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Mini-review: Brazilian fungi diversity for biomass degradation

Estela Y. Valencia [a], Felipe S. Chambergo [b,*]

[a] Departamento de Microbiologia Microbiana, Centro Nacional de Biotecnologia (CSIC), Madrid, Spain
[b] Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, Av. Arlindo Bettio 1000, Ermelino Matarazzo, São Paulo, Brazil

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ABSTRACT

Brazil houses over 10% of the total number of known species on Earth, with a great diversity of plants and fungi. The collection, isolation, identification and conservation of filamentous fungi with relevance to agriculture, pharmaceutical, food and biotechnological industries in Biological Resource Centers (CRBs) is very important to the development of a nation's scientific and technological infrastructure. In Brazil, 36 fungal collections are registered in the database of International Collections. Several federal and state programs have encouraged the formation of a researcher's network in order to study natural resources and the nation's biodiversity. In this context, Brazilian researchers have been on the frontiers of knowledge, investigating the enzymatic systems from native filamentous fungi with potential for biomass degradation and biotechnological application. In this review, we address recent progress in Brazilian fungal research, focusing on the identification and study of fungi and enzymes with potential for biomass degradation and application in bioenergy.

1. Introduction

Brazil spans 8.5 million km^2, most of it located between Tropic of Capricorn and the equator, displaying several climatic zones. These climatic differences lead to great ecological variations, forming distinct biogeographical zones or biomes: Amazon, the world's biggest rainforest (which spans 49% of the territory); Pantanal (1.7%), the biggest flood plain; Cerrado (23.9%), with savannahs and woods; Caatinga (9.9%), with semiarid forests; Pampas' meadows (2%); and the Atlantic rainforest (13%). Brazil also has a coastline of 3.5 million km^2, which includes ecosystems such as coral reefs, dunes, mangroves, lakes, estuaries and swamps (IBGE, 2010). The diversity of biomes results in a great richness in flora and fauna: Brazil houses over 10% of the total number of known species on Earth, with a great diversity of plants and fungi, as well as animals (Alho, 2008).

It has been estimated that there may be from 1.5 to 5.1 million of fungal species in the world and about 100,000 species have been described (Hibbett et al., 2011). Lewinsonh and Prado (2005) estimated the total number of fungal species known (13,090–14,510) and predicted (150,300–263,900) to occur in Brazil.

There is no register of the total number of fungal species native of Brazil that have been identified or collected in all country. In the book "Catálogo de Plantas e fungos do Brasil" (Catalogue of plants and fungi from Brazil) (Forzza et al., 2010), there is register of 78 orders, 924 genera and 3608 species of fungi, and in the website "Fungos do Brasil" (Fungi from Brazil) (Minter and da Silva, 2007, www.cybertruffle.orguk/brazfung, access on 03/28/2013), 4325 species of Brazilian fungi are registered.

Geopolitical, economic, and employment concerns have promoted researchers, entrepreneurs, and legislators to focus on harnessing the potential of lignocellulosic feedstock for biotechnological application. The carbohydrate skeleton of plant cell walls needs to be depolymerized into simpler sugars for their application in industrial process, including ethanol production. The role of lignocellulolytic enzymes in the degradation of the plant cell wall is very important and cellulase accounts for nearly 20% of the total world enzyme market. In order to satisfy the market demand for cellulases, fungal microorganisms (*Hypocrea jecorina*, *Aspergillus niger* and *Fusarium* sp.) or bacteria (*Cellulomonas* sp., *Bacillus* sp.) are being employed (Chandel et al., 2012).

Here, we address recent progress in the Brazilian fungal research with a focus on identification and study of fungi and enzymes with potential for biomass degradation and application in bioenergy production.

2. Fungi collections

The Earth's biological resources are vital to humanity's economic and social development. In 1992, the Convention on Biological Diversity (CBD) established an strategic plan for Biodiversity 2011–2020. Amongst the strategic goals were: (i) to enhance the benefits to all from biodiversity and ecosystem services and (ii) to enhance implementation through participatory planning,

* Corresponding author. Fax: +55 11 3091 1020.
E-mail addresses: evalencia@cnb.csic.es (E.Y. Valencia), fscha@usp.br (F.S. Chambergo).

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knowledge management and capacity building (Convention on Biological Diversity, http://www.cbd.int/idb/, access on 03/29/2013).

Studies on fungal diversity have varying estimates. As the numbers differ greatly between 1.5 million species (Hawksworth, 2001), 3.5–5.1 million species (O'Brien et al., 2005) or 712,000 species (Schmit and Mueller, 2007) in the world. Filamentous fungi have traditionally been used to produce various substances of relevance to pharmaceutical, food and biotechnological industries; they have proved to be extremely useful to produce industrially applicable primary and secondary metabolites such as peptides, enzymes, organic acids and antibiotics (Lange et al., 2012).

The CBD gives sovereign rights to the country of origin and aims at fair and equitable benefit sharing, especially with regard to the country of origin in the case of successful economic exploiting of these genetic resources. The establishment of Biological Resource Centers (CRBs), which contain collections of culturable organisms (microorganisms, plants, animals, human cells, etc.), replicable parts of culturable organisms (genomes, plasmids, viruses, cDNA, etc.), non-culturable viable organisms, cells and tissues, as well as databases and bioinformatic resources related to these collections, is very important as key components of the scientific and technological infrastructure of a country.

In 2007, the Ministério do Meio Ambiente/IBAMA, through the Normative Instruction 160, creates the National Record of Biological Collections (CCBio) in order to gather the scientific collections from research institutions from Brazil, which constitute an information heritage regarding fauna, flora and genetics, important for conservation, studies, exploration and propagation of Brazilian species.

The establishment of the Brazilian Center of Biological Material (CBMB), an authorized center for storage of microorganisms with patent purposes, as part of the national policy for biotechnology development established by Decreto no 6.041/07 (Brazil, 2007), is ongoing and currently there is no second institution of the kind in all Latin America. Several collections from Brazilian institutions are registered on the World Federation for Culture Collections (WFCC, www.wfcc.info/home/, access in 03/28/13) (Table 1a) or on the Biodiversity Collection Index (www.biodiversitycollectionsindex.org/) (Table 1b). Other collections can be found at the Brazilian herbaria network (Sociedade Botânica do Brasil, www.botanica.org.br), SICOL data system (www.sicol.cria.org.br) and species link (www.splink.org.br), which are mostly collections from research centers and/or universities.

Enterprises such as INCT, "Herbário Virtual da Flora e dos Fungos" (Virtual Herbarium of Flora and Fungi) (www.inct.florabrasil.net/), species link (www.splink.org.br/), "Centro de Referência em Informação Ambiental" (Reference Center for Environmental Information) (CRIA, www.cria.org.br/), BIOTA/FAPESP – SinBiota (www.biota.org.br) – environmental information system of the State of São Paulo – and the information system for collections of biotechnological interest "Coleções de Interesse Biotecnológico" (SICol, http://www.sicol.splink.org.br/), have as a goal and strategy the propagation of electronic, public access information about the Brazilian biological resources centers and the biological collections, so that they serve as an integrated element of the diverse biological collections. However, several collections and subcollections do not have an online catalogue due to lack of resources, which limits the knowledge and propagation of their material and collected species.

3. Research

The systematic and organized study of the biodiversity of Brazil's native fungi should allow not only the collection and identification of species, but also bioprospecting. Hence, the diverse fungal collections from Brazil (Tables 1a and b) harbor an important biological material that can be explored, for example, to search for fungi capable of efficiently hydrolyzing biomass and to identify enzymes and genes of potential biotechnological interest.

Bioethanol, a product obtained by the fermentation of sugars present in sugar cane or corn, is a renewable clean product, whose utilization as fuel may contribute to the reduction of the greenhouse effect and air pollution, thus benefiting public health in the long term (Zaldivar et al., 2001). In the 1970s, Brazil started a program to substitute gasoline for ethanol; in this program, sugarcane was chosen as the feedstock to produce ethanol. However, only part of the biomass produced is used for bioenergy production; one-third of the plant is used for sugar production, one-third is bagasse, which is burnt for electricity production, and the

Table 1a
Selected Brazilian genetic resource collections.[a]

Acronym	WDCM number	Collection
BCCCp	WDCM 921	Brazilian Culture collection of Crinipellis perniciosa
CBMAI	WDCM 823	Brazilian Collection of Microorganisms from the Environment and Industry
CCB	WDCM 713	Coleção de Culturas de Basidiomicetos
CEMM	WDCM 880	CEMM – Centro Especializado em Micologia Médica
CG	WDCM 712	Collection of Fungi of Invertabrates
CM-UFMG	WDCM 1029	Collection of Microorganisms, DNA and Cells of Universidade Federal de Minas Gerais (UFMG)
CMM	WDCM 923	Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes
CNEN-LABPC	WDCM 710	Laboratorio de Pocos de Caldas
COAD	WDCM 989	Coleção Octávio de Almeida Drumond
ESAP	WDCM 294	Instituto Zimotecnico-Z
Fiocruz/CCFF	WDCM 720	Coleção de Culturas de Fungos Filamentosos
Fiocruz/CFAM	WDCM 957	Coleção de Fungos da Amazonia
Fiocruz/CFP	WDCM 951	Coleção de Fungos Patogenicos
Fiocruz/CMRVS	WDCM 575	Coleção de Microrganismos de Referencia em Vigilância Sanitária
Fiocruz/CMT	WDCM 948	Coleção Micológica de Trichocomaceae
FTI	WDCM 716	Centro de Biotecnologia e Química – CEBIQ
IAL	WDCM 282	Núcleo de Coleção de Microrganismos
IALMIC	WDCM 717	Micoteca do Instituto Adolfo Lutz
IGESALQ	WDCM 902	Coleção Microorganismos
IMT	WDCM 718	Micoteca do Instituto de Medicina Tropical de São Paulo
IPT	WDCM 721	Agrupamento de Biotecnologia, Culture Collection of Microorganisms
Micoteca IAL	WDCM 869	Micoteca do Instituto Adolfo Lutz
MMBF	WDCM 942	Micoteca Mario Barreto Figueiredo

[a] Registered in World Federation for Culture Collections (http://www.wfcc.info/home/): Collection of Fungi.

