Role of Thermophilic Enzymes and their Applications in Industry

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Abstract:- The exploration of thermophilic and hyperthermophilic microorganisms to utilize their enzymatic system has been done by the research scientist from middle age era. Such examinations are currently a day acing the cloning and mechanical abuse of a wide assortment of qualities encoding chemicals like thermophilic cellulases, amylases, xylanases, polymerases, galactosidases and proteinases etc. A number of thermophilic microorganisms like bacteria, fungi, archaebacteria and actinomycetes known to producing thermophilic enzyme. These enzymes have some structural specificity to show optimum activity in thermophilic environment. Use of thermophilic enzymes is beneficial than using mesophilic enzymes during industrial processes because some of these processes are occur at high temperature. Thermophilic enzymes have wide range of applications in different industries like paper and pulp industry, biofuel industry, textile industry and food industry. To make easy production of these enzymes, genes for thermophilic enzymes have been cloned in mesophilic host. In this text, we review the function of various thermophilic enzymes in different industrial methods from hyperthermophilic and extreme thermophilic microorganisms, the industrial application of thermophilic enzymes are mainly discussed.

Keywords: *Thermophilic; hyperthermophilic; Microorganisms; Industrial application.*

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I. INTRODUCTION

Thermophilic Cellulase

Cellulose is the most plentiful organic basis of nourish, fuel and fodder, synthetic structure of cellulose is comprises of glucose units connected by β-1,4-glycosidic bonds in a direct manner. Cellulose-corrupting chemicals are basic in nature and are transcendently delivered by various microorganisms for example microbes, archaea, and fungi.Three different classes of enzymes including endoglucanases, exoglucanases and β-glucosidases plays a efficient role in cellulose hydrolysis. Internal glycosidic linkages are hydrolyze by Endoglucanases , which results quick diminish in length of polymer and a slow but sure boost in liberated reducing ends counts [1] together with monosaccharide's from cellulose and hemicellulose. Glycoside hydrolases enzymes selectively catalyze reactions that produce smaller carbohydrate units from polysaccharides [2]. Glycoside hydrolases proteins are eminent impetuses which increment the rate of hydrolysis by up to 17 arranges over the unanalyzed hydrolysis of glycosidic linkages [3]. There is some cellulase producing microorganism described in table 1.

II. THERMOPHILIC XYLANASE

Xylan is the major constituent of hemicellulose which is one of the a large amount profuse macrobiotic substance on earth. Xylan has wide application in paper & pulp industry [4,5]. The pulp is produced by the use of wood and then treats the paper at high temperature and alkaline pH medium, which requires that enzyme, should be thermostable and should have wide pH range [6]. Complete hydrolysis of xylan need a huge variety of courteously acting enzymes [7,8]. Thermophilic xylanase were isolated from a number of bacterial and fungal sources. Members of the Bacillus sp., Streptomyces sp., Xylanases is produced from Thermoascus aurantiacus and Fusarium proliferatum at temperature between 50 to 80ºC[9] ,[10].Inferior quality Paper is act as an tremendous carbon source and also inducer for xylanase in Thermoascus aurantiacus [11], Humicola lanuginosa [12] and Paecilomyces *variot*i. In Melanocarpus albomyces and Thermomyces lanuginosus [13].

THERMOPHILIC AMYLOLYTIC ENZYMES

Amylose & Amylopectin are two major components of starch which have characteristics of high molecular weight components. Amylose is a linear polymer while Amylopectin is a branched, Amylose is made of repetition of α-glucose units which are linked by α-1,4 glycosidic linkage while Amylopectin is consists of α-glucose units and also containing α-1,4 glycosidic bonds additionally to α-1,6. Starch polymer needs a proper grouping of enzymes for its entire hydrolysis [14]. Amylose & Amylopectin enzymes are plentiful in hyperthermophiles and thermophiles, telling an significant responsibility in their metabolism. The 30 percent of enzyme consumption is done with starch hydrolytic enzymes[15]. The renovation of all starch by the use enzymes includes gelatinization which dissolve the starch granules and forms a viscous suspension, which involves decrease in viscosity and fractional hydrolysis, and relating the creation of glucose & maltose via back up hydrolysis[16]. The foremost thermophilic α-amylases were cut off from Bacillus subtilis, Bacillus amyloliquefaciens and Bacillus licheniformis [17]. Bacterium Thermus sp., Bacillus sp. strain SMIA-2 and fungal isolate *Thermomyces lanugenosus* found good thermophilic amylase producer (Table 1)

