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著者	MOWLAH Aleya H., KAKIMOTO Daiichi
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Microflora in the Alimentary Tract of Gray Mullet-VII

Growth temperature of *Vibrio*

Aleya H. MOWLAH*² and Daiichi KAKIMOTO*¹

Abstract

The minimum and maximum growth temperatures of five isolates of *Vibrio* and three biotypic strains (standard) of *Vibrio anguillarum* were determined in peptone broth containing 2% or 2.5% NaCl in static and shaking temperatures at 8, 10, 12, 14, 16, 18, 30, 32, 34, 36, 38, and 40°C. Each minimum temperature for growth of various *Vibrio* strains ranged from 11 to 12°C in both media, although cell growth was greater in 2% NaCl containing medium. All strains grew well at 35°C. Only *Vibrio* isolates grew at 38°C but failed to grow at 40°C. Growth behavior of the organisms at temperatures between minimum and maximum growth temperatures are discussed.

Five isolates of *Vibrio* isolated from the alimentary tract of gray mullet (*Mugil cephalus*) living in sea and fresh water, were found similar to *Vibrio anguillarum* in the previous paper¹⁾. Isolates of *Vibrio* had a capacity to grow in both low and high temperatures. *Vibrio* species predominated in fish intestine were resistant to low pH and high temperature²⁾. The present study was undertaken to obtain definite minimum and maximum temperatures of the isolates and also to know whether these temperatures for growth of standard *Vibrio anguillarum* and *Vibrio isolates* of fish mullet, which was supposed to be introduced into the tract came from drainage water were same or different.

Materials and Methods

Bacteria and their growth conditions. Details of experimental methods, chemicals and media used have been published³⁻⁷⁾ or are in press. Three strains of *Vibrio anguillarum* as standard strains were used which were received from Hokkaido University. The cultures numbered A (V-1), B (V-1), C (V-1), D (V-1) and E (V-1) were of gray mullet origin and F (NCMB 6), G (NCMB 828) and H (NCMB 829) were the standard strains.

Preparation of inocula. The test organisms were transferred twice before use

*¹ Laboratory of Microbiology, Faculty of Fisheries, Kagoshima University, Japan.

*² Aleya HAMID has been changed into Aleya HAMID MOWLAH. All her previous papers can be read as Aleya H. MOWLAH.

in the growth studies. A subculture was grown in Erlenmeyer flask containing 100 ml medium at 25 C in a shaking condition. A second subculture was made after 24 hours and incubated for a further 24 hours.

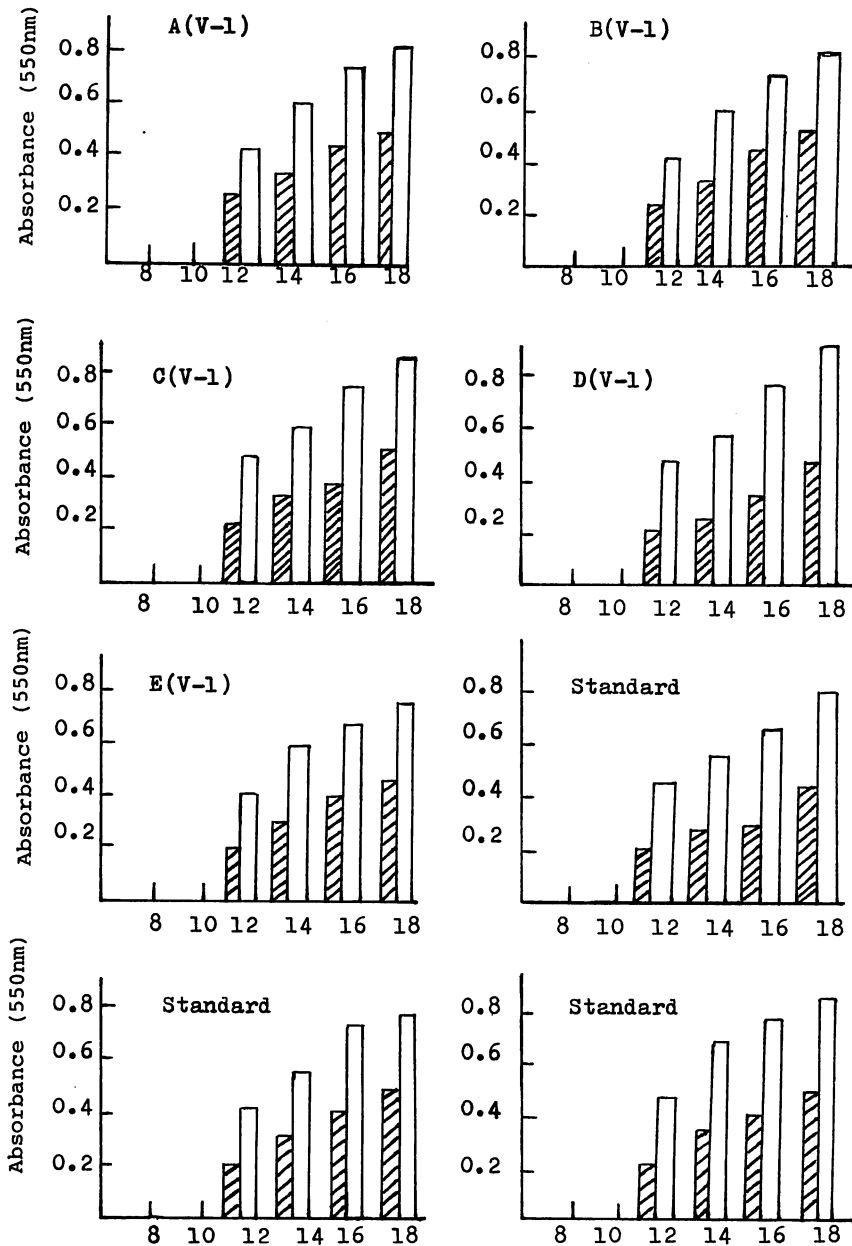


Fig. 1 Growth of five isolates of the intestinal *Vibrio* in 2.5% NaCl (shaded bar) and 2% NaCl (open bar) and growth of standard strains in 2.5% NaCl (shaded bar) and 2% NaCl (open bar) in static condition.

