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ORIGINAL ARTICLE

Cloning, heterologous expression, and comparative characterization of a mesophilic α-amylase gene from *Bacillus subtilis* JN16 in *Escherichia coli*

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Abstract A gene encoding mesophilic α-amylase from *Bacillus subtilis* JN16 was identified and designated as *AmyQ*. The *AmyQ* gene was cloned, sequenced, and expressed in *Escherichia coli*. *AmyQ* is 1,980 bp in length and encodes a protein of 660 amino acids. *AmyQ* was cloned in plasmid pET20b (+) on an *Nco*I–*Bam*HI fragment, and used to transform competent *E. coli* amylase-negative cells (BL21); ampicillin-resistant transformants were screened for the production of α-amylase. The recombinant α-amylase encoded in *E. coli* was designated AmyQ A, and α-amylase from wild-type *B. subtilis* strain JN16 was designated AmyQ B. AmyQ A was characterized biochemically and showed maximal activity at pH 7.0 and maximal stability at pH 5.5; the optimum temperature for enzymatic activity was close to 70°C. The optimal pH for purified AmyQ B was 7.5. With soluble starch as substrate, the K_m and V_{max} of AmyQ A were 3.40 g/L and 15.70 g/(L min^{-1}), respectively, and the K_m and V_{max} of AmyQ B were 2.01 g/L and 6.95 g/(L min^{-1}), respectively. The activity of AmyQ A was enhanced by K^+, Mn^{2+}, Co^{2+} and Ca^{2+}, and the activity of AmyQ B was enhanced in the presence of K^+, Co^{2+} and Ca^{2+}.

Keywords Cloning · Heterologous expression · Characterization · Mesophilic α-amylase

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Introduction

α-Amylase (EC.3.2.1.1) is classified as belonging to family 13 of the glycosyl hydrolases and catalyzes the hydrolysis of α-1,4 glycosidic linkages of glycogen, starch and related polysaccharides to produce glucose, oligosaccharides and dextrins (Fukusumi et al. 1988). Considerable attention has been focused on starch-degrading enzymes from mesophilic prokaryotes as they are likely to produce enzymes with activity and stability characteristics suitable for industries such as starch processing, food, fermentation, detergent, textile and paper and so on (Ahmadia et al. 2010). Mesophilic enzymes are optimally active at the moderate temperatures of 20–60°C (Clark and Kelly 1990; Ludlow and Clark 1991; Vieille et al. 1996). A number of mesophiles also produce α-amylase that is stable and active above 60°C (Khajeh and Nemat-Gorgani 2001). For example, the α-amylase from *Bacillus licheniformis* is thermostable at 75°C (Asther and Meunier 1990), and the optimal temperature for the amylase from *Dictyoglomus thermophilum* is around 90°C (Fukusumi et al. 1988). Numerous α-amylases from eubacteria, fungi, plants and animals have been characterized, and their genes have been cloned. With the exception of eubacterial enzymes, they all belong to the same α-amylase family, having similar

structures, similar catalytic sites, and the same catalytic mechanism (Fukusumi et al. 1988; Gupta et al. 2003; Jespersen et al. 1993). α-Amylases contain three domains, and four highly conserved regions form an active center, a substrate binding site, and a Ca^{2+} binding site (Buisson et al. 1987; Dong et al. 1997; Violet and Meunier 1989). A common feature of α-amylases is that a metal ion is required for their structural integrity, as well as their enzymatic activity (Nielsen and Borchert 2000; Sajedi et al. 2007).

There are a few reports about the cloning, heterologous expression, and comparative characterization of mesophilic α-amylase genes from *Bacillus subtilis* in *Escherichia coli* (Ahmadia et al. 2010; Dong et al. 1997; Gupta et al. 2003). In the present work, a putative α-amylase gene from *B. subtilis*, designated as *AmyQ*, was cloned based on the genome sequence of *B. subtilis*, and the α-amylase gene was expressed successfully in *E. coli* BL21. The biochemical properties of mesophilic α-amylase from *B. subtilis* and *E. coli* were characterized and compared. The results obtained here may be useful for the industrial production of mesophilic α-amylase.

Materials and methods

Strains and plasmids

All strains and plasmids used were obtained from the Laboratory of Biosystem and Bioprocess Engineering of Jiangnan University. *AmyQ* was obtained from *Bacillus subtilis* JN16. *Escherichia coli* JM109 was used for the construction and proliferation of plasmid DNA. Plasmid pET20b (+) was used for subcloning. *Escherichia coli* BL21 was used for recombinant α-amylase expression. The recombinant α-amylase expressed in *E. coli* was designated as AmyQ A, and the α-amylase from the wild-type *B. subtilis* JN16 strain was designated as AmyQ B.

