International Committee on Nomenclature of Bacteria

Subcommittee on the Taxonomy of Lactobacilli and Closely Related Organisms

Minutes of Meeting, 7-9 August 1970

Hotel Maria Isabel, Mexico City, Mexico

Minute 1. *Call to order and record of attendance.* The meeting was called to order at 7:30 PM on 7 August by the Chairman, G. Mocquot. Present were P. A. Hansen, O. Kandler, K. Kitahara, T. Mitsuoka, G. Mocquot, G. Reuter, M. Elisabeth Sharpe, and P. H. A. Sneath.

Minute 2. *Approval of minutes of previous meetings.* The minutes of the meetings held on June 30 and July 1, 1968 were approved.

Minute 3. *Status of the generic name Lactobacillus Beijerinck.* Sneath reported that the Judicial Commission was in favor of conserving the generic name *Lactobacillus* Beijerinck with *L. delbrueckii* as the type species and that Hansen's request for such conservation would be published as soon as possible.

Minute 4. *Status of certain Lactobacillus species names.* Hansen went into the question of the correct author citation for some species names of lactobacilli after which the name Holland has appeared in various publications. The matter is dealt with in three papers: (1) by Hansen and Mocquot and (2) by Hansen and Lessel, which appear in this issue, and (3) by Rogosa and Hansen, to appear shortly.

Minute 5. *New species of Lactobacillus.* Sharpe presented a paper on slime-forming lactobacilli and suggested a new species, *L. saccharum*, which Kandler thought should be named *L. confusus*, raising *L. caprophilus* subsp. *confusus* to species status.

Minute 6. *Slime-forming lactobacilli.* Reuter wished to subdivide further the slime-forming species and suggested that more strains should be isolated. Kandler mentioned that he has about 100 strains of *L. confusus*. It was deemed desirable to distribute representative strains to members. Sharpe warned against carrying subdivision of species too far by separating on the basis of action on a single carbohydrate. She has been able to produce non-maltose fermenting strains of *L. helveticus* by UV irradiation. Such mutants would be indistinguishable from *L. jugurti*.

Adjournment. The meeting was adjourned at 10:00 PM.

Minute 7. *Second session.* The second session of the meeting was called to order at 7:00

PM on 8 August by the Chairman. Present were H. Beerens, F. Gasser, Hansen, Kandler, Kitahara, Mitsuoka, Mocquot, Reuter, M. Rogosa, and Sharpe.

Minute 8. *Report on the genus Lactobacillus.* Rogosa gave a comprehensive view of the genus *Lactobacillus* and of the relationships between the subgenera of Orla-Jensen using several newer characteristics: gas and acid in gluconate, ribose fermentation, and GC content. The division into the three well-known subgenera seems sound. *L. salivarius* is an intermediate species, but closest to *Thermobacterium*. Some organisms previously considered as lactobacilli may possibly better be placed with *Leuconostoc*. It is not possible to give here the numerous interesting details, some of which have already appeared while others will be published in the future.

Minute 9. *Report on Sporolactobacillus inulinus.* Kitahara read a paper, illustrated by slides, on auto-spheroplastizing in *Sporolactobacillus inulinus*. Tadpole-like cells could be formed in which the cell walls of the expanded part were broken, resulting in a spheroplast. Kitahara gave a list of GC ratios for strains of lactobacilli isolated.

Minute 10. *DNA hybridization.* Hansen reported on DNA hybridization in some species of *Lactobacillus* using the hydroxy apatite technique and studying the kinetics of reassociation as suggested by Kohne. DNA of *L. bulgaricus* ATCC 11842 was labeled with ^{32}P and, after denaturation, was hybridized with unlabeled denatured DNA's of the following strains: *L. bulgaricus* ATCC 11842, *L. lactis* ATCC 12315, *L. jugurti* ATCC 521, and *L. helveticus* ATCC 15009. Better than 98% of the input-labeled DNA was recovered double-stranded in the homologous reassociation. In the heterologous reactions, no ^{32}P -labeled hybrid was detected with *L. jugurti* ATCC 521, 3 to 5% with *L. helveticus* ATCC 15009, and approximately 86% with *L. lactis* ATCC 12315. These results leave no doubt about the close relationships between *L. bulgaricus* and *L. lactis*, and the lack of such between *L. bulgaricus* and *L. jugurti*, although the fermentation of

the carbohydrate series is identical for the two latter species and not at all for *L. bulgaricus* and *L. lactis*. It is indicated that it is not safe to rely on fermentation reactions without taking other characters into consideration (also see Minute 11).

Minute 11. *New species of Lactobacillus.* Gasser described homofermentative lactobacilli behaving phenotypically as *L. leichmannii* (GC ratio: 51) but having an entirely different GC ratio: 36. The motility of the lactic dehydrogenase in these strains was quite different from that of *L. leichmannii*. Consequently, the lactobacilli isolated must be assigned to a new species, to which is given the name *Lactobacillus jensenii*.

Minute 12. The following strains were recommended, subject to the approval by the Judicial Commission, as neotypes for the respective species: *Lactobacillus buchneri* ATCC 4005 (although the original description calls for growth at 45 C, ATCC 4005 does not develop at that temperature); *Lactobacillus bulgaricus* ATCC 11842 (Orla-Jensen's strain No. 14); and *Lactobacillus jugurti* ATCC 521. However, the members of the Subcommittee did not yet agree on recommending a neotype for *Lactobacillus leichmannii*.

Adjournment. The meeting was adjourned at 10:15 PM.

