Fungal microbiota of sugarcane straw and their ability to produce hydrolytic enzymes

Microbiota fúngica de paja de caña de azúcar y su capacidad para producir enzimas hidrolíticas

Microbiota fúngica de palha de cana-de-açúcar e a sua capacidade de produzir enzimas hidrolítica

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Abstract

The microbiota presents in sugarcane (*Saccharum officinarum* L.) straw can have benefits to produce sustainable crops, also can be used for the development of alternative processes to produce molecules of industrial interest and valorization of biomass and residues unexploited. Therefore, the objective of the present work was the isolation of the fungal microbiota present in the sugarcane straw (CP 72-2082) and its capacity to produce hydrolytic enzymes. The fungal microbiota was isolated by sampling for four months one sampling for month the straw in fields of the "El Potrero" sugar mill in the Veracruz state, Mexico, and soil was also sampled to determine the effect of straw chili on the organic matter content. Furthermore, the capacity of the strains to produce xylanases and cellulases was determined in a Petri dish using birch xylan and carboxymethylcellulose as substrates. Thirty-four strains were isolated from the samples, in all was identified the genera *Trichoderma*, *Fusarium* in three and *Aspergillus* and *Penicillum* in two. The results indicate that if sugarcane straw is reincorporated into soils where sugarcane is grown, it can have a beneficial impact, 22 isolated strains showed the ability to produce hydrolytic enzymes. The organic matter content in the soils with both shredded and unshredded crop residues showed that chili does not present a benefit to the soil but can contribute beneficial fungal microbiota for various purposes.

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Resumen

La microbiota presente en la paja de caña de azúcar (*Saccharum officinarum* L.) puede tener beneficios para la producción de cultivos sostenibles, también, puede utilizarse en el desarrollo de procesos alternos que permitan producir moléculas de interés industrial y al mismo tiempo valorizar biomasa y residuos no aprovechados. El objetivo del presente trabajo fue aislar la microbiota fúngica presente en la paja de caña de azúcar (CP 72-2082) y su capacidad para producir enzimas hidrolíticas. La microbiota fúngica fue aislada muestreando una vez por mes durante cuatro meses la paja en campos del ingenio "El Potrero" en el estado de Veracruz, México, también se muestreó suelo para determinar el efecto del ahile de la paja sobre el contenido de materia orgánica. Asimismo, se determinó la capacidad de las cepas para la producción de xilanasas y celulasas en caja Petri utilizando xilano de abedul y carboximetilcelulosa como substratos. En los cuatro muestreos realizados, se aislaron 34 cepas, identificándose en todos, el género *Trichoderma*, *Fusarium* en tres de ellos y *Aspergillus* y *Penicillum* en dos. Los resultados indican que la reincorporación de la paja de caña en los suelos donde es cultivada caña de azúcar puede tener impacto benéfico, encontrándose que 22 de las cepas aisladas, mostraron la capacidad de producción de enzimas hidrolíticas. El contenido de materia orgánica, en los suelos donde se tenían residuos de cosecha ahilados y no ahilados, demostró que el ahile no presenta un beneficio al suelo, pero puede aportar microbiota fúngica benéfica para diversos objetivos.

Palabras clave: *Saccharum officinarum*, *Trichoderma*, hongos, celulasas, xilanasas.

Resumo

A microbiota presente na palha da cana de açúcar (*Saccharum officinarum* L.) pode ter benefícios para a produção de culturas sustentáveis, e também pode ser utilizada para o desenvolvimento de processos alternativos para produzir moléculas de interesse industrial e valorização da biomassa e dos resíduos não explorados. Portanto, o objectivo do presente trabalho foi o isolamento da microbiota fúngica presente na palha da cana de açúcar (CP 72-2082) e a sua capacidade de produzir enzimas hidrolíticas. A microbiota fúngica foi isolada por amostragem uma vez por mês durante quatro meses a palha nos campos do moinho de açúcar "El Potrero" no estado de Veracruz, México, e o solo também foi amostrado para determinar o efeito da palha chili sobre o conteúdo de matéria orgânica. Além disso, a capacidade das estirpes para produzir xilanases e celulases foi determinada numa placa de Petri, utilizando carboximetilcelulose e xilan de bétula como substratos. Nas quatro amostragens realizadas, foram isoladas 34 estirpes, identificando o género *Trichoderma* em todas elas, *Fusarium* em três delas e *Aspergillus* e *Penicillum* em duas delas. Os resultados indicam que se a palha da cana de açúcar for reincorporada em solos onde a cana de açúcar é cultivada, pode ter um impacto benéfico. Além disso, 22 estirpes isoladas mostraram a capacidade de produzir xilanases e celulases. O teor de matéria orgânica nos solos com resíduos de culturas triturados e não triturados mostrou que a pimenta não apresenta um benefício para o solo, mas pode contribuir com microbiota fúngica benéfica para vários fins.

