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REVIEW OF AMYLASE PRODUCTION BY MICROORGANISMS AND THEIR INDUSTRIAL APPLICATION

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ABSTRACT

The enzyme amylase is responsible for catalyzing the conversion of starch into sugars. There are three different kinds of amylase: gamma, beta, and alpha. It has been reported that microbes produce all the three types of amylase. Microbial amylases derived from fungi and bacteria are primarily employed in a variety of sectors and have enormous potential application in industries. This review documents the application of microbial amylase across different industries.

Keywords: Microbial Enzymes; Amylase; Industrial Applications; Sustainability; Microorganisms.

INTRODUCTION

In recent times, amylase-producing microorganisms have garnered significant attention as a result of their pivotal role in various industrial and environmental applications. Amylase, an enzyme that is known to catalyze the hydrolytic breakdown of starch as well as glycogen into simpler sugars, holds immense significance in industries ranging from food processing to biofuel production (Gupta *et al.*, 2013). The production of this enzyme by microorganisms, particularly in unconventional habitats such as waste dump sites, has emerged as an intriguing area of study. Waste dump sites, characterized by their unique environmental conditions, are often considered inhospitable for most forms of life. However, they serve as reservoirs of microbial diversity, housing organisms that have adapted to thrive in extreme or contaminated environments. Among these, amylase-producing bacteria represent a valuable resource with the potential for biotechnological exploitation. This article offers a thorough summary of the current body of information regarding the uses of amylase-producing bacteria in various industries.

Amylase and its Significance

Amylases have been described as a group of hydrolyzing enzymes capable of causing the chemical decomposition of moieties entailing bond splitting as well as the addition of the hydrogen cation coupled with water based hydroxide anion. As hydrolyzing enzymes, amylases are proteins that catalyze the breakdown of specific biopolymers (complex sugars, proteins) through a chemical process that results in the formation of monomers or smaller polymers, like amino acids or monosaccharides (Arekemase *et al.*, 2020). According to Singh *et al.* (2013), amylases function by breaking down the connections that currently exist between neighbouring glucose moieties, resulting in the creation of unique metabolic products that are particular to the implicated enzyme. These proteins are recognised as belonging to a class of commercial enzymes that account for 25–30% of the market for enzyme synthesis worldwide. The synthesis and use of amylases has opened up a wide range of new and exciting possibilities for the commercialization of biotechnological processes. These include the production of renewable energy, medicines, detergents, food products, warp sizing of textiles or fibres, paper manufacturing facilities, baking, and pre-treatment of animal feed to increase digestibility (Abalaka and Adetunji, 2017; Fentahum and Kumari, 2017).

Two primary techniques are utilized for commercial scale production process involving α-amylase namely submerged and solid state fermentation. Molasses and broths are two examples of freely flowing liquid substrates that are used in submerged fermentation (SmF). The

fermentation broth is an elaborated form of the metabolic end products generated during fermentation (Prameela *et al.*, 2016; Sundarram and Murthy, 2014). Because the substrates are often used quite quickly, a constant supply of new substrates is needed. Microorganisms like bacteria that require higher moisture content for growth and multiplication can be accommodated by SmF. The primary use of SmF is the extraction of secondary metabolites intended for liquid utilization (Sundarram and Murthy, 2014).

Bacteria that produce amylase are vital to the cycling of nutrients, the breakdown of organic materials, and the preservation of ecological equilibrium in natural ecosystems. Their ecological significance is profound, as they influence the availability of carbon and other nutrients to various organisms and impact the health and functionality of ecosystems. Amylase-producing bacteria are integral members of the soil microbiota, forming diverse communities in terrestrial ecosystems. These bacteria are well-adapted to the soil environment and are often found associated with plant roots, organic matter, and microbial aggregates in the rhizosphere as well as the detritusphere [soil associated with decomposing organic matter] (He *et al.*, 2020).

One of the main ecological functions of bacteria that produce amylase is the breakdown of complex carbohydrates found in organic debris. According to He *et al.* (2020), they release amylases, which catalyze the breakdown of starch and related substances into simpler sugars like glucose and maltose. Bacteria generating amylase aid in the breakdown of leaf litter in forest habitats. As a result of this breakdown process, nutrients, including carbon are released back into the soil where they can be absorbed by plants and other microbes (Sinsabaugh *et al.*, 2016). Additionally essential to the breakdown of root exudates (organic substances secreted by plant roots) are bacteria that produce amylase. This disintegration promotes the establishment of advantageous soil microorganisms and aids in controlling the rhizosphere's carbon and energy source availability (Gyaneshwar *et al.*, 2022).

The activities of amylase-producing bacteria are central to carbon cycling in ecosystems. By converting complex carbohydrates into simpler compounds, they contribute to both the input of organic carbon into soils (from plant litter and root exudates) and the release of carbon through decomposition (Sinsabaugh *et al.*, 2016). Amylase-producing bacteria influence the accumulation and turnover of soil organic carbon, a critical component of soil fertility and ecosystem resilience. Plant and other organism growth are impacted by the soil's carbon cycle (He *et al.*, 2020). The balance between carbon input and carbon release in ecosystems is essential for maintaining overall ecosystem health. Amylase-producing bacteria participate in this balance by mediating carbon turnover processes (Sinsabaugh *et al.*, 2016).

Bacteria that produce amylase interact with a variety of different soil microbes, such as fungi, archaea, and other bacteria. These interactions can be cooperative or competitive and influence nutrient cycling dynamics and microbial community structure (Rousk *et al.,* 2010). In some cases, bacteria and fungi collaborate in the decomposition of complex organic matter. For example, bacteria may produce amylases to break down starch, while fungi produce enzymes like cellulases to degrade cellulose (Rousk *et al.,* 2010). Amylase-producing bacteria may compete with other microorganisms for resources, including carbon sources and nutrients. These competitive interactions can shape microbial community composition in soils (He *et al.,* 2020).

