Screening, Characterization and Production of Thermostable Alpha-Amylase Produced by a Novel Thermophilic Bacillus megaterium Isolated from Pediatric Intensive Care Unit

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Abstract

This study aimed to isolate the thermophilic Bacillus strain capable of producing a high amount of thermo-stable α-amylase. The screening and isolation of amylase producing bacteria were done on selective media. The identification of bacteria was made using routine biochemical and molecular 16s rRNA techniques. The amylase activity assay was performed by using dimitsraliclyc acid (DNS) method, and finally, the optimum temperature, \( K_m \) (Michaelis constant) and maximum rate of reaction \( (V_{\text{max}}) \) of the enzyme was calculated. Results: The newly thermo-stable amylolytic enzymes of Bacillus megaterium designated AGHO1 was isolated from pediatric intensive care unit through a selective enrichment procedure. This isolate was identified based on biochemical and morphological traits along with 16s rRNA partial sequence analysis. This isolate showed the highest amylolytic activity (19.2 U/mL after 24 h) as compared to other isolates. The optimum temperature for the enzyme activity was achieved at 90°C and pH 7.0. At this condition, \( K_m \) of the enzyme for the degradation of starch was 3.36 mg/mL and \( V_{\text{max}} \) was 0.177 mM/min. The enzyme was stable at 90°C and 80°C in 33.4% and 35.7% of its original activity at pH 7.0 after 30 minutes and 9.1% and 48% after 60 minutes of incubation, respectively. Bioinformatics analysis showed the enzyme had no disulfide bond and had a 35 amino acid signal sequence. The aliphatic index (69.14) and grand average of hydropathicity index (-0.399) calculated by ProtParam server indicated that this α-amylase may be stable for a wide range of temperature and has excellent solubility in water. Conclusions: The high-thermal stable enzyme produced by Bacillus megaterium AGHO1 could be considered as an excellent alternative source for bioethanol production and pharmaceutical applications. Moreover, our data provides such beneficial information for a better industrial formulation with proper stability in extreme conditions.

Keywords: Amylase, Amylolytic activity, Bacillus megaterium, Thermo-stability

1 Introduction

Amylases take part as an essential class of industrial enzymes having about 25-33% of the world enzyme market. Initially, the term amylase was used originally to designate an extracellular enzyme capable of hydrolyzing α-1,4-glucosidic linkages in polysaccharides containing three or more glucose units (1). The enzyme acts on starches, glycogen and oligosaccharides randomly, leading to liberating reducing groups of sugars. It has potential applications for a large number of industrial purposes such as textile, bread and parchment paper, detergent, food, pharmaceutical and fine-chemical industries (2). However, with the advent of the new frontier in biotechnology, the amylase application has also expanded in numerous other fields such as clinical, analytical and medicinal chemistry (3-6), as well as their extensive usage in the distilling and brewing industries and starch saccharification (7, 8). It is necessary to have more thermo-active and thermostable amylases because these types of enzyme should be applicable at the high temperature of liquefaction (80–90 °C) and gelatinization (100–110 °C) to economize processes (9). Since the geographic region where the microorganisms are isolated, affect and determine enzyme behaviour, many attempts have been made to isolate some potential thermostable amylase producing strains from the harsh ecological area in the world (10-12). Geothermal sites, hot spring, compost, and soils were recognized as suitable habitats for microorganisms which can provide the source of thermostable enzymes (13). Because of degrading the starch...
constitutes (the highest substrate of these media), amylolytic bacteria are supposed to be usually available in there(14-18). Numerous studies have proven that Bacillus sp. found in natural areas (19-21) (especially those grown at harsh conditions) was recognized as the leading producer of exotic amylase with high economic importance (22, 23). This work is focused on the isolation and identification of a bacterial strain, which can generate thermophilic and thermo-stable amylase with excellent characteristics and could be applicable for commercial purposes.

2 Material and methods

2.1 Screening and isolation of microorganisms

During the present investigation, the samples were collected from pediatric intensive care unit, Namazi hospital in Shiraz, Iran. One gram of each sample was suspended in 9 mL of sterile saline (9 g/L NaCl), serially diluted up to 10² and cultured on starch nutrient agar (25 g/L nutrient agar and 3 g/L starch at pH 7.0) plate. After 24 h of incubation at 37°C and further subculturing to obtain pure culture, particular colonies were isolated, and colonies were measured in diameter.

