

*ISOLATION OF MALTOGENIC AMYLASE GENE FROM  
Bacillus licheniformis TH-1*

MOHD ANUAR JONET<sup>1</sup>, ROSLI MD ILLIAS<sup>2</sup>

*Faculty of Chemical and Natural Resources Engineering,  
University Teknologi Malaysia<sup>1,2</sup>.*

**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  $\alpha$ -(1,4)- and  $\alpha$ -(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 programme.

## 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.

```
result sequence Amy28: 00GAACGAGCCTTCCGATGCGGAAATTCGCGCAACAGCACCOCATGATTACTGGTTTCCGGAAAGTGGCGCCCTCCATTCCAGGGCTCTCCAA
result sequence Amy28: 100 150 200 250 300 350 400 450 500 550
result sequence Amy28: TACGGATTATTCCTGACAGCCGCTGATGATCGGACACTTTTTAAGGAAAGCAATGGTGCATGTCCCTTTCCCGGGAALGGCGCCGATATG
result sequence Amy28: 500 550 600 650 700 750 800 850 900 950
result sequence Amy28: CCGAALCACTCTTTTAAATTTCCGTTTGTCTGAGGACAGACAGCTTTGATGACACTGGCTGGCTCAAATCAACCGTCTGGTATCAAAAT
result sequence Amy28: 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450
result sequence Amy28: YTTCCGGAGCCCTTTCCGACGGGGCGGGAAGATTGTCTCCGGAAAGCCCTTTCCCATCGGGAAGGAAAGATCCCTGAGCGCGCCGATTTT
result sequence Amy28: 1500 1550 1600 1650 1700 1750 1800 1850 1900 1950
result sequence Amy28: TTCCGAGGGGATTTCCGACGGGATGATGAGCAAGCTGGACTATTGGGAAAGACTTTGGGGCTTCCGGGAATGATATTGACCGCCCATOTTTCGC
result sequence Amy28: 2000 2050 2100 2150 2200 2250 2300 2350 2400 2450
result sequence Amy28: GCGCCTTCGAACCATAAATAGGACACATTTGGACTATTGCTCCATCGATCGCCCATTTTCCGGATGAGGAGCTCTTTCCGACCGCTGGTCCAGG
result sequence Amy28: 2500 2550 2600 2650 2700 2750 2800 2850 2900 2950
result sequence Amy28: CCGATTCCAGAGCCGGGAAATGAAAAYCAGCTTGTATCTCTGTCTTAAACCATTCGGAGGGCTTCCGACAGCTCCGACGATGTTTGTCAA
result sequence Amy28: 3000 3050 3100 3150 3200 3250 3300 3350 3400 3450
result sequence Amy28: AAGGGTGAACCTCCCGGTATAAAGACTGCTTCCATATTCATCTCTTCCCTCTTAAAGAAAGCCAGCTATCATACATTTGGCTTTACTCCG
result sequence Amy28: 3500 3550 3600 3650 3700 3750 3800 3850 3900 3950
result sequence Amy28: GAGATGCCGAAGCTCAATACGCCCAACCCGGAAATTCAGCCCTTATTTCCTTGCATATTCCGGTGTACTCCATCCCGGAAATTTGATATCCGAC
result sequence Amy28: 4000 4050 4100 4150 4200 4250 4300 4350 4400 4450
result sequence Amy28: GCGTCCGCTTTGGATGTGGCAAAATGAAATGATCTGCTGCTTTTGGAAAGAAATTCGGGAAAGCGGTCAAGCCGACAAAAGCCCGACATTTT
result sequence Amy28: 4500 4550 4600 4650 4700 4750 4800 4850 4900 4950
result sequence Amy28: ATATTGGGCAAAATCTGGCATCAGGCTGATCGGTTGGCTTAGAGGAGAGGAAATTCATTCGGTGCATGAAGTACCCGTTACACAGAACCGATG
result sequence Amy28: 5000 5050 5100 5150 5200 5250 5300 5350 5400 5450
result sequence Amy28: ATTTGACTATTTGCGAGCGGATCGATTTCCAGCTTCCCAATGCGCCACCCGCATCAATTCGCATTTAATGAGCGGGATGAGGACGTCGAAAT
result sequence Amy28: 5500 5550 5600 5650 5700 5750 5800 5850 5900 5950
result sequence Amy28: GAGGTGATCTTTAATTTGCTCGCAGCTGATGACACAGAAAGCGATTTTGAGCAAGATCGGGAGGAGATGACAAAGAGGTGGGCTCTCTTTTG
result sequence Amy28: 6000 6050 6100 6150 6200 6250 6300 6350 6400 6450
result sequence Amy28: GCAATTCATGTTGCTCAGACAGGCTCCGCTTGCATTTATTACGGAGCGGCACTCGGCTTTCAGCGCGGCGATGATCCATTTGTCGGCGAGG
result sequence Amy28: 6500 6550 6600 6650 6700 6750 6800 6850 6900 6950
result sequence Amy28: TGCATGCTTTCAGGAGAGGCAAAAACAGATGAAAGAGATGCTCCATTTATGAAAACCGCTGATCGCTTTCCGAAAGCAAGGCAATGATGCTG
result sequence Amy28: 7000 7050 7100 7150 7200 7250 7300 7350 7400 7450
result sequence Amy28: TTGACTTATCGCGGCTTCAGTGGAAAGCTGCTTCATGACCAAAAAGGATTTTGTCAATTTTTCTCGAACCGATGAGGCAAGAGCTGATC
result sequence Amy28: 7500 7550 7600 7650 7700 7750 7800 7850 7900 7950
result sequence Amy28: TACTTCTTCACCAAGGCAAGGAGGCTGCTCAGATTCGCGGATTTGAAAGATTCGACAGCGATAAAGCAATGATAGATGCGTGGAGCG
result sequence Amy28: 8000 8050 8100 8150 8200 8250 8300 8350 8400 8450
result sequence Amy28: GAAGAAGCGCTTCAGCATGATGATGCTGCTCCGACATTCAGCCAGGCGGATTTTTCATTTTGGCGCGCTGTAAAATTCGGTAAAT
```

