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Thermostable Alpha-Amylase from Geothermal Sites of Ethiopia (Afar Region): Isolation, Purification and Characterization

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ABSTRACT

One thousand six hundred and thirty bacterial colonies were isolated from hot springs of Afar region (Ethiopia). Of all the species tested, two bacterial strains designated as AGMSB1 and AGLSB5 were selected and produced the highest level of enzyme with an optimum growth temperature of 55^{O}C and 45^{O}C, respectively. They were identified as rod shaped gram positive bacteria belonging to *Bacillus species.* The optimum temperature of alpha-amylase produced by AGMSB1 was found to be in the range of 75^{O}C to 95^{O}C with 0.85 U/ml activity at 85^{O}C. The optimum pH was in the range of 5 to 7. The enzyme maintained 78% of its original activity (0.66U/ml) after 60 minutes of incubation at 85^{O}C with more or less stable pH values of 5 to 9. Zn^{+2} strongly inhibited its activity but it was calcium independent. The optimum temperature of the enzyme produced by AGLSB5 was found to be between 65^{O}C and 85^{O}C with 1.96 U/ml activity at 75^{O}C. The optimum pH was in the range of 5 to 7. The enzyme maintained 60% of its original activity (1.18U/ml) after 50 minutes of incubation at 85^{O}C and it was stable at pH values of 5 to 9. Its activity decreased significantly by the presence of Zn^{+2} but it was independent of calcium ion.

INTRODUCTION

Extremophiles are microorganisms that can grow and thrive in extreme environments, like high or low temperature, high or low pH, high salinity, very low water activity, high pressure, low oxygen tension, etc. They are structurally adapted at the molecular level to withstand these harsh conditions and among them biocatalysts play a major role which are called as extremozymes produced by these microorganisms that function under extreme conditions. There are various extremozymes like cellulases, amylases, xylanases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases and phytases, which have great potential for application in various biotechnological processes. Currently, only 1-2 % of the microorganisms on the Earth have been commercially exploited and amongst these there are only a few examples of extremophiles (Gome *et al.,* 2004).

Amylases are enzymes that break down starch or glycogen which are produced by a variety of living organisms, ranging from bacteria to plants and humans, Alpha amylases (endo-1,4-α-D-glucan glucohydrolase, EC 3.2.1.1) belongs to the enzyme class of hydrolases which randomly cleaves the 1,4-α-D-glucosidic linkages between the adjacent glucose units in linear amylose chain of starch. Most of the alpha-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes (Bordbar *et al.,* 2005). Currently, two major classes of alpha-amylases are commercially produced through microbial fermentation. Based on the points of attack in the glucose polymer chain, they can be classified into two categories, liquefying and saccharifying. Bacterial alpha-amylase randomly attacks only the alpha-1,4 bonds, it belongs to the liquefying category. On the other hand, the fungal alpha-amylase belongs to the saccharifying category and attacks the second linkage from the non reducing terminals (i.e. C4 end) of the straight segment, resulting in a disaccharide called maltose. Thus, amylases have emerged as one of the leading biocatalyst with proven potential to find usage in a wide array of industrial applications, such as additives in processed food industries, additives in detergents, waste-water treatment, biopulping, bioremediations and molecular biology. These enzymes account for about 30 % of the world's enzyme production (Maarel *et al.,* 2005).

Afar region is one of the nine regional states of Ethiopia and the Afar depression, also known under the outdated name Danakil depression, is a part of the Great Rift Valley of Ethiopia. It is one of the lowest point in Africa. The Afar depression, a plate tectonic triple junction is found in the Afar regional state. This geologic feature is that it is one of the earth's great volcanic areas. It is believed that microorganisms found in this area are capable of producing enzymes which can function at harsh conditions. The present investigation is aimed at production, partial purification and characterization of starch hydrolyizing thermostable alpha-amylase from thermophilic microorganisms (Bacteria) of geothermal sites of Afar region (Ethiopia).

MATERIALS AND METHODS

Microbial Sample Collection

Water and soil samples were collected from geothermal sites, located at Afar region. Temperature and PH of the water was measured directly.

Screening and Isolation of alpha amylase producing thermophilic bacteria

Samples were transported to the Food Science and Nutrition Laboratory located at Addis Ababa University, Ethiopia where screening of the microorganisms were accomplished. Enrichment of well mixed soil and water samples were done using nutrient broth supplemented with 0.5% (w/v) starch; the nutrient broth were mixed with the water samples in the ratio of 1:1 in test tubes. For soil samples, the mixture was 1% soil in the nutrient broth and was mixed in test tubes and incubated at 65^{O}C overnight. A serial dilution of enriched samples (10^{-1} up to 10^{-6}) was made by distilled water. Replicate plates of starch agar were spread plated with 0.1ml of diluted samples and incubated at 55^{O}C for about 24h. After counting the colonies, plates were flooded with gram's iodine solution and promising amylase producers showing different morphology were taken and independently maintained in a nutrient broth to check the purity of the colonies.

Selection of high amylase producers

Test tubes with nutrient broth and independent promising alpha amylase producers were incubated at 55^{O}C for about 24h. From each test tube samples were streak plated on to starch-agar plates and incubated overnight at 55^{O}C. Then Petri plates were flooded with two drops of iodine solution and those showing a clear halo were isolated. The colonies were cheeked by inoculating them in to test tubes, incubated at the same temperatures, flooded with two drops of iodine solution and the time taken to decolorize the iodine solution was obtained.

Separation of enzyme sample

A liquid culture medium containing 0.5% meat extract, 1% polypeptone and 1% soluble starch was prepared. One hundred milliliters of medium in 500ml Erlenmeyer flasks was inoculated with 4ml of an overnight bacteria culture and it was incubated at different temperatures (45, 50, 55 and 60^{0}C) with rotary shaking of 130rpm. The culture was harvested at fixed time intervals (3hrs

interval) and cells were separated by centrifugation (2500rpm for 15min). The cell free culture supernatant was used as the crude enzyme source.

Enzyme Assay

Alpha amylase activity was monitored using 3,5-dintrosalicylic acid reagent (Bernfeld, 1995). A volume of 0 .5 ml of enzyme solution was added to 0.5ml of soluble starch solution (1% W/V) in 0.02M sodium phosphate buffer, pH 6.5 and it was incubated for 3m at 25^0C. After adding 1 ml of 3,5-dinitrosalicylic acid reagent, tubes were placed in a boiling water bath for 15min after which they were cooled at room temperature & 10ml of distilled water was added to stop the reaction. The blank consisting of distilled water instead of enzyme solution was used. Finally, the absorbance at 540nm was measured after zeroing the spectrophotometer. One alpha-amylase activity unit is defined as the amount of enzyme that produces one micromole of maltose per minute under the assay condition.