2.2 Identification of amylase-producing strains

Distinct colonies were identified based on morphological features like size, colour, elevation, shape, margin, gram stain, motility, spore stain and cell shape and then, the amylolytic activity of each isolated strain was measured by adding Gram’s iodine reaction mixture (0.2% KI, 0.2% I₂) (24). Colonies with clear and transparent zone were selected as amylase producing strains. The selected colonies were purified by replica plating on starch-nutrient agar slant.

2.3 Selection of desirable enzyme producer strain

In order to select the best thermo-tolerant enzyme producer, the selected bacteria were cultured at different temperatures (30, 37, 42, 45, 50 and 55°C) in the fermentation medium and incubated at 140 rpm.

2.4 Identification of microorganisms

Various biochemical, physiological and morphological characterizations of the selected amylase-producing isolates were determined by using Bergey’s Manual of Determinative Bacteriology (25). Biochemical tests such as catalase and Voges-Proskauer and oxidase test, oxygen requirement and motility were also studied. Colony morphology such as form, margin, elevation, colour and diameter (mm) after 24 h were observed by light microscopy, and finally, the genus of the selected strain was determined (26). For further identification, the analysis of 16s rRNA gene sequence was done following Gholami et al. method (27). Concisely, the bacterial genomic DNA was extracted by the heat shock method. The universal prokaryotic (16s rRNA) primers, 5’-ACGGGCGGTGTGTAC-3’ as the forward primer and 5’-CAGCCCGGTAAAATC-3 as the reverse primer was used to amplifying the partial sequence of 16s rRNA gene of isolates by PCR, which amplify ~ 800 base pair region of the 16s rRNA gene. The PCR products were purified and then determined by Cinnagen Company (Tehran, Iran) and then resulting 16s rRNA gene sequences were aligned and the sequence similarity compared to some known microorganisms in GenBank database of the National Center for Biotechnology Information by using Basic Local Alignment Search Tool (BLAST).

2.5 Inoculum preparation

Purified bacterial strains were selected based on the capability to cultivate in the selective medium containing starch as an individual carbon and energy source at room temperature. The activation media had the following composition: Soluble starch (3.0 g/L) and nutrient broth (25 g/L) at pH 7.0. Ten ml of this activation medium was inoculated by 100µl of 0.5 McFarland of the bacterial strain that grown on starch nutrient agar plates and incubated for 12 h in a rotary incubator shaker at 42°C at 140 rpm (28).

2.6 Seed culture preparation

A two-day-old culture was used for the preparation of cell suspension. 25 ml of seed medium was added to 250 ml Erlenmeyer flask. The medium contained soluble starch (2 g/L), peptone (5 g/L) and yeast extract (3 g/L) at pH 7.0. The flasks were autoclaved at 105 Pa pressure (121 °C) for 20 min and then gradually cooled at 25°C. Under sterile environments, 1ml of inoculum was added to the flask, and the flask was incubated in a rotary incubator shaker (which is kept at 140 rpm) overnight at 37°C.

2.7 Production of amylase in the bioreactor system

Enzyme production was carried out using a 10 L bioreactor (Biotron Inc., South Korea) containing 5L of fermentation medium. A 20% (v/v) microorganism suspension was transferred from seed culture to the fermentation medium containing: NaCl (2 g/L), (NH₄)₂SO₄ (2 g/L), K₂HPO₄ (2.5 g/L), KH₂PO₄ (1 g/L), MgSO₄ . 7H₂O (0.01 g/L), FeCl₃ (1 g/L), CaCl₂ (0.01 g/L), peptone (2 g/L) and soluble starch (2 g/L) at pH 7.0. Subsequently, the bioreactor was set up at 140 rpm at temperatures ranging from 30 till 90°C for 12, 24, 36, 48 and 60 h. After the fermentation period, the cells were discarded by centrifugation, and the Cell-free filtrate was used for enzyme assay.

