REVIEW ARTICLE

Bacterial Amylase: A Review

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ABSTRACT

Amylase can be obtained from several fungi, yeast, bacteria and actinomycetes; however, enzyme from fungal and bacterial sources has dominated applications in industrial sectors. The application of an amylase in industrial reactions depends on its unique characteristics, such as its action pattern, substrate specificity, major reaction products, optimal temperature, and optimal pH12. Bacterial α-amylase preferred for application in starch processing and textile industries due to its action at higher temperature (75- 105^{0}C) and neutral to alkaline pH13. Generally, production of this enzyme has been carried out by submerged fermentation. Among the bacterial sources *Bacillus subtilis*, *Bacillus staerothermophilus, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus acidocaldarius, Bifidobacterium bifidum* and *Bifidobacterium acerans* are important species. The present review was focused on bacterial amylase and this review assesses the following chapters: Amylase, Microorganisms and amylases, Physiology of amylases, Fermentation studies on bacterial amylase production and Commercial application of amylases.

Key words: Enzyme, Amylase, Bacteria, Physiology, Fermentation and Applications.

1. INTRODUCTION

Microorganisms had significant contribution in production of various industrial enzymes. The global market for industrial enzymes estimated at $2 billion in 2010 and expected to rise at an average annual growth rate of 3.3%. These starch - degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile, paper, pharmaceutical to sugar industries [1]. Conversion of starch into sugar, syrups and dextrins forms the major part of starch processing industry [2]. Starch degrading enzymes like amylase have received great deal of attention because of their perceived technological significance and economic benefits [3].

Nowadays, amylases (α-amylases, β- amylases and glucoamylases) represent one of the most important enzyme groups within the field of biotechnology [4]. α-amylase (EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase, endoamylase) is a classical calcium containing enzyme catalyze hydrolysis of starch and related carbohydrates by randomly cleaving internal α-D-(1-4) glycosidic linkage, yielding glucose, maltose, maltotriose, and other oligosaccharides [5]. It belongs to family thirteen in the classification of glycoside hydrolases. This family is the most varied of all glycoside hydrolase families, containing many enzymes able to catalyze various reactions, such as hydrolysis, transglycosylation, condensation and cyclization [6].

The use of enzymes in industrial processes is beginning to deliver its promise. Enzymes have high catalytic rates and work in aqueous solution. By industrial standards, only moderate temperatures and pressures are required. Thus, industrial exploitation of enzymes is making the development of cleaner, environmentally friendly processes possible. Furthermore, their exquisite specificity keeps unwanted side reactions to a minimum, maximizing yield. In industrial processes, however, we are still asking enzymes to perform under conditions for which they have not evolved. Because of this, a major problem in the industrial exploitation of enzymes is lack of stability. An example that illustrates both the possibilities, and also the limitations of the industrial use of enzymes, is starch processing, which is considered an unqualified success of modern industrial biotechnology.

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Amylase has a great deal of application in starch saccharification. The amylolytic enzymes find a wide spectrum of applications in food industry for production of glucose syrups, high fructose corn syrups, maltose syrups, reduction of viscosity of sugar syrups, reduction of haze formation in juices, solubilization and saccharification of starch for alcohol fermentation in brewing industries, and also find a wide range of application in baking, paper, textile and detergent industry [7]. In most cases the enzymatic process is inhibited by high substrate and product concentration and also instability of the enzyme under repetitive or prolonged use.

2. AMYLASES

Amylases are enzymes which hydrolyze starch molecules to give diverse products including dextrin and progressively smaller polymers composed of glucose units [8]. These enzymes are of great significance in present day biotechnology with applications ranging from food, fermentation, textile to paper industries [9]. Although amylases can be derived from several sources, including plants, animals and microorganisms, microbial enzymes generally meet industrial demands. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry [9].

The history of amylases began in 1811 when the first starch degrading enzyme was discovered by Kirchhoff. This was followed by several reports of digestive amylases and malt amylases. It was much later in 1930, that Ohlsson suggested the classification of starch digestive enzymes in malt as a- and b-amylases according to the anomeric type of sugars produced by the enzyme reaction. α-Amylase (1,4-α-D-glucan-glucanhydrolase, EC. 3.2.1.1) is a widely distributed secretary enzyme. α-Amylases of different origin have been extensively studied.

Amylases can be divided into two categories, endoamylases and exoamylases. Endoamylases catalyze hydrolysis in a random manner in the interior of the starch molecule. This action causes the formation of linear and branched oligosaccharides of various chain lengths. Exoamylases hydrolyze from the non-reducing end, successively resulting in short end products. Today a large number of enzymes are known which hydrolyze starch molecule into different products and a combined action of various enzymes is required to hydrolyze starch completely. A number of reviews exist on amylases and their applications, however, none specifically covers α-amylases at length. α-Amylases are one of the most popular and important form of industrial amylases and the present review highlights the various aspects of microbial α-amylases.

α - Amylases belong to family 13 of the glycoside hydrolase group of enzymes [10] that catalyze the hydrolysis of the internal α-1,4-glucosidic bond in starch. The enzymes are widely used in food and detergent industries [11]. The three dimensional structures of α-amylases from various sources such as *Aspergillus oryzae*, *Aspergillus niger*, porcine pancreas, barley, human saliva, *Bacillus licheniformis*, *Bacillus stearothermophilus* and *Alteromonas haloplanctis* have been determined [12].

The α-amylases from *Bacillus licheniformis* and *Bacillus stearothermophilus* are reported to have two additional calcium binding sites [13], while a fourth site that is also likely to bind a calcium ion has been identified in the chimeric protein constructed from the genes encoding *Bacillus licheniformis* and *Bacillus amyloliquefaciens* α-amylases [14].

The α-amylases from *Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus stearothermophilus* are among the most widely studied amylases and are highly homologous with respect to primary and tertiary structure [15]. However, despite their structural similarities, these reported amylases vary significantly in their thermal stability. The thermal inactivation of *Bacillus* α-amylases has been suggested to involve a two-step process, with the first step of reversible unfolding, followed by an irreversible conformational change [16]. Calcium ions have also been implicated in mechanisms involving the thermal inactivation of the *Bacillus* α-amylases, whereby it has been proposed that the first step involves the reversible dissociation of calciumions from the native enzyme, followed by irreversible denaturation at high temperatures [17].

3. MICROORGANISMS AND AMYLASES

Amylases can be derived from several sources, such as plants, animals, bacteria and fungi. Because of the short growth period, biochemical diversity and the ease with which enzyme concentrations might be increased by environmental and genetic manipulation, the enzymes from microbial sources generally meet industrial demands [18, 19]. The majority of enzymes used to date have been obtained from

mesophilic microorganisms. The applications of these enzymes are restricted because of their limited stability to extreme temperature, pH and ionic strength [20]. Therefore, efforts were made on the enzymes of thermophilic and halophilic bacteria, which could be used in many harsh industrial processes where the concentrated salt solution and high temperatures used would inhibit many enzymatic conversions [21, 22].

