MICROBIAL FLORA OF GULF OF MEXICO AND POND SHRIMP[1, 2]

CARL VANDERZANT, EVA MROZ, AND R. NICKELSON

Animal Science Department
Texas A&M University
College Station 77843

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ABSTRACT

Bacterial counts of shrimp delivered by fishing vessels to processing plants varied greatly. Aerobic plate counts at 28 C ranged from 870-1,300,000 per gram. Either natural seawater or distilled water could be used in media preparation. The use of artificial seawater usually resulted in lower counts. The microbial flora of Gulf shrimp was dominated by coryneforms and species of *Pseudomonas, Moraxella,* and *Micrococcus.* Refrigerated storage usually caused an increase in *Pseudomonas* species. Bacterial counts of pond shrimp were much lower than those of Gulf shrimp. In some samples of pond shrimp *Bacillus* and *Lactobacillus* species were predominant.

The number and types of microorganisms on fish at time of landing depends on many factors such as species, season, fishing grounds, methods of catching, handling on board, and time and temperature of storage. Microbial activity is one of the main causes of quality deterioration of shrimp. To determine the significance and role of individual microbial species in this process, a detailed analysis of the microbial flora is useful. Information on this subject is limited. Green *(5, 6)* reported on the quantitative changes in bacterial populations of shrimp from catching to landing. She determined the influence of various handling procedures such as washing, heading, icing, and storage on the bacterial count. In general, if shrimp was handled under sanitary conditions and iced promptly, low bacterial counts could be maintained for several days. Campbell and Williams *(3)* and Williams et al. *(10)* showed that species of *Achromobacter, Bacillus, Micrococcus, Flavobacterium,* and *Pseudomonas* predominated in Gulf Coast shrimp. In Pacific shrimp, *Acinetobacter-Moraxella* species were predominant *(7).*

Raising shrimp in artificial ponds has become increasingly important in recent years. Texas has about 200,000 acres of coastal marshland, part of which could be used for this purpose. The objective of this study was to determine the level and type of microbial population of shrimp from the Gulf of Mexico and from marshland ponds.

MATERIALS AND METHODS

Samples of shrimp were collected monthly during 1969 and were taken directly from commercial boats at Galveston just before they were unloaded. The boats had been in the Gulf of Mexico for periods ranging from 1-8 days. A representative sample of the middle layer of iced shrimp in a bin was obtained, avoiding the top and bottom layers. The exact age of the sample was not established. Shrimp were either brown *(Penaeus aztecus)* or white *(Penaeus setiferus)* or a mixture of the two types. All shrimp were headed and iced after being caught. The samples were brought to the laboratory in sterile jars packed in ice and were plated within 4-5 hr.

Five samples of white shrimp were obtained from artificial ponds located in a marshland area on the West Galveston bay shore in Brazoria County. Ponds were stocked with postlarvae shrimp (about 3/4-inch long) at a rate of 20,000 per acre. They were harvested 80-120 days later. These shrimp were headed immediately and placed in sterile containers packed in ice. The time interval between obtaining the samples and plating was 4 to 5 hr.

Bacterial counts were determined by plating appropriate dilutions of blended shrimp on Standard Methods agar (SMA, Difco) by the spreadplate method. Approximately 50 g of shrimp (3 shrimp) were placed in 450 ml of sterile phosphate buffer *(1)* and blended. Further dilutions were made with sterile phosphate buffer. Plating media were prepared with three different types of water, artificial seawater, natural seawater, and distilled water. The artificial seawater was prepared from synthetic sea salts (Aquarium Systems, Inc., Wickliffe, Ohio) according to the directions supplied by the manufacturer. The natural seawater was obtained from the Gulf of Mexico at Galveston Bay. Duplicate sets of plates were incubated aerobically and anaerobically at 5 C for 7 days and at 28 C for 2 days. To obtain anaerobic conditions, jars with GasPak Lids (BBL) were used. The GasPak envelope (BBL) was used to produce hydrogen and carbon dioxide gases. The same procedure was followed for stored shrimp. Stored shrimp consisted of fresh shrimp (shrimp from boats at time of landing) stored for 7 days at 1 C. Bacterial counts on shrimp from artificial ponds were obtained in the same way.

