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Optimum Temperatures for the Peak Growth of Some Selected Bacterial Fish Pathogens

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Abstract

The optimal growth temperatures for 24 strains of 15 bacterial fish pathogens were determined under uniform cultural conditions. For *Flavobacterium psychrophila*, peak growth temperature (t_{peak}) was 21±0.4ºC for the strain tested. *Flavobacterium columnaris* exhibited 29.8±0.2 and 29.9±0.3ºC, respectively, as t_{peaks} for two strains. The t_{peaks} of *Flavobacterium branchiophilum* were 24.9±0.2 and 24.1±0.5ºC, respectively, for two strains. Three strains of *Edwardsiella tarda* were examined and they showed t_{peaks} at 36.1±0.4, 35.5±0.2 and 36±0.3ºC, respectively. *Citrobacter freundii* showed its t_{peak} at 36.1±0.4ºC. For *Aeromonas hydrophila*, two strains were tested and it was found that t_{peaks} were 32.9±0.6 and 34.8±0.5ºC, respectively. The t_{peaks} of *Aeromonas salmonicida* were 26.1±0.4 and 25.6±0.2ºC, respectively. Temperature 27.8±0.3ºC was found as t_{peak} of a *Vibrio anguillarum* strain while *Vibrio ordalii* strain exhibited it at 26.5±0.4ºC. Peak growth at temperatures of 26.7±0.5 and 27.6±0.4ºC, respectively, was found for *Photobacterium damselae* (formerly *Pasteurella piscicida*). In *Pseudomonas fluorescens*, the peak growth occurred at temperatures of 29.9±0.2 and 28.5±0.3ºC, respectively. *Pseudomonas* sp. showed t_{peaks} at 24.9±0.1 and 29.2±0.5ºC, respectively. The peak growth for the two strains of *Lactococcus garvieae* (formerly *Enterococcus seriolicida*) was at temperatures of 29.7±0.4 and 31.1±0.2ºC, respectively where the *Streptococcus* sp. showed t_{peak} at 28.3±0.5ºC.

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Introduction

Bacterial fish disease is one of the major constraints in the development of aquaculture. Particularly in intensive aquaculture, basic hygiene which is necessary for maintaining good water quality is often overlooked and may lead to the start of a disease cycle (McCarthy 1977). Water temperature is one of the vital factors for growth, reproduction, disease and other biological activities of fish. The progress of fish disease is influenced by environmental temperature. *Flavobacterium psychrophila* has been well documented as a fish pathogen (Borg 1960). *Flavobacterium columnaris* is the causative agent of columnaris disease (Davis 1922). The effect of water temperature on columnaris disease in Oriental weather fish was studied by Wakabayashi & Egusa (1972). All of the exposed fish held at 20-35ºC died. The relationship of water temperature with the number of fish infections induced by *Aeromonas hydrophila* had been reported (Groberg et al. 1978). The seasonality in the occurrence of disease is thought to be due to the change in environmental temperature. In disease, environmental temperature can influence not only fish but also the pathogen. Many bacteria are known to cause fish disease and their growth temperatures are different from genus to genus. The most appropriate temperature for the growth of pathogen is not the same with the temperature occurring disease and pathogenicity. It is known that the environmental temperature which causes disease has a tendency to be lower than the optimal growth temperature of pathogen but the details are not known. In this study we compared the optimal growth temperature of some important bacterial fish pathogens. Knowledge of the factors affecting growth (temperature, NaCl concentration for marine bacterium, pH etc.) is indispensable before embarking on susceptibility testing. For the production of highly effective bacterins, it is essential to know the cultural conditions of a particular bacterium.

However, many investigators worked on different bacterial fish pathogens and in most cases only a single bacterium was completely examined. Use of different media, different growth conditions and a variety of assay made the literature reports very difficult to interpret and compare. In this study, optimal growth temperatures of some selected strains of bacterial fish pathogens were determined under the uniform cultural conditions.

