# Succession of Bacterial Genera During Iced Storage of Three Species of Tropical Prawns, *Penaeus indicus, Matapenaeus dobsoni* and *M. affinis**

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The native bacterial flora of ocean fresh tropical prawns, *Penaeus indicus, Metapenaeus dobsoni* and *M. affinis* was more or less similar, mainly consisting of *Pseudomonas, Acinetobacter, Moraxella* and *Arthrobacter*. A definite succession of bacterial genera during iced storage was observed in these prawns. As the day of ice storage increased, the proportion of *Acinetobacter* and *Moraxella* also increased considerably and consituted 70–78% of the flora at the time of spoilage. Spoilage by *Pseudomonas* was very insignificant in prawns under iced storage.

Iced storage is one of the most prevelent methods of retarding spoilage of fish and shell fish in India. By using ice as preservative, the rate of growth of bacteria originally present on the fish and shell fish is delayed or slowed down. After a few days of storage, the lag phase of growth of bacteria present on fish/shellfish passes into the logarithmic phase, which by the 12th to 14th day, when the bacterial count per gram muscle has increased by some thousand to ten thousand times, passes in to the stationary phase (Shewan, 1961). During these phases, marked changes occur not only in the numbers, but also in the types of the bacteria present.

A lot of studies have been reported regarding the development of successive bacterial groups during the iced-storage of fish, both from colder regions and tropical seas. (Castell *et al.*, 1959; Shewan & Hobbs, 1967; Shewan, 1961; Surendran, 1980; Surendran & Iyer, 1976; Surendran & Gopakumar, 1981, 1982). Similar work on shellfish are rather scanty. Qualitative changes in the bacterial flora of Gulf of Mexico shrimp during icedstorage have been studied by Campbell & Williams (1952). Changes in the flora during storage of scampi *(Nephrops norvegicus)* from North Sea were reported by Hobbs *et al.* (1971) and Walker *et al.* (1970). In tropical prawns from Mosambique, Cann (1974) found that the spoilage flora after 12 days in ice consisted of 67% *Achromobacter*. Surendran & Gopakumar (1981, 1982) have given some preliminary information on the spoilage flora of the Indian prawn *M. dobsoni*. In this paper, the succession of the bacterial genera during iced storage in three commercially important species of tropical Indian prawns are presented.

*Formed part of the Ph.D. Thesis of the first author (University of Kerala, Trivandrum)

## Materials and Methods

Prawns, *Penaeus indicus, Metapenaeus affinis* and *M. dobsoni* caught by trawl nets off Cochin were collected, washed with seawater, transferred to sterile glass containers and kept under ice, until brought to the laboratory (usually within 3–6 h). Prawns for storage studies, were packed in ice, (prawn to ice ratio 1:1) in thermocole insulated boxes. During the 20 days storage, ice losses were made up by addition of ice, usually on alternate days. Total viable bacterial counts (TPC) were estimated on the first day and subsequently at intervals of 3–5 days during storage. TPC was determined using seawater agar medium (SWA). The plates were incubated at 28 ± 1°C for 3 days and counts taken. Cultures were isolated from TPC plates, morphologically and biochemically characterised and classified as described by Surendran (1980) and Surendran & Gopakumar (1981).

## Results and Discussion

The initial bacterial load on all the three species was in the range of $10^3$ to $10^4$/g muscle, the bacterial load on *P. indicus* being the lowest and that on *M. affinis* the highest (Fig. 1). During iced storage,

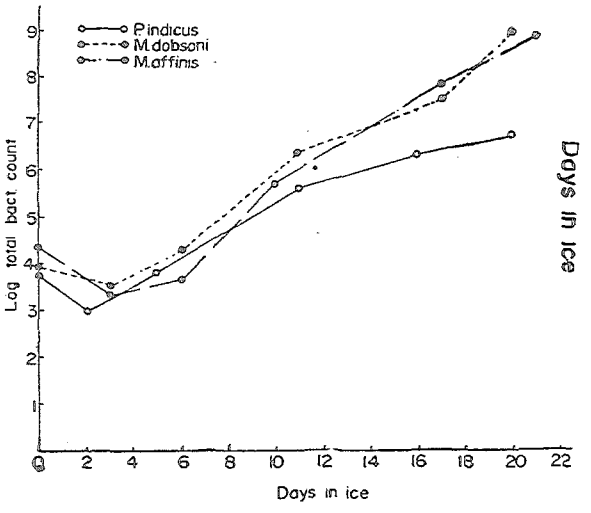


Fig. 1. Changes in the total bacterial count of *P. indicus; M. dobsoni* and *M. affinis* during iced storage.