III. Thermophilic Protease

For the economic point of view the fabrication of proteases is much beneficial than any other enzyme group of biotechnological significance. They are vigorously rising as the mainly chief industrial enzymes which make a large part of enzymes market, approx 65% of the total market [18]. Proteases are mainly categorised in two parts: endopeptidases; these are the proteases which have peptide linkage within the amino acid chain of protein and the other one is exopeptidases; theses are those proteases which have amino acids group at the ends of the protein chain. These enzymes are mainly utilized in industries like food, pharmaceutical, leather and textile [19,20]. The most basic isolated thermophilic *Bacillus* sp. that manufactures proteases is formed from *Bacillus stearothermophilus* which is steady at 60ºC. One more bacterial strain B. licheniformis 50b has formed thermophilic proteases which have best possible movement at 60ºC. *Chaetomium thermophilum* is a fungal isolates producing thermostable proteases (Table 1)

IV. Thermophilic Polymerase

The PCR (polymerase chain reaction) has lead to a gigantic progress in heritable engineering because of its property to magnify DNA fragment. Denaturation, Renaturation and synthesis are the succeeding steps of the process. Denaturation which is also known as melting of the DNA strand is obtained at a very high temperature range about 90-95ºC, Renaturation which is also known as primer annealing is done at 55ºC and in the last Synthesis which is also known as primer extension is done at approximately 75ºC [21,22,23]. This process has been developed by thermostable DNA Taq polymerase which is from bacterium *Thermus aquaticus* (table 1). This was the first thermostable DNA polymerase characterized [24,25]. Continual revelation to 98ºC in a reaction shield had slight cause on the enzyme action and after exposure to 99ºC enzyme activity remained was significant.

Table 1: Some microorganism producing Thermophilic enzymes

Enzyme	Producing microorganism	Optimum Temperature	Type of microorganism	Refrence
Cellulase	*Clostridium thermocellum* *Thermoascus aurantiacus*	60 ºC 50 ºC	Bacterium Fungus	[Peng, *et al.*, 2011] [Romanelli *et al*., 1975]
Xylanase	*Bacillus stearothermophilus* T-6 *Bacillus sp*. JB 99 *Thermomyces lanuginosus* SS8	65 ºC 70 ºC 60 ºC	Bacterium Bacterium Fungus	[Khasin *et al.*, 1993] [shrinivas *et al.*, 2010] [Shrivastava *et al*., 2011]
Amylases	*Thermus sp.* *Bacillus sp* strain SMIA-2 *Thermomyces lanugenosus*	60 ºC 40-50 ºC 50 ºC	Bacterium Bacterium Fungus	[Fantoni *et al*., 2012] [De Carvalho., 2008] [Kunamneni *et al*., 2005]
Proteases	*B. subtilis* 50a *B. licheniformis* 50b *Chaetomium thermophilum*	50 ºC 60 ºC 60 ºC	Bacterium Bacterium Fungus	[Azlina and Norazila, 2013] [Azlina and Norazila, 2013] [Li *et al*., 2007]
Polymerase	*Thermus aquaticus*	70 ºC	Bacterium	[Huang and Ito, 1999]

V. Industrial Application of Thermostable Enzymes

Thermophilic enzymes are widely used in industrial processes because of their specific properties like high temperature stability with large tolerance zone. Thermophilic enzymes are utilized in different industries like chemical, pharmaceutical, paper and pulp, textile and food [26-29]. Some of important industrial application described below.

VI. Textile Industry

Thermostable enzymes plays an important role in textile industries as the most important degumming process needs higher temperature which leads to melting of enzymes like mesophilic xylanase and proteases, these enzymes acts most actively in the temperature range of 30-40°C which is not suitable for the compete melting process, so the enzyme which are not stable at higher temperature are not used for this process. On other hand in some process a number of chemicals are used to complete the process [30].

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Thermostable enzymes are used for those industrial process which needs higher temperature range for large spam of time and silk melting is one of the process which used these thermostable proteases enzymes [31].The impotant thing regarding these thermostable protease is their high temperature range for any process to take place i.e. 50-100`C and for a large spam of time.. The properties like bacterial strain, isolation and purification of these proteases will advance biological silk degumming by making the process environment friendly [32]. So also, Desizing of cotton and micropoly textures was finished utilizing thermostable xylanase from Bacillus pumilus ASH. Micropoly texture demonstrated preferable desizing over cotton under same conditions. Enzymatic desizing at pH 7.0 and at 60°C demonstrated the readings falling in the scope of showing great desizing proficiency [33].