α-Amylases are universally distributed throughout the animal, plant and microbial kingdoms. Over the past few decades, considerable research has been undertaken with the extracellular a-amylase being produced by a wide variety of microorganisms [23]. The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and microbes are easy to manipulate to obtain enzymes of desired characteristics. α -Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors [24].

Several *Bacillus* sp. and thermostable Actinomycetes including *Thermomonospora* and *Thermoactinomyces* are versatile producers of the enzymes [25]. The genus *Bacillus* produces a large variety of extracellular enzymes of which amylases and proteases are of significant industrial importance. An highly thermostable and alkaline α-amylases is available from the mesophile *Bacillus* sp. [26]. The thermophilic bacterium *Bacillus stearothermophylus* offers an alternative for commercial production of thermostable α-amylases.

Alkaline and thermotolerant amylases were produced by *Bacillus* spp., *Bacillus licheniformis*, and *Bacillus halodurans* [27]. These enzymes were purified and characterized. Some of them have been reported to be maltooligosaccharide producing enzymes [28]. Production of oligosaccharides with the enzymes is attractive because they are more stable than general amylase showing high activity at a region of 30-40°C. The enzyme was not suitable for the production of oligosaccharides because a major product produced from soluble starch was glucose, and maltose (G2) and maltotriose (G3) were observed as minor components. Hence, we newly tried to isolate producers of thermotolerant amylases, showing activity under alkaline conditions, and examined reaction products.

The advantages of using thermostable amylases in industrial processes include the decreased risk of contamination and cost of external cooling, a better solubility of substrates, a lower viscosity allowing accelerated mixing and pumping [29]. The development of saccharifying amylolytic enzymes that are active at high temperatures (90°C) would directly benefit the starch-processing industries. However, running α-amylase production processes at higher temperatures will require new process design and improved knowledge of thermophilic bacteria [30]. Processes using thermophiles still lack the maturity of classical processes with mesophilic bacteria and yeasts [31]. Alkaliphilic *Bacillus* strains often produce enzymes active at alkaline pH, including alkaline α-amylase, protease and carboxy methyl cellulase [32].

4. PHYSIOLOGY OF AMYLASE PRODUCTION

The production of α-amylase by submerged fermentation (SmF) and solid state fermentation (SSF) has been thoroughly investigated and is affected by a variety of physicochemical factors. Most notable among these are the composition of the growth medium, pH of the medium, phosphate concentration, inoculum age, temperature, aeration, carbon source and nitrogen source [33]. Most reports among fungi have been limited to a few species of mesophilic fungi where attempts have been made to specify the cultural conditions and to select superior strains of the fungus to produce on a commercial scale [34, 35].

4.1. Physicochemical parameters
The role of various physico-chemical parameters, including carbon and nitrogen source, surface acting agents, phosphate, metal ions, temperature, pH and agitation have been studied [35].

4.1.1.1. Substrate sources
α -Amylase is an inducible enzyme and is generally induced in the presence of starch or its hydrolytic product, maltose [36]. Most reports available on the induction of α-amylase in different strains of *Aspergillus oryzae* suggest that the general inducer molecule is maltose. There is a report of a 20-fold increase in enzyme activity when maltose and starch were used as inducers in *Aspergillus oryzae* [37]. Similarly, strong α-amylase induction by starch and maltose in the case of *Aspergillus oryzae* has been reported [38]. Apart from maltose, in some strains, other carbon sources as lactose, trehalose, α-methyl-D-glycoside also served as inducers of amylase. Not

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only the carbon source, but also the mycelial condition/age affects the synthesis of a-amylase by *Aspergillus oryzae* [39, 35].

There are reports that 5 days starved non-growing mycelia were the most appropriate for optimal induction by maltose. α - Amylase production is also subjected to catabolite repression by glucose and other sugars, like most other inducible enzymes [40]. However, the role of glucose in the production of a-amylase in certain cases is controversial. α - Amylase production by *Aspergillus oryzae* was not repressed by glucose rather; a minimal level of the enzyme was induced in its presence. However, xylose or fructose has been classified as strongly repressive although they supported good growth in *Aspergillus nidulans* [41, 35].

The carbon sources as glucose and maltose have been utilized for the production of α-amylase. However, the use of starch remains promising and ubiquitous. A number of other non-conventional substrates as lactose, casitone, fructose, oilseed cakes [42] and starch processing waste water [35].

Cui YQ *et al*. [43] have also been used for the production of α-amylase while the agro-processing by-product, wheat bran has been used for the economic production of α-amylase by SSF. The use of wheat bran in liquid surface fermentation (LSF) for the production of α-amylase from *Aspergillus fumigates* and from *Clavatia gigantea*, respectively, has also been reported. High α-amylase activities from *Aspergillus fumigatus* have also been reported using α-methyl-D-glycoside (a synthetic analogue of maltose) as substrate.

Use of low molecular weight dextran in combination with either Tween 80 or Triton X-100 for a-amylase production in the thermophilic fungus *Thermomyces lanuginosus* has been reported [44]. Triton X-100 had no effect, whereas Tween 80 increases the α-amylase activity 27-fold [35].

4.1.1.2. Nitrogen sources
Organic nitrogen sources have been preferred for the production of α-amylase. Yeast extract has been used in the production of α-amylase from *Streptomyces* sp., *Bacillus* sp. and *Halomonas meridian* [45, 35].

Yeast extract has also been used in conjunction with other nitrogen sources such as bactopeptone in the case of *Bacillus* sp., ammonium sulphate in the case of *Bacillus subtilis*, ammonium sulphate

and casein for *C. gigantea* and soybean flour and meat extract for *Aspergillus oryzae*. Yeast extract increased the productivity of α-amylase by 156% in *Aspergillus oryzae* when used as an additional nitrogen source than when ammonia was used as a sole source [46, 35].

Various other organic nitrogen sources have also been reported to support maximum α-amylase production by various bacteria and fungi. However, organic nitrogen sources *viz*. beef extract, peptone and com steep liquor supported maximum α-amylase production by bacterial strains soybean meal and amino acids by *Aspergillus oryzae* [47]. CSL has also been used for the economical and efficient production of α-amylase from a mutant of *Bacillus subtilis*. Apart from this, various inorganic salts such as ammonium sulphate for *Aspergillus oryzae* and *Aspergillus nidulans*, ammonium nitrate for *Aspergillus oryzae* and Vogel salts for *Aspergillus fumigatus* have been reported to support better α-amylase production in fungi [48, 35].

Amino acids in conjunction with vitamins have also been reported to affect α-amylase production. However, no conclusion can be drawn about the role of amino acids and vitamins in enhancing the a-amylase production in different microorganisms as the reports are highly variable. α-Amylase production by *Bacillus amyloliquefaciens* increased by a factor of 300 in the presence of glycine [49]. The effect of glycine was not only as a nitrogen source rather it affected a-amylase production by controlling pH and subsequently amylase production increased. Alanine, DL-nor valine and D-methionine were effective for the production of alkaline amylase by *Bacillus* sp. However, the role of amino compounds was considered to be neither as nitrogen nor as a carbon source, but as stimulators of amylase synthesis and excretion. It has been reported that only asparagine gave good enzyme yields while the importance of arginine for α-amylase production from *Bacillus subtilis* has also been well documented [50, 35].

