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Utilization of Channels Digestion Golden Snail (*Pomacea Canaliculata*) as Lytic Enzyme and Application on Yeast *Pichia Manshurica* DUCC-Y15

Wijanarka[1,*], Jafron W.Hidayat[1]; Sarjana Parman[1]

[1]Departement of Biology, Faculty of Science and Mathematics UNDIP Semarang

*Email: wikasmara@yahoo.co.id

ABSTRACT. Mollusks were one of invertebrate animals that have not been studied and used in in the field of enzyme. One type of mollusk that has not been exploited significantly is golden snail (*Pomacea canaliculata*), especially in the digestive tract for the production of lytic enzymes. Lytic enzymes can be used as a microbial cell wall-breaking agents that will produce protoplasts, besides that this enzyme plays an important role in protoplast isolation technique producing good and healthy protoplasts. Protoplasts derived from the yeast *Pichia manshurica* DUCC-Y15is capable of producing the inulinase enzyme. The aim of this study was to use the digestive tract golden snail (*Pomacea canaliculata*) as lytic enzymes and to determine the amount of the released protoplasts at a concentration level of lytic enzymes different from the digestive tract golden snail (*Pomacea canaliculata*). Lytic enzyme concentrations used in this study was 75% (E3) and 100% (E4). The results showed that the digestive tract golden snail (*Pomacea canaliculata*) can produce lytic enzymes, the higher the concentration of lytic enzymes digestive tract golden snail given, then the higher the protoplasts were released. At a concentration of 75% lytic enzyme (E3) liberate protoplasts of 6.7 x 10^{17} (33.4%) and 100% (E4) of 9.9 x 10^{17} (45%).

Key words: lytic enzyme, Mollusk, golden snail (*P. canaliculata)*

Introduction

Golden Snail (*Pomacea canaliculata*) as well as snails, classified as slimy and soft animals known as molluscs. The mucus secreted on the surface of the body of the golden snail serves to support the survival like to eat, move, reproduction and osmoregulation. According Berniyanti (2007) that Glycosaminoglycans (GAGs), also known as glycoprotein as a constituent achasin contained in Golden Snail mucus or Snail (*Achatina fulica* Ferussac). Achasin serves as antibiotics. On the other hand, one of the Golden Snail was agricultural pests that have a high ability to damage agricultural crops. This suggests that the Golden Snail able to digest forage also and fiber produces digestive enzymes [1]. Castro *et. al.* [2] states that the midgut gland Golden Snail capable of producing cellulase enzymes

According Ezeronye and Okerentugba [3] that the intestinal tract ataupu snail groups other Mollusks produce the enzyme β glukoronidase, endo and β glucanase and arylsulphatase. These enzymes are normally present in the digestive tract (gut) and capable of being used for protoplast isolation. Golden snail is one of a group of Class Mollusks.

Pichia manshurica DUCC-Y15 was a type of yeast inulinolytic. The yeast capable of producing the enzyme inulinase (EC 3.2.1.7) [4]. This enzyme plays an important role to hydrolyze inulin into fructose polymer and doctoring agent. Relative enzyme produced by small, so it was necessary to genetic manipulation by protoplast fusion techniques [5, 6].

One of stage in the process was the isolation protoplast fusion. Protoplasts were cells that have lost the cell wall and still have a life activity. In the process of protoplast isolation, the use of the type and concentration factor of the amount of lytic enzyme plays an important role, so it will be perfect protoplasts [6, 7]

The aim of this study was to get a lytic enzyme derived from the Golden snail (*Pomacea canaliculata*), and to determine the amount of Pichia manshurica protoplast DUCC-Y15 liberated at a concentration level of 75% lytic enzyme (E3) and 100% (E4)

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Methods

Yeast strains and media.

P. manshurica DUCC-Y15 was obtained from our microbiology laboratory at the Faculty of Sciences and Mathematics, Diponegoro University [8]. This yeast was grown at 28 °C on Yeast Peptone Dextrose Broth (YPDB) This medium containing (w/v) yeast extract 1%, peptone 2%, and glucose 2%, with a pH of 5.5–6.0 [9].

Lytic enzyme of golden Snail (*Pomacea canaliculata*) preparation

Lytic enzyme extraction of gold snail according to the method Agogbua et al. (1978). Some golden snail that average-sized (120 g) was dissolved in 50 ml osmotic stabilizer sorbitol solution in sodium phosphate buffer pH 5.8. Later in the blender, the supernatant obtained was then filtered with a membrane filter. The filtrate enzymes were then stored in the refrigerator. This enzyme will be used for protoplast isolation.

Protoplast preparation .

P. manshurica DUCC-Y15 was grown on YPDB medium until the log phase, when culture was harvested and suspended in sorbitol osmotic stabilizer 1 M in 0.2 M H_2PO_4/Na_2HPO_4 buffer with a pH of 5.8. After washing with the same buffer, cells were incubated at 28°C for 90 min with lytic enzyme 75% and 100% to achieve protoplast isolation [10]

Result and discussion

From the observations that have been made to the growth of the yeast *P. manshurica* DUCC-Y15 in the media YPDB apparent that log phase took place at the 6 h until the 30 h (Figure 1).

