Molecular properties are included in the definition of a ‘species’. Exciting new findings announced here by Erko Stackebrandt and Jonas Ebers show that a 16S rRNA gene sequence similarity range above 98.7–99 % should be mandatory for testing the genomic uniqueness of a novel isolate. This overturns the old value of 97 % and will greatly facilitate the work of taxonomists.

With the inclusion of defined genomic properties in ‘minimal standards’ of taxon descriptions, molecular data are now fully acknowledged in systematic studies of prokaryotes. Depending on the rank of a taxon, these approaches are either mandatory or optional. At the taxonomic level of ‘species’, molecular properties serve two requirements: first, to verify the morphological, biochemical and chemotaxonomic coherence of strains of a ‘species’ by their similarities (preferably identity) at the genomic level and, second, to delineate this taxon from phylogenetically neighbouring species of the genus (Wayne et al., 1987; Rossello-Mora & Amann, 2001). As the taxon ‘species’ represents populations that themselves are the result of different mechanisms and tempi of evolution (Stackebrandt et al., 2002; Gevers et al., 2005), the degree of deviation from nearly absolute phenotypic and genomic identity (as expected to occur in clones) requires from taxonomists a balanced judgement of evolutionary processes that they may possibly not be aware of. In order to facilitate and harmonize taxonomic decisions in a field in which the Biological Species Concept does not apply, an arbitrary and artificial definition has evolved over a century of bacterial taxonomy (Stackebrandt & Krieg, 1984; Stackebrandt, 2000); today, the description of the construct ‘species’ is more stringently controlled by recommendations than that of any other taxon. While in the pre-Approved Lists era, taxonomists were allowed to follow their own subjective judgements, the past 25 years have witnessed a more objective and internationally controlled verification process of ‘species’ descriptions. The predictability of the uniqueness of a ‘species novum’ has been largely strengthened by the universal applicability of molecular data. Methods applied, to name a few, embrace approximate characterization of the chromosome by determination of the base composition (mol% G+C content) and degree of reassociation of single-stranded DNA (DNA-DNA hybridization) as well as comparison of one-dimensional restriction and PCR patterns (Pukall, 2005); other methods focus on genes and operons, encoding rRNA and proteins, including typing and sequencing. Each of the methods applied has its strength in elucidating a defined range of the 4-billion-year evolution of prokaryotes. Though several molecular methods have their merits in taxonomy, two approaches, the ‘gold standards’, play a dominant role: DNA-DNA hybridization for ‘species’ delimitation, and 16S rRNA gene sequence similarities for unravelling more distant relationships among strains. DNA-DNA hybridization can be expressed as percentage reassociation similarity or $\Delta T_m$ of reassociated DNA strands (Wayne et al., 1987), but only the first parameter is in general use. This judgement appears objective when browsing through the past 15 or so volumes of the International Journal of Systematic and Evolutionary Microbiology (IJSEM) (formerly International Journal of Systematic Bacteriology), the official publication of the International Union of Microbiological Societies (IUMS). Almost every species description contains a phylogenetic analysis of the type strain based on 16S rRNA gene sequence similarity comparison and many novel species are delineated from their phylogenetic neighbours by DNA–DNA reassociation values below 70 %.
Despite the importance of the DNA-DNA reassociation approach, most microbial taxonomists are not in a position to perform these studies and need collaboration with specialized laboratories. Experience is needed in isolation and purification of DNA, and although one can choose from a variety of different hybridization methods (Roselló-Mora, 2003), none of these is straightforward to apply without thorough training. But these are not the only reasons for the aversion to this technique: the method of reassociation of denatured DNA strands of two different strains unfolds the homologous genome stretches that are involved in the reassociation process. In these times of complete genome sequences and the teaching of sequence techniques to undergraduates, this failure to examine the mechanisms underlying this technique, e.g. De Ley et al. (1994), summarizing an overall evaluation. In 'Bacterial classification I. Classification of prokaryotic organisms: an overview', in Bergey's Manual of Systematic Bacteriology, vol. 1, pp 1–48. Edited by N.R. Krieg & J.G. Holt. Baltimore: Williams & Wilkins. 1984.

References

Adapted from Wayne et al. (1987), summarizing an overall concern of these authors 'that any phylogenetically based taxonomic schemes that result must also show phenotypic consistency.

Figs. 1. Comparison of 16S rRNA gene sequence similarities and DNA–DNA reassociation values. Data have been compiled from publications containing species descriptions from IJSEM 55 (2005). The different colours refer to broad categories of reassociation methods, red, microtitre plate technique, e.g. Ezaki et al. (1999); dark blue, spectrophotometric technique, e.g. De Ley et al. (1970); light blue, membrane filter method, e.g. Tourova & Antonov (1987); black, other methods, e.g. dot hybridization (Amaluata et al. 2005), or not defined. Horizontal rules between squares indicate data obtained by two different reassociation methods. Arrows point to the position of the xin-xin-axially isolated 16S rRNA gene sequence similarity values of sequences deposited by Amaluata et al. (2005). The horizontal blue bar indicates the threshold range above which it is now recommended to perform DNA-DNA reassociation experiments; the horizontal red bar indicates the threshold values published previously (Stackebrandt & Goebel, 1994). E. Stackebrandt & E. Ebers