Media and culture conditions

Bacillus subtilis JN16 was cultured in medium containing (%, w/v): lactose 14.0, corn steep liquor 3.0, fish meal 1.5, and $CaCO_3$ 1.1. The pH of the medium was adjusted to 7.0 with 1 M NaOH. The fermentation was performed in a 3.6 L vessel (INFORS, Bottmingen, Switzerland) with a working volume of 2.0 L at 35°C for 60 h.

Luria-Bertani (LB) medium was used for seed cultivation of *E. coli*. *Escherichia coli* was cultured on a rotary shaker at 200 rpm and 37°C for 10 h. Terrific Broth (TB) medium was used for the production of α-amylase. The fermentation was performed in a 3.6 L vessel (INFORS) with a working volume of 2.0 L at 37°C for 36 h.

Cloning of *AmyQ*, construction of expression cassette, transformation and bioinformatics assay

The PCR amplification of *AmyQ* sequence from the cleavage site of the putative signal peptide to a region downstream of the termination codon was carried out using the oligonucleotides A_1 (ATATGACCATGGCGTTTGCAAAACGATTCAAAACCTC) and A_2 (GAATTCGGATCCTCTATGGGGAAGAGAACCGC) with bases from the template sequence modified in order to generate restriction sites (*Nco*I and *Bam*HI; underlined). The PCR product was analyzed on an agarose gel and sequenced. The *AmyQ* was digested with the restriction endonucleases *Nco*I and *Bam*HI. The fragments generated by PCR were isolated using an agarose gel DNA purification kit (TakaRa, Dalian, China). PCR fragments and isolated plasmids were digested by the restriction enzymes *Nco*I and *Bam*HI at 37°C for 2.5 h. The resulting fragments were then isolated from agarose gels as noted above. The fragments isolated (4 μL PCR fragments, 1 μL fragments from digested plasmids, and 5 μL T4 DNA ligase) were incubated at 16°C for 3 h. Then, 10 μL of reaction liquid was added to 100 μL competent cells of *Escherichia coli* JM109 and spread on an LB plate (including 100 μL ampicillin and 2% agar). Colonies harboring plasmid were selected on ampicillin, and *E. coli* harboring recombinant plasmid pET-20b (+) was selected by colony PCR. The selected *E.coli* were cultured in LB medium (containing 100 μL ampicillin) at 37°C for 10 h. The recombinant plasmid pET-20b (+) was isolated using an EZ-10Spin Column Plasmid Mini-Prep kit (TakaRa, Dalian, China). The plasmid pET-20b (+)-*AmyQ* was purified and transformed into competent cells of *E. coli* BL21.

The composition and properties of the nucleotide sequence and deduced amino-acid (aa) sequence of *AmyQ* were analyzed using the software Vector NTI. Distance trees were

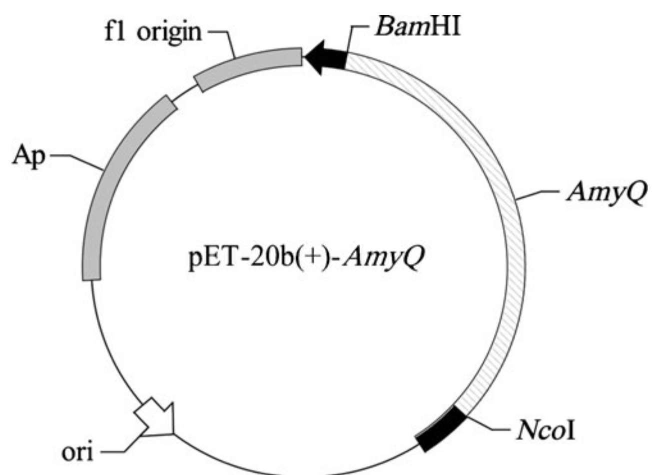

Fig. 1 Map of expression plasmid pET-20b(+)-*AmyQ*

generated via neighbor-joining methods by Mega version 3.1 (Kumar et al. 1994), after construction of preliminary multiple alignments of the sequences with Clustal W version 1.83 (Thompson and Higgins 1994).

Expression of recombinant AmyQ A

TB medium was used for the expression of recombinant AmyQ A. Fermentation was performed in a 3.6 L vessel (INFORS) with a working volume of 2.0 L at 37°C for 36 h. When the fermentation ended, the crude culture broth was centrifuged at 8,000 *g* at 4°C for 30 min and the supernatant containing α-amylase was recovered.

