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Production and optimization of α-amylase from thermo-halophilic bacteria isolated from different local marine environments

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Abstract

Background: Amylases are among the most important enzymes which are of great significance for biotechnology and have almost completely replaced chemical hydrolysis of starch in the starch processing industry. The present study was concerned with the production and optimization of extracellular α-amylase from *Bacillus* sp. NRC22017.

Results: The effect of various fermentation conditions on α-amylase production through shake-flask culture was investigated. Bacterial strain produces α-amylase was isolated from water in Wadi El-Natron. Based on microbiological, biochemical tests, and 16S rRNA gene sequences, the isolate was identified as *Bacillus* sp. NRC22017 and was later used for further studies. Maximum yield of α-amylase is 15.15 ± 0.47 U/ml from *Bacillus* sp. NRC22017; this strain is characterized with high temperature and high salinity in cultivated culture, and achieved maximum yield of α-amylase at pH 6.0 with inoculum size of 500 μl at 45 °C and aerobically incubation period of 72 h. The optimum volume of the fermentation medium was found to be 20 ml in 100 ml Erlenmeyer flask; the best starch and meat extract plus peptone concentration that provided the highest enzyme production from *Bacillus* sp. NRC22017 were found to be 2% and 1.05% (*w/v*) respectively.

Conclusion: Enzyme production was higher after optimizing the production conditions as compared to the basal medium.

Keywords: α-Amylase, Starch hydrolysis, Thermo-halophilic bacteria

Background

Alpha amylase (EC: 3.2.1.1), randomly attack α-1, 4-glycosidic bond of starch, maltodextrins, maltose, and glucose units were formed as a result of α-amylase action (Maity et al. 2015). Alpha amylase has gained a great attention due to its broad spectrum of applications and economic merits (Bansode 2010). Currently, amylase production has reached up to 65% of enzyme market in the world and is continuously increasing (Abdullah et al. 2014). It has a wide range of applications in starch liquefaction, paper, desizing of textile fabrics, preparing starch coatings of paints, removing wallpaper, brewing industry, sugar induction by the production of sugar syrups, and pharmaceuticals. Alpha amylase can be produced by micro- and macroorganisms (Simair et al. 2017). When the origins are in comparison, amylases from animal and plant origins have low resistance under acidic, basic, and high temperature conditions. On the other hand, bacterial and fungal amylases have good stability under such conditions and have more economic production operations. So, microbial enzymes have been applied in a large number of applications.

Recently, starch saccharification, the main use of amylase, has totally displaced chemical utilization with amylase enzyme hydrolysis. Saccharification is executed at raised temperature and thermophilic microorganisms could be most hopeful candidates for amylase production because these strains will produce thermostable amylase. This is why, still, search for new microbial strains is continued to achieve industrial requirements of enzymes.

In addition, amylase is supplemented in local detergents due to high alkaline pH stability needed for industries (Asad et al. 2011). Moreover, thermophilic amylase

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is required for other applications in the production of sweeteners from starch and saccharification of starch for biochemical production (Castro et al. 1999).

Bacillus genus is famous for alpha-amylase production and several *Bacillus* strains such as *B. stearothermophilus*, *B. subtilis*, *B. cereus*, *B. licheniformis*, and *B. amyloliquefaciens* are isolated and screened for amylase production (Sivaramakrishnan et al. 2006). Some *Bacillus* strains are involved in raw starch degradations (Demirkan et al. 2005; Goyal et al. 2005; Puspasari et al. 2013).

Still, many are being searched to obtain the maximum output with unique industrial properties. Enzyme production could be improved through the optimization of various physical and nutritional growth parameters; the important factors that determine the bioprocess are incubation period, temperature, pH, aeration, inoculum size, carbon, and nitrogen sources. Therefore, in this study, the effects of physical and nutritional parameters were investigated for the optimum production of extracellular enzyme α-amylase from *Bacillus* sp. NRC22017.

Results

Isolation, screening, and identification of amylolytic bacteria

In the present investigation, a pure strain of *Bacillus* sp. NRC22017 was isolated from Wadi El-Natron and we found bacteria isolated from such places may have better potential to produce enzyme under adverse conditions. The isolate was inspected for the qualitative production of α-amylase on a starch agar plate supplemented with 2% soluble starch using iodine solution (Fig. 1). Finally, NRC22017 strain was selected as the best amylase producer. The promising isolate (NRC22017) was identified based on its microscopic, morphological, and biochemical characterization and it was proven to be *Bacillus* sp. (Table 1).

