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BACTERIOLOGICAL STATUS OF FISH AND SHRIMPS AT LANDING ON FISH HARBOUR AND LOCAL RETAIL MARKETS IN KARACHI, PAKISTAN

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Bacterial counts on two popular varieties of fish *(Cybium* and *Stromateus sp.)* and shirmps *(Penaeus* sp. at the time of landing at fish harbour and at local retail markets revealed no seasonable variation during a period of sixteen months. Total numbers of bacteria were found to be remarkably equal both at 20^{o} and 37^{o}. Market samples gave a higher count than harbour samples. Strains of typical psychrophilic organisms of *Flavobacterium, Pseudomonas, Acinetobacter* and *Morexlla* genera were isolated from harbour samples but never encountered in market samples. Some of the probable factors that determine the number and type of bacteria on these fish are discussed.

INTRODUCTION

Bacterial flora of fish landing at the harbour reflect not only the environment from which it is captured, but also the manner in which these fish are handled after harvest. It is well recognized that microbiological factors play an important role in the fish spoilage during handling prior to processing and during processing and storage of processed fish.

In Pakistan, marine fish catch is landed at the fish harbour where it is auctioned and passed to retailers and processors. Not much emphasis is placed on careful handling aboard fishing vessels and ashore at the harbour and markets. Fishermen go for fishing trips ranging from 5 to 10 days duration dependeing upon the capacity of the vessel. On board fish is stored with ice. The catch is landed at the fish harbour and auctioned. No ice is used when the fish is handled at the harbour and markets. The handling of catches at sea and ashore leaves much to be desired. Despite the fact that fish and fishery products are increasingly important as food in Pakistan, no information is available on the bacteriological status of fish and shellfish marketed for local consumption or meant for export.

Microbiological specifications for fishery products have either been or are being introduced in countries having fishery resources [7]. Such guidelines are increasingly applied in international trade. Many processors are using microbiological standards or specifications as a quality control in their own processing factories [3]. Sooner or later the export-oriented Pakistan fish industry will have to introduce the microbiological standards for its products. In order to arrive at a realistic and practical guideline it is extremely important to obtain a thorough understanding of the numbers and types of bacteria present on the raw fish and the effect of processing on these bacteria.

