Current Research in Microbiology

Chapter 3

Amylase: A Magnificent Enzyme Tool

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1. Introduction

Since past couple of decades, enzymes have been consistently used as an efficient industrial tool to synthesize a variety of products. Industries are highly dependent upon some basic enzymes viz Amylase, Carboxymethyl cellulase and proteases. Such pioneering enzymes find their applications in a wide range of industries, including pharmaceuticals, food and beverages industry and textile industry. Amylase were initially reported from the buds of tuber by Bailey [1].

1.1. Sources of enzymes

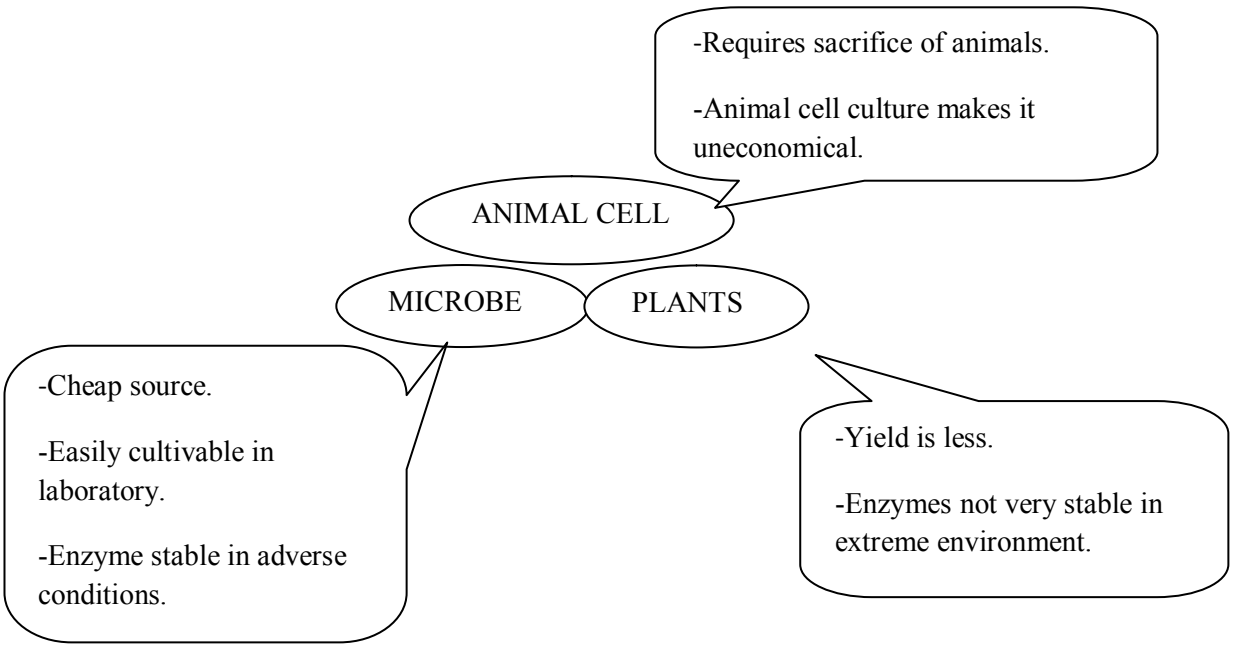

Figure 1: Sources of Amylases

In nature amylases are derived from three main sources:

(i) Animals
(ii) Plants
(ii) Microbial sources

Microbial sources of enzymes are more preferred over plants and animals because of their stability and high productive yield.

1.2. Amylases: An ancient enzyme

Amylase is well known enzyme and its potential industrial application gained recognition since 1970s. All amylases act upon the α–1,4–glycosidic bonds. As a class of diastase, Amylase is the first enzyme to be discovered and isolated in 1833 by Anselme Payen. Amylase initiates the chemical process of digestion of starchy food in mouth as it is present in saliva of humans and some animals. Plant cells also contain Amylase. The basic principle behind Amylase action is conversion of starch into sugars. Amylases are classified into three types-

(i) α–Amylase (EC3.2.1.1) also known as 1,4-α-D-glucan glucanohydrolase, glycogenaseis a calcium dependent metallo-enzyme and unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0. In human physiology, both the salivary and pancreatic amylases are α-amylases. The α-amylases form is also found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (Bacillus).

(ii) β–Amylase (EC3.2.1.2) also known as 1,4-α-D-glucan maltohydrolase, glycogenase, saccharogen amylase) is also synthesized by bacteria, fungi, and plants. Working from the nonreducing end, β-amylase catalyzes the hydrolysis of the second α-1,4glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. The optimum pH for β-amylase is 4.0–5.0.