The health of soil and the efficiency of ecosystems are influenced by the existence and activity of bacteria that produce amylase. Healthy soils rich in microbial diversity and activity are essential for nutrient cycling, plant growth, and the overall sustainability of terrestrial ecosystems (He *et al.*, 2020). Amylase-producing bacteria contribute to plant-soil feedback mechanisms, where plants influence the compositional profile of edaphic microbial communities, and in turn, soil microorganisms impact floral growth as well as nutrient availability (van der Putten *et al.*, 2013). Bacterial communities, including amylase-producing bacteria, contribute to the resilience of ecosystems by maintaining nutrient availability and soil structure, which helps ecosystems recover from disturbances (Gonzalez *et al.*, 2018).

Amylase-producing bacteria have significant ecological significance in natural ecosystems, contributing to organic matter decomposition, carbon cycling, and nutrient availability. Their activities impact soil health, plant growth, and overall ecosystem functionality, making them essential components of terrestrial ecosystems.

Classification of Amylases

These enzymes are grouped with reference to their catalytic attributes, which entail substrate and metabolic end product specificities. Amylases are classified into three EC classes: isomerases (EC 5), hydrolases (EC 3), and transferases (EC 2). The majority of amylases are found in the EC 3 class. Based on how they function, enzymes are divided into two categories: retaining and inverting enzymes. After the retaining enzymes' catalytic activity is finished, the anomeric arrangement in the substrate is preserved; nevertheless, the arrangement undergoes inversion following the inversion enzymes' catalytic activity. All other enzymes harbouring amylase (EC 3.2.1.1) are known examples of retaining enzymes, whereas two specific forms of amylases—glucoamylase (EC 3.2.1.3) and amylase (EC 3.2.1.2)—are known inverting proteins.

The specificity of the glucan chain is another criterion used in the classification of amylases. Enzymes are typically classified as either exo-acting or endo-acting based on this characteristic. Because of this, some amylases and glucoamylase are categorized as exoenzymes that can liberate glucose or maltose from the non-reducing portion of the glucan chain (Tiwari *et al*., 2015).

α-amylase (EC 3.2.1.1)

This protein is essential for starch hydrolysis in both nature and the starch industry (Tiwari *et al*., 2015). As starch has been described as the most critical source of energy for all living biota, these enzymes can be found in a wide variety of living biota. This group of proteins has accumulated an extensive quantity of data from fauna, flora as well as microorganisms. The enzyme from *Aspergillus oryzae* has drawn the greatest interest as a microbial amylase with the majority of our knowledge of amylase activity obtained from this strain (Tiwari *et al*., 2015).

The α-1,4 linkages in glucan are hydrolyzed by α-amylase, but the α-1,6 linkages are not. Dextrins with known elevated molecular weights are created early in the hydrolysis process, culminating in a quick reduction in the viscosity associated with the starch solution. It is well known that as the hydrolysis proceeds, the average DP of dextrins rapidly decreases. The hydrolysis products at the terminal stage of the process include high concentrations of glucose, maltose, and maltotriose as well as oligosaccharides (limit dextrins) with the -1,6 linkage. The hydrolysis products all share the same configuration.

The composition of the product created in the course of hydrolysis is known to differ with respect to the origin of the protein. Some enzymes are known to display a marked reduction in viscosity at the onset of the hydrolysis process, whilst other proteins exhibit a relatively delayed decrease at the same step of the process. The proteins in this category have been isolated and characterized from a variety of microorganisms such as bacteria (e.g. *Bacillus* sp.), fungus (e.g. *Aspergillus* sp.), and archaea (e.g. *Sulfolobus* sp.). While most of these microbes are most active when the temperature is between 30 and 37 ºC and the pH is neutral, some are most active when the temperature is over 100 ºC and the pH is between 3 and 10 (Tiwari *et al*., 2015). Particularly important for fundamental research as well as practical applications are the enzymes that have been extracted from a variety of microbes, including *Aspergillus niger*, *A. oryzae*, *Bacillus amyloliquefaciens*, *B. circulans*, *B. licheniformis*, *B. stearothermophilus*, and *B. subtilis*. According to Tiwari *et al.* (2015), every protein in this category is a known member of the GH 13 family.

Structural and functional attributes of α-amylase

It has been noted that microorganisms, flora as well as higher organisms all harbor α-amylase (-1,4-glucan-4-glucanohydrolase) (Kandra, 2013). The enzyme is an endo-amylase that can cleave -D-(1-4) glycosidic linkages to catalyze the first hydrolysis of starch into shorter oligosaccharides (Kandra, 2013). Glucose residues or -1,6-linkages at the end of the substrate cannot be broken down by α-amylase (Whitcomb and Lowe, 2017). oligosaccharides with varying sizes and a -

configuration combined with -limit dextrins, an assembly of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units carrying both -1,4 and -1,6 linkages, are the metabolic products of α-amylase activity (Whitcomb and Lowe, 2017). While it is known that other amylolytic proteins also contribute to the breakdown of starch, -amylase has been found to be the most crucial for the start of the process (Tangphatsornruang *et al.*, 2015).

