Mem. Fac. Fish., Kagoshima Univ.
Vol. 29 pp. 349~354 (1980)

# Microflora in the Alimentary Tract of Gray Mullet—VIII

## Utilization of Amino Acids by *Vibrio* and *Enterobacter* Isolates

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**Abstract**

Bacterial isolates of *Vibrio*, similar to *Vibrio anguillarum* and isolates of *Enterobacter*, similar to *Enterobacter aerogenes* which were isolated from gray mullet intestines, grew in media containing several amino acids upon incubation at different temperatures. All of the isolates utilized glycine as a sole carbon source. Glutamic acid, aspartic acid, lysine and ornithine supported the growth of *Vibrio* isolates at 20°C and 25°C, but the isolates of *Enterobacter* totally failed to grow in the same amino acids at all tested temperatures. Arginine supported the growth of *Enterobacter* isolates at 30°C and 35°C. These isolates also grew in serine at all of the tested temperatures, but *Vibrio* isolates did not grow in serine. Glutamic acid failed to support the growth of *Vibrio* at 30°C and 35°C, but supported the growth of *Vibrio* isolates at 20°C and 25°C. It was found that these isolates grew in glutamic acid at 30°C and 35°C when serine was added to the medium. No specific growth was detected when other amino acids were added.

The normal intestinal microflora contains microorganisms capable of producing or utilizing amino acids. This has been demonstrated in a wide variety of microflora (HALL)[1]. *Vibrio* species have been shown to be capable of growing in fish at a low temperatures, but very little is known concerning the nutritional determinants for their growth at a low temperature (MATCHES and LISTON)[2]. JEZESKI and OLSEN[3] observed that *Pseudomonas* spp. have a preference for amino acids during growth at low and high temperatures. The purpose of this study was to determine whether or not isolates of *Vibrio* and *Enterobacter* possessed a preference for utilization of amino acids during growth at low and high temperatures. These two bacterial species are considered to be the most important intestinal microflora of the gray mullet which lives in both sea water and fresh water.

## Materials and Methods

### Bacteria and Their Growth Condition:

Details of experimental methods are the same as those described previously[4-5].

### Characterization of Bacteria:

Identification of bacterial species and their characterization have been followed as stated in previous paper of this series[6].

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**Culture Media:**

Strains of *Vibrio* isolates and *Enterobacter* isolates were cultured in ZoBell broth and nutrient broth at 25°C and 30°C respectively and placed on a shaker for 18 hours. Cells were harvested, washed and resuspended in 0.9% NaCl. An aliquot of 0.1 m*l* of the suspension was inoculated into test tubes containing 10 m*l* of modified basal medium. This medium contains $KH_2PO_4$: 1 gm., $Na_2HPO_4$: 3 gm., NaCl: 5 gm., $CaCl_2$: 0.1 gm., $MgSO_4 \cdot 7H_2O$: 2 gm., $MnSO_4 \cdot H_2O$: 0.006 gm., $FeSO_4 \cdot 7H_2O$: 0.015 gm., and distilled water 1 liter, pH adjusted to 7.0 (ZACHARIAH and LISTON)[7]. Amino acids of 0.015 M concentration were then added. The tubes were inoculated and incubated on a shaker at 20°C, 25°C, 30°C and 35°C for 96 hours. Growth of these isolates was determined by measuring their absorbance on the spectrophotometer (Hitachi/Model 101) at a wavelength of 550 nm.

## Results and Discussion

The ability of the bacterial isolates to utilize various amino acids at different temperatures is shown in Table 1. All of the strains grew in glycine at 30°C to 35°C. Aspartic acid and glutamic acid individually supported the growth of all the isolates of *Vibrio* at 20°C and 25°C, but they did not support the growth of *Enterobacter*. Glutamic acid did not support the growth of *Vibrio* isolates at 30°C and 35°C. *Enterobacter* isolates were able to grow in serine at all tested temperatures, but *Vibrio* isolates totally failed to grow in serine. Lysine supported the growth of *Vibrio* isolates at 20°C and 25°C. It did not support the growth of *Enterobacter* isolates at any of the tested temperatures. Growth of *Vibrio* isolates occurred at 30°C and 35°C upon addition of serine to the medium containing glutamic acid. The inability of *Vibrio* strains to grow in serine-glutamic acid medium at 30°C and 35°C while they grew at 20° and 25°C indicates their incapability to utilize the medium at high temperatures. This is due either to a loss of the enzyme activity of amino acid synthesis or to a failure of transport mechanisms. The growth which occurred at 30°C and 35°C upon the introduction of serine into glutamic acid medium be due to protein synthesis by different metabolic path ways which yield various amino acids, but the actual reason for the growth in serine-glutamic acid medium is not yet been known.