(iii) γ–Amylase (EC3.2.1.3) also known as glucan 1,4-α-glucosidase, amyloglucosidase, exo-1,4-α-glucosidase, glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase cleaves α-1,6 glycosidic linkages, as well as the last α-1,4glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose. The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3.

2. Recent Advances in Enzyme Technology

With the recent advent of biotechnology, there has been a growing interest and demand for enzymes with novel properties. Considerable efforts have been devoted to the selection of microorganisms via sophisticated screening techniques and process methodology for the production of enzymes with new physiological/physical properties and tolerance to extreme conditions used in the industrial processes (e.g. temperature, salts and pH). The marine environment has proven to be a rich source of both biological and chemical diversity [3]. The optimum activity of marine bacterial enzymes usually occurs at high salinity, making these enzymes utilizable in many harsh industrial processes, where the concentrated salt solutions used would otherwise inhibit many enzymatic transformations. In addition, most marine bacterial enzymes are considerably thermo tolerant, remaining stable at room temperature over long periods.

3. Current Research on Amylase Catalyzed Starch Hydrolysis

Starch processing industries mainly derive starch from major crops like potato, rice and wheat. Conventionally, acid hydrolysis method is used for its degradation. Starch processing industries which are springing fast in past decades witnessed a paradigm shift from chemical hydrolysis to enzymatic hydrolysis for production of maltodextrins,modified starches, glucose or fructose syrups.

According to Marrel, 30% of the enzyme production in the world is focused on starch converting enzymes. The total starch converting enzymes essentially belonging to the amylase are reported to be 13 glycosyl hydrolases [4]. The studies on these thirteen enzymes by Crystallization and X- ray crystallography revealed a common structural architecture having 8 barrel structure protein.

Marine bacteria, corals and soft algae are reported to be novel sources of Amylase. The investigation revealed eight marine sedentary organisms, six marine bacteria, one soft coral and one algal isolates. All were reported to produce industrially important enzymes [5].

4. Future Prospects in Organic Matter Recycling in Marine Environment

Novel bacterial isolates were reported to produce either amylase or α-Carboxy methyl cellulaseor protease. Alcaligenes and Bacillus were reported to show highest activities amongst the isolates. These enzymes may find their commercial applications in catalyzed organic matter recycling. Hot springs were investigated for isolation of bacterial strains by Asif and Rasool [6]. It revealed Bacillus WA 21 had maximal yield at 55 ºC and pH 6.

5. Economical Approach Towards Enzyme Production

Since amylases and associated enzymes have great demand in sectors of pharmaceuticals, leather, laundry, food and waste processing industries, effectiveness of their production is very much targeted.

Agro industrial waste is the upcoming and widely accepted alternative due to its easy availability, diversity, high nutritive value and extremely low cost. Many reports of usage of Baggasse, molasses, cassava waste water, tea, coffee leaves, banana leaves, rice husk and paddy straw for amylase production have been recorded [7].

6. Experimental Designing for Optimization of Enzyme Yield

Designing of experiments was a newer approach towards optimization of enzyme production. Designing helps to evaluate the effect of interactions of different physicochemical parameters on enzyme production. Two approaches were taken by researchers for optimization: viz Smf and SSF. Negi and Banergee employed Evolutionary Operation (EVOP) factorial design technique in amylase production [8]. Experimental runs were carried out in a bioreactor by modified solid state fermentation. The EVOP technique was applied on indigenously isolated strain *Aspergillusawamori* MTCC 6652 . The result supported amylase production upto levels of 9420.6 IU/g at 37ºC, pH 4.00 and relative humidity 85%.

Smf approaches were also taken up by various scientists [9,10]. Experiments on influence of various organic and inorganic nitrogen sources are carried under optimization. Ammonium nitrate (450/ mg), maltextracttryptone are some of the preferred Nitrogen sources supporting amylase production. Optimization of thermal stability of the enzyme was also carried out. The thermal denaturation studies in presence of trehalose, sorbitol, sucrose and glycerol were carried out due to their industrial relevance [11]. The denaturation stabilities are aided by techno based intrinsic and 8–anilinonaphathalene 1–sulphonic acid fluorescence studies revealing exposure of hydrophobic cluster on protein surface.