Table 1 Cultural and biochemical characteristic of the promising bacterial isolate

Test	*Bacillus* sp. NRC22017
Morphological characterization	
Gram stain	Positive
Motility	Positive
Spore-former	Positive
Appearance of the colony surface	Wrinkled
Color	Creamy
Elevation	Umbonate
Edge	Entire
Whole colony	Circular
Pigmentation	No pigment
Opacity of the bacterial colony	Opaque
Biochemical characterization	
Starch hydrolysis	Positive
Voges-Proskauer	Negative
Citrate utilization	Negative
Nitrate reduction	Negative
Oxidase	Positive
Catalase	Positive
Acid production from	
Glucose	Positive
Fructose	Positive
Galactose	Negative
Lactose	Negative
Maltose	Negative
Sucrose	Positive
Mannose	Positive
Xylose	Negative

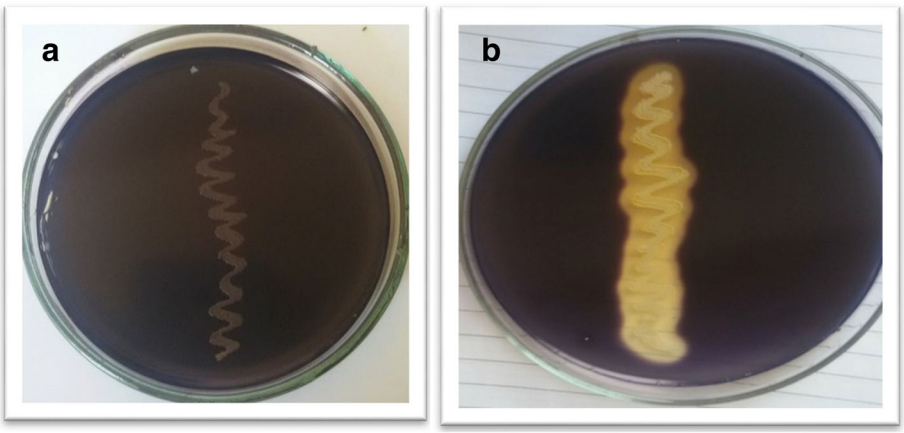

Fig. 1 Bacterial isolate negative for starch hydrolysis test (**a**). Zone of clearance around NRC22017 (**b**) due to starch hydrolysis

A molecular technique was used to prove and further confirm the identification of the isolate to the species level. The partial 16S rDNA sequence was determined and was compared to the GenBank databases. The isolate was identified as *Bacillus* sp. NRC22017. The sequence was submitted to GenBank in NCBI (https://www.ncbi.nlm.nih.gov/nuccore/KY614074) with the accession number KY614074. The phylogenetic tree based on different species of *Bacillus* was constructed using neighbor joining method (Fig. 2).

Optimization of α-amylase production conditions

Though different *Bacillus* species have similar growth patterns and enzyme profiles, but their optimized conditions vary, depending upon the strain. Optimization of the process parameters is needed for the improved production of the enzyme to make the process cost effective. Selected strain *Bacillus* sp. NRC22017 was subjected to various culture conditions to investigate the optimum culture conditions for α-amylase production.

Effect of five different fermentation media on alpha amylase production

Studies on bacterial growth and α-amylase production from *Bacillus* sp. NRC22017 in shake-flask cultures were carried out using five different media at pH 7.0 and incubated for 72 h at 50 °C. Results in Fig. 3 illustrated that among the culture media, the medium 5 exhibited a significant impact on the microorganism development (2.15 ± 0.067) and enzyme production (11.02 ± 0.17 U/ml), while the lowest growth rate (0.089 ± 0.001) and α-amylase synthesis (3.20 ± 0.12 U/ml) were obtained when medium 3 was used.

