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## SHORT COMMUNICATION

# Preliminary Gene Characterization of α-Amylase from *Bacillus amyloliquefaciens* UMAS 1002

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### ABSTRACT

Characterization of α-amylase gene sequence produced by *Bacillus amyloliquefaciens* UMAS 1002, a cellulolytic and amylolytic bacilli isolated from sago pith waste is described here. The *amyE* gene encoding the α-amylase was isolated by polymerase chain reaction. The 1,980 bp of *amyE* gene corresponding to 660 amino acids showed 99% homology to the α-amylase sequence from *Bacillus subtilis* X-23 (GenBank: BAA31528). The α-amylase sequence of *B. amyloliquefaciens* UMAS 1002 (GenBank: KC800929) differs from that of *B. subtilis* X-23 by 5 amino acids. *In silico* analysis of α-amylase from *B. amyloliquefaciens* UMAS 1002 showed similar characteristics compared to α-amylase from *B. subtilis* X-23.

Keywords: *Bacillus amyliquefaciens*, starch degrading, amylase, *in silico*, sago waste

Starch is among the most abundant polysaccharides on earth and a very important source of energy for most organisms (van der Maarel *et al*., 2002). However, for starch to be transformed into usable energy it needs to be hydrolyzed to its monomer, i.e. glucose. Enzymes responsible for this action are the starch-degrading enzymes. Among them is α-amylase (EC 3.2.1.1). This enzyme catalyses random hydrolysis of α-1,4-glycosidic linkages in starch polymers, thus suitable for conversion of starch into glucose, dextrins and limit dextrins. Different amylases have a large number of different substrate specificities in addition to a huge variation in optimal temperature and pH (Pandey *et al*., 2000). In biotechnological application, this enzyme is of great importance for use in various industries such as food, fermentation, textile and paper production (Pandey *et al*., 2000). For industrial application, generally these amylases are derived from animal and microbes.

Amylase group of enzymes are commonly found in eubacteria and eukaryotes. Bacterial amylases especially from *Bacillus*, and fungal amylases have a widespread use in industry because of the ease of manipulation for the type of work they are involved in (Svensson & Søgaard, 1992). In general, bacterial α-amylases have been grouped into two; for saccharification and liquefaction of soluble starch (Gangadharan *et al*., 2009; Matsuzaki *et al.,* 1974; van der Maarel *et al*., 2002). The α-amylases from *B. amyloliquefaciens, B. licheniformis* and *B. stearothermophilus* belong to the liquefaction group of α-amylase. *B. amyloliquefaciens* is one of the most extensively studied among all of the *Bacillus* species due to its ability to secrete amylase at relatively high concentrations (Gangadharan *et al*., 2009; Priest, 1977). A previous study of *B. amyloliquefaciens* UMAS 1002 showed an interesting capability to degrade starch as well as cellulose (Apun *et al*., 2000). This unique characteristic of dual enzyme capability has not been described elsewhere before, although it is common to find description of either amylase (Demirkan *et al*., 2005) or cellulase (Singh *et al*., 2013) in a single strain. In this study, the nucleotide sequence of α-amylase from *B. amyloliquefaciens* UMAS 1002 is described for the first time.

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*B. amyloliquefaciens* UMAS 1002 used in this study was originally isolated from sago pith waste (Apun *et al.,* 2000). The inoculum was revived from glycerol stock stored at -80°C, and grown in Luria–Bertani (LB) broth medium consisting of (g/l): peptone, 10.0; yeast extract, 5.0; NaCl, 5.0. For the growth medium used for α-amylase production, inoculum was grown in the medium consisting of (g/l): 5.0; soluble starch, 15.0; yeast extract, 1; $MgSO_4$, 2.0; $KH_2PO_4$. The pH of the medium was adjusted to 6.0. Following this, the media were autoclaved at 120°C for 20 min. Bacterial culture was grown in 100 ml of growth medium in 250 ml conical flasks at 40°C. Incubation was carried out with agitation at 180 rpm for 12 h. The bacterial culture was harvested by centrifugation for use in further analysis.

