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PRODUCTION OF AN EXTRACELLULAR HALOPHILIC AMYLASE FROM THE EXTREMELY HALOPHILIC ARCHAEON *NATRIALBA AEGYPTIACA* STRAIN 40^{T}

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Abstract

The extremely halophilic archaeon, *Natrialba aegyptiaca* strain 40^{T}, which was isolated from a salty soil close to Aswan, Egypt is able to produce a halophilic extracellular, raw starch-digesting amylase. Optimization of medium components and culture conditions to enhance amylase production was investigated. Maximum production of this amylase was achieved in a medium contained (%, w/v): NaCl, 15; KCl, 1.5; $MgSO_4.7H_2O$, 0.1 and soluble starch, 0.05 at a pH range between 6-8 and a temperature range between 45-47 ^{o}C after incubation period of 144 hr. under static conditions and 48 hr. with shaking at 150 rpm. The enzyme could efficiently hydrolyze raw starches from different plant sources.

Key words: halophilic, *Natrialba aegyptiaca*, amylase, production, optimization, raw starches

Introduction

Extremely halophilic archaea are microorganisms that can grow best in media containing 2.5-5.2 M (i.e. 15-32 %, w/v) NaCl (Kushner & Kamekura, 1988). They dominate the microbial populations of hypersaline environments (Ventosa, 2006). Enzymes from organisms grown in extreme environments have proven to be useful for industrial processes (Niehaus *et al.*, 1999). Amylases are one of the most important industrial enzymes, they are extracellular hydrolytic enzymes which hydrolyze starch polymers to give diverse products including dextrin and progressively smaller polymers composed of glucose units (Windish & Mhatre, 1965; Horváthová *et al.*, 2000). They are considered as a class of industrial enzymes, having approximately 25 % of the world enzyme market. These enzymes are of great importance in the present day biotechnology and they cover many industrial applications such as food fermentation, sugar, brewing, distilling, textile, paper industries, and pharmaceuticals (Pandey *et al.*, 2000; Saxena *et al.*, 2007; Rajagopalan & Krishnan, 2008). Although amylases can be obtained from several sources including plants, animals and microorganisms, the microbial enzymes generally meet the expanding industrial demands. Today a large number of microbial amylases are commercially available and they have, nearly, completely replaced the chemical hydrolysis of starch in starch processing industry (Pandey *et al.*, 2000). Although many amylases of various microorganisms have been studied, there are relatively fewer studies focused on archaeal amylases (Jones *et al.*, 1999; Hutcheon *et al.*, 2005). Halophilic archaeal amylases have been characterized, e.g.,

from *Halobacterium halobium*, *Natronococcus amylolyticus*, *Haloferax mediterranei*, *Haloarcula sp. S-1* and *Haloarcula hispanica* (Good & Hartman, 1970; Kobayashi *et al.,* 1992; Pérez-Pomares *et al.,* 2003; Fukushima *et al.,* 2005; Hutcheon *et al.,* 2005). Optimization of fermentation conditions, particularly physical and chemical parameters, is important in the development of fermentation processes due to their impact on the economy and practicability of the process (Francis *et al.,* 2003). The effect of various factors such as NaCl, substrate, KCl and $MgSO_4.7H_2O$ concentration as well as incubation period, temperature and pH have been previously studied (Good & Hartman, 1970; Oren, 1983; Kobayashi *et al.,* 1992; Pérez-Pomares *et al.,* 2003; Fukushima *et al.,* 2005; Hutcheon *et al.,* 2005). The physical and chemical parameters that affect the production of amylases by the halophilic archaea, *Halobacterium halobium*, *Natronococcus amylolyticus*, *Chromohalobacter sp. TVSP101* and *Haloarcula hispanica* have been widely studied and described (Gupta *et al.,* 2003). Raw starches are the dominant amylase substrates amongst different substrates that exists in nature. It is therefore surprising that few α-amylases appear to be capable of hydrolyzing this substrate in its native state (Hamilton *et al.,* 1999; Nidhi *et al.,* 2005). Amylases are used extensively in the industry, particularly in the liquefaction of starch but due to the inability of these enzymes to hydrolyze the native substrate efficiently, starch is firstly gelatinized prior to enzymatic degradation (Hamilton *et al.,* 1999; Nidhi *et al.,* 2005). This paper deals with production capability as well as productivity's optimization of the raw starch-digesting amylase from the halophilic archaeon *Natrialba aegyptiaca* strain 40^T.

