



LIGNOCELLULOLYTIC FUNGI AND THEIR ENZYMES: BIOTECHNOLOGICAL POTENTIAL IN CUBA

HONGOS LIGNOCELULOLÍTICOS Y SUS ENZIMAS: POTENCIAL BIOTECNOLÓGICO EN CUBA

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The revaluation of lignocellulosic biomass for use in animal production has been studied as a solution to the food shortage in this sector. This review deals with aspects related to lignocellulolytic fungi, their enzymes, and their biotechnological potential in Cuba. Information is collected on the progress made in bioconversion processes using solid-state fermentation with strains that are highly productive of bioactive compounds. The diversity and versatility of cellulases and ligninases with the ability to degrade complex substrates and phenolic compounds are described. This constitutes an interesting challenge today, which involves elucidating the complex biochemical and physiological mechanisms involved in fungal degradation. The design of strategies for the production of lignocellulolytic enzymes will allow improving digestibility and nutritional quality of alternative sources, which can achieve more efficient agricultural production in a sustainable and ecological way.

La revalorización de la biomasa lignocelulósica para su uso en la producción animal, se ha estudiado como una solución ante el déficit de alimentos en este sector. Esta reseña aborda, fundamentalmente, los aspectos relacionados con los hongos lignocelulolíticos, sus enzimas y su potencial biotecnológico en Cuba. Se recopila información acerca de los avances alcanzados en los procesos de bioconversión mediante fermentación en estado sólido con cepas altamente productoras de compuestos bioactivos. Se describe la diversidad y versatilidad de las celulasas y ligninasas con la capacidad para degradar sustratos complejos y compuestos fenólicos. Lo anterior constituye un interesante reto en la actualidad, que pasa por la elucidación de los complejos mecanismos bioquímicos y fisiológicos involucrados con la degradación fúngica. El diseño de estrategias para la producción de enzimas lignocelulolíticas permitirá la mejora de la digestibilidad y la calidad nutritiva de fuentes alternativas, que de forma sostenible y ecológica puedan lograr producciones agropecuarias más eficientes.

Key words: monogastric animals, cellulose, fiber, lignin

Palabras clave: animales monogástricos, celulosa, fibra, lignina

Introduction

The ever-increasing need to achieve efficient and low-cost monogastric animal production in the tropics (Korver 2023 and Wlazlak *et al.* 2023) determines the selection of alternative raw materials with acceptable bioavailability, and which compete as little as possible with human nutrition.

Agricultural by-products have become important components of the circular economy with their use in animal feeding (Plouhinec *et al.* 2023). One of the ways to implement it was the use of microorganisms that produce exogenous enzymes, capable of improving nutritional value, reducing fiber content and antinutritional factors.

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Lignocellulolytic fungi are microorganisms suitable for developing bioconversion processes through solid-state fermentation (SSF), due to their ability to grow in substrates with low water content (Zwinkels *et al.* 2023). The new applications of fungal SSF have gained significant interest in recent decades to produce valuable organic compounds and to valorize various agri-food wastes and discards. This reduces their environmental impact (Cebrián and Ibarruri 2023).

The range of industrial products that can be obtained through fungal SSF includes enzymes, organic acids, biofuels and various active and other compounds such as antibiotics, pigments or biological control agents, with applications in foods, feeds, pharmaceuticals, cosmetics, biofuels or agronomic sectors (Boondaeng *et al.* 2024). The SSF offers several advantages, including lower energy requirements and higher productivity. However, several operational aspects remain without an effective technical solution, so only a few compounds are industrially implemented (Kuhad *et al.* 2016).

The bioconversion of fibrous substrates of different nature has as a critical point the access to the polysaccharide matrix. This aspect depends largely on the fiber composition and, in particular, its cellulose and lignin content (Saldarriaga-Hernández *et al.* 2020). The structural complexity of the fiber, as well as its close association and chemical cross-linking, make them recalcitrant biomolecules.