VII. PAPER AND PULP INDUSTRY

Amid the dying of wood mash for the paper business, a lot of chlorinated sweet-smelling mixes are delivered and discharged into nature. These mixes are to a great degree harmful and are a noteworthy wellspring of contamination. The paper and mash industry is looking for elective strategies for blanching mash. One such technique includes the utilization of hemicellulases to discharge the shaded lignohemicellulose. Second, a large portion of the responses in this industry did at higher temperature and high pH that is the reason the alkalistable and thermostable xylanases are popular for these industry and creating xylooligosaccharides by hydrolyzing xylan segment of agro-buildups. Some noval xylanases known to date are ideally dynamic at over 50°C and at antacid pH. To date, few xylanases are accounted for to be dynamic and stable at soluble pH and hoisted temperature [34] revealed two thermostable antacid xylanases from an alkaliphilic Bacillus sp. An another extremely proficient strain Thermomyces lanuginosus SS-8 was disconnected by [35]from soil tests that had been gathered from close self-warming plant material which were wellspring effective xylanase maker to use in paper and mash industry.

VIII. BIOFUEL INDUSTRY

Lignocellulose is the most plenteous starch source in plants and has huge potential for transformation into fluid powers or biofuels. Biofuels give a way to decrease the reliance on petroleum derivatives and in addition to diminish worldwide outflows of ozone depleting substances into nature [36]. In any case, the generation of biofuels, which depend on the maturation of corn starch or unadulterated sweetener, are neither monetarily nor biologically feasible. Lignocellulose is the most inexhaustible sugar source in plants and has critical potential for transformation into fluid fills or biofuels. Lignocellulose comprises fundamentally of three noteworthy polymers: cellulose, hemicellulose and lignin. There are requirement for proteins that can change over this lignocellulosic waste to type of sugar which can be used by microorganism to deliver ethanol or biofuel. Enzymatic arrival of monosaccharides from cellulose and hemicelluloses is interceded by glycoside hydrolases. The materialness of thermophilic compounds as biocatalysts for the depolymerization of lignocellulosic feedstock in the creation of biofuels is increasing wide modern and biotechnological intrigue. Different microorganisms, archaea, and growths have gotten significant consideration as potential hotspots for thermostable cellulosic proteins. The broadness of thermophilic organisms with enzymatic qualities agreeable to lignocellulose deconstruction has been explored [37]; be that as it may, thermostable catalysts are created both by thermophilic and mesophilic microorganisms. The expenses related with process cooling are diminished or wiped out permitting the volatilization of items, for example, ethanol to be streamlined, thermostable proteins will bring about a change to the general economy of the procedure [38]. Along these lines, thermophilic protein assumes a critical part in biofual industry .

IX. FOOD INDUSTRY

Thermostable compounds particularly appropriate for nourishment preparing are proteases, β-galactosidases, and distinctive hydrolazes, catalyzing transformation of starch into oligosaccharides, cyclodextrins, and maltose or glucose [39-41]. Some of these catalysts can catalyze transglucosylation or switch responses, which prompt the arrangement of another protein or sugars subsidiaries, e.g., trehalose, maltulose. As of late, the thermostable α-amylase from Bacillus licheniformis was utilized for starch absorption to maltodextrins. Glucose or maltose syrup generation from starch is a two-advance process including α-amylases, glucoamylases, or α-glucosidases. Additionally, pullulanases are vital for debranching of amylopectin atoms containing α-1,4-and α-1,6-glucosidic linkages [42] During liquefaction the concentrated slurry of starch granules is gelatinized at hoisted temperatures (90– 110◦C). Expansion of thermostable endoamylase at this phase of the procedure secures against a fast increment of the starch arrangement consistency[43]. created mutant variations of Bacillus sp. 406 delivering thermostable α-amylase by site-coordinated mutagenesis. Another essential use of thermophilic chemicals in sustenance industry is the planning of low lactose drain and dairy items can be expert utilizing a few β-galactosidases or β-glucosidases, which regularly indicate β-galactosidase action, and are broadly disseminated in microorganisms. The use of these compounds permits to maintain a strategic distance from lactose prejudice,

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thermostable β-galactosidase has been sanitized and portrayed from Basidiomycete fomitopsis and Aspergillus fumigatus Z5 to be conceivably utilized as a part of nourishment industry [44,45].

