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Microbial amylases and their potential application in industries: A review

Indrakshi Bhattacharjee, Deepika Mazumdar and Shyama Prasad Saha

Abstract
Amylases are starch hydrolysing enzymes which breakdown starch to dextrin and smaller polymers composed of glucose units. Amylases have application in various industries including food, textile, paper, pharmaceutical industries. Enzymes are good alternative over chemical catalysts based on the growing environmental concern. Enzymes from the microbial sources have dominated applications in industries. The native starch has many undesired properties such as tendency to retrograde, instability at high temperature and low pH and poor water solubility. Enzymatic modification of starch may offer a more favourable and linear structure. This review focuses on the structure and mechanism of action of amylases and their various industrial application and enzymatic modification of starch.

Keywords: Amylases, starch, enzymatic modification

Introduction
Amylases are hydrolytic enzymes which hydrolyses starch molecules. α-amylases catalyses the hydrolysis of α-1,4 glucan linkages in starch. They can hydrolyze starch molecules into maltose, dextrins and progressively smaller polymers composed of glucose units (Gupta *et al*., 2003; Kandra 2003; Rajagopalan and Krishnan 2008) [1, 2, 3]. The amylases are widely distributed in the microbial, plant and animal kingdoms (Kandra 2003) [2]. They are most commonly derived from mammalian saliva and pancreas. Amylases are one of the most important enzymes used in the industry and they constitute approximately 25% of the world enzyme market (Rajagopalan and Krishnan 2008; Reddy *et al*., 2003) [3, 4]. At present, a large number of microbial amylases are commercially available which has almost completely replaced the chemical hydrolysis of starch in the starch processing industry. The microbial amylases have a broad spectrum of applications in the industries as they are more stable than the amylases prepared with plant and animal sources (Tanyildizi *et al*., 2005) [5]. There are some major advantages of using microorganisms for the production of amylases. One of these advantages includes economic bulk production capacity. Also, the microorganisms are easy to manipulate to obtain enzymes with required characteristics. Fungal and bacterial amylases have dominated applications in the industrial sectors (Gupta *et al*., 2003) [1]. Amylases stand out for their useful application in food brewing, textile and pharmaceutical industries. Bacterial and fungal amylases are very useful in pharmaceutical and fine chemical industries. They are employed for starch liquefaction, production of maltose, oligosaccharide mixtures and high fructose syrup. Along with the technological progress microbial amylases played wide range of application in various industries like in starch saccharification, textile, food, brewing and various medicinal industries (Gupta *et al*., 2003; Kandra 2003; Pandey *et al*., 2000) [1, 2, 6].
Starch is an abundant natural resource. It is biodegradable, environmentally friendly and very cost effective (Jane, 1995) [7]. However, the application of native starch in industries is limited as it has many undesired properties such as tendency to retrograde, instability at high temperature and low pH and poor water solubility. The approaches to overcome these problems include physical, chemical and enzymatic modifications. The chemical modification reduces the tendency of starch of retrogradation and increases solubility whereas the enzymatically treated starch may offer a more favourable and linear structure thus increasing the complexing capability (Conde, 2017) [8]. The present review focuses on the different types of bacterial and fungal amylases and their various applications in industries.

Starch
Starch is a major reserve carbohydrate of all higher plants and they occur as water insoluble granules. The size and shape of these granules are sometimes characteristic of the plant from

which the starch is extracted (Prasanna, 2005) [9]. Among the carbohydrate polymers starch is gaining more attention due to its useful applications in different food products. It has many applications in food industry as thickener, gelling agent, water retention agent etc (Jaspreet *et al*., 2007) [10]. In native state, however, it shows limited applications because of its low shear stress resistance, high retrogradation and synthesis, thermal decomposition and low solubility in the common organic solvents (Kavlani *et al*., 2012) [11]. Starch is an important constituent of human diet. Many economically important crops (such as wheat, rice, maize, tapioca, potato etc) contain starch as their major storage products. Starch contains amylose which is a straight chain of glucose molecule and amylopectin which is branched chain. Most starches consist of 20% amylose and 80% amylopectin.

Starch is a heterogeneous polysaccharide. It is a polymer of glucose linked to another one through glycosidic bonds. It is composed of two high molecular weight entities called amylose and amylopectin (Prasanna, 2005) [9]. Amylose consists of single unbranched chains of 500-20000 α-1, 4-D-glucose units. A very few α-1,6 branches and also linked phosphate groups may be found (K. R Aneja 4th edition) [12]. Amylopectin contains α-1,4-D-glucose liner chain which is branched through α-1,6 linkages (Ellis and ring, 1985; Kerr, 1950) [13, 14].Branching after every 30 residues of glucose in a chain is governed by the branching enzyme. Millions of glucose residues are there in each amylopectin among which only 5% residues form the branch point.

