Research on *Aeromonas* and *Plesiomonas*

Introduction

A. von Graevenitz

Department of Medical Microbiology, University of Zürich, CH-8028 Zürich (Switzerland)

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This workshop grew out of a seminar on *Aeromonas* and *Plesiomonas* held at the Annual Metting of the American Society for Microbiology on March 6, 1985 with the author and Dr Sally Jo Rubin, St. Francis Hospital, Hartford, Conn. (USA), as conveners. Dr Rubin died in May 1985. The reader is referred to her contribution to the field (Abstr. A. Meet. Am. Soc. Microbiol. 1985, p. 318).

The workshop held on September 5 and 6, 1986, as a satellite meeting at the 14th International Congress of Microbiology in Manchester, England, was convened by P.C.B. Turnbull and J.V. Lee with A. von Graevenitz as Chairman and T. Wadström as Vice-Chairman. Its purpose was to review the 'state of the art', to discuss controversial issues, and to give impetus to new inquiries. International authorities in the field were asked to contribute. Some overlap as well as some omissions in the papers are inevitable. The inclusion of two chapters on toxins was deliberate since this area is still controversial.

The first strain of a bacterium which today we would call *Aeromonas* was isolated in 1890 from tap water in Chemnitz by Zimmermann[17] and named *Bacillus punctatus*. One year later, Sanarelli[12] isolated a similar strain from a frog and called it *B. hydrophilus fuscus*. A trout pathogen called *Bacterium salmonicida* was isolated in 1894 by Emmerich and Weibel[4]. Food as a source of *Aeromonas* was described by Hammer[8] in 1917, who isolated a bacterium from spoiled milk called *Bacillus ichthyosmius*. The first human isolate dates back only 32 years; in 1954, Hill et. al.[9] described a strain from septicemia which Caselitz[1] called *Vibrio jamaicensis*. These names underwent subsequent changes while new isolates from the above sources were described under different names – developments traced by Ewing et al.[5] and by Caselitz[2], which will not be repeated here. The name *Aeromonas*, first proposed by Kluyver and van Niel[10], was promoted by Stanier[16]; the genus was introduced into the 7th edition of Bergey's Manual[15] (1957) when the identity of previous strains had become sufficiently clear.

The first isolate of *Plesiomonas*, described as 'C 27' by Ferguson and Henderson[6], was from the feces of a patient whose clinical history was not available. Following different designations, among them *A. shigelloides*[2], Habs and Schubert[7] proposed a new genus, *Plesiomonas*.

The 7th edition of Bergey's Manual[15] classified *Aeromonas* as one genus in the family Pseudomonadaceae (with three species selected mainly on grounds of animal pathogenicity which are not recognized as separate entities today). The 8th and 9th editions list *Aeromonas* as well as *Plesiomonas* as genera in the family Vibrionaceae. As for species, the 8th edition followed Schubert who used mainly biochemical criteria[13], whereas the newest one followed Popoff who used DNA hybridization data[11]. This symposium should provide further insights into the taxonomy of the group.

Following the characterization of animal and environmental strains of *Aeromonas* by several groups[3,5,7,13] clinical microbiologists began to isolate more strains (which earlier had presumably been misidentified as *Escherichia coli*). After an association with diarrheal disease had been found, selective media were devised for stool cultures. Subsequently, carrier rates and, particulary from 1975 on, toxins and other virulence factors exhibited by aeromonads were investigated. In this endeavor, prior studies on toxigenicity, invasiveness, adherence and outer membrane proteins of other intestinal pathogens provided models. Aeromonads were also recognized as agents of septicemia (in mostly immunocompromised hosts and in patients with liver disease) as well as of water-borne wound infection. The interest in ecology and epidemiology led to proposals for serotyping and biotyping systems. The number of publications on *Aeromonas* is increasing rapidly. In the years before 1970 and between 1971 and 1975, approximately 55 papers per year had appeared. For the period between 1981 and 1985, that figure has tripled; much of the increase had been due to publications on toxic factors and enteritis. We shall hear more about this in the individual presentations.

Plesiomonas[14] may not have received the attention that it deserves, which is probably due to geographically restricted detection. But even the number of publications on *Plesiomonas* has significantly increased over time. In the period before 1975, approximately 20 papers had appeared that dealt with *Plesiomonas* only. Fifteen appeared between 1976 and 1980, and 30 between 1981 and 1985. The present emphasis is on ecology, toxin production, serology, and extraintestinal disease. The latter is still rare, whereas fecal isolates do not seem uncommon any more even in temperate climates.

