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Microflora in the Alimentary Tract of Gray Mullet—IX

Vitamin Requirement for the Growth of *Vibrio* and *Enterobacter* Isolates

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Abstract

Isolates of *Vibrio*, similar to *Vibrio anguillarum* and isolates of *Enterobacter*, similar to *Enterobacter aerogenes* isolated from the alimentary tract of Gray mullet (*Mugil cephalus*) were grown in the presence or absence of eight vitamins. Omission of vitamins from the medium confirmed that biotin, riboflavin, pantothenic acid, folic acid, pyridoxal and nicotinic acid were not essential for the growth of the isolates of *Vibrio* and *Enterobacter*. It was found that cobalamin was non essential and inhibited the growth of *Enterobacter* isolates. Although a little requirement of thiamin for the growth of *Vibrio* isolates was detected, but it seems to be non essential for the growth of the isolates. No requirement of the other essential growth factors could be detected.

The normal intestinal microflora including bacteria are a source of vitamins in animal rations to improve feed efficiency and increase the rate of growth of animal (HALL)[1]. Riboflavin is synthesized in varying amounts by many bacteria (PRYDHAM)[2]. The production of B_{12} by microorganisms has been studied extensively (BURTON and LOCHEAD)[3]. Indigenious bacteria in the gastrointestinal tract influence profoundly the lives of their hosts by producing or utilizing vitamins and other important nutrients (GORDON)[4]. The purpose of this communication was undertaken to determine the requirement of vitamins by *Vibrio* and *Enterobacter* during the growth at various temperatures, since, these two bacterial spp. are supposed to be the most important and useful for the growth of gray mullet.

Materials and Methods

Bacteria, their Growth Conditions and Characterizations:

The methods for enumaration of bacteria, procedures for isolation, compositions of the media used, incubation temperatures, methods for morphological and biochemical examinations of the species of bacteria, process of estimation of enzymic activities of the bacteria and the assessment for the requirement of amino acids by the intestinal bacteria of gray mullet were the same as described previous papers of this series[5)-11)].

Culture Media:

Strains of *Vibrio* isolates and *Enterobacter* isolates were cultured in ZoBell and nutri-

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ent broth at 25°C and 30°C respectively on a shaker for 18 hours. Cells were harvested, washed and resuspended in 0.9% NaCl. An aliquot of 0.1 m*l* of suspension was inoculated in test tubes containing 10 m*l* basal medium[12] containing KH_2PO_4: 1 gm., Na_2HPO_4: 3 gm., NaCl: 5 gm., $CaCl_2$: 0.1 gm., $MgSO_4 \cdot 7H_2O$: 0.2 gm., $MnSO_4 \cdot H_2O$: 0.006 gm., $FeSO_4 \cdot 7H_2O$: 0.015 gm. and vitamin free casamino acid or amino acids: (except glutamic acid, glycine, lysine and serine because the growth of the isolates were positive in these amino acids) 0.5 gm. each in distilled water 1 liter (pH adjusted to 7.0). The tubes were then incubated on a shaker at temperatures at 20°C, 25°C, 30°C and 35°C for 72 hours respectively. Growth of the test organisms was determined by measuring the absorbance at 470 nm wave length by spectrophotometer (Hitachi/Model 101).

Vitamin Mixture:

This contained (μg ml^{-1} final concentration): riboflavian, 0.5; nicotinic acid, 1.0; thiamine, 1.0; biotin, 0.5; folic acid, 2.5; cobalamin, 1.0; pyridoxal, 1.0; and pantothenic acid, 0.5; dissolved in water. The mixture was diluted to half strength when used in growth media.