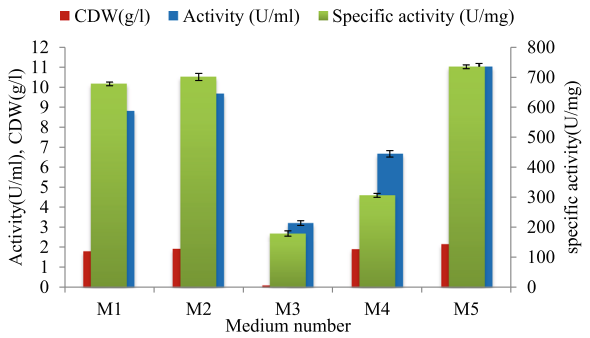

Fig. 3 Production of α-amylase by *Bacillus* sp. NRC22017 in five different fermentation media. Data were expressed as mean ± standard error

Effect of physical parameters on alpha amylase production

Incubation period The results obtained from the incubation time study in submerged culture cleared that bacterial growth and α-amylase productivity was gradually raised with the increment in the incubation period. Incubation for 3 days was the best for both α-amylase productivity (11.56 ± 0.20 U/ml) and bacterial growth (2.04 ± 0.09 g/l). The activity of α-amylase was declined by further increasing the incubation period. At 5 days of incubation, it was extremely reduced and the minimum amount of α-amylase was obtained after 5 days of incubation (6.96 ± 0.17 U/ml) (Fig. 4a).

Inoculum size Data in Fig. 4b show the effect of inoculum size (200 to 700 μl *v*/*v*) on cell growth and extracellular amylase secretion. The highest amylase yield was achieved when 500 μl (14.24 ± 0.2 U/ml) of 24-h-old seeds culture was inoculated into the fermentation medium.

Incubation temperature Data in Fig. 4c show the effect of fermentation temperature (40–55 °C) on the synthesis of amylase by *Bacillus* sp. NRC22017 grown in fermentation medium containing 20 g/l starch and 14 g/l peptone plus yeast extract at pH 7.0 and incubated for 72 h. Enzyme concentration increased with incubation temperature and maximum amylase yield was noted at

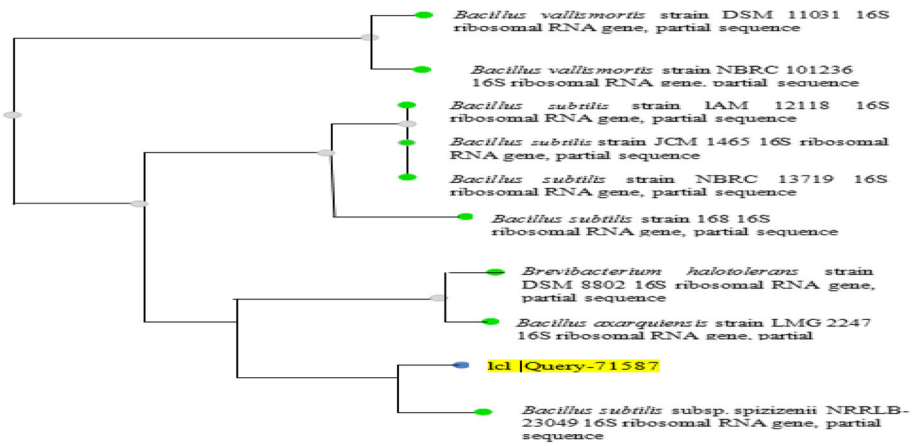

Fig. 2 Phylogenetic tree based on the 16S rRNA sequences of isolate and its closest *Bacillus* sp.

