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Isolation of Thermoalkalophilic-α-amylase Producing Bacteria and Optimization of Potato Waste Water Medium for Enhancement of α-amylase Production

Sulaiman A. Alrumman[1*], Yasser S. Mostafa[1], Saleh A. Eifan[2], Saad A. Alamri[1], Abd El-Latif Hesham[1,3]

1. Department of Biology, College of Science, King Khalid University, P.O. Box 9004, Abha 61321, Saudi Arabia
2. Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
3. Department of Genetics, College of Agriculture, Assiut University, Assiut 71516, Egypt

* E-mail of the corresponding author: salrumman@kku.edu.sa

ABSTRACT

Sixty one thermoalkalophilic bacteria were isolated from soil samples in Saudi Arabia's southern region. Isolate TA-38, obtained from the Tanomah region, showed the best performance for enzyme production and was submitted for further study. It was identified as *Bacillus axarquiensis* based on 16S rRNA gene sequencing studies. The feasibility of using potato waste water as a simple and cheap medium for the production of α-amylase was evaluated compared with starch broth medium. The production of α-amylase in the potato waste water medium was only 13.8% less than that of the starch medium. Maximum enzyme production was achieved after 48 hours of cultivation at the beginning of the stationary phase at pH 10.0 and 50 ^{0}C. The appropriate addition of starch; nitrogen; phosphate; and calcium to potato waste water significantly enhanced the production of α-amylase. The enzyme production reached a maximum of 64.5 Uml-1 with the potato wastewater adding with 0.5 % starch; 0.4 % yeast extract; 0.04% $CaCl_2$-$2H_2O$ and 0.05 % KH_2PO_4. The optimization of the potato waste water medium led to an approximately 4.02 fold increase in the production of α-amylase compared to starch broth medium. Data indicated that the potato waste water contained substrates which could be used by bacterial isolate for the production of α-amylase production and the developed procedure was cost effective since it requires only a slightly addition of nutrients to the medium.

Keywords: Isolation; α-amylase; 16S rRNA; Production; Potato waste water; Thermoalkaliphilic bacteria.

1. Introduction

Amylases have potential biotechnological applications in a wide range of industrial processes and account for nearly 30% of the world's enzyme market (Rajagopalan and Krishnan, 2008). α-amylase (1,4-α-D-glucan glucanohydrolase; EC3.2.1.1) is the endo-acting enzyme which hydrolyze starch by cleaving α-1,4-glucosidic linkages (Tester *et al*., 2004). These have been used widely in food; paper; detergent; textiles; and in the pharmaceutical industries (Acourene and Ammouche, 2010). Each application, of α-amylase, requires unique properties with regard to specificity and thermal stability (Konsula and liakopoulou-Kyriakides, 2006). Irrespective of the wide range of applications, one major limitation, of the amylases, is their ineffectiveness in the detergent industry (Khan and Briscoe, 2011). Alkaline α-amylases have high alkaline pH catalytic efficiency and stability which ranges from 9 to 11 and, it has potential applications in the starch, textile and detergents industries (Yang *et al*., 2011). The use of enzymes, in formulation of detergents, enhanced the detergents' ability to remove tough stains and to make the detergent environmentally safe. Amylases are the second type of enzymes used in the formulation of enzymatic detergent, and 90% of all liquid detergents contain these enzymes (Hmidet *et al*., 2009). Because of the industrial importance of amylases, there is on-going interest in the isolation of new thermo-alkaline bacterial strains producing amylases (Vaseekaran *et al*., 2010). The α-amylase is produced by a wide variety of microorganisms; however, the best commercial producers were *Bacillus licheniformis*; *Bacillus stearothermophilus* and *Bacillus amyloliquefaciens* (Goyal *et al*., 2005; Mitidieri *et al*., 2006). The main reasons for selecting enzymes, from alkaliphiles, were their stability in the presence of detergent additives such as bleach activators; softeners; and perfumes. In order to meet the industries' demands, a low-cost medium was required for the production of α-amylase. There was considerable interest in improving the productivity and product economy of α-amylase by using residues and by-products as production media (Hassan and Karim, 2012). The production of potato chips and frozen French fries entailed the use of high volumes of water for unit operations such as washing; peeling; trimming; slicing; blanching and cooking. This leaves enormous amounts of starch-rich wastes which could be detrimental to the quality of the environment (Azab, 2008). Because potato processing waste water contained high concentrations of starch; protein and total suspended solids, it could be used as substrates for the production of useful biomaterials (Hung *et al*., 2004; Jin *et al*., 2005). This study was designed to isolate thermoalkalophilic-α-amylase producing bacteria from the soil

sample of starchy plants and from the cement industry in Saudi Arabia's southern region, and to optimize the production of α-amylase using potato waste water as an economical medium.

2. Material and Methods

2.1. Potato waste water

Potato waste water, used in this investigation, was collected from Saudi Snack Foods Company Ltd, Jeddah, Saudi Arabia. The chemical analyses of waste water were performed according to Evers *et al* (1984) and Van Koningsveld *et al.*, (2002). The potato waste water was valuable waste, it contain organic compounds as nitrogen (0.27%) and starch (1.7%). Total ash was also determined by dry evaporation and dry mineralization at 600^{o}C for 3 hours, it reached to about 0.22%.

2.2. Isolation and screening of thermoalkalophilic α-amylase producing bacteria

Various soil samples were collected from the rhizosphere of starchy plants and from the cement industry in Saudi Arabia's southern region. The isolation of alkaliphilic bacteria was achieved particularly through the addition of sterile Na_2CO_3 to autoclaved media in order to obtain a pH in excess of 9.5 to 10. The medium was composed of (g l^{-1}): yeast extract 5.0; peptone 5.0; potato starch 10.0; K_2HPO_4 1.0; $MgSO_4$ $7H_2O$ 0.20; and agar 20.0 (pH 10.0). The plates were incubated at 50 ^{O}C and examined after 24 to 48 hours. The α-amylase producing colonies were selected by flooding the plates with iodine solution (1% iodine in 2% potassium iodide w/v). The clearance zone-forming ability on starch agar plates was used for the primary selection of isolates (Kaur and Vyas, 2012). The bacterial isolates were screened for their α-amylase productivity after 48h in the starch broth medium using an incubator shaker at 50 ^{O}C and 200 rpm. The cultures were maintained on nutrient agar slants at 4 ^{O}C.

