AZOTOBACTERACEAE: The Taxonomy and Ecology of the Aerobic Nitrogen-Fixing Bacteria

J. P. Thompson and V. B. D. Skerman

Queensland Wheat Research Institute, Toowoomba, Queensland, Australia

Department of Microbiology University of Queensland, St. Lucia, Brisbane, Australia

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

London · New York · Toronto · Sydney · San Francisco

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road,
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

British Library Cataloguing in Publication Data
Thompson, John Purvis
Azotobacteraceae.
1. Azotobacter—Classification
I. Title II. Skerman, V. B. D.
589.9'5 QR82.A9 79-41053

ISBN 0-12-689050-1

Printed by J. W. Arrowsmith Ltd., Bristol BS3 2NT

Foreword

It was in 1885 that Berthelot demonstrated that the gain in nitrogen often seen in fallow soil was due to the action of living agents and in 1893 Winogradsky applied his newly developed principle of elective or enrichment culture to isolating the nitrogen-fixing agents. He prepared fluid media devoid, as far as possible, of nitrogen compounds but containing all other elements necessary for growth and with glucose as a source of carbon and energy. He added an excess of calcium carbonate and distributed the medium in layers 8–9 mm deep in conical flasks before inoculating them with soil and incubating them in a stream of air.

Winogradsky isolated the strictly anaerobic nitrogen-fixing bacterium, *Clostridium pastorianum.* It is not at all clear why Beijerinck decided some five years later to repeat Winogradsky's work, nor is it entirely clear why he obtained different results although his use of a wider range of carbon/energy sources, some of which did not support the growth of the anaerobe, was certainly important. Whatever the reasons, he isolated "einer noch nicht beschriebenen oligonitrophilen Bakteriengattung, welche zu den Aërobien gehört. *Azotobacter chroococcum* he isolated from garden soil and *Azotobacter agilis* from "Kanalwasser zu Delft". It seems certain, in retrospect, that Winogradsky had already seen the typical cells of *A. chroococcum* some seven years earlier but had failed to recognize their significance. Shortly after his publication Beijerinck with van Delden clearly demonstrated the nitrogen-fixing capabilities of *Azotobacter.*

Since these important publications at the turn of the century *Azotobacter* has played a prominent role in microbiology for a variety of reasons; as a model for the investigation of the biochemistry of nitrogen-fixation, as an important component of the ecosystem and as some of the largest bacterial

cells known Azotobacters have attracted the attention of microbiologists interested in cell structure. Some Azotobacters produce cysts and provide a model for studies in cell differentiation. Perhaps most important they and the later described members of the Azotobacteraceae represent a largely untapped gene pool potentially of immense value to agriculture and microbiological industry.

Beijerinck's isolations were followed by the description of a handful of other species and a definitive account of the Family Azotobacteraceae was written by H. L. Jensen in 1954; one of the classic papers of microbial taxonomy.

The authors of the present volume have produced a monograph on this fascinating and important family which is surely destined to take its place among the outstanding publications in microbiology. As an account of the family it is quite outstanding in its detailed coverage of the development of our understanding and of the organisms themselves; but it is far more than an academic record in the classical style. The authors describe the application of new methods and new concepts to the classification of the family relating in a penetrating and highly informative way the results of these newer ideas to the traditional view of the relationships between the organisms involved. The result is a work the importance of which transcends the actual group of micro-organisms with which it is concerned. It relates developing technology to the principles of microbial classification, providing a reference point for all taxonomists and a readable essay on an important aspect of microbiology for the general microbiologist. Above all it is a valuable signpost to the future pattern of taxonomic work; a model for similar studies of other families.

J. R. Norris
Agricultural Research Council
Meat Research Institute
Langford, Bristol

August 1979

Preface

Nitrogen is the nutrient required in greatest quantity for plant growth and productive agricultural systems. The nitrogen-fixing symbiosis between *Rhizobium* spp. and legumes is used to great advantage in agriculture and the desire for a similar, inexpensive source of nitrogen for cereals and pasture grasses has focussed attention on non-symbiotic nitrogen-fixing microorganisms since late last century. Despite this long and increasingly intense interest in non-symbiotic nitrogen fixation, much is still unknown and research is proceeding on a number of fronts.

