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Review Article

A Review of the Microbiological Aspect of α-amylase Production

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Abstract

The hydrolysis of starch to low molecular weight products, catalyzed by the enzyme, α-amylase is one of the most important and successful commercial enzyme-catalyzed reactions used in the processing industry. From an industrial point of view, mostly bacterial and fungal sources have been used for the production of α-amylase. α-amylase can be obtained from plants, animals and microorganisms. The properties of each α-amylases such as thermostability, pH optimum and their other physico-chemical properties are important in the development of most suitable fermentation processes. α-amylases can be produced by fungi in large amount but they are usually not heat stable beyond 40°C. On the other hand, bacterial species such as *Bacillus subtilis, B. megaterium, B. amyloliquefaciens* and *B. licheniformis* produce more heat stable enzymes. Bacterial species, which produce α-amylase enzymes that can withstand a temperature of 70°C have been reported previously. There is often a need to isolate species of microorganisms that can grow at high temperatures and whose enzymes can function at temperature up to 95-100°C. The purpose of this manuscript was to review the literature on the microorganism associated by the production of α-amylase on using different substrate, thermostability profile and its industrial application.

Keywords: α-amylase; Thermostability; *Bacillus licheniformis;* Hydrolysis; Characterization

Introduction

Kirchhoff was the first scientist to report the discovery of α-amylase in 1811. It was much later in 1930 that Ohlsson suggested the classification of starch hydrolyzing enzymes as α and ß-amylases according to the anomeric type of sugars produced by the enzyme reaction (Gupta *et al*., 2003). Starch is the primary storage compound of a large number of economically important crops such as rice, maize, wheat and potatoes. Starch is an abundant source of carbohydrate. It consists of amylopectin and amylose. Amylopectin is formed from linked α-1, 4 chains of glucose with linked (α, 1-6) branch points and amylose consists of chains of glucose α, 1-4 linked. From the last century, the trend of establishing the starch processing industry is increasing, in which the practices like the passaging of the acid hydrolysis of starch and production of maltose and glucose syrup have been carried out by using starch-converting enzymes (Arikan, 2008; Gupta *et al*., 2008). α-amylase (α, 1-4-glucan-glucanhydrolase, EC 3. 2. 1. 1) is an amylolytic enzyme. This enzyme breaks down α, 1-4-glucosidic linkages of starch and related products in an endo fashion and produces oligosaccharides. The mode of action, properties and products of hydrolysis differ somewhat, depending on the source of enzyme (Parka and Son, 2007).

Microorganisms Associated with α-amylase Production

Several reports have been published on the isolation, identification and characterization of α-amylase from a variety of sources (Pederson and Niclson, 2000; Kılıc *et al*., 2005; Muralikrishna and Nirmala, 2005; Nidhi *et al*., 2005; Hernandez *et al*., 2006; Asghar *et al*., 2007; Bakri *et al*., 2009; Hmidet *et al*., 2010; Asad *et al*., 2011; Ahmad *et al*., 2013). Industrial enzymes have been produced from plants, animals and micro-organisms, but the production from the first two sources is rather limited for several reasons. The concentrations of enzymes in the plant materials are generally low but starch processing industries require large quantities of enzymes. On the other hand, the enzyme of animal origin is from a by-product of the meat industry and therefore its supply is also limited. However, α-amylase from microbial sources can be produced in abundant quantities to meet the necessary industrial market requirements, because the diversity of microbes as the source material producing α-amylase is great. Microbial enzymes have a wide range of features that makes them quite useful in a variety of applications (Saroja *et al*., 2000; Teodoro and Martin, 2000; Shiau and Hung 2003; Sajedi *et al*., 2005; Sodhi *et al*., 2005; Ten *et al*., 2005; Sajitha *et al*., 2010; Mohammadabadi and Chaji, 2012; Ahmadi, 2012).

