Microbial Flora of Pond-Reared Brown Shrimp (Penaeus azte cus)

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Agar plate counts and microbial types are reported for brown shrimp reared in 2-acre natural marshland and in 0.5-acre artificial ponds during June to October 1970. Bacterial counts of pond-reared shrimp ranged from \(5 \times 10^4\) to \(5.5 \times 10^6\) per g. At final harvest in October, bacterial counts ranged from \(2 \times 10^6\) to \(5.5 \times 10^6\) per g. In marsh ponds, bacterial counts of shrimp and pond water were lowest in August when both water temperature and salinity were high. Coryneform bacteria and to a lesser extent Vibrio were the predominant isolates from fresh pond shrimp. Shrimp stored at 3 to 5°C for 7 days were acceptable as judged by appearance and odor. Between 7 and 14 days of refrigerated storage, bacterial counts increased sharply and about 50% of the samples became unacceptable. Refrigerated storage of pond shrimp caused increases in coryneform bacteria and micrococci and decreases in Vibrio, Flavobacterium, Moraxella, and Bacillus species. Pseudomonas species were not significant in fresh or stored pond shrimp. The microbial flora of pond water usually was dominated by coryneform bacteria, Flavobacterium, Moraxella, and Bacillus species.

Nearly all shrimp harvested commercially in the United States are caught in nets by trawlers on near-shore fishing grounds. In some other countries, particularly in Asia and the Far East, cultivation of shrimp in ponds is a common practice (4, 6). Although pond cultivation of shrimp in the United States is almost entirely limited to experimental trials, research on shrimp mariculture has become increasingly important in recent years (1, 2, 5, 7, 8, 11). Along the Texas Gulf Coast, natural or artificial ponds filled with brackish water are stocked with either postlarval or young shrimp (juveniles) collected from the Gulf of Mexico. Ponds also have been stocked with postlarvae obtained from females spawned in the laboratory. In ponds, shrimp usually are fed a variety of foods rich in animal proteins. They are harvested after approximately 80 to 120 days.

Most published information on shrimp mariculture has dealt with (i) pond construction, (ii) mass production of postlarvae in the laboratory, (iii) mass culture of algae to supply food for postlarvae reared in the laboratory, and (iv) the development of suitable food for raising shrimp in ponds. Little or no information is available on the microbial flora of pond-reared shrimp. Vanderzant et al. (10) reported that Pseudomonas species were less predominant on pond than on Gulf or Pacific shrimp. Since microbial activity is one of the main causes of quality deterioration of shrimp, information on the microbial flora of pond-reared shrimp can be useful to assess the potential shelf life of this food. This paper reports on the microbial flora of water and shrimp from marshland and artificial ponds along the Texas Gulf coast. In addition, data are provided on changes in level and types of bacteria in pond shrimp during refrigerated storage.

MATERIALS AND METHODS

Ponds. The five ponds used in this study are located on the West Galveston Bay shore in Brazoria County, Tex. Two of the ponds (A, B) are natural marshland ponds and were filled and stocked by natural tidal waters through flood gates. At the beginning of the experiment, the shrimp (Penaeus azte cus) population in pond A (2 acres) was estimated at 20,000 per acre and that of pond B (1 acre) was estimated at 6,000 per acre. Pond A had been treated with rotenone (Chem Fish Kill, Dow Chem. Co.) to remove competitive and predatory fish. Shrimp in these ponds were fed three times a week at a rate of 10% of their body weight. Pelleted feeds were prepared by B & D Mills Inc., Grapevine, Tex. Animal products such as fish meal, poultry meal, blood meal, and meat and bone meal were important ingredients in these feeds.

The others (C, D, E) were 0.5-acre ponds and were located above high tide. In construction of these ponds with the aid of bulldozers, the natural soil was used to make levees. These ponds were filled by

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Microbial Flora of Brown Shrimp

Fig. 1. Agar plate counts of fresh pond shrimp and pond shrimp harvested in October and stored at 3 to 5°C for 14 days (A, B, marshland ponds; C, D, E, artificial ponds).

Fig. 2. Agar plate counts of water samples from ponds.