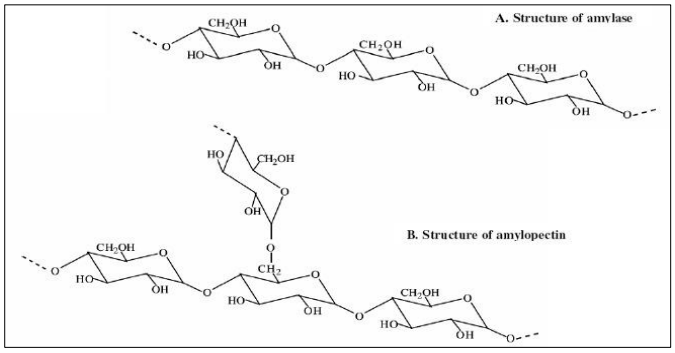

Fig 1: Structure of amylose (A) and amylopectin (B) present in starch (Muralikrishna *et al*., 2005) [15].

Types of amylase

α-Amylase

α-amylases (EC 3.2.1.1) are extracellular enzymes. They hydrolyses the α-1,6 glycosidic linkages (Kuriki and Imanaka, 1999) [16]. The α-amylases are calcium metalloenzymes and cannot function in the absence of calcium. Long chain carbohydrate was degraded at random location by them and produce maltotriose and maltose from amylose or maltose, glucose and limit dextrins from amylopectin (Rani, 2015) [17]. The α-amylases are faster acting enzymes than β-amylases due to their ability to act anywhere on the substrate. It is a major digestive enzyme of animals. In human, the salivary and pancreatic amylases are α-amylases. They are also found in plants (barley), fungi (basidiomycetes and ascomycetes) and bacteria (*Bacillus*). (Rani, 2012a; Rani, 2012d; Rani, 2012c) [18, 19, 20].

Structure of α-amylase

The amylase has a three-dimensional structure which is capable of binding to substrate and promotes the cleavage of the glycoside links through the action of highly specific catalytic groups (Iulek *et al*., 2000) [21]. The α-amylase of human is a calcium-containing enzyme which is composed of 512 amino acids in a single oligosaccharide chain and has a molecular weight of 57.6 kDa (Whitcomb and Lowe, 2007) [22]. It contains 3 domains: A, B, and C (Figure 2). The domain A is the largest and has a barrel shaped $(\beta/\alpha)_8$ super structure. The domain B is inserted between the domain A and C and it is attached to the A domain by disulphide bond. The C domain is linked to domain A by a simple polypeptide chain and it has a β sheet structure. The active site of α-amylase is situated between the carboxyl end of the domains A and B. The calcium (Ca^{2+}) that is located between the domains A and B acts in the stabilization of the three-dimensional structure and as allosteric activator. There are 5 subsites in the substrate-binding site and the catalytic site is present at subsite 3. Substrate binds to the first glucose residue in subsite 1 or 2 which allows cleavage to occur between the first and second or second and third glucose residues (Whitcomb and Lowe, 2007) [22].

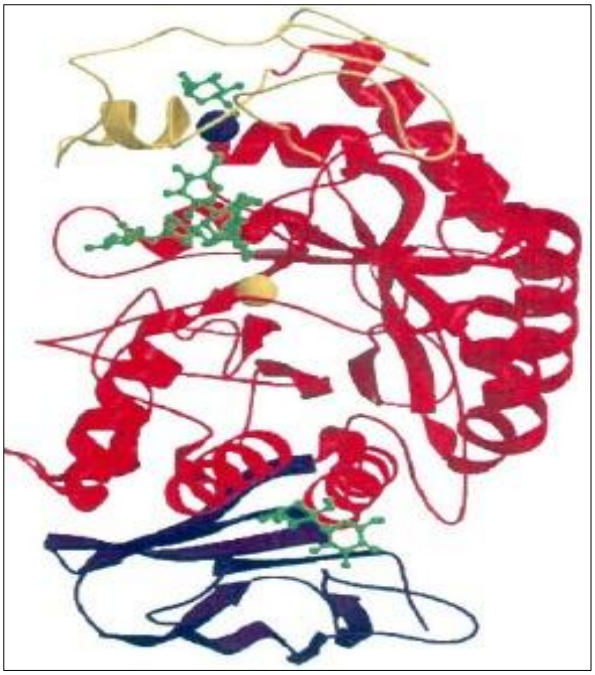

Fig 2: Structure α-amylase. A domain is shown in red, B domain is shown in yellow and C domain is shown in purple. In the catalytic centre, calcium ion is shown in blue sphere and chloride ion in the yellow sphere. The green structures are bound to active site and to surface binding sites (Payan, 2004) [23].

