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Review Article

MICROORGANISMS AND α-AMYLASE: A CONCISE REVIEW

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ABSTRACT

Amylases are one of the most industrially important enzymes and hold the maximum market share of enzyme sales. The article surveys the α-amylase family and the major structural characteristics, microbial sources, and industrial applications. This review is, therefore, an attempt to execute a detailed study of the literature on the development made in research on microbial α-amylase production, a highly important and demanded industrial enzyme in different fields such as starch processing, detergent, alcohol, textile, food, paper, and pharmaceutical.

Keywords: α-amylase, Enzyme, Microorganisms.

INTRODUCTION

Starch is the major polysaccharide, produced by plants as an energy store, and it is considered as the third biomass source on earth after lignocelluloses and chitins [1]. Amylases are extracellular enzymes that catalyze the hydrolysis of internal α-1,4-glycosidic linkages in starch to dextrin, and other small carbohydrate molecules constituted of glucose units [2]. Based on the cleavage site, they are classified into three types: α-amylase, β-amylase, and γ-amylase. The α-amylase acts at random locations along the starch chain, β-amylase hydrolysis second α-1,4-glycosidic bond, leading to cleaving off two glucose units, whereas γ-amylase cleaves α-1,6-glycosidic linkages, in addition to cleaving the last α-1,4-glycosidic linkages at the non-reducing end of amylose and amylopectin, resulting in the formation of glucose (Fig. 1) [3,4].

Amylases have attracted global enzyme market due to their vast applications in starch processing, detergent, alcohol, textile, food, paper, and pharmaceutical industries [6]. The use of amylase enzyme in industries, particularly in pharmaceutical industries, requires complete purification [7]. These amylases can be procured from various natural resources such as plants, animals, and microorganisms [6,8]. Microorganisms are the predominant source of amylase production due to optimum growth requirements, accessibility, efficient, eco-friendly, and cost-effective as compared to other resources such as animal and plant. The use of efficient production strategies, the microorganisms have established their potential to contribute in industrial applications [9,10]. In the starch liquefaction process, enzymatic hydrolysis through amylases are preferred over other chemical hydrolysis processes and contributing a major share (25%) of total enzymes presently use in the worldwide [11,12]. Major industries, which use 75% of industrially produced enzymes, are detergents (37%), textiles (12%), starch (11%), baking (8%), and animal feed (6%) [3,13,14]. Indeed, amylases are reported to occur in archaea [15,16], actinomycetes [17,18], bacteria [19,20], and fungi [21,22]. Bacterial strains are the significant resources of amylase enzyme. α-amylases are one of the most popular and important form of industrial amylases and the present review point out the microorganisms that produce this enzymes.

STRUCTURAL CHARACTERISTICS OF α-AMYLASE

The α-amylase catalyzes the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of α-D-1,4-glycosidic bonds belongs to endo-amylases family [23,24]. Neither α-1,6-linkages nor terminal glucose residues can be broken by α-amylase [25]. The end products of α-amylase activity are oligosaccharides with different length with an α-configuration and α-limit dextrins, which form a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α-l,6 and α-1,4 oligoglucans [25-28]. Different other enzymes participate in starch breakdown process, but among them, α-amylase is the most important for the initiation of this process [24]. Molecular weights of α-amylases vary from about 10 to 210 kDa. The lowest and highest molecular weight of 10 and 210 kDa has been reported for *Bacillus caldolyticus* and *Chloroflexus aurantiacus,* respectively [29,30].

MICROORGANISMS AND α-AMYLASE

α-amylases are globally dispersed throughout the animal, plant, and microbial kingdoms. Over the past few decades, marked research has been undertaken with the α-amylase being produced by a wide variety of microorganisms. The benefit of using microorganisms for amylases the production is the economical bulk production capacity and microbes are easy to culture to get enzymes of required characteristics. α-amylase has been derived from several bacteria, actinomycetes, archaea, and fungi (Fig. 2); however, enzymes from bacterial and fungal sources have vast applications in different industrial sectors.