Table 1b
Selected Brazilian genetic resource collections.[a]

Collection	Code	Year founded	Web-site
Fundação Oswaldo Cruz Mycological Herbarium	IOC	1922	http://ccff.fiocruz.br/
Instituto Agronômico Mycological Herbarium	IACM	1932	http://herbario.iac.sp.gov.br/
Instituto Biológico Herbário	IBI	1931	http://www.biologico.sp.gov.br/
Instituto de Botânica Herbário	SP	1917	http://www.ibot.sp.gov.br/
Universidade de Brasília Herbário	UB	1963	http://www.florescer.unb.br/janela4.html
Universidade de Mogi das Cruzes Herbarium Mogiense	HUMC	2004	http://herbario.umc.br/pages/index.faces
Universidade de Santa Cruz do Sul Herbário	HCB	1984	ND
Universidade do Rio Grande Herbário	HURG	1982	ND
Universidade Estadual de Feira de Santana Herbário	HUEFS	1980	http://herbario.uefs.br/
Universidade Federal de Pernambuco Herbário	URM	1954	http://www.ufpe.br/
Universidade Federal de Santa Catarina Herbário	FLOR	1964	http://herbario.paginas.ufsc.br/
Universidade Federal do Piauí Herbário Graziela Barroso	TEPB	1977	ND
Universidade Federal do Rio de Janeiro Herbário	RFA	1953	www.museunacional.ufrj.br
Universidade Federal do Rio Grande do Norte Herbario	UFRN	ND	ND
Coleção e Herbário Fitopatológico "Verlande Duarte Silveira"	UFRRJ	1916	http://www.fito2009.com/fitop/fitopcol.htm

ND: Not determined.
[a] Biodiversity Collection Index http://www.biodiversitycollectionsindex.org/. Search using words-key: Fungi + Brazil (access in 03/28/2013).

remaining one-third is left in the field, which is decomposed by microorganisms (Soccol et al., 2010).

In the Brazilian sugarcane industry, large amounts of sugarcane bagasse is produced, sugarcane bagasse consists of approximately 50% cellulose and 25% each of hemicellulose and lignin and one renewable source for production of biofuels is the conversion of biomass-derived carbohydrates from sugarcane bagasse into bioethanol (Pandey et al., 2000; Zaldivar et al., 2001). Bioethanol production from lignocellulosic materials [second generation (2G) bioethanol] include pre-treatment processes and enzymes technology for conversion of cellulose/hemicellulose to fermentable sugars (Dias et al., 2011).

Over the years, a large number of microorganisms including bacteria, yeasts and fungi have been studied and characterized by their ability to degrade lignin, hemicelluloses, and cellulose. However, filamentous fungi are the preferred microorganisms on account of their production and secretion capacity of lignocellulosic enzymes for biomass degradation. In order to use lignocellulosic materials or sugarcane bagasse for bioethanol production, Brazilian research groups organized in networks are developing technology for conversion of biomass to fermentable sugars.

In 1999, the São Paulo Research Foundation (FAPESP, www.fapesp.br) created the BIOTA program, with focus not only on discovering, mapping and analyzing the origins, diversity and distribution of the flora and fauna of the State of São Paulo, but also on evaluating the possibilities of sustainable exploitation of plants, fungi or animals with economic potential and assisting in the formulation of conservation policies. In 2004, the Biota Network of Bioprospection and Biotrials (BIOprospecTA – www.bioprospecta.org.br) was created with the goal of bioprospecting new compounds of economic interest in the biodiversity of the State of São Paulo, including microorganisms, fungi, flora, and fauna, through bioassays (BIOTA-FAPESP, 2008).

The National Institute of Science and Technology of Bioethanol (INCT-Bioetanol, www.inctdobioetanol.com.br/), financed by the National Council of Science and Technology (CNPq, www.cnpq.br), comprises a network of 31 laboratories from five Brazilian states, divided into five research centers: gene expression and sugarcane transformation, plant physiology and cellular biology, genetics and sugarcane improvement, fungi prospection and enzyme engineering, enzyme characterization and processes engineering. The goal of the fungal prospecting center is to identify new species of fungi with ability to degrade biomass, to test the action of enzymatic cocktails and isolated enzymes, enzyme improvement, production of multifunctional enzymes, fungal transformation to enhance the production of one or more enzymes and heterologous enzyme expression. The objective of the center for enzyme characterization and processes engineering is to study the structure (amino acid sequencing and X-ray crystallography) and production of enzymes at industrial level.

In 2008, FAPESP created the Bioenergy Research Program (BIOEN, www.bioenfapesp.org/). The Bioenergy Program, BIOEN, supports public and private projects to advance and apply knowledge in fields related to bioenergy production and has funded 89 individual projects and approved 241 research fellowships (BIOEN, 2012). The BIOEN program comprises five divisions: biomass research, ethanol industrial technologies, bio-refinery technologies and alcohol chemistry, ethanol applications for motor vehicles and impacts. The focus of ethanol industrial technologies division is on engineering, processing and equipment design related to bioethanol production including research in cellulosic ethanol (development of high performance cellulases and hydrolases, reduction of the impact of fermentation inhibitors, development of microorganism strains capable of efficient fermentation of pentoses and hexoses and by-products recovery) (BIOEN, 2010).

In January 2010, the Brazilian Bioethanol Science and Technology Laboratory (CTBE – Laboratório Nacional de Ciência e Tecnologia do Bioetanol, www.bioetanol.org.br) was launched to contribute to the Brazilian leadership in the renewable energy sources and chemical industry raw material production sectors, mainly by improving the sugar cane bioethanol production chain and using sugar cane as a carbon source that can be efficiently transformed into fuels and others products for the food, chemical and pharmaceutical industries, and by consolidating plants in biorefineries, through state-of-the-art research, development and innovation. Brazilian researchers outside the network obtain resources through public call of CNPq or through direct submission of proposals to foundations that support research in their states.

4. Fungi and biomass degradation

Lignocellulosic materials are formed by three main polymeric constituents: lignin, cellulose and hemicellulose. Lignin is a recalcitrant polymer which is highly resistant towards chemical and biological degradation, present in the layers of the cell wall, forming, together with hemicelluloses, an amorphous matrix in which the cellulose fibrils are embedded and protected against biodegradation. Basidiomycetes fungi are well characterized by their ability to degrade or modify lignin in an enzymatic process catalyzed by ligninolytic peroxidase enzymes, and only a small number of ascomycetous fungi can perform lignin degradation, although very limited. Because

of their efficient and simultaneous degradation of lignin, hemicelluloses, and cellulose, basidiomycetes fungi are prime candidates for biomass degradation (Martinez et al., 2005; Floudas et al., 2012).

Polysaccharides cellulose and hemicellulose are the major constituents of plant biomass. The enzymatic hydrolysis of cellulose to glucose and other fermentable carbohydrates catalyzed by cellulases is a key stage in the process of production of second-generation bioethanol. Filamentous fungi are the major source of commercial cellulases. Cellulolytic fungi belonging to the genera *Trichoderma* (*T. viride*, *T. longibrachiatum*, *T. reesei*) have long been considered the most productive, and commercial cellulase preparations based on mutant strains of *T. reesei* (also known as *Hypocrea jecorina*) are produced on an industrial scale by many companies worldwide (Gusakov, 2011). However, new data demonstrate that fungi belonging to the genera *Penicillium*, *Acremonium* and *Chrysosporium* might represent alternatives because they are competitive to *T. reesei* on some important parameters, such as protein production level and cellulase hydrolytic performance per unit of activity or milligram of protein (Gusakov, 2011).

A complex of enzymes is necessary for biomass degradation; for example, the white-rot Brazilian fungus *Pycnoporus sanguineus* PF2, show cellulase and hemicellulase (FPase, endoglucanase, β-glucosidase, xylanase, mannanase, α-galactosidase, α-arabinofuranosidase, and polygalacturonase) activities, when cultivated under submerged fermentation using corncobs as carbon source (Falkoski et al., 2012). However, the major bottleneck in the fungi selection for enzyme production is the need for a complex of enzymes, thus these fungi are able to secrete large or relatively low amounts of one or other enzyme and this ability can be influenced by different substrate composition and/or nutritional availability of carbon source. de Almeida et al., 2011 showed that the highest FPase, endoglucanase, and xylanase activities were produced by *Acremonium* sp. EA0810 in medium containing sugarcane bagasse, the highest β-glucosidase activity was produced by *Acremonium* sp. EA0810 cultivated using D-xylose and that *A. zeae* EA0802 had the highest α-arabinofuranosidase and α-galactosidase activities in medium containing xylan.

The establishment of Brazilian research networks and the widespread availability of inexpensive lignocellulosic biomass such as sugarcane bagasse or agroindustrial residues stimulate the search for native fungi which efficiently use such waste. The focus of this review is in material published in journals available at PubMed database from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using a combination of key words (e.g., Brazil plus fungi, cellulose, lignocelluloses, lignin, xylan) and selected papers showing the identification and analysis of lignocellulosic enzymes from fungi collected in Brazil.

