Hindawi
Journal of Chemistry
Volume 2020, Article ID 6092165, 7 pages
https://doi.org/10.1155/2020/6092165

Research Article

Agave Leaves as a Substrate for the Production of Cellulases by *Penicillium* sp. and the Obtainment of Reducing Sugars

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Received 9 February 2020; Accepted 2 March 2020; Published 24 April 2020

Academic Editor: Thomas R. Caulfield

Lignocellulosic biomass can be used to obtain fermentable sugars by enzymatic hydrolysis, and also it serves as a carbon source to produce cellulases by solid-state fermentation. In this study, we propose the use of leaves of *Agave salmiana* as a carbon source to produce cellulases by the fungus *Penicillium* sp., isolated from the same plant. The crude enzymatic extract was used to obtain sugars from the hydrolysis of the parenchymal cells of the leaves. The enzymes produced were characterized (endoglucanase 14.4 U/g; exoglucanase 3.5 U/g; β-glucosidase 4.14 U/g). The enzymes showed activities at elevated temperatures: 50°C for endoglucanase and exoglucanase and 70°C for β-glucosidase. Furthermore, the crude enzymatic extract obtained was able to hydrolyze the parenchyma in 51.6% in 48 h. The evidence presented in this paper shows the potential of the *agave* leaves as a source of carbon in the production of enzymes by fermentation with the consequent production of reducing sugars. In addition, the enzymes produced by *Penicillium sp.* could be used in the production of bioethanol, since they work at high temperatures.

1. Introduction

With an annual production from land plants of more than 100 billion tons, lignocellulosic biomass has considerable potential to produce biofuels and high value-added by-products [1]. To obtain these by-products, it is necessary to degrade lignocellulosic biomass from cellulose to glucose. Cellulases, an enzymatic system generally composed of endoglucanase, exoglucanase, and β-glucosidase, are the enzymes responsible for hydrolyzing the cellulose chain to glucose monomers [2]. Cellulases are the third most produced industrial enzymes, representing about 20% of the total market of enzymes in the world [3]. Nevertheless, the high price of cellulases and a large amount of these enzymes needed compromise the industrial degradations of cellulose cost. Strategies have been implemented to improve the efficiency of known cellulases and to search for new cellulase-producing microorganisms. Alternatively, cellulases can be produced by solid-state fermentation (SSF) [3]. Several microorganisms capable of producing cellulases have been reported using lignocellulosic residues as a carbon source [4–6].

Plants from the genus *Agave* represent a potential feedstock for enzymatic production of biofuels and high value-added by-products, with advantageous characteristics over other crops, such as low water requirement, high productivity in arid or semi-arid lands, adaptability to high temperatures, and resistance to drought [7]. There are more than 200 species of the genus *Agave*, of which the majority are native to México [8].

Recently, *Agave* species have been found to be a good source of polysaccharides (i.e. cellulose and fructans), used to obtain different products such as nanocellulose and fermentable sugars and as a substrate for enzymes

production [9–13]. During the industrial production of fermented beverages from *A. salmiana*, leaves are discarded. These leaves contain a low amount of lignin (approximately 9–13 wt%) that provides low recalcitrance [14, 15]. These leaves are covered with a waxy layer of cutin and a thick layer of chlorophyll, that protects the plant (Figure 1(a)) from adverse environmental conditions. Inside, the cellulose fibers serve as support for the leaves, while the parenchyma cells are actively involved in photosynthesis, secretion, and food storage (Figure 1(b)). The cell wall in the parenchyma of the *Agave* leaves is thinner as compared with other plants [12, 15].

Previously, we isolated cellulose layers in the form of nanoplatelets with a thickness of less than 90 nm from the parenchymal cell wall of *A. salmiana* leaves [16]. We have also reported that these cellulose nanoplatelets are easier to hydrolyze than larger cellulose, due to their low lignin content, poor crystallinity, and greater surface area [13]. Also, several strains of enzyme-producer fungi have been isolated from *Agave* species [17, 18]. For instance, in earlier studies, we evaluated the enzymatic activity of different fungal strains previously isolated from *A. salmiana* leaves, with the highest enzymatic activity resulting from a fungus preliminarily classified as belonging to the genus *Penicillium* (unpublished data). For the above reasons, the aim of the present study was therefore to produce cellulases by solid-state fermentation using *A. salmiana* leaves as the carbon source. In addition, the enzymes obtained by this method were characterized and used to obtain fermentable sugars from the parenchymal cells of the same plant species.

