MICROBIAL ENZYMES WITH SPECIAL REFERENCE TO α-AMYLASE

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ABSTRACT

The microbial enzymes particularly the bacterial enzymes have gained more popularity in the industrial sector as they are more stable than the enzymes prepared from plants and animal resources. Modern molecular techniques, such as metagenomics modified by different strategies are used for analysis of microbial enzymes. Bacteria and fungi produce the majority of industrial microbial enzymes. Production of enzyme is carried out using submerged fermentation and also solid state fermentation in some cases. α-Amylase (1,4-α-D-glucanohydrolase; EC3.2.1.1) is responsible for hydrolysis of the α-1,4-glucosidic linkage in starch and other similar molecules. It is one of the many enzymes involved in starch degradation. The production of α-amylase is essential for the conversion of starch to oligosaccharide. Enzymes like amylases—starch converting enzymes, are important in production of glucose and fructose syrups, modified starches. For isolation and optimization of α-amylase from bacillus species, an α-amylase producing bacillus species is isolated from a soil sample and the soluble starch amylase was characterized. Then, the optimum pH activity, temperature activity and substrate concentration for bacterial growth is observed. The purification of the enzyme is then carried out and the enzyme growth is then recorded.

Keywords: Microbial enzymes, α-Amylase, Starch, α – 1, 4 – glycosidic bonds,

INTRODUCTION

Enzymes are proteins that act as catalysts which can speed up chemical reactions that occur in the cells. An enzyme can add atoms to a molecule, move atoms from a molecule, split a large molecule into two smaller molecules, or join together smaller molecules to form a large molecule. Enzymes have unique shapes and reactive sites that allow them to bind with specific molecules namely substrate molecules.

Digestive enzymes turn the food we eat into energy and unlock this energy for the use in the body. Our bodies naturally produce both digestive and metabolic enzymes. It is believed that taking enzymes to supplement cooked food lack of enzymes will promote better digestion and preserve your own limited enzyme production. Once enzyme production ceases in the body, life ends. Enzymes are present in each living cell thus also in all microorganisms.

Hydrolytic enzymes also called as hydrolases are the enzymes that carry out the hydrolysis of a chemical bond. Hydrolysis is the biochemical reaction that involves the addition of water molecule and the cleavage of the covalent bond holding the two monomers together.

A-B + H₂O → A-OH + B-H

Amylases, proteases, lipases are some examples of hydrolytic enzymes.

MICROBIAL ENZYMES

Microbial enzymes term represent the enzymes that are present in the various species of bacteria, fungi and yeast. These enzymes play a crucial role in industrial biochemical processes. Each single strain of these organisms produces a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature. But the absolute and relative amounts of the various individual enzymes produced vary markedly between species and even between strains of the same species. Hence, for the
commercial production of specific enzymes the strain of microbes which have the capacity for producing highest amounts of the particular enzymes is desired.

**BACTERIAL ENZYMES**

The enzymes obtained and isolated from bacteria are the numerous and have vast variety of applications. The organisms like Bacillus subtilis, Bacillus licheniformis, Bacillus amylofaciens and other species of Halobacillus are the main produces of bacterial enzymes. Also various acidophilic and thermo acidophilic bacteria produce enzymes like amylases. Thermostable enzymes have increased use in industries as they are able to function even in harsh extreme temperature conditions. Enzymes produced by some halophilic microorganisms have optimal activity at high salinities and thus can be used in difficult industrial environments, where the concentrated salt solutions used would interfere with enzyme activity. In addition, most halobacterial enzymes are considerably thermostolerant and remain stable at room temperature over long periods. Halophilic amylases have been characterized from halophilic bacteria such as Chromohalobacter sp., and Bacillus dipsosauri.

**FUNGAL ENZYMES**

Species of Aspergillus like Aspergillus niger, Aspergillus oryzae, Aspergillus flavus are the major fungal species that are capable of producing industrially functional enzymes. Also these filamentous fungi, produce large quantities of amylases which can then be used commercially. Aspergillus oryzae has been largely used in the production of food such as soy sauce, organic acid such as citric and acetic acids and enzymes including α-amylase. Aspergillus niger has significant hydrolytic capacities in the α-amylase production. Also, due to its tolerance of acidity (pH < 3), it allows the avoidance of bacterial contamination.

**AMYLASES**

Amylases are enzymes that hydrolyse starch molecules to give diverse products including dextrans and progressively smaller polymer composed of glucose units. Amylases are enzymes which hydrolyze starch molecules to give diverse products including dextrans and progressively smaller polymers composed of glucose units. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry.