The microorganisms themselves constitute a gene pool or genetic resource that is as yet only partially documented. One of the most commonly isolated and researched microorganisms is the aerobic nitrogen-fixing bacterium *Azotobacter chroococcum*, but other aerobic nitrogen-fixers that constitute the family Azotobacteraceae are less well known. Indeed, some have been isolated only rarely and because many have not been systematically compared with one another their classification is somewhat controversial. In view of their potential economic value in agriculture and industrial biochemistry, it is important that we learn more about them, what they are as bacteria, their characteristic properties, how many species and subspecies there are worth recognizing for further research, and how we can readily identify each.

This work was undertaken with these considerations in mind. It was designed to thoroughly reappraise the taxonomy of the Azotobacteraceae by means of a fresh experimental approach with modern methods of numerical taxonomy.

The work commences with a review that attempts to unravel the taxonomic literature and to display objectively the various concepts as they have developed since 1901. Original publications and type descriptions have been sought for this purpose and the whole is summarized in flow-chart form. This review is supplemented by another in which the autoecology of the Azotobacteraceae and the methods for their selective isolation are considered.

The experimental work proceeds from the isolation of Azotobacteraceae from various natural sources to the systematic characterization of a

comprehensive collection of strains. This Collection (Number 447 in the World Directory of Collections of Cultures of Microorganisms) was established with the local isolates and with cultures received from many collections throughout the world following attempts to obtain extant type and other representative strains of all named taxa within the Azotobacteraceae. The strains were examined systematically by a wide range of morphological, nutritional, physiological, biochemical and resistance tests, many of which have not previously been applied to this family of bacteria. Emphasis was placed on test methods that are amenable to routine use with large numbers of strains. From these examinations variant attributes were derived and the data matrix, containing some 35 000 entries, was numerically analysed by hierarchical classification, ordination and diagnostic procedures. These results, comprising groupings of the strains and attendant diagnostic attributes, were compared with the various classificatory concepts revealed in the literature review and recommendations for classification within the Azotobacteraceae were made. The taxa that were recognized are then fully described and new keys are presented for their identification. New methods for the selective isolation of the various species of Azotobacteraceae are also given.

The work represents a classical systematic treatment of a bacterial family carried out with modern computer-assisted methodology and as such may form a model for similar treatments of other families.

Acknowledgements. The authors sincerely thank the many colleagues and friends who expressed interest in this work and who assisted in many ways. In particular we thank the following: Professor J. R. Norris, ARC Meat Research Institute, Bristol, for writing the Foreword; Mr. A. J. Stevenson and Mr. D. N. Orange, formerly of the Queensland Wheat Research Institute, Toowoomba, for technical assistance; Dr. A. C. Hayward, Department of Microbiology, University of Queensland, for advice on systematic methods; Dr. A. W. Moore, C.S.I.R.O. Division of Soils, Brisbane, and Mr. P. W. Milne, C.S.I.R.O. Division of Computing Research, Canberra, for assistance with CSIRONET computing facilities; Dr. H. Doelle and Mrs. M. Roubichek for translations from German; Mr. A. Diatloff for translations from Russian; Mrs. L. Boyett for typing the manuscript; and the many colleagues mentioned in the text who so generously provided bacterial cultures.

J. P. Thompson expresses special gratitude to his wife, Lynne, for her personal support throughout the course of this work from its first inception.

Kind permission to quote copyright material was given by the following authors and/or copyright owners for the following descriptions:

Veb Gustav Fischer Verlag Jena for original descriptions of *Azotobacter* Beijerinck 1901, *A. chroococcum* Beijerinck 1901, *A. agilis* Beijerinck

1901, *A. smyrnii* Lipman & Burgess 1915, and *Azotomonas insolita* Stapp 1940.

New Jersey Agricultural Experiment Station, Rutgers University, for original descriptions of *Azotobacter vinelandii* Lipman 1903, *A. beijerinckii* Lipman 1904 and *A. woodstownii* Lipman 1904.

The American Society for Microbiology for diagnoses of *Azotobacter* by Winslow *et al.* (1917) and of *Azotobacter* and *Beijerinckia* by Jensen, H. (1954).

Franz Deuticke, Wein for the original definition of Azotobacteraceae Pribram 1933.

Williams and Wilkins Co., Baltimore for the original description of *Azotobacter indicus* Starkey & De 1939, and for Hofer's (1948) diagnosis of the Azotobacteraceae.

