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TAXONOMIC NOTE

Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology

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An ad hoc committee for the re-evaluation of the species definition in bacteriology met in Gent, Belgium, in February 2002. The committee made various recommendations regarding the species definition in the light of developments in methodologies available to systematists.

Keywords: bacterial systematics, species definition, housekeeping genes, ICSP

The conclusions and recommendations of the ad hoc committee on reconciliation of approaches to bacterial systematics (Wayne *et al*., 1987) have provided bacteriologists with a uniform definition of prokaryotic species that has been widely used in systematic studies (Stackebrandt, 2000; Rosselló-Mora & Amann, 2001). However, since 1987 the introduction of innovative methods has provided new opportunities for prokaryotic systematics, some of which have already been realized. Developments of particular interest include:

- The ability to order prokaryotic taxa hierarchically among the ranks of genera and kingdoms has been improved by replacing 16S rRNA cataloguing and reverse transcriptase sequencing of 16S rRNA by high quality 16S rDNA sequence analyses (Ludwig & Klenk, 2001; Garrity & Holt, 2001).
- Determination of inter- and intraspecies relatedness has been facilitated by rapid DNA typing methods (for reviews, see Vaneechoutte, 1996; Rademaker *et al*., 2000; Gürtler & Mayall, 2001; van Belkum *et al*., 2001), such as those targeting whole genomes (AFLP, RAPD, Rep-PCR, PFGE), gene clusters (ribotyping of *rrn* operons), individual genes (ARDRA of 16S rDNA) and intergenic 16S–23S rDNA spacer regions (ISR).
- Multilocus sequence typing (MLST) has brought a new dimension into the elucidation of genomic relatedness at the inter- and intraspecific level by sequence analyses of housekeeping genes subjected to stabilizing selection (Maiden *et al*., 1998). To date, this technique has been mainly used in epidemiology; but it offers the opportunity to incorporate the insights available from population genetics and phylogenetic approaches (Maynard Smith *et al*., 1993, 2000; Istock *et al*., 1996; Achtman, 1998) into bacterial systematics and, as already recommended by Wayne *et al*. (1987), provides microbiologists with the tools to search for phylogenetic markers independent of rDNA genes (Gupta, 1998; Eisen, 1995). The role of DNA sequence data, especially those of protein-coding genes, in

Comments on the conclusions and recommendations included in this report are welcome and should be sent to Erko Stackebrandt.

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ecology and classification have been dealt with in theory and practice by the research group of Frederick M. Cohan (Palys *et al.*, 1997, 2000). The former publication already recommended that 16S rDNA gene sequences, protein-coding gene sequences and DNA–DNA hybridization should be considered as molecular criteria for species delineation.

- Sequence analyses of complete genomes, starting with the analysis of *Haemophilus influenzae* (Fleischmann *et al.*, 1995) has provided scientists with an immeasurable wealth of information, ranging from sequences to chromosome architecture, including the position and nature of episomal elements, the gene products of which have usually been excluded from the classification process. These data permit the identification of genes that are conserved across taxa and enable the analysis of these genes in a wide range of bacteria.
- The characterization and/or identification of isolates has been improved by applying physical methods to prokaryotic cells, such as Fourier-Transformed Infrared Spectroscopy (FTIR) (Helm *et al.*, 1991; Oberreuter *et al.*, 2002), pyrolysis-mass spectrometry (Goodacre, 1994; Colquhoun *et al.*, 2000), and Matrix-assisted Laser Desorption/Ionization with Time-of-flight (Maldi/Tof) (Claydon *et al.*, 1996; Conway *et al.*, 2001) or spray-ionization mass spectrometry (Vaidyanathan *et al.*, 2001).