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Table 1: Microorganism utilized different Nitrogen Sources for the Production of α-amylase

Microorganisms	Nitrogen source	References
Pancillium camemberti; Streptomyces sp.; Bacillus sp. IMD 434; Halomonas meridian	Yeast extract	Natasha *et al.*, 2011
Bacillus sp. IMD 434	Peptone	Kanwal *et al.*, 2004
Aspegillus niger; Corynebacterium gigantean	Ammonium sulphate Casein	Riaz *et al.*, 2007
Aspergillus flavus	Ammonium nitrate	Sajitha *et al.*, 2010
Bacillus subtilis	Arginine	Haq *et al.*, 2010
Bacillus licheniformis NH1; Bacillus megaterium	Chicken feathers	Nouadri *et al.*, 2010

Table 2: Microorganism used different Carbon Sources for the Production of α-amylase

Microorganisms	Carbon source	References
Bacillus subtilis	Corn starch	Lene *et al.*, 2000
Bacillus subtilis; Bacillus licheniformis	Potato starch	Bilal and Figen, 2007
Aspergillus flavus; Aspergillus oryzae	Maize starch	Niazi *et al.*, 2010
Bacillus licheniformis	Wheat starch	Hmidet *et al.*, 2010
Bacillus subtilus KCC103	cane sugar	Patel *et al.*, 2005
Bacillus subtilis; Bacillus licheniformis	Rice starch	Saroja *et al.*, 2000

Table 3: Effect of various Temperatures and pH on α-amylase

Microorganisms	Maximum temperature (°C)	Maximum pH	References
Bacillus sp. ANT-6	37	7	De-Souza and Martins, 2000
Bacillus subtilis	50	7	Shiau and Hung, 2003
Bacillus halodurans	60	8	Nagamine *et al.*, 2003
Bacillus sp. A3-15	65	8.5	Arikan, 2008
Bacillus amyloliquefaciens	37	7	Francis *et al.*, 2003
Bacillus sp. I-3	70	7	Gangadharan *et al.*, 2006
B. licheniformis NH1	75	6.5	Haq *et al.*, 2002
Bacillus sp. Ferdowsicous	70	7	Shafique *et al.*, 2009
Aspergillus niger	30	5	Gomes *et al.*, 2005
Penicillium camemberti PL21	30	5	Leveque *et al.*, 2000
Aspergillus niger	37	5	Hunter *et al*., 2011
Aspergillus niger	40	5.5	Patel *et al.*, 2005
Aspergillus niger	30-40	3.87	Haq *et al.*, 2010
Penicillium olsonii	30	5.5	Afifi *et al.*, 2008
Bacillus subtilis JS-2004	50	8	Asgher *et al.*, 2007
Bacillus licheniformis	70	6.5	Bozic *et al.*, 2011
Bacillus licheniformis	60	7	Muralikrishna and Nirmala, 2005

Bacteria as a source material for α-amylase production: α-amylase is produced by bacterial species of *Bacillus* (Muralikrishna and Nirmala, 2005; Asghar *et al*., 2007), *Pseudomonas* (Haq *et al*., 2002; Shiau and Hung, 2003) and *Clostridium* (Kılıc *et al*., 2005). Bacterial species such as *Bacillus subtilis* (Sumrin *et al*., 2011; Rajput and Li, 2012; Rajput *et al*., 2013), *B. licheniformis* and *Bacillus a.* are generally preferred for the production of α-amylase because they appear to be very productive (Nidhi *et al*., 2005; Kokab *et al*., 2007; Reda, 2007; Niazi *et al*., 2010). For the thermal stabilities of their α-amylase enzymes to be utilized in various fermentation processes, extreme thermophilic bacteria such as *Rhodothermus marinus* and mesophilic bacteria such as *B. megaterium*, *B. macerans* and *B. coagulans* are generally selected and utilized (Saroja *et al*., 2000; Gimbi and Kitabatake, 2002). Most thermostable α-amylase utilized in the industry is produced from *B. licheniformis* (Reda, 2007; Hmidet *et al*., 2010). Highly thermostable α-amylases are also obtained in hyperthermophilic and thermophilic Archaea such as *Pyrococcus furiosus*, *Thermococcus hydrothermalis*, *T. profundus*, *Sulfolobus acidocaldarius* and *S. solfataricus* (Goyal *et al*., 2005; Hernandez *et al*., 2006; Arikan, 2008).