Materials And Methods

Archaeal strain, growth conditions and media used

The extremely halophilic archaeon *Natrialba aegyptiaca* strain 40^T was isolated from a salty soil close to Aswan, Egypt. It was obtained kindly from Dr. Francis F. Hezayen (coauthor). It is deposited in the DSMZ as DSM 13077^T and in Japan Collection of Microorganisms, Wako, Saitama, Japan as JCM 11194^T (Hezayen *et al.,* 2001; Hezayen, 2002).

Growth and maintenance medium: The strain was grown and maintained on proteose peptone salt medium which contained (%, w/v): NaCl, 25; KCl, 0.2; $MgSO_4.7H_2O$, 0.5; proteose peptone, 0.75 and yeast extract, 0.5; incubation period was 3 days.

Amylase production medium: which contained (%, w/v): NaCl, 25; KCl, 0.2; $MgSO_4.7H_2O$, 0.5; emulsified soluble starch, 0.5 and agar, 2. incubation period was 5 days.

Starch salt agar medium: this medium was used to determine the amylase activity and contained (%, w/v): NaCl, 25; Bacto-agar, 1.5 supplemented with emulsified soluble starch, 0.5.

If otherwise is not indicated, pH of the media was adjusted to 7.0 with 0.1 N NaOH, sterilized by autoclaving and the incubation temperature was 40 ^oC.

Ability of *Natrialba aegyptiaca* strain 40^T to produce extracellular amylase:-

Amylase production was tested qualitatively in both, agar and broth cultivations, following the methods described by Amoozegar *et al.,* (2003) and Ammar *et al.,* (1998), respectiely. Agar plates and the broth of the amylase production medium were inoculated, and incubated for 5 days. Amylase production in agar plates was tested by flooding their surfaces with Lugol's iodine solution (0.3 % I_2, 0.6 % KI) (Cowan, 1974), and in liquid growth by clear zone technique (CZT) as mentioned below. Appearance of clear zones indicates amylase production. This test indicated that strain 40 is strong amylase producer as previously mentioned (Hezayen *et al.,* 2001; Hezayen, 2002).

Factors affecting productivity was studied as follows**:** the amylase production medium was dispensed in 250 ml capacity Erlenmeyer conical flasks, 50 ml in each inoculated with 2 ml of 3-days old pre-culture containing 1 x 10^8 CFU/ml and incubated for 5 days (If otherwise is not indicated). Then, growth was harvested, centrifuged for 30 min. at 6000 rpm, and the amylase activity was determined in the supernatants.

Determination of amylase activity:-

Amylase activity from broth cultivations was determined according to the clear zone technique (CZT) of Elwan *et al.,* (1986), standardized later by Ammar *et al.,* (1998). In the plates of starch salt agar medium mentioned above, three cups were made in each plate using a sterile cork borer, each cup was filled with 0.25 ml of the cell free supernatant and the plates were incubated at 40 ^oC for 48 hr., then flooded with Lugol's iodine solution. The diameters of clear zones were measured, the average was calculated and expressed in terms of units/ml using amylase standard curve (Ammar *et al.,* 1998).

Factors affecting amylase production:-

Effect of various factors such as NaCl, KCl, $MgSO_4.7H_2O$, substrate concentration, pH, incubation temperature, incubation period, inoculum size and shaking rate as well as carbon and nitrogen sources on amylase production was tested. If otherwise is not indicated, factors were tested on the amylase production medium and amylase productivity was assayed as mentioned above.

Effect of NaCl, KCl and $MgSO_4.7H_2O$:-

To determine the effect of these salts on the amylase productivity of strain 40^T, different concentrations from each was tested i. e. for NaCl, 5.0, 8.0, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, 30.0 and 32.0; for KCl,. 0.0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5 and 3.0 and for $MgSO_4.7H_2O$, 0.0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0 (g %, w/v). Amylase productivity was assayed as mentioned above.

Effect of substrate (soluble starch) concentration:-

This was performed, applying different concentrations i.e. 0.0, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 (g %, w/v) from soluble starch. Amylase productivity was assayed as mentioned above.

