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BACTERIOLOGICAL CHANGES IN KARACHI COASTAL WATER SHRIMP (*PENAEUS MERGUIENSIS* AND *METAPENAEUS MONOCEROS*) DURING ICE STORAGE

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Changes occurring in total bacterial populations and in generic composition of bacterial flora of two shrimp species *(Penaeus merguiensis* and *Metapenaeus monoceros)* during ice storage period of 20 days were studied. No significant quantitative or qualitative bacterial differences were observed between the two species of the shrimps during storage. The total bacterial population increased from 10^6 to 10^9 CFU (colony forming unit)/g after 20 days. Irrespective of the initial flora, the flora that emerged at the end of the storage was remarkably similar. In both species of the shrimp *Vibrio, Micrococcus, Pseudomonas* or *Moraxella* genera were initially dominated. During storage *Vibrio* spp. disappeared and *Alteromonas* increased steadily to become the dominant genus.

At the end of the storage period *Alteromonas putrefaciens, Pseudomonas* Groups II and III, *Moraxella* and *Micrococcus* constituted the bulk of the flora of the stored shrimp species.

INTRODUCTION

Microbial activity is one of the main causes of quality deterioration of shrimp. Information on the changes in numbers and types of microbial populations during refrigerated storage may contribute to a better understanding of the role of microorganisms in the process of spoilage.

Several reports are available on microbiological and/or chemical changes in shrimp species stored in ice for various lengths of time [1–10].

It was reported by Campbell and Williams [1] that during storage of the Gulf of Mexico shrimp, *Micrococcus* and *Flavobacterium* decreased and *Achromobacter* increased steadily. Shewan [11] reported that during the storage of herring and haddock fillets, the mesophilic population was suppressed and a psychrophilic population increased which was dominated by *Pseudomonas* and *Achromobacter.* Cobb and Vanderzant [6] showed that when different microbial species previously isolated from shrimp were reinoculated into shrimp *(Penaeus setiferus)* different spoilage patterns were produced.

In a study [12] of freshly caught *Jaira (Penaeus merguiensis)* and *Kalri (Metapenaeus monoceros)* shrimp, the main flora was found to be composed of *Vibrio, Micrococcus, Pseudomonas* and *Moraxella* species. A seasonal effect is noticeable in the presence of different groups of organisms. Mesophilic *Vibrio* dominates during May through July and October which are the hottest months of the Karachi summer (24-36°) while *Pseudomonas* and *Moraxella* form the bulk of flora during December, through February, the coldest months of the Karachi winter (10-27°). The temperature of water, from which these shrimps were derived ranges between 25 and 30.5° throughout the year [13].

The purpose of this study was to determine the effect of the initial microflora on the ultimate flora that emerges during ice storage. Since the flora may vary during different seasons, the study was extended to cover the whole year in order to derive some tentative conclusions about the spoilage flora of the test shrimp species.

MATERIALS AND METHODS

Shrimp samples. Two shrimp genera of commercial importance *Penaeus merguiensis* locally known as *Jaira* and *Metapenaeus monoceros* locally known as *Kalri* were selected for study. A total of 12 samples of *Jaira* and 8 samples of *Kalri* were collected between June 1980 and June 1981 at approximately monthly intervals. The samples of both shrimp were collected directly from fishing boats

from the same source, Ibrahim Hydri, a small harbour at Karachi. Fishermen here, generally make short trips, leaving in the morning and returning at 4-5 pm in the afternoon. The whole trip does not take more than 10 hrs. The uniced shrimp were packed in ice immediately after purchase and transported to the laboratories. Beheaded shrimp were packed in perforated polythene bags with Jaira and Kalri, packed separately. These bags containing 50-100g were covered with sterile crushed ice and stored in the refrigerator for 20 days. The shrimp were constantly checked for ice during storage period and reiced when required.

