African Journal of Biotechnology Vol. 10(77), pp. 17831-17836, 5 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2194
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Isolation of soil thermophilic strains of actinomycetes for the production of α-amylase

Khwaja Salahuddin[1]*, Ram Prasad[2], Santosh Kumar[3] and Manish D. Visavadia[4]

[1]Department of Botany, Bahauddin Science college, Junagadh-362001, Gujarat, India.
[2]Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida-201303, India.
[3]Department of Botany, BRA Bihar University, Muzaffarpur – 842001, India.
[4]Department of Zoology, Bahauddin Science College, Junagadh-362001,Gujarat, India.

Accepted 21 October, 2011

Two thermophilic strains of actinomycetes, KS-52 and KS-60, were induced to produce α-amylase. α-Amylase activity was determined on solid medium supplemented with starch. The detection was based on the formation of clear or transparent zones around colonies. The size of transparent zone was found to be proportional to the amount of α-amylase enzyme produced by the strains. Extraction of α-amylase was done in liquid medium. The assay was observed by measuring the release of reducing sugar (RS) by 3,5-dinitrosalicylic acid (DNS) method and expressed in International Units (IU). The thermophilic strains were further tested for their ability to produce α-amylase enzyme by growing them on two different substrates - soluble starch (1%) and corn starch (1%). Optimization of the α-amylase production activity was achieved through variations of parameters including pH, incubation period, temperature, and carbon sources.

Key words: Actinomycetes, α-amylase, thermophilic.

INTRODUCTION

Enzymes are complex biological catalysts produced by living organisms in their cells to regulate the physiological processes of body. Enzymes are among most important products acquired for human needs in the area of industrial, environmental and food technology through microbial sources (Boing, 1999; Gurudeeban et al., 2011).

The soil is the main hub for microbial population, especially the actinomycetes. Actinomycetes constitute a formidable group of industrially important microorganisms that have been explored for the production of thermostable enzymes. α-Amylase is been derived from several fungi, yeast, bacteria and actinomycetes. It is used in food, textile, baking, pharmaceutical and detergent industries. It is also used in starch processing industry to convert starch to high fructose (Pandey et al., 2000; Asgher et al., 2007).

*Corresponding author. E-mail: khwajasalahuddin@yahoo.com. Tel: +91-2852675731 or +91 9067721514.

Abbreviations: **RS,** Reducing sugar; **IU,** international units; **DNS,** 3,5-dinitrosalicylic acid

Several species of actinomycetes such as *Streptomyces limosus* and *Thermomonospora curvata* have been found to be potent source of α-amylase enzyme. Other microorganisms such as *Bacillus coagulans*, *B. licheniformis* and *B. amyloliquefaciens* are great sources of α-amylase enzyme. Several fungi have been reported to synthesize amylase (Cherry et al., 2004).

Starch digestion is mainly done through the enzymatic method. Degradation of starch is carried out mainly and commonly by the action of amylolytic enzyme of bacterial origin (Sohail et al., 2005). Microbial amylases are mostly used in industries due to their high enzyme activity and thermostability (Burhan et al., 2003). Their thermostability can be increased by pH, temperature or substrates. Enzymes involved in hydrolyses of starch are categorized into the following four groups based on their mode of action: i) α- amylase, ii) β-amylase, iii) glucoamylase, and iv) disbranching enzymes (Fatma and El-Refai, 1991). α-Amylase (α-1,4 glucan, 4 glucanohydrolase, (E.C. 3.2.1.1.) is an extracellular enzyme which randomly hydrolyses α-1,4 glucosidic linkage situated anywhere in either amylose or amylopectin chains.

MATERIALS AND METHODS

Microorganism

Soil samples were randomly collected from 20 different locations in Muzaffarpur, Bihar, India from rhizoplane of herbaceous plants. The thermophilic strains of actinomycetes were isolated on different media by using serial dilution technique. Isolation was conducted in triplicates per soil sample per dilution level.

Culture media

The following media were used for isolating thermophilic strains of actinomycete as well as for the extraction of enzyme. The pH of the media was adjusted to between 6.8 and 7.2 either by addition of 1 N NaOH or 1 N HCl. Then the media were autoclaved at 121°C for 15 min.

