WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 631-641. Research Article ISSN 2277– 7105

AMYLASE ENZYME PRODUCTION USING TAPIOCA WHITE PEELS

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Article Received on
30 April 2015,

Revised on 25 May 2015,
Accepted on 16 June 2015

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ABSTRACTS

Amylases are one of the most important enzymes in present-day biotechnology. The present study was aimed to produce amylase under optimized conditions utilizing White Tapioca peels as substrate. The objectives of the present study includes the analysis of various physical factors that influence the growth and the production of the amylase such as temperature and pH, substrateconcentration,Incubation,effecet of different Carbonsources& Nitrogen sources. Substrate cmc for obtaining maximum yield. *B. amyloliquefaciens*strain was obtained from (MTCC1488) and organism exhibited higher amylase activity .On analyzing the physical parameters the optimum incubation time for amylase production is 72 hrs and pH found to be 8.Optimum temperature was found to be 40°C because activity was higher at this temperature. Sucrose and casein were effecetcarbon sources and nitrogen sources respectively. On above mentioned optimum conditions amylase enzyme was produced by *B. amyloliquefaciens*in higher amount of 2.890 (U/ml).

KEYWORDS: *B. amyloliquefaciens*strain,

INTRODUCTION

Enzymes are biological catalysts; they are highly specialized catalytic proteins with extraordinary catalytic power and also have remarkable specificity. They are essential for all forms of life by catalyzing the various chemical reactions in the cells. **(Sankaralingam*et al.*, 2012).**Starch is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as an energy store. It is the most common carbohydrate in human diets and is contained in large amounts in such staple foods as potatoes, wheat, maize (corn), rice and cassava. Pure starch is white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two molecules:

the linear and helical amylase and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight **(Brown *et al.*, 2005).**

Amylases [α-amylase, β-amylase and glucoamylase (GA)] are among the most important enzymes in present-day biotechnology. The enzymes of amylase family have great significance due to its wide area of potential application. The spectrum of amylase application has widened in many other fields, such as clinical, medical and analytical chemistry**. (Suman. and Ramesh,2010).**The amylases can be derived from several sources such as plants, animals, and microbes. The major advantage of using microorganisms for production of amylase is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics. The microbial amylases meet industrial demands; a large number of them are available commercially; and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry. Although many microorganism produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis*, *Bacillus amyloliquefaciens*and *Aspergillusniger*. Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries. They are mainly employed for starch liquefaction to reduce their viscosity, production of maltose high fructose syrup and maltotetraose syrup. In detergent production, they are applied to improve cleaning effect and also used for starch de-sizing in textile industry **(Vidyalakshmi*et al.*, 2009)**.

Tapioca is a starch extracted from Manioc (Manihotesculenta). This species is native to the Northeast of Brazil but spread throughout the South American continent. The plant was spread by Portuguese and Spanish explorers to most of the West Indies, Africa and Asia, including the Philippines and Taiwan, being now cultivated worldwide.In Brazil, the plant (cassava) is named "mandioca", while its starch is called "tapioca" **(Thomson*et al.*, 2005**) Amylase from plant and microbial sources have been employed for centuries as food additives. Barely amylases have been used in the brewing industry. Fungal amylase have been widely used for the preparation of oriental foods. In spite of the wide distribution of amylase, microbial sources, namely fungal and bacterial amylase, are used for the industrial production due to the advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimation.**(Burhan*et***

***al.*,2003)**Sources of amylase in yeast and moulds have been reported and their properties have also been described by **(Adebiyi*et al*.,1998;Buzzini *et al*., 2002).**

MATERIALS AND METHOD

Substrate collection

Tapioca white peels were collected from in and around Kanyakumari District. The substrate was dried in sun shade & made into powder by means of mechanical blenders.

Bacterial Strain

Bacillus amyloliquefaciens(MTCC 1488) was obtained from Microbial Type Culture Collection, Chandigarh. The culture was maintained in Luria broth.

