

Temperature Growth Response of Spoilage Bacteria Isolated from Indian Oil Sardine (*Sardinella longiceps*) Stored in Chilled Sea Water

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The optimum growth temperature of different bacterial genera, important in the spoilage of Indian oil sardine (*Sardinella longiceps*) stored in chilled sea water was studied. Isolates belonging to different bacterial genera, selected from plates incubated at $28 \pm 2^\circ\text{C}$ and $2 \pm 1^\circ\text{C}$, were tested for their growth response at different temperatures. The results indicated that the spoilage bacteria, irrespective of their primary isolation temperatures, showed good growth response only at the temperature ranging from $2 \pm 1^\circ\text{C}$ to $28 \pm 1^\circ\text{C}$, indicating their facultative psychrophilic nature.

In order to know the bacteriological quality of fish stored at refrigerated conditions, a through study of the temperature requirements is necessary. Different incubation temperatures have been recommended for the bacteriological examination of tropical fish (Surendran & Iyer, 1976; Anand & Setty, 1977; Thampuran & Iyer, 1979; Surendran & Gopakumar, 1982; Devaraju & Setty, 1985). However there are difference of opinions with regard to incubation temperatures and techniques to be adopted for the bacteriological examination of tropical fish. Thus, the present investigation was undertaken to study the temperature growth response of different bacterial genera important in the spoilage of fish preserved in chilled sea water.

Materials and Methods

Fresh Indian oil sardines (*Sardinella longiceps Valenciennes*) were transferred to chilled sea water (CSW) immediately after catch. Temperature of the fish was maintained at $2 \pm 1^\circ\text{C}$ throughout the storage period.

Samples of fish were drawn periodically and total plate counts (TPC) were taken on plate count agar containing 0.5% NaCl, using spread plate technique. The plates

were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for two days and another set of plates at low temperature ($2 \pm 1^\circ\text{C}$) for ten days. For the purpose of establishing the temperature growth response of spoilage bacteria, the plates obtained from the 10th day (spoiled fish) sampling of CSW stored fish were used. About 250-300 colonies were picked up, using random tables, from each set of plates incubated at room temperature and low temperature. All these selected isolates were identified up to their generic level making use of the scheme suggested by Shewan *et al.* (1960) and Lechevallier *et al.* (1980). Care was taken not to expose the low temperature isolates to higher temperature, during the whole course of the experiment.

The optimum growth temperatures for representative isolates of major genera were determined qualitatively by streaking them on to nutrient agar (0.5% w/v of NaCl) and incubating at $2 \pm 1^\circ\text{C}$, $8 \pm 1^\circ\text{C}$, $28 \pm 2^\circ\text{C}$ and $37 \pm 1^\circ\text{C}$ for both the sets of isolates, till the appearance of visible colonies. At least 10 to 20 isolates of each genera were used for the study.

Results and Discussion

The initial total plate count of fish was 1.3×10^4 and 2.9×10^5 per g of flesh at the incubation temperatures of $28 \pm 2^\circ\text{C}$ and

$2 \pm 1^\circ\text{C}$ respectively (Table 1). It was noticed that the initial counts at low temperature incubation were less than that at room temperature incubation by nearly one log. It was reported (Surendran & Gopakumar, 1982) that the TPC were markedly affected by the incubation temperatures. They observed that incubation at $28 \pm 2^\circ\text{C}$ gave higher count than at $36 \pm 1^\circ\text{C}$ and $8 \pm 1^\circ\text{C}$. Gillespie & Macrae (1975) also found a difference of nearly one log in the counts at 20°C and 2°C . The lower counts at low temperature incubation in the present study is assumed to be due to the elimination of true mesophiles which failed to grow at this temperature.

Table 1. Total plate counts (cfu/g) of fish during storage in chilled sea water

Temperature of incubation	Days of storage				
	0	2	5	8	10*
$28 \pm 2^\circ\text{C}$	1.3×10^4	7.8×10^4	8.3×10^5	6.5×10^6	4.3×10^7
$2 \pm 1^\circ\text{C}$	2.9×10^3	8.2×10^3	5.7×10^4	7.6×10^5	5.7×10^7

* Fish was unacceptable

The TPC, during the storage of fish in CSW, increased to 4.3×10^7 and 5.7×10^7 per g on the day of spoilage at $28 \pm 2^\circ\text{C}$ and $2 \pm 1^\circ\text{C}$ respectively (Table 1). There was an initial lag period of 2 to 3 days, during which time the rate of increase was very marginal but towards the end of the storage period, the counts at two incubation temperatures were found to be almost equal. This may be attributed to the fact that the bacteria thriving at the end of the storage period were mostly cold tolerant or facultatively psychrophilic. The chilled and almost anaerobic conditions that prevail in CSW medium may eliminate most of the obligate mesophiles, which inhabit the fish through handling, water, ice etc. as contaminants.