4.1.1.3. Role of phosphate
Phosphate plays an important regulatory role in the synthesis of primary and secondary metabolites in microorganisms and likewise it affects the growth of the organism and production of α-amylase. A significant increase in enzyme production and condition in *Aspergillus oryzae* above 0.2 M phosphate levels has been reported [51]. Similar findings were corroborated in *Bacillus*

amyloliquefaciens where low levels of phosphate resulted in severely low cell density and no α-amylase production. In contrast, high phosphate concentrations were inhibitory to enzyme production by *Bacillus amyloliquefaciens* [52, 35].

4.1.1.4. Role of other ions

K^+, Na^+, Fe^{2+}, Mn^{2+}, Mo^{2+}, Cl^+, SO_4^{2+} had no effect while Ca^{2+} was inhibitory to amylase production by *Aspergillus oryzae*. Mg^{2+} played an important role and production was reduced to 50% when Mg^{2+} was omitted from the medium. Na^+ and Mg^{2+} show coordinated stimulation of enzyme production by *Bacillus* sp. [53]. Addition of zeolites to control ammonium ions in *Bacillus amyloliquefaciens* resulted in increased yield of α-amylase. An inverse relationship between α-amylase production and growth rate was observed for *Streptomyces* sp. in the presence and absence of Co^{2+}, the presence of Co^{2+} enhancing the final biomass levels by 13-fold, albeit with a reduction in enzyme yield [54, 35].

4.1.1.5. pH

Among the physical parameters, the pH of the growth medium plays an important role by inducing morphological change in the organism and in enzyme secretion. The pH change observed during the growth of the organism also affects product stability in the medium. Most of the *Bacillus* strains used commercially for the production of bacterial α-amylases by SmF have an optimum pH between 6.0 and 7.0 for growth and enzyme production. This is also true of strains used in the production of the enzyme by SSF. In most cases the pH used is not specified excepting pH 4.2 in the case of *Aspergillus oryzae*, 8.0 in *Aspergillus oryzae* and 6.8 for *Bacillus amyloliquefaciens* [55, 35].

In fungal processes, the buffering capacity of some media constituents sometimes eliminates the need for pH control. The pH value also serves as a valuable indicator of the initiation and end of enzyme synthesis. It is reported that *Aspergillus oryzae* accumulated amylase in the mycelia when grown in phosphate or sulphate deficient medium and was released when the mycelia were replaced in a medium with alkaline pH [56, 35].

4.1.1.6. Temperature

The influence of temperature on amylase production is related to the growth of the organism. Among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25 to 35°C. Optimum yields of α-amylase were achieved at 30 to 37°C for *Aspergillus oryzae* [57]. α-Amylase production has also been reported at 55°C by the Thermophilic fungus *Thermomonospora fusca* and at 50°C by *Thermomonospora lanuginosus* [58]. α-Amylase has been produced at a much wider range of temperature among the bacteria. Continuous production of amylase from *Bacillus amyloliquefaciens* at 36°C has been reported. However, temperatures as high as 80°C have been used for amylase production from the hyperthermophile *Thermococcus profundus* [59, 35].

4.1.1.7. Agitation

Agitation intensity influences the mixing and oxygen transfer rates in much fungal fermentation and thus influences mycelial morphology and product formation [60]. It has been reported that a higher agitation speed is sometimes detrimental to mycelial growth and thus may decrease enzyme production. However, it is reported that the variations in mycelial morphology as a consequence of changes in agitation rate do not affect enzyme production at a constant specific growth rate. Agitation intensities of up to 300 rpm have normally been employed for the production of amylase from various microorganisms [61, 35].

5. FERMENTATION STUDIES ON BACTERIAL AMYLASE PRODUCTION

The effect of environmental conditions on the regulation of extracellular enzymes in batch cultures is well documented. A lot of work on the morphology and physiology of α-amylase production by *Aspergillus oryzae* during batch cultivation has been done. Accordingly, morphology of *Aspergillus oryzae* was critically affected by the growth pH [62]. In a series of batch experiments, authors observed that at pH 3.0 to 3.5, freely dispersed hyphal elements were formed. In the pH range 4 to 5, both pellets and freely dispersed hyphal fragments were observed whereas at pH higher than 6 pellets were the only growth forms recorded. Other groups have recorded similar observations for other strains of *Aspergillus oryzae* [63]. The optimum growth temperature was found to be 35°C. It is demonstrated that when glucose was exhausted the biomass production stopped whereas the secretion of α-amylase increased rapidly [64, 35].

One report states that inoculum quantity did not affect morphological changes in *Aspergillus oryzae* in air-lift bioreactors and that pellet size decreased considerably as the air velocity increased [65]. In the case of α-amylase production by *Bacillus flavothermus* in batch cultivation in a

20 L fermentor, α-amylase production and biomass peaked twice and highest activity was obtained after 24 hours. It was observed that the kinetics of enzyme synthesis was more of the growth associated than non-growth associated type. Similar findings were cited in another report with *Bacillus amyloliquefaciens* [66, 35].

Continuous and fed-batch cultures have been recognized as most effective for the production of the enzyme and several groups have studied the effectiveness of these cultures. The production of α-amylase from *Bacillus subtilis* was enhanced substantially by extending batch cultivation with fed-batch operation [67]. The bulk enzyme activity was nearly 54% greater in a two-stage fed-batch operation at a feed rate of 31.65 ml h^{-1} of medium, than that attained in the single stage batch culture. The effects of controlled feeding of maltose at a feed rate of 4 g h^{-1} for α-amylase and glucoamylase production from *Aspergillus oryzae* in a rotary draft tube fermentor (RTF) have been studied [68]. At a feed rate of 1 g h^{-1} the yields of α-amylase were twice than those obtained in batch cultures. When fed-batch cultivations were performed on a pilot scale RTF at a feed rate of 24 g h^{-1}, the biomass and amylase yields was higher than those obtained in a laboratory scale jar fermentor [35].

A model to simulate the steady-state values for biomass yield, residual sugar concentration and specific rate of α-amylase production has been proposed which simulated experimental data very well. Furthermore, it was found in chemostat experiments that the specific rate of α-amylase production decreased by upto 70% with increasing biomass concentration at a given dilution rate. Shifts in the dilution rate in continuous culture could be used to obtain different proportions of the enzymes, by the same strain [69]. It was further demonstrated that maximum production of α-amylase occured in continuous culture at a dilution rate of 0.15 h^{-1} and amylase activity in the culture was low at dilution rates above 1.2 h^{-1}. In contrast, in *Bacillus* sp. the switching of growth from batch to continuous cultivation resulted in the selection of a non α-amylase producing variant [70, 35].

