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A polyphasic taxonomic study of thermophilic bacilli from a wide geographical area

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Abstract

Two hundred and thirty-four thermophilic *Bacillus* strains isolated from geographically widespread locations were examined by phenotypic characterisation followed by numerical analysis. The strains were distributed between eighteen cluster-groups which were subsequently evaluated in DNA base composition and DNA sequence homology studies. The inclusion of type and reference strains unambiguously identified strains related to *B. licheniformis, B. pallidus, B. smithii, B. stearothermophilus, B. thermocloacae* and *B. thermoglucosidasius*. Other reference strains included in distinctive groups were '*B. caldotenax*', together with '*B. caldovelox*' and '*B. caldolyticus*', *B. kaustophilus* and '*B. thermodenitrificans*'. An emended description of *B. kaustophilus* is provided. It is proposed that '*B. caldotenax*' and '*B. thermodenitrificans*' should be accepted as validly described species. Members of other clusters that appeared to have distinctive characteristics, including beta-glucanase production and the ability to degrade tyrosine, may provide the nuclei of further novel species.

Introduction

The first isolation of a thermophilic *Bacillus* has been attributed to Miquel who in 1888 described a thermophilic sporeforming strain isolated from the river Seine. This report was followed by the isolation and description of numerous *Bacillus* strains, from soil, sewage and spoilt food products, that were able to grow at or above 60°C. Many of these early isolations originated from work at the National Canners Association in Washington (Cameron & Esty 1926).

Ruth Gordon & Nathan Smith (1949) examined 216 of these early isolates using cultural, microscopic and physiological analyses and in so doing significantly rationalized the taxonomy and nomenclature of the thermophilic bacilli. Numerous strains were considered to be thermophilic variants of species normally regarded as mesophilic; the remainder were assigned to two species, *Bacillus coagulans* and *Bacillus stearothermophilus*. The original description of *B. stearothermophilus* (Donk 1920) was largely based on morphological and physiological characteristics and, although the original strain was lost, Gordon and Smith (1949) considered the description was typical of most obligately thermophilic species of *Bacillus*. Only two species of thermophilic bacilli with optimum growth temperature of 55°C or above, *B. coagulans* and *B. stearothermophilus*, were described in the seventh and eighth editions of *Bergey's Manual of Determinative Bacteriology* (Smith & Gordon 1957; Gibson & Gordon 1974).

Three major subdivisions were recognized among thermophilic bacilli by Walker & Wolf (1969) and Klaushofer & Hollaus (1970). Members of one of these were characterized by their inability to hydrolyse starch. *Bacillus stearothermophilus* strains unable to hydrolyse starch had been described previously (Daron, 1967; Epstein & Grossowicz 1969). Subsequently, *Bacillus* species that were unable to hydrolyse starch (e.g. *B. thermocloacae*; Demharter & Hensel

1989) or were only able to do so weakly were described (e.g. *B. pallidus*; Scholtz et al. 1987). Walker & Wolf (1971) and Klaushofer & Hollaus (1970) also described groups able to denitrify nitrate to nitrogen gas, these were considered to comprise strains of '*B. thermodenitrificans*'; originally named '*Denitrophilum*' by Ambroz (1913) and later renamed by Mishustin (1950).

Since these pioneering studies, several new species of thermophilic bacilli have been validly described including *B. acidocaldarius* (Darland & Broek 1971), *B. pallidus* (Scholtz et al. 1987), *B. thermocloacae* (Demharter & Hensel 1989), *B. thermoglucodasius* (Suzuki et al. 1983) and *B. thermoleovorans* (Zarilla & Perry 1987). Moreover, some other species such as '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*' (Heinen & Heinen 1972), '*B. thermocatenulatus*' (Golovacheva et al. 1975) and '*B. thermoflavus*' (Heinen et al. 1982) were excluded from the Approved Lists of Bacterial Names and have lost their standing in bacterial nomenclature. This proliferation of species suggests that the thermophilic bacilli may be more diverse than originally considered and yet they have not been subject to comprehensive, taxonomic studies to the extent of their mesophilic counterparts (Priest 1993). This is surprising given the biotechnological importance of these bacteria as sources of thermostable enzymes and other products of industrial interest (Sharp & Munster 1986) and the value of phenetic classifications for the development of computerized identification schemes and for the formulation of selective isolation programmes for novel strains (Bull et al. 1992).

In this study, the phenetic diversity of thermophilic bacilli from geographically widespread locations has been examined in order to clarify the classification and identification of these important bacteria and to provide a physiological database for their further exploitation.

Materials and methods

Strains

The 234 thermophilic strains of *Bacillus* comprised type strains, reference strains from previous studies, and environmental isolates from a wide range of geographical locations (Table 1, see p. 370). Isolations were made from both naturally heated and temperate environments by plating samples on tryptone soya agar (TSA) (Oxoid CM131) and incubating at 55°C for 16 hours. Strains were routinely maintained at 4°C on TSA slopes. For longer term maintenance, cell suspensions were stored at - 70°C in broth cultures supplemented with 10% glycerol.

Phenotypic tests

Each strain was examined for 96 unit characters (Table 2, see p. 376). Tests based on agar-solidified media were inoculated from overnight plate cultures and those on liquid media from 4 hour peptone broth cultures (Cowan 1974). All tests were carried out in duplicate and repeated when inconsistent results were observed. Unless otherwise stated all test media were incubated at 55°C for 1–3 days.

Morphological analysis and degradation tests were generally carried out using agar plates, the exceptions were gelatin, hippurate and tyrosine degradation which were carried out in MacCartney (20 ml) bottles. Sugar fermentation and organic acid utilisation tests were carried out in 25-well Replidishes and inoculated with a 4 hour peptone water broth culture using a multipoint inoculator (Denley). Physiological and growth tests were carried out in 10 ml broth cultures.

Degradative tests

Casein (10% skim milk) and tributyrin (1% v/v) were incorporated in TSA; clearing of the agar under and around the growth was scored as positive. The hydrolysis of carboxymethylcellulose (CMC, 0.5% w/v) was examined in the same basal medium but detected by flooding with 1 M HCl and ethanol and noting zones of clearing. The hydrolysis of gelatin, hippurate and tyrosine was determined by the methods of Gordon et al. (1973) after incubation for 5 and 10 days. The production of a brown pigment from tyrosine was also noted after incubation for 10 days. The hydrolysis of chitin (0.5% w/v), xylan (0.5% w/v) and *Saccharomyces cerevisiae* cells (OD 600 nm about 0.6) for beta-glucanase activity, was detected by the presence of clear zones around growth in a basal medium of ammonium salts solution (ASS) (Gordon et al. 1973) solidified with agar. The hydrolysis of pustulan (0.3% w/v) and pullulan (0.8% w/v) was detected in ASS medium after 3 days incubation followed by flooding with ethanol. Hydrolysis of DNA (Bacto DNase test agar; Difco) and RNA (0.3% w/v) in nutrient agar was detected by precipitation with 1 M-HCl. Pectin degradation was based on the use of 0.5% (w/v) polygalacturonic acid in ASS agar; after incubation for 5 days plates were flooded with 10% $CuSO_4 \cdot 4H_2O$ in

10% acetic acid and left for 3 to 4 hours. A heavy white halo against a blue background was considered positive. Starch hydrolysis was determined according to Gordon et al. (1973).

Utilisation of organic acids
Citrate and lactate (0.5%) were provided as carbon sources in the medium of Gordon et al. (1973). Positive results were indicated by a change of the indicator colour to green.

Tolerance tests
Tryptone soya broth (TSB) supplemented appropriately was examined for growth of the test strains at pH 5.5, 6.0 and 6.5, at various temperatures (Table 2) and in the presence of lysozyme (10 μg/ml Sigma L 6876), potassium tellurite (0.3% w/v), sodium azide (0.2%) and sodium chloride 2 and 10% (w/v) after incubation for 3 days.

Acid from carbohydrates
The production of acid from carbohydrate was examined after incubation for 3 days using the medium of Gordon et al. (1973).

Miscellaneous tests
The reduction of nitrate and nitrite, the oxidase reaction, the production of catalase, the methyl red and Voges-Proskauer tests and the oxidation/fermentation of glucose were detected using the methods of Cowan (1974).

Morphology and pigmentation
Colonial morphology (Wilson & Miles 1964) was examined after growth on TS agar for 16 hours.

Data analysis
Thirty-four strains were duplicated and the results analysed according to Sneath & Johnson (1972) to estimate test variance and the average probability (p) of an erroneous test. The final matrix comprised data for 234 strains and 96 tests. Data were scored in binary code and analysed using the CLUSTAN 3 package (Wishart 1987) on a DECVAX system computer version 5.1. Three proximity coefficients (Jaccard, S_J, simple matching S_{SM}, pattern difference D_P) were calculated and the similarity values were clustered using the unweighted pair group method with arithmetic averages (UPGMA) algorithm (Sneath & Sokal 1973). Cophenetic correlation values were determined for the three dendrograms produced using the CLUSTAN program. Principal component analysis (PCP) of the data was also carried out using this package.

Identification procedures
The frequency matrix was examined using the CHARSEP (Sneath 1979a) and DIACHAR (Sneath 1980a) programs which calculate the character separation indices and the most diagnostic tests for clusters, respectively. The matrix was further tested for cluster overlap using OVERMAT (Sneath 1980c) and hypothetical median organisms were calculated and identified using MOSTTYP (Sneath 1980b). Strains were identified using the MATIDEN program (Sneath 1979b).

DNA extraction
Chromosomal DNA was extracted from the test strains (Tables 3 and 4) by the method of Rodriguez & Tait (1983). Cultures were grown in either brain heart infusion or tryptone soy broth (both Oxoid) to yield ca. 3 to 4 g wet weight of biomass (culture OD600 = 1.2). All DNA samples were dialysed against the buffer to be used for that particular experiment with at least 4 changes of buffer over 3 days. The DNA concentration was determined by using the diphenylamine assay (Johnson 1981) with salmon testes DNA as standard in the range of 5 to 100 μg/ml.

Determination of Tm
DNA samples were diluted in 0.1 $\times$ SSC until the A_{260} = 0.4–0.6. The change in absorbance at 260 nm was continuously monitored using a PYE-Unican SP8-100 spectrophotometer equipped with a programmable thermal cycler operating an electrically heated cell block. All samples were initially tested with a temperature gradient of 1°C/minute to provide an estimate of the *T*m. The runs were then repeated at a gradient of 0.5°C/minute at an interval that was close to the denaturation curve. The *T*m was determined from the point of inflexion of the heating curve. All *T*m determinations were repeated until three results were obtained that were within 1°C of one another. The mol % G + C ratio was calculated from the equation: mol % G + C = 2.44 (*T*m-53.9) (Fahmy et al. 1985).