Fungi as a source material for α-amylase production: Several α-amylase producing strains of yeast, fungi and actinomycetes were isolated from soil. *Aspergillus* and *Rhizopus spp.* were mainly studied in the developing countries because of their ubiquitous nature and wide distributions and the fact that the nutritional requirements of these organisms are not very stringent and relatively easily met (Piao *et al*., 1999; Asghar *et al*., 2000; Bilal and Figen, 2007; Afifi *et al*., 2008; Pascoal *et al*., 2010). α-amylase from fungal sources, especially *Aspergillus spp.* has gained more attention because of the easy availability and high productivity of the fungi, which are also suitable for genetic manipulations. Different species of the genus *Aspergillus* such as *A. niger*, *A. oryzae*, *A. flavaus*, *A. tamarie*, *A. fumigatus* and *A. kawachii* have been frequently used for the production of α-amylase (Asghar *et al*., 2000; Haq *et al*., 2002; Shiau and Hung, 2003; Nagamine *et al*., 2003; Ramachandran *et al*., 2004; Hussein and Janabi, 2006; Rasooli *et al*., 2008; Bakri *et al*., 2009; Bhutto and Umar,

2011; Natasha *et al*., 2011; Hunter *et al*., 2011). *Penicillium spp*. such as *P. chrysogenum* and *P. camemberti* was reported to have been used for the production of α-amylase and also in cheese production (Bilal and Figen, 2007). α-amylase was obtained from some thermophilic fungus spp. such as *Hemicola insolens*, *H. lanuginosa*, *H. stellata*, *Mucour pusillus* and *Talaromyces thermophilus* (Khajeh *et al*., 2004). From industrial point of view, some species of yeast such as *Candida tsukubaensis*, *Filobasidium capsuligenum*, *Lipomyces kononenkoae*, *Saccharomycopsis caspularis*, *S. fibuligera* and *Sachhromyces cerevisae* have been used for the production of α-amylase (Gupta *et al*., 2008; Valaparla, 2010).

The Production Process of α-amylase

There are two major methods for large scale production of α-amylase: (a) solid state fermentation (SSF) process and (b) submerged fermentation (SmF) process (Soni *et al*., 2003; Akpan and Adelaja, 2004). Initially, submerged fermentation process was the chosen technique for the production of α-amylase due to the easy control of different physico-chemical parameters (Coronado *et al*., 2000; Gangadharan *et al*., 2006). However, now-a-days, SSF is the preferred method for the production of α-amylase in the industry due to several reasons, including better quality of production, easy follow through procedures, lesser production cost, energy savings and no formation of foam. Many factors are involved in the production and optimization of α-amylases such as nitrogen and carbon sources supplied, metal ions, pH and temperature (Konsula and Kyriakides, 2004; Bilal and Figen, 2007; Shafique *et al*., 2009; Bhutto and Dahot, 2010). Some of these will be discussed in the following:

Nitrogen sources used for the production of α-amylase: Various nitrogen sources including corn steep liquor, casein, yeast extract, tryptone, ammonium nitrate, potassium nitrate, sodium nitrate and ammonium chloride are utilized for the production of α-amylase in basal medium. It has been reported that organic nitrogen sources like peptone, yeast extract usually have stimulating effect. It has been observed that among all the nitrogen sources, peptone is the best candidate for the maximum production of α-amylase (Fogarty and Kelly, 1980; Pederson and Niclson, 2000; Bhutto and Umar, 2011). Utilization of different nitrogen sources by microorganisms is shown in Table (1).

