

Applications and uses of enzymes

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Abstract

Enzymes are biological components produced from amino acids by digesting food proteins. They play major role in control of major chemical reactions occurring in our body. Many enzymes are produced by microorganisms. Researchers have isolated specific microorganisms from extreme sources under extreme culture conditions, with the objective that such isolated microbes would possess the capability to synthesize special enzymes. The microbial enzymes act as biocatalysts to perform reactions in bio-processes in an economical and environmentally-friendly way as opposed to the use of chemical catalysts. Such enzymes have proven their utility in industries such as food, leather, textiles, animal feed, and in bio-conversions and bioremediations. Microbial enzymes play an important role in the diagnosis, cure and monitoring of different diseases. In this review, we have discussed the main properties of enzymes, types of enzymes including proteases, amylases and many others along with their screening methods and their known biotechnological applications.

Keywords: Biocatalyst, culture, microbial enzymes, proteases, microorganisms

Introduction

Enzymes are the biological substance that can act as catalyst to initiate biochemical reactions inside and outside of the cell. They are proteinaceous in nature and make up the largest and most highly specialized class of protein molecules. Use of enzyme has been seen in ancient Egyptians where they were used for the preservation of food and beverages. Enzymes are considered as a potential biocatalyst for a large number of reactions. The manufacture or processing of enzymes for use as drugs is an important facet of today's pharmaceutical industry [1]. Particularly, the microbial enzymes have widespread uses in industries and medicine. The microbial enzymes are also more active and stable than plant and animal enzymes. In addition, the microorganisms represent an alternative source of enzymes because they can be cultured in large quantities in a short time by fermentation and owing to their biochemical diversity and susceptibility to gene manipulation. Industries are looking for new microbial strains in order to produce different enzymes to fulfill the current enzyme requirements. Several pancreatic enzymes can be used to treat cancer in 1902 [2]. He proposed that pancreatic proteolytic enzymes represent the major body's defense against cancer. These enzymes can be further used as anticancer agents. These complex proteins, produced from living cells, are the primary instruments for the expression of gene action since they catalyze thousands of biochemical reactions. These large globular proteins act as catalysts for biochemical reactions, providing the lower-energy pathway between reactants and products.

Naturally found enzymes have been used widely since ancient times and in the manufacture of products such as linen, leather, and indigo. All of these processes dependent on either enzymes produced by microorganisms or enzymes present in added preparations such as calves' rumen or papaya fruit. The recombinant DNA technology has further improved

production processes and helped to produce enzymes commercially that could not be produced previously. Furthermore, the developments in biotechnology, such as protein engineering and directed evolution, further revolutionized the commercialization of industrial important enzymes. This advance in biotechnology is providing different kinds of enzymes displaying new activities, adaptability to new conditions leading to their increase use in industrial purposes. Various carbohydrases, primarily amylases and cellulases, used in industries such as the starch, textile, detergent, and baking industries, represent the second largest group [3]. Enzymes can be used in various fields, including food manufacturing, animal nutrition, cosmetics, medication, and mostly as tools for research and development. At present, almost 4000 enzymes are known and of these, approximately 200 microbial original types are used commercially. However, only about 20 enzymes are produced on truly industrial scale. According to some studies, enzymes are specific biological catalysts, they can make the most desirable therapeutic agents for the treatment of metabolic diseases. Enzymes are too large to be distributed easily within the body cells.

Enzymes with Special Characteristics

Special characteristics of microbial enzymes include their capability and appreciable activity under abnormal conditions, mainly of temperature and pH. Hence, certain microbial enzymes are categorized as thermophilic, acidophilic or alkalophilic. Microorganisms with systems of thermostable enzymes that can function at higher than normal reaction temperatures would decrease the possibility of microbial contamination in large scale industrial reactions of prolonged durations [4, 6]. The quality of thermostability in enzymes promotes the breakdown and digestion of raw materials; also the higher reaction temperature enhances the penetration of enzymes [7]. Thermophilic xylanase are considered to be of

commercial interest in many industries particularly in the mashing process of brewing. The thermostable plant xerophytic isoforms of laccase enzyme are considered to be useful for their applications in textile, dyeing, pulping and bioremediation [8, 9].

