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Growth of Cross Contaminating Bacterial Pathogens in Fish Muscle at Different Temperatures of Storage

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Growth of cross contaminant bacterial pathogens such as enteropathogenic *Escherichia coli* (EPEC) and *Salmonella typhimurium* were examined in fish muscle maintained at 5°C, 20°C and 35°C to mimic the different conditions prevailing in the retail fish markets. While the cells hardly grew in fish muscle kept at 5°C, there was 5 log and 4 log increase in number of cells of EPEC and *S. typhimurium* respectively at 35°C. The growth of these cells at 20°C was more gradual and the increase in the number of cells was around 3 log. The results indicate potential danger posed by these contaminant bacterial pathogens to the consumers of temperature abused fish, which is a very common sight in the retail markets of India. Implementation of minimum standards for the fish markets, regular monitoring and enforcement are suggested to ensure food safety.

Key words: ***Escherichia coli, Salmonella typhimurium,*** **Contamination, Temperature, Growth, Fish**

Fish is a widely preferred food item worldwide. In those countries that maintain useful record of food borne diseases, fishery products account for a significant proportion of outbreaks reported. Compared to Gram-positive pathogens, Gram-negative intestinal pathogens such as *Salmonella* spp., pathogenic strains of *E. coli, Shigella* spp., *Vibrio* spp., *Campylobacter* spp., and *Yersinia* spp. are most frequently implicated in outbreaks of food borne illness (Bean & Griffin, 1990).

E. coli is a valuable indicator of faecal contamination. In addition to this, *E. coli* strains especially belonging to enteropathogenic serotypes are known to cause gastroenteritis of children and adults. Based on epidemiological studies throughout the world, *E. coli* is judged to be a significant pathogen in cases of traveller's diarrhoea and in gastrointestinal illness in developing nations and other localized areas characterized by poor personal hygiene.

Fishery products for human consumption must be free from pathogenic microorganisms. The extent of contamination can be reduced by proper preservation of fish and fishery products. Low temperature preservation (icing/refrigeration) is the most common practice in all parts of the world. Hatha & Lakshmanaperumalsamy, (1997) isolated a number of sero types of *Salmonella* from retail fish.markets. During the investigation it was found that the fish were kept without proper icing or chilling . The present study was taken up to find out the growth pattern of enteropathogenic *E. coli* (EPEC) and *S. typhimurium* in fish muscle stored at different temperatures such as 5°C, 20°C and 35°C in order to mimic various situations prevailing in retail fish markets, i.e., well iced, poorly iced and kept at ambient temperature.

Materials and Methods

Fish samples of *Scomberomorus commersoni,* commonly known as Spanish mackerel, collected from local fish landing centre were brought to the laboratory in iced condition.

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Skin surface of fish was decontaminated by wiping with ethanol. The fish was then filleted aseptically using a sharp knife. The fillets were made into pieces and fish muscle cubes weighing about 10 g were transferred into individual sterile petridish.

Two bacterial strains, viz. enteropathogenic *E. coli* and *S. typhimurium*, isolated from market fish samples were used for the study. Serotyping of the strains were done at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh.

The cells were inoculated into nutrient broth (Himedia) and incubated at 37°C overnight. The cells were then harvested by centrifugation at 3000 rpm for 10 min. The cells were washed in sterile isotonic saline and serially diluted to get an inoculum density of 10^2 cfu/ml. Aseptically obtained fish muscles kept in individual sterile petri dishes were surface inoculated with 1 ml of the respective inoculum using sterile pipette. Dishes were randomly allocated into incubation temperatures of 5°C, 20°C and 35°C.

One sample per treatment was withdrawn from respective incubation temperatures at every 2 h till the 10^{th} h. The samples were then homogenized in a sterile tissue homogenizer, serially diluted and 0.2 ml of the samples were spread plated on well dried sterile nutrient agar plates in duplicate. All inoculated plates were incubated at 37°C for 24 h. Uninoculated controls of fish muscles were also tested for sterility of the sample. After the incubation the plates were counted and the results were expressed as cfu/g. Mean was expressed as the average of duplicate platings of each sample using two replicate experiments.

Results and Discussion

Growth of EPEC in fish muscles kept at different temperatures is given in Table 1. The results revealed a 5 log build up of EPEC cells in the fish muscles maintained at 35°C and 3 log build up in those maintained at 20°C. There was no significant growth in the fish muscle maintained at 5°C.

Table 1. Growth of enteropathogenic *Escherichia coli* (EPEC) in fish muscle kept at different temperatures.