Media preparation. Diluent was prepared by adding 0.1% peptone and 0.9% NaCl to distilled water. Nutrient agar (Merck) with 1% NaCl added was used for the plating medium and agar slants. All media and diluent were heat sterilized at 121°C for 15 minutes.

Bacteriological analysis. The samples of Jaira and Kalri were removed for bacteriological examination after 0, 4, 8, 12, 16 and 20 days of storage, 50 grams of shrimp samples were removed and blended with 450 ml diluent and 0.1 ml of appropriate dilutions were spread evenly over the surface of nutrient agar with bent glass rods. Duplicate plates were incubated at 25°C for 48 or 72 h.

Identification of organisms. At least 20-30 colonies were picked at random from appropriate plates for each sample. Isolated colonies were streaked on nutrient agar slants with 1% NaCl, identified as described previously [12] and percentages of various genera were determined.

RESULTS AND DISCUSSION

Quantitative aspects. Table 1 shows the changes in bacterial counts during ice storage of Jaira. The count of fresh shrimp ranged from 5.3×10^5 to 8.3×10^7 CFU/g (Median 3.1×10^6 CFU/g). In general the counts increased with the time of storage. After 4 days the count was 3.1×10^5 to 5.4×10^7 CFU/g showing a slight decrease in half of the samples, while the remaining samples showed slight to one log increase as expected. This increase and decrease in bacterial counts may be due to the nature (psychrotrophic and/or mesophilic) of initial flora. After 8 days storage, the count ranged between 2.8×10^5 and 4.9×10^8 CFU/g with a 2 log increase in most of the samples when compared with the 0 day counts. However, 2 of the 12 samples showed a decrease in the count. The same was true, upto some extent, after 12 days of storage. The count ranged from 6.4×10^5 to 8.8×10^8 CFU/g. After 16 days, a general increase in the count was obtained in all the samples, except No. 2 which decreased to 2.5×10^4 CFU/g from 6.4×10^5 CFU/g obtained after 12 days of storage. After 20 days storage almost a steady state was noticeable showing very little or no change in bacterial count during the third week of storage. The counts ranged from 8.4×10^6 to 3.5×10^{10} CFU/g. Samples No. 2 & 3 showed a 1 log increase in the count while sample No. 8 showed a decrease in the count which may be due to the death phase of some organisms.

Table 2 shows the changes in bacterial count during ice

Table 1. Changes in bacterial count/g of *Jaira* shrimp *(Penaeus merguiensis)* during ice storage

Experiment S.No.	Months 1980-81	Days of storage					
		0	4	8	12	16	20
1.	Jun.	3.2×10^7	2.5×10^6	1.4×10^8	7.2×10^8	3.9×10^9	*X
2.	Jul.	5.3×10^5	3.1×10^5	2.8×10^5	6.4×10^5	2.5×10^4	8.4×10^6
3.	Sep.	9.8×10^5	2.4×10^6	1.4×10^7	3.1×10^7	4.0×10^7	1.6×10^9
4.	Oct.	2.8×10^6	5.3×10^6	2.7×10^7	X	X	X
5.	Nov.	2.1×10^6	4.7×10^6	2.0×10^7	8.4×10^7	7.0×10^8	4.7×10^9
6.	Nov.	3.7×10^7	1.3×10^7	6.4×10^7	2.2×10^8	2.7×10^9	X
7.	Dec.	8.3×10^7	5.4×10^7	1.5×10^7	2.9×10^8	1.1×10^9	1.5×10^9
8.	Jan.	1.2×10^6	3.6×10^7	2.2×10^8	3.3×10^8	5.4×10^8	4.0×10^7
9.	Feb.	3.4×10^6	1.1×10^7	9.1×10^7	1.1×10^8	3.8×10^8	1.8×10^9
10.	Mar.	1.7×10^6	3.4×10^7	X	7.2×10^7	1.6×10^8	5.1×10^9
11.	Apr.	7.3×10^6	3.7×10^6	6.5×10^6	2.6×10^8	1.1×10^9	3.5×10^{10}
12.	May	6.5×10^6	5.8×10^6	4.9×10^8	8.8×10^8	1.9×10^9	1.0×10^9