Enzyme assay in all cases was done in triplicate. A standard curve for maltose was prepared to calculate the amount of maltose liberated.

Identification of bacterial strains

The bacteria were identified up to its genus level according to morphological and gram staining test at the Ethiopian Health and Nutrition Research Institute (EHNRI).

Effect of incubation temperature on enzyme production

The optimum incubation temperature for the production of alpha amylase from the selected bacterial strains were identified by incubating the enzyme production media at 45, 50, 55 and 60^OC.

Characterization of alpha-amylase

Effect of temperature and pH

The optimum temperature for the activity of alpha amylase was determined by incubating the mixture of 0.5ml enzyme solution and 0.5ml starch solution in 0.02M phosphate buffer (pH 6.5) at 45-100^OC for about 10min assaying the enzyme activity at various reaction temperatures (45-100^0C). Optimum pH was determined by conducting enzyme assay at optimum temperature using different buffers {acetate buffer (pH 3 to 5), sodium potassium buffer (pH 6 to 8) and Tris–hydro chloride buffer (pH 9 to 11)}, all at the concentration level of 50mM. Enzyme activity in each case was measured by the method described in section 2.5.

Effect of metal ions

Chloride salts of Ca^{2+}, mg^{2+} and Zn^{2+} each at a concentration level of 1mM, 5mM & 10mM were used to determine their effects on the activity of alpha amylase. To 0.5 ml of enzyme solution, 1ml of the respective salt solution were added and incubated for 1h in 20mM sodium phosphate buffer (pH 6.0). The remaining amylase activity was analyzed following the procedures indicated in section 2.5.

Temperature and pH stability

Thermostability of alpha amylase was done by incubating 0.5ml enzyme solution at temperature interval of 45 to 105^oc in 1ml of sodium phosphate buffer (pH 6.5) for 10, 20, 30, 40, 50 and 60min. The remaining enzyme activity was then measured and compared to the initial enzyme activity. The initial enzyme activity was assayed under optimum temperature conditions without heating the enzyme. To determine the pH stability, enzymes were maintained at 4^OC for about an hour in different buffers of various pH (3-11) and the residual activities were measured under the standard assay condition.

RESULTS AND DISCUSSION

In the present work, a study has been made to assess the existence of thermostable alpha-amylase producing microorganisms in three geothermal areas of Afar region (Meteka, Logia and Awash National Park) and a number of microorganisms were isolated.

Total microbial count

A total of 38.5 x 10^6, 49.5 x 10^5, 18.5 x10^5, 15.5 x10^6 and 14.0 x10^6 CFU/ml were encountered from Meteka water (MW), Meteka soil (MS), Soil at every sixty kilometer interval from Logia to Meteka (Log60), Awash National Park water (ANPW) and Awash National Park soil (ANPS) respectively (Table 3.1). The Table also indicates that a large number of thermophilic microbial colonies (38.5 x 10^6 CFU/ml**)** were recorded from MW as compared to the other sample sites. This result confirmed the thermophilic species richness of the geothermal sites in Afar region of Ethiopia.

Table 3.1: Total microbial count of the sample

Sample	Number of colonies (CFU/ml or gm)
MW	38.5×10^6
MS	49.5×10^5
Log60	18.5×10^5
ANPW	15.5×10^6
ANPS	14.0×10^6

Thermostable alpha-amylase producing bacteria

A total of 1630 (One thousand six hundred thirty) bacterial colonies were isolated. The respective number of bacterial colonies isolated from MW, MS, Log60, ANPW and ANPS were 420, 310, 250, 300, and 350.

This particular finding is comparable with the number of total isolates of thermophilic microorganisms obtained from hydrothermal areas of Ethiopia like Arbaminch, Hawasa, Abijata & Shalla and Wendo Genet (Haki , 2003).

Even though all the isolated bacterial colonies were able to utilize the growth medium, only 93 (about 6%) of the total isolates (25(6%), 18(6%), 12(5%), 17(6%) and 21(6%)) from the respective sample areas showed good amylolytic halo in a starch agar plates stained with iodine; showing the consumption of starch by the growing bacterial colonies (Figure 3.1). Of these 93 isolates, those exhibiting a halo with a diameter of 0.6-1.4mm were selected for further confirmatory experiment.

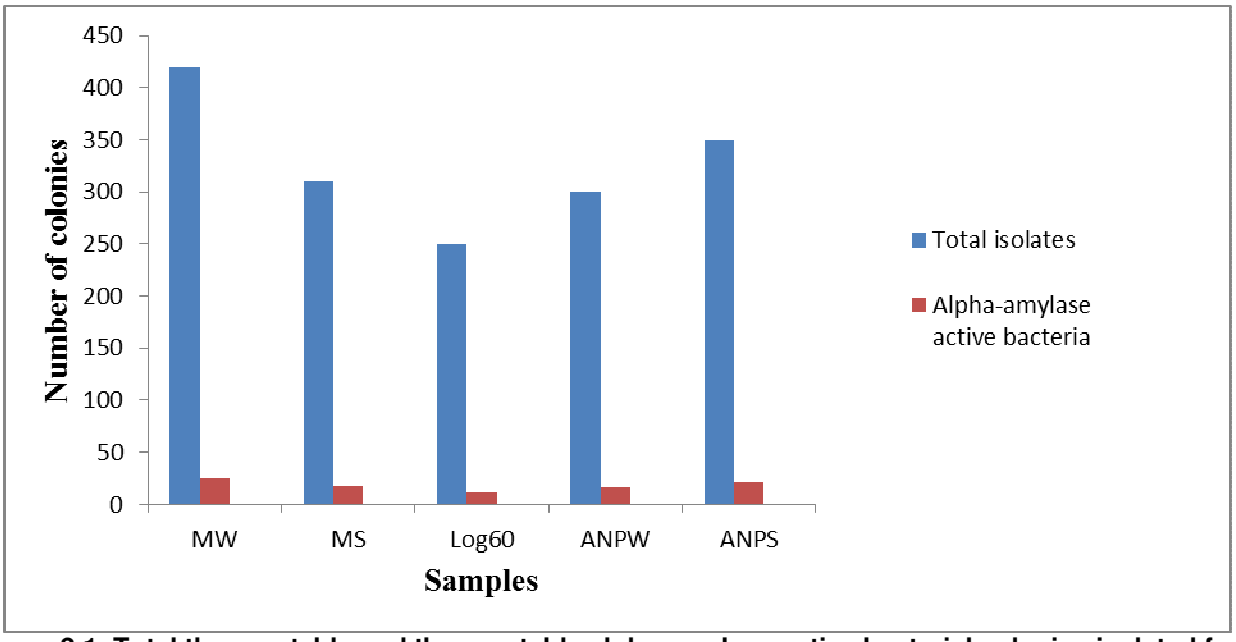

Figure 3.1: Total thermostable and thermostable alpha-amylase active bacterial colonies isolated from hydrothermal areas of Afar region, Ethiopia