2. Materials and Methods

2.1. Feedstock and Reagents. All reagents were of analytical grade. Salts and commercial carbohydrates were purchased from Sigma-Aldrich and culture media from BD Bioxon. The leaves were pulled away from the base of plant grown in soil and washed and decorticated to eliminate the epidermis and cuticle. The remaining material was cut into small pieces and frozen at −20°C until use. This biomass obtained from inside the *Agave* leaf was used to produce enzymes, nanocellulose, and sugars in the following steps.

2.2. Inoculum Preparation. Previously, *Penicillium* sp. was isolated from a leaf of decaying *A. salmiana*. Potato dextrose agar (PDA) was used for the isolation and storage of the strain. Unless otherwise mentioned, the inoculum was prepared as follows: fungus was inoculated in tubes filled with 5 mL PDA and incubated by 6 days at 28°C. After, spores were recovered with saline 0.85% w/v. The inoculum was adjusted to a concentration of 1×10^6 spores/mL using a Neubauer chamber for counting the spores.

2.3. Agave salmiana Leaves as a Carbon Source. Agave biomass was lyophilized to facilitate the mechanical separation of the fibers from the parenchyma. Fibers were washed with hot water to remove residual sugars, and the parenchyma was used unmodified. A subsample of the parenchyma was used to obtain nanocellulose by a method previously described [12]. The isolated *Penicillium* sp. strain was cultivated in 125 mL Erlenmeyer flasks containing 500 mg of fiber or parenchyma, 1 mg $(NH_4)_2SO_4$, and 1 mL acetate buffer pH 5. Flasks were sterilized by autoclaving and subsequently inoculated with 1 mL of the inoculum prepared previously. Flasks were incubated at 28°C under static conditions for 7 days. After incubation, enzymes were recovered by adding 25 mL acetate buffer pH 5 and transferred at 50 mL tubes. Tubes were centrifugated at 4,500 rpm for 20 min. The supernatants were separated and used as crude cellulase extracts to hydrolyze 3 substrates: fiber, parenchyma, and nanocellulose. In 2 mL tubes, 100 μL crude extract, 900 μL acetate buffer pH 5, and 10 mg of each substrate were added. Tubes were incubated at 50°C, 150 rpm for 24 h. After hydrolysis, sugars were measured for the 3,5 DNS acid method [19].

2.4. Scanning Electron Microscopy (SEM). The morphology was observed by SEM using a HITACHI UHR Cold-Emission FE-SEM SU8020 with an acceleration voltage of 1 kV and a working distance of 9 mm. A subsample of the lyophilized biomass (previously obtained) was suspended in ethanol. A drop of the suspension was deposited on a silicon wafer and then dried at 40°C for 2 h. Then, the silicon wafer was glued with an adhesive carbon tape to an aluminum holder.

2.5. Cellulase Production by SSF. Agave biomass was defrosted and washed with hot water to remove inorganic compounds, colorants, and sugars (the reducing sugars were measured in the wash water until being completely removed from the biomass). The resulting bagasse was oven-dried for 2 days at 70°C and ground (Krups mill, model GX410011). Fermentations were carried out in 125 mL Erlenmeyer flasks, each having 3 g of the ground bagasse and 12 mL of a solution (g/L: 2.0 KH_2PO_4, 1.4 $(NH_4)_2SO_4$, 0.3 $MgSO_4$, 0.3 $CaCl_2$, 1.0 peptone, 1.0 yeast extract, 1 mL Triton X–100, and pH 5.5). The flasks were sterilized by autoclaving and subsequently inoculated with 3 mL of inoculum prepared previously at a concentration of 1×10^6 spores/mL. Flasks were incubated at 28°C for 0, 2, 4, 6, 8, 10, 12, and 14 days without agitation. Enzymes were recovered by adding 30 mL acetate buffer at pH 5 by the previously described method.