Types of Enzymes

A number of different enzymes exist with various functions. Microbial proteases are hydrolytic enzymes that have been extensively studied. [10, 15]. Proteases prepared from microbial systems are of three types: acidic, neutral and alkaline. Alkaline proteases are efficient under alkaline pH conditions and consist of a serine residue at their active site. Alkaline proteases have shown their capability to work under high pH, temperature and in presence of inhibitory compounds [16, 17]. Insoluble and fibrous proteins consisting of feathers and wool are considered as keratinases. The protein is abundantly available as a by-product from keratinous wastes, representing a valuable source of proteins and amino acids that could be useful for animal feeds or as a source of nitrogen for plants [18]. Many significant enzymes like amylases can be used in industry for starch conversion [19]. Amylolytic enzymes act on starch and related oligo- and polysaccharides [20]. The baking industry uses amylases to delay the staling of bread and other baked products; the paper industry uses amylases for the reduction of starch viscosity to achieve the appropriate coating of paper. Amylase enzyme is used in the textile industry for warp sizing of textile fibers, and used as a digestive aid in the pharmaceutical industry [21]. Another class of enzymes named as xylanases have established their uses in the food, pulp, paper and textile industries, agri-industrial residues utilization, and ethanol and animal feed production [22, 23].

Sources of therapeutic Enzymes

Therapeutic enzymes are widely distributed in plant and animal tissues and microorganisms including bacteria, yeasts and fungi. Although, microorganisms are potential sources of therapeutic enzymes.

Trypsin and Pepsin

There are two types of protease enzymes: trypsin and pepsin. The principal advantage of enzymes is their specificity, which enables only one kind of material, such as a starch or protein, to be rapidly acted upon. Each enzyme has specific bond-cleaving mechanisms and activity units. The enzyme's speed of action and its potential for use depends on a number of factors that may not be well defined for specific use in paper conservation.

Enzymes in paper and pulp industry

The paper and pulp industry requires a step of separation and degradation of lignin from plant material, where the pretreatment of wood pulp using ligninolytic enzymes is important for a milder and cleaner strategy of lignin removal compared to chemical bleaching. Bleach enhancement of mixed wood pulp has been achieved using co-culture strategies, through the combined activity of xylanase and laccase. Fungi are the most potent producers of lignin degrading enzymes.

Screening of Enzymes

With recent advances, enzyme screening has led to the commercialization of a number of cold-adapted enzymes, like

an alkaline phosphatase from New England Biolabs and lipase 435 from Novozymes. Patents have also be filed for cold-adapted enzymes that include a β -galactosidase that efficiently hydrolyses lactose in milk at low temperature and a xylanase for use in the baking industry [24, 25]. Many other potentially valuable proteases, polysaccharide degrading enzymes, lipases and β -galactosidases have been discovered by screening psychrophilic microorganisms directly on diagnostic media or by PCR amplifying and cloning genes expressed heterologously in *E. coli* [26, 27]. The availability of complete genome sequences for a limited number of cultured psychrophiles also provides a rational means of *in silico* bioprospecting. While screening enzymes from axenic cultures is unquestionably valuable, this approach is limited as a result of the small fraction of culturable microorganisms [28]. As a result, the use of recombinant DNA methods to characterize enzymes from microorganisms offers potential benefits.

Uses

Enzymes are being used in many areas for production of useful products. In the textile industry lipases are used for the removal of size lubricants, which increases fabrics absorbance ability for improved levelness in dyeing. The hydrolytic lipases are commercially very important, and their addition to detergents is mainly used in laundries and household dishwashers [29]. On the other side, to modify the food flavour by synthesis of esters of short-chain fatty acids and alcohols lipases have been frequently used. Lipases play a major role in the fermentative steps during manufacturing of sausage and also to measure changes in long-chain fatty acid liberated during ripening. Previously, lipases of different microbial sources were used for refining rice flavour, modifying soybean milk, and for enhancing the aroma and speed up the fermentation of apple wine [30].

