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Identification of *Aspergillus species* using morphological characteristic and the effect of temperature on the protease activity

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ABSTRACT

Aspergillus sp. is a fungus that can produce protease enzyme. A proteolytic enzyme of *Aspergillus sp* is enzymes group that attack the peptide bonds of proteins. In this study, we emphasize on morphological methods including macroscopic and microscopic characteristics for identification of *Aspergillus sp*. *Aspergillus sp* was isolated from saffron bulb. Protease was produced by *Aspergillus sp.* and the temperature effect was evaluated on its activity, at 20, 25,30°C with casein as a substrate. The results obtained show that isolates selected have the green color colony (Fig.1. A,B,C),grown evenly on Czapex medium were incubated at 37°C for 4 days, round-shaped vesicles(Fig.1.1), conidiophores somewhat yellowish green nodes, and conidia spore round and light green. The data of protein contents is shown on Table 1. The average protein contents of the crude enzyme protease extract were 0.95 mg/ml of isolates A, 0.98 mg/ml of isolates B, and 1.03 mg/ml of isolates C. Isolate C was selected to produce enzyme and analyze enzyme activities. The enzyme activities of isolate C were 85.85 U/ml at 20°C, 101.87 U/ml at 25°C and 179.00 U/ml at 30°C,. The optimum activity was 179.00 IU/ml and it was reached at 30°C**.**

Key words: *Aspergillus,* identification, temperature, protease activity

INTRODUCTION

Aspergillus sp. is a fungus that can produce protease. In some Asian countries, some fungus are widely applied to producing traditional fermented food (Fogarty, 1983). Proteases from fungi have more profit than the protease from bacteria in the separation of the enzyme because the mycelium can be removed simply by filtration. Proteases produced by *A. sp.* is more important because the ability of the resulting higher protease. Solid-state fermentation has many advantages, including large-scale productivity; substrate used is not expensive, simple process, low energy requirements and wastes little (Malathi and Chakraborty, 1990).

Generally identification of the Aspergillus species is based on the morphological characteristics of the colony and microscopic examinations (McClenny, 2005). Although molecular methods continue to improve and become more rapidly available, copy and culture remain commonly used and essential tools for identification of *Aspergillus sp*. The use of microorganisms for enzyme production has several advantages, including easy producing on a large scale; production time is relatively short and is produced simultaneously with a relatively low cost.Protease-producing microorganisms can be bacteria, fungi, and yeast (Such as, *B. licheniformis, B. subtilis, A. niger, A. sp., A. oryzae* etc). The enzymes produced by microorganisms were separated by centrifugation to separate the cells and subsequent purification is done by precipitation, gel filtration and ion exchange chromatography (Smith, l990). The most common simple method is precipitation in cold temperatures with a high concentration of ammonium salt specific (different) or the use of organic solvent, the precipitate was dissolved in buffer (Deutcher, 1990). Proteases are enzymes that catalyse hydrolytic reactions in which protein molecules are degraded to peptides and amino acids (Sumantha et al., 2006). They constitute one of the most important groups of industrial enzymes and have applications in different industries such as detergent, food, feed, pharmaceutical, leather and waste processing. Proteolytic enzymes are important in the industry, about 60% of the total enzyme traded in the world (Woods et al., 2001). In Indonesia, the needs of protease enzymes also increased but the need is still dependent on imported production. One way to anticipate dependence on imports is necessary to attempt to produce protease enzymes (Thomas, 1984; Suhartono, 1989). This study deals to obtain protease-producing *Aspergillus* in solid-state fermentation conditions (SSF) saffron bulbs and find the optimum Temperature

MATERIALS AND METHODS

Sample preparation

Samples were collected from saffron bulbs obtained from pampore area of kashmir . moist sterile swab was rub on the surface of saffron bulb and streaked on plate containing czapeks medium in sterile conditions and then the samples were incubated 27°at C for 4days in a shaker (Nadem et al., 2007).

Culture and identification

Inoculated samples on czapeks medium were enriched with 1% casein, and then incubated at 37°C for 7 days (Hanjhani and Setyaningsih, 2006). Isolates obtained were observed on colony and cell morphology. *Aspergillus* was identified according to its specific colony characteristics, slides were also prepared for identification of mycelium and hyphial arrangement with lactophenol blue staining method (Darise, 1987). Isolate produced highest protein content were selected for protease enzyme production.

Protease Production

Isolates were grown in the medium as much as 10 ml of molasses plus 15 ml of saline solution.Medium was sterilized at 121°C for 20 min. Incubation was carried out at room temperature for 7 days (Malathi and Chakraborty,

Table 1. Protein content from saffron bulb isolates (mg/ml)	
Name of isolate	Protein content (mg/ml)
A	0.92
B	0.98
C	1.03
Average	0.98

1990). Prepared substrate plus 200 ml phosphate buffer pH 7, and then shaked until homogeneous and filtered. The filtrate obtained is a source of crude protease enzymes.