Cellulose-modifying enzymes have the ability to hydrolyze β -1.4-glucosidic bonds to low molecular weight products, including hexoses and pentoses. Its complex organization consists of three main components: endo- β -glucanase, exo- β -glucanase and β -glucosidase. The sequential stages of cellulolysis require a synergistic sequence of events (Singh *et al.* 2021). However, the enzymes that modify lignin are oxidative, non-specific, and act through non-protein mediators (Dias *et al.* 2022). Among the main ligninolytic enzymes, laccases (oxidoreductases capable of degrading the phenolic and aromatic units of lignin with the reduction of molecular oxygen to water) are the most widely applied in industry (Coêlho *et al.* 2021). For this reason, the design of strategies for the production of lignocellulolytic enzymes will improve the digestibility and nutritional quality of alternative sources so that, in a sustainable and ecological way, more efficient agricultural production can be achieved. This review deals with aspects related to lignocellulolytic fungi, their enzymes, and their biotechnological potential in Cuba.

Cell wall structure and composition of lignocellulosic material

The characteristics, composition, and structure of plant cell walls are described by various authors in the international literature. According to Quiroz and Folch-Mallol (2011), the structure of the cell wall is responsible for tensile strength, as well as shaping the cell and conferring resistance to pathogens. It is a highly organized structure composed

of cellulose, hemicellulose, and the phenolic polymer lignin. The composition and percentages vary among plant species, depending on their age, tissue, and growth stage (Zhang and Lynd 2004).

Cellulose: It is the main component of plants cell wall. It is the most abundant polymer in nature, it constitutes 50 % of their dry weight. Depending on the degree of polymerization, it can have 2,000 to 25,000 glucose units. It is composed of D-glucose monomers, linked by β -1.4 glycosidic bonds that form cellobiose molecules. The β -glucosidic bond configuration forms a crystalline structure. The crystalline regions are separated by amorphous cellulose. The cellulose is intertwined with hemicellulose and lignin, forming a structure that is highly resistant to degradation, so very few microorganisms can hydrolyze it (Kameshwar and Qin 2016).

Hemicellulose: Is a complex polymer of heteropolysaccharides, formed by pentoses (D-xylose and L-arabinose) and hexoses (D-glucose, D-mannose and D-galactose), which form branched chains, in addition to the acid sugars 4-O-methylglucuronic acid, D-galacturonic and D-glucuronic, linked by β -1.4 glycosidic bonds and others by β -1.3 bonds. It has branches formed by sugars (xylans, xyloglucans, mannans, glucomannans and glucans). Xylan constitutes more than 70 % of the composition of hemicellulose. It is the most abundant and is formed by β -1.4 bonds of D-xylose units. It acts as a connection between lignin and cellulose through ester-type bonds, and with cellulose through hydrogen bonds (Sajith *et al.* 2016).

Lignin: It is a polymer highly resistant to chemical and biological degradation. Few organisms can mineralize the hydrolysis of the polysaccharides it protects. It is part of the cell wall, as a structural support and impermeability. Structurally, it is an irregular, insoluble, branched heteropolymer, formed by three aromatic alcohols of the phenylpropane type: coumaryl, coniferyl and sinapyl alcohol, joined by C-C bonds and ether bonds between the aromatic rings. Lignin is the most difficult component of lignocellulosic material to degrade (Saldarriaga-Hernández *et al.* 2020).

Main glycolytic enzymes involved in lignocellulose degradation

Cellulases are O-glucoside hydrolases that hydrolyze the β -1.4 bond of cellulose. They are characterized by their modular structure: catalytic, peptide-binding, and cellulose-binding. They are classified according to their enzymatic activity: endoglucanases, exoglucanases, and β -glucosidases (Escuder *et al.* 2018).

The endoglucanases (EC 3.2.1.4; 1.4- β -D-glucan-4-glucanohydrolase) are monomeric enzymes, whose molecular mass ranges between 22 and 45 kDa. These enzymes initiate the attack of the amorphous regions of the cellulose fiber, followed by the action of cellobiohydrolases on the reducing and non-reducing ends. The endoglucanases are

not glycosylated; however, they may contain a relatively low amount of carbohydrates (1 to 12 %). The optimal pH is between 4 and 5. The optimal temperature ranges from 50 to 70 °C.