Estimation of DNA:DNA sequence homology
Probe DNA was labelled with ^{32}P-dCTP (Feinberg & Vogelstein 1983) and hybridized to target DNA immobilized on nitrocellulose filters using the slot blot sys-

tem of Schleicher and Schuel (Alexander & Priest 1989). The filters were hybridized under non-stringent conditions (20–25°C below *T*m); % homology was calculated from the radioactivity of the hybrids relative to non-homologous (salmon testes) and homologous controls (Johnson 1981). All determinations were performed in triplicate and the results represent the average values.

Results

Numerical classification

Previous studies (unpublished) had revealed that antibiotic resistance tests were unreliable when used at the high temperature necessary for the growth of thermophilic bacilli, hence such tests were not included in this study. Endospore morphology was also excluded from the database as several strains failed to produce spores. Where endospores were observed, they were elliptical and formed slightly or prominently swollen sporangia.

Estimation of test error from the 34 duplicated strains revealed total variance of 0.057 which corresponds to a probability of test error of 5.7%. There was no evidence that any particular type of test was especially unreliable, but DNA hydrolysis (Pi, 0.21) and casein hydrolysis (Pi, 0.20) were the least reproducible tests and the sugar fermentation tests the most reliable (average Pi 0.05). The final $n \times t$ table comprised data for 340 strains and 96 tests. The cophenetic correlation values were 0.872, 0.723 and 0.587 for the S_J, S_{SM} and D_p, UPGMA analyses respectively. Eighteen multimembered phena were recovered at 0.54–0.61 S_J and 0.78–0.8 S_{SM} and 17 in the Dp analysis together with numerous single membered clusters. Minor strain rearrangements were noticed between the three dendrograms but these did not affect the identity of the clusters. The only major differences were the fusing of clusters 17 and 18 from the S_J/UPGMA analysis when analysed using the S_{SM} and D_P/UPGMA combinations and phenon 9 of the S_J/UPGMA classification was split into two groups, represented by the two reference strains (12060 and 12061), at the 79% S level by the S_{SM} coefficient. Detailed results are presented for the S_J/UPGMA classification (Fig. 1) given the relatively high cophenetic correlation value.

The integrity of the 18 defined clusters were analysed using OVERMAT (Sneath 1980c) which revealed that no pair showed significant overlap at $V_o < 0.05$ but the standard deviation of some clusters was high (> 0.3) indicating heterogeneity. The properties of the defined taxa are given in Table 2.

Taxonomy of the established species

Seven clusters in the S_J/UPGMA classification could be identified unambiguously with validly described *Bacillus* species and are described below in the order in which they appear in the dendrogram (Fig. 1).

Phenon 1. *Bacillus pallidus [Cluster standard deviation (SD) 0.28]*
This phenon, which was defined at the 54% S_J level contained the type strain of *B. pallidus* and 29 other isolates including several strains from group 2 of Walker & Wolf (1971). In the PCP analysis, the type strain was recovered near the centre of the phenon which was homogeneous (data not shown). The inclusion of five outliers, strains 10527, 11147, 10316, 10326 and 12253, reduced the similarity level at which the cluster was delineated. Members of the cluster tended to be less thermotolerant than other test bacteria, and were unable to grow at 70°C. They were non-proteolytic and generally did not hydrolyse starch or reduce nitrate as a terminal electron acceptor (Table 2). The DNA analyses fully supported the integrity of this phenon. Chromosomal DNA base composition was 32 to 40% G + C for five strains (Table 3, see p.379) and three representative strains showed high levels of homology to the type strain (Table 4, see p. 382).

Phenon 2. *Bacillus kaustophilus (Cluster SD 0.31)*
Phenon 2 was defined at the 60% S_J level and contained the type strain of *B. kaustophilus*, a strain of '*B. thermoproteolyticus*', two strains from the study of Walker & Wolf (1971) and 10 environmental isolates. Members of this group hydrolysed casein, gelatin, starch and pullulan, and produced acids from various sugars. The DNA base content of the two strains examined was in the region of 40% but the type strain, ATCC 8005, had the much higher G + C content of 54% (Table 3). Moreover, DNA from *B. kaustophilus* ATCC 8005 showed limited homology with any other of the test strains including the other members of cluster 2 (Table 4). In the PCP plot this strain lay near the periphery of the group (Fig. 2a).

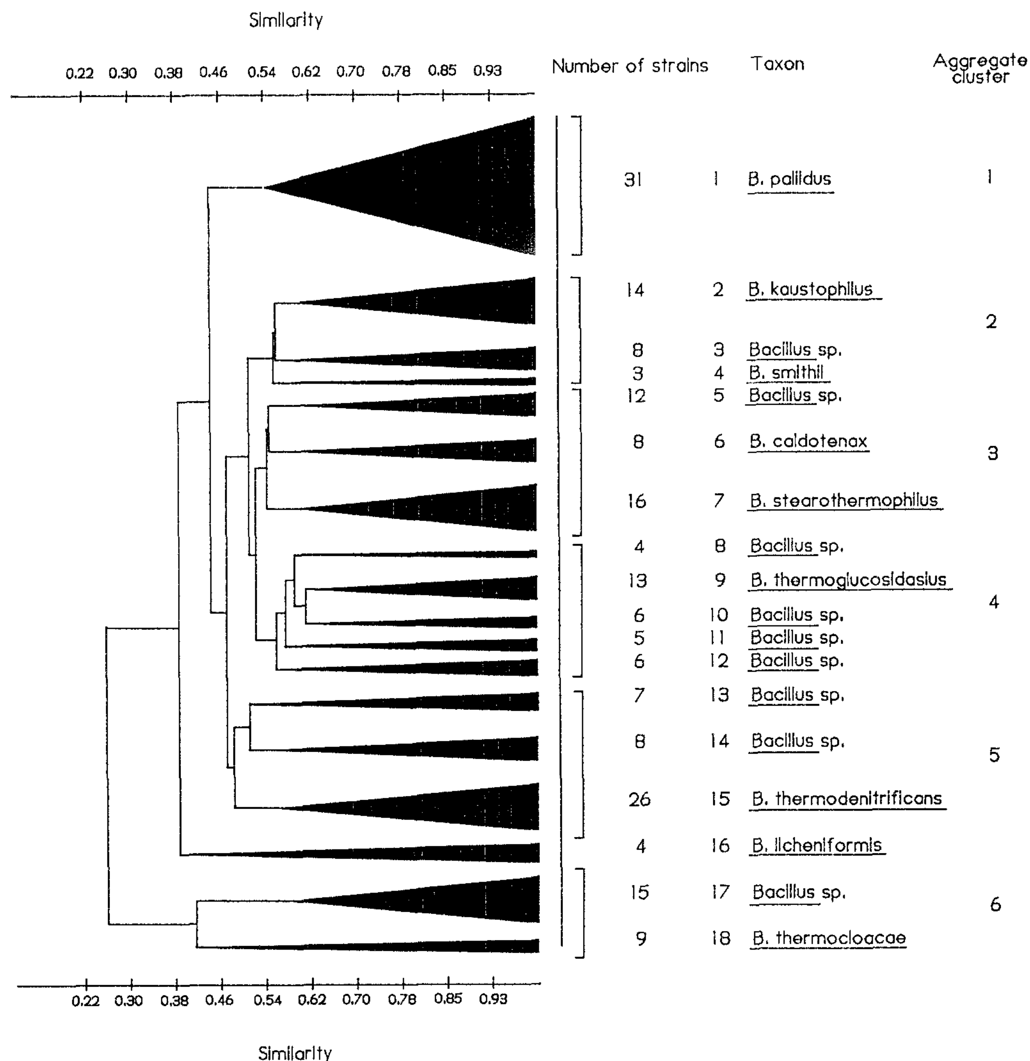

Fig. 1. Simplified dendrogram showing the relationships between clusters of thermophilic *Bacillus* strains based on the S_J coefficient and average linkage (UPGMA) cluster analysis.

Phenon 4. *Bacillus smithii (Cluster SD 0.25)*
This cluster, which was well separated from its neighbours in the dendrogram (Fig. 1) and PCP plot (Fig. 2a), contains two environmental isolates and a strain of *B. smithii*. The strains were non-proteolytic, unable to grow at 68° C or to grow in the presence of 2% NaCl but did grow at pH 5.5. The base composition of DNA from *B. smithii* 12052 (DSM 460) was 38% G + C (Table 3). The strain showed low homology with DNA from all of the other reference strains (Table 4).

Phenon 7. *Bacillus stearothermophilus sensu stricto (Cluster SD 0.22)*
This phenon was homogeneous in both the cluster analysis (low SD) and PCP plot (Fig. 2b). It contained 10 strains from culture collections, including the type strain of *B. stearothermophilus*, *'B. calidolactis'* ATCC 10149 and five strains from group 3a of Walker & Wolf (1971). The strains were typical of *B. stearothermophilus* and were sensitive to sodium azide with negative oxidase and catalase reactions. Members of this cluster hydrolysed pullulan, starch and tributyrin. The DNA base composition for six strains was in the range of 51.5 to 59% G + C, but strain 12005 (DSM 494) was atypical with a base composition of 43%; similar to the published figure of 46% (Manachini et al. 1968). It was therefore surprising that this strain showed high homology (73%) to the reference strain (Table 4). Strain 12769 (a beta-glucanase positive isolate from Iceland) was misplaced in this group