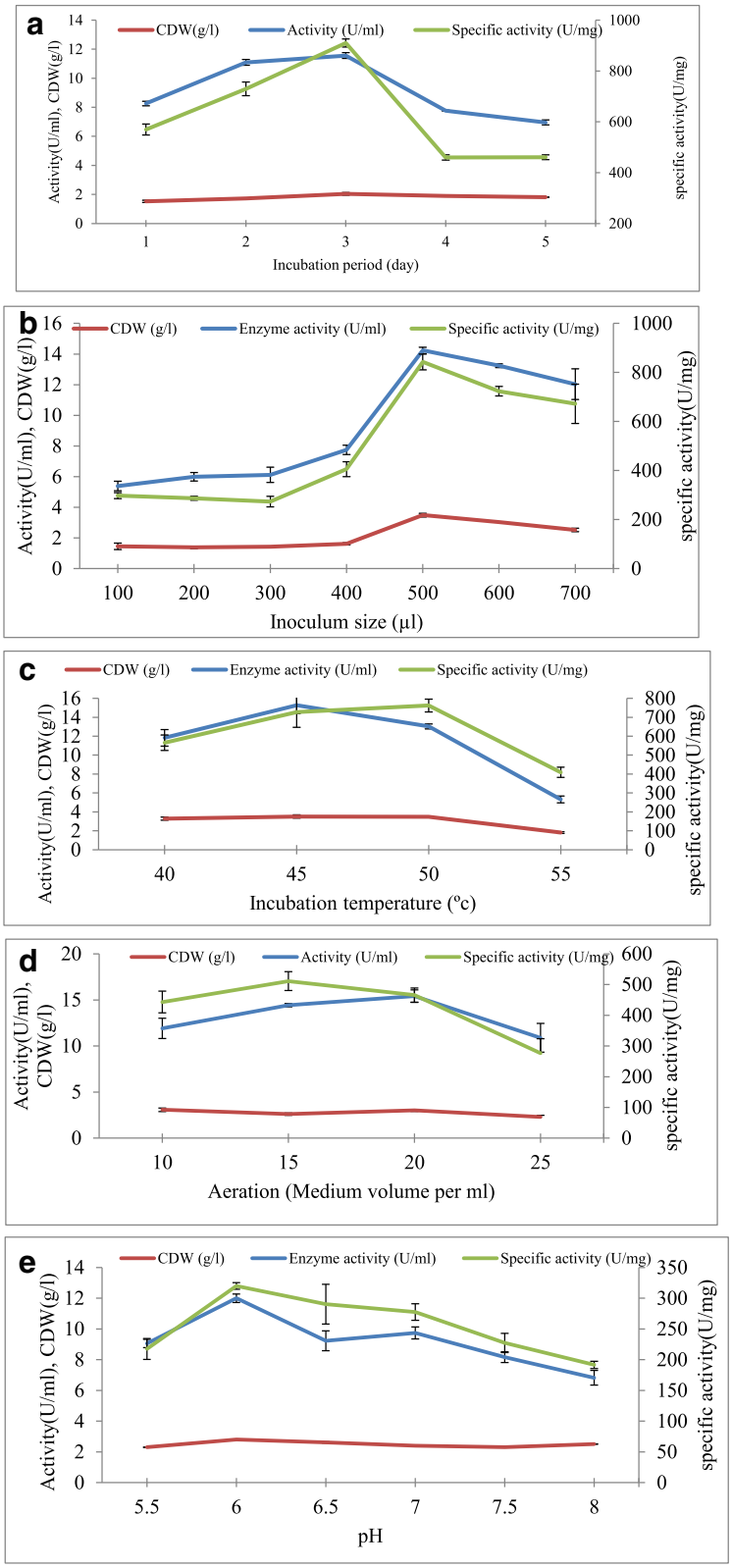

Fig. 4 Effect of incubation period (**a**), inoculum size (**b**), incubation temperature (**c**), medium volume in 100 ml conical flask (**d**), and different pH values (**e**) on α-amylase production by *Bacillus* sp. NRC22017. Data were expressed as mean ± standard error

45 °C (15.27 ± 0.88 U/ml) and a further increase of temperature reduced amylase synthesis. The production of the enzyme was greatly inhibited at 55 °C (5.32 ± 0.36).

Aeration In our study, the highest productivity of α-amylase was recorded at 20 ml broth medium in 100 ml conical flask (15.42 ± 0.68 U/ml), and the lowest production level was reported at 25 ml broth medium in a conical flask of 100 ml capacity (10.89 ± 1.57 U/ml) (Fig. 4d).

pH In our study, the maximum bacterial growth and synthesis of α-amylase were found at pH 6.0 (12.00 ± 0.28 U/ml) (Fig. 4e).

Effect of nutritional factors on α-amylase production

Various carbon sources Different carbon sources (starch, sucrose, fructose, xylose, and maltose) at a concentration of 2.0% (*w*/*v*) were individually tested in the basal medium at optimal temperature, incubation period, and pH to observe the effect on enzyme synthesis by *Bacillus* sp. NRC22017. Out of these carbon sources, maltose was found to be the best for amylase production (13.74 ± 0.02 U/ml) within 72 h (Fig. 5a). Even though the maximum activity of α-amylase enzyme was observed in the existence of maltose as a carbon source, starch is used for supplementation in the production process, because it works as an inexpensive source as compared with maltose. From the present findings, it was evident that the enhancement of α-amylase needs substrates with α-1,4 glycosidic bonds, including starch and maltose.

The influence of starch concentration as a carbon source on α-amylase production from *Bacillus* sp. NRC22017 was clarified in Fig. 5b. The amount of α-amylase increase until it reaches the highest level at a starch concentration of 2% (*w*/*v*) (12.42 ± 0.06 U/ml), then it starts to decrease.