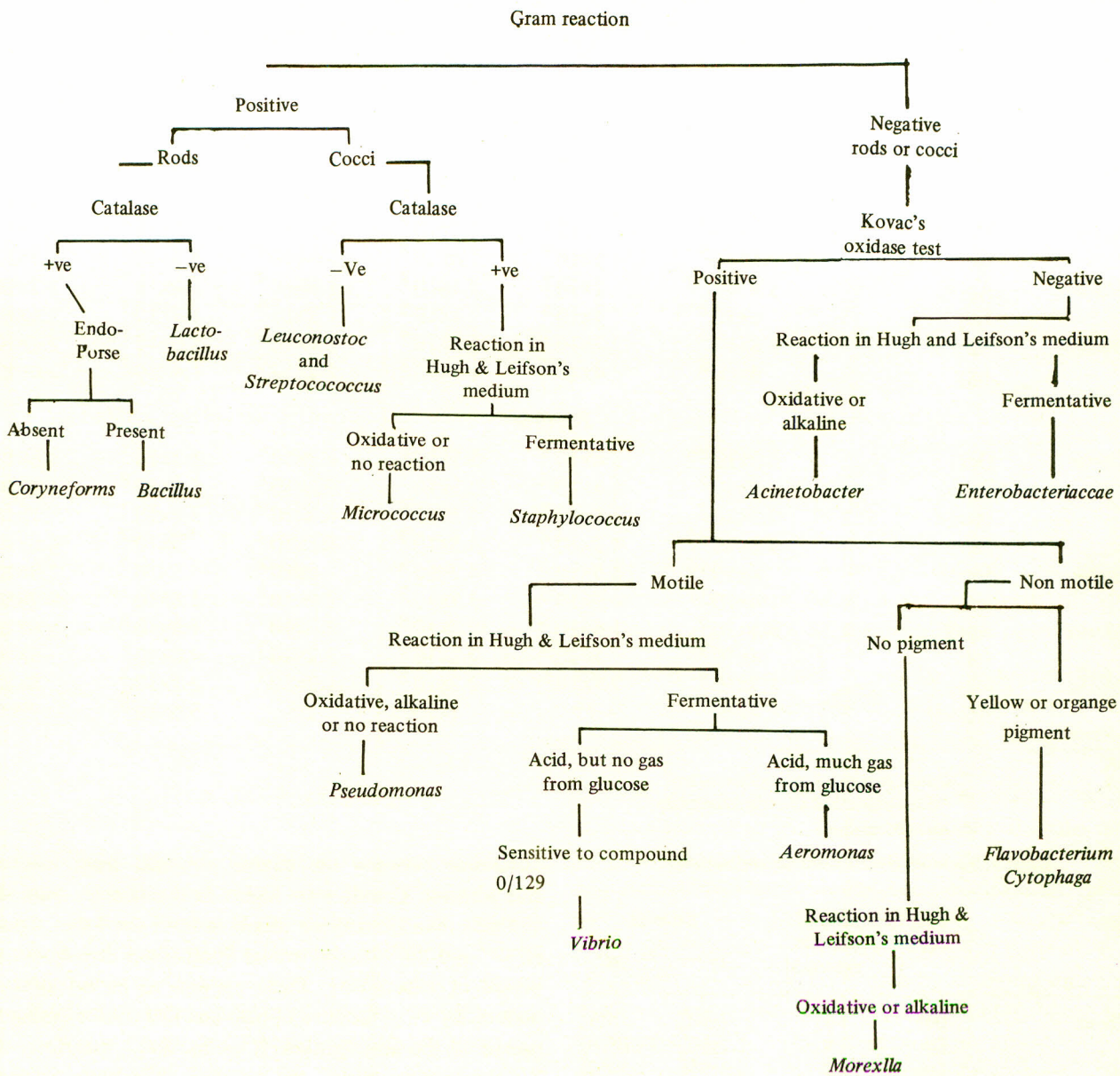
The purpose of the present study is to evaluate quantitatively and qualitatively the actual bacteriological conditions of fresh fish and shrimps landed at the harbour and those sold in various local markets. Such information will help us to recommend better methods for the transport and storage of fish in Pakistan.

MATERIALS AND METHODS

During the period March 1975 – June 1976 several specimen of raw pomfret, mackerel and whole shrimps were collected in clean plastic bags at regular intervals from the local retail markets, as well as from the fish harbour and brought to the laboratory within 1–2 hr from collection. During transit the fish and shrimps were surrounded with ice. On arrival fish skin samples of known uniform area and weighed whole shrimp, fish gut and gill samples were excised. Immediately after the samples were ready, 10g each of the shrimps, gut and gills or 10^{2} cm of skin were blended for 90 sec in Waring blender with 90 ml 0.1% sterilized peptone-water and further serial dilutions made using the same diluent.

Aerobic plate counts of the samples were determined with the spread plate method, by placing 0.1 ml of appropriate dilutions on nutrient agar (Difco, Merck) plates. In each count, duplicate series of plates were incubated at 37^{o} and 20^{o} and the resulting colonies counted after 48 and 72 hr respectively. To determine microbial types a certain number of colonies was picked up at random from countable plates. Gram-positive organisms were identified according to the method of Baird and Paker [1] and

Scheme for the Indentification of Bacteria



gram-negative rods were classified by the use of an identification scheme set up by Shewan *et al.* [4] and modified with the use of Bergey's Manual of Determinative Bacteriology [2]. The above scheme was used for the identification of bacteria.

RESULTS AND DISCUSSION

The results of aerobic counts carried out at 37^{o} and 20^{o} on samples of mackerel and pomfret are recorded in Table 1 and those of raw shrimp are given in Table 2.

It is clear from Tables 1 and 2 that values obtained for bacterial counts carried out at incubation temperatures of 37^{o} and 20^{o} are not different from each other. In some instances the 37^{o} count was lower than that obtained at 20^{o} and in some cases the reverse was true. In warmer water (25^{o} – 30^{o}) around or coast large number of mesophilic bacteria are to be expected, in contrast to Northern waters (2^{o}–12^{o}) where the viable count of 37^{o} rarely exceed 5% of the counts at 20^{o} or 0^{o} which is approxi mately equal [5].