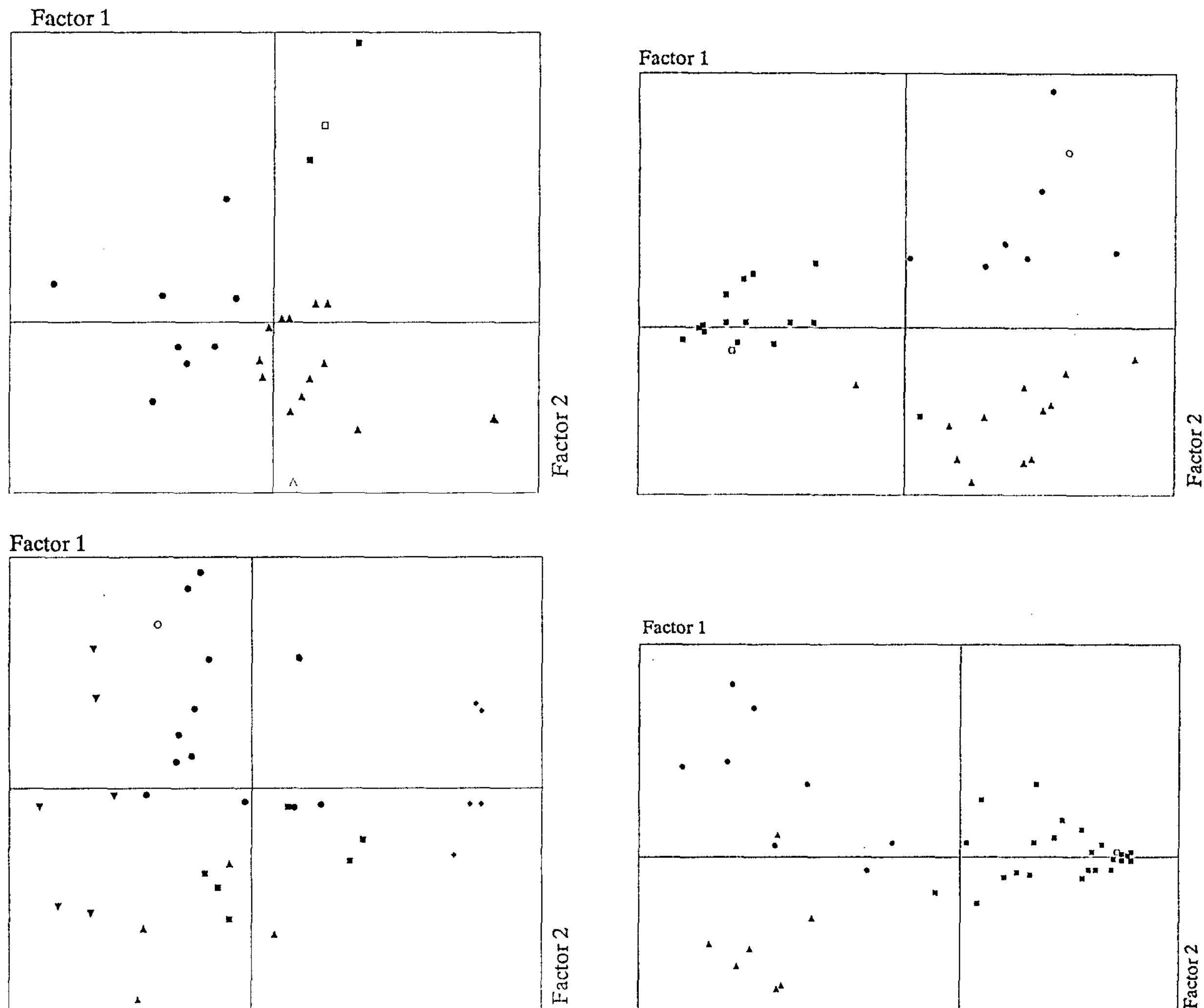

Fig. 2. Principal component analysis of; (a) aggregate cluster 2 (see Fig. 1) strains; ▲, taxon 2 (*B. kaustophilus*, △, type strain ATCC 8005); •, taxon 3 (*Bacillus* sp.); ■, taxon 4 (*B. smithii*, □, type strain DSM 460). (b) aggregate cluster 3 strains; ▲, taxon 5 (*Bacillus* sp.); •, taxon 6 (*B. caldotenax*, ○; type strain DSM 406); ■, taxon 7 (*B. stearothermophilus sensu stricto*, □; type strain NCA 26). (c) aggregate cluster 4 strains; ▲, taxon 8 (*Bacillus* sp.); •, taxon 9 (*B. thermoglucosidasius*, ○; type strain DSM 2542); ■, taxon 10 (*Bacillus* sp.); ♦, taxon 11 (*Bacillus* sp.); ▼, taxon 12 (*Bacillus* sp.). (d) aggregate cluster 5 strains; ▲, taxon 13 (*Bacillus* sp.); •, taxon 14 (*Bacillus* sp.) and ■, taxon 15 (*B. thermodenitrificans*, □; type strain DSM 466).

by cluster analysis and should have been assigned to cluster 6 in the dendrogram (see Fig. 2b). In support of this, DNA from strain 12769 did not hybridize with DNA from the reference strain of phenon 7.

Phenon 9. *Bacillus thermoglucosidasius (Cluster SD 0.3)*

Isolates from China, Thailand and USA clustered with *B. thermoglucosidasius* 12060 (DSM 2542) and 12061 (DSM 2543) in this cluster which was defined at the 63% S_J level. Principal components analysis (Fig. 2c) revealed a widely scattered plot which is reflected in the high SD of the cluster. Most of the strains hydrolysed gelatin, pullulan and starch and produced acid from numerous sugars including ribose and trehalose. DNA homology between *B. thermoglucosidasius* 12060 and strain 11099 (Walker & Wolf 1971; group 1b) was high (74%) and there was negligible homology between DNA from *B. thermoglucosidasius* 12060 and DNA from any other test strains. The G + C contents of DNA from strains 12060 and 11099 were both 43%.

Phenon 16. *Bacillus licheniformis (Cluster SD 0.23)*
This cluster contained the type strain of *B. licheniformis* and three environmental isolates. These thermotolerant organisms were characterized by CMC hydrolysis, growth in 10% sodium chloride and at 25°C.

Phenon 18. *Bacillus thermocloacae (Cluster SD 0.22)*
Phenon 18 was united with phenon 17 in the S_{SM} and Dp/UPGMA analyses but separated when the negative correlation was removed in the S_J analysis. Indeed, the two taxa were also delineated on the PCP plot (data not shown). The nine strains in phenon 18 resembled *B. thermocloacae* closely. These generally unreactive organisms produced an alkaline reaction in the oxidation/fermentation test and, with few exceptions, did not produce acid from carbohydrates. The DNA base composition for two strains was 40%. DNA from strain 10531 hybridized extensively with DNA from strains of cluster 17 but not with DNA from any other test strain.

Taxonomy of species incertae sedis

Two clusters equated with thermophilic *Bacillus* species which are not included in the Approved Lists of Bacterial Names or their supplements and therefore have lost standing in bacterial nomenclature.

Phenon 6. *'Bacillus caldotenax ' (Cluster SD 0.32)*
This taxon encompassed eight strains including the three 'caldo-active' bacteria, '*B. caldotenax*', *B. caldovelox*' and '*B. caldolyticus*' together with isolates from Iceland and New Zealand. The PCP plot (Fig. 2b) of these strains revealed heterogeneity which was reflected in the relatively high SD of the cluster. The strains grew well at 72°C, reduced nitrate to nitrite and degraded tyrosine. The base composition of DNA from '*B. caldotenax*' 00263, '*B. caldolyticus*' 12028 and an isolate from Iceland was in the range 53 to 56% G + C. The high intragroup DNA homology among these strains coupled with low homology with other probe DNAs supports the integrity of this taxon.

Phenon 15. *'Bacillus thermodenitrificans' (Cluster SD 0.26)*
The 26 strains forming this cluster included '*B. thermodenitrificans*' DSM 465 and DSM 466, numerous strains from group 1a of Walker & Wolf (1971) and environmental isolates from France, Iceland and Thailand. The low SD of the cluster was supported by the close scatter of the strains in the PCP plot (Fig. 2d). The members of the group were characterised by the fermentation of arabinose and melibiose, the reduction of nitrate to gas and a rough non-spreading colony morphology with fimbriate colony margins. The average G + C content of the DNA from strains 10079, 10513, 11024 and 12019 was 51.5% and there was high homology between DNA from '*B. thermodenitrificans*' DSM 466 and a member of Walker & Wolf's (1971) group 1a (Strain 11024) thereby supporting the integrity of this taxon.

Taxonomy of unnamed clusters

Half of the clusters recovered in the S_J/UPGMA analysis could not be identified with known *Bacillus* species and probably represent previously undescribed taxa. The lack of homology between recipient DNAs from strains representing clusters 3, 5, 8, 11, and 12, and probe DNAs from reference strains confirms the novelty of the clusters. Brief descriptions of some of these taxa are presented below.

Phenon 3 was well defined in the S_J/UPGMA analysis (cluster SD 0.3) and the PCP plot (Fig. 2a). It contained eight environmental isolates which were proteolytic, grew well at 70°C and reduced nitrite to gas (Table 2).

Phenon 5 (Cluster SD 0.32) contained twelve strains, 10 of which were from Iceland (Figs 1 and 2b). Most of these strains hydrolysed yeast cell walls, which is an indication of beta-glucanase activity and is an unusual attribute among the thermophilic bacilli (strains 12058, 12755 and 12789 were beta-glucanase negative). The DNA base composition of strain 12757 (beta-glucanase positive) was 55% and strain 12755 (beta-glucanase negative) was 43% G + C.

Phenon 8 (cluster SD 0.29) encompassed three strains from group 1b of Walker & Wolf (1971) and an environmental isolate. These strains produced a diffusible brown pigment when grown on tyrosine agar and acid from xylose. The base composition of DNA from strain 11103 was 41% G + C.

The six strains in phenon 10 (cluster SD 0.3) were all originally assigned to group 1b of Walker & Wolf (1971). Strains in this fairly dispersed group were generally unable to hydrolyse casein and gelatin (although some strains did so weakly) but they did produce acid from melibiose, and mannose, and grew at 72°C and in the presence of 5% sodium chloride.

Phenon 11 (cluster SD 0.3) contained members of Walker & Wolf's (1971) group 1b and two strains from

group $1b_2$. Despite the high SD of the cluster, the PCP plot revealed a homogeneous group. These bacteria hydrolysed gelatin but not casein and most produced acid from dextrin, melibiose, raffinose and ribose. All but one of the strains (11072) utilized tyrosine. The G + C content of DNA from strain 11101 was 42%.

Phenon 12 (cluster SD 0.28) contained five environmental isolates from East Asia which clustered at the 62% S_J (84% S_{SM}) level. They were joined by *B. stearothermophilus* EP 262 at 55% S_J (83% S_{SM}). The inclusion of EP 262 was justified by the low SD of the cluster and reasonable scatter in the PCP plot (Fig. 2c). The strains hydrolysed gelatin and starch but not casein and grew at 72°C. The DNA base composition of strain 12657 was 40% G + C.

Phenon 13 (cluster SD 0.33) contained six strains from China and Thailand with two members of Walker & Wolf's (1971) group 1b. Most of these strains hydrolysed hippurate and produced acid from ribose and trehalose.

