Biochemical and Biophysical Research Communications 426 (2012) 289–293

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mini Review

Biocatalysts: Beautiful creatures

Walid Saibi [a,*], Salma Abdeljalil [b], Khaled Masmoudi [a], Ali Gargouri [b]

[a] Plant Protection and Improvement Laboratory, Centre of Biotechnology of Sfax (CBS), University of Sfax–Tunisia, Tunisia
[b] Laboratory of Biomass Valorization and Protein Production in Eucaryotes, Centre of Biotechnology of Sfax (CBS), University of Sfax–Tunisia, Tunisia

ARTICLE INFO

Article history:
Received 29 June 2012
Available online 15 July 2012

Keywords:
Biocatalysts
Bifunctionality
Biotechnology
Enzyme like
Enzyme classification

ABSTRACT

The chemical industry has come under increasing pressure to make chemical production more eco-friendly and independent to fossil resources. The development of industrial processes based on micro-organisms can especially help to eliminate the use or the generation of hazardous substances and can support the transition from dependence on fossil resources towards real sustainable and eco-safety industrial processes. The biocatalysts are the best solution given by nature that can be used to improve some biotechnological applications. In this research review, we report some peculiar properties of biocatalysts, implicated in a range of metabolic pathways and biotechnological tools.

1. Introduction

Catalysis is a process that increases the speed with which a reaction reaches equilibrium. Since the reaction rate depends on the free energy of activation, a catalyst causes the decrease of the energy barrier and accelerates the catalytic stage [1]. Enzymes are molecules which can reduce the activation energy of reaction and thus accelerate the biochemical reactions that take place in the cell. Therefore, the functionality of a cell, its reproduction requires that all reactions occur in coordinated and controlled synergy. The enzymes are thus key players in life [2].

2. Biocatalysts: activity assay and classification

2.1. Enzymatic activity

The catalytic activity of an enzyme is measured by determining the increase in the reaction rate under defined conditions, the difference between the turnover of the catalyzed reaction and uncatalyzed reaction in a specific time interval. Normally, reaction rates are expressed as the change in product concentration per unit of time. Since the enzymatic activity is independent of the volume, the unit used for enzymes is usually turnover per unit time, expressed in katal (kat, mol s^{-1}). However, the international unit U is still more commonly used (μmol turnover min^{-1}; 1 U = 16.7 nkat).

2.2. Enzyme classes

More than 2000 enzymes are currently known. A system of classification has been developed that takes into account both their reaction and substrate specificity. Each enzyme is entered in the enzyme catalog with a four-digit enzyme commission number (EC number). The first digit indicates membership of one of the six major classes. The next two indicate subclasses and subsubclasses. The last digit indicates where the enzyme belongs in the subsubclass. For example, lactate dehydrogenase has the EC number 1.1.1.27 (class 1, oxidoreductases; subclass 1.1, CHOH group as electron donor; subsubclass 1.1.1, NAD (P^+) as electron acceptor). Enzymes with similar reaction specificities are grouped into each of the six major classes: (1) the oxidoreductases catalyze the transfer of reducing equivalents from one redox system to another; (2) the transferases catalyze the transfer of other groups from one molecule to another. Oxidoreductases and transferases generally require coenzymes; (3) the hydrolases are also involved in group transfer, but the acceptor is always a water molecule; (4) the Lyases (also named as "synthases") catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing; (5) the isomerases move groups within a molecule, without changing the gross composition of the substrate and (6) the ligases (also named synthetases) are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates. They catalyze the ligation reactions.

The enzymes thus have characteristics that make them vital molecules within the living world. Indeed, two closely related properties make powerful enzymes: specificity of substrate

* Corresponding author.
E-mail address: saibiwalid@gmail.com (W. Saibi).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.028

binding associated with the optimal arrangement of their catalytic site. The optimal arrangement of the provision of liaison groups and catalytic groups is the result of centuries of evolution. Those biocatalysts operate on a variety of mechanisms that fall into six categories [2]: (1) acid–base catalysis; (2) covalent catalysis; (3) metal ion catalysis; (4) electrostatic catalysis; (5) catalysis by proximity effect and orientation and (6) catalysis by preferential binding to the complex of the transition state.

