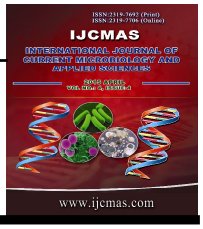
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 17-26**
http://www.ijcmas.com



Review Article

Overview of Microbial Therapeutic Enzymes

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A B S T R A C T

Enzymes possess specificity, greater affinity, and high catalytic efficiency. They are required for many chemical inter conversions that support life and speed up all the metabolic processes. These entire characteristics discern them from all other types of drugs. Due to these, enzymes are widely been used for different therapeutic purposes and enzyme therapies are acquiring much attention. Both digestive and metabolic enzymes can be used either separately or in combination with other therapies for the treatment of several diseases such as leukemia, skin ulcers, Pompe's disease, cardiovascular diseases, celiac disease, Parkinson's disease, Fabry's disease, inflammation, digestive disorders, pancreatic disorders etc. They are also employed in diagnosis, biochemical investigation and monitoring of many alarming diseases. Medically important enzymes produced by microorganisms have advantage of being economically feasible and consistent. They have high yield and are easy for product modification and optimization. The present review compiles the information on the sources and application of medically important enzymes produced by microorganisms and future prospects of these enzymes as drugs.

Keywords

Enzymes, Therapeutic, Digestive, Metabolic, Drugs

Introduction

The enzyme technology is applied to pharmaceutical research, development and manufacturing and is a growing field. Therapeutic enzymes have been in use for around at least 40 years. For example, a therapeutic enzyme was described as a part of replacement therapies for genetic deficiencies in 1960s by de Duve (Vellard, 2013). Attempts are made to capitalize on the advantages of enzymes as drugs at every pharmaceutical research center in the world (Gonzalez and Isaacs, 1999).

Regular consumption of enzymes and enzyme-rich foods contributes to vibrant health, prevention of disease, and anti-ageing process. Each cell in our body needs enzymes for its biochemical functions, and a deficiency of these enzymes will accelerate the aging process. Some of the important functions of enzymes are regulation of the growth of the body from a single cell to a mature organism, conversion of food to energy to fulfill the body's needs, and break down or buildup of certain substances within the cell (Kaur and Sekhon, 2012).

Enzymes were largely ignored as drugs other than digestion aids. In the later years of 19th century, crude proteolytic enzymes were used to treat gastrointestinal disorders only e.g. pepsin for dyspepsia. Later, researchers observed that an extra cellular secretion i.e. nuclease (enzymatically degrades nucleic acid) of *Bacillus pyocyaneus* kills anthrax bacilli and protects mice from otherwise lethal bacterial inoculum. This became a milestone in the use of parental enzyme in the treatment of infections, cancers and finally diverse spectrum of diseases. Supplements of enzymes are available in pills, capsules and powder form and often consist of a combination of different enzymes (Gonzalez and Isaacs, 1999). Enzymes have chiral selectivity property which is employed to prepare enantiomerically pure pharmaceuticals (Underkofler *et al.,* 1957).

Majority of medically important enzymes are obtained from a limited number of fungi, yeast and bacteria. These organisms are also considered when a new enzyme is required (Teal and Wymer, 1991). Medically important enzymes are required in very less quantity as compared to the industrially important enzymes. But they should have a high degree of purity and specificity. The kinetics of these enzymes are low Km and high Vmax, therefore it has maximum efficiency even at low concentrations of enzymes and substrates. The sources of these kinds of enzymes should be selected with great care and precautions to prevent any possibility of undesirable contamination by incompatible material and also to enable ready purification. Medically important enzymes are usually marketed as lyophilized pure preparations with biocompatible buffering salts and mannitol diluent. The cost of these enzymes is high but do not exceed or are comparable to those of therapeutic agents or treatments (Gurung *et al.,* 2013).

Different types of therapeutic enzymes

Medically important enzymes (digestive and metabolic) can be used either alone or in combination with other therapies for treating a variety of diseases safely. These enzymes have two important features, a) they often bind and act on their targets with a high affinity and specificity; b) they have catalytic property and convert multiple target molecules to the desired products. These two features are exploited to make enzymes specific and potent drugs for a numerous disorders (Cooney and Rosenbluth, 1975).