Effect of pH, incubation temperature and incubation period:-

The effect of temperature on amylase productivity was measured in the range of 10 ^{o}C to 70 ^{o}C while the effect of pH was measured in the range of 4 to 10 (0.5 intervals), pH was adjusted by either HCl or NaOH (0.1 N) and for the incubation period, different incubation periods were applied i.e. 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hour. Amylase productivity was assayed as mentioned above.

Effect of inoculum size:-

Different inocula sizes of a standard suspension of 3-days old culture of *Natrialba aegyptiaca* strain 40^{T} were tested i.e. 0.1, 0.2, 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 ml^{-1}/flask. Each 1 ml^{-1} of suspension contained 1 x 10^{8} CFU/ml. Amylase productivity was assayed as mentioned above.

Effect of shaking rate:-

This was performed by incubating the inoculated flasks in a shaking incubator (optic ivymen® system, 100 D) at different shaking speeds i.e. 50, 100, 150, 200, 250, 300 and 350 rpm at 40 ^{o}C. Amylase productivity was assayed as mentioned above.

Effect of different carbon sources:-

Various carbon sources such as soluble starch, corn, rice, potato, wheat, beans starch (*Phaseolus vulgaris*) as well as dextrin, glycogen, carboxymethyl cellulose, glucose, lactose, maltose, sucrose, xylose, glycerol and mannitol were tested, where the amylase production medium was supplemented with 0.5 % (w/v) of the desired substance. Then the enzyme productivity was assayed as mentioned above.

Effect of different nitrogen sources:-

The effect of different inorganic and organic nitrogen on amylase productivity was determined. The amylase production medium was supplemented with 0.1 % (w/v) of the inorganic sources; NH_4Cl, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, $(NH_4)_3PO_4$ as well as of the organic sources; peptone, protease peptone, gelatin, yeast extract, casamino acids, casein, skim milk, fresh milk, meat casein, beef extract, tryptone and fibrin. Productivity was assayed as mentioned above.

Effect of crude amylase on raw starch from different plant sources:-

The ability of amylase produced by *Natrialba aegyptiaca* strain 40^{T} to digest raw starch from different sources was tested following the method described by Hamilton *et al.* (1999) with some modifications as follows; 0.5 gm of raw starch grains i.e. of each of corn, rice, potato, wheat and beans was ground and incubated with 5 ml of crude amylase in 5 ml of 0.1 M citrate buffer at 40 ^{o}C for 1 hr. Soluble starch was used as control. After that the reaction mixture was boiled to stop amylase action and then centrifuged at 6000 rpm for 30 min. The amylase activity was assayed in the supernatant using the iodometric method (Pantschev *et al.*, 1981) as follows; 1 ml of the supernatant was taken in a clean test tube and 1 ml of 0.01 N iodine solution was then added. The amylase activity was measured spectrophotometrically at 580 nm. The activity was calculated in unit/ml using

amylase standard curve. One unit of activity was defined as the amount of enzyme that hydrolyzes 0.5 gm of starch to dextrin per minute.

Results And Discussion

The main aim of this work was to optimize the medium components and cultural conditions for amylase production by the extremely halophilic archaeon *Natrialba aegyptiaca* strain 40^T. This optimization plays a significant role in enhancing amylase production by various microorganisms (Good & Hartman, 1970; Kobayashi *et al.,* 1992; Pérez-Pomares *et al.,* 2003; Fukushima *et al.,* 2005; Hutcheon *et al.,* 2005; Sajedi *et al.,* 2005; Couto & Sanromán, 2006; VijayAnand *et al.,* 2010).

Amylase production

The amylase production by *Natrialba aegyptiaca* strain 40^T was detected and determined on both solid (Fig.1.) and liquid (Fig.2.) production medium.

Fig.2.

Fig.1.

Fig.1. detection of amylase productivity according to Amoozegar *et al.*, (2003).
Fig.2. detection of amylase productivity using the CZT.

Factors affecting amylase production:-

The effect of each of NaCl, KCl, $MgSO_4.7H_2O$, substrate concentration, pH, incubation temperature, incubation period, inoculum size and shaking rate as well as carbon and nitrogen sources on amylase production was tested as mentioned above.