there was an initial decline in the bacterial counts, due to death of the cold sensitive species of bacteria. But, subsequently, the bacterial counts gradually increased and by about 10 to 14 days, the total count surpassed the one million mark $g^{-1}$ muscle. The increase was rapid in *M. dobsoni* and *M. affinis,* where as, it was slow in *P. indicus.* Also, by about 9 to 10 days of iced-storage, spoilage started in *M. dobsoni* and *M. affinis,* where as, *P. indicus* did not show any spoilage up to 12 days. After 16 to 20 days of storage, all the three species were completely spoilt.

The succession of bacterial genera during, iced-storage in *P. indicus, M. dobsoni* and *M. affinis* is presented in Tables 1, 2 and 3 respectively. The initial flora of ocean fresh *P. indicus* consisted of *Pseudomonas* (12%), *Acenetobacter* (24%), *Moraxella* (28%), *Vibrio* (5%), *Flavobacteria/Cytophaga* (6%) and *Arthrobacter* (12%). The native flora of *M. dobsoni* and *M. affinis* was more or less similar, the major genera being *Pseudomonas, Acenetobacter, Moraxella* and *Arthrobacter, Vibrio* predominating in *M. dobsoni.*

**Table 1.** *Successive changes in the bacterial flora of P. indicus during iced storage*

| Bacterial genera | % of the microorganisms at different intervals during iced-storage, | | | | | |
|---|---|---|---|---|---|---|
| | Days in ice | | | | | |
| | 0 | 3 | 6 | 11 | 17 | 21 |
| *Pseudomonas* | 12 | 8 | 12 | 16 | 22 | 18 |
| *Acenetobacter* | 24 | 24 | 30 | 28 | 34 | 38 |
| *Moraxella* | 28 | 26 | 28 | 32 | 36 | 40 |
| *Vibrio* | 5 | 5 | 2 | 2 | 2 | 0 |
| *Flavobacteria/ Cytophaga* | 6 | 8 | 6 | 2 | 0 | 0 |
| *Micrococcus* | 2 | 2 | 4 | 2 | 0 | 0 |
| *Arthrobacter* | 12 | 10 | 10 | 6 | 2 | 2 |
| Others | 11 | 17 | 10 | 12 | 4 | 2 |
| No. of cultures identified | 99 | 94 | 54 | 48 | 51 | 46 |

**Table 2.** *Successive changes in the bacterial flora of M. dobsoni during iced storage*

| Bacterial genera | % of the microoragnisms at different intervals during iced-storage, | | | | | |
|---|---|---|---|---|---|---|
| | Days in ice | | | | | |
| | 0 | 4 | 7 | 12 | 16 | 20 |
| *Pseudomonas* | 10 | 8 | 14 | 15 | 14 | 16 |
| *Acenetobacter* | 16 | 20 | 18 | 27 | 27 | 32 |
| *Moraxella* | 22 | 20 | 27 | 36 | 39 | 38 |
| *Vibrio* | 16 | 12 | 12 | 8 | 4 | 4 |
| *Flavobacteria/ Cytophaga* | 7 | 8 | 6 | 4 | 0 | 0 |
| *Micrococcus* | 8 | 8 | 5 | 2 | 3 | 2 |
| *Arthrobacter* | 14 | 10 | 8 | 0 | 1 | 1 |
| Others | 7 | 14 | 12 | 8 | 12 | 7 |
| No. of cultures identified | 60 | 62 | 52 | 56 | 70 | 67 |

The native flora changed significantly during iced-storage. The proportion of *Acenetobacter* and *Moraxella* steadily increased on storage and by the time, the prawns began to spoil, *Moraxella* and *Acenetobacter* together constituted the major group forming 78% in *P. indicus,* 70% in