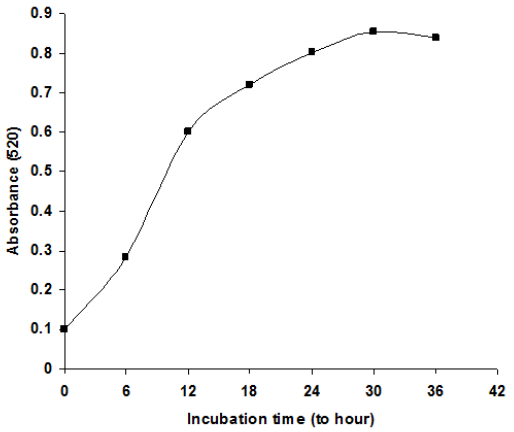


Figure 1. Growth Curve of Cells of *P.manshurica* DUCC-Y15 for Protoplast Isolation on YPDB Medium [11]

Harvesting the cells and ranges in this phase needs to be done, this was due to the phase of cell division activity was having a very high and fast. And thus the number of cells can be increased very quickly anyway. In this phase it was suitable for harvesting cells and the expected time of isolation protoplast good yield. In the log phase of cell age was still young enough and easy to do protoplast. According to Peberdy and Santiago [12] and Santopietro et al. [9] that the microbial culture yeast especially in the exponential phase (log phase) produces the number of protoplasts good and healthy [13]

The number of protoplasts were released directly proportional to the lytic enzymes are given. The higher the concentration of lytic enzymes is given, then the protoplasts are released also greater. This is evidenced in the treatment of E3 (the concentration of lytic enzymes 75%) and E4 (concentration of lytic enzymes 100%) respectively liberate protoplasts of 6.7 x 10^{17} (33.4%) and 100% (E4) of 9.9 x 10^{17} (45%) (Figure 2 and 3).

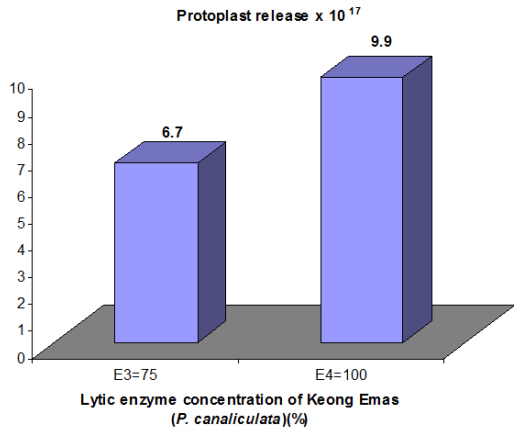


Figure 2. Effect of Lytic Enzyme Concentrations from golden Snail (*P. canaliculata*) on protoplast release

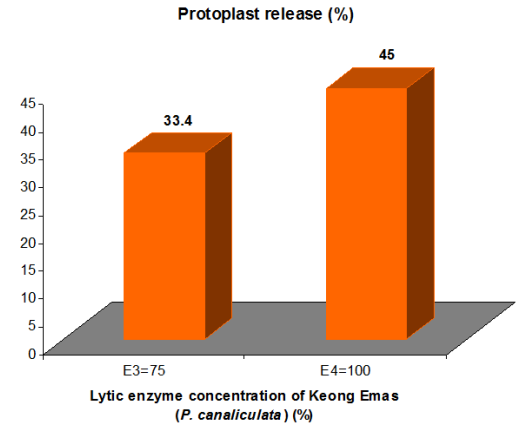
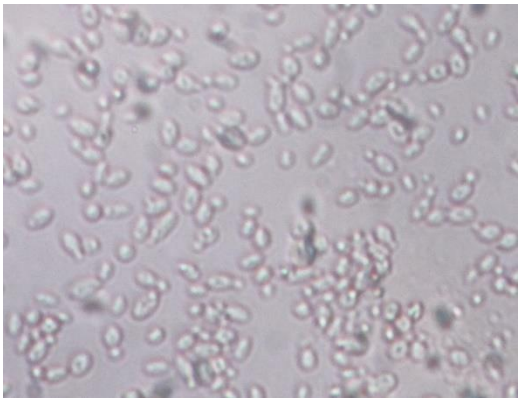


Figure 3. Persentage of protoplast release after effect of Lytic Enzyme Concentrations from golden Snail (*P. canaliculata*)

Based on research that has been done, changes oval shape into a round protoplasts from intact (Figure 4a and b). This is in accordance with the opinion of Santopietro et al. [9] that the results of spherical protoplasts healthy and intact. Protoplast any deformities may be caused by the lytic enzymes contained in the digestive tract golden snail that was able to change the shape of protoplasts. According Ezeronye and Okerentugba [3] that the intestinal tract, snail, snail gold or other mollusk groups were able to produce the enzyme β glucoronidase, endo and β glucanase and arylsulphatase.

a.



b.

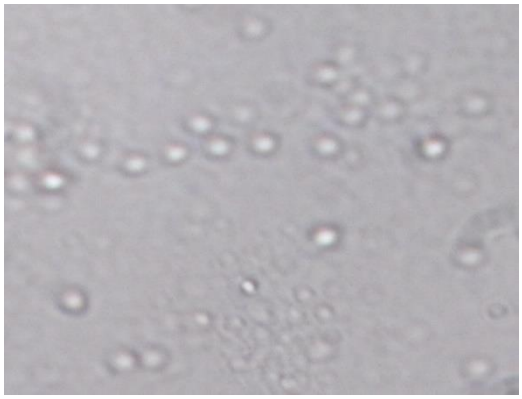


Figure 4. *Pichia manshurica* DUCC-Y15. (a) Cell normal (oval) (b) Protoplasts (rounded)

Conclusions

Based on research that has been done, it can be concluded:

1. That the lytic enzymes can be produced from the digestive tract golden snail (Pomacea canaliculata).
2. Lytic enzyme golden snail (*Pomacea canaliculata*) can be used to destroy the cell wall of the yeast *Pichia manshurica* DUCC-Y15
3. The concentration of lytic enzymes golden snail (*Pomacea canaliculata*) 100% (E4) was able to liberate the highest protoplast 9.9 x 10^{17} (45%).

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