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Kinetic Properties of α-amylase Produced by *Bacillus megaterium* RAS103 under Optimum Conditions in Submerged Fermentation

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THE CURRENT investigation is aimed to optimize the culture conditions for α-amylase production by *Bacillus megaterium* isolated from rabbit manure as well as to study the kinetic properties of the produced amylase. Out of 38 bacterial isolates recovered from 10 rabbit manure samples, 7 isolates were selected as highly amylase producers onto starch agar medium. Interestingly, the bacterial isolate RAS103 was selected as the most highly producer for amylase with activity 81.76±0.12U/ml in starch mineral broth medium using submerged fermentation method. This isolate was identified based on the phenotypic and genotypic characteristics as *Bacillus megaterium* and deposited in the GenBank database with the accession number MH817142.1. Amylase activity was maximized to 106.39±2.36U/ml under the optimized culture conditions of a fermentation medium of 2% starch supplemented by 3g/L yeast extract, adjusted at pH 8.0, inoculated with 1% bacterial inoculum and incubated at 35°C for 24h. The V_{max} (maximum reaction velocity) and K_m (Michaelis constant) values of the produced amylase were 81.30U/ml and 0.878mg/ml, respectively, for hydrolysis of starch in a reaction mixture of pH 6.0 at 45°C for 20min. These findings suggest the applicability of using the bacterial isolate *B. megaterium* RAS103 as a potential producer of α-amylase for industrial purposes.

Keywords: Amylase, Fermentation, Optimization, Kinetics, Genotypic.

Introduction

Enzymes are an imoprtant class of proteins produced by living cells of microorganisms, plants and animals to catalyze specific biochemical reactions of the metabolic pathways of the cells (Rasmey et al., 2017). Among the produced enzymes, amylases are the most important group for biotechnology and account approximately 65% of enzyme market in the world (Balkan & Figen, 2007 and Abd-Elhalem et al., 2015). α-amylase (endo-1,4-α-d-glucan glucohydrolase EC 3.2.1.1) is endo-acting enzyme that catalyses the hydrolysis of α-1,4 glycosidic bonds in starch and leads to the formation of low molecular weight oligosaccharidesas glucose, maltose and maltotriose units (Rajagopalan & Krishnan, 2008).

Nowadays, amylases find potential widespread applications in different industrial processes especially in food industry for liquefaction and saccahrification of starch into fructose and glucose syrups (Prakash & Jaiswal, 2010 and Khusro et al., 2017). In enzymatic detergents to remove tough stains and to degrade the residues of starchy foods on clothes (Hmidet et al., 2009 and Mukherjee et al., 2009), in ethanol fermentation for saccharification of cereal grains starch into monosaccharaides applicable for fermentation by the used yeast strain (Tokosy Öner, 2006). Also it can be used in backing industry to degrade the flour starch into simple oligosaccharides in the dough of bread which enhance the fermentation rate and reduce the consumed time (Gupta et al., 2003), in textile industry for desizing and removing starch from the fabric without breaking of the fibers during the weaving process (Ahlawat et al., 2009). Moreover, in paper industry for modification of the coated paper starch to produce high molecular weight with low viscosity starch (van der Maarel et al., 2002) and in preparing cold water dispersible laundry starches (Gupta et al., 2003). To face the demands of these different

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applications, low cost source with high amount of the enzyme is required for amylases production on industrial scale.

Amylases can be extracted from several sources such as plant, animal and microorganisms such as fungi and bacteria (Hasan et al., 2017), however the bacterial amylases are today available commercially in starch processing industry and preferred to other organisms due to some advantages such as cost effectiveness, plasticity, consistency, less time and large capacity production (Tanyildizi et al., 2005). The large scale production of α-amylases from *Bacillus* species such as *B. subtilis*, *B. stearothermophilus*, *B. macerans*, *B. megaterium* and *B. amyloliquefaciens*is of special interest for detergents industry due to their remarkable activity and stabilityunder high temperatures and alkaline pH (Enhasy, 2007 and Chi et al., 2009).