The Copyright Agency of the USSR for original descriptions of *Azotobacter nigricans* Krasil'nikov 1949, *A. agilis* subsp. *jakutiae* Krasil'nikov 1949, *Azotomonas fluorescens* Krasil'nikov 1947 *accord. to* Krasil'nikov 1949 and for Krasil'nikov's (1949) diagnoses of other *Azotobacter* spp.

Koninklijke Nederlandse Akademie van Wetenschappen for original descriptions of *Beijerinckia* Derx 1950a and *Azotobacter insignis* Derx 1951.

Treub Laboratory, Bogor for original descriptions of *Beijerinckia mobilis* Derx 1950b, and *B. indica* subsp. *alba* Derx 1950b.

Dr. J. Kauffmann and Museum National d'Histoire Naturelle, Paris for the original description of *Azotobacter lacticogenes* Kauffmann & Toussaint 1951.

Dr. Y. T. Tchan and the Linnean Society of New South Wales for original descriptions of *Azotobacter beijerinckii* subsp. *acidotolerans* Tchan 1953a *Beijerinckia derxii* Tchan 1957 and for Tchan's (1953b) descriptions of the Azotobacteraceae.

Dr. V. Jensen and The Royal Veterinary and Agricultural University, Copenhagen for the original description of *Azotobacter beijerinckii* subsp. *achromogenes* Jensen, V. & Petersen 1954 and for Jensen, V. and Petersen's (1955) diagnoses of *A. chroococcum* and *A. beijerinckii.*

Acta Agriculturae Scandinavica for the original description of *Azotobacter macrocytogenes* H. Jensen 1955.

Dr. Johanna Döbereiner and respectively Revista de Biologia, Lisboa and Pesquisa Agropecuaria Brasileira, Rio de Janeiro for original descriptions of *Beijerinckia fluminensis* Döbereiner & Ruschel 1958 and *Azotobacter paspali* Döbereiner 1966.

Dr. A. B. Roy and Nature, London for original descriptions of *Azotobacter acidus* Roy 1958 and *Derxia indica* Roy & Sen 1962.

Professor J. R. Norris and respectively Laboratory Practice, Journal of Applied Bacteriology, Academic Press, London and State Laboratory for Soil and Crop Research, Lyngby for Norris' (1959) key to *Azotobacter* spp., for a taxonomic statement by Baillie *et al.* (1962), for characteristics of *Azotobacter* spp. given by Norris and Chapman (1968) and for a description of the Azotobacteraceae and a key to *Azotobacter* spp. given by Norris & Kingham (1968).

Springer-Verlag, Heidelberg for the original description of *Derxia gummosa* Jensen, Petersen, De & Bhattacharya 1960.

Dunod, Paris for the original description of the class Azotobacterales Prévot 1961.

Drs E. Afrikian, A. V. Kirakosyan and Zh. S. Melkonyan and the Copyright Agency of the USSR for original descriptions of *Azotobacter agilis* subsp. *armeniae* Kirakosyan & Melkonyan 1964 and *A. vitreus* subsp. *armeniae* Kirakosyan & Melkonyan 1964.

Dr. L. N. Pshenin and Plenum Publishing Corporation, New York for an English translation of the original description of *Azotobacter miscellus* Pshenin 1964.

Dr. F. Hilger and respectively Faculté des Sciences Agronomiques de l'Etat, Gembloux and Annales de Microbiologie de l'Institut Pasteur for the original description of *Beijerinckia congensis* Hilger 1963 and for Hilger's (1965) descriptions of *Beijerinckia* spp.

Dr. G. Florenzano and Annali di Microbiologia ed Enzimologia for the original description of *Beijerinckia venezuelae* Materassi, Florenzano Balloni & Favilli 1966.

Dr. V. F. Coty and Interscience Publishers Ltd., New York for the original descriptions of *Azotobacter oleovorans* Coty 1967 and *A. aromaticus* Coty 1967.

Dr. E. G. Mulder and North Holland Publishing Co. Amsterdam for characteristics of species of Azotobacteraceae given by Mulder and Brotonegoro (1974).

Dr. D. B. Johnstone and Williams and Wilkins Co., Baltimore for descriptions of *Azotobacter* and *Azomonas* and Dr. J. H. Becking and Williams and Wilkins Co., Baltimore for descriptions of Azotobacteraceae, *Beijerinckia* and *Derxia* from 8th ed. Bergey's Manual of Determinative Bacteriology.

June 1979

J.P.T.
V.B.D.S.

