# Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria

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Minimal standards for describing new taxa within the aerobic endospore-forming bacteria are proposed, following Recommendation 30b of the Bacteriological Code (1990 Revision). These minimal standards are recommended as guidelines to assist authors in the preparation of descriptions for novel taxa. They encourage broad polyphasic characterization and the construction of descriptions that are practically useful in routine diagnostic laboratories. The proposals have been endorsed by the Subcommittee on the Taxonomy of the Genus *Bacillus* and Related Organisms of the International Committee on Systematics of Prokaryotes.

A detailed listing of the aerobic, endospore-forming bacteria, together with a brief historical account of the taxonomy of these organisms, and a list of relevant curated 16S rRNA gene sequences of aerobic endospore-formers and relatives are available as supplementary material with the online version of this paper.

## Introduction

This paper proposes minimal standards for describing new genera and species of aerobic, endospore-forming bacteria, in order to address Recommendation 30b of the Bacteriological Code (Lapage *et al.*, 1992) and also the

remit of the Subcommittee on the Taxonomy of the Genus *Bacillus* and Related Organisms of the International Committee on Systematics of Prokaryotes (ICSP). It is important first to clarify the scope of these minimal standards. They cover all of the aerobic, endospore-forming bacteria among the low-G+C Gram-positive bacteria (*Firmicutes*); that is to say all species of *Bacillus* (including those that are described as strictly anaerobic, and those for which sporulation has not been demonstrated, but that are seen to be placed phylogenetically in the vicinity of aerobic endospore-forming bacteria), all those genera accommodating species originally allocated to *Bacillus* and all the other genera that have been proposed to accommodate new species of aerobic endospore-formers that were not previously accommodated within *Bacillus*. At one time, the family *Bacillaceae* encompassed all of the taxa with which the Subcommittee was concerned, but these taxa are now found, sometimes along with non-spore-formers, in several families of the order *Bacillales*: *Bacillaceae*, '*Alicyclobacillaceae*', '*Paenibacillaceae*', *Pasteuriaceae*, *Planococcaceae* and '*Sporolactobacillaceae*'. A detailed listing of the aerobic, endospore-forming bacteria, together with a brief historical account of the taxonomy of these organisms, appears in a document that is available as supplementary material to this paper in IJSEM Online.

Species should be identifiable by readily available methods, but published descriptions too often lack both adequate representation of within-taxon diversity and sufficient features to allow a taxon's recognition and distinction. Phenotype, along with genotype, 'continues to play a salient role in the decision about cut-off points of genomic data for species delineation ... [and] ... description of species ... should be based on the use of well-documented criteria, laboratory protocols and reagents which are reproducible' (Stackebrandt *et al.*, 2002). The written description of a species or genus must be seen as a practically essential, integral part of any taxonomic proposal, and care needs to be taken to ensure that it is as comprehensive and practically useful as possible; the diagnostic characters should not be seen merely as adjuncts to the delineation of a taxon that has been recognized primarily on the basis of genomic data. A widespread problem is that comparisons are often made with descriptions given in the literature, rather than by laboratory study of reference cultures, so compilations as well as original descriptions should never be relied upon entirely.

Against a background of ever-increasing numbers of taxa and rapid advances in characterization methodologies, we propose this set of minimal standards for the written descriptions of novel genera and species of aerobic endospore-formers.

## Minimal standards

### Isolates

It is **strongly recommended** that the number of strains to be studied for each taxon should be at least five, and ideally ten or more; observations on a minimum of three strains are necessary to have some indication of natural variability. Preferably, strains should have been isolated from more than one location and, ideally, from several locations. If strains are from a single source, their non-clonality should be checked using a nucleic acid fingerprint technique. Proposals based upon single isolates should be avoided wherever possible. Each habitat, or source of isolates, must be described in sufficient detail, and geographical locations must be specified, in order to allow the isolation work to be repeated. Isolation procedures must be fully specified and include details of medium formulation, pH and inoculation methods, of the gaseous composition of the atmosphere for incubation and of the temperature and duration of incubation. If several isolates are available for study, they should all be studied; the selection for study of a single strain from such a collection and the disregarding of the other strains is **strongly discouraged**. A proposal based upon a single isolate should state this explicitly in the description. The description must outline the source(s) of the isolates. Maintenance procedures must be adequately specified. The type strain must be deposited in two publicly accessible culture collections in different countries, as required by the Judicial Commission of the ICSP (De Vos & Trüper, 2000), and it is strongly recommended that another, reference, strain of the same taxon is deposited at the same time in order to allow confirmation of authenticity. For the practice of valid publication of names and deposit of type material, authors should consult Tindall *et al.* (2006), Tindall (2008) and Tindall & Garrity (2008).

