Use of powder-formed probiotics in extensive tiger shrimp culture

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Abstract. To know the effectivity of powder-formed probiotics compared to the liquid-formed probiotics t-test experiment was carried out in the Marana Experimental Pond Installation of the Research Institute for Coastal Aquaculture and Fisheries Extension, Maros. Ten 250-m² concrete-wall ponds with 80 cm water depth and aerated with supercharge blower were stocked with 8 pcs of tiger shrimp post larvae/m². Treatments tested here were A) The liquid-formed of alternate use of Bacillus subtilis BM12, B. licheniformes BM58, and Brevibacillus laterosporus BT951 and B) The powder-formed of alternate use of Bacillus subtilis BM12, B. licheniformes BM58, and Brevibacillus laterosporus BT951. There were five replications in each treatment. After 67 days of culture showed that the use of powder-formed probiotics tended to be better than the use of the liquid ones, even though not significantly different (P>0.05). The average production and survival rate of powder-formed probiotics were 430.4 ± 37.6 kg/ha and 72.1 ± 9.8%, while those of the liquid-formed probiotics were 385.6 ± 73.6 kg/ha and 66.6 ± 10.8%. Both forms of probiotics could effectively maintain water quality parameters like total organic matter, NH₃-N, NO₂-N, and TBV/TPC ratio.

1. Introduction

Tiger shrimp culture in the world has been devastated for more than two decades by shrimp diseases that is mainly caused by vibriosis and white spot syndrome virus (WSSV) [1-6]. Therefore some farmers change to culture white leg shrimp to get more productivity in their ponds [7]. Using probiotic bacteria is an alternative to maintain good water quality in the shrimp culture pond [6-9], while using Chlorella sp is also a possible way to prevent white spot syndrome virus in tiger shrimp culture [10]. Many species of bacteria probiotic has been applied for tiger shrimp larvae rearing [9, 11]. Five strains of local probiotic bacteria that were isolated from macroalgae, sea sediments, brackish water ponds, and surrounding areas have been developed by the Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE) in Maros, South Sulawesi. They are Brevibacillus laterosporus strain BT951, Serratia marcescens strain MY1112, Pseudoalteromonas sp. Edeep-1 strain BL542, Bacillus subtilis strain BM12, and Bacillus licheniformis strain BM58 [6-9]. Since these five probiotic bacteria are developed by the RICAFE, then they were named as RICA probiotics.

These five RICA probiotics have been tested in laboratory scale and applied in several extensive and intensive shrimp farms, but so far, they were applied in liquid form after fermented for about three days [6-9]. Since most shrimp farmers want to use probiotic easily without culturing, then three
probiotic bacteria strains (*Bacillus subtilis* strain BM12, *Bacillus licheniformis* strain BM58, and *Pseudoalteromonas* sp. Edeep-1 strain BL542) were made in powder form and tested in extensive tiger shrimp culture ponds to know whether this form is effective in maintaining water quality parameters in tiger shrimp ponds as well as for production and survival rate of the cultured tiger shrimp.

## 2. Materials and Methods

### 2.1. Materials

Eight pieces of tiger shrimp PL17 (with about 10 mg in average body weight) were stocked per square meter at ten experimental ponds of 250 m$^2$ in size with 80 cm water depth. Prior to stocking, the tiger shrimp post-larvae were tested using PCR (IQ2000 test kit) to make sure that they were negative WSSV. Three different RICA probiotics were used in this experiment, that was *Bacillus subtilis* strain BM12, *Bacillus licheniformis* strain BM58, and *Pseudoalteromonas* sp. Edeep-1 strain BL542. These three RICA probiotics were made into powder form separately by adding some cassava meal before dried in low temperature sterile oven. These three powder-formed probiotics (B) were applied alternately into the shrimp pond one time per week for about 0.1 ppm (20 g/pond containing about 200 m$^3$ of brackish water pond). While for liquid-formed probiotics (A), each RICA probiotic (100 mL) was fermented for three days before used in the shrimp ponds. These probiotics were fermented using 500 g of rice bran, 200 g of fish meal, 250 g of molasses, and 50 g of yeast for 10 L of brackishwater pond water [12]. Alternating use of probiotic bacteria for both treatments was done every two weeks until 10 weeks.