Palavras-chave: *Saccharum officinarum*, *Trichoderma*, fungos, celulases, xilanases.

Introduction

From an economic and social point of view, nowadays the cultivation of sugar cane is the most important in Mexico, since it contributes 0.5% of the gross domestic product and is present in 15 states of the country (Cervantes-Preciado *et al*., 2019). Similarly, according to the Comité Nacional Para el Desarrollo Sustentable para la Caña de Azúcar (CONADESUCA, 2019), 57,036,700 t of sugar cane were harvested per hectare during the 2018-2019 harvest.

There is a need to increase the productivity of the cultivation of sugarcane (*Saccharum officinarum* L.) in a sustainable way, which has promoted the development of alternatives to improve crop yield, with the microbiota being a potential option that can allow the exploration of untapped diversity to modulate growth, development, defense against pathogens, nutrient acquisition and resistance to stress, as well as its use in industrial processes for the generation of new products (De Souza *et al*., 2016). It is currently known that the native microflora of sugarcane is diverse and can beneficially influence its development and health, such information invites the use of microbial and industrial technologies for its correct exploitation (Armanhi *et al*., 2018; De Souza *et al*. , 2016). For this reason, the objective of the present research work is to generate information on the diversity of fungal strains present in aligned sugarcane straw in sugarcane fields, in addition to evaluating the potential of these strains for hydrolytic enzymes production.

Materials and methods

Sampling and harvesting of sugarcane straw

The sugarcane straw (harvest residues), variety CP 72-2086 were sampled by the practice of align in fields of "El Potrero" sugar mill in the state of Veracruz. The sampling was carried out using the five of golds method (Martínez *et al*., 2013) taking six samples per point (identified with a Garmin® GPS) with and without align to compare the content of organic matter (OM) and pH in soil. The sampling coordinates were: Point one: Latitude: 18°54'4.93"N, Longitude: 96°46'58.55"W, Point two: Latitude: 18°54'5.69"N, Longitude: 96°46'56.64"W, Point Three: Latitude: 18°54'11.48"N, Longitude: 96°46'54.80»W, Point Four: Latitude: 18°54'11.95"N, Longitude: 96°46'57.04»W, Point 5: Latitude: 18°54'9.42"N Longitude: 96° 46'56.91"W.

Soil analysis

The soil samples were processed for the determination of organic matter (OM) by the Walkley-Black method (Yarce and Castillo, 2014). For the determination of OM to 0.5 g of ground soil, 10 mL of a 1 N solution of potassium dichromate ($K_2Cr_2O_7$) in acid medium (20 mL of 98 % H_2SO_4) were added, after 45 min of In reaction, distilled water and a few drops of ferroin indicator were added (0.696 g of ferrous sulfate with 1.485 g of orthophenanthroline monohydrate ($C_{12}H_8N_2.H_2O$) in 100 mL of distilled water). The quantification was carried out by titration of Cr^{+6} with Mohr's salt ($Fe(NH_4)_2(SO_4)_2$ $.6H_2O$) 0.5 N. For the determination of pH, dry soil samples were sieved (2 mm) and 2.5 parts of water were added for each part of soil, the mixture was allowed to settle and the pH was determined with a Hanna® potentiometer (Rozas *et al*., 2011).

Isolation, purification and morphological characterization of native fungal strains

The fungal strains were isolated by placing small fragments of the sugarcane straw samples in humid chambers and incubated at 28 °C for 72 h. The fungal strains were purified by the hyphal tip technique in the culture medium Dicloran Rosa de Bengal Chloramphenicol (Millipore®) (Lacerda *et al*., 2018) and kept at 28 °C for 48 h.

Once the pure strains were obtained, they were characterized morphologically (macroscopically and microscopically) by staining with cotton blue (Merck®), identifying them according to the Barnett and Hunter guide (1972). Strain purification was performed with samples collected from March, April, May and June 2018.

Strain growth on plate sugarcane straw

Disks of the purified fungal strains were taken and placed in the center of Petri dishes containing culture medium prepared with Bacteriological Agar (Bioxon®) at a concentration of 15 $g.L^{-1}$ and sugar cane straw of particle size smaller than 0.595 mm at a concentration of 13 $g.L^{-1}$ and incubated at 28 °C for seven days, during which radial growth was measured with the help of a vernier every 12 h (Lizardi-Jiménez *et al*., 2019).

