The Natural Relationships of *Aeromonas formicans*

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Summary. The nature and manner of regulation of the enzymes of the tryptophan pathway in *Aeromonas formicans* indicate that its tryptophan genes are arranged on the chromosome like those of the enteric bacteria, not *Pseudomonas*. When viewed with other similar information, this leads to the conclusion that aeromonads are more closely related to the *Enterobacteriaceae* than to the *Pseudomonadaceae*.

Organisms presently classified in the genus *Aeromonas* (KLUYVER and VAN NIEL, 1939) were first observed before the turn of the century (rev. EDDY, 1960; EWING et al., 1961), but only recently have they received appreciable attention. They present an interesting problem for microbial taxonomists, for they possess some of the characteristics of each of two large and well-studied bacterial groups, the pseudomonads and the enteric bacteria. Typically, aeromonads are cephalotrichous, gram-negative rods, morphologically indistinguishable from many members of the genus *Pseudomonas*. Biochemically, aeromonads are characterized by a vigorous fermentation of carbohydrates giving rise to end products typical for enteric organisms (STAHEL and ADAMS, 1944; CRAWFORD, 1954). Aeromonads give a strongly positive test for cytochrome oxidase by Kovacs' method, a trait that is absent among enteric bacteria but quite common in pseudomonads (STANIER et al., 1966).

Numerical taxonomists have placed aeromonads in a position intermediate between pseudomonads and enteric organisms (COLWELL and LISTON, 1961; LYSENKO, 1961). Studies of DNA base ratios show aeromonads to have guanine-cytosine contents between 58 and 62\%/o (SEBALD and VÉRON, 1963), at the upper end of the range for enteric bacteria (COLWELL and MANDEL, 1964) and lower than most pseudomonads.

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(MANDEL, 1966). DNA-RNA hybridization has suggested a weak homology between certain *Aeromonas* strains and enteric species (McCarthy and Bolton, 1963). It is also noteworthy that LEIFSON (1960) has observed that young *Aeromonas* cultures frequently contain cells having several lateral flagella in addition to their polar ones.

The senior author's interest in aeromonads began in the summer of 1952 when he was a student in C. B. VAN NIEL's general microbiology course. There he isolated an organism from Pacific Grove sewage which subsequently, with VAN NIEL's help, was characterized as a fermentative, polarly monotrichous organism (CRAWFORD, 1954). Recent authors have favored the name *Aeromonas formicans* for this strain (PYVICK and SABINA, 1957), though some, notably EWING et al. (1961) and EDDY and CARPENTER (1964), feel it is only a variant of *A. hydrophila*. It is not the purpose of the present work to consider the validity of the species designation. Instead, we hoped to use this organism, a fairly typical aeromonad, to indicate the natural relationships between *Aeromonas* and the pseudomonad and enteric bacterial groups.

It has recently become apparent that a difference in the organization of the structural genes for the tryptophan synthetic pathway in the enteric and pseudomonad bacterial groups results in enzymological patterns that are striking and distinctive. It was our intention to observe whether *A. formicans* follows the enteric or the pseudomonad pattern, hoping that we could predict the chromosomal arrangement of the tryptophan genes of this organism even before a satisfactory system for genetic analysis is found.

The pathway of tryptophan synthesis (Fig. 1) has been well studied in many microorganisms. In the enteric bacteria the six steps of the pathway (when TS-A and TS-B are considered two steps) are catalyzed by five gene products. The AS and PRT gene products combine to form an aggregate catalyzing the AS and PRT reactions (ITo and YANOFSKY, 1966); the TS-A and TS-B gene products have been known for a longer time to do the same (rev. YANOFSKY, 1960). The PRAI and InGPS reactions are catalyzed by a bifunctional protein consisting of a simple polypeptide chain of about 45,000 molecular weight (CREIGHTON and YANOFSKY, 1966). These five gene products are coordinately regulated (ITo and CRAWFORD, 1965) and are synthesized on a single, polycistronic segment of messenger RNA (IMAMOTO et al., 1965).

The tryptophan enzymes of *Pseudomonas putida* (and probably *P. aeruginosa* also) are quite different. Here there are six gene products, with evidence for aggregation between only two of them, TS-A and TS-B (ENATSU and CRAWFORD, 1967). The AS, PRT and InGPS enzymes vary together according to the amount of tryptophan available to the organism, but PRAI is apparently unregulated (CRAWFORD and GUNSALUS, 1966). Moreover, TS-A and TS-B are regulated independently of the others by substrate induction, not repression. Recent studies with transducing phages (CHAKRABARTY et al., 1967) have shown that