Table 2. Effect of incubation temperature on protease activity (U/ml)	
Incubation temperature (°C)	Enzyme activity (U/ml)
20	85
25	101
30	179
Average	121

Protein Determination

Protein was estimated according to the method of Lowry *et al*. (1951). So,1 ml enzyme solution plus 5 ml Lowry B, then shaked and left for 10 minutes. The solution was added with 0.5 ml of Lowry A, then shaked and left for 20 minutes. The solution was read with a spectrophotometer at 600 nm absorbance.

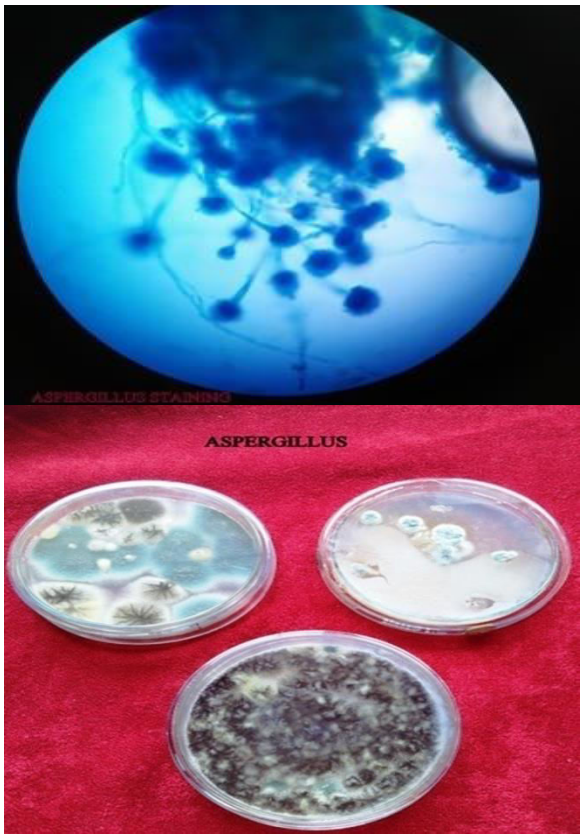
Protease activity assays at different temperatures

It was determined using casein as a substrate according to the method reported by Pagare *et al.* (2009). 0.2 ml of crude Enzyme extract plus 2 ml casein 0.2% and 1.8 ml phosphate buffer pH 7, then incubated at 20, 25, 30°C for 10 min. At the mixture we added 4 ml of 5 % TCA and allowed to stand at room temperature for 20 minutes. The mixture was filtered with Whatman paper No. 1, which was read by spectrophotometer at 280 nm absorbance (Pagare et al., 2009).

RESULTS AND DISCUSSION

Isolates selected have the green color colony (Figure.1. A, B, C), grown evenly on Czapeks medium were incubated at 37°C for 4 days, round-shaped vesicles (Fig.1. 1), conidiophores some what yellowish green nodes (Fig.1. 2), and conidiaspore round and light green (Fig.1. 3). Based on the results of cell morphology observers, these isolates belong to the group of *Aspergillus sp*. that have the same characteristics, namely globular vesicles, conidiophores shaped translucent yellowish green, semi conidiaspore round to round-shaped light green to brownish green (Yunasfi, 2008). Coniodiaspore colors caused by lipid compounds red, brown, yellow on the walls of spores, while the dark Spores caused by walls containing melanin (Schenck, 1982).

The average protein contents of the crude enzyme protease extract were 0.95 mg/ml of isolates A, 0.98 mg/ml of isolates B, and 1.03 mg/ml of isolates C (Table 1). According to Harlis (2008), different types of mold provide different levels of protein. The differences in levels of a



protein produced in each mold was due to different populations of fungi that get each activity in the enzyme protease is also different. On the other hand, the results obtained shows that the temperature affect significantly the activity of proteolytic enzymes. The data of effect of temperatures on protease activity of *Aspergillus sp*.is shown on Table 2.The difference temperatures can affect the protease activity of *Aspergillus sp.* with a range between 200 U/ml and 1,000 U/ml with 3% casein as a substrate. (Table 2). So, the highest enzyme activity, i.e., 179 U/ml was obtained at an incubation temperature of 45°C ($P<0.01$). Similar results were reported by Hossain et al. (2006); Morimura *et al.* (1994).

According to Chaplin and Christopher (1990), the use of enzymes at high temperatures was better because it improved the response and able to protect against microbial contamination. However, enzymes are proteins that can undergo denaturation at temperatures above the action of the enzyme and usually are influenced by the environment.

CONCLUSION

Isolates selected are *Aspergillus* sp that have the green color colony, round-shaped vesicles, yellowish green nodes conidiophores, and round and light green conidiaspore. The highest protease enzyme activity of *Aspergillus sp*. isolated from saffron bulb (179 U / ml) was achieved at 30°C.

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