Purification of α-amylase

Solid ammonium sulfate was added to the recovered supernatant to 80% saturation at 0°C. The concentrated enzyme from ammonium sulfate precipitation was collected by centrifugation at 8,000 *g* and 4°C for 30 min, and the

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   1 M  A  F  A  K  R  F  K  T  S  L  L  P  L  F  A  G  F  L  L  L  F  H  L  V  L  A  G  P  T
   1 atggcgtttgcaaaacgattcaaaacctctttactgccgttattcgctggattttattgctgtttcatttggttctggcaggaccaacg
  31 A  A  N  A  E  T  A  N  K  S  N  E  L  T  A  P  S  I  K  S  G  T  I  L  H  A  W  N  W  S
  91 gctgcgaatgctgaaacggctaacaaatcaaatgagcttacagcgccatcgatcaaaagcggaaccattcttcatgcttggaattggtcg
  61 F  N  T  L  K  H  N  M  K  D  I  H  D  A  G  Y  T  A  I  Q  T  S  P  I  N  Q  V  K  E  G
 181 tttaatacgttaaaacacaatatgaaggatattcatgatgcaggatatacagccattcagacgtctccgattaaccaagtaaaggaaggg
  91 N  Q  G  N  K  S  M  S  N  W  Y  W  L  Y  Q  P  T  S  Y  Q  I  G  N  R  Y  L  G  T  E  Q
 271 aaccaaggaaataaaagcatgtcgaactggtactggctctatcagccgacatcgtaccaaattggcaaccgttacttaggaactgaacaa
 121 E  F  K  E  M  C  A  A  A  E  E  Y  G  I  K  V  I  V  D  A  V  I  N  H  T  T  S  D  Y  A
 361 gaatttaaagaaatgtgtgcagccgctgaagaatatggcataaaggtcattgttgacgcggtcatcaatcataccactagtgactatgcc
 151 V  I  S  N  E  I  K  S  I  P  N  W  T  H  G  N  T  Q  I  K  N  W  S  D  R  W  D  V  T  Q
 451 gtgatttccaatgagattaagagtattccaaactggacacatggaaacacacaaattaaaaactggtctgatcgatgggatgtcacgcag
 181 N  S  L  L  G  L  Y  D  W  N  T  Q  N  T  Q  V  Q  S  Y  L  K  R  F  L  E  R  A  L  N  D
 541 aattcattgctcgggctgtatgactggaatacacaaaatacacaagtacagtcctatttgaaacgtttcttagaaagagcattgaatgac
 211 G  A  D  G  F  R  F  D  A  A  K  H  I  E  L  P  D  D  G  S  Y  G  S  Q  F  W  P  N  I  T
 631 ggggcagacggttttcgatttgatgccgccaaacatatagagcttccggatgatgggagttacggcagtcaattttggccgaatatcaca
 241 N  T  S  A  E  F  Q  Y  G  E  I  L  Q  D  S  A  S  R  D  A  A  Y  A  N  Y  M  N  V  T  A
 721 aatacatctgcagagttccaatacggagaaatcctgcaggatagtgcctcaagagatgctgcctatgcgaattatatgaatgtgacagcg
 271 S  N  Y  G  H  S  I  R  S  A  L  K  N  R  N  L  G  V  S  N  I  S  H  Y  A  S  D  V  S  A
 811 tctaactatgggcattccataaggtccgctttaaagaatcgtaatctgggcgtgtcgaatatctcccactatgcatcagatgtgtctgcg
 301 D  K  L  V  T  W  V  E  S  H  D  T  Y  A  N  D  D  E  E  S  T  W  M  S  D  D  D  I  R  L
 901 gacaagctagtgacatgggtagagtcgcatgatacgtatgccaatgatgatgaagagtcgacatggatgagcgatgatgatatccgttta
 331 G  W  A  V  I  A  S  R  S  G  S  T  P  L  F  F  S  R  P  E  G  G  G  N  G  V  R  F  P  G
 991 ggctgggcggtgatagcttctcgttcaggcagtacgcctcttttcttttctagacctgagggaggcggaaatggtgtgagattcccgggg
 361 K  S  Q  I  G  D  R  G  S  A  L  F  E  D  Q  A  I  T  A  V  N  R  F  H  N  V  M  A  G  Q
1081 aaaagccaaataggcgatcgcgggagtgctttatttgaagatcaggctatcactgcggtcaatagatttcacaatgtgatggctggacag
 391 P  E  E  L  S  N  P  N  G  N  N  Q  I  F  M  N  Q  R  G  S  H  G  V  V  L  A  N  T  G  S
1171 cctgaggaactctcgaacccgaatggaaacaaccagatatttatgaatcagcgcggctcacatggcgttgtgctggcaaatacaggttct
 421 S  S  V  S  I  N  T  P  T  K  L  P  N  G  R  Y  D  N  K  A  G  A  G  S  F  Q  V  N  D  G
1261 tcctctgtttctatcaatacgccaacaaaattgcctaatggcaggtatgataataaagctggggcgggttcatttcaagtgaacgatggt
 451 K  L  T  G  T  I  N  A  R  S  V  A  V  L  Y  P  D  D  I  A  K  A  P  H  V  F  L  E  N  Y
1351 aaactgacaggcacgatcaatgccagatctgtggctgtgctttatcctgatgatattgcaaaagcgcctcatgttttccttgagaattac
 481 K  T  G  V  T  H  S  F  N  D  Q  L  T  I  T  L  R  A  D  A  N  T  T  K  A  V  Y  Q  I  N
1441 aaaacaggtgtaacacattctttcaatgatcaactgacgattaccttgcgtgcagatgcaaatacaacaaaagccgtttatcaaatcaat
 511 N  G  P  E  T  A  F  K  D  G  D  Q  F  T  I  G  K  G  D  P  F  G  K  T  Y  T  I  M  L  K
1531 aatggaccagagacagcgtttaaggatggagatcaattcacaatcggaaaaggagatccatttggcaaaacatacaccatcatgttaaaa
 541 G  T  N  S  D  G  V  T  K  T  E  E  Y  S  F  V  K  R  D  P  A  S  A  K  T  I  G  Y  Q  N
1621 ggaacgaacagtgatggtgtaacgaagaccgaggaatacagctttgttaaaagagatccagcttcggccaaaaccatcggctatcaaaat
 571 P  N  H  W  S  Q  V  N  A  Y  I  Y  K  H  D  G  G  R  A  I  E  L  T  G  S  W  P  G  K  P
1711 ccgaatcattggagccaagtaaatgcttatatctataaacatgatgggggccgggcaattgaattgaccggatcttggcctggaaaacca
 601 M  T  K  N  A  D  G  I  Y  T  L  T  L  P  V  D  T  D  T  T  N  A  K  V  I  F  N  N  G  S
1801 atgactaaaaatgcagatggaatttacacgctgacgctgcccgtggacacggatacaaccaacgccaaagtgatttttaataatggcagc
 631 A  Q  V  P  G  Q  N  Q  P  G  F  D  Y  V  Q  N  G  Y  N  D  E  G  L  S  G  S  L  P  H  R
1891 gcccaagtgcccggtcagaatcagcctggctttgattatgtgcaaaatggatataatgacgagggcttaagcggttctcttccccataga
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Fig. 2 Nucleotide sequence of *AmyQ* (*lower case letters*) and deduced amino acid sequence (*upper case letters*)

