

## A REVIEW OF LITERATURE ON ISOLATION OF BACTERIA $\alpha$ - AMYLASE

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**Abstract** - Alpha-amylases are enzymes that catalyses the hydrolysis of starch into oligosaccharides, maltose and glucose. They are widely distributed in microbial, plant and animal kingdoms.  $\alpha$ -amylases are one of most important and used enzymes whose range of application has widened in many sectors, which include manufacture of High Fructose Corn Syrup (HFCS). This study is aimed at reviewing related literature on isolating and production of amylase from bacteria. Literature reviewed in this article include those on isolation of bacteria producing amylase, production and optimization processes involved and other closely related and recent literature.

This study also seeks to discover research gap in this field of study that needs to be filled by researchers and appropriate recommendations made. A thorough literature was conducted to come up with the conclusions in this review article.

**Key Words:** Bacillus Subtilis,  $\alpha$ - Amylase, amylolytic enzyme, bacteria, isolation.

### 1. INTRODUCTION

Amylases are enzyme that catalyses the hydrolysis of starch into sugars. They are present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth. The pancreas and salivary gland makes amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. Amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833). (Richard, 2002)

The history of amylases began in 1811 when the first starch degrading enzyme was discovered by Kirchoff this was followed by several reports of digestive amylases and malt amylases. It was much later in 1930 that Ohlsson suggested the classification of starch digestive enzymes in malt as  $\alpha$  and  $\beta$  amylases according to the anomeric type of sugars produced by the enzyme reaction.

Amylases can be classified into different types; alpha, beta amylase and debranching enzyme. The  $\alpha$ -amylases (EC 3.2.1.1) (alternative names: 1,4- $\alpha$ -D-glucan glucanohydrolase; glycogenase) are calcium metalloenzymes, completely unable to function in the absence of calcium. They act at random locations along the starch chain,  $\alpha$ -amylase breaks down long-chain carbohydrates, yielding maltotriose and maltose from amylose, maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate,  $\alpha$ -amylase tends to be faster-acting than  $\beta$ -amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0. The  $\alpha$ -amylases form is also found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (*Bacillus*).

Another form of amylase,  $\beta$ -amylase (EC 3.2.1.2) (alternative names: 1,4- $\alpha$ -D-glucan maltohydrolase; glycogenase; saccharogen amylase) is also synthesized by bacteria, fungi, and plants. They act from the non-reducing end,  $\beta$ -amylase catalyzes the hydrolysis of the second  $\alpha$ -1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit,  $\beta$ -amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. Both  $\alpha$ -amylase and  $\beta$ -amylase are present in seeds;  $\beta$ -amylase is present in an inactive form prior to germination, whereas  $\alpha$ -amylase and proteases appear once germination has begun. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain  $\beta$ -amylase, although it may be present in microorganisms contained within the digestive tract. The optimum pH for  $\beta$ -amylase is 4.0–5.0

Alpha amylases are one of most important and used enzymes whose range of application has widened in many sectors. They have found applications in food, baking, brewing, textile and paper industries. Increasing utility and consumption of alpha amylase in different industries has placed a greater stress on increasing indigenous enzyme production and search of more rapid processes (Carlsen *et al.*, 1996; Ramachandran *et al.*, 2004; Kathiresan and Manivanan, 2006; Gupta *et al.*, 2008).

Though  $\alpha$ -amylase can be derived from several sources such as plants, animals and microorganisms, the production from plants and animals is limited because of low concentration of enzymes in the plant material, thus requiring the processing of large amount of plant material; on the other hand enzyme of animal origin is by-product of meat industry. However bacteria can produce large amounts of the enzyme through fermentation to meet the market demands.

### 1.1 STUDY OBJECTIVES

This study is aimed at reviewing related literature on isolating and production of amylase from bacteria and also production and optimization processes involved. Literature reviewed in this article include those on isolation of bacteria producing amylase, production and optimization processes involved and other closely related and recent literature.