The Czapek-Dox Agar Medium (CAM) contained 2.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4$. $7H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4.7H_2O$, 30.0 g of Sucrose, 15.0 g of agar and 1000 mL of distilled water.

Starch Agar Medium (SAM) consisted of 2.0 g of soluble starch, 5.0 g of peptone, 3.0 g of beef extract, 15.0 g of agar and 1000 mL of distilled water.

Detection of enzyme

Firstly, Starch Agar Medium containing soluble starch as carbon source was prepared and autoclaved at 121°C for 15 min. The sterilized medium was poured into sterile Petri plate and allowed to solidify. Later, the strains of microorganism were inoculated on Petri plate. The inoculated plate was subjected to incubation at 37°C in an inverted position for 3-5 days. The plates were flooded with iodine solution for 1 min until the entire medium became colored in blue. Formation of a clear yellow zone around colonies in blue medium indicated hydrolysis of starch, and confirmed production of α-amylase enzyme (Shaw and Ou-Lee, 1984).

Extraction of enzyme

α-Amylase enzyme was extracted in crude form. For this, 100 ml of sterile starch broth (liquid medium) containing starch was prepared. 5 ml of bacterial spore suspension $1x10^5$ spores ml^{-1} was prepared and inoculated into flask and then subjected to shaking incubation for 5 days at 37°C. After incubation, flask was harvested and contents were filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 10,000xg for 15 min to remove spores. The supernatant was collected in the form of crude enzyme and used for estimation of amylolytic enzyme (Fossi et al., 2005; Ashwini et al., 2011)

Enzyme activity measurement

α-Amylase activity was assayed by measuring the increase in reducing sugars formed by the enzymatic hydrolysis of starch. A mixture of 5 ml of 1% soluble starch, 1 ml of 0.2 acetate buffer and 1 ml of enzyme solution was prepared. This assay mixture was subjected to incubation at 37°C for 4 h. The reaction was stopped by adding 1 ml of DNS and then kept in boiling water bath for 5 min. The amount of reducing sugars liberated was quantified by Nelson's modification of Somogyi after diluting with 1 ml of distilled water and absorbance absorbed at 575 nm in spectrophotometer (Nahas and Waldermarin, 2002). The enzyme activity was expressed in International Units (IU). One unit of α-amylase activity is defined as the amount of α-enzyme required to liberate 1 mole of reducing sugar (as maltose) per ml of enzyme extract per unit time.

Culture conditions

To observe the effect of different culture conditions on α-amylase enzyme production, the present investigation was conducted using liquid starch medium at varying incubation periods (24, 48, 72, 96 and 120 h), pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and temperatures (45, 50, 55, 60 and 70°C) (Ramesh and Lonsane, 1989). The effect of each factor on enzyme production and reducing sugars was observed. The effect of different carbon sources on α-amylase production was investigated in liquid medium. Five carbon sources such as wheat straw, corn, potato, rice and soluble starch were amended to liquid starch medium.

Factors affecting enzyme activity

Different concentrations of starch solution ranging from 0.5 to 3.0% were used to investigate the effect of substrate concentration on α-amylase activity; substrates like soluble starch and corn starch were used to observe the substrate specificity for the enzyme. Temperature and pH are the most limiting factors for the activity of α-amylase (Morgan and Prist, 1981) and these were investigated in this study.

RESULTS AND DISCUSSION

α-Amylase production by these thermophilic strains could easily be detected on solid medium. However, the ability of these strains to produce hydrolysis zone in plates correlates with α- amylase production in shaking condition (Vihinen and Mantsala, 1989).

Two thermophilic strains, KS-52 and KS-60 were tested for their ability to produce α-amylase enzyme by growing on various concentrations of substrates, like soluble starch and corn starch. α- Amylase enzyme showed the highest activity in the presence of soluble starch. Corn starch responded to enzyme activity not so heavily (Freer, 1993) (Figures 1 and 2).

The microbial α-amylase is an inducible enzyme and has catabolic repression effect of glucose concentration (Buonocove et al., 1976). The elevation of α- amylase production is observed between late positive log phase to initial stationary phase of bacterial growth. The starch content of the production medium acts as an inducer for the expression of bacterial gene of enzyme production but its catalytic product, glucose has repressive effect on enzyme synthesis (Melasniemi, 1987; Brown et al., 1990; El-Assar et al., 1992).

