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European Journal of Experimental Biology, 2015, 5(10):49-53

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Utilization digestive tract of golden snail (*Pomacea canaliculata*) as lytic enzyme for protoplast isolation *Pichia manshurica* DUCC-Y15

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ABSTRACT

Mollusc is one of invertebrate animals groups that have not been studied and used in particular in the field of enzymatic process. One mollusc that has not been exploited for the production of lytic enzymes is in snail's digestive tract golden snail (Pomacea canaliculata). Lytic enzymes can be used as a microbial cell wall-breaking agents (yeasts) that will produce protoplasts. Besides that, such enzyme plays an important role in protoplast isolation technique that will produce good and healthy protoplasts. Protoplasts derived from the yeast Pichia manshurica DUCC-Y15 were capable of producing the inulinase enzyme. The aims of this study are to use of the digestive tract of 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 were 75% (E3) and 100% (E4). The results showed that the digestive tract of golden snail (Pomacea canaliculata) can produce lytic enzymes. The higher the concentration of lytic enzymes in digestive tract of golden snail given, the higher the protoplasts were released. At a concentration of 75%, lytic enzyme (E3) liberated protoplasts as much as 6.7 x 10^{17} (33.4%) and at concentration 100% (E4) was 9.9 x 10^{17} (45%).

Key words: lytic enzyme, golden snail, protoplast isolation, digestive tract

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INTRODUCTION

Keong Mas or golden snail is terrestrial mollusc with spherical shell, conical, herbivores, eating very greedy and golden yellow color [9]. 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; including to move, eat, reproduction and osmoregulation. The Glycosaminoglycans (GAGs), also known as glycoprotein is a achasin constituent contained in Golden Snail mucus or land snail (*Achatina fulica* Ferussac). Achasin serves as antibiotics for snail [16]. 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 enable to digest forage and produce fiber digestive enzymes [2]. Mentiones that the midgut gland of Golden Snail capable of producing cellulase enzymes [4]. According to The intestinal tract of golden snail or other Molluscs can produced the enzyme β glukoronidase, endo and β glucanase and arylsulphatase [7]. These enzymes are normally present in the digestive

tract (gut). These enzyme are capable of being used for protoplast isolation. Golden snail was one of a group of Class Molluscs.

Pichia manshurica DUCC-Y15 was a type of inulinolytic yeast. The yeast are capable to produce inulinase enzyme (EC 3.2.1.7) [6]. This enzyme plays an important role to hydrolyze inulin into fructose polymer and doctoring agent. Relatively small in number of such enzyme produced, therefore it is necessary to manipulate genetically by protoplast fusion techniques [5][14]. One of stage in the process was the isolation protoplast fusion. Protoplast were cells that have lost the cell wall but still have a life activity. In the process of protoplast isolation, type and concentration factors of the amount of lytic enzyme plays an important role, to bear a perfect protoplast [1] [14]. Protoplas is very important to use the fusion process. Protoplasts of different microbial cells or species can be fused even if they are not closely linked taxonomically. With the help of protoplasts fusion, genetic information is transferred and recombinant features constructed. Industrially, important cells such as yeasts have cell walls which make it difficult to fuse with cells of different species hence retarding the aim of improving some industrial products by producing good hybrids through genetic manipulation.This problem is overcome by obtaining protoplasts and using them instead of using intact cells with cell walls [10].

The aims of this study are to use of the digestive tract of 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*)

MATERIALS AND METHODS

Yeast strains and media

P. manshurica DUCC-Y15 was obtained from Microbiology Laboratory of Faculty of Sciences and Mathematics, Diponegoro University, Indonesia [8]. This yeast was grown at 28 ^{o}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 [12].

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

Lytic enzyme extraction of golden snail were done according to the method Ezeronye, O.U and Okerentugba. Some golden snail that had average-sized about 120 g were dissolved in 50 ml osmotic stabilizer of sorbitol solution in sodium phosphate buffer pH 5.8. Later, it was blended, the supernatant obtained was then filtered using a membrane filter. The filtrate of the enzymes were then stored in the refrigerator with temperature of 4^{o}C. This enzyme were ready to be used for protoplast isolation [7].

