M.S.57. CHANDRASEKARAN, M.–Studies on the Microbial spoilage of Penaeus Indicus'–1985–Dr. D. Chandramohan and Dr. Lakshmana Perumal Samy, P.

The present investigation was carried out mainly to build up the knowledge on the role of commensal bacteria present on the prawns during storage at various temperatures. The main objectives of the study includes evaluation of sopilage in raw untreated penaeid prawns Penaeus indicus, immediately after catch, at room temperature and at 4°C and −18°C, changes in the proximate composition during spoilage, isolation and characterization of bacterial flora and their growth kinetics.

The thesis contains five major chapters. First chapter forms the Introduction part covering preface, review of relevant literature and research approach. Second chapter presents in detail the Materials and Methods employed in the present investigation - Third chapter gives an exhaustive account of the results of the various studies conducted in the present investigation. Fourth chapter forms the Discussion part and fifth chapter presents the Summary. Apart from these, references cited and list of publications are also included.

In the preface section, the relevance of undertaking the present investigation, in the lights of commercial importance of *Penaeus indicus* in the Seafood Industry and the need for a study to find out the role of commensal bacteria in spoilage is discussed. The Review section presents the quantum of scientific knowledge accumulated in the past on spoilage studies carried out with regard to microbiology of *Penaeus indicus* in and out of India. The research approach section highlights the main objectives of the present study which includes (1) evaluation of spoilage of prawns by organoleptic assessment, estimation of Total Heterotrophic Bacterial

Population (THB), trimethylamine (TMA), Ammonia and pH, (2) changes in the proximate components such as protein, lipid, carbohydrates, ash and moisture content, (3) generic composition of THB, (4) hydrolytic enzyme producing heterotrophs, (5) determination of spoilage potential of bacteria, (6) growth kinetics and (7) mixed population studies.

In the second chapter 'Material and Methods', details regarding the sample; collection, transportation and treatment of sample; procedures for organoleptic assessment; THB; TMA; ammonia; pH; isolation; identification; testing of hydrolytic enzyme production; determination of spoilage potential in flesh media and Trimethylamine oxide (TMAO) reduction to Trimethylamine (TMA) are presented. Also, methods by which, effect of temperature, pH and sodium chloride concentration on growth and survival of selected strains; generation time and behaviour of mixed cultures in flesh borth studied are described in detail.

The third chapter which forms the 'Results' gives an account of the major findings of the present investigation. *Penaeus indicus* was used in the present study in four types namely 'whole', 'Headless', 'Peeled and undeveined' (PUD) and 'Peeled and deveined' (PD). They were stored as raw unprocessed prawns at 28 ± 2°C, 4°C and −18°C. Organoleptic assessment suggested rapid spoilage of all the four types of prawns by 8 hours at 28 ± 2°C, by 10 days at 4°C and 100 days at −18°C. THB was found to be increased to a maximum level exceeding a log of 10^9 at 28 ± 2°C and 4°C and a log of 10^8 at −18°C, in all the four types of samples. TMA was found to be increased significantly from O u g/g to around 20.87 ug/g at 28±2°C, 20.59 ug/g at 4°C and 16.66 ug/g at −18°C in all the samples. Ammonia level also increased from O ug/g to around 298.4 ug/g at 28 ± 2°C, 576.8 ug/g at 4°C and 280.3 ug/g at −18°C for the samples. The level of pH, which was initially 6, was found to be increased to around pH 7.55 at 28 ± 2°C, pH 8.55 at 4°C and pH 8.5 at −18°C for the samples.

Protein, lipid, carbohydrate and ash contents showed significant reduction. Also a significant increase of moisture from their initial level in the samples at all the three storage temperatures, was recorded.

Species of *Pseudomonas, Vibrio, Acinetobacter, Micrococcus, Bacillus, Corynebacterium* and members of Enterobacteriaceae were found to constitute the bacterial flora in the fresh prawns. During storage at 28 ± 2°C, *Vibrio* sp showed dominance over *Pseudomonas, Acinetobacter* and others in all the samples. At 4°C *Pseudomonas* showedd dominance followed by *Vibrio* and *Acinetobacter*. At −18°C *Pseudomonas, Acinetobacter* and *Micrococcus* showed dominance at various occasions followed by *Vibrio* and *Bacillus*. Among the various hydrolytic enzyme producers proteolytic forms were dominant followed by lipolytic, amylolytic and ureolytic forms at all the three storage temperatures. Among the 219 isolates used for the determination of potential spoilers in flesh media about 63% of the isolates were found to be potential spoilers. Species of *Vibrio, Pseudomonas* and *Acinetobacter* formed the major flora. Among 178 isolates tested for TMAO - TMA reduction, 92.1% of the isolates were TMAO reducers. Species of *Vibrio, Pseudomonas* and *Acinetobacter* were found to be the major TMAO reducers.

Eight isolates representing species of *Pseudomonas, Vibrio* and *Acinetobacter* were studied for their growth kinetics. They were tested in flesh broth and Zopell's broth. When tested for the effect of temperature, pH and NaCl conc. on growth and survival, it was observed that they grow well at temperatures

between 15°C and 45°C, pH 6 and pH 10 and at 1 - 6% NaCl conc. irrespective of the culture media. They survived well at 45°C and 5°C than at −15°C and 60°C and at pH 6 - pH 8.6 and at 0 - 6% NaCl conc. However their percentage of survival decreased with increase of time. When tested for the effect of temperature, pH and NaCl conc. Combined with each other at various levels on the growth of bacteria, it was found that all the isolates could grow at pH 6 – 10 at alll the temperatures (5°, 15°, 30° and 45°C) and at moderate sodium chloride conc. However incubation at 30°C with 1% and 3% NaCl conc. and pH 6 and pH 8 in the media was observed to favour the growth of almost all the isolates than any other combination. It was observed that generation time of the isolates was not same in both the media viz. flesh broth and ZoBell's, tested. They varied according to media for each strain.

When strains of different species were mixed together at various inoculum size and grown in flesh broth the recovery of individual strains were observed to be influenced by the type of species than the inoculum size. When *Pseudomonas* sp and *Vibrio* sp, isolated from prawns stored at 28+2°C were mixed, *Vibrio* sp was recovered in higher percentage. Similarly, when *Pseudomonas* sp, *Vibrio* sp. and *Acinetobacter* sp, isolated from prawns stored at 4°C, were mixed at various proportion, on most of the occasions *Pseudomonas* sp out numbered others.

The fourth chapter includes the discussion of the results obtained in the present investigation drawing an insight on the facts and features of various factors governing spoilage and related studies undertaken.

Fifth chapter summarises the whole thesis work in a nutshell.