3. The cellulose hydrolysis requires a multienzymatic system: cellulases

Cellulose represents the most abundant carbohydrate substance in nature. It is a bio-polymer of glucose units related by β1, 4 glucosidic linkages [3]. Its degradation requires a multi-enzymatic system composed of three enzymes: the endoglucanases which randomly attack cellulose in amorphous zones releasing cellooligomers; the cellobiohydrolases which liberate cellobiose from reducing and none reducing ends and finally the β-glucosidases which hydrolyze cellobiose and other cellooligosaccharides into glucose units. β-glucosidases (BGL) are present in all living organisms (bacteria, archaea, and eukarya) and perform wide range of functions [4]. In bacteria and fungi, β-glucosidases play an important role in cellulose degradation, that is, cellobiohydrolases and endoglucanases catalyze the cellulose hydrolysis to produce cellobiose and short cellooligosaccharides, which are ultimately hydrolyzed to glucose by β-glucosidase [4,5]. In the process of saccharification of lignocellulosic biomass, cellooligosaccharides and cellobiose are often produced by the limited hydrolysis of cellulose, leading to inefficient ethanol fermentation. The hydrolytic product, glucose, is the best substrate for ethanol production.

In addition, β-glucosidases have potential roles in various fields such as food industries, pharmacology, cosmetic, and valorisation of some products, owing the properties of this enzyme to convert and to synthesize bio-molecules of high added value [4].

Therefore, many trials have been carried to produce highly efficient BGL and more precisely "cellobiase". Most BGLs have a lower affinity and conversion efficiency for cellobiose [6]. *In vivo*, the last one represents the physiological substrate of the most β-glucosidases. However, *in vitro*, this ability becomes a non apparent criterion; the ability of β-glucosidase to split cellobiose gives him the denomination of cellobiase, if not, it is named aryl/alkyl β-glucosidase [6].

The cellulasic systems are generally inducible and repressible. Cellulose and their derivatives (CMC, HEC) represent their most inductors. In addition, there are other inductors like disaccharides (sophorose, gentiobiose, lactose) and monosaccharides (xylose). However, glucose constitutes the most popular repressor of hydrolases, especially cellulases [5].

Like various biocatalysts, the inhibition of β-glucosidases has been the subject of several studies [7]. We mentioned the role played by the hydroxyl groups of glucose in β-glucosidase inhibition. We have demonstrated the importance of the distribution of these hydroxyls, not only in the inhibition efficiency but also in the inhibition severity. The type, the nature of the linkage and the state of cyclization of the sugar influence the presence and the levels of inhibition of those biocatalysts [7].

According to the thermodynamics of inhibitor binding, the increased affinity for the transition state over the ground state by an enzyme is primarily derived from enthalpy. Therefore, it could be argued that inhibitors which truly mimic the transition state should also bind with large enthalpic contributions. Measurements were made using various strategies and methods such as ITC, which gives a direct read-out on the affinity of the inhibitor, stoichiometry of binding between inhibitor and enzyme and the enthalpic contribution to binding, from which the Gibbs free energy and entropy can be calculated. All of the inhibitors examined in this case displayed a negative and therefore favorable enthalpic contribution to binding [1].

4. The cellobiose dehydrogenases (CDH)

CDH is an extracellular redox enzyme produced by various wood degrading fungi and ascomycetes fungi. It catalyzes the oxidation of cellobiose, higher water soluble cellodextrins, lactose and mannobiose to their corresponding lactones. Efficient and complete degradation of woody plant cell walls is generally ascribed to certain basidiomycetes collectively referred to as white rot fungi [8]. Commonly associated with woody debris and forest litter, these fungi can depolymerize, degrade, and fully mineralize all cell wall polymers, including cellulose, hemicelluloses and the normally rather recalcitrant polymer lignin. Such plant cell wall deconstruction requires complex extracellular oxidative and hydrolytic systems. Mechanistic aspects of the degradative processes remain uncertain, but the field has attracted interest because woody feedstocks are increasingly viewed as potential sources for high-value low-molecular-weight products. The involvement of low-molecular-weight, diffusible oxidants, especially hydroxyl radicals, has long been suspected.

Repression and inhibition of biocatalysts can be affected by other factors. In this case, we demonstrated that production of β-glucosidase (bglG) is negatively affected by CDH. Indeed, on cellobiose-based medium, bglG time course production decreases drastically. This effect is the consequence of the release in the culture medium of a secondary metabolite (Δ-gluconolactone) that is known for its inhibitory effect on β-glucosidase activity. So we can say that inhibition of β-glucosidases and enzymes in general may be influenced by the presence of an enzyme or a metabolite secreted during kinetics production.