SSF was reported to be superior during Amylase production experiments based on wheat bran and rice bran as substrates for enzyme production. Oyeleke et al tried to utilize Africa lowest beans as a substrate for amylase production coupled with protease [12]. Optimization of pH and temperature revealed highest yield of 0.87 mg / 1ml/sec. Enzyme characterization experiments revealed Km and Vmax values. Influence of activators and inhibitors studies helped to explore the Kinetic behavior of the enzyme.

7. Optimization of Amylase Production

The general optimal parameters investigated by various researches were pH of medium, incubation temperature, influence of various carbon and Nitrogen sources on enzyme produc-

tion. Different sources of enzymes have exhibited different optimal parameters with respect to pH, temperature, carbon sources and nitrogen sources as given in the following **Table**.-

Table 1: Different microorganism and optimization parameter for the production of α-Amylase

Bacteria strain		
Organism	**Optimization parameter**	**Authors**
B. amyloliquefaciens (MTCC 1270)	calcium (Ca^{+2}), Nitrate (NO_3^-), and chloride ions (Cl^-)	Saha et al., 2014 [13]
Bacillus amyloliquefaciens	Wheat bran	Abd-Elhalem et al., 2015 [14]
Bacillus amyloliquefaciens	37ºC, pH =7	Francis et al., 2003 [15]
Bacillus cereus	Cow dung	Vijayaraghavan et al., 2015 [16]
Bacillus cereus	35ºC	Kumari et al, 2017[17]
Bacillus cereus KR9	Starch, Peptone, pH = 10	Krishma and Radhathirumalaiarasu, 2017 [18]
B. licheniformis NH1	75ºC, pH = 6.5	Haqet al., 2002 [19]
Bacillus licheniformis	Wheat starch	Hmidetet al., 2010 [20]
Bacillus licheniformis	70ºC, pH = 6.5	Bozicet al., 2011 [21]
Bacillus licheniformis	60ºC, pH = 7	Muralikrishna and Nirmala, 2005 [22]
Bacillus sp. A3-15	65ºC, pH = 8.5	Arikan, 2008 [23]
Bacillus sp. ANT-6	37ºC, pH = 7	De-Souza and Martins, 2000 [24]
Bacillus sp. BBXS-2	Lignocellulosic biomass	Qureshi et al.,2016 [25]
Bacillus sp. IMD 434	Peptone	Kanwal et al., 2004 [26]
Bacillus sp. IMD 434	Yeast extract	Natasha et al., 2011 [27]
Bacillus strain	50°C and pH 8.	Simair, 2017 [28]
Bacillus subtili *Bacillus licheniformis*	Rice starch	Sarojaet al., 2000 [29]
Bacillus subtilis	Arginine	Haq et al., 2010 [30]
Bacillus subtilis	Corn starch	Lene et al., 2000 [31]
Bacillus subtilis *Bacillus licheniformis*	Potato starch	Bilal and Figen, 2007 [32]
Bacillus subtilis	50ºC, pH = 7	Shiau and Hung, 2003 [33]
Bacillus subtilis	50°C	Hassan and Khairiah, 2014 [34]
Bacillus subtilus KCC103	Cane sugar	Patel et al., 2005 [35]
Bacillus MJK1, MJK2, MJK6 and MJK10	pH = 8.0, 50°C and 72 hrs	Kanimozhi et al, 2015 [36]
Yeast strain		
Saccharomyces cerevesiae	pH = 6.0, initial temperature 20ºC	Jayalakshmi and Umamaheswari, 2015 [37]

Mold strain		
Aspergillusflavus	Ammonium nitrate	Sajitha et al., 2010 [38]
Aspergillusflavus;		
Aspergillusoryzae	Maize starch	Niaziet al., 2010 [39]
Aspergillusniger	pH = 6.0, initial temperature 30ºC	Ire et al., 2017 [40]
Aspergillusniger	30ºC, pH = 5.5	Afifi et al., 2008 [41]
Aspergillusniger BTM-26	Wheat bran	Abdullah et al., 2014 [42]
Aspegillusniger, Corynebacterium gigantean	Ammonium sulphate Casein	Riaz et al., 2007 [43]

8. Applications of Amylases

The major industry which requires starch hydrolysis with various product implications is food industry. The differential hydrolysis of starch can be facilitated through novel enzyme source combined with variable hydrolytic conditions. The main products resulted through the amylases are glucose syrups, fructose syrups, clarified beverages and fruit juices, dextrose powder, dextrose crystals, malto-oligosaccharides, maltosyrups, and Alo- mixtures.

Amylases are also used for imparting sweetening to the dough, acceleration rates of fermentation in bakery products, improving palatability of bakery items. Other than food and bakery industry, amylases are utilized in detergents, direct ethanol production and textile industry.

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