Table 2 shows that the introduction of glycine into the medium contained growth supporting amino acids maintained a good growth of the bacterial isolates at 25°C, while no growth was detected upon the addition of glycine to non-growth supporting amino acids such as, alanine, valine, leucine, isoleucine, proline, histidine, methionine, threonine, tryptophan, phenylalanine, cysteine, tyrosine and hydroxyproline. It is possible that these amino acids inhibited the growth of bacteria in glycine. The lysine, serine, glutamic acid, aspartic acid, ornithine and arginine were used in combination to examine for the growth of *Vibrio* and *Enterobacter* isolates at 25°C. From the results which are shown in Table 3, is evident that, with one exception, there was no growth in these combination media. *Vibrio* isolates grew in serine and

Table 1. Utilization of some amino acids by *Enterobacter* and *Vibrio* isolates at various temperatures.

| Substrate used (0.015 M) | Temperatures °C | *Vibrio* isolates | *Enterobacter* isolates |
|---|---|---|---|
| Basal medium | | − (0.00) | − (0.00) |
| Casamino acids | | + (0.30~0.34) | + (0.25~0.28) |
| Glycine | | + (0.22~0.24) | + (0.23~0.25) |
| Lysine | | + (0.19~0.20) | − (0.01) |
| Arginine | 20°C | − (0.02) | − (0.02) |
| Ornithine | | + (0.14~0.16) | − (0.02) |
| Serine | | − (0.02) | + (0.10~0.13) |
| Glutamic acid | | + (0.19~0.20) | − (0.02) |
| Aspertic acid | | + (0.20~0.23) | − (0.01) |
| Basal medium | | − (0.00) | − (0.00) |
| Casamino acids | | + (0.35~0.38) | + (0.29~0.31) |
| Glycine | | + (0.24~0.26) | + (0.27~0.29) |
| Lysine | | + (0.21~0.23) | − (0.02) |
| Arginine | 25°C | − (0.02) | − (0.02) |
| Ornithine | | + (0.18~0.20) | − (0.07) |
| Serine | | − (0.02) | + (0.14~0.16) |
| Glutamic acid | | + (0.24~0.26) | − (0.02) |
| Aspertic acid | | + (0.22~0.25) | − (0.01) |
| Basal medium | | − (0.00) | − (0.00) |
| Casamino acids | | + (0.21~0.23) | + (0.31~0.36) |
| Glycine | | + (0.16) | + (0.32~0.34) |
| Lysine | | − (0.03) | − (0.02) |
| Arginine | 30°C | − (0.01) | + (0.17~0.20) |
| Ornithine | | − (0.02) | − (0.01) |
| Serine | | − (0.02) | + (0.18~0.20) |
| Glutamic acid | | − (0.03) | − (0.02) |
| Aspertic acid | | − (0.02) | − (0.01) |
| Basal medium | | − (0.00) | − (0.00) |
| Casamino acids | | + (0.09~0.12) | + (0.31~0.32) |
| Glycine | | + (0.09~0.10) | + (0.28~0.32) |
| Lysine | | − (0.02) | − (0.02) |
| Arginine | 35°C | − (0.02) | + (0.18~0.19) |
| Ornithine | | − (0.01) | − (0.02) |
| Serine | | − (0.02) | + (0.15~0.16) |
| Glutamic acid | | − (0.01) | − (0.02) |
| Aspertic acid | | − (0.02) | − (0.02) |

(−) No growth, (+) Positive growth.

Table 2. Growth of *Vibrio* and *Enterobacter* isolates in amino acids with or without glycine at 25°C for 24 hours.

| Amino acids used | *Vibrio* | | *Enterobacter* | |
|---|---|---|---|---|
| | With glycine | Without glycine | With glycine | Without glycine |
| Glycine | ++ | | ++ | |
| Lysine | ++ | + | + | − |
| Serine | + | − | ++ | + |
| Glutamic acid | ++ | + | + | − |
| Aspertic acid | ++ | + | + | − |
| Ornithine | ++ | + | + | − |
| Arginine | + | − | ++ | + |
| Histidine | − | − | − | − |
| Metheonine | − | − | − | − |
| Tryptophan | − | − | − | − |
| Cysteine | − | − | − | − |
| Threonine | − | − | − | − |
| Proline | − | − | − | − |
| Hydroxyproline | − | − | − | − |
| Tryosine | − | − | − | − |
| Alanine | − | − | − | − |
| Valine | − | − | − | − |
| Leucine | − | − | − | − |
| Isoleucine | − | − | − | − |

(−) No growth, (+) weak growth, (++) good growth.