5. Bifunctionality's concept of some enzymes: evolutive aspect and necessity to modulate metabolic pathways

5.1. Fructose-1,6-bisphosphate aldolase/phosphatase

Some enzymes are referred to as being bifunctional; they consist of either two distinct catalytic domains or a single domain that displays promiscuous substrate specificity. Thus, one enzyme active site is generally responsible for one biochemical reaction. In contrast to this conventional concept, archaeal fructose-1,6-bisphosphate (FBP) aldolase/phosphatase (FBPA/P) consists of a single catalytic domain, but catalyzes two chemically distinct reactions of gluconeogenesis: (1) the reversible aldol condensation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P) to FBP; (2) the dephosphorylation of FBP to fructose-6-phosphate (F6P). Thus, FBPA/P is fundamentally different from ordinary enzymes whose active sites are responsible for a specific reaction. However, the molecular mechanism by which FBPA/P achieves its unusual bifunctionality remains unknown. Here we report the crystal structure of FBPA/P at 1.5-Å resolution in the aldolase form, where a critical lysine residue forms a Schiff base with DHAP. A structural comparison of the aldolase form with a previously determined phosphatase form revealed a dramatic conformational change in the active site, demonstrating that FBPA/P metamorphoses its active-site architecture to exhibit dual activities. Thus, these results expand the conventional concept that one enzyme catalyzes one biochemical reaction [9].

This concept of bifunctionality observed with some enzymes is also implicated with the hemicellulases such as xylanases. These categories of enzymes follow various bifunctionality aspects.

5.2. Bifunctional xylanase–arabinosidase

Xylanases are extracellular enzymes that hydrolyze the internal β-1,4-xylosidic linkages of the xylan backbone structure. Xylanase action is restricted by the presence of side chains. Removal of side-chain substituents requires additional enzymatic activities of arabinofuranosidase, uronidase, glucosidase, mannosidase, and acetyl esterase. The xylanase gene of *Streptomyces halstedii* was used to isolate a DNA fragment from a gene library of *Streptomyces chattanoogensis*. Nucleotide sequence analysis revealed a gene encoding a bifunctional biocatalyst bearing two independent xylanase and α-L-arabinofuranosidase domains separated by a Ser/Gly-rich linker. The N terminus of the predicted protein showed high homology to family F xylanases. The C terminus was homologous to amino acid sequences found in α-L-arabinofuranosidase from *Streptomyces lividans*. To our knowledge, this is the only report that described xylanase and arabinofuranosidase domains in the same open reading frame.

5.3. Bifunctional xylanase–deacetylase

Pseudobutyrivibrio xylanivorans has a potent xylanolytic enzyme system. Xyl 11A isolated from this bacterium belongs to the glycosyl hydrolase family 11. The gene encoding for Xyl 11A contains N-terminal carbohydrate binding type-6a domain, which exhibits xylanolytic activity and C-terminal domain coding for putative polysaccharide deacylase implicated in removing acetyl groups from acetylated xylan, and thus it is probably capable of hydrolyzing acetylated xylan debranching in the xylan backbone. *Cytophaga hutchinsonii* is reported to produce bifunctional xylanase/esterase encoded by CHU-1239 gene CHU-1240, where xylanase belongs to glycoside hydrolase family 8 proteins and esterase belong to carbohydrate esterase family 4 proteins. Furthermore, CHU-1239 gene is responsible for bifunctional acetylxylan esterase/xylanase enzyme, where xylanase belongs to glycoside hydrolase family 10 protein and carbohydrate esterase family 6 one [10].

5.4. The biocatalysts implicated in proline metabolism

Proline is a proteinogenic amino acid with an exceptional rigidity of conformation, and is essential for primary metabolism. The metabolism of proline is of interest both to those seeking to better understand the stress physiology of plants as well as those seeking to understand the metabolic regulation While in the past, the metabolism of proline has been studied primarily by those interested to drought and other abiotic stresses, there is increasing evidence that proline is also relevant for the interactions between plant pathogens and is involved in programmed cell death and development [12].