This progress in methodology and insights into population structure, with its high potential for bacterial systematics, was the stimulus to form an ad hoc committee of the International Committee for the Systematics of Prokaryotes, which convened for a Workshop on the Re-evaluation of the Species Definition in Bacteriology at the University of Gent, Belgium, on 6–8 February 2002. This ad hoc committee evaluated the present polyphasic (Colwell, 1970) and pragmatic species definition in view of the recent innovations listed above, many of which yield information on the genetic basis of heredity and relatedness. The committee came to the conclusion that despite certain drawbacks with respect to reproducibility, workability, and rigid application of DNA–DNA hybridization values for species delineation, the present system is sound. The current species definition is pragmatic, operational and universally applicable, and serves the community well: as described by Rosselló-Mora & Amann (2001), 'a species is a category that circumscribes a (preferably) genomically coherent group of individual isolates/strains sharing a high degree of similarity in (many) independent features, comparatively tested under highly standardized conditions'. For the time being, the parameters DNA–DNA similarity and, whenever determinable, ΔT_m (Wayne *et al.*, 1987; Grimont, 1981) remain the acknowledged standard for species delineation. The committee, however, also recognizes that these two approaches, currently applied to the delineation of species, cannot be improved.

Therefore, the following recommendations are made that maintain the pragmatic delineation of species in the genomic era whilst integrating new techniques and new knowledge.

Investigators are encouraged to propose new species based upon other genomic methods or techniques provided that they can demonstrate that, within the taxa studied, there is a sufficient degree of congruence between the technique used and DNA–DNA reassociation. In addition, investigators are encouraged to develop new methods to supplement or supplant DNA–DNA reassociation. These methods should be validated by the following criteria:

- The method should be quantitative and the results amenable to appropriate statistical analysis.
- The method should be validated with collections of strains for which extensive DNA–DNA similarity and, by preference, thermal stability data are available. Investigators are encouraged to make such collections of strains available for this purpose. Ideally, these strain collections should be evaluated by more than one method.
- Strain collections representative of the phylogenetic lineage(s) of the species should be studied.

Methods of great promise

The adoption of techniques such as DNA–DNA reassociation and 16S rDNA sequence analyses has established the major role of the relationships among nucleotide sequences in the definition of bacterial species. The committee considers the following to be techniques which show great promise in further developing this approach.

Sequencing of housekeeping or other genes. The ad hoc committee recommends evaluation of protein-coding gene sequence analysis for its applicability to genomically circumscribe the taxon species and differentiating it from neighbouring species detected by, for example, rDNA sequences. The current consensus is that an informative level of phylogenetic data would be obtained from the determination of a minimum of five genes under stabilizing selection for encoded metabolic functions (housekeeping genes). Such genes should be at diverse chromosomal loci and widely distributed among taxa. Similar levels of information would be obtained by the determination of a larger number of gene fragments of defined length and position. The levels of information obtained will be dependent on the level of genetic diversity present within a given taxon, therefore the absolute number of genes to be analysed should be evaluated on the basis of the robustness of clusters obtained by a variety of phylogenetic analyses. Additional strains could be affiliated to the species on the basis of partial sequences or a complete gene sequence of one gene of the gene set. In order to validate this approach, organisms

should be chosen for which extensive DNA–DNA reassociation data are available and the intraspecific diversity has been evaluated by DNA profiling methods. It is expected that the level of divergence between strains of a species may differ from taxon to taxon.

This is an extension of the MLST approach which is increasingly used for the indexing and organizing of within-species genetic variability.

DNA profiling. DNA profiling should be validated for its ability to discriminate at the subspecific level. Methods of promise include AFLP (Vos *et al.*, 1995; Mougel *et al.*, 2002), ribotyping, Rep-PCR, PCR-RFLP. As a general principle, any method used should yield complex fragment patterns. To maximize reproducibility among laboratories (Clerc *et al.*, 1998), the ad hoc committee recommends methods employing restriction as their only or final step, which generate reproducible fragments of equal intensity, above amplification under stringent conditions which gives better reproducibility than low stringency PCR. In case of patterns of lower complexity, results of different profiling methods should be combined. It is strongly recommended that dendrograms resulting from DNA fragment patterns are documented with a measure of the statistical significance of the genomic clusters, by using methods such as bootstrap (Felsenstein, 1985; Mougel *et al.*, 2002).