Phenon 14 (cluster SD 0.33) strains were largely environmental isolates from Hong Kong and Iceland and differed from those of phenon 13 in the degradation of aesculin, hydrolysis of tributyrin and acid production from salicin and galactose. The looseness of clusters 13 and 14 (relatively high SDs) was apparent in the PCP analysis of this group (Fig. 2d).

Phenon 17 (cluster SD 0.22) contained 16 environmental isolates which were generally unreactive in the selected tests. These strains were distinguished by an alkaline reaction in the oxidation/fermentation test, hydrolysis of DNA and a low G + C content (average 35% for three strains).

Minor clusters

Forty-one strains formed single or two-membered clusters at the 51% S_J level and for clarity these organisms have been excluded from Fig. 1. Most of these were environmental isolates, but of the named strains, *B. pallidus* H14 failed to join phenon 1 in the S_J analysis although it was included in this cluster in the D_P analysis indicating that it may be atypical in growth rate or reactivity in the tests used. '*Bacillus flavothermus*' DSM 2641, a yellow pigmented bacterium, remained a single membered cluster in all analyses as did the tyrosine-degrading isolate '*B. thermotyrovorans*' (Sharp et al. 1989).

Computer-assisted identification

The data in Table 2 were evaluated using CHARSEP, a program which calculates separation indices for the characters in a frequency matrix (Sneath 1979a). Twenty-three characters are highlighted in Table 2 of which all but reduction of nitrate to nitrogen and 'spreading-4 morphology' had VSP indices > 25% as recommended by Sneath. Using these data for the 18 taxa, the OVERMAT program indicated that overlap exceeded 5% in only one case, that between taxa 1 (*B. pallidus*) and 8 (*Bacillus* species).

Previous studies with mesophilic bacilli had indicated that an acceptable identification was obtained with Willcox probability scores of 0.95 or greater preferably when associated with a figure for standard error of taxonomic distance (SED) below 7.0 (Priest & Alexander 1988). These criteria were therefore adopted in the present study. When hypothetical median organisms were calculated they were correctly identified using MOSTTYP (Sneath 1980b) with Willcox probabilities of 0.999 to 1.0 and SED values below -2.0. Twenty-seven strains were selected randomly and all but two (*B. stearothermophilus* 11200 and strain 12810 from phenon 14) were correctly identified using the classification test data with Willcox probabilities of > 0.95 and SED scores < 2.0. Interestingly, strain 12058, originally misclassified in phenon 5, was correctly identified as *B. stearothermophilus sensus stricto* with a Willcox probability of 0.999. However, when the test data for 20 strains were re-determined and the identifications repeated, the Willcox probabilities were generally reduced (Table 5, see p. 386). Nevertheless, 13 strains (65%) identified to the correct taxon with Willcox probabilities above 0.95 and SED scores below 3.12 but three strains identified to the wrong taxon and four strains were not identified. Of 13 environmental isolates examined for the battery of tests, 2 identified to *B. smithii* and three to *B. kaustophilus* with Willcox probabilities greater than 0.95 and SED scores below 3.3. The remainder were not identified.

Discussion

Numerical classification has proved to be an invaluable approach to the classification of large heterogeneous taxa such as *Bacillus* (Priest et al. 1988) and *Streptomyces* (Williams et al. 1983a). In general, taxa defined phenotypically have subsequently proven to be genomically homogeneous by DNA reassociation.

Some examples from the mesophilic bacilli include phena 4, 9, 16 and 47 of Priest et al. (1988) which were raised to species status as *B. glucanolyticus* (Alexander & Priest 1989), *B. amylolyticus* (Nakamura 1984), *B. atrophaeus* (Nakamura 1989) and *B. smithii* (Nakamura et al. 1988) respectively when DNA reassociation studies confirmed homogeneity among clusters and distinctiveness from other taxa. In some circumstances, numerically defined clusters have been shown to be genomically diverse, particularly in cases where the bacteria are physiologically inert and have responded negatively to many of the tests used in numerical classification studies. In these instances it is important to adopt testing regimes appropriate to the metabolic capabilities of the strains under study (Alexander & Priest 1991).

The congruence between the numerical classification and DNA reassociation studies reported here is particular encouraging and confirms the phenetic homogeneity of taxa such as '*B. caldotenax*', *B. pallidus, B. stearothermophilus sensu stricto* and *B. thermodenitrificans*. Moreover, the enormous diversity of the thermophilic bacilli has been emphasized. Of the 9 unnamed phena shown in Fig. 1, DNA from strains representing phena 3, 5, 8, 11 and 12 did not hybridize strongly with any of the probe DNA molecules thereby endorsing the separateness of these taxa and hence indicating that they do not represent physiologically atypical variants of established species but in fact comprise independent taxa. Similarly, the 41 single membered clusters presumably denote numerous other centres of variation which may be equated with species when more strains are isolated and characterized.

This study has also revealed the value of including non-hierarchic analyses with cluster analysis. For example, the mis-classification *B. stearothermophilus* 12058 and the phenon 5 strain 12769 by cluster analysis (Fig. 1) was highlighted in Fig. 2b. Moreover, diffuse clusters such as those in Fig. 2c or well-circumscribed taxa such as *B. stearothermophilus sensu stricto* were readily indicated. Logan and Berkeley (1981) also noted the value of PCP analysis for the separation of *Bacillus* clusters derived from phenotypic analysis. The general correlation of cluster standard deviation with PCP analysis suggests that the former is also a useful indicator of cluster heterogeneity. The ensuing discussion focuses on the taxonomy of the major taxa recovered in this study beginning with those associated with validly described species.

Bacillus pallidus (Scholtz et al. 1987) has been placed in RNA group 5 (Ash et al. 1991) close to, but distinct from *B. stearothermophilus* (Ash, personal communication). The properties for this species given in Table 2 are in agreement with the original description (Scholtz et al. 1987) with the exception that negative results were recorded in the present study for growth in the presence of 10% sodium chloride and tributyrin hydrolysis and positive results for acid production from cellobiose and mannose. The base composition for the type strain was 40% G + C which is in accordance with the published figure (Scholtz et al. 1987) but other strains showed a slightly lower value (32 to 39 mol %). The starch-negative strains of Daron (1967) and Epstein & Grossowicz (1969) were included in the *B. pallidus* phenon indicating that this species had been described several years before the published description of Scholtz et al. (1987).

Bacillus kaustophilus presents some taxonomic problems. The type strain (ATCC 8005) was isolated by Prickett (1928) from pasteurised milk and was assigned to group IIIb in the numerical classification of Klaushofer and Hollaus (1970) and to group 1B by Walker & Wolf (1971). It was recovered as a single membered cluster in an earlier numerical classification (Priest et al. 1981). Here it was recovered in cluster 2 with numerous environmental isolates but it was located on the periphery of this group in the PCP analysis. *Bacillus kaustophilus* 12002 (ATCC 8005) had a much higher DNA base composition than other members of phenon 2 and the DNA did not reassociate with that from another member of the group. Small subunit rRNA sequences place strain ATCC 8005 as a distinct member of the thermophilic branch (group 5) of the *Bacillus* phylogenetic tree (Ash, personal communication). There is therefore no doubt that *B. kaustophilus* ATCC 8005 represents a species which is separate from other thermophilic species but its affinity with phenon 2 of this study is dubious. An emended description of *B. kaustophilus* based on the reactions of the type strain is given at the end of this Discussion.

Bacillus stearothermophilus sensu stricto (phenon 7) was composed almost entirely of culture collection strains with a single environmental isolate which was discovered to be a misclassified strain from phenon 5. This taxon equates with Walker & Wolf's (1971) group 3a and the Klaushofer & Hollaus (1970) group IIIA. It is difficult to compare the properties outlined in Table 2 with those mentioned in the original description by Donk (1920) since only six tests are common to the two studies. However, there is consistency between the data in Table 2 and those from other studies (Sharp et al. 1980; Logan & Berkeley 1981; Priest et al. 1981,

1988) so this profile can be considered authoritative. The base composition of the DNA is consistently in the range 52 to 54 mol G + C (Table 3; Sharp et al. 1980; Fahmy et al. 1985) but *B. stearothermophilus* strains 12005 and 12065 are atypical in having low (Table 3; Manachini et al. 1968) and high G + C contents respectively.

Thermophilic strains which secreted α-glucosidases with preference for the 1,6 glycosidic bond have been classified as *B. thermoglucosidasius* (Suzuki et al. 1975). It is reassuring that these organisms formed a reasonably homogeneous taxon (phenon 9, Fig. 1) both phenotypically (with some scatter in the PCP plot, Fig. 2b) and genotypically (high reassociation and close base composition of two strains) as this species has not been examined in previous numerical studies.

Bacillus licheniformis is a thermotolerant organism which is often cited in studies of thermophilic bacilli (e.g., Debartolomeo et al. 1991) because of the ability of many strains belonging to this species to grow at 60°C. However, the ability of such strains to grow at 25°C readily distinguishes them from the truly thermophilic organisms as does their hydrolysis of CMC an apparently rare property of true thermophilic bacilli.

The final two clusters (17 and 18) in the S_J/UPGMA dendrogram contained generally unreactive strains which were combined in the other cluster analyses. Although the difference in DNA base composition between representatives of the two phena indicates separate taxa, the DNA reassociation data are consistent with a single species and the standing of these taxa requires further investigation. It is unfortunate that authentic strains of *B. thermocloacae* were not available when this study was conducted because both taxa 17 and 18 conform with the published description of this species being uniformly negative in most tests. It seems likely that members of both clusters are closely related to, if not identical with *B. thermocloacae.* A thermophilic strain of *B. sphaericus* was recovered in cluster 18 but it is likely that this was a function of negative correlation as *B. sphaericus* strains are notably unreactive in most physiological tests based on sugar fermentations (Alexander & Priest 1991).

Two clusters contained named strains from species which are not included in the Approved Lists of Bacterial Names. The three 'caldo-active' strains of Heinen & Heinen (1972) were recovered together in both the cluster analysis and the PCP plot. The production of extracellular amylase and proteases were considered to be distinguishing features of these taxa but Sharp et al. (1988) reported strong amylase production from '*B. caldolyticus*' and restricted production from strains of the other two species. This is in accordance with the data presented in Table 2, although there is also variation in protease synthesis. The DNA base composition for the three representatives were uniform (54.6 ± 1.7) and much lower than the values previously reported by Sharp et al. (1980) for '*B. caldotenax*' (64.8%) and '*B. caldovelox*' (65.1%), but similar to that reported for '*B. caldolyticus*' (52.3%). Moreover, although extensive reassociation between the DNA from the three strains was found, Sharp et al. (1980) reported high homology between '*B. caldotenax*' and '*B. caldovelox*' only, '*B. caldolyticus*' shared only 38% homology with '*B. caldotenax*'. The reasons for these discrepancies are not clear at present hence the revival of *B. caldotenax* is recommended to include strains previously labelled as '*B. caldotenax*' and '*B. caldovelox*' with '*B. caldolyticus*' remaining as *species incertae sedis* until further DNA data are available.