Protoplast preparation

P. manshurica DUCC-Y15 was grown on YPDB medium culture until the log phase, then it 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 being washed with the same buffer, cells were incubated at 28^{o}C for 90 min with 75% of lytic enzyme and 100%, respectively, to achieve protoplast isolation [3].

RESULTS AND DISCUSSION

Growth of *P. manshurica* DUCC-Y15

Based on the observations that have been made on the growth of the yeast *P. manshurica* DUCC-Y15 in the media YPDB, it showed that log phase took place from 6 h until 30 h without a lag phase (Figure 1). In this study, the starter reduced the lag phase, and the log phase was reached quickly. In this research, the starter reduced the lag phase, and the log phase was reached quickly.

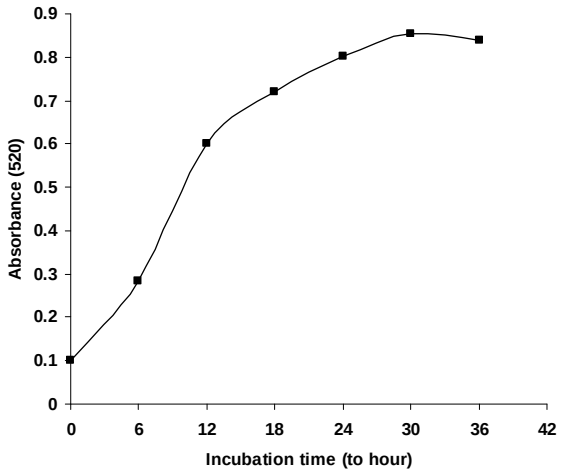


Figure 1. Growth curve of cells of *P.manshurica* DUCC-Y15 for protoplast isolation on YPDB medium [15]

Results showed that the cells were harvested, however the ranges in this phase needed to be done. This was due to the phase, cell division activity were very high and fast. Thus, the number of cells can increase very quickly. In this phase, it was suitable for harvesting cells and the expected time of isolation protoplast resulted in good yield. In the log phase, cell age was still young enough and easy to do protoplast isolation. According to The microbial of a culture, especially in yeast, the exponential phase (log phase) produces numbers of good and healthy protoplasts [12][14].

Lytic enzyme of keong emas and protoplast isolation

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

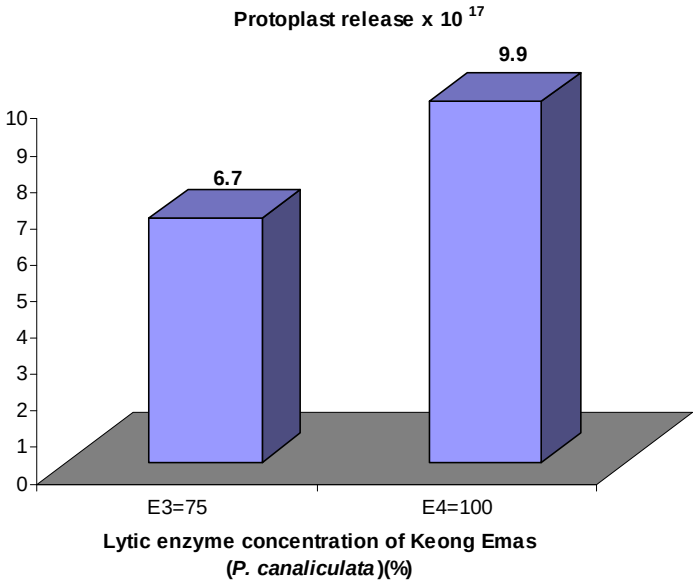


Figure 2. Effect of Lytic Enzyme Concentrations from digestive tract of golden snail Keong emas (*P. canaliculata*) on protoplast released

