International Committee on Systematic Bacteriology

Subcommittee on the taxonomy of *Vibrionaceae*

Minutes of the meetings, 17 August 1999, Sydney, Australia

Session 1. Closed meeting

Minute 1. Call to order. The closed meeting was held from 09:00 on 17 August 1999 at the Skyline Room 2, Level 3 of the Sydney Convention Centre. The Chairman, B. Holmes, called the meeting to order.

Minute 2. Record of attendance. Those members present were R. R. Colwell, B. Holmes, Y. Kosako, G. B. Nair, R. Sakazaki, R. Schubert and S. Shinoda. K. Kita-Tsukamoto was welcomed to the meeting as an observer.

Minute 3. Apologies for absence. Apologies were received from M. Altwegg, D. J. Brenner, A. Carnahan, J. J. Farmer III, A. Huq, J. M. Janda, S. Joseph, M. Kelly and J. V. Lee. B. Holmes chaired the meeting and the Secretary, G. B. Nair, recorded the minutes.

Minute 4. Approval of agenda. No additional matters were proposed for discussion.

Minute 5. Minutes of the previous meeting. The minutes of the previous meeting, which was held on 19 May 1998 in Atlanta, USA, had been approved by mail and have been published [*Int J Syst Bacteriol* **49** (1999), 1945–1947]. A copy was given to each member and a written agenda was also circulated.

Minute 6. To receive proposals for changes in officers and membership. R. Sakazaki expressed the wish to retire from the subcommittee after having served for 35 years since its inception. He mentioned that the first subcommittee had only three members, J. C. Feeley, R. Hugh and himself, and they were extremely active. Prior to the meeting, J. J. Farmer in correspondence with the Secretary had suggested that R. Sakazaki be made an honorary (non-voting) member of the subcommittee for life, a proposal readily accepted by those present. Echoing a further suggestion from J. J. Farmer, R. R. Colwell, B. Holmes and other members requested R. Sakazaki to write recollections of his time in the subcommittee, which could be appended to the minutes [regrettably, R. Sakazaki passed away before he had an opportunity to do so; an obituary was published in *Int J Syst Evol Microbiol* **52** (2002), 1435]. All members expressed their sincere appreciation to R. Sakazaki both for his 35 years of dedicated and efficient services to the subcommittee and for his excellent contributions to the taxonomy of the family *Vibrionaceae*.

Minute 7. Consideration of change of name of subcommittee. Following the proposal of the separate family *Aeromonadaceae*, it was suggested by J. J. Farmer that the name of the subcommittee be changed to '... of *Vibrionaceae* and related organisms (Véron 1965)'. This name would reflect the original definition of this group of microbes by Véron in 1965 and thereby facilitate retention of *Aeromonas* and *Plesiomonas* within the responsibility of the present subcommittee. Although clearly a proposal with much to commend it, it was felt a decision should be deferred to the next meeting of the subcommittee to allow for consideration by members not present.

Minute 8. Minimal standards. At the subcommittee meeting held on 7 July 1994 at the Postgraduate Medical and Pharmaceutical Institute, Budejovicka, Prague (Czech Republic), J. J. Farmer was unanimously requested to prepare a draft document from the information already to hand. J. J. Farmer has collected voluminous data and had agreed to prepare a draft of the minimal standards to include *Vibrio*, *Aeromonas* and *Plesiomonas*. R. R. Colwell said that the standards are likely to evolve. B. Holmes said that whilst this was appreciated, it was nevertheless a duty of taxonomic subcommittees to prepare such standards.

Minute 9. Report on the Chicago Meeting of the Working Group on *Aeromonas* Taxonomy. The Working Group on *Aeromonas* Taxonomy held a meeting on 30 May 1999 in Chicago, IL, USA to consider certain outstanding issues, including various pertinent suggestions and concerns raised previously by the *Vibrionaceae* subcommittee. The principal issues discussed were:

9.1. Use of the epithet 'sobria' in *Aeromonas veronii* biovar (bv) sobria. There is much room for confusion in the use of the name 'sobria' as both a bv of the species *A. veronii* (hybridization group [HG] 8/10) and as the species name sobria for HG7 *Aeromonas sobria*. Following elaborate discussions, it was decided that a vote be taken to determine if the present terminology should be supported, or if it should be proposed to be replaced by one of three alternatives suggested at the meeting. It was decided that the voting would be done by e-mail and the majority recommendation would be accepted by all committee members and forwarded to the *Vibrionaceae* subcommittee.

9.2. Validity of retaining the subspecies designations within *Aeromonas salmonicida*. As there are insufficient data available, especially on the 'atypical' strains, it was decided to reconsider the question at the next meeting.