The 26 strains assigned to phenon 15, the '*B. thermodenitrificans*' cluster, formed a homogeneous taxon with respect to the cluster analysis, the PCP plot (Fig. 2c), the base composition of four strains (51.5 ± 1.45 mol % G + C) and DNA reassociation data. Probe DNA from '*B. thermodenitrificans*' DSM 466 hybridized less than 40% with all other DNA samples in the study with the exception of that from strain 11024, a second member of this taxon and a member of Walker & Wolf's (1971) group 1a. This cluster can be equated with '*B. thermodenitrificans*', an organism which was originally described and named by Ambroz (1913) as '*Dentribacterium thermophilum*'. Subsequently, the species was neglected and the original strains lost, although Allen (1953) considered the taxon to merit species status. Two isolates resembling the original description were deposited in the DSM by Klaushofer and Hollaus (1970) and were examined in the present study. Of these two strains, '*B. thermodenitrificans*' DSM 466 more closely resembles the published description and reduces nitrate more strongly than '*B. thermodenitrificans*' DSM 465. Moreover, the former strain lies nearer to the centre of the cluster (Figs 1 and 2c) than the latter. It should be noted that the capacity to reduce nitrate to gas is not unique to members of this taxon, it is also a feature of some strains assigned to phena 3 and 13.

The nine clusters which could not be identified with existing species were phenetically well distinguished and it seems likely that most of these represent new species. Where DNA homology data were obtained (Table 4) strains from these taxa showed limited homol-

ogy with DNA from reference strains supporting their distinction from named thermophilic species. Nevertheless, additional DNA base composition and reassociation data are needed before these taxa can be proposed as new species.

Most of the single membered clusters consisted of new environmental isolates with the single exception of the yellow pigmented '*B. flavothermus*' (Heinen et al. 1982). This bacterium presumably forms the nucleus of a new taxon as do many of these isolates.

Phenetic databases have important uses beyond the initial circumscription of taxa. Williams et al. (1984) showed how phenetic information derived from numerical studies could be used to design selective media for the isolation of rare streptomycetes with the potential for synthesizing novel antibiotics, and this taxonomic approach to selective isolation has been used for the isolation of insect-pathogenic strains of *B. sphaericus* (Guerineau et al. 1992). The data in Table 2 should therefore be of assistance in the formulation of selective media for particular thermophilic bacilli, such as those of phenon 5 which degrade yeast cell walls or the tyrosine-degrading strains of phenon 11.

Identification

Identification of thermophilic bacilli has been severely hampered by the absence of a comprehensive identification table or data matrix in which the properties of all relevant taxa are compared. The data in Table 2 rectifies this deficiency and the selection of the most important diagnostic features into a frequency matrix for probabilistic identification should aid identification. The threshold scores for identification used here are similar to, or more stringent than those adopted for other Gram positive bacteria (reviewed in Priest & Williams 1993) and served well for the identification of strains from the original study. Moreover, the close agreement between identification scores using the original classification test results and repeated tests (Table 5) suggests that test error is not a serious problem. However, the poor success rate (37%) for identification of new isolates, albeit a small sample of strains, is much lower than for similar studies of coryneform (Hill et al. 1978), *Streptomyces* (Williams et al. 1983b) and mesophilic bacilli (Priest & Alexander 1988) in which more than 50% of strains were identified. It is likely that most of the unidentified isolates represent undescribed species (for example the numerous single-membered clusters in this study). Poor identification derives from inadequate classification and this inability to identify environmental isolates emphasizes the need for more extended taxonomic studies of the thermophilic bacilli.

Nomenclature

From the results of this polyphasic classification, the reintroduction of '*Bacillus caldotenax*' and '*Bacillus thermodenitrificans*' is recommended and an emended description of *Bacillus kaustophilus* is given.

Description of *Bacillus caldotenax* (Heinen & Heinen 1972) sp. nov. nom. rev. cald'o. tenax. L. adj. *caldus* warm, hot; L. adj. *tenax* tightly holding, adherent; heat-requiring bacteria resistant to breakage. The description given below is taken from the present and earlier studies (Heinen & Heinen 1972; Sharp et al. 1989). Strains of this species are characterized by the production of oval, terminal endospores which distinctly distend the mother cell. Strains produce acid from cellobiose, dextrin, glucose, fructose, melibiose, salicin and raffinose. They also hydrolyse starch, pullulan and tributyrin and are obligate thermophiles, that grow at 72°C. Citrate and to some extent lactate are used as carbon sources. Growth is inhibited by 10% NaCl and lysozyme. Base composition of chromosomal DNA is 54.6 ± 1.7 mol % (two strains). Source: Soil and water from geothermal areas. Type strain: DSM 406.

Description of *Bacillus thermodenitrificans* (Ambroz 1913) sp. nov. nom. rev. ther. mo. de. nitrifi.cans Gr. n. *therme* heat; M.L. inf. *denitrificare* to denitrify; M.L. part. adj. *denitrificans.* denitrifying. Heat-requiring bacteria capable of reducing nitrate to nitrogen. The description is taken from this study and from several others (Klaushofer & Hollaus 1970; Walker & Wolf 1971; Priest et al. 1988; Sharp et al. 1989). The strains in this species are characterised by the ability to hydrolyse starch, RNA, tributyrin and xylan. Nitrate and nitrite are strongly reduced to gas. Acid is produced from arabinose, cellobiose, melezitose, melibiose and trehalose but not from galactose or rhamnose. The strains are (moderately) thermophilic with a maximum growth temperature of 70°C. Flat colonies with a fimbriate colonial margin are produced on tryptone soya agar at 55°C. The G + C content of the four strains examined is 51.48 ± 1.45 mol %; the figure for the type strain is 52.2 mol %. Source: Soil. Type strain: DSM 466.

Emended description of *Bacillus kaustophilus* (Prickett 1928; Priest, Goodfellow & Todd 1988). Strains in this species hydrolyse casein, DNA, gelatin,

hippurate, pullulan, starch and tributyrin but not aesculin. Nitrate is reduced to nitrite. Pigment is produced on tyrosine medium. Acid is produced from adonitol, cellobiose, inositol, maltose, mannose, salicin, sucrose, trehalose and xylose, but not from arabinose, glycerol or ribose. Grows in the presence of 2% sodium chloride, 0.3% potassium tellurite and lysozyme and at 68°C, but not in the presence of 5% sodium chloride or at 70°C. Citrate but not lactate utilized. Colonies are convex and transparent. The G + C content of the DNA of the type strain is 53.9 mol %. Source: pasteurised milk, soil. Type strain: ATCC 8005.

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Table 1. Designation and source of strains assigned to clusters defined at the 60 to 65% S_J/UPGMA level

Strain	Source
Strains assigned to cluster 1. (*Bacillus pallidus*)	
11138, 11157	LUDA T138, LUDA T157, Walker and Wolf Group 2
12243	Yellowstone National Park
12064	*B. stearothermophilus* DSM 2349
12606	Soil from Sha Tin, Hong Kong
10517, 10503, 10534, 10527	Soil from Barbados
00136	"*B. stearothermophilus*" DSM 2334
10018	Airbourne contaminant, CAMR
11152	LUDA T152, Walker and Wolf Group 2
10503	Soil from Barbados
11167	LUDA T167, Walker and Wolf Group 2
12613	Kowloon, Hong Kong
05169, 05170, 10218	Poole, UK
11132	LUDA T132, Walker and Wolf Group 2
12672	Soil from Thailand
10053, 10208, 10057	Soil from France
12716	Soil from Skalholt, Iceland
12621	Soil from Shiqi, South China
12068	*B. pallidus* DSM 3670
10093	Hot water supply, CAMR
11147	LUDA T147, Walker and Wolf Group 2
10316, 10326	Soil from New Zealand
12253	Soil from Nigeria
Strains assigned to cluster 2 (*B. kaustophilus*)	
12612	Soil from Hoi Ha, Hong Kong
10058	Soil from France
12725	Skalholt, Iceland
10332, 10315	Near Lake Taupo, New Zealand
12663	Soil from Kanchanaburi, Thailand
12059	"*B. thermoproteolyticus*" (Rokko)
12728	Soil from Mosfell, Iceland
10007	Soil from Wales, UK
11127	LUDA 127, Walker and Wolf Group 1b3
12002	*B. kaustophilus* ATCC 8005 (NCIB 8547)
12734, 12794	Isolate from Hveragerthi, Iceland
11082	LUDA T82, Walker and Wolf Group 1b
Strains assigned to cluster 3 (*Bacillus* sp.)	
12660, 12661	Water from River Kwai, Thailand
12791	Isolate from Hveragerthi, Iceland
12604	Isolate from Sai Kung, Hong Kong
12731	Isolate from Laugaths, Iceland

Table 1. continued

Strain	Source
12615	Isolate from Hoi Ha, Hong Kong
12701	Isolate from Iceland
10242	Compost from Salisbury
Strains assigned to cluster 4 (*B. smithii*)	
10067	Soil from La Rochelle, France
12052	*B.coagulans* DSM 460
12662	Isolate from Kanchanaburi, Thailand
Strains assigned to cluster 5 (*Bacillus* sp.)	
12755, 12758, 12741, 12813, 12764	Isolate from Hveragerthi, Iceland
12745	Isolate from Mosfell, Iceland
12757	Geyser outlet, Iceland
12796	Isolate from Mosfell, Iceland
12789	Isolate from Skalholt, Iceland
12058	LUDA T214, Walker and Wolf Group 3a
10303	Geothermal area, New Zealand
10338	Icelandic isolate SM-4
Strains assigned to cluster 6 ("*B. caldotenax*")	
10331	Near Lake Taupo, New Zealand
12793, 12737	Isolate from Hveragerthi, Iceland
00263	"*B. caldotenax*" DSM 406
12028	"*B. caldolyticus*" DSM 405
10304	Geothermal area, Iceland
12025	"*B. caldovelox*" DSM 411
11125	LUDA T125, Walker and Wolf Group 1b1
Strains assigned to cluster 7 (*B.stearothermophilus sensu stricto*)	
11210	*B. stearothermophilus* NCA 26 (type strain)
00170	*B. stearothermophilus* NCA 1503
11182, 11168, 11220	LUDA T183, LUDA T168, LUDA T220, Walker and Wolf Group 3a
12045	*B. stearothermophilus* ATCC 7953-1
12057	LUDA T210, Walker and Wolf Group 3a
12026, 12027	*B. stearothermophilus* ATCC 12016, ATCC 12980
12003	"*B. calidolactis*" ATCC 10149
12005, 12001, 12004, 12065	*B. stearothermophilus* DSM 494, DSM 456, ATCC 12976, DSM 1550
11200	LUDA T200, Walker and Wolf Group 3a
12769	Isolate from Hveragerthi, Iceland

