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Effect of Humidity and Fermentation Duration on Amylase Enzyme by *Trichoderma harzianum*

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Abstract

The effects of humidity and duration of fermentation on amylase enzyme by *Trichoderma harzianum* were investigated in this work. Different Humidity were used as to test the best one to produce the amylase enzyme from this isolate. Also different duration of fermentation were test. Humidity increase from 15 to 20 and also won an award in both enzymatic activity and specific activity at humidity rises above 20. The best duration of fermentation was at 21 days.

Keywords: Proteolytic activity; Wheat bran; Rice hulls; Enzyme activity

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1. Introduction

Microorganisms are an ideal source for enzymes production that are reproductive very quickly and in a short time [1]. Amylase is one of the most Worthwhile enzymes for biotechnology and its global market has been growing significantly. Amylases are the efficient executioners of a common chemical reaction: the hydrolysis of peptide bonds [2]. Amylase is obtained mainly from *Aspergillus* and *Penicillium*. The different hydration rates were used in material fermentation Solid, the humidity effect on amylase production of *A. flavus* was ranged between 35-80% while the effect on *A. oryzae* was at 50% [3]. The wheat bran medium is better than soybean medium in production amylase enzyme by *A. flavus*. It gives a productivity higher than that of soybeans by approximately a half times [4]. Also It was found that wheat bran medium is in development amylase production by *A. oryzae* was the best among solid fermentation media [5]. This study evaluate the effect of agro-waste for potential amylase product by *Trichoderma harzianum* (Fig. 1).

2. Experimental Part

Trichoderma harzianum was used to produce amylase. Two waste materials were used: bran and rice hulls as basic materials for the production of amylase enzyme. Available solids of 10 g/flask were used. The above-mentioned solids are moistened using them Including phosphate at a concentration of 0.2 molar and pH = 7, with a hydration ratio of 1:5 by volume/weight by adding 50 mL of buffer Phosphate to 10 g of solid and binder Wet the oil with solids and sterilize the flasks. It is sealed with a humidity resistance of 121 °C and a pressure of 15 psi for 15 minutes. The flasks were inoculated with the suspension Spores: 610 spores/10 g and incubated for 72 min.

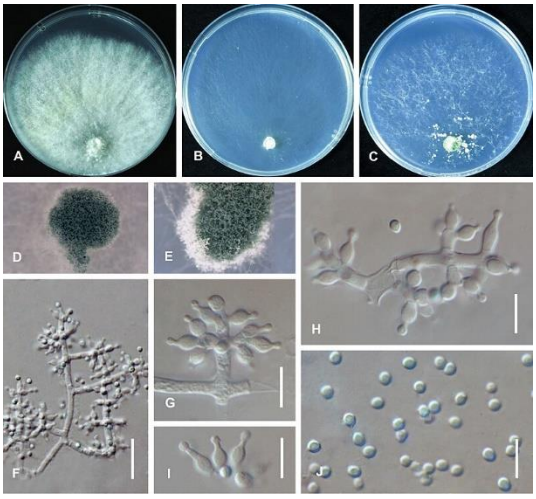

Fig. (1) *Trichoderma zelobreve* (CGMCC 3.19695). Cultures at 25 °C after 3 days (A on PDA B on CMD C on SNA) D conidiation pustules on CMD after 10 days E conidiation pustules on SNA after 10 d F conidiophores G, I conidiophores and phialides H phialides with conidia J conidia. Scale bars: 25 µm (F); 10 µm (G–J)

Murachi method [6] was applied to evaluate Amylase using casein %0.0 with different types of basic materials and defines the unit Enzymatic: It is the

amount of enzyme that is amplified in optical wavelength at wavelength 220 nm/min under standard conditions. Protein concentration can also be measured by strength loss according to the method described by Bradford [7].

3. Optimal Conditions of Amylase Production

Determine the optimal conditions for amylase production in the medium Solid fermentation. A study was conducted that affects some factors as a percentage hydration and nutritional value of the medium vary humidity and duration of fermentation affect the production of amylase by local isolation of *T. harzianum* at medium bran, 10 g per flask and the use of phosphate buffer in humidification. All the circumstances referred to above were recorded in the judgment. The required factor has a message and its effect. For determine the optimal humidity for enzyme production, inoculate wheat bran medium with spore extraction 1×610 spore/10 g is a very hot substance and was incubated at high humidity various (20, 28, 30, 32 and 35%) for 72 hours, then the enzyme was extracted and estimated enzymatic activity.