α-amylase is a primary metabolite secreted as extracellular by the bacterial cells in presence of starch as a substrate and its production in the fermentation medium is reported to be growth associated. Therefore, optimization of the culture conditions, such as the physical and chemical parameters, is important due to their impact on the bacterial growth and enzyme production (Francis et al., 2003). The most important factors are the fermentation medium constituents, carbon source, nitrogen source, pH of the medium, incubation time, inoculum size and incubation temperature (Couto & Sanromán, 2006).

The aim of the present study is to optimize the α-amylase production by *Bacillus megaterium* and to perform its kinetic characterization.

Materials and Methods

Samplescollection

Ten samples of rabbit manure were collected aseptically in sterilized polyethylene bags from different ten rabbit farms located at Suez Governorate, Egypt. The collected samples were transferred immediately to the laboratory and used for isolation of starch hydrolyzing bacteria during one hour of collection.

Isolation of amylolytic bacteria

Isolation of starch hydrolyzing bacteria was performed by the soil dilution plate method using nutrient agar base containing 1% soluble starch based on the method of Clark et al. (1958). The developed individual colonies were picked up and cultured onto fresh solid medium. The purified isolates were transferred to tryptone soya agar (TSA) slants, grown at 37°C for 24h and then refrigerated at 4°C.

Screening foramylolytic bacteria

The isolated bacteria were individually screened to determine their amylase production capability by streaking onto starch agar platesand incubated for 24h at 37°C (Dash et al., 2015). The composition of starch agar medium was as follows (gL^{-1}): Soluble starch, 10; peptone, 5; yeast extract, 5; $MgSO_4.7H_2O$,0.2; K_2HPO_4, 1; agar, 15 and adjusted to pH 7.0. After incubation, the plates were flooded with 1% Lugol's iodine reagent for 20min. The appearance of a clear halo-zone around colonies indicated the starch hydrolysis and amylase production.

α-amylase production in submerged fermentation

The bacterial inoculum was prepared by inoculation of 100ml of nutrient broth by a loopful of the tested bacterial isolate and grown on a shaker incubator (150rpm) at 37°C for 24h until reach to 10^6cfu/ml. One ml (10^6cfu/ml) of bacterial inoculum was inoculated to 100ml of the fermentation medium. The used fermentation medium was composed as follows (gL^{-1}): soluble starch, 10; KNO_3, 0.5; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.1; $FeCl_3$, 0.001and adjusted to pH 7.0. After 48h of incubation at 37°C on 150rpm, the fermentation medium was centrifuged at 4000 for 20min and the resulted clear supernatant was used as the extracellular crude enzyme.

Amylase activity assay

Amylase activity was determined based on the method of Miller (1959). Substrate solution 1% was prepared by adding 1.0g of soluble starch in 100ml of 0.1M phosphate buffer (pH 6.5). Also, dinitrosalycilic acid (DNS) solution was prepared by adding 1.0g of DNS in 20ml of 2M NaOH, then 30g of sodium potassium tartarate was mixed and completed to 100 ml with distilled water. A reaction mixture of 0.5ml of substrate and 0.5ml of crude enzyme was incubated for 30min at 50°C on an orbital shaker (150rpm). A control was prepared by adding 0.5ml deionized water instead of the crude enzyme. After incubation, the reaction was stopped by adding 1.0ml of dinitrosalycilic acid (DNS) solution and boiled for 5min to give brown color. The final mixture

was filled to 5ml with distilled water and its absorbance was recorded at 540 $_{nm}$. One unit (U) of α-amylase activity was defined as the amount of enzyme that liberated 1µg glucose per minute, under the used assay conditions.

Characterization and identification of the highly amylase producer isolate

Phenotypic characterization

The colour, elevation, form, surface, margin and opacity of the bacterial colony were recorded onto nutrient agar. The Gram stain was performed on 24h culture and the shape of cells was recorded under the oil lens of light microscope. Biochemical characteristics of the isolate were detected according to the standard methods in Bergey's Manual of Systematic Bacteriology (Niall & Paul, 2009). In addition, the bacterial culture was grown on nutrient agar and incubated for 24h at different temperatures (5, 25, 37, 45 and 50°C) to determine its temperature profile.

