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Review Article

A Review: Characteristics and Application of Amylase

Kumari Sweta[1], Swati Tyagi[2], Anchal Bamal[3].

[1-3]Faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India

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ABSTRACT

Amylases are enzymes that hydrolyze starch in plants as a reserve of carbohydrate and glycogen in animals as a reserve of carbohydrate to reduce fermentable sugars, primarily maltose, and restrict dextrins. Amylases are classified as saccharifying (β-amylase) and as dextrinizing (α-amylases). Alpha-amylases are enzymes catalyzing starch hydrolysis into oligosaccharides, maltose, and glucose. They're widely distributed in kingdoms of microbials, plants and animals. α-amylases are one of the most essential and commonly used enzymes in many industries, including the manufacture of High Fructose Corn Syrup (HFCS). The aim of this study is to review related literature on the isolation and amylase development from bacteria. The literature reviewed in this article includes those involved in isolating amylase-producing bacteria, manufacturing and optimizing processes, and other literature closely related to them and recent ones.

1. INTRODUCTION

1.1. Microorganisms

Microorganisms are the most important enzyme-production sources. Choosing the right species plays a key role in generating high yields of suitable enzymes for industrial use. Bacteria, fungi, actinomycetes, algae, and protozoa may be classified as soil microorganisms. Each of these groups possesses characteristics that characterize them and their soil work. The most common microorganisms in soil are the bacteria and Archea. Enzymes are biological catalysis of a macromolecular nature. At the start of the cycle, the molecules are called substrates, and the enzyme transforms these into various molecules called products [9]. The definition of a microorganism has allowed a real understanding of the microbial environment as one that can be analyzed using similar methods and strategies, even though it reflects very different forms of reproductive units and cell organizations. Biologists began to understand the commonality of all species in the late 20th century following the work of Carl Woese and other molecular evolutionists, all consisting of cells with remarkable resemblance to each other and having a common evolutionary heritage, and consequently with major features of a widely shared genetic code and molecular biology. Microbiology and biology as a whole were united in this context, as they had never been before [86, 11].

1.2. Enzyme

To many the term enzyme is a common phrase. Enzymes as described are biological compounds which accelerate biochemical and biological reactions. Our function is to act as chemical catalysts that accelerate the time of these internal and external reactions to the cell of an organism [75]. Biological catalysts are enzymes which regulate different biochemical reactions. In recent years, the possibility of using microorganisms as biotechnological sources of industrially important enzymes has sparked interest in many microorganisms exploring extracellular enzymatic action.Proteases and amylases are considered to be the most prominent enzymes among the industrially significant enzymes as they are commonly used in the brewing, detergent and food industries [16]. Enzyme uses increased after World War II because of advances in industrial microbiology and biochemical engineering. [26].For several different

fields, such as beef, feed, detergent, textiles, clothing, tanning, as well as pharmaceutical, cosmetics, and finechemical industries, enzymes are now being used for days. Industrial applications constitute more than 80 per cent of the global enzyme market [80]. At least 50 percent of the enzymes sold today are derived from GMOs, using genetic manipulation and protein manipulation. Feed enzymes are the most commonly used, and are still the largest market share of enzymes. [52]. They are composed of amino acid chains that are connected to peptide bonds [50].The specificity comes about because every type of enzyme has a selective binding site for their respective substratum. The substratum binds to the binding site of the enzyme, known as the 'active site.' There, various chemical bonds are broken and formed in the substratum, which is transformed into a product/s.

Different enzymes need additional components to function, called 'co-factors.' They are critical during conformation in many catalytic steps or in changes to the substrates. These additional components include various metal ions, prosthetic groups and chemical groups called co-enzymes which are very similarly bound to the enzyme. [Figure 1]

1.3. Classes and functions of enzymes

Enzymes perform quite a variety of functions. Mostly, they are responsible for the chemical interconversion that an organism wants to live [75]. Within nature different types of enzymes are found. Every enzyme has a different functional mode on its particular substratum. Some enzymes break up polymeric compounds into their constituent monomers while others do the reverse. On top of that, some enzymes turn a molecule's conformational structure into another shape. They are given specific names based on the type of role an enzyme has. The enzymes are classified into 6 different groups according to the Commission on Enzymes [75] [Table 1].