Genomic DNA was extracted from *B. amyloliquefaciens* UMAS 1002 cells and purified according to Sambrook *et al.* (1989). Two oligonucleotide primers were synthesized based on the α-amylase genes of *B. amyloliquefaciens* FZB42 (GenBank: YP001419958) (Chen *et al.*, 2009). These were used for amplification and determination of the α-amylase gene sequence (*amyE*) of *B. amyloliquefaciens* UMAS 1002. The complete sequence of *amyE* was amplified using upstream primer designated as FZ Forward (5'-ATGTTTGAAAAACGATTCAAAACCTCTT TACTG-3') and the downstream primer FZ Reverse (5'-TTAATGCGGAAGATAACCAT TCAAACC-3') resulting in a fragment of approximately 2.0 kb. Amplification of DNA was carried out using a PCR thermocycler in the following condition: 34 cycles of 94°C for 1 min, 65°C for 1 min 30 s and 72°C for 1 min. The PCR products were analyzed on agarose gel and purified. It was later cloned in *E. coli* JM109 and sent for automated double-stranded DNA sequencing service (Research Biolabs Technologies, Singapore).

The nucleotide sequence of the *amyE* gene and the deduced primary structure of the protein encoded by this gene are shown in Figure 1. Analysis of the nucleotide sequence of *amyE* gene and its flanking DNA regions showed an open reading frame (ORF) with the size of 1,980 bp, starting with an ATG codon at nucleotide position 1 and terminating with a TAA stop codon at the position 1,980. This is similar to the α-amylase gene from *B. subtilis* X-23 (Chen *et al.*, 2009) and *B. amyloliquefaciens* FZB42 (Ohdan *et al*., 1999), with the size of 1,979 bp and 1,980 bp respectively. Analysis of the ORF revealed a codon usage typical of *B. amyloliquefaciens* with a G + C content in the *amyE* gene of 46.21%, which is very close to the values reported for choromosomal DNA of other *B. amyloliquefaciens* (Welker & Campbell, 1967). The nucleotide sequence of the *amyE* gene from *B. amyloliquefaciens* UMAS 1002 (GenBank: KC800929) showed 98% and 96% homology with the α-amylase gene from *B. subtilis* X-23 (GenBank: AB015592) and *B. amyloliquefaciens* FZB42 (GenBank: CP000560), respectively. The amino acid sequence deduced from the nucleotide sequence contained 660 amino acids with a calculated molecular weight of 72.281 kDa. The size is similar to the molecular weight of α-amylase from *B. subtilis* X-23, which is 72.280 kDa. *In silico* analysis of polypeptide sequence of both α-amylases showed that although the size is very similar, they have different isoelectric point (pI). The pI of α-amylase from *B. subtilis* X-23 is 6.0 while the pI for α-amylase from *B. amyloliquefaciens* UMAS 1002 is 5.63. Difference in the pI is due to changes in various parameters such as number of positively- and negatively-charged amino acid residues, number of amino acids and molecular weight of the enzymes (Panda & Chandra, 2012). Analysis of the charge of protein at pH 7.0 also showed differences between these α-amylases. For α-amylase from *B. subtilis* X-23, the charge at pH 7.0 is -9.4 while for α-amylase from *B. amyloliquefaciens* UMAS 1002, the charge at pH 7.0 is -14.2.

From this research work, we were able to isolate and characterize the α-amylase gene of *B. amyloliquefaciens* UMAS 1002. Based on this study, the gene will be used for future work on optimizing the conditions for recombinant enzyme production in an expression host. Even more interesting is this new knowledge on *B. amyloliquefaciens* UMAS 1002 α-amylase and cellulase would provide great potential for protein engineering which can increase the enzyme efficiency and stability at extreme pH

and temperature. Future work will include heterologous expression of α-amylase and characterization of cellulase gene and enzyme from *B. amyloliquefaciens* UMAS 1002.