The core of proline metabolism involves two enzymes catalyzing proline synthesis from glutamate in the cytoplasm or chloroplast, two enzymes catalyzing proline catabolism back to glutamate in the mitochondria, as well as an alternative pathway of proline synthesis via ornithine.

The Δ^1-pyrroline-5-carboxylate synthetase (P5CS) is a key enzyme involved in the synthesis of the amino acid proline in both mammals and plants. Proline level is tightly regulated by its de novo synthesis and degradation [12]. Glutamate, a precursor of proline synthesis in the cytosol, is first phosphorylated to gamma-glutamyl phosphate, which is then reduced to glutamate semialdehyde (GSA) by a bifunctional ATP and NAD(P)H-dependent P5CS enzyme that consists of two domains, an N-terminal gamma-glutamyl kinase (gamma-GK) domain and a C-terminal GSA dehydrogenase domain. GSA, spontaneously cyclizes to P5C, which is finally reduced to proline by the P5C reductase (P5CR). In prokaryotes and lower eukaryotes like *Saccharomyces*, the P5CS activity is carried out by a complex of distinct gamma-GK and GSA dehydrogenase enzymes [13]. Besides that proline is essential for primary metabolism, several other roles have been postulated for this amino acid in plants. Proline accumulation is a widespread response to water stress to which it may play a role as an osmolyte. Proline may also act as a chaperone to protect macromolecules from degradation, as a sink for energy and reducing power, as a transient source of carbon and nitrogen, or as a hydroxyl radical scavenger. Upon relief from stress, proline is rapidly oxidized by the sequential action of two mitochondrial enzymes proline dehydrogenase and P5C dehydrogenase to produce glutamate [13]. In this context, development of an accurate, reliable, and simple method for measuring P5CS activity is of considerable interest. Several methods have been used to assay P5CS activity. The most commonly used method involved the incorporation of labeled glutamate into proline [11]. Another method was also reported based on the P5CS reverse reaction. Indeed GSA reductase activity was measured by phosphate-dependent reduction of NADP+, using P5C as the substrate. Here, we describe a new method to measure the specific P5CS activity based on the quantification of inorganic phosphate. Indeed, Pi is formed after the phosphorylation of glutamate to -glutamyl phosphate by the gamma-GK activity of P5CS and its reduction by the GSA dehydrogenase activity. Pi determination is based on a malachite green colorimetric assay. This method is sensitive enough, requires small amount of biological material, and does not use any radio labeled compounds [14].

6. Recent patents involving the importance of biocatalysts as biotechnological tools

The use of biomass is increasingly seen as a convenient means for sustainable energy supply and environment of long-term care throughout the world. Regarding food security, bio-ethanol based on starch or sugar and petroleum products are very limited for large-scale production. However, the conversion of lignocellulosic residues of food crops is a potential alternative. Because of his reluctance, the process of current biomass is very expensive; we note that the selection of energy crops is a promising solution. So to meet the needs, energy crops are defined with high efficiency for both food and biofuels.

Lignocellulose biomass, a source of fermentable sugar, has not hitherto been used on an industrial scale. The resistance of the lignocellulose biomass to enzymatic hydrolysis occurs particularly at the crystalline phase of the cellulose, at the accessible surface, at the lignin coating and finally at the hemicelluloses screening the cellulose.

The Patent entitled ''Method for producing ethanol by fermentation from lignocellulosic biomass'' [15] relates to a method of producing bioethanol by separating lignin from a crushed lignocelluloses biomass and obtaining cellulose and, if required, hemicelluloses and further processing of the cellulose or mixture of cellulose and hemicellulose to obtain sugars and subsequently obtain bioethanol.

The patent ''Compositions and methods for biofuel crops'' [16] is based on the use of the natural variation of sweet and grain sorghum to uncover genes that are conserved in rice, sorghum, and sugarcane, but differently expressed in sweet versus grain sorghum. The present invention relates to compositions and methods to increase the sugar content and/or decrease the lignocellulose content in plants such as corn, rice, sorghum, Brachypodium, Miscanthus and switchgrass. The invention involves identifying genes responsible for sugar and lignocellulose production and genetically altering the plants to produce biofuels in non-food plants as well as the non-food portions of food crop plants to use as biofuel.