Identification of the bacterial isolate RAS103using 16S rRNA

PCR amplification: DNA extraction was carried out using Bacterial DNA Preparation kit (Jena Bioscience) according to the method of Rasmey et al. (2017). The PCR amplification of purified 16S rRNA gene was carried out by using Qiagen Proof-start Tag Polymerase kit (Qiagen, Hilden, Germany). The two specific primers 16SF: 5′-GAGTTTGATCCTGGCTTAG-3′ and 16SR: 5′-GGTTACCTTGTTACGACTT-3′ were used. The reaction mixture(25µL) including 2µL of template DNA(20ng/µL), 12.5µL PCR Master Mix, 20pmol (2µL) each of forward and reverse primers and the total reaction volume was completed by 8.5µL of DNAase free water. The reaction mixture was incubated in automated thermocycler TC-3000. The reaction conditions were: An initial denaturation at 94°C for 5min, 37 cycles of denaturation at 94°C for 30sec, annealing at 51°C for 30sec and extension at 72°C for 30s. A final extension was conducted at 72°C for 5min. PCR products of about (1500bp) were purified from gel with QIA quick gel extraction kit (Qiagen, Hilden, Germany).

Nucleotide sequence analysis: The purified PCR product was sequenced by cycle sequencing with didesoxy mediated chain-termination (Sanger et al., 1977). The full length sequences obtained were matched with previously published sequence available in NCBI using BLAST at the NCBI website: http:// www.ncbi.nlm.nih.gov/ BLAST/ in order to assess the degree of DNA similarity. Multiple sequence analysis was carried out using CLUSTALX (http://clustalw.ddbj.nig.ac.jp/top-ehtml) and further MP (Maximum parsimony) plot was constructed using MEGA 7.2.2. The phylogenetic tree derived from 16S rRNA gene sequence of the isolate RAS103 with other related sequences in GenBank database was carried out by MEGA 7 program and displayed using the TREEVIEW program.

Optimization of culture conditions for α-amylase production

The influences of different culture conditions such as nitrogen source, starch concentration, inoculum size, incubation temperature and incubation period on amylase production in the fermentation medium were studied to determine the optimum conditions. The fermentation medium was supplemented individually with organic and inorganic nitrogen substances (Yeast extract, ammonium sulfate, urea, peptone, malt extract and sodium nitrate) at 0.3% level. Effect of various starch concentrations (0.25, 0.5, 1, 1.5, 2 and 2.5 %) was studied in the fermentation medium. Also, the effect of inoculum size (0.5, 1, 2, 3, 4, and 5%) was tested. To determine optimum pH for amylase production, initial pH of the medium was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The bacterial culture was incubated at different temperatures (20, 25, 30, 35, 40 and 45°C) to determine the optimum temperature. The incubation period was optimized by incubating the culture at various times (4, 8, 12, 16, 20, 24, 32, 40 and 48h). At the end of each condition the amylase activity was assayed.

Kinetic properties of amylase

Kinetic properties of the produced α-amylase were determined by studying the effects of substrate concentration, pH, incubation time and temperature on amylase activity. The maximum reaction velocity (V_{max}) and Michaelis constant (K_m) were determined by testing various concentrations of substrate (0.5, 1.0, 1.5 and 2.0%) in the reaction mixture. The effect of pH of the reaction on amylase activity was studied at different values using different buffers (phosphate pH 7-11, KCl- NaOH pH 12-13 and acetate pH 3-6). Different incubation temperatures (30, 35, 40, 45 and 60°C) of the reaction were studied on amylase activity.

Results and Discussion

A total of 38 bacterial isolates were recovered from the collected 10 samples of rabbit manure and further were screened for their potentiality to produce amylase and hydrolyze starch using starch agar medium. The hydrolysis of starch was indicated by formation of a clear zone around the producer colony after addition of iodine reagent. Among 38 bacterial isolates,seven were selected as the highest amylase producers observed by the formation of the larger clear zones (2.0–2.8mm) onto starch agar medium. Using of starch agar plates and iodine reagent for determining the amylase producing bacteria was reported by many researchers (Alariya et al., 2013; Abd-Elhalem et al., 2015; Khusro et al., 2017 and Padmavathi et al., 2018). The selected seven isolates were secondary screened for their potentiality to produce amylase into starch minerals broth medium using submerged fermentation method (Table 1). The most highly producer isolate was RAS103 with amylase activity of 81.76±0.12U/ml, therefore this isolate was selected for phenotypic characterization and genotypic identification using 16S rRNA gene sequencing.