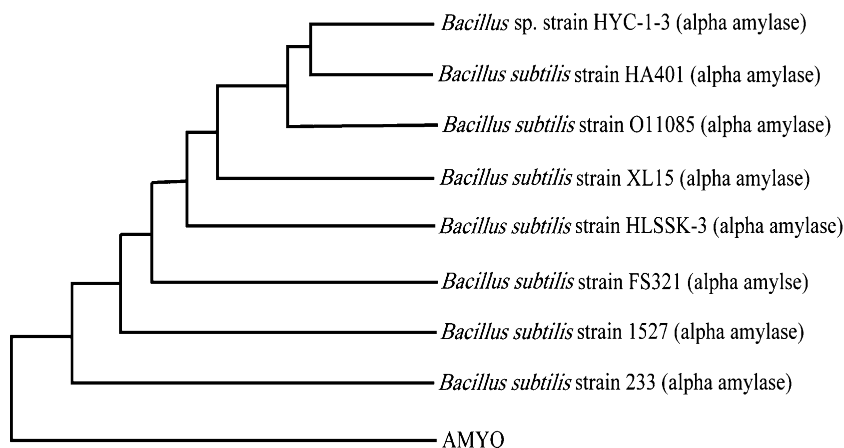

Fig. 3 Phylogenetic tree of AmyQ A and α-amylases reported from bacteria in GenBank

precipitate was dissolved in 5 mL phosphate buffer (0.1 M, pH 6.5). The mixture was loaded into dialysis bags and dialyzed against the same buffer at 0°C for 8 h. The dialyzed enzyme was loaded onto an anion exchanger (Q-Sepharose HP) already equilibrated with phosphate buffer (20 mM, pH 7.5). The bound protein was eluted using 50, 100, 150, 200, 250, and 300 mM NaCl. The active fractions were combined and used as the purified enzyme in subsequent experiments.