Pumping brackish water through 1/4-inch (0.31 cm) wire screen from canals in the marshland. They were stocked with 10,000 juveniles (P. aztecus) per pond. Shrimp in these ponds were fed the same feeds at the same times as those in the marsh ponds but at a rate of 3% (pond C), 5% (pond D), and 10% (pond E) of their body weight. All ponds were approximately 3 feet (91 cm) in depth. Shrimp samples were collected by seining. Temperature and salinity of the water in each pond were determined at each feeding period. Salinity was determined by measurement of refractivity.

Microbiological procedure. Samples of shrimp and water were collected in June, July, August, and October 1970. Samples were held in sterile containers packed in ice and were plated within 5 hr of collection. Before plating, shrimp were headed by using sterile rubber gloves. Bacterial numbers of pond waters and shrimp homogenates were determined with the spread plate method by placing 0.1 ml of appropriate dilutions on Standard Methods agar (BBL) plates. Plates were incubated at 28°C for 2 days. To determine microbial types, approximately 40 colonies were picked at random from countable plates. Diagnostic procedures and schemes for identification of the microbial flora were presented previously (9). Bacterial counts and microbial types were also determined after storage of shrimp at 3 to 5°C for 7 and 14 days.
Table 1. Distribution of microbial flora of pond-reared shrimp

| Pond | Sample | Coryneform | Vibrio | Flavobacterium | Achromobacter | Moraxella | Alcaligenes | Bacillus | Micrococcus | Unidentified |
|------|--------|------------|--------|----------------|--------------|------------|-------------|----------|-------------|-------------|
| A    | June   | 90         | 2.5    | 7.5            |              |            | 2.5         |          |             |             |
|      | July   | 72.5       | 10     | 12.5           | 2.5          |            |             | 2.5      |             |             |
|      | August | 55         | 22.5   | 10             | 7.5          | 15         |             |          |             |             |
|      | October| 55         | 20     | 10             |              | 15         |             |          |             |             |
|      | Stored | 91.3       |        |                |              |            |             |          | 8.7         |             |
| B    | June   | 77.5       | 1.5    | 7.5            |              |            | 5           |          |             |             |
|      | July   | 85.7       | 7.1    |                |              |            | 3.6         |          |             |             |
|      | August | 67.5       | 20     | 2.5            | 5            |            |             | 5         |             |             |
|      | October| 30         | 20     | 45             |              |            |             |          |             |             |
|      | Stored | 54         |        |                |              |            | 15.4        |          | 23          | 7.6         |
| C    | June   | 82.5       | 7.5    | 2.5            | 5            | 2.5        |             |          |             |             |
|      | July   | 80.2       | 6.6    | 6.6            |              |            | 6.6         |          |             |             |
|      | August | 45         | 30     | 2.5            | 7.5          | 12.5       | 2.5         |          |             |             |
|      | October| 45         | 15     | 5              | 10           | 20         |             |          | 2.5         |             |
|      | Stored | 100        |        |                |              |            | 15          |          | 5           |             |
| D    | June   | 82.5       | 7.5    | 2.5            | 5            | 2.5        |             |          |             |             |
|      | July   | 72.5       | 17.5   | 5              | 2.5          | 2.5        |             |          |             |             |
|      | August | 57.5       | 20     | 5              | 5            |            |             | 2.5      |             | 30          |
|      | October| 50         | 20     |                |              |            |             |          |             |             |
|      | Stored | 100        |        |                |              |            |             |          |             |             |
| E    | June   | 82.5       | 7.5    | 2.5            | 5            | 2.5        |             |          |             |             |
|      | July   | 87.5       | 2.5    | 7.5            |              |            |             | 2.5      |             |             |
|      | August | 60         | 5      | 2.5            |              |            |             | 2.5      | 30          |             |
|      | October| 50.4       | 8.4    | 16.8           |              |            |             | 16.8     | 8.4         | 5.5         |
|      | Stored | 94.5       |        |                |              |            |             |          |             |             |

The water used for dilutions and in the preparation of the plating medium was seawater diluted with distilled water to approximate the salinity of the pond water. Seawater was obtained from Sea-Arama Marine World in Galveston. Salinity was determined by measurement of refractivity.