A decline in enzyme production was also accompanied by morphological and metabolic variations during continuous cultivation. The industrial exploitation of SSF for enzyme production has been confined to processes involving fungi and it is generally believed that these techniques are not suitable for bacterial cultivation. The use of SSF technique in α-amylase production and its specific advantages over other methods has been discussed extensively [71, 35].

Lynn Hamilton *et al.* [72] tested the *Bacillus* sp. producing a raw starch-digesting but non-raw starch-adsorbing α-amylase. Maximum amylase yield was achieved in a medium containing lactose (4%, w:v) as the carbon source and yeast extract (2%, w:v) as the nitrogen source. Although highest amylase yields were achieved with lactose, the biomass levels were notably less than those with starch or b-cyclodextrin. The enzyme was purified in a single step using an a-cyclodextrin (CD) Sepharose 6B column, it had an *M*r of 63 000 and displayed maximum activity at pH 6.0 and 65°C. Glucose and maltose were released as the main end-products on hydrolysis of both soluble starch and raw corn starch.

Gashaw Memo and Amare Gessesse [73] purified two α-amylases *Amy*I and *Amy*II, from the cell-free culture supernatant of the Thermophilic *Bacillus* sp. The two amylases completely adsorb to potato and corn starch granules. At an enzyme dose of 1 U/mg raw starch and an incubation temperature of 60°C, the percent hydrolysis of raw potato and corn starch, respectively, was 77 and 44% for *Amy*I and 82 and 37% for *Amy*II. The optimum temperature for the activity of both enzymes was at 75–80°C, and '50% of the original activity was retained after 4 hours of incubation at 80°C. The two enzymes were optimally active at pH 5.5 and stable in the pH range of 5.5–9.0. Activity was inhibited in the presence of Hg_21, Cu_21, and Fe_31, but no inhibition was observed in the presence of Zn_21.

Arikan Burhani *et al.* [74] isolated a thermostable alkaline α-amylase producing *Bacillus* sp. was isolated from soil samples. Enzyme synthesis occurred at temperatures between 25 and 45°C with an optimum of 37°C. There was a slight variation in amylase synthesis within the pH range 7 and 11 with an optimum pH of 9. The optimum temperatures for amylase production and growth were the same. The enzyme showed optimum activity at pH 10.5 and 80 8C. The partial purified enzyme was highly active in the alkaline range of pH (9.5 to13), and it was completely active up to 100°C retaining 85.5% initial activity at pH 10.5. Enzyme activity was enhanced in the presence of 5 mM $CaCl_2$ (110%) and 3 mM PMSF (103%), and inhibition with 5 mM by Zn, Na, Na-sulphide,

EDTA (10 mM), Urea (8 M) and SDS (0.1%) was obtained 36.9, 21.5, 22.2, 4.90, 86% and 10.27, respectively. The enzyme was stable (55%) at high alkaline pH for 24 hours.

Ben Messaoud *et al*. [75] isolated and identified a new bacterial strain *Bacillus subtilis* from soil and selected for its potential production of an atypical amylase. The identification was founded on physiological tests and molecular techniques related to the 16S rRNA, 23S rRNA genes and intergenic sequences showing the highest similarity of 98% with regions in the complete genome of *Bacillus subtilis*. The molecular mass of the enzyme is about 60 kDa as determined by SDS–PAGE. Optimal conditions for the activity of the purified enzyme are pH 6 and 65°C. The half-life duration is about 3 hours at 70°C and 5 hours at 65°C. This enzyme belongs to the endo-type amylases according to the hydrolytic mode study using Ceralpha and Betamyl methods. It is classified as a maltoheptaose- and maltohexaose-forming amylase since it generates about 30% maltohexaose (DP6) and 20% maltoheptaose (DP7) from starch.

Damodara Rao Mendu *et al*. [76] conducted an affinity chromatographic method with a novel eluant from *Bacillus licheniformis* is described. Amylase was bound to starch, starchcelite, starch-Sepharose columns and the bound amylase was rapidly eluted with 2% (w/v) white dextrin. The binding capacity of amylase to starch column is 380 µmol/g of starch. The purified enzyme showed a single polypeptide on SDS-polyacrylamide gel electrophoresis with a molecular weight of 58 kD. The specificity of purified enzyme was confirmed by immunodiffusion, immunoelectrophoresis. Single radial immunodiffusion and western blotting studies analyzed the synthesis of enzyme at different time points.

Eva Bernhardsdotter *et al*. [77] selected an alkaliphilic amylase producing bacterium, *Bacillus* sp. from 13 soda lakes isolates. When grown at pH 10.5 and 37°C produced multiple forms of amylases in the culture broth. The molecular weight of BAA was determined to be 51 kDa by SDS gel electrophoresis. The pH optima for activity below and above 40°C were 9.5–10.0 and 7.0–7.5, respectively. BAA was stable in the pH range 6–11 and was completely inactivated at 55°C. Thermostability was not increased in the presence of Ca^{2+}. The enzyme was strongly inhibited by Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+} and Cu^{2+}, whereas the presence of Na^{2+}, Co^{2+} and EDTA (10 mM) enhanced enzymic activity. The Km and specific activity of BAA on soluble starch were 1.9 mg/ml and 18.5 U/mg, respectively.

Zoe Konsoula and Maria Liakopoulou [78] immobilized the *Bacillus subtilis* cells by entrapment in calcium alginate gel capsules and the immobilized biocatalyst was used for the semi-continuous production of amylase. The amylase yield and operational stability of the immobilized system were increased by tailoring the capsules' characteristics. Capsules prepared from 2% (w/v) sodium alginate and 3.5% (w/v) $CaCl_2$ were the best support for cell immobilization, providing 2.5-fold higher amylase production in comparison to the freely suspended cells. Immobilized biocatalysts sustained 90% of their initial productivity over five sequential batches in a 10 day period, while amylase production by free cells declined sharply after the second use. Even higher operational stability was achieved when the capsules were treated with 2% (w/v) $CaCl_2$ for 30 min before each batch.

Rohban *et al*. [79] isolated 231 moderate halophilic and 49 extremely halophilic bacteria from Howz Soltan Lake, among which there were 172 Gram positive rods, 56 Gram negative rods and 52 Gram positive cocci. They found that 132 strains from Gram positive rods, 35 strains from Gram negative rods and 26 strains from Gram positive cocci were amylase producer. These data are similar to our findings and reveal that, Gram positive bacteria are the dominant amylolytic strains in the saline environments. Meanwhile the bacterium *Rheinheimera aquimaris* was introduced as a producer of amylase with the highest ranking among the studied bacteria for the first time in this investigation.

Sasmita Mishra and Niranjan Behera [80] isolated from soil sample receiving kitchen waste and growth pattern as well as optimum growth condition was determined. Characteristic feature of the strain indicates that it belongs to the genus *Bacillus*. The optimum temperature for this strain was 37°C, whereas maximum growth was observed at 2% starch concentration. The pH range was found to be 6.8 - 7.2 for optimum growth. Amylase activity was maximum in the temperature range of 50 - 70°C, whereas this temperature range was deleterious for this bacterial strain. Also maximum enzyme activity was observed at 2% of starch concentration.