In general it appears from the 37^{o} counts that the number of viable bacteria on the skin of mackerel and pomfret vary between 1.8×10^{6} – 5.8×10^{10} and 1.7×10^{7} – 1.4×10^{11} org/ cm^{2}. On the gills the range is between 1.4×10^{7}–1.0×10^{11} organisms/g for mackerel and between 5.6×10^{6} – 1.8×10^{10} org/g for pomfret. The number of

Table 1. Bacterial counts from mackerel and pomforet samples

Source	Month 1975	No. of fish examined	Skins count* at		Gut count† at		Gills count** at	
			37°	20°	37°	20°	37°	20°
				Mackerel				
Harbour	Oct	1	8×10^6	9.7×10^7	7.4×10^6	5.2×10^6	4.3×10^7	8.2×10^6
	Nov	1	2.3×10^7	8.8×10^7	4.1×10^6	4.2×10^6	3.5×10^7	6.5×10^7
S. Bazar	March	1	4.8×10^8	2.2×10^9	1.5×10^9	2.2×10^7	1.1×10^8	5.4×10^7
	June	1	9.6×10^9	1.6×10^9	1.6×10^8	1.6×10^9	4.3×10^8	1×10^9
L. Market	April	1	5.8×10^{10}	2×10^9	5.1×10^8	1.8×10^7	1.4×10^9	1.7×10^8
	July	1	6×10^6	4×10^6	1×10^8	1.2×10^9	1.4×10^7	2.6×10^8
E. Market	May	1	1.8×10^9	2.2×10^{10}	1.6×10^9	2.1×10^9	1.0×10^{11}	2.1×10^{11}
	July	1	6×10^6	4×10^6	1.1×10^8	1.2×10^9	1.4×10^7	2.6×10^8
				Pomfret				
Harbour	Oct	1	8.3×10^8	2.6×10^8	5.7×10^6	6.2×10^6	1.1×10^7	8.8×10^6
	Nov	1	1.7×10^7	1.4×10^7	2.5×10^7	6.1×10^6	5.8×10^8	1.2×10^7
	March	1	4.7×10^7	3.6×10^8	7.3×10^6	6.3×10^7	3.1×10^7	1.1×10^8
	June	1	3.6×10^8	4.2×10^8	4.5×10^7	1.1×10^7	2×10^7	8.6×10^7
S. Bazar	March	1	5×10^{10}	5.3×10^{10}	4.7×10^8	6×10^6	5×10^7	2.8×10^7
	June	1	1.4×10^{11}	4.5×10^{10}	3.7×10^7	2.9×10^8	1.8×10^{10}	9.9×10^9
L. Market	April	1	9.5×10^9	1.4×10^8	2.7×10^9	3.6×10^7	1.8×10^8	6×10^7
	July	1	4×10^9	1×10^8	2.1×10^8	3.2×10^7	4.8×10^8	5×10^7
E. Market	May	1	1.7×10^9	2.7×10^8	2.2×10^8	1.2×10^9	2.1×10^8	8×10^8
	August	1	2.7×10^7	**	7×10^5	**	5.6×10^6	**

* Count expressed as organisms/cm^2
† Count expressed as organisms/gram
** Not determined

Table 2. Bacterial counts from shrimp sample.

Source		Month (1975)	No. of samples examined	Count/g	
				37°	20°
Harbour		Oct	1	7×10^6	5×10^5
		Dec	4	1.1×10^7	1.02×10^8
		Jan	2	2.2×10^8	1.5×10^8
		June	2	1.6×10^8	7.15×10^7
	Total harbour samples		9 av	9.9×10^7	8.1×10^7
S. Bazar		March	3	9.1×10^9	1.8×10^{10}
		June	3	1.1×10^{11}	3.4×10^{11}
L. Market		July	3	8.10^8	1.5×10^8
		May	3	5.4×10^8	5.9×10^8
		August	3	3.2×10^8	1.2×10^9
	Total market samples		15 av	2×10^{10}	6×10^{10}