α-Amylase enzyme activity was observed on different incubation periods. Strains showed rapid increase in enzyme activity starting from 48 h. Its activity was found to be maximum at 96 h. The slight decline in enzyme production was noticed afterwards (Maitin et al., 2001) (Figure 3).

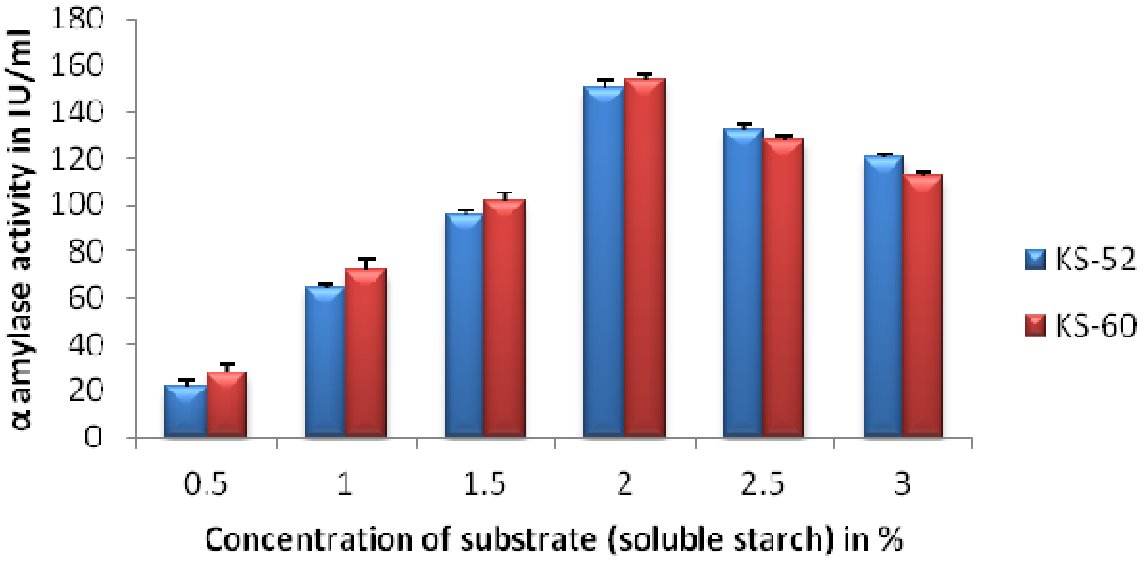

Figure 1. Effect of different concentration of soluble starch on α- amylase activity of thermophilic strains (KS-52 and KS-60).