Burhan Arikan [81] isolated a thermostable alkaline α-amylase producing *Bacillus* sp. from compost samples. There was a slight variation in amylase synthesis within the pH range 6.0 and 12.0 with an optimum pH of 8.5 on Starch agar medium. Analyses of the enzyme for molecular mass and amylolytic activity were carried out by starch SDS–PAGE electrophoresis, which revealed two independent bands (86,000 and 60,500 Da). Enzyme synthesis occurred at temperatures between 25 and 65°C with an optimum of 60°C on petridishes. The partial purification enzyme showed optimum activity at pH 11.0 and 70°C. The enzyme was highly active (95%) in alkaline range of pH (10.0 – 11.5), and it was almost completely active up to 100°C with 96% of the original activity remaining after heat treatment at 100°C for 30 min. Enzyme activity was enhanced in the presence of 5 mMCaCl$_2$ (130%) and inhibition with 5 mM by ZnCl$_2$, NaCl, Na–sulphide, EDTA, PMSF (3 mM), Urea (8 M) and SDS (1%) was obtained 18%, 20%, 36%, 5%, 10%, 80% and 18%, respectively. The enzyme was stable approximately 70% at pH 10.0 – 11.0 and 60°C for 24 hours.

Dhanya Gangadharan *et al.* [82] immobilized *Bacillus amyloliquefaciens* calcium alginate beads and used for the effective hydrolysis of soluble and raw potato starch which was comparable to the free enzyme. The levels of parameters (sodium alginate, calcium chloride and curing time) that significantly influence the immobilization of a-amylase in calcium alginate were analyzed and optimized using response surface methodology. Reactor studies were performed to study the reusability and operational stability of the beads. The alginate beads retained more than 60% of their initial efficiency after five batches of successive use and 40% of efficiency was exhibited in the 6th and 7th batch run of 6 hours duration.

Oueleke and Oduwole [83] obtained ten grams (10 g) of soil sample obtained from a cassava waste and analyzed bacteriologically. One gram (1.0 g) of the sample inoculated into a liquid soluble starch medium generated reducing sugar with a concentration of 1.65 mg/ml after 72 hours. Characterization of the soluble starch amylases revealed an optimum temperature of activity of 70°C. Optimum pH for activity was between 6.5 and 7.5. The most frequently occurring amylolytic bacteria were *Bacillus subtilis* (37.5%), followed by *Bacillus subtilis*. *Bacillus megaterium* and *Bacillus coagulans* (18.75% each). The least

occurring isolates were *Merus* and *Bacillus pumilus* (6.25% each). The mean zone of amylolytic activity for the isolates ranged between 2.1 mm for *Bacillus subtilis* and 1.1 mm for *Bacillus pumilus*.

Yu Chieh Liao and Mei Jywan Syu [84] purified α-Amylase from *Bacillus amyloliquefaciens* by the immobilized metal ion affinity adsorbent, b-CDcl-IDA-Cu^{2+}. The adsorbent was prepared by reacting the cross-linked b-cyclodextrin (b-CD) with the ligand, iminodiacetic acid (IDA). The copper ion was further linked to the adsorbent. Poly (ethylene glycol) (PEG) was added to the fermentation broth to improve the adsorption efficiency of the adsorbent toward α-amylase. The effort was to provide hydrophobic interactions with the impurities which might interfere with the adsorption of α-amylase. It also provided a polymer shielding effect to prevent non-specific interactions. With the addition of PEG, the adsorption efficiency could be increased to 98%.

Vijayalakshmi *et al.* [85] optimized the production of extracellular amylase by *Bacillus* sp. in a submerged fermentation. The production of the enzyme was maximum at 10 hours after inoculation. The effect of incubation period, pH of the medium and incubation temperature was optimized. The maximum productions of enzyme were obtained at 35°C and pH 7.

Sujata Bansode [86] produced the thermostable α-amylase using the bacteria isolated from soil in a liquid media. Screening of important nutrient parameters from selected seven medium components *viz.* starch, yeast, extract, tryptone, K$_2$HPO$_4$, CaCl$_2$.2H$_2$O, MgSO$_4$.7H$_2$O and KCl were carried out in shake flask cultures for enzyme production using Plackett-Burman experimental design of twelve trials. Among these starch, yeast extract and CaCl$_2$.2H$_2$O contributes to large extent; tryptone and K$_2$HPO$_4$ have moderately significant; while MgSO$_4$.7H$_2$O and KCl shows minute effect for amylase production by submerged fermentation.

Ghasemi *et al.* [87] examined the amylase production capability of halophilic bacteria isolated from Maharloo hypersaline lake. In a primary screening program, 50 colonies were isolated being capable of using starch as a sole carbon source. To determine the amylase activity, starch digestion was measured using the iodometric methods. Among them, 13 strains with more amylase activity were identified by biochemical and morphological characterization

and 16S rRNA gene sequence as a molecular marker. In this study, the bacterium *Rheinheimera aquimaris* was reported to have the highest capability for production of amylase.

Senthilkumar *et al*. [88] isolated the bacteria from the soil samples and the bacteria isolated was identified as *Bacillus* sp. based on Staining techniques, motility test, plating on selective media and biochemical tests. Amylase production by *Bacillus* sp. was detected by the disappearance of blue colour in the starch agar medium around the microbial colonies after incubation. Cassava was used as the substrates for the amylase production. Solid state fermentation was carried out for the production of amylase using *Bacillus* sp. The effect of different carbon and nitrogen source, Temperature and pH was determined on enzyme production by *Bacillus* sp. Amylase activity was determined by four methods such as DNSA method, Dextrinizing activity method, decrease in starch-iodine color intensity and Plate assay.

7. COMMERICAL APPLICATIONS AMYLASES

S. No	Industry	Applications
1.	Baking & milking	Reduction of dough viscosity acceleration of fermentation process, increase in loaf volume, improvement of crumb score and softness maintenance of freshness and softness
2	Beer	Mashing
3	Cereals	Precooked baby foods, breakfast foods
4	Chocolate, coco	Manufacture of syrups
5	Confectionery, candy	Sugar recovery from scrap candy
6	Corn syrup	Manufacture of high maltose syrup
7	Distilled beverages	Mashing
8	Feeds, animal	Pig starter rations
9	Flavour	Clarification (starch removal)
10	Pharmaceutical and Clinical	Digestive acids
11	Textiles	Digesting fabrics
12	Vegetables	Liquefying soups

8. CONCLUSION

As an evident from various reports, amylases are among the most important enzymes used in various industries. Research on amylase has progressed very rapidly over the last five decades and potential industrial applications of the enzyme especially in solid waste management have been identified. Major impediments to exploit the commercial potential of amylase are the yield, stability and cost of amylase production. Although, the bacterial isolates have been extensively studied by many researchers. Further, there arises a need for more efficient amylases in various sectors, which can be achieved either by chemical modification of the existing enzymes or through protein engineering. In the light of modern biotechnology, amylases are now gaining importance in biopharmaceutical applications.