From a structural perspective, amylase is known to have a three-dimensional form that permits the enzyme to attach to substrate and facilitate the dissolution of glycoside links *via* the activity of highly specific catalytic assemblages (Iulek *et al.,* 2020). According to Whitcomb and Lowe (2017), human α-amylase is a protein that contains calcium and has a molecular weight of 57.6 kDa. It is composed of 512 amino acids in a single oligosaccharide chain. Three domains of the enzyme are known to exist: A, B, and C. (Figure 1). With a (/)8 superstructure structured like a barrel, Domain A is the largest. Positioned between the A and C domains, the B domain is linked to the A domain through a disulphide bond. The C domain is connected to the A domain by a short polypeptide chain and is thought to exist as a separate domain with an unidentified function. It is known to have a sheet structure. The lengthy gap between the carboxyl terminus of the A and B domains houses the substrate-binding active site of the enzyme α-amylase. Typically located between the A and B domains, calcium (Ca^{2+}) can stabilize the three-dimensional structure and function as an allosteric activator. Figure 1 depicted the α-amylase structure.

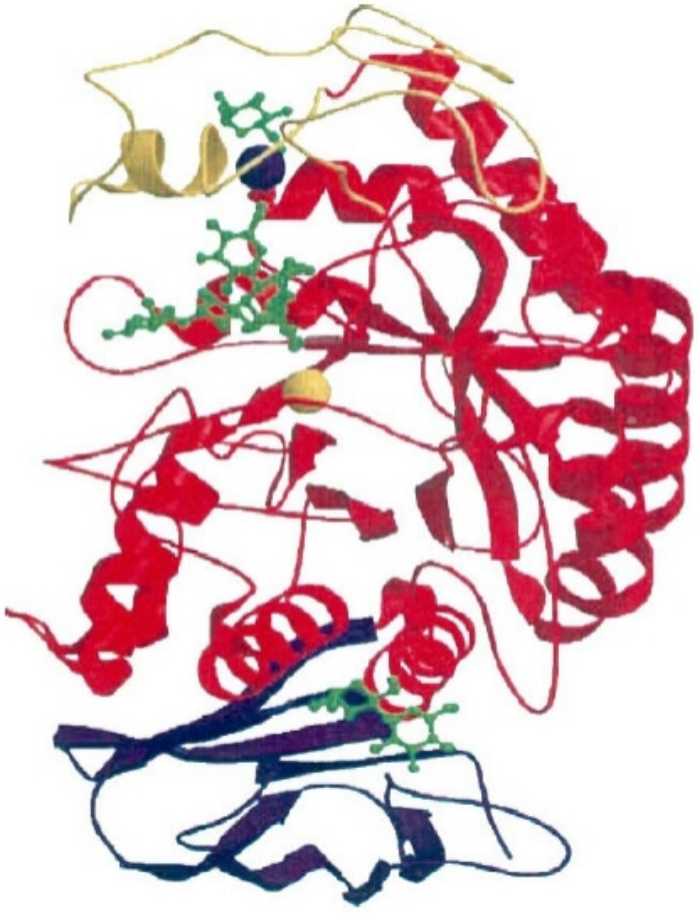

Figure 1: Structure of α-amylase. Domain B is symbolized by yellow, Domain C by purple, and Domain A by red. In the catalytic centre, the calcium ion is visible as the blue sphere and the chloride ion as the yellow sphere. Both the surface binding sites and the active site are connected by green structures. Source: (Payan, 2014)

α-amylase production

Numerous physico-chemical factors have been found to have a direct impact on the production of α-amylase through both solid-state fermentation (SSF) and submerged fermentation (SmF).

Based on the ease with which different parameters ranging from pH, temperature, aeration as well as moisture can be controlled; SmF has traditionally been used to produce industrially important enzymes (Gangadharan *et al*., 2018). SSF systems would appear promising as a result of their inherent potential and benefits. Because SSF is recognised to mimic the natural habitat of microorganisms, it is an excellent choice for fostering their growth and production of highly valuable products with added value. Particularly in relation to mycoflora, SmF might be seen as an aberration of their native habitat (Singhania *et al*., 2019).

In accordance with the water activity theory, mycoflora such as fungi and yeast have been regarded as suitable microorganisms for SSF, whilst prokaryotes were viewed as an unsuitable choice. However, previous situations have revealed that effective management and manipulation of prokaryotic strains for SSF process is feasible. Additional benefits of SSF over SmF include higher production, easier processes, fewer energy requirements, less capital investment, less water output, better product recovery, and no accumulation of foam. It is also regarded to be the most effective procedure for developing countries. Researchers have recently looked into whether SSF is a better system for producing enzymes. When compared to SmF, they found that SSF produced greater product yields, suggesting its suitability for the manufacture of microbial enzymes and other thermolabile products (Couto and Sanromán, 2016).

The optimization of current fermentation conditions, particularly physical and chemical parameters, is crucial for the development of optimal fermentation processes due to their influence on the economics and practicability of the methods (Francis *et al*., 2013). Numerous parameters, including pH, temperature, metal ions, carbon and nitrogen sources, surface active moieties, phosphate, and agitation, have been examined in relation to -amylase production. Each -amylase's thermostability, pH profile, stability, and Ca independence should be suited to the application for which it is intended. For example, in the starch industry, α-amylases should be active and stable at low pH concentrations, but not at high pH levels in the detergent manufacture sector. The most significant of these variables include the microbial growth medium's composition and pH, phosphate value, inoculum age, temperature, aeration, and sources of carbon and nitrogen (Couto and Sanromán, 2016). Numerous bacterial and fungal sources of -amylases have been the subject of in-depth investigation and study on their physical and chemical characteristics (Gupta *et al*., 2013). Table 1 displays the characteristics of several amylases derived from microorganisms.