Various nitrogen sources The various organic nitrogen sources impact on bacterial growth and α-amylase production from *Bacillus* sp. NRC22017 was shown in Fig. 5c. Supplementation of yeast extract plus meat extract to the fermentation medium gave the highest bacterial growth and enzyme productivity (14.17 ± 0.20 U/ml).

Figure 5d demonstrated that *Bacillus* sp. NRC22017 gave the highest α-amylase productivity at 1.05% meat plus yeast extract (15.15 ± 0.47 U/ml). Also, from the obtained data, it was obvious that bacterial growth increase with increasing nitrogen source concentration and the highest cell dry weight was obtained at the highest nitrogen source concentration (1.4%) (4.67 ± 0.18 g/l), but α-amylase synthesis decreased with increasing nitrogen source concentration above the optimum concentration.

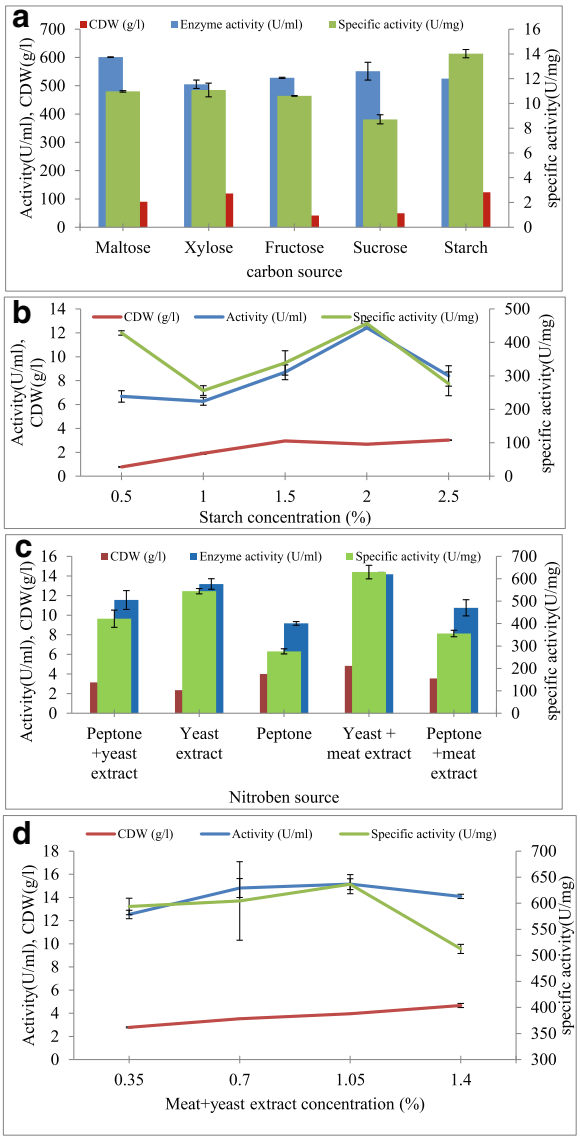

Fig. 5 Effect of different carbon sources (**a**), different concentrations of starch (**b**), different nitrogen sources (**c**), and different concentrations of yeast extract plus meat extract (**d**) on α-amylase production by *Bacillus* sp. NRC22017. Data were expressed as mean ± standard error

Discussion

The initial screening of *Bacillus* sp. NRC22017 strain revealed zones of hydrolysis on starch agar plates. This clear zone indicated that NRC22017 α-amylase can hydrolyze starch.

In the present study, we observed 72 h as the optimum growth period for the presently reported *Bacillus* strain and longer incubation period supported less number of colonies and lower enzyme activity. A prolonged incubation period extremely reduced α-amylase possibly due to the exhaustion of nutrients, death of microorganisms, gathering of byproducts in the medium

such as toxins, inhibitors, proteolysis of α-amylase by proteases enzymes, and also the cells may attained the decline phase and showed diminished amylase synthesis (Teodoro and Martins 2000; Aiyer 2005). These findings were closely similar to the results of *B. licheniformis* ATCC 12759 (Nurullah 2011) and *Bacillus* isolates (Kanimozhi et al. 2014). While, Kaur and Vyas (2012), Deb et al. (2013), Singh et al. (2016), and Paul et al. (2017) found that an extended period of incubation beyond 48 h did not increase the enzyme production from *Bacillus* sp. DLB, *B. amyloliquefaciens* P-001, *Bacillus* sp. strain B-10, and *Bacillus* sp. MB6, respectively. These results were in contradiction with our results in the present study.

