Minutes

R. A. Whiley Secretary
M. Kilian Chairman

International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of staphylococci and streptococci

Minutes of the closed meeting, 31 July 2002, Paris, France

Minute 1. Call to order. The Chairman, Mogens Kilian, called the closed meeting to order at 15:30 on Wednesday 31 July 2002 at the Palais de Congrès, Paris, France.

Minute 2. Record of attendance. The members present were A. Bouvet, T. Ezaki, R. R. Facklam, M. Kilian, K.-H. Schleifer and R. A. Whiley. Apologies for absence were received from J. Freney, J. Jelínková, D. Mossel, L. M. Teixeira and P. Vandamme.

Minute 3. Minutes of the meeting held in Auckland, New Zealand, on 13 October 1999. In accordance with Minute 3 of the meeting held in Auckland, the minutes of that meeting had previously been checked via e-mail and subsequently approved for publication [*Int J Syst Evol Microbiol* **53** (2003), 913–914].

Minute 4. Retirement of members. The members were informed of the retirement from the subcommittee of members J. Henrichsen, V. Hájek, W. Köhler and J. M. Hardie. A formal letter will be sent to each expressing thanks for the services they have given to the subcommittee.

Minute 5. Current position regarding species epithet changes recently proposed within *Streptococcus* and *Staphylococcus*. Following several name (species epithet) changes effected on the grounds of grammatical correctness, particularly within the genus *Streptococcus* [Trüper & de' Clari, *Int J Syst Bacteriol* **47** (1997), 908–909; Trüper & de' Clari, *Int J Syst Bacteriol* **48** (1998), 615], and subsequent arguments against this, particularly with regard to older established names [Kilian, *Int J Syst Evol Microbiol* **51** (2001), 723–724], M. Kilian reported that his formal request for the retention of older, well-established names had been rejected by the Judicial Commission of the International Committee on Systematics of Prokaryotes and that, inevitably, a line would be drawn under the matter with the eventual appearance of the new names in the forthcoming 2nd edition of *Bergey's Manual of Systematic Bacteriology*.

It was also noted that, in future, all names published on the Approved Lists of Bacterial Names and appearing in Validation and Notification Lists would be regarded as

permanent, as stated in the Amendment to Rule 61 of the Bacteriological Code [Minute 7, Session 1, of the meeting of the Judicial Commission held on 14, 15 and 18 August 1999, Sydney, Australia; *Int J Syst Evol Microbiol* **50** (2000), 2239–2244].

It was agreed that there is a need for any nomenclatural changes within the remit of the subcommittee to be voted by the members, and the collective opinion passed on to the International Committee on Systematics of Prokaryotes.

Minute 6. Species described since the previous meeting (October 1999). An overview was given of the species listed within *Macrococcus*, *Staphylococcus* (K.-H. Schleifer) and *Streptococcus* (M. Kilian), in particular those species described since the previous meeting. The new taxa included:

(i) *Macrococcus* gen. nov., including *Macrococcus bovicus* sp. nov., *Macrococcus carouselicus* sp. nov., *Macrococcus equipercicus* sp. nov. (type species) and *Macrococcus caseolyticus* comb. nov. [Kloos *et al.*, *Int J Syst Bacteriol* **48** (1998), 859–877];

(ii) *Staphylococcus carnosus* subsp. *carnosus* subsp. nov. and subsp. *utilis* subsp. nov. and *Staphylococcus condimenti* sp. nov., from soy-sauce mash [Probst *et al.*, *Int J Syst Bacteriol* **48** (1998), 651–658], *Staphylococcus fleurettii* sp. nov., isolated from goat's milk cheeses [Vernozy-Rozand *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 1521–1527], *Staphylococcus hominis* subsp. *hominis* subsp. nov. and subsp. *novobiosepticus* subsp. nov., from human blood [Kloos *et al.*, *Int J Syst Bacteriol* **48** (1998), 799–812], *Staphylococcus lutrae* sp. nov., isolated from otters [Foster *et al.*, *Int J Syst Bacteriol* **47** (1997), 724–726] and *Staphylococcus succinus* sp. nov., isolated from 25–35 million year old Dominican amber [Lambert *et al.*, *Int J Syst Bacteriol* **48** (1998) 511–518]. *Staphylococcus pulvereri* has been reported as probably synonymous with *Staphylococcus vitulinus* on the basis of biochemical characteristics, but requiring molecular analyses for confirmation [Petráš, *Int J Syst Bacteriol* **48** (1998), 617–618];

(iii) *Streptococcus australis* sp. nov., from the human oral cavity [Willcox *et al.*, *Int J Syst Evol Microbiol* **51** (2001), 1277–1281], *Streptococcus constellatus* subsp. *constellatus* subsp. nov. and subsp. *pharyngis* subsp. nov., from the human oral cavity, respiratory tract and clinical specimens