Exoglucanases or cellobiohydrolases (EC 3.2.1.74; 1,4- β -D-glucan-glucanohydrolase and EC 3.2.1.91; 1,4- β -D-glucan-cellobiohydrolase) act on the reducing and non-reducing ends of cellulose chains, releasing cellobiose. These enzymes represent 40 to 70 % of the total component of the system, and can hydrolyze crystalline cellulose. They are enzymes with a molecular mass between 50 and 65 kDa. Their glycosylation is very low (less than 12 %), their optimal pH is between 4 and 5, and their optimal temperature is between 37 and 60 °C.

The β -glucosidases weigh between 35 and 640 kDa and are mostly glycosylated. They have an optimal pH between 3.5 and 5.5 and an optimal temperature between 45 and 75 °C. The cellulase enzyme systems act synergistically. Although the synergism between the different components of the cellulase complex is not yet fully clarified, it clearly depends on several factors, such as the nature of the substrate, the affinity of the cellulase component for the substrate, the stereospecificity of the component, the concentration of the enzyme, and the ratio between the enzyme components (Zhao *et al.* 2019).

The hemicellulases are glycoside hydrolases; but some may be carbohydrate esterases. Xylanases are the main enzymes involved in the degradation of hemicellulose. This group includes endoxylanases (EC 3.2.1.8; endo-1,4- β -D-xylanases) and β -xylosidases (EC 3.2.1.37; xylan 1,4- β -xylosidase). They also require accessory enzymes, such as xylan esterases, ferulic and coumaric esterases, α -arabinofuranosidases, and α -4-methyl glucuronosidases, among others, which act synergistically (Hu *et al.* 2013).

Lignin depolymerization involves extracellular oxidative enzymes, which release highly unstable products that subsequently have oxidation reactions, peroxidases and laccases (Viswanath *et al.* 2014). Among the peroxidases are lignin peroxidase (LiP; EC 1.11.1.14) and manganese-dependent peroxidase (MnP; EC 1.11.1.13), which are oxidoreductase enzymes. Lignin peroxidase is a glycoprotein that can oxidize phenolic and non-phenolic compounds, amines, aromatic ethers and polycyclic aromatics and is the most effective. Manganese-dependent peroxidase uses manganese as a substrate and oxidizes it from Mn^{2+} to Mn^{3+} and acts as an oxidant of lignin compounds. In other studies, a third type, the versatile peroxidase, was found that combines both activities.

Laccases (p-diphenol oxygen oxidoreductases; EC 1.10.3.1) are polyphenol oxidase enzymes. They contain four copper ions in their active center and catalyze the oxidation of phenolic compounds by reducing molecular oxygen to water, generating insoluble compounds that are easily recovered.

They also catalyze the oxidation of many phenolic and non-phenolic compounds in the presence of mediators (Brink *et al.* 2019).

Fungi that produce cellulolytic enzymes

Cellulolytic enzymes are a complex that catalyzes the progressive conversion of cellulose. The catalytic mechanisms of this enzyme complex are synergistically develop, which ensure the efficiency of bioconversion. Cellulolytic fungi produce these enzymes under conditions of a lack of alternative carbon sources with greater degradability and absorption, and of other nitrogen sources that are also rich in metabolizable energy and provide carbon chains. This characteristic is due to the strict catabolic repression control to which cellulase genes are subject (Beier *et al.* 2022). Under such conditions, these microorganisms use enzymes to separate simple sugars from the solid substrate and use them as a carbon source (Singh *et al.* 2021).

There are complementary activities between the types of enzymes they have and they are considered responsible for synergism, since the action of two or more combined enzymes is greater than the sum of the individual activities in the degradation (Sajith *et al.* 2016). Synergism may occur between and within different types of cellulolytic enzymes. Although the synergism between the different components of the cellulase complex is not yet fully elucidated, it is clear that it depends on different factors, such as the nature of the substrate, the affinity for the substrate, the stereo-specificity, the concentration of the enzyme and the ratio between the enzymatic components (Saidi *et al.* 2024). In addition, the performance of cellulase mixtures in biomass conversion processes depends on several of their properties, including stability, product inhibition, specificity, synergy between different enzymes, productive binding to cellulose, physical characteristics, and the composition of cellulosic biomass (Sajith *et al.* 2016).