Mechanism of action

The α-retaining double displacement mechanism is the generally accepted catalytic mechanism of α-amylase enzyme. This catalytic mechanism involves two catalytic residues in the active site; a glutamic acid as acid/base catalyst and an aspartate as the nucleophile (Fig 3). This involves five steps: (a) When the substrate is bound in the active site, the glutamic acid residue in the acid form donates a proton to the glycosidic bond oxygen, that is, the oxygen between two glucose molecules at the subsites −1 and +1 and the nucleophilic aspartate then attacks the C1 of glucose at subsite −1; (b) then an oxocarbonium ion-like transition state is formed which is followed by the formation of a covalent intermediate; (c) after that the protonated glucose molecule at subsite +1 leaves the active site while a water molecule or a new glucose molecule moves into the active site and then

attacks the covalent bond between the glucose molecule at subsite −1 and the aspartate; (d) an oxocarbonium ion-like transition state is then formed again; (e) the base catalyst glutamate accepts a hydrogen from an incoming water molecule or the newly entered glucose molecule at subsite +1, the oxygen of the incoming water or the newly entered glucose molecule at subsite +1 replaces the oxocarbonium bond between the glucose molecule at subsite −1 and the aspartate and forms a new hydroxyl group at the C1 position of the glucose at subsite −1 (hydrolysis) or a new glycosidic bond between the glucose at subsite −1 and +1 (transglycosylation) (Koshland, 1953) [24].

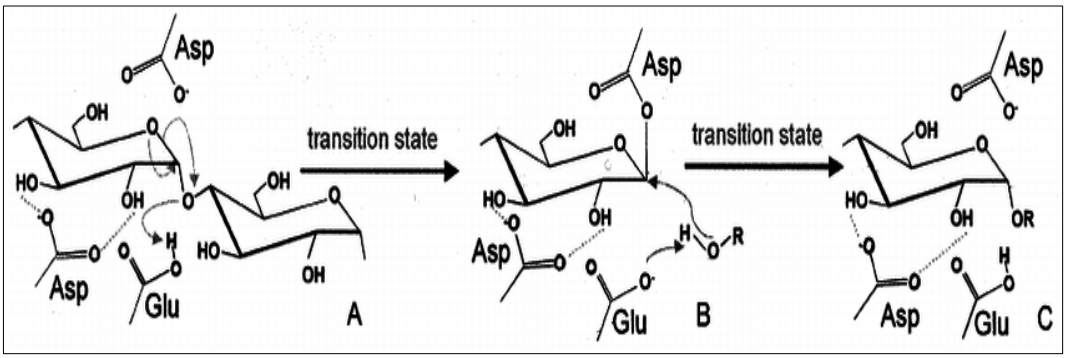

Fig 3: Double displacement mechanism and formation of covalent intermediate by which the retaining glycosyl hydrolases act (Marc *et al*., 2002) [25].

β-amylase

β-amylases (EC 3.2.1.2) is synthesized by bacteria, fungi and plants. Working from the non-reducing end, they hydrolyses the second α-1, 4 glycosidic bond and cleave off two glucose units (maltose) at a time. β-amylases breakdown the starch into maltose during the ripening of fruits which gives sweet flavour of ripe fruit (Rani, 2015) [17]. Both α-amylase and β-amylase are present in seeds. β- amylase is present in inactive form prior to germination and α-amylase appears after germination has begun. Many microbes produce amylase to degrade extracellular starches. Animal tissues do not contain β-amylase, although it may be present in microorganisms that are present within the digestive tract (Rani, 2012a; Rani, 2012b; Rani, 2012d) [18, 26, 19].

Structure of β-amylase

The β-amylase is found in the GH family 14. The x-ray crystallography derived structures of beta amylases are known for sweet potato (Cheong *et al*., 1995) [27], soybean (Adachi *et al*., 1998; Mikami *et al*., 1993; Mikami *et al*., 1994) [28, 29, 30], and barley (Mikami *et al*., 1999) [31]. The β-amylase of sweet potato consists of four identical subunits of 498 amino acids. Each subunit has a large core $(\beta/\alpha)_8$ barrel catalytic domain, three long loops which are associated with a subdomain, an extended C terminal loop. The overall structure β-amylase from sweet potato is similar to that of soybean and barley. In soybean β-amylase glu186 and glu380 residues play important roles in enzymatic reactions as an acid and a base catalyst respectively (Mikami *et al*., 1993) [29]. These two conserved residues occupy the positions above and below the bound polyglucan chain.

