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White-rot fungi, new biotechnological tools for a cleaner environment

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ABSTRACT

White-rot fungi are key regulators of the global C-cycle; they are so far unique in their ability to complete degrade all components of our principal renewable resource, lignocellulose. These filamentous wood decay fungi, common inhabitants of forest litter and fallen trees, can attack all the wood components, the lignin as well as the polysaccharides. They produce an impressive array of enzymes (cellulases, hemicellulases, pectinases and ligninases) with potential in a wide range of biotechnological applications, including hazardous waste remediation and in the industrial processing of paper and textiles. Interest in these fungi has increased during the past two decades spurred by the ability of these organisms to degrade a wide variety of hazardous compounds (including polychlorinated biphenyls, pesticides, explosives, polycyclic aromatic hydrocarbons and industrial dyes). To be able to use the white rotters in these processes it is necessary to learn how their enzymes are secreted, how they operate and under what conditions they are active. Our current research is focused on these subjects, and in the screening of new isolates for their ability to degrade xenobiotics, in the search of more efficient strains.

KEYWORDS: white-rot fungi, lignocellulolytic enzymes, bioremediation

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INTRODUCTION

White-rot fungi are unique in their ability to completely degrade wood and other lignocellulosic substrates. Interest in this group of fungi and their lignocellulolytic enzyme system has increased during the past two decades spurred by their biotechnological applications, among them, biodegradation of numerous environmental pollutants. The present paper reviews our current research, focused in learning how their enzymes are secreted, how they operate and under what conditions they are active, and in the screening of new isolates for their ability to degrade xenobiotics, in the search of more efficient strains.

White-rot fungi, new biotechnological tools for a cleaner environment

White-rot fungi (WRF) are so far unique in their ability to completely degrade all components of our principal renewable resource, lignocellulose. Lignocellulose degradation is a central step for carbon recycling in land ecosystems. Moreover, fungal decay of wood in service results in billion-euro losses [1]. Plant biomass represents the key natural raw material for many current biotechnological processes and a sustainable source of future fuels, chemicals and materials [2]. The use of lignocellulose as a source of fuel, food and fiber is of major economic significance. Some of these uses require fractionation, e.g. chemical pulping; in others the resource is used inefficiently (e.g. animal nutrition), and in many there are by-products for which uses should be found (e.g. urban wastes, forest wastes, straw). In these cases

biodegradation might contribute to improved utilization, so a better understanding of this complex process could be economically and environmentally important [3].

Wood and other lignocellulosic materials are formed by three main polymeric constituents: cellulose, hemicellulose and lignin. Cellulose is a linear and highly ordered (often crystalline) polymer of cellobiose (D-glucopyranosyl-ß-1,4-D-glucopyranose) that represents over 50% of wood weight. By contrast, lignin is a three-dimensional network built up of dimethoxylated (syringyl), monomethoxylated (guaiacyl) and non-methoxylated (*p*-hydroxyphenyl) phenylpropanoid units, derived from the corresponding *p*-hydroxycinnamyl alcohols, which give rise to a variety of subunits including different ether and C-C bonds. Lignin is highly resistant towards chemical and biological degradation, and confers mechanical resistance to wood. It forms together with hemicelluloses, an amorphous matrix in which the cellulose fibrils are embedded and protected against biodegradation. The third structural component, hemicelluloses (polyoses), has an intermediate degree of complexity and is made up of different pentose and hexose residues, which are often acetylated, and generally form branched chains. Typically, hemicelluloses in softwood are glucomannans, whereas those in hardwoods are mainly xylans together with variable percentages of galactose, arabinose, rhamnose and methylglucuronic acid units, and acetyl groups [1].

