Note

Cloning and Sequencing of the Maltoolhaxaose-producing Amylase Gene of Klebsiella pneumoniae

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The molecular characterization of the maltoolhaxaose-producing amylase gene of Klebsiella pneumoniae revealed an open reading frame in which 2,031 base pairs encode a protein of 677 amino acids with a calculated molecular weight of 75,921. The amylase gene had high similarities of 73.6% in DNA sequence and 79.3% in deduced amino acid sequence with the periplasmic α-amylase MaS gene of Escherichia coli.

Key words: gene cloning; amylase; maltoolhaxaose; Klebsiella pneumoniae

The maltoolhaxaose-producing amylase of Klebsiella pneumoniae is an α-d-1,4-glucan exo-maltoolhaxaohydrolase (EC 3.2.1.98; here abbreviated G6-amylase), which can produce α-anomeremic maltoolhaxaose from the non-reducing end of α-d-1,4-glucosidic polymers such as starch, amylose, and glycogen. There have been reported several kinds of bacterial exo-glycosytic α-amylases that could produce α-anomeremic malto-oligodextrins such as maltotetraose, maltopentaose, and maltoolhaxaose. Although malto-oligodextrins are expected to be useful for food industries and fine chemical industries as raw materials, the physiological roles of these amylases in bacteria have not been clearly understood. The genes of maltotetraose-producing amylase (G4-amylase) from Pseudomonas saccharophila, maltopentaose-producing amylase (G5-amylase) from Pseudomonas sp., and G6-amylase from Bacillus sp. are cloned and their nucleotide sequences have been reported. In this paper, the cloning and characterization of the G6-producing amylase gene from K. pneumoniae are reported to describe the structure and function of the gene.

A chromosomal DNA library of the K. pneumoniae IFO-3321 mutant was constructed in the phage λ-EMBL3 BamHI site after partial digestion by Sau3AI, and then transfected into E. coli LE392 on an LB plate containing 0.5% starch. An amylase positive plaque was selected from approximately 500 plaques by staining with 0.02% I2-KI solution. The 22 kilo base pairs (bp) insertion was deleted and subcloned into plasmid pUC19. The amylase positive plasmid pG6IK containing a 3.3-kb Sau3AI-Kpnl fragment was characterized further. The reaction products from soluble starch with the culture supernatant of E. coli JM109 carrying pG6IK were analyzed by thin-layer chromatography to confirm the G6-amylase gene was cloned. The main product of the reaction was maltoolhaxaose showing that the G6-amylase gene was cloned (Fig. 1).

The nucleotides of the 3,303-bp fragment containing the G6-amylase gene were sequenced. The sequence had an open reading frame (ORF) of 2,031 bp encoding the 677 deduced amino acid sequence starting at the nucleotide number of 815, a possible ATG initiation codon (Fig. 2). The 75,921 of the estimated molecular weight agreed with the SDS polyacrylamide gel electrophoresis data of purified G6-amylase (data not shown). The similarity search

Fig. 1. Thin-layer Chromatogram of Reaction Products from Soluble Starch.

Twenty-five μl portions of culture broth were incubated with the same volume of 2% soluble starch at 37°C for 3 hours. Four μl of each reaction product was put on the chromatogram as follows: R, 2% maltoolhaxaose; 1, E. coli JM109/pUC19; 2, K. pneumoniae IFO3321; 3, E. coli JM109/pG6IK.

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in the EMBL database revealed that the *K. pneumoniae* G6-amylase gene had high homologies with the MaLS periplasmic α-amylase gene of *E. coli.* However, the gene had rather low similarity with the *Bacillus* sp. G6-amylase and other malto-dextrin forming amylases except highly conserved regions.

Fig. 2. Nucleotide and Deduced Amino Acid Sequences of the G6-Amylase Gene of *K. pneumoniae.*

The figure illustrates the nucleotide sequence of the Sau3AI-KpnI fragment encoding G6-amylase. The −35 and −10 regions of a possible promoter sequence and the possible Shine-Dalgarno (SD) sequence are underlined. The possible initiation codon ATG and stop codon are also underlined. The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB026834.
The similarities of the open reading frame DNA sequence and the deduced amino acid sequence between *K. pneumoniae* G6-amyrase and *E. coli* MalS were 73.6% and 79.3%, respectively. The deduced amino acid sequence aligned with *E. coli* periplasmic α-amyase MalS is shown in Fig. 3. The MalS has a 17-amino-acids-long signal peptide, but it is not clear whether G6-amyrase has the same processing because of some modification in the NH2-terminal of the mature protein. Vihinen et al. have identified four highly conserved regions (I to IV) which are found in α-amylasses, and related enzymes. Svensson has also proposed six conserved regions (1 to 6) of which four (3 to 6) overlap with regions I to IV. There exists an amino acid residue difference in these regions between *K. pneumoniae* G6-amyrase and MalS of *E. coli*. The G6-amyrase has a 160 amino acids extension in the NH2-terminal end as well as MalS, which has no similarity to other amyrases but to the proposed peptide-binding domain of GroEL of *E. coli*.

In conclusion, the 3.3-kbp fragment containing G6-amyrase gene of *K. pneumoniae* was cloned and sequenced. The 2,031-bp open reading frame of the G6-amyrase had high similarities in the deduced amino acid sequence and the DNA sequence with a periplasmic α-amyrase gene of *E. coli*. These results suggested that the *K. pneumoniae* G6-amyrase corresponds to the *E. coli* periplasmic α-amyrase MalS, which is an enzyme belonging to the maltose and maltodextrin usage system.

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**References**


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