A total of 51 articles fully referenced published in the 2000–2013 period were analyzed. Table 2 provides a list of native fungi isolated in Brazil, lignocellulosic enzymes identified, sample/collection site and culture collection deposited.

A total of 136 isolates belonging to 23 genera and 45 species were reported. Ascomycetes fungi of the genera *Paecilomyces* (1 strains), *Aspergillus* (9 strains), *Acremonium* (3 strains), *Pestalotiopsis* (1 strain), *Penicillium* (4 strains), *Nigrospora* (1 strain), *Neosartorya* (1 strain), *Thermomyces* (1 strains), *Trichoderma* (83 strains), *Thermoascus* (3 strains), *Colletotrichum* (1 strains), *Fusarium* (1 strain), *Cladosporium* (2 strains), *Monodictys* (1 strain), *Phoma* (1 strain), *Tetraploa* (1 strain), *Xylaria* (2 strains) and basidiomycetes of the genera *Agaricus* (1 strains), *Pycnoporus* (1 strains), *Pleurotus* (2 strains), *Marasmiellus* (1 strains), *Tinctoporellus* (1 strains) and *Peniophora* (1 strains), were identified and studied.

The potential of biodiversity of soil fungi present in the Atlantic rainforest (Banhado Grande, SP, Brazil) was studied and 1211 strains were isolated and divided into the groups: Hyphomycetes, Ascomycetes, Zygomycetes, Coelomycetes, and fungi-like organisms (Oomycetes). From these, 112 species were identified, 8 of which down to the genus level; among the strains, 67 were cellulolytic (Tauk-Tornisielo et al., 2005). A microbial screening of xylanase producers was carried out in Brazilian Cerrado area in Selviria city, Mato Grosso do Sul State, Brazil. About 50 bacterial strains and 15 fungal strains producing xylanase were isolated from soil sample. Amongst these isolated microorganisms, a fungus, *Neosartorya spinosa* (strain P2D16), was identified as a good xylanase producer, using as substrate source wheat bran and corncobs (Alves-Prado et al., 2010). The data suggest a considerable potential for the identification of fungi with lignocellulosic enzyme activity in the Brazilian biodiversity.

The most studied fungi have been isolated through the screening of the soil, plants, sugarcane bagasse, decomposed wood, or obtained from Brazilian culture collection (Tables 1a and 1b). Isolated strains from *Trichoderma* showing potential biotechnological applications of enzymes have been studied: cellobiohydrolase I and endoglucanase of *T. harzianum* IOC 3844 (de Castro et al., 2010a; Colussi et al., 2011), β-glucosidase, FPase, and endoglucanase of *T. harzianum* IOC-4038 (de Castro et al., 2010b), FPase, xylanase and β-glucosidase of *T. harzianum* P49P11 (Delabona et al., 2012), and xylanase of *T. inhamatum* (de Oliveira da Silva and Carmona, 2008). Lignocellulosic enzymes identified in *Aspergillus* strains were: β-D-xylosidase of *A. ochraceus* (Michelin et al., 2012c), endoglucanase, FPase and xylanase of *A. niger* A12 (Cunha et al., 2012; Vitcosque et al., 2012), endoglucanase and endo-β-1,4-xylanase of *A. fumigatus* FBSPE-05 (Grigorevski-Lima et al., 2009; Souza et al., 2012), endoglucanase, β-glucosidase and xylanase of *A. niger* (Farinas et al., 2010), β-glucosidase, cellulase and endoglucanase of *A. japonicus* URM5620 (Herculano et al., 2011), xylanase of *A. terricola* and *A. ochraceus* (Michelin et al., 2010), and pectinase and xylanase of *A. niger* F3 (Rodriguez-Fernandez et al., 2011).

The habitat of fungi is diverse; Bezerra et al. (2012) studied endophytic fungi (*Cladosporium cladosporioides*, *C. sphaerospermum*, *Acremonium terricola*, *Monodictys castaneae*, *Penicillium glandicola*, *Phoma tropica*, *Tetraploa aristata*, *Aspergillus japonicas*) isolated from *Opuntia ficus-indica* with xylanolytic, proteolytic, pectinolytic and cellulolytic activity. Laccase activity was detected in marine basidiomycetes fungi: *Marasmiellus* sp. CBMAI 1062, *Peniophora* sp. CBMAI 1063 and *Tinctoporellus* sp., isolated from the Brazilian sponges *Amphimedon viridis* and *Dragmacidon reticulate*, collected in the city of São Sebastião on the northern coast of the State of São Paulo, Brazil (Bonugli-Santos et al., 2010).

One strategy of mutagenesis for the improvement of cellulase production was developed in a strain of *Penicillium echinulatum* using ultraviolet (UV) light and hydrogen peroxide (H_2O_2), having resulted in the isolation of two new cellulase-secreting *P. echinulatum* mutants: strain 9A02S1 and strain 9A02D1 (Dillon et al., 2006). The strain 9A02S1 (DSM18942) showed high production of cellulase, endoglucosidase, β-glucosidase, and xylanases enzymes (Camassola and Dillon, 2010) and the secretome profile after growth on integral sugarcane bagasse, microcrystalline cellulose and three types of pre-treated sugarcane bagasse revealed that its enzymatic repertoire is geared mainly towards the production of cellulases (endogluganases, cellobiohydrolases and β-glucosidases), xylanases and pectinases, showing that this fungus has a potential biotechnological application (Ribeiro et al., 2012).

The secretome analysis of *T. harzianum* T4 grown on cellulose or sugarcane bagasse medium identified chitinases, endo-N-acetylglucosaminidases, hexosaminidases, galactosidases, xylanases, exo-1,3-glucanases, endoglucanases, xylosidases, α-L-arabinofuranosidase, N-acetylhexosaminidases, cellobiohydrolase I-II, xylan 1,4-β-xylosidase, acetyl xylan esterase, endochitinase arabinogalactan, cutinase, α-N-arabinofuranosidase, glucoamylase, endo-beta-1,4-glucanase, swollenin, and α-1,3-glucanase (do Vale et al., 2012; da Silva et al., 2012).

Table 2
Selected Brazilian fungi and biomass degradation enzymes.

Fungi strain	Enzymes	Isolated/Brazilian culture collection	Refs.
Acremonium terricola, *Aspergillus japonicus Saito*, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Fusarium lateritium*, *Monodictys castaneae*, *Nigrospora sphaerica*, *Penicillium aurantiogriseum*, *P. glandicola*, *Pestalotiopsis guepinii*, Isolate PF103, 104, 108, 117, 202, 208, 300, 303, 304, *Phoma tropica*, *Phomopsis archeri*, *Tetraploa aristata*, *Xylaria* sp. 1, *Xylaria* sp. 2	Pectinase, Cellulase, Xylanase, Protease	The samples of forage cactus were collected in the municipality of Itaíba, Pernambuco	Bezerra et al. (2012)
Acremonium. zeae EA0802 and A. sp. EA0810	Activity—FPase, endoglucanase, β-glucosidase, xylanase, α-arabinofuranosidase and α-galactosidase	Corn seeds/Laboratory of Biochemical Technology at Universidade Federal de Viçosa, Viçosa, Minas Gerais	de Almeida et al. (2011)
Agaricus brasiliensis CS1, *Pleurotus ostreatus* H1, *Aspergillus flavus*	Xylanase, polygalacturonase	Edible and Medicinal Mushroom Laboratory, Federal University of Lavras; National Research Centre for Genetic Resources and Biotechnology, Cenargen, and Enzymology Laboratory, University of Brasília. Distrito Federal	de Siqueira et al. (2010)
Aspergillus ochraceus	Xylanase and β-Xylosidase	Decomposing fruits and leafs, in the Ribeirão Preto region, São Paulo/Mycology Culture Collection URM from Federal University of Pernambuco, Recife	Michelin et al. (2012a)
Aspergillus fumigatus RP04 *A. niveus* RP05	Xylanase	Soil and decomposing leaves of the Campus of São Paulo University at Ribeirão Preto, São Paulo./Deposited in Pernambuco Federal University, Pernambuco	Peixoto-Nogueira et al. (2009)
Aspergillus japonicus URM5620	β-Glucosidase, total cellulase, endoglucanase	Mycology Department's Mycoteca—URM, at Federal University of Pernambuco. Pernambuco	Herculano et al. (2011)
Aspergillus niger A12	Endoglucanase, FPase, endoglucanase, xylanase	Black pepper/Embrapa Food Technology collection, Rio de Janeiro, Rio de Janeiro	Cunha et al. (2012) and Vitcosque et al. (2012)
Aspergillus niger	Endoglucanase, b-glucosidase and xylanase	Embrapa Food Technology collection, Rio de Janeiro, Rio de Janeiro	Farinas et al. (2010)
Aspergillus niger F3	Pectinase, xylanase	Biotechnology and Bioengineering, Division of the Federal University of Paraná. Paraná	Rodriguez-Fernandez et al. (2011)
Aspergillus niger, *A. niveus*, *A. ochraceus*	Xylanases	soil with decomposing fruit and leaves from the Ribeirão Preto region, São Paulo/André Tosello Foundation, São Paulo	Betini et al. (2009)
Aspergillus niveus	Pectin Lyase	*Mangifera indica*/Pernambuco Federal University, Pernambuco	Maller et al. (2012)
Aspergillus ochraceus	β-D-Xylosidase	Decomposing fruits and leaves, in the Ribeirão Preto regiao, São Paulo/Mycology Culture Collection URM at Federal University of Pernambuco. Recife	Michelin et al. (2012c)
Aspergillus terricola	Xylanase and *β*-xylosidase	Tree trunk surface (*Hovenia dulcis*), Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, São Paulo/Mycology Culture Collection URM at the Federal University of Pernambuco, Pernambuco	Michelin et al. (2011)
Aspergillus terricola, *A. ochraceus*	Xylanase	Nature, in São Paulo State and classified by Mycology Culture Collection URM at the Federal University of Pernambuco. Pernambuco	Michelin et al. (2012b)
Aspergillus. fumigatus FBSPE-05	Endo-β-1,4-xylanase; endoglucanases	Sugarcane bagasse sample, collected from a sugar mill	Grigorevski-Lima et al. (2009) and Souza et al. (2012)
Colletotrichum graminicola	β-Glucosidase, β-Xylosidase, Xylanase	Amazon rainforest soil, Amazonas	Zimbardi et al. (2013)
Marasmiellus sp. CBMAI 1062, *Tinctoporellus* sp. CBMAI 106, *Peniophora* sp. CBMAI 1063	Laccases	Brazilian sponges Amphimedon viridis and Dragmacidon reticulate, São Sebastião on the northern coast of São Paulo state/Brazilian Collection of Microorganisms from Environment and Industry (CBMAI)	Bonugli-Santos et al. (2010)
Neosartorya spinosa	Xylanase	Soils from wood and organic material in decomposition from the Cerrado area in Selviria city, Mato Grosso do Sul State	Alves-Prado et al. (2010)
Paecilomyces variotii	Polygalacturonase	Decaying hemicellulosic material collected in the state of São Paulo/Collection of the Recife Mycology University—PE, Brazil, WFCC, number 604.	de Lima Damásio et al., 2010
Penicillium viridicatum RFC3	Pectate Lyase, Exopolygalacturonase	Decaying vegetables in São José do Rio Preto, São Paulo	Gomes et al. (2009) and Ferreira et al. (2010)
Penicillium echinulatum 9A02S1 (DSM	β-Glucosidases, Endoglucanases. Xylanases	Division of Enzyme and Biomass, Institute of	Sehnem et al.