Enzyme assays

α-Amylase activity was determined by measuring the amount of reducing sugar released during enzymatic hydrolysis of 1% soluble starch in phosphate buffer (pH 6.5) at 60°C for 5 min. A control without enzyme addition was used. The amount of reducing sugar was measured by a modified dinitrosalicylic acid method (Fuwa 1954). One unit of α-amylase activity was defined as the amount of enzyme that released 1 mg reducing sugar as maltose per minute under the assay conditions. The basis of active enzyme used in all experiments was 0.8 U/mL.

Determination of kinetic parameters

The reaction was performed in sodium phosphate buffer (pH 6.5) at 60°C for the determination of kinetic parameters. Assays were performed with active enzyme and soluble starch of different concentrations from 1 to 10 g/L. The Lineweaver-Burk equation $(1/\mathrm{V} = K_m/(V_{max} \cdot [s]) + 1/V_{max})$ was used to calculate kinetic parameters K_m and V_{max} according to the enzyme reactions (Lineweaver and Burk 1934).

Effects of temperature and pH on activity

The optimal reaction temperature of α-amylase was analyzed from 30 to 90°C in sodium phosphate buffer (pH 6.5). The thermal stability of α-amylase was determined at the temperatures indicated in sodium phosphate buffer (pH 6.5) for 10 min. To estimate the optimal pH for α-amylase, the purified protein was incubated in various buffers. The buffer used for the determination of optimal pH was as follows: citrate buffer for pH 3.5–5.5, sodium phosphate buffer for pH 6.0–8.0, and Tris-HCl buffer for pH 8.0–10.0. The pH stability of α-amylase was determined over pH values ranging from 3.5 to 10.0 at 25°C for 24 h. After incubation, α-amylase was added to 1% soluble starch in phosphate buffer (pH 6.5) and activity was measured at 60°C.

Effects of metal ions on enzyme activity

The effects of metal ions on α-amylase activity were examined by adding 1 mM KCl, $CaCl_2$, $MgCl_2$, $FeCl_3$, $FeCl_2$, $CoCl_2$, $ZnCl_2$, $MnCl_2$ and $CuCl_2$ to the reaction mixture. The relative activity (%) was calculated on the basis of the activity that was determined in phosphate buffer (pH 6.5) without the addition of any metal ions at 60°C.

Protein content assay

The protein content of samples was measured by the Bradford method with BSA as a standard (Bradford 1976).

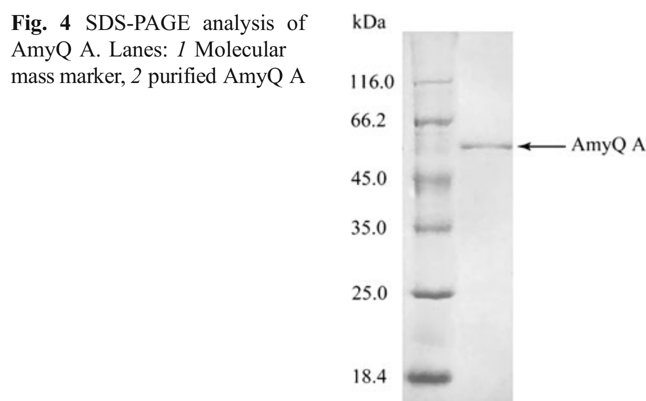

Fig. 4 SDS-PAGE analysis of AmyQ A. Lanes: *1* Molecular mass marker, *2* purified AmyQ A