Still, their application in food and starch based industries is the major market and thus the demand of amylases would always be high in these sectors.

REFERENCES

1. Kunamneni, A., Permaul, K and Singh, S. 2005. Amylase production in solid state fermentation by the thermophillic fungus *Thermomyces funginosus*. *Journal of Bioscience and Bioengineering*, 100(2): 168 - 171.
2. Aiyer, P.V. 2005. Amylase and their applications. *African Journal of Biotechnology*, 4(13): 1525 - 1529.
3. Gupta, A., Gupta, V.K., Modi, D.R and Yadava, L.P. 2008. Production and characterization of α-amylase from *Aspergillus niger*. *Biotechnology*, 7(3): 551-556.
4. Rodriguez, V.B., Alameda, E.J., Gallegor, J. F and Requena, A. R. 2006. Modification of the activity of α-amylase from *Bacillus licheniformis* by several surfactants. *Electron Journal of Biotechnology*, 9(5): DOI: 10.225/vol9-issue5-fulltext-16.
5. Ryan, S. M., Fitzgerald, G. F and Sinderen, D. 2011. Screening for identification of starch, amylopectin and pullulan degrading activities in *Bifidobacteria* strains. *Applied Environmental Microbiology*, 72(8): 5289 - 5296.
6. Ali, M. B., Khemakhem, B., Robert, X., Haser, R and Bejar, S. 2006. Thermostability enhancement and change in starch hydrolysis profile of the maltohexaose forming amylase of *Bacillus stearothermophilus* strain. *Biochemistry Journal*, 394: 51 - 56.
7. Sivaramakrishnan, S., Gangadharan, D., Nampoothiri, K. M and Pandey, A. 2006. Amylases from microbial sources – An overview on recent developments. *Food Technology and Biotechnology*, 44(2): 173 - 184.
8. Windish, W. W and Mhatre, N.S. 2012. Microbial amylases. In: Wayne WU, editor. Advances in applied microbiology, vol. 7. New York: Academic Press, 2005: 273 - 304.
9. Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D and Mohan, R.

2000. Advances in microbial amylases. *Biotechnology and Applied Biochemistry*, 31: 135 - 152.

10. Kadziola, A., M. Sogaard, B. Svensson, R. Haser. 1998. Molecular structure of a barley a-amylase-inhibitor complex: implications for starch binding and catalysis. *Journal of Molecular Biology*, 278: 205– 217.

11. Aghajari, N., Feller, G., Gerday, C and Haser, R. 1998. Structures of the psychrophilic *Alteromonas haloplanctis* amylase give insights into cold adaptation at a molecular level. *Structure*, 6: 1503 – 1516.

12. Suvd, D., Fujimoto, Z., Takase, K., Matsumura, M., and Mizuno, H. 2001. Crystal structure of *Bacillus stearothermophilus* amylase: possible factors determining thermostability. *Journal of Biochemistry (Tokyo)*, 129: 461 – 468.

13. Tanaka, A and Hoshino, E. 2002. Calcium binding parameter of *Bacillus amyloliquefaciens* amylase determined by inactivation kinetics. *Biochemistry Journal*, 364: 635 – 639.

14. Hashim, S. O., O. Delgado, M. A. Martinez, R. Hatti-Kaul, F.J. Mulaa and B. Mattiasson. 2004. Alkaline active maltohexaose-forming a-amylase from *Bacillus halodurans* LBK 34. *Enzyme Microb. Technol*., 36: 139–146.

15. Nielsen, A.D., Pusey, M.L., Fulsgang, C.C and Westh, P. 2003. A proposed mechanism for the thermal denaturation of a recombinant *Bacillus halmapalus* amylase - the effect of calcium ions. *Biochim. Biophys. Acta*., 1652: 52– 63.

16. Fitter, J., Herrmann, R., Dencher, N.A., Blume, A and Hauss, T. 2001. Activity and stability of a thermostable amylase compared to its Mesophilic homologue: mechanisms of thermal adaptation. *Biochemistry*, 40: 10723 – 10731.

17. Simons, J.W.F.A., Kosters, H.A., Visschers, R.W and Jongh, H.H.J. 2002. Role of calcium as trigger in thermal h-lactoglobulin aggregation. *Archives of Biochemistry and Biophysics*, 406: 143– 152.

18. Oliveira, A., Oliveira, L., Andrade, J and Junior, A. 2007. Rhizobial amylase production using various starchy substances as carbon substrates. *Brazilian Journal of Microbiology*, 38: 208 - 216.

19. Mishra, S and Behera, N. 2008. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *African Journal of Biotechnology*, 7: 3326 - 3331.

20. Utong, J., Al-Quadan, F and Akel, H. 2006. Effect of various growth conditions on production of extracellular amylase from thermotolerant *Bacillus* species isolated from hot springs in Jordan. *Journal of Biological Science*, 6: 621 - 625.

21. Saxena, R., Dutt, K., Agarwal, L and Nayyar, P. 2007. A highly thermostable and alkaline amylase from a *Bacillus* sp. PN5. *Bioresource Technology*, 98: 260 - 265.

22. Amoozegar, M., Malekzadeh, F and Malik, K. 2003. Production of amylase by newly isolated moderate halophile, *Halobacillus* sp. strain. *Journal of Microbiological Methods*, 52: 353 - 359.

23. Lonsane, B. K and Ramesh, M. V. 1990. Production of bacterial thermostable amylase by solid state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis. In: Advances in applied microbiology, vol. 35. San Diego: California Academic Press, 19:1 -56.

24. Manonmani, H. K and Kunhi, A. A. M. 1999. Interference of thiol compounds with dextrinizing activity assay of amylase by starch iodine color reaction: modification of the method to eliminate this interference. *World Journal of Microbiology and Biotechnology*, 15: 485 - 487.

25. Ben Massoud, E., Ben Ali, M., Elluch, N and Bejar, S. 1999. Purification and properties of maltyoheptose and maltohexose forming amylase produced by *Bacillus subtilis*. *Enzyme and Microbial Technology*, 34: 662 - 666.

26. Saxena, R., Dutt, K., Agarwal, L and Nayyar, P. 2007. A highly thermostable and alkaline amylase from a *Bacillus* sp. *Bioresource Technology*, 98: 260 - 265.

27. Setyorini, E., Takenaka, S., Murakami, S and Aoki, K. 2006. Purification and characterization of two novel halotolerant extracellular proteases from *Bacillus*

subtilis strain. *Bioscience, Biotechnology and Biochemistry*, 70: 433 – 440.

28. Hashim, S. O., Delgado, O., Martinez, M. A., Hatti Kaul, R., Mulaa, F. J and Mattiasson, B. 2004. Alkaline active maltohexaose forming amylase from *Bacillus halodurans*. *Enzyme Microbiology Technology*, 36: 139 – 146.

29. Lin, L. L., Chyau, C. C and Hsu, W. H. 1998. Production and properties of a raw starch-degrading amylase from the thermophilic and alkaliphilic *Bacillus* sp. *Biotechnology and Applied Biochemistry*, 28: 61 – 68.