Enzymes have long been obtained from natural sources for industrial use. Specific enzymes were extracted and generated for the manufacture, breakdown and conversion processes of various compounds in mass quantities in industrial fermenters. The amylase enzyme is a very common one among the many forms of enzymes that are used. [75]

1.4. Amylases

The amylases which have a vial function in the starch [63, 49, and 13] are thus the most commonly used thermostable enzymes. Amylases are enzymes that hydrolyze starch molecules into a range of products, including maltose, dextrin limits, and increasingly smaller glucose-composed polymers [85]. Often, amylase is called 'glycoside hydrolases'[30].

Amylases are enzymes that catalyze the hydrolysis of starch into a sugar. These are present in human saliva and a few other species, where the process of chemical digestion starts. Foods containing large amounts of starch but little sugar, such as rice and potato, taste slightly sweet when chewed, as amylase converts some of their starch into sugar in the mouth. The pancreas and the salivary gland make amylase (alpha amylase) into disaccharides and trisaccharides to hydrolyze dietary starch, which other enzymes turn to glucose to provide the body with energy.Also, amylase is produced by plants and some bacteria. Amylase was the first enzyme discovered and isolated (in 1833, by AnselmePayen) [59]. The International Commission on Enzymes has classified six separate groups of enzymes according to their catalytic reactions: EC1 Oxidoreductases; EC2 Transferases; EC3 Hydrolases; EC4 Lyases; EC5 Isomerases; and EC6 Ligases [44].

Biologically active enzymes are typically obtainable from plants, animals, and microorganisms. For the development of extracellular hydrolases, amylase has been isolated from various sources [4,5,19].

The history of amylases began in 1811 when the first starch degrading enzyme was discovered by Kirchhoff this was followed by several reports of digestive amylases and malt amylases. In 1930 Ohlsson suggested classifying starch digestive enzymes in malt as α and β amylases according to the anomeric form of sugars formed by the enzyme reaction, much later. Amylases, hydrolyte enzymes, catalyze starch hydrolysis into sugar molecules of low molecular weight. In nature, starch is the most abundant food store for polysaccharides after

cellulose and the Earth's main accessible source of carbon and energy [12, 69].

It is plant synthesized and used in the milk, fiber, paper, alcohol, pharmaceutical industries [69]. Amylases are omnipresent and spread throughout the plants, animals and microbes. Nonetheless, amylas-producing microorganisms have successfully replaced chemical processing methodology in various industries because of cost-effectiveness and technological advantages [46, 81].Starch, a raw, sustainable and biodegradable polymer, is provided by many plants as a source of stored energy and can be found in plant roots, stalks, crop seeds and staple crops such as rice, corn, wheat, tapioca, potatoes and amylase-derived micro-organisms [12, 73, and 38]. Starch consists of two elements, one being a linear glucose polymer, amylose containing α-1, 4 connections and the other a branched polymer, amylopectin with linear chains of α-1, 4 residues interlinked by α-1,6 connections [26].

The depolymerization of starches by amylases is the basis of many industrial processes [60]. Studied the thermokinetic activity of α-amylase in the process of starch hydrolysis under various temperature, pH and metal ion conditions using the principle of thermocinetics and reduced extent method [28]. Reported the bioinformatics and biochemical study in the hyperthermophilicarchaeonThermococcussp of a novel maltose-forming α-amylase of the family GH57. CL1-CL1. Maltose-forming α-amylase is a member of the 57 (GH57) family of glycoside hydrolase and is unusual in that it exhibits dual hydrolysis activity against α-1, 4- and α-1, 6-glycosidic bonds and recognizes only maltose.This enzyme was previously only recognized in Pyrococcus sp. IS 04. [39]. [47].

Amylases (E.C:3.2.1.0), a term referring to α-amylase, ß-amylase and π-amylase, are among the most common and significant enzymes due to their wide variety of applications and are also the second largest group on the protease market [47]. They are one of the most important industrial enzymes that covers about 30 percent of the world's total enzyme market [2, 21].