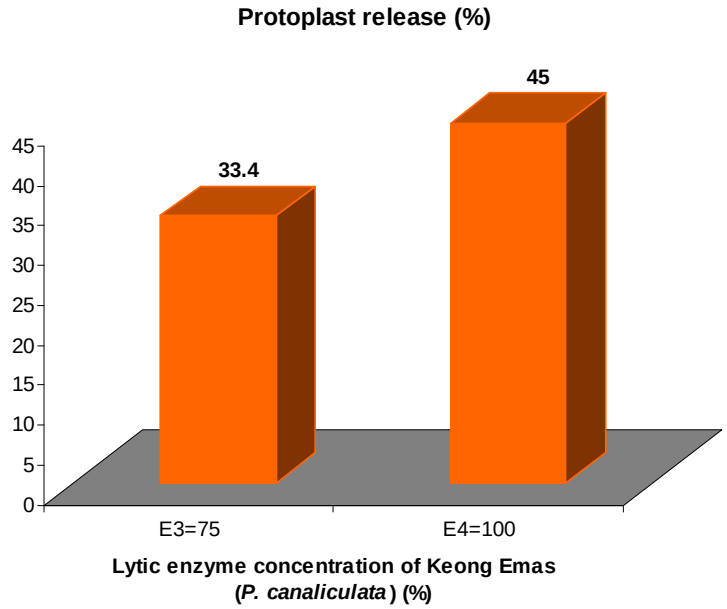
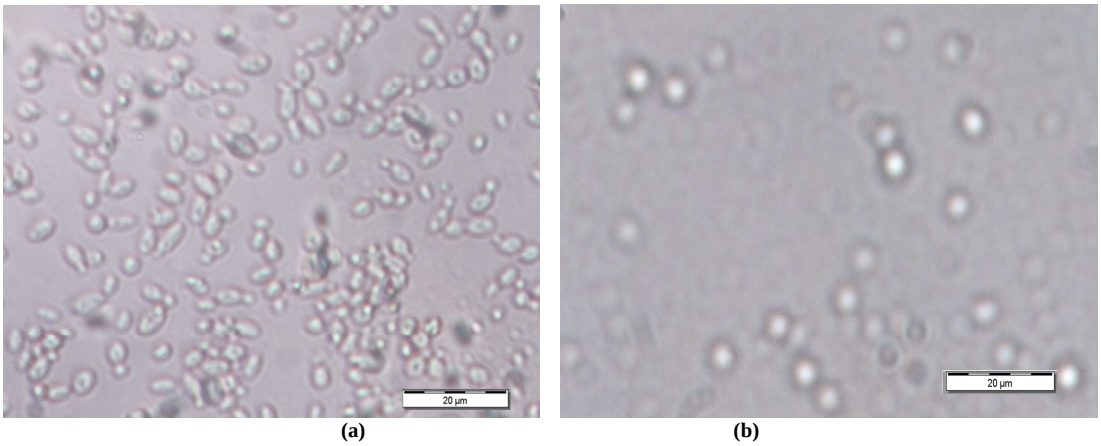


Figure 3. Percentage of protoplast released after being treated with Lytic Enzyme from digestive tract of golden snail Keong emas (*P. canaliculata*) on protoplast released

Based on research that has been done, there was a change in general shape of the protoplast from oval shape into a spherical during intact (Figure 4 a and b). This was in accordance with the research of showed spherical protoplasts, which indicated that a success to produce healthy protoplast and intact. If protoplast performed any deformities, it may be caused by the lytic enzymes contained in the digestive tract of golden snail, had responsibility on changes in the shape of protoplasts. In the intestinal tract of snail, golden snail or other mollusc groups were able to produce the enzyme β glukoronidase, endo and β glucanase and arylsulphatase [7] [11]. In the otherhand, the protoplasts appear more rounded, an effect enhanced by the osmotic stabilizer solution [14].



(a) **(b)**

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

CONCLUSION

Based on research that has been done, it can be concluded that: The lytic enzymes can be produced from the digestive tract of golden snail (*Pomacea canaliculata*), lytic enzyme of golden snail (*Pomacea canaliculata*) can be used to destroy the cell wall of the yeast *Pichia manshurica* DUCC-Y15, the concentration of 100 % lytic enzymes golden snail (*Pomacea canaliculata*) (E4) was able to release the highest protoplast 9.9 x 10^{17} (45%).

Acknowledgements
The authors gratefully acknowledge the financial support received from the University of Diponegoro that financed the research througt Source of Funds Budget Undip. PNBP DIPA 2015, contract number: 042.04.2.400049/ 2015. On 15 April 2015.

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