Medically important enzymes produced by microorganisms find their application in removal of cytotoxic substances within the blood circulation, treatment of life threatening disorders as oncolytics, thrombolytics, anti-coagulants and as replacements for metabolic deficiencies (Kaur and Sekhon, 2012). There is very less information about the utilization of microbial enzymes for therapeutic purposes except for some anticancer enzymes and the enzymes active against cystic fibrosis (Sabu, 2003).

There major application is in the treatment of cancer (prodrug activator enzymes and antineoplastic enzymes) and various other diseases as genetic diseases including Gaucher, Fabry, MPS I, Pompe, MPS VI, SCID, CF and PKU & infectious diseases caused by protozoa, fungi or bacteria. They

can also be used to aid digestion where they are used to supplement lipase, protease and amylase in lactose intolerant people who require lactose as their body is unable to produce it.

Asparginase is employed for the treatment of acute lymphocytic leukemia. Tumor cells lack aspartate-ammonia ligase activity, which stops the synthesis of nonessential amino acid L-asparagine. The activity of asparginase is based on this fact. The asparginase does not affect the normal cells which have the capability to synthesize L-asparagine for their own need, but they cause a decline in the free exogenous concentration, which causes a state of fatal starvation in the tumor cells. The enzyme can be administered intravenously and is effective only when the asparagine levels within the bloodstream are extremely low (Gurung *et al.,* 2013).

Chitinase has antimicrobial property. Chitin is the component of cell wall of many pathogenic organisms, including fungi, protozoa, and helminthes and is a good target for antimicrobials (Fusetti *et al.,* 2002). The cell walls of *Streptococcus pneumonia*, *Bacillus anthracis,* and *Clostridium perfringens* are targeted using lytic enzyme derived from bacteriophage (Zimmer *et al.,* 2002). These lytic enzymes derived from bacteriophages can be used for the treatment of several infections and also shows activity against new drug-resistant bacterial strains. Proteolytic enzymes have anti-inflammatory actions. Huge number of these proteolytic enzymes of bacterial origin can also be employed in the removal of dead skin of burns (Gurung *et al.,* 2013).

Collagenase helps in the healing of burns and skin ulcers. It helps to break up and remove dead skin and tissue and thus help in repair mechanism. This in turn helps antibiotics to work better and speed up an individual's body's natural healing process (Ostlie *et al.,* 2012).

Lipase is used as digestive aids. It is also used in the treatment of malignant tumors as they have the ability to activate tumor necrosis factor. Lipases were used in the treatment of dyspepsia, gastrointestinal disturbances, cutaneous manifestations of digestive allergies, and many more such infections in the past. Lipase from *Candida rugosa* synthesizes lovastatin, a drug that has the ability to lower serum level of cholesterol. The hydrolysis of 3-phenylglycidic acid ester, which is asymmetric, is a key intermediate in the synthesis of diltiazem hydrochloride. It is a widely used coronary vasodilator and is synthesized using *S. marcescens* lipase (Matsumae *et al.,* 1993).

Nattokinase is a serine proteinase obtained from *Bacillus subtilis*. It can reduce some factors of blood clotting and lipids that are associated with an increased risk for cardiovascular disease (CVD). Oral administration of nattokinase could be considered as a CVD neutraceutical. It decreases the plasma levels of fibrinogen, factor VII, and factor VIII (Hsia *et al.,* 2009). Nattokinase shows prolonged action of preventing coagulation of blood and dissolving existing thrombus (Milner, 2008).

Serratiopeptidase is useful in the treatment of pain and inflammation. It has three mechanisms to reduce inflammation. It breaks down fibrin, the insoluble protein byproducts of blood coagulation and thins the fluids formed from inflammation and injury. It also facilitates their drainage which increases the speed of the tissue repair process. It also alleviates pain as it inhibits the release of bradykinin, a specific pain inducing peptide (Rothschild, 1991; Esch and Fabian, 1989).