2.3. DNA extraction, 16S RNA gene amplification, sequences and phylogenetic analysis

The extraction of total bacterial genomic DNA was performed according to procedures described by Hesham *et al*., (20006). Molecular genetics identification of the bacterial isolate was performed by amplification of 16S rRNA gene with bacterial universal primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-CGGCTACCTTGTTACGACTT-3) as described by Lane (1991). PCR reaction was carried out in a final volume of 50 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, each dNTP at a concentration of 0.2 mM, 1.25 IU of Taq polymerase, each primer at a concentration of 0.2 mM, and 1 µl of the DNA template PCR was performed with the following program: 5 min denaturation at 95°C, followed by 36 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1.5 min extension at 72°C, and a final extension step of 7 min at 72°C. Five µl of the amplified mixture was then analyzed using 1.5% 0.5 TBE agarose gel electrophoresis. The gel was stained with ethidium bromide, visualized under UV light, and photographed. PCR product sequenced in both directions using an ABI 3730 automated sequencer. 16S rDNA sequences obtained were then aligned with known 16S rDNA sequences in Gen bank database using BLAST search. A phylogenetic tree was constructed with MEGA version 4.0 using a neighbour-joining algorithm, plus the Jukes-Cantor distance estimation method with bootstrap analyses for 1,000 replicates was performed.

2.4. Enzyme production and optimization of culture conditions

Firstly, the enzyme production by promising strain was evaluated using starch broth as synthetic medium and potato wastewater without supplementation. The inoculum was prepared by growing the bacterial culture, in the nutrient broth medium, at 50 ^{O}C for 24 hours. Erlenmeyer flasks (250 ml) containing 50 ml of starch broth medium[17] or potato wastewater medium were inoculated with 2% inoculum (approximately 2 x 10^8 CFU/ml) and incubated at 50 ^{O}C in an incubator shaker at 200 rpm for 12 to 96 hours. At regular (12 hour) intervals, the triplicate samples were harvested and the obtained growth was measured as turbidity at 600 nm. Cells were removed by centrifugation (6000 rpm for 20 minutes) and supernatant was used for enzyme assay. The optimization of the potato waste water medium was investigated to enhance the production of α-amylase, the addition of starch; yeast extract; $CaCl_2 2H_2O$; KH_2PO_4 and $MgSO_4.7H_2O$; was optimized. All the experiments were conducted in triplicate and the data used in the figure are mean values of three experiments.

2.5. Alkaline α-amylase assay

α-amylase activity was assayed by adding 0.5 ml of supernatant to 0.5 ml soluble starch (1%) in a 100 mM glycine-NaOH buffer, pH 10.0, and incubating at 50ºC for 30 minutes. The reaction was stopped by the addition of 1ml of 3,5-dinitrosalicylic acid reagent. The absorbance was measured using a double beam UV/Vis scanning spectrophotometer (Model: Shimadzu, 1601PC) at 550 nm. One unit of enzyme activity was defined, as the amount of enzyme releasing1 µmol glucose per minute, under the standard assay conditions (Aygan *et al*., 2008).

2.6. Statistical analysis

Analyses of data were done by analysis of variance (ANOVA) using the MSTATC program. The Least Significant Difference (*LSD*) at $P \leq 0.05$ was used to detect differences among treatments.

3. Results and Discussions

3.1. Isolation and screening of thermoalkalophilic α-amylase producing bacteria

Sixty one thermoalkalophilic bacteria were isolated from rhizosphere of starchy plants and from the cement industry in Saudi Arabia's southern region, as one of the most important sources for isolation of these bacteria (Aygan *et al.*, 2008; Nielsen *et al.*, 1995). The results indicated clearly that the highest number of thermoalkalophilic bacteria were isolated from soil samples in the Jazan (17), Najran (12) and Tanomah (7) regions whilst low number of isolates were collected from the soil samples of Khamis Mushayt (3); Rijal almaa (5); Mahail (6); Bellasmar (6); and Abha (5). The results indicated also that no isolates were detected in water samples collected from hot springs in the Jazan and Rijal almaa regions. The occurrence of isolates in the different localities might be affected by the prevailing environmental conditions and the nature of the soil. The clearance zone-forming ability was used for the primary selection of amylolytic isolates using an iodine test. Forty-seven isolates, showed positive results with a clear zone. Of these, the regional results were as follows: Jazan (12); Najran (9); Bellasmar (5); Rijal almaa (4); Tanomah (7); Mahail (4); Abha (3); and Khamis Mushayt (2). Only ten isolates (21.27 %) were found to produce a high clearance zone, as a result of starch hydrolysis. Highest amylolytic ability was detected by isolate TA38 which obtained from Tanomah region. Mishra *et al.*, (2008) and Cordeiro *et al.*, (2002) used the same method for the screening of amylolytic bacteria. Thermoalkalophiles were adapted to survive in alkaline and hot regions. These bacteria produced unique biocatalysts which functioned under extreme conditions comparable to those prevailing in various industrial processes as reported by Haki and Rakslit (2003). The production of α-amylase by promising thermoalkalophilic isolates were screened quantitatively using starch broth medium. The results mentioned in Fig. 1, showed a broad range of enzyme productivity amongst the tested isolates. The strain TA-38 gave the highest amylase production (12.3 Uml^{-1}); therefore, it was selected for further studies.

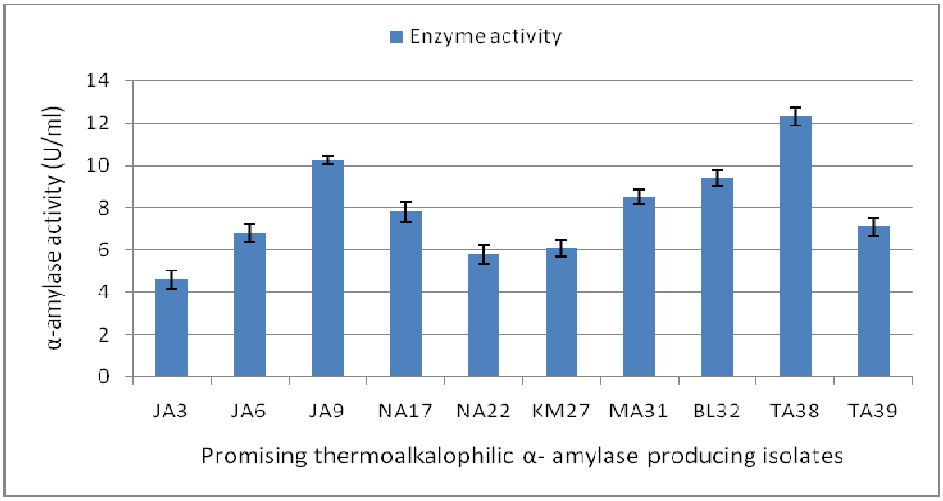

Figure 1. Screening of promising isolates for thermoalkaline production of α-amylase. (Isolates JA were obtained from Jazan region, isolates NA from Najran region, isolates KM from Khamis Mushayt region, isolates MA from Mahail region, isolates BL from Belasmar region and isolates TA from Tanomah region). Bars indicate standard errors of the mean (n=3). (*LSD*, 1.714143).