7. Improvement of the catalytic and thermostability of the biocatalysts: excellent biotechnological tools

7.1. Immobilization: one of the best tools

The biogenous iron oxide (BIO) from *Leptothrix ochracea* represents an excellent example to follow one of the tools used to improve the enzymatic efficiency for biotechnological applications. Indeed, it was transformed to an organic–inorganic hybrid support to prepare an excellent immobilized enzyme showing high catalytic performance.

Indeed, living things create fascinating materials with unique morphologies and nanostructures that cannot be synthesized artificially. In particular, the extracellular materials produced by the iron-oxidizing microorganism have attractive characteristics. Although the biological and morphological aspects of BIO have been investigated, no studies have been reported that explore the application of BIO to new functional materials. Because materials with a specific structure, size, shape, and chemical composition are the key to the creation of innovative functions. Large amounts of BIO sediment, which cause problems of pipe clogging and rusty water, have been considered to be waste. BIO is an environmentally benign, sustainable, and unused natural resource consisting of ubiquitous elements [17].

7.2. Wheat déhydrine (DHN-5) exerts chaperon molecular role during enzymatic processes

In other case, improvement of thermoactivity and thermostability of GOD, LDH and bglG was proved using an LEA protein, isolated from wheat (DHN-5) [18]. Brini et al. proved for the first time that DHN-5 enhances thermoactivity and thermostability of GOD and bglG at high temperatures. Indeed, the half-life-time of bglG is upgraded from 15 to 50 min. According to these findings, we reported the efficiency conferred to the biocatalysts to improve the recovery, during biotechnological processes.

7.3. Directed mutagenesis

Directed mutagenesis represents one of the most efficient tools used to enhance activity and /or stability of enzymes under described conditions (pH and/or temperature). Indeed, L-arabinose isomerases catalyze the bioconversion of D-galactose into D-tagatose. In order to generate an efficient enzyme for the production of the D-tagatose, various mutants were constructed and characterized. The first one was significantly more acidotolerant and more stable at acidic pH than the wild-type enzyme. The second mutant has a broad optimal temperature range from 50 to 65 °C to make the process of production of D-tagatose more effective, they have generated the double mutant having an optimum pH of 6–7 and an optimum temperature of around 50–65 °C, at which the enzyme is stable [19].

8. Peculiar biocatalysts

8.1. The case of "ribozymes"

Ribozymes are RNAs that possess the property of catalyzing a specific chemical reaction. The term "ribozyme" is a chemeric expression formed from the words "ribonucleic acid" and "enzyme". The discovery of these molecules in the 1980s, independently by Tom Cech and Sidney Altman, was a surprise because until then, the proteins were the only biological macromolecules known to catalyze chemical reactions. The catalytic properties of ribozymes are linked to the ability of RNA to fold into a compact structure well defined, which, as in the case of proteins, allows the formation of cavities forming binding sites for ligands [20].

Today we know a number of natural RNAs or ribozymes that are proven or probable ribozymes, RNA is still autocatalytic, which are their own substrate:(1) RNase P involved in tRNA maturation has demonstrated a catalytic activity. It is a natural ribozyme present in all cells [20]. It is composed of a catalytic RNA and one or more accessory proteins. Its function is to catalyze the maturation of transfer RNA; (2) the ribosome is a ribozyme, which was established indirectly but clearly by resolution of its crystallographic structure. The active site of the ribosome, which catalyzes the synthesis of peptide bonds, is in fact composed by ribosomic RNA [21]; (3) the self-épissables introns, able to specifically cleave in the absence of protein. Even if they are able to promote their splicing, they are not catalysts in the strict sense, since it is a single reaction without catalyst recycling; (4) the riboswitches found in some mRNAs have an autolytic activity: they cleave themselves when bound to a ligand activator. This is the case for example of specific riboswitch glucosamine-6-phosphate; (5) the spliceosome, responsible for the cytoplasmic splicing of introns in eukaryotes is also probably a ribozyme, although the formal demonstration to do yet been performed; and (6) the RNA of some viruses contain structures capable of autocatalytic cleavage activity, as the ribozyme "hammerhead" characterized in the plant infecting viroid avocado.

According to these data, RNA can both act as a catalyst and support of genetic information, like DNA, it became possible to imagine a world where prebiotic RNA was the precursor of all biological functions. This is the so-called RNA world hypothesis or RNA world [22].