This study also seeks to discover research gap in this field of study that needs to be filled by researchers in this field and appropriate recommendations made.

## 2.0 LITERATURE REVIEW

### 2.1 Historical Background

Amylase is one of the oldest enzymes known, the history of amylase began in 1815 when Kirchhoff laid the foundation for the discovery of amylase, Kirchhoff performed an experiment, which converted four parts of water, two parts of starch, and malt into a starch paste. This paste began to liquefy into sweet syrup; his results showed that gluten had the capacity to convert a larger quantity of starch into sugar. In 1831, Erhard Friedrich Leuchs described the hydrolysis of starch by saliva, due to the presence of an enzyme in saliva, "ptyalin", an amylase (Erhard 1831). In 1833, Anselme Payen and Jean-François Persoz further describe and isolate diastase (amylase) in powder form from barley malt, showing it to be heat labile (Encyclopedia.com).

Enzymes capable of hydrolysing starch and related saccharides are produced by both prokaryotes and eukaryotes, i.e. by organisms belonging to all the three domains of life: Bacteria, Archaea and Eucarya. In other words, different amylolytic enzymes are known to be of animal, plant and microbial origins. Since degradation of starch usually requires co-operation of several amylolytic enzymes, starch-degrading organisms often have several amylolytic activities (Vihinen & Mäntsälä, 1989). On the other hand, not all the living organisms able to utilise starch and/or related saccharides always produce all the enzymes necessary for complete degradation of these substrates.  $\alpha$ -Amylases and related enzymes (e.g.  $\alpha$ -glucosidases, pullulanases) as well as glucoamylases have been reported to occur in a wide variety of organisms, especially of microorganisms (Fogarty, 1980; Vihinen & Mäntsälä, 1989). They are produced also by animals and plants (Janeček & Balaz, 1992; Pandey, 1995). On the other hand,  $\beta$ -amylases have been found to be distributed in higher plants and some microorganisms only (Adachi *et al.* 1998; Mikami *et al.*, 1999).

Due to the improvement of the industrial starch degradation process there has been a great interest in extremely thermostable amylolytic enzymes, especially in glucoamylase involved in the second step, i.e. in conversion of starch dextrin to glucose (Legin *et al.*, 1998; Reilly, 1999). In the first step, degradation of starch into the limit dextrin, the highly thermostable  $\alpha$ -amylase from *Bacillus licheniformis* is used at about 95°C (Legin *et al.*, 1998). The used glucoamylase, which is produced by filamentous fungi, works at 60°C (Reilly, 1999). Having thermostable glucoamylase suitable for the industrial use would reduce the cost of the starch degradation process by reducing the process to a single step. Since Archaea were found to be a good source of hyperextremostable enzymes (Woese, 1987), many efforts have been aimed at finding, isolation and biochemical characterisation of amylolytic enzymes, especially glucoamylase, of archaeal origin.

### 2.2 Different forms amylases

At present there are more than about 30 different amylolytic and related enzymes (Janeček, 1997). Degradation of starch is essentially performed by the four groups of enzymes (Guzmán-Maldonado & Paredes-López, 1995): endo and exo-amylases acting primarily on  $\alpha$ -1,4-linkages, debranching enzymes attacking mainly the  $\alpha$ -1,6-linkages, and cyclodextrin glycosyltransferases that degrades starch by catalysing mainly cyclisation and disproportionate reactions.

Endoamylases cleave only the  $\alpha$ -1,4-bonds in starch in the inner regions of the starch molecule by passing the  $\alpha$ -1,6-branching points of amylopectin (Vihinen & Mäntsälä, 1989).

The  $\alpha$ -amylase (EC 3.2.1.1) is the best known endoamylase. It causes a rapid loss of viscosity of the starch solution. These enzymes are often divided according to degree of hydrolysis of substrate into two categories: liquifying (30-40%) and saccharifying (50-60%). This division is widely used to describe the properties of  $\alpha$ -amylases (Vihinen & Mäntsälä, 1989). Thus the products of endoamylases are oligosaccharides of varying lengths.

