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Bio Chemical Characteristics of Phenaloxidase in *Machrobrachium Rosenbergii*, "South Tailake No.2"

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Abstract *Regarding the serum phenol oxidase (PO) of the Macrobrachium rosenbergii, "South Tailake No.2" as the material, the properties of PO were studied by using L-DOPA as the reaction substrate and spectrophotometer to determine the PO activity under different temperature and pH conditions. The results showed that the temperature of PO activity was highest at 25 ℃, Activity decreased gradually below and above 25 ℃. The serum PO with vitality when the pH ranged from 5 to 8.4 and the best pH was 6. Therefore, the optimum parameters for the determination of PO activity of Macrobrachium rosenbergii were the reaction medium with pH 6 as the reaction medium at 25.*

Keywords: *Macrobrachium rosenbergii*; "South Tailake No.2"; phenol oxidase; biochemical characteristics

1. INTRODUCTION

The Macrobrachium rosenbergii is also known as the Malaysian prawn, which is mainly distributed in Southeast Asia and has the characteristics of large size, wide food and rapid growth. Since 1976 China's introduction, the Freshwater Fisheries Research Institute of Zhejiang province introduced the Burma population and domestic hybrid breeding of Macrobrachium rosenbergii, "South Tailake No.1" before and after 2002. They are greatly improved resistance, disease resistance increased, the survival rate increased 8.87%, 64.5kg per mu increased [1], in 2009 and further breeding better meat, faster growth "South Tailake No.2" [2-3], In Zhejiang, Jiangsu and other areas of extensive farming in mainland China, has gradually become one of China's major cultured shrimp, has important economic value. In 2016, the breeding area of Macrobrachium rosenbergii in China was over 40 thousand hectares, and the area of Lixia river area was nearly 20 thousand hectares, which accounted for half of the whole country. Gaoyou area, Jiangsu province introduced from Zhejiang to carry out the production of seed breeding of Macrobrachium rosenbergii, as the main supply base of Macrobrachium rosenbergii larvae in East china. Although many manufacturer claims to be, the South Lake 2 on the Macrobrachium rosenbergii, but the main use of pond culture into the shrimp as a parent, in recent years there has been slow growth, mature early, shrimp disease frequent, once suffered shrimp dilemma ,thus seriously affecting the economic efficiency.

Prophenoloxidase activating system is not specific immune function of crustacean's important defense and recognition system. It is composed of serine protease and other related factors, which have the ability to recognize foreign objects, release the regulating hormone, promote the phagocytosis and capsule of blood cells and produce the immune function such as killing and removing foreign objects. Phenoloxidase (PO) is the product of the activation system of phenoloxidase, which is involved in the immune response, and it is an important index to measure the immune function of the body. Studies on the PO of crustaceans, such as Japanese biogas, Chinese prawns, Chinese mitten crab, and crustacean crayfish, have found that there are certain differences in the biochemical characteristics of different kinds of PO. Liu Kai reported that phenoloxidase from *Macrobrachium rosenbergii* can be activated by lipo polysaccharide, SDS, Ca^{2+}, Mg^{2+} and so on in vitro [13], but the biochemical characteristics of phenoloxidase from *Macrobrachium rosenbergii* at different temperatures and pH have not been reported. Therefore, in this study, the changes of phenoloxidase activity in different

temperature and pH environment were studied in order to study the effects of the activities of phenoloxidase on the culture of *Macrobrachium rosenbergii* and the resistant population Breeding provided basis.

2. MATERIALS AND METHODS

2.1. Test Materials and Preparation Methods

In this study, the sera of *Macrobrachium roxburghii* (18.46 ± 8.18 g) were collected from the pond of Taizhou area. The seedling originated from Jiangsu Gaoyou nursery field. It's kept in the tank of 100 cm x 30 cm x 50 cm for 7 days. Feed the fresh snail meat one time a day, change the water 1/5, and siphon the stool in time. Breeding water by aeration 72 h dechlorination of tap water, the hardness is 123 mg/L, pH 7.4, temperature 25 - 2 DEG C. During normal feeding, shrimp foster vigorous natural mortality was less than 5%. Choose the vibrant roche spermatogenesis 186 tail, with 5 ml syringes punctured head cuirass draw blood from the heart to the lymph in 10 ml centrifuge tube 4 ℃ place overnight, after waiting for serum precipitation 5 000 r/min 4 ℃ centrifuge for 10 min, take that used in experiments, the supernatant fluid to collect all the serum mixed together, forming a sample 4 ℃ under test.