Summary

The literature on the taxonomy of the nitrogen-fixing bacteria in the family Azotobacteraceae Pribram 1933, on their occurrence in nature and on the methods for their isolation was reviewed. An attempt was made to display objectively and chronologically the taxonomy of the Azotobacteraceae as it developed in the literature starting from when Beijerinck (1901) isolated and described the first of the species now in this family.

It was found that some five generic, thirty-six specific and eleven subspecific names that are both validly published and legitimate have been applied to taxa placed in this family by various authors. Many differing opinions on the classification of these taxa were found to exist in the literature. There were few points of universal agreement, nearly every classificatory concept having been challenged by at least one subsequent author. Many of the more controversial problems centred around the classification of taxa that were first described in relatively recent times, but other differences of opinion arose quite early this century and were recurrent through most of the literature. It was further apparent that most of the classifications presented for the entire family were based not on systematic comparisons of representative strains in the laboratory but on comparisons of published descriptions that varied in detail. There was no published attempt at applying the principles of numerical taxonomy (Sokal and Sneath, 1963) to the classification of these bacteria. It was considered that numerical taxonomic methods could be profitably applied to assess the various published classifications of the Azotobacteraceae and subsequent experimental work was designed and conducted with this purpose.

Attempts were made to obtain type strains and or other strains representative of all named taxa within the Azotobacteraceae from the culture collections of institutions and individual bacteriologists throughout the world. Strains of the following taxa were thus obtained: *Azotobacter chroococcum* Beijerinck 1901, 567.; *A. agilis* Beijerinck 1901, 577. objective synonym *Azomonas agilis* (Beijerinck) Winogradsky 1938, 400.; *A. vinelandii* Lipman 1903, 238., as well as some strains labelled *A. vinelandii* (*agilis*); *A. indicus* Starkey & De 1939, 337. objective synonym *Beijerinckia indica* (Starkey & De) Derx 1950 a, 146.; *Azotomonas insolita* Stapp 1940,

18., *Azotomonas fluorescens* Krasil'nikov 1947 *accord. to* Krasil'nikov 1949, 420.; *A. nigricans* Krasil'nikov 1949, 506.; *A. agilis* subsp. *jakutiae* Krasil'nikov 1949, 508.; *B. indica* subsp. *alba* Derx 1950b, 7.; *B. mobilis* Derx 1950b, 7.; *A. insignis* Derx 1951a, 344. objective synonym *Azomonas insignis* (Derx) V. Jensen 1955, 156.; *A. lacticogenes* Kauffmann & Toussaint 1951, 710. objective synonym *B. lacticogenes* (Kauffmann & Toussaint) Tchan 1953b, 88.; *A. beijerinckii* subsp. *acidotolerans* Tchan 1953 a, 83.; *A. beijerinckii* subsp. *achromogenes* Jensen & Petersen 1954, 106.; *A. macrocytogenes* H. Jensen 1955, 280. objective synonyms *Azomonas macrocytogenes* (H. Jensen) Baillie, Hodgkiss & Norris 1962, 118. and *B. macrocytogenes* (H. Jensen) Rubenchik 1959, 333.; *B. derxii* Tchan 1957, 315.; *B. fluminensis* Döbereiner & Ruschel, 1958, 269.; *Derxia gummosa* Jensen, Petersen, De & Bhattacharya 1960, 193.; *B. congensis* Hilger 1963, 150.; *A. agilis* subsp. *armeniae* Kirakosyan & Melkonyan 1964, 41.; *A. vitreus* subsp. *armeniae* Kirakosyan & Melkonyan 1964, 41.; *A. miscellus* Pshenin 1964, 684.; *A. paspali* Döbereiner 1966, 364.; *B. venezuelae* Materassi, Florenzano, Balloni & Favilli 1966, 210.

In spite of requests to many likely sources, viable strains of certain taxa could not be obtained. It is probable there are no extant strains of these taxa which are as follows: *Azotobacter woodstownii* Lipman 1904, 250.; *A. vitreus* Löhnis & Westermann 1909, 236.; *A. hilgardii* Lipman 1909, 942.; *A. smyrnii* Lipman & Burgess 1915, 504.; *A. agilis* subsp. *atypica* Kluyver & van den Bout 1936, 263.; *A. unicapsulare* Bachinskaya & Kondrat'yeva 1941, 100.; *A. galophilus* Suschkina 1945 *accord. to* Krasil'nikov 1949, 506.; *A. araxii* Panosyan 1950 *accord. to* Rubenchik 1959, 332.; *A. acidus* Roy 1958, 121.; *A. agilis* subsp. *mannitovorum* Becking 1962, 189.; *Derxia indica* Roy & Sen 1962, 605.; *A. oleovorans* Coty 1967, 30.; *A. aromaticus* Coty 1967, 30.

