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Agave Biomass is Excellent for Production of Bioethanol and Xylitol Using *Bacillus* Strain 65S3 and *Pseudomonas* Strain CDS3

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A high abundance of cellulose and low content of lignin make *Agave americana* an ideal feedstock for the biorefining industry. Eighteen cellulose-decomposing bacterial isolates were examined for their ability to decompose agave biomass and production of bioproducts. In agave-agar plate qualitative assay, 3 bacterial strains displayed better hydrolysis ability than the positive control, *Cellumonas xylanilytica* XIL11. In microplate quantitative assay using 3,5-dinitrosalicylic acid as substrate, a *Bacillus* strain (65S3) and a *Pseudomonas* strain (CDS3) were found superior for bioethanol and xylitol production. Bioethanol output of 65S3 was as high as 0.92 g/g, while CDS3 degraded agave and produced xylitol as high as 0.98 g/g. Significant structural degrading changes have been observed using scanning electron microscopy, after 14- and 28-day treatments by using CDS3 and 65S3. CDS3 and 65S3 have great potentials in the biorefining industry for the production of value-added bioproducts.

Keywords: Agave, Biomass, Fermentation, Ethanol, Xylitol.

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1. INTRODUCTION

Given the emergence of concerns surrounding energy crises and severe environmental pollution on a global scale, the development and use of new energy is entering a new era and is undergoing a critical change from fossil fuels to renewable energy sources. Biofuels offer benefits due to their high sustainability and low carbon dioxide emissions, while they promise not to contribute to the rise in pricing of non-renewable energies. As a result of these benefits, the demand for biofuel feedstock is soaring.[1–3] It is estimated that by 2020, the production of biofuels will skyrocket to 125 billion liters.[4] Thus, bioethanol has become the subject of immense focus in the field of biotechnology research. Currently, the production of bioethanol relies mainly on the hydrolysis of grains with high sugar and starch contents, such as corn and sugarcane, consequently resulting in the rise of food prices.[5] More recently, attempts are being made to utilize agricultural waste and other cellulose-based waste materials for the production of biofuels; however, the current conversion efficiency remains slow. Seeking a more efficient bioconversion of biomass for the production of bioethanol is now an attractive endeavor.

Natural plant fibers can be completely biodegraded to produce large quantities of sugars and alcohols, and thus they have become an ideal substitute for starch-based biomass in the production of bioethanol. Agave, a plant fiber that is well known the world over, is a readily renewable resource. It grows at a rapid rate even with limited water supply; it is also capable of growing in dry land where corn and sugarcane rarely survive.[6] Additionally, the biofuel produced from agave plants has extraordinarily low CO_2 emissions at only 35 g/J; whereas in contrast, the CO_2 emission of corn-based biofuel production can be as high as 85 g/J.[7] It is evident therefore that agave-based biofuel is more environmentally friendly when compared with other types of biofuel; while at the same time, the growth of agave requires less water and land than that of other crops, and as such food prices will not be significantly affected.

Fibers of *Agave americana* are composed of cellulose (~68%), hemicellulose (~15%), moisture (~8%), lignin (~5%) and wax (~0.26%).[8] Wherein, cellulose is a chain polymer, formed by the β-1,4glycosidic bond of D-glucose. It is the founding structure of lignocellulose,

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and is generally insoluble in water andorganic solvents. It can be hydrolyzed by acid.[9] On the other hand, hemicellulose is a heterogeneous polymer constituted by different types of monosaccharides which are usually pentoses and hexoses.[3,9] Hemicellulose does not dissolve in water but it is soluble in alkali. When heated in diluted acids, it is more easily hydrolyzed than celluloses.[10] Finally, lignin is a complex phenolic polymer and is not soluble in water. In cellulosic biomass, lignin plays a key role in supporting the structure, preventing oxidation, and defending against the invasion of microorganisms. It is reported that fungi and bacteria, particularly those belonging to the phylum Actinomycete, are the main players in the degradation of cellulose, hemicellulose, and lignin. However, the major obstacle of microbial degradation of lignocellulosic biomass in industry centers on the high cost due to the recalcitrance of lignin.[11] Recent studies have shown that some aerobic bacteria, such as *Cellulomonas* and *Pseudomonas*, can degrade cellulose; while halophilicthermophilic *Bacillus* NCIM59[12] is capable of degradingxylan, a major backbone of many types of hemicellulose.

