Amino Acid Requirements of *Aeromonas*

M. A. ROUF, PHILLIP L. WEBER, AND MARY M. RIGNEY

Department of Biology, Wisconsin State University, Oshkosh, Wisconsin 54901

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Four of the 12 cultures of *Aeromonas hydrophila*, 5 of the 10 *A. shigelloides*, and 9 of the 10 *A. salmonicida* that were studied required arginine and lysine, among other amino acids, for their growth.

For immunological studies of *Aeromonas*, attempts were made to grow the various strains in simple synthetic medium, and it was noted that certain strains would not grow in this medium unless Casamino Acids were added.

The literature search did not show any study on the amino acid requirements of *Aeromonas*. However, *Berger's Manual* (7th ed.) noted from unpublished data of Griffin that arginine and methionine were essential for the growth of *A. salmonicida*. The genus *Aeromonas* is known to cause diseases in warm-water fish and frogs and even in humans (1–5, 7, 8, 10). The ability of *Aeromonas* to grow at 0 °C also makes them important in the food industry (2). In the light of the importance of this genus, we took it upon ourselves to establish the amino acid requirements for 32 different strains of *Aeromonas* belonging to three different species, so that others beginning work with *Aeromonas* would not encounter similar difficulty. A secondary benefit derived from this work is that our data help to clarify the taxonomy of *Aeromonas* and may aid in the problem of identification of certain *Aeromonas*.

Of the 32 strains used in this study, 30 were kindly provided by W. H. Ewing and W. J. Martin of the Center for Disease Control, Atlanta. Two strains of *A. hydrophila* were isolated and identified in this laboratory. The names, numbers, and sources of these strains are given in Table 1. All cultures were maintained in Brain Heart Infusion-agar. For amino acid requirement studies, cultures were first tested for their ability to grow in the "C" medium (6) with 1% glucose as energy source. The cultures which did not grow in this medium, unless 0.5% vitamin-free Casamino Acids were added (Table 1), were tested further for their amino acid requirements.

For inoculum, a loopful of culture was taken carefully from the top part of the agar slant, washed, and suspended in physiological saline to eliminate the possibility of carry-over of nutrients. For the amino acid requirement studies, two types of media were employed. At first, all strains were tested for growth in C medium with

| Species of Aeromonas | Sources and strain no. | Without organic nitrogen | With organic nitrogen |
|----------------------|------------------------|--------------------------|-----------------------|
| A. hydrophila        | "Red leg" isolates     | -                        | +                     |
| A. hydrophila        | A-1                    | -                        | +                     |
| A. hydrophila        | A-3                    | -                        | +                     |
| NIH (CDC) strains    |                        |                          |                       |
| A. hydrophila        | 3402-68                | +                        | -                     |
| A. hydrophila        | 3910-68                | +                        | -                     |
| A. hydrophila        | 2195-68                | +                        | -                     |
| A. hydrophila        | 5244-68                | +                        | -                     |
| A. hydrophila        | 3325-68                | +                        | -                     |
| A. hydrophila        | 3337-68                | +                        | -                     |
| A. hydrophila        | 3326-68                | +                        | -                     |
| A. hydrophila        | 3067-68                | +                        | -                     |
| A. hydrophila        | 3527-68                | +                        | -                     |
| A. hydrophila        | 3890-68                | +                        | -                     |
| A. shigelloides      | 2413-68                | +                        | -                     |
| A. shigelloides      | 3428-67                | -                        | +                     |
| A. shigelloides      | 389-69                 | +                        | -                     |
| A. shigelloides      | 1432-68                | +                        | -                     |
| A. shigelloides      | 4337-69                | +                        | -                     |
| A. shigelloides      | 882-69                 | +                        | -                     |
| A. shigelloides      | 2154-68                | +                        | -                     |
| A. shigelloides      | 2418-69                | -                        | +                     |
| A. shigelloides      | 1383-69                | -                        | +                     |
| A. shigelloides      | 4332-69                | -                        | +                     |
| A. salmonicida       | 3001-60                | -                        | +                     |
| A. salmonicida       | 3002-60                | -                        | +                     |
| A. salmonicida       | 3004-60                | -                        | +                     |
| A. salmonicida       | 3005-60                | -                        | +                     |
| A. salmonicida       | 2933-60                | -                        | +                     |
| A. salmonicida       | 3010-60                | -                        | +                     |
| A. salmonicida       | 3008-60                | -                        | +                     |
| A. salmonicida       | 3000-60                | -                        | +                     |
| A. salmonicida       | 2907-60                | -                        | +                     |

