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A comparative Study on The Applied Enzymatic Properties of Catalase Produced by *Aspergillus niger* 1102 and *Strepiococtus hemolyiicus* 2107

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ABSTRACT

Catalases are important antioxidant enzymes that can catalyze hydrogen peroxide, most of which are distributed in plant chloroplasts, endoplasmic reticulum, mitochondria, liver and red blood cells of animals. The enzymatic activity of catalase can remove hydrogen peroxide in organisms, eliminate the harmful effects of hydrogen peroxide and provide an antioxidant defense mechanism for the cells of the body. *Aspergillus niger* 1102 and *Strepiococtus hemolyiicus* 2107 are isolated from the soil and successfully mutagenized in the laboratory. By changing one of the many factors, keeping the other factors unchanged, conducting parallel experiments, obtaining multiple sets of experimental data, and sorting and comparing them, more accurate experimental results were obtained. This study mainly compared the enzymatic properties of catalase produced by *Aspergillus niger1102* and *Streptococcus hemolyticus* 2107 through the effects of temperature, pH, and metal ions on the enzyme activity. This research mainly focused on the enzymatic properties of catalase produced by *Aspergillus niger* 1102 and *Streptococcus hemolyticus* 2107.

INTRODUCTION

Catalase is widely used in clinical medicine, food processing, textile printing and dyeing, environmental protection and many other fields. So far, China's capacity to produce catalase has grown steadily at a rate of 17.7% per year (Wu, 2012). As a decomposing enzyme with an antioxidant effect, catalase exists in almost all known animals, some anaerobic microorganisms, most aerobic microorganisms and plants, but it does not include fungi (Yu, 2016). In studying the function of catalase in microorganisms, it was found that catalase can help pathogenic microorganisms to survive safely in the host. During the process of microbial fermentation, a large amount of catalase will be produced and released outside the cell. The higher purity catalase extracted by the biological preparation method can be used in many aspects such as the pharmaceutical industry, food processing and industrial production (Li, 2007).

The thermal stability and pH stability of catalase from different sources and different tissues are also different (Zhang, 2005; Zhao, 2007). In the process of the textile industry with serious pollution, catalase is more time-saving, energy-saving and water-saving than traditional hydrogen peroxide removal methods, and it is safer and protects the environment, which is very conducive to the implementation of sustainable development (Liu, 2009; Zhang, 2010). Catalase can quickly eliminate hydrogen peroxide. It only needs to be washed once with cold water, or even without washing. And it can also be carried out at the same time as dyeing.

MATERIALS AND METHODS

All the chemicals used were of analytical grade and were mainly purchased from Sigma Chemical Company. *Aspergillus niger* 1102 and *Strepiococtus hemolyiicus* 2107 were kept in our laboratory and the identification of this strain was performed according to the descriptions of Buchanan (Buchanan, 1984).

Culture Medium Used for The Seeds (g/100mL):

(1) *Aspergillus niger* 1102: Potato 20.0 , glucose 2.0, agar 2.0 and adjusted to pH = 7.0.
(2)*Strepiococtus hemolyiicus* 2107: Glucose 2.0, peptone 1.0, yeast extract 1.0, beef extract 0.5, NaCl 1.0 and adjusted to pH = 7.5.

The Culture Medium Used for The Fermentation (g/100mL):

(1) *Aspergillus niger* 1102: Glucose 1.0, soybean meal 2.0, $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 0.1 and adjusted to pH = 7.0.
(2) *Strepiococtus hemolyiicus* 2107: Peptone 0.3, yeast extract 2.5, beef extract 1.0, NaCl 0.5 and adjusted to pH = 7.2.

Determination Of Catalase Enzyme Activity:

The principle of UV rate direct method is to use hydrogen peroxide to have strong absorption at 240nm (Nakayama, 2008). The reaction system in the reference cuvette: the total volume is 3.0mL, 2.9mL is the phosphate buffer solution with pH 7.0, and 0.1mL is the enzyme solution to be tested. Reaction system in sample cuvette: The total volume is 3.0mL, 2.9mL is the phosphate buffer solution containing 30% hydrogen peroxide, and 0.1mL is the enzyme solution to be tested. Enzyme activity determination: The amount of enzyme required to decompose 1μmol/L of hydrogen peroxide measured at 240nm in 1 minute is one unit of enzyme activity.

