Probiotic enriched *Daphnia* sp: the nutritional profile and enzymatic activities

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**Abstract.** Live food played an important role in aquaculture especially hatcheries. This study was carried out to evaluate the effect of enriched different probiotic doses on total length, nutritional profile and enzymatic activities of *Daphnia* sp. This research used 3 treatments and 3 replications. The experimental treatments are 0, 5 and 10 ml L⁻¹ and enriched period was 0, 12 and 24 hours. Probiotic consortia bacteria of *Bacillus subtilis* (10⁷ CFU ml⁻¹) and *Lactobacillus casei* (10⁷ CFU ml⁻¹) which is fermented by the addition of spices and molasses. *Daphnia* sp. was cultured in fresh water with a density of 20 ind ml⁻¹. Observed parameters include total length, nutritional profile and enzymatic activities. The results showed that probiotic doses effect significantly different (*p*<0.05). In this research, the best performance enriched at 10 ml L⁻¹ doses of probiotic and 12 hours incubation period included total length of 1.8 mm, and moisture, protein, lipid, ash amounted to 98.43±0.03, 42.72±0.3, 6.34±0.07, 11.72±0.39 %, protease and lipase activity were 21.92±0.90 U ml⁻¹ and 0.82±0.06 U ml⁻¹. Enriched *Daphnia* sp. has live food potential for hatcheries.

**1. Introduction**

The ecological success of a species is most likely dependent on its ability to efficiently adjust food assimilation through positive regulation [1]. Recent studies have shown that *Daphnia* sp. can selectively assimilate nutritional compounds from its food [2, 3], because it shows a strong regulatory ability that can selectively absorb food nutrition compounds. Some freshwater *Daphnia* sp. species are able to maintain particle size bacteria [4]. Live food is used as a carrier of compounds in various nutritional or therapeutic values for the larval stage of aquatic animals [5]. *Daphnia* allows it to be a vector in the delivery of different substances, such as nutrients and probiotics [6] through the enrichment process.

Probiotics are organisms and substances that contribute to intestinal microbial balance with the aim of improving host health [7]. The use of probiotics has an effect on the host in providing nutrients and contributes to enzymatic reactions [8]. The use of probiotics in daphnia culture can improve health, nutritional profiles and the presence of digestive microflora which can influence organisms at higher trophic levels. This study aims to evaluate the enrichment of *Daphnia* sp. with probiotics on nutritional profile and enzymatic activities.
2. **Materials and methods**

2.1 **Preparing of probiotic**

Probiotic ingredients prepared from spices, molasses, coconut water and freshwater up to 30 liters. The mixture is boiled at 100°C. After 48 hours, *Bacillus subtilis* and *Lactobacillus casei* were inoculated as much as 4% (10^7 CFU ml^-1) into the media. Probiotic fermentation is carried out for 10 days. Previous probiotics tested total bacterial counts in Nutrient Agar (NA) and DeMan Rogosa Sharpe Agar (MRSA) and bacterial biochemical tests.

2.2 **Daphnia culture**

*Daphnia* sp. in this experiment is the teenage stage which is 1.5 mm filtered in. *Daphnia* sp. stock was cultured in an aquarium with a capacity of 100 L.

![Figure 1.a. Morphology of Daphnia sp.](image)

![Figure 1.b. Restocking Daphnia sp.](image)

2.3 **Experimental design**

This experimental design using 2 factors included factor 1 (probiotic doses: 0, 5, 10 ml L^-1) and factor 2 (enrichment period: 0, 12, 24 hours) with 3 replications. *Daphnia* sp. density enriched at 20 ind ml^-1 and culture capacity of 2 liters. Light intensity is set to 2000 lux with a temperature of 28°C [9]. Data analysis was carried out for several parameters including total length, nutritional profile (protein, lipid, moisture, and ash content), and enzymatic activity (proteases and lipases).