(*continued on next page*)

Table 2 (*continued*)

Fungi strain	Enzymes	Isolated/Brazilian culture collection	Refs.
18942)		Biotechnology, Caxias do Sul, Rio Grande do Sul	(2006) and Camassola and Dillon (2010)
Pleurotus sajor-caju PS-2001	Laccases	Institute of Biotechnology, University of Caxias do Sul, Rio Grande do Sul	Bettin et al. (2009)
Pycnoporus sanguineus PF-2	Endoglucanase, β-glucosidase, Glucosidase Cellobiase, Xylanase Mannanase, α-Galactosidase, β-Xylosidase, β-Mannosidase α-Arabinofuranosidase Polygalacturonase	Laboratory of Forest Pathology and Genetics of Plant Pathogen Interaction, Federal University of Viçosa, Minas Gerais	Falkoski et al. (2012)
Strains of *Trichoderma*	Endoglucanase, β-glucosidase, and xylanase	Embrapa's mycology collection. Embrapa Meio Ambiente, Jaguariuna, São Paulo and Embrapa Recursos Geneticos, Brasilia, DF	Florencio et al. (2012)
T. harzianum P49P11	FPase, xylanase, and β-glucosidase	Soil and decomposed wood samples from the Amazon forest reserve of Embrapa (Belém, PA)	Delabona et al. (2012, 2013)
Thermoascus aurantiacus CBMAI-756	Polygalacturonase	Decaying hemicellulosic material collected in the state of Amazonas/Coleção Brasileira de Microrganismos de Indústria e Meio Ambiente-CBMAI, Unicamp, Campinas, São Paulo	Martins et al. (2007)
Thermoascus aurantiacus 179–5	α-Glucosidase	Decaying hemicellulosic material collected in the State of Amazonas	Carvalho et al. (2006)
Thermoascus aurantiacus CBMAI 756	Xylanase	Lab. of Applied Biochemistry and Microbiology of Sao Paulo State Univ. (UNESP), São Paulo	Oliveira et al. (2010)
Thermomyces lanuginosus IOC-4145	Xylanase	Soil at IBILCE-UNESP, São Paulo/Fundação Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro	Damaso et al. (2004)
Trichoderma inhamatum	Xylanase	Environmental Studies Center-CEA/UNESP. São Paulo	de Oliveira da Silva and Carmona (2008)
Trichoderma harzianum strain T4	Chitinases, endochitinases, endo-N-acetylglucosaminidases, hexosaminidases, galactosidases, xylanases, exo-1,3-glucanases, endoglucanases, xylosidases, α-L-arabinofuranosidase, N acetylhexosaminidases, and other enzymes	Fungal culture collection of Laboratory of Enzymology University of Brasilia, Brasilia. Distrito Federal	Do Vale et al. (2012)
Trichoderma harzianum IOC-3844	Cellobiohydrolase I, Endoglucanase, *β*-glucosidase and FPase activities	Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Rio de Janeiro	de Castro et al. (2010a) and Colussi et al. (2011)
Trichoderma harzianum IOC-4038	β-Glucosidase, FPase, and endoglucanase	Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Rio de Janeiro	de Castro et al. (2010b)
78 preselected strains of the genus *Trichoderma*, *Trichoderma* sp. CG 104NH	Endoglucanase	Embrapa Meio Ambiente, Jaguariuna, São Paulo, and Embrapa Recursos Geneticos, Brasilia, Distrito Federal	Florencio et al. (2012)
1211 strains were isolated *(*groups: *Hyphomycetes*, *Ascomycetes*, *Zygomycetes*, *Coelomycetes*, and *Oomycetes).* 67 were cellulolytic: *Aspergillus giganteus*, *A. niger*, *A. fumigatus*	Cellulases, xylanases,	Soil samples from Banhado Grande in the state of São Paulo	Tauk-Tornisielo et al. (2005)

The data shown in Table 2 indicate that the enzymatic complexes that have the ability to degrade lignocellulosic materials or agricultural wastes are present in native fungi from Brazil.

5. Enzymes

Several pretreatment processes can be used to make the biomass more accessible to the action of enzymes and to obtain fermentable sugars such as xylose, arabinose and glucose. The pretreatment of biomass can be physical (milling, pyrolysis), physicochemical (steam explosion, hydrothermal, ammonia fiber explosion, CO_2 explosion), chemical (diluted acid hydrolysis, concentrated acid hydrolysis, alkaline hydrolysis, organosolv) and biological (enzymatic hydrolysis by microorganisms). The principal advantages of physical, physicochemical and chemical pretreatment is that inhibitors are not produced, the short time process and the use of low or medium temperatures, respectively, and the disadvantages are the high cost, inhibitors formation and the fact that it is not environmentally friendly (Alvira et al., 2010).

Enzymatic hydrolysis is the most feasible, convenient and ecofriendly method for lignocellulose hydrolysis, resulting in appreciable sugar yields of more than 90% under optimized hydrolytic conditions (Chandel et al., 2012). The enzymatic degradation of biomass or lignocellulosic materials to simpler sugars is a process that requires the cooperative action of enzymes that act synergistically for degradation of cellulose (endoglucanases, cellobiohydrolases, β-glucosidase), hemicellulose (xylanases, mannanases, β-xylosidases, β-mannosidases, α-arabinofuranosidases, α-galactosidases, acetyl xylan esterase, feruloyl esterases, p-coumaryl esterases, and α-glucuronidase) and lignin (manganese peroxidase, lignin peroxidase and laccases) (Martinez et al., 2005; Pauly and Keegstra, 2008). The crystallinity of cellulose, its accessible surface area and protection by lignin and hemicellulose, the degree of cellulose polymerization, the degree of acetylation of hemicelluloses, the topochemical distribution and varied content of lignin and hemicellulose that determine to recalcitrance of the biomass are the main factors considered as affecting the rate of biological degradation of lignocelluloses by the enzymes (Taherzadeh and Karimi, 2008).

de Souza et al. (2012) analyzed the composition and structure of sugarcane cell wall, having revealed that ~30% of the cell wall content is cellulose, ~50% hemicelluloses, and ~10% pectins. In this study, it has been proposed that, in the absence of a pre-treatment, the intact cell walls would have to be first treated with pectinases

(endopolygalacturonase, pectin-methyl-esterase, α-arabinofuranosidase, and β-galactosidase) together with lichenase to hydrolyze β-glucan, feruloyl esterase to fully release pectins, and feruloyl- and acetyl-esterases to break the ferulic bridges among hemicelluloses. After this enzymatic pre-treatment, the cellulose is available for enzymatic attack by endo-β-glucanases, cellobiohydrolases, and β-glucosidases.

The ability of native fungal strains to show lignocellulosic activity was tested using different media, conditions, time points or carbon source, and quantified using different assays.