Materials and Methods

Bacterial strains

A total of 24 strains, one to three from each of 15 different bacterial species of fish or pond water origin, were selected for this study. The details of the bacterial strains studied are summarized in table 1. All these bacterial strains were collected from France, Japan, KSA and USA. After collection, they were preserved at -80ºC on a long term basis. Stock cultures of the organism were maintained on tryptone-soya broth "Nissui" (TSB) or tryptone yeast extract (TYE) broth at 18 or 25ºC incubation temperature. The TYE broth was used for the culture of *F. psychrophila*, *F. columnaris* and *F. branchiophilum*. An extra 1.5% NaCl was added to TSB for *V. anguillarum*, *V. ordalii*, *P. damselae* and *L. garvieae* while the rest of the bacteria were cultured in TSB. For inoculum, a loopful of actively growing broth culture was inoculated in 18 ml of each broth in an L-shaped tube and subsequently incubated at different points of temperature gradient incubator (TGI: Toyo Co.,Tn-3), over a range of 3-48ºC. The shaking speed was $30 \cdot min^{-1}$. Media composition: TSB = peptone (17 g), bean peptone (3.0 g), NaCl (5.0 g), glucose (2.5 g), $KHPO_4$ (2.5 g) in a total amount of 30 g media dissolved in 1000 ml distilled water having pH 7.3±0.1. TYE = tryptone (0.4%), yeast extract (0.05%), $CaCl_2.2H_2O$ (0.02%), $MgSO_4.7H_2O$ (0.05%) and pH 7.2±0.1.

Determination of growth

Growth of organisms was monitored by measuring the optical density of the culture by a spectrophotometer (Hitachi, U-2000) at 480 nm. In a pre-study, time course effect on the growth of several bacteria at different temperatures was determined and it was found that similar growth patterns were followed up to an OD 0.8 within log phase culture (Tables 2 and 3). So, samples were collected when the OD was lower than 0.6 (within log phase) to determine the growth after respective incubation hours of different strains.

Results

The main objective peak growth optimum temperatures of 24 strains of 15 bacterial species were determined from the mean of three

Table 1. Optimum growth temperatures of different bacterial fish pathogen strains used in this study

Bacterial species	Strain/ Isolation year	Source (Fish / Pond water)	Locality	Initial culture temperature (ºC)	[a]Peak growth temperature (ºC)	Growth range temperature (ºC)	Incubation period (h)
Flavobacterium psychrophila	FPC805/1988	*Oncorhynchus mykiss*	France	18	21.0±0.4	12-24	20
Flavobacterium columnaris	FPC74/66	*Misgurnus anguilli-caudatatus*	Japan	25	29.8±0.2	22-23	22
	FPC667/87	*Carassius auratus*	Japan	25	29.9±0.3	19-33	18
Flavobacterim	ATCC35035/77	*Oncorhynchus masou*	Japan	18	24.9±0.2	13-30	40
branchiophilum	ATCC35036/78	*Oncorhynchus tshawytcsha*	USA	18	24.1±0.5	12-28	53
Edwardsiella	FPC22/80	*Anguilla japonica*	Japan	25	36.1±0.4	24-41	6
tarda	FPC611/81	Eel-pond water	Japan	25	35.5±0.2	23-40	6
	FPC802/79	*Oreochromis niloticus*	Japan	25	36.0±0.3	26-40	6
Citrobacter freundii	FPC349/ -	Eel-pond water	Japan	25	36.1±0.4	27-41	5
Aeromonas	FPC868/78	*Anguilla japonica*	Japan	25	32.9±0.6	20-39	6
hydrophila	AHC02/2001	Hybrid tilapia	KSA	25	34.8±0.5	19-42	6
Aeromonas	NCMB2020/ -	-	-	18	26.1±0.4	17-33	13
salmonicida	FPC367/78	*Oncorhynchus mykiss*	Japan	18	25.6±0.2	18-31	14
Vibrio anguillarum	FPC92/66	*Plecoglossus altivelis*	Japan	25	27.8±0.3	19-35	11
Vibrio ordalii	FPC676/74	*Plecoglossus altivelis*	Japan	25	26.5±0.4	19-35	9
Photobacterium damselae	FPC851/87	*Seriola quinquera-diata*	Japan	25	26.7±0.5	23-32	35

Table 1. Optimum growth temperatures of different bacterial fish pathogen strains used in this study (continued)