Attempts were also made to isolate Azotobacteraceae strains by non-selective methods and by the selective methods of Derx (1951b), Jensen, V. (1961) and Becking (1961a) from a number of soil, rhizosphere, phyllosphere and water samples collected in south-east Queensland. The strains thus obtained were initially identified simply as *Azotobacter* spp. or *Beijerinckia* spp. *Azotobacter* spp. were not detected in any phyllosphere samples and *Beijerinckia* spp. were not detected in any phyllosphere or water samples.

A more extensive survey of soils was then made for *Beijerinckia* spp. by using the selective medium of Becking (1961a). *Beijerinckia* spp. were detected in 46% of 155 samples collected in a subtropical area (26°40′–30°30′ S. Lat.) of Australia but in only 9% of forty-one samples from more temperate areas. The highest latitude at which *Beijerinckia* was detected was 38°40′ S in Victoria.

The occurrence of *Beijerinckia* in soil samples from the subtropical area was examined in relation to certain edaphic factors. *Beijerinckia* occurred most frequently in soils with reactions in the range pH 5.0–6.4 and was not detected in any sample more acid than pH 4.5 or more alkaline than pH 6.9. In a grumusol with gilgai microtopography, *Beijerinckia* was detected in the slightly acidic soil of the depressions but not in the alkaline soil of the mounds. At least one sample from nineteen of the twenty great soil groups that were sampled contained *Beijerinckia*. However, lateritic soils (57% positive) were more favourable than other soils (31% positive). This difference was partly associated with a difference in the pH distribution of samples in the lateritic and non-lateritic categories. The frequency of occurrence of *Beijerinckia* was unrelated to four broad categories of vegetative cover.

A collection of 151 strains consisting of the representatives of the various taxa that were obtained from culture collections and of strains that were isolated in Australia was subjected to a comprehensive systematic examination. Various test methods were used to determine a wide range of the organisms' morphological, physiological, nutritional, biochemical and resistance attributes. The results of these determinations were translated into binary and multistate attributes of which 230 were variant.

The data matrix of 151 strains × 230 variant attributes was numerically analysed by several procedures. The strains were hierarchically classified by information analysis (Lance and Williams, 1967) and were ordinated by principal co-ordinates analysis (Gower 1966, 1967). From these analyses, groups of strains were delimited and identified with the most appropriate of the named taxa. The diagnostic methods of Lance *et al.* (1968) were applied to the higher level fusions in the hierarchical classification and to the major vectors from the principal co-ordinates analysis in order to find *a posteriori* which attributes most contrasted the groups of strains delimited.

A system of classification of genera, species and subspecies was then proposed from these results considered in conjunction with the various classificatory concepts revealed in the literature review. It was considered that the bacteria examined should be classified in seven distinct genera of which five can be identified with the following named genera: *Azotobacter* Beijerinck 1901, 567.; *Azomonas* Winogradsky 1938, 391.; *Azotomonas* Stapp 1940, 18.; *Beijerinckia* Derx 1950a, 145.; and *Derxia* Jensen, Petersen, De & Bhattacharya 1960, 193. Neither *Azotobacter macrocytogenes* H. Jensen 1955, 280. nor *Azotobacter paspali* Döbereiner 1966, 364. was closely similar to the type species or other species of *Azotobacter* Beijerinck 1901 or to the species in any other of the above five genera. Therefore the establishment of two new genera was proposed. The first was *Azomonotrichon* gen. nov. with *Azomonotrichon macrocytogenes* (H.

Jensen 1955) comb. nov. as the type species by monotypy. The second was *Azorhizophilus* gen. nov. with *Azorhizophilus paspali* (Döbereiner 1966) comb. nov. as the type species by monotypy.

The following five species were delimited within the genus *Azotobacter* Beijerinck 1901.:

A. chroococcum Beijerinck 1901, 567. which is the type species of the genus *Azotobacter.*

A. nigricans Krasil'nikov 1949, 506.

A. armeniacus (Kirakosyan & Melkonyan) sp. nov. Basionyms: *A. agilis* subsp. *armeniae* Kirakosyan & Melkonyan 1964, 41. and *A. vitreus* subsp. *armeniae* Kirakosyan & Melkonyan 1964, 41.