### Cultivation

Cultivation procedures must be fully specified and include details of medium formulation and pH. Attempts should be made to grow organisms on standard media, but care should be taken that specific characters are not thereby lost. Failure to grow on standard media should be indicated, and alternative media that satisfy the needs of the organisms should be given. The formulation of these should be fully described, as should the inoculation methods and incubation conditions. Select an incubation temperature that matches the optimum growth temperature of the organism(s) as closely as is practical. Certain media and methods may be adapted for acidophilic and alkaliphilic organisms by adjusting the pH. Similarly, a medium with NaCl or a mixture of salts should be used for the cultivation of halophiles. The description **must state** the preferred growth medium for strains of the proposed taxon, and indicate the incubation conditions required.

### Standardization

Whatever methods are used for the description, standardization of methodologies and inclusion of reference strains is crucial. The methods used to generate the characters included in species descriptions have not usually been

standardized between laboratories, and the test results shown in differentiation tables often include information lifted from the literature, so that data are frequently not comparable. As the number of taxa with validly published names increases, the task of studying related and reference organisms in parallel becomes more demanding, and authors may be tempted to lean ever more heavily on data presented in the literature. In addition, standardization of methodologies for many phenotypic tests is inherently impossible between those organisms whose conditions for growth do not overlap. Miniaturized versions of traditional biochemical tests (e.g. API kits, VITEK cards and Biolog plates) offer standardized methods for a range of biochemical characters; the first named offers some versatility in temperature and pH, while the last named can be incubated at a range of temperatures.

Several strains should be duplicated within any taxonomic analysis, so as to allow determination of error in all kinds of characterization methods; the determined errors should be cited in the results, so as to indicate the significance of the findings.

## Reference strains

It is **strongly recommended** that the original and emended descriptions of related species are consulted wherever possible, and that cultures of appropriate phylogenetic and phenetic relatives are included for laboratory comparison. There is no substitute for making direct laboratory comparisons with authentic reference strains. It must be appreciated that published species descriptions may be based upon very few strains, so that within-species diversities are unknown, and that typographic errors may have occurred during their compilation. Studies that use type strains alone for reference purposes are exposed to the danger of ignoring intraspecies variability. Wherever possible, the type and one or more other reliable reference strains of a related species should be included in the study, so as to confirm the authenticities of these reference strains and to take into account at least some of the intraspecies variability. Nowadays, culture collections or BRCs (Biological Resource Centres) increasingly provide cultures under quality control certification (ISO 9001, for example), so that users may be confident of the authenticity of the type or reference material.

The descriptive characters given below are summarized in Table 1.

## Microscopic morphology

Phase-contrast microscopy at $\times 1000$ magnification is strongly recommended for such observations; it is superior to bright-field microscopy of stained smears, including spore staining, and is much more convenient. Magnifications higher than $\times 1000$ are available with some microscopy systems, and are valuable when making observations of parasporal bodies and measurements of cell dimensions. Electron microscopy may be of value to reveal additional morphological information, including flagellation.

## Vegetative cells

It should be borne in mind that not all species belonging to the *Firmicutes* stain Gram-positive, and so the Gram reaction should be tested. Even if cell-wall composition is analysed, the Gram reaction remains a valuable diagnostic character. Cellular morphology should ideally be studied in relatively young (e.g. 18–24 h at 30 °C) cultures grown in broths aerated by shaking. Morphologies of cells that have been raised on agar plates or slopes may be heterogeneous owing to varying conditions of oxygen supply within colonies. Wet preparations may be viewed by phase-contrast microscopy at $\times 1000$ magnification and observed for cell size (width and length), shape, shapes of ends of cells (rounded, squared, tapered), chains, filaments and motility (motility might not always be observed by microscopy, because some organisms can lose their flagella when slides are prepared; the use of motility agar is recommended); for cells grown on glucose agar, observe for storage inclusions (use the type strains of *Bacillus cereus* and *Bacillus subtilis* as positive and negative controls, respectively). Capsules may be revealed by India ink negative-staining. For motile organisms, stating the type of flagellation is desirable; this may be determined by flagella staining or electron microscopy.

