Effects of salinity on intestinal flora composition of Penaeus vannamei

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Abstract. Based on IonS5TMXL sequencing platform, the effects of salinity on intestinal flora composition of Penaeus vannamei were studied. The results showed that the total number of OTUs in intestinal tract of P. vannamei in groups 6‰, 18‰ and 30 was 99, the unique number of OTUs in group 6‰ was 18, the unique number of OTUs in group 18‰ was 110, and the unique number of OTUs in group 30‰ was 53. At phylum level, the dominant bacteria in intestinal tract of three groups shrimp were Proteobacteria, Bacteroides, Firmicutes, Cyanobacteria, Actinobacteria, Tenericutes and Fusobacteria. At genus level, the main predominant bacteria in intestinal tract of shrimps were Defluviimonas, Stenotrophomonas, Shinella, Acinetobacter, Ruengeria, Rhodobacter, Nautella, unidentified Cyanobacteria and Pseudoalteromonas, and the relative abundance of intestinal microflora in different salinity groups shrimp was quite different. Simpson, Chao l, ACE and PD total tree indices of shrimps in salinity 18‰ group were higher than those of shrimp in 6‰ and 30‰ groups, while Shannon index was lower than that of shrimp in salinity 30‰ group, indicating that diversity and richness of intestinal bacterial community in salinity 18‰ group were higher than those of shrimp in 6‰ and 30‰ group. The results laid a foundation for further study on the effects of salinity on growth and metabolism of P. vannamei.

1. Introduction

Penaeus vannamei is one of the three major shrimp aquaculture in the world, and it is also the shrimp with the widest area and the highest yield in China. The shrimp is a broad-salt crustacean that can live in the water salinity of 0.5 ~ 45‰. However, in recent years, it has been found that the slow growth rate, slow molting and susceptibility of P. vannamei have gradually become prominent under low salinity conditions, and hindered the development of P. vannamei aquaculture industry. Studies have shown that salinity changes directly affect molting, growth, physiological and molecular changes of crustaceans [3-7]. In order to further elucidate the mechanism of the effects of salinity on growth and immunity of P. vannamei, we studied the effects of salinity on intestinal flora composition of P. vannamei.
2. Materials and Methods

2.1. Experimental materials

2.1.1. Penaeus vannamei. Shrimps (3.15 ± 1.03 g) were obtained from Tianjin Baodi Fishermen Aquatic Technology Development Co., Ltd., Tianjin, China.

2.1.2. Experimental Instrument. Bioprep-6 biological sample homogenizer, Hangzhou Austrian Sheng Instrument Co., Ltd.; vertical steam sterilizer, Shanghai Bo Xun Industrial Co., Ltd. medical equipment factory; PCR amplify instrument, American Bole company; Gel Doc EZ Imager gel imaging system, American Bole company; MIKRO 200R centrifuge, Germany Hettich Scientific Instruments Inc; UNIVERSAL 320R desktop refrigeration centrifuge, Hettich Scientific Instrument Company, Germany; Precision pH Meter, Shengbang Scientific Instrument Technology Company, Tianjin; Electronic Analytical Balance, Metler-Toledo Company (Switzerland).

2.1.3. Feed. The experimental feed was produced by Tianjin Kaiyuan Feed Co., Ltd.

2.2. Experimental Method

2.2.1. Experimental design. Three salinity gradients, 6‰, 18‰ and 30‰ were set up in this study. There were three parallel treatments in each group. Shrimps were fed in aquariums (730 × 530 × 450 mm), and 40 shrimps were placed in each aquarium.

2.2.2. Feeding Management. The feeding amount was based on shrimp weight (4%–6%). The shrimps were fed four times a day (i.e., 6:00, 11:00, 16:00, and 21:00). Water temperature, salinity, pH, and dissolved oxygen values were 27-29 ℃, 18-20, 7.8-8.1, and >6 mg/L, respectively.

2.2.3. Intestinal flora composition analysis. Six shrimps in each group were randomly selected and their intestinal contents were obtained. Samples in each group were labeled SAL6, SAL18 and SAL30 respectively. Samples were sent to Tianjin Nohe Bioinformatics Technology Co., Ltd. for high throughput sequencing.

3. Result and Analysis

3.1. Cluster Analysis of Intestinal Microflora of P. vannamei at Different Salinity

Under salinity of 6‰, 18‰, and 30‰, 80166, 67978 and 71560 Tags were obtained from the intestinal tract of P. vannamei. The valid Tags were clustered by OTUs with 97% consistency. The results of cluster analysis were shown in Fig. 1 and 2. The total number of OTUs shared by three groups of shrimp was 99, the unique number of OTUs in 6‰ group, 18‰ and 30‰ was 18, 110 and 53, respectively.
Figure 1. OTUs Cluster Analysis of intestinal tract of *P. vannamei*

Figure 2. Venn diagram

3.2. Comparison of relative abundance of intestinal flora at phylum and genus levels in shrimp with different salinity

At phylum level, the dominant intestinal flora of three groups of shrimp were Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Actinobacteria, Tenericutes and Fusobacterium. The
The relative abundance of intestinal flora of shrimp in different salinity groups varied greatly. The relative abundance of Proteobacteria was 90.33%, 36.61% and 84.59% in 6‰, 18‰ and 30‰ groups, respectively. The relative abundance of Bacteroidetes, Phytophthora and Cyanobacteria in 18‰ group shrimp was significantly higher than that in 6‰ and 30‰ groups (Table 1). At genus level, the predominant bacteria in intestinal tract of shrimp in three groups were Defluviimonas, Stenotrophomonas, Shinella, Acinetobacter, Ruegeria, Rhodobacter, Nautella and Pseudoalteromonas, the relative abundance of intestinal flora in shrimp with different salinity groups was quite different (Table 1).