Concerning inoculum size, this study proposed that α-amylase output from *Bacillus* sp. strain depends on the growth. Our results are almost in harmony with literature for enzyme production from different strains concerning inoculum size. The size of inoculum plays a notable role in the fermentation rate. It is noteworthy that there is no precise bacterial inoculum volume suitable for amylase production. It can vary from 0.5% for *B. amyloliquefaciens* (Haq et al. 2010) to 2.95% (Zambare 2011) for *Bacillus* sp. and 8% for *B. cereus* (Sivakumar et al. 2012).

Temperature is a highly sensitive parameter for α-amylase productivity and so it needs to be controlled and this is usually varied from organism to another (Sivakumar et al. 2011). Temperature can affect an enzyme in two ways. One is a direct influence on the reaction rate constant, and the other is in thermal denaturation of the enzyme at elevated temperatures (Demirkan et al. 2017). Similarly, Vijayaraghavan et al. (2015) and Asad et al. (2011) obtained maximum amylase secretion from *Bacillus cereus* IND4 and *Bacillus* sp. WA21 at 45 °C on starch agar medium, whereas Hasan et al. (2017) reported maximum amylase production was at moderate temperature (37 °C) for both *Chryseobacterium* and *Bacillus* isolates.

During fermentation, the aeration rate indirectly indicates the dissolved oxygen level in the fermentation broth. Yegneswaran and Gray (1991) reported that a high concentration of oxygen had an influencing effect on microbial growth and enzyme production. The biosynthesis of α-amylase was diminished with the increase in the broth volume; this behavior might probably because of a reduction in air supply as a result of the drooping in the agitation rate of medium that happened with a high volume of fermentation medium (Narang and Satyanarayana 2001). Riaz et al. (2003) and Dash et al. (2015) conveyed that the maximum yield of α-amylase from *B. subtilis* GCBUCM-25 and *B. subtilis* BI19 was gained at 25 and 50 ml of enzyme production medium in 250 ml Erlenmeyer flask respectively.

Different enzymes have different optimum pH values. This is the pH value at which the bonds within them are affected by H^+ and OH^- ions in a way that the shape of the active site is the most complementary to the shape of the substrate. At the ideal pH, the rate of reaction is optimum. Any alteration in pH above or below the optimum will quickly cause a decline in the reaction rate, since more of the enzyme molecules will have active sites whose shapes are not (or at least are less) complementary to the shape of their substrate (Demirkan et al. 2017). Similar result has been recorded for *Bacillus* sp. and *Brevibacillus borstelensis* R1 (Singh et al. 2012, Suribabu et al. 2014). The production optimization studies showed that maximum enzyme production by *Penicillium notatum* IBGE 03 was obtained at pH 5.5 (Ahmed et al. 2015). In neutral conditions (pH 6.5–7.5), the α-amylase production was reported in *Bacillus* sp. (Asgher et al. 2007), and *Bacillus* sp. NRC12017 (Elkady et al. 2017). In alkaline conditions (pH 7.5–11.0), the α-amylase production was reported in *Bacillus* sp. (Saxena et al. 2007). It was stated that at high pH, the metabolic action of bacterium may be suppressed and thus it inhibits the enzyme production (Ellaiah et al. 2002).

Regarding the effect of different carbon sources on alpha amylase production by *Bacillus* sp. NRC22017, results obtained were in agreement with results of NandLal et al. (2017) in *Bacillus licheniformis* JAR-26; Thippeswamy et al. (2006) in *Bacillus* species (B_3) where highest amylase production (0.464 U/ml) was induced by maltose. Suribabu et al. (2014) also found maltose better than other carbon sources examined for α-amylase production with *Brevibacillus borostelensis* R1 under submerged fermentation. Whereas Elkady et al. (2017) and Gangadharan et al. (2006) reported that *Bacillus* sp. NRC12017 and *Bacillus amyloliquefaciens* gave the highest enzyme yield with soluble starch, followed by maltose. *Bacillus* sp. NRC22017 could grow and produce α-amylase in the presence or absence of starch from the fermentation medium. Sucrose, fructose, and starch have almost similar effect on α-amylase synthesis from *Bacillus* sp. NRC22017 (12.61 ± 0.72, 12.07 ± 0.05, and 12.01 ± 0.17 U/ml), respectively.