RESULTS AND DISCUSSION

The Influence of pH Value on Catalase Enzyme Activity and Stability：

Diluted the catalase to the optimal dilution factor in a 50mmol/L phosphate-citrate buffer solution with pH 4~6, a 50mmol/L phosphate buffer solution with pH 7~8, and a 50mmol/L pH 9~11 in carbonate buffer solution , and Enzymatic reactions were carried out in salt buffer solution. The effect of pH on catalase activity was measured with an ultraviolet spectrophotometer and the optimal pH value was obtained. The results were shown in Figure 1.

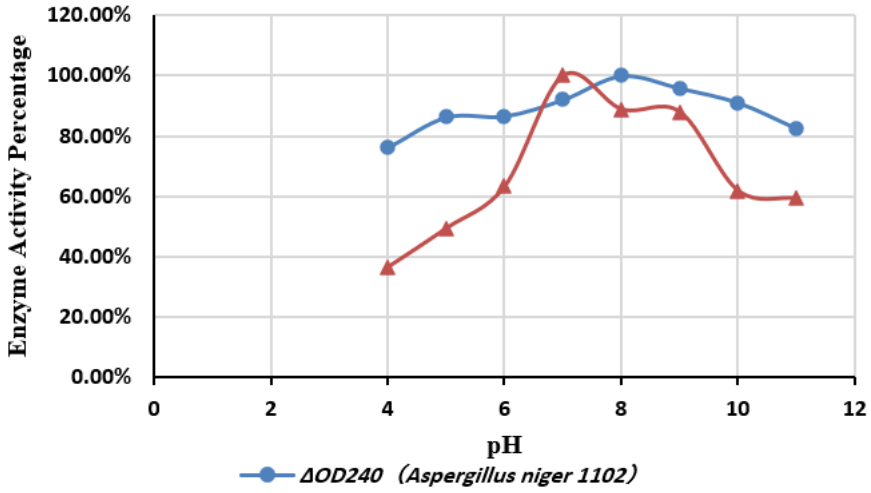

Fig.1. The effect of pH on CAT enzyme activity.

Based on data in Fig.1, when the pH was 8, the catalase activity produced by *Aspergillus niger 1102* was the highest; when the pH was 7, the catalase activity produced by *Streptococcus hemolyticus* 2107 was the highest.

After incubating the enzyme solution with the best dilution multiple in the above buffer for 120 min at 30°C, the remaining enzyme activity was determined to study the pH stability of catalase (Song, 2020). The results were shown in Figure 2.

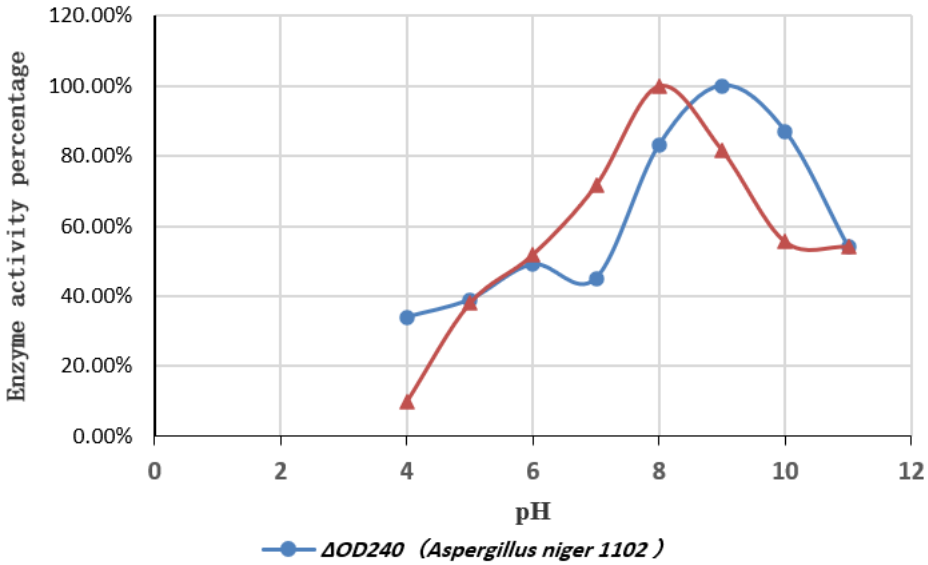

Fig.2. The effect of pH on CAT enzyme stability.

