Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

GROWTH AND ACTIVITY OF CELLULASE-AMYLASE ENZYME *PENICILLIUM NALGIOVENSE* AND *ASPERGILLUS TAMARII* MOLDS ISOLATED FROM COW RUMEN FLUID

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Abstract

Cow rumen fluid is a fluid rich in cellulolytic microbes that play a role to help digest food that contains high crude fiber. Two species of moulds have been isolated from the cellulolytic rumen fluid of local cattle and have identified as Penicillium nalgiovense and Aspergillus tamarii. Cellulolytic Index both of Penicillium nalgiovense and Aspergillus tamarii were 2.33 and 1.24 respectively. The purpose of this study is to characterize their growth and activities of cellulase and amylase. Research include: 1) growth of mould 2) macroscopic description, and 3) activities of cellulase and amylase enzyme using the DNS (3,5-dinitrosalicylic acid) method. Based on the growth curve is known that the logarithmic phase of growth of Penicillium nalgiovense peak occurred on day 6 with a population of 9.696 x 10^3 cfu cells, whereas Aspergillustamarii logarithmic phase, occurred on day 5 with a population of 4.65 x 10^2 cfu cells. Cell morphological characters of P. nalgiovense as conidium colour milky white, and spore powdery, in the early growth of Penicillium nalgiovense white colonies after a while the colonies get old is also white and conidiophores with smooth stipe. As macroscopic A.tamarii is yellow-brownish, at the beginning of growth colonies colour is a bright yellow growth after several days, color were changed to dark brownish yellow. This mold has a conidial head yellow-brown, not columnar and have ornaments that are not clear. Cellulase enzyme activity in Penicillium nalgiovense amounted to 2.420 units / ml, and amylase enzyme activity of 2.146 units / ml. Aspergillustamariii to be used for degradation of fiber for feed.

Keywords: *description, characteristic, cellulose and amylase enzymes activity, Penicilium nalgiovense, Aspergillus tamari, cow rumen fluid.*

INTRODUCTION

Superior microorganisms which can be used as a source of enzymes as well as fermentative microorganisms can be isolated from a natural source. Natural sources are sources such as cow rumen fluid derived from the Slaughterhouse waste (RPH), because they contain different types of enzymes that can break down the most complex structure in the fodder. Jovanovic and Cuperlovic (1977) states rumen microbes can increase the nutritional value of food due to microbial protein, resulting in increasing digestibility. In addition, rumen is a source of polysaccharide degrading enzymes due to synergistic effects and interactions of complex microorganisms, mainly producers of cellulase and xylanase (Trinci et al., 1994).

Andriani (2010) isolated a fungus cellulotik of local cattle rumen fluid, and got the results that based on cellulotic index (CI), *Penicillium nalgiovense* and *Aspergillus tamarii* are molds that have the largest cellulotic ability among all aerobic fungi isolated, respectively 2.33 and 1.24. The cellulolytic potential can be utilized in the process of pre-digestion of the coarse fiber in the form of cellulose, hemicellulose and lignin, which are abundantly found in waste cellulose, so that at the time of entry into the body of the fish it is already available in a form that is more easily digested, for example in the form of poly or oligosaccharides.

Enzymes have a specific ability to degrade the material according to its kind. These

capabilities can be measured by conducting an enzyme activity test. Similarly, the use of enzymes from bovine rumen fluid microorganisms origin requires testing first, so it can be measured in its ability to degrade feedstuffs containing high crude fiber, cellulose in this case. Testing will then become the basis for the use of natural more economical enzymes as an alternative to commercial enzymes on the pre-digestion of agricultural waste in-vivo, with the goal of lowering coarse fiber, particularly cellulose agricultural waste based fish feed ingredients.

MATERIALS AND METHODS

The tools used in this study include incubator oven, UV-Vis spectrophotometer, centrifuges, vortex, autoclave, incubator shaker, laminar air flow, balance scales, water heaters, micropipette, tip micropipette, polypropylene tubes, Eppendorf tubes, and glass tools. The sample used in this study were *Penicilium nalgiovense*, *Aspergilus tamarii* fungal isolate, PDA and SBA agar, glucose, distilled water, soluble starch, CMC (Carboxy Methyl Cellulose), phosphate buffer, 3.5-Dinitro-salisilat acid reagent (DNS).