Effect of NaCl concentration:-

As shown in figure (3) amylase production occurred within a broad range of NaCl concentrations, i.e. between 8 and 32 (%, w/v). Production began at 8 (%, w/v), and increased gradually till it reached its maximum rate (30 unit/ml) at 15 (%, w/v), then decreased gradually. From 17.5 to 32 NaCl (saturation) (%, w/v), production rate was stable. This was corresponding to NaCl concentrations required for the strain's growth (Hezayen, 2002; Hezayen *et al.,* 2001). On the other hand and in contrast with our foundation, the maximum amylase production by the extremely halophilic archaea *Haloferax mediterranei* and *Haloarcula sp.* strain S-1 occurred at 25 (%, w/v) NaCl, and 18.5 (%, w/v) for *Haloarcula hispanica* (Pérez-Pomares *et*

al., 2003; Fukushima *et al.,* 2005; Hutcheon *et al.,* 2005). This may be because *Natrialba aegyptiaca* strain 40^T is adapted for growth at lower NaCl concentratins than most other extremely halophilic archaea which require more than 12 (%, w/v) to grow (Hezayen, 2002; Hezayen *et al.,* 2001).

Effect of KCl concentration:-

It is known that K^+ and Cl^- play a very important role in the physiology and growth behavior of the extremely halophilic archaea, consequently enzymes' production. Halophilic archaea accumulate K^+ intracellularly, 4 M KCl, for osmoregulation, cells integrity and adaptation of intracellular and extracellular enzymes for maintaining their activity and stability (Zaccai & Eisenberg, 1990; Madern *et al.,* 2000; Marhuenda-Egea & Bonete, 2002). As shown in (Fig.4) amylases was produced in absence of KCl, because KCl is important for the growth of halophilic archaea, we guess that the strain could use KCl which is present as contaminants in the other components of the production medium. Productivity was slightly stimulated by KCl until 1 (%, w/v) and exhibited its maximum amylase production at 1.5 (%, w/v) KCl. The effect of K^+ ion on amylase production was observed also by Fukushima *et al.,* (2005) who reported that the maximum amylase production by the extremely halophilic archaeon *Haloarcula sp.* S-1 occurred at 0.2 (%, w/v) KCl, while Hutcheon *et al.* (2005) found that the optimum KCl concentration required for the maximum amylase production by the extremely halophilic archaeon *Haloarcula hispanica* was 0.5 (%, w/v).

Effect of $MgSO_4.7H_2O$ concentration:-

Various systems of halophilic enzymes require divalent ions such as Mg^{2+} for their activity and stability (Norberg *et al.,* 1973; Lanyi, 1974). As shown in (Fig.5), the present strain showed maximum amylase production between 0.1 and 0.2 (%, w/v) $MgSO_4.7H_2O$. Lower and higher concentrations inhibited slightly amylase production. The explanation of amylase production in the absence of $MgSO_4.7H_2O$ is that the strain could use $MgSO_4.7H_2O$ which is present as contaminants in the other components of the production medium. In, contrast, it was found that the maximum amylase production was exhibited at 2 and 2.7 (%, w/v) $MgSO_4.7H_2O$ for the extremely halophilic archaea *Haloarcula sp.* S-1 and *Haloarcula hispanica*, respectively (Fukushima *et al.,* 2005; Hutcheon *et al.*, 2005).

Effect of substrate (soluble starch) concentration:-

It was found that substrate concentration has a great effect on amylases production by halophilic archaea. Interestingly, as shown in (Fig. 6), the present strain could produce amylase in the absence of starch. Although productivity was low, it indicates that strain 40 can synthesize amylase constitutively. It was observed that nearly, any addition of starch stimulates amylases production (Fig. 6). The maximum production was exhibited at 0.05 (%, w/v) starch, This was in contrast with the foundations of Pérez-Pomares *et al.* (2003) and Fukushima *et al.* (2005) who reported that that the maximum amylase production by the extremely halophilic archaeon *Haloferax mediterranei* and *Haloarcula sp.* S-1 was exhibited at 0.2 and 1 (%, w/v) soluble starch respectively.