Table 1. continued

Strain	Source
Strains assigned to cluster 8 (*Bacillus* sp.)	
12609	Isolate from Hong Kong
11060, 11077, 11103	LUDA T60, LUDA T77, LUDA T103, Walker and Wolf Group 1b1
Strains assigned to cluster 9 (*B. thermoglucosidasius*)	
11099	LUDA T99, Walker and Wolf Group 1b2
12668, 12670, 12673	Soil from Bangkok, Thailand
12678	Soil from Canton Zoo, China
12664	Isolate from Kanchanaburi, Thailand
12061, 12060	*B. thermoglucosidasius* DSM 2543, DSM 2542
12641	Isolate from Ayttahya, Thailand
Strains assigned to cluster 9 (*B. thermoglucosidasius*) (Cont'd)	
11056	LUDA T56, Walker and Wolf Group 1b1
12633	Soil from Thailand
12251	Yellowstone National Park, USA
12656	Soil from Lon Chin, South China
Strains assigned to cluster 10 (*Bacillus* sp.)	
11040, 11080, 11058, 11057	LUDA T40, LUDA T80, LUDA T58, LUDA T57, Walker and Wolf Group 1b1
11061, 11041	LUDA T61, LUDA T41, Walker and Wolf Group 1b2
Strains assigned to cluster 11 (*Bacillus* sp.)	
11068, 11073, 11072	LUDA T68, LUDA T73, LUDA T72, Walker and Wolf Group 1b1
11101, 11111	LUDA T101, LUDA T111, Walker and Wolf Group 1b2
Strains assigned to cluster 12 (*Bacillus* sp.)	
12617	Isolate from Hoi Ha, Hong Kong
12620	Isolate from Shiqi, South China
12608	Isolate from Hong Kong
12650	Soil from South China
12657	Soil from Lon Chin, South China
00262	"*B. stearothermophilus*" EP262 (Sharp et al. 1980)

Table 1. continued

Strain	Source
Strains assigned to cluster 13	
12635	Soil from Thailand
11074	LUDA T74, Walker and Wolf Group 1b1
12646	Isolate from Chung Kai, Thailand
12630	Isolate from Dali Peoples Commune, China
11071	LUDA T71, Walker and Wolf Group 1b1
12637	Isolate from Lamma Island, Hong Kong
Strains assigned to cluster 14	
12618, 12619	Soil from Tai Mo Shau, Hong Kong
10205	Soil from Pammakale, Turkey
12810, 12788, 12815	Isolate from Hveragerthi, Iceland
12820	Isolate from Mosfell, Iceland
12651	Soil from Foshan, South China
Strains assigned to cluster 15 ("*B. thermodenitrificans*")	
11016, 11019, 11025	LUDA T16, LUDA T19, LUDA T25, Walker and Wolf Group 1a
12806	Isolate from Hueragerdi, Iceland
11038, 11033	LUDA T38, LUDA T33, Walker and Wolf Group 1a
10513, 10501	Soil from Barbados
11024	LUDA T24, Walker and Wolf Group 1a
Strains assigned to cluster 15 ("*B. thermodenitrificans*") (Cont'd)	
12019	"*B. thermodenitrificans*" DSM 466
11028	LUDA T28, Walker and Wolf Group 1a
10097	River Cam, Cambridge, UK
12823	Soil from Iceland
10211, 10209	Soil from Paris, France
11022, 11020	LUDA T22, LUDA T20, Walker and Wolf Group 1a
12628	Isolate from Deli Peoples Commune, China
12647	Isolate from Chung Kai, Thailand
12643	Isolate from Ayutthaya, Thailand
10079, 10078	Soil from La Mont St Michel, France
10150	Soil from Greece
12669	Soil from Bangkok, Thailand
10168	Soil from Calcutta, India
12018	"*B. thermodenitrificans*" DMS 465

Table 1. continued

Strain	Source
Strains assigned to cluster 16 (*B.licheniformis*)	
12020, 12066	*B. licheniformis* LO2, DSM 13
10227, 10229	Pond mud from Zaire
Strains assigned to cluster 17 (*Bacillus* sp.)	
10013	Water sample from Boston, UK
10035, 10030, 10042, 10026, 10031, 10040, 10019, 10036	Soil from Bracknell, UK
10241, 10240	Compost from Salisbury, UK
10508	Isolate from Barbados
10105	Spring sample St Albans, UK
10182	Soil from Calcutta, India
10124	Compost from Surrey, UK
Strains assigned to cluster 18 (*B. thermocloacae*)	
10531	Isolate from Barbados
10139, 10135, 10136, 10138	Compost from Salisbury, UK
12084	RJ13c, CAMR
10177	Soil from Calcutta, India
12041	*B.sphearicus* DSM 463
10158	Soil from Greece
Single membered clusters	
12069	*B. pallidus* H14,
10172, 10169, 10160, 10173, 10174	Soil from Calcutta, India
18312, 18302, 18305, 18314, 18309 18327, 18303, 18317	Soil from Wilton, UK
10190	Soil from India
12062	"*B. flavothermus*" DSM 2641
12212	Yellowstone National Park
10327, 10337	Near Lake Taupo, New Zealand
10073, 10074	Plate contaminant, CAMR
12715	Isolate from Skalholt, Iceland
10087	Soil from Granville, France
12638, 12639	Isolate from Lamma Island, Hong Kong
11085	LUDA T85, Walker and Wolf Group 1b1
12247	White Creek, Yellowstone National Park
12766	Isolate from Skalholt, Iceland
11222	LUDA T222, Walker and Wolf Group 3b2
12666	Soil from Bangkok, Thailand
12623	Isolate from Shiqi, S. China

Table 1. continued

Strain	Source
10518, 10525, 10533	Isolate from Barbados
10080	Soil from La Mont St Michel, France
10223, 10228, 10226	Pond mud from Zaire
10005, 10006	Soil from Wales

Strains listed in inverted commas are not in the Approved Lists of Bacterial Names.

Abbreviations: ATCC - American Type Culture Collection, CAMR - Centre for Applied Microbiology and Research, DSM - Deutsche Sammlung von Mikroorganismen, LUDA - Leeds University Deptartment of Agriculture, NCA - National Canners Assocation.

Table 2. Percentage distribution of positive characters to clusters defined at 60 to 65% S_J in the UPGMA analysis

	B. pallidus	B. kaustophilus	Bacillus sp	B. smithii	Bacillus sp	B. caldotenax	B. stearothermophilus	Bacillus sp	B. thermoglucosidasius	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp	B. thermodenitrificans	B. licheniformis	Bacillus sp	B. thermocloacae
Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. strains	31	14	8	3	12	8	16	4	13	6	5	6	6	8	26	4	15	9
Hydrolysis of:																		
Aesculin	97	71	62	99	58	75	75	50	85	50	20	99	33	99	77	99	1	22
Casein	3	99	99	1	42	25	81	25	77	33	20	1	88	87	19	99	1	1
CMC*[1]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	1	1
DNA	59	50	12	99	75	75	75	1	39	50	20	1	50	38	58	99	99	11
Gelatin	**13**	**71**	**62**	**1**	**25**	**12**	**81**	**50**	**99**	**17**	**99**	**99**	**83**	**99**	**19**	**66**	**13**	**33**
Hippurate	13	29	12	33	33	13	1	50	23	50	99	33	67	12	12	1	1	1
Pectin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pullulan	16	79	75	99	92	88	99	75	92	99	80	66	37	87	96	1	1	45
Pustulan	1	7	1	1	1	1	1	1	1	1	1	1	17	1	1	1	1	1
RNA	97	93	99	67	92	63	1	75	92	83	80	99	67	37	99	99	47	22
Starch	23	93	62	67	92	99	99	99	39	99	40	99	99	88	96	99	7	11
Tributyrin	**1**	**79**	**99**	**33**	**83**	**88**	**99**	**25**	**23**	**1**	**60**	**1**	**50**	**1**	**96**	**66**	**1**	**1**
Tyrosine	3	7	1	1	1	63	1	50	23	33	80	1	33	12	27	1	1	1
Pigment*[2]	13	7	25	1	25	75	1	99	15	50	40	1	50	25	42	1	67	11
Xylan	1	1	12	1	25	25	1	1	23	50	1	1	1	87	88	1	1	1
ß-glucanase	1	36	1	1	75	38	1	1	1	1	1	1	1	12	1	1	1	1
Acid from:																		
Adonitol	74	14	12	1	1	1	1	1	7	1	40	1	1	1	1	1	1	1
Arabinose	52	21	25	1	42	12	1	1	46	1	40	1	17	12	85	33	1	1
Cellobiose	**84**	**64**	**1**	**67**	**67**	**75**	**6**	**1**	**69**	**1**	**40**	**16**	**67**	**75**	**88**	**99**	**1**	**1**
Dextrin	**7**	**93**	**12**	**1**	**58**	**62**	**99**	**1**	**31**	**67**	**80**	**33**	**1**	**1**	**27**	**1**	**1**	**1**
Dulcitol	32	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Erythritol	26	7	1	1	1	1	1	1	1	1	1	1	1	25	1	1	1	11
Fructose	99	99	75	99	99	88	99	75	99	99	99	84	67	99	99	99	1	1
Galactose	1	36	12	1	75	63	12	50	38	83	80	16	67	1	69	33	7	1
Glucose	99	99	88	99	99	99	99	99	99	99	99	99	99	87	99	99	40	67
Glycerol	**97**	**79**	**50**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**1**	**99**	**87**	**92**	**99**	**7**	**11**
Inositol	74	21	1	33	8	12	1	1	15	1	20	16	1	37	62	33	1	1
Inulin	3	7	1	1	1	1	1	1	1	1	1	1	1	12	1	1	1	1
Lactose	1	7	1	1	75	1	1	1	7	1	1	1	1	1	8	1	1	1
Maltose	94	99	99	99	99	99	99	50	99	99	99	84	83	99	99	99	1	1
Mannose	90	86	88	99	99	88	99	99	99	99	99	50	67	87	92	99	1	11
Melezitose	3	7	1	33	75	38	64	1	1	50	1	1	1	25	85	1	1	1
Melibiose	**1**	**1**	**1**	**1**	**83**	**75**	**81**	**25**	**15**	**99**	**99**	**1**	**33**	**75**	**96**	**1**	**1**	**1**
Raffinose	**3**	**1**	**1**	**33**	**92**	**50**	**88**	**1**	**15**	**50**	**80**	**1**	**17**	**75**	**62**	**1**	**1**	**1**
Rhamnose	26	1	25	67	8	12	1	1	15	1	1	16	1	12	1	33	1	1
Ribose	**36**	**71**	**75**	**99**	**92**	**75**	**6**	**1**	**99**	**67**	**99**	**84**	**57**	**62**	**81**	**66**	**1**	**1**
Salicin	**97**	**64**	**38**	**1**	**83**	**88**	**19**	**1**	**46**	**33**	**20**	**1**	**1**	**75**	**85**	**66**	**1**	**1**
Sorbitol	87	7	1	1	8	1	6	1	15	1	1	1	17	25	1	66	1	1
Sucrose	94	64	25	67	33	88	94	50	70	67	99	64	33	99	81	99	1	1
Trehalose	**90**	**86**	**99**	**99**	**33**	**99**	**31**	**75**	**92**	**99**	**99**	**99**	**83**	**99**	**96**	**99**	**1**	**1**
Xylose	**48**	**86**	**75**	**99**	**83**	**12**	**1**	**75**	**69**	**83**	**80**	**16**	**17**	**37**	**81**	**1**	**1**	**11**