5.1. Cellulases

Total cellulase activity (FPase) (0.55 and 0.51 U mL^{-1}) was measured in *Acremonium* sp. EA0810 and *A. zeae* EA0802, respectively, when cultivated in submerged culture containing sugarcane bagasse as carbon source (de Almeida et al., 2011); 97 U L^{-1} were obtained in *Trichoderma harzianum* IOC-4038 cultivated in submerged fermentation with pre-treated sugarcane bagasse (namely, cellulignin) (de Castro et al., 2010b); 121 FPU/g, in *T. harzianum* strain P49P11 grown in delignified steam-exploded bagasse plus sucrose as carbon source (Delabona et al., 2012); 953.4 U/g dry substrate, in *Aspergillus japonicus* URM5620 on solid-state fermentation using castor bean meal as substrate (Herculano et al., 2011); 180.4 ± 8.1 IU/L were obtained in *Agaricus brasiliensis* CS1 using sugarcane bagasse as substrate (de Siqueira et al., 2010); 54.3 ± 1.3 IU/L in *Pleurotus ostreatus* H1 in culture containing sugarcane bagasse (10%) (de Siqueira et al., 2010); 199.2 ± 8.5 IU/L in *Aspergillus flavus* in culture containing corn residue (10%) (de Siqueira et al., 2010).

Exoglucanase activity (cellobiohydrolase) was determined for *Trichoderma harzianum* IOC-3844 in Avicel (1.25 U/mg) or p-nitrophenyl-β-D-cellobioside (1.53 U/mg) (Colussi et al., 2011); 5.7 ± 0.9 IU/L were obtained in *Agaricus brasiliensis* CS1, 74.9 ± 4.5 IU/L in *Pleurotus ostreatus* H1 grown in sugarcane bagasse (10%), and 11.4 ± 4.9 IU/L in *Aspergillus flavus* grown in corn residue (10%) (de Siqueira et al., 2010).

β-glucosidase activity (0.17 U mL^{-1}) was obtained in the submerged culture of *Acremonium* sp. EA0810 using D-xylose as carbon source (de Almeida et al., 2011); 40.13 ± 5.10 U gdm^{-1} were obtained in *Penicillium echinulatum* 9A02S1 in media containing different ratios of sugarcane bagasse and wheat bran (Camassola and Dillon, 2010); 745 U L^{-1} in *Trichoderma harzianum* IOC-4038 (de Castro et al., 2010b); 1730 IU/g in *T. harzianum* strain P49P11 (Delabona et al., 2012); and 88.3 U/g dry substrate in *Aspergillus japonicus* URM5620 (Herculano et al., 2011).

Endoglucanase activity (67.44 ± 0.25 U mg^{-1}) was identified in *Pycnoporus sanguineus* PF-2 (Falkoski et al., 2012); 1052 ± 34 IU/L were observed in *Aspergillus niger* A12 (Cunha et al., 2012); 290.47 ± 43.57 U gdm^{-1} in *Penicillium echinulatum* 9A02S1 (Camassola and Dillon, 2010); 559 U L^{-1} in *Trichoderma harzianum* IOC-4038 (De Castro et al., 2010a,b); 365 U L^{-1} in *A. fumigatus* FBSPE-05 using sugarcane bagasse (1%) (Grigorevski-Lima et al., 2009); 191.6 U/g dry substrate in *Aspergillus japonicus* URM5620 (Herculano et al., 2011); 315.3 ± 30.7 IU/L in *Agaricus brasiliensis* CS1 (de Siqueira et al., 2010); 138.6 ± 9.6 IU/L in *Pleurotus ostreatus* H1 growth in sugarcane bagasse (10%) (de Siqueira et al., 2010); 432.7 ± 11.7 IU/L in *Aspergillus flavus* grown in corn residue (10%) (de Siqueira et al., 2010).

The secretion and activity of cellulosic enzymes is an indicative that the Brazilian fungi are well equipped to digest cell wall components of plants, and the production of enzymes by fungi grown in various substrates, including low-cost agro-industrial residues such as sugar cane bagasse and corn straw, indicates that these can be good carbon sources for cellulases production.

5.2. Hemicellulases

Xylanase activity were found to be 2.46 U mL^{-1} and 2.16 U mL^{-1} in the submerged culture of *Acremonium* sp. EA0810 and *A. zeae* EA0802, respectively, using sugarcane bagasse as carbon source (de Almeida et al., 2011); 80.08 ± 2.80 U mg^{-1} in *Pycnoporus sanguineus* PF-2 (Falkoski et al., 2012), 90 to 126 U/mL after 90 h of induction in *Thermomyces lanuginosus* IOC-4145 (Damaso et al., 2003); 850 U/mL in *Thermomyces lanuginosus* IOC-4145 (Damaso et al., 2004); 8000 IU/g in *T. harzianum* strain P49P11 (Delabona et al., 2012); 11.00 ± 0.16 and 10.95 ± 0.25 U/mL) in *Aspergillus terricola marchal* and *A. ochraceus* when grown using Segato Rezzatti or Adams culture medium, respectively (Michelin et al., 2010); 244.02 U/mL in *Trichoderma inhamatum* grown with xylan as carbon source (de Oliveira da Silva and Carmona, 2008); 1226.0 ± 80.2 IU/L and 4008.0 ± 139.5 IU/L, in *Pleurotus ostreatus* H1 grown in sugarcane bagasse (10%) and *Aspergillus flavus* grown with corn residue (10%), respectively (de Siqueira et al., 2010); 130 U/mL in *Theroascus aurantiacus* CBMAI 756 (Oliveira et al., 2010). *Aspergillus fumigatus* RP04 and *A. niveus* RP05 produced high levels of xylanase (368 total U) on agricultural residues (corncob or wheat bran) or birchwood xylan (100.3 total U), respectively (Peixoto-Nogueira et al., 2009).

Mannanase activity of 34.00 ± 2.16 U mg^{-1} and 206.4 ± 53.9 IU/L in *Pycnoporus sanguineus* PF-2 and *Agaricus brasiliensis* CS1, respectively, was determined (de Siqueira et al., 2010; Falkoski et al., 2012); an α-arabinofuranosidase activity of 0.045 U mL^{-1} was achieved in *A. zeae* EA0802 in submerged culture using oat spelt xylan as carbon source (de Almeida et al., 2011).

5.3. Ligninases

Laccases activity was 37 U mL^{-1} in *Pleurotus sajor-caju* PS-2001 after culture with fructose (Bettin et al., 2009); 971.2 U L^{-1}, 709.03 U L^{-1} and 751.54 U L^{-1} in *Marasmiellus* sp. CBMAI 1062, *Peniophora* sp. CBMAI 1063 and *Tinctoporellus* sp. CBMAI 1061, respectively (Bonugli-Santos et al., 2010).

5.4. Other enzymes

Polygalacturonase activity was 148.24 ± 3.36 U mg^{-1} in *Pycnoporus sanguineus* PF-2 (Falkoski et al., 2012); 219.0 ± 78.4 IU/L in *Agaricus brasiliensis* CS1 (de Siqueira et al., 2010); 3965.4 ± 105.3 IU/L and 4547.6 ± 158.3 IU/L in *Pleurotus ostreatus* H1 grown in sugarcane bagasse (10%) and *Aspergillus flavus* grown in corn residue (10%), respectively (de Siqueira et al., 2010). Pectinase activity with zone of activity of 0.84 and 0.61 cm was detected in *Aspergillus japonicus* and *Penicillium glandicola*, respectively (Bezerra et al., 2012). *Penicillium viridicatum* RFC3 displayed a pectate lyase activity of 1500 U/mL (Ferreira et al., 2010).

The data analysis shows that the value of activity enzymes are diverse (one needs to consider the differential assays used), but it is necessary to improve the evaluation of the best conditions for enzymes production. In this context, different studies reporting the conditions of enzymes production were published. Cunha et al. (2012) obtained an endoglucanase productivity of 57 ± 13 IU/L/h in *Aspergillus niger* A12 using 5-L bubble column bioreactor and culture medium supplemented with 10 g/L glucose and 1% (w/v) of sugarcane bagasse; a high level of endo-β-1,4-xylanase (219.5 U g^{-1}) production by *Aspergillus fumigates* FBSPE-05 was obtained in solid-state fermentation using sugarcane bagasse (Souza et al., 2012); production of xylanases from *Aspergillus niveus* (927.7 total U), *A. niger* (794.7 total U) and *A. ochraceus* (752.6 total U) was obtained under solid-state fermentation using wheat bran or a mixture of corncob and wheat bran, respectively (Betini et al., 2009); production of laccases was studied in liquid cultures

of *Pleurotus sajor-caju* strain PS-2001, in medium with fructose or glucose as carbon sources, in which the maximum enzyme activities obtained were 37 and 36 U mL^{-1}, respectively (Bettin et al., 2009). A response surface methodology (RSM) was used for the optimization of β-glucosidase, β-xylosidase and xylanase production by *Colletotrichum graminicola* in sugarcane trash, peanut hulls and corncob, yielding 159.3 ± 12.7 U g^{-1}, 128.1 ± 6.4 U g^{-1} and 378.1 ± 23.3 U g^{-1}, respectively (Zimbardi et al., 2013); the production of cellulases in *Trichoderma harzianum* IOC-3844 (6358 U L^{-1} of endoglucanase activity) was studied using a pre-treated sugarcane bagasse (namely, cellulignin), by submerged fermentation (de Castro et al., 2010a); production of cellulases and xylanases by *Penicillium echinulatum* 9A02S1, in solid-state fermentation was performed using different ratios of sugarcane bagasse and wheat bran (Camassola and Dillon, 2010); a procedure for on-site production was developed for *Trichoderma harzianum* strain P49P11, when enzymatic activities were found to be up to 121 FPU/g, 8000 IU/g, and 1730 IU/g of delignified steam-exploded bagasse plus sucrose for cellulase, xylanase and β-glucosidase, respectively (Delabona et al., 2012); *Aspergillus niveus saito* produced high levels of pectin lyase under submerged fermentation in medium supplemented with citrus pectin, without agitation (Maller et al., 2012); xylanase production was higher when cultures from *Aspergillus terricola* were grown in an airlift bioreactor using wheat bran as carbon source (Michelin et al., 2011); better conditions for xylanase and β-xylosidase production were observed when *Aspergillus ochraceus* was cultivated with 1% wheat bran adding 10% wheat straw liquor in a stirred tank bioreactor (Michelin et al., 2012c). Solid-state fermentation and citrus peel were used for producing pectinase and xylanase enzymes from *Aspergillus niger* F3, with activities of 265 U/g and 65 U/g, respectively, with air flow intensity of 1 V kg M (Rodriguez-Fernandez et al., 2011).