Screening of amylase producing bacteria

Luria agar medium was supplemented with 0.1% starch and culture was streaked onto the plate incubated for 24 hrs. After 24 hrs the plates were flooded with iodine-KI solution. The whitecolour zone formation indicates the utilization of starch **(Thippeswamy*et al.*, 2006).**

Media optimization

Effect of incubation time on amylase production

The production media was prepared with all the nutritional components and the culture was inoculated and kept for incubation at varying time duration (24, 48 and 72hrs). After incubation the amylase activity was estimated using Iodine-Potassium solution **(Palanivelu, 2004).**

Effect of pH

The production media was prepared with all the nutritional components with the alteration in the pH alone varying from (4, 6, and 10) was inoculated with the culture and incubated for 72 hrs. After incubation the amylase activity was assayed using Iodine-Potassium Iodide solution **(Palanivelu, 2004).**

Effect of Temperature

The production media was prepared with all the nutritional components with the alternative in the temperature alone varying from (20°C ,30°C ,40°C and 50°C) was inoculated with the culture and incubated. After incubation the amylase activity was estimated using Iodine-Potassium Iodide solution **(Palanivelu, 2004).**

Effect of Carbon source

Amylase production media was prepared for 100ml with different carbon sources, 0.5 g of maltose, 0.5g of sucrose, and0.5g of lactose. The culture was inoculated and incubated. The amylase activity was determined using iodine-Potassium Iodide solution **(Palanivelu, 2004).**

Effect of Nitrogen source

Amylase production media was prepared for 100ml with different nitrogen sources, 0.5g of beef extract, 0.5g casein and 0.5g ammonium sulphate. The culture was inoculated and incubated at 72 hrs .The amylase activity was assayed using iodine solution **(Palanivelu, 2004**).

Effect of substrate concentration on amylase production

To find the suitable concentration of substrate for amylase production, it was carried out using different concentrations of substrate such as 0.5,1, 1.5, 2g/100ml of the production medium. The enzyme activity was determined using iodine solution (**Palanivelu, 2004).**

Effect of inoculum size on amylase production

Amylase production was carried out using different inoculum size of 0.5, 1, 1.5 and 2 ml/100ml of the production medium. The enzyme activity was assayed using iodine solution **(Palanivelu, 2004).**

Amylase enzyme production

The liquid medium containing 0.6% peptone 0.5% Mg SO_4, 0.5% KCl and 2g of Tapioca peels as substrate, pH 8.0 was prepared. To the media 0.5g of sucrose as carbon source and white 0.5g of casein as nitrogen sources were added and inoculated with a 1.5ml of culture. Culture was grown in 250 ml Erlenmeyer's flasks with 100ml of medium in a rotary shaker (100rpm) at 40 ° C and incubated for 72 hrs. After 72 hrs the supernatant was separated by centrifugation and used to evaluate total amylase activity.

Total amylase activity

Maltose (100mg / 100ml) was used as standard and different concentrations of maltose between 0.2 to 1ml was taken and made up to 10 ml with distilled water from stock solutions. From the above test tubes, 0.5ml of working standard was taken in fresh test tubes. 5 ml of starch solution was added and incubated 90 °C at 10 min .The reaction was stopped by

addition of 5 ml of 0.1N HCl. One ml of iodine solution was added to all tubes. Read absorbency at 640 nm

RESULTS

Collection of substrate

Tapioca White peels was collected from Kanyakumari District made into powder.

Screening of amylase producing bacteria

Luria agar medium was supplemented with 0.1% starch and culture was streaked onto the plate incubated for 24 hrs. After 24 hrs the plates were flooded with iodine solution. The whitecolourzone formation indicates the utilization of starch by the organism. According to screening, *Bacillus* sp has ability to produced amylase.

Effect of incubation time on amylase production

In the present study amylase activity was higher in Tapioca white peels at 48hrs of incubation.

Effect of pH on amylase production

The amylase production by *B. amyloliquefaciens*was found maximum at pH 8 (Fig.1).The medium containing white peel as substrates and pH 8, the amylase activity was 0.950 (U/ml).