4. Results and Discussion

For the purpose of study the efficiency of selected local isolate *T. harzianum* to produce the amylase enzyme using fermentation technology. When planting hardwood, waste of plants were used as a culture media to test the best solid fermentation medium to produce the amylase enzyme from this isolate. Wheat bran and rice hulls A (solid media, separately). It turns out that the center of wheat bran is better fermentation medium for enzyme production compared to another medium. With wheat bran medium the enzymatic activity was at 1600 units/mL and effectiveness was at 1200 units/mg Protein. While the enzymatic activity with rice hulls medium was at 350 units/mL and effectiveness 290 units/mg protein. The reason for the superiority of wheat bran over another medium (rice hulls) was may be to Class A (for the high content of planets, as its protein content 14.62% compared while rice hulls contains 9.93 of protein. Also, the physical properties of materials that used in the fermentation of solid culture effects the production of various enzymes such as the size of particles. The surface area exposed to the action of living organisms Microstructure and porosity of the medium [8,9]. Previous study found that the wheat bran medium is better than soybean medium in production amylase enzyme by *A. flavus*. It gives a productivity higher than that of soybeans by approximately a half times [4]. Also It was found that wheat bran medium is in development amylase production by *A. oryzae* was the best among solid fermentation media [5].

Amylase enzyme productivity from T. *harzianum* elevated when humidity increase from 15 to 20 and also won an award in both enzymatic activity and specific activity at humidity rises above. For a long time The optimum humidity for enzyme production was 20, at enzymatic activity is 1610 units/mL and is effective 1500 units/mg protein and varies, changes in both the enzymatic activity and specificity of the enzyme at humidity rises above 20. The drop in humidity below the optimum level. It leads to slow growth and delayed enzyme synthesis. This humidity is suitable for mushroom growth. On the other hand enzyme stability on the other hand, the optimum humidity for the production of amylase enzyme. This study is 20 m in length, along with the results of other studies. It dealt with the production of amylase enzymes by species where *Aspergillus* humidity s rise 20. The optimum for the production of these enzymes between 28. These studies indicated a decrease in the effectiveness of the enzyme.

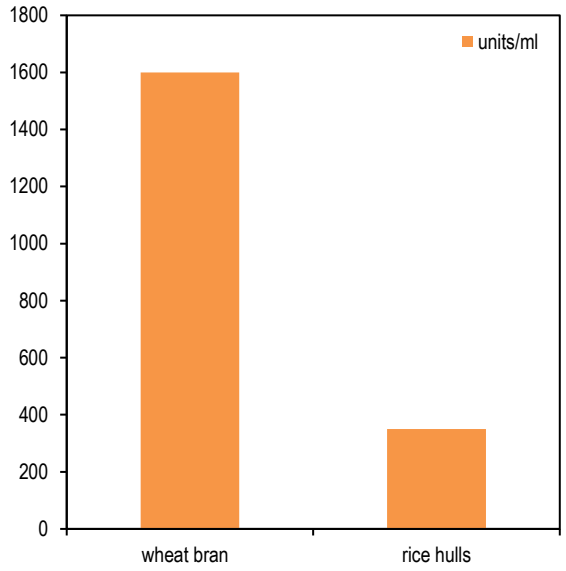

Fig. (1) Effect of formation media on amylase enzyme activity

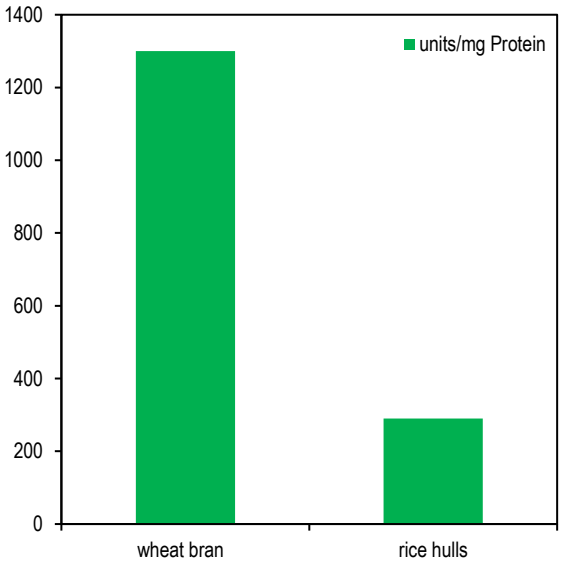

Fig. (2) Effect of formation media on amylase enzyme activity

5. Conclusion

The effects of humidity and duration of fermentation on amylase enzyme by *Trichoderma harzianum* were investigated in this work. Different Humidity were used as to test the best one to produce the amylase enzyme from this isolate. Also different duration of fermentation were test. Humidity increase from 15 to 20 and also won an award in both enzymatic activity and specific activity at humidity rises above 20. The best duration of fermentation was at 21 days.

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