Table 3. *Successive changes in the bacterial flora of M. affinis during iced-storage*

| Bacterial genera | % of microorganisms at different intervals during iced-storage | | | | | |
|---|---|---|---|---|---|---|
| | Days in ice | | | | | |
| | 0 | 3 | 7 | 10 | 15 | 21 |
| *Pseudomonas* | 10 | 12 | 16 | 20 | 18 | 20 |
| *Acenetobacter* | 24 | 24 | 30 | 32 | 36 | 30 |
| *Moraxella* | 22 | 18 | 28 | 36 | 34 | 44 |
| *Vibrio* | 8 | 8 | 4 | 0 | 2 | 0 |
| *Flavobacteria/ Cytophaga* | 6 | 8 | 6 | 2 | 0 | 0 |
| *Micrococcus* | 8 | 12 | 6 | 4 | 4 | 2 |
| *Arthrobacter* | 14 | 10 | 6 | 2 | 0 | 0 |
| *Others* | 6 | 8 | 4 | 4 | 6 | 6 |
| No. of cultures identified | 49 | 54 | 46 | 51 | 57 | 46 |

*M. dobsoni* and 74% in *M. affinis*. Initially *Pseudomonas* ranged between 10 to 12% which gradually reached 16 to 20% of the total flora at the time of spoilage. This indicates that *Pseudomonas* is less significant in the spoilage of ice-stored tropical prawns. *Vibrio, Flavobacteria/ Cytophaga* and *Arthrobacter* gradually decreased and finally disappeared by the time the prawns began to show symptoms of spoilage.

The succession of bacterial flora of the Gulf of Mexcio shrimp during iced storage was found to be similar to the changes observed by the authors in this study. In Gulf of Mexico shrimp, irrespective of the composition of initial flora being heterogeneous in character, the flora of shrimp after 16 days in ice was composed of 82% of *Achromobacter* (the present *Acinetobacter-Moraxella* group) and 16.5% of *Pseudomonas* (Campbell & Williams, 1952). Also, Walker *et al.* (1970) have found that even though 80% of the initial flora of the Dublin Bay prawn *(Nephrops norvigicus)* was constituted by 'coryneforms', the flora changed during iced storage and finally consisted of 70% *Achromobacter* spp. and 8% *Pseudomonas*. In tropical prawns from Mosambique (Cann, 1974) the native flora underwent successive changes and after 12 days in ice, consisted mainly of *Achromobacter* (67%), *Coryneforms* (19%) and *Flavobacteria/Cyptophaga* (8%). Similarly, the Malasian prawns after 12 days in ice contained 48% *Achromobacter*, 41% *Coryneforms* and 8% *Pseudomonas*.

The succession of the flora of the Indian prawn *P. indicus, M. dobsoni* and *M. affinis* during iced storage, leading to the development of the *Moraxella - Acinetobacter* group (the earstwhile *Achromobacter* group) as the main spoilage flora, is different from the flora of ice stored Gulf of Thailand prawn (*Penaeus* spp.), where 90% composed of *Pseudomonas* spp. and only 4% by *Achromobacter* spp. (Cann, 1971). In Indian prawns, 18% of the flora of *P. indicus*, 16% of *M. dobsoni* and 20% of *M. affinis* were constituted by *Pseudomonas* spp. at the time of spoilage under iced storage.

The succession of bacteria in ice-stored prawns is quite different from that found in fish. In oil sardine and mackerel from the same waters, even though their initial flora was a heterogenous population consisting of *Pseudomonas, Moraxella, Acinetobacter, Vibrio, Micrococcus* and *Flavobacteria/Cytophaga*, the predominant flora at the time of spoilage was constituted by *Pseudomonas* alone (Surendran & Gopakumar, 1981 & 1982). In fishes from cold and temperate waters also, the inital flora consisting of *Pseudomonas, Achromobacter, Flavobacterium, Coryneforms* and *Micrococcus* underwent significant change during iced storage and at the time of spoilage, *Pseudomonas* was the predominant group (Shewan *et al.*, 1960; Shewan & Stewart, 1958).

The significant difference noted in the spoilage flora of prawns and fish would indicate that the end products of bacterial spoilage in both cases might be both qualitatively and quantitatively different. However, more detailed study is necessary to ascertain this.

The authors are thankful to the Director, Central Institute of Fisheries Technology, Cochin - 682 029 for the facilities provided for the work.

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