The microbes were cultured for 7 days using the submerged fermentation method. The medium used was Potato Dextrose Agar, Sabouroud Dextrose Agar, to which has been added to roomates CMC 1% and Medium cellulose to which tetracycline has been added respectively.

After 24 hours, colonies were growth and then calculated using dillution series methods (Total Plates Count). Cellulase and amylase enzyme activitiy were carried out during in the peak of logaritmic phase (4 days). Research observed include: 1) growth of mold, 2) macroscopic of mould description, and 3) The activity of cellulase and amylase enzyme by using the DNS (3,5-dinitrosalicylic acid) method.

RESULTS AND DISCUSSIONS

The results can be described if the macroscopic identification of *P. nalgiovense* is milk-white in color, and its spores resemble milk powder, initially white and also white after aging, but less bright. While *A.tamarii* was macroscopic yellow-brownish, at early rejuvenation bright yellow and after a few days it turned stark contrast to dark brownish yellow. Results of microscopic identification key, based on the identification of the book *Introduction of Food Borne Fungi*, indicated that *A. tamarii* colonies were yellow, conidial head yellow-brown, non-columnar head conidial yellow or brown, conidial heads had the real chocolate ornaments, while *P.nalgiovense* colony was white and conidiophores with smooth stipes.

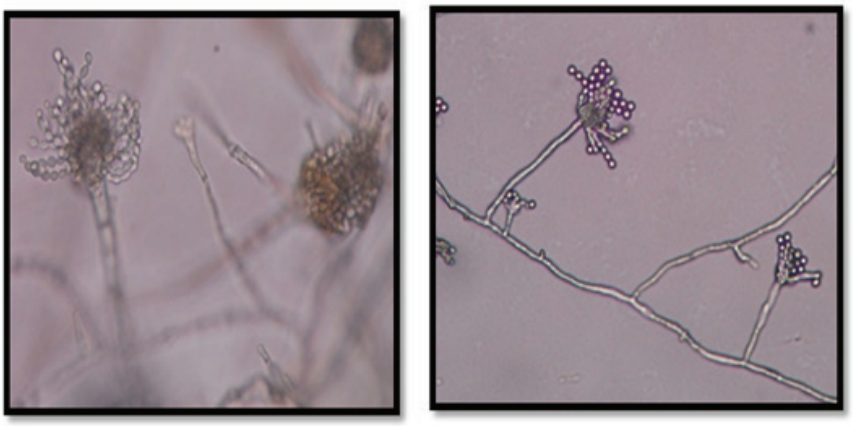


Figure 1. Macroscopic identification (left-right: *P. nalgiovense-A. tamari* 400x)

Judging from the genus of microorganisms selected and identified, the genus of microorganisms selected is known to be in accordance with the several studies previously conducted. The fungi isolates of cellulotic aerob of bovine rumen origin are among others *Aspergillus*, *Geotrichum*, *Penicillium*, *Rhizopus*, and *Trichoderma* (Ogimoto, 1981; Suhardini, 2008). However, further review to the species, no report was found stating the isolation of species *Aspergillus tamarii* and *Penicillium nalgiovense* from cow rumen fluid.

The types of microorganisms are commonly found in soil substrate, rice straw, corn leaves and plants (Moreira, 1999).

The type and amount of microorganisms in cow rumen, is very much influenced by the fodder consumed (Hungate, 1973; Ogimoto, 1981). The process liquidifying and the type of feed given to cattle feed allows entry of other microorganisms in the digestive tract along with the green fodder consumed. Based on the asumption in Ogimoto (1981), that a diversity of rumen microorganisms is very dominantly influenced by microorganisms carried through cattle feed. It is further stated that the feed follwing of microorganisms, particularly fungi, are able to survive in the conditions of the rumen, and there are some types that are functional in the digestion of cattle.

One of the requirements to be selected as microorganisms feed biodegradator is that it must be in the category of facultative aerobic microorganism; because the biodegradation process feed material will take place aerobically. The circumstance in which the microorganisms live which is crucial in energy metabolism of microorganisms is oxygen. The *Aspergillus tamarii* and *Penicillium nalgiovense* microorganisms are cow rumen fluid microorganisms that survive in aerobic culture conditions. At the time it was isolated the microorganisms was living and was capable of breeding on selective media.

