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International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of Gram-negative anaerobic rods

Minutes of the meetings, 9 and 10 July 2000, Manchester, UK

Minute 1. Call to order. Chairman H. N. Shah called the open meeting to order at 09:50 on 9 July 2000; the meeting ended at 15:30 on 9 July 2000. The meeting resumed as an open session at 10:00 on 10 July and was followed by a closed session.

Minute 2. Record of attendance. Members present were H. N. Shah (Chairman), I. Olsen (Secretary), B. I. Duerden, S. M. Finegold, S. E. Gharbia, H. Jousimies-Somer and A. Tanner. Invited guests and observers were H. M. Bianchini (Argentina), M. Claydon (UK), E. Könönen (Finland), G. Kulechii (Turkey) and S. Thomas (USA).

Minute 3. Changes in membership. K. Bernard (Canada) was voted in as a new subcommittee member to fill the vacant position left by M. Sebald who, prior to the meeting, informed the subcommittee of her retirement. Her contributions over the years were acknowledged.

Minute 4. Status of subcommittee mandate. Committee members were of the common opinion that the subcommittee should be expanded to include experts with interest in environmental and veterinary anaerobes.

Minute 5. Criteria for acceptance of new species. In recent years there have been a number of taxonomic proposals and reclassification of existing species based solely on 16S rDNA sequence analysis. This situation was debated at length and members were concerned that this was not a taxonomically sound approach. The importance of a polyphasic taxonomic approach was strongly recommended.

Minute 6. Reliable phenotypic tests for identification. B. I. Duerden reported that there has been a distinct lack of innovative developments in conventional phenotypic approaches for over a decade. 'Biochemical tests' coupled with GLC are the basis of most identification protocols in reference laboratories. There is still an emphasis on the traditional tests of sugar fermentation, protein degradation and the like; some of these tests detect the presence of metabolic pathways (although the breakdown of the same substrate to acidic products does not necessarily mean that the same pathway is present) or of individual enzymes. These tests, which are based on the expression of metabolic activity during growth, have formed the basis of identification schemes and tables for over 20 years. The phenotypic tests include inhibition/stimulation tests (e.g. through bile, dyes) and the antibiotic resistance tests. Phenotypic markers derived for these tests remain useful, but do not provide definitive information for classification and identification. Several attempts have been made to incorporate these tests into commercial formats, but none of them have been particularly successful for identifying more than the most obvious and common anaerobes. More recent approaches have aimed to provide a more precise determination of phenotype by the detection of specific enzymes that hydrolyse chromogenic substrates. These systems are focused on detecting pre-formed enzymes in short (4 h) assays, and are independent of the growth of the organisms. Identification to the genus level is mostly reliable, but identification to the species level is less so. A serious problem with these tests is that they are aimed at the whole range of anaerobes. Improved phenotypic tests are needed to support the genetic basis of taxonomy, as the traditional tests are time-consuming and remain in use only in reference laboratories, while commercial systems are still inadequate.

Minute 7. Checkerboard DNA probe assay. The checkerboard DNA–DNA hybridization technique for analysing clinical samples was reviewed by A. Tanner and B. Thomas. Using this method, bacteria in 28 clinical samples, usually from oral subgingival plaques, are identified by hybridization to digoxigenin-labelled whole-genomic probes to 40 species on a single membrane. Reactions can be conveniently visualized using a chemifluorescent scanner. The method allows for quantitation of bacterial levels present in samples by comparing signal intensities with those of known standards. Advantages of the checkerboard DNA probe method to analyse clinical samples include the rapid and accurate detection of members of fastidious, and slow-growing, species, including Gram-negative anaerobes, since growth of samples is not required. Checkerboard DNA probe analysis [Socransky *et al.*, *Biotechniques* **17**, 788–792 (1994)] detects species above the assay threshold (frequently 10^4 cells) compared with cultural analyses which detect species primarily as a percentage of the (cultivable) microbiota.

Minute 8. Subdivisions of *Bacteroides*. H. N. Shah reported that proposals to restructure the genus *Bacteroides* (which, up to the1980s, comprised some 60 species) into three major genera, *Bacteroides sensu stricto*, *Prevotella* and *Porphyromonas*, were made prior to 16S rRNA sequence analysis and relied largely on biochemical, chemotaxonomic, mol% G+C values and DNA–DNA hybridization. Over the years, several clearly unrelated taxa, such as *Bacteroides hypermegas*, *Bacteroides multiacidus* and *Bacteroides termitidis*, have been assigned to several monospecific genera. However, a large number of taxa still remain unclassified while several are retained in misplaced lineages. In general, 16S rRNA sequence analyses have supported the proposals made using phenotypic criteria. *Bacteroides* and *Cytophaga* are placed in division 2.15.1 of the *16S rDNA phylogenetic* tree with the following relevant (anaerobic) subdivisions:

2.15.1.2. *Bacteroides* group.

2.15.1.2.1. *Rikenella microfusus* (*Bacteroides putredinis*).

2.15.1.2.6. *Bacteroides splanchnicus*.