9.3. Prioritization between *Aeromonas trota* and *Aeromonas enteropelogenes*. *A. enteropelogenes* is a senior subjective synonym of *A. trota*. Nevertheless, the name *A. trota* has seen much wider use by the scientific community. The issue was deliberated and it was decided that more time should elapse to determine if the trend towards the greater acceptance of *A. trota* continues in the future.

9.4. Is *Aeromonas ichthiosmia* a junior synonym of *A. veronii* biovar sobria? After considerable discussion, it was decided that the Chair would assemble and send relevant written DNA–DNA hybridization data obtained from the Centers for Disease Control and Prevention (CDCP) in Atlanta to Dr Schubert. He in turn would attempt the appropriate DNA hybridizations, i.e. the type strain of *A. ichthiosmia* ATCC 49904^T against the type strain of *A. veronii*, ATCC 35624^T (HG 10) and reference strain ATCC 9071 (HG 8Y). To establish agreement, Dr Schubert will also send the strains in question to both Drs Huys and Stackebrandt for interlaboratory comparison of the results of all the analyses.

9.5. *Aeromonas encheleia*/DNA Group 11/*Aeromonas eucrenophila* subgroup II controversy. This taxonomic issue

concerns the recent proposal to emend the original description of the species *A. encheleia* Esteve *et al.* 1995 to include members of *A. eucrenophila* Subgroup II and DNA HG 11. This proposal was made by Geert Huys with his colleagues. As he was present at the meeting, Geert gave a brief review of his polyphasic taxonomic data which supported the proposed emendment and which included chemotaxonomic, FAME, AFLP, ribotyping and DNA–DNA hybridization data. A counter view was presented by Dr Martinez-Murcia, a co-author on the original paper describing *A. encheleia*, who was also a Working Group member. His 16S rRNA sequencing data suggest that these taxa represent three separate phylogenies and should not be combined. Further, his data also included DNA–DNA hybridization of these strains and the values obtained were in sharp disagreement with those of Huys *et al.* After much discussion, it was decided by the Working Group members, that since each laboratory had used a different method of hybridization, albeit with nominally the same group of strains, that this matter could best be resolved by a third independent laboratory evaluating the strains in question by the ‘gold standard’ hydroxyapatite method of DNA–DNA hybridization. The Chair, Amy Carnahan, proposed approaching D. J. Brenner at CDCP through J. J. Farmer as a possible independent laboratory to confirm the true genomic status of these strains in relation to each other and nearby species. This was agreed upon by the Working Group and the parties concerned. Amy Carnahan and J. J. Farmer would draft a small proposal for this project with the approval of Drs Huys and Martinez-Murcia, for Dr Brenner’s consideration.

9.6. Citation of Kluver and Van Niel (1936) for the genus *Aeromonas*. There is a discrepancy between the Opinion 48 rendered by the Judicial Commission in 1973 and the Approved Lists of Bacterial Names. Opinion 48 clearly states that Kluver and Van Niel are no longer to be associated with the genus designation of *Aeromonas*. In the future, Stanier (1943) should be associated with both the genus *Aeromonas* and the type species, *Aeromonas hydrophila*. The Working Group’s view was that the Judicial Opinion must stand and the incorrect inclusion of Kluver and Van Niel for the genus *Aeromonas* in the Approved Lists should be corrected.

9.7. Opinions 1 and 2 for Judicial Commission (these Opinions were merely presented to the Working Group and were not open for discussion by Group members as they are being submitted to the Judicial Commission following the ASM Meeting in Chicago).

Opinion 1 was read by Dr Barry Holmes. This Opinion essentially addressed the current situation in which *Aeromonas punctata* and *Aeromonas caviae* are considered objective synonyms as they share the same type strain, ATCC 15468^T. A Judicial Opinion will be requested to make *A. caviae* the single legitimate name for the type strain ATCC 15468, which is the definition strain for HG 4.

Opinion 2 was read by Dr Jim Farmer. This Opinion addressed the issue of *A. punctata* and *A. eucrenophila* as objective synonyms and sharing the same type strain NCIMB 74^T (ATCC 23309^T). An Opinion will be requested to replace *A. punctata* with *A. eucrenophila* because of the more widespread scientific usage of the latter.

9.8. Miscellaneous agenda items: (i) Martin Altwegg brought to the Working Group’s attention the purported proposal of a new species, ‘*Aeromonas pastore*’, as part of an unknown

journal reference that based the proposal on a Malaysian PhD thesis published in 1990. In view of the lack of any further effort to establish this organism as a new species, the Working Group decided to table the issue.