2.2. Test Equipment and Drugs

Instruments used are: UV spectrophotometer (Pharmacia Biotech ultrospec 2000), HHS type constant temperature water bath (Shanghai Bo News Industry Co., Ltd.), analytical balance (accuracy of 0.0001 g, Shanghai balance instrument factory), centrifuge (Eppendorf) Acidity meter (pHS-3C) and pipette (Eppendorf). The reagents used are: L-DOPA (Singma), citric acid, KH2PO4 and Na2HPO4 reagents are analytical pure (Sinopharm Group).

2.3. Determination of Phenol Oxidase Activity

2.3.1. Effect of pH on phenol oxidase activity

The buffer of pH 5.0 ~ 8.4 was prepared with citric acid, KH2PO4 and Na2HPO4 at room temperature. Take clean 10 ml calibration tube, join the pH of the 1/15 mol/L potassium phosphate buffer 3 ml, join mL0.01 0.1 mol/L L - DOPA and 0.1 ml serum under test, fully blending, rapid determination of initial absorbance wavelength of 490 nm value, timing starts at the same time, the determination of 6 min change absorbance value (OD). Each group is parallel to 3 times. The concentration of L-DOPA and the absorbance at 490 nm were ODL and OD0 as control groups. (U), PO = (OD-ODL-OD0) / t [14] as an enzyme activity unit (mean) per minute (t) OD490nm.

2.3.2. Effect of temperature on the activity of phenol oxidase

Take clean 10 ml calibration tube, add 1/15 of the 3 ml pH 6.0 mol/L potassium phosphate buffer, in 0 ~ 45 ℃ water bath pretreatment buffer temperature reaches the preset temperature for 5 min. Other steps are performed at 1.3.1.

2.4. Data Analysis

Using SPSS19.0 software for statistical analysis, ANOVA and Duncan were used to compare the differences of mean value between groups, and P was less than 0.05 as significant difference, with P greater than 0.05 as no significant difference.

3. RESULTS

3.1. The Effect of Buffer Ph on the Activity of Phenol Oxidase

Changes of serum PO activity in *Macrobrachium rosenbergii* in different pH buffer environments are shown in Fig. Serum PO in the experiment set the range of 5 ~ 8.4 are dynamic. The activity of PO was the highest at pH 6, which was significantly higher than that of other groups ($P < 0.05$). The activity of PO decreased rapidly at pH 6, and only 34.29% at 6 and 5 ($P < 0.05$). When the pH was higher than 6, the activity of PO decreased gradually, and there was no significant difference between them at 6.5 and 7 ($P > 0.05$). There was no significant difference between 7.5 and 8.8 ($P > 0.05$). The results showed that the pH of the buffer was the highest when the buffer pH was 6.

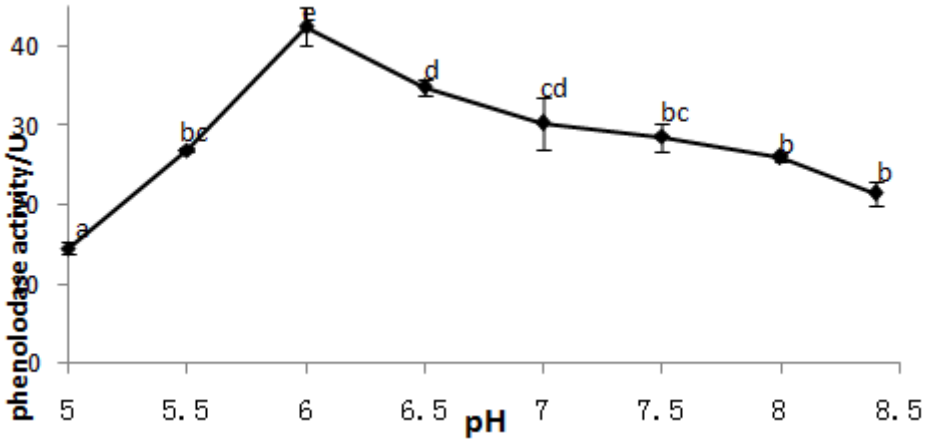

Fig1. *The effect of pH on phenoloxidase activity in serum of Macrobrachium rosenbergii*

3.2. Effect of Temperature on the Activity of Phenol Oxidase

The changes of serum PO activity in *Macrobrachium rosenbergii* at different temperatures were as follows: (2) the serum PO was stable at 2.0 ℃ ~ 45 ℃, and the highest activity was at 25 ℃. The serum PO increased gradually with the temperature increasing at 0 ℃ ~ 25 ℃, except for the difference between 15 and 20 (P> 0.05), and there was significant difference (P <0.05). The activity of PO was decreased from 30 ℃ to 45 ℃, but there was no significant difference (P> 0.05).