2.15.1.2.7. *Porphyromonas macacae*, *Bacteroides forsythus*, *Porphyromonas catoniae*, *Porphyromonas circumdentaria*, *Porphyromonas endodontalis*, *Porphyromonas cangingivalis*,

Porphyromonas levii, *Porphyromonas canoris*, *Porphyromonas asaccharolytica*, *Bacteroides merdae*, *Bacteroides distasonis*.

2.15.1.2.8. *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, *Bacteroides caccae*, *Bacteroides acidifaciens*, *Bacteroides eggerthii*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Bacteroides stercoris*, *Prevotella zoogleoformans*, *Prevotella heparinolytica*.

2.15.1.2.9. *Prevotella tannerae*.

2.15.1.2.10. *Prevotella bryantii*.

2.15.1.2.11. *Prevotella ruminicola*, *Prevotella brevis*.

2.15.1.2.12. *Prevotella buccae*, *Prevotella loescheii*, *Prevotella dentalis*, *Hallella seregens*, *Prevotella oralis*, *Prevotella buccalis*.

2.15.1.2.13. *Prevotella nigrescens*, *Prevotella oris*, *Prevotella oulora*, *Prevotella melaninogenica*, *Prevotella denticola*, *Prevotella veroralis*, *Prevotella disiens*, *Prevotella corporis*, *Prevotella intermedia*, *Prevotella albensis*, *Prevotella bivia*, *Prevotella pallens*.

The subcommittee recommended the following: *Prevotella heparinolytica* and *Prevotella zoogleoformans* be designated *Bacteroides heparinolyticus* and *Bacteroides zoogleoformans* but studies other than 16S rRNA sequence analysis be undertaken before the status of the remaining taxa is accepted.

Minute 9. Pigmented species. H. Jousimies-Somer reviewed the current taxonomy of black-pigmented anaerobic rods. Intestinal bile-resistant *Bacteroides distasonis* and periodontitis-associated *Bacteroides forsythus* cluster close to *Porphyromonas*. The bile-resistant, pigment-producing Gram-negative rods, which fall close to *Bacteroides putredinus* and *Rikenella microfusus*, form a separate genus. The recently described indole-negative, *Porphyromonas levii*-like bacteria, non-pigmented *Porphyromonas catoniae*, and indole- and lipase-positive zoonotic *Porphyromonas macacae* (formerly *Porphyromonas salivosa*) are weakly assacharolytic and *N*-acetyl *β*-glucosaminidase-positive. Several new *Prevotella* spp. of animal origin, recovered from bite infections have been described. *Prevotella pallens* forms a pale pigment, is lipase-negative and is distinct from the lipase-positive *Prevotella intermedia* and *Prevotella nigrescens*. *Prevotella tannerae* closely resembles *Prevotella melaninogenica* but is α-galactosidase-negative. The oral *Prevotella enoeca*, a water-drop-like colony-forming *Prevotella dentalis* (formerly *Mitsuokella dentalis* and *Hallella seregens*), pentose-fermenting species of animal origin (*Prevotella albensis*, *Prevotella brevis*, *Prevotella bryantii*) and the reclassified *Prevotella ruminicola*, form part of the non-pigmented *Prevotella* spp.

Minute 11. *Fusobacterium–Leptotrichia* group. S. Gharbia provided an update of the taxonomic and phylogenetic status of species belonging to the *Fusobacterium–Leptotrichia* group. These organisms are currently described as fusobacteria and relatives and belong to division 2.29 of the bacterial phylogenetic group, with five subdivisions. The *Leptotrichia buccalis* subgroup contains *Leptotrichia buccalis*, '*Leptotrichia sanguinegens*' ('*Leptotrichia microbii*'), *Sebaldella termitidis* and *Streptobacillus moniliformis*. The *Propionigenium* subgroup is composed of two species, *Propionigenium maris* and *Propionigenium modestum*, while two monospecific groups are proposed for *Cetobacterium ceti* and *Fusobacterium perfoetens*. All other *Fusobacterium* species are phylogenetically similar and belong to a single group.

Minute 12. *Leptotrichia*. I. Olsen gave a brief review of an ongoing study of *Leptotrichia* isolates based on partial 16S rDNA sequence analysis, long-chain cellular fatty acid profiles and biochemical reactions (ATB bioMérieux). Partial sequencing showed that of 60 strains examined, 25 could be assigned to *Leptotrichia buccalis* while only 22 could be described as *Leptotrichia* sp.; the remainder did not belong to this species. Good congruence was found between the results derived from 16S rDNA sequence and fatty acid analyses but the phenotypic tests based on the API system gave discordant data.

Minute 13. Current membership of the subcommittee. H. N. Shah (Chairman), UK; I. Olsen (Secretary), Norway; K. Bernard, Canada; B. I. Duerden, UK; S. M. Finegold, USA; S. E. Gharbia, UK; H. Jousimies-Somer, Finland; D. N. Love, Australia; T. Mitsuoka, Japan; B. J. Paster, USA; A. Tanner, USA; K. Ueno, Japan.

Minute 14. Adjournment. The meeting was adjourned at 11:30 on 10 July 2000.

Ingar Olsen, *Secretary*
Haroun N. Shah, *Chairman*