WRF, the most frequent wood-rotting organisms, are a physiological rather than taxonomic group comprising those fungi characterized by their ability to degrade lignin, hemicelluloses, and cellulose often giving rise to a cellulose-enriched white material [4]. Cellulose and hemicellulose are hydrolyzed by cellulases and hemicellulases, whereas lignin is oxidized by various oxidases and peroxidases. The name white-rot derives from the appearance of wood attacked by these fungi, in which lignin removal results in a bleached appearance to the substrate. Most known WRF are basidiomycetes, although a few ascomycete genera within the Xylariaceae are also capable of white-rot decay. Due to the ability of WRF to degrade lignin selectively or simultaneously with cellulose, two white-rot patterns have been described in different types of wood, namely selective delignification and simultaneous rot [1]. The ability to catabolize cellulose and hemicellulose, the polysaccharides forming the other main components of lingo-cellulose, is fairly common as a primary metabolic process among fungi and other organisms, and occurs under a range of environmental conditions. As a result, it is not regarded as a rate-limiting step in carbon flux. Lignin, however, is extremely recalcitrant and is mineralized in an obligatory aerobic oxidative process, carried out appreciably only by the WRF. This recalcitrance, possession of ligninolytic ability among relatively few taxa, and annual lignin production estimated at 20.3×10^{12} kg, contribute to lignin degradation being regarded as the rate-limiting step to carbon turnover in lignocellulose-dominated environments. Interestingly, the oxidation of lignin yields no net energy gain, and so lignin is not a substrate in primary metabolism [4]. As lignin cannot be degraded as a sole source of carbon and energy, ligninolysis only occurs when other readily biodegradable substrates are available. *Phanerochaete chrysosporium* (studied extensively, as a model organism) initiates ligninolysis only after primary growth has ceased due to carbon, nitrogen or sulfur limitation. Lignin is degraded during secondary metabolism. The physiological importance of lignin biodegradation is destruction of the lignin matrix so that the microorganisms can gain better access to the real substrates hemicellulose and cellulose [5].

WRF secrete one or more of the three enzymes essential for lignin degradation. Two glycosylated hemecontaining peroxidases, lignin peroxidase (LiP), and Mn-dependant peroxidase (MnP), and of a copper containing phenoloxidase, laccase (Lac), constitute the lignin-degrading enzyme systems of WRF. Together with an H_2O_2-generating system, secondary metabolites and cellulolytic and hemicellulolytic enzymes, they may act synergistically during the decay of wood. LiP action is an H_2O_2 (endogenously generated)-dependent one-electron oxidation of a variety of lignin-related aromatic structures, giving rise to the formation of aryl cation radicals. These can perform several non-enzymatic reactions like: benzylic alcohol oxidation, carbon-carbon bond cleavage, hydroxylation, phenol dimerization and/or polymerization, and demethylation, and

produce large quantities of end products. The LiPs are particularly attractive as pollutants degraders. They are relatively non-specific, have a very high oxidation-reduction potential, and can potentially oxidize xenobiotics usually difficult to be affected by other peroxidases. MnPs catalyze the H_2O_2-dependent oxidation of Mn(II) to Mn(III), and this latter mediates the oxidation of a variety of phenolic substrates. Molecular oxygen is the oxidant involved in the laccase-mediated oxidation of phenolic substrates to phenoxy radicals. Laccases can also oxidize non-phenolic aromatics when mediators such as 2,2'-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 1-hydroxybenztriazole (HBT) and others (even natural cooxidants) are present in the reaction mixture [6].

WRF, have received considerable attention for their bioremediation potential. WRF appear unique and attractive organisms for the bioremediation of polluted sites for several reasons. Most of them are robust organisms and may tolerate higher concentrations of pollutants than bacteria. They posses a very powerful extracellular oxidative enzymatic system: the same unique nonspecific mechanisms that give these fungi the ability to degrade lignin also allow them to degrade a wide range of pollutants. Other non-ligninolytic enzymes like cellobiose-dehydrogenase (heme-flavin enzyme which has been implicated in both cellulose- and lignin degradation, oxidizes cellobiose and some other carbohydrates and reduces quinones and the radicals produced by the ligninolytic enzymes [7]), may participate in the transformation of polluting substances. They are usually secreted under non-ligninolytic conditions when cellulose is the nutrient carbon, and either directly or indirectly may oxide several contaminants. As a consequence, a vast range of toxic environmental, including low soluble compounds, can be mineralized or degraded by WRF. WRF may grow using inexpensive substrates such as agricultural crop wastes that can be easily added as nutrients to the contaminated site. WRF, being filamentous fungi, can reach the soil pollutants in ways that bacteria cannot. The lignin degrading enzymes are mainly of constitutive nature. This feature prevents WRF to be adapted to the chemical being degraded [6].

