International Committee on Systematic Bacteriology

Subcommittee on the taxonomy of *Vibrionaceae*

Minutes of the closed meeting, 19 May 1998, Atlanta, GA, USA

Minute 1. Call to order. The closed meeting was held at 18:15 on 19 May 1998 at the Georgia World Congress Center, Atlanta, GA, USA, in conjunction with the American Society for Microbiology Annual Meeting. The meeting was called to order by the Chairman, B. Holmes.

Minute 2. Record of attendance. The members present were M. Altwegg, D. J. Brenner, A. Carnahan, R. R. Colwell, J. J. Farmer, III, B. Holmes (Chairman), A. Huq, S. W. Joseph and G. B. Nair (Secretary).

Minute 3. Apologies for absence. Apologies were received from P. Desmarchelier and J. M. Janda. P. Desmarchelier stated that in view of the nature of her current duties she would like to withdraw from membership of the subcommittee. Her withdrawal was accepted and the members expressed their appreciation for her contribution to the subcommittee.

Minute 4. Minutes of previous meetings. The minutes of the Prague, Czech Republic meetings held on 4 and 7 July 1994 and the Jerusalem meeting held on 21 August 1996 have now been published [*Int J Syst Bacteriol* **48** (1998), 1467–1469 and 1471, respectively].

Minute 5. Proposals for changes in officers and membership. Several members opined that no additional membership to the subcommittee should be sought for the present. It was also expressed that appropriate letters be sent to members who have been unable to attend the past two consecutive meetings to enquire about their continued interest in subcommittee affairs. All other current members expressed an interest to continue and contribute to the working of the subcommittee.

The current voting membership and officers of the subcommittee is as follows: M. Altwegg, Zürich, Switzerland; D. J. Brenner, Atlanta, GA, USA; A. Carnahan, Baltimore, MD, USA; R. R. Colwell, Arlington, VA, USA; J. J. Farmer, III, Atlanta, GA, USA; B. Holmes (Chairman), London, UK; A. Huq, Baltimore, MD, USA; J. M. Janda, Berkeley, CA, USA; S. W. Joseph, College Park, MD, USA; M. T. Kelly, Vancouver, Canada; Y. Kosako, Saitama, Japan; J. V. Lee, Nottingham, UK; G. B. Nair (Secretary), Calcutta, India; R. Sakazaki, Tokyo, Japan; R. Schubert, Frankfurt, Germany; and S. Shinoda, Okayama, Japan. In addition, R. Hugh, Washington, DC, USA, is an honorary member.

Minute 6. *Vibrio vulnificus* biogroup 3. J. J. Farmer drew attention to an interesting group of marine vibrios associated with severe wound infection and bacteremia isolated from different locations in Israel in 1996 and 1997. Initially, the isolates were identified as several different species, including *Vibrio alginolyticus*, *Vibrio vulnificus* and *Vibrio* spp. [N. Bisharat & R. Raz (1996). *Lancet* **348**, 1585–1586]. Further work at Centers for Disease Control and Prevention (CDC), USA, showed that phenotypically, the *Vibrio* isolates were most similar to *V. vulnificus*, but differed by negative

reactions in citrate, lactose, salicin, cellobiose and ONPG. However, all isolates were positive in a species-specific PCR for the cytotoxic haemolysin gene of *V. vulnificus*. Nine isolates representing cases from both years were compared by two DNA-based subtyping techniques. By PFGE, all isolates were non-typable. By restriction endonuclease digestion (using *Dde*I, *Hha*I and *Hpa*II) of the fragment generated by PCR of the haemolysin gene, all nine isolates had identical RFLP patterns. Based on clinical, epidemiologic, phenotypic and molecular data, CDC has designated this new vibrio as '*Vibrio vulnificus* biogroup 3'.

Minute 7. *Vibrio cholerae* whole-genome sequencing project.

R. R. Colwell reported on the progress of the whole-genome sequencing of a selected vaccine strain of *Vibrio cholerae* belonging to the El Tor biotype, the one responsible for the current and seventh pandemic of cholera. Preliminary comparisons of the 3.2 Mb genome of *Vibrio cholerae* indicate that *V. cholerae* and *Escherichia coli* share about 50% of their genes and there are a few genes in common with other species, e.g. *Aeromonas hydrophila*, *Bacillus* spp. and *Pseudomonas aeruginosa*. About one-third of the genes of *V. cholerae* were not shared by *E. coli* or *Shewanella putrefaciens*. The project is jointly undertaken by Craig Venter and Rebecca Clayton at The Institute of Genomic Research (TIGR), R. R. Colwell, University of Maryland, and John Mekalanos at Harvard University. It was indicated that the whole-genome sequence of *V. cholerae* would be available around September/October 1998.

Minute 8. Status of *Vibrio cholerae* serovars O1 and O139 and recent additions of new serotypes.