## Sporangia

As sporangial appearance is valuable in identification, full details should be given: study cultures grown for 24 h and up to 7 days on a medium that encourages sporulation. Supplementing the medium with 5 mg $MnSO_4\ l^{-1}$ is often successful, and several formulations for other sporulation media are available; failure to sporulate may be due to the use of too rich a medium (where organisms may die and lyse, rather than sporulate), and reduction of the nutrient content may help. The description must include observations of sporangia for spore shape (spherical, cylindrical, ellipsoidal), position (central or paracentral, subterminal, terminal), presence of parasporal bodies (use the type strain of *Bacillus thuringiensis* as a positive control) and for swelling of the sporangium. A **photomicrograph of sporangial morphology**, with a bar marker for size, should be provided, and details of sporulation conditions should be given. Sporulation has not been observed in several recently described species, but the potential to form endospores may be detected using a PCR method based upon certain genes for sporulation (Brill & Wiegel, 1997).

Microscopic characters that are **essential** and **strongly recommended** in any description accompanying the proposal of a new taxon of aerobic endospore-former are detailed in Table 1. Comment on any observed variations of spore size, shape and position and sporangial swelling. A

**Table 1.** Summary of standards for description of new taxa of the aerobic endospore-forming bacteria

| Essential feature | Recommended feature |
|---|---|
| **Microscopic morphology** | |
| Cell size (width and length), overall shape, shapes of ends of cells, chains, filaments | |
| Gram reaction of young culture | |
| Motility | Type of flagellation (by flagellar staining or electron microscopy) |
| Presence of storage inclusions | |
| Sporangial morphology | |
| Spore shape and position | |
| Sporangial swelling | |
| Presence of parasporal bodies and other inclusions or extracellular deposits | |
| Photomicrograph of sporangia | |
| **Macroscopic morphology** | |
| Growth medium used | |
| Cultural conditions (temperature, pH, salinity, incubation time) | |
| Colony diameter after a given period of incubation | |
| Colony shape, elevation, mention of any tendency to spreading or colonial motility, morphology of edges, surface texture, colour, consistency and mention of any tendency to be adherent to the medium | |
| **Physiological characters** | |
| Recommended growth medium | |
| Optimum, maximum and minimum growth temperatures | |
| Optimum, maximum and minimum pH for growth | |
| Oxygen requirements* | |
| NaCl tolerance or requirement (minimum, optimum and maximum concentrations), or stimulation of growth by NaCl | |
| Catalase | |
| Oxidase | |
| Any known special nutritional requirements | Ability to grow on minimal medium (state composition and conditions) |
| **Biochemical characters** | |
| Acid production from D-glucose | Gas production from D-glucose |
| | Acid production from L-arabinose, D-mannitol, D-xylose and other specified carbohydrates of differential value† |
| | Voges–Proskauer reaction |
| Hydrolysis of casein, gelatin and starch | Hydrolysis of aesculin and urea |
| | Nitrate reduction, and reduction of nitrate or nitrite to nitrous oxide or nitrogen gas |
| | Arginine dihydrolase |
| Citrate utilization | Utilization of propionate and other organic acids as sole carbon sources |
| | Utilization of amino acids as sole carbon sources |
| | Utilization of carbohydrates as sole carbon sources |
| | Egg yolk (lecithinase) reaction‡ |
| | Indole production from tryptophan |
| | ONPG (*o*-nitrophenyl $\beta$-D-galactopyranoside) hydrolysis |
| **Chemotaxonomic characters** | |
| Fatty acid profile | |
| Cell wall diagnostic diamino acid | Murein structure§ |
| | Polar lipid analysis§ |
| | Quinone analysis§ |
| **Nucleic acid studies** | |
| 16S rRNA gene sequence (>1400 nt, <0.5 % ambiguity) and phylogenetic analysis | Phylogenetic analysis based on housekeeping protein-coding genes |
| | G+C content of DNA§ |
| DNA–DNA relatedness with closely related species‖ | $\Delta T_m$ of hybrid DNA |

**Table 1.** cont.

*Is the organism strictly aerobic, microaerophilic, facultatively anaerobic or strictly anaerobic? The unqualified word 'aerobic' is insufficient.
†Note that a carbohydrate utilization test in which a positive reaction is revealed by evidence of growth is not equivalent to an acid formation test. Many organisms may not produce detectable acid from carbohydrate metabolism.
‡This character is especially important within the *B. cereus* group.
§Features that are considered as essential for the description of a new genus.
||Essential when 16S rRNA gene sequences of the novel strains show 97 % similarity or more with existing taxa.

photomicrograph of sporangial morphology should accompany the description.

