


MICROBIAL ENZYMES: THERAPEUTIC APPLICATIONS

Abstract

Enzymes are biomolecules with highly specialized catalytic functions produced by all living organisms and are responsible for biochemical reactions in plants, animals, microorganisms and human beings. Nowadays enzymes are considered as core of biotechnology because they are the main tools for the application of basic biotechnological techniques, **the target of the therapeutic drugs and are indispensable intermediates in all biotechnological processes.** The concept of the therapeutic enzymes has been around for at least 40 years. Microbial enzymes are preferred over other sources and in this review different types of microbial enzymes are discussed for their therapeutic applications. 

MICROBIAL ENZYMES: THERAPEUTIC APPLICATIONS

Enzymes are biomolecules with highly specialized catalytic functions produced by all living organisms and are responsible for biochemical reactions in plants, animals, microorganisms and human beings. The use of enzyme in processing raw materials from plants and animals have been practiced for a long time. The first observation of the enzymatic degradation was in 1783 by Spallanzani. In 1814 Kirchhoff found that the barley contain a substance that convert starch into sugars. The term enzyme was coined by Kuhne in 1878. Enzyme preparations were used in ancient times without much knowledge about the nature and properties of enzymes. Today industrial application of enzymes began with Jokichi Takamine, who developed an enzyme preparation takadiastase a mixture of carbohydrases and proteases(Uhlig, 1998).

Enzymes have been used as catalysts in various industries like brewing, tanning, bakery, dairy etc along centuries. Nowadays the enzymes are

considered as core of biotechnology because they are the main tools for the application of basic biotechnological techniques, the targets of the therapeutic drugs and the indispensable intermediates in all biotechnological processes. Apart from the function as targets in therapy, enzymes are novel in that they find application as therapeutic molecule itself (Vitolo, 2009). This review focuses on the application of various microbial enzymes as therapeutic agents.

Microbial therapeutic enzymes

Enzymes were largely ignored as drugs until Emmerich and his associates observed in 1902 that an extracellular secretion of *Bacillus pyocyaneus* was capable of killing anthrax bacilli. He deduced that the secretions contain nucleases which is the responsible element for the bacterial lysis. This milestone study gradually opened the way for the use of enzymes in the treatment first of infections, then of cancer and finally of a diverse spectrum of diseases (Gonzalez and Issacs, 1999).


The concept of the therapeutic enzyme has been around for at least 40 years. For example, a therapeutic enzyme was described as part of replacement therapies for genetic deficiencies in the 1960s by de Duve (1966). Enzymes as drugs have two important features i) they often bind and act on their targets with great affinity and specificity and ii) they are catalytic and convert multiple target molecules to the desired products. These two features make enzymes specific and potent drugs for a wide range of disorders (Vellard, 2003).

Sources of therapeutic enzymes include animals, plants and microorganisms (bacteria and fungi). Microbial enzymes are preferred because they are generally cheaper to produce, their enzyme content is more predictable and controllable and

the availability of reliable supplies of raw materials of constant composition. As they are foreign in nature, some of them are incompatible with the human body. Plant and animal sources contain more harmful materials than microbes which include phenolic compounds (from plants), endogenous enzyme inhibitors and proteases (Kaur and Sekhon, 2012).

Microbial enzymes have found wide application in medicine and pharmacology and their use in this field is recognized recently. Therapeutic enzymes have a wide variety of specific uses such as oncolytics, thrombolytics, or anticoagulants and as replacements for metabolic deficiencies, anti-inflammatory agents etc (Gurung et al., 2013). Various microbial therapeutic enzymes are described below.

L- asparaginase

L-Asparaginase  is broadly distributed among the plants, animals and microorganisms. Microbes are a better source of L-asparaginase, because they can be cultured easily and the extraction and purification of L- asparaginase is also convenient with the large-scale production. A wide range of bacteria, fungi, yeast, actinomycetes and algae are very efficient producers of L-asparaginase (Savitri et al ,2003; Verma et al ,2011).