The recent status of *V. cholerae* O1 and O139 in Calcutta and other parts of the world was presented by G. B. Nair. The O139 serogroup is a recent addition which appeared abruptly in September 1992 in Southern India and rapidly spread to virtually all cholera endemic areas in India and neighbouring countries. The extent and rapidity of spread of the O139 serogroup led cholera workers to conclude that this event was the beginning of the eighth pandemic of cholera. In February 1994, the O1 serogroup again replaced the O139 serogroup and became the dominant serogroup causing cholera in Calcutta and in other parts of the Asian subcontinent. Subsequent studies showed that the O1 serogroup which replaced the O139 serogroup was a new clone of the O1 El Tor biotype. A quiescent period followed and it was thought that the appearance of O139 was a one-time event. However, a resurgence of *V. cholerae* O139 was observed in Calcutta in August 1996 and by September 1996 the O139 serogroup again became the dominant serogroup causing cholera. Molecular studies showed that the O139 strains which re-emerged in August 1996 were indistinguishable by ribotyping from the O139 strains isolated in 1992–1993, but showed a unique change in the structure and organization of the CTX genetic element. The O139 serogroup continues to be restricted to the Asian continent and has not yet spread to

the African or American continents [World Health Organization (1998), *Wkly Epidemiol Rec* 73, 201–208].

The serogrouping scheme for *Vibrio cholerae* is being maintained by Toshio Shimada, National Institute of Infectious Diseases, Tokyo, Japan. The updated serogrouping system for *Vibrio cholerae* contains 198 O antigens (O1–O198; O194–O198 are still unpublished). Serogroups from O156 to O193 have been reported in the *J Jpn Assoc Infect Dis* [71 (1997), 1037–1045; in Japanese with English abstract]. The reference strains representing each serogroup of *Vibrio cholerae* have not been deposited in the Japan Collection of Microorganisms (JCM) because JCM does not deal with human pathogenic organisms at present. Many members expressed concern over the fate of reference cultures of *V. cholerae* serogroups upon the retirement of T. Shimada. The subcommittee recommended that the investigator involved in developing serogrouping schemes should make appropriate arrangements to deposit the reference cultures in a well-established culture collection so that these valuable strains are not lost to science.

Minute 9. Resistance to pteridine (O/129 vibriostatic agent).

In view of the reports of the high incidence of resistance to pteridine, especially in developing countries, A. Huq asked whether the test should still be used for differentiation between the genera *Aeromonas* and *Vibrio*. D. Brenner stated that the test could still be used in some circumstances and should not be removed altogether.

Minute 10. Present status of *Plesiomonas shigelloides* serotyping. The serotyping scheme of *Plesiomonas shigelloides* is currently being maintained by Dr Eva Aldova who submitted the following report.

The two antigenic schemes (Aldova's, and that of Shimada and Sakazaki) were now unified. In 1992, at a taxonomic conference in Prague, R. Sakazaki offered Eva Aldova the right to allocate numbers to new antigens because T. Shimada had started to deal exclusively with vibrios. At present, the unified scheme has 101 O and 51 H antigens (H1ab and H1ac, included). Because of weak sera, Eva Aldova has made two corrections: O90 was O17 and H47 was H11. These two must be left out so that the last O antigen is O102 and the last H antigen H51. Recently a revision of new H sera (H42–H51) was made with known H antigens [Aldova, E. (1997). *Centr Eur J Publ Health* 5, 21–23; Bravo, L., Monte, R., Ramirez, M., Garcia, B., Urbaskova, P. & Aldova, E. (1998). *Centr Eur J Publ Health* 6, 67–70].

In addition to the above-mentioned antigenic scheme, the sources of which are predominantly isolates from man and warm blooded animals, another scheme has been created with strains of Professor R. H. W. Schubert originating exclusively from surface water and water insects [Aldova, E. & Schubert, R. H. W. (1996). *Med Microbiol* 5, 33–39]. Its 32 O and 7 H antigens designated Schubert O1 ..., Schubert H1 ... are distinct and after an agreement with the subcommittee they could/should be included in the unified scheme. Professor Schubert's strains were serotyped by Eva Aldova and the sera were produced by SEVAC Prague. The sera are with Professor Schubert and the reference strains are with E. Aldova. The incorporation of Schubert's antigens into the official antigenic scheme can be made only by Professor Schubert.

Minute 11. Minimal standards. The members felt that a general document which is not dictatorial but instead is a guideline, should be prepared to serve as a reference for those who wish to describe a new species in the family

Vibrionaceae. The purpose of the minimal standards would be to assist description of a new species in a reasonable way. J. J. Farmer has agreed to compile such a document which will be placed before a future subcommittee meeting for discussion.

Minute 12. Interpretation of 16S ribosomal sequence data.

Amy Carnahan raised concern over the lack of consistency in published RNA trees. D. Brenner mentioned that the breadth of the difference in the tree did not matter as long as no other species came in between. There was agreement that 16S rRNA sequence data are appropriate for taxonomic delineation at the level of genus and higher taxa, but that relatedness of total DNA is necessary to determine species, especially if 16S rRNA sequence similarity is 97–100%.

Minute 13. Working group on *Aeromonas* taxonomy.