WRF and their ligninolytic enzymes are able to degrade many pollutants, among them: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other halogenated aromatics (including dioxins), trinitrotoluene (TNT) and other nitroaromatic explosives, many pesticides, and a range of other toxic pollutants such as dyes of different chemical structure, cyanides, azide, carbon tetrachloride and pentachlorophenol [4]. Besides the ligninolytic enzymes, the involvement of other enzymes was recognized to be crucial to the mineralization process. For instance, some pollutants such as TNT, or carbon tetrachloride (CCl_4) were significantly transformed by cellobiose dehydrogenase [8]. Intracellular systems that are generally present in most fungi, such as cytochrome P-450 monooxygenase, may also be involved in organopollutant degradation. However, as ligninolytic enzymes are active extracellularly, white rot fungi are better candidates for the bioremediation of highly apolar pollutants than non-ligninolytic microorganisms. For example: bioremediation of PAH-polluted soil is severely hampered by the low rate of degradation of higher PAHs, particularly the four- and five-ring PAHs. These higher PAHs have very low water solubility and are often tightly bound to soil particles. This results in very low bioavailability for bacterial degradation. The observation that white rot fungi can oxidize PAHs rapidly with their extracellular ligninolytic enzyme systems has therefore raised interest in the use of these organisms for bioremediation of PAH-polluted soils [6, 9]. Oxidation of PAHs by white rot fungi to more water-soluble products with greater bioavailability resulted in higher rates of mineralization by bacteria than those of the parent compounds [10].

Despite the obvious potential of WRF in bioremediation, our understanding of the complex physiology of these unique organisms remains limited. All three ligninolytic enzymes (MnP, LiP, Lac) are produced in multiple isoforms and encoded by gene families with complex regulation. Nutrient levels, mediator compounds and required metal ions (Mn (II) for MnP, Cu (II) for Lac) affect transcription of respective genes [11]. The utilization of fungi for bioremediation requires an understanding of the factors that enhance their ability to detoxify, and clarification of the enzyme mechanisms used [12].

A majority of the previous studies have focused primarily on the lignin-degrading enzymes of *P. chrysosporium* and *Trametes versicolor*. Both species proved their ability to metabolize a wide range of xenobiotic compounds. The white-rot fungus *P. chrysosporium* has been used extensively as a model organism to study the physiological requirements and enzymes required for lignin biodegradation. Completition of its genome sequence is an important starting point toward a comprehensive understanding of its genetics and physiology [2, 13]. However, the practical application of this fungus in waste treatment and bioremediation does not always enable the culture conditions to be fulfilled (i.e. low nitrogen, high temperature, secondary metabolism and static-culturing conditions). It may therefore be beneficial to screen a variety of WRF for the ability to degrade xenobiotics under a wide range of environmental conditions [5].

Recently, there has been a growing interest in studying the ligninolytic enzymes of a wider array of white-rot fungi, not only from the standpoint of comparative biology but also with the expectation of finding better lignin-degrading systems for use in various biotechnological applications [14]. Other fungi have begun to be evaluated for their pollutant-degrading abilities, and notable differences with regard to the extent of their pollutant transformation ability have been demonstrated. An important number of ligninolytic fungi have been described for the Southern Hemisphere, but few reports deal with the enzymatic systems implicated in biodegradation by these species. *Trametes trogii* is a white rot basidiomycete, distributed worldwide. *T. trogii* strain BAFC 463, an Argentinean strain, besides efficiently degrading lignin in wood [15], has been tested successfully in biomechanical pulping experiments [16] and also demonstrated to be a good producer of cellulases [17], pectinases [18], xylanases [19] and ligninases [20]. The simultaneous presence in this fungus of high ligninolytic and hydrogen peroxide producing activities, essential for peroxidase activity and rate limiting for pollutant degradation, make it an attractive microorganism on which to base future biotechnological applications. On the other hand, in contrast to the well studied model organism *P. chrysosporium*, *T. trogii* is N-unregulated. Sufficient or excess N-nutrients stimulate high MnP and laccase titres in parallel with the high biomass production. This characteristic of the fungus makes it an outstanding candidate for large-scale fermentation to produce ligninolytic enzymes in bulk for bioremediation. It produces high amounts of MnP, and higher laccase levels than those reported for most other white rot fungi under favorable conditions, accompanied by high levels of the hydrogen peroxide- producing enzyme glyoxal oxidase. Its ability to almost completely remove high priority pollutants such as PCBs and PAHs [21], nitrobenzene and anthracene [22], and a wide range of textile dyes [23, 24], has been recently demonstrated. In view of the results currently being obtained in our laboratory on the biodegradation of different organopollutants by *T. trogii*, this strain seems promising for detoxification.