α-amylase-producing bacteria, actinomycetes, and archaea

α-amylase can be produced by different species of bacteria, actinomycetes, and archaea (Tables 1-3). Al-Qodah [31] isolated and studied α-amylase-producing thermophilic bacterium *Geobacillus stearothermophilus* JT2 from a hot spring in Jordan. A thermostable extracellular amylase-producing *Bacillus* strain was isolated from dhal industry red gram waste [32]. *Alrumman et al.* [33] isolated thermoalkalophilic-α-amylase producing bacterium *Bacillus axarquiensis* from soil samples in Saudi Arabia's southern region. Their study indicated that the potato wastewater contained substrates which could be used by bacterial isolate for the α-amylase production, and the developed procedure was cost-effective since it requires addition of very minute amount of nutrients to the culture medium. Amylase enzyme-producing *Brevibacillus parabrevis* and *Bacillus licheniformis* was also isolated in the proximal, middle, and distal segments of the gastrointestinal tracts of brackish water fishes *Scatophagus argus* and *Terapon jarbua* [34]. De *et al.* [35] isolated potential amylase-producing fish gut bacteria *Bacillus* sp. DDKRC1 and *Bacillus subtilis* DDKRC5 from Asian sea bass (*Lates calcarifer*) and milkfish (*Chanos chanos*), respectively. These extracellular enzymes from the intestinal microbiota potentially could have a significant role in digestion. The α-amylase produced by *Geobacillus stearotermophilus* was also purified and characterized by Fincan

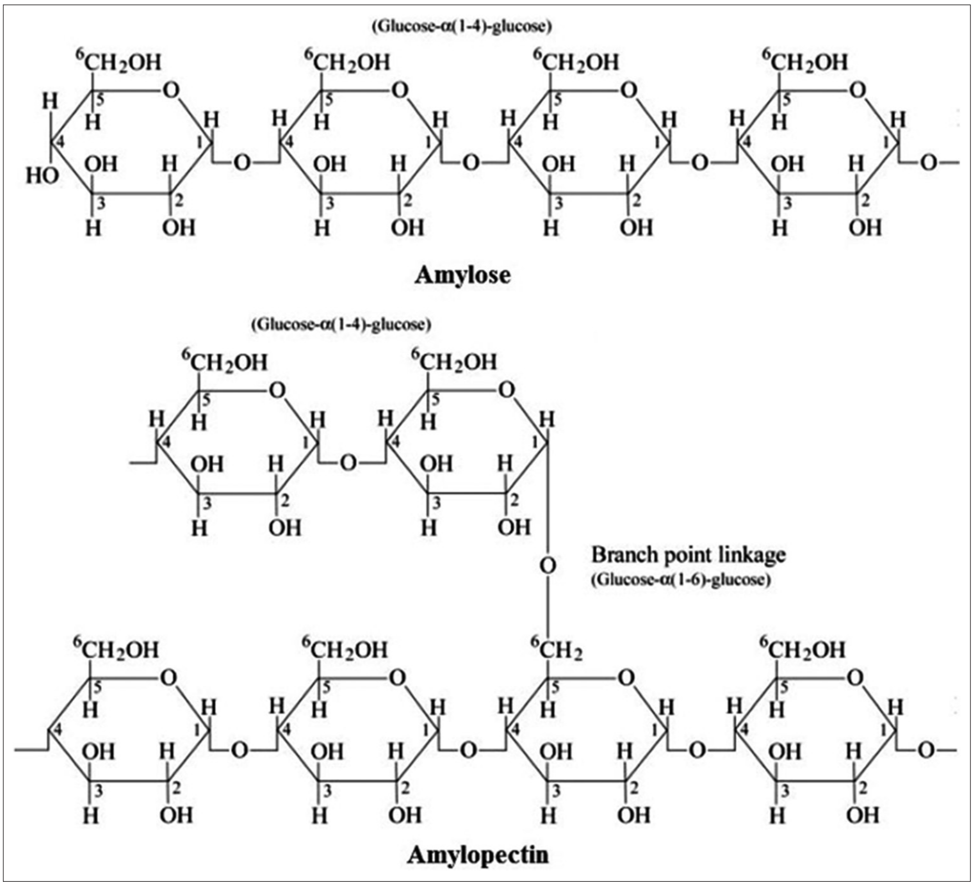

Fig. 1: Structure of amylose and amylopectin [5]

Table 1: Amylase-producing bacteria isolated from different sources

Bacteria	Isolated from	References
B. thermooleovorans	Hot spring	[44]
B. pseudofirmus, B. cohnii, B. vedderi, B. agaradhaerens, N. halobia	Soil and water of soda lakes	[45]
L. plantarum, L. fermentum	Traditional fermented foods	[46]
Halobacillus sp.	Saline soil	[47]
B. subtilis	Traditional fermented food	[14]
C. taiwanensis	Hot spring	[48]
A. amylolyticus	Geothermal soil of active fumaroles	[49]
B. sphaericus	Hot spring	[50]
S. marcescens	Seas and lake	[51]
Chromohalobacter sp.	Solar evaporated saltern pond	[52]
B. licheniformis, B. subtilis	Digestive tract of fish	[53]
B. megaterium	Soil	[54]
B. agaradhaerens	Salt-enriched soil	[19]
B. licheniformis, Gracilibacillus sp.	Salt lake	[55]
P. luteola	Olive washing wastewater contaminated soil	[20]
B. amyloliquefaciens	Rhizosphere of plant	[56]
C. alkanolyticum	Intestine of freshwater fish	[43]