*X Not determined.

storage of Kalri samples. The count of fresh shrimp ranged from 7.3 x 10^5 to 5.1 x 10^7 CFU/g (Median 3.2 x 10^6 CFU/g). After the 4th day of storage the count ranged from 4.6 x 10^5 to 3.5 x 10^7 CFU/g showing a slight decrease in 3 samples, while the remaining 5 samples showed a slight to one log increase in the count, similar to the pattern of Jaira. After 8 days storage counts ranged from 2.9 x 10^6 to 2.8 x 10^8 CFU/g. The counts after 12 days storage ranged from 1.1 x 10^6 to 4.6 x 10^8 CFU/g and increased up to 10^8 CFU/g after 16 days of storage. One exception was sample No. 2 which had lower count than the other samples. After 20 days the counts ranged from 1.4 x 10^7 to 1.9 x 10^9 CFU/g, and all the samples were completely spoiled showing pinkish black colour, putrid odours and slimy appearance. Increases in the aerobic plate counts during ice storage of shrimp species has also been reported by other workers (1, 4, 6, and 15).

Qualitative aspects: Table 3 summarizes the changes in generic composition of bacterial flora of 12 raw Jaira samples during ice storage for 20 days. The microbial flora of fresh shrimp in order of predominance was composed of *Vibrio, Micrococcus, Pseudomonas, Moraxella, Flavobacterium, Bacillus* and *Alteromonas putrefaciens.* During storage *Vibrio* and *Flavobacterium* disappeared and *Alteromonas* and *Pseudomonas* Group II gradually increased to dominate the flora at the end of storage period, while *Moraxella* and *Micrococcus* made up predominant proportions of the populations throughout the storage. A gradual increase and then decrease was found with *Bacillus.* At the end of the storage period the flora in order of predominance was composed of *Alteromonas, Pseudomonas* Group II, *Micrococcus, Pseudominas* Group III, *Moraxella* and *Bacillus.*

Mesophilic *Vibrio* was the major component of the population of organisms of fresh shrimp. *Vibrio* usually

Table 2. Changes in bacterial count/g of Kalri shrimp *(Metapenaeus monoceros)* during ice storage

Experiment S. No.	Months	Days of storage					
	1980-81	0	4	8	12	16	20
1.	Jun.	*X	X	X	X	X	X
2.	Jul.	$7.3x10^5$	$4.6x10^5$	$3.0x10^6$	$1.1x10^6$	$4.7x10^5$	$1.4x10^7$
3.	Sep.	$4.1x10^6$	$4.1x10^6$	$2.9x10^6$	$2.1x10^7$	$5.3x10^7$	$1.6x10^9$
4.	Oct.	$3.6x10^6$	$3.1x10^6$	$1.8x10^8$	X	X	X
5.	Nov.	$2.5x10^6$	$8.9x10^6$	$4.0x10^7$	$8.6x10^7$	$2.1x10^8$	$1.3x10^9$
6.	Nov.	$5.1x10^7$	$3.4x10^6$	$2.6x10^7$	$3.7x10^7$	$2.1x10^9$	X
7.	Dec.	$9.8x10^6$	$1.3x10^7$	$2.5x10^8$	$4.6x10^8$	$2.6x10^8$	$2.9x10^8$
8.	Jan.	$2.9x10^6$	$3.5x10^7$	$2.8x10^8$	X	X	X
9.	Feb.	$2.8x10^6$	$9.3x10^6$	$2.1x10^7$	$7.6x10^7$	$7.2x10^8$	$1.9x10^9$

*X Not determined

Table 3. Summary of changes in bacterial flora of Jaira shrimp *(P. merguiensis)* during ice storage