Based on data in Fig.2, when the pH is 7-9, the catalase activity produced by *Streptococcus hemolyticus* 2107 was the highest; when the pH was 8-10, the catalase activity produced by *Aspergillus niger* 1102 was the highest.

The Influence of Temperature on Catalase Activity and Thermal Stability:

Diluted the catalase to the optimal dilution factor in a 50mmol/L phosphate buffer solution with pH 7, and Enzymatic reactions were carried out at different temperatures (0℃~70℃) (Zhou, 2013). An ultraviolet spectrophotometer was used to measure the effect of temperature on catalase activity and the optimum temperature was obtained. The results were shown in Figure 3.

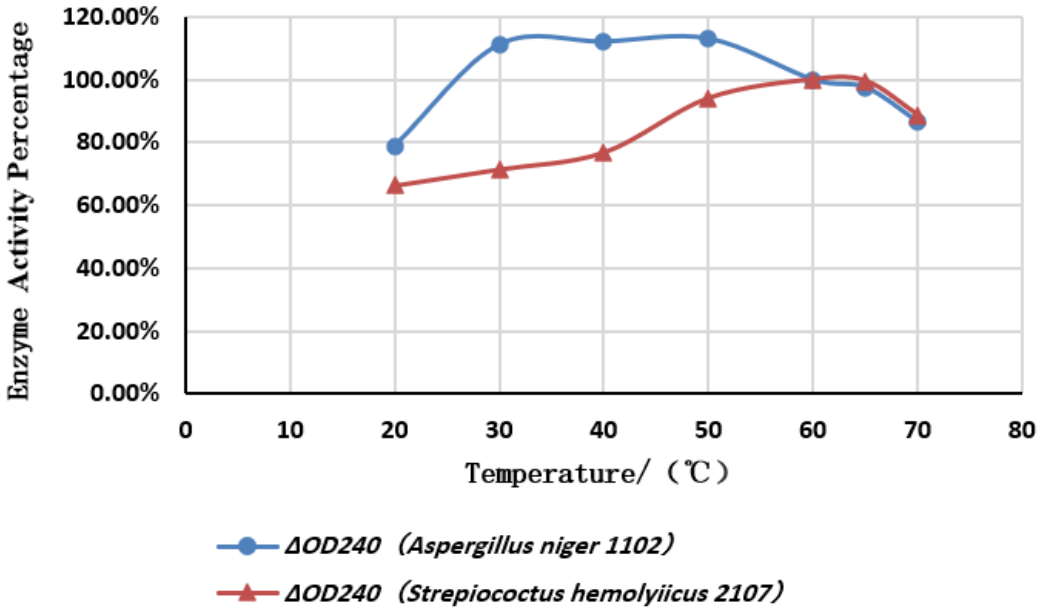

Fig.3. The influence of temperature on catalase enzyme activity.

Based on data in Fig.3, when the temperature was 50°C, the catalase activity percentage produced by *Aspergillus niger* 1102 was the highest; when the temperature was 60°C, the catalase activity percentage produced by *Streptococcus hemolyticus* 2107 was the highest.

After treating the enzyme solution at different temperatures (60°C, 65°C, 70°C) for 120 minutes in a buffer solution with pH 7, the thermal stability of catalase was studied by measuring the remaining enzyme activity (Wu, 2014). The results were shown in Figure 4-1, Figure 4-2, and Figure 4-3.