Bacterial species	Strain/ Isolation year	Source (Fish / Pond water)	Locality	Initial culture temperature (ºC)	[a]Peak growth temperature (ºC)	Growth range temperature (ºC)	Incubation period (h)
Photobacterium damselae	FPC852/87	*Seriola quinqueradiata*	Japan	25	27.6±0.4	22-31	22
Pseudomonas fluorescens	FPC96/73	*Cyprinus carpio*	Japan	25	29.9±0.2	19-34	12
	FPC348/ -	*Salvelinus pluvinus*	Japan	25	28.5±0.3	18-32	13
Pseudomonas sp. (fluorescens-like)	FPC78/73	*Cyprinus carpio*	Japan	25	24.9±0.1	15-29	11
Pseudomonas sp. (putida-like)	FPC951/94	*Plecoglossus altivelis*	Japan	25	29.2±0.5	23-31	7
Lactococcus garvieae	FPC137/75	*Seriola quinqueradiata*	Japan	25	29.7±0.4	16-42	9
	FPC871/90	*Paralichthys olivaceus*	Japan	25	31.1±0.2	20-38	14
Streptococcus sp.	AHC92/92	Hybrid tilapia	KSA	25	28.3±0.5	15-40	14

[a]=mean ± standard deviation (SD) of three replicates

Table 2. Time course effect on the growth response of *Flavobacterium psychrophila* at different temperatures

Temperature	Growth Optical Density					
(ºC)	11 h	14h	17 h	20 h	23 h	26 h
12.5	0	0	0.02	0.03	0.13	0.21
15.5	0	0	0.07	0.19	0.43	0.75
18.5	0	0.04	0.17	0.40	0.54	0.92
19.5	0	0.08	0.23	0.44	0.72	1.04
20.7	0	0.12	0.28	0.51	0.80	1.05
21.6	0	0.07	0.19	0.38	0.51	0.73

Table 3. Time course effect on the growth response of *Aeromonas hydrophila* (a representative of mesophilic bacteria) at different temperatures

Temperature	Growth Optical Density					
(ºC)	0 h	4h	5 h	6 h	7 h	8h
30.3	0	0.09	0.20	0.39	0.55	0.73
31.8	0	0.14	0.28	0.53	0.80	1.10
33.2	0	0.17	0.32	0.58	0.84	1.10
34.6	0	0.13	0.25	0.52	0.73	1.02
35.9	0	0.11	0.27	0.46	0.57	0.82
37.2	0	0.07	0.22	0.37	0.45	0.59

replications in each case. The growth temperature ranges were also recorded though the incubation period was limited. The effect of temperature on the growth of all investigated strains is summarized in table 1. Growth of all the strains was profoundly affected by incubation temperature as a whole. Peak growth temperature (t_{peak}) of *F. psychrophila* strain was 21±0.4ºC. The growth temperature range was 12-24ºC for an incubation period of 20 h. The t_{peaks} for the two strains of *F. columnaris* were 29.8±0.2 and 29.9±0.3ºC, respectively. Growth temperature ranges were 19-33ºC for 18-22 h culture. The tpeaks of *F. branchiophilum* were 24.9±0.2 and 24.1±0.5ºC, respectively, where two strains were tested. A 40-53 h culture showed growth temperature ranges as 12-30ºC. Three strains of *E. tarda* were examined and they showed t_{peaks} at 36.1±0.4, 35.5±0.2 and 36±0.3ºC, respectively. Temperatures 23-41ºC were found as growth ranges for 6 h cultivation period. A strain of *C. freundii* stood at 36.1±0.4ºC for its tpeak. Growth temperature range was 27-41ºC for 5 h. For *A. hydrophila* two strains were tested and it was found that t_{peaks} were 32.9±0.6 and 34.8±0.5ºC, respectively. The growth temperature ranges were 19-42ºC for 6 h. The peak growth temperatures for two strains of *A. salmonicida* were at 26.1±0.4 and 25.6±0.2ºC, respectively. The growth temperature ranges were 17-33ºC for 13-14 h cultures. The temperature 27.8±0.3ºC was found as t_{peak} of *V. anguillarum* strain while *V. ordalii* strain gave t_{peak} at 26.5±0.4ºC. In both cases, growth temperature range was 19-35ºC at 9-11

