Review Article

AN EXTENSIVE REVIEW: INDUSTRIALLY IMPORTANT ENZYMES, THEIR CLASSIFICATION AND DEPICTING THE CONSORTIUM OF INDUSTRIAL APPLICATIONS

ABSTRACT

Microorganism has been used since the start of human society. Enzyme processing process are quickly gaining the attention because of their short time of processing, cost effective, nontoxic, low energy input and environment friendly characters as well. Moreover through protein engineering and recombinant DNA technology a microorganism can be easily manipulated and cultured in large scale to meet increased demand in different sectors. Therapeutic enzymes have a huge variety of selective uses such as anticoagulants or thrombolytics, oncolytics and also as replacement for various metabolic deficiencies. Proteolytic enzymes are excellent anti-inflammatory agents. There are various factors that decrease the potential of microbial source enzymes, once we enter to medical sciences due to high molecular size of catalyst which stop their distribution within somatic cells. In industrial processes the quantity of enzymes should be high, while in therapeutic case the purity and specify should be excellent, if the quantity level is less no matter. The kinetics of such enzymes is high and low so that it is maximally efficient even at low concentration of substrate and enzymes. The source of such enzymes should be designated with high care to minimize or prevent the chances of undesirable growth by mismatched material and also to enable ready purification.

INTRODUCTION

Microorganism has been used since the start of human society. The first reported microorganism is the uses of yeast to make alcoholic products from barley by the Sumerians and Babylonians as early as 6000 BC. Microbes enzymes catch their attention globally for their great application in distinguish sectors of industries, such as in chemical, energy, food, medicine and agriculture.

Enzyme processing process are quickly gaining the attention because of their short time of processing, cost effective, nontoxic, low energy input and environment friendly characters as well. Moreover through protein engineering and recombinant DNA technology a microorganism can be easily manipulated and cultured in large scale to meet increased demand in different sectors. Related driving factors that's enhance the use of microbes enzyme products in industrial uses are increasing demand of user, natural resources reduction, necessity of cost reduction and eco-friendly. World market for industrial microbial enzymes was projected nearly about $4.2-4.5 billion in 2014 and predictable to make at a compound AGR of nearly 8% over the time from 2016 to 2020 to influence $6.4 billion(Kour et al., 2019). Microbial enzymes are biological compounds, protein in nature except catalytic ribonucleic acid molecules and act catalyst to speed up the chemical reaction and to support almost all of the chemical reaction needed for the life (Gurung, Ray, Bose, & Rai, 2013). Enzymes are highly specific in nature; speedup the chemical reaction by decreasing the activation energy of the chemical reaction with any permanent change in the reaction, and therefore major molecules to support life (Anastas, Bartlett, Kirchhoff, & Williamson, 2000). For enzyme activity they need optimal temperature and pressure for catalyzing reaction, and are uses in substitute toxic chemical pollutant owing to their nontoxic and biodegradable nature (Anastas et al., 2000).

In addition to advantages of enzymes over conventional methods, there are some drawbacks of using enzymes in healthcare and other industries. For many mammalian enzymes, 37°C and 7.4 are the optimal temperature and pH, respectively, and their activity is highly sensitive to any change in these parameters. Higher temperature (>40°C), and a large deviation from the physiological pH (7.4) lead to their denaturation, which limits the use of these macromolecules in non-physiological conditions. Additionally, they are susceptible to substrate or product inhibition and their products may cause allergic reactions. The high cost of isolation and purification of enzymes and their difficult recovery for subsequent reuse may discourage their use(Singh, Kumar, Mittal, & Mehta, 2016).

ENZYMES

Enzyme are large molecules consist of AA attached through amide NH2 bonds, range between kilo-Dalton to mega-Dalton in molecular mass. The specificity of enzymes molecules to catalyze reaction between one kinds of chemical compound over the offers the center of its name and classification. In biochemistry area a great advancement achieved after 1940, a huge number of enzyme isolate and identified, for this purpose it was mandatory to legalize enzyme nomenclature. IUBMB in discussion with International Union of Pure and Applied Chemistry develop an enzyme Commission to in charge of managing systemic classification and naming to enzymes. Enzyme was classified into six different groups on the basis of the reaction they catalyzed. For industrial enzymes microbes are the best choice because of their easily availability, and fast growth rate of microbes. Through recombinant DNA technology genetic changes can easily be done in microbes for the production of enzymes and scientific development (Liu). Microbial enzymes production is mandatory event in industrial zone, due to the loftier and great performance of enzymes from various microorganism, because they performed well in wide range of chemical and physical condition (Anbu, Gopinath, Chaulagain, Tang, & Citartan, 2015). Microbial enzymes help in the cure of many disorder linked with the absence of human enzymes caused due to genetic changes. Some enzymes are greater than the substrate they act on, and only a very small part of enzymes is directly involved in catalysis (Almeida, Serra, Silveira, & Moura, 2010). Enzymes also have a site that bind to the cofactors, which are required for catalysis of the reaction (Bartlett, Porter, Borkakoti, & Thornton, 2002).