Table 2. continued

	B. pallidus	B. kaustophilus	Bacillus sp	B. smithii	Bacillus sp	B. caldotenax	B. stearothermophilus	Bacillus sp	B. thermoglucosidasius	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp	B. thermodenitrificans	B. licheniformis	Bacillus sp	B. thermocloacae
Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. strains	31	14	8	3	12	8	16	4	13	6	5	6	6	8	26	4	15	9
Growth in:																		
2% NaCl	97	64	50	1	83	88	94	99	85	99	99	50	99	87	99	99	99	99
5% NaCl	**90**	**7**	**1**	**1**	**8**	**1**	**1**	**75**	**31**	**83**	**60**	**16**	**83**	**12**	**88**	**99**	**93**	**78**
10% NaCl	6	1	1	1	1	1	6	1	1	1	1	1	1	1	1	99	1	1
0.02% Na azide	94	93	88	66	75	88	1	75	85	67	99	99	99	75	81	99	40	89
Kuhn's medium	36	57	25	1	33	52	1	1	62	17	99	33	1	12	42	99	7	1
K_2 tellurite	97	86	99	99	99	88	99	99	99	99	99	99	99	99	99	99	99	99
Lysozyme	3	36	1	1	8	12	6	25	15	17	1	1	1	1	4	66	13	1
Growth at:																		
pH 6.0	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	27	99
pH 5.5	**3**	**64**	**75**	**99**	**83**	**88**	**88**	**50**	**77**	**33**	**20**	**1**	**33**	**38**	**12**	**99**	**1**	**1**
72° C	**1**	**7**	**25**	**1**	**25**	**63**	**38**	**50**	**85**	**99**	**60**	**99**	**83**	**12**	**1**	**1**	**1**	**1**
70° C	**3**	**36**	**99**	**1**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**38**	**85**	**1**	**7**	**1**
68° C	45	71	99	67	99	99	99	99	99	99	99	99	99	99	99	1	7	33
65° C	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	1	55	99
25° C	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	1	1
Utilisation of:																		
Lactate	32	36	12	33	75	38	6	1	69	1	40	50	17	62	12	33	13	22
Citrate	1	28	1	1	25	75	1	1	1	1	1	1	1	12	1	1	1	1
Reduction of:																		
NO_3-NO_2	1	43	25	1	1	87	1	1	7	33	1	33	1	1	12	66	1	1
NO_3-NO	1	7	1	1	58	13	12	99	92	67	99	50	1	25	4	99	1	1
NO_3-N_2	1	7	1	1	25	13	12	1	1	1	1	1	99	50	85	1	1	1
NO_2-NO	1	7	88	1	58	25	1	99	92	50	99	66	1	38	27	1	1	1
NO_2-N_2	**3**	**7**	**99**	**1**	**17**	**1**	**18**	**1**	**1**	**1**	**20**	**1**	**99**	**50**	**92**	**1**	**1**	**1**
Miscellaneous:																		
Catalase	99	99	99	99	99	99	1	99	99	99	99	99	99	99	99	99	99	99
Oxidase	99	93	99	99	83	99	1	75	99	67	80	99	99	99	99	99	99	99
V.P.*[3]	13	1	1	1	33	38	6	1	1	1	1	1	1	1	1	1	1	1
Methyl-red	58	86	88	99	58	88	99	99	85	99	99	33	67	75	31	66	1	1
Aerobic O/F	1	1	12	1	1	1	1	1	8	1	1	16	17	1	1	1	1	1
Anaerobic O/F	97	99	88	99	99	99	99	99	85	99	80	84	67	87	99	66	1	1
Alkaline O/F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	78
Colonial morphology:																		
Circular	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**13**	**62**	**12**	**1**	**73**	**1**
Convex	39	71	62	1	75	62	83	99	38	17	60	99	50	75	1	1	33	1
Crenated	13	1	1	1	42	1	69	50	77	84	1	66	1	1	1	1	1	1
Entire	**84**	**93**	**88**	**99**	**33**	**1**	**12**	**50**	**8**	**1**	**1**	**33**	**33**	**12**	**1**	**1**	**99**	**1**
Erose	1	1	1	1	17	62	6	25	31	1	80	33	33	12	1	1	1	1
Fimbriate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	99
Flat	**48**	**21**	**12**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**96**	**1**	**67**	**99**
Lobate	1	1	12	1	1	38	12	1	15	17	40	1	33	75	1	99	1	1

Table 2. continued

	B. pallidus	B. kaustophilus	Bacillus sp	B. smithii	Bacillus sp	B. caldotenax	B. stearothermophilus	Bacillus sp	B. thermoglucosidasius	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp	B. thermodenitrificans	B. licheniformis	Bacillus sp	B. thermocloacae
Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. strains	31	14	8	3	12	8	16	4	13	6	5	6	6	8	26	4	15	9
Low-convex	10	1	12	1	25	38	1	1	46	50	40	1	67	1	1	1	1	1
Pellicle	3	7	1	1	1	1	1	1	1	50	40	1	17	1	1	1	7	11
Pulvinate	1	1	1	99	1	1	1	1	1	1	1	1	1	25	1	1	1	1
Raised	26	1	12	1	1	1	1	1	1	1	1	1	1	1	1	99	1	1
Rough	**1**	**1**	**12**	**1**	**1**	**62**	**1**	**1**	**1**	**1**	**60**	**1**	**50**	**75**	**96**	**1**	**1**	**1**
Smooth	**97**	**99**	**88**	**99**	**99**	**1**	**66**	**99**	**92**	**84**	**1**	**99**	**1**	**25**	**1**	**99**	**99**	**1**
Sediment	13	7	62	33	83	13	12	25	23	1	1	16	67	75	8	99	7	11
Spreading 1	3	1	12	99	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Spreading 2	1	7	12	1	1	1	1	1	8	1	60	1	33	25	1	1	1	1
Spreading 3	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	27	22
Spreading 4	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**7**	**78**
Transparent	32	36	50	1	1	83	6	1	15	1	80	16	83	75	8	1	7	78
Turbid	87	99	62	99	75	87	88	75	92	83	99	99	99	38	96	1	88	99
Umbonate	3	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Undulate	10	7	1	1	95	1	1	1	54	67	1	1	1	12	1	1	1	1

Chitin hydrolysis was not detected in any strains. All strains grew at pH 6.5. Four types of spreading colony morphology were distinguished by (1) colonies chaining together but not true spreaders; (2) a margin of cells spreading away from the main colony; (3) most of the plate covered but with evidence of original streak and (4) all of plate covered with no evidence of original streak. Characters printed in bold are those used for the identification matrix.

*[1] Carboxymethyl cellulose.

*[2] Pigment production from tyrosine hydrolysis.

*[3] Voges Proskauer.

Table 3. Base composition (mol% G+C) of representative strains circumscribed in the numerical phenetic survey based on the S_J UPGMA analysis

Cluster	Strain	Ave. Tm (±SD)	mol% G+C (±SD)
1	*B. pallidus*		
	12064	70.0	39.3
	00136	68.7	36.1
	10018	67.0	31.9
	12068	70.3	40.0
	12069	69.0	36.8
	Group Average	69 ± 1.31	36.8 ± 3.19
2	*B. kaustophilus*		
	12725	71.5	42.9
	12059	69.8	38.8
	12002	76.0	53.9
	Group Average	72.4 ± 3.20	45.2 ± 7.81
3	*Bacillus* sp.		
	12701	71.5	42.9
	10242	67.6	33.4
	Group Average	69.6 ± 2.76	38.2 ± 6.74
4	*Bacillus* sp.		
	12052	69.4	37.8
5	*Bacillus* sp.		
	12755	71.5	42.9
	12757	76.5	55.1
	Group Average	74 ± 3.52	49 ± 8.63
6	"*B. caldotenax*"		
	12793	76.2	54.4
	00263	77.1	56.6
	12028	75.7	53.2
	Group Average	76.3 ± 0.71	54.6 ± 1.73

Table 3. continued

Cluster	Strain	Ave. Tm (±SD)	mol% G+C (±SD)
7	*B. stearothermophilus*		
	11210	75.0	51.5
	00170	75.2	51.9
	11182	77.5	57.6
	12005	71.5	42.9
	12001	75.0	51.5
	12065	78.0	58.8
	12796	75.9	53.7
	Group Average	75.4 ± 2.12	52.6 ± 5.18
8	*Bacillus* sp.		
	11103	70.8	41.2
9	*B. thermoglucosidasius*		
	11099	71.5	42.9
	12060	71.6	43.2
	Group Average	71.6 ± 0.92	43.1 ± 0.25
10	*Bacillus* sp.		
	11068	71.4	42.7
11	*Bacillus* sp.		
	11101	71.0	41.8
12	*Bacillus* sp.		
	12657	70.2	39.7
15	"*B. thermodenitrificans*"		
	10513	75.3	52.2
	11024	75.3	52.2
	12019	74.1	49.3
	10079	75.3	52.2
	Group Average	75 ± 0.60	51.5 ± 1.45