2.8 Amylase assay

Amylase assay was performed by using a reaction solution consisting of 0.5 ml of the substrate (20 g/L soluble starch in 50 mM phosphate buffer pH 7.2 and 0.5 ml of the supernatant as a crude enzyme) (8). The reaction was done at 90°C for 5 min. The released sugars from starch were estimated using dinitrosalicylic acid (DNS) reagent according to the Miller method. Subsequently, the product was heated to 100°C for 10 min and then cooled. The optical density of samples was taken at 575 nm in a spectrophotometer (Shimadzu, Japan). The activity of the enzyme was measured in units (1 unit was defined as the quantity of enzyme which produces 1mmole glucose under assay condition.

2.9 Characterization of amylase and kinetic properties

0.25 ml of the diluted crude enzyme was combined with a solution of starch (0.25 mL, 0.02%) at 90°C and the quantity of resulting glucose over the time was measured. The effects of temperature, pH and substrate concentration on enzyme activity were considered, and Km and Vmax values of the enzyme were measured by using double-reciprocal (Lineweaver-Burk) plot.

2.10 Evaluation of thermo-stability of the enzyme

At 80 and 90 °C, the stability of the enzyme produced by Bacillus sp. was assessed at without any additives at pH 7.0, and the half-life of the enzyme was estimated.
2.11 Bioinformatics Study of α-amylase

Specific primers for α-amylase was designed according to the available α-amylase nucleotide sequences in the NCBI database (Forward: 5'-GTGCTAAGTGGTCCACGCCCATC-3' and Reverse: 5'-CAAAGCCATGCCACCAACCGT-3') and then sequenced. The multiple sequence alignment (MSA) of amylase sequences was performed using the CLC free Workbench, and then the evolutionary tree was constructed by using the Neighbour Joining algorithm (27, 29). Percentages of hydrophobic and hydrophilic residues were computed from the primary structure analysis outcomes. The physicochemical traits, theoretical isoelectric point (pl), molecular weight, extinction coefficient, the total number of positive and negative residues, aliphatic index, instability index and grand average hydropathy (GRAVY) were calculated using the Expasy’s ProtParam (http://us.expasy.org/tools/protparam.html) prediction server. The putative amino acid sequence was inspected for a signal sequence by the SignalP 4.0 server (http://www.cbs.dtu.dk/services/SignalP/). The existence of disulfide bonds was predicted by CYS_REC and DISULFIND servers (27, 30).

3 Results and discussion

It is so necessary to screen bacteria for their amylase production, which could be a significant source of the enzyme after pure culture on a large scale. The productions of enzymes for industrial consistency and cost-effectiveness can be adopted with the selection of proper strain (31). The preliminary screening of environmental bacteria has prime importance as the starch-rich soil and water are the primary sources for varieties of bacteria as well as their amylase producing activity (32). Bacteria that produce amylase may be isolated from locations like vermin compost sites (33), processing firms, fermenting roots of cassava and naturally fermented alfalfa (34) and honey processing areas (35) as well as sewage surrounding mills (36).

At the present investigation, amylase producing strains were isolated from pediatric intensive care unit in a hospital. Among fifty-five different colony which produced bright halos with iodine solution, only three strains produced amylase at between 10.0 and 20.0 U/mL, and the rest of them produced amylase activity less than 10.0 U/mL. The high amylase producing bacteria labelled as strain N15, strain N32, and strain N7. The halo diameters of the strains N15, N32 and N7, were 31.86, 30.83 and 21.48 mm and their halo diameter to colony diameter ratios were 6.37, 6.17 and 4.3, respectively (Table 1). Among the three strains, N15 which was isolated from pastry waste produced the highest amylase activity (19.8 U/mL) after 24 h at pH 7.0 and 90°C and indicated the highest value for halo diameter to colony diameter ratio (7.4), which demonstrated highest amylase activities. Therefore, it was chosen for additional evaluations.

3.1 Effect of temperature on enzyme production

The effect of temperature on enzyme production was studied to find the best thermostable enzyme-producing strain, at the temperature ranging from 30 to 55°C (Fig. 1). The best temperature for the highest amylase production of all three strains is 37°C, and the more increase in temperature the less production of amylase was achieved. The results showed that N15 had the highest activity at all temperatures among the three selected strains.

![Figure 1: Effect of temperature on amylases production of three selected strains (N15, N32 and N7) after 24h at pH 7.0 in the fermentation medium](image1)

3.2 Effect of fermentation time on enzyme production

The suitable fermentation time for the highest enzyme activity was considered. Two strains depicted high enzyme activities after 24 h [N15 (19.2 U/ml), N32 (16.08 U/ml), and one of them produced high amylase activity after 36h (N7 (14.6 U/ml))] (Fig. 2).