Effect of pH:-

pH of the production medium plays an important role in enzyme secretion. Change in pH during the growth of the organism affects product stability in the medium (Gupta *et al.,* 2003). The pH values also serve as a valuable indicator of the initiation and the end of enzyme's synthesis (Friedrich *et al.,* 1989). As it is shown in (Fig. 7), the amylase production by the strain 40 occurred at a wide pH range i.e. between 5 and 9, with maximum production between 6 to 8 pH. On the other hand, the maximum amylase production by the extremely halophilic archaeon *Haloferax mediterranei* was exhibited at 7.2 pH (Pérez-Pomares *et al.,* 2003). Fukushima *et al.* (2005) observed that pH 7.0 stimulated the maximum production of amylase by the extremely halophilic archaeon *Haloarcula sp.* S-1. Moreover, Hutcheon *et al.* (2005) reported that the pH 6.5 exhibited the maximum amylase production by the extremely halophilic archaeon *Haloarcula hispanica*.

Effect of incubation temperature:-

Temperature has a great effect on growth and amylase production by halophilic archaea. It was interesting to find that amylase production by the present strain occurred within a temperature range from 20 to 60 ^{o}C and maximum production was exhibited between 45 and 47 ^{o}C (Fig. 8). This was in contrast with the foundations of other workers who found that maximum amylase production by the extremely halophilic archaea *Haloferax mediterranei* and *Haloarcula sp.* strain S-1 was observed at 37 ^{o}C and for *Haloarcula hispanica*, was 50 ^{o}C (Pérez-Pomares *et al.,* 2003; Fukushima *et al.,* 2005; Hutcheon *et al.,* 2005). This may be because the present strain adapted to the relatively high temperature which is characteristic to the location of isolation, Aswan in Upper Egypt.

Effect of incubation period:-

In contrast to eubacteria, extremely halophilic archaea are characterized by its long incubation periods, ranging from 4 to 21 days, or more in rare cases (Tomlinson & Hochstein, 1972; Tindall *et al.,* 1984; Montalvo-Rodriguez *et al.,* 1998; Xin *et al.,* 2000; Xu *et al.,* 2001; Gutiérrez *et al.,* 2007). As shown in (Fig.9), production of amylase by strain 40 was detected after an incubation period of 24 hr, it increased gradually with increasing incubation until it reaches its maximum within 144 hr (6 days) under static condition This was in complete accordance with the foundations of Hutcheon *et al.* (2005) who found that the maximum amylase production by the extremely halophilic archaeon *Haloarcula hispanica* occurred within 6-7 days of incubation, and Fukushima *et al.* (2005), who reported that the maximum amylase production by the extremely halophilic archaeon *Haloarcula sp.* S-1 was observed after 7 days. Under shaking, at 150 round per minute (rpm), the maximum production was achieved after 48 hr incubation, above which, productivity was constant. This because presence of high salt concentration in the production medium causes oxygen tension which affects significantly the growth of the strain and therefore the enzyme production, while with shaking, the aeration of the medium increased, leading to sufficient oxygen supply. This increased the rate of growth and subsequently the enzyme production increased (Kumar & Takagi, 1999; Beg *et al.,* 2003).

Effect of inoculum size:-

As it is shown in (Fig. 10), inoculums' size has a great effect on amylase productivity. Production increase strongly with increasing the inoculums' size until it becomes constant. This may be because large inoculums decrease the lag or preparatory phase of growth. It was observed that 4 ml^{-1}/flask (i.e. 8 %, v/v), 1 ml^{-1} contained 1 x 10^8 CFU/ml of 3-days old inoculums of strain 40^T, exhibited the maximum amylase production, above which, productivity was constant.

Effect of shaking rate:-

As mentioned above, shaking accelerates reaching maximum productivity. As shown in Fig. (11), maximum amylase productivity was exhibited at 150 rpm, above this the enzyme production decreased. Productivity increased because shaking leads to sufficient supply of dissolved oxygen in the medium, consequently, nutrient uptake by the strain increased and subsequently the enzyme production increased (Kumar & Takagi, 1999; Beg *et al.,* 2003). Above 150 rpm, the amylase production decreased, this may be because, the higher shaking rates could increase the oxygen supply to the medium but did not bring about the increase in enzyme production, probably because at high shaking rates, the structure of the enzyme would be altered (Roychoudhury *et al.,* 1988). It was reported that the maximum amylase production by the halophilic *Micrococcus halobius*, *Halomonas meridiana*, *Halobacillus sp.* Strain MA-2, *Nesterenkonia sp.* Strain F and *Halomonas sp.* AAD21 was exhibited at agitation rates of 140, 200, 200, 220 and 180 rpm respectively (Onishi & Sonoda, 1979; Coronado *et al.,* 2000; Amoozegar *et al.,* 2003; Shafiei *et al.,* 2010; Uzyol *et al.,* 2012).