TABLE 1. Screening of amylase production by the selected bacterial isolates.

Isolate code	Amylase activity (U/ml)
RAS101	72.4 ± 0.14^{d}
RAS103	81.76 ± 0.12^{a}
RAS112	68.07 ± 0.28^{e}
RAS119	75.48 ± 0.15^{c}
RAS125	78.34 ± 0.08^{b}
RAS129	77.82 ± 0.11^{b}
RAS130	73.68 ± 0.07^{d}

The morphological and biochemical characteristics of the bacterial strain RAS103 are shown in Table 2. Morphologically, the colonies of this bacterial isolate was white, raised, circular, entire margin and opaque. Microscopically, this isolate was Gram positive with rod shaped cells. Biochemically, the isolate was positive for catalase, urease, methyl red (MR) and could be utilize sugars such as: Citrate, glucose, sucrose, maltose, lactose and mannitol. While, it was negative for indole and Voges-Proskaure (VP)tests. The isolate was able to grow at 5, 20, 25, 37 and 45°C, but was unable to grow at 50°C. Based on the described phenotypic characteristics, the isolate RAS103 was assigned to the bacterial species *Bacillus megaterium* according to the standard method of Bergey's Manual of Systematic Bacteriology (Niall & Paul, 2009). This identification was confirmed by 16SrRNA gene sequencing, which indicated that this isolate was 93% similar to *Bacillus megaterium* available in Genbank database. Comparison between 16S rRNA gene sequence of the isolate RAS103 and 16S rRNA gene sequences in GenBank database was determined by using Blast search analysis. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length= 0.12244580 is shown in Fig. 1. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions having gaps and missing data were removed. There were 824 positions in the final dataset. Evolutionary analyses were carried out in MEGA7 (Kumar et al., 2016). The nucleotide sequence of *Bacillus megaterium* RAS103 was deposited in the GenBank database with accession number MH817142.1.

TABLE 2. Morphological and biochemical characteristics of the bacterial isolate RAS103.

Test	Result
Colony morphology	**White, raised, circular,entire and opaque**
Cells shape	Rod shaped
Gram stain	Positive
Urease	Positive
Catalase	Positive
Indole	Negative
VP	Negative
MR	Positive
Citrate	Positive
Glucose	Positive
Lactose	Positive
Maltose	Positive
Sucrose	Positive
Mannitol	Positive
Temperature profile	
5°C	Positive
25°C	Positive
37°C	Positive
45°C	Positive
50°C	Negative