Days of storage	Vibrio	Micrococcus	Pseudomonas Group III	Pseudomonas Group II	Moraxella	Acinetobacter	Flavobacterium	Alteromonas	Bacillus
0	29.7	18.2	16.5	0	20.0	1.3	6.0	4.0	4.3
4	5.6	16.3	18.6	6.1	29.8	0	3.0	6.5	14.1
8	3.7	12.5	14.2	7.7	33.5	0	0	15.4	12.9
12	0	20.9	6.6	16.3	10.5	0	0	36.4	9.3
16	0	17.8	12.5	20.4	19.0	0	0	28.9	2.4
20	0	23.3	14.0	23.3	12.0	0	0	26.5	3.7

disappeared during initial storage and could be isolated after 7 days of storage only from samples 1 and 4. The disappearance of *Vibrio* during ice storage is possibly due to the low temperature sensitivity of this organisms [16, 17 and 18]. *Vibrio* isolates did not show any growth at 5°, but grew well at 41°, showing the true mesophilic nature of this genus. Cobb *et al.* [15] also reported the disappearance of *Vibrio* during ice storage and attributed it to reduction in the salinity of the shrimp because of the washing effect of melting ice. Loss of salinity coupled with low storage temperature possibly resulted in the disappearance of the organism in this study.

Pseudomonas Group II were neither encountered in fresh sample nor appeared during the second week of storage in most of the samples. These organisms increased as the storage time progressed and dominated the flora at the end of the storage. *Pseudomonas* Group III were often present in fresh samples and remained a major part of the spoilage flora. It has generally been assumed that members of the genus *Pseudomonas* are of prime importance in the spoilage of fishery products. Shewan, Hobbs and Hodgkiss [19] reported that during storage of fish in ice, the *Pseudomonas* gradually predominate and after 12 days may contribute between 70-90% of the flora. Other studies have demonstrated that at chilled temperatures the non fluorescent *Pseudomonas* types II and III are active in spoilage [6, 18 and 19]. Castell and coworkers [22, 23, and 24] have shown that some of the spoilage odours occurring in spoiling fillets can be reproduced by inoculating sterile pieces of fish with a pure culture of *Pseudomonas* strain.

Alteromonas putrefaciens were not generally encountered in fresh samples and only one of 12 samples was found positive for these organisms. However, *Alteromonas* were frequently isolated as storage progressed, becoming the dominant organism at the end of the storage. This organism, formerly classified as *Pseudomonas putrefaciens* is the subject of several spoilage studies [22 and 25] and the spoilage potential of this organism has been confirmed by the production of NH_3, H_2S, TMA, HX and extracellular deoxyribonuclease by the work of Sadovski and Levin [26] and Van Spreekens [27]. Chai *et al.* [28] reported that *Pseudomonas putrefaciens* constituted one of the major spoilage bacteria on haddock fillets. The initial population of this organism on fillets is generally uniformly below 4% and most frequently no greater than 1%. During refrigerated storage the organisms increased at a more rapid rate than other competing psychrophilic population and constituted 50-90% of the total population at the time of spoilage.

Castell *et al.* [22] reported that *Pseudomonas putrefaciens* were not abundant in the initial flora of freshly cut fillets, but if present they multiply rapidly during storage in ice or at a temperature close to freezing. Van Spreekens [29] reported that a mixed flora generally developed on commercially caught cod during ice storage for 11 days but the predominant organism finally was *Pseudomonas putrefaciens.* In another communication (27) she further reported that *Alteromonas putrefaciens* could be considered as an important spoiler of shrimp.