## Macroscopic morphology

There is a very wide range of colonial morphology, both within and between species, and of course medium composition and other incubation conditions have strong influences. Despite this diversity, however, colonies of many aerobic endospore-formers on routine media are not generally difficult to recognize, and so details of colony morphologies in descriptions are valuable, on the condition that the medium used and the cultural conditions (temperature, gaseous conditions and period of incubation) are indicated. Colonial descriptions may include details of diameter, overall plan shape, elevation, any tendency to spreading or colonial motility, morphology of edges, surface texture (such as glossy or matt), colour, consistency, any tendency to be adherent to the medium and haemolysis (absent, slight or marked, partial or complete). Sporulation may be strongly associated with the spatial development of the bacterial community, and full details of the cultural conditions employed when making observations of colonial morphology are therefore essential.

Cultural characters that are **essential** in any description accompanying the proposal of a new taxon of aerobic endospore-former are detailed in Table 1.

Bacteriologists may not be able to obtain all the characters listed below in their own laboratories, and it is **strongly recommended** that collaborators experienced with specialized techniques be sought. The Subcommittee may be able to help in finding suitable collaborators.

## Physiological and biochemical characters

The tests for *Bacillus* described by Gordon *et al.* (1973) and updated by Claus & Berkeley (1986) and by Logan & De Vos (2009) are still valuable for distinguishing aerobic endospore-formers and are widely applicable across the many genera. The most widely used commercially available methods for identifying members of the genus *Bacillus* and its relatives are still based upon miniaturized developments of traditional, routine biochemical tests: the API 20E and 50CHB systems (bioMérieux), the VITEK system (bioMérieux) and Biolog plates. It is emphasized that tests done using traditional, home-made media and those performed with commercially available kits may often give different results for a particular test. As tables of differential characters accompanying proposals for new taxa are often partly compiled from the literature, it is important to be aware of this potential lack of comparability and the kinds of tests used for all the taxa included in such tables should be declared. For all characterization tests the methodologies **must** be stated explicitly, and it is **essential** that results be verified using reference strains as positive and/or negative controls.

Physiological and biochemical characters that are **essential** and **strongly recommended** in any description accompanying the proposal of a new taxon of aerobic endospore-former are listed in Table 1.

Note that reduction of nitrate has been used widely and reliably in the characterization of aerobic endospore-formers for many years, and several authorities consider it to be an **essential** test; it is again emphasized that the methodology must be stated explicitly. However, others consider the tests for nitrate and nitrite reduction to be unreliable and liable to give ambiguous results (Heylen *et al.*, 2006).

## Chemotaxonomic characters

Chemotaxonomic fingerprinting techniques applied to aerobic endospore-formers include fatty acid methyl ester (FAME) profiling, PAGE analysis of whole-cell proteins, polar lipid analysis, quinone content, cell-wall diamino acid content, pyrolysis mass spectrometry, Fourier-transform infrared spectroscopy, Raman spectroscopy and matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. Several of these techniques generate profiles that may be valuable in classification and identification; however, if such profiles do not reveal the chemical compositions of the organisms, or the data are not portable, they may not be useful for inclusion in descriptions.

FAME analysis is presently the only chemotaxonomic technique that is linked to a commercial database for identification purposes. A disadvantage is that growth and other conditions need to follow strictly standardized protocols. This makes the commercial system unsuitable for the identification of organisms that require special growth conditions (e.g. acidophiles, alkaliphiles, halophiles, thermophiles). However, fatty acid profiles are

perfectly suitable for taxon description and also for comparative analyses of profiles that have been obtained under identical growth conditions. Fatty acid profiles are therefore very useful in descriptions of new taxa, and it is **strongly recommended** that the fatty acid profile should be available and the minor compounds that are characteristic of a novel taxon should be stated in the description.

## Cell-wall composition

Murein structure is known for only a small proportion of aerobic endospore-formers, and rather few laboratories are equipped to analyse it. More data are accessible concerning the diagnostic diamino acid in the peptidoglycan and, so far, three different diamino acids have been detected in members of the *Bacillaceae*: *meso*-diaminopimelic acid, ornithine and lysine. For descriptions, determination of the diagnostic diamino acid is **essential**, and determination of murein structure is **essential** for description of new **genera** and **strongly recommended** for all novel **species**.