2.6. Enzymatic Activity Assays. Exoglucanase, endoglucanase, and β-glucosidase activities were determined by using microcrystalline cellulose type 20 (MCC20), carboxymethyl cellulose (CMC), and p-nitrophenyl-β-D-glucopyranoside (pNPG) as substrates, respectively. Assays to determinate exoglucanase and endoglucanase activities were carried out in 2 mL tubes containing 900 μL acetate buffer pH 5, 100 μL enzyme extract, and 10 mg substrate (MCC20 or CMC). Tubes were incubated at 50°C, 150 rpm for 2 h and 20 min for exoglucanase and endoglucanase, respectively. Sugars were measured for the 3,5 DNS acid method. β-glucosidase activity was determined by incubating 50 μL enzyme extract

(a) (b)

Figure 1: *Agave* plant (a) and a micrograph (own image) of the interior of the leaf showing the morphology of the fibers (arrows) and the parenchymal cells (circles) (b).

and 200 μL 0.2 mM pNPG (in acetate buffer pH 5) at 50°C for 10 min. After incubation, 1 mL of 0.2 M Na_2CO_3 was added to stop the reaction, and the absorbance was measured at 405 nm. Once the optimal day of production of each enzyme was determined, the influence of pH and temperature on enzymatic activity was evaluated. Assays were performed at pH ranging from 3 to 10 and at temperatures ranging from 30 to 90°C. One unit of activity is defined as the amount of crude enzyme extract that releases 1 μmol of glucose or p-nitrophenol (pNP) per min per gram of biomass under assay conditions.

2.7. Obtainment of Sugars. Ground bagasse was obtained from the frozen *Agave* biomass as previously described. This bagasse was sieved to separate the parenchyma from the fibers. The fibers were discarded, and the parenchyma was subjected to enzymatic hydrolysis. The assays were carried out in 2 mL Eppendorf tubes containing 1000 μL of acetate buffer (pH 5), 500 μL of crude cellulase extract, and 10 mg of parenchyma. The tubes were incubated at 50°C and 150 rpm for 0, 12, 24, and 48 h. After each time, sugars were measured using the 3,5 DNS acid method. The assays were performed in triplicate.

3. Results and Discussion

3.1. Agave salmiana Leaves as a Carbon Source. Due to the morphological dissimilarities between the cellulose from the fibers and the parenchyma (Figure 1(b)), the ability of *Penicillium* sp. to use each type of cellulose as a carbon source was evaluated. The fungus grew faster using the parenchyma as the carbon source (Figure S1); nevertheless, when evaluating enzymatic activity, the enzymatic extract alone was able to hydrolyze the parenchyma (Table 1). It is possible that the tested *Penicillium* strain produced inulinases to hydrolyze the fructans from the parenchyma instead of the cellulose; for this reason, the hydrolysis of the fibers or nanocellulose was absent. Using fibers as the carbon source, slowed *Penicillium* growth (Figure S1), however, the enzymatic extract was able to hydrolyze both substrates (Table 1). It has to be noted that fibers were thoroughly washed before *Penicillium* was inoculated, avoiding the presence of any carbon source other than cellulose; therefore, it seems unlikely that the fungus produced alternative hydrolytic enzymes.

The nanocellulose and parenchyma are known to be easier to hydrolyze as compared to fibers due to their lower crystallinity and small size that increases surface area and enzymes contact with the substrate, thus causing greater hydrolysis [13]. The slight differences in enzymatic activities between using either nanocellulose or parenchyma may be related by the structural differences among both carbon sources. Pretreatment given to the nanocellulose are reported to eliminate its lignin cover and makes nanocellulose easier to hydrolyze [20].

In natural habitats, microorganisms regulate their enzyme production for the most efficient usage of the available carbon source [21]. Previously, it has been reported that the addition of glucose to the culture medium favors rapid growth of fungi but suppresses the cellulolytic activity [22]. Since a parenchymal cell contains simple sugars rapidly used by fungi before cellulose, fungi production of cellulases was limited (Table 1). On the other hand, fungi were able to produce cellulases to hydrolyze fibers and grow. It is noted the importance of minimizing sugar presence in the biomass before carrying out fermentation to induce cellulose production by fungi from the culture start. *Agave* leaves contain high amounts of soluble sugars [10, 14] which are mostly removed by squeezing the leaves after a freeze-thaw process. These sugars were recovered and preserved for future experiments as they can be used directly for fermentation.