The most commonly used fungal genera in the biodegradation of lignocellulolytic biomass are the *Trichoderma*, *Aspergillus* and *Penicillium* genera, corresponding to more than 50 % of the studies related to cellulases, their more productive use is conditioned by the use of highly efficient enzyme mixtures (Passos *et al.* 2018). In the available scientific literature are showed different genera and species involved in the production of cellulase enzymes (table 1). Most of these studies only consider *in vitro* assays to show their enzymatic potential.

Ligninolytic enzymes are produced by a large number of fungi belonging to the *Ascomycota* and *Basidiomycota* divisions (de Oliveira Rodrigues *et al.* 2020). Most of ligninolytic fungi belong to *Basidiomycetes* group, microorganisms that are more efficient at completely degrading lignin. These fungi secrete several extracellular enzymes that are essential for the initial transformation

Table 1. Fungal species involved in the production of cellulase enzymes in lignocellulosic biomass

Genera	Species	References
<i>Aspergillus</i>	<i>A. niger</i> ; <i>A. oryzae</i> ; <i>A. fumigatus</i> ; <i>A. nidulans</i> , <i>A. heteromorphus</i> , <i>A. acculeatus</i> , <i>A. terreus</i> , <i>A. flavus</i>	Kuhad et al. (2011), Sajith et al. (2016), Ma et al. (2024)
<i>Penicillium</i>	<i>P. brasilianum</i> ; <i>P. occitanis</i> ; <i>P. decumbans</i> ; <i>P. fusiculosum</i> ; <i>P. janthinellum</i> ; <i>P. pinophilum</i> , <i>P. echinulatum</i>	Schneider et al. (2014), Sajith et al. (2016), Lodha et al. (2020), Bhandari et al. (2021)
<i>Trichoderma</i>	<i>T. reesei</i> , <i>T. longibrachiatum</i> ; <i>T. harzianum</i> ; <i>T. koningii</i> , <i>T. viride</i> , <i>T. branchiatum</i> ; <i>T. atroviride</i>	Kuhad et al. (2016), Passos et al. (2018), Hamdan and Jasim (2021), Liu et al. (2021)
<i>Fusarium</i>	<i>F. solani</i> , <i>F. oxysporum</i>	Kuhad et al. (2011), Cruz-Davila et al. (2022), Devi et al. (2024),
<i>Neurospora</i>	<i>N. crassa</i>	Alhomodi et al. (2022)
<i>Thermoascus</i>	<i>T. aurantiacus</i>	Jain et al. (2017), Singh and Bajar (2019)
<i>Curvularia</i>	<i>C. lunata</i> , <i>C. indicum</i>	Yadav and Vivekanand (2020)
<i>Lichtheimia</i> (thermotolerant)	<i>L. ramosa</i>	Schwab et al. (2021)

of lignin and together achieve its mineralization (Iram et al. 2021). Laccases are among the most discussed enzymes in scientific papers, hence the focus on the characteristics of the fungi that produce these proteins. However, fungi belonging to *Ascomycota* division are decomposing microorganisms with versatile repertoires of extracellular and intracellular enzymes with high potential for lignin depolymerization (Jain et al. 2017). Studies on the production of ligninolytic enzymes in the different species that include the *Ascomycota* division are recent, so their application in the different fields of biotechnology is barely considered. Despite this, there are several reports in the scientific literature that discuss the enzymatic production of these microorganisms, mainly on the production of laccase enzymes (Pérez-Grisales et al. 2019).

Fungi producing ligninolytic enzymes

Laccase-producing fungi can secrete different isoforms of the protein. The number of isoenzymes depends on each species and their expression depends on the culture components, the presence of specific inducers and the growth conditions. The stability characteristics, pH, optimal temperature and affinity for different substrates can considerably differ between isoenzymes (Xie and Liu 2024).