Plaque xylanase and cellulase production capacity

The fungal strains that grew in the medium supplemented with cane straw were sown in Petri dishes with culture medium composed of agar agar (Sigma®), birch xylan and carboxymethylcellulose (Sigma®) as the only carbon source (Ramírez-Lozano *et al*., 2016). The Petri dishes of the fungal strains that showed growth after seven days at 28 °C were stained with a 0.4 % solution of Congo red (Merck®) and after 15 min, the cultures were washed with 10 mL of 1 M NaCl (Fermont®) to reveal the enzyme halo. The enzymatic activity of xylanase and cellulase were determined by the presence of a hydrolysis halo (Adesina and Onilude, 2013; Florencio *et al*., 2012; Youssef *et al*., 2016).

Statistical design and analysis

The experimental design was completely randomized blocks with two blocks (with and without align), two treatments (OM and pH) and four replications (March, April, May and June). The results obtained were analyzed by an analysis of variance (ANOVA) using the Minitab® software (Borges *et al*., 2012).

Results and discussion

Soil analysis

Regarding the soil analysis, the statistical analysis reflected that there are no significant differences between treatments ($p > 0.05$), however, the furrows that contained cane straw (align) presented an average pH of 7 and a content of average organic matter of 16 %, while the rows without the cane straw had an average pH of 6 and an organic matter content of 14 %. In the treatments with align the OM increased 2 % with respect to the soil without align and the pH decreased from 7 with align to 6 without align which is indicative of the benefit of this practice. The result obtained agrees with results where it has been reported that harvest residues were stable in the OM content in the soil (Bojórquez-Serrano *et al*., 2015; Rivera-Cruz *et al*., 2017). Likewise, Quiroz and Pérez (2013) reported that the use of chemical fertilizers caused acidity in the soil, but after filtering crop residues, the OM and pH content did not show variations. On the other hand, it has been shown that harvesting practices that include burning, generate an appreciable decrease in the OM content in the long term, which is considered an aspect to control to prevent soil degradation (Graham *et al*., 2002), making the disposal of sugarcane harvest residues even more relevant.

Isolation and characterization of fungal strains

Thirty-four fungal strains were isolated from the sugarcane straw samplings in March, April, May and June. The results indicated that in the March sampling, strains of the genera *Fusarium* and *Trichoderma* were isolated, in April *Fusarium*, *Trichoderma* and *Penicillum*, in May *Trichoderma*, *Penicillum* and *Aspergillus* and in June *Fusarium*, *Trichoderma*, *Penicillum* and *Aspergillus*. The results obtained in the present work show that as the age of the plant increased, a greater number of fungal genera were isolated, this result agrees with that reported by Deshmukh *et al*. (2013) who indicated that the microbial activity of the plant increases as its age increases.

Figure 1 shows different representative morphological structures (macroscopic and microscopic) for each fungal strain isolated and characterized by month of sampling. Likewise, it is important to mention that the predominated genus in the four samplings was *Trichoderma* spp., Followed by *Fusarium* spp. and *Penicillum* spp., which were detected in three of the four samplings carried out.