X. FUTURE PROSPECTIVE OF THERMOPHILIC ENZYMES

The relevance of thermophilic catalysts in paper and mash industry, material industry in the creation of biofuels and generation of nourishment items is increasing wide modern and biotechnological intrigue. Their powerful thermostabilities improve them suited for the brutal preparing conditions in businesses to fermentable items. The thermostabilities of these chemicals have been credited to numerous variables, Researchers are correspondingly creating techniques to upgrade the warm security and action of thermophilic catalyst utilizing both arbitrary and coordinated methodologies. Analyst are endeavoring to create thermophilic compounds by mesophilic host to make simple generation of these proteins by hereditary designing systems. Researcher can expand thermostability of mesophilic compound by protein building and bioinformatical instruments. The bioengineering of fermentative creatures, for example, yeast to deliver "one-stop biorefineries" with thermotolerant cellulolytic and hemicellulolytic abilities.

REFERENCES

[1] Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production, a review. Biores Technol 83:1–11.

[2] Kobata A, (2001) The history of glycobiology in Japan. Glycobiology 11:99–105.

[3] Wolfenden R, Lu X, Young G (1998). Spontaneous hydrolysis of glycosides. J Am Chem Soc 120:6814–6815

[4] Chen C, Adolphson R, Dean F, Eriksson K, Adamas M, Westpheling J (1997) Release of lignin from kraft pulp by a hyperthermophilic xylanase from *Thermotoga maritema*. Enzyme Microb Technol 20:39–45.

[5] Beg QK, Kapoor M, Mahajan L, Hoondal G (2001) Microbial xylanases and their industrial applications: review. Appl Microbiol Biotechnol 56:326–338.

[6] Jacques G, Frederic DL, Joste LB, Viviane B, Bart D, Fabrizio G, Benoit G, Jean-marie F (2000) An additional aromaticinterac tion improves the thermostability and thermophilicity of a mesophilic family 11 xylanase: structural basis and molecular study. Protein Sci 9:466–475.

[7] Biely P (1985) Microbial xylanolytic systems.Trends Biotechnol 3:286–290.

[8] Puls J, Schmidt O. Granzow C (1987) Glucuronidase in two microbial xylanolytic systems. Enzyme Microb Technol 9:83–88.

[9] Kohilu U, Nigam P, Singh D, Chaudhary K (2001) Thermostable, alkaliphilic and cellulase free xylanases production by Thermoactinomycesthalophilus subgroup C. Enzyme Microb Technol 28:606–610.

[10] Swaroopa D, Krishna N (2000) Production of thermostable cellulase- free xylanases by *Clostridium absonum* CFR-702. Proc Biochem 36:355–362.

[11] Khandke KM, Vithayathil PJ, Murthy SK (1989) Purification of xylanase, beta-glucosidase, endocellulase, and exocellulase from a thermophilic fungus, *Thermoascus aurantiacus*. Arch Biochem Biophys 274(2):491-500.

[12] Anand L, Krishnamurthy S, Vithayathil PJ (1990) Purification and properties of xylanase from the thermophilic fungus, *Humicola lanuginosa* (Griffon and Maublanc) Bunce. Arch Biochem Biophys 276(2):546-53.

[13] Purkarthofer H, Sinner M, Steiner W (1993) Effect of shear rate and culture pH on the production of xylanase by *Thermomyces lanuginosus*. Biotechnol Lett 15:405–410.

[14] Poonam N, Dalel S (1995) Enzyme and microbial systems involved in starch processing. Enzyme Microb Technol 17:770–778.

[15] Van der Maarel M, Van der Veen B, Uitdehaag H, Leemhuis H, Dijkhuizen L (2002) Properties and applications of starchconverting enzymes of the a-amylase family. J Biotechnol 94:137–155.

[16] Rakshit SK (1998) Utilization of starch industry wastes. In: Martin, A. (Ed.), Bioconversion of Waste Materials to Industrial Products, second ed. Blackie Academic and Professional, London.

[17] Underkofler L (1976) Microbial enzymes. In: Miller, B., Litsky, W. (Eds.), Industrial Microbiology. McGraw-Hill, New York.

[18] Rao M, Tankasale A, Ghatge M, Desphande V, (1998) Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev 62:597–634.

[19] Fan Z, Zhu Q, Dai J (2001). Enzymatic treatment of wool. J Dong Hua University 18:112–115

[20] Mozersky S, Marmer W, Dale AO (2002) Vigorous proteolysis: Relining in the presence of an alkaline protease and bating (Post-Liming) with an extremophile protease. JALCA 97:150–155.

[21] Mullis K, Faloona F, Saiki R, Horn G, Erlich H (1986) Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp Quant Biol 51:263–273.

[22] Erlich H, Gelfand D, Saiki R (1988). Specific DNA amplification product review. Nature 33:461–462.