![Figure 2: Effect of fermentation time on amylase production of three selected strains (N15, N32 and N7) at pH 7.0and at 37°C](image2)

However, the growth rate of three strains showed the same trend and after 12 h reached the maximum growth (Fig. 3). According to the highest enzyme-producing capability at high temperature, the strain N15, which was nominated as the best strain, was further characterized.

Table 1: Comparing the amylase activities of three selected strains (N15, N32 and N7), amylolytic power (diameter of clear halo) and its ratio to colony diameter at pH 7.0 and 90°C after 24 h

<table>
<thead>
<tr>
<th>Strain</th>
<th>Amylolytic power (mm)</th>
<th>Halo diameter to colony diameter ratio</th>
<th>Amylase activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N15</td>
<td>37</td>
<td>7.4</td>
<td>19.8</td>
</tr>
<tr>
<td>N32</td>
<td>30</td>
<td>6.0</td>
<td>16.3</td>
</tr>
<tr>
<td>N7</td>
<td>24</td>
<td>4.8</td>
<td>14.5</td>
</tr>
</tbody>
</table>
3.3 Identification of the microorganism

In order to identify the genus of the strain, morphological, cultural and biochemical (Table 2) characteristics were used. The strain N15 was rod-shaped, Gram-positive, motile, and with negative results for oxidase test and positive results for catalase test. According to results, as shown in Table 2, the strain N15 was similar to those of the genus *Bacillus*. Finally, according to the widely used of 16s rRNA gene analysis, we sequenced the 16s rRNA gene of amylase producing strain named as AGH01 (16s rRNA accession no. KP437334). The obtained sequence was blasted against various genomic databases in NCBI, which indicated 99% homology with *B. megaterium*. Therefore, based on perceptions and analysis, strain AGH01 was classified as *B. megaterium* AGH01.

3.4 Characterization of amylase and kinetic properties

The pattern of the kinetics of the resulting enzyme from *B. megaterium* in the first 5 min presented zero-order patterns and for this reason, in the subsequent investigation, the reaction time was established on 5 min (Fig. 4). In order to assess the activity of the enzyme produced by *B. megaterium*, different temperatures ranging from 30-95ºC was studied. The optimal temperature for amylase activity of this strain was 90 ºC, at pH 7.0 (Fig. 5). In this study, amylase showed 92.7, 96.4 and 97.0% of its maximal activity at 80, 95 and 85ºC, respectively, at pH 7.0. In contrast, Hayashida et al. obtained the maximum activity of amylase for *B. subtilis* 65 at 60ºC and pH 6.0 (37). Therefore, our result showed that this strain produced more heat-stable amylase than previous similar works.

![Figure 3: Effect of fermentation on the growth of three selected strains (N15, N32 and N7) at pH 7.0 and 37°C](image)

![Figure 4: Effect of fermentation time on activity of the enzyme produced by *B. megaterium* with soluble starch](image)

![Figure 5: The activity of amylase obtained from *B. megaterium* at different temperature at pH 7.0](image)

