International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of *Campylobacter* and related bacteria

Minutes of the meetings, 2 and 4 September 2001, Freiburg, Germany

Session 1. Closed meeting

Minute 1. Call to order. The Chairman, P. Vandamme, called the meeting to order at 12:00 on 2 September 2001 in Meeting Room 5 of the Konferenzhal in Freiburg, Germany.

Minute 2. Record of attendance. The members present were P. Vandamme (Chairman), J.-P. Butzler, J. G. Fox, A. J. Lastovica, F. Mégraud, S. L. W. On (Secretary), R. J. Owen and I. V. Wesley. Apologies for absence were received from F. E. Dewhirst, A. Lee and T. J. Trust.

Minute 3. Agenda. The proposed agenda was approved.

Minute 4. Minutes of the previous (1999) meeting. The minutes of the meeting held in Baltimore, USA, in 1999 were approved.

Minute 5. Matters arising from the previous meeting. Descriptions of putative new species outside the IJSEM: P. Vandamme had contacted members of several other subcommittees to determine whether there was any concern about the quality of new species descriptions in journals other than the IJSEM. The quality of such descriptions had concerned members of the present subcommittee for several years, but this concern was not shared by members of other subcommittees that had been contacted. Nonetheless, the subcommittee strongly reaffirms its dedication to 'good taxonomic practice', as shown by the publication of two sets of minimal standards for the description of novel *Campylobacter* and *Helicobacter* species since 1994. The subcommittee continues to recommend high quality taxonomic studies for the accurate delineation of new species within the epsilon subclass of the class *Proteobacteria*.

Taxonomic status of *Helicobacter nemestrinae*: J. G. Fox described results of a study that confirmed that the type strain of *H. nemestrinae* held at the American Type Culture Collection (ATCC 49396^T) belonged to the species *Helicobacter pylori*. The results indicated that *H. nemestrinae* should therefore become an inferior synonym of *H. pylori*. Full details of the investigation have now been published [Suerbaum *et al.*, *Int J Syst Evol Microbiol* **52** (2002), 437–439].

Minute 6. Minimal standards for describing new species of the genus *Helicobacter*. The recommended minimal standards paper had been published in IJSEM [Dewhirst *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 2231–2237]. S. L. W. On agreed to circulate reprints to all subcommittee members.

Minute 7. Recommendations of the subcommittee. The views of the subcommittee on a number of contemporaneous issues concerning the taxonomy and nomenclature of

Campylobacter and related bacteria had been published [Vandamme & On, *Int J Syst Evol Microbiol* **51** (2001), 719–721]. One such recommendation concerned the taxonomic status of '*Campylobacter hyoilei*', which was considered to be the same species, and thus a subjective synonym, of *Campylobacter coli*. This recommendation had been challenged by Dep *et al.* [*Int J Syst Evol Microbiol* **51** (2001), 819–826] who had found differences between members of these taxa with respect to the activity of several enzymes, and by the presence of a genetic marker (detectable by PCR) in strains of '*C. hyoilei*'. Dep and colleagues thus claimed their data upheld the taxonomic distinction between '*C. hyoilei*' and *C. coli*. The subcommittee reviewed these, and other relevant findings, and concluded that:

(i) Unequivocal differences between strains of extant species were already known (e.g. *Campylobacter jejuni* subsp. *jejuni* strains deficient in hippuricase or catalase), hence the quantitative enzymic differences observed were not necessarily indicative of interspecific distinctiveness.

(ii) The taxonomic value of the described PCR test could also be questioned, since the genetic marker may simply represent a trait present in some, but not all strains of a species and thereby reflect infraspecific diversity, not inter-species divergence.

(iii) Two separate investigations using different methods for resolving AFLP patterns in whole-cell DNA of *Campylobacter* species had clearly clustered '*C. hyoilei*' strains with the type and reference strains of *C. coli* [On & Harrington *FEMS Microbiol Lett* **193** (2000), 161–169; Duim *et al.*, *Microbiology* **147** (2001), 2729–2737].

After consideration of the available data, the subcommittee members concurred with their prior recommendations, although further investigations on the matter were suggested.

