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Practical Considerations with the Proposal of New Bacterial Taxa

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Species is the basic unit for constructing a taxonomic classification system providing a stable identification framework for different microbiological disciplines. Despite the recurrent controversy founded on the synonymy of the term species, taxonomy requires a unit that provides a pragmatic, operational and universally applicable classification system. The latest formulation of the concept: *"A prokaryotic species is a category that circumscribes a monophyletic (preferably) genomically coherent group of individual isolates/strains sharing a high degree of similarity in (many) independent features, comparatively tested under highly standardized conditions"* (Stackebrandt *et al*., 2002, IJSEM 52), provides a framework for a universally applicable concept of species. However, the definition (often misunderstood as concept), is a dynamic set of parameters and values that bounder species in the frame of the hitherto methods in use, and being improved in parallel to the technological developments.

In the last years the numbers of species and new genus descriptions have been increased tremendously. One reason for this is the detailed insight into the microbial diversity in various environments. But species and genera descriptions are now largely based on the 16S rRNA gene sequencing approach, and the methods necessary for sequencing are now widely (and easily!) used in many laboratories. Despite the advantages of the sequence based approach, there appears to be a tendency to allow comparative sequence analyses of 16S rDNA to determine classification contrary to the indications of other data. In several cases, classification is based solely on 16S rDNA data.

As gene reconstructions, and especially 16S rRNA gene comparisons provide evidences for organism's phylogenetic coherence, genomic coherence has been historically tested by data as GC mol% content and DNA-DNA hybridization experiments. Despite the important criticisms, DNA-DNA hybridization has importantly influenced the way to recognize species and its validity as circumscribing parameter is being validated by genometric data as ANI (average nucleotide index). These observations were already foreseen by Wayne *et al*. (1987) who wrote: "There was general agreement, that the complete deoxyribonucleic acid (DNA) sequence would be the reference standard to determine phylogeny and that phylogeny should determine taxonomy."

At that time, sequencing of full genomes was far beyond imagination, and hence, this statement was made without deeper knowledge on genome organization, and differences in the phylogenetic history of different genes. Other details, like gene losses, gene duplication, horizontal gene transfer, homologous recombination and chromosomal rearrangements could also not be considered in this statement, processes which are now known to shape the prokaryotic genome to a far wider extent than previously supposed

Nevertheless, in 2002, the value of DNA-DNA hybridization was again acknowledged by an Ad hoc committee in 2002 (Stackebrandt *et al*., 2002), and this method was recommended as the standard for species delineation. When making this recommendation, the ad hoc committee was well aware of the pitfalls and problems of this method, but due to the lack of a better alternative; there was agreement that it cannot be replaced until another approach has been evaluated as equivalent or superior.

The most frequently cited sentence in papers in where authors justify the independent circumscription of their new species is that of Wayne *et al*. (1997): "*The phylogenetic definition of a species generally would include strains with approximately 70% or greater DNA-DNA relatedness and with 5°C or less* ΔT_m". However, the ad hoc committee also emphasized the fact of recognizing phenotypic coherence of the members of a single species writing: "*Phenotypic characteristics should agree with this definition…* " and " *… it is recommended that a distinct genospecies that cannot be differentiated from another genospecies on the basis of any known phenotypic property not be named until they can be differentiated by some phenotypic property*". The ad hoc committee supported this view stating: "*More emphasis should be placed on discriminating markers. Description of species should be based on the use of well-documented criteria, laboratory protocols and reagents which are reproducible*" (Stackebrandt *et al*., 2002). "*In practice descriptive and diagnostic characters should be described in sufficient detail to permit comparisons between taxa and allow reproduction of observations*" (Stackebrandt *et al*., 2002). This is also a basic element, which is clearly documented in the Bacteriological Code. Recommendation 29 covers genera and in principle recommendation 30b covers species.

One of the problems coming along with this development is the increasing number of taxa, which cannot be differentiated phenotypically. Again, phenotype is of major biological importance for taxonomy. A lot of complex genotypic information is behind phenotype (still to be discovered), especially in features which we call chemotaxonomic features, but only phenotyping shows that these genes are really expressed and thus biologically important.

At present (more or less) similar criteria are used for species and genus descriptions. This may change in the future, when we have a full insight into the complexity of the genomes of microorganisms and the biological meaning behind this information, but we should wait with taxonomic rearrangements until we have this full insight.

References

1. **Stackebrandt, E. et al. (2002).** *Int J Syst Evol Microbiol* **52**, 1043-1047.

2. Wayne, L.G. et al. (1987). *Int J Syst Bacteriol* **37**, 463-464.