Approximately 40 representative colonies were picked from countable plates to determine microbial types isolated from shrimp. Colony characteristics were recorded before transfer to slants of the same medium. Smears for microscopic examination were prepared using the Gram stain (Hucker's modification). Diagnostic procedures and schemes for identification were the same as presented by Vanderzant and Nickelson *(9).* Analysis of variance, assuming a fixed model, was employed in the statistical treatment of the data. The individual plate counts were transformed to logarithms before analysis of variance was applied.

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TABLE 1. AEROBIC PLATE COUNTS (APC) OF FRESH AND STORED GULF SHRIMP WITH PLATE INCUBATION AT 28 C FOR 2 DAYS.

Sample	APC/g of fresh shrimp[a]	APC/g of stored shrimp[b]	Period at sea (days)
1	670,000	13,000,000	2
2	150,000	7,900,000	3
3	870	34,000	1
4	8,100	8,500,000	7
5	51,000	480,000	2
6	65,000	2,500,000	5
7	12,000	4,000,000	5
8	3,800	2,100,000	7
9	1,300,000	15,000,000	6
10	63,000	8,800,000	1
11	130,000	70,000,000	8
12	23,000	2,000,000	2

[a]Fresh shrimp: shrimp as sampled from the boat at time of landing.
[b]Stored shrimp: fresh shrimp stored at 1 C for 7 days.

TABLE 2. ANALYSIS OF VARIANCE RESULTING FROM EFFECTS OF CONDITIONS OF PLATE INCUBATION AND COMPOSITION OF PLATING MEDIA ON THE BACTERIAL COUNT OF FRESH AND STORED GULF SHRIMP.

Source of variation	d.f.	Mean squares: Fresh shrimp	Mean squares: Stored shrimp
Plate incubation (P)	3	3.76368**	2.88180**
Plating media (M)	2	0.33031	1.44117*
P x M	6	0.07802	0.05722
Residual	132	0.78792	0.36564
Total	143		

*<0.05 level of probability
**<0.01 level of probability

RESULTS AND DISCUSSION

The bacterial counts of fresh Gulf shrimp varied greatly. They ranged from 870-1,300,000 per gram on SMA plates prepared with distilled water and incubated at 28 C for 2 days (Table 1). In addition to factors such as season, area of catch, and length of trawling, these differences in count most likely reflect differences in the handling of shrimp on the boat, sanitary conditions of hold and ice, and time and temperature of storage. For example, an inspection of the boats revealed that sample 1 was poorly iced and that samples 9 and 11 were from dirty bins in need of repair. These conditions probably contributed to the relatively high bacterial counts of these samples (Table 1).

Aerobic plate counts of fresh shrimp were generally somewhat lower at 5 C than at 28 C and ranged from 400-1,100,000 bacteria per gram on SMA plates prepared with distilled water. However, 5 of the 12 samples gave similar counts at both incubation temperatures. The same was true but to a lesser extent on SMA plates prepared with either regular seawater or artificial seawater. When fresh shrimp was stored on ice for 7 days, the bacterial counts increased greatly. Aerobic plate counts on stored shrimp ranged from 34,000 to 70,000,000 per gram on SMA agar prepared with distilled water with plate incubation at 28 C for 2 days. With stored shrimp, a majority of the samples showed similar aerobic plate counts at 28 and 5 C. The bacterial counts at both plate incubation temperatures usually were highest on media with distilled water or natural seawater and lowest on those with artificial seawater. Counts on plates incubated under anaerobic conditions were usually lower than comparable plates incubated under aerobic conditions.

Based on the data on fresh shrimp, the analysis of variance (Table 2) indicated that highly significant differences in viable count resulted from conditions of plate incubation. By Duncan's test (8), aerobic plate counts with plate incubation at 28 C differed significantly from the others and ranked highest (Table 3). No statistically significant differences were observed among media.

With respect to the data on stored shrimp, signifi-

TABLE 3. MEAN LOG COUNT OF FRESH AND STORED GULF SHRIMP WITH DIFFERENT CONDITIONS OF PLATE INCUBATION AND MEDIA

Effect	Means[1]: Fresh shrimp	Means[1]: Stored shrimp
Plate incubation at:		
28 C - 2 days - aerobic	4.55622[a]	6.58151[a]
5 C - 7 days - aerobic	4.14004[b]	6.54894[a]
28 C - 2 days - anaerobic	3.98131[b]	6.12481[b]
5 C - 7 days - anaerobic	3.79794[b]	6.03491[b]
Media (SMA) with:		
Seawater	4.17344[a]	6.43274[a]
Artificial seawater	4.02341[a]	6.12282[b]
Distilled water	4.15978[a]	6.41207[a]

[1]Means with different superscript are significantly different (P<0.05).