Effect of different carbon sources:-

Although, strain 40^T could secrete amylase constitutively, i.e. in the absence of a substrate, it was interestingly found that soluble starch, corn, rice, potato, wheat and beans starch as well as dextrin and glycogen enhanced amylase production strain. The highest amylase productivity was recorded for glycogen followed by rice starch (Fig 12). These natural substrates may be useful as cheaper alternative sources for halophilic amylase production. The other tested sources, i.e. carboxymethyl cellulose, glucose, lactose, maltose, sucrose, xylose, glycerol and mannitol did not enhance the amylase production by the strain. Amoozegar *et al.,* (2003) investigated that the moderately halophilic *Halobacillus* sp. strain MA-2 produce amylase was in the presence of soluble starch, dextrin, maltose, sucrose, lactose, and glucose exhibiting its maximum productivity with dextrin followed by starch. Prakash *et al.,* (2009) reported that few substrates, i.e. maltose and soluble starch induced amylase production by the halotolerant eubacterium *Chromohalobacter* TVSP 101. *Halomonas sp.* AAD21 produced amylase using soluble starch, sucrose, lactose, and potato starch as substrates (Uzyol *et al.,* 2012).

Effect of different nitrogen sources:-

Comparing with the control; NH_4NO_3, casein, meat casein, tryptone, and fibrin inhibited productivity, while no effect of beef extract and nearly casamino acids.

Other tested sources stimulated productivity in variable extents but the maximum was exhibited by yeast extract, followed by gelatin and then $(NH_4)_2SO_4$ (Fig. 13).

It was reported that the addition of inorganic nitrogen sources such as ammonium nitrate, sodium nitrate and ammonium sulfate reduced productivity but peptone exhibited the maximum productivity of amylase by the moderately halophilic bacterium *Halomonas* sp. strain AAD21 (Uzyol *et al.*, 2012). The maximum productivity of amylase by the extremely halophilic archaeon Halobacterium halobium occurred when peptone was used as nitrogen source (Patel *et al.*, 1993).

Ideal conditions for maximum amylase production:-

From studying the factors affecting amylase productivity by strain 40^T, the optimum nutritional and environmental requirements for maximum amylase production were as follows (%, w/v): NaCl, 15; soluble starch, 0.05; KCl, 1.5; $MgSO_4.7H_2O$; 0.1; pH 6-8; inoculum size 8 % (v/v) of $1x10^8$ CFU/ml; incubation period 48 h, shaking rate, 150 rpm and the incubation temperature 45 - 47 ^oC.

Digestion of raw starches from different plant sources:-

As it is shown in (Fig. 14), the present amylase could hydrolyze all tested raw starches, mentioned above. Compared with soluble starch as a control, it was found that corn, wheat and bean were hydrolyzed at the same degree of soluble starch hydrolysis. Amylase exhibited the maximum effect in case of rice as a substrate, while potato starch was less hydrolyzed, because potato starch is hardly digested by amylases because of the larger size of its granules (Hamilton *et al.*, 1999; Nidhi *et al.*, 2005). It is known that corn, wheat and rice are among the main cereals used for the preparation of fermented foods and beverages (KyalAkond, 2005). Subsequently, it is suggested that the present amylase can be used in the first stage of industrial starch hydrolysis instead of using chemicals such as hydrochloric acid, as enzymatic pre-treatment of rice starch as the first step in rice wine preparation process to produce dextrins and reducing sugars which could be, later, fermented by yeast to produce alcohol.