Like protein enzymes have linear chain of AA, long that fold to make a 3D products. Each AA sequence make a selective structure, which has unique properties. Mostly enzymes are denatured that is outspread and deactivated by chemical or heating, which disrupt 3D structure of the protein. Denaturation may be irreversible or reversibledependent on the enzymes. Enzymes are classified into six groups on the basis of reaction they catalyze(Kotera, Okuno, Hattori, Goto, & Kanehisa, 2004).

Amylase enzyme

Amylase is an enzyme that break starch molecule into sugars. Amylase enzyme is commonly found in saliva that breakdown the food and help in digestion. Some foods contain high amount of starch and contain slight sugar, such as potato and rice, taste somewhat as they are masticated because amylase cracks a few of their starch into sugar in human mouth. Pancreas also produce amylase which hydrolyze dietary starch into disaccharide carbohydrates and trisacchrides which are rehabilitated by other enzymes. Bacteria and some plants also produce amylase. In 1833 amylase was the first enzyme isolated and identified by Anselme Payen. Entirely amylases act on α-1,4-glycosidic and are glycoside hydrolases. Amylases has nearly 25% enzyme market and widely used in industry (Edner et al., 2007). In present area chemical hydrolysis of starch in starch processing industry is almost replaced by amylases. Amylases isolated from microbes are more stable than plants and animal and for that’s reason they have broad spectrum of industrial uses. Microbes are easy to change their nature and desired enzymes, so the major benefit of microbes for the production of enzymes is the mass production and economical. In biotechnology sector starch hydrolyzing amylolytic enzymes are of high advantages ranging from paper, textile, fermentation and food industries. Amylases can be extracted from several sources such animals and plants but enzymes obtained from microbes generally satisfied industrial burdens and had made greater contribution to the beravges and food industry(Souza, 2010).

Types

Amylase has three sub-classes —*α*- *β*- *γ*-amylase.

***α*-Amylase**

Alpha amylase enzymes help in the hydrolysis or breakdown of the internal *α*-1,4-glycosidic linkages in low molecular weight compound such as maltotriose, maltose and glucose (Sundarram & Murthy, 2014). *α*-Amylase are important form of amylase found in human, cow, sheep, and in other mammals (Butterworth, Warren, & Ellis, 2011). It is also present in those seeds of plants that comprise of starch as food reserve and is released by numerous fungus. Amylyse are also present in high amount in saliva and pancreatic juice, each of which has itself isoform of human amylase. In human amylase are connected to 1p21 chromosome.

Uses of *α*-Amylase

α-Amylase uses in production of ethyl alcohol by break down the starch in grains into sugars. The intital phase in the making of high fructose corn syrup is the treatment of cornstarch with *α*-amylase, making smaller chain of sugars known as oligosaccharides. Some microbial source alpha amylases help in removing starch detergent (Butterworth et al., 2011).

***β*-Amylase**

β-amylase is another form of amylases synthesize by plants, fungi and bacteria. The alternative name for β-amylase is 1, 4-α-D-glucan maltohydrolase; saccharogen amylase; glycogenase). β-amylase working from non-reducing end, catalyzes the breakdown of the 2nd α-1,4 glycosidic bond, breaking of two glucose unit at the same time. β-amylase breaks the starch into maltose during the ripening of fruits, resulting in the sweet flavor of ripe fruits. α-amylase and β-amylase are found in seeds, β-amylase are inactive before the germination while proteases and α-amylase seen once germination has started. For the production of malt cereal grain amylase is the key. Different microorganism also produce amylases that help in the degradation of extracellular starches. Animal cell and tissue do not have β-amylase, although the microorganism present in gut of animal produce β-amylase. The best pH for β-amylase is 4-5.

Use of β-amylase

Both alpha and beta amylases are significant in makingliquor and beer prepared from sugars consequent from starch. In microbial fermentation process mushroom ingest sugars and defecateethyl alcohol. In liquors and beer, the sugars exist at the start of fermentation have been formed by “squashing" grains or other starch cradles.

γ-Amylase

Gamma-amylase is an amylase which break α(1-4) glycosidic and α(1-6) glycosidic linkages at the non-reducing end of amylopectin and amylose, resulting in glucose. The γ-amylase has high acidic pH because they are highly active at pH 3. The alternative name for γ-amylase is 1, 4-α-glycosidase.