Table 1: Properties of amylases produced by various microorganisms

Microorganism	Type of fermentation	Optimal pH	Optimal temperature (ºC)
Aspergillus niger	SSF	5.5	70
Penicillium fellutanum	SmF	6.5	30
Bacillus amyloliquefaciens	SmF	7.0	33
Thermomyces lanuginosus ATCC 58160	SSF	6.0	50

Source: (Tanyildizi *et al*., 2017; Kathiresan and Manivannan, 2016)

β-amylase

These proteins are called exo-amylases, and they can either target either α,1-4 glycosidic bonds, like β-amylase, or both α,1-4 and α,1-6 glycosidic bonds, like α-glucosidase (E.C. 3.2.1.20) and amyloglucosidase (E.C. 3.2.1.3). Exoamylases are recognised for their ability to react with the external glucose residues linked to amylose or amylopectin. Consequently, they solely produce glucose (glucoamylase and α-glucosidase), maltose, and β-limit dextrin. It is also known that β-amylase and glucoamylase catalyse the change from α- to β- of the anomeric structure linked to the released maltose. The substrate preferences of glucoamylase and glucosidase are known to differ; glucoamylase is known to prefer long-chain polysaccharides, whilst glucosidase is known to prefer short malto oligosaccharides and can liberate glucose with a -configuration. It has also been noted that a broad variety of microorganisms are capable of producing glucoamylases and α-amylases (Couto and Sanromán, 2016).

γ-amylase

The ability of the enzyme γ-amylase to cleave the last α-(1-4) glycosidic connections in the non-reducing region of both the amylose and amylopectin moieties, generating glucose units, is well known. It also attacks α-(1-6) glycosidic bonds. γ-amylase has been reported to function best in acidic environments and have an ideal pH of 3, in contrast to the other forms of amylase (Tiwari *et al.*, 2015).

Amylase substrates

The bulk of all living things on Earth use starch as an energy source. Starch is a naturally occurring carbohydrate that is produced by photosynthesis and accumulates in green vegetation. Two recognised components of starch are amylose and amylopectin. Amylose is a polyglucan that has been defined as having a degree of polymerization (DP) between 700 and 4000. Its moiety is primarily made up of α-1,4-linked glucans, and it typically makes up 20–25% of starch. DP 104–105 describes amylopectin, the opposite component, as a considerably larger molecule made up of α-1,4-linked glucans that link via α-1,6 linkages to form a highly branched structure. Regular branching allows for the formation of a crystalline moiety in some areas of the amylopectin moiety. It is known that 75–85% of all starch is amylopectin. The sole component of waxy starch found in some waxy flora, such as waxy rice and waxy maize, is amylopectin. Starch is found in nature as crystalline, insoluble granules known as raw starch.

Glycogen is a type of α-glucan that is stored in microbes and animals. It is known to consist of α-1,4-linked glucan connected by the α-1,6 linkage (branching), just as amylopectin. The solubility of glycogen in water is increased by this branching, which is known to happen often and to be equally distributed across the glycogen molecule. Amylase is known to act on both α-polyglucans (Whitcomb and Lowe, 2017).

Microbial synthesis of α-Amylases

Worldwide, α-amylases are synthesized by a wide variety of members of the fauna, flora as well as microbial phyla respectively. In the course of time, studies focused mainly on the microbial production of α-amylases have been undertaken in various nations. Several advantages of utilizing microorganisms for the biosynthesis of amylases have been documented and they include; the economical bulk production capacity as well as the ease of culturing microorganisms with the objective of producing enzymes having desired attributes. α-amylases has been recovered from several prokaryotes, archaea as well as fungi (Tables 2 to 4); however, α-amylases from bacterial and fungal organisms have been observed to have vast applications in various industrial settings.

Despite the large range of amylase sources, industrial scale enzyme production processes use microbial sources, primarily bacterial and mycological amylases, for a number of reasons. These advantages include: financial feasibility, consistency, quicker processing timelines, and ease of process alteration as well as optimization (Burhan *et al.*, 2013). Al-Qodah (2016) recovered and examined α-amylase synthesizing thermophilic prokaryote named; JT2 *Geobacillus stearothermophilus* from a Jordanian hot spring. From the solid waste of the dairy industry, a thermostable extracellular *Bacillus* strain that synthesizes amylase was found. Alrumman *et al.*

(2014) cultivated *Bacillus axarquiensis,* a thermoalkalophilic α-amylase synthesizing prokaryote, from soil samples taken from the southern part of Saudi Arabia. The researchers discovered that the wastewater from potatoes contained substrates that prokaryotic cultures could use for the biosynthesis of α-amylase. Additionally, the technique that was developed was economical because it only needed a small amount of nutritional additives to be added to the growth medium. *Brevibacillus parabrevis* and *Bacillus licheniformis*, which synthesize amylase, were cultivated from various segments of the gastrointestinal tracts of two estuarine fish species*, Terapon jarbua* and *Scatophagus argus* (Das *et al.*, 2014). *Bacillus* sp. DDKRC1 and *Bacillus subtilis* DDKRC5, which are likely amylase-synthesizing bacteria, were grown by De *et al.* (2014) from Asian sea bass (*Lates calcarifer*) and milk fish (*Chanos chanos*), respectively. These extracellular enzymes that were cultivated from the gut microbiota may play a major part in the process of digestion. The α-amylase that *Geobacillus stearotermophilus* synthesized was found and examined by Fincan and Enez (2014). In 2014, Khannous and colleagues cultivated a new strain of *Pseudomonas luteola* that synthesizes amylase from top soil samples contaminated by olive wastewater that were taken from Sfax, Tunisia.