Thrombolytic drugs (Fibrinolytics) are used to lyse thrombi/clot to regain the normal flow of occluded blood vessels. Instead of being prophylactic, they are curative. Their action involves the activation of the natural fibrinolytic system and thus the activation of plasminogen. Some plasminogen activators are Streptokinase, Urokinase, etc. Streptokinase is inactive but when it combines with the circulating plasminogen, it forms an activator complex which then results in limited proteolysis of other plasminogen molecules to plasmin. It is antigenic, has ability to cause hypersensitivity reactions and anaphylaxis. Urokinase directly activates plasminogen and it is non-antigenic (Banerjee *et al.*, 2004; Olson *et al.*, 2011).

L-amino acid α-ligase obtained from *Empedobacter brevis* catalyzes the ligation of two amino acids L-alanine and L-glutamine. This dipeptide i.e. Ala-Gln is easily digested in human body and hence is used in nutritional therapy. In multiple-trauma patients, parenteral supplementation of alanyl-glutamine dipeptide was associated with better insulin sensitivity. It also prevents muscle wasting and increases synthesis of protein in muscle (Bohumil and Frantisek, 2006). In *Bacillus subtilis*, a novel enzyme coded by a gene YwfE was identified, which catalyzed this dipeptide formation from unprotected amino acids in an ATP-dependent manner. This novel enzyme was classified into the category of L-amino acid α-ligase (LAL) (Kazuhiko and Shin-ichi, 2005).

Industrial production of therapeutic microbial enzymes

Industrial expansion in the 19th up till mid-20th century, well known as industrial revolution, resulted in a great increase in population and its demand for survival. This created a great impact in the medicinal, industrial, dairy, and agricultural sectors. Many chemical processes were developed by a number of scientists to meet this raising demand. But in the later years many harmful effects were observed due to the use of these chemical catalysts to fast up the processes. The drawbacks of chemical transformation processes were both commercial and environmental. Poor product yield may be obtained due to nonspecific reactions. High temperatures and/or high pressures required for reactions and large volumes of cooling water downstream, increases the cost of the process. High capital investment and specially designed equipment and control systems are needed for harsh and hazardous processes involving high pressures, temperatures, alkalinity or acidity. The reaction may result in unwanted and harmful by-products which can induce negative impact on the environment, as well as the chemicals and energy consumption.

Hence there is a need of environment friendly process/biocatalyst, for which a biotechnology came into picture, where different live organisms were utilized to obtain desirable products in an ecofriendly way. Industrial enzymes are obtained from biological systems hence effectively contribute to sustainable development as they are isolated from microorganisms which are fermented using primarily renewable resources. Both solid and liquid enzyme preparations take up very little storage space as only small amounts of enzymes are needed. Uncomplicated and widely available equipment can be used as the operating conditions are mild. It also reduces the impact of manufacturing on the environment by reducing the utilization of energy, chemicals, and water, and the subsequent waste production (Gram *et al.*, 2001; Raven, 2002; Clark and Dickson, 2003). In pharmaceutical industry, manufacture or processing of enzymes for

use as drugs is an important aspect. The advantages of enzymes as drugs, is now been capitalized at virtually every pharmaceutical research center in the world (Cassileth, 1998).

Medically important enzymes are broadly distributed in animal tissues, plants, and microorganisms including bacteria, yeast, and fungi. The use of microbial enzymes in medical field is limited, although they have a good potential, because they are incompatible with the human body. But the use of microbial enzymes is still increasing because of their economic feasibility. Various methods involving fermentation technology are available for the production of microbial enzymes (Sabu *et al.,* 2000). These include solid statefermentation, submerged fermentation, and immobilization, etc. These methods are utilized for bulk production of therapeutic enzymes on commercial scale than liquid cultures in huge bioreactors (Lozano *et al.,* 2012). Various production techniques and downstream processing of large-scale production of microbial therapeutic enzymes have been reported (Sabu *et al.,* 2005).