L- asparaginase from *Erwinia carotovora* or *Escherichia coli* is used in the treatment of acute lymphocytic leukemia (Eden et al., 1990). Its activity depends upon the fact that tumour cells lack aspartate-ammonia ligase activity, which stops the synthesis of nonessential amino acid L-asparagine(Gurung et al., 2013). Hence, they are extracted from body fluids. Thus leukemic cells require L –asparagine, unlike normal cells, for their survival (Kidd and Sobin, 1966). By injecting L-

asparaginase the availability of the aminoacid is reduced, so the leukemic cells fail to survive(Mashburn and Wriston, 1964).

Collagenase

True bacterial collagenases are consensually described as enzymes that cleave helical regions of fibrillar collagen molecules under physiological conditions (Harrington, 1996). Microbial collagenases belong to the MEROPS peptidase family M9 (INTERPRO: IPR002169; PFAM: PF01752), which comprises bacterial metalloproteinases (predicted to be zinc-dependent) from *Vibrio* and *Clostridium* with presumable collagenolytic activity (Rawlings et al., 2012). Collagenases are applied in the pharamaceutical world for the treatment of various disorders listed below.

Treatment of damaged tissues


For treating damaged tissues, several studies, comparing the use of enzymatic methods with surgical/ mechanical procedures as well as comparing the effect between several enzymes were made. Data are controversial, while some studies state that wound debridement is more effective by using enzymatic procedures, reducing hospital staying and the need for surgical debridement (Karagol et al., 2011).

Enzymatic debriding agents are effective alternative for removing necrotic material from pressure ulcers, leg ulcers, and partial-thickness wounds. They may be used to debride both adherent slough and eschar. Enzymatic agents may be used as the primary technique for debridement in certain cases, especially when alternative methods such as surgical or conservative sharp wound debridement (CSWD) are not feasible owing to bleeding disorders or other

106 considerations(Ramundo and Gray, 2008).

107 For the removal of dead skin of burns, the use of a large number of bacterial
108 and plant enzymes have been studied. Among the microbial enzymes, a proteolytic
109 enzyme from *Vibrio proteolyticus* was found to be effective, and it successfully
110 finished phase1b clinical trials in 2004. Now it is used under the trade name
111 Vibrilase TM, especially for the serious secondary burn treatments(Gurung, 2013).

112 **Dupuytren's disease (DD)**

113 Clostridium  collagenase is also applied in the treatment of Dupuytren's
114 disease. .Dupuytren's disease is a fibroproliferative disorder of the palmar fascia
115 that limits hand functions, ultimately disabling the hand, and diminishing life's
116 quality (Hurst et al., 2009). This progressive disorder results in the permanent and
117 symptomatic flexion contracture of the digits.

118 Although some side effects have been reported for injectable *C. histolyticum*
119 collagenase like skin lacerations, edema, hemorrhage, injection site pain and
120 bruising and less frequently tendon and pulley rupture (Hallock, 2012; Kaplan,
121 2011), its effectiveness has been proved by several in vivo studies (Foissac et al.,
122 2013; Martin-Ferrero et al., 2013) with contracture reduction in more than 60% of
123 the patients injected with injectable clostridial collagenase (Hurst et al., 2009;).
124 Collagenase injection is more cost- effective than surgical fasciotomy (Martin-
125 Ferrero et al., 2013), has less and milder side-effects, and demonstrated a better
126 total reduction of Dupuytren's contracture leading to higher patient satisfaction
127 (Vollbach et al., 2013).

128 **Chronic total occlusions (CTO)**

129 Microbial collagenases (more precisely clostridial collagenases) have also

130 been applied to the treatment of chronic total occlusions (CTO) in animal models
131 (Segev et al., 2005; Strauss et al., 2003). CTO is defined as a 3-month-old total
132 obstruction of a coronary artery, and is one of the more difficult challenges for
133 coronary interventionists (Aziz & Ramsdale, 2005). It consists of various degrees of
134 fibroatheromatous plaque and thrombus, depending on the occlusion mechanism
135 and its duration, and occurs in approximately 30% of the patients with coronary
136 artery disease. Presently, clinical trials have showed that in human subjects, local
137 delivery of collagenase into coronary chronic total occlusion is feasible and safe
138 (Strauss et al., 2012).