Organoleptic examination. Pond shrimp were examined organoleptically at time of harvest and after storage at 3 to 5°C for 7 and 14 days. Acceptability was judged on the basis of appearance and odor.

RESULTS

Bacterial counts of fresh pond shrimp sampled from June to October ranged from 5 x 10^4 to 5.5 x 10^6 per g (Fig. 1). At harvest (October), the counts ranged from 2 x 10^4 to 5.5 x 10^6 per g. Counts decreased sharply in July (except for sample B), reached their lowest level in August, and increased in October. A similar pattern in bacterial counts, although at a lower count level, was observed in water from marsh ponds (Fig. 2) but not in artificial ponds. In the latter, the counts in October were slightly higher than in June. The bacterial counts of fresh pond shrimp stored at 3 to 5°C for 7 days changed little. However, after 14 days the counts increased sharply except in shrimp from pond B. The increase in log of viable count after storage of shrimp harvested in October (Fig. 1) ranged from 0.7 to 1.6. An organoleptical examination showed that nearly all of the pond samples stored for 7 days were acceptable as judged by appearance and odor. About 50% of the samples stored for 14 days were judged acceptable.

Although variations in distribution of microbial types were noted between samples, coryneform bacteria and, to a lesser extent, Vibrio were the predominant isolates from fresh pond shrimp (Table 1). The microbial flora of stored pond shrimp was dominated by coryneform bacteria. A comparison of the microbial flora of shrimp at the beginning and end of the experimental period (June versus October) showed a decrease in coryneform bacteria and an increase in Vibrio and Flavobacterium species. No pattern could be detected in the changes of the other bacterial types. Refrigerated storage of pond shrimp...
usually caused increases in the percentage of coryneform bacteria and micrococci and decreases in *Vibrio*, *Flavobacterium*, *Moraxella*, and *Bacillus* species. A similar pattern of changes in microbial types was observed in shrimp collected and stored in June, July, and August.

The microbial flora of pond water (Table 2) usually was dominated by coryneform bacteria and species of *Flavobacterium*, *Moraxella*, and *Bacillus*. From June to October, the percentage of coryneform bacteria usually decreased. At the same time, increases were noted in *Vibrio*, *Moraxella*, *Flavobacterium*, and *Micrococcus*.

**DISCUSSION**

The changes in bacterial counts of shrimp and water from marsh ponds from June to October probably are related to some extent to changes in characteristics of the water such as temperature, salinity, oxygen level, phytoplankton activity, and pH. In August, when the counts were low, the salinity of the water was high (Fig. 3)

### Table 2. Distribution of microbial flora of pond waters

| Pond | Sample | Coryneform | Vibrio | Flavobacterium | Aeromonas | Moraxella | Alcaligenes | Bacillus | Micrococcus |
|------|--------|------------|--------|---------------|-----------|-----------|-------------|----------|-------------|
| A    | June   | 90         | 2.5    | 2.5           | 2.5       | 2.5       | 2.5         | 2.5      | 2.5         |
|      | July   | 80         | 7.5    | 5             | 7.5       |           |             | 15       |             |
|      | August | 87.5       | 2.5    | 2.5           | 2.5       |           |             | 2.5      | 2.5         |
|      | October| 50         | 5      | 10            | 20        |           |             | 15       |             |
| B    | June   | 90         | 2.5    | 2.5           | 2.5       | 2.5       | 2.5         | 2.5      | 2.5         |
|      | July   | 90         | 2.5    | 2.5           | 2.5       | 2.5       | 2.5         | 5        |             |
|      | August | 87.5       | 2.5    | 2.5           | 2.5       |           |             | 2.5      |             |
|      | October| 40         | 10     | 15            | 10        |           |             | 20       | 5           |
| C    | June   | 90         |        | 2.5           | 2.5       |           |             | 7.5      |             |
|      | July   | 87.5       | 2.5    | 7.5           | 2.5       |           |             | 5        |             |
|      | August | 57.5       | 7.5    | 7.5           | 2.5       | 5         |             | 20       |             |
|      | October| 50         | 40     | 10            |           |           |             |          |             |
| D    | June   | 87.5       |        |               |           |           |             |          |             |
|      | July   | 80         | 2.5    |               |           |           |             | 10       | 2.5         |
|      | August | 82.5       | 7.5    |               |           |           |             | 17.5     | 5           |
|      | October| 55         | 5      | 15            |           |           |             | 5        | 5           |
| E    | June   | 92.5       |        | 2.5           |           |           |             |          |             |
|      | July   | 67.5       |        |               |           |           |             |          |             |
|      | August | 47.5       | 27.5   | 2.5           | 5         | 2.5       | 2.5         | 30       |             |
|      | October| 45         | 5      | 25            | 10        | 10        | 5           | 10       | 5           |