Recombinant DNA technology in the production of therapeutic microbial enzymes

The production of large amount of therapeutic enzymes is made feasible due to the development of recombinant DNA technology. This enhances the activity and stability of an enzyme prior to its production (Kaul, 2008). It also reduces the cost and facilitates easy production. The modification in protein activity by rDNA can be overcome by site directed mutagenesis and shuffling functional domains. This modifies activity and regulation of enzyme and avoids unwanted side effects (Sabu, 2003). In rDNA technology, the gene coding for the enzyme with required characteristics is transferred into a selected microbial production strain. This strain has all the desired features such as safety, high expression levels and for which the growth medium is optimum. Hence the individual enzyme producing strains need not be optimized (Kaul, 2008). The principle underlying rDNA technology is cloning of cDNA protein, insertion of cloned cDNA into expression vector, transformation of *E. coli* it's over expression and finally purification (Sabu, 2003). Genetic engineering techniques allows enzyme manufacturer to produce sufficient amounts of enzyme from any microbial source. Protein engineering facilitates enzyme manufacturer to adjust the properties of enzymes prior to production (Kaul, 2008).

Future Prospects

The use of microbial enzymes in various fields such as industrial and pharmaceutical have increased greatly during the past few years. More biopharmaceuticals are entering the drug discovery and development pipelines in the recent times (John, 2009). Enzymes are already been used in clinical test reagents and further development in this field can be expected. Development in the field of clinical application of enzymes is also seen. Proteolytic enzymes are used for debridement of wounds. Injection of certain enzymes such as streptokinase also promises positive clinical results. The use of small molecular pharmaceuticals may increase, once the diseases are better understood at molecular level. Crystalline and extremely purified enzymes will be necessary for clinical and therapeutic uses. Rapid advances may be expected in the availability of high purity enzymes on an industrial scale. Various industries including enzyme manufacturers are carrying out enzyme research currently to find new and improved methods for using enzymes, to improve

yields of industrial microbial enzymes, &find new enzymes for industrial and medical purposes. This research will help in continuous usage of old and new enzymes (Underkofler *et al.*, 1957).

There is a great and growing market for therapeutic enzymes. Many diseases have increased the demand of enzymes as therapeutic agents. Presently, therapeutic enzymes are available as pills, capsules, powders and food supplements. Studies are been conducted to utilize the varied microbial resources including both marine and terrestrial microorganisms. Medically important enzymes produced by microorganisms are used as anticoagulants, oncolytics, thrombolytics, fibrinolytics, mucolytics, anti-inflammatories, antimicrobial and digestive aids. Diseases which are resurging after acquiring resistance to antibodies can also be treated using microbial enzymes. Combinations of enzymes and drugs also have the ability to induce synergistic effects and can treat various diseases by counteracting their side effects. Hence, it can be concluded that there is an indeed need of research in the near future in these biomolecules which will later on prove beneficial to mankind in their relevance.

Table.1 Microbial therapeutic enzymes and their application

S. No.	Enzyme	Source	Use
1	Acid protease (Kaur and Sekhon, 2012)	*Aspergillis niger* & *Aspergillus oryzae*	Stomach disorders
2	Alkaline protease (Vishalakshi *et al.*, 2009)	*Streptomyces gulbargensis*	Bio cleaning agent for washing surgical instruments
3	Amylase (Kaur and Sekhon, 2012)	*Aspergillus* sp.	Easy digestion
4	Arginase (Kaur and Sekhon, 2012)	*Bacillus subtilis* & *E. coli*	Antitumor
5	Asparginase (Jain *et al.*, 2012)	*E. coli*	Leukemia
6	Bacilysinsynthetase (Torsten, 2005)	*Bacillus subtilis*	Antibiotic
7	Bacitracin synthetase (Pfaender *et al.*, 1973; Dirk and Andrea, 1997)	*Bacillus licheniformis*	Antibiotic
8	Collagenase (Dolynchuk *et al.*, 2000)	*Clostridium perfringens*	Skin ulcers
9	Glucose oxidase (Bankar *et al.*, 2009)	*Aspergillus*, *Penicillium*, & *Saccharomyces* sp.	Biosensors, Antimicrobial
10	Glucosidase (Kaur and Sekhon, 2012)	*Aspergillus niger*	Antitumor
11	Glutaminase (Spiers and Wade, 1976)	*E. coli* SFL-1	Leukemia
12	Gramicidin synthetase (carboxyl-activating synthetases) (Edward and Arnold, 1988)	*Bacillus brevis*	Antibiotic
13	L-amino acid α-ligase (Makoto and Shin-ichi, 2008; Kazuhiko and Shin-	*Empedobacterbrevis*& *Bacillus subtilis*	Patient infusion