DNA arrays. The committee believes that this methodology (Schena *et al.*, 1998) shows great promise (Akman & Aksoy, 2001; Salama *et al.*, 2000) and recommends that taxonomists carefully follow developments in this field for applications in rapid identification and determination of novel taxa.

Species should be identifiable by readily available methods (phenotypic, genomic). Efforts should be made to establish standardized methods of reporting phenotypic and genomic data.

- All species descriptions should include an almost complete 16S rDNA sequence (>1300 nt, $<0{\cdot}5\%$ ambiguity).
- Phenotype (together with genotype) continues to play a salient role in the decision about cut off points of genomic data for species delineation. More emphasis should be placed on discriminating markers. It is recommended that a common format be developed to facilitate the coherent description of species. The format should be based on the use of well-documented criteria, laboratory protocols and reagents which are reproducible without recourse to proprietary kits.
- In practice descriptive and diagnostic characters should be described in sufficient detail to permit comparisons between taxa and allow reproduction of observations. Given the availability of electronic publication of supplementary material by the IJSEM and other journals or by the web servers of public organizations, this requirement is neither onerous nor expensive.
- Diagnostic or differentiating properties should be obtained by comparable methods applied to reference strains of closely related taxa.
- The mol% G+C content of DNA should be part of the description of the type strain of the type species of a new genus. Indication of the DNA G+C content of a type strain of a new species in an established genus is optional.

Minimal characteristics should be provided and follow the guidelines set forth by various subcommittees of the ICSP. Where such guidelines do not exist, descriptions should follow guidelines for closely related taxa. Comparisons should always include type material from closely related species.

Microbiologists are encouraged to base a species description on more than a single strain on the basis of the arguments in Christensen *et al.* (2001).

Phenotype, including chemotaxonomic markers, will remain important diagnostic properties in a species description. The ecological role can, in certain cases, decide on the species status. For example, medical organisms with defined clinical symptoms may continue to bear names that may not necessarily agree with their genomic relatedness so as to avoid unnecessary confusion among microbiologists and non-microbiologists ['*nomen periculosum*' according to Rule 56a(5) of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1990)].

Efforts should be made to establish standards for the electronic exchange of taxonomic information through the development of XML schemas, topic maps or ontologies that provide links to other resources including federated databases, literature resources and repositories of raw and curated data (Anonymous, 2001; Zehetner & Lehrach, 1994). Curated sequence data, such as those provided by the MLST databases (http://campylobacter.mlst.net and http://neisseria.mlst.net) are crucial for their use in species delineation and determination of intraspecific substructure.

Microbiologists are encouraged to use the '*Candidatus*' concept for well-characterized but as-yet uncultured organisms (Murray & Schleifer, 1994; Murray & Stackebrandt, 1995).

The committee reinforced the earlier statement of Wayne *et al.* (1987) that new recommendations should be compatible with the current classification. The underlying basis of systematics is evolution and the process of doing systematics requires periodic adjustment to scientific advances. Experimental and theoretic evidence is compelling that the 'lumpy diversity' present in prokaryotes (Dykhuizen & Green, 1991; Maynard-Smith *et al.*, 1993; Achtman *et al.*, 1999; Lan & Reeves, 2001) is recognizable as discrete centres of variation when appropriate methods are

applied. One of the acknowledged mechanisms involved in the formation of recognizable discrete genomic units is sexual isolation, controlling inter- and intraspecific recombination by the mismatch repair system in relation with genome similarities (Vulic *et al.*, 1997; Radman & Wagner, 1993; Matic *et al.*, 1995; Majewski *et al.*, 2000; Denamur *et al.*, 2000). Other mechanisms may be identified as a result of intensified studies of ecological forces on populations (Majewski & Cohan, 1999; Cohan 2001). The dialogue among systematists, population and evolutionary geneticists, ecologists and microbiologists will be to the benefit of bacterial systematics in general, and of a more transparent species concept in particular.

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