Selection for best Amylase producers

From the primary screened bacterial isolates, those designated as AGMSB1 and AGLSB5 exhibit a higher halo diameter (1.4 and 1.1mm) and less time to decolorize the iodine solution (6 and 26seconds), respectively than the other screened strains (Table 3.2) and selected for further study. They were grown in the same broth and maintained on starch-agar slants at 4^0C.

AGMSB2 was better than AGLSB5 as it can be seen from Table 3.2, but priority is given for the AGLSB5 because from Meteka soil AGMSB1 strain was already selected. So AGLSB5 was selected from the other sample site (Logia) instead of AGMSB2.

Table 3.2: Halo diameter formed by selected bacteria and the time taken to decolorize iodine solution

Code	Diameter of halo formed by the organism (mm)	Time to decolorize iodine solution (s)	Remark
AGMSB1*	1.4	6	Bacteria isolated from Meteka soil (MS)
AGMSB2	1.2	18	
AGMSB5	0.8	49	
AGMWB2	1.0	29	Bacteria isolated from Meteka water (MW)
AGMWB3	1.0	25	
AGMWB4	0.8	50	
AGLSB3	1.0	33	Bacteria isolated from soil collected every 60km interval between Logia and Meteka (Log60)
AGLSB4	0.6	150	
AGLSB5*	1.1	26	
AGANPSB3	0.8	90	Bacteria isolated from awash National park (ANPW)
AGANPSB4	0.7	120	
AGANPSB5	0.9	39	

*selected for further study

Amylolytic activity of AGMSB1 and AGLSB5

These strains of bacteria originally designated as AGMSB1 and AGLSB5 isolated from Meteka and Logia were selected for further study based on the criteria mentioned above. The crude alpha-amylase extracts of both the organisms were heated at 65^{O}C and further incubation on starch-agar media at 45, 50, 55, and 60^{O}C confirmed the thermostability of the isolated enzymes (Table 3.3). The maximum zone of diameter obtained by the extracted enzyme of AGMSB1 and AGLSB5 were 12.4 and 9.3 at 55 and 45 ^{O}C, respectively. The minimum zone of diameter of the extracted enzyme recorded 10.0 and 8.4 at 45 and 55^{O}C by strains of AGMSB1 and AGLSB5, respectively. This clearly proved the thermophilic nature of the selected bacterial strains.

Table 3.3: Halo diameter formed by the crude amylase extract of selected bacteria

Code	Zone diameter of the extracted enzyme (mm)			
	45^{O}C	50^{O}C	55^{O}C	60^{O}C
AGMSB1	10.0	11.2	12.4	12.3
AGLSB5	9.3	9.0	8.4	9.2

Taxonomic assessment of the microorganisms

Taxonomic study of AGMSB1 and AGLSB5 were conducted according to the morphological and gram staining test at The Ethiopian Health and Nutrition Research Institute (EHNRI). Following the identification, both of them (AGMSB1 and AGLSB5) were recognized that they are gram positive road -shaped bacteria belonging to the genus *Bacillus.*

Alpha amylase production

Optimum incubation temperature and time for alpha-amylase production from AGMSB1 and AGLSB5

In the present study, only the effect of incubation temperature and time on the production of enzymes by the two selected bacterial strains (AGMSB1 and AGLSB5) was studied. Maximum enzyme production was affected by different incubation temperatures and time. Figures 3.2 and 3.3 shows enzyme activity of AGLSB5 and AGMSB1, respectively.

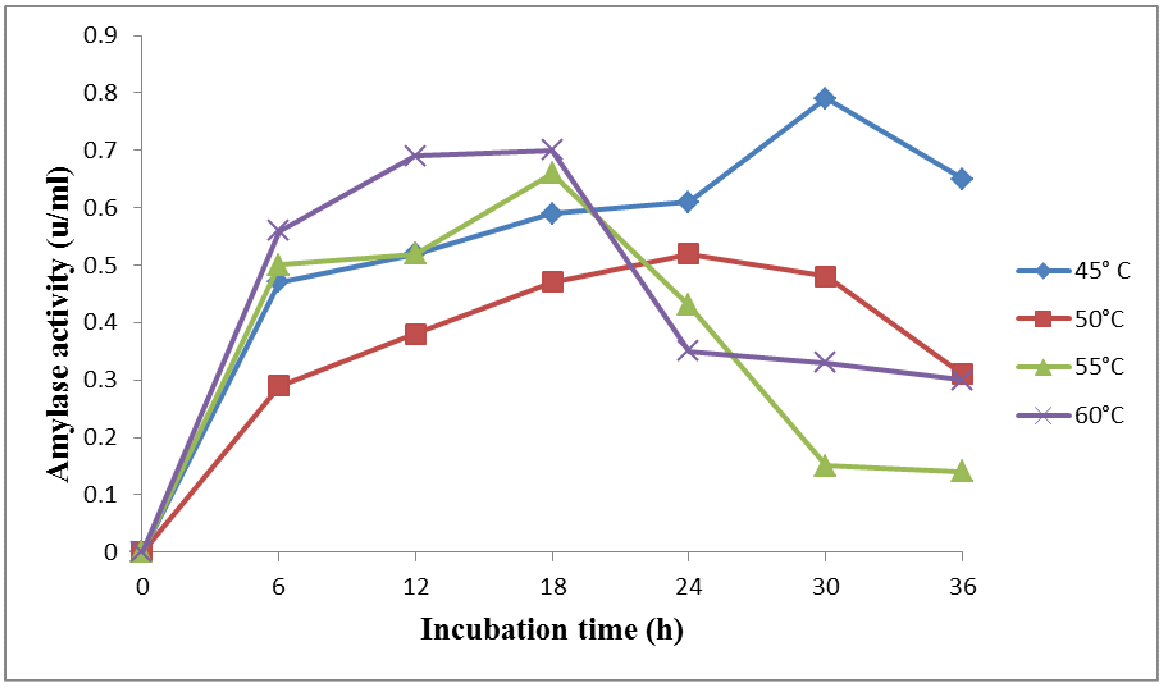

Figure 3.2: Effect of incubation temperature on amylase production of AGLSB5. Initial pH 6.5