viable bacteria in gut samples from both fish species varied from $1.6\times10^6 - 1.6\times10^9$ in case of mackerel and $7.0\times10^5 - 2.7\times10^9$ in pomfret. In general there appears to be no difference between the counts obtained from mackerel and pomfret. Counts were higher in the samples from skin and gills than from those of gut in both the fishes. Counts at 37° and 20° showed similar fluctuations throughout the period of experiment. There appears to be no apparent periodicity in occurrence of high and low counts during the period of the investigation. It seems likely, therefore, that the similarity between the number of bacteria occurring on the two type of fish is due to the selective effect exerted on bacteria of the environment provided by the exposed surfaces of fish body.

Bacterial counts of shrimps (Table 2) ranged between $7.0\times10^6 - 1.1\times10^{11}$ at 37° between $5\times10^5 - 3.4\times10^{11}$ at 20°, again showing no difference between the counts at the two different temperatures. It may be noted that the counts from harbour samples both at 37° and 20° were somewhat lower than those obtained from retail markets. In market samples averages of 2×10^{10} and 6×10^{10} were found at 37° and 20° respectively. The corresponding averages in harbour samples were found to be $9.9\times10^7 - 8.1\times10^7$ at 37° and 20° respectively.

Table 3. Summary of total aerobic counts from market and harbour samples.

Source	No. of samples tested	Count at 37°			Count at 20°		
		Min	Max	Av	Min	Max	Av
Market	75	2×10^5	3.1×10^{11}	1.4×10^{10}	4×10^{10}	1×10^{12}	2.42×10^{10}
Harbour	39	1×10^5	8.3×10^8	9.8×10^8	5×10^5	4.2×10^8	9.9×10^7

Table 4. Aerobic bacterial flora of fish and shrimps as landed and at retail markets.

Source	Incubation temp.	Species	*Bacillus* sp.	*Staphylococcus* sp.	*Vibrio* sp.	*Micrococcus* sp.	*Corayba-certium* sp.	*Entero-bacteria-ceae* sp.	*Flavobac-terium* sp.	*Pseudo-monas* sp.	*Aeromo-nas* sp.	Misc
Retail markets	37°	Mackerel	40	30	6	10	14	–	–	–	–	1
		Pomfret	46	40	8	2	–	–	–	–	–	4
		Shrimps	40	24.4	31.1	4.5	–	–	–	–	–	–
	20°	Mackerel	30	30	17.5	10	12.5	–	–	–	–	–
		Pomfret	34	32	10	12	10	–	–	–	–	2
		Shrimps	48.8	24.4	23.4	3.5	–	–	–	–	–	–
Fish harbour	37°	Mackerel	10	25	50	–	–	–	–	10	–	5
		Mackerel	10	27.5	12.5	17.5	–	12.5	5	10	5	–
		Shrimps	15	46.6	10	5	–	–	6.8	16.6	–	–
	20°	Meckerel	5	35	45	–	–	–	–	15	–	–
		Pomfret	–	37.5	15	10	2.5	2.5	5	2.6	25	–
		Shrimps	10	63.5	–	3.3	–	10	–	6.6	6.6	–

In order to determine the effect of the source of samples the summary of aerobic count is presented in Table 3.

The counts of market samples ranged between $2.0\times10^5 - 3.1\times10^{11}$ (mean 1.41×10^{11}) and $4\times10^6 - 1\times10^{12}$ (mean 2.42×10^{10}) at 37° and 20° respectively, whereas the counts of harbour samples ranged between $1\times10^5 - 8.3\times10^8$ (mean 9.8×10^7) and $5\times10^5 - 4\times10^8$ (mean 1.1×10^7) at 37° and 20° respectively, indicating that the market sample had higher bacterial loads than the harbour samples. This may be due to increase in the bacterial population during transit from harbour to market, since most of the catch is transported unchilled and the high temperatures prevailing in most part of the year promotes the multiplication of the organisms. Little difference was found among the counts obtained from different markets whether the samples were obtained from Soldier Bazar, Empress Market or Lea Market.

