merit species status given the good congruence between the DNA homology and numerical phenetic data. The name *B. fusiformis* has been proposed for this taxon (see below). A strain of '*B. rotans*' was assigned to DNA homology group III by Krych *et al.* (1980). The recovery of '*B. sphaericus* var. *rotans*' (NCIB 8867) as a single-member cluster is in support with the view that this organism may also represent a new taxospecies.

The integrity of *B. azotoformans* (Pichinoty *et al.*, 1983) was supported by the assignment of six representatives of this species to cluster 38. A few psychrophilic strains were also recovered in cluster-group D. The numerical phenetic data support the current taxonomic status of *B. globisporus*, *B. insolitus* and *B. psychrophilus* (Larkin & Stokes, 1967; Ruger, 1983; Nakamura, 1984b).

Cluster-group E. The clear separation of *B. lentus* (cluster 44) from *B. firmus* (cluster 25, cluster-group C) confirms the independent status of these species. The recovery of two strains of *B. macquariensis* in cluster-group E casts doubt on the reported affinity between *B. circulans* and *B. macquariensis* (Gibson & Gordon, 1974; Logan & Berkeley, 1981).

Cluster-group F. The recovery of the *B. coagulans* and *B. stearothermophilus* strains in a single aggregate group is in good agreement with the earlier study of Logan & Berkeley (1981) which showed that these bacteria have many features in common beyond their ability to grow at high temperature. *B. coagulans* comprises at least two phenetic groups (Wolf & Barker, 1968), and although limited DNA reassociation studies indicated genetic homology (Seki *et al.*, 1978), two DNA homology groups have subsequently been revealed (I. Blumenstock, personal communication: quoted by Claus & Berkeley, 1986). In the present study, strains of *B. coagulans* were similarly assigned to two clusters, cluster 46 representing *B. coagulans sensu stricto*.

The recovery of the *B. stearothermophilus* strains in two major clusters and one single-member cluster provides yet further evidence for the heterogeneity of this taxon. It is generally accepted that *B. stearothermophilus* encompasses at least three distinct taxa (Baillie & Walker, 1968; Klaushofer & Hollaus, 1970; Walker & Wolf, 1971; Sharp *et al.*, 1980). Cluster 48 contained strains of *B. stearothermophilus sensu stricto* (Walker & Wolf, 1971; group 3) although it also encompassed strains assigned by these workers to their group 2. Cluster 49, which was particularly well defined, corresponds to group 1 ('*B. kaustophilus*') of Walker & Wolf (1971). This taxon is phenetically and genotypically distinct from *B. stearothermophilus* (Sharp *et al.*, 1980) and merits species status (see below).

The genus Bacillus, the emerging taxonomy

It is appropriate in a wide-ranging study such as the present one, to draw some general conclusions and suggest priorities for the future. It is now evident that the genus *Bacillus* encompasses some 80 taxa of approximate species rank that can be assigned to five or more cluster-groups. The latter should be used as a framework for redefining the current genus and splitting it into several genera. An indication of how this might best be achieved has been revealed by Stackebrandt *et al.* (1987), who have shown that *B. sphaericus* and other species containing round-spored organisms can be distinguished from other bacilli on the basis of rRNA oligonucleotide sequencing, spore morphology and cell-wall composition studies. However, we agree with these authors that many more strains need to be studied by similar techniques before 'a formal dissection of the genus *Bacillus* with consequent description of new genera is proposed'.

It is also evident from the present study that several clusters merit species status given the appropriate supporting data from the literature, and formal proposals are given below. It is also highly likely that taxa such as '*B. aneurinolyticus*', '*B. apiarius*', '*B. cascainensis*', '*B. thiaminolyticus*' and the various halotolerant isolates described as '*B. firmus*–*B. lentus* intermediates' should be raised to valid species status. Supporting DNA base composition and reassociation data are required before this can be recommended.

Further comparative studies are needed to revise and clarify the classification of heterogeneous species such as *B. brevis*, *B. circulans*, *B. coagulans*, *B. sphaericus* and *B. stearothermophilus*. It is also possible that strains carrying names such as '*B. cirroflagellosus*', '*B. epiphytus*', '*B. filicolonicus*', '*B. freudenreichii*', '*B. globigii*', '*B. loehnisii*', '*B. longissimus*', '*B. macroides*', '*B. maroccanus*', '*B. pacificus*' and '*B. repens*' represent new centres of variation, but additional representatives of these taxa need to be examined to determine their taxonomic status.

NOMENCLATURE

Description of *Bacillus flexus* (Batchelor, 1919) nom. rev.

flex'us. L. adj. *flexus*, flexible.

The description given below is taken from the present and earlier studies (Hunger & Claus, 1981; Claus & Berkeley, 1986). Strains in this species have similar properties to *B. megaterium* but differ from typical members of that species as cells are smaller (mean cell width 0.9 µm), poly-β-hydroxybutyrate is not formed, phenylalanine is not deaminated, neither is aesculin hydrolysed nor acid formed from pentoses. Strains of this species degrade casein, elastin, gelatin, pullulan and starch, are urease positive, but give a negative Voges–Proskauer reaction and do not reduce nitrate to nitrite. Additional properties are given in Table 5.