139 **PROTEASES**

140 Proteases constitute the single most important group of industrially important
141 microbial enzymes which are capable of hydrolysing peptide bonds in to
142 aminoacids based on the size of the molecules they can attack or preferably attack.
143 These may be proteinases or petidases. Since the later years of the nineteenth
144 century, crude proteases are used for the treatment of gastrointestinal disorders.
145 Microbial proteases are used either directly or indirectly in the field of medicine for
146 diagnostic or therapeutic purposes (Moriyama et al., 1950).

147 **Streptokinase**

148 Pathologies involving a failure of hemostasis and the development of clot
149 require clinical intervention consisting of intravenous administration of thrombolytic
150 agents (Collen et al., 1988; Collen, 1990; Francis and Marder, 1991). Streptokinase
151 is one such agent.

152 Streptokinase is an extracellular enzyme produced by β hemolytic
153 streptococci. Streptokinase, produced by certain strains of streptococcus, is used

as a therapeutic agent in the treatment of cardiovascular diseases. It is a single chain polypeptide that exhibits its fibrinolytic action by indirectly activating the circulating plasminogen. Streptokinase is used in the treatment acute myocardial infarction , it is certainly more cost effective, however its use is not risk free.

When Streptokinase binds with circulatory plasminogen or plasmin, the resulting 1:1 stoichiometric complex is a high specificity protease that proteolytically activate other plasminogen molecules to plasmin (Bajaj and Castellino, 1977). Comparative clinical trials and cost effective considerations suggest that streptokinase is the drug of choice for thrombolytic therapy(Mucklow, 1995). Streptokinase is a non human protein and its introduction in to the circulatory system can elicit severe anaphylatic response including death (Lee, 1995). This immunogenicity restricts multiple applications of the streptokinase.

Staphylokinase

Staphylokinase is a protein produced by certain strains of staphylococcus and possesses fibrinolytic activities. Staphylokinase is a single polypeptide chain with a molecular weight of approximaltely 15.5 KDa and length of 163 amino acids. Natural staphylokinase has been purified from S. aureus strains that were transformed with bacteriophages containing the staphylokinase gene, or that had undergone lysogenic conversion to staphylokinase production (Lijinen et al., 1992).

Staphylokinase converts inactive proteolytic enzyme plasminogen to its active form, plasmin. Staphylokinase is used for the treatment of myocardial infarction. It can stimulate the lysis of both erythrocyte rich and platelet rich clots(Szarka etal., 1999).

Serrazime

Serrazyme, a proteolytic enzyme from *Aspergillus oryzae* and *Aspergillus*
mees, is used as an alternative of serratiopeptidase and is used as a dietary
 supplement for cardiovascular, antiinflammatory or immune support.

Serrapeptase

Serrapeptase is available for clinical use more than a decade.
 Serratiopeptidase binds to alpha -2-macroglobulin in the blood in the ratio of 1:1,
 which helps to mask its antigenicity but retains its enzymatic activity and is slowly
 transferred to site of inflammation. Serratiopeptidase hydrolyses bradykinin,
 histamine and serotonin responsible for the oedematic status. Serratiopeptidase
 reduces swelling, improves microcirculation and expectoration of sputum,
 etc(Mohankumar, 2009).

Serrapeptase or serratiopeptidase from *Serratia marcescens* is used as a
 therapeutic enzyme and possesses applications, as antiinflammatory agent, for
 treating carpal tunnel syndrome, for fibrocystic treatment and as agent to enhance
 the activity of antibiotics against biofilm formation(Preethi,2012).

Glutaminase

L-glutaminase (EC.3.5.1.2) is an amidohydrolase which catalyses the
 hydrolytical deamination of L-glutamine resulting in the production of L-glutamic
 acid and ammonia. L-Glutaminases are ubiquitous in the biological world (Ohshima
 et al., 1976; Iyer and Singhal, 2010) and organisms ranging from bacteria to human
 beings have the enzyme.