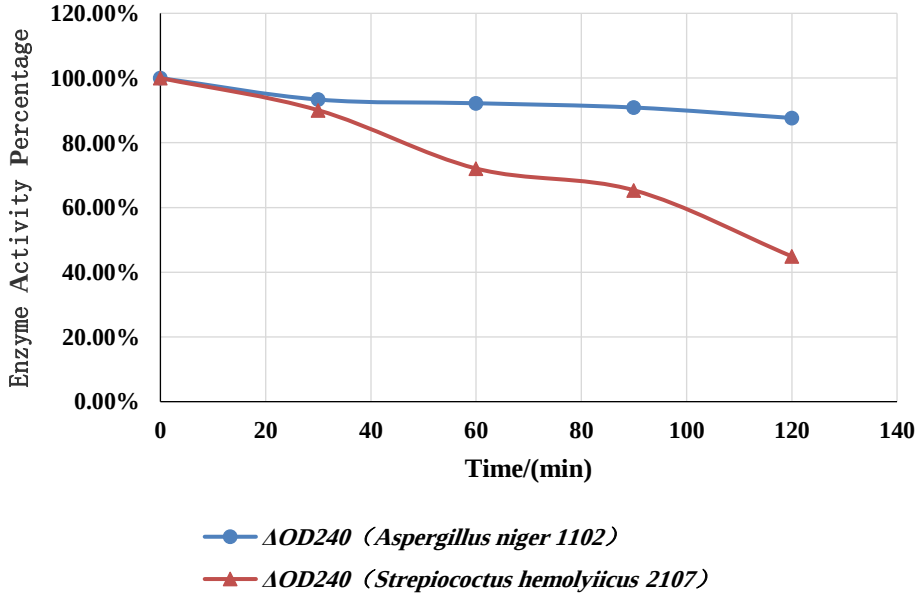

Fig.4-1. Thermal stability of catalase enzyme at 60°C

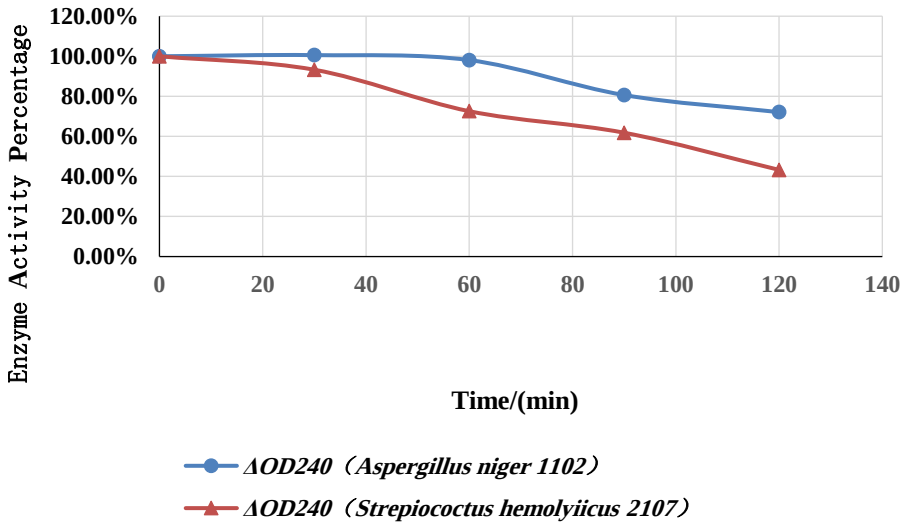

Fig.4-2. Thermal stability of catalase enzyme at 65℃.