(ii) In Dr Janda’s absence, the Chair, Amy Carnahan, presented a request on his behalf regarding the establishment of a standard for the minimum number of strains (not isolates) necessary to propose a new species. In reply, Dr Schubert considered that, in the pure context of systematics, the definition of a species is based on only one strain, the type strain. Further discussion by the Working Group members focused on the history of ‘atypical’ type strains and the lack of phenotypic markers for new species proposed on the 16S rRNA sequence of 2–3 strains. Overall, the Working Group appeared to be in agreement with Dr Janda’s view that a critical number of strains should be required for new species proposals in the future. However, it was difficult to arrive at a minimal number of strains since this number could vary among different genera and within particular families. This item will be incorporated into Dr Farmer’s work on establishing Minimal Standards for the description of new members of the Family *Vibrionaceae*.

The next meeting of the Working Group on *Aeromonas* Taxonomy was tentatively scheduled for the next ASM Annual Meeting to be held in Los Angeles, CA, USA, in May 2000.

Minute 10. Working Group on *Vibrio* Taxonomy. As the Working Group on *Vibrio* Taxonomy had been established for some time, it was expected that a report of the Group’s activities would be available for presentation at the subcommittee meeting. As no report had been received for the past two subcommittee meetings, the Chairman and other members felt that a new Chairperson should take over the activities of this group. R. R. Colwell proposed K. Kita-Tsukamoto, who was present at this meeting, as the new Chairperson of this Working Group in view of her recent contributions on the taxonomy of vibrios. With the agreement of the members present, the Chairman invited K. Kita-Tsukamoto to take over as the Chairperson of this working group and she agreed. The Chairman requested the Secretary to write to J. V. Lee about this change and also to find out if he still maintained an interest to be a member of the subcommittee. K. Kita-Tsukamoto was requested to initiate the activities of the working group as soon as possible.

Minute 11. Classification of *Vibrio*. J. J. Farmer had written regarding the many proposals in the literature to subdivide the genus. In preparing the relevant chapter for the forthcoming edition of *Bergey’s Manual*, he had examined these proposals and observed that there was much confusion, poor data and many different trees based on 16S rRNA sequencing that are in conflict with each other. It was felt that this was a clear area that needed to be addressed by the Working Group on *Vibrio* Taxonomy. Based on 5S sequence data, R. R. Colwell stated that the family *Vibrionaceae* may soon be split into two and it was clear that the family was not homogeneous.

Minute 12. Working group on serotyping. Report on present status of respective schemes for *Vibrio cholerae*, mesophilic *Aeromonas* spp., *Plesiomonas shigelloides* and *Vibrio vulnificus*. R. Sakazaki presented the current status on each of the serotyping systems.

***Vibrio cholerae*:** Based on the serotyping scheme of *V. cholerae* by Sakazaki, Tamura & Gomez [*Jpn J Med Sci Biol* 23 (1970), 13–20], Shimada and his co-workers have ex-

tended the scheme to 204 O serotypes [Yamai *et al.*, *Kansenshougaku Zasshi* **71** (1997), 1037–1045; in Japanese]. Of the 204 serogroup strains, O114 to 117, O135, O194, O201 and O202 were specified as *Vibrio mimicus*. Although *V. mimicus* is taxonomically different from *V. cholerae*, their O antigens are apparently identical to the O antigens of *V. cholerae*.

***Vibrio vulnificus*:** Since Shimada & Sakazaki established 18 O serogroups within *V. vulnificus* [*Jpn J Med Sci Biol* **37** (1984), 241–246], about 30 strains isolated from patients were forwarded to the National Institute of Health (now the National Institute of Infectious Diseases), Tokyo. However, no new O serogroups were found among those strains.

Mesophilic *Aeromonas* spp.: Sakazaki & Shimada, in 1984, described an O serogrouping scheme including 45 O antigen groups for mesophilic *Aeromonas* strains [*Jpn J Med Sci Biol* **37** (1984), 247–255]. The scheme has been extended to 97 O serogroups to date by Shimada and his co-workers (unpublished data).