The member of the genus *Micrococcus* were quite dominant in the flora of fresh samples and 9 out of 12 *Jaira* samples were positive for the organism. However, during ice storage their survival varied and they were generally in low number after two week-storage in some samples, but made up a major portion of the population of other samples after 20 days. They were in lower numbers than *Pseudomonas* and *Moraxella.*

These organisms do not appear to contribute much to the spoilage of shrimp; however, pigmented micrococci are often found on fresh shrimp. Farber and Lerke [30] also reported that fresh rock fish fillets had a relative predominance of pigmented micrococci.

Moraxella made up a major proportion of the flora of fresh sample. These organisms increased during storage and constituted a permanent part of the spoilage flora at the end of storage. In literature this group is descirbed as nonmotile coccobacilli that are biochemically less active than *Pseudomonas* spp. [19 and 31], and produce little or no odour [19]. The *Bacillus* group was initially present on both species of shrimp in low numbers and persisted throughout the storage in varying proportions. The dominant flora of one sample from each shrimp genus i.e. *Jaira* and *Kalri* (Sample 2, Tables 1 and 2) was composed of Gram +iv organisms, *Bacillus* and *Micrococcus.* These samples had relatively lower counts at each sampling period than other samples where the proportion of these organisms was initially low.

Table 4 summarizes the changes in the generic composition of bacterial flora of 8 raw *Kalri* samples during ice storage. The microbial flora of fresh samples in order of predominance was composed of *Vibrio, Micrococcus, Moraxella, Pseudomonas, Alteromonas putrefaciens, Bacillus, Staphylococcus,* and *Flavobacterium.* At the end of storage period the flora that emerged was composed of *Alteromonas putrefaciens, Moraxella, Micrococcus, Pseudomonas* Group II, *Pseudomonas* Group III and *Bacillus.* A comparison with the flora on *Jaira* samples (Table 3) show no significant difference in the bacteria from both genera of

Table 4. Summary of changes in bacterial flora of Kalri shrimp *(M. monoceros)* during ice storage

Days of storage	Vibrio	Micrococcus	Pseudomonas Group III	Pseudomonas Group II	Moraxella	Flavobacterium	Alteromonas	Staphylococcus	Bacillus
0	25.5	21.0	13.0	2.5	18.5	2.3	5.1	5.1	6.8
4	10.1	20.0	22.2	2.0	28.3	1.5	6.9	0	9.0
8	4.6	18.8	12.1	2.5	37.1	0	10.1	0	14.6
12	5.4	12.5	25.7	10.7	10.7	11.4	19.8	0	14.2
16	3.2	11.0	23.1	22.2	15.3	0	25.2	0	0
20	9.6	17.6	3.5	16.6	18.8	0	31.5	0	2.1

shrimp. A slight difference in the percentage composition and order of predominance of organisms was noticed.

Staphylococcus was not encountered in *Jaira* samples. The presence of *Staphylococcus* on fresh samples of *Kalri* indicates unsatisfactory sanitary practices during handling on the boat. This organism could not be isolated at any stage of the storage.

An examination of the data presented enable a number of observations to be made.

(a). The flora of Karachi coastal water fresh shrimp *Jaira (Penaeus merguiensis)* and *Kalri (Metapenaeus monoceros)* is dominated by the genera, *Vibrio, Micrococcus, Pseudomonas,* and *Moraxella. (b). Vibrio* disappears during ice storage due to its sensitivity to low temperature and reduction in salinity due to the washing effect of melting ice. (c). The genus *Alteromonas*; shows a gradual increase during storage and dominates the flora as storage progresses. (d). *Alteromonas putrefaciens, Pseudomonas* Group II and III, *Moraxella* and *Micrococcus* form the bulk of the flora of ice stored samples at the time of spoilage and (e). *Micrococcus* contributes little to spoilage, as mentioned earlier.

It may be concluded that although the initial flora of Karachi coastal water shrimp *Penaeus merguiensis* and *Metapenaeus monoceros* is different from the shrimp caught in colder waters, during ice storage they follow a course similar to that found in areas where air and water temperatures are much lower. Similar findings have been reported for fish from India and Australia [32 and 33].

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