Results and Discussion

Growth of *Enterobacter* and *Vibrio* isolates in eight vitamins at different temperatures are shown in Table 1. It has been shown that all the bacterial strains grew well at all the tested temperatures in a medium containing tryptic digest of casein and amino acids. The nutritional requirements for the growth of these intestinal isolates were not unusual. The unique features of the growth of these isolates in the media were

Table 1. Growth of the isolates of *Vibrio* and *Enterobacter* at different temperatures. The composition of the medium were sterilized in an autoclave for 20 min at 121°C, except for vitamins which were added after sterilization by filtration.

Vitamin used	Temperatures °C	*Vibrio* isolates	*Enterobacter* isolates
Basal medium		− (0.00)	− (0.00)
Vitamin free			
Casamino acids		++ (0.98)	++ (0.65)
Amino acids		+ (0.76~0.78)	+ (0.56)
Thiamine		++ (0.86~0.89)	+ (0.54~0.57)
Riboflavin	20°C	+ (0.71~0.74)	+ (0.54~0.55)
Pyridoxal		+ (0.76~0.78)	+ (0.57)
Cobalamin		+ (0.71~0.75)	± (0.49~0.51)
Folic acid		+ (0.70)	+ (0.54~0.56)
Nicotinic acid		+ (0.76)	+ (0.55)
Pantothenic acid		+ (0.71~0.74)	+ (0.51)
Biotin		+ (0.74~0.76)	+ (0.57)

Table 1. (Continued)

Vitamin used	Temperatures °C	*Vibrio* isolates	*Enterobacter* isolates
Basal medium		− (0.00)	− (0.00)
Vitamin free			
Casamino acids		++ (1.10~1.15)	++ (0.78~0.80)
Amino acids		+ (0.85~0.87)	+ (0.68~0.70)
Thiamine		++ (0.92~0.95)	+ (0.65~0.68)
Riboflavin	25°C	+ (0.82~0.85)	+ (0.61~0.64)
Pyridoxal		+ (0.85)	+ (0.65)
Cobalamin		+ (0.83)	± (0.55~0.57)
Folic acid		+ (0.84~0.85)	+ (0.62~0.64)
Nicotinic acid		+ (0.81~0.83)	+ (0.65)
Pantothenic acid		+ (0.83~0.85)	+ (0.66)
Biotin		+ (0.85)	+ (0.67~0.70)
Basal medium		− (0.00)	− (0.00)
Vitamin free			
Casamino acids		++ (0.85~0.87)	++ (1.00~1.10)
Amino acids		+ (0.65)	+ (0.89~0.92)
Thiamine		++ (0.70)	+ (0.89~0.90)
Riboflavin	30°C	+ (0.62~0.64)	+ (0.80)
Pyridoxal		+ (0.60~0.64)	+ (0.85~0.87)
Cobalamin		+ (0.65)	± (0.70~0.71)
Folic acid		+ (0.67)	+ (0.89)
Nicotinic acid		+ (0.66)	+ (0.91)
Pantothenic acid		+ (0.64~0.67)	+ (0.87)
Biotin		+ (0.61~0.64)	+ (0.87~0.89)
Basal medium		− (0.00)	− (0.00)
Vitamin free			
Casamino acids		++ (0.65~0.67)	++ (0.95~0.97)
Amino acids		+ (0.45~0.47)	+ (0.75~0.79)
Thiamine		++ (0.45~0.48)	+ (0.71~0.74)
Riboflavin	35°C	+ (0.41)	+ (0.76)
Pyridoxal		+ (0.43~0.45)	+ (0.73~0.75)
Cobalamin		+ (0.47)	± (0.65)
Folic acid		+ (0.45)	+ (0.74~0.78)
Nicotinic acid		+ (0.43)	+ (0.73~0.75)
Pantothenic acid		+ (0.45~0.46)	+ (0.76)
Biotin		+ (0.43)	+ (0.71~0.74)

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

not specifically dependant on the presence or absence of vitamins, (except thiamine and cobalamin). Each vitamin was omitted in turn from the medium, no specific change of growth yield was detected, except in thiamine, where the growth yield of *Vibrio* isolates was a little higher at all the tested temperatures (Table 2). But when the thiamine was omitted, the growth of *Vibrio* isolates continued, although slow growth occurred after a relatively long lag period (Fig. 1). Addition of thiamine shortened the lag period and improve the growth, but the growth rate remained poorer than that of in control culture where the bacteria had been grown with tryptic digest of casein. Here thiamine could be supposed to be stimulant and probably non essential for the growth of *Vibrio* isolates.

