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Listeria spp. in Fish and Fish-Handling Areas, Mangalore, India

Y.B. MANOJ
G.M. ROSALIND
INDRANI KARUNASAGAR
IDDYA KARUNASAGAR

Department of Fishery Microbiology
College of Fisheries
Mangalore - 575 002
India

Abstract - *Listeria innocua, L. grayi, L. seeligeri* and *L. murrayi* were isolated from fish, shrimp and fish contact surfaces. Five of the eight strains isolated required seven-day enrichment. None of the isolates were hemolytic on sheep blood agar. Two strains of *L. grayi* and one strain of *L. innocua* were virulent for mice.

During recent years, *Listeria monocytogenes*, a food-borne pathogen, has become a major concern to the food industry. This organism is of special significance since it can grow at refrigerator temperatures (Doyle 1988). Noting that *L. monocytogenes* was present in trout, Gray and Killinger (1966) suggested that other fish might also be contaminated. So far, only one outbreak of listeriosis has been linked to the consumption of shellfish and raw fish (Lennon et al. 1984). The presence of *L. monocytogenes* in frozen seafood was recorded by Weagant et al. (1988) while Fuchs and Surendran (1989) noted the absence of *L. monocytogenes* in fish and fisheries products from Cochin, India. This paper describes a study of fish and fish-handling areas around Mangalore for the presence of *Listeria* spp.

Samples consisted of different species of fishes and shrimp from the Mangalore fish-landing center, fish market and shrimp-processing units around Mangalore, swabs from decks of fishing vessels, fish-carrying baskets, floors of shrimp-processing units and other shrimp-contact surfaces in these units. The U.S. Food and Drug Administration method (Lovett 1988) was used for isolation. Briefly, 25-g samples were homogenized in 225 ml selective enrichment broth and incubated for seven days at 30°C. Swab samples were dipped in

enrichment broth and incubated. At 24 hours and seven days, undiluted cultures and cultures diluted 1/10 in 0.5% KOH were streaked on modified McBride's *Listeria* agar and incubated at 35°C for 24 hours. Colonies appearing blue or bluish gray under incident light were picked out, purified and subjected to biochemical tests. *L. monocytogenes* NCTC 11994 was used as a reference culture. Species differentiation was made using the criteria described by Lovett (1988).

All the *Listeria* isolates were tested for their pathogenicity to mice. One ml of 24-hour culture in Brain Heart Infusion (BHI) broth or tenfold dilutions thereof in the same medium was injected intraperitoneally into six 20-g white mice (IISc strain) and observed for one week. LD_{50} values were calculated by the technique of Reed and Muench (1938) wherever undiluted culture caused death in more than 50% of the mice. Strains causing death in less than 50% mice on injecting undiluted cultures were regarded as having low pathogenicity and strains causing death in none of the mice were regarded as nonpathogenic.

As shown in Table 1, *Listeria* spp. were isolated from two of the fifty-one fish samples, two of nineteen shrimp samples and four of the fifteen swab samples. *L. innocua, L. seeligeri, L. grayi* and *L. murrayi* were the species encountered. *Listeria* spp. were isolated from all

Table 1. Incidence of *Listeria* spp. in various samples.

Site and samples	No. of samples	No. positive for *Listeria*	Species of *Listeria* isolated
Fish-landing center			
Fish	12	Nil	
Shrimp	1	Nil	
Swabs	6	1	*L. innocua*
Fish market			
Fish	39	2	*L. seeligeri*
Shrimp	2	Nil	*L. grayi*
Processing plant			
Shrimp	6	1	*L. murrayi*
Processed shrimp	10	1	*L. innocua*
Swabs	9	3	*L. grayi*
Total	95	8	

three sites examined *viz.*, fish-landing center, fish market and shrimp-processing plants. Of the eight strains isolated, four were *L. grayi,* suggesting that this might be the commonest species associated with tropical seafood, followed by *L. innocua* in two samples.

Apart from *L. monocytogenes,* the only other *Listeria* species suspected to be involved in human disease is *L. ivanovii* (WHO 1988). Both these species were absent in the samples examined. Fuchs and Surendran (1989) observed only *L. innocua* in ten of thirty-five samples of tropical fish and fisheries products. Results in Table 2 suggest that three of our isolates are pathogenic to mice; two were *L. grayi* and one *L. innocua.* Of particular significance is the virulence of *L. grayi* isolated from a shrimp-grading tray.

Table 2. Characteristics of *Listeria* species isolated.

Species	Source	Enrichment time required for isolation (days)	Pathogenicity to mice
L. innocua	Fish-carrying basket	7	Low pathogenicity
	Processed shrimp	1	LD_{50} 2.0×10^9 cells
L. grayi	Floor of processing plant	7	LD_{50} 1.5×10^8 cells
	Tank holding water	7	Nonpathogenic
	Grading tray	7	LD_{50} 4.4×10^7 cells
	Fish	7	Nonpathogenic
L. seeligeri	Fish	1	Low pathogenicity
L. murrayi	Raw shrimp	1	Nonpathogenic

Factors contributing to the virulence of these strains are not known. In the case of *L. monocytogenes,* a cytolytic hemolytic listeriolysin has been implicated as an important virulence factor (Chakraborty and Goebel 1988). Leimeister-Wachter and Chakraborty (1989) demonstrated that listerio-lysin is produced also by *L. seeligeri.* In view of this, the pathogenicity of *Listeria* spp. needs to be evaluated. None of the strains isolated in this study produced hemolysins.

Detection of *Listeria* spp. in this study is highly significant since there is a newly developed gene-probe technique assay for all *Listeria* spp. in food and environmental samples (Klinger et al. 1988). It has been suggested that *Listeria* other than *L. monocytogenes* may be present as indicator organisms (King et al. 1989).

Results in Table 2 also emphasize the need for seven-day enrichment for isolation of *Listeria* spp. from seafood. Five of the eight strains isolated were after seven-day enrichment. Fuchs and Surendran (1989) also made a similar observation with tropical fish and fisheries products.

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