***Plesiomonas shigelloides*:** Preliminary studies on the serology of *Plesiomonas shigelloides* were conducted by Shimada & Sakazaki in 1978 [*Jpn J Med Sci Biol* **31** (1978), 135–142]. Subsequently, E. Aldova and T. Shimada have collaborated to extend the serotyping system. In 1994, Shimada and co-workers added 26 new O (O51–O76) and 24 new H (H18 to H41) antigens to the international antigenic scheme of *Plesiomonas shigelloides*. This scheme has now been further expanded by Aldova & Shimada (E. Aldova & T. Shimada. New O and H antigens of the international antigenic scheme for *Plesiomonas shigelloides*. *Folia Microbiol (Praha)* **45** (2000), 301–304] after the addition of 26 new O (O77–O102) and 10 new H (H42–H50 and H1ald) antigens. The reference strains for production of antisera were chosen from strains sent to the Prague Laboratory by Czech, Slovak and other microbiologists from around the world. All the reference strains are kept at the Czechoslovak National Collection of Type Cultures (CNCTC) and at the Japan Collection of Microorganisms (JCM). Eva Aldova has made two corrections because of weak sera. The original O90 antigen was in fact O17, and the original H47 was H11 and these have been omitted. These numbers are now allocated to Shimada's antigens, so that O90 now belongs to the reference strain 843-89 (CNCTC 5384, JCM 8307) while the H47 reference strain number is 891-89 (CNCTC 5377, JCM 8289). Aldova and Shimada also found a partial antigenic relationship of the ab, ac type between O102 (strain 31612) and O60 (strain 766-87). A similar relationship was also found in the H antigens of these reference strains. Absorption experiments revealed a different antigenic relationship, on the basis of which this H antigen was labelled as H1ald.

R. Sakazaki expressed his desire to retire from the Working Group on Serotyping. He further accepted the view that serotyping was on the decline and molecular techniques were likely to replace this traditional method. G. B. Nair pointed out, however, that a recent pandemic due to *V. cholerae* O3: K6, which spread from Asia to the USA, would not have been recognized without serology. G. B. Nair went on to state that T. Shimada, a previous member of the Working Group, had retired. Regretfully, the winding up of this Working Group appeared inevitable. In doing so, however, the subcommittee members expressed their sincere thanks for all the efforts put in over the years by the Working Group members and also to CNCTC and JCM for preserving the

various serotype reference strains for study by future generations of microbiologists.

Minute 13. Report on whole genome sequencing of *Vibrio cholerae* and future plans for sequencing strains of *V. cholerae* (classical) and O139. R. R. Colwell reported on the efforts that were ongoing to complete the whole genome sequence of an El Tor *V. cholerae* O1 isolate (N16961), which is representative of strains causing the current pandemic. At the time of presentation, the work related to the whole genome sequence of the *V. cholerae* isolate and of its two circular chromosomes was complete. The sequencing team discovered a total of 3885 open reading frames: 2770 on the larger chromosome 1 (2.9 million base pairs) and 1115 on the smaller chromosome 2 (1.1 million base pairs). Slightly more than 50% of these open reading frames encode proteins homologous to protein of known function; the remainder encode proteins without ascribed functions or that are not homologous to any known proteins. The whole genome sequence of *V. cholerae* O1 El Tor has now been published [*Nature* **406** (2000), 477–483]. R. R. Colwell stated that efforts are in progress to sequence the whole genome of a classical and O139 isolates of *V. cholerae* and rough drafts of classical and possibly O139 are under way. This work is ongoing at The Institute for Genomic Research.

Minute 14. Definitions for describing new clones of *V. cholerae* O1 and O139 using molecular typing methods. G. B. Nair presented data on a set of *V. cholerae* O1 and O139 strains typed by ribotyping, RAPD and pulsed field gel electrophoresis and underscored the need for developing standards for integrating information available by these techniques in taxonomic terms. Genetic typing is the means by which the microbiologist is provided with the ability to discriminate between and catalogue microbial nucleic acid molecules. Since genetic characterization forms the basis that allows researchers to classify isolates of microorganisms, the most detailed form of typing should essentially integrate taxonomy. The members agreed and said that this topic should be tabled at the next meeting where the details could be considered.

Minute 15. Instability of names. In a written communication, J. J. Farmer had recorded his concerns with efforts being made to change ('correct') the spelling of names and felt the subcommittee should develop a position statement on this subject. He drew attention to J. P. Euzéby's bacterial nomenclature web site (<http://www.bacterio.cict.fr/>) for a listing of all the 'possible corrections', which showed the extent of the problem. J. J. Farmer's view was that he opposed name changes just to have 'correctly' formed Latin words. The members felt this was an issue to be reconsidered at the next meeting once all subcommittee members had had a chance to consider it.

Minute 16. Adjournment. The meeting was adjourned at 12:55.

Session 2. Open meeting

Although it had been planned to hold an open meeting, the proposed time and venue had not been advertised in the general IUMS Congress programme. As interested parties would have no way of knowing of the meeting and therefore of being able to attend, it was decided to cancel it and cover all issues in the closed meeting.

G. Balakrish Nair, *Secretary*
Barry Holmes, *Chairman*