After test of activity and production, the next step is to construct the enzymatic cocktails containing a proper ratio of enzymes so that they have synergic activity. Synergic action of *C. graminicola* crude extract and *T. reesei* cellulases on raw sugarcane showed potential to constitute an efficient cocktail for lignocellulosic materials hydrolysis (Zimbardi et al., 2013), and supplementation with two selected accessory enzymes (pectinase and arabinofuronidase) on *T. harzianum* P49P11 enzymatic extract led to an increase in saccharification of pre-treated sugarcane bagasse (Delabona et al., 2013).

In nature, fungi are able to secrete large or relatively low amounts of one or other enzyme. To solve this problem, an homologous or heterologous expression system is necessary for the production of proteins. The *Pichia pastoris* system with the inducible promoter AOX1 was used for efficient production of β-1,4-xylanase, endoglucanase and endoglucanase III from *Thermomyces lanuginosus* IOC-4145, *Penicillium echinulatum* 9A02S1 (DSM18942) and *Trichoderma harzianum* IOC-3844, respectively (Damaso et al., 2003; Rubini et al., 2010; Generoso et al., 2012).

Extracellular polygalacturonase produced from *Paecilomyces variotii* grown in medium with 1.0% citric pectin or other carbon sources was purified to homogeneity through two chromatography steps using DEAE-Fractogel and Sephadex G-100 (de Lima Damásio et al., 2010); exopolygalacturonase from *Penicillium viridicatum* RFC3 was produced in submerged fermentation with orange bagasse and wheat bran as carbon sources and purified using Sephadex G-75 and Q-Sepharose (Gomes et al., 2009); β-xylosidase from *Aspergillus ochraceus* produced in medium with 1% (w/v) oat spelt xylan as carbon source was purified using DEAE-cellulose ion exchange chromatography, Sephadex G-100 and Biogel P-60 gel filtration (Michelin et al., 2012a); polygalacturonase produced in medium containing citrus juice processing waste from *Thermoascus aurantiacus* CBMAI-756 was purified by gel filtration and ion-exchange chromatography (Martins et al., 2007); α-amylase secreted by *Aspergillus niveus* was purified using DEAE fractogel ion exchange chromatography and Sephacryl S-200 gel filtration and thermal stability was improved by covalent immobilization on glyoxyl agarose (Silva et al., 2013).

Chimeric enzymes have been created with bifunctional hydrolytic activity that combine laccase – xylanase (Furtado et al., 2013) and laccase – β-1,3–1,4-glucanase (Ribeiro et al., 2011) activities. Crystal or X-ray structure was determined to endoglucanase 3 from *Trichoderma harzianum* (Prates et al., 2013), cellobiohydrolase I from *Trichoderma harzianum* IOC 3844 (Textor et al., 2013), endoglucanase III from *T. harzianum* (Vizona Liberato et al., 2012) and cellobiohydrolase I from *T. harzianum* (Colussi et al., 2010).

6. Concluding remarks and future directions

In nature, the breakdown of plant materials is done primarily by fungi, by means of secreted fungal enzymes, and after many years of research the conclusion is that the most efficient and gentle way of converting recalcitrant lignocellulosic materials into high value products for industrial purposes is through the use of fungal enzymes (Lange et al., 2012). Brazilian research needs increase in the understanding of the biology of the Brazilian fungi, through the study of how fungi degrade biomass in nature, as well as through the study of the biodiversity, proteome, metabolome, protein expression (homologous as well as heterologous), physiology (especially of extremophiles), secretome (regulation, composition and function), structure and discovery or creation of new enzymes and new molecular/bioinformatics tools. It is important to note that, in Brazil, sugarcane bagasse is a significantly cheaper carbon source for biotechnological process and that the use of agricultural residues as alternative carbon sources reduces the production costs and the price of the final product.

The key issues related to the implementation of enzymes to improve the biomass use in Brazil are: (i) search of novel and potent strains producing lignocellulosic enzymes and their improvement by classical approaches such as physical and chemical mutagenesis; (ii) development of recombinant strains for protoplast fusion, manipulation of inducer forming pathways, signaling cascades and/or activation of transcription of the cellulase genes, improved cellulase titers or plant-based expression of biomass degrading enzymes; (iii) design of highly active enzymes by adopting protein re-engineering techniques, directed evolution, rational design, bifunctional activity with increasing activity, thermostability and inhibitors resistance; (iv) design of a fine cocktail of enzymes (exoglucanase, endoglucanase, beta-glucosidases, xylanases and other ancillary enzymes) and development of novel measurement assays of enzymes activity; and (v) development of conditions of culture and carbon source for increased enzyme production.

Over the last years, research groups in Brazil have achieved significant advancement in the study of fungi and enzymes involved in biomass degradation. Analyses of data show some diversity in fungi that have been studied; however, it is still a small number. It is important to conserve the collected native fungi; it is also important that these collections have the necessary support to make their online strain catalogues available, which allow the knowledge and study of the isolated fungi. The financial support for students and researches, given by funding agencies, is important; however, a long-term strategy needs to be established with the scientific community to reach the goal of bioenergy generation from biomass, with incentive to a better interaction between research groups, international diffusion of the results obtained, incentive to innovation, register of patents and exchange of experience with international research groups.

Our suggestions of what we need to do and which questions should be answered to achieve a better understanding of the

roadmap of bioenergy production from biomass using native filamentous fungi are as follows: (i) intensify collaborations between mycologists and molecular biologists to answer the basic question of classical or molecular identification of fungi. It is possible that many fungal strains are stored in several collections waiting to be classified; (ii) develop analysis procedures in large scale to identify fungi with considerable activity of enzymes useful for biomass degradation; (iii) investigate all habitats in several regions of Brazil to increase the number of known fungal species, so that isolates can be available in collections to perform lignocellulosic enzyme activity assays; (iv) establish standard conditions for fungal cultures and lignocellulosic enzyme production, using waste biomass as carbon source, or establish an homologous/heterologous expression system which allows an efficient production and activity of recombinant enzymes; (v) support the formation of more research groups for the formulation of enzymatic cocktails, enzyme improvement, chimeric protein design and structural analyses; and (vi) produce and analyze the sequence data from Brazilian fungi.