Laccases obtained from ligninolytic fungi have wide applications, due to their ease of separation, purification and production in bioreactors (Hernández et al. 2019 and Liu et al. 2022), which allows their large-scale production. The laccases from these fungi have a higher redox potential (up to 0.8 V) than plant and bacterial laccases (0.4-0.5 V), which is why they have greater biotechnological applications (Osma et al. 2014). However, the commercial application of laccases is limited because these enzymes are produced in small quantities. This problem is the fundamental cause that determines the increase in studies of new laccase-producing

strains, as well as more efficient methods for their obtaining and purification, which guarantee an adequate quantity, specificity and catalytic activity (Xie and Liu 2024).

Given the high structural complexity of lignin (Reid 1995), the natural mechanisms that carry out its degradation are enzymes that do not recognize the substrate in a stereospecific way, but that allow the formation of oxidizing agents able of reacting with the aromatic rings of the phenylpropane residues and with the carbon chains linked to these rings, which provoke oxidation reactions that proceed through free radicals. The aliphatic chains obtained later are degraded into carbon dioxide and water by some microorganisms. In other organisms, what occurs is the depolymerization of the units. The cooperation between diverse enzymes and their precise regulatory mechanisms become evident when it is noted that *in vitro* lignin degradation has not been possible (Cullen 1997). Table 2 shows different types of genera and species of fungi involved in the production of ligninolytic enzymes in lignocellulosic biomass.

Biotechnological potential in Cuba

In recent decades, research aimed at increasing the biological value of sugar industry wastes through the inoculation of filamentous fungi has been carried out in Cuba by research groups from the University of Havana (UH), the Cuban Institute of Sugarcane Derivatives (ICIDCA), the Technological University of Havana (Cujae), and the Institute of Animal Science (ICA). These latter institutions concentrated their efforts on the search for new microorganisms for productive purposes.

The ICA has collections of microorganisms with potential use in animal feeding. One of them, referring to lignocellulolytic mutant strains of fungi for enzymatic action on highly fibrous substrates, such as sugarcane bagasse (Sosa et al. 2017), and the other of fungal strains,

Table 2. Different types of genera and species of fungi involve on the ligninolytic enzymes production

Genera	Species	References
<i>Phanerochaete</i>	<i>P. chrysosporium</i> , <i>P. sordida</i>	Mori <i>et al.</i> (2021)
<i>Pleurotus</i>	<i>P. ostreatus</i>	Liguori <i>et al.</i> (2015), Asensio-Grau <i>et al.</i> (2020), El-Ramady <i>et al.</i> (2022)
<i>Ganoderma</i>	<i>G. lucidum</i>	de Oliveira Rodrigues <i>et al.</i> (2020)
<i>Xylaria</i>	<i>X. polymorpha</i>	Wattanakitjanukul <i>et al.</i> (2020)
<i>Phlebia</i>	<i>P. radiata</i>	Liu <i>et al.</i> (2021)
<i>Physisporinus</i>	<i>P. rivulosus</i>	Alhujaily <i>et al.</i> (2024)
<i>Ceriporiopsis</i>	<i>C. subvermispora</i>	Khan <i>et al.</i> (2024)
<i>Trametes</i>	<i>T. versicolor</i>	Dao <i>et al.</i> (2023)

with production of β -glucosidase enzymes, belonging to ICIDCA and Cujae (Dustet and Izquierdo 2004). The strains were molecularly identified, and their nucleotide sequences were registered in the Genbank. Table 3 shows the degradative activity of these microorganisms on different fibrous substrates used in animal feeding.

The ICA mutant strains belong to the species *Trichoderma viride*, *Penicillium implicatum* and *Aspergillus fumigatus*, producers of endo, exo β 1-4 glucosidase and β -glucosidase enzymes. These strains are resistant to catabolic repression with hydrolytic activity on sugarcane bagasse, using a solid-state fermentation system, with potential for the saccharification of other grasses. The strains of *Aspergillus* (J-1, 6, 21, 27) and *Neurospora* (E623), belonging to Cujae and ICIDCA, were identified as *A. niger*, *A. fumigatus* and *N. crassa*. These are also efficient in the process of saccharification and simultaneous fermentation of bagasse, but with a higher β -glucosidase production than the mutants. So, they could be used in synergy with cocktails of enzymatic crudes or microorganisms (Menéndez *et al.* 2015). The main enzymes of the first group endo, exo β 1-4 glucosidase, β glucosidase act here, and a second group endo xylanase and β xylosidase, which yield glucose and xylose respectively, as well as the accessory ones: α arabinofuranosidase, endo-mannanases, pectinases, pectate lyase, α and β galactosidase, which yield arabinose, mannose, galacturonic acid and galactose respectively, which are not quantified. However, it was confirmed by polyacrylamide gel electrophoresis that the main enzymes have well-defined bands between 25 and 66 kDa, compared with the commercial standards of NOVOZYMES (Valiño *et al.* 2020).