The tiger shrimp were fed with a commercial pellet diet, containing 37-38% crude protein and 4-4.5% fat, from 50% of total biomass per day (given twice a day) at the beginning of the experiment, decreasing to 2% of total biomass/day (given 3 times/day) at the end of the culture period (10 weeks).

### 2.2. Methods

This experiment was carried out at the Marana Experimental Ponds of the Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE), Maros, South Sulawesi, using ten 250-m$^2$ ponds aerated with "supercharge blower". Each pond was stocked with 8 pcs m$^{-2}$ of PL17 black tiger shrimp fry. T-test experiment was applied to see the effectivity of the powder-formed RICA probiotics (B) compared to the liquid-formed RICA probiotics (A), each with five replications. Cultured tiger shrimp were harvested after 67 days and calculated for their survival rate and production. Data were then analyzed using a t-test analysis.

Total bacterial count (TPC) in the pond sediment and pond water was observed and analyzed using tryptic soy agar (TSA) media, while total Vibrio count (TVB) was observed and analyzed using thiosulphate citrate bile salt sucrose agar (TCBSA) media every other week [13-14]. The ratio of total vibrio count to total bacterial count (TPC) was then calculated in the percentage of TVB/TPC ratio. Data were then analyzed graphically and discussed descriptively.

Water quality parameters of the shrimp pond water were monitored and measured every two weeks. Total organic matter (TOM) and total alkalinity were measured titrimetrically, while nitrite-nitrogen (NO$_2$-N) and nitrate-nitrogen (NO$_3$-N) were measured using spectrophotometer [15-16]. Water temperature and dissolved oxygen (DO) were monitored using the DO-meter YSI model. Water pH was checked with pH-meter, while salinity was checked with Atago hand refractometer. Data were then analyzed graphically and discussed descriptively.

### 3. Results and Discussion

Table 1 shows that the use of powder-formed RICA probiotics resulted in better tiger shrimp production and their survival rates than the use of liquid-formed RICA probiotics, even though there were not significantly different (P>0.05) among them. This might be caused that the powder-formed
probiotics that have been deactivated by aeration for about 24-hrs before applied in the shrimp culture ponds. Therefore the three kinds of bacteria in this powder-formed could work properly just like in the liquid ones.

Both forms of the RICA probiotics here consisted of Bacillus subtilis strain BM12 that mostly works for organic demineralization and preventing the growth of pathogenic Vibrios, then Bacillus licheniformis strain BM58 that works on changing ammonia (NH$_3$-N) to nitrate-nitrogen (NO$_3$-N), and finally the bacteria of Pseudoalteromonas sp. Edeci-1 strain BL542 that has a role as nitrification bacteria by changing nitrite-nitrogen (NO$_2$-N) to nitrate-nitrogen (NO$_3$-N), which is less toxic than the previous ones. These processes could be well done by the bacteria since dissolved oxygen in the pond water were quite enough, that we're more than 3.0 mg L$^{-1}$ for the whole shrimp culture ponds (Table 2).

Table 1. Production and survival rate of tiger shrimp cultured in experimental extensive shrimp ponds of Marana, Maros, Indonesia Oct 7th – Dec 13th 2019

| Probiotic Form | Variable | 67-d Production* ($\text{kg ha}^{-1}$) | Survival Rate* (%) |
|---------------|----------|-------------------------------------|-------------------|
| Liquid        | Range    | 264 - 448                           | 52.0 - 82.0       |
|               | Average  | 385.6*                              | 66.58*           |
|               | SD       | 73.6                                | 10.81            |
| Powder        | Range    | 392 - 480                           | 60.5 - 78.8       |
|               | Average  | 430.4*                              | 72.06*           |
|               | SD       | 37.6                                | 7.06             |
| South Sulawesi| Range    | 50 - 150                            | 7 - 20           |

*) Based on the data of five replications per treatment; the same superscript means Not significantly different (P>0.05)

Table 2 shows that dissolved oxygen (DO) in the shrimp culture ponds treated with liquid-formed probiotics was 3.62 – 4.88 mg L$^{-1}$ in ranges, while in the ponds treated with powder-formed probiotics were 3.59 – 5.36 mg L$^{-1}$ in ranges. Based on this table 2 shows that pond water salinity ranges during the experiment (October 7 to December 13, 2019) were quite high that was 41 – 49 ppt. This high water salinity might be dangerous to the cultured tiger shrimp or at least could inhibit the shrimp growth, because some of the energy taken from the feed was used for the shrimp osmoregulation. Water pH during the experiment was relatively stable between 8.0 – 9.0. However, the total alkalinity of the shrimp culture pond water decreased much during the fifth sampling because of the heavy rain one day before sampling. Fortunately, the values of total alkalinity in the ponds here were still higher than the dangerous level (80 mg L$^{-1}$ equivalent to CaCO$_3$).