The results presented in Table 2 indicate that at an incubation temperature of $2 \pm 1^\circ\text{C}$, all the room temperature isolates belonging to different genera showed visible growth

within 120 to 240 h (5-10 days) except *Alcaligenes* spp. which showed no growth, whereas all the low temperature isolates took 96 to 168 h (4-7 days) except those of *Alcaligenes* spp. and *Vibrio* spp. which took 120 to 192 h. In the case of the incubation temperature of $8 \pm 1^\circ\text{C}$ all the room temperature isolates showed visible growth within 72 to 144 h except 50% of *Alcaligenes* spp. and 100% *Vibrio* spp. which took 120 to 192 h while all the low temperature isolates took 72 to 120 h with the exception of *Vibrio* spp. which took 120 to 144 h. When incubated at $28 \pm 2^\circ\text{C}$, both the room temperature and low temperature isolates belonging to different genera showed good

growth within 24 to 48 h. However, when incubated at $37 \pm 1^\circ\text{C}$, very few genera showed good growth within 24 h, while others showed a poor growth or no growth at all.

Devaraju & Setty (1985), in their study on the bacterial isolates (selected from a primary isolation temperature of $28 \pm 2^\circ\text{C}$) obtained from fishes caught off Mangalore, reported that all the isolates could show visible growth after 9 days at $2 \pm 1^\circ\text{C}$, after 3 days at $8 \pm 1^\circ\text{C}$ and after 1 day at $28 \pm 2^\circ\text{C}$, and at $37 \pm 1^\circ\text{C}$ only the isolates belonging to genera *Moraxella*, *Corynebacterium* and *Alcaligenes* showed growth after a day. They also observed that the optimum growth temperature for majority of the groups was 28°C and their growth was poor at 37°C . This observation is in good agreement with the present work, where all the isolates irrespective of their primary isola-

Table 2 *Growth response of bacterial isolates from oil sardine stored in CSW, to different incubation temperatures*

Bacterial genera	Number of isolates	Temperature of incubation °C	Room temperature Isolates		Low temperature Isolates	
			Period of incubation h	Isolates showing growth, %	Period of incubation, h	Isolates showing growth, %
<i>Moraxella</i> spp.	12	1 ± 1	144-168	100	96-120	100
		8 ± 1	72-120	100	72-96	100
		28 ± 2	24-36	100	24-36	100
		37 ± 1	24	0	24	0
<i>Acinetobacter</i> spp.	16	2 ± 1	120-144	100	120-168	100
		8 ± 1	96-120	100	72-84	100
		28 ± 2	24-36	100	24-36	100
		37 ± 1	24	50	24	25
<i>Pseudomonas</i> spp. Group I	16	2 ± 1	144-166	100	120-168	100
		8 ± 1	96-120	100	72-96	100
		28 ± 2	24-36	100	24-48	100
		37 ± 1	24	25	24	25
<i>Pseudomonas</i> spp. Group II	14	2 ± 1	120-168	100	120-144	100
		8 ± 1	96-120	100	96-120	100
		28 ± 2	24-48	100	24-36	100
		37 ± 1	24	0	24	0
<i>Pseudomonas</i> spp. Group III	20	2 ± 1	120-144	100	120-144	100
		8 ± 1	72-96	100	72-96	100
		28 ± 2	24-36	100	24-48	100
		37 ± 1	24	25	24	0
<i>Pseudomonas</i> spp. Group IV	17	2 ± 1	120-144	100	96-120	100
		8 ± 1	96-120	100	72-96	100
		28 ± 2	24-48	100	24-36	100
		37 ± 1	24	0	24	0
<i>Flavobacterium</i> spp.	20	2 ± 1	192-240	100	120-144	100
		8 ± 1	120-144	100	96-120	100
		28 ± 1	24-48	100	24-48	100
		37 ± 1	24	75	24	20
<i>Alcaligenes</i> spp.	15	2 ± 1	192	0	120-192	100
		8 ± 1	144-192	50	96-120	100
		2 ± 8	124-48	100	24-48	100
		37 ± 1	24	100	24	33.33
<i>Aeromonas</i> spp.	21	2 ± 1	144-192	100	120-168	100
		8 ± 1	96-144	100	96-120	100
		28 ± 2	24-48	100	24-48	100
		37 ± 1	24	100	24	33.33
<i>Vibrio</i> spp.	24	2 ± 1	144 -192	100	120-192	100
		8 ± 1	120-192	100	120-144	100
		28 ± 2	24-48	100	24-48	100
		37 ± 1	24	100	24	75

tion temperatures showed good growth at $28 \pm 2^{\circ}\text{C}$. Anand & Setty (1977) pointed out that the nature of microflora from tropical marine waters was facultatively psychrophilic, since none of their isolates could show growth at 37°C , but all the isolates showed good growth from 0 to 28°C . Subsequently, the facultative psychrophilic nature of tropical marine bacteria was confirmed (Singh, 1978). In the present study also, all the isolates irrespective of primary isolation temperatures showed good growth at $28 \pm 2^{\circ}\text{C}$, $8 \pm 1^{\circ}\text{C}$ and $2 \pm 1^{\circ}\text{C}$, except the room temperature isolates of *Alcaligenes* spp. which showed no growth at $2 \pm 1^{\circ}\text{C}$. Surendran & Iyer (1976) observed that only 30% of their isolates from mackerel were capable of growth at 0°C and Surendran & Gopakumar (1982) observed that only 2.2% of the isolates from oil sardine, 7% from mackerel and 12% from prawn were capable of growth at 0°C .

From the results of this study, it looks evident that the nature of fish spoilage bacteria is facultatively psychrophilic rather than psychrophilic or mesophilic. Also in the enumeration of marine fish bacteria, it is advisable to adopt spread plate or drop plate techniques and incubate the plates at room temperature ($28 \pm 2^{\circ}\text{C}$).

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