2.4 **Analytical method**

Evaluation of growth (total length) is measured using a micrometer microscope. There were 20 samples taken from each experiment. Proximate analysis includes protein content using the Lowry method [10], lipid analysis of the modified method [11], ash content of the combustion method and moist [12].

Enzyme extraction by preparing a physiological saline solution (0.9% NaCl) and homogenized to a total volume of 1.6 ml per sample. The homogenized solution was centrifuged at 5,000 rpm for 5 minutes. The supernatant is used for enzymatic activities analyst [13]. Enzymatic activity (protease) using 2% casein hydrolysis [14] and lipase were measured using an Arabic olive-Gum oil emulsion substrate through sample titration [15]. Data analyst by ANOVA test.

3. **Result and Discussion**

3.1 **Total length**

Total length measurements of 20 individuals are shown in Table 1. The average total length *Daphnia* sp. ranged from 1.23 to 1.81 mm in the total treatment. The results of probiotic enrichment tests with different doses and periods of total length of *Daphnia* sp. are shown in Table 1.
Table 1. Total length Daphnia sp. at different doses and enrichment periods

| Enrichment Treatments | 0 ml L⁻¹ (hours) | 5 ml L⁻¹ (hours) | 10 ml L⁻¹ (hours) |
|------------------------|------------------|------------------|-------------------|
|                        | 0       | 12    | 24    | 0       | 12    | 24    | 0       | 12    | 24    |
| Total length (mm)      | 1.25    | 1.67  | 1.77  | 1.23   | 1.78  | 1.78  | 1.76   | 1.80  | 1.81  |
|                        | ±0.07   | ±0.01 | ±0.01 | ±0.02  | ±0.01 | ±0.01 | ±0.02  | ±0.01 | ±0.00 |

Note: averages with different notations show significant differences and averages with the same notations show insignificant differences.

ANOVA test results showed that probiotic enrichment had a significant effect on the total length of Daphnia sp. (P <0.05) at doses of 0 and 5 ml L⁻¹ with a dose of 10 ml L⁻¹, while doses of 0 and 5 ml L⁻¹ were not significant differences (P> 0.05). The total length at 10 ml L⁻¹ dose in the 12 and 24 hour periods also showed no significant differences (P> 0.05) presented in Table 1. Daphnia sp. including herbivorous zooplankton that can utilize bacteria directly as a food source to promote growth [16]. Bacteria can also contribute to the removal of toxic metabolic substances in living media, such as ammonia, which can affect the growth and survival of Daphnia sp. [17].

3.2 Nutritional profile
The nutritional profile results include protein, fat, moisture, and ash content of Daphnia sp. with probiotic enrichment shown in Table 2.

Table 2. Nutritional value of Daphnia sp. at different doses and enrichment

| Enrichment Treatments | 0 ml L⁻¹ (hours) | 5 ml L⁻¹ (hours) | 10 ml L⁻¹ (hours) |
|------------------------|------------------|------------------|-------------------|
|                        | 0       | 12    | 24    | 0       | 12    | 24    | 0       | 12    | 24    |
| Moisture (%)           | 92.31   | 98.00 | 98.22 | 97.76   | 98.42 | 98.01 | 97.86   | 98.43 | 97.95  |
|                        | ±0.29   | ±0.00 | ±0.00 | ±0.03   | ±0.00 | ±0.00 | ±0.13   | ±0.01 | ±0.05  |
| Protein (%)            | 41.63   | 38.47 | 36.20 | 41.56   | 41.68 | 40.05 | 41.41   | 42.72 | 41.60  |
|                        | ±0.09   | ±0.06 | ±0.31 | ±0.19   | ±0.29 | ±0.34 | ±0.19   | ±0.14 | ±0.14  |
| Lipid (%)              | 4.59    | 4.61  | 4.68  | 4.57    | 5.30  | 5.93  | 4.56    | 6.43  | 6.84   |
|                        | ±0.08   | ±0.01 | ±0.03 | ±0.31   | ±0.09 | ±0.02 | ±0.21   | ±0.03 | ±0.05  |
| Ash (%)                | 10.14   | 10.26 | 9.18  | 10.17   | 11.62 | 11.61 | 10.13   | 11.72 | 11.39  |
|                        | ±0.02   | ±0.01 | ±0.16 | ±0.14   | ±0.03 | ±0.15 | ±0.00   | ±0.08 | ±0.04  |

Note: Averages with different notations show significant differences and averages with the same notations show insignificant differences.