The enzymes of these fungal strains were also shown to produce a series of positive changes in the nutrient content of legumes (*Canavalia ensiformis*, *Lablab purpureus*, *Vigna unguiculata* and *Mucuna pruriens*) through solid-state fermentation of grain meals (Valiño *et al.* 2015) and foliage meals (Savón *et al.* 2014, Valiño *et al.* 2016 and Scull *et al.* 2018). These changes included: increased essential amino acid

content, soluble protein, and *in vitro* dry matter digestibility; significant decrease in alpha-galactoside and penta- and hexaphosphate inositol levels; reduction in protease inhibitors and lectins, as well as in the degree of tannin polymerization. Other studies were conducted to deepen our understanding of the physiological and biochemical characteristics of these fungal strains, as well as to identify new, more efficient fungal strains.

Vázquez *et al.* (2019) isolated 35 fungal strains, according to the morphological characteristics of each crop, they were characterized as to the generic level, which allowed them to be grouped into 11 genera: *Trichoderma*, *Curvularia*, *Fusarium*, *Aspergillus*, *Penicillium*, *Neurospora*, *Hypoxylon*, *Cladosporium*, *Paecilomyces* and *Mucor*. Isolates of *Trichoderma* sp., *Hypoxylon* sp., *Aspergillus fumigatus*, *Curvularia kusanoi*, and *Curvularia lunata* showed the greatest lignocellulolytic potential. The nucleotide sequences of these strains were registered in the Genbank. The strain *Curvularia kusanoi* L7 developed the highest induction of laccase enzymes, with growth in co-culture, carbon mineralization, production of high concentrations of cellulase and laccase enzymes with the ability to degrade fibrous substrates. The isolation, identification, characterization, and conservation of these microorganisms became fundamental links in obtaining agricultural products through biotechnology way for subsequent use in livestock or in the bioindustry (Vázquez *et al.* 2022).

Fungal evaluations and their enzymatic activity in fibrous sources for productive interest species

The use of lignocellulosic biomass in animal feeding is presented as an important solution to the lack of food for livestock. Rising prices for cereals and other dietary components create a need to seek more economical alternatives that provide a food product with adequate nutritional value. In lignocellulosic biomass, several agro-industrial residues have a chemical and physical composition that allows their use with satisfactory results in this field.

Table 3. Biodegradation of lignocellulosic biomass with lignocellulolytic fungi isolated in Cuba