Minimum growth temperature under static condition. Tubes containing 10 ml of medium were incubated at temperatures ranging from 8°C~18°C for 24 hours. Each tube was inoculated with a loopful of the test organism. The minimum growth temperature was measured by the absorbance at 550 nm in a spectrophotometer (Hitachi/Model 101).

Minimum growth temperature under shaking condition. Tubes containing 10 ml of medium were incubated in shaking incubator at various temperatures from 8°C~18°C for 24 hours. The growth was assessed by measuring the absorbance at 550 nm in a spectrophotometer.

Maximum growth temperature. 10 ml of cultures were grown in a shaking incubator at temperatures ranging from 30°C~40°C for 24 hours. Growth was assessed by measuring the absorbance.

Results and Discussion

Minimum growth temperature. Intestinal isolates of five strains of *Vibrio* strains A (V-1), B (V-1), C (V-1), D (V-1) and E (V-1) and three standard strains of *Vibrio anguillarum* F (NCMB 6), G (NCMB 828) and H (NCMB 828) were incubated in static condition in 2% or 2.5% NaCl. The minimum growth temperatures of intestinal *Vibrio* ranged from 11 to 12 C under this condition. In all the cases the patterns of the bar graphs is very similar as shown in Fig. 1. The minimum growth temperatures in both media were also the same. Fig. 2

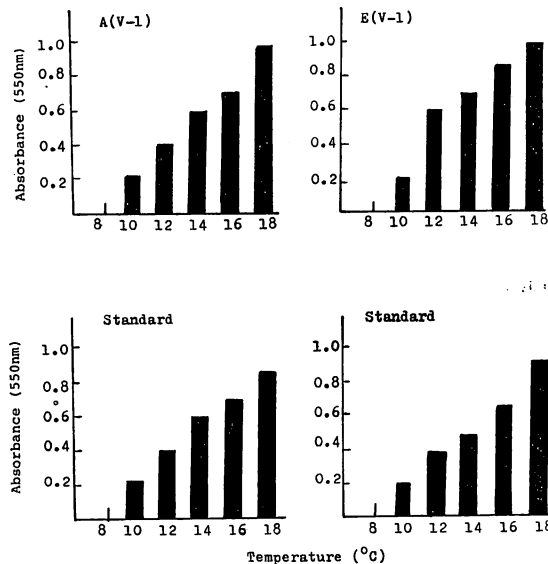


Fig. 2 Growth of two representative isolates of the intestinal *Vibrio* and two standard strains in 2% NaCl in shaking condition.

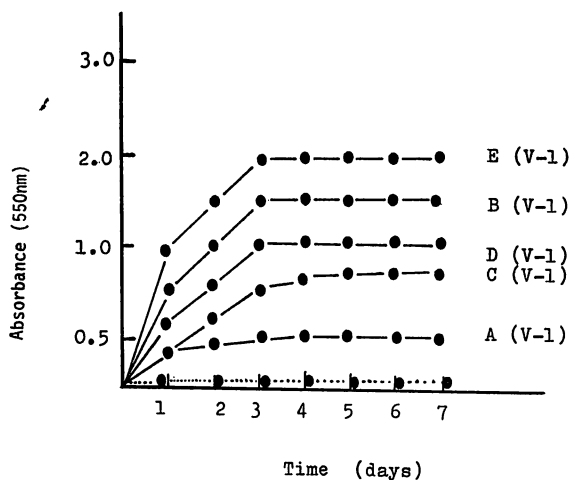


Fig. 3 Growth of five isolates of intestinal *Vibrio* in 2% NaCl at 38C.

—●— 38C
 ...●... 40C

shows the growth for minimum temperatures of two representative strains from two sources in 2% NaCl containing medium in shaking condition. All strains grew at lower temperatures in the shaking condition than in the static condition.

Maximum growth temperature. The maximum growth temperatures of the strains from intestine were similar to each other but differed from the three standard strains of *Vibrio anguillarum*. All the strains grew well at 30 to 35 C. Only five isolates of intestine grew at 38 C but none grew at 40 C (Fig. 3). To the contrary, the strains of *Vibrio anguillarum* grew well at 35 C but failed to grow at 38 C.

The study on the growth temperatures of *Vibrio* strains isolated from the alimentary tract of gray mullet indicates that the organisms show a typical mesophylic nature. The minimum growth temperature of fish intestinal *Vibrio* isolates were the same as the standard *Vibrio anguillarum*, but the maximum temperature for their growth was slightly different.

Thus from the above data it can be concluded that the strains of intestinal origin and standard strains were similar in minimum growth temperature but differed in maximum growth temperature, although their other characteristics were the same. It seems most likely that a *Vibrio anguillarum* like species to be introduced into the alimentary tract of gray mullet come from the surrounding sea water where they live in and gradually adapted to the environmental conditions encountered in the intestine.

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