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I. Taxonomy, ecology, isolation and identification

Aeromonas taxonomy

S. W. Joseph, R. R. Colwell and M. T. MacDonell*

Department of Microbiology, University of Maryland, College Park (Maryland 20742, USA)

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The taxonomy of the aeromonads has been the subject of scrutiny and reclassification by several investigators in an attempt to clarify the position of this group of organisms and their relationship to similarly described but obviously separate genera. Recommendations have varied from recognition of one genus comprising three species to the proposal of a complex subspeciation[3, 10]. Other than the obvious need for accurate nomenclature and classification, there are pragmatic reasons to seek improvement in the classification of *Aeromonas*, e.g., in clinical medicine, where physicians tend to refer to all aeromonads as *Aeromonas hydrophila* and, in fact, most of the early medical literature concerning *Aeromonas* used this nomenclature only. We now know such simplification to be unrealistic in light of Popoff and Véron's contributions[9], which showed that *A. hydrophila* and *A. punctata* constitute a single species, *A. hydrophila*. A second, previously undescribed group of strains was also described and named *A. sobria*. The human medical significance of *A. sobria* was demonstrated when it was isolated from a scuba diver's wound infection[5], and in a study of the comparative occurrence and virulence of *A. hydrophila* and *A. sobria* from human and environmental sources[2].

The species on which there is agreement is *A. salmonicida*. It differs from other aeromonads as follows: non-motile, no growth at 37 °C, produces a brown, water-soluble pigment on nutrient agar. In Bergey's Manual of Systematic Microbiology[7] this species is subspeciated into *salmonicida, achromogenes,* and *masoucida*.

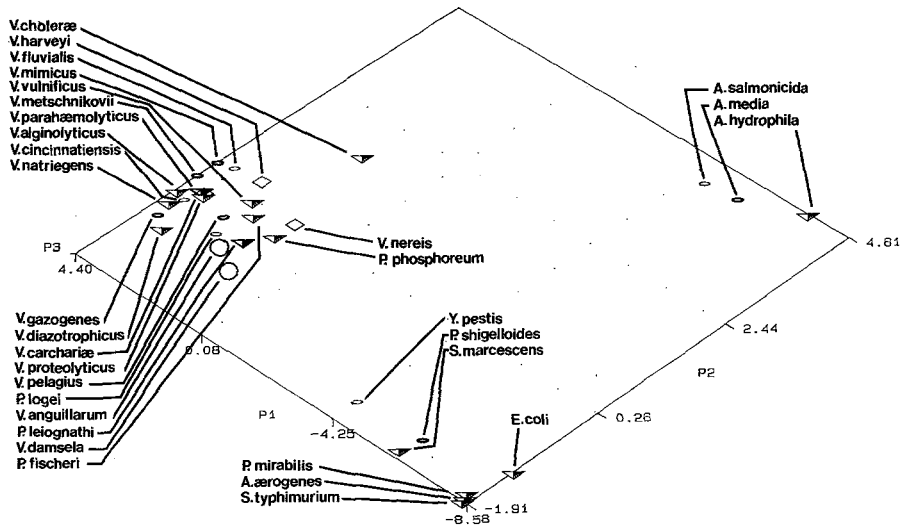
Based upon differential, phenotypic, and genetic characteristics, Popoff and Véron recommended establishment of a third species, *A. caviae,* which is included amongst the motile aeromonads[8]. In summary, five species and three sub-species of *Aeromonas* are recognized at the time of this writing.

Definition

Aeromonas consists of straight cells, rod-shaped and with rounded ends to coccoid. Resting stages are not known. Generally motile by a single flagellum. Some species are non-motile. Metabolism of glucose is both respiratory and fermentative. Oxidase positive and resistant to the vibriostatic agent 2,4,diamino-6,7-diisopropylpteridine (0/129). The mol% G + C of the DNA is 57–63 (Bd,T_m).

Minimal identifying and differential characters

The common characteristics which define the motile aeromonads are possession of motility, morphology, growth in nutrient broth at 37 °C, indole production, fermentation of sucrose and



Overhead view of a four-dimensional plot of evolutionary relationships amongst species of the gamma subdivision of the Rhodobacteria[6, 11]. In this projection, principal components 1 and 2 are given by the X and Y axes, respectively, while principal component 4 is indicated through the use of symbols. Principal component 3, i.e., the Z axis, is lost in this projection.