[23] Saiki R, Gelfand D, Stoffe S, Scharf S, Higuchi R, Horn G, Mullis K, Erlich H (1988) Primer directed

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enzymatic amplification of DNA with thermostable DNA polymerase. Science 239:487–491

[24] Chien A, Edgar D, Trela J (1976) Deoxyribonucleic acid polymerase from the extreme thermophilile *Thermus aquaticus*. J Bacteriol 127:1550–1557.

[25] Kaledin A, Sliusarenko A, Gorodetski S (1980). Isolation and properties of DNA polymerase from extreme thermophilicbac teria *Thermus aquqticus* YT-1. Biochimiya 45:644–651.

[26] Zaks A (2001) Industrial biocatalysis. Current Opinion in Chemical Biology. 5:130–136

[27] Van Beilen JB, Li Z, (2002) Enzyme technology: an overview. Current Opinion in Biotechnology. 3:338–344.

[28] Demirijan D, MorisVaras F, Cassidy C (2001). Enzymes from extremophiles. Curr Opin Chem Boil 5:144–151.

[29] Gomes J, Steiner W (2004). The biocatalytic potential of extremophiles and extremozymes. Food Technol Biotechnol 42:223–235.

[30] Johnvesly B, Naik GR (2001) Studies on production of thermostable alkaline protease from thermophilic and alkalophilic *Bacillus sp JB-99* in a chemically defined medium, Proc Biochem 37:139-144.

[31] Rajasekhar A, Ravi V, Reddy MN, Sambasiva Rao KRS (2011) Thermostable Bacterial Protease - A New Way for Quality Silk Production. International Journal of Bio-Science and Bio-Technology 3(4):43-58.

[32] Adinarayana K, Ellaiah P, Prasad DS (2003) Purification and partial characterization of thermostable serine alkaline protease from a newly isolated *Bacillus subtilis* PE-11. AAPS Pharm Sci Tech 4(4):440-468.

[33] Battan B, Dhiman SS, Sharma J (2012) Application of Thermostable Xylanase of *Bacillus pumilus* in Textile Processing. indian J Microbiol 52(2):222-229.

[34] Verma D, Kawarabayasi Y, Miyazaki K, Satyanarayana T (2013) Cloning, expression and characteristics of a novel alkalistable and thermostable xylanase encoding gene (Mxyl) retrieved from compost-soil metagenome. PLoS One 8(1):e52459. doi: 10.1371/journal.pone.0052459.

[35] Shrivastava S, Shukla P, Mukhopadhyay K (2011) Purification and preliminary characterization of a xylanase from*Thermomyces lanuginosus* strain SS-8. 3 Biotech 1(4):255-259.

[36] Hill J, Nelson E, Tilman D, Polasky S, Tiffany D (2006). Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuels. Proc Natl Acad Sci USA 103:11206–11210.

[37] Blumer-Schuette SE, Kataeva I, Westpheling J, Adams, MW, Kelly RM (2008). Extremely thermophilic microorganisms for biomass conversion, status and prospects. Curr Opin Biotechnol 19:210–217.

[38] Haki GD, Rakshit SK (2003). Developments in industrially important thermostable enzymes, a review. Biores Technol 89:17–34.

[39] Coolbear T, Daniel RM, Morgan HW (1992) The enzymes from extreme thermophiles: bacterial sources, thermostabilities and industrial relevance. Adv Biochem Eng Biotechnol 45:57–98.

[40] Covan D, Daniel R, Morgan H (1985) Thermophilic proteases: properties and potential applications. Trends Biotechnol 3:68–72.

[41] Crabb WD, Shetty JK (1999) Commodity scale production of sugars from starches. Curr. Opin. Microbiol 2:252–256.

[42] Guzman-Maldonado H, Paredes-Lopez O (1995). Amylolytic enzymes and products derived from starch: a review. Crit Rev Food Sci Nutrit 35:373–403.

[43] Alexandr VK, Anatoliy NE (2013) Thermostable mutant variants of Bacillus *sp.* 406 α-amylase generated by site-directed mutagenesis. Central European Journal of Biology 8(4):346-356.

[44] Yoon JJ, Kim KY, Cha CJ (2008) Purification and characterization of thermostable beta-glucosidase from the brown-rot basidiomycete Fomitopsis palustris grown on microcrystalline cellulose. J Microbiol 46(1):51-55.

[45] Liu D, Zhang R, Yang X, Zhang Z, Song S, Miao Y, Shen Q (2012) Characterization of a thermostable β-glucosidase from *Aspergillus fumigatus Z5*, and its functional expression in *Pichia pastoris* X33. Microb Cell Fact. 17:11-25