3.2. Molecular identification of α-amylase producing Bacteria

For molecular identification, genomic DNA was extracted from the isolated bacterial strain TA38, and universal primers 27F and 1492R were used for the amplification and sequencing of the 16S rRNA gene fragment. An almost complete sequence (about 1.5 kb) of 16S rRNA gene was obtained for the Bacillus strain ASU7 and compared with the sequences of 16S rRNA regions in GenBank database by means of BLAST search. Results show that the 16S rRNA sequence of the TA38 isolated strain was highly homologous to *Bacillus axarquiensis*, (Strain, Alrumma-001) with 99 % sequence similarity. To confirm the position of the strain Alrumma-001 in phylogeny, a number of sequences representative some *Bacillus* sp. were selected from Gen bank database for the construction of a phylogenetic tree. As shown in Fig. 2, the phylogenetic tree indicated that strain Alrumma-001and *B. axarquiensis* shared one clade cluster. Therefore, the strain Alrumma-001was identified as *B. axarquiensis*. The 16S ribosomal DNA sequence of strain Alrumman-001 reported in this study has been

deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under Accession No. KC904794. Recently, Bacillus sp. was identified based on 16S rRNA gene sequencing and phylogenetic analysis (Wei *et al*., 2011; He *et al*., 2012; Hesham *et al*., 2012).

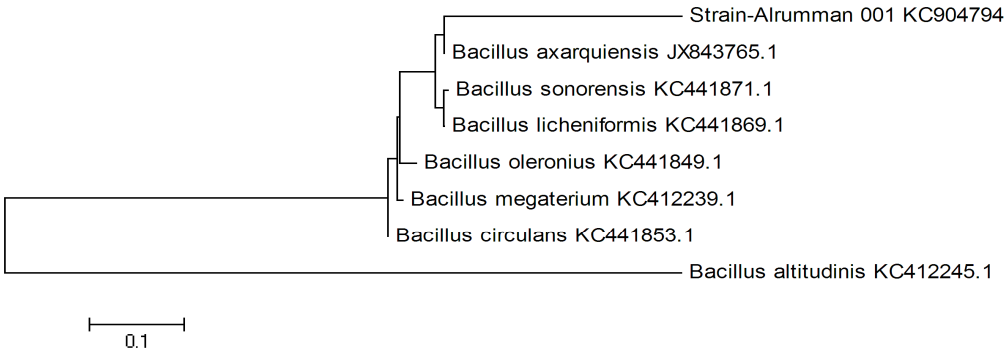

Figure 2. Phylogenetic tree for *Bacillus axarquiensis* strain Alrumman 001 and related species constructed by the neighbor-joining method based on 16rRNA gene sequences. Segments corresponding to an evolutionary distance of 0.1 are shown with bars. Accession numbers for sequences are as shown in the phylogenetic tree.

3.3. Effect of time course on production of α-amylase using starch broth medium and potato waste water as cheap medium

In order to understand the potential of potato wastewater as a growth and production medium for *Bacillus axarquiensis*, α-amylase production on potato waste water was compared with that of the starch broth medium (Fig. 3). The results indicated that there was little difference in α-amylase production between the two media. The enzyme production in potato waste water medium was only 13.8 % less than that of starch medium. These different patterns of enzyme production and cell growth were probably caused by the different characteristics of the carbon sources available in the two media. Cellular growth and α-amylase production were affected by the time of incubation and the growth dependent α-amylase production was observed by this strain. It was observed that maximum α-amylase production occurred when the bacterial growth reached to its maximum, this suggests that this organism may be sensitive to metabolite repression (Cordeiro *et al*., 2002). In the starch broth medium, maximum α-amylase production (16.01 Uml^{-1}) was obtained after 36 hours of cultivation whilst, in the potato waste water medium, optimum enzyme production was achieved after 48 hours of cultivation. In the active growth phase, the enzyme was secreted early and reached its maximum toward the beginning of the stationary phase. Extension beyond the optimum time course was generally accompanied by a decrease in the growth rate and enzyme productivity. The decreased activity in the later phase of growth was probably due to catabolite repression by glucose released from starch hydrolysis and proteolysis of α-amylase. Similar observations were reported by Krishna and Chandrasekaran (1996) and Kaur and Vyas (2012). They reported that the effective production of α-amylase may not occur until the stationary phase has been reached and the readily available carbon source has been depleted. The short incubation period for *Bacillus* sp. compared with other bacteria and fungi offers the potential for inexpensive enzyme production. Furthermore, Barampouti *et al* (2005) and Huang *et al*., (2003) found that the potato waste water was rich in biodegradable materials such as starch and proteins; this made them suitable for the production of bio-chemicals. In conclusion, the starch broth medium was expensive synthetic medium and after some supplementation for the reduction of the medium cost, it could be replaced by potato waste water as a cheap medium.

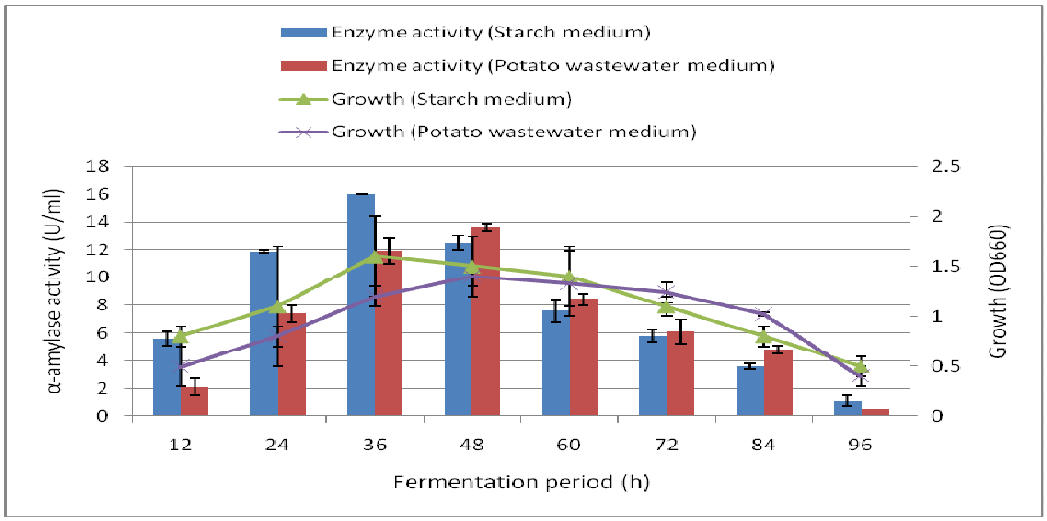

Figure 3. Production of α-amylase using starch broth and medium of potato waste water
(*LSD* (Starch medium): enzyme activity, 1.533139; growth, 1.05841)
(*LSD* (Potato waste water medium): enzyme activity 1.014196; growth, 0.549273)

3.4. Effect of initial pH on α-amylase production

The initial pH of the culture broth was one of the most critical environmental parameters affecting both growth and α-amylase production. The influence of initial pH on production of α-amylase was determined in the pH range of (6.0 – 13.0). The results presented in Fig. (4) showed that pH 10.0 was the most favourable for α-amylase production and bacterial growth. The pH dependent changes in the amount of enzyme production might have been due to pH control over the growth of bacteria or pH dependent control of amylase gene expression (Yang *et al*., 2011). Several investigators reported that the optimum pH for α-amylase productivity was at a broad value between 5.0 and 11.0 and it plays an important role by inducing enzyme production and morphological changes in the microbes (Burhan *et al*., 2003; Horikoshi 1971; Kaur and Vyas 2012).

