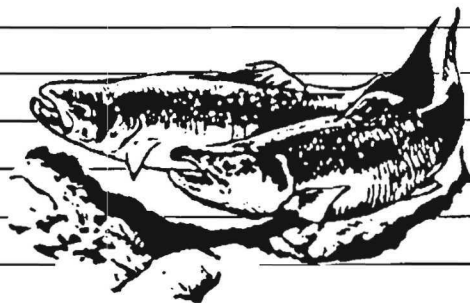


Research

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Identification Scheme for Bacteria of the Genus *Aeromonas*

Motile *Aeromonas* septicemia (MAS) is an important bacterial disease that affects all species of freshwater fish. The disease can cause high mortalities or can manifest itself in a more chronic, slightly virulent form. Motile *Aeromonas* spp. are generally considered secondary pathogens requiring some debilitating predisposing stressor to trigger an outbreak.

Over the years, the taxonomy of the motile aeromonads has been in constant flux and confusing to the diagnostician. Motile aeromonads have recently been implicated in human disease and, as a result, a number of new species of *Aeromonas* have been proposed. The naming of new species will add to the confusion in identification of MAS in fisheries and aquaculture. Our objective is to present criteria that will facilitate the identification of species within the genus *Aeromonas*.

Present Taxonomy of Motile Aeromonads

The genus *Aeromonas* belongs to the family Vibrionaceae along with the genera *Vibrio*,

Photobacterium, and *Plesiomonas*. Members of this family are gram-negative rods that ferment glucose and are cytochrome oxidase positive.

Aeromonas spp. are differentiated from the *Vibrio* spp. by salt requirements for growth and by resistance to O/129 or novobiocin. *Aeromonas* spp., which is starch-hydrolysis positive, can be separated from *Photobacterium* and *Plesiomonas*, which are starch-hydrolysis negative.

The genus *Aeromonas* is divided into two major groups based on motility—motile aeromonads and the *Aeromonas salmonicida* group, which is nonmotile. Bergey's *Manual of Determinative Bacteriology* presently lists three species of motile aeromonads: *A. hydrophila*, *A. sobria*, and *A. caviae*. Many older taxonomic schemes are still in use among aquacultural diagnosticians, compounding the nomenclature confusion (i.e., *A. liquifaciens*, *A. punctata*, and others). Recently, six new species have been described from human clinical isolates. The creation of these new species is based on DNA hybridization studies and shows that they are genotypically distinct from the three species as set forth in Bergey's manual. A listing of the current genotypes of *Aeromonas* is presented in Fig. 1.

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Motile *Aeromonas* Septicemia

Motile *Aeromonas* septicemia in freshwater fish can be caused by any one of the many species or strains of motile aeromonad bacteria. Identification to genus is all that is needed for a practical diagnosis of MAS. However, identification to the species level may be important to the diagnostician in evaluating virulence of an isolate or in identifying potential human pathogens from aquacultural sources. At present there is no universal scheme for sorting out motile aeromonads using a finite set of biochemical tests; the group is too varied to allow such a scheme. Our objective was to develop a usable set of biochemical criteria that would give the clearest delineation between proposed species of motile aeromonads. This scheme will assist the clinical diagnostician in sorting through this complex group of fish pathogens.

Establishment of the Data Base

Biochemical data on motile aeromonads were compiled from the literature and from isolates obtained from the American Type Culture Collection, Rockville, Maryland; the Southeastern Fish Cultural Laboratory Fish Pathogen Repository, Marion, Alabama; and from case records at the Mississippi Cooperative Extension Service Fish Diagnostic Laboratory, Belzoni, Mississippi. More than 300 isolates were screened by using a panel of

53 biochemical tests commonly available in microbiology laboratories. Thirty-six of the biochemical tests were run using the MINITEK system (Becton-Dickinson Microbiology Systems, Cockeysville, Maryland).