[Whiley *et al.*, *Int J Syst Bacteriol* **49** (1999), 1443–1449], *Streptococcus didelphis* sp. nov., from opossums with suppurative dermatitis and liver fibrosis [Rurangirwa *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 759–765], *Streptococcus pluranimalium* sp. nov., from clinical specimens of several animals and birds [Devriese *et al.*, *Int J Syst Bacteriol* **49** (1999), 1221–1226], *Streptococcus entericus* sp. nov., from the intestines of cattle suffering from catarrhal enteritis [Vela *et al.*, *Int J Syst Evol Microbiol* **52** (2002), 665–669], *Streptococcus infantarius* subsp. *infantarius* subsp. nov. and subsp. *coli* subsp. nov., responsible for endocarditis and isolated from human blood and faeces and from food products [Schlegel *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 1425–1434], *Streptococcus lutetiensis* sp. nov. and *Streptococcus pasteurianus* sp. nov., within the *bovis* species group or ‘complex’, reported as sp. nov. on the basis of the superoxide dismutase gene (*sodA*) sequences [Poyart *et al.*, *Int J Syst Evol Microbiol* **52** (2002), 1247–1255], although the former is synonymous with *S. infantarius* subsp. *coli*; *Streptococcus orisratti* sp. nov., from the mouths of laboratory rats [Zhu *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 55–61], *Streptococcus ovis* sp. nov., isolated from clinical specimens from sheep [Collins *et al.*, *Int J Syst Evol Microbiol* **51** (2001), 1147–1150], *Streptococcus sinensis* sp. nov., isolated from patients with endocarditis [Woo *et al.*, *J Clin Microbiol* **40** (2002), 805–810; included in Validation List no. 87, *Int J Syst Evol Microbiol* **52** (2002), 1437–1438], and *Streptococcus urinalis* sp. nov., from human urine of patients with cystitis [Collins *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 1173–1178].

The emergence by 16S rDNA sequence analysis of a hitherto unnamed species group centred on *Streptococcus hyovaginalis* and also including *Streptococcus thoralensis* and *Streptococcus pluranimalium* was noted.

Within the *Streptococcus bovis*/*Streptococcus equinus* ‘complex’, it was agreed that *Streptococcus bovis* is a junior synonym of *Streptococcus equinus*. *Streptococcus waius* is a synonym of *Streptococcus macedonicus* [Manachini *et al.*, *Int J Syst Evol Microbiol* **52** (2002), 945–951] and *Streptococcus intestinalis* is indistinguishable from *Streptococcus alactolyticus* on the basis of biochemical characteristics and SDS-PAGE of whole-cell proteins [Vandamme *et al.*, *Int J Syst Bacteriol* **49** (1999), 737–741]. There was some discussion as to the validity of the proposal by Poyart *et al.* [*Int J Syst Evol Microbiol* **52** (2002), 1247–1255], for the inclusion of strains previously named *Streptococcus gallolyticus*, *Streptococcus macedonicus* and *Streptococcus waius* within a single species, to be named *Streptococcus gallolyticus*. It was agreed that the data presented in the paper, particularly from the DNA–DNA hybridization experiments, were somewhat open to reinterpretation, as relatedness values consistently below 70% were reported, and that it is more likely that, while *Streptococcus macedonicus* and *Streptococcus waius* together represent a single species, *Streptococcus gallolyticus* should remain a separate species. The latter interpretation had in fact been considered to be

a possibility by the authors themselves in the discussion within that paper.

Minute 7. Author for *Staphylococcus* chapter in *Bergey’s Manual of Systematic Bacteriology*, 2nd edition, vol. 3. K.-H. Schleifer reported that at present there is an outstanding requirement for someone to undertake the chapter on *Staphylococcus* for vol. 3 of the 2nd edition of *Bergey’s Manual of Systematic Bacteriology*. The subcommittee notes that this is a matter of some urgency and that a suitable author needs to be found as soon as possible.

Minute 8. Streptococcal serogroup reference strains.

M. Kilian pointed out the continuing requirement for a list of reference strains of streptococci as representatives of serological groups from which standard antisera could be derived. R. Facklam said that he would assist by sending his list of candidate strains from the CDC and pointed out that three new serological group antigens had been found within *Streptococcus porcinus*. M. Kilian remarked that work carried out by himself and colleagues had revealed that the Lancefield group O antigen of *Streptococcus mitis* biovar 1 was, in fact, identical to the C-polysaccharide antigen of *Streptococcus pneumoniae* [Bergström *et al.*, *Eur J Biochem* **267** (2000), 7147–7157].

R. Facklam raised the issue of the problem of serological cross-reaction shown by some strains (i.e. non-monospecific reactions) and gave the example of Lancefield group B reaction with strains of *Streptococcus porcinus*. He considered that adsorption of antisera was a prerequisite for reliable use and that, in his opinion, one could not have confidence in some commercial antisera due to inadequate adsorption procedures. R. Facklam stated that he has raised this issue in several of his published papers.

T. Ezaki pointed out that, in Japan, there was less emphasis on Lancefield serological grouping for routine purposes and that strains were usually tested only against groups A, B, C, D, F and G.