Amylases can be divided into two types on the basis of the manner of catalysis, endoamylases and exoamylases. Endoamylases catalyze hydrolysis in a random manner in the interior of the starch molecule. This action causes the formation of linear and branched oligosaccharides of different chain lengths. Exoamylases hydrolyze from the non-reducing end, successively resulting in short end products. A large number of enzymes are known which hydrolyze starch molecule into different products and a combined action of various enzymes is required to hydrolyze starch completely.

**STRUCTURE**

The structure of this enzyme is simple. There are different variations of the amylase molecule, called alpha (α) amylase and beta (β) amylase. The human body has two types of α-amylase, one in saliva and one in the pancreas. Both molecules are important in digestion of carbohydrates, since the body cannot digest complex sugars and starches without breaking them down first. β-amylase is not present in the human body, and is usually found in plants and seeds.

Even though the basic structure of amylase is not quite complex, there are some variants that are significantly more complicated than the others. Most α-amylases are very similar in length, but there are some that fold into more than three common domains. Many food crops have this type of α-amylase, including barley, soybeans, and sweet potatoes, but the size of the α-amylase molecule does not enhance or detract from the plant's nutritional properties.
SUBSTRATE HYDROLYSIS

The substrate for the amylase enzyme is starch. It is often found in seeds and tubers (e.g., potatoes). It is made up of two kinds of polymers of glucose. The simpler kind of polymer is called amylose, and it makes up about 20% of starch. It is basically a chain of glucose units linked by $\alpha-1, 4$ – glycosidic bonds. The other 80% of starch is a water insoluble molecule called amylpectin. It is a branched chain polysaccharide with $\alpha-1, 4$ – glycosidic links. This branching of glucose units occurs at every 25 glucose units. When treated with acid or enzymes, the components of starch are hydrolyzed to dextrins (mixture of low melting polysaccharides, made up of 3 – 8 glucose units), maltose and finally D-glucose.

![Figure 1- Starch (amylose)](image)

Starch is one of the major nutrients in the human diet. Its presence can be detected by the blue-black color produced when iodine solution is added to a sample of the material, like food, to be tested.

Starch + I$_2$ $\rightarrow$ blue-black color

TYPES OF AMYLASE

$\alpha$-AMYLASE (E.C.3.2.1.1) –

$\alpha$-amylase are enzymes that catalyses the hydrolysis of internal $\alpha-1,4$-glycosidic linkages in starch in low molecular weight products, such glucose, maltose and maltotriose units. A-Amylase can be derived from several fungi, yeasts and bacteria. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors.

$\beta$-AMYLASE (EC 3.2.1.2) -

$\beta$-amylase is another form of amylase synthesized by bacteria, fungi, and plants. $\beta$-amylase catalyzes the hydrolysis of the second $\alpha-1,4$-glycosidic bond. It works from the non-reducing end, cleaving off two glucose units at a time. During the ripening of fruit, $\beta$-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. Animal tissues do not have $\beta$-amylase.

$\gamma$- AMYLASE (EC 3.2.1.3) -

$\gamma$-amylase cleaves $\alpha-1,6$-glycosidic linkages, in addition to cleaving the last $\alpha-1,4$-glycosidic linkages at the non-reducing end of amylase and amylpectin, yielding glucose.

$\alpha$–AMYLASE

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF $\alpha$-AMYLASE

The $\alpha$-amylase belongs to the family of endoamylases which catalyse the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of $\alpha$-D-(1-4) glycosidic bond. The terminal glucose residues and $\alpha$-1, 6-linkages cannot be cleared by $\alpha$-amylase. The end products of $\alpha$-amylase enzyme action are oligosaccharides which constitute a mixture of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both $\alpha$-1,4 and $\alpha$-1,6 linkages.

The amylase has a three-dimensional structure capable of binding to substrate and, by the action of highly specific catalytic groups this enzyme promotes the breakage of the glycoside bonds. The human amylase is a classical calcium-containing enzyme composed of 512 amino acids in a single oligosaccharide chain with a molecular weight of 57.6 kDa. The protein contains 3 domains: A, B, and C.
INDUSTRIAL APPLICATION OF α-AMYLASE

STARCH PROCESSING INDUSTRY

The most wide applications of α-amylases are in the starch industry, which are used for starch hydrolysis in the starch liquefaction process that converts starch into fructose and glucose syrups. The enzymatic conversion of all starch includes: gelatinization, which includes the dissolution of starch granules, thus forming a suspension. The next process includes liquefaction, which involves partial hydrolysis and loss in viscosity; and the third is saccharification, involving the production of glucose and maltose via further hydrolysis.

Initially, the α-amylase of Bacillus amylo liquefaciens was used in starch processing but it has been replaced by the α-amylase of Bacillus stearothermophilus or Bacillus licheniformis. The enzymes from the Bacillus species hold special interest for large-scale biotechnological processes, the reason being their marked thermostability and efficient expression systems are available for these enzymes.