Time (h)	No. of *E. coli* (EPEC) cells (cfu/g)		
	35°C	20°C	5°C
0	2.19×10^2	2.19×10^2	2.19×10^2
2	2.14×10^4	3.83×10^3	8.45×10^2
4	6.33×10^4	2.23×10^4	8.37×10^2
6	2.03×10^5	4.18×10^4	1.24×10^3
8	5.54×10^5	7.91×10^4	1.87×10^3
10	2.82×10^7	5.94×10^5	2.60×10^3

Table 2 represents growth pattern of *S. typhimurium* in fish muscle kept at different temperatures. The growth was very rapid at 35°C and was almost stationary at 5°C. The results revealed clear growth of these organisms in fish muscles kept at ambient temperature. There was a build up of population at the rate of one log for every two hours till 8^{th} hour and then there was a build up of 2 log in the next 2 hours, i.e., at the 10^{th} h. The overall build up of the population during the experiment period was 5 log in the case of EPEC and around 4 log in the case of *S. typhimurium*. This is significant as the population at this level can cause definite food borne intoxication if the contaminated and temperature abused fish is consumed without proper cooking.

Table 2. Growth of *Salmonella typhimurium* in fish muscle kept at different temperatures

Time (h)	No. of *S. typhimurium* cells (cfu/g)		
	35°C	20°C	5°C
0	1.25×10^2	1.25×10^2	1.25×10^2
2	4.70×10^2	4.65×10^2	3.12×10^2
4	1.91×10^4	2.65×10^3	4.52×10^2
6	3.82×10^5	2.07×10^4	6.22×10^2
8	3.94×10^6	3.49×10^4	6.32×10^2
10	4.03×10^6	1.70×10^5	6.50×10^2

The comparative growth curves of EPEC and *S. typhimurium* in fish muscle

maintained at 35°C and 20°C is given in Fig. 1 and 2 respectively. The reason for the heavy build up of the cells may be the highly nutritious nature of the fish muscle (Fapohunda *et al,* 1994). The comparison also revealed the immediate acclimatization of *S. typhimurium* cells as evidenced by faster growth than EPEC in the first two hours. The easy acclimatization and rapid growth of *Salmonella* in fish muscles at 35°C is significant as temperature abuse during handling and storage is common (Mayer & Ward, 1991). Furthermore gross unsanitary practices have been reported from markets of tropical countries (Adesiyun & Omi, 1989). The growth of EPEC and *S. typhimurium* at 20°C revealed a similar pattern and showed a gradual acclimatization and growth. The build up of both cells was around 3 log.

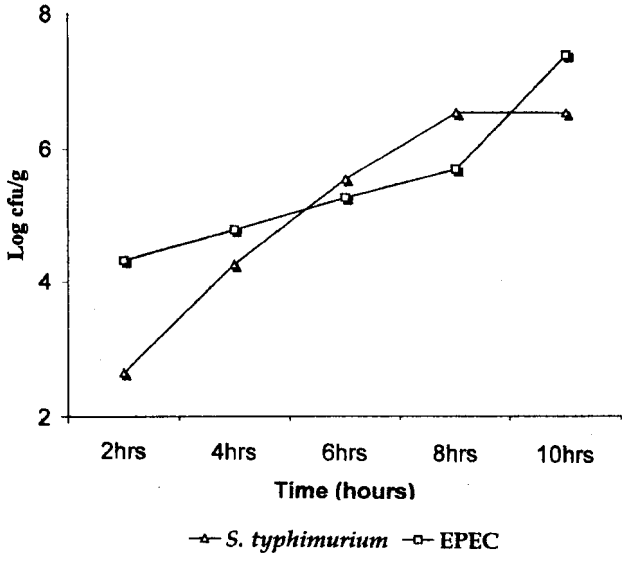

Fig. 1. Growth of enteropathogenic *Escherichia coli* (EPEC) and *Salmonella typhimurium* in fish muscle at 35°C

Compared to the growth pattern of EPEC and *S. typhimurium* at 35°C and 20°C, the growth of these cells was almost negligible (less than 1 log) in the fish muscle maintained at 5°C (Fig.3). This supports the findings that well iced fish muscle offers very little scope for bacterial multiplication (Matches & Liston, 1968; Fapohunda *et al.*, 1994). It is also reported that the minimum temperature at which salmonellae will grow