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1      M  F  E  K  R  F  K  T  S  L  L  P  L  F  A  G  F  L  L  L
1      ATGTTTGAAAAACGATTCAAAACCTCTTTACTGCCGTTATTCGCCGGATTTTTACTGCTG
20     F  H  L  V  L  S  G  P  A  A  A  N  A  E  T  A  H  K  S  N
61     TTTCATTTGGTTTTGTCAGGCCCGGCGGCTGCAAACGCTGAAACTGCACACAAATCGAAT
40     E  V  T  D  S  S  V  K  N  G  T  I  L  H  A  W  N  W  S  F
121    GAGGTGACCGATTCATCGGTCAAAAACGGGACCATCCTTCATGCATGGAATTGGTCATTC
60     N  T  L  T  E  N  M  K  E  I  R  D  A  G  Y  A  A  I  Q  T
181    AATACGTTAACAGAAAATATGAAAGAGATTCGTGATGCGGGTTATGCAGCCATTCAGACG
80     S  P  I  N  Q  V  K  E  G  N  Q  G  D  K  S  M  S  N  W  Y
241    TCTCCGATTAACCAAGTAAAGGAAGGGAACCAAGGAGATAAAAGCATGTCGAACTGGTAC
100    W  L  Y  Q  P  T  S  Y  Q  I  G  N  R  Y  L  G  T  E  Q  E
301    TGGCTCTATCAGCCGACATCGTACCAAATCGGCAACCGTTACTTAGGAACTGAACAAGAA
120    F  K  D  M  C  A  A  A  E  K  Y  G  V  K  V  I  V  D  A  V
361    TTTAAGGACATGTGTGCAGCCGCGGAAAAGTATGGCGTAAAAGTCATTGTCGATGCGGTT
140    V  N  H  T  T  S  D  Y  G  A  I  S  D  E  I  K  S  I  P  N
421    GTCAATCATACCACCAGCGATTATGGCGCGATTTCTGACGAGATTAAGAGTATTCCAAAC
160    W  T  H  G  N  T  Q  I  K  N  W  S  D  R  W  D  I  T  Q  N
481    TGGACCCATGGAAACACACAAATTAAAAATTGGTCGGACCGATGGGACATCACTCAAAAT
180    A  L  L  G  L  Y  D  W  N  T  Q  N  T  E  V  Q  A  Y  L  K
541    GCATTGCTTGGGCTGTATGATTGGAATACTCAGAATACTGAGGTGCAAGCCTACCTGAAA
200    G  F  L  E  R  A  L  N  D  G  A  D  G  F  R  Y  D  A  A  K
601    GGTTTCTTGGAAAGAGCATTGAATGACGGAGCAGACGGGTTCCGCTATGATGCCGCCAAG
220    H  I  E  L  P  D  D  G  N  Y  G  S  Q  F  W  P  N  I  T  N
661    CATATAGAGCTTCCGGATGATGGGAATTACGGCAGCCAATTTTGGCCGAATATCACAAAT
240    T  S  A  E  F  Q  Y  G  E  I  L  Q  D  S  A  S  R  D  T  A
721    ACATCGGCGGAGTTCCAATACGGAGAAATCCTGCAAGACAGCGCGTCCAGAGATACTGCT
260    Y  A  N  Y  M  N  V  T  A  S  N  Y  G  H  S  I  R  S  A  L
781    TATGCGAATTATATGAATGTGACGGCTTCTAACTATGGGCATTCCATCAGATCCGCTTTA
280    K  N  R  N  L  S  V  S  N  I  S  H  Y  A  S  D  V  S  A  D
841    AAGAATCGTAATCTGAGTGTGTCGAATATCTCCCATTATGCATCTGACGTGTCTGCGGAC
300    K  L  V  T  W  V  E  S  H  D  T  Y  A  N  D  D  E  E  S  T
901    AAGTTAGTCACATGGGTGGAATCACATGATACGTATGCCAATGATGATGAAGAGTCCACA
320    W  M  S  D  D  D  I  R  L  G  W  A  V  I  G  S  R  S  G  S
961    TGGATGAGTGATGACGATATTCGTTTAGGCTGGGCAGTGATTGGTTCCCGCTCAGGAAGC
340    T  P  L  F  F  S  R  P  E  G  G  G  N  G  V  R  F  P  G  K
1021   ACGCCTCTTTTCTTTTCCAGACCTGAGGGCGGAGGAAATGGTGTAAGATTTCCCGGAAAA