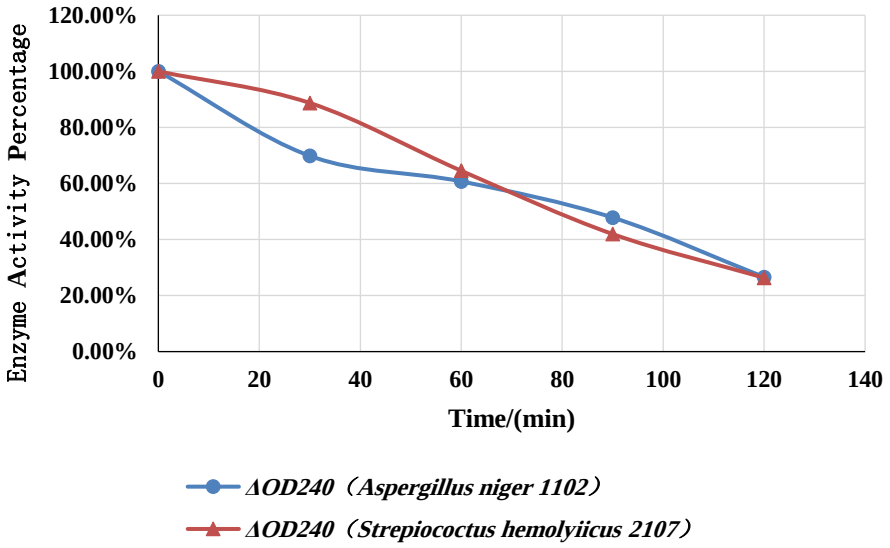

Fig.4-3. Thermal stability of catalase enzyme at 70℃

Based on data in Fig.4-1, Fig.4-2, and Fig.4-3, the thermal stability of catalase produced by *Aspergillus niger* 1102 was more stable than catalase produced by *Streptococcus hemolyticus* 2107 at 60℃ and 65℃. But when the catalase in the system was treated for less than 60 minutes, the thermostability of catalase produced by *Streptococcus hemolyticus* 2107 was more stable than catalase produced by *Aspergillus niger* 1102 at 70℃.

The Effect of Metal Ions on Catalase Enzyme Activity:

The effect of metal ions on catalase activity was studied in the system which was added different metal ions under the same conditions (Yang, 2014). At the same pH and temperature, sodium chloride solution, magnesium chloride solution, calcium chloride solution, and ferric chloride solution were respectively added (Huang, 2019), and their mass fractions were all 0.5 (g/L). The results were shown in Figure 5.

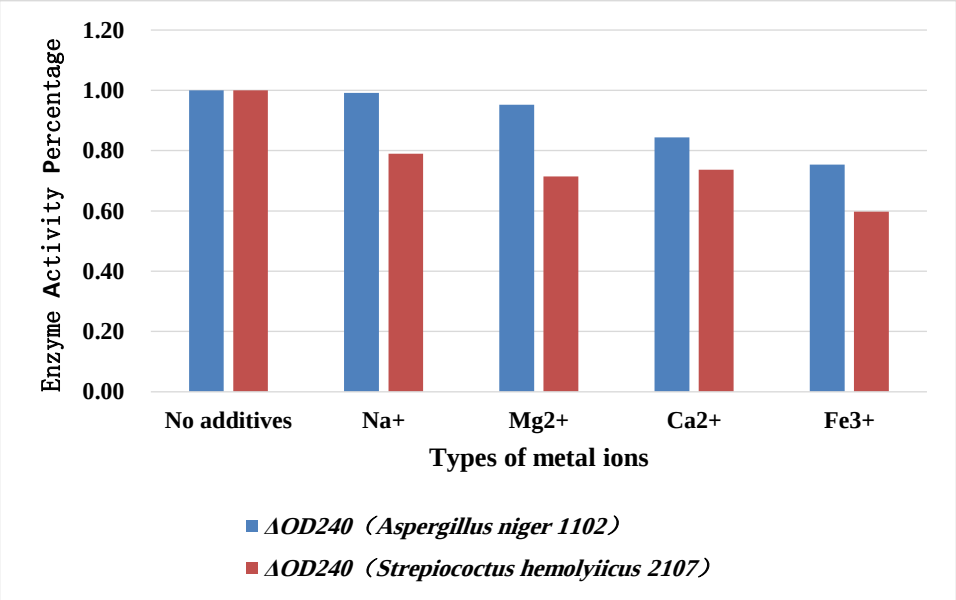

Fig.5. The effect of metal ions on catalase enzyme activity.

Based on data in Fig.5, catalase activity produced by *Aspergillus niger* 1102 was restrained from strong to weak : iron ion >calcium ion >magnesium ion >sodium ion; while catalase activity produced by *Streptococcus hemolyticus* 2107 was restrained from strong to weak: iron ion >magnesium ion> calcium ion in>sodium ion.

Conclusion

The optimum pH value of catalase produced by *Aspergillus niger* 1102 was 8, and the pH range for the more stable enzyme activity was between 7 and 9; while the optimal pH of catalase produced by *Streptococcus hemolyticus* 2107 was 7, and the pH range for stable enzyme activity was between 8-11. The optimum temperature of catalase produced by *Aspergillus niger* 1102 was 50℃; while the optimum temperature of catalase produced by *Streptococcus hemolyticus* 2107 was 60℃. Metal ions had an inhibitory effect on the activities of the two enzymes, of which trivalent iron ions had the strongest inhibitory effect.

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