Table 3. continued

Cluster	Strain	Ave. Tm (±SD)	mol% G+C (±SD)
16	*B. licheniformis*		
	12020	77.0	56.4
	12066	72.0	44.3
	Group Average	74.5 ± 0.60	51.5 ± 1.45
17	*Bacillus* sp.		
	10013	68.5	35.6
	10105	68.5	35.6
	10019	67.5	33.2
	Group Average	68.2 ± 0.58	34.8 ± 1.38
18	*B. thermocloacae*		
	10531	70.6	40.7
	10139	70.4	40.3
	Group Average	70.5 ± 0.14	40.5 ± 0.28

Table 4. Percent DNA:DNA homology of ^{32}P-labelled probe DNA against membrane filter bound test DNA

Probe DNA				
Species	*B.pallidus*	*B.kaustophilus*	*B.caldotenax*	*B.stearothermophilus*
Phenon	1	2	6	7
Strain	12068	12002	00263	11210
Phenon 1 (*B.pallidus*):				
12064	61 (3.2)*	2 (1.4)	5 (1.1)	4 (1.4)
00136	75 (2.1)	9 (4.9)	3 (1.1)	10 (2.1)
10018	71 (2.3)	3 (2.8)	4 (0.4)	14 (3.3)
12068	100 (-)	8 (6.2)	8 (1.1)	31 (5.7)
Phenon 2 (*B.kaustophilus*):				
12002	4 (1.4)	100 (-)	36 (6.4)	21 (4.4)
10242	5 (1.3)	16 (7.8)	9 (1.9)	7 (3.3)
Phenon 3:				
12701	9 (1.3)	11 (8.5)	18 (2.5)	11 (4.7)
10242	11 (1.6)	13 (12.7)	26 (3.5)	10 (4.0)
Phenon 4 (*B.smithii*):				
12052	6 0.8)	8 (6.4)	6 (1.6)	11 (4.9)
Phenon 5:				
12757	7 (1.7)	13 (6.4)	9 (1.6)	19 (3.9)
Phenon 6 ("*B.caldotenax*"):				
12793	8 (1.8)	27 (5.6)	79 (1.6)	12 (2.3)
00263	5 (1.9)	32 (6.4)	100 (-)	14 (3.8)
12028	4 (1.8)	20 (4.9)	93 (2.0)	14 (5.9)
Phenon 7 (*B.stearothermophilus*):				
11210	17 (5.9)	20 (11)	15 2.3)	100 (-)
00170	18 (1.8)	24 (9.0)	14 (2.0)	92 (7.1)
11182	14 (2.8)	13 (8.4)	17 (1.4)	67 (12.4)
12005	5 (1.3)	15 (2.8)	15 (2.0)	73 (8.1)
12001	8 (2.1)	4 (4.2)	12 (0.9)	71 (6.8)
12769	19 (3.7)	28 (9.9)	12 (2.8)	3 (2.3)

Table 4. continued

Probe DNA				
Species	*B.pallidus*	*B.kaustophilus*	*B.caldotenax*	*B.stearothermophilus*
Phenon	1	2	6	7
Strain	12068	12002	00263	11210
Phenon 8:				
11103	3 (2.3)	16 (8.3)	6 (1.4)	3 (1.0)
Phenon 9 (*B.thermoglucosidasius*):				
12060	3 (1.4)	7 (2.9)	10 (1.2)	12 (2.5)
11099	4 (2.1)	5 (5.6)	12 (1.9)	5 (3.1)
Phenon 11:				
11068	27 (4.2)	76 (12)	18 (2.7)	9 (2.3)
11101	10 (3.5)	6 (1.4)	12 (2.7)	5 (0.7)
Phenon 12:				
12657	15 (3.8)	14 (5.6)	5 (0.8)	8 (0.8)
Phenon 15 ("*B.thermodenitrificans*"):				
11024	9 (3.0)	12 (9)	4 (1.7)	13 (2.5)
12019	7 (1.1)	6 (4.9)	5 (0.9)	12 (3.3)
Phenon 16 (*B.licheniformis*):				
12020	6 (2.8)	4 (1.4)	5 (1.8)	4 (1.8)
DSM 13	5 (3.3)	2 (0.8)	3 (1.3)	3 (2.2)
Phenon 17:				
10013	6 (1.3)	6 (2.9)	19 (3.0)	19 (1.6)
10105	9 (1.9)	6 (3.3)	19 (3.0)	15 (2.8)
Phenon 18 (*B.thermocloacae*):				
10531	14 (2.3)	12 (9.5)	19 (0.8)	15 (3.5)
10136	12 (2.5)	9 (9.7)	13 (4.2)	13 (3.5)
Controls:				
E.coli	4 (1.5)	1 (2.1)	2 (1.3)	3 (1.6)
E.subtilis	4 (1.7)	3 (2.6)	4 (1.7)	2 (1.2)
B.coagulans	6 (2.3)	3 (2.1)	5 (2.1)	2 (1.1)

6 (2.9)			19 (3.0)	19 (1.6)
10105	9 (1.9)	6 (3.3)	19 (3.0)	15 (2.8)

Table 4. continued

Probe DNA			
Species	*B.thermo-glucosidasius*	*B.thermo-denitrificans*	*B.thermocloacae*
Phenon	9	15	18
Strain	12060	12019	10531
Phenon 1: (*B.pallidus*):			
12064	9 (1.9)	12 (2.8)	10 (1.6)
00136	7 (3.5)	8 (2.2)	8 (1.7)
10018	20 (8.5)	25 (2.8)	15 (1.9)
12068	20 (7.8)	22 (2.1)	16 (1.3)
Phenon 2 (*B.kaustophilus*):			
12002	5 (3.9)	5 (1.5)	11 (1.2)
12734	4 (3.5)	11 (4.2)	11 (1.4)
Phenon 3:			
12701	11 (2.6)	14 (1.1)	16 (1.4)
10242	15 (8.9)	11 (1.4)	11 (2.0)
Phenon 4:			
12052	11 (5.6)	14 (2.8)	12 (2.1)
Phenon 5:			
12757	11 (5.8)	36 (2.1)	13 (7.8)
Phenon 6 ("*B.caldotenax*")			
12793	7 (3.9)	15 (0.9)	15 (2.1)
00263	6 (3.7)	6 (2.9)	12 (4.2)
12028	7 (4.2)	9 (3.6)	16 (2.1)
Phenon 7 (*B.stearothermophilus*):			
11210	13 (7.5)	23 (2.9)	13 (6.4)
00170	19 (9.3)	28 (1.6)	22 (4.9)
11192	7 (5.2)	19 (1.6)	11 (4.7)
12005	10 (7.1)	21 (2.3)	15 (6.4)
12001	5 (4.2)	9 (5.7)	14 (4.9)
12796	4 (3.1)	6 (3.2)	6 (2.1)
Phenon 8:			
11103	4 (4.2)	18 (3.0)	5 (4.0)

Table 4. continued

Probe DNA			
Species	*B.thermo-glucosidasius*	*B.thermo-denitrificans*	*B.thermocloacae*
Phenon	9	15	18
Strain	12060	12019	10531
Phenon 9 (*B.thermoglucosidasius*):			
12060	100 (-)	31 (4.2)	8 (1.8)
11099	74 (6.4)	11 (3.6)	11 (2.8)
Phenon 11:			
11068	23 (13)	36 (2.1)	36 (2.1)
11101	5 (4.2)	6 (2.4)	9 (7.8)
Phenon 12:			
12657	9 (6.1)	5 (2.2)	14 (2.1)
Phenon 15: ("*B.thermodenitrificans*"):			
11024	14 (5.6)	85 (2.1)	15 (5.6)
12019	11 (8.5)	100 (-)	11 (5.6)
Phenon 16 (*B.licheniformis*):			
12020	3 (2.2)	4 (2.1)	5 (3.5)
DSM 13	4 (2.1)	2 (1.8)	5 (4.2)
Phenon 17:			
10013	22 (7.8)	36 (3.3)	129 (30.4)
10105	16 (11.3)	26 (0.9)	104 (9.2)
Phenon 18: (*B.thermocloacae*)			
10531	6 (5.6)	10 (1.1)	100 (-)
10136	11 (9.9)	15 (1.2)	78 (12.7)
Controls:			
E.coli	2 (1.3)	7 (3.2)	6 (1.4)
B.subtilis	3 (1.4)	3 (1.9)	15 (3.1)
B.coagulans	4 (1.2)	4 (1.7)	9 (1.8)

* Standard deviations are given in parenthesis.

Table 5. Some sample identifications using the probability matrix with classification test-data and re-tested data

Strain	Data[1]	Willcox Probability	Taxonomic distance	SE of taxonomic distance	Identity
B. caldotenax 00263	A	0.999	0.275	-0.85	*B. caldotenax*
	B	0.989	1.5	-0.54	*B. caldotenax*
B. kaustophilus 12002	A	0.999	0.345	0.44	*B. kaustophilus*
	B	0.718	0.450	2.88	unidentified
B. pallidus 12068	A	0.999	0.311	1.18	*B. pallidus*
	B	0.998	0.324	1.50	*B. pallidus*
B. thermoglucosidasius 12060	A	0.998	0.321	0.15	*B. thermoglucosidasius*
	B	0.970	0.319	0.10	*B. thermoglucosidasius*
B. thermocloacae 10531	A	0.999	0.103	-3.34	*B. thermocloacae*
	B	0.999	0.1	-3.33	*B. thermocloacae*
B. thermodenitrificans 12019	A	1.000	0.2	-2.2	*B. thermodenitrificans*
	B	0.999	0.395	2.16	*B. thermodenitrificans*
Phenon 10 11057	A	0.987	0.288	0.15	Phenon 10
	B	0.972	0.299	0.12	Phenon 10
Phenon 13 12646	A	0.978	0.381	0.77	Phenon 13
	B	0.631	0.385	1.5	Unidentified
Phenon 17 10031	A	0.999	0.165	1.54	Phenon 17
	B	0.999	0.172	1.32	Phenon 17

[1] In all examples, **A** refers to the use of test data from the original classification and **B** to the use of data from re-examination of the strains.