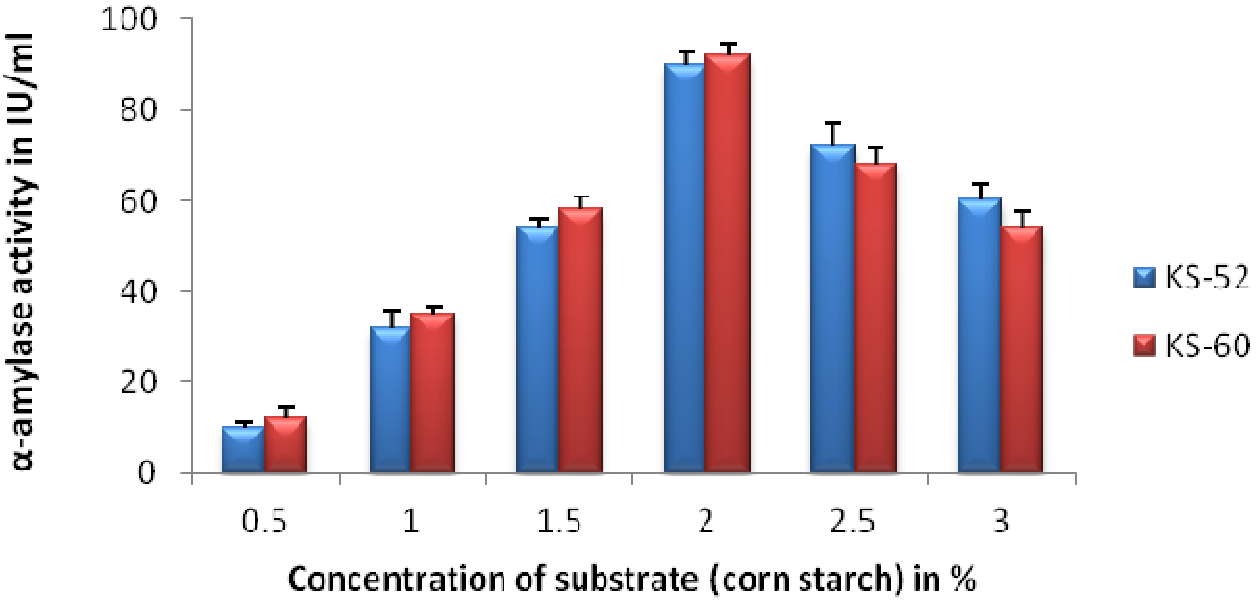

Figure 2. Effect of different concentration of corn starch on α- amylase activity of thermophilic strains (KS-52 and KS-60).

The effect of varying pH on α-amylase activity by these thermophilic strains was observed. α- Amylase tolerated a broad range of pH (from 4 to 10) at 52°C in the presence of starch as substrate. α- Amylase showed maximum activity at pH 6.0. As the pH shifts from optimum then its activity started decelerating (Shaw et al., 1989) (Figure 4).

Temperature was also considered a parameter to study the activity of α-amylase. α-Amylase showed the highest activity at 55°C in the presence of starch as substrate. Its activity started slowing down after 55°C. Temperature also gave its marked effect on the stability of α-amylase enzyme. The stability has been found concomitant with activity (Koch et al., 1980; Shaw et al., 1995) (Figure 5).

Different starch sources were also considered as one of the parameters to study their effect on α-amylase enzyme production. α- Amylase was found to be maximum when wheat straw (2% w/v) was supplied in the medium as carbon source. Corn straw is the second most inducer of a-

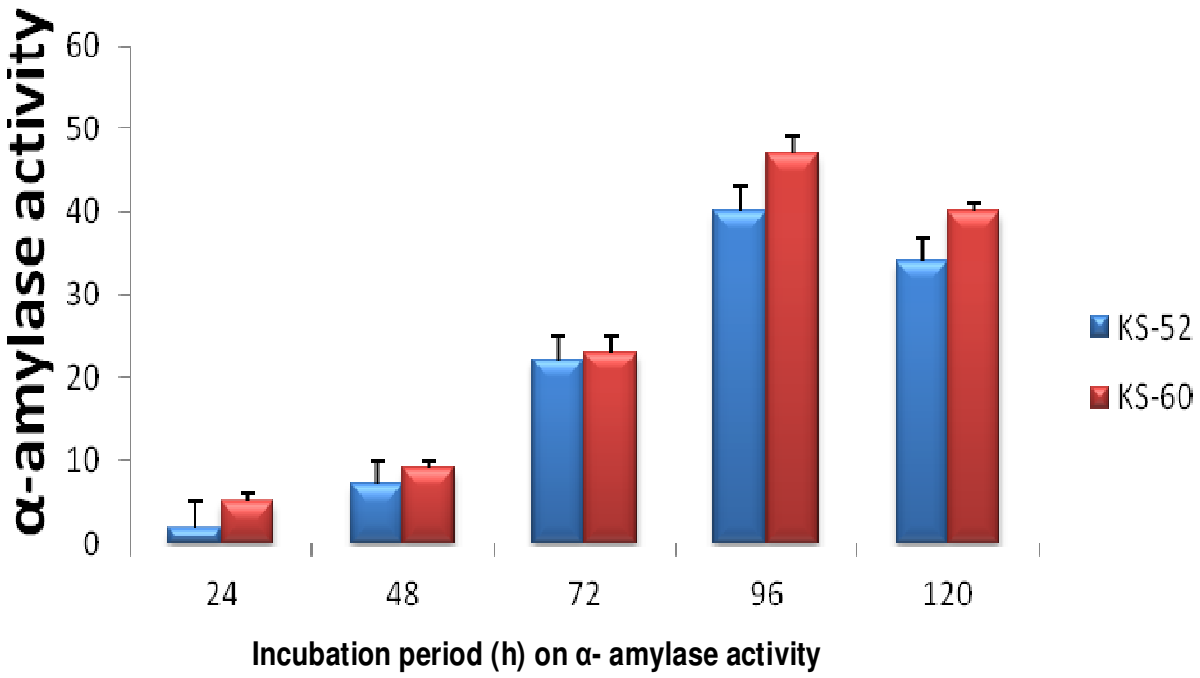

Figure 3. Effect of different incubation periods on α- amylase activity ($IUml^{-1}h^{-1}$).