Minute 8. Recent developments within the genera *Campylobacter* and *Helicobacter*. Five new species had been formally described since the previous meeting; *Campylobacter hominis*, *Campylobacter lanienae*, *Helicobacter mesocricetorum*, *Helicobacter ganmani* and *Helicobacter aurati*. Several putative new taxa had been described, but had still to be validated by publication in the IJSEM. These taxa included '*Helicobacter typhlonicus*' (subsequently '*Helicobacter typhlonius*'), '*Helicobacter canadensis*', '*Helicobacter winghamensis*', and two unnamed taxa, accommodating strains from dolphins [Harper *et al.*, *Appl Environ Microbiol* **66** (2000), 4751–4757] and rhesus monkeys affected with chronic idiopathic colitis [Fox *et al.*, *J Med Microbiol* **50** (2001), 421–429].

The nomenclature of '*Candidatus Helicobacter suis*' had been the subject of considerable debate. This uncultured organism shares over 99% sequence similarity in its 16S

rRNA gene with corresponding data determined for '*Helicobacter heilmannii*' type I strains; the latter name is in common use throughout the wider scientific community. The high level of sequence relatedness between representatives of these taxa strongly suggests they represent a single species, although their resistance to conventional culture prevents an accurate determination of their taxonomic status. Nevertheless, in a poster presented at the CHRO 2001 congress in Freiburg [*Int J Med Microbiol* **291** (2001) suppl. 31, 149, abstract no. P-40], O'Rourke and colleagues had proposed that '*Candidatus H. suis*' and '*H. heilmannii*' type I strains be referred to as '*Candidatus H. heilmannii*' to reflect the more frequent nomenclatural usage, and the non-specific host range of this organism. Further work is needed in order to resolve this issue.

Extensive sequence diversity in the 16S rRNA gene of *Helicobacter cinaedi* had now been documented in a polyphasic taxonomic study of members of this species [Vandamme *et al.*, *J Clin Microbiol* **38** (2000), 2261–2266]. Consequently, it should be noted that strains described previously as '*Helicobacter westmeadii*' and '*Helicobacter* sp. strain Mainz' represent additional strains of *H. cinaedi*.

Minute 9. Taxonomic status of species other than *Arcobacter*, *Campylobacter* and *Helicobacter* assigned to the epsilon subclass of the class *Proteobacteria*. Results from a recent review of the taxonomy of the epsilon subclass of the *Proteobacteria* [On, *Symp Ser Soc Appl Microbiol* **90** (2001), 1S–15S] were presented. A comprehensive phylogenetic analysis assigned '*Dehalospirillum multivorans*' to the *Sulfurospirillum* clade; the ecological and physiological characteristics of members of these taxa were similar, thereby indicating that '*D. multivorans*' should be considered as a *Sulfurospirillum* species. *Thiomicrospira denitrificans* is phylogenetically distinct from other *Thiomicrospira* spp. but appears to be broadly related to other members of the epsilon subclass of the *Proteobacteria*, most notably to *Thiovulum*. The free-living nature of *Thiomicrospira denitrificans* is similar to that of *Arcobacter nitrofigilis*, *Sulfurospirillum* spp. and *Thiovulum*. These data indicate *Thiomicrospira denitrificans* is misplaced in the genus *Thiomicrospira*. In addition, 16S rRNA gene sequences of bacterial symbionts in polychaete worms and shrimps represent a clade distinct from, but related to, *Arcobacter*, *Campylobacter*, *Helicobacter* and *Sulfurospirillum*. It was agreed that these bacteria be included under the remit of the subcommittee.

Minute 10. Taxonomic status of '*Flexispira rappini*' and '*Helicobacter ulmiensis*'. Extensive polymorphism in 16S rRNA gene sequences of '*Flexispira rappini*' strains has been described [Dewhirst *et al.*, *Int J Syst Evol Microbiol* **50** (2000), 1781–1787; Patterson *et al.*, *J Clin Microbiol* **38** (2000), 3722–3728]. *Helicobacter bilis*, *Helicobacter trogonum* and *Helicobacter aurati* are morphologically indistinguishable from strains first classified as '*F. rappini*'. It appears from the aforementioned studies that substantial taxonomic diversity exists among strains with this distinctive morphotype. A subsequent analysis has shown that '*F. rappini*' phylotypes 1, 4 and 5 represent the same species; it is presumed these strains will form part of a formal description of a novel *Helicobacter* species. Members of the subcommittee agreed that these results provided further evidence of extensive phylogenetic divergence within certain species assigned to the epsilon subclass of the class *Proteobacteria* and emphasized the need for a polyphasic taxonomic approach to the delineation of new *Helicobacter* species in particular.