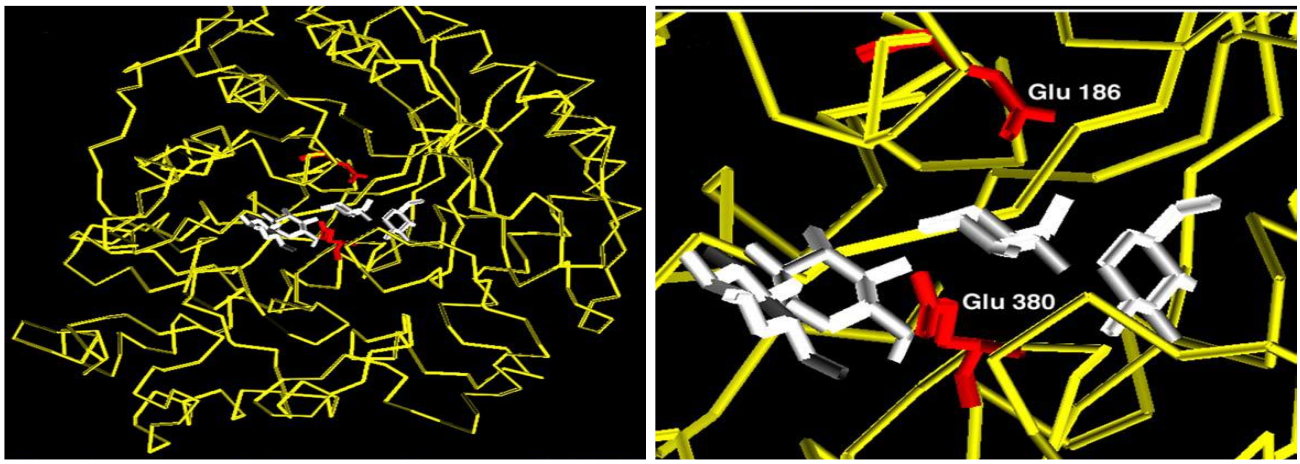

Fig 4: Structure of soybean beta-amylase (Mikami *et al*., 1993) [29]. A) Structure of beta-amylase. B) Close up of active site. Catalytic Glu186 and Glu380 residues are shown in red, glucose residues are shown in white, and the carbon backbone of the protein is shown in yellow.

Mechanism of action

B-amylase is an exoamylase which hydrolyses the α-1, 4 glycosidic linkages of polyglucan chain at non-reducing end and produce maltose (Fig 5). Two conserved glu residues are involved in the hydrolysis of the glycosidic bond which uses a general acid-base catalysis mechanism (Mikami *et al*., 1994) [30]. In the soybean β-amylase, Glu86 acts as a general acid and Glu380 acts as a general base (Mikami *et al*., 1994; Kang *et al*., 2004) [30, 32]. The carboxyl group of the Glu186 is present on the hydrophilic surface of the glucose which donates a proton to the glycosidic oxygen. The carboxyl group of the Glu380 is located on the hydrophobic surface of

the glucose at the subsite -1 and it activates an attacking water molecule. The deprotonated Glu186 is then stabilized by Thr342 after the cleavage of glycosidic bond (Mikami *et al*., 1994; Kang *et al*., 2004) [30, 32]. The reducing glucose of maltose product is in the β-form; hence it is named β-amylase.

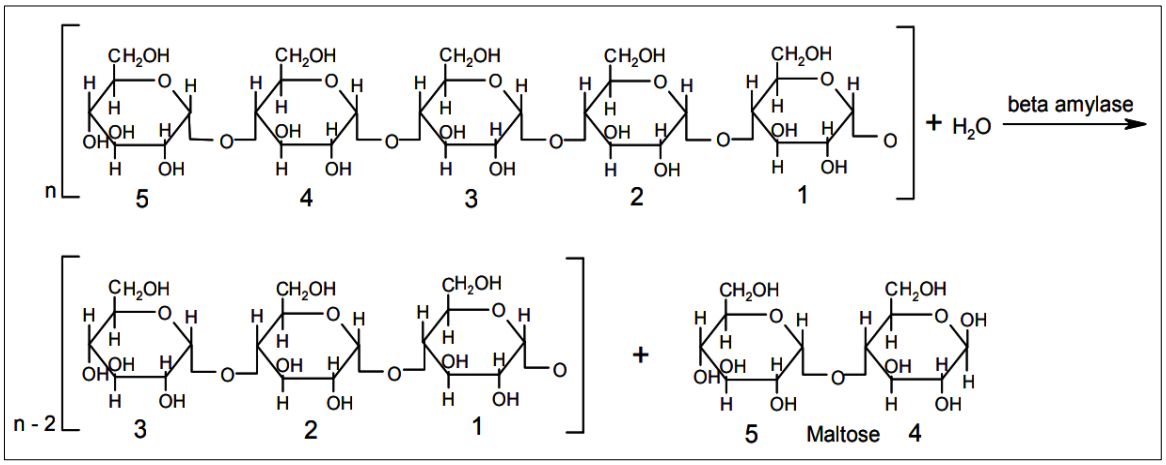

Fig 5: Hydrolysis of α-1,4 glycosidic linkage of polyglucan chain by β-amylase (Kaplan, 2004) [33].