8.2. The concept of "abzymes"

An abzyme, also called "catmab" (catalytic monoclonal antibody), is monoclonal antibody with catalytic activity. Molecules which are modified to gain new catalytic activity are called synzymes. Abzymes are usually artificial constructs, but are also found in normal humans (anti-vasoactive intestinal peptide autoantibodies) and in patients with autoimmune diseases such as systemic lupus erythematosus, where they can bind to and hydrolyze DNA. Abzymes are potential tools in biotechnology, e.g., to perform specific actions on DNA. They have a higher specificity relative to the substrate. Another advantage is that they can be produced to catalyze all sorts of reactions, such as the Diels–Alder reaction. Like all enzymes, they work by stabilizing the transition state of the reaction. In practice, they are developed and selected using a molecule-like state. Currently, research is conducted in the hope of using abzymes for therapeutic purposes [23].

In 2008 issue of the journal Autoimmunity Reviews, researchers of the University of "Texas Medical School at Houston" announced that they have engineered an abzyme that degrades the super antigenic region of the gp120 CD4 binding site. This is the one part of the HIV virus outer coating that does not change, because it is the attachment point to T lymphocytes, the key cell in cell-mediated immunity. Once infected by HIV, patients produce antibodies to the more changeable parts of the viral coat. The antibodies are ineffective because of the virus' ability to change their coats rapidly. Because this protein gp120 is necessary for HIV to attach, it does not change across different strains and is a point of vulnerability across the entire range of the HIV variant population. The abzyme does more than bind to the site, it destroys it, which makes HIV inert and may be related to other viruses. A single abzyme can destroy thousands of HIV viruses. Human clinical trials will be the next step in producing treatment and perhaps even preventative vaccines and microbicide [24].

8.3. Δ-Endotoxin plays their insecticide role via β-glucosidase activity

The crystals of Bacillus thuringiensis strain 1.1 consist of Δ-endotoxin, which exhibits β-glucosidase activity. When the crystals are reacted with specific antibodies directed either against the almond β-glucosidase or against the Δ-endotoxin, and then considerable reduction of enzymatic activity is observed almost at the same level with both antibodies. In another hand, the described β-glucosidase and the crystal polypeptide share antigenic similarities; in Western immunoblots, the crystal polypeptide is recognized by anti-β-glucosidase antibodies and the almond β-glucosidase is recognized by anti-crystal antibodies. The enzymatic properties of the almond β-glucosidase are similar to those residing in the crystals of *Bacillus thuringiensis*. Thus, both enzymes hydrolyze a wide range of substrates and have an optimum activity at 40 °C and pH 5. Both enzymes are relatively thermostable and are resistant to inhibition by glucose. Additionally, they show the same pattern of inhibition or activation by several chemical compounds. The Δ-endotoxin and the described β-glucosidase show almost equivalent levels of insecticidal activity against *Drosophila melanogaster* larvae [25].

Acknowledgments

This work was supported by grants from the Ministry of Higher Education, Scientific Research, and Technology of Tunisia.