30. Leveque, E., Janacek, S., Haye, B and Belarbi, A. 2000. Thermophilic archeal amylolytic enzymes. *Enzyme and Microbial Technology*, 26: 13 – 14.

31. Coolbear, T., Daniel, R.M and Morgan, H.W. 1992. The enzymes from extreme thermophiles: bacterial sources, thermostabilities and industrial relevance. *Advanced Biochemistry and Engineering Biotechnology*, 45: 57 – 97.

32. Horikoshi, K. 1999. Alkaliphiles: some applications of their products for biotechnology. *Microbiology and Molecular Biology Reviews*, 63 (4): 735 – 750.

33. Fogarty, W. M and Kelly, C. T. 1979. Starch degrading enzymes of microbial origin. *Progress Indian Microbiology*, 15: 87 - 150.

34. Mishra, S and Behera, N. 2008. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *African Journal of Biotechnology*, 7: 3326 - 3331.

35. Rani Gupta, Paresh Gigras, Harapriya Mohapatra, Vineet Kumar Goswami and Bhavna Chauhan. 2003. Microbial α – amylase: a biotechnological perspective. *Process Biochemistry*, 38: 1599 – 1616.

36. Morkeberg, R., Carlsen, M and Neilsen, J. 1995. Induction and repression of amylase production in batch and continuous cultures of *Aspergillus oryzae*. *Microbiology*, 141: 2449 - 2454.

37. Kelly, C. T., Bolton, D. J and Fogarty, W. M. 1997. Biphasic production of amylase of *Bacillus flavothermus* in batch fermentation. *Biotechnology Letters*, 19: 75 - 77.

38. Jin, B., Van Leeuwen, J. H and Patel, B. 1999. Mycelial morphology and fungal protein production from starch processing wastewater in submerged cultures of *Aspergillus oryzae*. *Process Biochemistry*, 34: 335 - 340.

39. Goto, C. E., Barbosa, E.P., Kistner, L.C.L., Moreira, F.G., Lenartoviez, V and Peralta, R.M. 1998. Production of amylase by *Aspergillus fumigatus* utilizing methyl D-glucoside, a synthetic analog of maltose as substrate. *FEMS Microbiology Letters*, 167: 139 - 143.

40. McMahon, H.E.M., Kelly, C. T and Fogarty, W. M. 1999. High maltose producing amylolytic system of a *Streptomyces* sp. *Biotechnology Letters*, 21: 23 - 26.

41. Hamilton, L.M., Kelly, C.T and Fogarty, W.M. 1999. Production and properties of the raw starch digesting amylase of *Bacillus* sp. IMD 435. *Process Biochemistry*, 35: 27 - 31.

42. Cordeiro, C.A.M., Martinas, M.L.L and Lucaino, A. 2003. Production and Properties of alpha amylase from thermophylic *Bacillus* species. *Brazilian Journal of Microbiology*, 33:1 - 3.

43. Cui, Y.Q., Van der Lans, R.G.J.M and Luyben, K.C.A.M. 2007. Effect of agitation intensities on fungal morphology of submerged fermentation. *Biotechnology and Bioengineering*, 55: 715 - 726.

44. Amanullah, A., Blair, R., Nienow, A. W and Thomas, C. R. 1999. Effects of agitation intensity on mycelial morphology and protein production in chemostat cultures of recombinant *Aspergillus oryzae*. *Biotechnology and Bioengineering*, 62: 434 - 436.

45. Spohr, A., Carlsen, M., Nielsen, J and Villadsen, J. 1997. Morphological characterization of recombinant strains of *Aspergillus oryzae* producing amylase during batch cultivations. *Biotechnology Letters*, 19: 257 - 261.

46. Agger, T., Spohr, A.B., Carlsen, M and Nielsen, J. 1998. Growth and product formation of *Aspergillus oryzae* during submerged cultivations: verification of a morphologically structured model using fluorescent probes. *Biotechnology and Bioengineering*, 57: 321 -329.

47. Hillier, P., Wase, D.A.J., Emery, A. N and Solomons, G. L. 2010. Instability of amylase production and morphological variation in continuous culture of *Bacillus amyloliquefaciens* is associated with plasmid loss. *Process Biochemistry*, 32: 51 - 59.

48. Wu, W.X., Mabinadji, J., Betrand, T. F and Wu, W. X. 2009. Effect of culture conditions on the production of an extracellular thermostable alpha-amylase from an isolate of *Bacillus* sp. *Journal of Zhejiang University Agriculture and Life Science*, 25: 404 - 408.

49. Delgado, G., Topete, M and Galindo, E. 2009. Interaction of cultural conditions and end product distribution in *Bacillus subtilis* grown in shake flasks. *Journal of Microbiology and Biotechnology*, 31: 288 - 292.

50. Dhanya Gangadharan, Madhavan Nambothari, Sweetha Ramakrishnan and Ashok Pandey. 2009. Immobilized bacterial amylase for effective hydrolysis of raw and soluble starch. *Food Research International*, 42: 436 - 442.

51. Chung, Y. C., Kobayashi, T., Kanai, H., Akiba, T and Kudo, T. 1995. Purification and properties of extracellular amylase from the hyperthermophilic archeon *Thermococcus profundus* DT5432. *Enzyme Technology*, 6(3): 115 – 120.

52. Justen, P., Paul, G. C., Nienow, A.W and Thomas, C. R. 2006. Dependence of mycelial morphology on impeller type and agitation intensity. *Biotechnology and Bioengineering*, 52: 672 - 684.

53. Bocking, S.P., Wiebe, M.G., Robson, G.D., Hansen, K., Christiansen, L. H and Trinci, A.P.J. 2009. Effect of branch frequency in *Aspergillus oryzae* on protein secretion and culture viscosity. *Biotechnology and Bioengineering*, 65: 638 - 648.

54. Cui, Y.Q., Van der Lans, R.G.J.M and Luyben, K.C.A.M. 2007. Effect of agitation intensities on fungal morphology of submerged fermentation. *Biotechnology and Bioengineering*, 55: 715 - 726.

55. Agger, T., Spohr, A.B., Carlsen, M and Nielsen, J. 1998. Growth and product formation of *Aspergillus oryzae* during submerged cultivations: verification of a morphologically structured model using fluorescent probes. *Biotechnology and Bioengineering*, 57: 321 -329.

56. Khoo, S. L., Amirul, A. A., Kamaruzaman, M., Nazalan, N and Azizan, M. N. 2004. Purification and characterization of amylase from *Aspergillus flavus*. *Folia Microbiology*, 39: 392 - 398.

57. Giri, N.Y., Mohan, A.R., Rao, L.V and Rao, C. P. 2000. Immobilization of amylase complex in detection of higher oligosaccharides on paper. *Current Science*, 59: 1339 -1340.

58. Ray, R. R., Jana, D.C., Nanda, G. 1995. Immobilization of b-amylase from *Bacillus megaterium* into gelatin film by cross - linking. *Journal of Applied Bacteriology*, 79: 157 - 162.