Carbon sources used for the production of α-amylase: α-amylase is produced from many sources of carbon (including fructose glucose, maltose, galactose, sucrose, lactose, dextrose), industrial waste (like date syrup and molasses), agricultural waste involving sugarcane bagasse and rice husk (Bhutto and Umar, 2011). Microorganisms used different sources of carbon for the production of α-amylase as presented in Table (2).

Metal ions: These ions play an important role for the production of α-amylase. The underlying reason for this is that most α-amylases are metalloenzymes. Ca^{2+} and $CaCl_2$ ions are significantly important for the production of these enzymes (Francis *et al*., 2003: Patel *et al*., 2005). 20 mM $LiSO_4$ and 1 mM $MgSO_4$ increased α-amylase production by *Bacillus* sp. I-3 (Sodhi *et al*., 2005), but 3 mM $FeCl_3$ and 12 mM $MgSO_4$ have shown negative influence on α-amylase production.

Amylase Action on Starch

Both bacteria and fungi are excellent producers of α-amylase (Omenue *et al*., 2005; Sidkey *et al*., 2011). α-amylase hydrolyzes the potato starch (Pandey and Nigam, 2000; Demirkan *et al*., 2005; Muralikrishna and Nirmala, 2005; Mendu *et al*., 2005; Riaz *et al*., 2007). The potato starch is the main source of carbohydrate (Goyal *et al*., 2005). Therefore, α-amylase is very important for digesting raw starches and is used in many starch based industries (Kanwal *et al*., 2004; Konsula and Kyriakides, 2004). Starch is broken down by using a mixture of α-amylase and glucoamylase. The first step is to solubilize starch. Starch is present in the form of insoluble starch granules (Khajeh *et al*., 2004).

Effect of Temperature and pH on α-amylase Activity

In any study of enzyme activity and temperature stability, it must be remembered that the action of enzymes are time dependent process. Increases in temperature will lead to an increase in activity reaction kinetics, but also accelerate the denaturation induced by higher physiological temperatures. This must be fully reflected in industrial applications, in which the enzyme is expected to be useful for long operating duration. It is widely known that at high temperatures enzymatic activity can be destroyed because enzymes are proteinaceous molecules. It is also important to note that regulatory authorities often require that no detectable enzymatic activity remain in the product. If a soluble enzyme is used in a manufacturing process, it is beneficial to operate the procedure at the maximum possible temperature. In recent years there has been an enhanced interest in enzymes obtained from extremophiles. These micro-organisms live in some of the most inhospitable places on earth, for example volcanic springs and possess enzymes with extreme thermotolerance. Thermostable α-amylases have been isolated for a long time from such organisms as *B. amyloliquefaciens*, *B. licheniformis* and *B. subtilis.* The enzymes obtained from *B. licheniformis* generally were stable than those from other *Bacillus* species (Fossi *et al*., 2005; Bozic *et al*., 2011). The effect of temperature on α-amylase action has been reported previously in such studies. In such studies optimum temperature was noted to be 65°C at low substrate concentration and 75°C at high substrate concentration, respectively and the optimum temperature was recorded at 50-60°C (Asghar *et al*., 2000; De-Souza and Martins, 2000; Leveque *et al*., 2000; Marc *et al*., 2002; Aquino *et al*., 2003). The effect of pH on enzyme stability and activity is also

dependent on time and temperature. In general, enzymes are less stable at high temperatures over time at pH values near the limit of the optimum. For this reason, the optimum pH should be determined to be under conditions close to those encountered in specific industrial applications. In such cases, it is important to choose an enzyme with as broad a pH optimum as possible. α-amylases are generally stable a pH ranges from 4 to 11 (Gimbi and Kitabatake, 2002; Gupta *et al*., 2003; Omenue *et al*., 2005; Kokab *et al*., 2007; Vidyalakshmi *et al*., 2009; Nouadri *et al*., 2010). Microorganisms utilized at different temperatures and pH for the production of α-amylase as described in Table (3).

