

# Growth Temperatures and Temperature Characteristics of *Aeromonas*

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Six of the 13 *Aeromonas hydrophila*, 1 of 10 *A. shigelloides*, and none of 10 *A. salmonicida* were found to be psychrophiles. All of the rest of the strains were mesophiles. The  $\mu$  values (temperature characteristics) could not be used to distinguish psychrophiles from mesophiles.

The optimum temperatures for *Aeromonas* species are ca. 20 C for some and 37 C for others (7). One strain, although it has an optimum temperature of about 37 C, is capable of some growth at near 1 C (6). Also, one strain of *A. salmonicida* has been termed as genuinely psychrophilic (2). In our laboratory, we noticed that the various strains of *Aeromonas* have a wide range of growth temperature. Eddy (5) also made similar observations and concluded that "it would clearly be of interest to know the lower limit of temperature which will permit the growth of these organisms."

The facts that *Aeromonas* species have been isolated from chilled meat (6), that they play an important role in the manufacture of raw sausage (3), and that they are able to grow at 0 C make them important in the food industry. The genus *Aeromonas* is also known to cause diseases in fishes, frogs, and even in humans (1, 4, 5, 7, 10, 12, 13, 15, 16).

In the present investigation, therefore, a thorough study of growth temperature and rate of 33 different strains of *Aeromonas* were made. Also, the temperature characteristics of growth ( $\mu$  value) of some strains were determined to see whether this would aid in classifying various strains of *Aeromonas* as psychrophile or mesophile. Since the range of growth for certain *Aeromonas* species is from 0 to 45 C or even 0 to 55 C, simple consideration of minimal, optimal, and maximal growth temperature may not be sufficient to classify them as psychrophiles or mesophiles. Furthermore, it would tell us whether the  $\mu$  value is meaningful in classifying bacteria as mesophile and psychrophile based on determinations made with closely related species of *Aeromonas*. The  $\mu$  value was found to be "markedly less for psychrophiles than for mesophiles" by Ingram (8).

## MATERIALS AND METHODS

**Organisms.** Of the 33 strains used in this study, 30 were kindly provided by W. H. Ewing and W. J. Martin of the Center for Disease Control, Atlanta. Three strains of *A. hydrophila* were isolated and identified in this laboratory. Thirteen of these 33 strains were *A. hydrophila*, 10 were *A. shigelloides*, and 10 were *A. salmonicida*. The names, numbers, and sources of these strains are given in Table 1. All strains were maintained in Brain Heart Infusion agar or Trypticase soy agar.

**Media and growth.** All strains were grown in Trypticase soy broth for growth and rate studies. Cultures were always pregrown at the appropriate temperature and in respective media before determination of growth rate to eliminate transient growth. Cultures were grown in 250-ml Erlenmeyer flasks in 40-ml volumes and incubated unshaken. Samples for Klett readings were withdrawn without removing the flasks from the incubators so that the growth rate would not be affected. We have noticed that the fastest and best growth of these strains was in unshaken shallow layers rather than shaken cultures. For inoculum, one drop of an actively growing culture from a 1-ml pipette was added to each flask containing 40 ml of medium. Duplicate flasks were always used in each experiment. Growth of the various strains was determined by Klett colorimeter by using a blue filter (400 to 465  $\mu$ m) at intervals of time. The amount of growth and the rate was determined at 5-degree intervals from 0 to 60 C.

**Calculation of growth rate and temperature characteristics.** A semilog plot of Klett units versus time in hours for each organism at each temperature was plotted. From this plot, the doubling time was determined by using the straight line portion of the graph. The specific growth rate was determined by taking the inverse of the doubling time.

Arrhenius plot of semilog of specific growth rate versus 1 per degree Kelvin was made for 12 organisms.

The temperature characteristic ( $\mu$ ) values for 12 organisms were determined from several ( $n$ ) of the