59. Prieto, J.A., Bort, B.R., Martinez, J., Randez – Gil, F., Buesa, C and Sanz, P. 1995. Purification and characterization of a new amylase of intermediate thermal stability from the yeast *Lipomyces kononenkoae*. *Biochemistry and Cell Biology*, 73: 41 - 49.

60. Mamo, G., Gashe, B. A and Gessesse, A. 1999. A highly thermostable amylase from a newly isolated thermophillic *Bacillus* sp. *Journal of Applied Microbiology*, 86: 557 - 560.

61. Marco, J. L., Bataus, L. A., Valencia, F. F., Ulho, C.J., Astolfi – Filho, S and Felix, C. R. 2006. Purification and characterization of a truncated *Bacillus subtilis* amylase produced by *Escherichia coli*. *Applied Microbiology and Biotechnology*, 44: 746 - 752.

62. Wind, R. D., Buitelaar, R.M., Eggink, G., Huizing, H.J and Dijkhuizen, L. 1994. Characterization of a new *Bacillus stearothermophilus* isolate: a highly thermostable amylase producing strain. *Applied Microbiology and Biotechnology*, 41: 155 - 162.

63. Eriksen, S.H., Jensen, B and Olsen, J. 2012. Effect of N-linked glycosylation on secretion, activity and stability of amylase from *Aspergillus oryzae*. *Current Microbiology*, 37: 117 - 122.

64. Laderman, K.A., Davis, B.R., Krutzsch, H.C., Lewis, M.S., Griko, Y.V., Privalov, P.L and Anfinsen, C.B. 2003. The purification and characterization of an extremely thermostable amylase from hypothermophilic archaebacterium

Pyrococcus furiosus. Journal of Biological Chemistry, 268: 24394 - 24401.

65. Aehle, W and Misset, O. 1999. Enzymes for industrial applications. In: Rehm HJ, Reed G, editors. Biotechnology, 2nd ed. Germany: Wiley-VCH: 189 - 216.

66. Hamer, R. J. 1995. Enzymes in the baking industry. In: Tucker GA, Woods LFJ, editors. Enzymes in food processing. Galsgow: Blackie Academic and Professional: 190 - 222.

67. Si, J. Q. 1999. Enzymes, baking, bread making. In: Flickinger MC, Drew SW, editors. Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation, vol. 2. Wiley: 947 - 958.

68. Monfort, A., Blasco, A., Preito, J.A and Sanz, P. 1996. Combined expression of *Aspergillus nidulans* endoxylanase X-24 and *Aspergillus oryzae* amylase in industrial baker's yeast and their use in bread making. *Applied Environmental Microbiology*, 62: 3712 - 3715.

69. Pritchard, P. A. 2002. Studies on the bread improving mechanisms of fungal amylase. *Journal of Biological Education*, 26: 12 - 18.

70. Van Dam, H. W and Hille, J.D.R. 2002. Yeast and enzymes in bread making. *Cereal Foods World*, 37: 245 - 252.

71. Spendler, T and Jorgensen, O. 1997. Use of a branching enzyme in baking. Patent Application WO97/41736.

72. Lynn M Hamilton, Catherine T Kelly and William M Fogarty. 1999. Production and properties of raw starch digesting amylase of *Bacillus* sp. *Process Biochemistry*, 35: 27 -31.

73. Gashaw Mamo and Amare Gessesse. 1999. Purification and characterization of two raw starch digesting thermostable amylase from a Thermophilic *Bacillus* sp. *Enzyme and Microbial Technology*, 25: 433 - 438.

74. Arikan Burhan, Unaldi Nisa, Coral Gohan, Colak Omer and Aygan Ashbil. 2003. Enzymatic properties of the thermostable, thermophilic, alkaline and chelator resistant amylase from a Thermophilic *Bacillus* sp. *Process Biochemistry*, 38:1397 - 1403.

75. Ben Massoud, E., Ben Ali, M., Elluch, N and Bejar, S. 1999. Purification and properties of maltyoheptose and

maltohexose forming amylase produced by *Bacillus subtilis. Enzyme and Microbial Technology*, 34: 662 - 666.

76. Damodara Rao Mendu, Rathnam, B. V. V., Purnima, A and Ayyanna, C. 2012. Affinity chromatography of amylase from *Bacillus licheniformis. Enzyme and Microbial Technology*, 37: 712 - 717.

77. Eva Bernhardsdotter, N., Eke, O. S and Oguntimehin, G. B. 2005. Partial purification and characterization of alpha amylase from *Bacillus cereus* BC. 19p. *Journal of Agricultural Science and Technology*, 2(2): 152 - 157.

78. Zoe Konsoula and Maria Liakopoulou - Kyriakides. 2006. Thermostable amylase production by *Bacillus subtilis* entrapped in calcium alginate gel capsules. *Enzyme and Microbial Technology*, 39: 690 - 696.

79. Rohban, R., Amoozegar, M. A and Ventosa, A. 2008. Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. *Journal of Microbiology and Biotechnology*, 36: 333 - 340.

80. Sasmita Mishra and Niranjan Behera. 2008. Amylase activity of starch degrading bacteria isolated from soil receiving kitchen wastes. *African Journal of Biotechnology*, 7(18): 3326 - 3331.

81. Burhan Arikan. 2008. Highly thermostable, thermophilic, alkaline, SDS and chelator resistant amylase from a Thermophilic *Bacillus* sp. *Bioresource Technology*, 99: 3071-3076.

82. Dhanya Gangadharan, Madhavan Nambothari, Sweetha Ramakrishnan and Ashok Pandey. 2009. Immobilized bacterial amylase for effective hydrolysis of raw and soluble starch. *Food Research International*, 42: 436 - 442.

83. Oyeleke, S. B and Oduwole, A. A. 2009. Production of amylase by bacteria isolated from cassava waste dumpsite in Minna, Nigeria. *African Journal of Biotechnology*, 3(4): 146-149.

84. Yu-Chieh Liao and Mei-Jywan Syu. Effect of salts on the binding of amylase from the fermentation broth of *Bacillus licheniformis* by affinity absorbent. *Carbohydrate Polymers*, 77: 334 - 350.

85. Vijayalakshmi Raja, Sasmita Mishra and Niranjan Behera. 2008. Amylase activity of starch degrading bacteria isolated from

soil receiving kitchen wastes. *African Journal of Biotechnology*, 7(18): 3326 - 3331.

86. Sujata Bansode. 2010. Screening of nutritional components for amylase production in submerged fermentation by bacteria isolated from soil. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(1): 93 - 98.

87. Ghasemi, Y., Rasoul - Amini, S., Ebrahiminezhad, A., Zarrini, G and Kazemi, A. 2010. Halotolerant amylase production by a novel bacterial strain, *Rheinheimera aquimaris*. *Research Journal Microbiology*, 5: 144 - 149.

88. Senthilkumar, P. K., Uma, C and Saranraj, P. 2012. Amylase production by *Bacillus* sp. using cassava as substrate. *International Journal of Pharmaceutical and Biological Archives*, 3 (2): 274 – 280.

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