Qin *et al.* (2014) used cloning to copy a novel gene (amyZ), which encoded an α-amylase (AMYZ) that is cold-active and halo-tolerant. The protein was expressed in *E. coli* and was barbored by a marine bacterium called *Zunongwangia profunda*. The gene is estimated to be 1785 bp in length and encodes an α-amylase with an estimated molecular mass of 66 kDa, consisting of around 594 amino acids. In 2015, Kanpiengjai and colleagues cultivated and found *Lactobacillus plantarum* S21, an amylolytic lactic acid bacterium that forms maltoses. Maltose (60%) and glucose (38%), respectively, are the main hydrolytic by-products linked to the breakdown of glycogen and starch, amylose, and amylopectin.

It is known that the amylase gene encodes a protein with 910 amino acids, along with a peptide sequence that serves as a signal moiety. Five *Bacillus* strains that produced amylases were found by Khusro and Aarti (2015) in soil samples infected with poultry excrement. Lee *et al.* (2015) discovered and identified a unique bacterial strain called *Microbulbifer thermotoleran*s DAU221, which is capable of generating α-amylase. Liaquat *et al.* (2015) extracted and partially characterized *B. subtilis* (RAS-1) and *Clostridium perfringenes* (RAS-4) from an anaerobic digester that was used to anaerobically co-digest a mixture of cow dung, fruit and vegetable waste, and agricultural residues. The process was carried out in a tank reactor that was constantly agitated. The procedure of anaerobic co-digestion was shown by the authors to be a financially efficient way to generate α-amylases from organic waste during the biogas synthesis process. With an estimated molecular weight of 80 kDa, the purified α-amylase shared a unique sequence motif with other members of the glycoside hydrolase family. Martins *et al.* (2011) described the use of column chromatography to purify extracellular amylase synthesized by *Corynebacterium alkanolyticum* ATH3 cultivated from the distal intestine of a freshwater fish, *Anabas testudineus*.

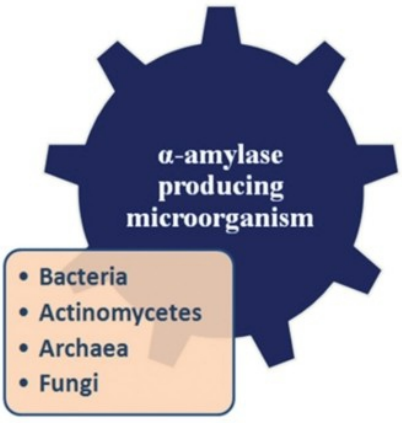

Figure 2: Different groups of α-amylase producing microorganism. Source: (Burhan *et al.,* 2013)

Bacterial amylases

It is known that certain microorganisms may synthesize α-amylase, but with respect to various commercial purposes, α-amylase is principally recovered from *Bacillus* spp. α-amylase recovered from *Bacillus licheniformis, B. stearothermophilus* as well as *B. amyloliquefaciens* are known to have the potential to be utilized in a broad spectrum of industrial processes which include; food and fermentation sectors as well as textile and paper production (Konsoula and Liakopoulou-Kyriakides, 2017). Thermostability has been described as a desirable attribute for the majority of industrial enzymes. Based on their stability, thermophilic proteins sequestered from thermophilic microorganisms have found a range of industrial applications. Several *Bacillus* spp. exemplified by *B. subtilis*, *B. stearotermophilus*, *B. licheniformis* as well as *B. amyloliquefaciens* have been described as popular heat stable amylase producers, and have been widely utilized for commercial scale synthesis of amylases that can be used for a range of applications (Asgher *et al.*, 2017). Several bacterial strains have been documented to synthesize heat stable amylases utilizing both SmF and SSF (Asgher *et al.,* 2017). Nevertheless, SSF has been found to be more advantageous than SmF, and the procedure may enable a more cost-effective method of producing amylase. It has been noted that only the species *Bacillus* can produce α-amylase by the SSF process; sources of enzymes included *B. subtilis*, *B. polymyxia*, *B. megaterium*, *B. vulgarus*, *B. megaterium* and *B. licheniformis* (Asgher *et al.*, 2017).

Table 2: Amylase-producing bacteria cultured from several habitats.

Bacterial isolate	Recovered from
Bacillus thermooleovorans	Hot spring
B. pseudofirmus, *B. cohnii*, *B. vedderi*, *B. agaradhaerens*, *Nesterenkonia halobia*	Sediment and water collected from soda lakes
Lactiplantibacillus plantarum, *L. fermentum*	Traditionally fermented food preparations
Halobacillus sp.	Saline soil samples
B. subtilis	Traditionally fermented food preparations
Cupriavidus taiwanensis	Hot spring
Anoxybacillus amylolyticus	Geothermal soil from active fumaroles
Bacillus sphaericus	Hot spring

Source: (Asgher *et al.,* 2017)

Table 3: Amylase-producing actinomycetes recovered from different habitats.

Actinomycetes isolates	Recovered from
Streptomyces albidoflavus	Soil samples
Nocardiopsis aegyptia	Marine sediments
Streptomyces gulbargensis	Soil samples
S. erumpens	Brick kiln soil samples
S. clavifer	Soil samples
S. lonarensis	Sediment collected from salt water lake
Nocardiopsis sp.	Marine sediments
Streptomyces sp.	Marine sponge

Source: (Asgher *et al.,* 2017)

Table 4: Amylase-producing archaea recovered from different habitats.

Archaea	Recovered from
Thermococcus profundus	Deep-sea hydrothermal vent
Thermus sp.	Hot spring sediments
Thermococcus sp.	Hot spring sediments

Source: (Asgher *et al.,* 2017)

α-amylase-producing fungi

The extensive use of fungal amylases in the preparation of oriental foods has been documented. Filamentous mycoflora have been widely utilized to synthesize amylases. These molds are frequently used in the synthesis of enzymes like α-amylases because they have been shown to be abundant makers of extracellular microbial proteins.