Fish with high viable counts indicate unhygienic handling [5]. Many cases are known where improved hygiene produced a dramatic lowering in the counts. This has certainly been the experience of the Canadian Fish Inspectorate who claim that counts of 10^6/g or more can easily be lowered to 2.5×10^5/g, their proposed standard. There is no reason to believe that the same cannot be done in Paksitan, but of course after adapting hygienic handling practices comparable to Canada and other developed countries.

Tables 4 and 5, show the distribution of the isolated bacteria among the genera from market and harbour samples. A high percentage of *Bacillus, Staphylococcus* and *Vibrio* species was found in market samples whereas *Staphylococcus* and *Vibrio* dominated the harbour samples. It is understandable that the gram-positive portion (50–80%) of the flora of fish living in warmer and shallower water are higher than those (2–30%) from colder and deeper waters of Scottland, Canada, Norway [5], and Denmark [9]. It is also reasonable to assume that the conditions are similar to those observed for fish caught off India [8], South Africa, Australia and in the Adriatic [5].

It may be noted from Table 4 that despite the variety of species of fish examined, namely mackerel, pomfret and shrimps, the bacterial flora appears to be similar. Similar

Table 5. Summary of bacterial flora from market and harbour samples expressed as percentage of total number of organisms isolated

Genus	Market samples isolates examined			Harbour samples isolates examined		
	20° (250) %	37° (350) %	Total (700) %	20° (250) %	37° (250) %	Total (500) %
Bacillus sp.	37.6	42	39.8	5	11.7	8.3
Staphylococcus sp.	29.5	31.5	30.0	45.3	33.0	39.1
Vibrio sp.	17.0	14.7	15.8	20.0	24.2	22.1
Micrococcus sp.	8.5	5.5	7.0	4.5	7.5	6.0
Cornybacterium sp.	7.5	4.5	6.1	0.8	–	0.4
Enterobacteriaceae sp.	–	–	–	4.2	4.2	4.2
Flavobacterium sp.	–	–	–	1.7	3.9	2.8
Pseudomonas sp.	–	–	–	8.1	12.2	10.1
Aeromonas sp.	–	–	–	10.5	1.7	6.2
Miscellaneous	–	1.7	0.9	0.7	1.7	1.2

findings have been reported from the fish species of colder waters [6]. However, flora of one species caught in different areas at approximately the same time of year, and investigated by identical techniques has shown clear-cut differences. Thus it is clear that environment is more important than species in its effect on flora.

Comparing the market and harbour samples (Table 5) the percentage of gram-negative flora is markedly higher in harbour samples. It may be noted that typical psychrophilic organisms namely *Flavobacterium* sp. *Pseudomonas* sp. and *Aeromonas* sp. were isolated from harbour samples but never detected in market samples throughout the study.

It is, therefore, reasonable to presume that the commercial handling of fish on a fish market in Pakistan results in an increase in the number of gram-positive mesophilic becteria either by contamination from other sources or due to an increase in the flora already present in prevailing high temperatures.

Most of the work on spoilage has been carried out on fish at or around 0°. As can be expected, spoilage at this temperature is due almost entirely to gram-negative bacteria, in particular the *Pseudomonas, Achromobacter* and *Morexlla* genera. However, with the existing higher temperatures in commercial practice in Pakistan gram-positive organisms especially *Micrococcus* sp. and *Bacillus* sp. would be expected to participate in the spoilage changes. The precise nature of the changes at higher temperatures remains to be investiagted.

SUMMARY

Bacterial counts made over a period of 16 months on skin, gut and gill samples of mackerel *(Cybium* sp.*)*, pomfret *(Stromateus* sp.*)* and whole shrimps *(Penaeus* sp.*)* obtained at the time of landing from Karachi fish harbour and from local retail markets revealed no seasonal variation throughout the studies. No difference was obtained in the number of bacteria at 20° or 37°. Higher counts were obtained from gill and skin samples as compared to gut samples of the fish examined Higher bacterial populations were obtained in market samples but little difference was found among the population from different markets. Strains of typical psychrophilic organisms of *Flavobacterium, Pseudomonas, Achromobacter* and Morexlla group were isolated from harbour samples but never detected in market samples during the study. Some of the probable factors that determine the number and type of bacteria on these fish are discussed.

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