Exoamylases also cleave the  $\alpha$ -1,4-bonds, e.g.  $\beta$ -amylase (EC 3.2.1.2), but some of them are able to attack the  $\alpha$ -1,6-bonds, e.g. glucoamylase (EC 3.2.1.3). These enzymes act externally on substrate bonds from the non-reducing end of starch and hence produce only low molecular weight products from starch, e.g. maltose and glucose, respectively (Wind, 1997).

Pullulanase (EC 3.2.1.41) and isoamylase (EC 3.2.1.68) may be the examples of debranching enzymes. Both are specific for  $\alpha$ -1,6-bonds in starch (amylopectin) and related polysaccharides and branched limit dextrins. According to the inability or ability to degrade also the  $\alpha$ -1,4- glucosidic bonds, pullulanases are classified into two categories (Wind, 1997): pullulanase I and pullulanase II, respectively. Pullulanase type II is usually referred to as  $\beta$ -amylase-pullulanase or amylopullulanase.

The fourth group of starch-degrading enzymes is the cyclodextrin glycosyltransferases (CGTases, EC 2.4.1.19). They produce cyclodextrins from starch, the rings which are composed of 6, 7 or 8 glucose units bound by  $\alpha$ -1,4- bonds (Pócsi, 1999). The CGTases catalyse intra and intermolecular reaction of glycosyl transfer (Svensson & Sogaard, 1993).