References

[1] T.M. Gloster, G.J. Davies, Glycosidase inhibition: assessing mimicry of the transition state, Organic and Biomolecular Chemistry 8 (2010) 305–320.
[2] A.M. Lieberman, Basic Medical Biochemistry: A Clinical Approach, Second Edition ed. (2004) 900 pages.
[3] B. Amouri, A. Gargouri, Characterization of a novel β-glucosidase from a *Stachybotrys* strain, Biochemical Engineering Journal 32 (2006) 191–197.
[4] W. Saibi, B. Amouri, A. Gargouri, Purification and biochemical characterization of a transglucosilating beta-glucosidase of *Stachybotrys* strain, Applied Microbiology and Biotechnology 77 (2007) 293–300.
[5] W. Saibi, S. Abdeljalil, A. Gargouri, Carbon source directs the differential expression of β-glucosidases in *Stachybotrys microspora*, World Journal of Microbiology and Biotechnology 27 (2011) 1765–1774.
[6] W. Saibi, A. Gargouri, Purification and biochemical characterization of an atypical β-glucosidase from *Stachybotrys microspora*, Journal of Molecular Catalysis B: Enzymatic 72 (2011) 107–115.
[7] W. Saibi, A. Gargouri, Hydroxyl distribution in sugar structure and its contributory role in the inhibition of *Stachybotrys microspora* beta-glucosidase (bglG), Carbohydrate Research 346 (2011) 1848–1854.
[8] W. Saibi, A. Gargouri, Cellobiose dehydrogenase influences the production of *S. microspora* β-glucosidase, World Journal of Microbiology and Biotechnology 28 (2012) 23–29.
[9] S. Fushinobu, H. Nishimasu, D. Hattori, H.J. Song, T. Wakagi, Structural basis for the bifunctionality of fructose-1,6-bisphosphate aldolase/phosphatase, Nature 478 (2011) 538–541.
[10] R. Khandeparker, M. Numan, Bifunctional xylanases and their potential use in biotechnology, Journal of Industrial Microbiology & Biotechnology 35 (2008) 635–644.
[11] P. Arellano, F.C. Alvarez, A. Martínez, J. Rodríguez-Díaz, J. Cervera, Pyrroline-5-carboxylate synthase and proline biosynthesis: from Osmotolerance to rare metabolic disease, Protein Science 19 (2010) 372–382.
[12] M. Spoljarevie, D. Agie, M. Lisjak, A. Gumze, I.D. Wilson, J.T. Hancock, T. Teklie, The relationship of proline content and metabolism on the productivity of maize plants, Plant Signaling and Behavior 6 (2011) 251–257.
[13] N.M. Cecchini, M.I. Monteoliva, M.E. Alvarez, Proline dehydrogenase contributes to pathogen defense in arabidopsis, Plant Physiology 155 (2011) 1947–1959.
[14] C.S. Zhang, Q. Lu, D.P.S. Verma, Removal of feedback inhibition of Δ1-pyrroline-5-carboxylate synthetase, a bifunctional enzyme catalyzing the first two steps of proline biosynthesis in plants, Journal of Biological Chemistry 270 (1995) 20491–20496.
[15] K. Ties, Method for producing ethanol by fermentation from lignocellulosic biomass, (2011).
[16] M. Joachim, C.T. Martin, B. Remy, Compositions and methods for biofuel crops, (2011).
[17] T. Sakai, Y. Miyazaki, A. Murakami, N. Sakamoto, T. Ema, H. Hashimoto, M. Furutani, M. Nakanishi, T. Fujii, J. Takada, Chemical modification of biogenous iron oxide to create an excellent enzyme scaffold, Organic and Biomolecular Chemistry 8 (2010) 336–338.
[18] F. Brini, W. Saibi, I. Amara, A. Gargouri, K. Masmoudi, M. Hanin, Wheat dehydrin DHN-5 exerts a heat-protective effect on beta-glucosidase and glucose oxidase activities, Bioscience, Biotechnology, and Biochemistry 74 (2010) 1050–1054.
[19] M. Rhimi, N. Aghajari, M. Juy, H. Chouayekh, E. Maguin, R. Haser, S. Bejar, Rational design of *Bacillus stearothermophilus* US100 l-arabinose isomerase: potential applications for D-tagatose production, Biochimie 91 (2009) 650–653.
[20] C. Guerrier-Takada, K. Gardiner, T. Marsh, N. Pace, S. Altman, The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme, Cell 35 (1983) 849–857.
[21] P. Nissen, J. Hansen, N. Ban, P.B. Moore, T.A. Steitz, The structural basis of ribosome activity in peptide bond synthesis, Science 289 (2000) 920–930.
[22] W. Gilbert, Origin of life: the RNA world, Nature 319 (1986) 618.
[23] D. Shabat, F. Grynszpan, S. Saphier, A. Turniansky, D. Avnir, E. Keinan, An efficient sol–gel reactor for antibody-catalyzed transformations, Chemistry of Materials 9 (1997) 2258–2260.
[24] H. Taguchi, S. Planque, Y. Nishiyama, J. Symersky, S. Boivin, P. Szabo, R.P. Friedland, P.A. Ramsland, A.B. Edmundson, M.E. Weksler, S. Paul, Autoantibody-catalyzed hydrolysis of amyloid β peptide, Journal of Biological Chemistry 283 (2008) 4714–4722.
[25] A. Papalazaridou, L. Charitidou, A. Sivropoulou, β-Glucosidase enzymatic activity of crystal polypeptide of the *Bacillus thuringiensis* strain 1.1, Journal of Endotoxin Research 9 (2003) 215–224.