TABLE 4. DISTRIBUTION OF MICROORGANISMS FROM FRESH SHRIMP SAMPLES ISOLATED FROM MEDIA WITH REGULAR SEAWATER.

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
Achromobacter	0	0	0	0	0	0	2.5	0	0	0	0	0
Alcaligenes	5.7	0	2.5	0	0	0	0	0	0	0	0	0
Bacillus	2.9	0	0	0	0	0	0	0	0	0	0	0
Coryneform	22.9	37.5	65.0	45.0	75.7	35.0	25.0	35.0	15.0	42.5	37.5	47.5
Flavobacterium	11.4	5.0	0	2.5	2.7	7.5	7.5	7.5	2.5	45.0	7.5	2.5
Lactobacillus	0	0	0	0	2.7	2.5	7.5	2.5	0	0	0	0
Microbacterium	0	0	0	0	0	0	2.5	0	0	0	0	0
Micrococcus	14.3	10.0	17.5	10.0	18.9	5.0	10.0	2.5	0	2.5	7.5	7.5
Moraxella	8.6	25.0	7.5	12.5	0	12.5	30.0	5.0	2.5	10.0	22.5	32.5
Pseudomonas	31.4	17.5	0	27.5	0	30.0	15.0	47.5	65.0	0	25.0	2.5
Staphylococcus	2.9	5.0	7.5	2.5	0	0	0	0	0	0	0	7.5
Vibrio	0	0	0	0	0	7.5	0	0	15.0	0	0	0

TABLE 5. DISTRIBUTION OF MICROORGANISMS FROM STORED SHRIMP SAMPLES ISOLATED FROM MEDIA WITH REGULAR SEAWATER.

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
Achromobacter	0	0	0	0	0	0	0	0	0	0	0	0
Alcaligenes	12.8	2.6	0	2.5	0	0	0	0	0	0	0	0
Bacillus	10.3	2.6	2.5	0	0	0	0	0	0	0	5.0	0
Coryneform	17.9	7.7	52.5	32.5	25.0	10.0	20.0	30.0	7.5	17.5	25.0	12.5
Flavobacterium	0	5.1	0	0	0	2.5	0	0	0	0	0	2.5
Lactobacillus	0	23.0	2.5	0	0	0	20.0	0	0	0	0	0
Microbacterium	0	0	0	0	0	0	0	0	0	0	0	0
Micrococcus	12.8	2.6	12.5	0	2.5	5.0	0	7.5	0	10.0	0	17.5
Mold	0	0	2.5	0	0	0	0	0	0	0	0	0
Moraxella	2.6	41.0	27.5	17.5	37.5	12.5	7.5	22.5	2.5	65.0	10.0	20.0
Pseudomonas	35.9	15.4	0	47.5	35.0	62.5	52.5	37.5	90.0	0	60.0	47.5
Staphylococcus	2.6	0	0	0	0	2.5	0	0	0	0	0	0
Vibrio	5.1	0	0	0	0	0	0	2.5	0	0	0	0

cant differences in bacterial counts resulted from conditions of plate incubation and composition of plating media. Counts on media with artificial seawater differed significantly from the others and ranked lowest. Aerobic plate counts differed significantly from anaerobic counts. Aerobic plate counts at 28 and 5 C did not differ significantly. This could be expected because during refrigerated storage of shrimp only the psychrotrophic bacteria will increase in number. These bacteria can be expected to show up on plating media at both 28 and 5 C.