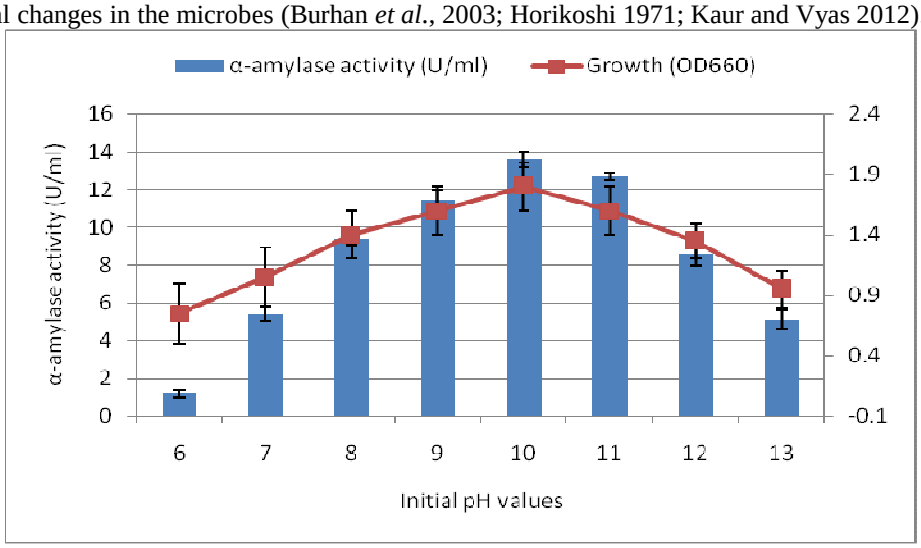

Figure 4. Effect of initial pH on production of α-amylase
(*LSD*: enzyme activity, 0.73971; growth, 0.16719)

3.5. Effect of incubation temperature on α-amylase production

The production of α-amylase was investigated in a temperature range of 35 to 65°C (Fig. 5). The results referred to a positive relationship between bacterial growth and enzyme production with incubation temperature up to 50 ^{O}C, and then gradual decrease was noticed. Many investigators have studied the correlation between α-amylase secretions with temperature which depends on the type of organism and culture conditions. Incubation temperature affects all the physiological activities in a living cell and it is an important environmental factor to control the growth, microbial activities, normal functioning of enzyme and many enzymes control the nutritional requirement of the cell and subsequently its composition (Kindle *et al*., 1986). Moreover, Saxena *et al*., (2007) had the

maximum enzyme production by Bacillus sp. at 60 ^{O}C, Konsula and Liakopoulou-Kyriakides, (2004) shows that a thermophilic Bacillus subtilis produced highest α-amylase production at 40 ^{O}C.

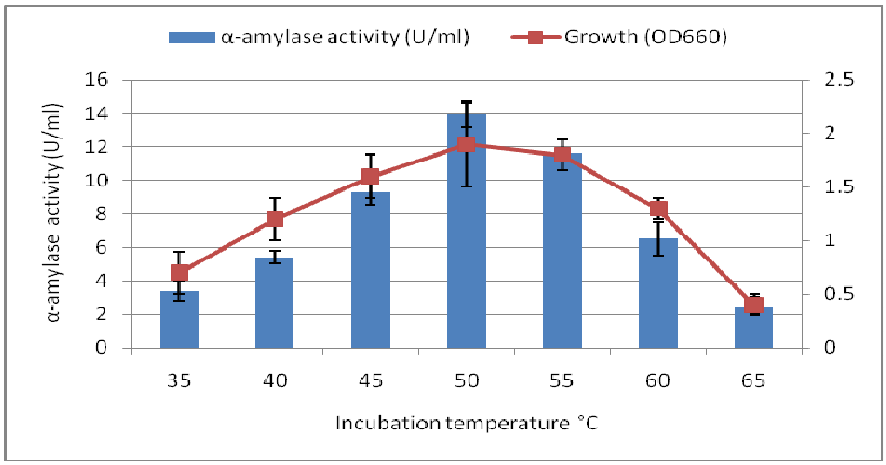

Figure 5. Effect of incubation temperature on production of α-amylase (*LSD*: enzyme activity, 1.69573; growth, 0.1829).

3.6. Optimization of potato waste water for production of α-amylase

The optimization and manipulation of the potato waste water medium was one of the most important techniques used for the over production of enzymes to meet industrial demands (Tanyildizi *et al*., 2005). To enhance the production of α-amylase using potato waste water, the microbial cultivation was established for 48h and the addition of nutrients was investigated.

3.6.1. Effect of starch concentration on production of α-amylase

Different concentrations of starch were used to elucidate the best concentration for maximum α-amylase production (Fig. 6). The results showed that 0.5 % starch concentration was optimum for production of α-amylase. This result might be explained by the potato waste water analysis which found that it had a large amount of starch (1.7 %). Further increasing the amount of starch was unfavourable for the production of α-amylase. This may be the effect of catabolic repression i.e. glucose which was formed during the hydrolysis of starch may influenced negatively on α-amylase gene expression (Haseltine *et al*., 1996). The declining productivity with high concentrations of starch could be also explained by the medium's high viscosity which affected the availability of oxygen needed in the growth of organisms (BOŽIĆ *et al*., 2011). These observations were accordance with finding of many investigators (Agger *et al*., 2001; El-Tayeb *et al*., 2000). Moreover, Mironescu (2011) reported that potato water was valuable waste, it contained organic compounds as proteins and starch which made it a good medium for fermentation processes. Furthermore, BOŽIĆ *et al*., (2011) found that the optimum production of α-amylase, by *Bacillus subtilis* IP 5832, was obtained with a starch concentration of 0.5%.