Enzymes in Medicine

Lipases are considered as important drug targets in the medical field. The presence or high levels of lipases can indicate certain infection or disease and can be used as diagnostic tool. They are used in the determination of serum triglycerides to liberate glycerol which is determined by enzyme-linked colorimetric reactions. Acute pancreatitis and pancreatic injury can be determined by the level of lipases in blood [72]. Few new developments have been made by using lipases for the diagnosis of pancreatitis.

Discussion

Various enzymes have a significant value in the list of microbial enzymes, which have established their applications in bio-industries. Lipases have been widely studied for their properties and utilization in many industries [31, 36]. Certain enzymes are specifically required in pharmaceutical industry for diagnostic kits and analytical assays [37, 40]. These innovations have played an important role in the establishment of current commercially successful level of bio-industries. As a result recent bioprocess-technology is capable of meeting future challenges and the requirements of conventional and modern industries, for example Trincone has reviewed the options for unique enzymatic preparation of glycosides [41].

Conclusion

Enzyme industry is one of the major industries of the world with great market. Enzymes are in great demand for use as

therapeutic agents against many harmful diseases. Microbial enzymes offer potential to treat many important diseases. With new discoveries and advances in isolation of proteins occurring at supersonic rates, we are endowed with a vast selection of highly purified enzymes that possess activities of up to twenty thousand times that of just a decade ago. Microbial enzymes have been studied for their special characteristics applicable in various bio-processes.