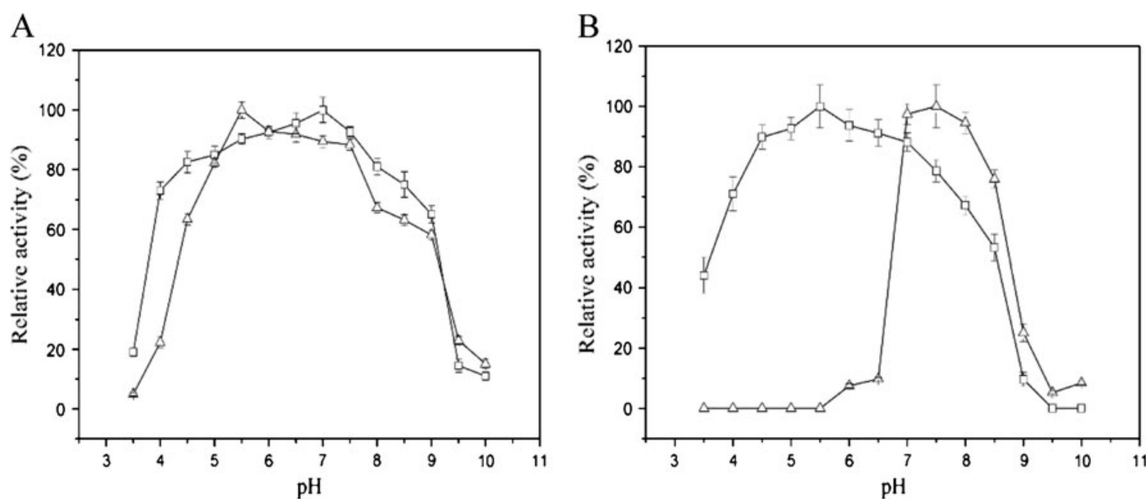

Fig. 5 Effect of pH on **a** activity and **b** stability of AmyQ A and AmyQ B. Δ AmyQ B, □ AmyQ A

Results and discussion

Cloning and sequencing of the gene *AmyQ*

The DNA fragment coding for mature α-amylase was amplified by PCR from the cleavage site of the putative signal peptide to a region downstream of the termination codon. The PCR product was analyzed on an agarose gel and sequenced. The *AmyQ* was digested with the restriction endonucleases *Nco*I and *Bam*HI. As shown in Fig. 1, the isolated fragments were joined to the plasmid pET-20b (+). The sequence of *AmyQ* from the start codon (ATG) to the termination codon (TGA) was analyzed with WebGeneMark heuristic approach software (Besemer and Borodovsky 1999), and the results revealed that *AmyQ* is 1,980 base pairs long and encodes a protein of 660 amino acids (Fig. 2). The analysis revealed the high similarity of α-amylases from *Bacillus subtilis* and *Bacillus* sp. The evolutionary relationships of AmyQ were constructed and the topology of the phylogenetic tree is shown in Fig. 3. Strains AMYQ and *Bacillus subtilis* 233 were clustered together as a small group separated from other α-amylases. Analysis of the signal peptide of the *AmyQ* gene with the program SignalP-3.0 indicated that the cleavage site was between Ala 34 and Glu 35. Three key residues (Asp218, Glu250 and Asp311) were important for the hydrolyzing activity of α-amylases (Abe et al. 2005).

Heterologous expression of AmyQ A in *Escherichia coli* BL21

The recombinant plasmid pET-20b (+)-*AmyQ* was transformed into competent cells of *E. coli* BL21. A transformant producing high amounts of α-amylase was isolated and used for the production of α-amylase in a 3.6 L fermentor. AmyQ A was expressed at pH 7.0 and 37°C, and 530 μg/mL protein was obtained at the end of fermentation.

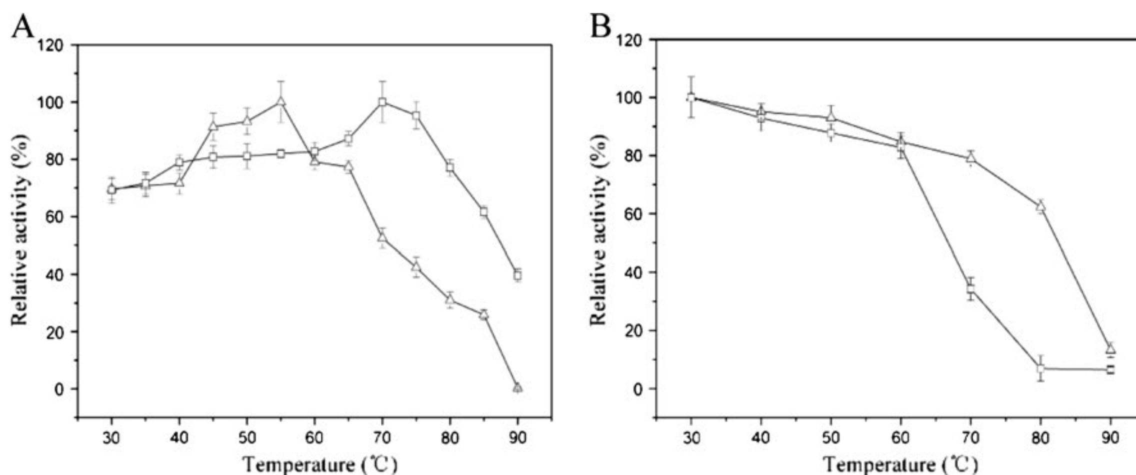

Fig. 6 Effect of temperature on the **a** activity and **b** stability of AmyQ A and AmyQ B. Δ AmyQ B, □ AmyQ A