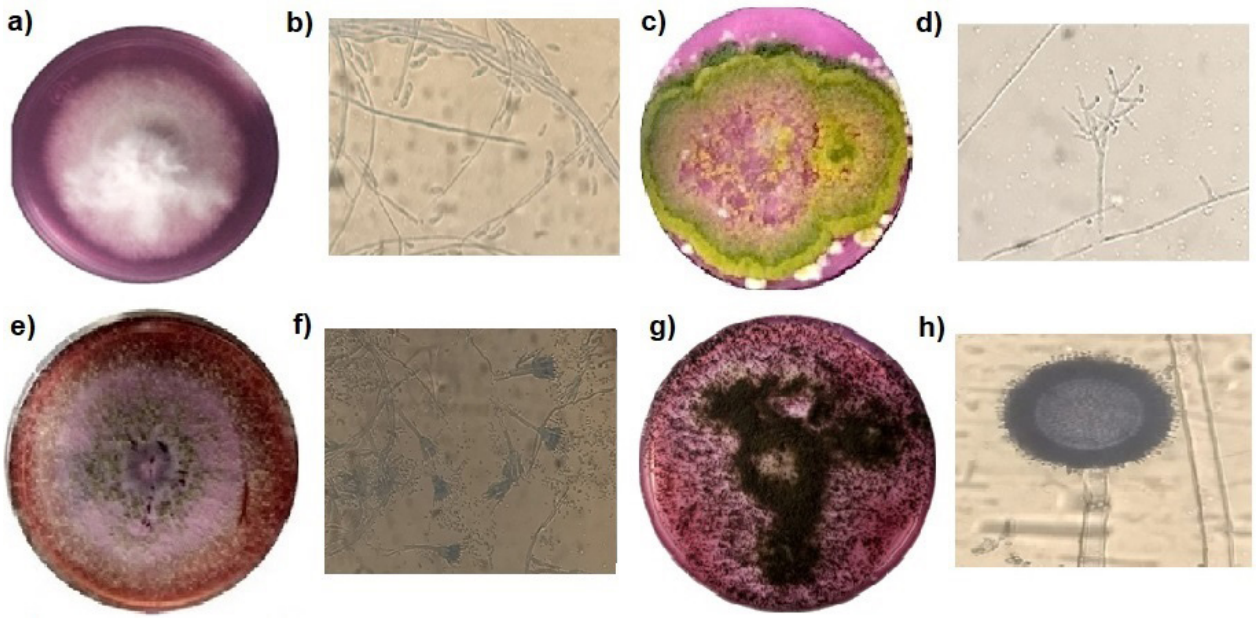

Figure 1. Morphological representation of the isolated strains of cane straw *Fusarium* spp. a) mycelium, b) oval conidia and septa; *Trichoderma* spp. c) cottony mycelium, d) hyaline conidiophores and ovoid conidia; Penicillum spp. e) mycelium and spores, f) branched hyaline conidiophores and globose conidia; *Aspergillus* spp. e) cottony mycelium and spores, f) hyaline conidiophores and conidia with globose vesicle.

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Strains of *Fusarium* spp. Isolated (figure 1a) presented white cottony hyphal growth, while the microscopic structure (figure 1b) showed extensive septate mycelium, simple conidiophores and oval conidia, these results agree with the macroscopic and microscopic structures reported for this fungal strain (Elias *et al*., 2016; Hsuan *et al*., 2011; Upadhyay *et al*., 2020). On the other hand, the strains of *Trichoderma* spp. (figure 1c) isolated showed greenish yellow cottony mycelial development, while in the microscopic structure (figure 1d) it showed hyaline conidiophores and ovoid conidia, these results agree with the macroscopic and microscopic structures reported by various authors (Kannangara and Dharmarathna 2017; Tegene *et al*., 2021). Strains of *Penicillum* spp. presented green cottony hyphal growth with diffusible pink pigmentation (figure 1e) with moderate sporulation, these characteristics are similar to a strain of *Penicillum* sp. characterized by Dhakar *et al*. (2014), on the other hand, the microscopic structure (figure 1f) showed branched hyaline conidiophores and globose conidia, characteristics similar to those reported by various authors (Dhakar *et al*., 2014; Saif *et al*., 2020). Lastly, the *Aspergillus* spp. isolates presented aerial mycelium with abundant sporulation and black spores (figure 1g) and the microscopic structure presented hyaline conidiophores and conidia with globose vesicle, these results coincide with what was indicated by Batista-García *et al*. (2014) for *Aspergillus* sp. cane bagasse isolated.

Romão-Dumaresq *et al*. (2016), indicated that the fungal community of the sugarcane rhizosphere contains at least 35 different species, in which the presence of the genera identified in the present investigation predominates. *Trichoderma* is known as an antagonist that can act as a controller of red rot (*Collecotricum falcatum*) in sugar cane and, its presence in soils has favored germination, yield and, therefore, its presence in cane straw. of sugar sampled may represent an opportunity to reintegrate it into the soils where sugar cane is produced (Joshi *et al*., 2016).

Studies with *Fusarium* spp. indicate that it is considered a phytopathogenic endophyte (Bertonha *et al*., 2018) and is associated with the disease known as red rot, which causes the inversion of sucrose reducing yields (Dela-Cueva *et al*., 2019), although it is also has indicated the resistance of sugarcane to this fungal strain (Mahlanza *et al*., 2013), so its presence can cause this disease in sugarcane stems, which is why careful handling of harvest residues. However, like *Trichoderma*, *Fusarium* is a strain that has been shown to be useful for the production of hydrolytic enzymes, however, as it is a phytopathogen, more studies are required to guarantee or rule out its use for this purpose.

Finally, there is research on the isolation of *Aspergillus* spp. and *Penicillum* spp. of sugar cane residues, mainly bagasse, which is attributed the capacity to produce hydrolytic enzymes, such as xylanases and cellulases (Agudelo *et al*., 2013; Batista-García *et al*., 2014), so its presence in crop residues does not represent any risk.

Growth on sugarcane straw in plate

On the other hand, the results of growth in a Petri dish supplemented with sugarcane straw showed that of the 34 isolated strains, 23 grew on the straw (as the only carbon source), indicating that the strains have the ability to incorporate nutrients from sugarcane straw to its metabolism and produce hydrolytic enzymes (Marques *et al*., 2018), so they can be used to design a solid state cultivation system for the production of xylanases and cellulases using cane straw of sugar as a substrate.