Amylases have a broad range of applications in starch liquefaction and saccharification[71] and also in various industries including food, beverage, biofuel, paper, pharmaceutical, sweetener, detergents, textiles, fermentation and starch processing [21,46,82,29,20,16,43,68,23]. Various catalytic activity of α-amylase was reported as a response to the nitrogen substance used in the production phase [23]. Reported molecular cloning and biochemical characterization in *Escherichia coli* of a thermoacidophilic, organic-solvent tolerant α-amylase from a Bacillus strain; [6]. [18].In plants, the term α-amylase materializes during the onset of seed germination and development and is promoted by abscisic acid and salicylic acid in response to gibberellins and repressed [87]. The principal role of ß-amylase is its involvement in the breakdown of starch in plants [32], proved the function of ß-amylase in the transitory breakdown of starch [66].

2. CLASSIFICATION OF AMYLASE

Depending on their mode of action, amylases can be divided into two classes, endo-and exo-amylases. The endo-amylases catalyze spontaneous hydrolysis of α-1, 4 glycosidic bonds present in the starch chain of structural components i.e. amylose or amylopectin.This catalytic activity contributes to the formation of linear and branched oligosaccharides of various lengths of chains. The exo-amylases hydrolyze from the non-reducing end, resulting in the formation of short end products in succession. Exo-amylase like β-amylase hydrolysis α-1, 4 glycosidic relation and glucoamylase catalyze α-1, 4 and α-1, 6 glycosidic bonds [1, 3, 7].

Endo and exo-amylases that primarily operate on α-1, 4-links, debranching enzymes that primarily target α-1, 6-links, and cyclodextringlycosyltransferases that kill starch by primarily catalyzing cycling and excessive reaction. [Table 2]

2.1. Endoamylases

Endoamylases merely cleave the α-1, 4-bonds in starch in the starch molecule's inner regions by passing the amylopectin α-1, 6-branching points. The most widely recognized endoamylase is the α-amylase (EC 3.2.1.1). This allows the starch solution to rapidly lose viscosity. These enzymes are mostly classified into two groups according to degree of substrate hydrolysis: liquifying (30-40%) and saccharifying (50-60%). This division is commonly used to describe the α-amylases

properties [83]. The endoamylases products are therefore oligosaccharides of varying lengths. Endoamylases catalyze spontaneous hydrolysis within the starch molecule, creating linear and branched oligosaccharides of varying lengths of the glucose chain. [21] [22]

2.2. Exoamylases

Exoamylases also cleave the α-1, 4-bonds, e.g. β-amylase (EC 3.2.1.2), but some of them are able to attack the α-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 [84].The exoamylases hydrolyze the substrate from the nonreducing end, resulting in successively shorter end products [21]. [Figure 2]

2.3. α-Amylase (EC 3.2.1.1)

The α-amylase family comprises a group of amylases with different substrate and metal ion specificities that function as glucose residues connected by glycosidic bonds α-1-1, α-1-4, α-1-6[79].

α-Amylase (EC 3.2.1.1),Calcium metalloenzyme, is also known as glycogenase or 1,4-α-D-glucanglucanohydrolase [7,14]. We arbitrarily hydrolyze α-1, 4 bonds, and cleave long-chain sugars to produce amylose and maltose maltotry and maltose, glucose, and restrict amylopectin dextrin [52]. α-Amylases come from plants, animals, bacteria (Bacillus) and fungi (ascomyests and basidomycetes)[7].

(EC 3.2.1.1) (1,4-α-D-glucanohydrolase; glycogenase) amylase is calcium metallo enzyme which works at random positions in the starch chain leading to the breakdown of long-chain carbohydrates into maltotriosis which amylose maltose, or amylopectin maltose, glucose and minimal dextrin. α-amylase appears to function quicker than β-amylase and is a major digestive enzyme of amylopectin blindness in animals.

2.4. β-amylase (EC 3.2.1.2)

β-amylase (EC 3.2.1.2) hydrolyzes α-1.4 glycosidic connections from non-reducing ends and cannot circumvent α-1.6 connections as compared to α-amylase It is produced by plants, bacteria, and fungi[29]. While in animal tissues β-amylase is absent, it may be present in the microbes that reside in the digestive tract. β-amylase starch hydrolyzes maltose in ripened fruits resulting in sweet flavour[7,20]. In seeds both forms of α- and β-amylase occur. β-amylase occurs in an inactive state before germination while αamylase occurs on germination.