Amongst mycoflora, the *Aspergillus* spp. are most popularly utilized to produce α-amylase (Table 5). Most researches focused on fungal synthesis of amylases have been constrained to the few mesophilic fungal species and efforts have been made to identify the situations and pick standout fungal strains for commercial scale enzyme synthesis (Gupta *et al*., 2013). According to Kathiresan and Manivannan (2016), these fungal strains that synthesize enzymes are restricted to terrestrial habitats, primarily *Aspergillus* and *Penicillium*. Although a wide range of extracellular enzymes are known to be synthesized by *Aspergillus* species, amylases are considered to be the most important from a commercial standpoint (Hernandez *et al*., 2016). Filamentous fungal strains exemplified by *A. oryzae* and *A. niger*, are known to synthesize several proteins that have several industrial applications. Based on its capacity to synthesize a large number of high-value proteins as well as commercial enzymes exemplified by α-amylase, Many people are interested in using *A. oryzae* as a viable microbial host for recombinant protein production (Kathiresan and Manivannan, 2016). *A. oryzae* is frequently used in the production of industrial enzymes like α-amylase, organic acids including citric and acetic acids, and soy sauce. In the production of α-amylase, *A. niger* is known to exhibit notable hydrolytic properties. Moreover, because of its acid tolerance (pH < 3), it can be used to neutralize possible prokaryote contamination. Based on their ability to colonize and multiply on the used solid substrate, filamentous fungus has been shown to be the perfect microorganisms for SSF (Kathiresan and Manivannan, 2016). Fungal α-amylases are highly recommended over other microbial enzyme producers due of their more widely approved GRAS status.

Majority of researches focused on mycological α-amylase producers has been limited to a few mesophilic fungal species whilst several steps have been made to further perfect prevailing cultural conditions with the aim of selecting optimal enzyme producing fungi on an industrial scale (Gupta *et al.,* 2013).

Table 5: Amylase-producing fungi cultured from several habitats.

Fungi	Recovered from
Penicillium fellutanum	Rhizospheric soil samples associated with mangrove plant
P. rugulosum	Soil samples
P. janthinellum	Soil samples
P. expansum	Waste fruit kernels
Aspergillus niger	Contaminated field soil samples
P. microspora, *A. oryzae*	Leaves of mangrove flora
P. chrysogenum, *Fusarium incarnatum*, *P. polonicum*	Saltwater lake
A. fumigatus	Soil samples
A. flavus	Soil samples collected from a solid waste dump site

Source: (Gupta *et al.,* 2013)

Amylase and its Significance

Amylase is an enzyme with a fundamental role in biological processes and various industrial applications (Smith and Johnson, 2020). This protein is primarily responsible for the hydrolysis of starch and glycogen, breaking down these complex polysaccharides into simpler monomeric sugars, such as maltose and glucose (Brown, 2020). Its significance can be understood from both biological and industrial perspectives.

Biological Significance of Amylase

Amylase, a family of enzymes with the primary function of breaking down complex carbohydrates into simpler sugars, plays a pivotal role in biological processes. Its significance can be understood through its contributions to digestion, energy metabolism, and overall nutrient absorption in various organisms.

Digestive Enzyme

In humans and many other animals, amylase is an essential digestive enzyme (Doe and Roe, 2018). Its production begins in the salivary glands, where it is secreted into the mouth (Smith and Johnson, 2018), here, it initiates the digestion of starch polymers present in foods. The enzyme catalyzes the hydrolysis of starch moieties into maltose, a disaccharide known to comprise of two glucose units. The initial breakdown of starch polymer into simpler sugars eases the subsequent stages of digestion in the gastrointestinal tract.

Within the small intestine, pancreatic amylase is known to take over the role of amylase digestion (Brown and Jones, 2019). It further cleaves maltose into individual glucose molecules and other disaccharides into their respective monosaccharaides, such as glucose, galactose, and fructose. These monosaccharaides can then be efficiently absorbed through the intestinal lining and transported to cells for energy production or storage. The complete digestion of starches into absorbable sugars is essential for extracting energy and nutrients from carbohydrate-rich foods like grains, legumes, and tubers. This energy is crucial for various physiological processes and the overall well-being of an organism.

Amylase in Energy Production

Amylase's role in carbohydrate digestion directly influences energy metabolism (White and Black, 2021). For cells, carbohydrates are a mainstay source of energy. The breakdown of complex carbohydrates into simpler sugars, facilitated by amylase, enables cells to access glucose, which is further processed through glycolysis and other metabolic pathways to produce adenosine triphosphate (ATP). ATP has been described as the cell's primary energy currency, and it is known to power virtually all cellular processes, including muscle contractions, nerve signaling, and biosynthesis.

Adaptations in Different Species

Amylase's biological significance varies across species due to their dietary habits and digestive systems. For instance, herbivores like cows and horses have evolved complex digestive systems and multiple stomach compartments to efficiently

digest cellulose and other plant polysaccharides. Carnivores, on the other hand, produce limited salivary amylase because their diets primarily consist of protein and fat (Gupta and Kapoor, 2017). In some animals, such as dogs, the production of amylase is minimal in comparison to omnivorous or herbivorous species, reflecting their evolutionary adaptations to diets rich in meat. These adaptations highlight the enzyme's biological significance in the context of an organism's dietary preferences and digestive needs.