**Table 1. Comparison of relative abundance of intestinal flora at phylum level in shrimp with different salinity (%)**

| Taxonomy       | SAL6       | SAL18      | SAL30      |
|----------------|------------|------------|------------|
| Proteobacteria | 90.33      | 36.61      | 84.59      |
| Bacteroidetes  | 0.31       | 27.94      | 7.93       |
| Firmicutes     | 5.41       | 14.49      | 1.89       |
| Cyanobacteria  | 0.08       | 10.29      | 0.29       |
| Actinobacteria | 2.02       | 5.19       | 3.66       |
| Tenericutes    | 0.06       | 2.67       | 0.16       |
| Fusobacteria   | 0.38       | 1.57       | 0.22       |
| Verrucomicrobia| 0.38       | 0.03       | 0.29       |
| Unidentified_Bacteria | 0.02 | 0.04 | 0.18 |
| Chloroflexi    | 0.13       | 0.01       | 0.15       |
| Planctomyces   | 0.04       | 0          | 0.01       |
| Acidobacteria  | 0.01       | 0          | 0.03       |
| Deinococcus-Thermus | 0 | 0.01 | 0.01 |
| Gemmatimonadetes| 0         | 0.01       | 0          |
| Nitrospirae    | 0          | 0.01       | 0          |
| Gracilibacteria| 0          | 0          | 0.01       |
| Others         | 0.83       | 1.14       | 0.58       |

**Table 2. Comparison of relative abundance of intestinal flora at genus level in shrimp with different salinity (%)**

| Taxonomy                  | SAL6       | SAL18      | SAL30      |
|---------------------------|------------|------------|------------|
| *Defluviimonas*           | 27.849     | 0.310      | 3.274      |
| *Stenotrophomonas*        | 10.434     | 21.045     | 21.423     |
| *Shinella*                | 18.479     | 0.182      | 0.944      |
| *Acinetobacter*           | 12.977     | 0.006      | 0.055      |
| *Ruegeria*                | 0.350      | 0.888      | 12.595     |
| *Rhodobacter*             | 9.069      | 0.019      | 0.118      |
| *Nautella*                | 0.102      | 0.069      | 9.943      |
| unidentified_Cyanobacteria | 0.082   | 10.286     | 0.295      |
| *Pseudoalteromonas*       | 0.002      | 0.010      | 9.852      |
| *Spongiimonas*            | 0.056      | 5.184      | 2.739      |
| *Moraxella*               | 0          | 5.119      | 0.017      |
| *Bifidobacterium*         | 0.004831852| 4.288230339| 0.007362144|
| *Tenacibaculum*           | 0.001610617| 0.02680144 | 3.859603917|
| *Marivita*                | 0.028991109| 0.003828777| 3.734447471|
| *Sulfitobacter*           | 0.06281407 | 0.015315108| 2.946698078|
| *Roseovarius*             | 0.148176781| 0.1837813  | 2.801295737|
| *Vibrio*                  | 0.167504188| 1.853128111| 2.418464257|
| *Candidatus_Bacilloplasma*| 0.057982219| 2.484876331| 0.110432158|
| *Ilumatobacter*           | 0.074088391| 0.028715828| 2.223367445|
| *Halomonas*               | 0.012884938| 0.011486331| 2.136862254|
| Others                    | 12.22619508| 37.29228884| 8.948685857|
3.3. Alpha -diversity analysis

The result was seen Table 2. In addition to the Shannon index, the other indices (species number, Simpson, Chaol, ACE, PD whole tree) of intestinal flora in 18‰ group shrimp were higher than those in other groups. Therefore, the diversity of intestinal flora of *P. vannamei* in 18‰ group was the best.

| Sample name | Number of species | Shannon | Simpson | Chao1 | ACE | Goods_coverage | PD_whole_tree |
|-------------|-------------------|---------|---------|-------|-----|----------------|---------------|
| SAL6        | 170               | 3.669   | 0.854   | 174.565 | 178.526 | 1.000          | 15.755        |
| SAL18       | 265               | 4.409   | 0.897   | 270.714 | 270.388 | 1.000          | 28.098        |
| SAL30       | 233               | 4.682   | 0.913   | 235.812 | 236.726 | 1.000          | 24.078        |

4. Conclusion

The total number of OTUs shared by three groups of *P. vannamei* was 99, the unique number of OTUs in 6‰ group, 18‰ and 30‰ was 18, 110 and 53, respectively. At the levels of phylum and genus, the relative abundance of dominant intestinal flora of the three groups of shrimp varied greatly. The diversity and richness of bacterial community in intestinal tract of shrimp in 18‰ group were higher than those of other two groups. The results laid a foundation for further study on the effects of salinity on growth and metabolism of *P. vannamei*.

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