TABLE 1. Growth temperatures of *Aeromonas*<sup>a</sup>

Species of <i>Aeromonas</i> , sources, and strain no.	Approximate growth temp			Conclusion
	Minimal <sup>b</sup> (c)	Optimal (c)	Maximal (c)	
<i>A. hydrophila</i>				
NIH (CDC) strains				
3402-68	10	35	55	Mesophile
3910-68	10	30-35	40	Mesophile
2195-68	15	30	45	Mesophile
5244-68	10	25-30	55	Mesophile
3325-68	10	30	55	Mesophile
3337-68	5	35	40	Mesophile
3326-68	0	15-20	45	Psychrophile
3067-68	0	20	45	Psychrophile
3572-68	0	30	45	Psychrophile
3890-68	0	20	55	Psychrophile
Red Leg-isolated				
A <sub>1</sub>	0	15-20	40	Psychrophile
A <sub>3</sub>	0	20	40	Psychrophile
A <sup>4</sup>	10	20-25	45	Mesophile
<i>A. shigelloides</i>				
NIH (CDC) strains				
2413-69	10	30-35	45	Mesophile
3428-67	10	30-35	45	Mesophile
389-69	10	30-35	55	Mesophile
1432-68	10	30-35	45	Mesophile
4337-69	5	30-35	45	Mesophile
882-69	0	35	45	Psychrophile
2154-68	10	30-35	55	Mesophile
2418-69	10	30	55	Mesophile
1383-69	10	25-30	40	Mesophile
4332-69	10	25-30	40	Mesophile
<i>A. salmonicida</i>				
NIH (CDC) strains				
3001-60	5	25	35	Mesophile
3002-60	5	20-25	35	Mesophile
3004-60	10	25-30	45	Mesophile
3005-60	10	30-35	40	Mesophile
2933-60	5	25	45	Mesophile
3010-60	15	35	40	Mesophile
3008-60	15	35	40	Mesophile
3000-60	15	30	40	Mesophile
2997-60	15	25-30	40	Mesophile
3007-60	15	30	40	Mesophile

<sup>a</sup> All cultures were grown in Trypticase soy broth.

<sup>b</sup> At these temperatures, the amount of growth in Klett units varied from 87 to 475. Organisms considered psychrophiles showed good growth (87 to 450 Klett units) in 6 to 11 days of incubation.

points that gave a straight line of the best fit by least square regression equation:

$$\mu = R \frac{\sum_{i=1}^n X_i \sum_{i=1}^n Y_i - n \sum_{i=1}^n (X_i - Y_i)}{\left( \sum_{i=1}^n X_i \right)^2 - \sum_{i=1}^n (X_i)^2}$$

where  $R = 1.98$  calories per degree per molecule,  $n =$  the number of points used;  $Y_i =$  natural log of

the growth rates, and  $X_i =$  the reciprocals of the corresponding temperatures.

## RESULTS AND DISCUSSION

The growth studies of all strains were made at 5-degree intervals of temperature starting from 0 to 60 C. At 0 to 15 C incubation temperature, cultures were incubated for a minimum of 14 days, at 20 to 60 C, for a minimum of 7 days,

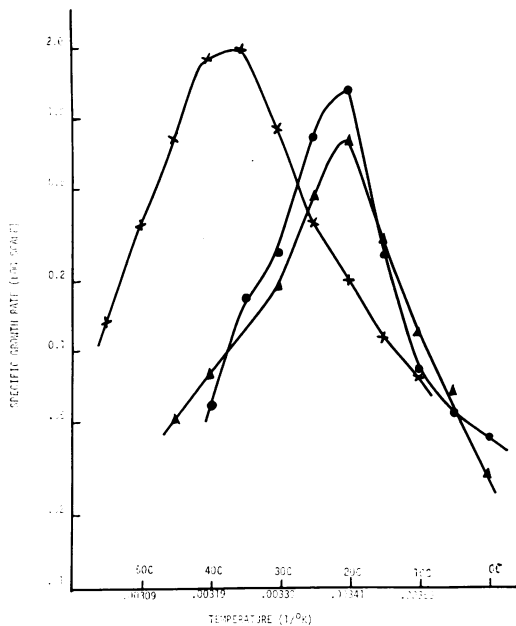


FIG. 1. Arrhenius plot of specific growth rates as a function of absolute temperature. Symbols: (●) *Aeromonas hydrophila* ( $A_3$ ), psychrophile; (▲) *A. hydrophila* (3326-68), psychrophile; (×) *A. hydrophila* (3402-68), mesophile.

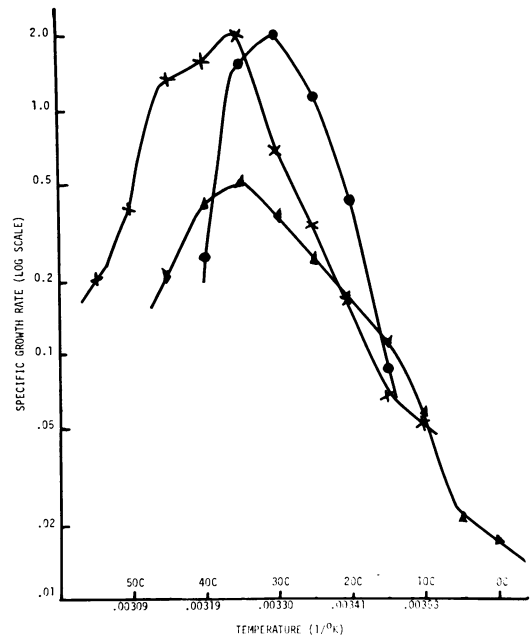


FIG. 2. Arrhenius plot of specific growth rates as a function of absolute temperature. Symbols: (●) *Aeromonas salmonicida* (3007-60), mesophile; (▲) *A. shigelloides* (882-69), psychrophile; (×) *A. shigelloides* (2418-69), mesophile.