3.2. Cellulase Production. The production of endoglucanase, exoglucanase, and β-glucosidase was determined in the SSF of *Penicillium* sp. at 28°C. Figure 2 shows that the activity of all enzymes was detected after 2 days of fermentation. Exoglucanase activity increased from day 2 and remained constant until 10 days, decreasing slowly after that. The

Table 1: Enzymatic hydrolysis of different sources of cellulose (expressed in mg/mL glucose).

Carbon source for enzyme production	Substrates for enzymatic hydrolysis		
	Fiber	Parenchyma	Nanocellulose
Fiber	0.17	0.90	1.06
Parenchyma	ND	0.91	ND

ND: not determined. Values below the measurement threshold.

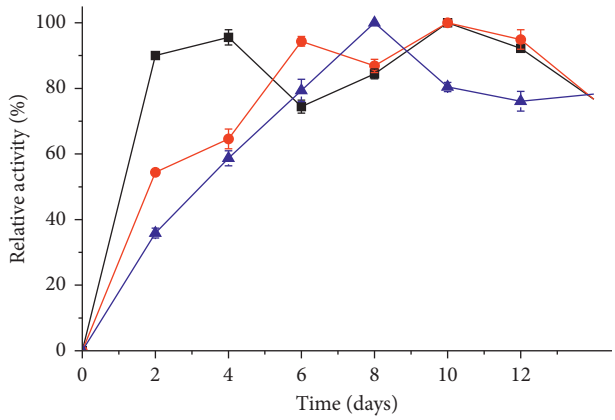

Figure 2: Production of endoglucanase (●), exoglucanase (■), and β-glucosidase (▲) by SSF of *A. salmiana* cellulose. The error bars represent the mean ± standard deviation (SD) of three values.

constant activity of the exoglucanase was probably motivated by the presence of *Agave* fibers in the medium. The exoglucanases are responsible for the hydrolysis of the crystalline portions of cellulose [2]. *Agave* fibers have a high crystallinity index similar to commercial cellulose; therefore, enzymes are required to be active throughout the fermentation to hydrolyze the fibers [16, 20]. The activities of endoglucanase and β-glucosidase increased until 6 and 8 days, respectively. The β-glucosidase showed a rapid decline after 8 days, while the endoglucanase remained active until 12 days. The synergy between endo- and exoglucanase generates cellobiose, which can be hydrolyzed by β-glucosidases [23]. This disaccharide is a strong inhibitor of endo- and exoglucanases; therefore, a constant activity of β-glucosidase is necessary to keep the concentration of cellobiose to a minimum so that there is no inhibition of the other enzymes. The rapid decrease in activity of glucosidase from day 10 caused a slow loss of activity of the other enzymes (Figure 2), so it was not needed to extend the fermentation time, since the maximum activity point for each enzyme was already reached.

3.3. Effect of pH and Temperature on Enzymatic Activity. The optimal pH for each enzymatic activity was 4 for endo- and exoglucanases and 6 for β-glucosidase. Variations in pH decreased these activities (Figure 3). Endo- and exoglucanases exhibited their highest activity at 50°C, while β-glucosidase up to 70°C. No enzyme showed activity above 80°C (Figure 4). The optimal pH values of each enzyme reported in this work are within the range of those already reported in other studies [24, 25]. Industrial enzymatic hydrolysis carried out at <50°C shows slow hydrolysis rates, generating low amounts of sugars from lignocellulosic biomass; in addition, they are prone to microbial contamination. In such cases, high doses of enzymes are required. For this reason, enzymes that work at high temperatures have been suggested to be used to overcome these limitations [26]. Microorganisms producing thermophilic enzymes have already been reported [25–27]. Enzymes produced by the tested *Penicillium* fungus have shown to have activity at high temperatures, so they could be used in enzymatic hydrolysis technologies.

The highest enzymatic activities obtained for endoglucanase, exoglucanase, and β-glucosidase were 14.4, 3.5, and 4.14 U/g of biomass, respectively, under the ideal conditions of pH and temperature. Table 2 compares the enzymatic activities of cellulases produced by different microorganisms under SSF using different carbon sources. The fermentation conditions, as well as the microorganism and the biomass source used, are the main factors that influence the enzymatic activity and the amount of cellulases produced under solid-state fermentation [3, 24, 28]; therefore, a realistic comparison of cellulase production by different other microorganisms and carbon sources as reported in the literature may not be possible due to the different fermentation conditions described by each research group [24]. Nevertheless, the enzymes produced by the isolated *Penicillium* fungus had a higher enzymatic activity compared with other *Penicillium* species (Table 2).