Plaque xylanase and cellulase production capacity

The strains that grew on the sugarcane straw were subjected to qualitative tests for the production of xylanases and cellulases in a Petri dish on birch xylan and carboxymethylcellulose. Of the 23 strains used in the test, 22 had the ability to grow on birch xylan, of which 8 were from the genus *Fusarium* (figure 2B c and d), 11 from *Trichoderma* (figure 2A a and b) and 3 from *Penicillum* (figure 2C e and f), the latter showed little growth as well as the hydrolysis halo. On the other hand, 22 strains grew on carboxymethylcellulose, 8 from the *Fusarium* genus (Figure 3B c and d), 12 from *Trichoderma* (Figure 3A a and b) and 2 from *Penicillum* (Figure 3C e and f). The strains corresponding to the *Trichoderma* genus with growth capacity on birch xylan and carboxymethylcellulose, showed their potential to produce extracellular xylanases and cellulases, as can be seen in figures 2 and 3 where it is observed that they formed a hydrolysis halo in the Petri dishes.

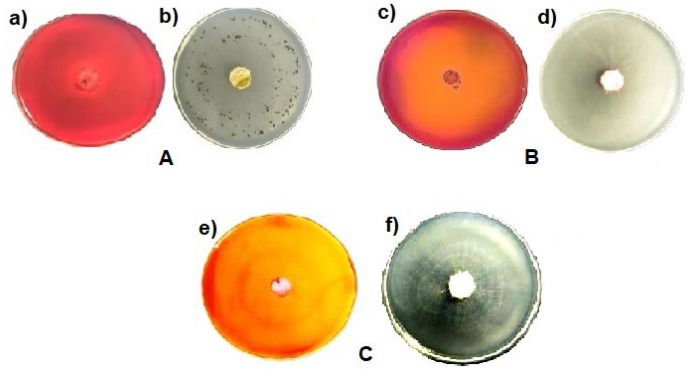

Figure. 2. Growth of the fungal strains on plates supplemented with birch xylan as the sole carbon source and the hydrolysis halo revealed with Congo red. A: *Trichoderma* spp. a) halo of hydrolysis, b) growth; B: *Fusarium* spp. c) halo of hydrolysis, d) growth; D: *Penicillum* spp. d) hydrolysis halo, e) growth.

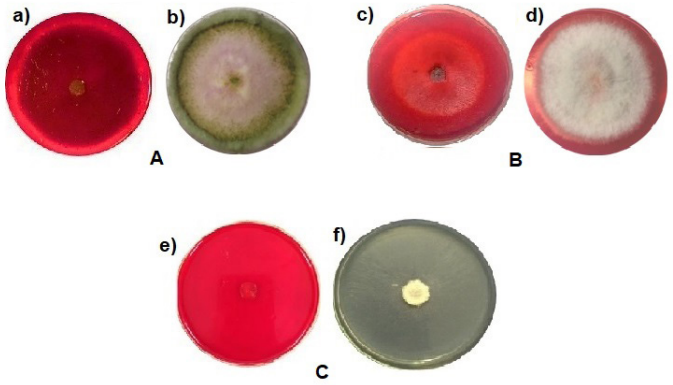

Figure 3. Growth of the fungal strains on plates supplemented with carboxymethylcellulose as the sole carbon source and the hydrolysis halo revealed with Congo red. A: *Trichoderma* spp. a) halo of hydrolysis, b) growth; B: *Fusarium* spp. c) halo of hydrolysis, d) growth; C: *Penicillum* spp. d) halo of hydrolysis, e) growth**.**

The growth results of *Trichoderma* spp. on carboxymethylcellulose and birch xylan demonstrate the potential of these fungal strains for the production of cellulases and xylanases, this fungal genus has been recognized for its ability to produce this type of enzymes extracellularly (Rahnama *et al*., 2013; Zhang *et al*., 2018). For this reason, the sugarcane straw and the fungal strain can be used for the design of a rational process for the production of hydrolytic enzymes, where the sugarcane straw can serve as a substrate and the fungal biomass can be the inoculum for the production of said enzymes (Farinas, 2015; Florencio *et al*., 2015), thus developing an integral process for the use of straw and native strains. It is very important to highlight that Marques *et al*. (2018) published that an endophytic strain of *Trichoderma viridae* presented an enzymatic activity of cellulase of 64 Ug^{-1} and xylanase of 351 Ug^{-1} in solid culture using cane bagasse as support, reaffirming the possibility of designing a process for the production of hydrolytic enzymes.

Likewise, investigations in corn harvest remains, indicate that *Fusarium oxysporum* (Panagiotou *et al*., 2003), as well as, in forest waste *Aspergillus niger* and *F. oxysporum* (Kaushal *et al*., 2012), have the ability to produce enzymes extracellular hydrolytics. Therefore, the strains of *Fusarium* spp. isolated in the present work, as well as sugarcane straw can be explored for the design of a process for the production of hydrolytic enzymes in solid culture, since they showed the growth capacity on carboxymethylcellulose and birch xylan. In addition, there are reports that indicate that hydrolytic enzymes can be produced efficiently using co-cultures of *Fusarium oxysporum* with *Aspergillus niger*, the second was also present in the sugarcane straw evaluated (Romão-Dumaresq *et al*., 2016).