There was a decrease in enzyme production at excessive starch concentration and this might be imputed to the rapid consumption of starch leading to the release of toxic metabolic wastes which suppress the growth of bacteria and α-amylase production. Also, high starch concentrations caused the broth culture to be more viscous, thus interfering with O_2 transfer resulting in restriction of dissolved O_2 required for the microbial growth. Similarly, Mishra and Behera (2008) found that raising the starch concentration increased both growth and α-amylase production by *Bacillus* strain from kitchen wastes and the maximum yield of the enzyme was reached at a starch concentration of 2%. While

Tiwari et al. (2014) informed that 4% soluble starch concentration was the best for amylase production by *B. tequilensis* RG-01 and above this concentration enzyme production was slightly decreased tested.

Nitrogen source is another essential nutrient that is required by the microorganisms in comparatively larger amounts. Nitrogen source type and relative concentration in the growth medium are important for both microbial growth and amylase production (NandLal et al. 2016). The nitrogen is metabolized to produce primarily amino acids, nucleic acids, protein, enzymes, and other cellular components that play a vital role in metabolism. The decrease in α-amylase production at excess nitrogen levels could be due to the pH depression or the stimulation of protease enzyme, which repress the amylolytic activity (Tonkova 2006). Both lower and higher nitrogen sources levels in the production broth are equally detrimental and cause inhibition of the enzyme (Sharma et al. 2012).

Conclusion

In this study, a combination of physical and chemical parameters was employed to maximize α-amylase synthesis. By optimizing the incubation conditions of α-amylase production from *Bacillus* sp. NRC22017 was enhanced with 37.63% enzyme yield as compared to control. Enzyme activities in modified medium and control medium were 15.15 ± 0.47 U/ml and 11.02 ± 0.17 U/ml, respectively. The results obtained in this study illustrated that the optimization of culture conditions played a pivotal role in influencing output through the fermentation bioprocess. Screening of microorganisms with higher α-amylase activities could therefore facilitate the discovery of novel amylases suitable for new industrial applications. Purification and characterization of the enzyme are in progress.

Materials and methods

Sample collection

Samples were collected from water and sediment of marine and salterns at different locations for isolation of bacteria. The samples were taken from Rashid, Sidi Bisher beach at Alexandria, Tiba rose village (El Sāhel Al-shamali), Hurghada, mangrove tree around the rhizosphere area (Marsa Alam), Safaga, El-Ain Elsokhna beach, and Wadi El-natron. Samples were collected in sterile bottles and brought to the lab, stored in the refrigerator at 4 °C until it was used.

Isolation and purification of α-amylase producing bacteria

Sample was suspended into 90 ml sterilized saline solution (0.85%, NaCl) and diluted using the dilution method (Hayakawa and Nonomura 1987). Then, 100 μl of diluted sample (10^{-4}–10^{-6}) concentrations was placed on the starch agar plate and spreads with a sterile L-shaped glass rod (Kanimozhi et al. 2014). The plate was incubated at 50 °C for 24 and 48 h, and single colonies of different sizes were selected. The colonies were subjected to purification to obtain single pure colonies.

Screening of isolated samples for α-amylase production

All isolates were curried using the starch plate (Moller et al. 2004). Bacteria were inoculated on starch plates to test for α-amylase secretion, incubated at 50 °C for 3 days, and stains with an iodine solution (0.5%). Amylase-positive stains were determined by the presence of a clear zone of starch hydrolysis around the colony on the starch plates, while presence of blue color around the growth indicated negative result (Hollo and Szeiti 1968). The bacterial isolates which produced a clear zone of hydrolysis in starch agar were selected as α-amylase producers for subsequent investigation. Selected colonies were maintained on nutrient agar slants at 4 °C and sub-cultured monthly.

Identification of potent amylase producer

Biochemical, morphological, and physiological characteristics of the potential producer (isolate number 16) was determined by adopting standard methods (Bergey and Holt 1994). The identification was confirmed with phylogenetic analysis. Briefly, genomic DNA of bacteria was extracted and universal primer 5′-TCCGTAGGT GAACTTTGCGG-3′ and 5′-TCCTCCGCTTATTGATA TGC-3′ primers was used for the amplification of DNA (Gardes and Bruns 1993). A single discrete PCR amplicon band was observed when resolved on agarose gel. The PCR amplicon was purified to remove contaminants. Sequencing products were resolved on an Applied Bio-systems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). Data were submitted to GenBank database. The DNA sequence was compared to the GenBank database in the national Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genbank/) using the BLAST program (Tamura et al. 2011). The sequencing was performed by Lab Technology Company.