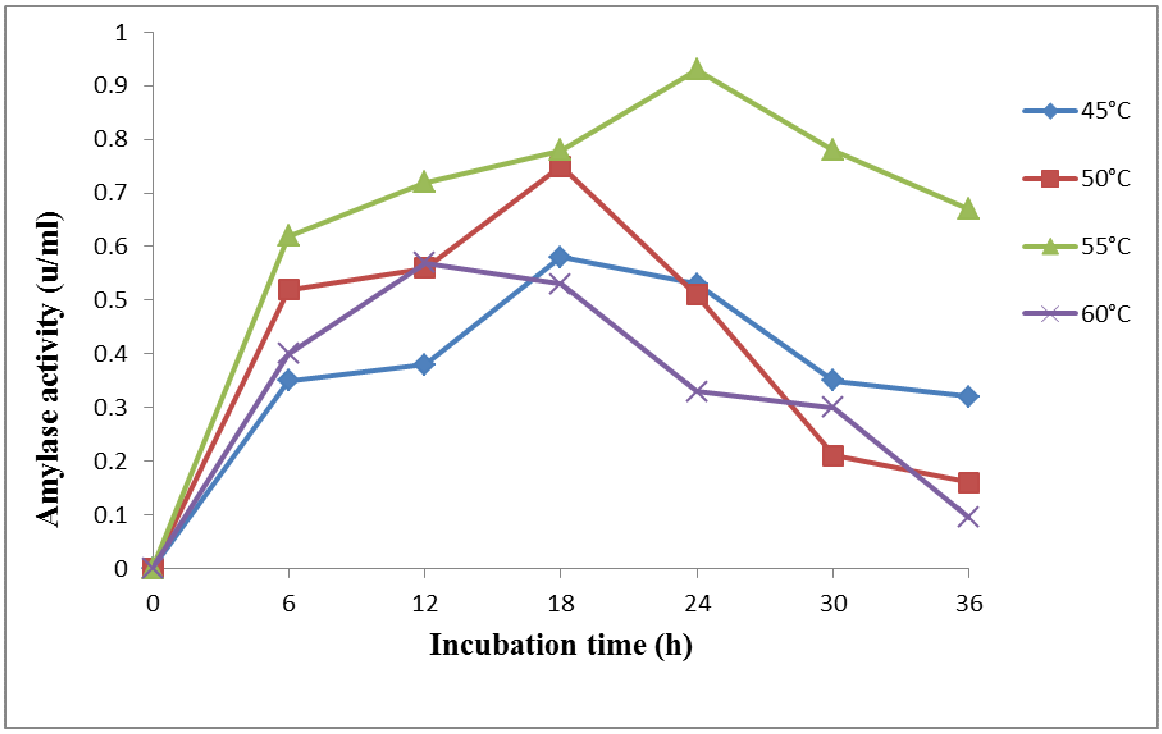

Figure 3.3 Effect of incubation temperature on amylase production of AGMSB1. Initial pH 6.5

The results obtained shows that the amylase activity of AGMSB1 reached its peak (0.93U/ml) at incubation temperature of 55^{O}C after 24hrs (Figure 3.3). On the other hand, high amylase production (0.79U/ml) by AGLSB5 was achieved at incubation temperature of 45^{O}C after 30hrs (Figure 3.2). The results indicated that further incubation did not show any increase in activity by the strain. Similar results were obtained by Ramesh and Lonsane (1991) concluding that alpha-amylase appeared to be growth related (in starch media) since the cell mass growth kinetics as exactly similar to the enzyme production rate.

Characterization of the partially purified thermostable alpha-amylase

The activities of enzymes can be affected by many factors like substrate concentration, incubation period and the concentration of chlorides of divalent metal ions (Dhanya *et al*., 2008). Having established that the protein produced is indeed an alpha-amylase, it was of interest to obtain some information about the operating characteristics of this enzyme. The results for the examination of such parameters as thermal stability, optimum temperature and pH yielded are shown in Figure 3.4 up to 3.11

Optimum temperature and pH

The results, represented in Figure 3.4 showed that the enzyme from AGMSB1 was optimally active between 75 and 95^{O}C, with a sharp decline above 95^{O}C. A similar result was also reported for *B. licheniformis* isolated from cassava steep water and activated sludge (Adeyanju *et al*,. 2007 and Hmidet *et al*., 2008). In addition, it was found that the enzyme had optimum pH in the range of 5-7 where more than 80% of its peak activity was displayed (Figure 3.5).

On the other hand, the optimum temperature for the activity of alpha-amylase from AGLSB5 was in the range of 65-85^{o}C (Figure 3.6). A similar result was reported on alpha-amylase activity of *B. stearothermophilus* by Vihinen and Mantasala (1990). The optimum pH was in the range of 5-7 (Figure 3.7). The maximum activity of most of the earlier reported enzymes is in its maximum activity in the range of 3.5-7.0 with its optimum pH range of 6.0-8.0 or pH 5.0-7.0 (Satyanarayana, 1994).