Figure 1: Nucleotide sequence of the maltogenic amylase gene from *Bacillus licheniformis* TH-1

maltogenic amylase <i>Bacillus licheniformis</i>	GVGGVITP	201	DAVFNK	251	HPKLN	300	GVRLDWANE	333	YILGETIWR	361	LL	LSHD	425	GSPTIYYGT	460
alpha amylase, catalytic region [ <i>Bacillus coagulans</i> 36D4]	GVGGVITP	205	DAVFNK	255	HPKLN	315	GVRLDWANE	346	YILGETIWR	376	LL	LSHD	440	GSPTIYYGT	475
alpha-amylase [ <i>Listeria vesikhimeri</i> serovar Gb_str._SLCC5334]	GVGGVITP	200	DAVFNK	250	HPKLN	310	GVRLDWANE	343	YILGETIWR	371	LL	LSHD	435	GSPTIYYGT	470
maltogenic amylase [ <i>Bacillus thermosthalophilus</i> ]	GVGGVITP	197	DAVFNK	247	HPKLN	299	GVRLDWANE	332	YILGETIWR	360	LL	LSHD	424	GSPTIYYGT	459
neopullulanase [ <i>Listeria monocytogenes</i> FSL N1-317]	GVGGVITP	200	DAVFNK	250	HPKLN	310	GVRLDWANE	343	YILGETIWR	371	LL	LSHD	435	GSPTIYYGT	470
amylase [ <i>Bacillus</i> sp. UP5616]	GVGGVITP	197	DAVFNK	247	HPKLN	299	GVRLDWANE	332	YILGETIWR	360	LL	LSHD	424	GSPTIYYGT	459
alpha-cyclodextrinase [ <i>Geobacillus_sesarothermophilus</i> ]	GVGGVITP	197	DAVFNK	247	HPKLN	299	GVRLDWANE	332	YILGETIWR	360	LL	LSHD	424	GSPTIYYGT	459
Consensus	gvngvityp		davfnk		hpkln		gvrlcwane		ylggetiwr		ll	lshd		gsptiyygt	

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).