Finally, it is important to mention that the isolated strains with morphological characteristics corresponding to *Penicillum* spp., Showed growth on carboxymethylcellulose and birch xylan, so it can also be explored for the production/design of hydrolytic enzymes, this species has been reported as part of the sugarcane rhizosphere (Romão-Dumaresq *et al*., 2016) and also as a producer of hydrolytic enzymes (Camassola and Dillon, 2010; Gong *et al*., 2015). However, for strains with the ability to produce hydrolysis halo in plates with culture medium supplemented with carboxymethylcellulose and birch xylan, as the only carbon source, additional studies are required in solid culture where the enzymatic activity is quantified and thus, know the real potential of the strains.

Conclusions

The sugar cane straw align favors that the organic matter content does not have significant variations in soils where sugar cane is grown. While the fungal microbiota isolated from the sugarcane straw was *Trichoderma* spp., *Fusarium* spp., *Penicillum* spp. and *Aspergillus* spp. Furthermore, three of the four isolated fungal strains showed potential to grow using carboxymethylcellulose and birch xylan as the sole carbon source indicative of their potential to produce extracellular hydrolytic enzymes.

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Literature cited

Adesina, F. & Onilude, A. (2013). Isolation, identification and screening of xylanase and glucanase-producing microfungi from degrading wood in Nigeria. *African Journal of Agricultural Research, 8*(34), 4414-4421. https://cutt.ly/Inh57Dh

Agudelo, J, Merchán, Z., Zapata, N., y Muñoz, O. (2013). Evaluación de las enzimas celulolíticas producidas por hongos nativos mediante fermentación en estado sólido (SSF) utilizando residuos de cosecha de caña de azúcar. *Revista Colombiana de Biotecnología, 15*(1), 108-117. https://cutt.ly/2nh54yM

Armanhi, J., de Souza, R., Damasceno, N., de Araújo, L., Imperial, J. & Arruda, P. (2018). A community-based culture collection for targeting novel plant growth-promoting bacteria from the sugarcane microbiome. *Frontiers in Plant Science, 8*, 2191. https://doi.org/10.3389/fpls.2017.02191

Barnett, H. & Hunter, B. (1972). Illustrated genera of imperfect fungi. *Illustrated genera of imperfect fungi* (3rd ed). Burges Publishing Company.

Batista-García, R., Balcázar-López, E., Miranda-Miranda, E., Sánchez-Reyes, A., Cuervo-Soto, L., Aceves-Zamudio, D. & Folch-Mallol, J. (2014). Characterization of lignocellulolytic activities from a moderate halophile strain of *Aspergillus caesiellus* isolated from a sugarcane bagasse fermentation. *PLoS One, 9*(8), e105893. https://doi.org/10.1371/journal.pone.0105893

Bertonha, L., Neto, M., Garcia, J., Vieira, T., Castoldi, R., Bracht, A. & Peralta, R. (2018). Screening of *Fusarium* sp. for xylan and cellulose hydrolyzing enzymes and perspectives for the saccharification of delignified sugarcane bagasse. *Biocatalysis and Agricultural Biotechnology, 16*, 385-389. https://doi.org/10.1016/j.bcab.2018.09.010

Borges, J., Barrios, M., Sandoval, E., Bastardo, Y. & Márquez, O. (2012). Características físico-químicas del suelo y su asociación con macroelementos en áreas destinadas a pastoreo en el estado Yaracuy. *Bioagro, 24*(2), 121-126. https://cutt.ly/jnh53mK

Bojórquez-Serrano, J., Castillo Pacheco, L., Hernández Jiménez, A., García Paredes, J. & Madueño-Molina, A. (2015). Cambios en las reservas de carbono orgánico del suelo bajo diferentes coberturas. *Cultivos Tropicales, 36*(4), 63-69. https://cutt.ly/jnh59Yk

Camassola, M. & Dillon, A. (2010). Cellulases and xylanases production by *Penicillium echinulatum* grown on sugar cane bagasse in solid-state fermentation. *Applied Biochemistry and Biotechnology, 162*(7), 1889-1900. https://doi.org/10.1007/s12010-010-8967-3

Cervantes-Preciado, J., Milanés-Ramos, N. & Castillo, M. (2019). Evaluación de 11 híbridos de caña de azúcar (*Saccharum* spp.) en la region central de Veracruz, México. *AGROProductividad, 12*(3), 69-74. https://doi.org/10.32854/agrop.v0i0.1085

Comité Nacional para el desarrollo sustentable de la caña de azúcar (CONADESUCA). 2019. Comparativo de días de zafra respecto al primer estimado de los ingenios, Ciudad de México: Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar. Fecha de consulta: agosto 2020.