The physical processes of cutting and grinding are useful for processing the lignocellulosic biomass as it manages to decrease its particle size, increasing the surface area [32]. Nevertheless, these physical pretreatments have no impact to eliminate other components such as waxes, lignin, and sugars nor do they improve the digestibility of cellulose considerably; this is why chemical pretreatments (alkaline or acid) have become necessary to improve the demineralization of cellulose [33]. However, it has been shown that these chemical pretreatments generate toxic compounds for yeasts during bioethanol production [34].

3.4. Enzymatic Hydrolysis of the Parenchyma. To favor the biomass hydrolysis to obtain a greater quantity of sugars, a high amount of crude enzyme extract was used, with which the parenchyma was hydrolyzed in 51.6% in a time of 48 h (Figure 5).

Although the parenchyma used to obtain sugars had no pretreatment, a high conversion of the biomass was achieved. The obtained percentage of hydrolysis was similar to that reported by other authors using *Agave* species [11, 13, 32]. As previously demonstrated, parenchyma was easier to hydrolyze than fibers due to its greater surface area.

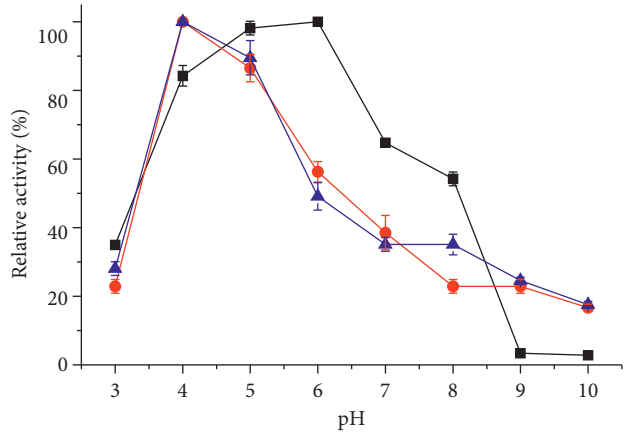

Figure 3: Effect of pH on the endoglucanase (●), exoglucanase (▲), and β-glucosidase (■) activity. The error bars represent the mean ± SD ($n = 3$).

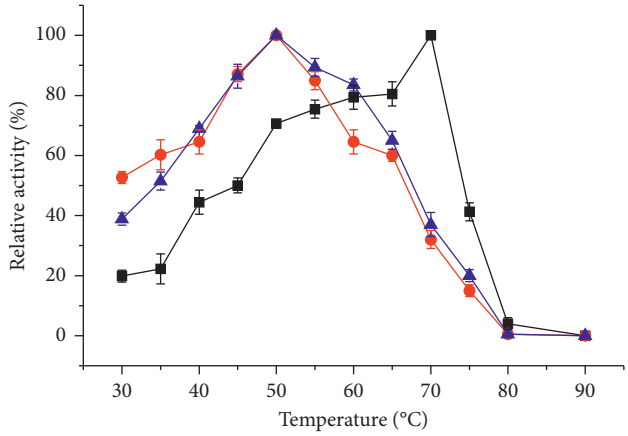

Figure 4: Effect of temperature on the endoglucanase (●), exoglucanase (▲), and β-glucosidase (■) activity. The error bars represent the mean ± SD ($n = 3$).

Table 2: Comparison of cellulase production by *Penicillium* sp. with other microorganisms under SSF.