γ-amylase

γ -amylases (*EC* 3.2.1.3) are also known as glucan 1, 4-α-glucosidase, amyloglucosidase, exo-1, 4-α-glucosidase, glucoamylase, lysosomal α-glucosidase, 1,4-α-D-glucanglucohydrolase (Tateno *et al*., 2007) [34]. They are most effective in acidic environments.

Structure of γ-amylase

γ-amylases are the members of GH family 15. They are generally multi-domain enzymes. Their catalytic domain is folded as a twisted (α/α)6 barrel and the central funnel-shaped active site is connected to the starch binding domain (Vaidya *et al*., 2015) [35].

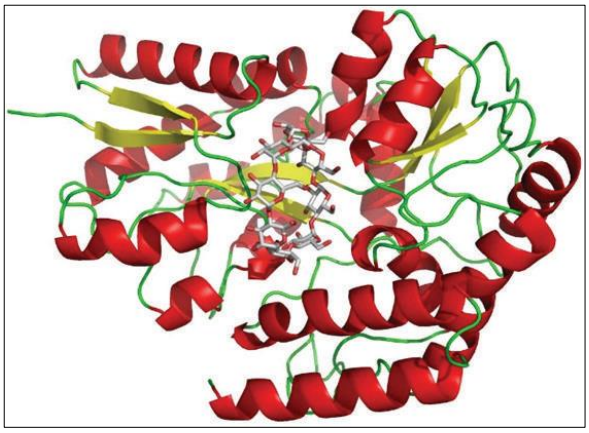

Fig 6: 3D image of gamma amylase from the *Thermoactinomyces vulgaris* R-47 cyclodextrin binding protein (2DFZ) (Vaidya *et al*., 2015) [35].

Mechanism of action

γ-amylases can cleave the last α-(1-4)-glycosidic linkages at the non-reducing end of the amylopectin and amylose and yields glucose with a single displacement mechanism. It can also cleave the α-(1-6)-glycosidic linkages (Tateno *et al*. 2007) [34].

Detection methods for alpha, beta and gamma amylases

Detection method for α-amylase

The production or secretion of α-amylase can be determined by various common methods which include solid-based and solution-based techniques. The solid-based method can be carried out on the nutrient agar plates which contain starch as the substrate. The solution-based methods include dinitro salicylic acid (DNSA) and Nelson-Somogyi (NS) methods.

In the solid agar method, the appropriate strain (bacteria or fungi) is inoculated into the starch containing agar plate. After proper incubation period, the plate is flooded with solution of iodine. It reveals a dark bluish colour on substrate region and a clear zone of hydrolysis around the inoculums which indicates the utilization of starch by the microorganism by amylase.

In the solution based DNSA method, at first the appropriate substrate and enzymes are mixed in proper proportion and reacted for 5 min at 50°C. After cooling to the room temperature, the absorbance of solution is taken at 540nm. In the NS method, starch and amylases are first mixed and then incubated for 5 min at 50°C. Then Somogyi cooper reagent is added to it to stop the reaction which is followed by boiling for 40min and cooling period. Then Nelson arseno-molybdate reagent is added to the mixture and incubated at room temperature for 10 min. Then the solution is diluted with water, centrifuged at high speed and then the supernatant is measured at 610nm (Gopinath *et al*., 2017) [36].

Detection method for β-amylase

In the detection of the beta-amylase method, substrate solution is preincubated at 40ºC for approximately 5 min, then crude enzyme extract is added to the substrate solution, mixed, and incubated at 40ºC for 10 min. After 10 min, stop buffer (1% (w/v) Trizma base) is added to stop the reaction. Production of p-nitrophenol is then measured at A_{410} spectrophotometric ally. In this method, the specific artificial substrate p-nitrophenyl maltopentaoside (PNPG5) is used, which is resistant to cleavage by the alpha-amylases for p-nitrophenol production (Kaplan, 2004) [33].

Detection method for γ-amylase

Gamma amylase activity can be determined by measuring the amount of glucose released from starch. At first, starch solution, 0.1M acetate buffer (pH- 4.5) and crude enzyme are mixed and then it is incubated at 55°C for 3 min. The glucose released can be then measured by the glucose oxidase peroxidase kit (Basma *et al*., 2015) [37].