	ichi, 2005)		
14	Lipase (Kaur and Sekhon, 2012)	*Aspergillus oryzae, Candida lipolytica,* & *Candida rugosa*	Digest lipids, Treatment of disorders of the pancreas
15	Maltase (Kaur and Sekhon, 2012)	*Aspergillis oryzae*	Therapy for Pompe's disease
16	Nattokinase (Hsia *et al.*, 2009)	*Bacillus subtilis*	Cardiovascular disease
17	Nonribosomal peptide synthetase (Deirdre and Claire, 2007)	*Aspergillus fumigatus*	Inhibitor of microtubule assembly hence anti-tumor
18	Penicillin acylase (Erickson and Bennett, 1965)	*Penicillium* sp.	Penicillin production/broad spectrum antibiotic production
19	Peptidase (Kaur and Sekhon, 2012)	*Bacillus polymyxa,* &*Beauveria bassiana*	Celiac disease, Clot formation, Inflammation, and Repair
20	Phenylalanine racemase (Edward and Arnold, 1988)	*Bacillus brevis*	Antibiotic
21	Protease (Izrael-živković *et al.*, 2010)	*Pseudomonas aeruginosa*	Antibacterial
22	Rhodanase (Kaur and Sekhon, 2012)	*Sulfobacillus sibiricus*	Cyanide poisoning
23	Ribonuclease (Lin *et al.*, 2013)	*Sacchromyces* sp.	Antiviral
24	RNase P ribozyme (Kaur and Sekhon, 2012)	*Bacillus subtilis*	Antiviral
25	Sacrosidase (Kaur and Sekhon, 2012)	*Saccharomyces cerevisiae*	Congenital sucrase-isomaltase deficiency
26	Serratiopeptidase (Kaur and Sekhon, 2012)	*Serratiamarcescens*	Anti–inflammatory
27	Staphylokinase (Kaur and Sekhon, 2012)	*Staphylococcus aureus,* &*Streptococci* sp.	Thrombolytic agent
28	Streptokinase (Banerjee *et al.*, 2004]	*Streptococci* sp.	Anticoagulant
29	Superoxide dismutase (Kaur and Sekhon, 2012)	*Mycobacterium* sp. & *Nocardia* sp.	Anti-oxidant, Anti-inflammatory
30	Tyrosinase (Kaur and Sekhon, 2012; Para *et al.*, 1984)	*Streptomyces glausescens,* &*Erwinia herbicola*	Antitumor, Treatment of Parkinson's disease
31	Urease (Banerjee andAggarwal, 2013)	*Lactobacillus* sp., & *Klebsiella aerogenes*	Nitrogen metabolism of ruminants
32	Uricase (Terkeltaub, 2009)	*Aspergillus flavus*	Gout
33	Urokinase (Zaitsev *et al.*, 2010)	*Bacillus subtilis*	Blood clots
34	VibrilaseTM (Ozcan *et al.*, 2002)	*Vibrio proteolyticus*	Treatment of damaged tissue
35	α-Galactosidase (Anisha *et al.*, 2008)	*Aspergillus*sp. &*Streptomyces griseoloalbus*	Fabry's disease, Prevention of xenorejection, Blood group transformation
36	β –Aminopeptidase (Jan and Nicolai, 2009)	*Ochrobactrum anthropi,* &*Sphingosinicellaxeno-peptidilytica*	Anti-oxidant
37	β-Galactosidase (Husain, 2010)	*Aspergillus* sp.	Removal of lactose from milk

38	β-Lactamase (Gupta *et al.*, 2012)	*Citrobacter freundii, Serratia marcescene* & *Klebsiella pneumonia*	Antibiotic resistance

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