The composition of the microbial flora of fresh and stored shrimp is presented in Tables 4 and 5. Although variations in microbial types were noted between samples, coryneforms and *Pseudomonas*, *Moraxella*, and *Micrococcus* species predominated in fresh and stored shrimp. The microbial flora of fresh shrimp was usually dominated by coryneforms; that of stored shrimp by *Pseudomonas* species. No consistent differences in type of bacterial flora were observed between the media prepared with seawater or distilled water. The present data do not allow any conclusions about the influence of season on the distribution of the microbial flora in shrimp. The experimental samples came from different boats and the trawling locations and duration of trawling were different. The composition of the microbial flora shown in this study is in some respect different from that of Gulf Coast shrimp reported in 1952 *(3, 10)* and that of Pacific shrimp *(7)*. According to Campbell and Williams, and Williams et al. *(3, 10)* species of *Achromobacter*, *Micrococcus*, *Pseudomonas*, *Flavobacterium*, and *Bacillus* predominated in Gulf coast shrimp. In Pacific shrimp *(7)* the initial flora in order of predominance was *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Pseudomonas*, gram-positive cocci, and *Bacillus* species. Some of the differences in microflora of shrimp can probably be attributed to differences in shrimp species, marine environment, shrimp handling on board, and time and temperature of storage. The small number of *Achromobacter* species reported in Gulf shrimp in this study and also in Pacific shrimp *(7)* most likely reflects a change in taxonomic status. Some of the biochemically inert, gram-negative, short stout rods, formerly classified as *Achromobacter* species are now frequently reclas-

TABLE 6. AEROBIC AND ANAEROBIC PLATE COUNTS OF "POND SHRIMP" ON SMA PREPARED WITH DIFFERENT WATERS.

Conditions of plate incubation	Agar plate count per g on medium prepared with		
	Seawater	Artif. seawater	Dist. water
2d at 28 C - aerobic	1400-6800	180-2000	72-2000
7d at 5 C - aerobic	<10-47	<10	<10-42
2d at 28 C - anaerobic	200-1900	130-1100	29-500
7d at 5 C - anaerobic	<10-67	<10	<10-21

TABLE 7. DISTRIBUTION OF MICROBIAL FLORA OF POND SHRIMP PLATED ON MEDIA WITH DIFFERENT WATERS.

Type	Percentage distribution of isolates from plates prepared with	
	Regular seawater	Dist. water
Achromobacter	0 (0)[a]	0-2.5 (0.5)
Alcaligenes	0-40.0[b] (11.5)	0-5.0 (1)
Bacillus	0-2.5 (0.5)	0-87.5 (23.4)
Coryneform	5-60.0 (43.0)	0-39.1 (19.5)
Flavobacterium	0-47.5 (24.5)	0-17.9 (5.6)
Lactobacillus	0-5.0 (1.0)	0-80.0 (32.2)
Microbacterium	0 (0)	0-2.6 (0.5)
Micrococcus	0-2.5 (1.5)	0-17.4 (5.0)
Moraxella	0-42.5 (15.0)	0-8.7 (2.8)
Pseudomonas	0-5.0 (2.0)	0-12.8 (2.6)
Staphylococcus	0-2.5 (1.0)	0-28.2 (5.8)
Vibrio	0 (0)	0-2.5 (0.5)

[a]Mean percentage
[b]Range of percentage distribution

sified as *Acinetobacter* or *Moraxella* species (2, 9). In the other studies, *Achromobacter* or *Acinetobacter-Moraxella* species predominated. In contrast, coryneforms usually were predominant in this study. Although some of the factors mentioned above may have been responsible for this difference in microflora, it is possible that recovery conditions (composition of plating medium and conditions of plate incubation) are involved. Some coryneforms isolated from shrimp were difficult to maintain on ordinary laboratory media because of their nutritional requirements. This may be another reason for the low numbers reported in other studies.