DETERGENT INDUSTRY

The primary consumption of enzymes is in detergent industries. Amylases are second in rank of enzymes that can be used in the formulation of enzymatic detergent. Amylase is used in detergents for laundry and automatic dishwashing. This leads to conversion of the residues of starchy foods such as potatoes, gravies, custard, chocolate, etc. to dextrins and other smaller oligosaccharides. Since, amylases have optimum activity at lower temperatures and at alkaline pH. Maintaining the necessary stability under washing conditions and the oxidative stability of amylases is a important criterion for use of amylase in detergents as the washing environment is very oxidizing. Examples of amylases used in the detergent industry are derived from Bacillus or Aspergillus.

FUEL ALCOHOL INDUSTRY

Ethanol is the most utilized liquid biofuel. For the ethanol production, starch is the most abundant substrate due to its low price and easy availability as a raw material. During this production, starch is solubilized and then subjected to two enzymatic steps in order to obtain fermentable sugars. The bioconversion of starch into ethanol involves liquefaction and saccharification, where starch is converted into sugar using an amylolytic enzyme such as α-amylase, followed by fermentation, where sugar is converted into ethanol using an ethanol fermenting microorganism such as yeast, for example Saccharomyces cerevisia. Among bacteria, α-amylase obtained from thermo resistant bacteria like Bacillus licheniformis or from engineered strains of Escherichia coli or Bacillus subtilis is used during the first step of hydrolysis of starch suspensions.

FOOD INDUSTRY

Amylases are extensively employed in processed-food industry such as brewing, preparation of digestive aids or supplements, production of cakes, fruit juices and starch syrups. The α-amylases are widely used in the baking industry. These enzymes can
be added to the dough of bread, this degrades the starch in the flour into smaller dextrins, which are then fermented by the yeast. The addition of α-amylase also lead to enhancing of the rate of fermentation and the reduction of the viscosity of dough, resulting in improvements in the volume and texture of the product formed. Also, it generates additional sugar in the dough, which improves the taste, crust color and toasting qualities of the bread.

TEXTILE INDUSTRY
Amylases are used in textile industry for desizing process. Sizing agents like starch are applied to yarn before fabric is produced to ensure a fast and secure weaving process. Starch is a very attractive sizing agent, because it is cheap, easily available in many regions of the world. Also it can be quite easily removed. Starch is then removed from the woven fabric in the textile finishing industry.

Desizing involves the removal of starch from the fabric. The starch acts as a strengthening agent to prevent breaking of the thread during the weaving process. The α-amylase remove selectively the size that is the starch and do not attack the fibers. Amylase from Bacillus stain was employed in textile industries for quite a long time.

PAPER INDUSTRY
The use of α-amylases in the pulp and paper industry is for the modification of starch of coated paper, i.e. for the production of low-viscosity, high molecular weight starch. The coating treatment serves to make the surface of paper sufficiently smooth and strong, to improve the writing quality of the paper. In this application, the viscosity of the natural starch is too high for paper sizing and this can be altered by partially degrading the polymer with α-amylases in a batch or continuous processes. Starch is a good sizing agent for the finishing of paper, improving the quality and erasebility, besides being a good coating for the paper. The size enhances the stiffness and strength in paper.

Examples of amylases obtained from microorganisms used in paper industry includes Amizyme® (PMP Fermentation Products, Peoria, USA), Termamyl®, Fungamyl, BAN® (Novozymes, Denmark) and -amylase G9995® (Enzyme Biosystems, USA).

CONCLUSION
Microbes are able to produce enzymes which will be commercial in nature and obtained from strains of molds, bacteria, and yeasts. Also, Microorganisms that can survive under extreme pH values could be good sources of thermoalkaliphilic enzymes, like proteases and lipases, particularly useful for applications as additives in laundry and dishwashing detergents. The reason for this increasing use of microbial enzyme in industry is the ease with which these enzymes can be obtained and with which enzyme concentrations can be increased by changes in environmental and genetic manipulation.

Amylases are one of the most widely used enzymes required for the preparation of fermented foods. Apart from food and starch industries, in which demand for them is increasing continuously, they are also used in various other industries such as paper and pulp, textile, etc. Knowing the promising applications of α-Amylase as industrial enzyme isolation, screening, characterisation and production of α-Amylase from Bacillus sp can be carried out.

Research is focused on developing thermotolerant and pH tolerant a-amylase from microbes, modifying them genetically. Also to acquire desired properties in the enzyme by applying the concept of site-directed mutagenesis. Production of α-amylase is generally obtained by submerged fermentation, but solid state fermentation can also be seen as a potential productive option. Using agroindustrial residues as substrate the production of bacterial amylase can be carried out.

REFERENCES