References

- Cassileth B. The Alternative Medicine Handbook. WW Norton & Co, New York, USA. 1998
- Gonzalez NJ, Isaacs LL. Evaluation of pancreatic proteolytic enzyme treatment of adenocarcinoma of the pancreas with nutrition and detoxification support. *Nutr cancer* 1999; 33:117-124.
- Underkofler LA, Barton RR, Rennert SS. Production of microbial enzymes and their applications, *Applied Microbiology*, 1957; 6(3):212-221,
- Wang X, Li D, Watanabe T, Shigemori Y, Mikawa T, Okajima T, Mao LQ, *et al* glucose/o-2 biofuel cell using recombinant thermophilic enzymes. *Int. J Electrochem. Sci.* 2012; 7:1071-1078.
- Banat IM, Nigam P, Marchant R. Isolation of a thermotolerant, fermentative yeasts growing at 52 °C and producing ethanol at 45 °C & 50 °C. *World J. Microbiol. Biotechnol.* doi: 10.1007/BF01201874. 1992; 8:259-263.
- Wati L, Dhamija SS, Singh D, Nigam P, Marchant R. Characterisation of genetic control of thermotolerance in mutants of *Saccharomyces cerevisiae*. *Genet. Eng. Biotechnol* 1996; 16:19-26.
- Zhang SB, Wu ZL. Identification of amino acid residues responsible for increased thermostability of feruloyl esterase A from *Aspergillus niger* using the PoP MuSiC algorithm. *Bioresour. Technol.* doi:10.1016/j.biortech.2010.08.019. 2011; 102:2093-2096.
- Pandey A, Selvakumar P, Soccol CR, Nigam P. Solid-state fermentation for the production of industrial enzymes. *Curr. Sci* 1999; 77:149-162.
- Ahmed S, Riaz S, Jamil A. Molecular cloning of fungal xylanases: An overview. *Appl. Microbiol. Biotechnol.* doi: 10.1007/s00253-009-2079-4. 2009; 84:19-35.
- Mukherjee AK, Adhikari H, Rai SK. Production of alkaline protease by a thermophilic *Bacillus subtilis* under solid-state fermentation (SSF) condition using *Imperata* cylindrical grass and potato peel as low-cost medium: Characterization and application of enzyme in detergent formulation. *J Biochem. Eng.* doi: 10.1016/j.bej.2007.09.017. 2008; 39:353-361.
- Rahman RNZRA, Basri M, Salleh AB. Thermostable alkaline protease from *Bacillus stearothermophilus* F1; Nutritional factors affecting protease production. *Ann. Microbiol.* 2003; 53:199-210.
13. Chudasama CJ, Jani SA, Jajda HM, Pate HN. Optimization and production of alkaline protease from *Bacillus thuringiensis* CC7. *J. Cell Tissue Res.* 2010; 10:2257-2262.
- Genckal H, Tari C. Alkaline protease production from alkalophilic *Bacillus* sp. isolated from natural habitats. *Enzym. Microb. Technol.* doi: 10.1016/j.enzmictec.2005.12.004. 2006; 39:703-710.
- Gupta R, Beg QK, Lorenz P. Bacterial alkaline proteases: Molecular approaches and Industrial Applications. *Appl. Microbiol. Biotechnol.* doi: 10.1007/s00253-002-0975-y. 2002; 59:15-32.
- Vijayalakshmi S, Venkat Kumar S, Thankamani V. Optimization and cultural characterization of *Bacillus* RV.B2.90 producing alkalophilic thermophilic protease. *Res. J Biotechnol.* 2011; 6:26-32.
- Gupta A, Joseph B, Mani A, Thomas G. Biosynthesis and properties of an extracellular thermostable serine alkaline protease from *Virgibacillus pantothenicus*. *World J Microbiol. Biotechnol.* doi: 10.1007/s11274-007-9462-z. 2008; 24:237-243.
- Johnvesly B, Naik GK. Studies on production of thermostable alkaline protease from thermophilic and alkaliphilic *Bacillus* sp. JB-99 in a chemical defined medium. *Process Biochem.* doi: 10.1016/S0032-9592(01)00191-1. 2001; 37:139-144.
- Gushterova A, Vasileva-Tonkova E, Dimova E, Nedkov P, Haertle T. Keratinase production by newly isolated Antarctic actinomycete strains. *World J Microbiol. Biotechnol.* doi: 10.1007/s11274-004-2241-1. 2005; 21:831-834.
- Nigam P, Singh D. Enzyme and microbial systems involved in starch processing. *Enzym. Microb. Technol.* doi: 10.1016/0141-0229(94)00003-A. 1995; 17:770-778.
- Pandey A, Soccol CR, Nigam P. Biotechnological potential of agro-industrial residues, II-Cassava Bagasse. *Bioresour. Technol.* doi: 10.1016/S0960-8524(99)00143-1. 2000; 74:81-87.
- Sivaramakrishnan S, Gangadharan D, Nampoothiri KM, Soccol CR, Pandey A. α -amylases from microbial sources – An overview on recent developments. *Food Technol. Biotechnol.* 2006; 44:173-184.
- Srinivasan MC, Rele MV. Cellulase free xylanase from microorganisms and their applications to pulp and paper biotechnology: An overview. *Indian J. Microbiol.* 1995; 35:93-101.
- Garg AP, Roberts JC, McCarthy A. Bleach boosting effect of cellulase free xylanase of *Streptomyces thermoviolaceus* and its comparison with two commercial enzyme preparations on birchwood Kraft pulp. *Enzym. Microb. Biotechnol.* doi: 10.1016/S0141-0229(97)00250-0. 1998; 22:594-598.
- Collins T, Hoyoux A, Dutron A, Georis J, Genot B, Dauvrin T. Use of glycoside hydrolase family 8 xylanases in baking. *J Cereal Sci.* 2006; 43:79-84.
- Hoyoux A, Jennes I, Dubois P, Genicot S, Dubail F, Francois J. Cold-adapted beta-galactosidase from the Antarctic psychrophile *Pseudoalteromonas haloplanktis*. *Appl Environ Microbiol.* 2001; 67:1529-1535.
- Wang F, Hao J, Yang C, Sun M. Cloning, expression, and identification of a novel extracellular cold-adapted alkaline protease gene of the marine bacterium strain YS-80-122. *Appl Biochem Biotechnol.* 2010a; 162:1497-1505.
- Ma C, Lu X, Shi C, Li J, Gu Y, Ma Y. Molecular cloning and characterization of a novel β -Agarase, AgaB, from marine *Pseudoalteromonas* sp. CY24. *J Biol Chem.* 2007; 282:3747-3754.

28. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and *in-situ* detection of individual microbial-cells without cultivation. *Microbiol Rev.* 1995; 59:143-169.
29. Applications of Lipases. AU-KBC Research Center, Life Sciences, Anna University, Chennai, India, <http://www.au-kbc.org/beta/bioproj2/introduction.html>.
30. EW Seitz, Industrial application of microbial lipases: a review, *Journal of the American Oil Chemists' Society.* 1974; 51(2):12-16.
31. Lott JA, Lu CJ. Lipase isoforms and amylase isoenzymes: assays and application in the diagnosis of acute pancreatitis, *Clinical Chemistry*, 1991; 37(3):361-368.
32. Reddivari M, Chirumamilla R, Nigam P. Understanding lipase stereoselectivity. *World J Microbiol. Biotechnol.* doi: 10.1023/A:1014417223956. 2002; 18:81-97.
33. Muralidhar R, Chirumamilla RR, Nigam P. Resolution of proglumide using lipase from *Candida cylindraceae*. *Bioorg. Med. Chem.* doi: 10.1016/S0968-0896(01)00409-6. 2002; 10:1471-1475.
34. Muralidhar R, Chirumamilla RR, Marchant R, Nigam P. A response surface approach for the comparison of lipase production by *Candida cylindraceae* using two different carbon sources. *Biochem. Eng. J* doi: 10.1016/S1369-703X(01)00117-6. 2001; 9:17-23.
35. Pandey A, Benzamin S, Soccol CR, Nigam P, Krieger N, Soccol VT. The realm of microbial lipases in biotechnology. *Biotechnol. Appl. Biochem.* 1999; 29:119-131.
36. Muralidhar R, Chirumamilla C, Marchant R, Nigam P. Lipases in racemic resolutions. *J Chem. Technol. Biotechnol.* doi: 10.1002/1097-4660(200101)76:1<3::AID-JCTB336>3.0.CO; 2-8. 2001; 76:3-8.
37. Zhou DM, Nigam P, Marchant R, Jones J. Production of salicylate hydroxylase from *Pseudomonas putida* UUC-1 and its application in the construction of biosensor. *J. Chem. Technol. Biotechnol.* doi: 10.1002/jctb.280640404. 1995; 64:331-338.
38. Banat IM, Marchant A, Nigam P, Gaston SJS, Kelly B, Marchant R. Production, partial characterization and potential diagnostic use of salicylate hydroxylase from *Pseudomonas putida* UUC-1. *Enzym. Microb. Technol.* doi: 10.1016/0141-0229(94)90087-6. 1994; 16:665-670.
39. Nigam P, Marchant R. Production of enzyme dihydrofolate reductase by methotrexate-resistant bacteria isolated from soil. *J Chem. Technol. Biotechnol.* 1993; 56:35-40. doi: 10.1002/jctb.280560107.
40. Nigam P, Banat IM, Kelly B, Marchant R. Dihydrofolate reductase synthesis in continuous culture using methotrexate-resistant *Escherichia coli*. *Enzym. Microb. Technol.* doi: 10.1016/0141-0229(93)90064-9. 1993; 15:652-656.
41. Trincone A. Angling for uniqueness in enzymatic preparation of glycosides. *Biomolecules.* doi: 10.3390/biom3020334. 2013; 3:334-350.