The use of natural solid substrates, especially lignocellulosic agricultural residues, as growth substrates of fungi has been enthusiastically studied for ligninolytic enzyme production in recent years [25, 26]. This approach is attractive because of anticipated effects in cost reduction, waste reuse and enhanced enzyme production. Various solid substrates were evaluated during the past few years, among them: wood chips, cereal grains, straw, and bran [26]. *Fomes sclerodermeus* BAFC 2752 is a white-rot basidiomycete that was isolated in Tucumán, Argentina. Besides degrading lignin in wood was able to grow and detoxify the fungicide malachite green [27]. It produces high levels of laccase and MnP when cultured in solid state fermentation in a natural medium based on wheat bran [28].The system wheat bran-*F. sclerodermeus* has proven to be effective in the removal and degradation of the fungicide dye malachite green from aqueous solution [29]. The ligninolytic activity of several other Argentinean white-rot strains is currently under investigation in our laboratory. Pine-needle degradation by *Stereum hirsutum* was studied under conditions of solid fermentation with the aim of accelerating its decomposition, avoiding the accumulation *in situ* and in view of the possible utilization of the residual organic matter [30]. The degradation of yard wastes by *Coprinus truncorum* was also evaluated [31]. Lignification protects polysaccharides from hydrolytic enzymes

and limits the digestibility of lignocellulosic materials by ruminant animals or by cellulases applied *in vitro*. Delignification of the raw materials by solid state fermentation with selective WRF increases their value as fodder for ruminants and as substrates for enzymatic saccharification and fermentation. Some lignin-degrading fungi, notably *Pleurotus* sp. and *Lentinus edodes* produce edible mushrooms and can directly convert lignocellulose into food for humans [7]. The production of lignocellulosic enzymes during growth and fruiting of the edible fungus *Lentinus tigrinus* on wheat straw was the matter of another of our investigations [32].

WRF are the most intensively studied dye-decolorizing microorganisms. As stated earlier, thanks to their nonspecific ligninolytic enzymes, these fungi are able to transform a wide range of organic compounds. Primarily, the decolorization of sulfonated polymeric dyes was used to assay ligninolytic activities and to assess the biodegradation capabilities of WRF. Later on, numerous WRF strains were used for the decolorization of distinct synthetic (textile) dyes and synthetic effluents, i.e., dye mixtures. Purified laccases, LiPs and MnPs are able to decolorize dyes of different chemical structure. Detailed studies on bioreactor performances are starting to emerge, seeking to extend the capacity of WRF to decolorize dyes in continuous or sequencing batch mode over long periods of time without the need for supplementation of new mycelium and, though a challenge, under non-sterile conditions. Other promising technological developments with potential for enzymatic treatment of effluents include the immobilization of laccase and the coimmobilization of MnP with glucose oxidase for *in situ* H_2O_2 formation [4, 11]. The ability to produce lignin-modifying enzymes and decolorize industrial dyes was also recently studied in various Argentinean WRF. Comparing the isolates with the well-known dye-degrader *P. chrysosporium*, a new fungus was identified: *Coriolus versicolor* f. *antarcticus*. Based on the magnitude of the decoloration obtained (whole cultures of this fungus were able to decolorize in an hour 28-100% of five different dyes added (Malachite Green, Remazol Brilliant Blue R; Xylidine, Indigo Carmine and Poly R-478), this strain seems potentially competitive in the bioremediation of textile processing effluents [33].

Two strategies for application in the degradation of recalcitrant compounds have been pursued: (1) direct transformation of pollutants by active cultures of white-rot fungi and (2) use of enzymes extracted from the culture media. The advantage of the second strategy is a higher independence from influences of wastewater composition that may affect the activity of the fungi, e.g. high salinity. A disadvantage is the higher costs resulting from the need for extraction and cleaning steps. But, crude culture filtrates offer additional advantages, their obtention process is not as expensive, and on the other hand, proteins or other factors present in the medium may stabilize crude enzymes [34].