Enzyme	Microorganism	Carbon source	Activity (U/g)	Reference
Endoglucanase	*Penicillium citrinum* MTSS 6489	Wheat bran	7.56	[29]
		Rice bran	10.2	
		Rice straw	3.72	
	Fomitopsis sp. RCK2010	Wheat bran	71.70	[24]
	Aspergillus niger	Soybean meal	35.10	[30]
	A. nidulans AKB25	Black gram	152.14	[28]
	P. citrinum CBMAI 1186	Wheat bran	1.93	[31]
	Penicillium sp.	*A. salmiana*	14.4	Present work
Exoglucanase	*P. citrinum* MTSS 6489	Wheat bran	6.88	[29]
		Rice bran	2.56	
		Rice straw	4.56	
	Fomitopsis sp. RCK2010	Wheat bran	3.49	[24]
	A. niger	Soybean meal	0.55	[30]
	A. nidulans AKB25	Black gram	3.42	[28]
	P. citrinum CBMAI 1186	Wheat bran	1.33	[31]
	Penicillium sp.	*A. salmiana*	3.50	Present work
β-glucosidase	*Fomitopsis* sp. RCK2010	Wheat bran	53.68	[24]
	A. nidulans AKB25	Black gram	35.11	[28]
	P. citrinum CBMAI 1186	Wheat bran	0.22	[31]
	Penicillium sp.	*A. salmiana*	4.14	Present work

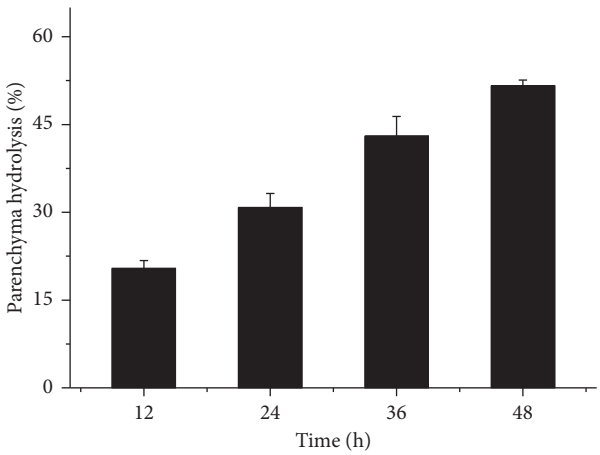

Figure 5: Percentage of hydrolysis of the parenchymal cells by the crude enzymatic extract at different times. The error bars represent the mean ± SD ($n = 3$).

Therefore, in addition to selecting the most appropriate pretreatment method and enzyme complex, it would also be convenient to consider the most digestible portion of the biomass as another factor to consider during enzymatic hydrolysis.

4. Conclusions

In this study, *Agave salmiana* leaves were evaluated both to produce enzymes and for obtaining sugars. The portion of the leaves corresponding to the parenchyma cells proved to be a material with promising potential to be used in obtaining reducing sugars. Nevertheless, for enzyme production, it is necessary to optimize the fermentation conditions to improve the yield. In addition, the *Penicillium* fungus isolated by the research group has not been evaluated or characterized in depth; however, it was observed that its enzymes have activity at high temperatures, so it will be convenient in the future to characterize the enzymes more thoroughly to be used in industrial enzymatic hydrolysis.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by the Universidad Autónoma de Nuevo León. JSM thanks the CONACYT for doctoral scholarship 514440.

Supplementary Materials

Figure 1: growth of *Penicillium* sp. on the parenchyma (left) and fibers (right). (*Supplementary Materials*)

References

[1] E. Espino, M. Cakir, S. Domenek, A. D. Román-Gutiérrez, N. Belgacem, and J. Bras, "Isolation and characterization of cellulose nanocrystals from industrial by-products of *Agave tequilana* and barley," *Industrial Crops and Products*, vol. 62, pp. 552–559, 2014.

[2] B. Henrissat and A. Bairoch, "New families in the classification of glycosyl hydrolases based on amino acid sequence similarities," *Biochemical Journal*, vol. 293, no. 3, pp. 781–788, 1993.

[3] L. W. Yoon, T. N. Ang, G. C. Ngoh, and A. S. M. Chua, "Fungal solid-state fermentation and various methods of enhancement in cellulase production," *Biomass and Bioenergy*, vol. 67, pp. 319–338, 2014.

[4] G. S. Dhillon, S. Kaur, S. K. Brar, and M. Verma, "Potential of apple pomace as a solid substrate for fungal cellulase and hemicellulase bioproduction through solid-state fermentation," *Industrial Crops and Products*, vol. 38, pp. 6–13, 2012.

[5] N. Y. Nava-Cruz, J. C. Contreras-Esquivel, M. A. Aguilar-González, A. Nuncio, R. Rodríguez-Herrera, and C. N. Aguilar, "*Agave atrovirens* fibers as substrate and support for solid-state fermentation for cellulase production by *Trichoderma asperellum*," *3 Biotech*, vol. 6, p. 115, 2016.