## Polar lipids

Although many polar lipids detected have not yet been structurally characterized, this disadvantage does not necessarily reduce the value of this analysis if, for an unknown lipid, a recognizable designation is used and the chromatographic behaviour is presented in an image of the two-dimensional thin-layer plate that shows all lipids. The taxonomic importance of polar lipids has now been demonstrated for some novel genera within the *Bacillaceae* and '*Paenibacillaceae*', such as *Cohnella*, *Ornithinibacillus* and *Viridibacillus*, and so this method of characterization is **essential** for description of new **genera** and **recommended** for all novel **species** (Table 1).

## Quinones

Menaquinones MK-7, MK-8 and MK-9 have so far been reported for representatives of the *Bacillaceae* and hence this method of characterization is **essential** for description of new **genera** and **recommended** for all novel **species**.

## Nucleic acid studies

**16S rRNA gene sequences.** 16S rRNA gene sequences form the phylogenetic basis for modern bacterial taxonomy and so it is **essential** that the sequence of the type strain, at least, of each novel species must be deposited in a database with public access, and its sequence similarity with related species represented in that database should be determined. A sequence similarity of 97 % or more between an almost-complete sequence (>1400 nt, <0.5 % ambiguity) of the type strain of the novel species and any sequence of a species with a validly published name available from the database (EMBL/GenBank/DDBJ) should lead to further genotypic (and phenotypic) analysis if the biological material is available, as it is well established that sequence similarity values higher than this do not guarantee conspecificity. As 16S rRNA gene sequences in public databases are often of low quality, we provide an overview (Supplementary Table S1) of many relevant sequences of aerobic endospore-formers and relatives that have been checked for their technical and functional (secondary structure) characteristics. It is recommended that only these sequences [or other thoroughly checked sequences from other sources, such as the SILVA, Living Tree Project (Yarza *et al.*, 2008) and EzTaxon (Chun *et al.*, 2007) databases] be used for comparative analyses of the aerobic endospore-forming bacteria.

DNA sequencing of other genes is now relatively rapid and inexpensive, highly reproducible and readily available from specialized sequencing services. Although 16S rRNA gene sequence comparisons are valuable in the determination of approximate phylogenetic relationships at the supraspecies and generic levels and higher, they are not always appropriate for the classification of strains at the species level (Stackebrandt & Goebel, 1994). The 16S–23S internal transcribed spacer (ITS) region is hypervariable in comparison with the more conserved 16S rRNA-encoding region, and ITS-PCR fingerprints have been used to investigate the relationships of endospore-formers (Xu & Côté, 2003).

Sequences of other highly conserved housekeeping or other protein-encoding genes can provide higher resolution than 16S rRNA gene sequences and can complement DNA–DNA relatedness or 16S rRNA gene sequence data for taxonomic analysis at the species level. Zeigler (2005) found *recN* sequence similarities to yield clustering patterns among *Geobacillus* very similar to those obtained with 16S rRNA gene sequences, and Mota *et al.* (2005) obtained clustering patterns for *Paenibacillus* based upon *rpoB* sequence comparisons that were similar to those obtained with 16S rRNA gene sequences. Wang *et al.* (2007a, b) included *gyrB* sequence comparisons in studies of the *B. subtilis* group and demonstrated the synonymy of *Bacillus axarquiensis* and *Bacillus malacitensis* with *Bacillus mojavensis*, and Cerritos *et al.* (2008) included *recA* sequence comparisons in the work that led to the proposal of *Bacillus coalhuilensis*. Priest *et al.* (2004), Sorokin *et al.* (2006) and Tourasse *et al.* (2006) used multilocus sequence typing (MLST) of housekeeping genes to investigate population structures within the *B. cereus* group. It is to be expected that multilocus sequence analysis (MLSA) and MLST schemes will become available through publicly accessible databases. However, it is essential that the species delineations claimed in MLSA schemes are validated by DNA–DNA hybridization data, thereby also validating the MLSA scheme itself. The discriminating power of an MLSA scheme can also be evaluated by electronic tools (such as TaxonGap; Slabbinck *et al.*, 2008) once validation has been performed.

**DNA base composition.** The mol% G+C of the type strain, at least, should be determined and included in the general description, with an indication of the method used.

The G+C content of the DNA is **strongly recommended** for the description of novel species, and **essential** for the description of new genera.