The mol% G + C content of the DNA of the two strains examined lies between 37 and 39 (T_m). The type strain has little in common with either '*B. carotarum*' or *B. megaterium*.

Source: Faeces and soil.

Type strain: DSM 1320 (= NRS 665).

Description of *Bacillus fusiformis* (Smith *et al.*, 1946) comb. nov. (*Bacillus sphaericus* var. *fusiformis* Smith, Gordon & Clark, 1946, 97)

fus.i.form'is. L. n. *fusus* spindle; L. n. *forma* shape, form; M.L. adj. *fusiformis* spindle-shaped.

The description is taken from the present study and from that of Krych *et al.* (1980). Strains in this species have similar properties to *B. sphaericus* but differ from typical members of that species as they are urease positive, grow in the presence of NaCl (7%, w/v) and are sensitive to tetracycline (1 µg ml⁻¹). They are oxidase positive, degrade gelatin and testosterone, but give a negative Voges–Proskauer reaction, and do not degrade starch or reduce nitrate to nitrite. Additional properties are given in Table 4.

The mol% G + C of the DNA falls within the range 35 to 36 (T_m) for the eleven strains examined. These strains form a distinct DNA homology group that is related to a second homology group which accommodates strains pathogenic for mosquitoes (Krych *et al.*, 1980).

Source: Soil.

Type strain: ATCC 7055.

Description of *Bacillus kaustophilus* (Prickett, 1928) nom. rev.

kau.sto.ph'il.us. Gr. n. *kaustos*, heat; Gr, adj. *philus* loving; M.L. adj. *kaustophilus* heat loving.

The description is taken from the present and several other studies (Prickett, 1928; Walker & Wolf, 1971; Sharp *et al.*, 1980). Strains in this species have similar properties to *B. stearothermophilus* but differ from members of this species by their ability to produce acid from cellobiose, *meso*-inositol and xylose, to degrade testosterone and to reduce nitrate to gas, and by their relative sensitivity to NaCl and failure to grow anaerobically. They produce oval to cylindrical spores that distend the sporangium to a greater or less extent, liquefy gelatin, degrade aesculin, arbutin, pullulan and starch (weakly), and grow optimally between 60 and 65 °C. Additional properties are given in Table 4.

The mol% G + C of the DNA of the five strains studied falls within the range 51 to 55 (T_m). There is evidence that these strains form a distinct DNA homology group (Sharp *et al.*, 1980).

Source: Pasteurized milk, deteriorated canned food and probably soil.

Type strain: ATCC 8005 (= N. R. Smith T281).

Description of *Bacillus psychrosaccharolyticus* (Larkin & Stokes, 1967) nom. rev.

psy.chro.sac.char.o.lyt'i.cus. Gr. adj. *psychros* cold; Gr. n. *saccharon* sugar; Gr. adj. *lytos* dissolvable; M.L. adj. *psychrosaccharolyticus* cold (adapted), sugar-fermenting.

The description is taken from the present and two other studies (Larkins & Stokes, 1967; Claus & Berkeley, 1986). Cells are distinctly pleomorphic, varying from coccoid to elongate. On glucose media they may contain globules that are unstainable with fuchsin. Growth and sporulation occur at 0 °C. If sporulation does not occur, the organism may swell and become faintly stainable, often forming pear-shaped bodies up to 2 µm in diameter. The spore frequently fills most of the sporangium; it may occur in a lateral position. Relatively thick opaque growth without spreading or outgrowths occurs on agar media. Overgrowth of laboratory cultures by asporogenous mutants appears to occur frequently. Glucose promotes anaerobic growth only slightly. Aesculin, allantoin and arbutin are hydrolysed, and elastin, gelatin, lecithin, pullulan and starch are degraded.

The mol% G + C of the DNA lies within the range 43 to 44 (T_m ; F.G. Priest, unpublished data).

Source: Soil and marshes.

Type strain: NCIB 11729 (= ATCC 23296 = DSM 6).

Direct plating of soil frequently yields organisms which have the characteristics of *B. psychrosaccharolyticus* except that some of them may diverge from that species in their action on nitrate (none or denitrification), proteins, starch, particular sugars, or in utilization of glucose for anaerobic growth. These organisms, which do not appear to have been named, have yet to be the subject of comparative studies to determine their possible relationship to *B. psychrosaccharolyticus*.

Description of *Bacillus simplex* (Gottheil, 1901) nom. rev.

sim'plex. L. adj. *simplex* simple.

The description is taken from the present study and that of Hunger & Claus (1981). Strains in this species have properties in common with *B. megaterium* but differ from typical members of that species as they reduce nitrate to nitrite, produce brownish colonies on tyrosine agar, fail to hydrolyse aesculin and urea, do not deaminate phenylalanine or form hydroxybutyrate and have cells that measure only 0.8 to 1.0 µm in diameter (a few broader cells are occasionally observed). They degrade arbutin, gelatin, starch and tyrosine but not chitin. They are negative for the Voges-Proskauer and egg-yolk tests and do not grow in the presence of lysozyme. Additional properties are given in Table 5.

The mol% G + C content of the DNA of the six strains examined lies between 40 and 41 (T_m). These strains form a distinct DNA homology group (Hunger & Claus, 1981).

Source: Soil.

Type strain: DSM 1321 (= NRS 960).

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