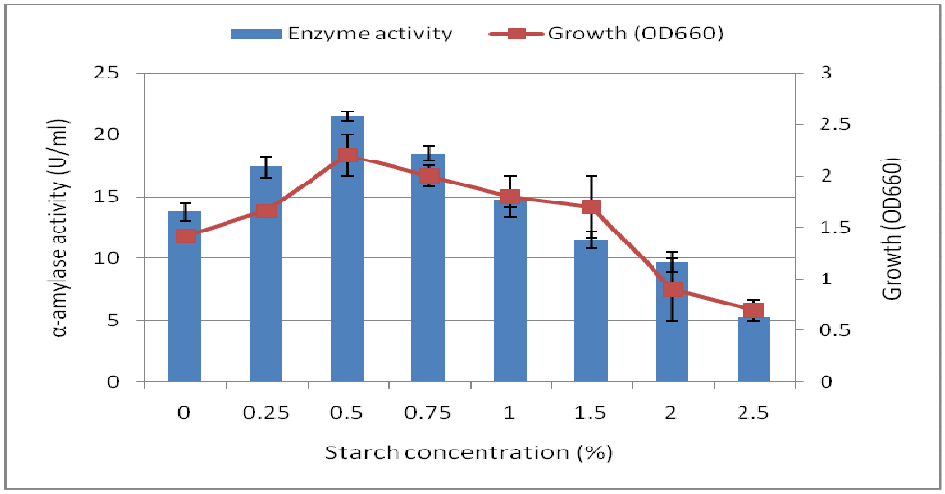

Figure 6. Effect of starch concentration on production of α-amylase (*LSD*: enzyme activity, 2.292743; growth, 0.653369).

3.6.2. Effect of yeast extract concentration on production of α-amylase

The concentration of nitrogen source is important for the formation of α-amylase. Yeast extract was found to be crucial for the production of α-amylase. The addition of yeast extract, at 0.4% to the potato waste water medium, increased significantly both growth and α-amylase synthesis (Fig. 7). This might be due to enhancing the microbial performances and the nature of potato waste water which contained about 0.27% nitrogen. Yuliani *et al*., (2011) reported that the potato waste water contained about 3.47 g l^{-1} total nitrogen and could be exploited for the production of industrial enzymes. The decline in α-amylase production at high yeast extract concentration could be explained by its effects on the surface charge; hydrophobicity; and the bacterial cell wall's nitrogen/carbon ratio as reported by Schar-Zammaretti *et al*., (2005). The hydrophobicity of the cell wall decreases as the concentration of yeast extract increase in the medium. This decreased hydrophobicity of bacterial cell wall may result in decreased extracellular release of enzyme. Furthermore, the high concentration of yeast decreased the pH of the medium during fermentation which ultimately destroys the enzyme produced in the medium (Babu and Satyanarayana, 1993). Many investigators reported that yeast extract was the best nitrogen source for production of α-amylase production. This was due probably to its high content in minerals; vitamins; amino acids; coenzymes; and nitrogen components (Riaz *et al*., 2009). Furthermore, Sivakumar *et al*., (2012) reported that maximum α-amylase production was observed using 5% yeast extract, whilst Saxena *et al*., (2007) reported that highly thermostable alkaline α-amylase, produced by *Bacillus* sp. PN5, was achieved at 0.3%. Moreover, in a recent study, Abdel- Fattah *et al*., (2013) reported that 0.75% yeast extract was found to be an important concentration for α-amylase synthesis by *Bacillus licheniformis*.

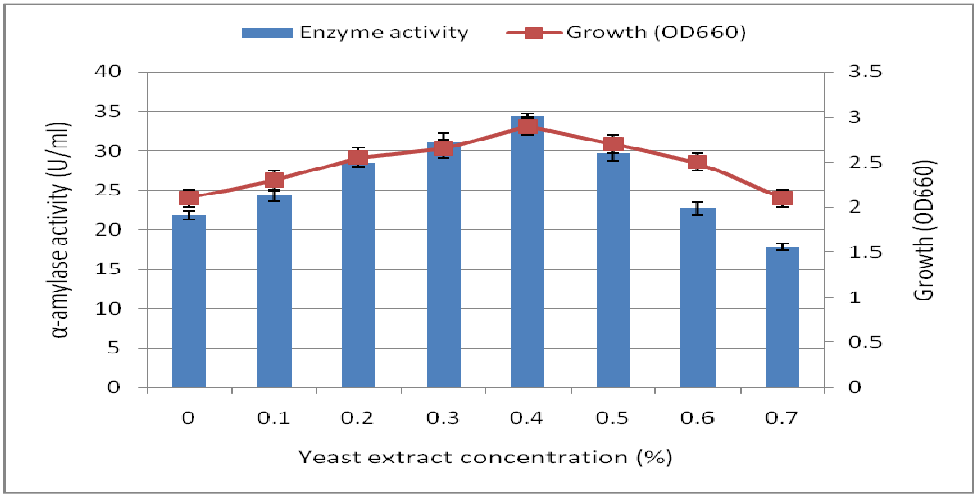

Figure 7. Effect of yeast extract concentration on production of α-amylase (*LSD*: enzyme activity, 2.52225; growth, 0.352538).

3.6.3. Effect of $CaCl_2$-$2H_2O$ on α-amylase production

The supplementation of potato waste water with 0.04 % $CaCl_2$-$2H_2O$, stimulated bacterial growth and enhanced production of α-amylase (Fig. 8). This low requirement for calcium ions suggests that thermostable α-amylase possesses high affinity for calcium ions (Kindle *et al*., 1986). The α-amylase was known to be a calcium metallo-enzyme having at least one calcium ion associated with its molecule. The enhanced bacterial growth and enzyme production might be the result of increased availability of calcium ions (Asgher *et al*., 2007). The results were in accordance with many investigators; Riaz *et al*., (2009) found that the addition, of 0.2 % $CaCl_2$ to the fermentation medium enhanced the production of α-amylase. Calcium ions are known to be the stabilizer and activator of α-amylase and it has been reported that requirement of Ca^{+2} are different for thermostable α-amylase as compared to thermolabile. In case of thermolabile α-amylase, Mamo and Gassesse (1999) reported that 0.1 g l^{-1} $CaCl_2$ was optimum for amylase production by Bacillus sp. WN 11 whereas Qader *et al*., (2006) reported that in case of Bacillus sp. AS-1, 0.2 g l-1 $CaCl_2$ was optimum for the maximum production of α-amylase. Furthermore, Kokab *et al*., (2003) reported that the addition, at 0.04%, of $CaCl_2.2H_2O$ to the fermentation media increased the enzyme production by *Bacillus subtilis*.