Dela-Cueva, F., De Torres, R., de Castro, A., Mendoza, J. & Balendres, M. (2019). Susceptibility of sugarcane to red rot caused by two *Fusarium* species and its impact on stalk sugar level. *Journal of Plant Pathology, 101*(3). https://doi.org/10.1007/s42161-019-00253-2

Dhakar, K., Sharma, A. & Pandey, A. (2014). Cold, pH and salt tolerant *Penicillium* spp. inhabit the high altitude soils in Himalaya, India. *World Journal of Microbiology and Biotechnology, 30*(4), 1315-1324. https://doi.org/10.1007/s11274-013-1545-4

De Souza, R., Okura, V., Armanhi, J., Jorrín, B., Lozano, N., Da Silva, M. & Arruda, P. (2016). Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports, 6*(1), 1-15. https://doi.org/10.1038/srep28774

Deshmukh, R., Dange, S., Jadhav, P., Deokule, S. & Patil, N. (2013). Studies on the mycoflora in the rhizosphere of sugarcane (*Saccharum officinarum* L.). *International Journal of Bioassays, 2*(4), 674-676. https://cutt.ly/enh50ew

Elias, F., Woyessa, D. & Muleta, D. (2016). Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. *International Journal of Microbiology, 2016*, 1-11. https://doi.org/10.1155/2016/5472601

Farinas, C. (2015). Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. *Renewable and Sustainable Energy Reviews, 52*, 179-188. https://doi.org/10.1016/j.rser.2015.07.092

Florencio, C., Couri, S. & Farinas, C. (2012). Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by *Trichoderma* strains. *Enzyme research, 2012*. https://doi.org/10.1155/2012/793708

Florencio, C., Cunha, F., Badino, A. & Farinas, C. (2015). Validation of a novel sequential cultivation method for the production of enzymatic cocktails from *Trichoderma* strains. *Applied Biochemistry and Biotechnology, 175*(3), 1389-1402. https://doi.org/10.1007/s12010-014-1357-5

Gong, W., Zhang, H., Liu, S., Zhang, L., Gao, P., Chen, G. & Wang, L. (2015). Comparative secretome analysis of *Aspergillus niger*, *Trichoderma reesei*, and *Penicillium oxalicum* during solid-state fermentation.

Applied Biochemistry and Biotechnology, 177(6), 1252-1271. https://doi.org/10.1007/s12010-015-1811-z

Graham, M., Haynes, R. & Meyer, J. (2002). Soil organic matter content and quality: effects of fertilizer applications, burning and trash retention on a long-term sugarcane experiment in South Africa. *Soil Biology and Biochemistry, 34*(1), 93-102. https://doi.org/10.1016/S0038-0717(01)00160-2

Hsuan, H., Salleh, B. & Zakaria, L. (2011). Molecular identification of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. *International Journal of Molecular Sciences, 12*(10), 6722-6732. https://doi.org/10.3390/ijms12106722

Joshi, D., Singh, P., Singh, A., Lal, R. & Tripathi, N. (2016). Antifungal potential of metabolites from *Trichoderma* sp. against *Colletotrichum falcatum* went causing red rot of sugarcane. *Sugar Tech, 18*(5), 529-536. https://doi.org/10.1007/s12355-015-0421-y

Kannangara, S. & Dharmarathna, R. M. (2017). Isolation, identification and characterization of *Trichoderma* species as a potential biocontrol agent against *Ceratocystis paradoxa*. *The Journal of Agricultural Science, 12*(1): 51-62. http://doi.org/10.4038/jas.v12i1.8206

Kaushal, R., Sharma, N. & Tandon, D. (2012). Cellulase and xylanase production by co-culture *of Aspergillus niger and Fusarium oxysporum* utilizing forest waste. *Turkish Journal of Biochemistry, 37*(1). http://doi.org/10.5505/tjb.2012.43434

Lacerda, L., Gusmão, L. & Rodrigues, A. (2018). Diversity of endophytic fungi in *Eucalyptus microcorys* assessed by complementary isolation methods. *Mycological Progress, 17*(6), 719-727. https://doi.org/10.1007/s11557-018-1385-6

Lizardi-Jiménez, M., Ricardo-Díaz, J., Quiñones-Muñoz, T., Hernández-Rosas, F. & Hernández-Martínez, R. (2019). Fungal strain selection for protease production by solid-state fermentation using agro-industrial waste as substrates. *Chemical Papers, 73*(10), 2603-2610. https://doi.org/10.1007/s11696-019-00814-w

Mahlanza, T., Rutherford, R., Snyman, S. & Watt, M. (2013). In vitro generation of somaclonal variant plants of sugarcane for tolerance to *Fusarium sacchari*. *Plant cell reports, 32*(2), 249-262. https://doi.org/10.1007/s00299-012-1359-0