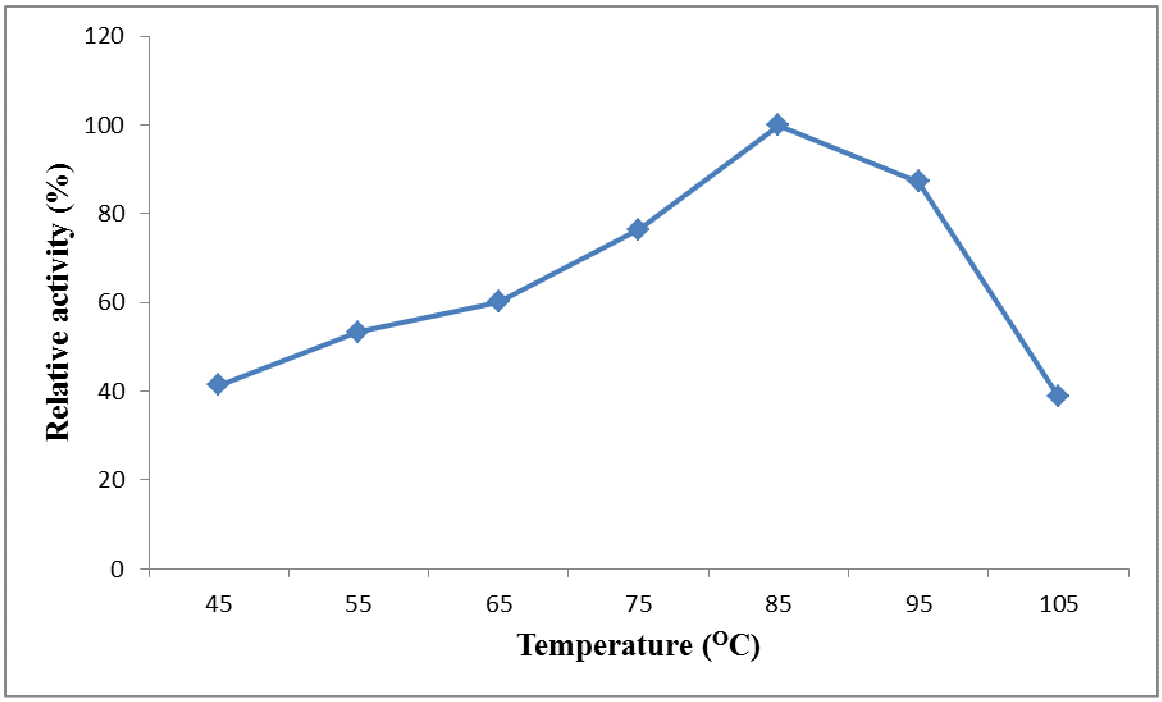

Figure 3.4: Effect of temperature on the activity of alpha amylase of AGMSB1

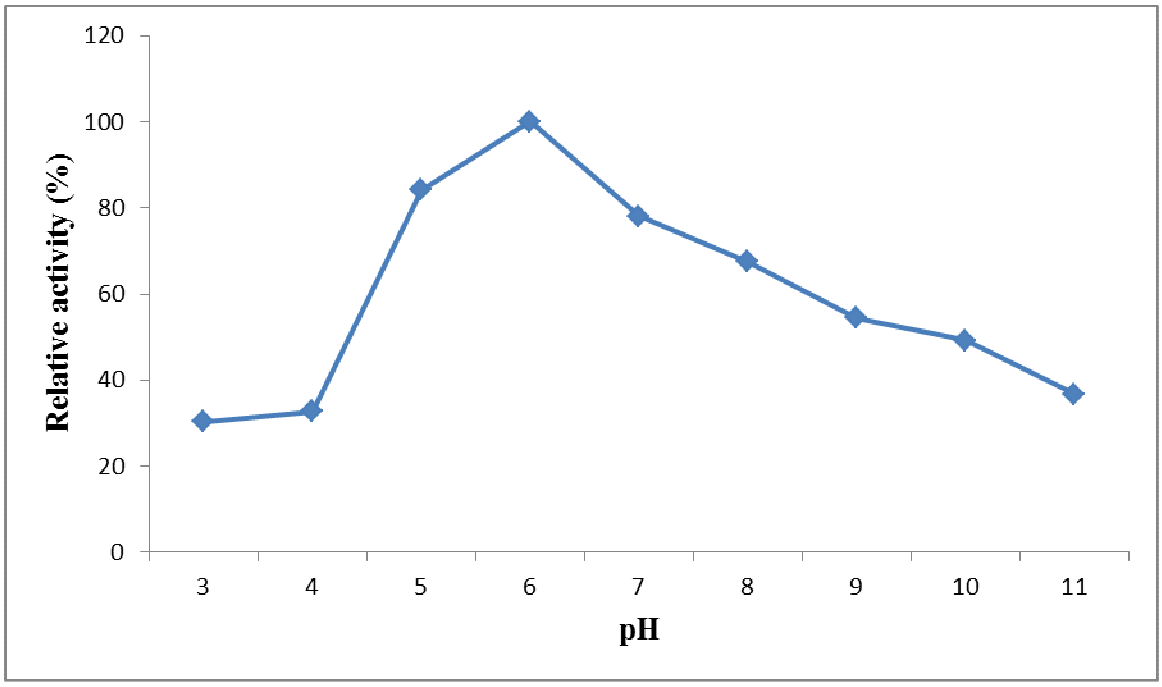

Figure 3.5: Optimum pH for alpha amylase activity of AGMSB1 at 85^{O}C

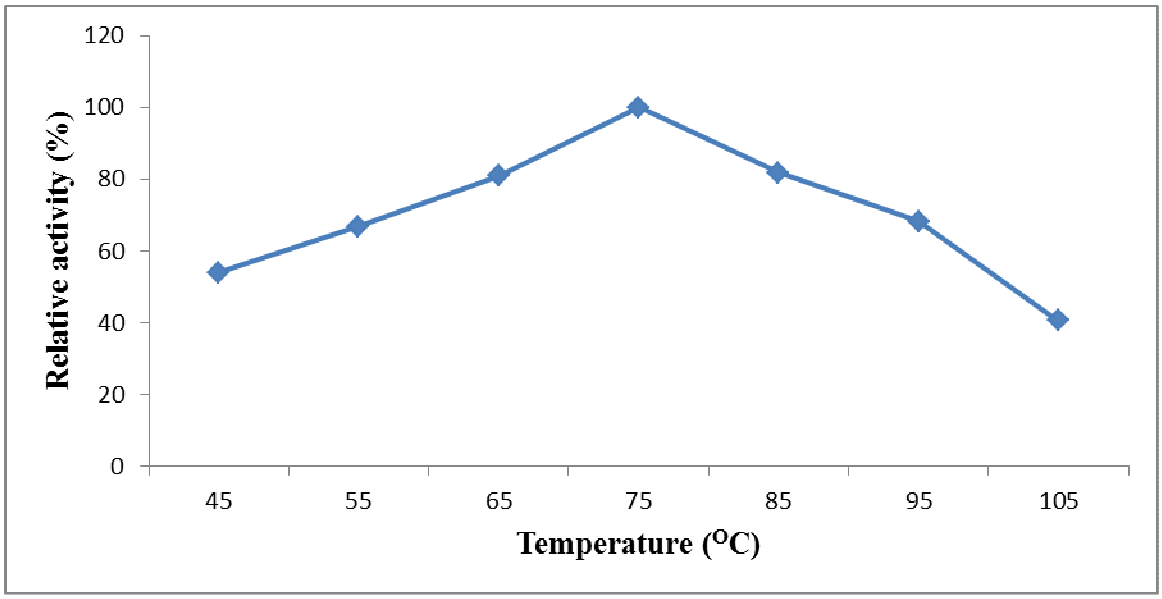

Figure 3.6: Effect of temperature on the activity of alpha amylase of AGLSB5

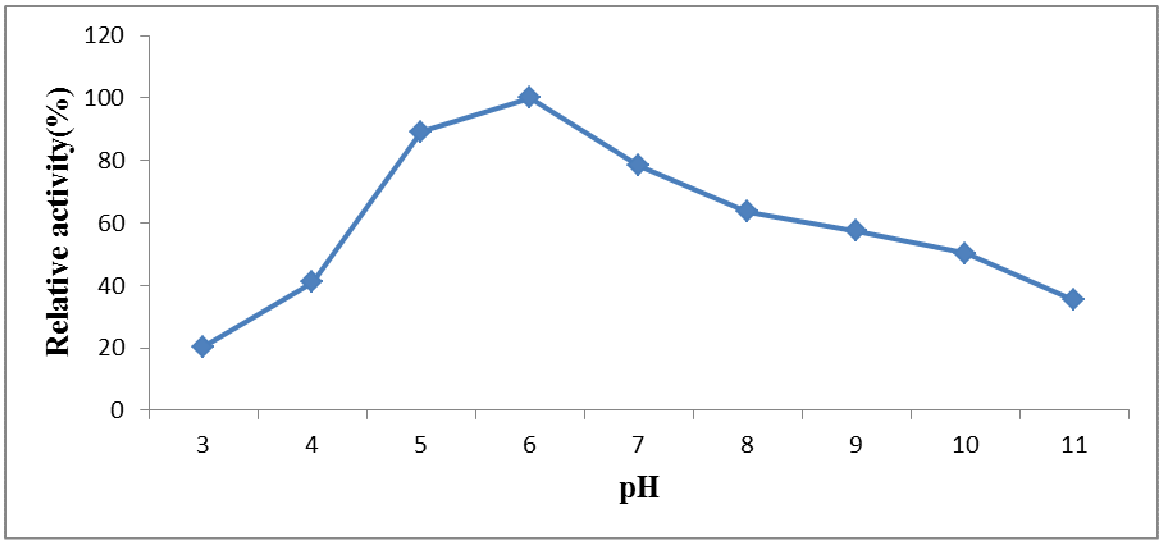

Figure 3.7: Optimum pH for alpha amylase activity of AGLSB5 at 75^{O}C

Temperature and pH stability

The enzyme from AGMSB1 has shown high thermostability in which it approximately maintained 78% of its original activity after 60 minutes of incubation at 85^{O}C (Figure 3.8). This enzyme is more stable than alpha-amylases produced from *B. stearothermophilus* GRE1 (Haki, 2003) and *B. subtilis* (Canganella *et al*., 1994).