The name '*Helicobacter ulmiensis*' had not, to the knowledge of members of the subcommittee, been proposed in any printed scientific literature in the English language, although this name has been assigned to a 16S rRNA gene sequence (GenBank accession no. AJ007931). P. Vandamme and R. J. Owen have examined isolates of this taxon by whole-cell protein profile comparisons and by PCR-RFLP analysis respectively and have identified them as strains of *Helicobacter hepaticus*. P. Vandamme volunteered to write to the depositors of the '*H. ulmiensis*' sequence to request that their sequence is renamed in order to avoid confusion.

Minute 11. Type strains of *Helicobacter* species. R. J. Owen reported on the difficulties of obtaining the type strains of certain fastidious *Helicobacter* species, including *Helicobacter bizzozeronii*, *Helicobacter felis* and *Helicobacter salomonis*. Although type strains of such *Helicobacter* species have been deposited in service culture collections, preservation and culture methods were generally poor and strains have frequently proved to be poorly viable or non-viable. The difficulty of obtaining viable cultures limited additional studies on these strains, including the development of new identification methods. This problem could result in a greater reliance being placed on the use of 16S rRNA gene sequence comparisons for identification, a practice that may give misleading results. P. Vandamme agreed to try and raise this problem of preserving such fastidious strains at the next meeting of the World Federation of Culture Collections.

In a separate issue relating to *Helicobacter* type strains, attention was drawn to a recent letter by Kuhnert *et al.* [(2001) *J Clin Microbiol* **39**, 2751–2752], in which it was suggested that the ATCC type strain of *H. cinaedi* that is currently being supplied to users was in fact the type strain of *Helicobacter fennelliae*. S. L. W. On agreed to raise this matter with the ATCC.

Minute 12. Agenda for the open meeting. It was agreed that P. Vandamme and S. L. W. On would prepare the agenda for the open meeting of the subcommittee.

Minute 13. Matters concerning membership. P. Vandamme and S. L. W. On were re-elected as Chairman and Secretary respectively of the subcommittee. One new member was proposed and would be invited by the Secretary to serve the subcommittee, pending approval from the Secretary for Subcommittees. The current members of the subcommittee are: J.-P. Butzler (Belgium), F. E. Dewhirst (USA), J. G. Fox (USA), A. J. Lastovica (South Africa), A. Lee (Australia), F. Mégraud (France), S. L. W. On (Secretary) (Denmark), R. J. Owen (UK), T. J. Trust (USA), P. Vandamme (Chairman) (Belgium) and I. V. Wesley (USA).

The members noted with deep regret the untimely death of the founding member and former Chairman of the subcommittee, Jan Ursing (25 September 1926–29 February 2000). The outstanding contributions of Jan to the subcommittee and to taxonomic research generally will be sorely missed by the scientific community. Members of the subcommittee expressed their sympathies to Jan's family and friends.

Minute 14. Next meeting. It was agreed that the next closed meeting of the subcommittee would be held in 2002. A subsequent ballot of members of the subcommittee identified the International Union of Microbiological Societies Congresses in Paris, France, as the preferred venue.

Minute 15. Adjournment. The meeting was adjourned at 13:32 on 2 September 2001.

Session 2. Open meeting

Minute 1. Call to order. The Chairman, P. Vandamme, called the meeting to order at 11:58 on 4 September 2001 in Meeting Room 1 of the Konferenzhal in Freiburg, Germany.

Minute 2. Record of attendance. The members present were P. Vandamme (Chairman), S. L. W. On (Secretary) and R. J. Owen, and apologies for absence were received from J.-P. Butzler, F. E. Dewhirst, J. G. Fox, A. J. Lastovica, A. Lee, F. Mégraud, T. J. Trust and I. V. Wesley. Fifteen delegates from the CHRO 2001 congress were present.