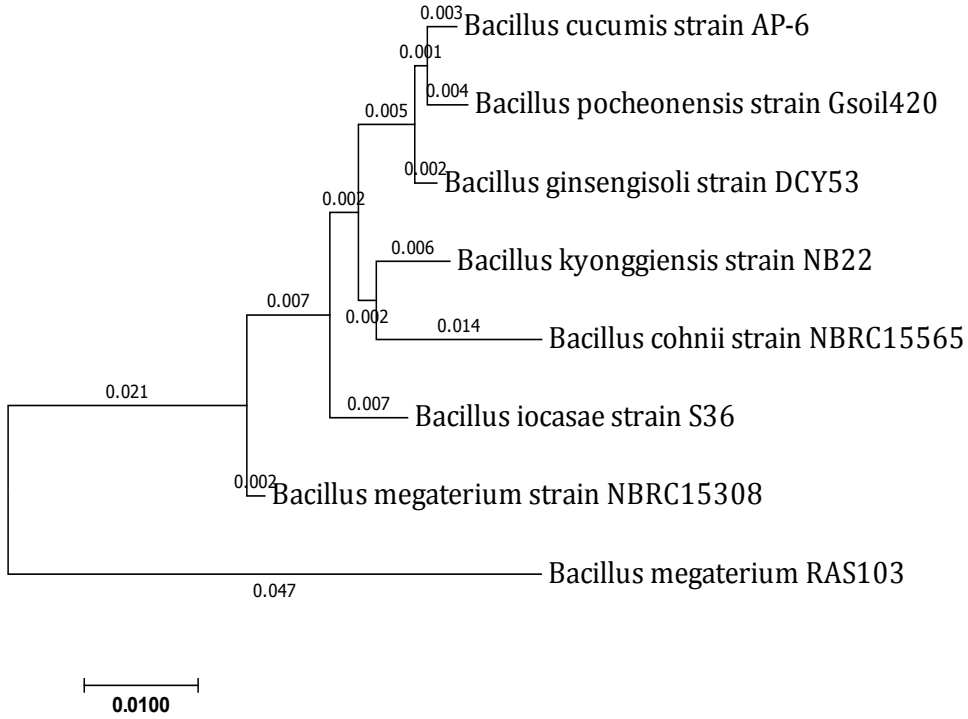

Fig. 1. Evolutionary relationships of *Bacillus megaterium* RAS103 with other species in GenBank database.

Optimization of culture conditions for α-amylase production

In order to maximize the production of α-amylase by the selected bacterial isolate *Bacillus megaterium* RAS103, the effects of some culture conditions were studied in submerged fermentation technique and the obtained data were shown in Table 3. The studied parameters were as follows:

pH

The effect of initial pH of the fermentation medium on amylase production was studied and the obtained data were shown in Table 3. The variation of pH of the medium showed a marked effect on amylase production and the maximum activity 83.04±0.79U/ml was achieved at pH 8. Further increase or decrease in pH, lead to decrease in amylase production. Behal et al. (2006) studied the production of amylase by *Bacillus* and revealed that the maximum enzyme activity was obtained at pH 8.0. Amylase production by bacteria significantly depends on the medium pH because it affects on the growth and many metabolic reactions as well as the movement of molecules across cell membrane (Nusrat & Rahman, 2007).

Starch concentration

Different starch concentrations in the fermentation medium for amylase production were tested and the optimum one was 2% with amylase activity 83.37±0.48U/ml. It has been reported that the biosynthesis of amylase in most *Bacillus* species, is subjected to catabolic suppression by readily metabolizable sugars such as glucose (Souza, 2010). Also, starch plays a main role as inducer in the fermentation medium for enhancement of amylase production to hydrolyze the starch in simple sugars such as glucose to be available for bacterial cells. The decrease in amylase biosynthesis at higher concentration of starch might be due to the inaccessibility of dissolved oxygen to the bacterial cells because of the high viscosity of carbon source in the fermentation medium.

Inoculum size

The size of inoculum is one of the greatest important parameters which affects amylase productionby bacteria. Maximum amylase production was 83.61±0.43U/ml which obtained with 1% inoculum size, while further increase or decrease in inoculum size decreasedthe amylase production. Initial microbial sizemay be affects the growth and metabolic pathways. The lower inoculum percent may lengthen the lag phase of bacterial growth, on the other hand the high inoculum size may be stimulating the growth, but reduce some metabolic activities of the culture (Aboseidah et al., 2017).

TABLE 3. Effect of some factors on amylase production by *B. megaterium* RAS103.

Factor	Amylase activity (U/ml)	Relative activity (%)
Initial pH		
4	34.86±0.51[d]	41.98
5	59.08±0.31[c]	71.15
6	75.33±0.59 [b]	90.71
7	82.96±0.71[a]	99.91
8	83.04±0.79[a]	100.00
9	82.28±0.71[a]	99.08
Starch concentration (%)		
0.25	31.86±0.70[e]	38.22
0.5	67.15±1.02[d]	80.55
1	77.54±0.08[b]	93.02
1.5	82.39±1.02[a]	98.84
2	83.37±0.48[a]	100.00
2.5	74.68±0.99[c]	89.58
Inoculum size (%)		
0.5	73.01±0.37[c]	87.31
1	83.61±0.43[a]	100.00
2	74.68±0.13[b]	89.33
3	71.79±0.10[cd]	85.87
4	71.86±0.56[cd]	85.95
5	71.08±0.58[d]	85.02
Temperature (°C)		
20	46.15±0.21[f]	49.51
25	58.30±0.84[e]	62.54
30	76.34±0.45[c]	81.89
35	93.23±0.76[a]	100.00
40	83.17±0.38[b]	89.21
45	73.63±0.36[d]	78.98
Incubation time (h)		
8	37.63±0.12[f]	37.42
12	48.14±0.96[e]	47.86
16	66.12±0.78[d]	65.75
20	95.32±1.46[b]	94.78
24	100.57±0.95[a]	100.00
32	94.04±0.40[b]	93.50
40	92.94±0.32[b]	92.41
48	89.98±0.26[c]	89.47
Nitrogen source (0.3%)		
Yeast extract	106.39±2.36[a]	100.00
Ammonium sulphate	42.21±0.17[e]	39.67
Urea	66.54±0.47[d]	62.55
Peptone	74.99±1.27[c]	70.48
Malt extract	82.60±0.68[b]	77.64
Sodium nitrate	84.24±0.31[b]	79.18