A. beijerinckii Lipman 1904, 248.

A. vinelandii Lipman 1903, 238.

Strains of *Azotobacter beijerinckii* subsp. *achromogenes* V. Jensen & Petersen 1954, 106. appeared more similar to *A. nigricans* than to *A. beijerinckii* and it was suggested that this subspecies be renamed *A. nigricans* subsp. *achromogenes* (V. Jensen & Petersen) comb. nov.

Two subgroups which differed from each other in several attributes were recognized within each of the three species *A. chroococcum, A. beijerinckii* and *A. vinelandii.* One of the subgroups in each of the first two species appeared to consist of variants obtained from specific localities. The strains in one of the subgroups of *A. vinelandii* appeared similar to *A. non-vinelandii* Derx 1951b, 626. Not Val. Pub. The type strain of *A. miscellus* did not differ appreciably from strains of *A. vinelandii* (including cotype strains) and thus *A. miscellus* Pshenin 1964, 684. should be regarded as a subjective synonym of *A. vinelandii* Lipman 1903, 238. Some strains obtained from culture collections as *A. agilis* were found to be *A. vinelandii* and one strain bearing the name *A. chroococcum* was found to be a strain of *A. beijerinckii.* This labelling is due to species mergers that have been suggested in the literature but which were not supported by the results of this investigation. Probably other strains bearing similar misleading labels are held in culture collections.

The following two species were delimited within the genus *Azomonas* Winogradsky 1938, 391.:

Azomonas agilis (Beijerinck) Winogradsky 1938, 400. which is the type species of this genus. Basionym: *Azotobacter agilis* Beijerinck 1901, 577.

Azomonas insignis (Derx) V. Jensen 1955, 156. Objective synonym. *Azotobacter insignis* Derx 1951a, 344.

Within *Azorhizophilus* gen. nov. only a single species was delimited i.e. *Azorhizophilus paspali* (Döbereiner 1966) comb. nov.

Likewise, within *Azomonotrichon* gen. nov. only a single species was delimited i.e. *Azomonotrichon macrocytogenes* (H. Jensen 1955) comb. nov.

A cotype strain of *Azotobacter agilis* subsp. *jakutiae* Krasil'nikov 1949 was very similar to cotype and other strains of *A. macrocytogenes* and the former subspecies should be regarded as an early subjective synonym.

The following two species were delimited within the genus *Azotomonas* Stapp 1940, 18.:

Azotomonas insolita Stapp 1940, 18.

Azotomonas fluorescens Krasil'nikov 1947, *accord. to* Krasil'nikov 1949, 420.

The only culture (NCIB 9884) of the latter species that was available to the author was a mixed culture; one of the strains obtained in pure culture from NCIB 9884 is possibly a member of some denitrifying species in the genus *Alcaligenes* Castellani & Chalmers 1919, 936.

The only species that was delimited within the genus *Derxia* Jensen, Petersen, De & Bhattacharya 1960, 193. was the type species *Derxia gummosa* Jensen, Petersen, De & Bhattacharya 1960, 193.

Four species and two subspecies were delimited within the genus *Beijerinckia* Derx 1950a, 145. These were as follows:

B. indica (Starkey & De) Derx 1950a, 146. which is the type species of this genus. Basionym: *Azotobacter indicus* Starkey & De 1939, 337.

B. indica subsp. *lacticogenes* (Kauffmann & Toussaint) comb. nov. Basionym: *Azotobacter lacticogenes* Kauffmann & Toussaint 1951, 710. Objective synonym *B. lacticogenes* (Kauffmann & Toussaint) Tchan 1953b, 88.

B. mobilis Derx 1950b, 7.

B. fluminensis Döbereiner & Ruschel 1958, 269.

B. derxii Tchan 1957, 315. Cotype strains of *B. congensis* Hilger 1963, 150. did not differ appreciably from a cotype strain of *B. derxii* and the former name should be regarded as a subjective synonym of the latter.

B. derxii subsp. *venezuelae* (Materassi, Florenzano, Balloni & Favilli) comb. nov. Basionym: *B. venezuelae* Materassi, Florenzano, Balloni & Favilli 1966, 210. The subspecies *B. indica* subsp. *alba* Derx 1950b, 7. can be regarded as a subjective synonym of *B. derxii* subsp. *venezuelae*.