In summary, amylase's biological significance is fundamental to the digestion of carbohydrates, the release of energy, and the overall nutritional well-being of organisms. Its presence and activity are tailored to an organism's dietary habits and contribute to the efficient utilization of dietary carbohydrates for growth, maintenance, and survival.

Industrial Significance of Amylase

Amylase enzymes hold immense industrial significance due to their versatility and ability to catalyze starch hydrolysis and similar carbohydrate moieties. They are used in many different industries, where they are essential to operations including waste management, biofuel production, and food production.

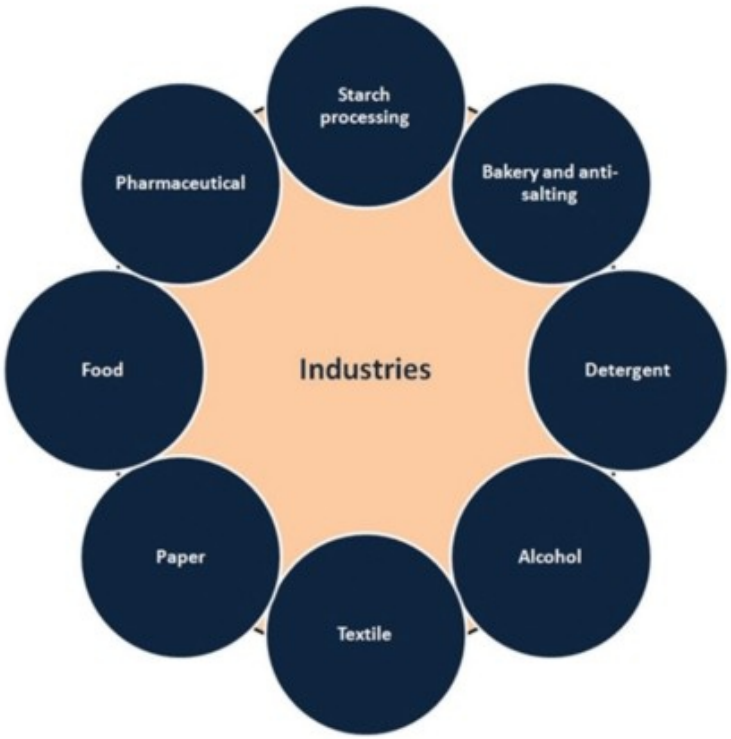

Figure 3: Industries where α-amylase are use. Source: (Brown and Jones, 2019)

Amylase in Food and Beverage Industry

With respect to the food and beverage industrial sector, amylases are indispensable for several critical processes. Amylases are used to convert starches in flour into fermentable sugars, such as maltose and glucose. This breakdown of starches enhances yeast fermentation, leading to dough leavening and the production of carbon dioxide gas, which causes bread to rise (Johnson and Smith, 2019). Brewers employ amylases to convert the starches in malted grains (e.g., barley) into fermentable sugars, which are essential for alcohol production during fermentation. Amylase enzymes play a crucial role in ensuring efficient sugar extraction from grains (Miller *et al.,* 2020). The starch industry relies on amylases for the efficient conversion of starch into various products, including sweeteners (e.g., high fructose corn syrup) and modified starches used as thickeners and stabilizers in food products (Gupta and Kapoor, 2017). Bacteria like *Bacillus subtilis* and *B. licheniformis* are utilized in the fermentation

industry to produce amylases. These enzymes are essential for converting starches into fermentable sugars, a key step in various food and beverage processes, such as brewing and baking (Johnson and Smith, 2019).

Amylases from bacteria are used in starch based industries to transform starch polymers into several end-products, which include; sweeteners like high fructose corn syrup and modified starches used in food products (Gupta and Kapoor, 2017).

Amylase in Textile Industry

For the textile industrial sector, it has been documented that amylases are employed for desizing processes. Starch-based sizing agents are often applied to fabrics to enhance their finish and appearance. Amylases break down these starch-based sizing, making fabrics softer and more comfortable to wear (Chen *et al.,* 2018).

The textile industry uses amylases generated from microbes to help in the desizing process. Before fabric is produced, sizing agents, such as starch, are added to yarn to provide a secure and dependable weaving process. Because it is readily available and relatively cheap in most parts of the world, starch is recognised for having an extremely enticing size. In the wet process used in textile finishing, starch is then extracted from the synthetic substance. The process of desizing has been defined as removing the starch from the fabric, which is known to function as a strengthening agent to prevent the warp thread from breaking during the weaving process. It is well known that the α-amylases stealthily eliminate the size without causing any intrusion into the fibres.

Amylase in Paper Industry

The paper industry uses amylases for de-inking processes. Amylases are utilized to remove starch-based inks and coatings from paper during recycling processes, contributing to more efficient paper recycling (Huang and Wang, 2016).

Amylases are used in the pulp and paper industry to modify the starch found in coated paper, resulting in low-viscosity, high molecular weight starch (Gupta *et al*., 2013). The coating technique is known to enhance the paper's writing quality by giving its surface an appropriate level of strength and smoothness. In this application, the viscosity of natural starch is too high for paper sizing; however, this can be changed by using amylases to partially degrade the polymer in batch or continuous operations. A good paper coating and the quality of the paper itself can be directly enhanced by the use of starch, an efficient optimized agent for paper finishing. Both the paper's strength and stiffness may increase with size (Gupta *et al*., 2013).

Amylase in Biofuel Production

Amylase enzymes are essential in biofuel production, particularly in the conversion of starch-rich feed stocks (e.g., corn, cassava) into bioethanol. Amylases facilitate the release of fermentable sugars from starch-containing plant materials. Microorganisms like yeast then ferment these sugars to produce ethanol, a renewable and sustainable biofuel (Lee and Lee, 2019).