Effect of Temperature of the medium on amylase production

The amylase activity was higher in 40°C so the optimum temperature was found to be 40°C (Fig.2)

Effect of Carbon sources on amylase production

In the current study amylase activity was higher in presence of 0.5g of sucrose as carbon source (Fig.3).So it is concluded that sucrose as carbon sources enhances the amylase production.

Effect of Nitrogen sources of amylase production

In the current study amylase activity was higher in presence of 0.5g of casein as Nitrogen sources (Fig.4). So it is concluded that casein as nitrogen sources enhances the amylase production.

Effect of substrate concentration on amylase production:

In the current study amylase activity was higher in presence of 2g of substrate (Fig.5). The medium containing white peels as substrate produced enzyme activity was 0.769 (U/ml).

Effect of Inoculum

In the current study amylase activity was higher in presence of 1.5ml of inoculums size. Optimum inoculum size was found to be 1.5 ml. (fig.6)

Amylase production

Strains presenting large clearing zones were used for amylase production assay on liquid medium. The liquid medium containing 0.6% peptone 0.5% Mg SO_4, 0.5% KCl and 2g of substrate pH 8.0 was prepared. To the media 0.5g of sucrose as carbon source and 0.5g of casein as nitrogen sources were added and inoculated with a 1.5ml of culture. Culture was grown in 250 ml Erlenmeyers flasks with 100ml of medium in a rotary shaker (100 rpm) at 40°C and incubated for 72 hrs. After 72 hrs, the enzyme was produced .The culture supernatant was separated by centrifugation and used to evaluate total amylase activity. The total activity of amylase enzymes produced was 2.890(U/ml).