Each of the foregoing species was completely described on the basis of the attributes determined in this investigation.

None of the strains of *Azotomonas* Stapp fixed nitrogen and it is doubtful if this capacity should have ever been ascribed to *Azotomonas*. Out of all the genera, *Beijerinckia* Derx was the least similar to *Azotobacter* Beijerinck, the type genus of the Azotobacteraceae. From these and other considerations it is suggested that the genera in the Azotobacteraceae are not closely related to one another. It is further suggested that the genus *Azotomonas* should be excluded from the Azotobacteraceae and that the remainder of the family should be recognized for what it is, i.e. a convenient and useful

grouping of the genera of aerobic, heterotrophic, nitrogen-fixing bacteria.

Diagnostic keys for identification of the genera and species of Azotobacteraceae are given. New methods for the selective isolation of most species of Azotobacteraceae and methods for some special purpose isolations are also given.

Comparisons with the Eighth Edition of Bergey's Manual of Determinative Bacteriology

Since this work was completed, a small number of publications on the taxonomy of the Azotobacteraceae have appeared. Pertinent information from these has subsequently been incorporated in the Literature Review (pp. 1–51) and in Fig. 1. The eighth edition of *Bergey's Manual of Determinative Bacteriology*, comprising contributions by Johnstone (1974) on *Azotobacter* and *Azomonas* and by Becking (1974b) on *Beijerinckia* and *Derxia*, contains taxonomic recommendations that differ from those of the authors. The following are some comparative notes on the two versions.

First, at the generic level, we considered that the Azotobacteraceae should be classified into six genera. We have proposed that *Azotobacter macrocytogenes* H. Jensen 1955 should be classified in a new monospecific genus *Azomonotrichon*, and not be placed in the genus *Azomonas* as proposed by Baillie *et al.*, 1962, and accepted by Johnstone (1974), or in the genus *Beijerinckia* as proposed by Rubenchik (1959).

On the basis of our numerical classification, we have further proposed that *Azotobacter paspali* Döbereiner 1966 be reclassified in a new monospecific genus *Azorhizophilus*, whereas Johnstone (1974) retained it in the genus *Azotobacter* Beijerinck. Apart from the DNA base composition of *A. paspali*, which does not of itself preclude this species from the genus *Azotobacter*, no new systematic data on this species were available to Johnstone.

At the level of species, our classification differs from Johnstone's (1974) in that we recognized *Azotobacter nigricans* Krasil'nikov 1949 and considered that *Azotobacter agilis* subsp. *armeniae* Kirakosyan & Melkonyan 1964 and *Azotobacter vitreus* subsp. *armeniae* Kirakosyan & Melkonyan 1964 are synonymous and merit specific rank. We therefore proposed a new species *Azotobacter armeniacus* sp. nov. to contain these two subspecies. Neither *A. nigricans* nor *A. armeniacus* have previously been comprehensively examined outside of U.S.S.R. explaining their lack of recognition in texts such as *Bergey's Manual of Determinative Bacteriology*. Cursory examinations of a strain of *A. nigricans* previously led others to the belief that it was only a slight variant of *A. chroococcum* Beijerinck (Jensen, H., 1954; Johnstone private communication in 1977).

Points of agreement between our work and *Bergey's Manual of Determinative Bacteriology* are in the recognition of the following species: *Azotobacter chroococcum* Beijerinck 1901; *Azotobacter beijerinckii* Lipman 1904; *Azotobacter vinelandii* Lipman 1903; *Azomonas agilis* (Beijerinck) Winogradsky 1938; *Azomonas insignis* (Derx) V. Jensen 1955; *Derxia gummosa* Jensen, Petersen, De and Bhattacharya 1960; *Beijerinckia indica* (Starkey & De) Derx 1950a; *Beijerinckia mobilis* Derx 1950b; *Beijerinckia fluminensis* Döbereiner & Ruschel 1958 and *Beijerinckia derxii* Tchan 1957.

At a lower taxonomic level, subspecific variation common to a number of strains was noted within four species of *Azotobacter* and two of *Beijerinckia.* No subspecific division has been attempted in *Bergey's Manual of Determinative Bacteriology.* This is a point of difference which is not, of course, a major one. Another minor difference is Becking's (1974b) listing of *Beijerinckia congensis* Hilger 1965 as a synonym of *B. indica* (Starkey & De) Derx whereas our experimentally derived results show the former species to be a synonym of *B. derxii* Tchan.

Contents