Amylase Producing Microorganisms

Amylases can be derived from several sources, for example from plants, animals, bacteria and fungi. Because of the short growth period, biochemical diversity and genetic manipulation, the enzymes from microbial sources generally have very high industrial demands (Oliveira *et al*., 2007; Mishra and Behera, 2008) [38, 39].

Bacterial amylases

α-amylases can be produced by the different species of microorganisms. α-amylases can be mainly produced from the genera Bacillus such as *Bacillus licheniformis, Bacillus stearothermophilus, Bacillus amyloliquefaciens, Bacillus subtilis.* Thermostable α-amylases can be produced by *Bacillus polymyxia, Bacillus vulgarus, Bacillus megaterium* (Mahmood *et al*., 1998) [40].

Halophilic α-amylase can be produced by *Chromohalobacter* sp., *Halobacillus* sp., *Haloarcula hispanica, Halomonas meridian, Bacillus dipsosauri* (Payan, 2004; Steiner *et al*., 2003) [23, 41]. Alkaline and thermotolerant amylases can be produced by the species *Bacillus licheniformis*, and *Bacillus halodurans* (Setyorini *et al*., 2006) [42]. Cold-active extracellular α-amylase can be produced from the bacteria *Micro bacteriumfoliorum* GA2 and *Bacillus cereus* GA6 (Kuddus and Roohi, 2014) [43].

Table 1: Properties of bacterial amylase

Bacteria	pH optimal/stability	Temperature optimal/stability	k_m	v_{max}	Reference
Bacillus sp. B-10	7	50	1.4 mg/ml	6.2 U/ml	Singh *et al*., 2016 [44]
Bacillus licheniformis SKB4	6.5	90	6.2 mg/ml	1.04 µmol/mg/min	Samanta *et al*., 2014 [45]
Bacillus sp. EF-TYK1-5	7	60	1.36 mg/ml	0.00074 mmol	Pathak and Rekadwad, 2013 [46]
Pseudomonas sp. K6-28-040	7	50	1.37 mg/ml	1.24 mg/ml/min	Liu *et al*., 2011 [47]
Bacillus subtilis	6	45	1.08mg/ml	151U/ml	Elif, 2011 [48]
Bacillus licheniformis	7	90	2.85g/l	238 U/L	Vaseekaran *et al*., 2010 [49]
Bacillus subtilis DM03	6.0-10.0	50	-	-	Mukherjee *et al*., 2009 [50]
Bacillus sphaericus JT3	7	50	0.96 mg/ml	260 µmol/mg/min	Al-Qodah *et al*., 2007 [51]
Bacillus circulans GRS 313	4.9	48	11.66 mg/ml	68.97 U	Dey *et al*., 2002 [52]
Enterobacter cloacae IIT-BT 08	4	60	0.15 mg/ml	18.18 U/ml	Kumar and das, 2000 [53]

Fungal amylase

Filamentous fungi are widely used for the production of amylases. Being efficient producers of extracellular proteins, they can be exploited for the production of different enzymes such as alpha amylases (Kazunari and Imanaka, 2011) [54]. Filamentous fungi are very suitable microorganisms for the solid state fermentation (SSF), mainly because their morphology makes them able to colonize and penetrate the solid substrates (Rahardjo *et al*., 2005) [55]. The fungal α-amylases are preferred because of their GRAS (Generally Recognized as Safe) status (Gupta *et al*., 2003) [1].

Amylase production is limited to a few species of fungi such as *Aspergillus* sp. and *Penicillium* sp. The major *Aspergillus* species are *Aspergillus oryzae* and *Aspergillus niger. Aspergillus niger* is an acid-tolerant microorganism and it is resistant to the contamination and hence it has important significance in the α-amylase production (Vaidya *et al*., 2015) [35]. The thermophilic fungus *Thermomyces lanuginosus* and *Thermoascus aurantiacus* are excellent producers of amylase (Manivannan and Kathiresan, 2006) [56].