[6] Z. K. Bagewadi, S. I. Mulla, and H. Z. Ninnekar, "Optimization of endoglucanase production from Trichoderma harzianum strain HZN11 by central composite design under response surface methodology," *Biomass Conversion and Biorefinery*, vol. 8, no. 2, pp. 305–316, 2018.

[7] J. A. Pérez-Pimienta, M. G. López-Ortega, and A. Sanchez, "Recent developments in Agave performance as a drought-tolerant biofuel feedstock: agronomics, characterization, and biorefining," *Biofuels, Bioproducts and Biorefining*, vol. 11, no. 4, pp. 732–748, 2017.

[8] A. Delgado-Lemus, A. Casas, and O. Téllez, "Distribution, abundance and traditional management of Agave potatorum in the Tehuacán Valley, Mexico: bases for sustainable use of non-timber forest products," *Journal of Ethnobiology and Ethnomedicine*, vol. 10, no. 1, p. 63, 2014.

[9] W. Praznik, R. Löppert, J. M. Cruz Rubio, K. Zangger, and A. Huber, "Structure of fructo-oligosaccharides from leaves and stem of Agave tequilana Weber, var. azul," *Carbohydrate Research*, vol. 381, pp. 64–73, 2013.

[10] D. Close, M. Rodriguez Jr., R. Hu, and X. Yang, "Disposition and bioavailability of inulin and free sugar in untreated and dilute acid pretreated *Agave tequilana* leaves," *Biomass and Bioenergy*, vol. 106, pp. 176–181, 2017.

[11] M. Láinez, H. A. Ruiz, A. A. Castro-Luna, and S. Martínez-Hernández, "Release of simple sugars from lignocellulosic biomass of *Agave salmiana* leaves subject to sequential pretreatment and enzymatic saccharification," *Biomass and Bioenergy*, vol. 118, pp. 133–140, 2018.

[12] J. Silva-Mendoza, M. E. Cantú-Cárdenas, S. Vazquez-Rodriguez, A. Toxqui-Terán, and L. Chávez-Guerrero, "Biopolymeric films obtained from the parenchyma cells of *Agave salmiana* leaves," *Cellulose*, vol. 26, no. 3, pp. 1869–1879, 2019.

[13] L. Chávez-Guerrero, J. Silva-Mendoza, S. Sepúlveda-Guzmán et al., "Enzymatic hydrolysis of cellulose nanoplatelets as a source of sugars with the concomitant production of cellulose nanofibrils," *Carbohydrate Polymers*, vol. 210, pp. 85–91, 2019.

[14] H. Li, S. Pattathil, M. B. Foston et al., "Agave proves to be a low recalcitrant lignocellulosic feedstock for biofuels production on semi-arid lands," *Biotechnology for Biofuels*, vol. 7, no. 1, pp. 50–60, 2014.

[15] K. Corbin, C. Byrt, S. Bauer et al., "Prospecting for energy rich renewable raw materials: agave leaf case study," *PLoS One*, vol. 10, no. 8, Article ID e0135382, 2015.

[16] L. Chávez-Guerrero, S. Sepúlveda-Guzmán, C. Rodríguez-Liñan, J. Silva-Mendoza, N. García-Gómez, and O. Pérez-Camacho, "Isolation and characterization of cellulose nanoplatelets from the parenchyma cells of *Agave salmiana*," *Cellulose*, vol. 24, no. 9, pp. 3741–3752, 2017.

[17] M. G. Contreras-Hernández, E. T. Aréchiga-Carvajal, M. R. Moreno-Jiménez et al., "Enzymatic potential of native fungal strains of agave residues," *BioResources*, vol. 13, pp. 569–585, 2018.

[18] G. Campos-Rivero, L. F. Sánchez-Teyer, E. A. De la Cruz-Arguijo, M. S. Ramírez-González, C. P. Larralde-Corona, and J. A. Narváez-Zapata, "Bioprospecting for fungi with potential pathogenic activity on leaves of *Agave tequilana* Weber var," *Journal of Phytopathology*, vol. 167, pp. 283–294, 2019.

[19] G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry*, vol. 31, no. 3, pp. 426–428, 1959.