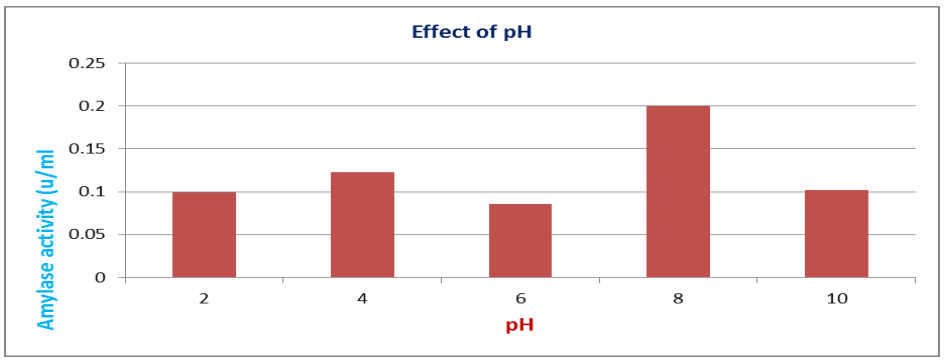

Fig.1: Effect of pH on amylase production

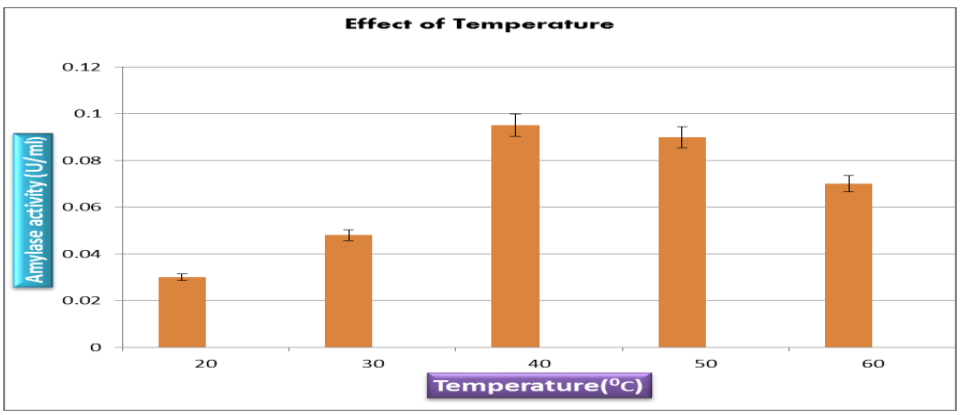

Fig.2: Effect of Temperature on amylase producti

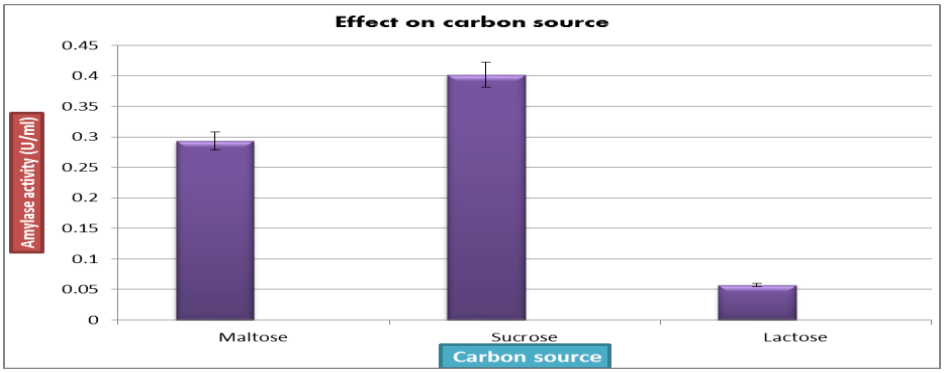

Fig.3: Effect of Carbon sources on amylase production

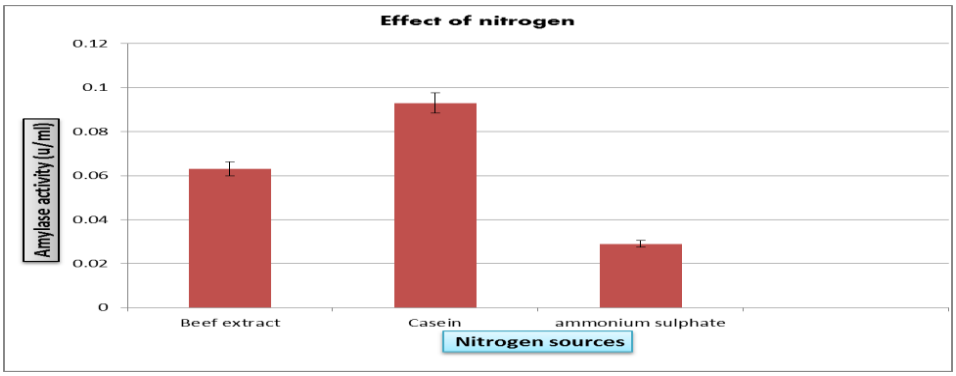

Fig.4: Effect of Nitrogen sources on amylase production

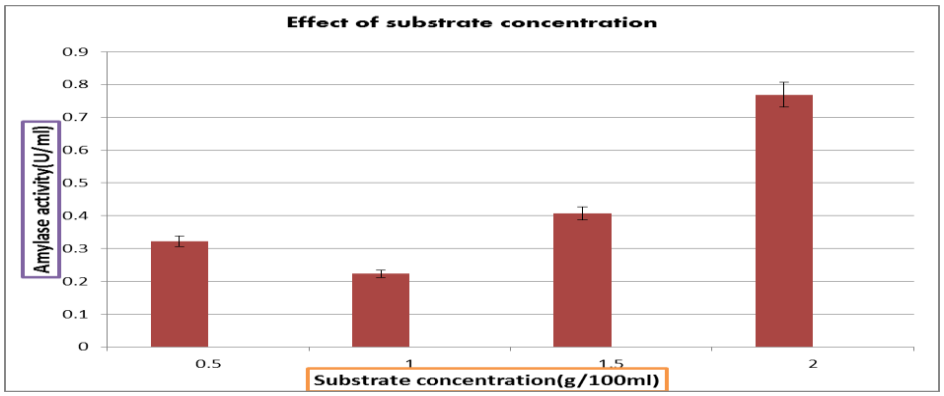

Fig.5: Effect of substrate concentration on amylase production

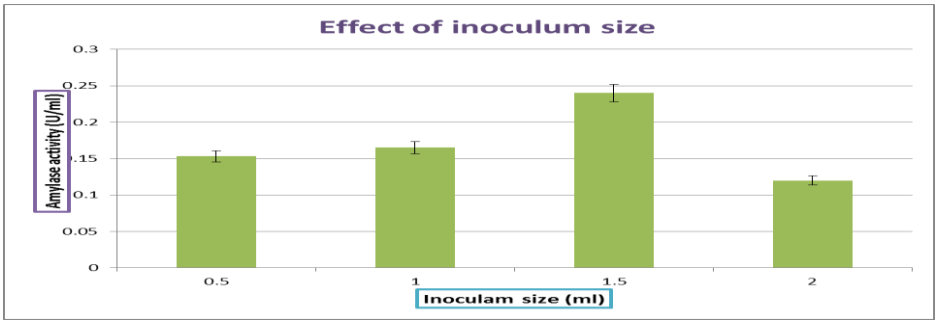

Fig.6: Effect of inoculum size on amylase productio

DISCUSSION

Amylases are widely distributed and are one of the most studied enzymes. These enzymes have wide scale application ranging from food to effluent treatment. Amylases are a class of enzymes (hydrolases) that are capable of digesting the glycosidic linkages found in starch or glycogen. Under aqueous conditions amylases act on glycosidic bonds present in starch. Starch degrading enzymes like amylase have received a great deal of attention because of their perceived technological significance and economic benefits. This enzyme is also useful for the commercial production of glucose. Nowadays, the renewed interest in the exploration of extracellular amylase production in bacteria and fungi is due to various industrial applications. Few attempts have been made to elucidate the control mechanism involved in the formation and secretion of the extracellular enzymes. The production of alpha amylase by moulds has been greatly reported. In present work *Bacillus amyloliquefaciens*was found as an effective enzymes producer though submerged fermentation process. In the present study the highest amount of amylase production was observed in lactose supplemented medium and conservation was that the production of amylase was stimulated by the presence of glucose, lactose and starch by *Bacillus* sp. Temperature is one of the important factors, which strongly affect the submerged fermentation **(Vidyalakhsmi*et al.*, 2009)**. Variation of the temperature brought about a change in metabolic pattern of the micro-organism; it exhibited its best amylase production in the mesophilic range **(Vidyalakhsmi*et al.*, 2009).**