Table 2. Ranges of “in situ” water quality parameters in the experimental extensive tiger shrimp ponds treated with either liquid-formed or powder-formed RICA probiotics

| Probiotic Form | Dissolved Oxygen (mg L$^{-1}$) | Salinity (ppt) | pH | Total alkalinity (mg L$^{-1}$ CaCO$_3$ equivalent) |
|---------------|-------------------------------|---------------|----|-----------------------------------------------|
| Liquid        | 3.62 – 4.88                   | 41 - 49       | 8.0 – 9.0 | 82.0 – 147.6                                |
| Powder        | 3.59 – 5.36                   | 41 - 46       | 8.0 – 9.0 | 90.2 – 143.5                                |

Table 3 shows the concentrations of ammonia-nitrogen (NH3-N), nitrite-nitrogen (NO2-N), and nitrate-nitrogen (NO3-N) during shrimp culture experiment were safe for the cultured tiger shrimp. However, concentrations of total organic matter (TOM) in the pond waters were quite high since the beginning of this culture experiment, which was 44.4 – 76.9 mg L$^{-1}$ in range. The availability of probiotic bacteria of Bacillus subtilis could not decrease TOM concentration in relation to the culture period because of a daily additional organic accumulation from uneaten feed and shrimp feces.
means that the three strains of the RICA probiotics both in liquid form and powder form could control but not decreasing TOM concentration. The increase of TOM concentration in the shrimp pond water might enhance the growth of some Vibrios in the pond sediment and pond water (Figures 1 and 2). These two figures show that the Vibrio population increase in relation to the culture time. Fortunately TBV/TPC ratios in both pond sediment and pond water were still less than 10%, because the dissolved oxygen concentration in the pond water was still more than 3 mg L\(^{-1}\). As we know that vibrio bacteria is a facultative anaerobic bacteria that prefer less dissolved oxygen. If TBV/TPC ratio was more than 10%, it would be dangerous for the cultured tiger shrimp [6-7,9]. This finding shows that both the powder-formed and the liquid-formed RICA probiotics could maintain good water quality parameters and control the pathogenic vibrio as well as.

**Table 3.** Ranges of “ex-situ” water quality parameters in the experimental extensive tiger shrimp ponds treated with either liquid-formed or powder-formed RICA probiotics

| Probiotic Form | Total Organic Matter (mg L\(^{-1}\)) | NH\(_3\)-N (mg L\(^{-1}\)) | NO\(_2\)-N (mg L\(^{-1}\)) | NO\(_3\)-N (mg L\(^{-1}\)) |
|---------------|-------------------------------------|--------------------------|--------------------------|--------------------------|
| Liquid        | 44.4 – 73.4                         | 0.0226 – 0.2679          | 0.0008 – 0.0705          | 0.0277 – 0.6753          |
| Powder        | 46.3 – 76.9                         | 0.0336 – 0.2225          | 0.0008 – 0.0129          | 0.0161 – 0.8458          |

**Figure 1.** TBV/TPC ratio (%) in the pond sediment treated with either liquid-formed RICA probiotics (A) or powder-formed RICA probiotics (B) in extensive tiger shrimp culture in Maros, Indonesia.
Figure 2. TBV/TPC ratio (%) in the pond water treated with either liquid-formed RICA probiotics (A) or powder-formed RICA probiotics (B) in extensive tiger shrimp culture in Maros, Indonesia.

4. Conclusion
It is concluded that the powder-formed probiotics of *Bacillus subtilis* BM12, *Bacillus licheniformes* BM58, and *Pseudoalteromonas* sp Edeep-1 BL542 applied weekly alternately could work as effective as the liquid-formed of the same bacteria in maintaining tiger shrimp pond water quality, especially ammonia and nitrite, as well as the ratio of *Vibrio* spp to total bacteria in the pond water. The use of powder-formed probiotics of three bacteria in extensive tiger shrimp culture tended to be better (P>0.05) than the liquid ones on the shrimp production and their survival rate.

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