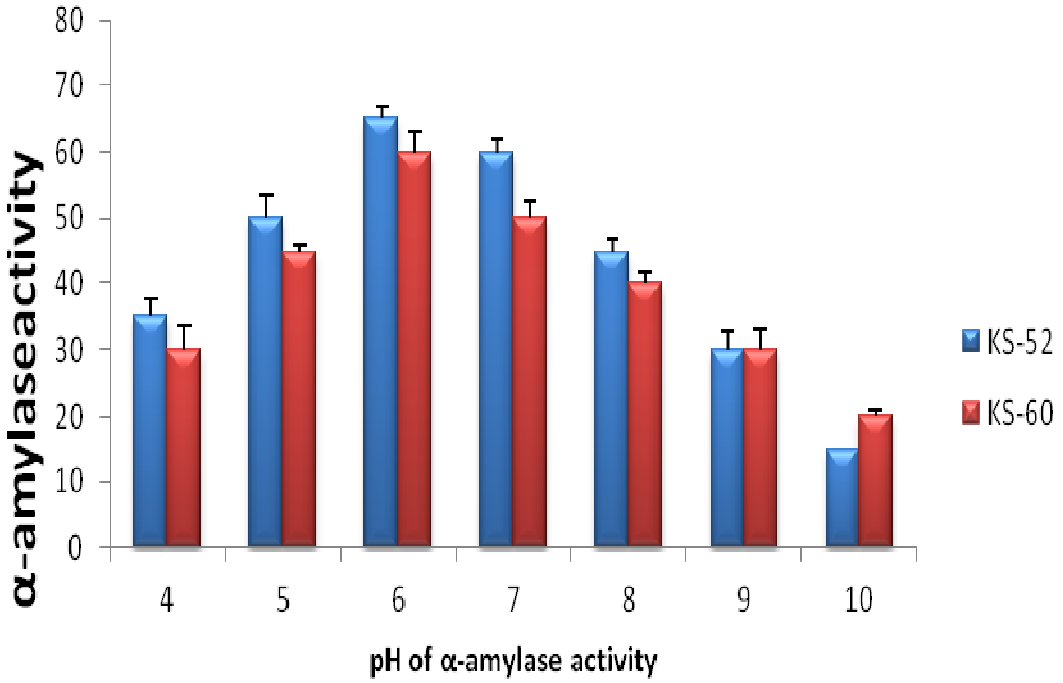

Figure 4. Effect of different pH on α- amylase activity ($IUml^{-1}h^{-1}$).

amylase enzyme. The other carbon sources such as potato, rice bran and soluble starch showed lesser α-amylase activity (Kochar and Katyal, 2003) (Figure 6).

The results of this study with respect to the thermophilic strains of Actinomycete suggested that the two isolated strains are the better microbial producers of thermostable