Table 2: Morphological, cultural and biochemical characteristics of N15

<table>
<thead>
<tr>
<th>Properties</th>
<th>Response of the strain</th>
<th>Biochemical test</th>
<th>Response of the strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Pale</td>
<td>Anaerobic growth</td>
<td>-</td>
</tr>
<tr>
<td>Form</td>
<td>Irregular</td>
<td>Growth in air</td>
<td>+</td>
</tr>
<tr>
<td>Margin</td>
<td>Irregular</td>
<td>Growth at 55ºC</td>
<td>+</td>
</tr>
<tr>
<td>Surface</td>
<td>Moist, shiny</td>
<td>Haemolysis on blood agar</td>
<td>β– haemolysis</td>
</tr>
<tr>
<td>Opacity</td>
<td>Opaque</td>
<td>Lactose fermentation</td>
<td>-</td>
</tr>
<tr>
<td>Elevation</td>
<td>Flat</td>
<td>Hyrolysis of tyrosine</td>
<td>-</td>
</tr>
<tr>
<td>Gram staining</td>
<td>Positive</td>
<td>KIA pattern</td>
<td>Acid slant, Acid butt,</td>
</tr>
<tr>
<td>Motility</td>
<td>Actively motile</td>
<td>H2S -, Gas -</td>
<td></td>
</tr>
<tr>
<td>Diameter of clear halo (mm)</td>
<td>31.86</td>
<td>Nitrate reduction test</td>
<td>+</td>
</tr>
<tr>
<td>Diameter of colony after 24 h (mm)</td>
<td>5</td>
<td>Urease test</td>
<td>+</td>
</tr>
<tr>
<td>Shape of vegetative cell</td>
<td>Rod</td>
<td>Oxidase production</td>
<td>+</td>
</tr>
<tr>
<td>Spore formation</td>
<td>Positive</td>
<td>Citrate production</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indole production</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catalase production</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voges-Proskauer test</td>
<td>+</td>
</tr>
</tbody>
</table>
Our results were in agreement with other works that indicated the optimal pH of amylase activity obtained from various *Bacillus* spp. such as *B. subtilis* KIBGE HAS (38), *B. licheniformis* (39), and *B. amylophilus* (40) and *B. coagulans* (41) is neutral. In order to estimate the $K_m$ and $V_{max}$, the activity of amylase was measured by the rate of substrate consumption. The activity became greater than 0.160 mM/min since the concentration of substrate was raised from 5 to 30 mg/mL at pH 7.0 and at 90°C (Fig. 7).

The $K_m$ (Michaelis constant) of the enzyme for the degradation of starch was 3.36 mg/ml, and maximum rate of reaction ($V_{max}$) was 0.177 mM/min at pH 7.0 and 90°C (Fig. 8). Inferior values of $K_m$ designate superior affinity of the enzyme for the substrate (42). In comparison to previous studies, our research clearly showed the lower Km values attributed to a higher affinity of the enzyme (40, 42). Because of variation in obtaining Km and Vmax values is completely related to the type of substrate and reaction condition, it is difficult to compare them. However, a similar work by Dragomirescu et al. showed the Km and Vmax values of 12.28 mg/ml and 0.82 mM/min at 37°C, respectively, for *B. amylophilus* (40).

### 3.5 Evaluation of enzyme stability

The thermal stability of amylase was assayed without any additives. After 30 min of incubation at 90°C, amylase obtained from *B. megaterium* kept 33.4% of its original activity at pH 7.0, and after one hour it decreased to 9.1% of its original activity. In comparison, at 80°C after 30 min of incubation, the enzyme retained 35.7% of its original activity at pH 7.0, and after one h, it reduced to 48% of its activity (Fig. 9). Asgher et al. reported that the amylase produced by *B. subtilis* JS-2004 was relatively stable at 60 and 70°C for one h, whereas at 80 and 90°C, 12% and 48% of its original activity was lost, respectively (43). In contrast, amylase obtained from *B. subtilis* AX20 in another study exhibited 60% and 35% of maximal activity at 40 and 70°C, respectively (44). In comparison to the above mentioned reports, our findings showed that the enzyme obtained from *B. megaterium* AGH01 had superior stability under the harsh conditions.