(EC 3.2.1.2) Alternative names: 1,4-α-D-glucanmaltohydrolase; glycogenase; saccharogen amylase) β-amylase is also synthesized by bacteria, fungi, seed pulses and plants which catalyze the hydrolysis of the second α-1,4 glycosidic bond, cleaving two glucose units (maltose) at a time. β-amylase breaks down starch into sugar during fruit ripening which results in sweetening of ripe fruit. Before germination β- amylase is present, while α-amylase and proteases emerge at the onset of germination. Animal tissues do not contain β-amylase although only [53, 56, and 54] can be found in microorganisms located within the digestive tract.

2.5. γ-Amylase (EC 3.2.1.3)

γ-Amylase (EC 3.2.1.3) catalyzes glycosidic hydrolysis of α-1,4 and α-1,6. Alternatively it is called glucoamylase, exo-1, 4-α-glucosidase, amyloglucosidase, glucan 1,4-αglucosidase, 1,4-α-D-glucanglucohydrolase or αglucosidaselysosomal. It amylase is optimally active in acidic conditions, as compared to α- and β-amylases [76, 36].

(EC 3.2.1.3) (alternative names: glucan 1,4-α-glucosidase; amyloglucosidase; exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucanglucohydrolase), last α(1-4)glycosidic interactions at the non-reducing end of amylose and amylopectin, yielding glucose along with α(1-6) glycosidic interactions. In comparison to other types of amylase, ÿ-amylase is most effective in acidic environments and has an optimum pH 3[54].

3. MECHANISM OF AMYLASE DEGRADATION

α-Amylodextrin is degraded to normal temperature by malt amylase in two fairly well-defined stages.
(a) A relatively fast reaction in which maltose is the main sugar produced but the α-effect predominates slightly in the mutarotation shown. A-dextrins and f-maltose are produced usually in the relative's decreasing (and mutarotating) proportions.

(b) A relatively slow, apparently linear reaction during which, if not the only reduced sugar formed, glucose is the chief, thus accounting for the linearity observed; At this point, there was no detectable mutarotation between the fission products.

There is no well-defined demarcation point between certain phases of the reaction unless low concentrations of enzymes are used. It describes a new technique for evaluating the sense of mutarotation in strongly colloidal enzyme-substrate mixtures [17].

4. AMYLASE ENZYME PRODUCING BACTERIA

Amylase is economical since it is easy to manipulate microbes to acquire enzymes that have different characteristics. *Bacillus sp*. Among bacteria; It is commonly used in the production of thermo-stable amylase to meet industrial needs, and is considered to be strong α-amylase producers [40]. Bacillus sp, is estimated to be. Enzymes account for approximately 50 per cent of the global enzyme market [65].

Different species of Bacillus were stated to be producing α-amylase by fermentation. Spp bacillus. KR-8104 produces α-amylase, which is Caindependent and active at low pH [62], used for the large-scale α-amylase development.Some *Bacillus* strains produce thermostable α-amylases while others develop acid-resistant α-amylases. An option for commercial production of thermostable α-amylases is the thermophilic bacterium *Bacillusstearothermophylus*. *Bacillus spp* produces alkaline, and thermotoleran*t* amylases. Such as *Bacillus licheniformis*[64, 67] and *Bacillus halodurans*. Production of cold-active extracellular αamylase from the novel bacteria *Microbacteriumfoliorum*GA2 and *Bacillus cereu*s GA6 was reported [34].

5. DETERMINATION OF AMYLASE ACTIVITY

Al1 the isolates of the *Bacillus subtilis* were tested with starch agar for amylase production. Starch agar medium inoculated with organism and then filled with a solution of iodine. The starch plate is flooded with the iodine solution above after 72 hrs of growth. Under blue backdrop, the clear zone is seen around amylase / generating colonies [48]

6. CALCULATION OF ENZYME ACTIVITY

The Enzyme activity was calculates as Concentration of the product produced/molecular weight of the product = 1/ incubation period.
The enzyme activity is expressed as µ moles / ml/ min [48].