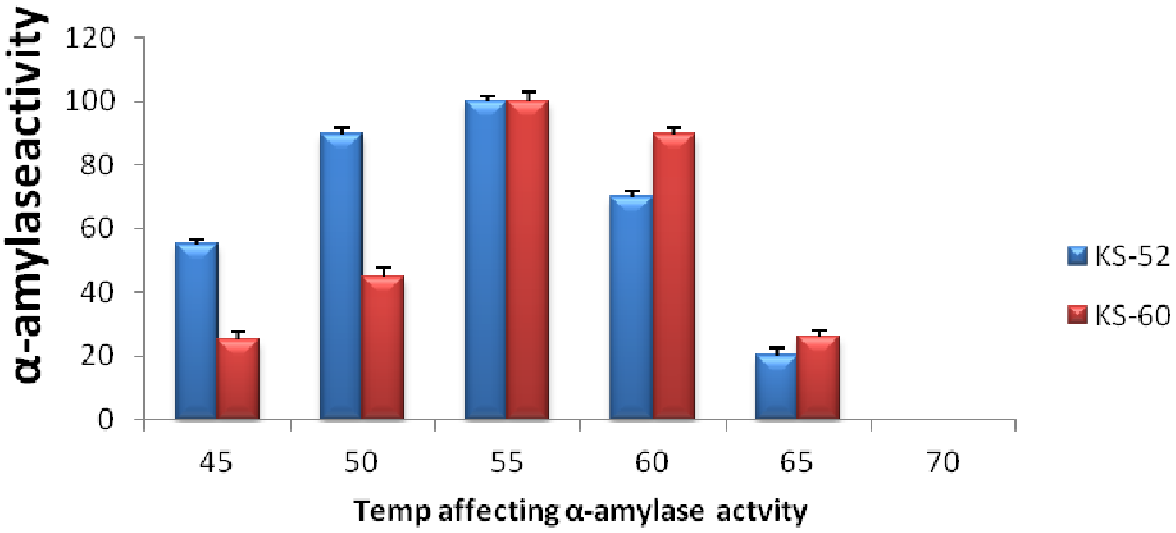

Figure 5. Effect of different temperature on α- amylase activity ($IUml^{-1}h^{-1}$).

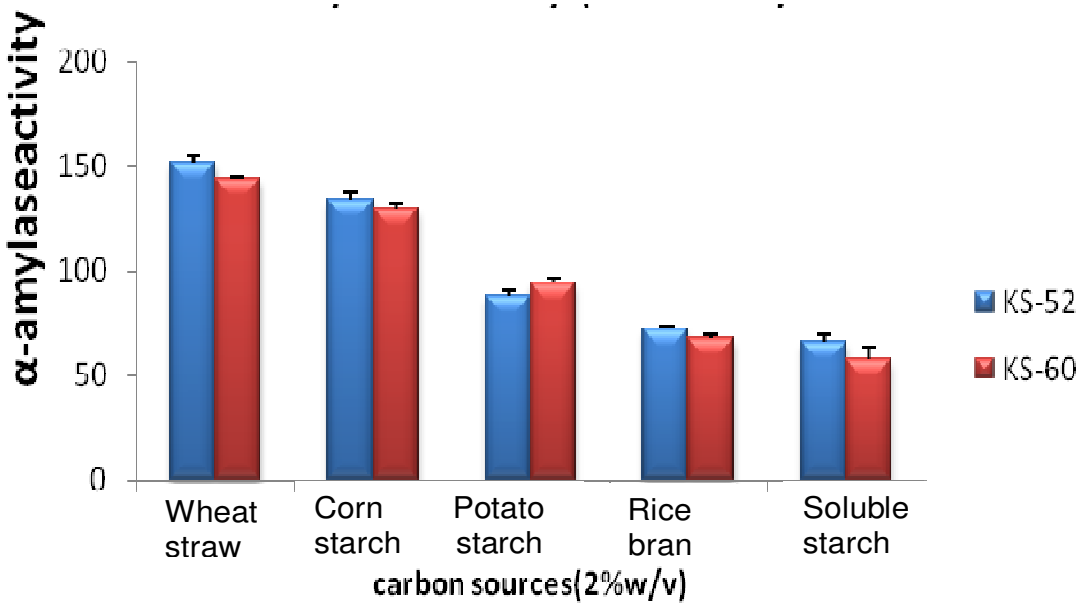

Figure 6. Effect of different carbon source on α- amylase activity ($IUml^{-1}h^{-1}$).

and extracellular enzymes which are useful in many industrial fields.

ACKNOWLEDGEMENT

Corresponding author is thankful to Council of Scientific and Industrial Research(CSIR New Delhi) for financial support in the form of Senior Research Fellowship during investigation. Author is also grateful to Farnaz Sheikh and Madhavi Thakar for statistical analysis of data.

REFERENCES

Asgher M, Asad MJ, Rehman SU, Legge RL (2007). A thermostable alpha amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing. J. Food Eng. 79: 950-955.

Ashwini K, Gaurav K, Karthik L, Bhaskara Rao K V (2011). Optimization, production and partial purification of extracellular α- amylase from *Bacillus* sp. m*arini*. Arch. Appl. Sci. Res. 3 (1): 33-42

Boing JTP (1999). Enzyme production in Industrial Microbiology 4th edn. (Reed G ed) CBS Publishers and Distributors, New Delhi, pp. 634-708.