The activity of AmyQ A obtained from fermentation was 30 U/mL. The AmyQ A was purified from the culture supernatant, and the purified AmyQ A was detected with 15% SDS-PAGE. The molecular weight of AmyQ A is around 57 kDa, as shown on SDS-PAGE (Fig. 4).

Effect of pH on the activity and stability of AmyQ A and AmyQ B

Purified AmyQ A displayed optimal activity at pH 7.0, and retained more than 70% of its activity at pH values ranging from 4.0 to 8.5, whereas activity declined rapidly out side of this range (Fig. 5a). The optimal pH for AmyQ B was between pH 5.0 and pH 7.5, and the activity decreased dramatically when the pH decreased to 4.5 or increased to 8.0 (Fig. 5a). The pH stability of the enzyme was tested after incubation at 25°C for 24 h. The AmyQ A displayed optimal stability at pH 5.5. AmyQ A was stable between pH 4.5 and pH 7.0 (Fig. 5b). However, the optimum stable pH for AmyQ B was 7.5, and it retained more than 70% relative activity at pH values ranging from 7.0 to 8.5. The enzyme activity declined rapidly outside of this range (Fig. 5b). The effects of pH on the activity of AmyQ A and AmyQ B were different. In addition, the effects of pH on the stability of AmyQ A and AmyQ B were also different. It is possible that the conformation of the α-amylase from *B. subtilis* might change when expressed and secreted in *E. coli* BL21. If the electrostatic environment of the active site (especially Glu) is altered, the sensitivity of α-amylase to pH might also change (Richèle et al. 1998; Abe et al. 2005).

Effect of temperature on activity and stability of AmyQ A and AmyQ B

The optimal temperature for AmyQ A was 70°C, and it retained more than 70% of its activity between 30°C and 80°C, while the activity declined rapidly outside of this range (Fig. 6a). The optimal temperature for AmyQ B activity was 55°C, and it retained more than 70% of its activity in temperatures ranging from 30 to 65°C. When the temperature rose higher than 65°C, the activity declined rapidly. AmyQ A and AmyQ B had the same trend for stable temperature below 60°C (Fig. 6b). The AmyQ A from *E. coli* BL21 was more thermostable than AmyQ B from *B. subtilis*. The thermostability of α-amylase can be influenced by different factors. Ionic interactions between residues make a large contribution to the thermostability of enzymes (Lin et al. 2008). Improved thermostability could be due to loop stabilization and the enhancement of calcium binding (Igarashi et al. 1999, 1998). Hydrophobic interactions inside protein molecules might be strengthened, thus affecting thermostability (Asther and Meunier 1990; Tomizawa et al. 1995a, b).

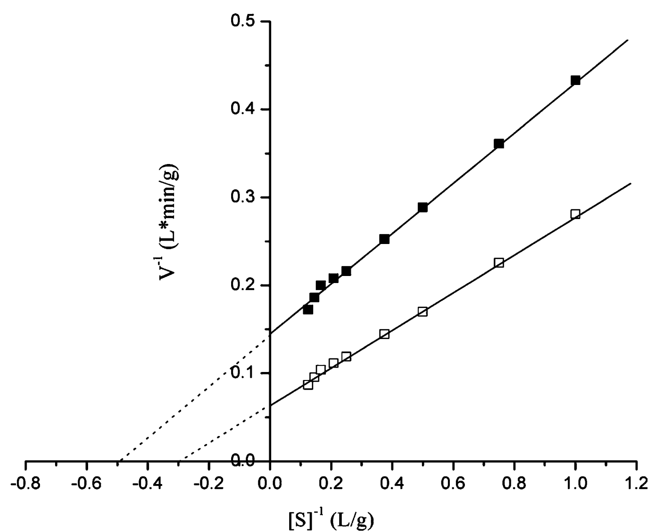

Fig. 7 Lineweaver-Burk plots for soluble starch degradation by AmyQ A and AmyQ B. ■ AmyQ B, □ AmyQ A

Differences in kinetic parameters between AmyQ A and AmyQ B

The K_m and V_{max} values were determined by nonlinear fit analysis based on the Lineweaver-Burk equation. The initial reaction rates were determined with soluble starch at concentrations of 1–10 g/L. As shown in Fig. 7, AmyQ A hydrolyzed soluble starch with a K_m of 3.40 g/L and V_{max} of 15.70 g/(L min^{-1}), and AmyQ B from *B. subtilis* hydrolyzed soluble starch with K_m of 2.01 g/L and V_{max} of 6.95 g/(L min^{-1}). The kinetic parameters of AmyQ A and AmyQ B could be different because the substrate combination regions of the enzyme may change subtly when α-amylase is expressed in *E. coli* BL21.