7. SCREENING FOR AMYLASE PRODUCING BACTERIA

Identified isolate was screened for amylolytic activity by stretching individual isolate to 1% starch nutrient agar and 1% starch dextrose agar size. For 2448hr, the agar plate was incubated at 37oC, and that of the fungi was incubated for 3-5days at room temperature. Culture plate was filled with lugol iodine to classify region around culture clearing. The diameter of clearing zone formed represents the isolated strain's amylolytic activity as defined in [9].

8. ISOLATION OF BACTERIA

Starch agar is a selective medium that was used to isolate *Bacillus subtilis* around 1.0gmof soil sample was diluted serially in sterile physiological saline and dilution was achieved by comprehensive mixing up to 10-5. 0.1ml of the 10-3 dilution sample was spread with the aid of L-rod on sterile petridishes containing starch agar, and the plates were incubated at 37^{o}C for 24-48 hours. The plates were observed for growth of bacteria after incubation [48].

9. APPLICATION OF AMYLASE

Amylases are one of the most commonly used enzymes needed to prepare fermented foods and the market for amylase is growing with an expanding range of uses. α-amylases was the first to be commercially produced and sold amongst all other enzymes. As digestive aid, the first industrial development of α-amylase from A. *Oryzae*, known as "Taka diastase," developed by Dr. J. Takamine [71] is used. The global market for enzymes is important for a broad variety of applications. Amylases have wide uses in food and non-food starch based industries. [Table 3]

9.1. Starch processing industry

Starch, the second largest food store in nature for polysaccharides, is the primary constituent of the majority of staple foods and is used in various food and non-food industries. Starch is created by

photosynthesis by plants and is an easily accessible source of carbon on Earth. Starch is used in various industries including food, alcohol, paper, textiles and so on. Starch processing is needed for most applications in the various industries. Chemical starch processing has drawbacks such as high temperature requirement, low pH, lower glucose yield, undesirable compound taste and unwanted color synthesis while enzyme-mediated hydrolysis has the capabilities to resolve these unfavorable features[69,27].α-amylase-mediated starch hydrolysis is used in glucose and fructose syrup production[45].

9.2. Food industry

The introduction of modern biotechnology has brought multiple improvements to the food industry. The use of enzymes in food industry is varied, and they are most often used as processing agents. In the processed food industry, amylases are commonly used for baking, drinks, starch syrups, etc. Bread production is one of the most common techniques in food processing globally [22]. The use of enzymes in bread making shows their importance in quality control and production efficiency. To extend the freshness and shelf-life of baking products, amylase is added to the flour, alone or in a combination of enzymes [70].For baking products such as bread, amylases are added to the dough to hydrolyze the flour polysaccharide into dextrins, fermented by the yeast [74]. In the clarification of juices, amylases are used to optimize the production of transparent juice [78, 71]. Amylases produced from microorganisms may also be used in alcoholic drinks by hydrolyzing starch prior to fermentation to reduce or remove turbidity. In conjunction with cellulases and pectinases, amylases are used for maceration, liquefaction, and clarification during juice processing to increase yield and cost-effectiveness [37, 18].

9.3. Detergent Industry

Recently, the use of enzymes as an additive in detergent formulations has become unavoidable due to milder conditions than the chemicals comprising detergents [61].

This company accounts for the leading use of industrial enzymes. The formulations of the enzyme detergent boost the efficacy of the detergents in stain removal in an eco-friendly manner [72]. Amylases catalyze glycosidic connection hydrolysis in stains and remove the starchy glue which combines with other stains and dirt. The α-Amylase, an endo-amylase, is mainly used for laundry detergents, since exo-amylase activity is inefficient for removing stain. Actually, α-amylases are used in the liquid detergent formulations of about 90 per cent [21, 72, 24, and 42].