Legend figures

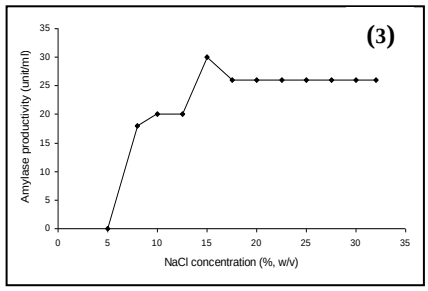

Fig.3. Effect of NaCl concentration

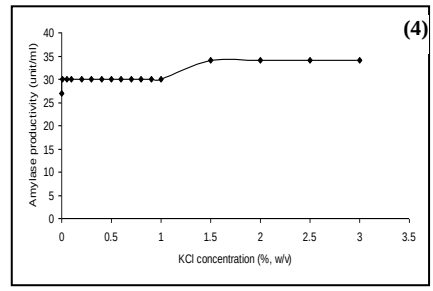

Fig.4. Effect of KCl concentration

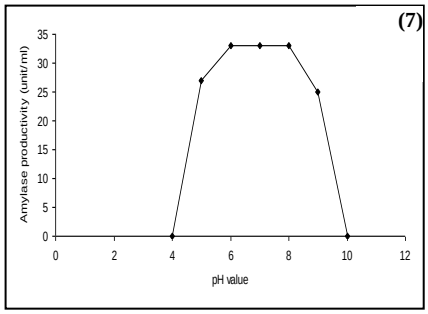

Fig.5. Effect of $MgSO_4.7H_2O$ concentration

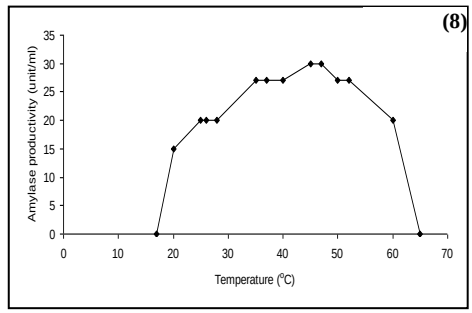

Fig.6. Effect of substrate concentration

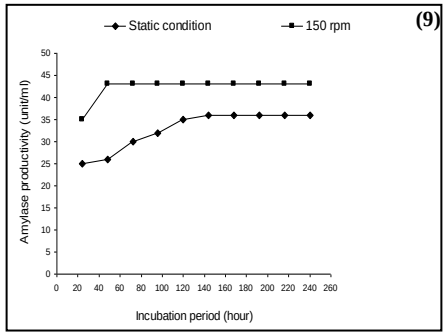

Fig.7. Effect of pH

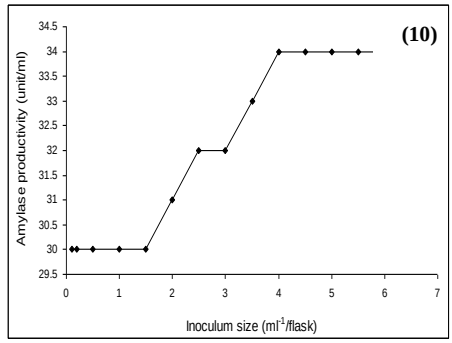

Fig. 8. Effect of incubation temperature

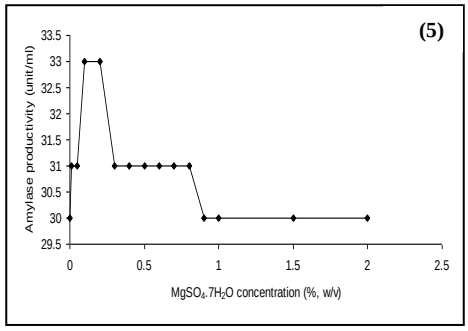

Fig.9. Effect of incubation period