360    S  Q  I  G  D  R  G  S  A  L  F  K  D  Q  A  I  T  A  V  N
1081   AGTCAAATAGGAGATCGCGGGAGCGCCTTATTTAAAGATCAGGCGATCACTGCGGTCAAC
380    Q  F  H  N  E  M  A  G  Q  P  E  E  L  S  N  P  N  G  N  N
1141   CAATTTCACAATGAAATGGCCGGGCAGCCTGAGGAACTCTCAAATCCGAATGGGAACAAT
400    Q  I  F  M  N  Q  R  G  S  K  G  V  V  L  A  N  A  G  S  S
1201   CAAATATTTATGAATCAGCGCGGCTCAAAAGGCGTTGTGCTGGCAAATGCAGGATCATCT
420    S  V  T  I  N  T  S  T  K  L  P  D  G  R  Y  D  N  R  A  G
1261   TCTGTCACCATCAATACTTCAACGAAATTACCTGACGGCAGGTATGATAATAGGGCCGGC
440    A  G  S  F  Q  V  S  N  G  K  L  T  G  T  I  N  A  R  S  A
1321   GCCGGTTCATTTCAAGTATCGAACGGCAAACTGACAGGTACGATCAATGCCAGATCCGCG
460    A  V  L  Y  P  D  D  I  G  N  A  P  H  V  F  L  E  N  Y  Q
1381   GCTGTCCTTTATCCTGATGATATTGGAAATGCGCCTCATGTCTTTCTTGAGAATTACCAA
480    T  E  A  V  H  S  F  N  D  Q  L  T  V  T  L  R  A  N  A  K
1441   ACAGAGGCAGTCCATTCTTTCAATGATCAGCTGACGGTCACCCTGCGTGCAAATGCGAAA
500    T  A  K  A  V  Y  Q  I  N  N  G  Q  E  T  A  F  K  D  G  D
1501   ACAGCAAAAGCCGTTTACCAAATCAATAATGGGCAGGAGACAGCATTTAAGGATGGAGAC
520    R  L  T  I  G  K  E  D  P  I  G  T  T  Y  N  V  K  L  T  G
1561   CGATTAACGATCGGGAAAGAAGATCCAATCGGCACGACATACAACGTCAAGTTAACCGGA
540    T  N  G  E  G  A  S  R  T  Q  E  Y  T  F  V  K  K  D  P  S
1621   ACGAACGGCGAGGGTGCATCGAGAACCCAAGAATACACGTTTGTCAAAAAAGACCCGTCC
560    Q  T  N  I  I  G  Y  Q  N  P  D  H  W  G  N  V  N  A  Y  I
1681   CAAACCAACATCATTGGCTATCAAAATCCGGATCATTGGGGCAATGTAAATGCTTATATT
580    Y  K  H  D  G  G  G  A  I  E  L  T  G  S  W  P  G  K  A  M
1741   TACAAACATGATGGAGGCGGGGCCATAGAATTAACCGGATCGTGGCCGGGGAAAGCCATG
600    T  K  N  A  D  G  I  Y  T  L  T  L  P  A  N  A  D  T  A  D
1801   ACTAAGAATGCAGATGGAATTTACACGCTGACGCTGCCTGCGAATGCGGATACGGCCGAC
620    A  K  V  I  F  N  N  G  S  A  Q  V  P  G  Q  N  H  P  G  F
1861   GCCAAAGTGATTTTTAACAATGGCAGCGCCCAAGTGCCCGGACAGAACCATCCCGGCTTT
640    D  Y  V  Q  N  G  L  Y  N  N  S  G  L  N  G  Y  L  P  H  *
1921   GATTATGTGCAGAATGGTTTGTATAACAACTCCGGTTTGAATGGTTATCTTCCGCATTAA
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**Figure 1.** Nucleotide sequence of *amyE* gene (GenBank: KC800929) and deduced amino acid sequence of the a-amylase of *B. amyloliquefaciens* UMAS 1002. Numbers on the right side of the amino acid and nucleotide sequences represent amino acid and nucleotide positions, respectively. Position 1 indicates start codon and asterisk (*) indicates a stop codon.

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