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References

Alho, C.J., 2008. The value of biodiversity. Braz. J. Biol. 68, 1115–1118.
Alves-Prado, H.F., Pavezzi, F.C., Leite, R.S., de Oliveira, V.M., Sette, L.D., Dasilva, R., 2010. Screening and production study of microbial xylanase producers from Brazilian Cerrado. Appl. Biochem. Biotechnol. 161, 333–346.
Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Biores. Technol. 101, 4851–4861.
Betini, J.H., Michelin, M., Peixoto-Nogueira, S.C., Jorge, J.A., Terenzi, H.F., Polizeli, M.L., 2009. Xylanases from *Aspergillus niger*, *Aspergillus niveus* and *Aspergillus ochraceus* produced under solid-state fermentation and their application in cellulose pulp bleaching. Bioprocess Biosyst. Eng. 32, 819–824.
Bettin, F., Montanari, Q., Calloni, R., Gaio, T.A., Silveira, M.M., Dillon, A.J., 2009. Production of laccases in submerged process by *Pleurotus sajor-caju* PS-2001 in relation to carbon and organic nitrogen sources, antifoams and Tween 80. J. Ind. Microbiol. Biotechnol. 36, 1–9.
Bezerra, J.D., Santos, M.G., Svedese, V.M., Lima, D.M., Fernandes, M.J., Paiva, L.M., Souza-Motta, C.M., 2012. Richness of endophytic fungi isolated from *Opuntia ficus-indica* Mill. (Cactaceae) and preliminary screening for enzyme production. World J. Microbiol. Biotechnol. 28, 1989–1995.
BIOEN, 2010. Brazilian research on bioenergy, in: Program, B.F. (Ed). FAPESP, pp. 136.
BIOEN, 2012. BIOEN News – Newsleeter. Fapesp Bioenergy Program, São Paulo, pp. 6.
BIOTA, 2008. Knowledge and Sustainable use of Brazilian Biodiversity: Biota-FAPESP Program. The State of São Paulo Research Foundation. – São Paulo: FAPESP, São Paulo, pp. 208.
Bonugli-santos, R.C., Durrant, L.R., Sette, L.D., 2010. Laccase activity and putative laccase genes in marine-derived basidiomycetes. Fungal Biol. 114, 863–872.
Brazil, 2007. Decreto No. 6.041 Institui a Política de Desenvolvimento da Biotecnologia, cria o Comitê Nacional de Biotecnologia e dá outras providências. In: Civil PdRC (Ed).
Camassola, M., Dillon, A.J., 2010. Cellulases and xylanases production by *Penicillium echinulatum* grown on sugar cane bagasse in solid-state fermentation. Appl. Biochem. Biotechnol. 162, 1889–1900.
Carvalho, A.F., Goncalves, A.Z., da Silva, R., Gomes, E., 2006. A specific short dextrin-hydrolyzing extracellular glucosidase from the *thermophilic fungus Thermoascus aurantiacus* 179–5. J. Microbiol. 44, 276–283.
Chandel, A.K., Chandrasekhar, G., Silva, M.B., Silverio da Silva, S., 2012. The realm of cellulases in biorefinery development. Crit. Rev. Biotechnol. 32, 187–202.
Colussi, F., Textor, L.C., Serpa, V., Maeda, R.N., Pereira Jr., N., Polikarpov, I., 2010. Purification, crystallization and preliminary crystallographic analysis of the catalytic domain of the extracellular cellulase CBHI from *Trichoderma harzianum*. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 66, 1041–1044.
Colussi, F., Serpa, V., Delabona, P.da S., Manzine, L.R., Voltatodio, M.L., Alves, R., Mello, B.L., Pereira Jr., N., Farinas, C.S., Golubev, A.M., Santos, M.A., Polikarpov, I., 2011. Purification, and biochemical and biophysical characterization of cellobiohydrolase I from *Trichoderma harzianum* IOC 3844. J. Microbiol. Biotechnol. 21, 808–817.
Cunha, F.M., Esperanca, M.N., Zangirolami, T.C., Badino, A.C., Farinas, C.S., 2012. Sequential solid-state and submerged cultivation of *Aspergillus niger* on sugarcane bagasse for the production of cellulase. Bioresour. Technol. 112, 270–274.
da Silva, A.J., Gomez-Mendoza, D.P., Junqueira, M., Domont, G.B., Ximenes Ferreira Filho, E., de Sousa, M.V., Ricart, C.A., 2012. Blue native-PAGE analysis of *Trichoderma harzianum* secretome reveals cellulases and hemicellulases working as multienzymatic complexes. Proteomics 12, 2729–2738.
Damaso, M.C., Almeida, M.S., Kurtenbach, E., Martins, O.B., Pereira Jr., N., Andrade, C.M., Albano, R.M., 2003. Optimized expression of a thermostable xylanase from *Thermomyces lanuginosus* in *Pichia pastoris*. Appl. Environ. Microbiol. 69, 6064–6072.
Damaso, M.C., de Castro, A.M., Castro, R.M., Andrade, C.M., Pereira Jr., N., 2004. Application of xylanase from *Thermomyces lanuginosus* IOC-4145 for enzymatic hydrolysis of corncob and sugarcane bagasse. Appl. Biochem. Biotechnol. 113–116, 1003–1012.
de Almeida, M.N., Guimaraes, V.M., Bischoff, K.M., Falkoski, D.L., Pereira, O.L., Goncalves, D.S., de Rezende, S.T., 2011. Cellulases and hemicellulases from endophytic *Acremonium* species and its application on sugarcane bagasse hydrolysis. Appl. Biochem. Biotechnol. 165, 594–610.
de Castro, A.M., Ferreira, M.C., da Cruz, J.C., Pedro, K.C., Carvalho, D.F., Leite, S.G., Pereira, N., 2010a. High-yield endoglucanase production by *Trichoderma harzianum* IOC-3844 cultivated in pretreated sugarcane mill byproduct. Enzyme Res. 2010, 854526.
de Castro, A.M., Pedro, K.C., da Cruz, J.C., Ferreira, M.C., Leite, S.G., Pereira Jr., N., 2010b. *Trichoderma harzianum* IOC-4038: a promising strain for the production of a cellulolytic complex with significant beta-glucosidase activity from sugarcane bagasse cellulignin. Appl. Biochem. Biotechnol. 162, 2111–2122.
de Lima Damásio, A.R., da Silva, T.M., Maller, A., Jorge, J.A., Terenzi, H.F., Polizeli Mde, L., 2010. Purification and partial characterization of an exo-polygalacturonase from *Paecilomyces variotii* liquid cultures. Appl. Biochem. Biotechnol. 160, 1496–1507.
de Oliveira da Silva, L.A., Carmona, E.C., 2008. Production and characterization of cellulase-free xylanase from *Trichoderma inhamatum*. Appl. Biochem. Biotechnol. 150, 117–125.
de Siqueira, F.G., de Siqueira, A.G., de Siqueira, E.G., Carvalho, M.A., Peretti, B.M., Jaramillo, P.M., Teixeira, R.S., Dias, E.S., Felix, C.R., Filho, E.X., 2010. Evaluation of holocellulase production by plant-degrading fungi grown on agro-industrial residues. Biodegradation 21, 815–824.
de Souza, A.P., Leite, D.C.C., Pattathil, S., Hahn, M.G., Buckeridge, M.S., 2012. Composition and structure of sugarcane cell wall polysaccharides: implications for second-generation bioethanol production. Bioenergy Res. 16.
Delabona, P.da S., Farinas, C.S., da Silva, M.R., Azzoni, S.F., Pradella, J.G., 2012. Use of a new *Trichoderma harzianum* strain isolated from the Amazon rainforest with pretreated sugar cane bagasse for on-site cellulase production. Bioresour. Technol. 107, 517–521.
Delabona, P.da S., Cota, J., Hoffmam, Z.B., Paixao, D.A., Farinas, C.S., Cairo, J.P., Lima, D.J., Squina, F.M., Ruller, R., Pradella, J.G., 2013. Understanding the cellulolytic system of *Trichoderma harzianum* P49P11 and enhancing saccharification of pretreated sugarcane bagasse by supplementation with pectinase and alpha-l-arabinofuranosidase. Bioresour. Technol. 131, 500–507.
Dias, M.O., Cunha, M.P., Jesus, C.D., Rocha, G.J., Pradella, J.G., Rossell, C.E., Filho, R.M., Bonomi, A., 2011. Second generation ethanol in Brazil: can it compete with electricity production? Bioresour. Technol. 102, 8964–8971.
Dillon, A.J., Zorgi, C., Camassola, M., Henriques, J.A., 2006. Use of 2-deoxyglucose in liquid media for the selection of mutant strains of *Penicillium echinulatum* producing increased cellulase and beta-glucosidase activities. Appl. Microbiol. Biotechnol. 70, 740–746.
Do Vale, L.H., Gomez-Mendoza, D.P., Kim, M.S., Pandey, A., Ricart, C.A., Ximenes, F.F.E., Sousa, M.V., 2012. Secretome analysis of the fungus *Trichoderma harzianum* grown on cellulose. Proteomics 12, 2716–2728.
Falkoski, D.L., Guimaraes, V.M., de Almeida, M.N., Alfenas, A.C., Colodette, J.L., de Rezende, S.T., 2012. Characterization of cellulolytic extract from *Pycnoporus sanguineus* PF-2 and its application in biomass saccharification. Appl. Biochem. Biotechnol. 166, 1586–1603.
Farinas, C.S., Loyo, M.M., Baraldo Jr., A., Tardioli, P.W., Neto, V.B., Couri, S., 2010. Finding stable cellulase and xylanase: evaluation of the synergistic effect of pH and temperature. New Biotechnol. 27, 810–815.
Ferreira, V., da Silva, R., Silva, D., Gomes, E., 2010. Production of pectate lyase by *Penicillium viridicatum* RFC3 in solid-state and submerged fermentation. Int. J. Microbiol..
Florencio, C., Couri, S., Farinas, C.S., 2012. Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by *Trichoderma Strains*. Enzyme Res. 793708.
Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., Henrissat, B., Martínez, A.T., Otillar, R., Spatafora, J.W., Yadav, J.S., et al., 2012. The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336, 1715.

Forzza, R.C., Leitman, P.M., Costa, A., de Carvalho Jr., A.A., Peixoto, A.L., Walter, B.M.T., Bicudo, C., Zappi, D, da Costa, D.P., et al. 2010. Catálogo de plantas e fungos do Brasil, Río de Janeiro.

Furtado, G.P., Ribeiro, L.F., Lourenzoni, M.R., Ward, R.J., 2013. A designed bifunctional laccase/beta-1,3–1,4-glucanase enzyme shows synergistic sugar release from milled sugarcane bagasse. Protein Eng. Des. Sel. 26, 15–23.

Generoso, W.C., Malago-Jr, W., Pereira Jr., N., Henrique-Silva, F., 2012. Recombinant expression and characterization of an endoglucanase III (cel12a) from *Trichoderma harzianum* (Hypocreaceae) in the yeast *Pichia pastoris*. Genet. Mol. Res. 11, 1544–1557.

Gomes, E., Leite, R.S., da Silva, R., Silva, D., 2009. Purification of an exopolygalacturonase from *Penicillium viridicatum* RFC3 produced in submerged fermentation. Int. J. Microbiol. 2009, 631942.

Grigorevski-Lima, A.L., Da Vinha, F.N., Souza, D.T., Bispo, A.S., Bon, E.P., Coelho, R.R., Nascimento, R.P., 2009. *Aspergillus fumigatus* thermophilic and acidophilic endoglucanases. Appl. Biochem. Biotechnol. 155, 321–329.

Gusakov, A.V., 2011. Alternatives to *Trichoderma reesei* in biofuel production. Trends Biotechnol. 29, 419–425.

Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105, 10.

Herculano, P.N., Porto, T.S., Moreira, K.A., Pinto, G.A., Souza-Motta, C.M., Porto, A.L., 2011. Cellulase production by *Aspergillus japonicus* URM5620 using waste from castor bean (*Ricinus communis* L.) under solid-state fermentation. Appl. Biochem. Biotechnol. 165, 1057–1067.

Hibbett, D.S., Ohman, A., Glotzer, D., Nuhn, M., Kirk, P., Nilsson, H.R., 2011. Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biol. Rev. 25, 9.