Amy Carnahan now chairs this working group. Members can take up with the chairperson any taxonomic problems that members feel the working group should address. There was extensive discussion on several matters of *Aeromonas* taxonomy which are itemized below.

Deprecation of the proposal of *Aeromonas veronii* biovar *sobria* – while several subcommittee members felt it was confusing to name a biovar after a different species by the same name, it was explained that this was initially proposed to keep a continuity in the literature between what were previously called *Aeromonas sobria*, but in actuality were genetically members of *A. veronii*. It was also noted that this biovar nomenclature has been used extensively in the literature over the last few years and is found in the major databases of bacterial identification systems with only one exception. No decision to discontinue the use of this nomenclature was reached by the subcommittee. Further discussions and recommendations about this matter will be forwarded by the working group.

The status of *Aeromonas allosaccharophila* and *Aeromonas encheleia* are both very controversial, and provoked discussions on the use of 16S RNA sequencing versus traditional DNA–DNA homology methods for delineation of new species. This matter will also be taken up by the working group as well as by the entire subcommittee when the Minimal Standards being written by J. J. Farmer are available for discussion.

The problem of *Aeromonas trota* and *Aeromonas enteropelogenes* was briefly discussed. Although *A. trota* is clearly entrenched in the literature as well as major identification systems, it is a 'junior synonym' and thus an illegitimate name because the name *A. enteropelogenes* was published and validated first, and this is the correct name under the Bacteriological Code. This nomenclatural problem will be discussed in the working group. The acceptance of *A. trota* over *A. enteropelogenes* would require an official Request for an Opinion from the Judicial Commission. The working group will ask the full subcommittee for its opinion on this problem.

There was also a very brief discussion about the validity of retaining the subspecies status of the various subspecies within *Aeromonas salmonicida*. This will be discussed by the working group also.

Finally, it was discussed that although Kluyver & van Niel (1936) have always been cited for the genus name *Aeromonas*, R. Schubert requested an Opinion from the Judicial Commission (Opinion no. 48 in 1973) that replaced the original Beijerinck type strain of Kluyver and van Niel with the Stanier 1943 *A. hydrophila* strain. The Opinion further

stated that Kluver and van Niel are no longer to be cited for the genus name *Aeromonas*. However, Schubert in his Chapter 'Aeromonas' in the 8th edition (1974) of *Bergey's Manual of Determinative Bacteriology*, and Popoff in 1984 in *Bergey's Manual of Systematic Bacteriology*, chose to cite Kluver and van Niel as the authors for *Aeromonas*. J. J. Farmer pointed out that since Kluver and van Niel were cited as the authors for *Aeromonas* in the 1980 Approved Lists of Bacterial Names, this citation (see p. 245) is in conflict with the earlier 1973 Opinion received by Schubert. The citation of 'Kluver and van Niel 1936' will be used in the forthcoming 2nd edition of *Bergey's Manual of Systematic Bacteriology* and it will be recommended to the working group that they likewise accept the citation in the Approved Lists. However, this problem will need future corrective action.

In their Request for an Opinion (manuscript in preparation) to the Judicial Commission to correct the type strain of *Aeromonas punctata* from ATCC 15468 to ATCC 23309, Holmes and Farmer also address another error in the 1980 Approved Lists. They request that the citation for *Aeromonas* be corrected from 'Kluver and van Niel 1936' to 'Stanier 1943'. This action would correct the disagreement between the Bacteriological Code and the 1980 Approved Lists.

The final decisions, Opinions and/or committee recommendations will await the report from the *Aeromonas* taxonomy working group. It is planned to have the first official meeting of the working group at the *Aeromonas/Plesiomonas* symposium to be held in Chicago in May 1999.

Minute 14. Working group on *Vibrio* taxonomy. This group had been established with John Lee as chairperson but no

major nomenclatural or other taxonomic problems were noted in the genus at the present time. There was a proposal to re-analyse the taxonomic difference between *Vibrio carchariae* and *Vibrio harveyi* which some members felt are the same. It was decided that any decisions, Opinions and/or committee recommendations will await a report from the *Vibrio* taxonomy working group.

Minute 15. Possible new working group on stability of bacterial names. J. J. Farmer opined that a working group on stability of bacterial names may be necessary because specialists in bacterial systematics seem to have forgotten the first principle of nomenclature which is the 'stability of bacterial names' (Bacteriological Code, p. 7). He felt that the members should be involved with the Code, which should be closely examined for any amendments. Anybody interested in the activity should contact J. J. Farmer.

Minute 16. Any other business. All the members expressed deep appreciation for the tireless efforts of B. Holmes in arranging this meeting and keeping the activities of the subcommittee abreast with recent developments in the family *Vibrionaceae*.

Minute 17. Date and venue of next meeting. The next subcommittee meeting will be held at the IUMS Congress in Sydney, during the Congress of Bacteriology and Applied Microbiology to be held between 16 and 20 August 1999 at the Sydney Convention Centre, Darling Harbour, Sydney, Australia.

Minute 18. Adjournment. The meeting was adjourned at 20:00 on 19 May 1998.

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