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Characterization of Starch Degrading Enzymes from *Bacillus amyloliquefaciens* UMAS 1002 Isolated From Sago Waste

***Muhammad Suhaib Mat Husin*, Mohd Hasnain Hussain, Awang Ahmad Sallehin Awang Husaini**

Proteomics Laboratory, Department of Molecular Biology,
Faculty of Resource Science and Technology, University Malaysia Sarawak,
94300 Kota Samarahan, Sarawak, Malaysia
Email: Suhaib.mh@gmail.com

ABSTRACT

Bacillus amyloliquefaciens UMAS 1002 isolated from sago pith waste was shown to have amylase and cellulase activity. DNA sequence encoding the cellulase gene has previously been isolated with the size of 1527 bp. Screening for amylase gene by polymerase chain reaction using primers based on conserved regions of amylase from *B.amyloliquefacien*s wild type (J01542.1) has yielded a 2.2kb PCR product. Sequence analysis of this fragment showed potential 14 Open Reading Frames. The longest ORF was 534 bp and the shortest ORF was 168 bp. Alignment of individual ORF using BLASTX showed that one of the ORF with a length of 249 bp has a highest similarity to alpha-amylase from *B. amyloliquefaciens* FZB2. This fragment is being cloned and expressed in *Escherichia coli* to determine whether it produces an active amylase.

INTRODUCTION

Sago palm is a high energy crops widely cultivated in peat soil land in tropical areas which is inhabitable for most other crops. Sago palm is also the world's highest starch producer. In the state of Sarawak 25 tonne per hectare of starch were produced every year (Ishizaki, 1997). Mean while, it is estimate that each sago factory can produce as much of 7 tons of sago pith waste every day. Sago pith waste has no significant industrial or commercial use and easily found in bulk and dumped at sago factory compound. Through biotechnological approach this unused sago waste can be utilized to produce value-added product such as reducing sugars.

Gram-positive bacteria species of the genus Bacillus are important sources of industrial enzymes such as amylases, cellulases and proteases. Much of the interest in these bacteria arises from their ability to secret important enzymes at relatively high concentration. Researchers in UNIMAS discovered one strain of *Bacillus amyloliquefaciens*, named UMAS 1002 which has the capability to degrade starch as well as cellulose (Apun et al. 2000).

Gene's manipulations for such enzymes are valuable for application in industrial biotechnology. The α-amylase gene have been expressed from various *Bacillus sp*. to *E.coli* for hyperproduction of amylase. A few foreign gene products have also been reported to be secreted into the growth medium by *E.coli* such as xylanase of *Bacillus sp*. Demirkan et al. (2004) has reported that the α-amylase from a new wild strain of *Bacillus amyloliquefaciens* isolated from soil has been cloned in *E.coli.*

In this study, α-amylase from *B.amyloliquefaciens* UMAS 1002 is genetically characterized. Expression of amylase and cellulase in *E.coli* will also be carried out. It is expected from this research work, that new knowledge about *B. amyloliquefaciens* UMAS 1002 would be obtained and described. In addition, new methods for sago waste utilization using transformed *E.coli* will be established.