Table 3. Assessment of growth of *Vibrio* and *Enterobacter* isolates in combination of positively responded amino acids at 25°C for 24 hours.

| Amino acids used | *Vibrio* isolates | *Enterobacter* isolates |
|---|---|---|
| Lysine+serine | − | − |
| Lysine+glutamic acid | − | − |
| Lysine+aspertic acid | − | − |
| Lysine+ornithine | − | − |
| Lysine+arginine | − | − |
| Serine+glutamic acid | + | − |
| Serine+aspertic acid | − | − |
| Serine+ornithine | − | − |
| Serine+arginine | − | − |
| Glutamic acid+aspertic acid | − | − |
| Glutamic acid+ornithine | − | − |
| Glutamic acid+arginine | − | − |
| Aspertic acid+ornithine | − | − |
| Aspertic acid+arginine | − | − |

(−) No growth, (+) positive growth.

glutamic acid combination at 25°C.

Fig. 1, shows the growth rate of one representative strain of *Vibrio* with glutamic acid at 20°C and 25°C. No growth was detected at 30°C and 35°C. Isolates of *Vibrio* grew at 30°C and 35°C when serine was added to the medium (Fig. 2). The lag period for growth of the strain at 30°C and 35°C was greatly extended. This suggests a slow utilization of the serine containing substrate at these temperatrues within the first several hours of incubation.

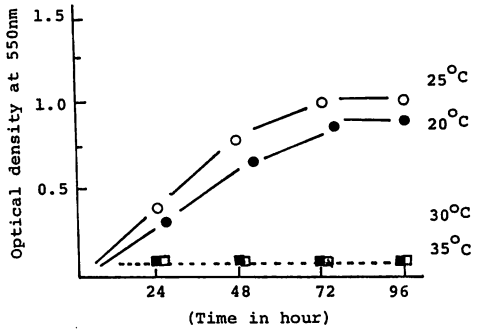


Fig. 1. Growth of a representative strain of *Vibrio* in glutamic acid.

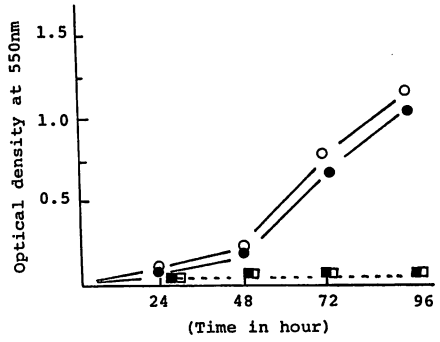


Fig. 2. Growth of same strain of *Vibrio* in glutamic acid with serine (○) at 30°C and (●) 35°C and without serine (□) and (■) at the same temperatures.

At various temperatures these strains could utilize a few amino acids which are non-essential for fish. They were not able to utilize properly and perfectly all of the essential amino acids which are commonly found in fish and which could be considered to be essential for the gray mullet (under investigation). Arginine, valine, lysine, leucine, isoleucine, methionine, phenylalanine, threonine and tryptophan were found to be essential amino acids for trout and salmon (HALVER, HALVER and SHANKS and HALVER et al.).[8-10] The inability of these strains to utilize these amino acids may indicate the possibility of the bacterial production of these amino acids and other nutrients in fish with an undeveloped stomach, as in gray mullet.

From these results it can again be concluded that *Vibrio* isolates and *Enterobacter* isolates are most important and probably indispensable for the gray mullet under sea and fresh water conditions.

It should be mentioned that in this study the *Vibrio* isolates were examined from their nutritional point of view only. Additional studies are needed in order to determine if these isolates are pathogenic or non-pathogenic. These studies are now in progress.

**Acknowledgement**

The authors would like to thank prof. TAKAHISA KIMURA of the Microbiology

Laboratory, Faculty of Fisheries, Hokkaido University for his kind discussion and important suggestions.

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