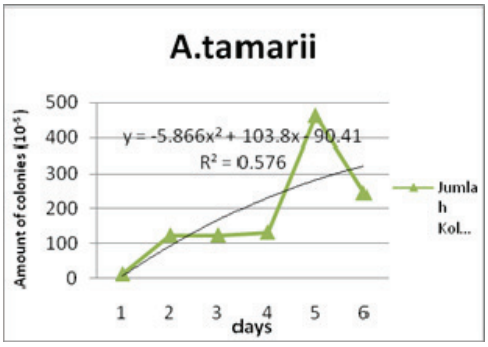
The growth curve was observed for best starter age for inoculum propagation. Mold growth curve was calculated by the method of *Total Plate Count* (TPC) (Table 1).When drawn, the mold growth curve of *P. nalgiovense* and *A. tamarii* has the same pattern as the general microbial growth curve (Figure 2).

Figure 2 showed when the peak growth of *A. tamarii* is on day 5, but on the 6th day a drastic decline is seen, until the effective time for *A.tamarii*was on day 5. In the meantime, *P. nalgiovense* experienced peak growth at day 6, as many cfu 9696.33.

Table 1. The average number of mold cells *A. tamarii* and P. nalgiovense in substrate

A. tamarii		*P. nalgiovense*
Days of culture	Total colonies in 10^6 cfu	Total colonies in 10^6cfu
1	1.2	195.8
2	12.4	99.9
3	12.5	188.2
4	13.2	201
5	46.5	697.3
6	24.6	969.6

TPC (Total Plate Count) results done singly for both types of fungi showed that *A. tamarii* and *P. nalgiovense* have exponential phase (log phase) on day 5. Exponential time is used as the optimum treatment time and the time of the start of the fermentation process, because the log phase is a period where microbial growth occurs very rapidly (Pelczar and Chan, 1986) so a lot of microbial activity occurred at this time.Mold growth trendline indicated a relationship between time (days) and the number of spores, the longer the treatment, the more spores produced.



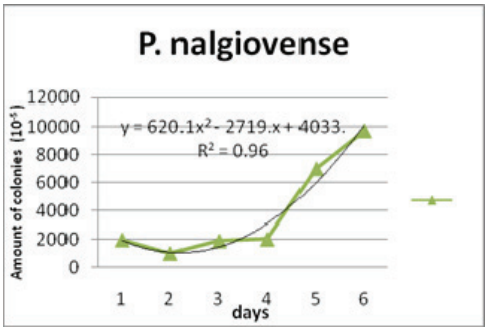


Figure 2. Growth curves of *A.tamarii* and *P.nalgiovense*

Based on enzyme activity assays, *A.tamarii* and *P.nalgiovense* fungi have cellulolytic ability / amylolytic capability measurable through the production of cellulase and amylase as secondary metabolites (Table 1). Cellulase activity of *P. nalgiovense* and *A. tamarii* increased from the first day of culture, reaching a peak on second day and its activities decreased on the third and fourth day. While the activity of amylase *P. nalgiovense* peaked at culture day 1 for 2.0165 units / ml and *A. tamarii* on day-3 of 4.110 units / ml.

These results were consistent with previous studies stating that mold of *A. tamarii* is known have amylase and glucoamylase activity (Moreira, 1999), and *P. nalgiovense* had a high cellulase activity, amounting to 0.027 units / ml (Nugroho, 2006).

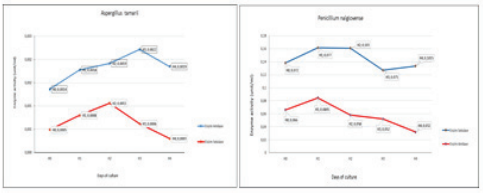


Figure 3. Activity of cellulase and amylase enzymes of *Aspergillus tamarii* fungi (left) and *Penicillium nalgiovense* (right)

CONCLUSIONS

Based on the description, growth and cellulase and amylase enzyme activity observed, molds *Aspergillus tamarii* and *Penicillium nalgiovense* isolated from bovine rumen fluid had quite high amylase and cellulase activity and were easily cultured, making them potential to be used as crude fiber degrading microbes in cellulose waste.

ACKNOWLEDGEMENTS

The author would like to thank DIKTI (Directorate General for Higher Education) for the financial support through the Post Graduate Grant Program and Ms. Diana Pasca Rahmawati for technical assistance in the implementation of activities in the laboratory.

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