Ethanol has been described as the most popularly utilized liquid biofuel. Based on its cost and ease of availability in most sections of the globe, starch has been regarded as the most popularly utilized substrate for ethanol synthesis (Chi *et al*., 2019). Starch can be dissolved and then put through two enzymatic processes in order to extract simple sugars. Saccharification and liquidification, in which starch is converted to sugar by an amylolytic microbe or enzymes like α-amylase, are followed by the fermentation of sugar into ethanol by *Saccharomyces cerevisiae*, an ethanol producer (Oner, 2016).

Amylase in Detergent Industry

In the detergent industrial sector, amylases are utilized as enzymes for stain elimination. Amylases are effective in breaking down starch-based stains, such as those from pasta, rice, and potatoes. They are incorporated into laundry detergents to enhance stain removal (Smith and Johnson, 2018). Amylases have been ranked as the second key enzyme utilized in the conceptualization of enzymatic detergent, and as such these proteins are present in ninety per cent of all liquid detergents (Gupta *et al*., 2013). According to Mukherjee *et al.* (2019), these proteins have the ability to break down starchy

food residues like potatoes, gravies, custard, chocolate, and so on into smaller oligosaccharides called dextrins. These can then be utilized in dishwashing machines and laundry detergents. Amylases' activity at lower temperatures and alkaline pH allow them to maintain the necessary stability in detergent conditions. The oxidative stability of amylases is a key factor to take into account when using them in detergents in strongly oxidizing washing environments. Additionally, as starch draws different kinds of dirt particles, it must be eliminated from surfaces in order to achieve the benefit of whiteness. According to Mitidieri *et al.* (2016), certain amylases used in the detergent business are taken from *Bacillus* or *Aspergillus*. Amylases are incorporated into laundry detergents as enzymes for stain removal. They help break down starch-based stains, improving the cleaning efficiency of detergents (Smith and Johnson, 2018).

Amylase in Pharmaceutical Industry

Amylases also find applications in the pharmaceutical industry. They are used in drug formulation processes for various purposes, such as controlling drug release rates or enhancing drug solubility (Brown *et al.*, 2020). Amylases from bacteria are used in pharmaceutical formulations for various purposes, including controlling drug release rates and enhancing drug solubility (Brown *et al.*, 2020).

Amylase in Waste Management

In waste management and environmental biotechnology, Amylase-producing microorganisms, including bacteria, are of interest due to their potential role in the biodegradation of organic materials, including starch-based waste. This can contribute to more efficient waste management and the reduction of organic pollution (Doe *et al.,* 2019). Amylase-producing bacteria, including those isolated from waste dump sites, have potential applications in biodegrading starch-based organic materials, contributing to efficient waste management and reducing organic pollution (Doe *et al.,* 2019). The presence of amylase-producing bacteria in soil can influence soil health and nutrient availability, which, in turn, affects plant growth and overall ecosystem dynamics (He *et al.,* 2020).

Bacteria in Waste Dump Sites

Waste dump sites, often referred to as landfills, are environments characterized by the deposition of solid waste materials, including household waste, industrial waste, and various discarded items. These sites are teeming with microorganisms, including bacteria, which play pivotal roles in waste degradation, nutrient cycling, and the overall dynamics of waste management ecosystems. Waste dump sites, commonly known as landfills, are not just repositories of discarded materials; they are dynamic ecosystems with microbial communities adapted to the extreme and challenging conditions presented by the waste environment. These microbial communities are distinctive due to the harsh physical and chemical characteristics of landfill sites, including high organic content, fluctuating environmental conditions, and limited oxygen availability. Landfills host diverse microbial communities, comprising bacteria, archaea, fungi, and viruses. Bacteria are often the dominant group due to their adaptability and capacity to degrade organic matter, a prominent component of landfill waste (Tchobanoglous *et al.,* 2013).

One of the defining features of waste dump sites is the lack of oxygen in the deeper layers due to the compaction of waste materials. As a result, anaerobic microorganisms thrive in these environments. Anaerobic bacteria are essential for processes like methanogenesis, where they convert organic matter into methane gas (CH_4) in the absence of oxygen (Hettiaratchi *et al.,* 2018). Methanogenic bacteria are a crucial group of microorganisms in landfill ecosystems. They specialize in producing methane by fermenting complex organic compounds, such as cellulose and fatty acids that are abundant in waste materials. These bacteria contribute significantly to landfill gas production, which can be harnessed as a renewable energy source if managed appropriately (Kjeldsen *et al.,* 2022). While anaerobic conditions dominate deeper layers, the upper, oxygen-rich zones of landfills host acid-producing bacteria. These microorganisms are responsible for generating acidic metabolic byproducts during the decomposition of waste, influencing the pH of the landfill environment (Tchobanoglous *et al.,* 2013).

Paper and cardboard, two materials high in cellulose, are frequently found in landfill debris. Through the release of cellulases, cellulolytic bacteria have gained the capacity to degrade cellulose into simpler carbohydrates. In landfills, these enzymes are essential to the breakdown of plant-based materials (Das and Chandran, 2011). In landfills, microbial communities frequently participate in intricate syntrophic interactions. For instance, some bacteria decompose complex organic molecules into simpler forms that are then utilized by other microbes in the neighbourhood. These cooperative interactions are vital for the overall degradation of organic matter in the waste pile (Hettiaratchi *et al.,* 2018). Microbial communities in landfills are instrumental in the biodegradation of organic materials. They release vital nutrients like phosphorus and nitrogen that other bacteria can use when they break down complex organic substances. According to Gautam and Tanti (2020), this procedure helps the landfill ecosystem cycle nutrients.

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