Minute 9. Discussion of ‘vitality’ of the subcommittee and its role. M. Kilian remarked on the danger of ‘dormancy’ in taxonomic subcommittees and the underlying problem of a lack of identity and awareness of the remit of subcommittees by their members and the problems they should be addressing.

The idea of setting up a properly functioning e-mail group for the dissemination of material in preparation (for example, key text-book chapters where opinions from the membership would be valuable) and for maintaining general discussion and group identity within this subcommittee was raised and agreed in principle.

Minute 10. Description of new species based on a single strain. The practice of publishing descriptions of new species based on single strains was discussed. It was generally agreed that this was a bad practice and that as

many strains as was practicable should be incorporated into proposals.

M. Kilian stated that, although strictly speaking the International Code of Nomenclature of Bacteria did not forbid the description of new species based on single isolates, it was unwise to do so in practice as there was no way of knowing whether a single strain could be guaranteed to constitute a representative of a separate species (i.e. a well-defined and genetically distinct population), particularly when considering the weight of scientific evidence that demonstrates that bacterial species comprise populations of genetically dissimilar clones, or strains, and that the overall population structure of species can be profoundly affected by horizontal gene transfer events.

T. Ezaki made the general point that, in the case of organisms isolated from remote environments, it may not be possible to obtain more strains and that the Judicial Commission was of the opinion that a case-by-case approach should be adopted. A. Bouvet pointed out that, where new, clinically important species were involved, it was right to publish a description even based on a single strain if that was all that was available. R. Facklam advised caution on this matter and quoted the example of the clinically important species *Aerococcus sanguinicola* [Lawson *et al.*, *Int J Syst Evol Microbiol* **51** (2001), 475–479], which had been described from a single isolate that was later found to be atypical when further strains were obtained and compared phenotypically. He pointed out that less haste would have resulted in a more accurate description in this case.

The subcommittee thought that the system practiced by the CDC, of numbering novel single strains until further isolates are found, instead of assigning a name, was a sensible one and makes the recommendation that this protocol should be generally adopted by those working with the taxa falling under the remit of the subcommittee.

A. Bouvet reminded members that the report of the ad hoc committee for re-evaluation of the species definition in bacteriology [Stackebrandt *et al.*, *Int J Syst Evol Microbiol* **52** (2002), 1043–1047] recommended polyphasic approaches and encouraged applicable genomic methods, provided that there was demonstrated congruence between the technique used and DNA–DNA reassociation using reference strains. Multilocus sequence typing (MLST) was one of the promising techniques that offered an alternative to DNA–DNA hybridization. M. Kilian was of the opinion that MLST provided a valuable technique that would eventually be widely used when databases were available. R. Facklam gave the example of M-protein gene (*emm*) typing and the CDC database available on-line, where approximately 2000 ‘hits’ per month were being recorded.

Minute 11. Use of commercially available test kits versus conventional testing for characterizing strains and for taxonomic descriptions. R. Facklam was of the opinion that commercial test kits should not be used for these purposes and that initial descriptions of species

should be based on characteristics determined using conventional biochemical and physiological tests, while commercial test kits could be used for routine identification purposes if required. He also pointed out that, in his professional capacity, he was not at liberty to endorse commercial products. M. Kilian was of the opinion that, whatever methods are employed in species descriptions, the reproducibility of the results is the decisive point and that methods should be clearly described. M. Kilian showed some unpublished data showing that, while there was often close agreement between commercial and conventional test formats, there were instances where marked disagreement occurred and, therefore, that these two sets of data thus derived should be treated as independent of each other while hopefully remaining consistent within individual species. These data would be sent to a leading commercial test kit manufacturing company with the suggestion that this cautionary note be included in the accompanying kit information.

Minute 12. Minimal standards for species descriptions. The minimum standards already published for staphylococcal species [Freney *et al.*, *Int J Syst Bacteriol* **49** (1999), 489–502] were discussed with regard to detail of characterization and the general availability of methods required. Some of the recommendations are possibly impractical in certain cases, particularly the requirement for peptidoglycan types to be determined, as this could probably only be carried out in a specialist laboratory. T. Ezaki commented that, in his laboratory, only molar ratios of peptidoglycan components were determined and not the type. R. Facklam reported that he had drafted the minimum standards for *Enterococcus* and would be grateful to receive comments. It was generally agreed that determination of G+C content should be included in the requirements and M. Kilian added that sequences of highly conserved housekeeping genes, such as those examined by MLST, are representative of the G+C content of DNA.

M. Kilian and R. Whiley are due to produce the minimum standards for streptococcal species descriptions and agreed to circulate a list of proposed tests for the subcommittee members to comment on.

Minute 13. Any other business. There was continuing concern over the increasing number of newly described genera with close relationships to those genera within the remit of this subcommittee. It was agreed that the taxa to be covered by the subcommittee needed clarification and that a list of the genera in question was required.

Minute 14. Next meeting of the subcommittee. A date for the next subcommittee meeting was not highlighted, and it was thought best if a list of relevant candidate scientific meetings be circulated to the membership before a decision is taken.

Minute 15. Adjournment. The meeting was adjourned at 17:15 on 31 July 2002.