- |a Medium contained (%): NH_{4}Cl, 0.2; Na_{2}HPO_{4}, 0.6; KH_{2}PO_{4}, 0.3; NaCl, 0.3; MgCl_{2}, 0.0010; Na_{2}SO_{4}, 0.0026; glucose, 1; and Casamino Acids, 0.5 (when added).
- |b = No growth (Klett units <3 in 7 days); + = growth (Klett units >50 in 5 days).
a mixture of 20 amino acids. The total amino acid concentration was 0.4%, and the individual amino acids added were in the concentration of 20 mg/100 mL. The procedure followed was that of Stokes et al. (9). For the determination of the individual amino acid requirements, in the first series, 50-ml flasks with 10 ml of medium were prepared; each amino acid was omitted in turn from a mixture containing all of them. In the second series of experiments, Difco media for the microbiological assay of amino acids were used. The results in both media were the same; subsequently, all strains requiring amino acids were further tested in Difco microbiological amino acids assay media. Duplicate flasks were always used in each experiment. Two series of controls were used. One series contained the C medium with 1% glucose and the other series contained 0.5% peptone plus 1% glucose in C medium.

The growth in the assay media was checked by Klett colorimeter with a blue filter (400 to 465 nm) at the end of 2, 5, and 7 days. For simplicity, the growth is represented in the table as + (Klett units greater than 50 in 5 days), and — (Klett units less than 3 in 7 days). The results are presented in Table 2.

Of the 12 strains of Aeromonas hydrophila studied, only 4 have amino acids requirement for growth. All four of these required arginine, isoleucine, and lysine, among other amino acids. One culture of A. hydrophila required all but alanine for growth.

Of the 10 A. shigelloides that were studied, 5 required certain amino acids for their growth. All five of these required both arginine and lysine, and one of them required all of the amino acids except tryptophan (Table 2).

All 10 strains of A. salmonicida needed certain amino acids for their growth. Among other amino acids, all 10 strains required arginine and 9 required lysine; two of them required all but glutamic acid (Table 2).

Stimulation of growth by certain amino acids for certain strains was noted; however, this aspect was not studied further.

None of the 32 strains studied showed any requirement for vitamins.

Of the many studies, including two thorough studies (2, 3), none mentions the amino acid requirements of the Aeromonas species. This aspect of growth requirement has perhaps been overlooked because of the similarities of Aeromonas to Escherichia and Aerobacter (as well as Pseudomonas), most of which do not have amino acid requirements. Unpublished data of Griffin mentioned in Bergey’s Manual (7th ed.) indicate that arginine and methionine are essential for growth of A. salmonicida. In our study, we found that all 10 strains of A. salmonicida do require arginine; however, only two of them require methionine.

| Amino acid       | Growth of A. hydrophila strain | Growth of A. shigelloides strain | Growth of A. salmonicida strain |
|------------------|--------------------------------|---------------------------------|--------------------------------|
|                  | A-1                            |                                 |                                |
| Alanine          | + -                            | + -                             | + -                             |
| Aspartic acid    | + -                            | + -                             | + -                             |
| Arginine         | - -                            | - -                             | - -                             |
| Cystine          | + +                            | + +                             | + +                             |
| Glutamic acid    | + -                            | + +                             | + +                             |
| Glycine          | + -                            | + +                             | + +                             |
| Histidine        | + +                            | + +                             | + +                             |
| Isoleucine       | + -                            | + +                             | + +                             |
| Leucine          | + -                            | + +                             | + +                             |
| Lysine           | + -                            | + +                             | + +                             |
| Methionine       | + -                            | + +                             | + +                             |
| Phenylalanine    | + +                            | + +                             | + +                             |
| Proline          | + -                            | + +                             | + +                             |
| Serine           | + -                            | + +                             | + +                             |
| Threonine        | + -                            | + +                             | + +                             |
| Tryptophan       | + -                            | + +                             | + +                             |
| Tyrosine         | + -                            | + +                             | + +                             |
| Valine           | + -                            | + +                             | + +                             |

* Indicates ability of organism to grow when specified amino acid was not incorporated into medium. All media were Difco assay media.
Also, all of these strains have other amino acid requirements.

For identification of *Aeromonas salmonicida* and their distinction from *Escherichia*, *Aerobacter* and *Pseudomonas*, one could use the amino acid requirements in conjunction with other biochemical characteristics. For preparation of endotoxin for immunological studies, the cells are normally grown in synthetic media without peptones or other organic matter of similar nature. This information, therefore, would be of help to others working on certain aspects of immunology of *Aeromonas*.

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