Industrial Uses of α-amylase

Bacterial amylases play one of the most important roles in the industrial production process. Enzyme production from microbial sources is extremely important for industrial competiveness, as well as our health, prosperity and well-being. Many *Bacillus* spp. produce commercially important enzyme, α-amylases and their derivatives. Many industrial processes involving manufacturing such as industrial, environmental processes and food biotechnology utilize this enzyme at some stage or the other. A few important industrial applications of α-amylase are given below.

Glucose and fructose industry: Many industries use α-amylase for the production of glucose. This enzyme hydrolyzes the starch and converts it into maltose and glucose. The glucosidic α-1, 4 linkages in the starch polymer is hydrolyzed in a randomly to produced maltose and glucose. Therefore, α-amylase is widely used in many starch processing industries for the production of glucose (Konsula and Kyriakides, 2004; Haq *et al*., 2010)**.** This process also can yield the water-soluble product dextrin. Many starches or barley materials are present in the feed. Accordingly, the nutritional value of the feed can be improved by the addition of α-amylase (Shiau and Hung, 2003; Demirkan *et al*., 2005; Saxena *et al*., 2007).

Bakery and anti-salting industries: In bakery industries, the α-amylase plays an important role in the improvement of quantity, aroma, taste and porosity of the product. This enzyme is the major part of the bread used in Russia, USA and the European countries. α-amylase can also affect antisalting in baking bread and help to improve the softness of the bread (Gupta *et al*., 2008).

Detergent industry: α-amylase is widely used and has significant role in the improvement of detergent quality by affecting bleaching. The addition of enzyme increases the stability and effectiveness of the bleach in laundry's detergents and soap bar composition (Haq *et al*., 2010).

Alcohol industry: Fermentable sugars are produced by the conversion of starches with the help of α-amylase. Starches such as grain; potatoes etc. are required for the manufacturing of ethyl alcohol, a major chemical having essential role in most of the biological and chemical reactions (Juge *et al*., 2006).

Textile desizing: Most of the textile industries extensively use α-amylase to hydrolyze and solubilize the starch. Starch increases the stiffness of the finished products after washing out of the clothes. Starch does not harm the fibers. α-amylase is used as a resizing agent. It has an essential role for removal of starch from the greasy clothes. Many garments especially the ubiquitous "Jeans" are more desirable after machining (Lene *et al*., 2000; Bozic *et al*., 2011).

Paper industry: α-amylase has been used for the manufacturing and modification of starches for coated paper. It improves the paper quality, protects against mechanical injury and increases the stiffness and strength in paper. The conversion of raw starch into glucose and fructose by the action of α-amylase is prerequisite for sizing and coating of the paper. So, α-amylase is widely used for some paper sizing (Bozic *et al*., 2011).

Feed industry: It has been reported that by the use of α-amylase in feed industry, the body weight gain and feed conversion ratio have increased. It readily hydrolyzes the starch polymers into fructose and glucose, which increases the digestibility of carbohydrates (Iji *et al*., 2003; Silva *et al*., 2006; Sidkey *et al*., 2011).

Conclusion

This review illustrates the importance of bacterial and fungal microbes in the production of α-amylase. From an industrial point of view, mostly organisms of the genus *Bacillus* have been utilized for the production of thermostable α-amylases. It has been reported that the enzymes produced from bacterial sources can withstand heat inactivation up to a temperature of 70°C. The fungal species especially, *A. niger, A. fumigatus* and *A. oryzae* are used for the production of α-amylase, but enzyme from fungal sources is not so capable of producing the thermostable variety and can withstand a temperature of only upto 40°C. On the other hand, among the bacterial spp. only *B. licheniformis* is the most promising strain to produce highly thermostable α-amylase. The maximal α-amylase stability from this organism's activity has been recorded at temperatures up to 100°C at pH 7.

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