B. thermooleovorans: *Bacillus thermooleovorans, B. pseudofirmus*: *Bacillus pseudofirmus, B. cohnii*: *Bacillus cohnii, B. vedderi*: *Bacillus vedderi, B. agaradhaerens*: *Bacillus agaradhaerens, N. halobia*: *Nesterenkonia halobia, L. plantarum*: *Lactobacillus plantarum, L. fermentum*: *Lactobacillus fermentum, B. subtilis*: *Bacillus subtilis, C. taiwanensis*: *Caldimonas taiwanensis, A. amylolyticus*: *Anoxybacillus amylolyticus, B. sphaericus*: *Bacillus sphaericus, S. marcescens*: *Serratia marcescens, B. licheniformis*: *Bacillus licheniformis, B. megaterium*: *Bacillus megaterium, P. luteola*: *Pseudomonas luteola, B. amyloliquefaciens*: *Bacillus amyloliquefaciens, C. alkanolyticum*: *Corynebacterium alkanolyticum*

and Enez [36]. Khannous *et al.* [20] isolated a novel amylase-producing *Pseudomonas luteola* strain from olive washing wastewater contaminated soil in Sfax, Tunisia. Li *et al.* [37] investigated the cultivable bacterial diversity and production of amylase in three Daqus of Chinese spirits such as Deshan, Baisha, and Wuling spirits. *Bacillus amyloliquefaciens*, *Bacillus cereus*, *B. licheniformis, B. subtilis,* and *Bacillus oleronius* were found to be good producers of α-amylase and glucoamylase. Qin *et al.* [38] cloned a novel gene (*amyZ*) encoding a salt-tolerant and cold-active α-amylase (AMYZ) from a marine bacterium *Zunongwangia profunda*, and the protein was expressed in *Escherichia coli.* The gene has 1785 bp length and encodes an α-amylase of 594 amino acids with an estimated molecular mass of 66 kDa. Choubane *et al.* [1] isolated α-amylase-producing *Bacillus* sp. R2 from Red Sea water at Hurghada, Egypt. They prepared readily available medium using pasta cooking water as basal medium for industrial amylase production. Kanpiengjai *et al.* [39] isolated and

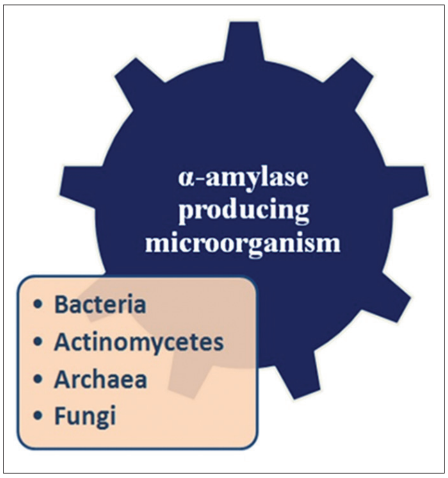

Fig. 2: Different groups of α-amylase producing microorganism

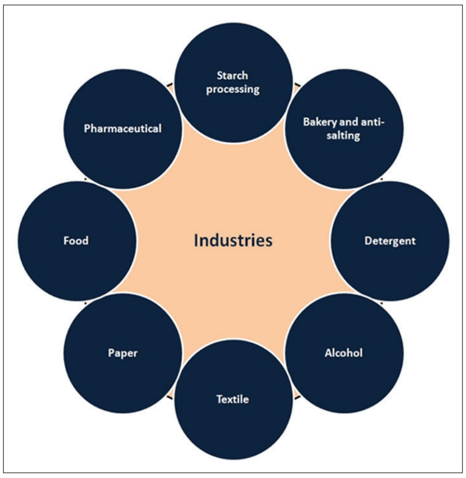

Fig. 3: Industrial applications of α-amylase

characterized a maltose-forming amylolytic lactic acid bacterium *Lactobacillus plantarum* S21. The main hydrolysis by-products from starch, amylose, amylopectin as well as glycogen were maltose (60%) and glucose (38%). The amylase gene encodes a protein consist of 910 amino acids including a signal peptide sequence. Khusro and Aarti [40] isolated five hyper-amylase-producing *Bacillus* strains from poultry feces soil sample. A maltotriose-producing α-amylase, from *Microbulbifer thermotolerans* DAU221, a newly isolated bacterial strain was purified and characterized by Lee *et al.* [41]. Liaquat *et al.* [42] isolated, partial characterized, and purified α-amylase by *B. subtilis* (RAS-1) and *Clostridium perfringens* (RAS-4) from anaerobic digester carrying anaerobic co-digestion of cow manure with fruit-vegetable waste and agricultural residues in continuous stirred tank reactor. Their results suggested the cost-effective production of α-amylases enzyme from organic waste during biogas production. The purified α-amylase with a molecular weight of approximately 80 kDa, shared a sequence motif characteristic of the glycoside hydrolase family 13. Purification of extracellular amylase produced by the bacterium, *Corynebacterium alkanolyticum* ATH3 isolated from the distal intestine of *Anabas testudineus*, a freshwater fish, was carried out using column chromatography [43].