[20] L. Chávez-Guerrero, S. Sepúlveda-Guzmán, J. Silva-Mendoza, C. Aguilar-Flores, and O. Pérez-Camacho, "Eco-friendly isolation of cellulose nanoplatelets through oxidation under mild conditions," *Carbohydrate Polymers*, vol. 181, pp. 642–649, 2018.

[21] N. Aro, T. Pakula, and M. Penttilä, "Transcriptional regulation of plant cell wall degradation by filamentous fungi," *FEMS Microbiology Reviews*, vol. 29, no. 4, pp. 719–739, 2005.

[22] A. Kobakhidze, M. Asatiani, E. Kachlishvili, and V. Elisashvili, "Induction and catabolite repression of cellulase and xylanase synthesis in the selected white-rot basidiomycetes," *Annals of Agrarian Science*, vol. 14, no. 3, pp. 169–176, 2016.

[23] M. Dashtban, H. Schraft, and W. Qin, "Fungal bioconversion of lignocellulosic residues; opportunities & perspectives," *International Journal of Biological Sciences*, vol. 5, pp. 578–595, 2009.

[24] D. Deswal, Y. P. Khasa, and R. C. Kuhad, "Optimization of cellulase production by a brown rot fungus *Fomitopsis sp*. RCK2010 under solid state fermentation," *Bioresource Technology*, vol. 102, no. 10, pp. 6065–6072, 2011.

[25] S. D. Kshirsagar, G. D. Saratale, R. G. Saratale, S. P. Govindwar, and M. K. Oh, "An isolatedAmycolatopsissp. GDS for cellulase and xylanase production using agricultural waste biomass," *Journal of Applied Microbiology*, vol. 120, no. 1, pp. 112–125, 2016.

[26] A. Bhalla, N. Bansal, S. Kumar, K. M. Bischoff, and R. K. Sani, "Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes," *Bioresource Technology*, vol. 128, pp. 751–759, 2013.

[27] H.-Y. Yu and X. Li, "Alkali-stable cellulase from a halophilic isolate, *Gracilibacillus sp*. SK1 and its application in lignocellulosic saccharification for ethanol production," *Biomass and Bioenergy*, vol. 81, pp. 19–25, 2015.

[28] A. Kumar, D. Dutt, and A. Gautam, "Production of crude enzyme from *Aspergillus nidulans* AKB-25 using black gram residue as the substrate and its industrial applications," *Journal of Genetic Engineering and Biotechnology*, vol. 14, no. 1, pp. 107–118, 2016.

[29] T. Dutta, R. Sahoo, R. Sengupta, S. S. Ray, A. Bhattacharjee, and S. Ghosh, "Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: production and characterization," *Journal of Industrial Microbiology & Biotechnology*, vol. 35, no. 4, pp. 275–282, 2008.

[30] G. L. Vitcosque, R. F. Fonseca, U. F. R. Rodriguez-Zuniga, V. B. Neto, S. Couri, and C. S. Farinas, "Production of biomass-degrading multienzyme complexes under solid-state fermentation of soybean meal using a bioreactor," *Enzyme Research*, vol. 2012, Article ID 248983, 9 pages, 2012.

[31] D. A. Santos, M. M. Oliveira, A. A. S. Curvelo, L. P. Fonseca, and A. L. M. Porto, "Hydrolysis of cellulose from sugarcane bagasse by cellulases from marine-derived fungi strains," *International Biodeterioration & Biodegradation*, vol. 121, pp. 66–78, 2017.

[32] M. A. Medina-Morales, O. Soto-Cruz, J. C. Contreras-Esquivel, R. Rodríguez-Herrera, H. De la Garza-Toledo, and C. N. Aguilar, "Study of enzymatic saccharification of *Agave* leaves biomass to yield fermentable sugars," *3 Biotech*, vol. 7, p. 55, 2017.

[33] Y. Sun and J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: a review," *Bioresource Technology*, vol. 83, no. 1, pp. 1–11, 2002.

[34] V. Mukherjee, J. Steensels, B. Lievens et al., "Phenotypic evaluation of natural and industrial *Saccharomyces* yeasts for different traits desirable in industrial bioethanol production," *Applied Microbiology and Biotechnology*, vol. 98, no. 22, pp. 9483–9498, 2014.