For a broad application, the cost of enzymes is one of the main factors determining the economics of a process. Reducing the costs of enzyme production by optimizing the fermentation medium is the basic research for industrial application. Enzyme overproduction can be achieved by media engineering. The use of different statistical designs for medium optimization has been recently employed for xylanase and laccase production by fungal cultures [35-37]. These statistical methods, as compared to the common one-factor-at-a-time method proved to be powerful and useful tools. Traditional methods of optimization involved changing one independent variable while fixing the others at a certain level. This single-dimensional search is laborious, time consuming, and incapable of reaching a true optimum due to the interaction among variables. Response surface methodology is an experimental strategy for seeking the optimum conditions for a multivariable system. Response surface optimization techniques were applied to maximize ligninolytic enzyme production by *T. trogii* and the decolorization efficiency of its crude culture filtrates. The ability of the culture fluids to decolorize different synthetic textile dyes and the role of ligninolytic enzymes in the decolorization process was also investigated [23, 24].

WRF and their ligninolytic enzymes have also potential for the treatment of pulp and paper industry effluents: for their decolorization as well as the reduction of adsorbable organic halides and the chemical oxygen demand [38, 39, 40]. Currently the most important application of

enzymes is in the prebleaching of kraft pulp. The removal of lignin from chemical pulps is called bleaching, and it is necessary for aesthetic reasons and for improvement of paper properties. Present-day bleaching of kraft pulp uses large amounts of chlorine and chlorine chemicals. Byproducts from using these chemicals are chlorinated organic substances, some of which are toxic and mutagenic. Enzymes provide a very simple and cost-effective way to reduce the use of chlorine compounds, and other bleaching chemicals. Xylanase prebleaching technology is now in use at several mills worldwide. MnP, laccase plus ABTS or other mediators, can partially reproduce the delignifying effect of the fungus and substantially increased the bleachability of kraft pulps. Pulp bleaching with a laccase mediator system has reached pilot plant stage and is expected to be commercialized soon. WRF and their enzymes (ligninases and xylanases) are considered also for the treatment of wood chips prior to pulping. While ligninases attack the lignin, xylanases degrade hemicelluloses and make the pulp more permeable for the removal of residual lignin. Termed biopulping, this process removes not only lignin but also some of the wood extractives, thus reducing the pitch content and effluent toxicity. When biopulping is followed by mechanical pulping, there is as much as 30% energy saving, whereas when it is followed by sulfite pulping, the cooking time is dramatically reduced. The paper strength properties have also improve after biopulping. However, this process is still in its infancy and no full-scale biopulping plants are currently in operation [41].

On the other hand, WRF seem to be a promising material for biosorption of heavy metal ions from polluted water, since they can be easily cultivated in high yields on various substrates. Their pellets formed during submerged shaken cultivation have high surface/volume ratio and good mechanical properties. Their cell walls similarly to other fungi consist mostly of polysaccharides, peptides and pigments that have a good capacity for heavy metal binding. During the last decade WRF have been tested for their ability to adsorb many heavy metals from solutions, including Cu, Cd, Cr, Ni, Pb, Hg and rare earth elements such as U and Th. In addition to mycelia from submerged cultivation, dried fruit bodies collected in nature and mycelia immobilized on various supports have been used [42].

WRF are also active producers of cellulases and xylanases. Enzymes hydrolyzing cellulose have been categorized as endoglucanases (EGs) or exocellobiohydrolases (CBHs) that act synergistically. In this model the internal glucosidic bonds in the ß 1-4 chain are first hydrolyzed by EGs to generate free non-reducing ends, from which the CBHs then remove cellobiose units that are converted to glucose by ß-glucosidase [3]. Total biodegradation of xylan requires endo-ß-1,4-xylanase, which attacks the main chains of xylans; ß-xylosidase, which hydrolyzes xylooligosaccharides to xylose; and several accessory enzymes, such as α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, ferulic acid esterase, and *p*-coumaric acid esterase, which are necessary for hydrolyzing various substituted xylans [43]. Traditionally, the application of xylanases in conjunction with cellulolytic enzymes has been mainly considered for the bioconversion of lignocellulosic materials to produce fuel and other chemicals [35]. Cellulases, hemicellulases and pectinases are used in various industries, including food, (for the extraction and clarification of fruit and vegetable juices, production of fruit nectars and purees, altering the sensor properties of fruit and vegetables, extraction of olive oil, improving the quality of bakery products), brewery and wine (for beer brewing and wine production), animal feed (to improve the nutritional quality of animal feed), textile and laundry (for bio-stoning and bio-polishing; in household washing powders, since they enhance the detergent performance), pulp and paper (in biomechanical pulping, biobleaching of kraft pulps, biodeinking), and agriculture (for controllimg plant diseases, as well as in enhancing plant growth and development). Today these enzymes, mostly from the deuteromycete fungi *Trichoderma* and *Aspergillus*, account for approximately 20% of the world enzyme market [44].