Temperature

The growth and amylase production depend strongly on incubation temperature of the culture, so effects of different incubation temperatures (20-45ºC with 5 intervals) on amylase production by *B. megaterium* RAS103 were studied. The optimum fermentation temperature for amylase production was 35ºC with activity 93.23±0.76U/ml. The optimum temperature stimulates the bacterial growth and enzyme production and might be improve the starch solubility, decrease the viscosity and limit contamination by other microbes (Thippeswamy et al., 2006). The decrease in amylase activity was obtained at higher temperatures might be due to inhibition of cell division and growth as well as protein denaturation of bacterial cells (Oyeleke et al., 2010).

Incubation time

Amylase production by *B. megaterium* RAS103 was sharply increased by the increase in fermentation period until to 24h of inoculation with maximum activity 100.57±0.95U/ml. Further increase of incubation time significantly decreased the enzyme production. The present investigation is in agreement with the results of Dash et al. (2015) who reported that the maximum amylase production from *Bacillus* species was achieved at 24h of inoculation. These results revealed that amylase was produced early in the active growth phase (log phase) and decreased towards the exponential growth phase. This ismay be due to nutrientd eficiency of the culture medium and accumulation of toxic metabolites (Shafique et al., 2009).

Nitrogen source

The effects of several organic and inorganic nitrogensubstrates (0.3%) on amylase production by *B. megaterium* RAS103 in submerged fermentation were tested. Yeast extract was the most applicable organic nitrogen source for enzyme production and gave amylase activity 106.39±2.36U/ml. The production of enzymes by bacteria is strongly influenced by the growth and metabolic activity of the culture which depends mainly on the provided nutrients particularly nitrogen source. Various studies have reported that yeast extract is the most suitable nitrogen sourcefor *Bacillus* species to achieve maximum amylase production (Valaparla, 2010; Oshoma et al., 2010; Ravindar & Elangovan, 2013 and Salman et al., 2016). It was reported by Khusro et al. (2017), that yeast extract plays avital role in production of enzymes by bacteria due to the presence of nitrogenous constituents, growth factors, coenzymes and essential elements.

Kinetic properties of the produced α-amylase

The effect of reaction temperature on amylase activity was considered in the range of 30-60ºC. The optimum activity was detected at 45ºC. But at 30ºC, 40ºC and 60ºC, remarkably decrease in enzyme activity was observed (Fig. 2). The decrease of enzyme activity at low temperatures is due to the decrease in atomic motion which decreases the activation energy of the reaction between the substrate and enzyme molecules. Also, the decrease of enzyme activity at high temperatures might be due to thermal denaturation of the enzyme (Bakare etal., 2005 and Krishma & Radhathirumalaiarasu, 2017).

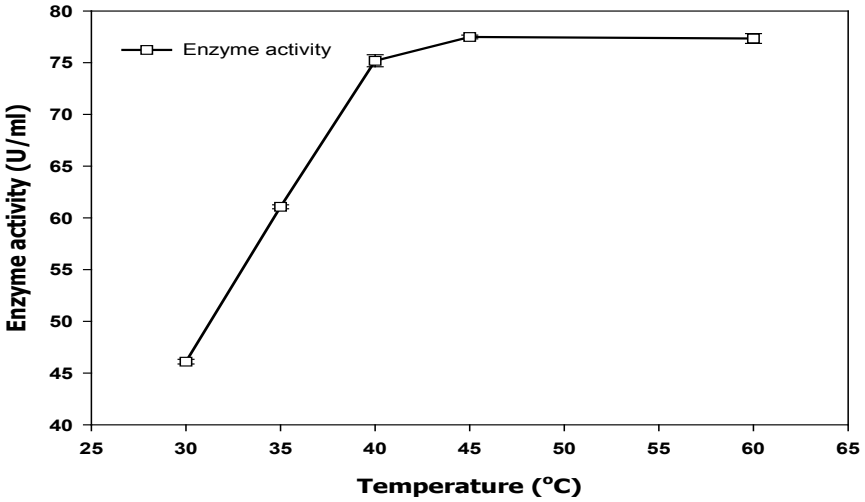

Fig.2. Effect of reaction temperature on amylase activity.

