ISOLATION OF MALTOGENIC AMYLASE GENE FROM
Bacillus licheniformis TH-1

MOHD ANUAR JONETI, ROSLI MD ILLIAS2

Faculty of Chemical and Natural Resources Engineering, University Teknologi Malaysia1,2.

ABSTRACT

Amylases with unique properties have been isolated and characterized for various applications in starch industry. Among the amylolytic enzymes isolated, maltogenic amylase has been identified to hydrolyze cyclodextrin to maltotriose and maltose. The maltogenic amylase gene was isolated and cloned from a locally isolated Bacillus licheniformis TH-1. Gene of about 1773bp encoding 591 amino acid sequence of maltogenic amylase was identified. Amino acid sequence of the maltogenic amylase from Bacillus licheniformis TH-1 showed the highest homology of 99%, with 99% identity to Bacillus licheniformis DSM 13.

INTRODUCTION

A number of amylases are needed for degradation of starch which usually found in a form of complex structure. Maltogenic amylases (EC 3.2.1.133) are a group of enzyme belonging to a subgroup of glycoside hydrolase family 13 along with neopullulanase (EC 3.2.1.135) and cyclomaltodextrinase (EC 3.2.1.54). The maltogenic amylase can hydrolyze substrates having α-(1,4)- and α-(1,6)-glucosidic linkages and transfer the hydrolyzed sugar moiety to another sugar molecule, which makes them useful for the preparation of branched oligosaccharide mixtures and novel carbohydrates. This study was designed to isolate the functional gene that encoded maltogenic amylase from a wild type Bacillus licheniformis TH-1.

Materials and Methods.

Bacillus licheniformis TH-1 was grown in Nutrient Broth at 37°C and 200 rpm for 16 hours. The culture was centrifuged at 13 000 rpm for 5 minutes and the cell pellet was kept to genomic isolation step. The genomic DNA was prepared according to the Ish-Horowitz, 1981 method. DNA manipulations were performed according to standard method as described by (Sambrook et al, 1982). Amplification reaction was carried out and the amplicons were then ligated with plasmid pTZ57R/T. The E.coli transformants were tested on LB-ampicillin (50μg/ml) containing 0.5mM IPTG and 40μg/ml X-Gal. After growth at 37°C for 16 hours, the white colonies were selected prior to plasmid isolation. The gene and deduced amino acid sequences were compared to those available at the Gen Bank and were aligned by using DNAsis/CLUSTAL X programe.
Result and Discussion

The gene encoding the maltogenic amylase from *Bacillus licheniformis* TH-1 was successfully cloned in *E. coli* JM109. It was found the gene was composed of 1773 nucleotides and encoded polypeptide with 585 amino acids.

Figure 1: Nucleotide sequence of the maltogenic amylase gene from *Bacillus licheniformis* TH-1
Figure 2: Comparison of deduced amino acid and sequence with other six alpha amylase families.

**Conclusion**

A maltogenic amylase gene from *Bacillus licheniformis* TH-1 was isolated and then cloned into *Escherichia coli* using pTZ57R/t as a cloning vector. The deduced amino acid sequence of the mature maltogenic amylase exhibited 99% homology with 99% identity to the maltogenic alpha-amylase sequence from *Bacillus licheniformis* (ATCC 14580).