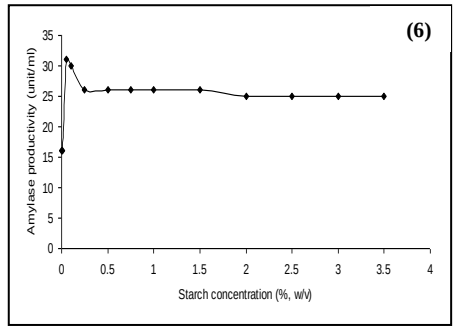

Fig.10. Effect of inoculum size

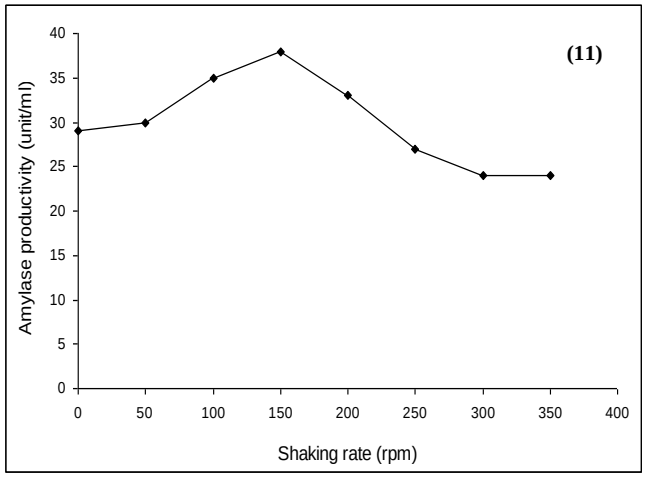

Fig.11. Effect of shaking rate

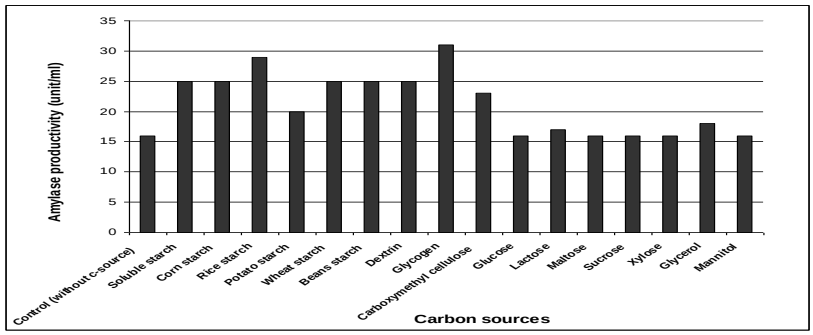

Fig. 12. The effect of different carbon sources on amylase productivity by strain 40^T

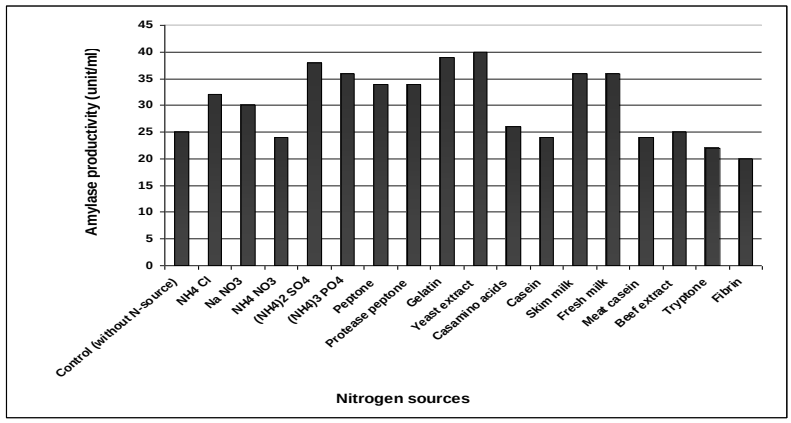

Fig.13. The effect of different nitrogen sources on amylase productivity by strain 40^T

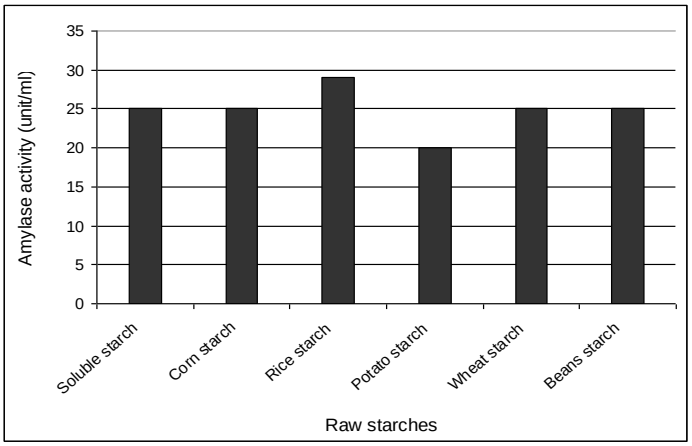

Fig.14. Digestion of raw starches from different plant sources by the crude amylase

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