The influence of pH of the reaction medium on amylase activity was detected and the obtained results revealed that pH 6.0 was the optimum (Fig. 3). The present result is in agreement with Daniel et al. (2010) and Oyeleke et al. (2010) who reported in their study that most bacterial enzymes function in a pH range between 6.0 and 8.0. The amylase activity is obviously affected by the pH of reaction medium, this is because the binding of substrate and enzyme is frequently dependent on charges distribution on both of them (Shah & Madamwar, 2005).

The α-amylase activity of *B. megaterium* RAS103 was markedly influenced by the substrate concentration. The increase in substrate concentration at a constant enzyme concentration led to an increase in amylase activity until reaching a saturation point, this is might be due to the saturation of active sites of enzyme molecules with substrate molecules as well as the increase of reaction viscosity. V_{max} (maximum reaction velocity) and K_m (Michaelis constant) were determined from Lineweaver–Burk plot equation, $1/V_0=K_m/V_{max}$ [1/[S]] + $1/V_{max}$ and when plotting $1/V_0$ against 1/[S], a straight line was obtained. The slope of this line represents K_m/V_{max}, which obtained as 0.0108 and the V_{max} was calculated as 81.30, so K_m was 0.878 (Fig. 4). In the present study, the $V_{max}$and $K_m$values of amylase for hydrolysis of starch at 45°C, pH 6.0 and 20min, were 81.30U $ml^{-1}$and 0.878mg ml^{-1}, respectively. This investigation is in agreement with the findings of Samanta et al. (2014). The present finding indicates the high maximum reaction rate of the produced α-amylase by the bacterial isolate *B. megaterium* RAS103.

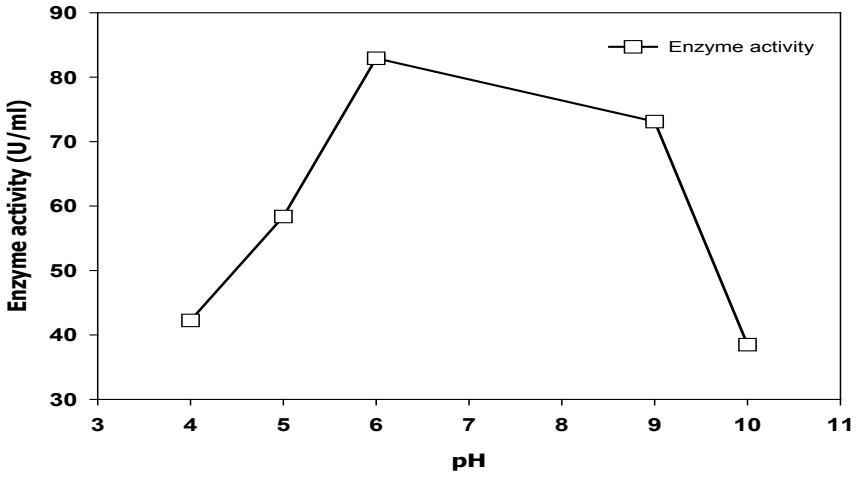

Fig. 3. Effect of reaction pH on amylase activity.