α-amylase-producing fungi

The majority of reports about α-amylase-producing fungi have been limited to a few species of mesophilic fungi, and attempts have been made to particularize the cultural conditions and to select superior strains of the fungus to produce on a commercial scale [7]. The thermophilic fungi are also excellent producer of α-amylase (Table 4).

Table 2: Amylase-producing actinomycetes isolated from different sources

Actinomycetes	Isolated from	References
S. albidoflavus	Soil	[57]
N. aegyptia	Marine sediment	[58]
S. gulbargensis	Soil	[59]
S. erumpens	Brick kiln soil	[60]
S. clavifer	Soil	[61]
S. lonarensis	Sediment of salt water lake	[62]
Nocardiopsis sp.	Marine sediment	[63]
Streptomyces sp.	Marine sponge	[64]

S. albidoflavus: *Streptomyces albidoflavus*, *N. aegyptia*: *Nocardiopsis aegyptia*, *S. gulbargensis*: *Streptomyces gulbargensis*, *S. erumpens*: *Streptomyces erumpens*, *S. clavifer*: *Streptomyces clavifer*, *S. lonarensis*: *Streptomyces lonarensis*

Table 3: Amylase-producing archaea isolated from different sources

Archaea	Isolated from	References
T. profundus	Deep-sea hydrothermal vent	[15]
Thermus sp.	Sediment of hot spring	[65]
Thermococcus sp.	Sediment of thermal spring	[66]

T. profundus: *Thermococcus profundus*

Table 4: Amylase-producing fungi isolated from different sources

Fungi	Isolated from	References
P. fellutanum	Rhizosphere soil of mangrove plant	[67]
P. rugulosum	Soil	[68]
P. janthinellum	Soil	[21]
P. expansum	Waste fruit kernels	[69]
A. niger	Contaminated field soil	[70]
P. microspora, *A. oryzae*	Leaves of mangrove plants	[71]
P. chrysogenum, *F. incarnatum*, *P. polonicum*	Saltwater lake	[72]
A. fumigatus	Soil	[22]
A. flavus	Soil sample of solid waste dump site	[73]

P. fellutanum: Penicillium fellutanum, P. rugulosum: Penicillium rugulosum, P. janthinellum: Penicillium janthinellum, P. expansum: Penicillium expansum, A. niger: Aspergillus niger, P. microspora: Pestalotiopsis microspora, A. oryzae: Aspergillus oryzae, P. chrysogenum: Penicillium chrysogenum, F. incarnatum: Fusarium incarnatum, P. polonicum: Penicillium polonicum, A. fumigatus: Aspergillus fumigatus, A. flavus: Aspergillus flavus

INDUSTRIAL APPLICATIONS OF A-AMYLASE

Microbial amylases play one of the most important roles in the industrial production process (Fig. 3). Enzyme production from microbial sources is extremely crucial for industrial competitiveness, as well as our health, prosperity, and well-being. Many industrial processes involving manufacturing such as industrial, environmental processes, and food biotechnology utilize this enzyme at some stage or the other. A few important industrial applications of α-amylase are given in Table 5.

CONCLUSION

This review illustrates the importance of bacterial and fungal microbes in the production of α-amylase. The search for new microorganisms that can be utilized for production of amylase enzyme is an ongoing process. More recently, many researchers have presented good results in advancing α-amylase production and purification techniques that enable applications in food, textile, paper, and pharmaceutical sectors, which need highly purify amylase enzymes.

Table 5: Detailed of industrial applications of α-amylase

Industries	Applications
Starch processing industry	Production of glucose and fructose
Bakery and antisalting industry	Improvement of quantity, aroma, taste, and porosity of the product; affect antisalting in baking bread and help to improve the softness of the bread
Detergent industry	Improvement of detergent quality by affecting bleaching; increases the stability and effectiveness of the bleach
Alcohol industry	Fermentable sugars are produced by the conversion of starches with the help of α-amylase
Textile industry	Digesting fabrics; resizing agent
Paper industry	Improves the paper quality, protects against mechanical injury, and increases the stiffness and strength in paper
Food industry	Increases the digestibility of carbohydrates
Pharmaceutical industry	Used as a digestive aid

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