9.4. Paper Industry

Owing to rising awareness of sustainability concerns and mitigating unfavorable environmental impact, the use of microbial origin enzymes has increasingly grown in the paper and pulp industry. Enzyme uses in this industry that the energy consumption, processing time and quantity of chemicals required for processing. Amylases uses in this industry include starch coating, de-inkling, enhancing drainage, and paper cleaning [35, 70]. The primary application of α-amylase in the paper industry is the manufacture of high molecular weight starch with low viscosity by adjusting the coated paper starch [22].This process makes the paper smooth and solid, to improve the quality of writing. α-Amylase is used to partly hydrolyze polymer in a batch or continuous cycle for paper sizing to preserve the starch viscosity. Paper sizing increases the consistency of the final product and boosts paper strength and rigidity. Active low temperature α-amylases are useful for reducing starch viscosity for proper paper coating [11, 33, and 43].

9.5. Textile Industry

Amylases are used in this industry for plant desizing, i.e. removal of starch to enhance uniform wet production. Starch is applied to the yarn, a low price and easily available sizing agent for quick and secure weaving operation. Starch paste is used for warping in textile weaving to provide strength and prevent the loss of cords [2].

After weaving, amylase is added which specifically catalyzes starch hydrolysis to water soluble dextrins. Effectively, amylase extracts starch without damaging the fabric [31].

The enzymatic desizing of cotton with α-Amylase is state of the art since many decades [41] the enzymatic desizing of cotton with α-amylases has been state of the art. Bacillus strain amylases are used in synthetic fibre warp sizing [72].

10. CONCLUSION

Due to their exploitable characteristics, amylases are immobilized into suitable organic, inorganic and non-toxic and biodegradable matrices to make them more viable in various industrial applications to enhance their thermal and storage stability as compared to free enzymes. Thus amylases have widened industrial applications especially in detergent, leather, paper, chemical and pharmaceutical industries.

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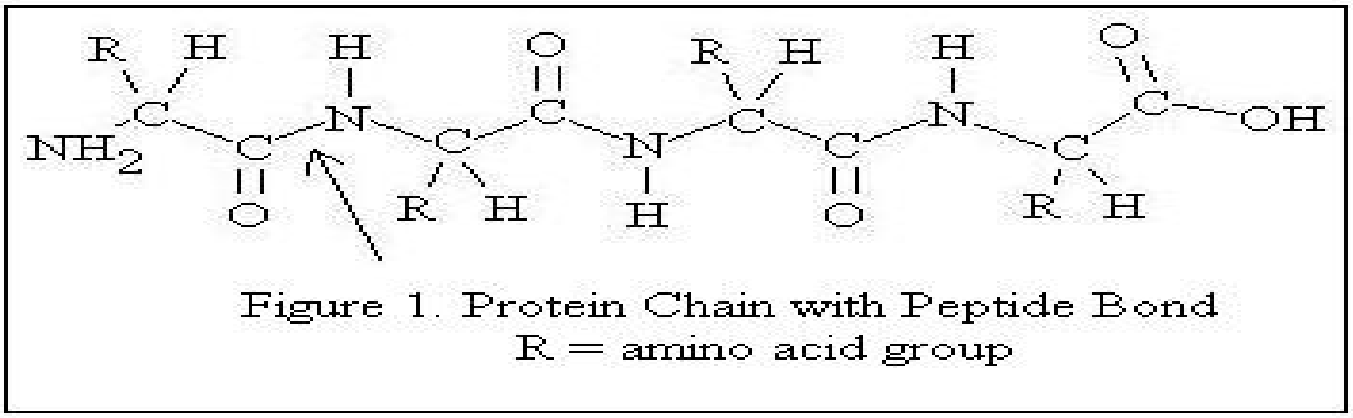

Figure 1: Typical protein structure: two amino acids joined by a peptide bond [50].

Table 1: Classes of enzymes with their functions [75]

Enzyme ommission Number	Class of enzyme	Function	Example
EC1	Oxidoreductases	Such enzymes catalyze the transfer of electrons between molecules. The transition of the hydrogen molecules takes place in biological systems. The enzymes that do this usually refer to as dehydrogenases.	Alcohol dehydrogenase
EC2	Transferases	Such enzymes move various forms of atoms between the different molecules.	Aminotransferase
EC3	Hydrolases	This class of enzymes breaks down molecules with the help of water.	Amylase
EC4	Lyases	Lyases remove groups to form double bonds, or add groups in substrates to double bonds.	Pectatelyase
EC5	Isomerases	These enzymes allow the same molecule to change structurally, by moving groups from one position to another.	Mutase
EC6	Ligases	Such enzymes bind molecules together through covalent bonds. Such reactions require energy input in the form of ATP co-factors.	DNA ligase