Flowchart Key to Species

The flowchart (Fig. 2) for motile aeromonads was created by using biochemical test information from the literature and from clinical data bases. Test results were recorded as positive if >85% of the isolates were positive for a given test; they were recorded as negative if <15% were positive. Test results between 15 and 85% were considered variable and placed under both positive and negative branches of the flowchart. A number of *Aeromonas* spp. appear more than once on the flowchart due to substantial variability in some of the biochemical tests.

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genotype	Bergey's *	new designation
1	<i>A. hydrophila</i>	<i>A. hydrophila</i>
2		un-named
3		<i>A. salmonicida</i> - motile
4	<i>A. caviae</i>	<i>A. caviae</i>
5A		<i>A. media</i> - motile
6		<i>A. eucrenophila</i>
7	<i>A. sobria</i>	<i>A. sobria</i>
8/10 **		<i>A. veronilbiovar sobria</i>
9	(<i>A. sobria</i>)	<i>A. jandaai</i>
10	(<i>A. sobria</i>)	<i>A. veronilbiovar veronii</i>
11	(<i>A. caviae</i>)	CDC group 77B
12	(<i>A. sobria</i>)	<i>A. schubertii</i>
13	(<i>A. sobria</i>)	<i>A. trota</i>
3S	<i>A. salmonicida</i>	<i>A. salmonicida</i> - non-motile
5B	(<i>A. salmonicida</i>)	<i>A. media</i> - non-motile

* = identification according to Bergey's Manual, 1984
** = genotypes 8 and 10 are identical
() = proposed new species identified according to Bergey's Manual, 1984

Fig. 1. Current taxonomy of the genus *Aeromonas*.

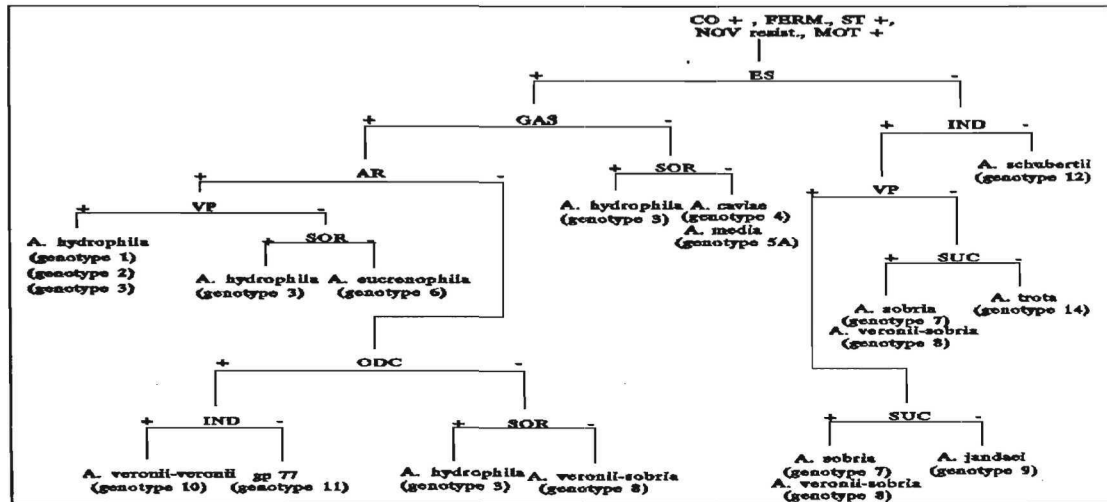


Fig. 2. Flowchart of biochemical reactions for motile aeromonads; CO = cytochrome oxidase; ferm = glucose fermentation; ST = starch hydrolysis; NOV = novobiocin resistance; MOT = motility; ES = exculin hydrolysis; GAS = gas from glucose; IND = indole; AR, arabinose; SOR = sorbitol; VP = Vokes-Proskauer; SUC = sucrose; and ODC = ornithine decarboxylase.