Fibrous substrate	Microorganism	Enzymatic activity			References
		Exo Glucanase	Endo Glucanase	Laccase	
SSF in bioreactor (30 L/min air flow)					
<i>Saccharum officinarum</i> (bagasse)	<i>Trichoderma viride</i> M5-2	19.21 UI/gDM	54.82 UI/gDM	-	Ibarra <i>et al.</i> (2002), García <i>et al.</i> (2002)
<i>S. officinarum</i> (hydrolyzed bagasse)	<i>T. viride</i> M5-2	8.82 UI/gDM	16.21 UI/gDM	-	Valiño <i>et al.</i> (2004)
<i>S. officinarum</i> (hydrolyzed bagasse)	<i>T. viride</i> 137	5.8 UI/gDM	6.10 UI/gDM	-	Valiño <i>et al.</i> (2003)
<i>S. officinarum</i> + <i>Vigna unguiculata</i> 80:20	<i>T. viride</i> 137 MCX1	1.84 UI/gDM	7.26 UI/gDM	-	Valiño <i>et al.</i> (2004)
<i>S. officinarum</i> (hydrolyzed bagasse)	<i>Aspergillus niger</i> (J1)	1 UI/mL	-	-	Dustet and Izquierdo (2004)
<i>S. officinarum</i> (hydrolyzed bagasse)	<i>Aspergillus niger</i> (J1) y <i>A. fumigatus</i> (6)	10 UPF/gDM of cellulose	-	-	Menéndez <i>et al.</i> (2015)
SSF with legume grains meals					
<i>Vigna unguiculata</i>	<i>T. viride</i> M5-2	12.71 UI/mL	18.10 UI/mL	-	Valiño <i>et al.</i> (2015)
<i>Lablab purpureus</i>	<i>T. viride</i> M5-2	12.23 UI/mL	17.08 UI/mL	-	
<i>Canavalia ensiforme</i>	<i>T. viride</i> M5-2	0.73 UI/mL	0.54 UI/mL	-	
<i>Mucuna pruriens</i>	<i>T. viride</i> M5-2	0.69 UI/mL	0.67 UI/mL	-	Valiño <i>et al.</i> (2016)
<i>Triticum aestivum</i> (wheat bran)	<i>T. viride</i> M5-2	0.22 UI/mL	0.21 UI/mL	0.22 U/mL	Valiño <i>et al.</i> (2020)
<i>M. pruriens</i> (follaje)	<i>A. fumigatus</i> (6)	0.20 UI/mL	-	-	Pérez-Soler <i>et al.</i> (2016)
<i>M. pruriens</i> (foliage)	<i>Aspergillus niger</i> (J1)	0.34 UI/mL	-	-	
<i>M. pruriens</i> (foliage)	<i>Neurospora crassa</i> (EC-623)	0.39 UI/mL	-	-	
SSF with different fibrous sources					
Grass hay	<i>Curvularia kusanoi</i> L7	0.80 UI/mL	2.36 UI/mL	-	Vázquez <i>et al.</i> (2019)
<i>T. aestivum</i> (wheat bran)	<i>C. kusanoi</i> L7	0.34 UI/mL	-	2800 U/L	
<i>S. officinarum</i> (bagasse)	<i>C. kusanoi</i> L7	0.802 UI/mL	2.73 UI/mL	0.06 U/mL	Vázquez <i>et al.</i> (2022)
<i>T. aestivum</i> (wheat bran)	<i>C. kusanoi</i> L7	0.535 UI/mL	0.340 UI/mL	1200 U/L	

Many of them are used in the production of animal food, such as ruminants, poultry, pigs, among other species of economic interest (Plouhinec *et al.* 2023). Table 4 summarizes the biotechnological potential of different fibrous sources biotransformed with cellulase and laccase enzymes from lignocellulolytic fungi and supplied to different species of monogastric animals in Cuba.

The use of whole dolicho forage meal, fermented with the strains *T. viride* 137 MCX1 and *T. viride* M5-2 at 10 % in broilers feeding, does not modify the final live weight and body composition (Martínez *et al.* 2016). However, it reduces abdominal fat content and has an effect on the apparent fecal retention of different nutrients and on the animal's cecum. Both strains had a similar performance for the mentioned indicators (Savón *et al.* 2014). Solid-state

fermentation with the mutant strains *T. viride* 137 MCX1 and *T. viride* M5-2 makes possible to improve the nutritional value and reduced fiber content. In addition, it reduced the content of antinutritional factors in creeping legume species, as tested in broilers with the following results: with the inclusion of 10 % in the diet, 30 % of flavonoids decreased, and lengthening of the intestinal villi at the level of the duodenum was observed, decreased the apparent digestibility of dry matter and crude protein, increased the bursa of Fabricius and decreased the polyphenol content (Scull *et al.* 2018). In addition to being ideal substrates for obtaining high inoculant production without the use of other nitrogen or mineral sources, it allowed their production to obtain fibrolytic enzyme crudes in different substrates, as well as a new product different from the enzyme as an alternative food. According to Alberto *et al.* (2024),

Table 4. Biotechnological potential of different fibrous sources biotransformed with cellulase enzymes and laccases from lignocellulolytic fungi, supplied to different species of monogastric animals