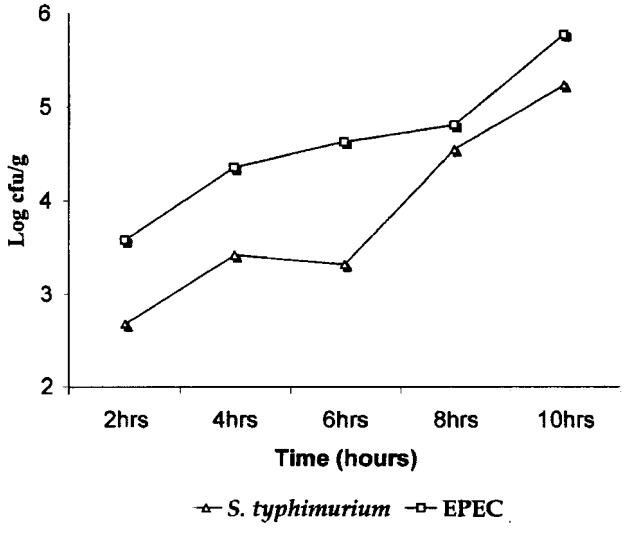

Fig. 2. Growth of enteropathogenic *Escherichia coli* (EPEC) and *Salmonella typhimurium* in fish muscle at 20°C

and increase in number is influenced by the growth medium as well as by competing microorganisms (Matches & Liston, 1968). Salmonellae are capable of growing on seafoods and other foods at 8°C, competing with normal spoilage bacteria and reaching high numbers. As the temperatures decreased towards minimum, the most obvious effect on the growth pattern of salmonellae is an extension of lag phase. The rate of increase of population during lag phase growth is very similar down to the minimum. This was clearly seen in the case of Salmonella, which showed a very short lag

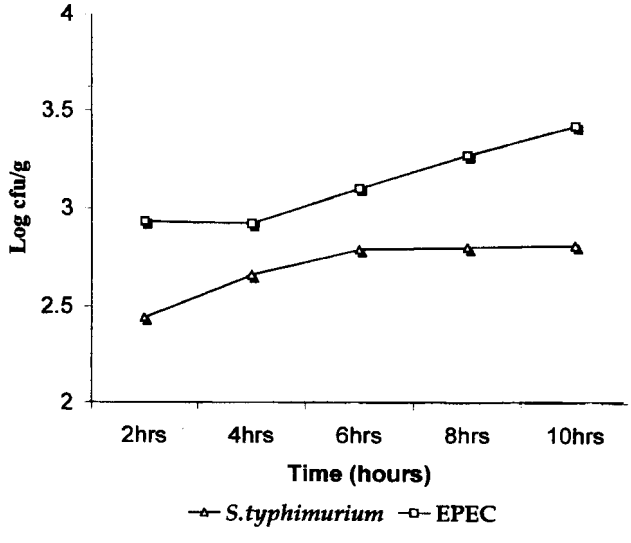

Fig. 3. Growth of enteropathogenic *Escherichia coli* (EPEC) and *Salmonella typhimurium* in fish muscle at 5°C

phase at 35°C and 20°C and then increasing lag phases at lower temperatures. This behavior suggests that some type of metabolic reorganization may be necessary for growth at the low temperatures.

Contamination of seafood by harmful Gram-negative bacteria is of great concern from public health viewpoint. Considering the degree of cross contamination prevailing in the retail markets of developing countries like India, further temperature abuse of the fish can result in increase of the contaminant pathogens to dangerous levels. As fish is becoming a very popular food item, such temperature abused contaminated fish can act as a potent vehicle of food borne infections in this country. Minimum prescribed standards for fish markets to be implemented with immediate effect and constant monitoring and enforcement is required to ensure food safety.

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References

Adesiyun, A.A. & Oni, O.O. (1989) *J. Food Prot.* **52**, 232

Bean, N.H. & Griffin, P.M. (1990) *J. Food Prot.* **53**, 804

Fapohunda, A.O., McMillan, K.W., Marshall, D.L. & Waites, W.M. (1994) *J. Food Prot.* **57**, 337

Hatha, A.A.M. & Lakshmanaperumalsamy, P. (1997) *Food Microbiol.* **14**, 111

Matches, J.R. & Liston, J. (1968) *J. Food Sci.* **33**, 833

Mayer, B.K. & Ward, D.R. (1991) *Microbiology of Fin fish and Shellfish Processing* in D.R. Ward and E.R. Hankreyel (ed.). Microbiology of Marine Products, Van Nostrand Rheinhold, New York

Mead, P.S., Slustker, L., Dietz, V., McCaig, L.F.,Bresee, J.S., Shapiro, C., Griffin, P.M. & Tauxe, R.V. (1999) *Emerg. Infec. Dis.* **5**, 607

Tauxe, R.V. (1991) *J. Food Prot.* **54**, 563