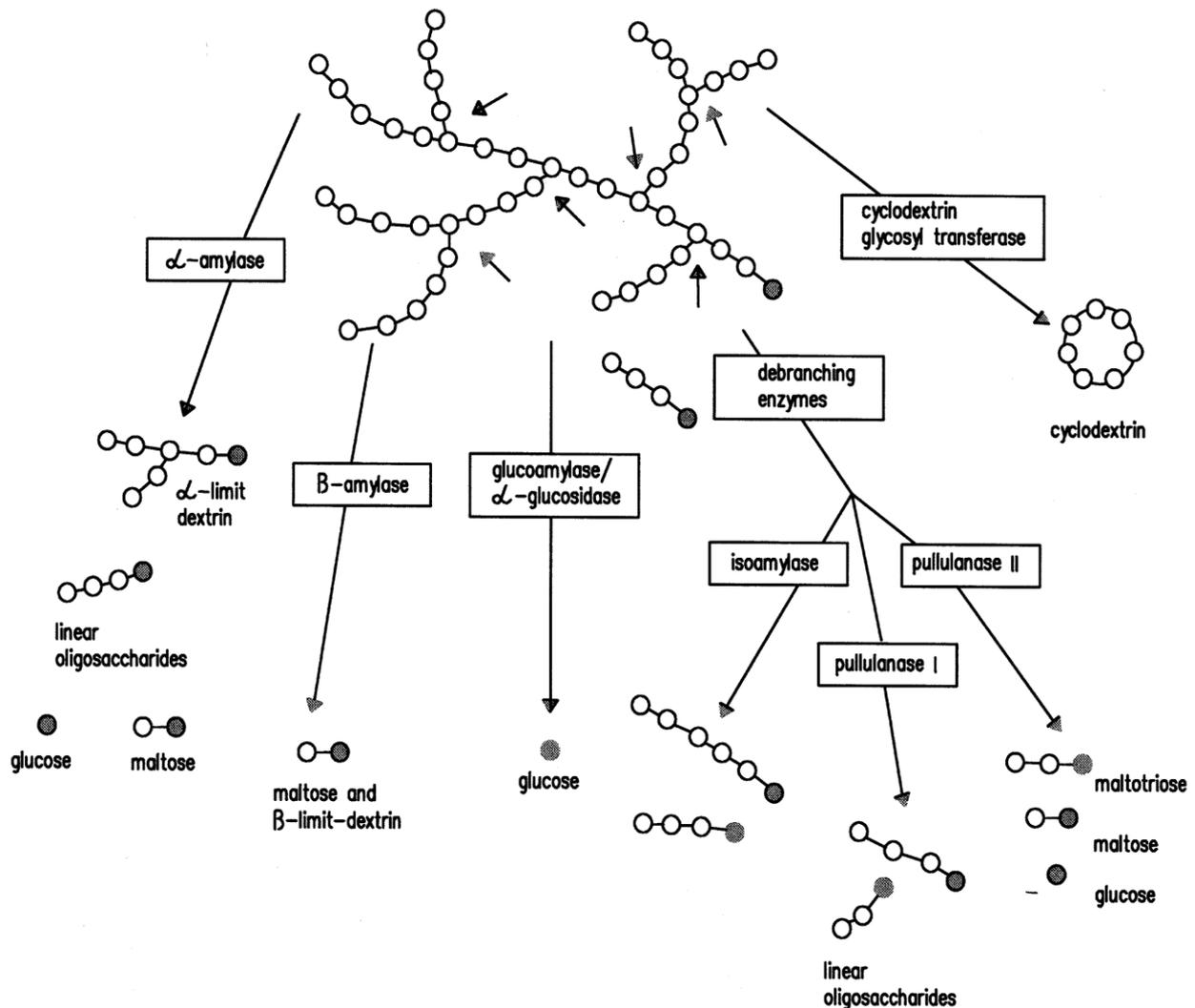


Figure 2.1. Action of starch-degrading enzymes on starch molecule. (Antranikian, 1991)

### 2.3 Sources of amylases

Amylases are ubiquitous enzymes produced by plants, animals and microbes, where they play a dominant role in carbohydrate metabolism (Varel *et al*, 1994). Amylases from plant and microbial sources are employed for centuries as food additives (Mabel *et al*, 2008). Barley amylases are used in Brewing industry. Fungal amylases are widely used in preparation of oriental foods (Popovic *et al*, 2009). Fungal and bacterial amylases are mainly used for industrial applications due to their cost effectiveness, consistency, less time and space requirement for production and ease of process optimization and modification (Ellaiah *et al*, 2002).

Among bacteria, *Bacillus.sp* is widely used for the production of amylases. Species like *B.subtilis*, *B.stearothermophilus*, *B.licheniformis*, and *B.amyloliquefaciens* are known to be good producers of alpha amylase (Harshemi *et al*, 2011). Similarly filamentous fungi have been widely used for the production of amylases for centuries (Juliana *et al*, 2011). As these moulds are known to be prolific producers of extracellular proteins, they are widely exploited for the production of different enzymes including alpha amylases (Kozunari *et al*, 2011). Fungi belonging to the genus *Aspergillus* have been most commonly employed for the production of alpha amylase.

## 2.4 Fermentative production of amylase

Low cost medium is required for the production of amylase, to meet the demand of industries (Aliyu *et al.*, 2011). Both Solid State Fermentation (SSF) and submerged fermentation (SMF) could be used for the production of amylase, although traditionally these have been obtained from submerged cultures because of easy handling and greater control of environmental factors such as temperature and pH (Xusheng *et al.*, 2011). Mostly synthetic media have been used for the production of bacterial amylase through SmF (Ajay *et al.*, 2010). The contents of synthetic media such as nutrient broth, soluble starch, as well as other components are very expensive and these could be replaced with cheaper agricultural by products for the reduction of the cost and the medium (Solange *et al.*, 2010). The solid substrate may provide only support and nutrition (Hashemi *et al.*, 2011). SSF is considered as an interesting alternative since the metabolites so produced are concentrated and purification is of less quality (Nasrin *et al.*, 2010). SSF is preferred to SmF because of simple technique, low capital investment, lower levels of catabolite repression and end product inhibition, low waste water output better product recovery and high quality has been reported to produce promising results (Ajay *et al.*, 2010) other substrates such as sunflower meal, rice husk, cotton seed meal, soybean meal, rice husk, cotton seed meal, soy bean meal, and pearl millet and rice bran have been tried for SSF (Maryam *et al.*, 2010).