Table 1 Effect of metal ions on the activity of AmyQ A and AmyQ B

Metal ion	Relative activity (%)[a]	
	AmyQ A	AmyQ B
Ca^{2+}	129.8±0.5	125.7±0.3
Mg^{2+}	96.3±0.1	82.7±0.9
Fe^{2+}	72.4±0.7	94.8±1.1
Fe^{3+}	96.1±1.2	66.2±0.7
Co^{2+}	138.8±2.3	120.1±0.4
Zn^{2+}	97.9±0.9	116.8±0.6
Mn^{2+}	118.1±0.8	41.5±0.9
Cu^{2+}	47.2±1.7	45.4±1.0
K^{+}	100.5±0.8	106.2±1.6

[a] Relative activity (%) was calculated relative to activity determined in phosphate buffer (pH 6.5) without the addition of any metal ions at 60°C

The K_m of AmyQ A was larger than that of AmyQ B from *B. subtilis*, indicating that the affinity of AmyQ A for soluble starch was lower than that of AmyQ B from *B. subtilis*. The V_{max} of AmyQ A was larger than that of AmyQ B in *B. subtilis*, yet this does not mean that the catalytic rate of AmyQ A for soluble starch is higher than that of AmyQ B because the catalytic rate matched the catalytic constant ($k_{cat} = V_{max}/[E]$). In this work, when the kinetic parameters were determined, the enzyme concentration ([E]=0.46 mg/L) of AmyQ A was three times that of AmyQ B. The catalytic constants (k_{cat}) of AmyQ A and AmyQ B were 5.7×10^2 s^{-1} and 7.6×10^2 s^{-1}, respectively. The catalytic constant (k_{cat}) of AmyQ A was lower than that of AmyQ B, and AmyQ B had a higher catalytic rate than AmyQ A.

Effect of metal ions on the activity of AmyQ A and AmyQ B

The effects of metal ions on the activity of AmyQ A and AmyQ B are shown in Table 1. The activity of AmyQ A was enhanced by K^+, Mn^{2+}, Co^{2+} and Ca^{2+}, whereas the metal ions Fe^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+} and Zn^{2+} were inhibitory to activity to a certain extent. However, for AmyQ B from *B. subtilis*, the effect of metal ions was the same as that on the activity of AmyQ A except for Mn^{2+} and Zn^{2+}, and the activity of AmyQ B was enhanced by Zn^{2+} and significantly inhibited by Mn^{2+}.

Conclusion

In this work, a putative α-amylase gene consisting of 1,983 bp from *B. subtilis* was cloned and identified. AmyQ A was expressed in *Escherichia coli* via the plasmid pET-20b(+). The purified AmyQ A displayed optimal activity at pH 7.0 and optimal stability at pH 5.5. However, the optimal pH for purified AmyQ B was observed between pH 5.0 and pH 7.5, and the optimum stable pH for purified AmyQ B was 7.5. The optimal temperature of AmyQ A was 70°C, and the optimal temperature of AmyQ B was 55°C. The AmyQ A and AmyQ B had the same trend for stable temperature below 60°C. The AmyQ A was more thermostable than AmyQ B. AmyQ A hydrolyzed soluble starch with a K_m of 3.40 g/L and a V_{max} of 15.70 g/(L min^{-1}), and AmyQ B hydrolyzed soluble starch with a K_m of 2.01 g/L and a V_{max} of 6.95 g/(L min^{-1}). The catalytic rate of AmyQ A for soluble starch was lower than that of AmyQ B. The activity of AmyQ A was enhanced by K^+, Mn^{2+}, Co^{2+} and Ca^{2+}, whereas it was inhibited by the metal ions Fe^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+} and Zn^{2+}. The effect of metal ions for the activity of AmyQ B was the same as that of AmyQ A except for Mn^{2+} and Zn^{2+}, and the activity of AmyQ B was enhanced by Zn^{2+} and inhibited significantly by Mn^{2+}.

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