Growth conditions for α-amylase production

To choose a proper culture medium for growth of the isolated strain and α-amylase production, primarily five different fermentation media were examined: Medium 1 (g/l): Na_2HPO_4 6.0, KH_2PO_4 3.0, NaCl 0.5, $MgSO_4$ 0.24, $CaCl_2$ 0.01, Peptone 3.0, Starch 10.0 (Burhan et al. 2003). Medium 2 (g/l): Starch 10.0, Yeast extract 2.0, Peptone 5.0, $MgSO_4$ 0.5, NaCl 0.5, $CaCl_2$ 0.15 (Swain et al. 2006). Medium 3 (g/l): Starch 20.0, Peptone 0.5, $MgSO_4.7H_2O$ 0.5, NaH_2PO_4 0.1, $(NH_4)_2SO_4$ 0.1, KCl 0.1 (Mishra and Behera 2008). Medium 4 (g/l): Peptone

10.0, Starch 5.0, Beef extract 10.0 (Amoozegar et al. 2003). Medium 5 (g/l): Starch 20, Peptone 10, Yeast extract 4, $MgSO_4$ 0.5, $CaCl_2$ 0.2 (Kanimozhi et al. 2014). The pH of the media was adjusted to 7 with 0.1N HCl and 0.1N NaOH. The media were sterilized and Erlenmeyer flasks of 100 ml capacity containing 20 ml of culture medium were inoculated with 1 ml of previously prepared inoculum and incubated at 50 °C in a rotary shaker at 140 rpm for 72 h. The samples were harvested after 72 h and the cells were separated by centrifugation (5000 rpm for 15 min at 4 °C) in centrifuge (SIGMA 3-18 KS). The cell dry weight (CDW) of culture broth was measured by harvesting the cells after centrifugation and drying them at 105 °C to a constant weight.

Enzyme assay

α-Amylase activity was determined by measuring the reduction in blue color intensity resulting from enzyme hydrolysis of starch (Palanivelu 2001). The reaction mixture consisted of 200 μl cell-free supernatant, 250 μl of soluble starch (1% *w*/*v*), and 500 μl phosphate buffer (0.2 M pH 7) incubated at 50 °C for 30 min. The reaction was stopped by adding 250 μl of 0.1 N HCl and color was developed by adding 250 μl of iodine solution. The optical density (OD) of the blue color solution was determined at 660 nm using (JASCO V-630) spectrophotometer. One unit (U) of enzyme activity is defined as the amount of enzyme that hydrolyzes 0.5 mg of starch per minute under assay optimum conditions. Specific activity was expressed as units of enzyme activity per mg of protein. All measurements were performed three times. The data were expressed as mean ± standard error of the mean.

Protein determination

Protein content was estimated by Bradford's method (Bradford 1976) using bovine serum albumin as standard.

Factors affecting α-amylase production from *Bacillus* sp. NRC22017

Flask cultures were performed in 100 ml Erlenmeyer flasks containing 20 ml of the medium 5. Factors affecting cell growth and α-amylase production were investigated using one factor at a time method. The optimized parameters were incubation time (1, 2, 3, 4, and 5 days) at 50 °C under shaking conditions at 140 rpm, inoculum size (100, 200, 300, 400, 500, 600, and 700 μl), medium volume (10, 15, 20, and 25 ml), temperature (40, 45, 50, and 55 °C), and initial pH of the medium (5.5, 6.0, 6.5, 7.0, 7.5, and 8.0). Studies were also performed to evaluate the influence of different carbon sources (maltose, sucrose, xylose, fructose, and starch 2% *w*/*v*) and different concentrations of starch (0.5, 1.0, 1.5, 2.0, and 2.5% *w*/*v*). Also different nitrogen sources were used (peptone, yeast extract, peptone plus yeast extract, yeast plus meat extract, and peptone plus meat extract 1.4% *w*/*v*) at different concentrations (0.35, 0.70, 1.05, and 1.40% *w*/*v*) of meat extract plus yeast extract were tested to get the best one for α-amylase production. The enzyme activity and protein concentration were determined.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Authors' contributions
This research was extracted from MSc thesis. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable (this study does not involve human participants, human data, or human tissue)

Consent for publication
Not applicable.

Competing interests
Purification and characterization of the enzyme are in progress.

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