Table 2: Properties of fungal amylase

Fungi	pH optimal/stability	Temperature optimal/stability (°C)	k_m	v_{max}	Reference
Trichoderma pseudokoningii	4.5-8.5	50	4mg/ml	0.74µmol	Abdulaal, 2018 [57]
Aspergillus oryzae	7	45	1.4 mg/ml	37.037 IU/ml	Shah *et al*., 2014 [58]
Aspergillus oryzae VB6	6	-	0.34 µg/ml	0.59 µg/ml/min	Joel and Bhimba, 2012 [59]
Pestalotiopsis microspora VB5	6.4	-	4 µg/ml	0.95 µg/ml/min	Joel and Bhimba, 2012 [59]
Penicillium camemberti PL21	6	30	0.92 mg/ml	38.5 µmol/min	Nouadri *et al*., 2010 [60]
Penicillium citrinum HBF62	5.5	55	0.2 mg/ml	5000 U/mg	Metin *et al*., 2010 [61]
Aspergillus niger	4	30	10.84 g/l	3.2 g/l/min	Spier, 2006 [62]
Penicillium fellutanum	6.5	30	-	-	Manivannan and Kathiresan, 2006 [63]
Aspergillus tamarii	4.5-6.5	50-55	2g/l	880 µg/mg/min	Moreira *et al*., 2004 [64]
Scytalidium thermophilum	6.5	60	0.28mg/ml	67.2 U/mg	Cereia *et al*., 2000 [65]

Enzymatic modification of starch

The enzymatic modification of starch involves the exposure of the suspensions of starch to a number of enzymes mainly the hydrolyzing enzymes that produce highly functional derivatives. The discovery of this technique can be dated back to the time during which glucose syrup or high fructose syrup was produced (Kavlani *et al*., 2012) [11].

The enzymes amylomaltases (α-1,4-α-1,4 glucosyl transferases) are found in eukaryotes, bacteria and archea. This enzymes break the α-1,4 bond between two glucose units and subsequently make a novel α-1,4 bond producing modified starch which can be used in the cosmetics, detergents, food stuffs, pharmaceutics, adhesives and drilling fluids. This is also a very good source of the plant derived substitute for gelatin although it forms a turbid gel while the gelatin gels are transparent (Kaper *et al*., 2003) [66].

The study on the gel texture formed in the modification of potato, maize, pea and high-amylose potato with the amylomaltase which is isolated from the hyperthermophilic bacteria *Thermus thermophilus* showed that there was an improvement in the gel texture compared to the parent starch (Hansen *et al*., 2008) [67]. All these modified starches showed broadened amylopectin chain length profiles.

Cyclomaltodextrinase (CDase; EC 3.2.1.54) which is isolated

from the alkalophilic bacteria *Bacillus* sp. I-5 (CDase I-5) was used to modify rice starch for the production of the low-amylose starch products. The amylose content of the starch was found to be decreased significantly while no significant change was observed in the side chain length distribution of the amylopectin. Storage of this modified rice starch at 4° C for 7 days showed that the retrogradation rate had significantly decreased compared to the control sample (Auh *et al*., 2006) [68].

Treatment of maize starch with the β-amylase, transglucosidase, maltogenic α-amylase, resulted into significant decrease in digestion rate, producing resistant starch with reduced glycemic index which can be used in the diabetes, prediabetes, cardiovascular disease and obesity. The increase in the starch branch density and in the crystalline structure in modified starches contributes to the slow digestion (Ao *et al*., 2007) [69].

Applications of Amylases

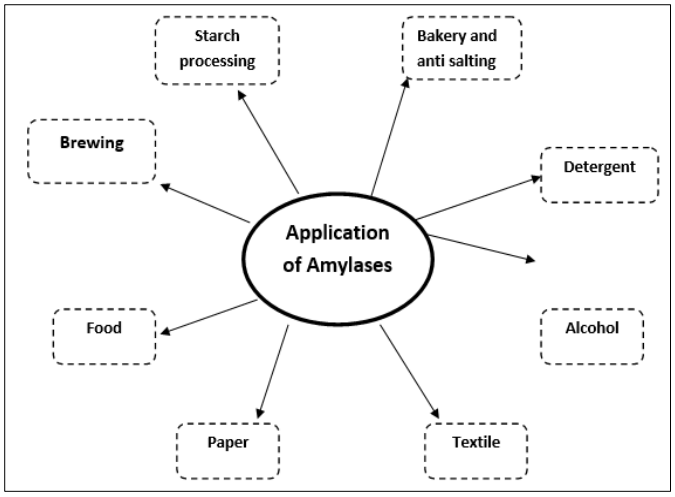

Fig 7: Applications of Amylases

Starch conversion

The α-amylases are widely used in the starch industry for starch hydrolysis in the process of starch liquefaction that results in the conversion of starch into fructose and glucose syrups (Nielsen and Borchert, 2000) [70]. The enzymatic starch conversion includes: gelatinization in which dissolution of starch granules takes place, thereby forms a viscous suspension; liquefaction, which causes partial hydrolysis and loss in viscosity; and saccharification, which involves the production of glucose and maltose via further hydrolysis (Gupta *et al*., 003; Praksh and Jaiswal, 2009) [1, 71]. Earlier, the α-amylase from *Bacillus amyloliquefaciens* was used which has been replaced by the α-amylase from *Bacillus stearothermophilus* or *Bacillus licheniformis* (van der Maarel *et al*., 2002) [72]. Because of their high thermostability and because of availability of efficient expression system, enzymes from *Bacillus* species are of special interest in the large scale biotechnological processes (Praksh and Jaiswal, 2009) [71].