### 3.6 Bioinformatics Study of α-amylase

α-amylase gene-specific primers were designated as detailed in materials and methods section. The sequences achieved from NCBI was analyzed using the BLASTP search, which disclosed only one large open reading frame, composed of 1647 base pairs and 549 amino acid residues. BLAST searches of the α-amylase protein sequence depicted 89% identity at the amino acid sequence level with *Geobacillus stearothermophilus*. However, it also showed inferior identity (24-86 %) with other bacterial α-amylase (data not shown). The amino acid sequence of α-amylase was introduced to CLC free Workbench software. Then multiple sequence alignment (MSA) (Fig. 10) and phylogenetic tree (Fig. 11) were drawn in order to reveal the taxonomical variation in α-amylase from various *Bacillus* species (45). The multiple sequence alignment of these protein sequences revealed conserved regions at different stretches, namely, from 113 to 150, 246 to 274 and 298 to 310. The constructed phylogenetic tree revealed three distinct clusters (Fig. 11).
Figure 10: Multiple sequence alignment of α-amylase protein sequences showing maximum homology from 113 to 150, 246 to 274 and 298 to 310 amino acid residues.
The computed physicochemical traits of α-amylase (by using the ProtParam tool) are demonstrated in Table 3. The calculated isoelectric point (pI value) for α-amylase was 7.98, which indicates its semi alkaline nature. The computed extinction coefficient was 141680, that could be detectable by UV spectrophotometer. The theoretical value for instability index was 39.21, which is stable in a test tube conditions (the index less than 40 are considered stable in the test tube). The aliphatic and GRAVY indexes were 69.14 and -0.399, respectively which implied that this α-amylase was almost thermostable and had excellent solubility in water (the negative GRAVY indicates solubility in water whereas a positive value suggests a hydrophobic nature). These data are inconsistent with our experimental data which confirmed that this enzyme is thermostable and could tolerate a wide range of pH, including alkaline. The result achieved from SignalP 4.0 server signified that α-amylase had signal sequences with 35 amino acids. The functional characterization revealed that α-amylase had seven cysteines (and one cysteine residue in signal peptide); however, our analysis did not show any disulfide bond in this protein (checked with DISULFIND and CYS_REC tools). This might be attributed to nature of Gram-positive bacilli secretion pathways which most of the secretory proteins are exported from the cytoplasm in an unfolded state that do not have disulfide bonds (REF A disulfide bond-containing alkaline phosphatase triggers a BdbC-dependent secretion stress response in Bacillus subtilis AND Bacillus subtilis as a cell factory for pharmaceutical proteins: a biotechnological approach to optimize the host organism).

### Table 3: In-silico physicochemical properties of recombinant α-Amylase obtained from ProtParam tool

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>α-Amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sequence length</td>
<td>549 aa</td>
</tr>
<tr>
<td>2</td>
<td>Molecular weight</td>
<td>62371.4 D</td>
</tr>
<tr>
<td>3</td>
<td>Theoretical pI</td>
<td>7.98</td>
</tr>
<tr>
<td>4</td>
<td>-R*</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>+R*</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>Extinction coefficients** (M-1 cm-1 at 260 nm)</td>
<td>141680-141180</td>
</tr>
<tr>
<td>7</td>
<td>Instability index</td>
<td>39.21</td>
</tr>
<tr>
<td>8</td>
<td>Aliphatic index</td>
<td>69.14</td>
</tr>
<tr>
<td>9</td>
<td>GRAVY index</td>
<td>-0.399</td>
</tr>
</tbody>
</table>

* -R: total number of negative residues. +R: total number of positive residues.
** First value is based on the assumption both cysteine residues form cystines, and the second assumes that both cysteine residues are reduced.

The cluster I consists B. subtilis, B. megaterium, B. thuringiensis, B. circulans and Anaerobacillus macyae. The cluster II consists of Pontibacillus Chungwensis and B. flexus. The cluster III consists B. stearothermophilus, B. megaterium AGH1 (our isolate), B. akibai and two other Bacillus species. The results illustrated that the optimum condition for the activity of this enzyme was the temperature of 90°C and pH 7.0. The Kₘ of the obtained amylase (Michaelis constant) to solubilize the starch was 3.36 mg/mL, and the maximum rate of reaction (Vsmax) was 0.177 mM/min. These properties especially the high thermal stability of the enzyme makes B. megaterium AGH01 an excellent candidate for industrial production of amylase in textile, detergent, pharmaceutical and fine chemical industries. In future by purification and study of biochemical traits of AGH01 isolate could determine further its potential for industrial application.

### 4 Conclusion

Based on a clear zone on selective medium containing starch as sole carbon and energy source, 55 bacterial strains were found that showed amylolytic activity. Among them, the highest enzyme producer was recognized as B. megaterium AGH01. The maximum amount of amylase production was achieved in 24 h after inoculation in the bioreactor. The results illustrated that the optimum condition for the activity of this enzyme was the temperature of 90°C and pH 7.0. The Kₘ of the obtained amylase (Michaelis constant) to solubilize the starch was 3.36 mg/mL, and the maximum rate of reaction (Vsmax) was 0.177 mM/min. These properties especially the high thermal stability of the enzyme makes B. megaterium AGH01 an excellent candidate for industrial production of amylase in textile, detergent, pharmaceutical and fine chemical industries. In future by purification and study of biochemical traits of AGH01 isolate could determine further its potential for industrial application.

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