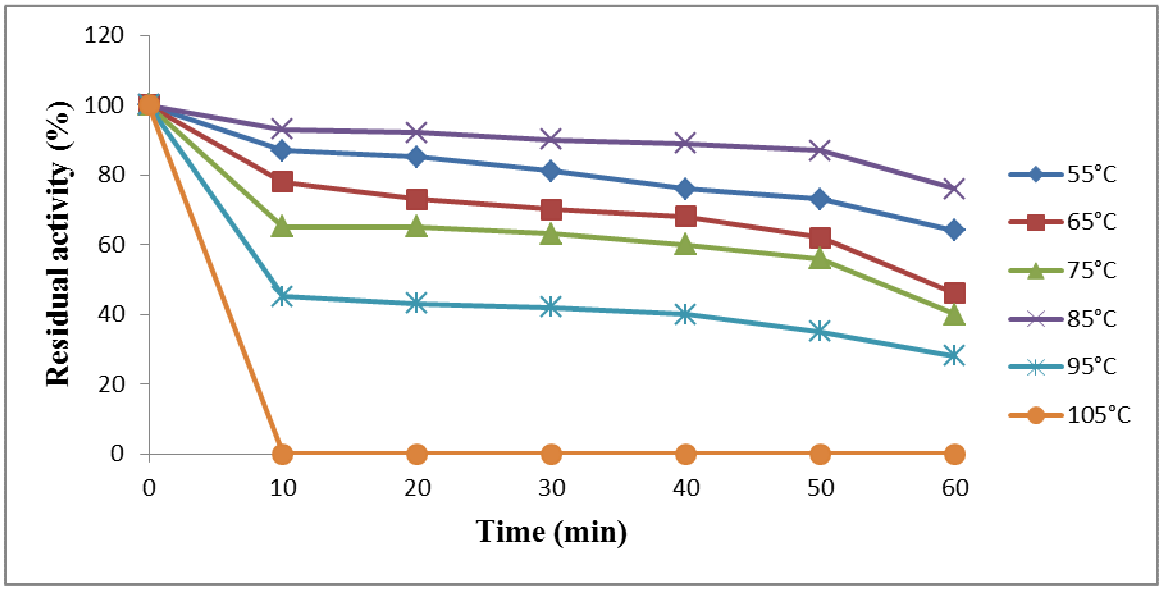

Figure 3.8: Thermostability of alpha-amylase from AGMSB1

Figure 3.9 shows that the enzyme from AGLSB5 exhibit a high thermostability, which is approximately 60% of its original activity after 50 minutes of incubation at 85^{O}C was maintained. Still, the enzyme has a capacity to maintain half of its original activity after 1 hour period of incubation at 85^{O}C. This enzyme still has a higher stability than alpha-amylases mentioned above. The result also indicates that alpha- amylase of AGLSB5 loses all of its original activity after 10 minutes of incubation period above 100^{O}C.