When the buffer temperature above 35 ℃ sample absorbance increases rapidly, viewed from the outside, Indicating that some of the protein degeneration, affecting the determination of the results. The higher the temperature is, the higher the temperature is. The activity of PO 0 C ice water bath is 25 degrees centigrade 28.84%.

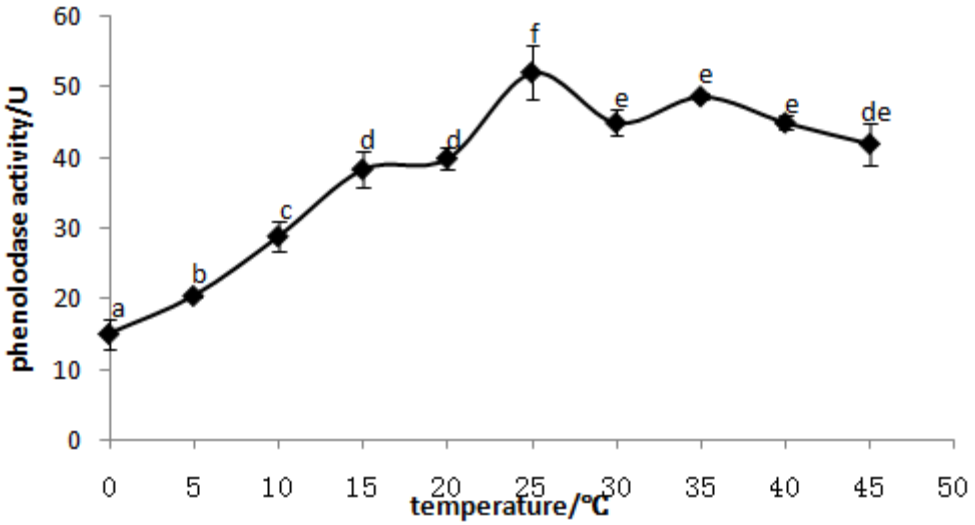

Fig2. *The effect of temperature on phenoloxidase activity in serum of Macrobrachium rosenbergii*

4. DISCUSSION

The results showed that the optimal pH value of serum PO was 6, and the activity was higher than that of 6 and below 6. In recent years, scholars have reported the optimum buffer pH for aquatic crustacean. It was found that the optimum pH of different species was different, and the optimum value was the highest in 6-7 [9-15], indicating that the optimum pH of most aquatic crustacean was pH slightly neutral.

Pang Qiu xiang and others believe that the PO vitality of some creatures will be affected by the acidic environment [16]. This experiment also found that the activity of PO in the more acidic environment will be greatly inhibited. During the experiment, the buffer with low pH of citric acid was used, and the activity was decreased rapidly. Wang Jianguo [12] reported that L-DOPA in acid and neutral

buffer in the more stable, when pH greater than 8.5, the solution will generate yellow brown substances, absorbance values considerably. Seriously interfere with the determination, the phenomenon is the substrate L - DOPA and environment redundant OH - reaction causes the absorbance changes, caused by measurement error.

The optimum temperature for the reaction between phenoloxidase and L-DOPA in aquatic crustaceans is between 20 and 50, and the difference among species is significant. Studies have shown that phenoloxidase is phenolic oxidase that cleaves the N-terminal peptides leading to conformational changes, and that a specific phenylalanine is removed from the active site after exposure to the substrate site of the active site is exposed [17]. Phenol oxidase catalyzes phenolic substances into black quinone compounds, which have antimicrobial effects and also facilitate the removal of phagocytes and thus achieve the role of immune defense. Different species encode the gene of the protein significantly [18]. Wang Qingqing and other person added up the phenol oxidase protein sequence of 2266 amino acid sites, of which 1056 variable sites (24 of the conserved loci), accounting for 46.6% of the total number of amino acids [19]. It was thought that the evolution of the phenoxygenase family and the evolution of species are also closely related.

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