Uses γ-Amylase

γ-Amylase are used in chemical, pharmacological, drugs distribution and food industry, as well as environmental and agriculture engineering. Hydroxypropyl beta cyclodextrin is the main compound present in Gamble's and Procterfreshening product.

Bacterial spp amylase

Amylase can be extracted from various species of microbes, but for commercial use, α-amylase derived from *Bacillus amyloliquefaciens*, *Bacillus licheniformis* and *Bacillus stearothermophilus*, has wide range of application in various industries such as in paper, textile, fermentation and food industries(Souza, 2010). Industrial enzymes has major and desirable characters known as thermostabality. Temperature stable enzymes have found huge number of commercial significance due to their stability in high temperature. The production of valuable industrial products such as gluose, dextrose syrup, maltose, crystalline dextrose and maltodextrins are improving by using thermos table amylolytic enzyme (Guzmán- Maldonado, Paredes- López, & Biliaderis, 1995).*Bacillus amyloliquefaciens*, *Bacillus subtilis*,*Bacillus licheniformis* and *Bacillus stearothermophilus* are widely used for industrial level production of enzymes for huge application. In present temperature resistance enzyme of *Bacillus licheniformis* or *Bacillus stearothermophilus* are used in starch degrading/ processing industry(Prakash & Jaiswal, 2010). In some industry high level of harsh processes occur and contain huge amount salt solution which stop/ inhibit the many enzymatic reaction. For this purpose halophilic microbes have excellent activity at high salt concentration and enzymes produced them could be used in such processes(Ventosa & Nieto, 1995). Beside such a good character halobacterial enzyme are temperature stable and can survive for longer period of time at high temperature. Halophilic amylases have been resulting from bacteria such as and *Bacillus* *dipsosauri*, *Chromohalobacter* sp., *Haloarcula hispanica*(Caton et al., 2004)and *Halomonas meridian*(Edbeib, Wahab, & Huyop, 2016).

Fungus Amylase

Many researcher work on fungus to produce amylases and to choose the best strains to produce amylase commercially. Previously mesophilic fungi reported that produce alpha-amylase. Terrestrial isolates of fungi such as *Penicillium* and *Aspergillus*produce various kinds of enzymes (Kango, Jana, & Choukade, 2019).*Aspergillus* spp mainly make a different varieties of extracellular enzymes, and amylases are the ones with most important industrial value. *Aspergillus niger*, and *Aspergillus oryzae* are filamentous fungi produce huge amount of enzymes that can be greatly use in industry. *A. oryzae* is well-thought-out to be the satisfactory host for the manufacture of heterologous protein as it has capability to conceal a huge amount of high valuable proteins and industrial products (Machida et al., 2005). *A. oryzae* has been greatly used in commercial enzymes containing α-amylase and food such as soy sauce and organic acid such as acetic and citric acid (Hajar-Azhari, Wan, Ab Kadir, Abd Rahim, & Saari, 2018). *A.niger* has a significant hydrolytic capacities in the α-amylase production, because of low pH/ acid tolerant, and they also avoid bacterial growth/contamination(Djekrif-Dakhmouche, Gheribi-Aoulmi, Meraihi, & Bennamoun, 2006). Fungal enzymes have generally safe status and that's why they are preferred over other microbial enzymes. The high temperature resistance fungus *Thermomyces lanuginosus* is an great producer of amylase (Tiwari et al., 2015).

LIPASE

Lipases are enzymes that breakdown the fats. Lipases are the sub-class of the esterase's (Arreaza et al., 1997). These enzymes plays significant role in processing of dietary lipids, digestion and transport. Certain viruses also contain genes that encodes lipases. Especially in small intestine most lipases enzymes act on precise position on glycerol backbone of lipid substrate(Rahman & Basri, 2006). Several other kinds of activities of lipase present in nature, such as sphingomyelinases and phospholipase, however these are frequently treated distinctly from orthodox lipases. Some pathogenic microbes also produce lipases during infection, especially *Candida albicans* contain huge number of lipases. In biotechnology sector lipases plays significant role and a valuable enzyme in these sectors(Saini, Saini, & Dahiya, 2017). Microbial enzymes have wide range of application at industrial level, because of their differentiated enzymatic properties and substrate specificity(Goodman, 2010). Bacterial lipases enzymes are more stable than plant and animal lipases (Burhan et al., 2003).

Types

There are no specific kinds of lipases, but mainly they classified according to its use, namely hepatic lipase, human digestive lipase and pancreatic lipase.