before they were discarded as unable to grow. All strains were always tested for purity at the end of each growth temperature experiment. Some strains formed slightly dark pigment in growth medium. For these, Klett readings for growth were made by using the centrifuged filtrate as control. The Klett readings and growth curves were made for all strains of *Aeromonas* to determine the approximate minimal optimal and maximal growth temperatures. The term approximate is used in describing minimal, optimal, and maximal growth because no lower or higher limits of temperature have been established within 1 to 2 degrees. The optimal growth temperature was the temperature which supported the largest cell yield at the beginning of the stationary phase (9). The psychrophiles were defined by the method of Ingram (8). The growth studies were not made below 0 C. Based on the growth temperatures, all of the *Aeromonas* were classified as either a mesophile or psychrophile (Table 1).

Of the 13 *A. hydrophila*, 6 were classified as psychrophile; all of these strains had optimal temperature between 15 and 20 C and in one case 30 C. All of these psychrophilic strains also had a maximal growth temperature of 40 to 45 C. The other seven strains were classified as mesophile.

One strain, although classified as mesophile, grew well at 5 C. Three of the mesophiles were able to grow at 55 C.

Of the 10 *A. shigelloides*, 9 were classified as mesophile and 1 psychrophile. The psychrophilic strain had an optimal temperature around 35 C. Another strain of *A. shigelloides* had an approximate minimal growth temperature of 5 C.

All 10 strains of *A. salmonicida* were classified as mesophile although 3 of the strains grew well at 5 C.

The growth temperatures of 33 *Aeromonas* species show that the psychrophilic strains are primarily found among *A. hydrophila*. Although 6 out of 13 *A. hydrophila* were psychrophilic, only 1 out of 10 of *A. shigelloides* and none out of 10 of *A. salmonicida* was found to be psychrophilic. All of the 33 species had rather wide ranges of growth temperature. A general conclusion could be that all of the organisms studied could grow well in 20 C.

The typical Arrhenius plots and temperature characteristics of six strains of *A. hydrophila*, three *A. shigelloides*, and three *A. salmonicida* were determined. To avoid duplicity, only six representative graphs are shown in Fig. 1 and 2. The selection of these strains for  $\mu$ -value determination was based on the fact that, within the

TABLE 2.  $\mu$  Values of certain *Aeromonas* species<sup>a</sup>

Organism and strain no.	$\mu$ Value (kcal/mole)	Temperature relationship			
		Minimal (c)	Optimal (c)	Maximal (c)	Conclusion
<i>A. hydrophila</i>					
3402-68	22.77	10	35	55	Mesophile
5244-68	23.45	10	30	55	Mesophile
3337-68	31.63	5	25	40	Mesophile
3326-68	24.85	0	20	45	Psychrophile
A <sub>1</sub>	42.39	0	15	40	Psychrophile
A <sub>3</sub>	45.34	0	20	40	Psychrophile
<i>A. shigelloides</i>					
2418-69	24.39	10	35	55	Mesophile
389-69	29.50	10	35	55	Mesophile
882-69	13.60	0	35	45	Psychrophile
<i>A. salmonicida</i>					
3001-64	32.88	5	25	35	Mesophile
3007-60	36.11	15	30	40	Mesophile
2933-60	25.97	5	25	45	Mesophile

<sup>a</sup> All cultures were grown in Trypticase soy broth.

species, these strains vary in growth temperature requirements. The  $\mu$  values are given in Table 2.

The data in Table 2 indicate that no definite conclusion about the psychrophiles having a lower  $\mu$  value than mesophiles, even within the same genus, can be made.

Among *A. hydrophila*, two organisms considered to be psychrophile had a higher  $\mu$  value than the ones considered mesophile. In *A. shigelloides*, however, the one considered psychrophile had a lower  $\mu$  value than the mesophiles. All three *A. salmonicida* were considered to be mesophile and to show somewhat similar  $\mu$  values.

The temperature coefficient ( $\mu$  values) determinations were meaningless in these cases to classify them as psychrophiles or mesophiles. Our data on this are in agreement with Shaw (14) and Hanus and Mortia (11) who also found no basis for using  $\mu$  values to distinguish mesophiles from psychrophiles.

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