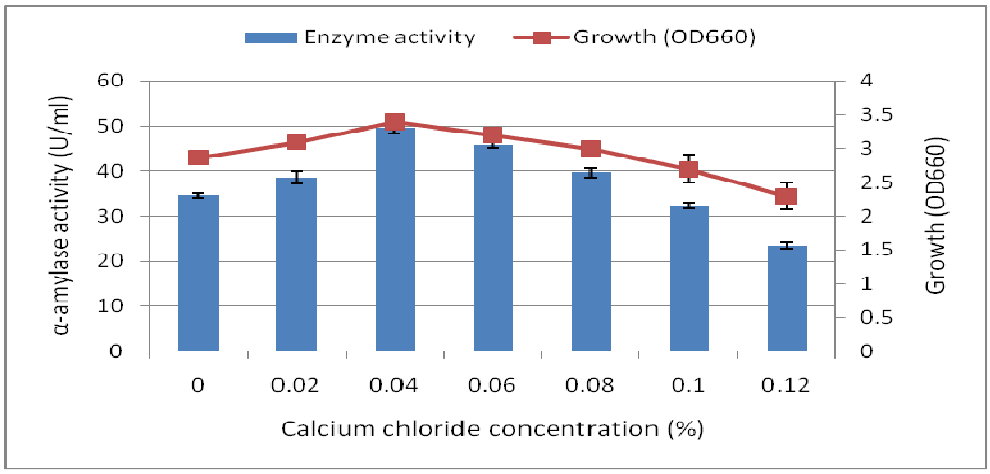

Figure 8. Effect of $CaCl_2.2H_2O$ concentration on production of α-amylase production (*LSD*: enzyme activity, 3.3236; growth, 0.455639).

3.6.4. Effect of KH_2PO_4 concentration on α-amylase production

The production of α-amylase using the medium of potato waste water was enhanced by addition of 0.05 % KH_2PO_4, and it was reduced by further additions of salt (Fig. 9). Yuliani *et al*., (2011) reported that the potato waste water contained about 0.52 g l^{-1} phosphorus; this made it a suitable medium for fermentation processes. Tanyildizi and Özer (2011) and Yoon *et al*., (1989) investigated the optimal phosphate level which maximizes α-amylase biosynthesis by *Bacillus amyloliquefaciens*. They observed that high concentration of phosphate level promotes maltose uptake and growth of the microbe, while high maltose uptake rate at the same time represses the enzyme biosynthesis presumably due to catabolite repression inside the microorganism. Many investigators recorded the important role of KH_2PO_4 in the production of α-amylase production, Kokab *et al*., (2003) reported that the maximum production of *Bacillus subtilis* α-amylase was observed using a medium containing 0.1% KH_2PO_4.

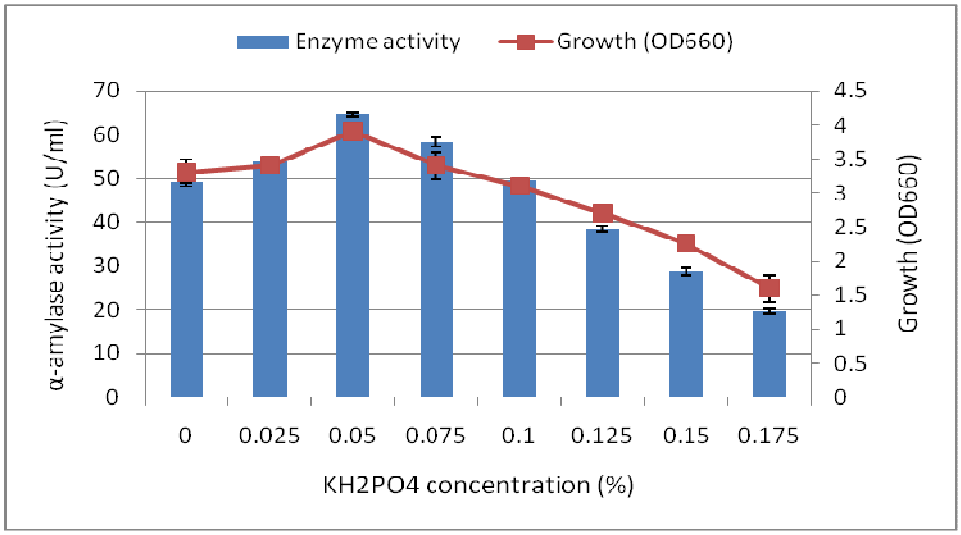

Figure 9. Effect of KH_2PO_4 concentration on production of α-amylase (*LSD*: enzyme activity, 2.336076; growth, 0.495976).

3.6.5. Effect of $MgSO_4 \cdot 7H_2O$ concentration on production of α-amylase

The results showed that, adding $MgSO_4.7H_2O$ to the medium had no significant effect on the production of α-amylase (Fig. 10). These results could be explained by the availability of magnesium salt in the potato waste water which covered the requirement for microbial growth and enzyme production. This was important in terms of the cost of producing enzymes Tanyildizi and Özer (2011). On the contrary, there were many reports indicating that $MgSO4.H_2O$ enhanced the bacterial production of α-amylase production (Goyal *et al*., 2005; Kokab *et al*., 2003). The interaction of α-amylase from *Bacillus amyloliquefaciens* with Mg^{2+} ion was studied by Saboury *et al*., (2005) They reported that α-amylase had eight identical and independent binding sites for Mg^{2+}

ion, which showed non-cooperatively in the binding process. The binding slightly destabilized the enzyme against thermal denaturation, as evident from absorption studies.

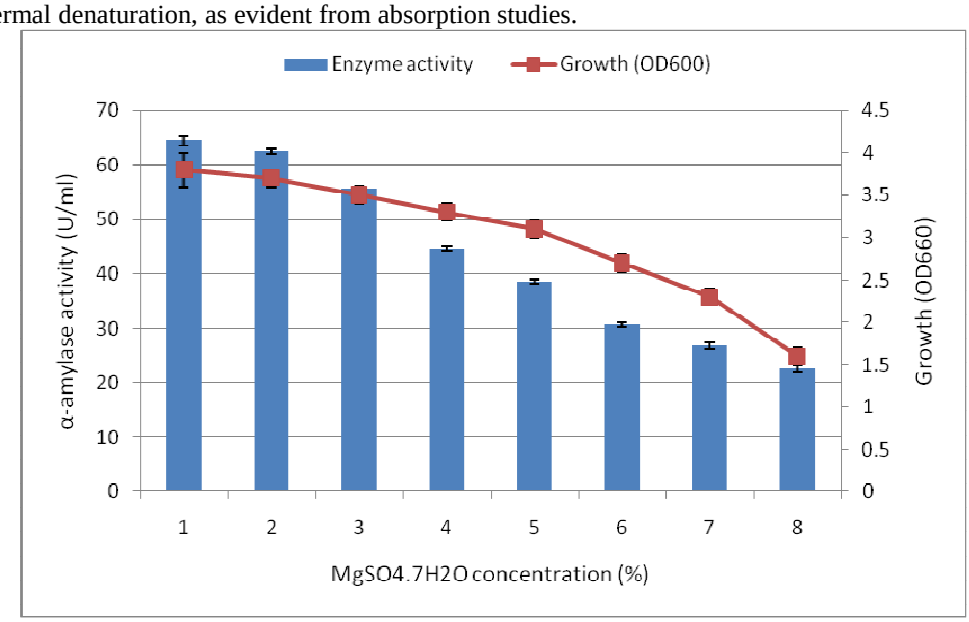

Figure 10. Effect of $MgSO_4.7H_2O$ concentration on production of α-amylase (LSD: enzyme activity, 2.021697; growth, 0.408066).

