Alpha-Amylase from *Bacillus amyloliquefaciens* UMAS 1002: Gene Characterization and Expression in *Escherichia coli*

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ABSTRACT

Alpha-amylase gene from locally isolated *B.amyloliquefaciens* UMAS 1002 was successfully cloned and expressed in *E. coli* BL21. 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 2.2 kb PCR product. Sequence analysis of the amplified PCR product revealed an open reading frame of 1929 bp and contain 643 amino acid residues. Alignment of the ORF using BLASTX showed it has a 96% homology to the sequence encoding alpha-amylase from *B. amyloliquefaciens* FZB2 (CP000560.1) Amino acids analysis revealed *B. amyloliquefaciens* UMAS 1002 α-amylase gene have different 25 amino acids compared to the same gene from the FZB2 strain. The complete ORF encoding α-amylase was isolated and expressed in *E.coli* cells using the pET 100 expression system. The transformant extracellular α-amylase activity was observed when it's colonies produce halo zone on minimal starch agar after Lugol's iodine staining test. Enzymatic assay using starch substrate showed the transformant has 18 fold increase in the amylase activity compared to the negative control.

Keywords: Alpha-amylase, *Bacillus amyloliquefaciens, Escherichia coli* expression.

INTRODUCTION

α-Amylase catalyses the hydrolysis of α-1,4 glycosidic linkages in starch to produce glucose, dextrins and limit dextrins. This enzyme is of great significance in present day biotechnology with application ranging from food, fermentation, textile to paper industries (Pandey *et al.*, 2003). Although amylases can be derived from several sources, including plants, animals and microorganisms, microbial enzymes generally meet industrial demands. Among the genus *Bacillus, B. amyloliquefaciens* and *B. licheniformis* are the two species used most frequently in the commercial production of α-amylase. In general bacterial α-amylase has been classified into two types; one is saccharifying and the other liquefying soluble starch. α-Amylase of *B. amyloliquefaciens, B. licheniformis* and *B. stearothermophilus* belong to the latter type.

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 manipulations for such enzymes are valuable for application in industrial biotechnology. The

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