**DNA–DNA relatedness.** As identical or highly similar 16S rRNA gene sequences do not guarantee species identity, DNA–DNA hybridizations are **essential** in cases of species descriptions when 16S rRNA gene sequences of the novel strains show 97 % or more similarity with existing taxa. Several widely used methods do not allow the determination of thermal stability (expressed as $\Delta T_m$) of the hybrid, but differences in $\Delta T_m$ between the hybrid and the homologous duplex are important and can be decisive for taxonomic conclusions, and determination of $\Delta T_m$ is **strongly recommended**. It should be borne in mind that, although 70 % or more DNA relatedness is recommended to delineate taxa at the species level (Wayne *et al.*, 1987), some strains of a species may show less than 70 % relatedness with the type strain or other strains of the same species (see Goris *et al.*, 2007). This threshold should not be rigidly applied – less than 70 % relatedness between two strains should not be taken automatically to mean that they belong to different species, and should not alone be considered sufficient evidence for the recognition of a novel species. Each DNA–DNA hybridization method may show inconsistencies between repeated measurements, indicating an appreciable standard deviation. Consequently, the 70 % threshold should be interpreted flexibly, and exceptions to following it should always be supported by distinctive phenotypic characters. It is **essential** that the integrities of the bacterial DNAs used in such studies always be checked before determining G+C content and DNA–DNA relatedness values, as poor-quality DNAs may yield misleading results. It is a prerequisite that reciprocal values and relevant controls are included. Investigators may use, instead, other validated genomic methods or techniques, provided that they can demonstrate that, for the organisms concerned, there is satisfactory congruence between that alternative approach and DNA–DNA hybridization (see Stackebrandt *et al.*, 2002).

**Nucleic acid fingerprinting.** Generally, these methods provide information at the subspecies and strain level. Examples are amplified fragment-length polymorphism (AFLP) analysis, macrorestriction analysis after pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) analysis, rep-PCR (repetitive element-primed PCR, directed to naturally occurring, highly conserved, repetitive DNA sequences, present in multiple copies in the genome), including REP-PCR (repetitive extragenic palindromic-PCR), ERIC-PCR (enterobacterial repetitive intergenic consensus sequences-PCR), BOX-PCR (derived from the *boxA* element) and $(GTG)_5$-PCR, and ribotyping. Strains of a manageable group defined by highly similar genomic fingerprints can be considered to be representatives of a single species. The most important disadvantage of some kinds of DNA fingerprint data is that they are not always portable and it is very difficult to compare them between different laboratories because of lack of standardization. Exceptions to the problem of portability include AFLP and ribotyping, and these approaches may provide sound taxonomic information. Also, some software for analysis of electrophoresis patterns enables rep-PCR data to be portable, and PFGE, if used with a standardized protocol such as PulseNet, is also portable. Members of the genus *Bacillus sensu lato* contain nine to twelve rRNA operons (e.g. Johansen *et al.*, 1996; Okamoto *et al.*, 1993), so ribotyping has been considered as a potentially useful approach to unravel their taxonomic structure. Taxonomic interpretation of the data is only possible when an extensive database is available. Although a commercialized ribotyping system is available, the relatively high cost of using such a system for routine characterization purposes has inhibited its widespread adoption for taxonomic work. AFLP has been used in various taxonomic studies and, under very standardized operating conditions, it allows the exchange of data between laboratories to some extent. If validated against DNA–DNA hybridization, AFLP patterns can be used to indicate the existence of novel species. In general, it may be concluded that these DNA fingerprinting methods are of limited value for species description but that, when used properly, they can be valuable for identification at species and subspecies levels. We also recommend the use of rep-PCR and RAPD for in-house studies, where they may be used to sort large numbers of strains into more manageable groups prior to polyphasic characterization. All of the above typing techniques may also be used to demonstrate whether isolates of a novel taxon are members of a clone or not.

Genomic data that are **essential** or **strongly recommended** in any description accompanying the proposal of a new taxon of aerobic endospore-former and closely related non-endospore-formers are detailed in Table 1.

## Final remarks

We recommend these minimal standards for adoption by those wishing to propose new taxa of aerobic endospore-forming bacteria and closely related non-endospore-formers. The intention is to guide the construction of species descriptions on the basis of current knowledge and to encourage some practically useful conformity, and so comparability, in the characterization of novel taxa. They are thus presented with the object of assisting rather than obstructing authors, and should not be seen as exhaustive or restrictive – indeed, the inclusion of new and useful taxonomic characters is strongly encouraged.

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