CONCLUSIONS

Although the ability of WRF and their ligninolytic enzymes to transform or mineralize a range of organopollutants has been confirmed, there are still unanswered questions about the catabolism of these compounds, particularly the precise role and regulation of the enzymes involved in these

processes. Bioremediation using microorganisms is already an established technology, although almost all currently employed treatments use prokaryotes. Treatments employing WRF offer the possibility to expand the substrate range of existing treatments via biodegradation of pollutants that cannot be removed by prokaryotes (or by chemical means). White-rot fungal bioremediation treatments may be particularly appropriate for *in situ* remediation of soils, where recalcitrant compounds (e.g. the larger PAHs) and bioavailability are problematic. A further application may lie in the operation of bioreactors for certain compounds (e.g. synthetic dyes) in liquid waste, where near-100% degradation efficiencies have been achieved using WRF [4]. Reducing the cost for enzyme production is still needed in order to develop enzymatic treatment processes for different industrial and environmental applications, which are more competitive with conventional and other novel treatment technologies. This may be achieved by means of process optimization using statistical experimental designs (process engineering), and the use of cheaper growth substrates such as agricultural and food wastes [25, 26]. Over expression of recombinant enzymes in heterologous systems, such as yeasts and bacteria, has been actively studied to enhance the production of fungal peroxidases and laccases, as well as to improve the catalytic activity and stability of these enzymes, during the last several years. Gene amplification and expression in appropriate hosts could be promising for abundant production and affordable price of ligninolytic enzymes, as is already the case with laccases used commercially in the pulp and paper industry [6, 26]. The ability of WRF to degrade a wide variety of environmentally persistent pollutants indicates their potential use in anti-pollution treatments. However, only a better understanding of the mechanisms used by these fungi will allow the development of technologies to apply these organisms to the cleaning-up of aquatic and terrestrial environments [45].

REFERENCES

1. Martinez, A. T., Speranza, M., Ruiz-Dueñas, F. J., Ferreira, P., Camarero, S., Guillén, F., Martinez, M. J., Gutierrez, A., and del Río, J.C. 2005, Int. Microbiol., 8, 195.
2. Teeri, T. T. 2004, Nat. Biotechnol., 22, 679.
3. Broda, P., Birch, P. R. J., Brooks, P. R., and Sims, P. F. G. 1996, Mol. Microbiol., 19, 923.
4. Pointing, S. B. 2001, Appl. Microbiol. Biotechnol., 57, 20.
5. Field, J. A., de Jong, E., Feijoo Costa, G., and de Bont, J. A. M. 1993, Trends Biotechnol., 11, 44.
6. Gianfreda, L., and Rao, M. A. 2004, Enz. Microbial Technol., 35, 339.
7. Reid, I. D. 1995, Can. J. Bot., 73, S1011.
8. Cameron, M. D., Timofeevski, S., and Aust, S. D. 2000, Appl. Microbiol. Biotechnol., 54, 751.
9. Kotterman, M. J. J., Vis, E. H., and Field, J. A. 1998, Appl. Environ. Microbiol., 64, 2853.
10. Meulenberg, R., Rijnaarts, H. H. M., Doddema, H. J., and Field, J. A. 1997, FEMS, Microbiol. Lett., 152, 45.
11. Wesenberg, D., Kyriakides, I., and Agathos, S. N. 2003, Biotechnol. Adv., 22, 161.
12. Cerniglia, C. E., Sutherland, J. B., and Crow, S. A. 1992, Microbial degradation of natural products, G. Winkelmann (Ed.), VCH Press, Weinheim, 193.
13. Martinez, D., Larrondo, L. F., Putnam, N., Gelpke, M. D. S., Huang, K., Chapman, J., Helfenbein, K. G., Ramaiya, P., Detter, J. C., Larimer, F., Coutinho, P. M., Henrissat, B., Berka, R., Cullen, D., and Rokhsar, D. 2004, Nat. Biotechnol., 22, 695.
14. D'Souza, T. M., Merritt, C. S., and Reddy, C. A. 1999, Appl. Environ. Microbiol., 65, 5307.
15. Levin, L., and Castro, M. A. 1998, IAWA J., 19, 169.
16. Planes, E., Bassi, M., Bensignor, J., and Burachik, M. 1986, ATIPCA, 25, 44.
17. Levin, L., and Forchiassin, F. 1997, Rev. Arg. Microbiol., 29, 1.
18. Levin, L., and Forchiassin, F. 1998, Acta Biotechnol., 18, 157.
19. Levin, L., and Forchiassin, F. 1998, World J. Microbiol. Biotechnol., 14, 443.
20. Levin, L., Ramos, A. M., and Forchiassin, F. 2002, Mycologia, 94, 377.
21. Haglund, C., Levin, L., Forchiassin, F., Lopez, M., and Viale, A. 2002, Rev. Arg. Microbiol., 34, 157.