Acinetobacter glutaminisificans , *Bacillus licheniformis*, *Bacillus subtilis*
 ,*Erwinia cartowora* ,*Microccus luteus* etc are some of the representatives of the
 microbial world with potential glutaminase production capacities(Holchenberg, 1976

202 ,Cook et al., 1981 , Shimizu et al., 1991).

203 L-Glutaminase, in combination with or as an alternative to asparaginase,
204 could be of significance in enzyme therapy for cancer especially acute lymphocytic
205 leukemia(Roberts et al., 1970). Glutaminase from microbes exhibit antitumour
206 activity and recombinant glutaminase from *Pseudomonas* is patented for its activity
207 against HIV and cancer therapy.

208 **Lysostaphin**

209 Lysostaphin is a 27 KDa zinc metalloenzyme secreted by certain strains of
210 *Staphylococcus simulans* which has a specific lytic action against *Staphylococcus*
211 *aureus*. It possess two functional domains an N terminal catalytic peptidase domain
212 and a C terminal targeting domain which bind to the peptidoglycan substrate.
213 Lysostaphin has activities of three enzymes namely, glycylglycine endopeptidase,
214 endo- β -N-acetyl glucosamidase and N-acetyl muramyl-L-alanine amidase.
215 Glycylglycine endopeptidase specifically cleaves the glycine–glycine bonds, unique
216 to the interpeptide cross-bridge of the *S. aureus* cell wall(Wu et al., 2003).

217 Due to its unique specificity, lysostaphin could have high potential in the
218 treatment of antibiotic-resistant staphylococcal infections(Kumar, 2008).
219 Lysostaphin is found to reduce surface colonization by *S.aureus* and *S.*
220 *epidermidis*. Thus the drug is more effective in preventing the nasal colonization of
221 *S. aureus*. Lysostaphin acts synergistically with some membrane active agents
222 polymyxin and ranalexin against MRSA. Recombinant lysostaphin was found
223 effective in the treatment of aortic endocarditis(Preethi et al., 2011).

224 **Laccases**

225 Laccase (EC 1.10.3.2) or p-diphenol oxidase is one of a few enzymes that have

226 been studied since the 19th century. Yoshida first described laccase in 1883 from
227 the exudates of the Japanese lacquer tree, *Rhus vernicifera* (Thurston 1994; Levine
228 1965). However in 1896, for the first time, both Bertrand and Laborde demonstrated
229 laccase to be a fungal enzyme (Thurston 1994; Levine 1965).

230 Laccases are copper-containing enzymes that catalyze the oxidation of a
231 wide variety of organic and inorganic substrates, including mono-, di-, and
232 polyphenols, amino phenols, methoxy phenols, aromatic amines and ascorbate
233 with the concomitant four electron reduction of oxygen to water (Galhaup et al.
234 2002). Laccase is a member of the large blue copper proteins or blue copper
235 oxidases (Thurston 1994). The ability of laccases to oxidize phenolic compounds as
236 well as their ability to reduce molecular oxygen to water has led to intensive studies
237 of these enzymes (Thurston 1994). Laccase activity has been reported only in few
238 bacteria, including *Azospirillum lipoferum*, *Marinomonas mediterranea*,
239 *Streptomyces griseus*, and *Bacillus subtilis* (Octavio et al. 2006).

240 The first bacterial laccase was detected in the plant root-associated
241 bacterium *Azospirillum lipoferum*, where laccase was associated with the melanin
242 production for cell pigmentation. Recently some bacterial laccases have also been
243 characterized from *Azospirillum lipoferum*, *Bacillus subtilis*, *Streptomyces*
244 *lavendulae*, *S.cyaneus* and *Marinomonas mediterranea*. Many products generated
245 by laccases are antimicrobial, detoxifying or active personal-care agents. Laccase
246 can be used in the synthesis of complex medical compounds as anesthetics, anti-
247 inflammatory agents, antibiotics, sedatives, etc, including triazolo(benzo)cycloalkyl
248 thiadiazines, vinblastine, mitomycin, penicillin X dimer, cephalosporins, and
249 dimerized vindoline (Pazarloglu , 2005, Shi, C., Clemmons, 2003).