IBGE IBdGeE, 2010. Censo 2010, in: IBGE (Ed).

Lange, L., Bech, L., Busk, P.K., Grell, M.N., Huang, Y., Lange, M., Linde, T., Pilgaard, B., Roth, D., Tong, X., 2012. The importance of fungi and of mycology for a global development of the bioeconomy. IMA Fungus 3, 87–92.

Lewinsonh, T.M., Prado, P.I., 2005. How many species are there in Brazil? Conserv. Biol. 19, 619–624.

Maller, A., da Silva, T.M., Damásio, A.R.L., Reis, V.R.A., Jorge, J.A., Polizeli, M.L.T.M., 2012. Production of pectin lyase by *Aspergillus niveus* under submerged and solid state fermentations using agro-industrial residues as carbon sources. Int. Res. J. Microbiol. 3, 029–035.

Martinez, A.T., Speranza, M., Ruiz-Duenas, F.J., Ferreira, P., Camarero, S., Guillen, F., Martinez, M.J., Gutierrez, A., del Rio, J.C., 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol. 8, 195–204.

Martins, E.S., Silva, D., da Silva, R., Leite, R.S., Gomes, E., 2007. Purification and characterization of polygalacturonase produced by thermophilic *Thermoascus aurantiacus* CBMAI-756 in submerged fermentation. Antonie Van Leeuwenhoek 91, 291–299.

Michelin, M., Peixoto-Nogueira, S.C., Betini, J.H., da Silva, T.M., Jorge, J.A., Terenzi, H.F., Polizeli, M.L., 2010. Production and properties of xylanases from *Aspergillus terricola* Marchal and *Aspergillus ochraceus* and their use in cellulose pulp bleaching. Bioprocess Biosyst. Eng. 33, 813–821.

Michelin, M., Polizeli Mde, L., Silva, D.P., Ruzene, D.S., Vicente, A.A., Jorge, J.A., Terenzi, H.F., Teixeira, J.A., 2011. Production of xylanolytic enzymes by *Aspergillus terricola* in stirred tank and airlift tower loop bioreactors. J. Ind. Microbiol. Biotechnol. 38, 1979–1984.

Michelin, M., Peixoto-Nogueira, S.C., Silva, T.M., Jorge, J.A., Terenzi, H.F., Teixeira, J.A., Polizeli Mde, L., 2012a. A novel xylan degrading beta-D-xylosidase: purification and biochemical characterization. World J. Microbiol. Biotechnol. 28, 3179–3186.

Michelin, M., Polizeli Mde, L., Ruzene, D.S., Silva, D.P., Ruiz, H.A., Vicente, A.A., Jorge, J.A., Terenzi, H.F., Teixeira, J.A., 2012b. Production of xylanase and beta-xylosidase from autohydrolysis liquor of corncob using two fungal strains. Bioprocess Biosyst. Eng. 35, 1185–1192.

Michelin, M., Polizeli, L.Mde, Ruzene, D.S., Silva, D.P., Vicente, A.A., Jorge, J.A., Terenzi, H.F., Teixeira, J.A., 2012c. Xylanase and beta-xylosidase production by *Aspergillus ochraceus*: new perspectives for the application of wheat straw autohydrolysis liquor. Appl. Biochem. Biotechnol. 166, 336–347.

Minter, D.W., da Silva, M., 2007. Fungos do Brasil.

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.M., Vilgalys, R., 2005. Fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbiol. 71, 5544–5550.

Oliveira, D.S., Meherb-Dini, C., Franco, C.M., Gomes, E., Da-Silva, R., 2010. Production of crude xylanase from *Thermoascus aurantiacus* CBMAI 756 aiming the baking process. J. Food Sci. 75, C588–594.

Pandey, A., Soccol, C.R., Nigam, P., Brand, D., Mohan, R., Roussos, S., 2000. Biotechnological potential of coffee pulp and coffee husk for bioprocesses. Biochem. Eng. J. 6, 153–162.

Pauly, M., Keegstra, K., 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. Plant J. 54, 559–568.

Peixoto-Nogueira, S.de C., Michelin, M., Betini, J.H., Jorge, J.A., Terenzi, H.F., Polizeli, M.de L., 2009. Production of xylanase by *Aspergilli* using alternative carbon sources: application of the crude extract on cellulose pulp biobleaching. J. Ind. Microbiol. Biotechnol. 36, 149–155.

Prates, E.T., Stankovic, I., Silveira, R.L., Liberato, M.V., Henrique-Silva, F., Pereira Jr., N., Polikarpov, I., Skaf, M.S., 2013. X-ray structure and molecular dynamics simulations of endoglucanase 3 from *Trichoderma harzianum*: structural organization and substrate recognition by Endoglucanases that lack cellulose binding module. PLoS One 8, e59069.

Ribeiro, L.F., Furtado, G.P., Lourenzoni, M.R., Costa-Filho, A.J., Santos, C.R., Nogueira, S.C., Betini, J.A., Polizeli Mde, L., Murakami, M.T., Ward, R.J., 2011. Engineering bifunctional laccase-xylanase chimeras for improved catalytic performance. J. Biol. Chem. 286, 43026–43038.

Ribeiro, D.A., Cota, J., Alvarez, T.M., Bruchli, F., Bragato, J., Pereira, B.M., Pauletti, B.A., Jackson, G., Pimenta, M.T., Murakami, M.T., Camassola, M., Ruller, R., Dillon, A.J., Pradella, J.G., Paes Leme, A.F., Squina, F.M., 2012. The *Penicillium echinulatum* secretome on sugar cane bagasse. PLoS One 7, e50571.

Rodriguez-Fernandez, D.E., Rodriguez-Leon, J.A., de Carvalho, J.C., Sturm, W., Soccol, C.R., 2011. The behavior of kinetic parameters in production of pectinase and xylanase by solid-state fermentation. Bioresour. Technol. 102, 10657–10662.

Rubini, M.R., Dillon, A.J., Kyaw, C.M., Faria, F.P., Pocas-Fonseca, M.J., Silva-Pereira, I., 2010. Cloning, characterization and heterologous expression of the first *Penicillium echinulatum* cellulase gene. J. Appl. Microbiol. 108, 1187–1198.

Schmit, J.P., Mueller, G.M., 2007. An estimate of the lower limit of global fungal diversity. Biodivers. Conserv. 16, 12.

Sehnem, N.T., Ramos de Bittencourt, L., Camassola, M., Dillon, A.J.P., 2006. Cellulase production by Penicillium echinulatum on lactose. Appl Microbiol Biotechnol. 72, 163–167.

Silva, T.M., Damasio, A.R., Maller, A., Michelin, M., Squina, F.M., Jorge, J.A., Polizeli, M.D., 2013. Purification, partial characterization, and covalent immobilization-stabilization of an extracellular alpha-amylase from *Aspergillus niveus*. Folia Microbiol (Praha).

Soccol, C.R., Vandenberghe, L.P., Medeiros, A.B., Karp, S.G., Buckeridge, M., Ramos, L.P., Pitarelo, A.P., Ferreira-Leitao, V., Gottschalk, L.M., Ferrara, M.A., da Silva Bon, E.P., de Moraes, L.M., Araujo, J.de A., Torres, F.A., 2010. Bioethanol from lignocelluloses: status and perspectives in Brazil. Bioresour. Technol. 101, 4820–4825.

Souza, D.T., Bispo, A.S., Bon, E.P., Coelho, R.R., Nascimento, R.P., 2012. Production of thermophilic endo-beta-1,4-xylanases by *Aspergillus fumigatus* FBSPE-05 using agro-industrial by-products. Appl. Biochem. Biotechnol. 166, 1575–1585.

Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int. J. Mol. Sci. 9, 1621–1651.

Tauk-Tornisielo, S.M., Garlipp, A., Ruegger, M., Attili, D.S., Malagutti, E., 2005. Soilborne filamentous fungi in Brazil. J. Basic Microbiol. 45, 72–82.

Textor, L.C., Colussi, F., Silveira, R.L., Serpa, V., de Mello, B.L., Muniz, J.R., Squina, F.M., Pereira Jr., N., Skaf, M.S., Polikarpov, I., 2013. Joint X-ray crystallographic and molecular dynamics study of cellobiohydrolase I from *Trichoderma harzianum*: deciphering the structural features of cellobiohydrolase catalytic activity. FEBS J. 280, 56–69.

Vitcosque, G.L., Fonseca, R.F., Rodriguez-Zuniga, U.F., Bertucci Neto, V., Couri, S., Farinas, C.S., 2012. Production of biomass-degrading multienzyme complexes under solid-state fermentation of soybean meal using a bioreactor. Enzyme Res. 2012, 248983.

Vizona Liberato, M., Cardoso Generoso, W., Malago Jr., W., Henrique-Silva, F., Polikarpov, I., 2012. Crystallization and preliminary X-ray diffraction analysis of endoglucanase III from *Trichoderma harzianum*. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 68, 306–309.

Zaldivar, J., Nielsen, J., Olsson, L., 2001. Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. Appl. Microbiol. Biotechnol. 56 (1–2), 17–34.

Zimbardi, A.L., Sehn, C., Meleiro, L.P., Souza, F.H., Masui, D.C., Nozawa, M.S., Guimaraes, L.H., Jorge, J.A., Furriel, R.P., 2013. Optimization of β-glucosidase, β-xylosidase and xylanase production by *Colletotrichum graminicola* under solid-state fermentation and application in raw sugarcane trash saccharification. Int. J. Mol. Sci. 14, 2875–2902.