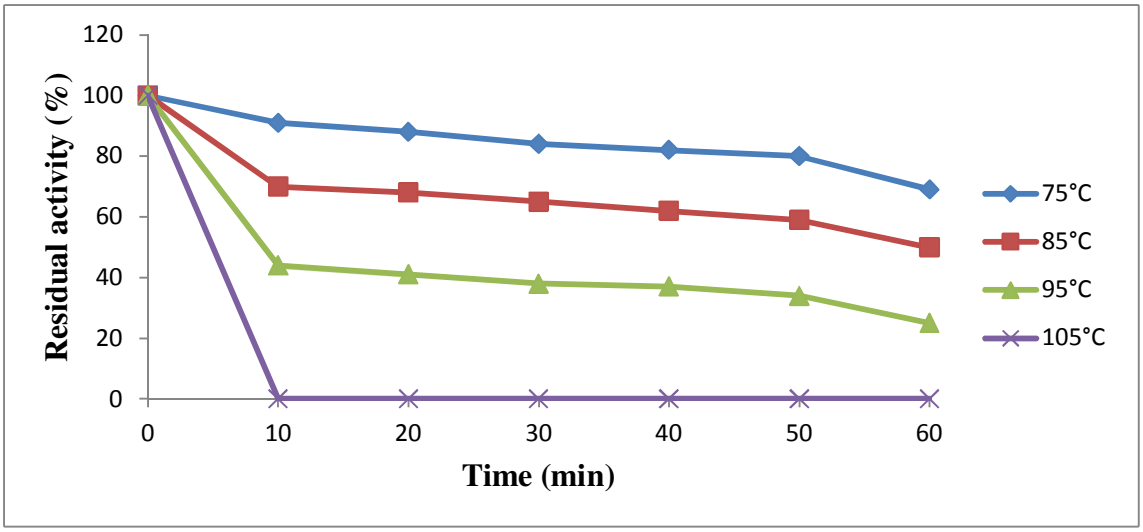

Figure 3.9: Thermostability of alpha-amylase from AGLSB5

Figure 3.10 and 3.11 shows that both of the enzymes from AGMSB1 and AGLSB5 were more or less stable in the range of 5.0 and 9.0 pH values. Similar pH stability has been found for previously reported *B. licheniformis* alpha-amylase (Pandey *et al*., 2000).

Alpha-amylase from AGMSB1 displayed 13% and 10% residual activities at pH 3 and 11, respectively (Figure 3.10) and that of AGLSB5 displayed 17% and 6% remaining activities at pH 3 and 11, respectively (Figure 3.11), which is an important feature in starch industry as far as starch liquefaction is concerned (Stranes,1990).

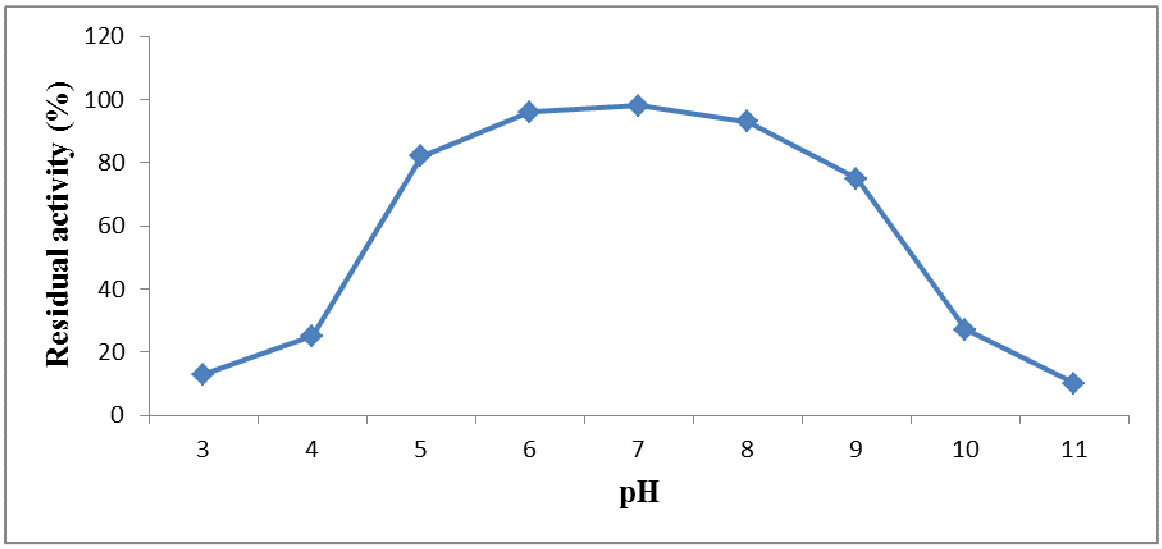

Figure 3.10: pH stability of alpha amylase from AGMSB1

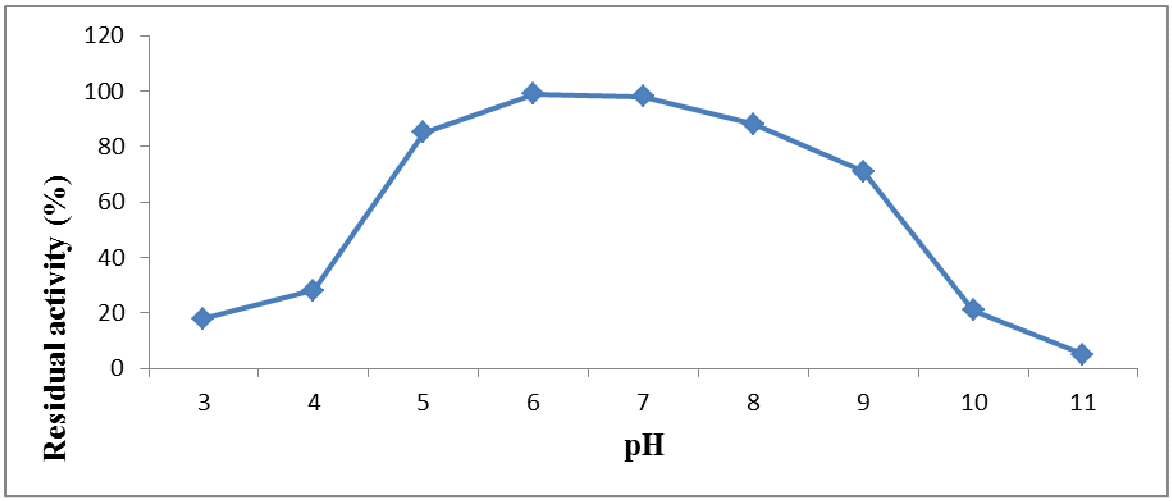

Figure 3.11: pH stability of alpha amylase from AGLSB5

Effect of divalent metal ions on the activity of alpha-amylase

External factors such as cations and additives have been known to affect the activity of enzyme. Many trace elements are needed by different microorganisms for their growth as well as for many enzyme catalyzed reactions. Ca^{2+} is found in alpha-amylase which is very essential to maintain the structural integrity of the active site (Whitaker, 1978).