22. Levin, L., Viale, A., and Forchiassin, F. 2002, Int. Biodet. Biodegr., 52, 1.
23. Trupkin, S., Levin, L., Forchiassin, F., and Viale A. 2003, J. Ind. Microbiol. Biotechnol., 30, 682.
24. Levin, L., Viale, A., and Forchiassin, F. 2005, Process Biochem., 40, 1381.
25. Pandey, A. Selvakumar, P., Soccol, C. R., and Nigam, P. 1999, Curr. Sci., 77, 149.
26. Ikehata, K., Buchanan, I. D., and Smith, D. W. 2004, J. Environ. Eng. Sci., 3, 1.
27. Papinutti, V. L., and Forchiassin, F. 2004, FEMS Microbiol. Lett., 231, 205.
28. Papinutti, V. L., Diorio, L., and Forchiassin, F. 2003, J. Ind. Microbiol. Biotechnol., 30, 157.
29. Papinutti, V. L., Mouso, N., and Forchiassin, F. 2006, Enz. Microbial Technol. (in press).
30. Mouso, N., Diorio, L., and Forchiassin, F. 2003, Rev. Arg. Microbiol., 35, 219.
31. Diorio, L., Mercuri, O., and Forchiassin, F. 2001, Rev. Arg. Microbiol., 33, 59.
32. Lechner, B. E., and Papinutti, V. L. 2006, Process Biochem., 41, 594.
33. Levin, L., Papinutti, V. L., and Forchiassin F. 2004, Bioresour. Technol. 94, 169.
34. Moreira, M. T., Palma, C., Mielgo, I., Feijoo, G., and Lema, J. M. 2001, Biotechnol. Bioeng., 75, 362.
35. Park, Y. S., Kang, S. W., Lee, J. S., Hong, S. I., and Kim, S. W. 2002, Appl. Microbiol. Biotechnol., 58, 761.
36. Ghanem, N. B., Yusef, H. H., and Mahrouse, H. K. 2000, Bioresour. Technol., 73, 113.
37. Vasconcelos, A. F. D., Barbosa, A. M., Dekker, R. F. H., Scarminio, I. S., and Rezende, M. I. 2000, Proc. Biochem., 35, 1131.
38. Bajpai, P. 1999, Biotechnol. Prog., 15, 147.
39. Wu, J., Xiao, Y., and Yu, H. 2005, Bioresour. Technol., 96, 1357
40. Selvam, K., Swaminathan, K., Song, M., and Chae, K. 2002, World J. Microbiol. Biotechnol., 18, 523.
41. Ali, M., and Sreekrishnan, T. R. 2001, Adv. Environ. Res., 5, 175.
42. Baldrian, P. 2003, Enz. Microbial Technol., 32, 78.
43. Saha, B. C. 2003, J. Ind. Microbiol. Biotechnol., 30, 279.
44. Bhat, M. K. 2000, Biotechnol. Adv., 18, 355.
45. Pereira Chagas, E., and Durrant, L. R. 2001 Enzyme Microb. Technol., 29, 473.