Table 2: Classification of amylases [77, 83]

Enzyme	Glycosidic Bond Specificity	Mode of action	Product
α-amylase (1,4-α- D-glucan glucanohydrolase)	α-(1-4)- glucosyl	Endo	oligosaccharides Linear and Branched
β-amylase (1,4-α- D-glucanmaltohydrolase)	α-(1-4)- glucosyl	Exo	Dextrins Maltose and limit
Amyloglucosidase (Exo-1,4-α- glucosidase; glucoamylase)	α-(1-4)- glucosyl and Glucose α-(1-6)-glucosyl	Exo/Endo	Glucose

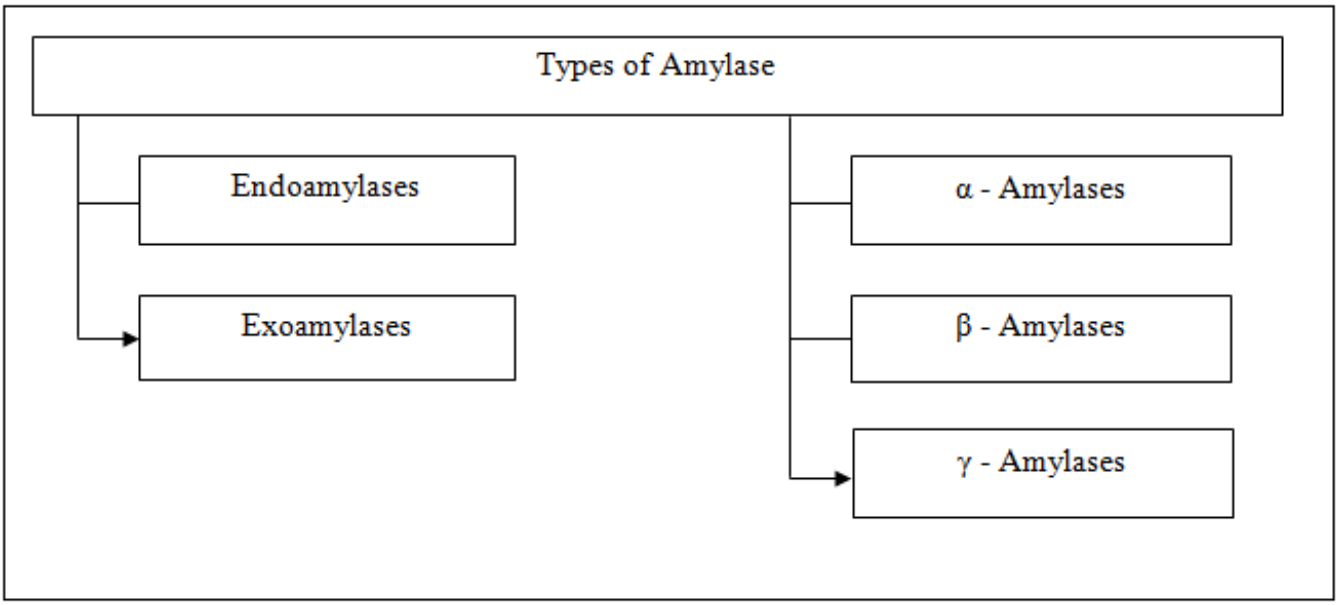

Figure 2: Different types of amylases

Table 3: Use of amylases in different industries [70, 21, 44]

Industry	Applications
Food	Starch liquefaction and saccharification; manufacturing of corn syrups; anti- staling in baking; Enhance shelf life of breads; reduction of chill haze formation in beverages
Detergent	Removal of starch based stains
Paper	Reduction of viscosity, Deinking, drainage improvement
Textile	Desizing, Warp sizing of textiles fibers
Biofuel	Ethanol production
Pharmaceutical	Digestive aid
Bioremediation	Bioremediation of vegetables wastes
Leather	Fiber splitting

Corresponding Author: Anchal Bamal
Faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India.
E-mail: anchalbamal@gmail.com

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