Minute 13. *Third session.* The third session of the meeting was called to order at 3:00 PM on 9 August by the Chairman. Present were: Gasser, Hansen, Kandler, Kitahara, Mitsuoka, Mocquot, Reuter, Rogosa, Sharpe, and a guest, F. Jimenez.

Minute 14. *Taxonomy of the lactobacilli using electrophoretic mobility of lactic acid dehydrogenase.* Gasser discussed taxonomic grouping in the genus *Lactobacillus*, particularly in the subgenus *Thermobacterium* using electrophoretic mobility of lactic acid dehydrogenase D(−) or L(+), NAD dependent or NAD independent, the immunological specificities of D lactic dehydrogenases based on three different specific antisera, and of L lactic dehydrogenase based on one antiserum, anti-L-LDH of *L. acidophilus*. These data were correlated with DNA base ratios previously published.

1. A homologous group of *L. bulgaricus*, *L. delbrueckii*, *L. lactis*, and *L. leichmannii*, having NAD-dependent D lactic acid dehydrogenase but not crossing with L lactic dehydrogenase of *L. acidophilus* III. The GC ratio is 50.

2. *L. jensenii*, phenotypically similar to *L. leichmannii* but with a GC ratio of 36. It also has NAD-independent lactic acid dehydroge-

nase which gives cross-reaction of partial identity with the group above.

3. *L. acidophilus* group having both D and L NAD-dependent lactic acid dehydrogenase. GC ratio between 35.7 to 38.1, depending on the groups I, II, III, each with its own immunological specificity for D and L-LDH. All of these cross-react for D-LDH with the two groups mentioned above.

4. *L. jugurti* and *L. helveticus* are close to *L. acidophilus* in the characters quoted above but with distinct immunological specificity and with a GC ratio of 39.

5. *L. salivarius* seems intermediate between *L. helveticus* and *L. casei*. It has NAD-dependent L lactic acid dehydrogenase and does not cross with D lactic dehydrogenase of *L. leichmannii* but with L lactic dehydrogenase of *L. acidophilus* III. GC ratio, 35.

6. *L. casei* shares the above characters with *L. salivarius*, but it has a GC ratio of about 46.

Gasser extended the discussion to *Streptobacterium* and *Betabacterium* and speculated on the phylogeny of these organisms and their relation to *Leuconostoc*. An extensive paper will be published in the near future. The classification by von Freudenreich and by Orla-Jensen and his later followers seems to receive support from these studies using the techniques of molecular biology. At the same time, we are warned against paying too much attention to certain phenotypic characters, e.g., fermentation of carbohydrates, a matter which Orla-Jensen pointed out years ago. A serious problem in constructing a family tree is the large gap between GC ratios of the groups.

Minute 15. *Neotype strain for Pediococcus cerevisiae Balcke.* A study group under this Subcommittee was organized to advise on the choice of a neotype strain for *Pediococcus cerevisiae* Balcke. This will replace the former ad hoc committee. The membership remains the same, but supplemented with Rogosa: Chairman, E. L. Garvie; other members, Hansen, Kitahara, C. F. Niven, and Rogosa. Kandler declared himself willing to obtain a copy of the thesis by Lindner which may be of importance in this case.

Minute 16. *Next meeting.* The members agreed that 4 years would be too long an interval until the next meeting. Kandler suggested a meeting during the summer of 1972 in Munich and graciously extended an invitation to the Subcommittee for a meeting at the Institut für Angewandte Botanik. It was accepted. The meeting was adjourned at 6:15 PM.

Minute 17. Election of officers. A meeting was called to order at 10:00 AM, 13 August by Gasser. Present were: Kandler, Kitahara, Rogosa, and Sharpe. The Chairman, Mocquot, had resigned as Chairman but remained a member. The Secretary, Hansen, had resigned as Secretary and as a member. The new officers are: Chairman, M. Rogosa (National Institutes of Health, Bethesda, Maryland, USA) and Secretary, F. Gasser (Institut Pasteur, Paris, France).

Minute 18. Membership of the Subcommittee. The present members of the Subcommittee are as follows: M. Rogosa (Chairman), Bethesda, Maryland, USA; F. Gasser (Secretary), Paris, France; H. Beerens, Lille, France; W. A. Clark (ex officio, ICNB), Rockville, Maryland, USA; H. G. Gyllenberg, Helsinki, Finland; O. Kandler, Munich, Germany; K. Kitahara, Tokyo, Japan; C. W. Langston, Kansas City, Missouri, USA; T. Mitsuoka, Yamato Machi Saitama, Pref., Japan; G. Mocquot, Jouy-en-Josas, France; C. S. Pederson, Geneva, New York,

USA; F. Petuely, Vienna, Austria; G. Reuter, Berlin-Dahlem, Germany; M. Elisabeth Sharpe, Reading, England; P. H. A. Sneath, Leicester, England; M. Sode Mogensen, Copenhagen, Denmark; and N. B. Williams, Philadelphia, Pennsylvania, USA.

Minute 19. Membership of the Subgroup on *Lactobacillus bifidus*. The present members of the Subgroup are: H. Beerens (Chairman), Lille, France; G. H. G. Davis, Herston, Queensland, Australia; F. Gasser, Paris, France; H. G. Gyllenberg, Helsinki, Finland; O. Kandler, Munich, Germany; T. Mitsuoka, Yamato Machi Saitama, Pref. Japan; W. E. C. Moore, Blacksburg, Virginia, USA; F. Petuely, Vienna, Austria; G. Reuter, Berlin-Dahlem, Germany; M. Rogosa, Bethesda, Maryland, USA; M. Elisabeth Sharpe, Reading, England; and H. Werner, Bonn, Germany.

P. Arne Hansen,
Secretary