Among tested nitrogen sources, organic nitrogen source supported maximum amylase yield when compared to inorganic nitrogen source. In this study the highest level of amylase yield was registered in tryptone added medium. This is correlated with earlier studies of **Nanag and Satyanarayana, (1994)** who reported that starch and tryptone have important source for maximizing the amylase production. The amylase synthesis by microorganisms has been correlated to the presence (or) absorbence of different nitrogen sources and various amino acids in the growth medium. The difference in nutritional requirements of various α-amylase producing organisms could be attributed to the differences in their genetics.

In this study, the maximum amylase production was recorded in ammonium nitrate supplemented medium within the tested inorganic nitrogen sources. Reported that the supplementation of inorganic nitrogen salts greatly increased the enzyme yields in *Aspergillusfoetidus*. The inhibitory effect of some of the salts may be related to the pH changes associated with their use in the medium. The enzyme is very sensitive to pH.

Therefore the selection of optimum pH is very essential for the production of α-amylase. In this study the highest yield of amylase was recorded in pH at 7. and temperature at C. **Narayana and Vidaylakshmi(2009)** also reported the op mum temperature and pH as C and 6.5, respectively, for the production of α-amylase from fungal species. The present study various surfactants were used for maximizing the amylase production. Among the tested surfactants, the maximum yield was recorded in Tween 80 supplemented medium over the control. In consistence with this present study, microbes such as *Bacillus* sp and *Thermomyces*sp were reported that the higher yield was registered in surfactants added medium. **Swain *et al* (2007)** were also registered that the increased enzyme yield might be due to cell membrane permeability. The addition of carbon source in the form of their monosaccharide or polysaccharides may influence production of amylase enzyme.

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