Marques, N., de Cassia Pereira, J., Gomes, E., da Silva, R., Araújo, A., Ferreira, H.& Bocchini, D. (2018). Cellulases and xylanases production by endophytic fungi by solid state fermentation using lignocellulosic substrates and enzymatic saccharification of pretreated sugarcane bagasse. *Industrial Crops and Products, 122*, 66-75. https://doi.org/10.1016/j.indcrop.2018.05.022

Martínez, L., Martínez, S. & Cuevas, R. (2013). Efecto de la tierra de diatomeas en las propiedades químicas del suelo en el cultivo de maíz (*Zea mays*, L.). *RIAA, 4*(2), 13-26. https://doi.org/10.22490/21456453.984

Panagiotou, G., Kekos, D., Macris, B., & Christakopoulos, P. (2003). Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. *Industrial crops and products, 18*(1), 37-45. https://doi.org/10.1016/S0926-6690(03)00018-9

Quiroz-Guerrero, I. & Pérez, A. (2013). Vinaza y compost de cachaza: efecto en la calidad del suelo cultivado con caña de azúcar. *Revista Mexicana de Ciencias Agrícolas, 4*(SPE5), 1069-1075. https://cutt.ly/Tnh5nVC

Rahnama, N., Mamat, S., Shah, U., Ling, F., Rahman, N. & Ariff, A. (2013). Effect of alkali pretreatment of rice straw on cellulase and xylanase production by local *Trichoderma harzianum* SNRS3 under solid state fermentation. *BioResources, 8*(2), 2881-2896. https://doi.org/10.15376/biores.8.2.2881-2896

Ramírez-Lozano, D., Soria, A., Gutiérrez, B., Baptista, R., Garza, Y. & Gaona Lozano, J. (2016). Producción de enzimas celulolíticas a partir de hongos aislados de muestras ambientales de Cuatro Ciénegas, Coahuila. *Mexican Journal of Biotechnology, 1*(1), 157-164. https://cutt.ly/Ynh5z7w

Rivera-Cruz, M., Magaña M. y Trujillo A. (2017). Modificaciones en materia orgánica y actividad enzimática del suelo por fuego usado en la quema de caña de azúcar. p. 393-397. *En*: Seguridad Alimentaria: Aportaciones Científicas y Agrotecnológicas. Primera edición.

Romão-Dumaresq, A., Dourado, M., Fávaro, L., Mendes, R., Ferreira A., & Araújo, W. (2016). Diversity of cultivated fungi associated with conventional and transgenic sugarcane and the interaction between endophytic *Trichoderma virens* and the host plant. *PloS one,11*(7), e0158974. https://doi.org/10.1371/journal.pone.0158974

Rozas, H., Echeverria, H., & Angelini, H. (2011). Niveles de carbono orgánico y ph en suelos agrícolas de las regiones pampeana y extrapampeana Argentina. *Revista Ciencia del Suelo, 29*, 29-37. https://cutt.ly/Znh5kAM

Saif, F., Yaseen, S., Alameen, A., Mane, S. & Undre, P. (2020). Identification of *Penicillium Species* of Fruits Using Morphology and Spectroscopic Methods. *Journal of Physics: Conference Series, 1644*(1), 012019. https://doi.org/10.1088/1742-6596/1644/1/012019

Tegene, S., Dejene, M., Terefe, H., Tegegn, G., Tena, E. & Ayalew, A. (2021). Evaluation of native *Trichoderma* isolates for the management of sugarcane smut (*Ustilago scitaminea*) in sugar plantations of Ethiopia. *Cogent Food & Agriculture, 7*(1), 1872853. https://doi.org/10.1080/23311932.2021.1872853

Upadhyay, M., Awasthi, A. & Joshi, D. (2020). Explorando la eficacia del biocontrol de *Trichoderma* spp. contra *Fusarium sacchari*, el agente causal de la marchitez de la caña de azúcar. *Biotecnología Vegetal, 20*(3), 237-247. https://cutt.ly/Mnh5jhI

Yarce, C. & Castillo, J. (2014). Validación no exhaustiva del método analítico de Walkley–Black, para la determinación de materia orgánica en suelos por espectrofotometría de UV-VIS. *Ingenium, 8*(19), 37-46. https://cutt.ly/Dnh5g03

Youssef, M., Saber, W., El-Taher, E., & El daiem, A. (2016). Isolation and Identification of the Highly Cellulolytic and P-Solubilizing Fungi. *Journal of Environmental Sciences, 45*(1), 75-84. https://cutt.ly/Gnh5fiL

Zhang, F., Zhao, X. & Bai, F. (2018). Improvement of cellulase production in *Trichoderma reesei* Rut-C30 by overexpression of a novel regulatory gene Trvib-1. *Bioresource Technology, 247*, 676-683. https://doi.org/10.1016/j.biortech.2017.09.126