4. Conclusion

The potential application of α-amylase in various industries, especially detergents, and the need to develop a cost-effective medium for improved production, made the potato waste water, an excellent alternative in enhancing the production of α-amylase. The contents of starch broth, was very expensive and after some supplementation for the reduction of the medium cost, these could be replaced cheaply by the medium of potato waste water. The optimization of the potato waste water medium by addition of some nutrients led to an approximately 4.02 fold increase in the production of α-amylase compared to starch broth medium.

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References

Abdel-Fattah, Y. R., Soliman, Nadia, A., El-Toukhy, N. M., El-Gendi, H. and Ahmed, Rania S. (2013). Production, purification, and characterization of thermostable α-amylase produced by Bacillus licheniformis isolate AI20. Journal of Chemistry, 1-11.

Acourene S. and Ammouche A. (2010). Optimization of culture medium for baker's yeast, ethanol, citric acid and α-amylase production from dates syrup. Research Journal of Agriculture and Biological Sciences, 6(6): 846-860.

Agger, T., Spohr, A. B. and Nielsen, J. (2001). α-amylase production in high cell density submerged cultivation of Aspergillus oryza and A. nidulans. App. Microbiol. and Biotechnol., 55: 81-84.

Asgher, M., Javaid Asad, M., Rahman, S. U. and Legge, R. L. (2007). A thermostable α-amylase from a moderately thermophilic Bacillus subtilis strain for starch processing. Journal of Food Engineering, 79, 950-955.

Aygan, A., Arikan, B., Korkmaz, H., Dinçer, S. and Çolak, O. (2008). Highly thermostable and alkaline α-amylase from a halotolerant-alkaliphilic Bacillus sp. AB68. Braz. J. Microbiol., 39 (3), 547-553.

Azab, M. S. (2008). Waste-waste treatment technology and environmental management using sawdust bio-mixture. J. of Taiba University of Science, 1, 12-23.

Babu, KR. and Satyanarayana, T. (1993). Extracellular calcium inhibited α-amylase of Bacillus coagulans B49. Enzyme Microbial Technol., 15, 1066-1069.

Barampouti, EMP., Mai, ST. and Vlyssides, AG. (2005). Dynamic modelling of biogas production in an UASB reactor for potato processing wastewater treatment. Chem. Eng. J., 106, 53–58.

Božić, N., Ruiz, J., Lopez-Santin, J. and Vujcić, Z. (2011). Optimization of the growth and α-amylase production of Bacillus subtilis IP 5832 in shake flask and laboratory fermenter batch cultures. J. Serb. Chem. Soc., 76 (7), 965–972.

Burhan, A., Nisa, U., Gokhan, C., Omer, C., Ashabil, A. and Osman, G. (2003). Enzymatic properties of a novel thermophilic, alkaline and chelator resistant amylase from an alkalophilic Bacillus sp. Isolate ANT- 6". Proc. Biochem., 38: 1397-1403.

Cordeiro, CAM., Martins, MLL., Luciano, AB. (2002). Production and properties of α-amylase from thermophilic Bacillus sp. Braz. J. Microbiol. 33, 57-61.

El-Tayeb, O., Hashem, A., Mohammad, F. and Aboulwafa, M. (2000). Optimization of the industrial production of bacterial alpha amylase in Egypt I. Strain selection and improvement. Proceedings of the 10th microbiology conference. Cairo, Egypt, Nov.11-14, 719-438.

Evers, A. D., Baker, G. L., and Stevens, D. J. (1984). Production and measurement of starch damage in flour. Staerke, 36,309-312.

Goyal, N., Gupta, J. K. and Soni, S. K. (2005). A novel raw starch digesting thermostable α-amylase from Bacillus sp. I-3 and its use in the direct hydrolysis of raw potato starch, Enzyme Microb. Technol., 37, 723–734.

Haki, G. D. and Rakslit, S. K. (2003). Developments in industrially important thermostable enzymes: a review, Bioresour. Technol., 89, 17-34.

Haseltine, C., Rolfsmeier, M., Blum, P. (1996). The glucose effect and regulation of α-amylase synthesis in the hyperthermophilic archaeon Sulfolobus solfataricus. J. Bacteriol., 178, 945-950

Hassan, H. and Karim, K. (2012). Utilization of agriculture by-product for alpha amylase production under solid state fermentation by Bacillus subtilis. Engineering Journal, 16 (5), 177-186.

He, X., Zhang, W., Ren, F., Gan, B., Guo, H. (2012). Screening fermentation parameters of the milk-clotting enzyme produced by newly isolated Bacillus amyloliquefaciens D4 from the Tibetan Plateau in China. Ann Microbiol DOI 10.1007/s13213-011-0270-1

Hesham, A., Wang, Z., Zhang, Y., Zhang, J., Lv, W., Yang, M. (2006). Isolation and identification of a yeast strain capable of degrading four and five ring aromatic hydrocarbons. Ann. Microbiol., 56,109-112.

Hesham, A., Mohammed, NH., Ismail, MA., Shoreit, AA. (2012). 16S rRNA gene sequences analysis of Ficus elastica rubber latex degrading thermophilic Bacillus strain ASU7 isolated from Egypt. Biodegradation, 23, 717-724.

Hmidet N., El-Hadj Ali N., Haddar A., Kanoun S., AlyaS & Nasri M. (2009). Alkaline proteases and thermostable α-amylase co-produced by Bacillus licheniformis NH1: Characterization and potential application as detergent additive. Biochemical Engineering Journal, 47, 71-79.

Horikoshi, K. (1971). Production of alkaline enzymes by alkalophilic microorganisms. II. Alkaline amylase produced by Bacillus No. A-40-2".Agric. Biol. Chem., 35: 1783-1791.

Huang, L. P., Jin, B., Lant, P. and Zhou, J. (2003). Biotechnological production of lactic acid integrated with potato wastewater treatment by Rhizopus arrhizus. J. Chem. Technol. Biotechnol., 78, 899–906

Hung, L. P., Jin, B., Lant, P., Qiao, X., Chen, J. and Sun, W. (2004). Direct fermentation of potato starch in wastewater to lactic acid by Rhizopus oryzae. Biotechnology and Bioprocess Engineering, 9 (4): 245-251.

