

M.S.76. –ROSAMMA PHILIP–Studies on proteolytic bacteria in fish spoilage–1987–Dr. P. Lakshmana Perumal Swamy

Total heterotrophic and proteolytic bacterial population of water, sediment, prawns (*Penaeus indicus* and *Metapenaeus dobsoni*), clams (*Sunetta scripta* and *Meretrix casta*) and fishes (*Etroplus suratensis* and (*Liza parsia*) were estimated for a period of one year. Analysis of the generic composition of the total heterotrophs showed that among the 1315 isolates majority were *Vibrio* (48.14%) followed by *Micrococcus* (13.23%) and *Pseudomonas* (10.95%). *Vibrio* was the dominant genus in water, sediment, prawns and fishes. In clams, besides *Vibrio*, the predominance of Enterbacteriaceae and *Micrococcus* was noticed. In general among the proteolytic forms also *Vibrio* was the dominant genus in the various samples followed by *Pseudomonas*, *Bacillus* *Micrococcus* and Enterbacteriaceae.

The spoilage potential of all the proteolytic bacteria were tested in various flesh media (prawn, clam and fish). It was observed that 91.73%, 75.33% and 32.02% of the isolates were capable of increasing the PH of the prawn, fish and clam flesh respectively. A majority could develop acidity in clam flesh and some maintained neutral PH in all the three media. An average of 71.21% were able to form ammonia in the flesh media. 54% of the proteolytic bacteria were capable of reducing TMAO to TMA - and 18.13% produced indole.

Four potential spoilers were selected for further study. They were *Pseudomonas* sp Ca 173, *Pseudomonas* sp Ca 386, *Vibrio* sp Ca 377 and *Vibrio* sp Ca 761. Generation time of these isolates were found to be comparatively shorter in the various flesh media depicting its fast multiplication rate and thereby reflecting the spoilage calibre. Growth and survival of these strains at different temperature, PH and NaCl in various media such as prawn, clam and fish flesh broth and nutrient broth were studied. Maximum survival was observed in different media for different strains and this suggest the varying protective effect offered by the different media.

Effect of temperature, PH and NaCl concentrations on the growth and protease

formation was noted. All the four isolates grew well and produced maximum protease at 30°C except *Pseudomonas* sp. Ca 173 which preferred 35°C. Optimum PH for growth and protease production was PH7. Maximum growth was observed at 1% and 2% and NaCl for both *Pseudomonas* spp and *Vibrio* spp. respectively. However, the protease production was maximum at 0% NaCl. Study on the time course of growth and protease production at different PH, temperature and NaCl concentrations showed that the enzyme production starts considerably when the cells enter the stationary growth phase.

The maximum protease activity was displayed at different temperature (30-50°C) by various strains. The protease of all the four strains were stable at 0 to 40°C for 120 M. At 50°C the proteases of *Pseudomonas* sp. Ca 173 and *Vibrio* sp. Ca 377 were stable. The optimum PH for the protease activity of the four isolates was around 7. All the proteases were generally stable at PH 6 to 8. Sodium chloride plays a major role in the activity and stability of the proteases. The protease of *Pseudomonas* sp. Ca 173 and *Vibrio* sp. Ca 377 did not show much variation in the enzyme activity with the addition of NaCl (upto 5%) and was found to be stable at this range where as *Pseudomonas* sp Ca 386 and *Vibrio* sp. Ca 761 were severely affected with the addition of NaCl and the maximal activity was observed at 0% NaCl.

Growth and flesh protein degradation by the selected strains were studied using prawn, clam and fish flesh media and casein media. The strains preferred different media for their maximum growth and protein degradation. During the first 12 hrs. of incubation, the growth of all the strains was found to be maximum in the prawn flesh broth which might be due to the increased level of free amino acid. It was further noticed that the protein degradation commenced considerably on the third day and progressed rapidly during the following days marking the first 2 days as a phase of inactivity as far as the proteolysis is concerned.

The present investigation suggests that majority of proteolytic bacteria associated with prawns, clams and fishes are potential spoilers and at ambient conditions they may cause serious damage to the flesh and bring down the nutritional quality of seafoods.