SSF technique is generally confined to the process involving fungi (Kiran *et al.*, 2010). However, successful bacterial growth in SSF is known much in natural fermentation (Lonsane *et al.*, 1990). The production of alpha amylase by SSF is limited to the genus *Bacillus* like *B.subtilis*, *B.polymaxa*, *B.mesentiricus*, *B.vulgarus*, *B.coagulans*, *B.megaterium* and *B.licheniformis* have been used for alpha amylase production in SSF (Natasa *et al.*, 2011). The production of bacterial amylase using alpha amylase technique requires less fermentation time which leads to considerable reduction in the capital and recurring expenditure (Li Zhuang *et al.*, 2011). Research on the selection of suitable substrates for SSF has mainly been centred around agro industrial residues due to their potential advantages for filamentous fungi which are capable of penetrating into the hardest of these solid substrates, aided by the presence of turgor pressure at the tip of the mycelium (Maryam *et al.*, 2010). In addition, the utilization of these agro industrial wastes, not only provides alternative substrates but also on the other hand helps in solving pollution problems (Priya *et al.*, 2011).

Table 2.1: Various agro-industrial residues (agrosubstrates) used for  $\alpha$ -amylase production

Substrate	Organism	Activity, U/g	Reference
Wheat bran	<i>Bacillus sp.</i> PS-7	464000	Sodhi <i>et al.</i> , 2005
Spent brewing grain	<i>A. oryzae</i> NRRL 6270	6 583	Francis <i>et al.</i> , 2003
Maize bran	<i>B. coagulans</i>	22 956	Babu & Satyanarayana 1995
Rice bran	<i>Bacillus sp.</i> PS-7	145 000	Sodhi <i>et al.</i> , 2005
Rice husk	<i>B. subtilis</i>	21 760	Baysal <i>et al.</i> , 2003
Coconut oil cake	<i>A. oryzae</i>	3 388	Ramachandran <i>et al.</i> , 2004
Mustard oil cake	<i>B. coagulans</i>	5953	Babu & Satyanarayana 1995
Corn bran	<i>Bacillus sp.</i> PS-7	97600	Sodhi <i>et al.</i> , 2005
Amaranthus grains	<i>Aspergillus flavus</i>	1920	Vishwanthan & Surlikar 2001
Gram bran	<i>B.coagulans</i>	8984	Babu & Satyanarayana 1995

### 2.4.1 Process optimization

Optimization of the various parameters and manipulations of media are one of the most important techniques used for the over production of amylase in large quantities (Balasubramanien *et al.*, 2011). To meet industrial demands production of alpha amylase in bacteria is known to depend on both morphological and metabolic state of the culture (Juliana *et al.*, 2011). Various physical and chemical factors have been known to effect the production of alpha amylase such as pH, temperature, Incubation period, carbon, nitrogen sources, surfactants, phosphate, different metal ions, moisture and agitation with respect to SSF and SmF (Ellaiah *et al.*, 2002).

### 2.4.2 Temperature

The influence of temperature on amylase production is related to the growth of the organism (Pandey *et al.*, 1990). Hence the optimum temperature depends on whether the culture is mesophilic or thermophilic. Among the fungi most amylase production studies have been done with mesophilic fungi within the temperature range of 25-37°C (Takahiro *et al.*, 2011). A raw starch degrading amylase was produced by *Aspergillus ficum* at 30°C by Hayashida *et al* in 1986. Amylase production at optimum level has been reported between 50-55°C for the thermophilic fungal cultures such as *Talaromyces emersonni*, *Thermomonospora fusca* etc (Ahmad *et al.*, 2010).