The data obtained on shrimp raised in ponds (Table 6) indicate that the bacterial counts of these samples were lower than those of most commercial samples from the Gulf of Mexico. Counts on media prepared with distilled water and incubated at 28 C ranged from 72 to 2,000 bacteria per gram. Psychrotrophic bacterial counts (5 C for 7 days) were very low. The low microbial load of pond shrimp compared to Gulf shrimp may be attributed in part to differences in environment. In addition, elimination of extensive handling and storage on board probably are responsible for the differences in counts. The microflora of pond shrimp on seawater medium (Table 7) was dominated primarily by coryneforms and *Flavobacterium* species and to a lesser extent by *Alcaligenes* and *Moraxella* species. On media prepared with distilled water, coryneforms and species of *Lactobacillus* and *Bacillus* predominated. This observation again emphasizes that the microbial flora recovered from foods might depend on many factors including the composition of the plating medium. A difference in microflora distribution between media prepared with different waters was not observed with Gulf shrimp. *Bacillus* and *Lactobacillus* species on both media constituted less than 2% of the microflora of Gulf shrimp. Hence, *Bacillus* and *Lactobacillus* probably were of greater significance on pond than on Gulf shrimp. Pond shrimp also contained fewer *Pseudomonas* species (mean % 2-2.6) as compared with Gulf shrimp (mean % 18-22). Differences in microbial flora of pond and Gulf shrimp might be attributed to differences in environmental conditions, amount of handling, and age of shrimp. In general, the artificial ponds were quite different from the marine conditions in the Gulf of Mexico. The ponds were shallow and muddy. The temperature of the water which is pumped into the ponds through filters is higher than that of the water in the Gulf of Mexico. Shrimp in the ponds are fed artificially with either fish meal or poultry by-products. Little is known about the effect of these feedstuffs on the microbial flora of pond shrimp. In view of the uncertain microbiological quality of some of these feedstuffs (4) it would be advisable to ascertain that no human pathogens are present in these materials.

The present study indicates that shrimp from ponds contained few psychrotrophic bacteria. With improved methods of harvesting shrimp from ponds, the level of psychrotrophic bacteria may be controlled which would benefit the shelf life of the product. Further studies on the effect of the microbial flora of pond shrimp on shrimp quality and shelf life as warranted.

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NUTRITIONAL ASPECTS OF DAIRY PRODUCTS

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one-half of the total solids-not–fat in milk, and contributes about 30% of the calories of whole milk.

Lactose is only about one-sixth as sweet as sucrose. Dr. John Yudkin, Professor of Nutrition, Queen Elizabeth College, University of London, England recently stated: "There is now quite strong evidence that dietary sugar—sucrose—is an important factor in the etiology of coronary heart disease" Again, as in so many things this is a theory. An important characteristic of lactose is its ability to promote the growth of certain beneficial lactic acid producing bacteria, with their possible displacement of undesirable forms in the intestinal tract.

That the milk sugar lactose stimulates absorption of calicum is well established although the exact mechanism is not understood. It is also known that this effect of stimulating absorption includes magnesium, barium, and phosphorus.

VITAMINS IN MILK

All the vitamins known to be required by man are found in milk, the water-soluble vitamins being in the nonfat portion of milk and others in the butterfat of milk; many in significant amounts and others at a lower level.

All the B vitamins occur in significant amounts in milk. Milk is an important source of riboflavin, and this vitamin is only slightly affected by the heat of pasteurization, and the riboflavin content of milk is only slightly reduced by exposure to light. Riboflavin, sometimes called vitamin B-2, helps cells use oxygen, helps the body use protein, and helps keep tissues healthy. Although niacin occurs in milk at a low level, it is considerably higher than indicated by a measure of its niacin content. This is evident from milk's effectiveness in curing pellagra, a disease that can be cured by administering niacin. Pasteurization does not reduce the niacin content of milk.

A significant amount of thiamine (B-1) occurs in milk, and in general about 90% of this thiamine is retained when milk is pasteurized.

MINERALS IN MILK

Milk contains seven minerals as major constituents and many more in minor or trace amounts. Calcium and phosphorus are considered dietary essentials.

Hoard's Dairyman in the May 25, 1969 issue, had a very interesting article in regard to calcium. Women, of course, received the bold black print— "WOMEN ARE IN BAD SHAPE—NUTRITIONALLY" Small print below—"And Adult Men Are Not Much Better Off."

Highlights in this article: *(a)* Millions of Americans are crumbling on the inside while showing no outward sign. The fault lies with a hidden hunger for calcium. *(b)* Seven million women are afflicted with osteoporosis, a bone deterioration disease. *(c)* It is estimated that more than one-half million women have fractures of their vertebrae without knowing it. *(d)* Hip and spine fractures in older people may be the result of bone failure, causing the fall. *(e)* Research with experimental animals suggests human reproduction may be impaired. *(f)* Life span may be shortened. *(g)* Height and strength may be reduced.

All of this and more may be the result of lower than recommended milk consumption. They are not drinking enough milk or eating enough cheese and ice cream. Neither are they getting enough whole grain or enriched breads and cereals, nor dark, green, leafy vegetables.

Two eminent nutritionists, Ruth M. Leverton and Millicent L. Hathaway, have written: "A body well

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