It may be concluded that *Vibrio* and *Enterobacter* isolates did not require cobalamin, since, it grew well without cobalamin. Moreover, growth of the isolates was not affected by the addition of cobalamin to the culture of *Enterobacter* isolates. The

Table 2. Determination of vitamin requirement for the growth of the isolates at 25°C for 24 hours.

Vitamin omitted from the basal medium containing some amino acids	*Vibrio* isolates	*Enterobacter* isolates
None	++ (0.90~0.93)	++ (0.70)
Thiamine	++ (1.00~1.10)	+ (0.68~0.70)
Riboflavin	+ (0.89)	+ (0.69~0.70)
Pyridoxal	+ (0.90)	+ (0.67~0.69)
Cobalamin	+ (0.88~0.90)	± (0.60)
Folic acid	+ (0.94)	+ (0.71)
Nicotinic acid	+ (0.92)	+ (0.70)
Pantothenic acid	+ (0.89)	+ (0.68)
Biotin	+ (0.91~0.92)	+ (0.70)

(±) Slow growth, (+) Good growth, (++) Very good growth.

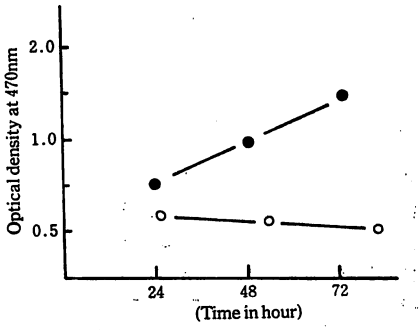

Fig. 1. Inhibition of a representative strain of *Enterobacter* isolate (●) without cobalamin and (○) with cobalamin.

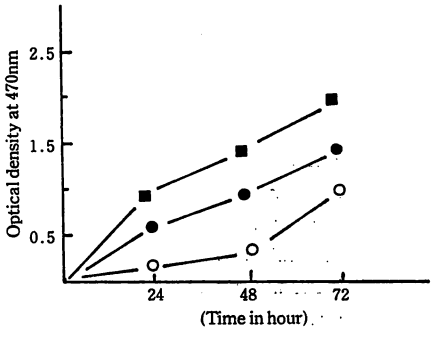

Fig. 2. Growth of a representative strain of *Vibrio* isolates with thiamine (●), without thiamine (○) and in tryptic digest of casein (■).

growth rate of *Enterobacter* isolates was reduced when cobalamin was added to the medium, that may inhibits the growth of bacteria in this medium (Fig. 1).

The results of the identification of essential and non essential components by studying the effect of their omission or addition, indicated that there was no specific requirement for biotin, riboflavin, pantothenic acid, folic acid, pyridoxal, cobalamin and nicotinic acid for the growth, under this experimental condition. It could be concluded that these vitamins are not essential for their growth.

Thus these strains could not utilize vitamins which could be assumed to be essential for gray mullet. PHILLIPS et al.[13] determined the need of 10 members of the vitamin B complex for Carp and Trout. DELONG[14] established the necessity of carp for pyridoxamine, riboflavin and pantothenic acid.

The inability to utilize these vitamins might be indicating the possibility of producing them together with other important nutrients in fishes with undeveloped stomach, like gray mullet.

Thus the result could be concluded that *Enterobacter* isolates and *Vibrio* isolates (studied only on their nutritional point of view) were most important and probably indispensable for gray mullet, living in fresh and sea water.

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