Minute 3. Description of activities, and areas of interest of the subcommittee. The Chairman described the activities and remit of the subcommittee to the delegates.

Minute 4. Recent developments. A number of key points were named by P. Vandamme. A summary of the novel species that had been described during the previous 24 months was given, and attention drawn to the mislabelled type strain of *H. cinaedi* that was currently being distributed by the ATCC. The ATCC type strain of *H. nemestrinae* had been re-examined by genotypic and phylogenetic methods and found to be a strain of *H. pylori*. Difficulties in the use of 16S rRNA gene sequence comparisons for identifying 'campylobacteria' were described, since it was clear that strains assigned to a distinct branch in a phylogenetic tree did not necessarily represent a distinct species. Current examples of species of the epsilon subclass of the class *Proteobacteria* that show exceptional divergence in the 16S rRNA gene sequences include *Campylobacter hyointestinalis*, *H. cinaedi* and '*Flexispira (Helicobacter) rappini*'.

Minute 5. Questions raised by delegates. The question was posed as to whether the taxonomic status of *C. lanienae* should be re-evaluated. The taxonomic history of this species was reviewed briefly; *C. lanienae* has been found to cluster between *C. hyointestinalis* subspecies when 16S rRNA gene comparisons were used. *C. lanienae* had not originally been compared with strains of *C. hyointestinalis* subsp. *lawsonii* but it was now evident that these taxa are phenotypically similar. Whole-cell protein patterns of *C. hyointestinalis* and *C. lanienae* strains are also similar, although AFLP analysis indicate that *C. lanienae* is a distinct species. It was conceded that further data were required to resolve the issue.

Recommendations for identifying unculturable *Helicobacter* species were requested. Reference was made to the minimal standards for describing *Candidatus* taxa but the resistance of members of several *Helicobacter* species to *in vitro* culture

methods continued to restrict the development of effective and accurate methods for identification. However, the application of PCR-based methods may help resolve this issue. Members of the subcommittee agreed to provide references describing such PCR-based methods to interested parties upon request.

Clarification of the taxonomic status of '*Flexispira rappini*' was requested. It was confirmed that this name presently refers to bacteria exhibiting a distinct cellular morphology and also encompasses three formally named species, namely *Helicobacter aurati*, *Helicobacter bilis* and *Helicobacter trogonum*. Extensive divergence in the 16S rRNA gene of '*F. rappini*'-like bacteria suggests considerable taxonomic diversity, although it has been shown via DNA-DNA hybridization studies that several distinct phylotypes belong to the same species. This subject has been considered in more detail elsewhere [Vandamme & On, *Int J Syst Evol Microbiol* **51** (2001), 719–721]. A comment was made concerning the possible revision of the current bacterial species concept by use of whole-genome sequence data. It was felt that such data might help to clarify the taxonomy of complex groups such as the '*F. rappini*' complex. However, at present, the impact of such methods in this area is entirely hypothetical.

A recommendation for the choice of appropriate DNA-DNA hybridization methods for taxonomic studies of *Campylobacter* and related bacteria was requested. P. Vandamme currently uses a microtitre-tray based method based on the work of Ezaki and colleagues [Ezaki *et al.*, *Int J Syst Bacteriol* **39** (1989), 224–229]. It is known that different DNA-DNA hybridization methods may give different absolute values, although the same overall conclusions result, provided the methods are of an appropriate quality. Reference to past and current collaborations with the University of Helsinki, Finland and the Centers for Disease Control, USA on *Helicobacter* spp. and *Campylobacter lari* were made to illustrate this point. It was also noted that the use of microarrays containing all ORFs identified from whole genome sequences provides a novel alternative to current whole-cell DNA hybridization methods [see Murray *et al.* *Proc Natl Acad Sci USA* **98** (2001), 9853–9858]. Professor Brendan Wren's research group in the UK has used a microarray based on the *C. jejuni* subsp. *jejuni* genome sequence to examine members of closely related *Campylobacter* species; the results appear to correlate well with extant results from conventional DNA-DNA hybridization methods.

Minute 6. Adjournment. The meeting was adjourned at 12:40 on 4 September 2001.

Stephen L. W. On, Secretary