Uses of Lipase

Lipases carried various biological processes ranging from daily metabolism process of dietary triglycerides to inflammation and cell signaling (Pascoal, Estevinho, Martins, & Choupina, 2018).Some lipases have specific selective function, while other plays significant role in extracellular compartment. In lysosome lipase enzyme is present in lysosomes, while other lipases such pancreatic lipases, they veiled into extracellular compartment where they done the breakdown of dietary lipids into simpler forms that can be easily transport throughout the body and absorbed. Bacteria and fungus may also conceal lipases to ease nutrient preoccupation from external medium. Certain bees and wasp contains phospholipases that increase biological processes of inflammation and wound delivered by sting. Dandruff in human is caused by a fungus known as *Malassezia globosa*, this fungus usually uses lipase to do the breakdown of sebum into oleic acid and enhance skin cell production causing dandruff. Lipases plays significant role in cheese and yogurt fermentation. In modern area lipases aye used as versatile and cheap source to degrade lipid.in biotechnology sector recombinant lipases are used in laundry, baking and even as catalyst, also used as alternative approaches to change vegetable oil into fuel. Lipases are safer and environmental friendly and also replaced traditional catalyst in biodiesel processing (Gurung et al., 2013).

Bacterial Lipase

The lipase producing bacterial genera comprise *Burkholderia*, *Pseudomonas and Bacillus*. The bulk production of bacterial lipase are easily and they are commercially very significant of because of extracellular production(Gupta, Gupta, & Rathi, 2004). Only a few bacterial species are used commercially for the production of lipases. The bacterial genera used commercialy include, *Pseudomonas*, *Achromobacter*, *Corynebacterium*, *Alcaligenes*, *Enterococcus*, *Arthrobacter*, *Chromobacterium*, *Burkholderia* and *Bacillus*, and in various biotechnology applications lipases from *Pseudomonas* are greatly used. Different products are launched in market are bacterial lipases based in few years, such as Lipomax and lumafast extracted from *Pseudomonas*, and their major application in detergent (Verma, 2019).

Fungal Lipase

Fungi that produce lipases are present in different habitats, such as dairy byproducts, soil contaminated with waste of vegetables, deteriorated food and seeds(Mehta, Bodh, & Gupta, 2017). One of the important fungus known as *Candida rugosa*lipases have been greatly use in biotechnology sector, because of their potential(Akoh, Lee, & Shaw, 2004).It is previously reported that *Thermomyces lanuginosus* lipases has wide range of applications in the field biotechnology and detergents. Other major lipase making fungi includes, *Rhizopus, Mucor,Humicola, Candida,Geotrichum, Aspergillus, Penicillium,Rhizomucor* and *Rhizopus* (Gurung et al., 2013).The temperature stable lipase is produced by thermophilic *Aspergillus terreu*s, *Mucor pusillus* and *Rhizopus homothallic.* A few study reported about molds with thermostable and alkaliphilic lipase (Mehta et al., 2017).

Applications of other different enzymes

Therapeutic enzymes have a huge variety of selective uses such as anticoagulants or thrombolytics, oncolytics and also as replacement for various metabolic deficiencies. Proteolytic enzymes are excellent anti-inflammatory agents. There are various factors that decrease the potential of microbial source enzymes, once we enter to medical sciences due to high molecular size of catalyst which stop their distribution within somatic cells. In industrial processes the quantity of enzymes should be high, while in therapeutic case the purity and specify should be excellent, if the quantity level is less no matter. The kinetics of such enzymes is high and low so that it is maximally efficient even at low concentration of substrate and enzymes. The source of such enzymes should be designated with high care to minimize or prevent the chances of undesirable growth by mismatched material and also to enable ready purification. In market therapeutic enzymes in usually in lyophilized from with biocompatible mannitol diluent and buffering salts. Cost of such enzymes are usually high in compare to treatment or therapeutic agents. Urokinase is an enzymes that that is extracted from urine and used for blood clots as ensample of such enzymes. In current research area therapeutic enzymes are used in cancer treatment such as acute lymphocytic leukemia, such as asparaginase enzyme has proved an excellent activity. The activity of such enzymes depend upon on the fact that tumour cells lack aspartate-ammonia ligase activity, which inhibit/stop the synthesis of non-essential amino acid. The asparaginase does not have impact on normal cells which are accomplished of manufacturing sufficient for their own necessities, but they reduce the free exogenous attentiveness, so in tumor cells they cause state of fatal starvation. The enzymes can be directed intravenously and is only operational in decreasing asparagine levels inside bloodstream, showing a half-life of about a day. This half-life can be enhanced by 20-fold with use of polyethylene glycol-modified asparaginase (Vellard, 2003).

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