In this particular work, among the divalent cation tested, the activities of alpha-amylases from both of AGMSB1 and AGLSB5 decreased significantly by all of the three concentrations (1mM, 5mM and10mM) of Zn^{2+} (Table 3.4 and 3.5). Similarly Adeyanju *et al.,* (2007) reported that Zn^{2+} and Cu^{2+} had inhibitory effect on amylase activity and Ahmad *et al.,* (2010) also reported that the enzyme activity of alpha-amylase from Bacillus species Ferdowsicous inhibited by Zn^{+2} and EDTA. In both enzymes, the addition of 5mM concentrations of Ca^{2+} very slightly activated enzyme catalysis (Table 3.4 and 3.5).

Table 3.4 shows that Mg^{2+} inhibited 18.5% and 20.4% of alpha-amylase activity of AGMSB1 with low and high concentrations, respectively. Whereas in the case of alpha-amylase of AGLSB5, Mg^{2+} inhibited only 6.2% and 7.3% of its activity with low and high concentrations respectively (Table 3.5).

Table 3.4: The effect of metal divalent cations on alpha-amylase from AGMSB1. Each value represents the mean of three independent assays (the standard errors were less than 5% of the means)

Cation	Concentration (mM)	Residual activity (%)
None		100.0
Ca^{2+}	1	92.6
	5	106.3
	10	87.3
Mg^{2+}	1	81.5
	5	80.4
	10	79.6
Zn^{2+}	1	50.2
	5	24.2
	10	19.8

Table 3.5: The effect of metal divalent cations on alpha-amylase from AGLSB5. Each value represents the mean of three independent assays (the standard errors were less than 5% of the means)

Cation	Concentration (mM)	Residual activity (%)
None		100.0
Ca^{2+}	1	96.6
	5	102.5
	10	95.6
Mg^{2+}	1	93.8
	5	93.5
	10	92.6
Zn^{2+}	1	48.5
	5	25.3
	10	24.5

CONCLUSIONS

The thermophilic microorganisms isolated, AGMSB1 and AGLSB5 produced alpha-amylases which offers interesting hydrolytic properties since the enzyme was active between pH 5 to 9. In addition to pH tolerance, thermostability of the enzyme make it potential for industrial applications such as starch liquefaction for sweeteners and syrups, textile desizing and paper industries, which requires the process to be carried out at high temperature. The enzyme was found to be calcium independent, which is a desirable characteristic in large-scale starch hydrolysis. Overall, the alpha-amylases obtained from both of the selected strains (AGMSB1 and AGLSB5) could be used for starch hydrolysis in different starch industries.

REFERENCES

Adeyanju M M, Agboola F K, Omafuvbe B O, Oyefuga O H, Adebawo O O (2007). A thermostable Extracellular α- amylase from *Bacillus Licheniformis* isolated from cassava steep water. Biotechnology, 6(4): 473-480.

Ahmad A , Jamshidkhan C, Milad L (2010). A novel thermostable, acidophilic alpha-amylase from a new thermophilic *Bacillus sp*. Ferdowsicous. *J. Biological macromolecules*. 46(3): 289-297.

Bernfeld P (1995). Amylases alpha and beta. Methods in Enzymology. 1, 149-158.

Bordbar A K, Omidiyan K, Hosseinzadeh R (2005). Study on interaction of alpha-amylase from *Bacillus subtilis* with cetyltrimethyl ammonium bromide. *Biointerfaces.* 40: 67–71.

Canganella F, Andrade C, Antranikian G (1994). Characterization of amylolytic and pullulytic enzymes from thermophilic archaea and from a new *Ferividobacterium species*. Appl. Microb. Biotechnol. 42: 239–245.

Dhanya G, Swetha S, Madhavan N K, Sukumaran KR, Ashok P (2008). Response surface methodology for the optimization of alpha-amylase produced by *B. amyloliquefaciens*. *J. Bioresource Technology*. 99(11): 4597-4602

Gome J Steiner W (2004). The biocatalytic potential of extremophiles and extremozymes. *Food Technol. Biotechnol*. 42(4): 223–235.

Haki G D, Rakshit S K. (2003). Developments in industrially important thermostable enzymes: a review. Bioresource Technol. 89(1): 17-34.

Hmidet N, Bayoudh A, Berrin J G, Kanoun S, Juge N, Nasri M (2008). Purification and biochemical charactrization of a novel alpha-amylase from *Bacillus licheniformis* NH1 Cloning, nucleotide sequence and expression of gene in *Escherichia coli*, ProcessBiochem. 43: 499–510.

Maarel van der, Van der Veen B, Uitdehaag Leemhuis H, Dijkhuizen L (2005). Properties and applications of starch converting enzymes of the alpha-amylase family, *J. Biotechnol*. 94: 137–155.

Pandey A, Nigam P, Soccol C R, Soccol V T, Singh D, Mohan R (2000). Advances in microbial amylases. Biotechnol Appl Biochem. 31: 135-152.

Ramesh M V & Lonsane B K (1991). Regulation of alpha-amylase production in *Bacillus licheniformis* M 27 by enzyme end-products in submerged fermentation and its overcoming in solid state fermentation system. Biotechnology. Letter. 13(5): 355- 360

Satyanarayana T (1994). Production of bacterial extracel-lular enzymes by solid-state fermentation, 122-129, In Pandey A.(ed.) solid-state fermentation, Wiley Eastern Limited, New Delhi

Stranes R (1990). Industrial potential of cyclodextrin glycosyl transferases. Cereal Foods World. 35: 1904-1100.

Vihinen M, Mantsala P (1990). Charactrization of a thermostable *Bacillus stearothermophilus* alpha-amylase. Biotech. Appl. Biochem. 12: 427– 435.

Whittaker R and Margulis L (1978). Protist classification and the Kingdom of organisms. Biosystems. 10: 3-18.

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