h incubation period. Peak growth at temperatures of 26.7±0.5 and 27.6±0.4ºC, respectively, was found in *P. damselae* for two strains. After 22-35 h cultivation, growth temperature ranges were between 22 and 32ºC. In *P. fluorescens* peak growth was evident at temperatures of 29.9±0.2 and 28.5±0.3ºC, respectively, for two strains. Temperature ranges for growth were at 18-34ºC after 12-13 h incubation. The strain of *Pseudomonas* sp. (capsulated, fluorescens-like) stood for t_{peak} at 24.9±0.1ºC while the putida-like *Pseudomonas* sp. strain showed it at 29.2±0.5ºC. Capsulated one showed the growth temperature range as 15-29ºC for 11 h while the putida-like gave 23-31ºC at 7 h culture. Temperatures of 29.7±0.4 and 31.1±0.2ºC, respectively, showed the peak growth for two strains of *L. garvieae*. The growth temperature ranges were 16-42ºC for 9-14 h incubation period. A strain of *Streptococcus* sp. gave peak growth temperature at 28.3±0.5ºC and growth range as 15-40ºC for 14 h of incubation.

Discussion

Temperature is a key factor for controlling the rate of development of microbial populations, a modulation of enzyme synthesis by the growth temperature having been observed in several microorganisms (Gugi et al. 1991). Pacha (1968) noted that the growth optimal of *F. psychrophila* was around 20ºC and growth was absent at 30ºC. These results fully coincided with our present findings. According to Holt et al. (1989) optimal growth of *F. psychrophila* was at 15ºC. Nomura & Ohara (1994) noted that the optimum growth temperature of *F. columnaris* was 25ºC. Our results showed a higher optimal growth temperature. The *F. branchiophilum* grew at temperatures between 10 and 25ºC, though some strains grew at a temperature of 5 or 30ºC (Wakabayashi et al. 1989). We observed a lightly higher temperature affinity for the growth, compared to literature.

Our *E. tarda* results followed the findings of Farmer & McWhorter (1984) where they noted the optimum growth temperature as 37ºC. The present *C. freundii* fully satisfied the results in Bergey's manual (Sakazaki 1984). Optimal temperature of *A. hydrophila* is considered to be 28ºC but Statner et al. (1988) mentioned a higher temperature than that. Rouf & Rigney (1971) noted it at 35ºC. These results are parallel to our findings. Popoff (1984) found optimum growth temperature for *A. salmonicida* at 22-25ºC, lower than the present work. Guerin-Faublee et al. (1995) found the optimum temperature range for *V. anguillarum* at 29.7-34ºC. Our

results verily differed with Larsen (1984) where optimum temperature was at 25ºC. The *V. ordalii* exhibited a higher growth temperature than the results, 15-22ºC found by Schiewe et al. (1981). According to Hashimoto et al. (1985) growth optimum temperature range of *P. damselae* was 22.5-30ºC. It is thought that infection takes place in sea water at a temperature of approximately 25ºC (Yasunaga et al. 1983). Our results supported these results. It is speculated that the growth optimal temperature might have some relation to infection. Literature showed that *P. fluorescens* grew well on nutrient agar at 22-25ºC. Putida-like *Pseudomonas* sp. causes bacterial haemorrhagic ascites of ayu, *Plecoglossus altivelis* (Wakabayashi et al. 1996). Capsulated, fluorescence-like *Pseudomonas* sp. was isolated from carp. The peak optimal temperatures in different *Pseudomonas* spp. were varied. Kusuda et al. (1991) stated that *L. garvieae* grew at 10-45ºC. The optimum temperature for the growth of B-hemolytic streptococcus was 25-30ºC (Ohnishi & Jo 1986). For *Streptococcus* sp., Al-Harbi (1994) reported the optimum growth temperature at 30ºC and in our study it was at 28.3±0.5ºC. It is noted that due to incubation time limit, we did not compare the present results of growth temperature ranges with the other findings.

Strains and temperature generally exerted a considerable influence on the growth of bacteria. The impact of temperature on growth of bacteria is in general highly significant. The appropriate temperatures for growth of bacterial fish pathogens differ in their genera and the range was 21±0.4 to 36.1±0.4ºC. The optimum temperatures for most of the examined bacteria were more or less similar to the literature available but some of them varied. In some cases, slightly higher temperatures than that of other reports for optimal growth of bacteria were observed. To some extent, data varied from literature to literature. All these variations may be due to strain variation, host differences, environmental factors, geographical distribution, setting of experiments and extensive sub-culture. The present findings would be helpful for future study to increase the knowledge in bacterial fish pathogens.

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