ANOVA test results showed that enrichment with different doses and periods gave a significant effect on the nutritional value of Daphnia sp.(P <0.05). ANOVA test are presented in Table 2. The nature of probiotics increases the process of absorption of food in digestion so that the nutrients obtained can be absorbed by Daphnia. Probiotics can provide benefits to the host by modifying the microbial community or associating with the host, improving the nutritional value and utilization of feed [17]. Bacteria, fungi, algae, and yeast are types of microbes that can produce mainly single cell protein both Bacillus sp. and Lactobacillus sp. groups [18,19] so that it can increase organisms that use it as feed.

3.3 Enzymatic Activity
The results of probiotic enrichment on protease and lipase activity of Daphnia sp. shown in Table 3.
Table 3. Enzymatic activities *Daphnia* sp. at different doses and enrichment periods

| Enrichment Treatments | 0 ml L⁻¹ (hours) | 5 ml L⁻¹ (hours) | 10 ml L⁻¹ (hours) |
|-----------------------|------------------|------------------|-------------------|
|                       | 0 12 24          | 0 12 24          | 0 12 24           |
| Proteases (U ml⁻¹)    | 17.16⁺ 17.19⁺ 19.20⁺ 17.11⁺ 19.51⁺ 20.47⁺ 17.36⁺ 21.92⁺ 21.05⁺ |
| Lipases (U ml⁻¹)      | 0.55⁺ 0.58⁺ 0.57⁺ 0.52⁺ 0.65⁺ 0.73⁺ 0.55⁺ 0.82⁺ 0.79⁺ |

Note: Averages with different notations show significant differences and averages with the same notations show insignificant differences.

ANOVA test results showed that enrichment with different doses and periods had a significant effect on the activity of the enzyme *Daphnia* sp. (P <0.05) as presented in Table 3. The best treatment of enzymatic activity is at 10 ml L⁻¹ dose of enrichment with 12 hours. Enrichment of probiotics shows the activity of the enzyme *Daphnia* sp. which is higher than the control. This is most likely caused by the colonization of a number of beneficial bacteria in the body of *Daphnia* sp. and the ability of probiotics to produce a number of exogenous enzymes, including proteases, lipases, amylases, and mannanases [1]. Bacteria will induce secretion of the digestive enzyme Daphnia [20]. Bacillus and Lactobacillus, including bacteria that can produce proteolytic enzymes [21]. Proteolytic enzymes are produced by lactic acid bacteria around the cell wall, cytoplasmic membrane, or in cells that function in protein hydrolysis [22]. Bacteria also produce lipase as a form of survival for growth that replaces the function of carbon as energy [23].

4. Conclusion

This study evaluates probiotic enrichment with different doses and periods that can affect the total length, nutritional profile, and enzymatic activities of *Daphnia* sp. before being given to organisms with higher trophic levels. ANOVA test showed that enrichment of *Daphnia* sp. showed significant differences (P <0.05). The interaction between probiotic doses and length of enrichment showed significantly different results with the best results on enrichment of 10 ml L⁻¹ with a duration of 12 hours. As a result of the study, *Daphnia* sp. has the potential as living food with body content can be increased through the enrichment process. On the other hand, further research on the use of enriched *Daphnia* sp. is also important for analyzing the relationship of enriched *Daphnia* sp. with a consortium of probiotic bacteria to nutritional efficiency and growth of predatory organisms.

5. References

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