Brown SH, Costantino HR, Kelly RM (1990). Characterization of amylolytic enzyme activities associated with hyperthermophilic archibacterium, *Pyrococus furiosus*. Appl. Environ. Microbiol. 56: 1985-

1991.
Buonocove VC, Caporale MDR, Gambacorta A (1976). A stable inducible thermoacidophilic α-amylase from *Bacillus acidocaladarius*. J. Bacteriol. 128: 515-521.
Burhan A, Nisa U, Gokhan C, Omer C, Ashabil A, Osman G (2003). Enzymatic properties of a novel thermostable, thermophile, alkaline and cheater resistant amylase from an alkaline *Bacillus* sp. Process Biochem. 38: 1397-1403.
Cherry HM, Hossain MT, Anwar MN (2004). Extracellular glucoamylase from *Aspergillus fumigatus*. Pak. J. Biol. Sci. 7 : 1988-1992.
El-Assar SA, Omar SH, Gouda MK, Isah AM, Abdel Fattah BF (1992). Purification of α-amylase from *Bacillus lentus* cultures. Appl. Microbiol. Biotechnol. 38: 312-314.
Fatma JE, El-Refai A (1991). Purification and Characterization of α-amylase by a thermophilic isolate of *Bacillus coagulans*. Chem. Microbiol. Technol. Lenbensm.13: 102-110.
Fossi BT, Tavea F, Ndjouenkeu R (2005). Production and partial characterization of a thermostable amylase from ascomycetes yeast strain isolated from starchy soils. Afri. J. Biotechnol. 4 (1):14-18.
Freer NS (1993). Purification and characterization of extracellular α-amylase from *Streptococcus bovis* LBI. Appl. Environ. Microbil. 59: 1398-1402.
Gurudeeban S, Satyavani K, Ramanathan T (2011). Production of extra cellular α-amylase using *Bacillus megaterium* isolated from White Mangrove (*Avicennia marina*). Asian J. Biotechnol. 3 (3): 310-316.
Koch R, Zablowski P, Spreinat A, Antranikian G (1980). Extremely thermostable amylolytic enzyme from the archaebacterium, *Pyrococcus furiosous*. FEMS Microbiol. Lett. 71: 21-26.
Kochar GS, Katyal P (2003). Use of potato starch for extracellular amylase production by a yeast isolates. Deptt of Microbiology, Punjab Agriculture University, Ludhiana.
Maitin V, Kavita R, Kumar S U (2001). Properties of an extracellular amylase enzyme purified from a *Bacillus* species. W.J. Microbiol. Biotechnol. 17: 823-826.
Melasniemi H (1987). Characterization of α-amylase and pullunase activities of *Clostridium thermohydrosulfuricum*. Biochem. J. 246: 193-197.
Morgan FJ, Prist FG (1981). Characterization of α -amylase and pullunase activities of a thermostable α-amylase from *Bacillus licheniformis* NCIB 6346. J. Appl. Bacteriol. 50: 107-114.
Nahas E, waldermarin MM (2002). Control of amylase production and growth characteristic of *Aspergillus ochraceous*. Rev. Latinoam. Microbiol. 44: 5-10.
Pandey A, Nigam P, Soccol C R, Soccol V T, Singh D, Mohan R (2000). Advances in microbial amylases. Biotechnol. Applied Biochem. 31: 135-152.
Ramesh MV, Lonsane BK (1989). Solid state fermentation for production of high titers of thermostable α-amylase with two peaks for pH optima by *Bacillus licheniformis* M 27. Biotechnol. Lett. II (I): 49-52.
Shaw FJ, Ou-Lee TM (1984). Studies on the α-amylase from the germinated rice seeds. Bot. Bull. Acad. Sin. 23:41-46.
Shaw FJ, Linn PF, Chen CS, Chen CH (1995). Purification and properties of an extracellular α-amylase from *Thermus* sp. Bot. Bull. Acad. Sin. 36: 195-200.
Shaw JF, Pan RS, Hsu WH (1989). Influence of pH on the inactivation of isoamylase. Bot. Bull. Acad. Sin. 30: 91-95.
Sohail M, Ahmad A, Shahzad S, Khan SA (2005) A survey of amylolytic bacteria and fungi from native environmental samples. Pak. J. Bot. 37(1): 155-161.
Vihinen M, Mantsala P (1989). Microbial amylolytic enzymes. Crit. Rev. Biochem. Mol. Biol. 24: 329-418.