Jin, B., Yin, P., Ma, Y. and Zhao, L. (2005). Production of lactic acid and fungal biomass by Rhizopus fungi from food processing stream. J. of Indian Microbiology and Biotechnol., 32: 678-686.

Kaur, P. and Vyas, A. (2012). Characterization and optimal production of alkaline α-amylase from Bacillus sp. DLB 9. African Journal of Microbiology Research, 6(11): 2674-2681.

Khan JA. and Briscoe S. (2011). A study on partial purification and characterization of extracellular alkaline amylase from Bacillus megaterium by solid state fermentation. Int. J. Appl. Biol. Pharma. Technol., 2, 3.

Kindle, KL., Mainzer, SE., Marlatt, DL., Sawyer, CB. (1986). Thermostable alpha amylase having a low requirement for calcium ions, derived from a bacillus microorganism. United States Patent 4600693.

Krishna, C. and Chandrasekaran, M. (1996). Banana waste as substrate for α-amylase production by Bacillus subtilis (CBTK106) under solid-state fermentation. Appl. Microbiol. Biotechnol., 46, 106-111.

Kokab, S. M., Asghar, K., Rehman, M. J. and Adedyo, O. (2003). Bio-processing of banana peel for α-amylase production by Bacillus subtilis. International J. of Agriculture & Biology, 5 (1): 36–39.

Konsula, Z. and liakopoulou-Kyriakides, M. (2004). Hydrolysis of starches by the action of α-amylase from Bacillus subtilis. Process Biochem., 39: 1745-1749.

Konsoula Z. and Liakopoulou-Kyriakides M. (2006)). Thermostable α-amylase production by Bacillus subtilis entrapped in calcium alginate gel capsules. Enzyme and Microbial Technology, 39, 690-696.

Lane, DJ., 1991. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–175.

Mamo, G. and Gessesse, A. (1999). Effect of cultivation conditions on growth and α-amylase production by a thermophilic Bacillus sp. Letter in Applied Microbiology, 29: 61-65.

Mironescu, M. (2011). Investigations on wastewaters at potato processing and starch recovery and characterisation. Journal of Agroalimentary Processes and Technologies, 17(2): 134-138.

Mishra, S. and Behera, N. (2008). Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. African J. of Biotechnol., 7 (18):3326-3331.

Mitidieri, S., Souza Martinelli, A. H., Schrank, A. and Vainstein, M. H. (2006). Enzymatic detergent formulation containing amylase from Aspergillus niger: a comparative study with commercial detergent formulations. Bioresour Technol, 97: 1217-1224.

Nielsen, P., Fritze, D., Priest, F. G. (1995). Phylogenetic diversity of alkaliphilic Bacillus strains: proposal for nine new species. Microbiol., 141:1745-1761.

Rajagopalan G. and Krishnan C. (2008). Alpha-amylase production from catabolite derepressed Bacillus subtilis KCC103 utilizing sugarcane bagasse hydrolysate. Bioresour Technol., 99: 3044-3050.

Riaz, A., Qadar, S.A.U. Anwar, A. Iqbal, S. and Bano, S. (2009). Production and characterization of thermostable α-amylase from a newly isolated strain of Bacillus subtilis KIBGE-HAR. The Internet Journal of Microbiology., 6 (1):

Qader, SA., Bano, S., Aman, A., Syed, N. and Azhar, A. (2006). Enhanced production and extracellular activity of commercially important amylolytic enzyme by a newly isolated strain of Bacillus sp. AS-1. Turk. J. Biochem., 31(3): 135-140.

Saboury, AA., Ghasemi, S. Dahot, MU., (2005). Thermodynamic study of magnesium ion binding to alpha-amylase.Indian J. Biochem. Biophys., 42 (5): 326-329.

Saxena, RK., Dutt, K., Aqarwal, L. and Nayyar, P. (2007). A highly and thermostable alkaline α-amylase from a Bacillus sp. PN5. Bioresource Techno., 98: 260-265.

Schar-Zammaretti, P., Dillmann, ML., D'Amico, N., Affolter, M. and Ubbink, J. (2005). Influence of fermentation medium composition on physicochemical surface properties of Lactoibacillus acidophilus, Appl. Envior. Microbiol., 71: 8165-8173.

Sivakumar, T., Shankar, T., Vijayabaskar, P., Muthukumar, J. and Nagendrakannan, E. (2012). Amylase production using Bacillus cereus isolated from a vermicompost site. International Journal of Microbiological Research, 3 (2):117-123.

Tanyildizi, M. S., Ozer, D. and Elibol, M. (2005). Optimization of α-amylase production by Bacillus sp. using response surface methodology, Process Biochem., 40: 2291–2296.

Tanyildizi, M. S. and Özer, D. (2011). An Investigation of α-amylase production in semi solid substrate fermentation by using corn bran with Bacillus amyloliquefaciens. Turkish Journal of Science & Technology, 6(1): 47-52.

Tester R. F., Karkalas J. and Qi X. (2004). Starch–composition, fine structure and architecture. Journal of cereal science, 39: 151- 165.

Van Koningsveld, G. A., Gruppen, H., de Jongh, H., Wijngaards, G., van Boekel, M., Walstra, P., Voragen, A. (2002). The solubility of potato proteins from industrial potato fruit juice as influenced by pH and various additives, Journal of the Science of Food and Agriculture, 82(1): 134–142,

Vaseekaran, S., Balakumar, S. and Arasaratnam, V. (2010). Isolation and identification of a bacterial strain producing thermostable α- amylase. Tropical Agricultural Research, 22 (1):1-11.

Wei, Z., Jia, Z., Yu-guang, W., Hong-bo, Z. (2011). A marked enhancement in production of amylase by Bacillus amyloliquefaciens in flask fermentation using statistical methods. J. Cent. South Univ. Technol., 18: 1054−1062.

Yang H., Liu L., Li, J., Du, G. & Chen, J. (2011). Heterologus expression, biochemical characterization and overproduction alkaline α-amylase from Bacillus alcalophilus in Bacillus subtilis. Microb. Cell Fact., 10: 77.

Yoon, M. Y., Yoo, Y. J. and Gadman, T. W. (1989). Phosphate effects in the fermentation of α-amylase by Bacillus amyloliquefaciens. Biotchnology Letters, 11(1): 57-60.

Yuliani, E., Imai, T., Teeka, J., Tomita, S. and Suprayogi, ST. (2011). Exopolysaccharide production from sweet potato-shochu distillery wastewater by Lactobacillus sakei CY1. Biotechnol. & Biotechnol., 25(2): 2329-2333.

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