250 **Lipases**

251 Lipases(tri acyl glycerol acyl hydrolases E.C 3.1.1.3) are hydrolases that
252 catalyse the hydrolysis of tri glycerides to glycerol and free fatty acids over an oil
253 water interface. Bacterial lipases are glycoproteins but some extracellular lipases
254 are lipoproteins. In addition to this, the enzyme catalyzes the transesterification
255 and hydrolysis of other esters and also synthesis of some others. Such
256 transformations enable them to be used in food, cosmetic and especially in
257 pharmaceutic industry.

258 Among bacteria, *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp.,
259 *Pseudomonas* sp., *Staphylococcus* sp., and *Chromobacterium* sp. have been
260 exploited for the production of lipases.

261 Microbial lipases are used to enrich PUFAs from animal and plant lipids, and
262 their mono and diacylglycerides are used to produce a variety of pharmaceuticals.
263 Many PUFAs are essential for normal synthesis of lipid membranes and
264 prostaglandins. Free PUFAs and their mono and diacylglycerides are subsequently
265 used to produce a variety of pharmaceuticals. Considerable effort is being made to
266 obtain optically pure compounds, which are pharmacologically more active than its
267 antipode. Profens, a class of nonsteroidal anti-inflammatory drugs, are active in the
268 (s)-enantiomer form.

269 Lee et al(1995) and Xie et al (1988) synthesized pure (s)-ibuprofen using
270 lipase-catalyzed kinetic resolution via hydrolysis and esterification, respectively.
271 Optically active homochiral intermediates for the synthesis of nikkomycin-B, non
272 steroid anti-inflammatory drugs (naproxen, ibuprofen, suprofen and ketoprox), the
273 potential antiviral agent lamivudine, and for the enantiospecific synthesis of

alkaloids, antibiotics, vitamins, and anti- arteriosclerotic, anti tumour and antiallergic compounds(Pandey et al., 1999). Lipase from *Candida rugosa* is used to synthesize lovastatin, a drug that lowers serum cholesterol level. The asymmetric hydrolysis of 3-phenylglycidic acid ester which is a key intermediate in the synthesis of diltiazem hydrochloride is a widely used coronary vasodilator and is synthesized using *S. marcescens* lipase(Matsumae et al., 1993).

Alginate lyase

Alginate lyase can digest alginate through the beta elimination of the glycosidic bond(Wong et al., 2000). They yield various oligosaccharides with unsaturated uronic acid at the non reducing terminus and unsaturated duronic acid monomers. The oligosaccharides released by the enzyme seems to possess biological activities like enhancing the growth of endothelial cells and stimulate secretion of cytokines from human macrophages. The enzyme possesses pharmaceutical activity(Courtois, 2009., Iwamoto et al., 2005, Kawada et al, 1999).

One of the leading causes of illness and death in cystic fibrosis (CF) patients is *Pseudomonas aeruginosa* infection of the respiratory tract. Patients colonised by mucoid, alginate-producing strains have a particularly poor prognosis (Govan and Deretic,1996), and the infection is rarely eliminated by antibiotic treatment. Co-administration of alginate lyase with gentamicin increased the killing of biofilms of mucoid *P. aeruginosa* growing in conditions similar to those found in the CF respiratory tract (Cotton et al., 2009).


Microbial enzymes also find their application in various lysosome storage diseases. The lysosomal storage diseases are due to the deficiency of a particular enzyme such as β - glucuronidase or sphingomyelinase which lead to incomplete

298 digestion of particles and results in clinical symptoms.

299 CONCLUSION

300 Enzymes are known to mankind since the ancient times. Even in the period
 301 when there was no much knowledge on enzymes, people used them in various
 302 forms in fields like brewing etc,. Later on, in 18th century, the entity was identified
 303 as enzymes. Now the global use of enzymes is estimated to be worth \$ 3.3 billion
 304 in 2010, and by the year 2015 it is estimated to reach at \$ 4.4 billion. Microbial
 305 enzymes are considered as the highly effective therapeutic agents of this century.
 306 To discover more and more new enzymes and also to explore their novel
 307 applications, research is going on world wide. To achieve this goal, intense
 308 research in the field is necessary.

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