Detergent industry

The primary consumers of enzymes are detergent industries in terms of both volume and value. The use of enzymes in detergents formulations increases the ability of the detergents to remove tough stains and makes them environmentally safe. 90% of all liquid detergents contain amylases (Gupta *et al*., 2003; van der Maarel *et al*.. 2002; Mitidieri *et al*., 2006) [1, 72, 73]. These enzymes are used in detergents for dishwashing to degrade the residues of starchy foods such as potatoes, gravies, custard, etc. to dextrins and other smaller oligosaccharides (Mukherjee *et al*., 2009; Olsen and Folhalt, 1998) [74, 75]. Amylases have their optimum activity at lower temperatures and alkaline pH. The most important criteria for their use in detergents are their oxidative stability where the washing environment is oxidizing (Chi *et al*., 2009; Kirk *et al*., 2002) [76, 77]. Mainly the amylases which are used in the detergent industries are derived from *Bacillus* or *Aspergillus* (Mitidieri *et al*., 2006) [73].

Fuel alcohol production

Starch is the most used as a substrate for ethanol production because of its low price and easy availability raw in most regions of the world (Chi *et al*., 2009) [78]. In this production, starch is first solubilized and then submitted to two enzymatic steps to obtain fermentable sugars. Liquefaction and saccharification are involved in the bioconversion of starch into ethanol where starch is converted into sugar by amylolytic microorganisms or by *α*-amylase. This is followed by fermentation where ethanol is produced from sugars by ethanol fermenting microorganisms such as yeast *Saccharomyces cerevisiae* (Moraes *et al*., 1999; Oner, 2006) [79, 80].

Food industry

Application of amylases were also reported in processed food industries like in brewing, baking, production of cakes, fruit juices preparation of digestive aids and starch syrups (Couto and Sannoman, 2006) [81]. The α-amylases have been widely used in the baking industry for decades. These enzymes are added to the bread dough where they degrade the starch in the flour into smaller dextrins, which are further fermented by the yeast. The α-amylase in the dough increases the rate of fermentation and reduces the viscosity of dough that improves the volume and texture of the product. It also generates additional sugar in the dough such as glucose and maltose that results in the improvement of the taste, crust colour and toasting qualities of the bread. The enzymes also have an anti-staling effect in bread baking, and improve the softness retention and shelf life of baked goods (Gupta *et al*., 2003; van der Maarel *et al*., 2002) [1, 72]. Currently, a thermostable amylase from *Bacillus stearothermophilus* is commercially used in the bakery industry (van der Maarel *et al*., 2002) [72]. Amylases are also used in the clarification of beer or fruit juices. (Gavrilescu and Chisti, 2005; Ghorai *et al*., 2009; van der Maarel *et al*., 2002) [82, 83, 72].

Textile industry

In textile industry amylases are used for desizing process. Sizing agents like starch are applied to yarn before the production of fabric to ensure a secure and fast weaving process. As starch is cheap, easily available in most regions of the world, and can be removed quite easily, they are very attractive size. Desizing process involves the starch removal from the fabric that serves as a strengthening agent to prevent breaking of the warp thread during the weaving process. The α-amylases selectively remove the size and do not attack the fibres (Ahlawat *et al*., 2009; Feitkenhauer, 2003; Gupta *et al*., 2003) [84, 85, 1]. Amylase from *Bacillus* strain has been employed in textile industries for a long time.

Paper industry

The α-amylases are used in the pulp and paper industry to modify starch of coated paper, that is, to produce low-viscosity, high molecular weight starch (Gupta *et al*., 2003; van der Maarel *et al*., 2002) [1, 72]. For the preparation of smooth and strong writing paper the coating treatment is essential. In this application, the viscosity of the natural starch is very high for the paper sizing which can be altered by partial degradation of the polymer with α-amylases in a batch or continuous processes. Starch is a very good sizing agent for the finishing of paper, improving the quality of the paper, besides being a good coating for the paper. The size increases the stiffness and strength in paper (Bruinenberg, 1996; van der Maarel *et al*., 2002) [86, 72].

Chocolate industry

For the production of chocolate syrup, amylases are treated with cocoa slurries where the chocolate starch is dextrinizing and therefore the syrup does not become thick. Amylolytic enzymes are used to produce cocoa flavoured syrups that have a high cocoa content and excellent stability and flow properties at room temperature (Saini *et al*., 2017) [87].

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