### 2.4.3 pH

pH is one of the important factors that determine the growth and morphology of microorganisms as they are sensitive to the concentration of hydrogen ions present in the medium (Ellaiah *et al.*, 2002). Earlier studies have revealed that fungi required slightly acidic pH and bacteria required neutral pH for optimum growth. pH is known to effect the synthesis and secretion of alpha amylase (Yakup *et al.*, 2010). Fungi of *Aspergillus* sp. such as *A.oryzae*, *A.ficum* and *A.niger* were found to give significant yields of alpha amylase at pH equal to 5.0 to 6.0 in SmF (Parveen *et al.*, 2011). Alpha amylase producing yeast strains such as *S.cerevisiae* and *S.kluyveri* exhibited maximum enzyme production at pH 5.0 (Samrat *et al.*, 2011).

### 2.4.4 Carbon sources

Carbon sources such as galactose, glycogen and Inulin have been reported as suitable substrates for the production of amylases by *B.licheniformis* and *Bacillus*.sp.1-3 (Xusheng *et al.*, 2011). Starch and glycerol were known to increase enzyme production in *B.subtilis* IMG22, *Bacillus* sp, PS-7 and *Bacillus* sp.1-3 (Maryam *et al.*, 2010). Soluble starch has been found as the best substrate for the production of alpha amylase by *B.stearothermophilus* (Srivastava *et al.*, 1986). *Bacillus* sp. was noted to give a maximum raw starch digesting amylase in a medium containing lactose (1%) and yeast extract (15%). Agricultural wastes are being used for both liquid and solid fermentation to reduce the cost of fermentation media. The waste consists of carbon and nitrogen sources necessary for the growth and metabolism of organism. These nutrients sources include orange waste, peer millet starch, potato, corn, tapioca, wheat and rice as flours (Lin Hui *et al.*, 2011).

### 2.4.5 Nitrogen sources:

Soybean meal was found as the best nitrogen source for alpha amylase by *Bacillus* sp. 1-3. Tanyildizi *et al* reported that peptone increased enzyme activity while yeast extract exhibited no effect on alpha amylase production (Arpana *et al.*, 2011). Strains of *Bacillus* *stearothermophilus* and *B.amylolyticus* secreted maximum alpha amylase in a medium supplemented with 1% peptone, 0.5% yeast extract and 0.5% maltose under vigorous shaking conditions (Elif *et al.*, 2005). L-asparagine was reported to be one of the most promising nitrogen sources for alpha amylase production by *Thermomyces lanuginosus* (Adinarayana *et al.*, 2005).

### 2.4.6 Moisture content:

Moisture is one of the most important parameters in SSF that influences the growth of the organism and thereby enzyme production (Pandey *et al.*, 2000). Low and high moisture levels of the substrate effect the growth of the microorganisms resulting (Ellaiah *et al.*, 2002). High moisture content leads to reduction in substrate porosity, changes in the structure of substrate particles and reduction of gas volume. Bacteria are generally known to require initial moisture of 70-80%. Alpha amylase production by *Bacillus* *licheniformis* M27 was highest with 65% initial moisture content in an SSF system (Namita *et al.*, 2007). Significant decrease in enzyme production was observed with high increase in moisture content which was due to the decrease in the rate of oxygen transfer. A thermotolerant *B.subtilis* requires initial moisture of 30% for its growth and maximum enzyme production (Ahmad *et al.*, 2010).

### 3.0 RECOMMENDATION AND CONCLUSION

Among the microorganisms, the *Bacillus spp* are one of the extracellular enzyme producing bacteria and from this review it has been seen that *bacillus spp* are great producers of  $\alpha$ -amylase. This study shows a critical literature review and from the study, it is recommended further research should be carried out using other agricultural products for the isolation and production of amylase. While there is so much study on the isolation of *Bacillus Subtilis*  $\alpha$ -amylase, there is limited literature on other related bacteria such as *Bacillus amyloliquefaciens* etc.

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