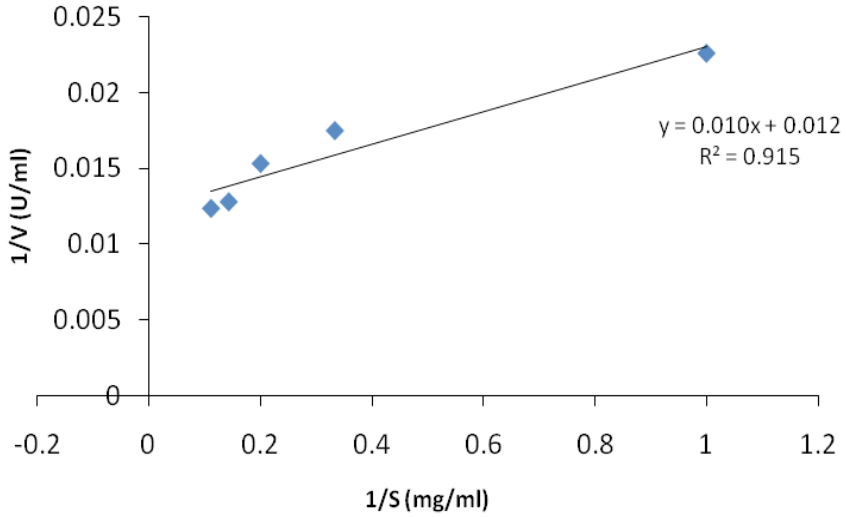

Fig. 4. Lineweaver–Burk plot for calculating V_{max} and K_m of the produced amylase.

Conclusion

The current work describes the isolation and identification of highly α-amylase producer bacterial species from rabbit manure as well as the optimization of fermentation conditions for enzyme production by the selected isolate. A total of 38 amylolytic bacterial isolates were recovered from 10 samples of rabbit manure. Out of them, 7 isolates were selected as highly amylase producers and the bacterial isolate RAS103 was the most potential one. This isolate was identified phenotypically and genotypically as *Bacillus megaterium* RAS103 and was assigned the accession number MH817142.1 in GenBank. The fermentation conditions for enzyme production was optimized and the obtained data revealed that the optimum conditions were pH 8.0, 2% starch concentration, 1% inoculum size, 35°C incubation temperature, 24h incubation time and 0.3% yeast extract as nitrogen substrate. At the optimized culture conditions, the maximum amylase activity was 106.39±2.36U/ml. In addition, the V_{max} and K_m values of the produced amylase were determined from Lineweaver–Burk plot equation as 81.30U/ml and 0.878mg/ml, respectively, for hydrolysis of starch at 45°C, pH 6.0 and 20min. The present findings indicate the highly potentiality of *Bacillus megaterium* RAS103 for amylase production in submerged fermentation.

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الخصائص النشطة لانزيم ألفا أميليز المنتج بواسطة باسيلس ميجاتريم رقم RAS103 تحت الظروف المثلى فى التخمير المغمور

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هدفت الدراسة الحالية إلى تحسين الظروف المزرعية لإنتاج ألفا أميليز بواسطة باسيلس ميجاتريم المعزولة من روث الأرانب وكذلك لدراسة الخصائص النشطة للأميليز المنتج. من بين 38 عزلة بكتيرية تم عزلها من 10 عينات من روث الأرانب، تم اختيار 7 عزلات الأكثر انتاجية للإنزيم على بيئة أجار النشا. ومن المثير للإهتمام، تم اختيار العزلة البكتيرية رقم RAS103 كأفضل عزلة منتجة للأميليز بنشاط 81.76 ± 0.12 وحدة لكل مللى على بيئة نشا الأملاح السائلة بطريقة التخمير المغمورة. وقد تم تعريف هذه العزلة على أساس الخصائص المظهرية والوراثية على انها باسيلس ميجاتريم وتم تسجيلها في قاعدة بيانات بنك الجينات برقم MH817142.1. كذلك تم زيادة نشاط الأميليز إلى 106.39 ± 2.36 وحدة لكل مللى تحت الظروف المثلى للمزرعة لوسط التخمير بنسبة 2% من النشا و3 جرام مستخلص خميرة، عند الرقم الهيدروجيني 8، وتم تلقيحها بنسبة 1% من اللقاح البكتيري وتم تحضينها عند 35 درجة مئوية لمدة 24 ساعة. وكانت قيم V_{max} (أقصى سرعة رد فعل) و K_m (ثابت ميشيلس) للأميليز المنتج هى 81.30 وحدة/مل و 0.878 وحدة/مل ، على التوالي، لتكسير النشا في خليط التفاعل برقم هيدروجينى 6.0 وعند 45 درجة مئوية لمدة 20 دقيقة. وتشير هذه النتائج إلى إمكانية استخدام العزلة البكتيرية باسيلس ميجاتريم رقم RAS103 كعزلة قوية لإنتاج ألفا أميليز للأغراض الصناعية.