Strains	Animal	Fibrous source	Results	References
<i>T. viride</i> 137 MCX1	Broilers	<i>L. purpureus</i> wholemeal	Decreased apparent fecal retention and fibrous fraction except for hemicellulose Nitrogen retention was similar to the corn/soybean control	Savón <i>et al.</i> (2014)
<i>T. viride</i> M5-2	Broilers	<i>L. purpureus</i> wholemeal	Replacing corn/soybean meal with 10 % whole dolicho forage meal improves physiological indicators and immune response.	
<i>T. viride</i> 137 MCX1	Broilers	<i>L. purpureus</i> wholemeal forage	The inclusion of 10 % fermented whole wheat forage meal was similar to the control in apparent fecal nitrogen retention and decreased that of organic matter. The NDF, ADF and cellulose were lower than the control and hemicellulose did not vary. There were not differences in the empty cecums compared to the control.	Martínez <i>et al.</i> (2016)
<i>T. viride</i> M5-2	Broilers	<i>L. purpureus</i> wholemeal forage	The inclusion of 10 % fermented whole wheat forage meal decreased apparent fecal retention of nitrogen and organic matter compared to the control. Regarding the fibrous fraction, all indicators decreased, except hemicellulose. The weight of the empty cecum increased compared to the control.	
<i>C. kusanoi</i>	Broilers	<i>S. officinarum</i> (bagasse)	Enzymatic pretreatment increased the digestibility of sugarcane bagasse fiber in birds diets with native enzymes.	Alberto <i>et al.</i> (2024)
<i>C. kusanoi</i> + <i>T. pleurotica</i>	Broilers	<i>S. officinarum</i> (bagasse)	Enzymatic pretreatment with induced enzymes increases the <i>in vivo</i> digestibility of sugarcane bagasse fiber in bird diets.	
<i>C. kusanoi</i>	Rabbits	<i>S. officinarum</i> (bagasse)	Enzymatic pretreatment with native L7 laccases increases the <i>in vivo</i> digestibility of sugarcane bagasse fiber with native enzymes.	
<i>C. kusanoi</i> + <i>T. pleurotica</i>	Rabbits	<i>S. officinarum</i> (bagasse)	Enzymatic pretreatment with induced laccases increases the <i>in vivo</i> digestibility of sugarcane bagasse fiber in rabbit diets.	

laccase and cellulase enzymes from *Curvularia kusanoi*, and the consortium *C. kusanoi*, *T. pleurotica* and *T. viride* M5-2, native and induced, modified the lignin structure of raw wheat straw, improved the nutritional quality and digestibility of sugarcane bagasse and increased the *in vivo* digestibility of sugarcane bagasse in diets for birds and rabbits. At the same time, they constituted an alternative for pretreatment of fibrous sources for animal production.

Final considerations

Lignocellulosic biomass has been considered an important source with great potential for the sustainable production of biofuels and bioproducts. However, the economic viability of these processes depends on the efficient conversion of structural polysaccharides into fermentable oligosaccharides and monomeric sugars. To achieve this, it is necessary to have high-yielding strains, develop technologies for using enzyme cocktails or inducers, which can come from different lignocellulolytic organisms, and optimize these processes to improve agricultural waste for animal production. The use

of appropriate enzymes in monogastric nutrition allowed reducing corn intake, as the most important component of feed, resulting in considerable savings for farmers and improved diets use. Although studies must be conducted to determine the inclusion levels of the raw extracts obtained from these productions, nutritionists knowledgeable in the subject see as an advantage not only the improvement in feed conversion, but also in digestibility and other factors caused by the high fiber content, which would have great benefits: the use of crude extracts for their added value in enzymes for different industries, as well as the improvement of conventional foods. The production of fibrolytic enzyme extracts for other industrial sectors would also ensure benefits in the quality of products obtained in the Cuban textile, paper, leather, pharmaceutical, and animal food markets. For this reason, the development of strategies for the production of lignocellulolytic enzymes will improve the digestibility and nutritional quality of alternative sources, thereby in a sustainable and ecological way; they can achieve more efficient agricultural production.

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