**Fig. 3.** Salinity of pond water during experimental period.
and the temperature of the water had been 26 to 31°C for at least 2 months (Fig. 4). These factors also may have been responsible for the gradual reduction in coryneform bacteria. A marked decrease in water temperature and salinity preceded a rise in counts in October. The percentage of coryneform bacteria, however, remained the same or continued to decline. Similar reasons might be given for the changes in bacterial counts of shrimp in artificial ponds. On the other hand, the bacterial counts of water from artificial ponds did not change markedly from June to October. Oxygen level and pH of the pond waters were not measured in this study. In small ponds (1/40 acre) with excess feed, pH values of as high as 9.0 have been recorded (8). Aeration of these ponds was necessary to maintain a desirable oxygen level.

The bacterial counts of pond shrimp in the present study were considerably higher than those reported previously (10). Changes in ponds and characteristics of water and shrimp stock could have caused these differences. In addition, minor changes in the agar plate method could have been involved. In the present study, the water used in diluents and plating medium was adjusted to approximate the salinity of the pond water. In the previous study (10), natural seawater, artificial seawater, and distilled water were used.

In the present study, coryneform bacteria were the predominant isolates from fresh pond shrimp. All gram-positive, catalase-positive, nonsporeforming pleomorphic rods were placed in this group. The taxonomic status of these isolates is still uncertain. A taxonomic study of 100 isolates belonging to this group is in progress and will be reported later. In fresh and stored Gulf Coast shrimp, coryneform bacteria, *Pseudomonas*, *Moraxella*, and *Micrococcus* predominated (10). In fresh Pacific shrimp (3), the microbial flora in order of predominance consisted of *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Pseudomonas*, gram-positive cocci, and *Bacillus* species. Differences in microbial flora of Gulf Coast, Pacific, and pond-reared shrimp might be attributed to differences in environmental conditions, amount of handling, and, to some extent, to differences in plating procedures and identification techniques and schemes used by various investigators. The water temperature of the ponds usually was higher and the salinity usually was lower than the temperature and salinity of the Gulf of Mexico. In addition, the salinity of pond waters varied more because of evaporation and rainfall. Furthermore, the addition of shrimp feed to the ponds may have influenced the microbial flora of pond water and shrimp. The aerobic plate counts of two pelleted shrimp feeds were 27,000 and 47,000 per g. *Bacillus* species predominated in both feeds. No particular effect of rate of feeding on either total bacterial count or distribution of microbial types could be established.

Although bacterial counts of pond shrimp in this study were comparable to many commercial boat samples at time of landing, the striking difference in microbial flora between Gulf and pond-reared shrimp was the lack of *Pseudomonas* in the latter. With similar plating techniques and identification procedures, large numbers of pseudomonads were isolated from Gulf shrimp. The lack of significance of *Pseudomonas* in pond shrimp also was noted in the spoilage characteristics of pond shrimp when held for 14 to 21 days at 3 to 5°C. A putrid, slimy condition frequently associated with *Pseudomonas* spoilage in shrimp stored for excessive periods was not observed in pond shrimp. Spoiled pond shrimp usually showed ammoniacal off-odors.

The microbial flora of the water and feed is the main factor controlling the initial microbial flora of marine species. Although purely speculative at present, it is possible that, with increased interest in commercial culture of crustaceans, techniques will be devised to control the microbial population in ponds. This together with improved sanitary methods of harvesting, handling, and storage could possibly lead to production of shrimp with a controlled microbial population and hence enhanced shelf life.

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