Hemicellulose is the second richest component in agave biomass. According to the report by Nong et al.[13] the bacteria *Paenibacillus* sp. has the ability to decompose hemicellulose into bioethanol with low cost and high efficiency. At the same time, the hydrolysis of hemicellulose can produce D-xylitol, which has been widely used in food and medicine. D-Xylitol is a 5-carbon sugar alcohol produced by hydrogenation of the pentose xylose. Currently, the demand for xylitol is increasing globally; the annual consumption of xylitol is more than 125,000 tonnes per year with an estimated market value of more than US $537 million.[14] Traditional chemical catalytic hydrogenation methods are not only complex and unsafe, but are also high-cost as they use pure xylose as raw material. The conversion rate from xylose to xylitol is about 50–60%.[15] It has been necessary and worthwhile to explore new methods for the effective production of xylitol using microbial conversion, as it may overcome the shortcomings in chemical methods. The principle of producing xylitol by biological means is to use xylose reductase as a catalyst through the deoxidization of xylose to xylitol; extra reducing coenzyme and hydrogen are unnecessary, as they are from inside the cells. Biological methods can be carried out under normal temperature and pressure and have the characteristics of low energy consumption, simple equipment and safe operation. Some *Trichoderma* and yeast (such as *Candida guilliermondii*, *Candida tropicalis*, *Candida pelliculosa*, *Candida boidinii*, etc.), which produce xylose reductase, can digest xylose into xylitol. Additionally, some bacteria such as *Enterobacterliquefaciens* can hydrolyze hemicellulose into xylitol.[16]

In this study, cellulase-producing bacteria isolated from municipal wastes and peats were used to degrade agave fiber and produce bioethanol and xylitol.

2. MATERIALS AND METHODS

2.1. Chemicals and Bacterial Strains

The chemicals used in this study were purchased from Sigma-Aldrich and were of analytical or HPLC grade. *Agave americana* biomass (kindly provided by Jeffrey Phelps of Redding, California, USA) was dried at 70 °C in a drying oven (Thermo Fisher Scientific, Canada) for 24 hours, grounded in a Wiley mill, and then sieved through 20 mm pore mesh. Eighteen cellulase-producing bacterial isolates, originally isolated from a variety of municipal waste and peat samples,[17] were selected to test their activity towards degradation of agave biomass. The eighteen isolates are listed in Table I.[17]

2.2. Screening for Carboxymethylcellulase Activity

To examine carboxymethylcellulase activity, the eighteen bacterial isolates and two controls were grown overnight in 5 ml fresh LB broth at 30 °C, shaking at 180 rpm. *Cellumonasxylanilytica* XIL11 was used as a positive control[18] and *Escherichia coli* BL21 was used as a negative control. Five microliters of each resulting broth culture was inoculated on carboxymethyl cellulose (CMC) agar plates containing: 5.0 g/L agave, 1.0 g/L $NaNO_3$, 1.0 g/L K_2HPO_4, 1.0 g/L KCl,0.5 g/L $MgSO_4$, 0.5 g/L yeast extract, 1.0 g/L glucose, 15.0 g/L agar. All bacteria were incubated for 48 h at 30 °C; following this, plates were flooded with Gram's iodine solution (6.7 g/L KI and 3.3 g/L I) for 5 min.[19] After the addition of Gram's iodine solution the diameter (D, cm) of halos, represented by transparent zones, as well as colony diameter (d, cm) were measured to show the ratio of diameter of hydrolysis ring to clone (HC value). HC value represents hydrolysis ability and can be expressed as $(D/d)^2$. The cellulase activity was also visualized by imaging.

2.3. Determination of Reducing Sugars from Agave Degradation

Three bacterial isolates which possess the highest hydrolysis ability (*Duganella* 55S2, *Bacillus* 65S3 and *Pseudomonas* CDS3) and two controls were grown overnight in 5 ml of fresh LB broth at 30 °C, shaking at 200 rpm. Subsequently, 200 μl of overnight cultures were transferred to 250 mL flasks containing 50 mL minimal salts medium containing: 1 g/L $NaNO_3$, 1 g/L K_2HPO_4, 1 g/L KCl, 0.5 g/L $MgSO_4$, 0.5 g/L yeast extract, 3 g/L peptone and 5% agave, for each bacterial strain. The flasks were placed in an incubator shaker at 30 °C, shaking at 200 rpm. After 7 days of incubation, the drop plate counting method was used to confirm survival of all bacterial strains. Microtitre plate method using 3,5-dinitrosalicylic acid (DNS) as a substrate was used to detect reducing sugars.[20] Briefly, 1 ml of each bacterial culture was centrifuged for 1 min at 17000 g. Then, 60 μl of the culture supernatant and 120 μl of 3,5-dinitrosalicylic acid (DNS) were added to each well of the microtitre plate. The plate

Table I. CMC hydrolytic ability of 20 different bacteria and 2 controls.

Source	Isolate	Transparent circle diameter (D, cm)	Colony diameter (d, cm)	Hydrolysis ability $(D/d)2$	Species
Peat	55S1	2.6	0.8	10.6	*Bacillus sp.*
	55S5	3.2	0.9	12.7	*Bacillus sp*
	65S3	3.1	0.7	19.6	*Bacillus sp*
	6S1	3.3	0.9	13.5	*Bacillus sp*
	6S4	2.6	0.7	13.7	*Duganella sp*
	55S2	3.1	0.8	15.0	*Duganella sp*
	65S5	2.9	0.8	13.1	*Paenibacillu sp*
Municipal	AS1	2.4	1.0	5.8	*Pseudomonas sp*
waste	CDS3	3.2	0.7	20.9	*Pseudomonas sp*
	CH2OS1	3.1	1.2	6.7	*Pseudomonas sp*
	GH2OS1B	3.6	1.2	9.0	*Pseudomonas sp*
	CTS1A	3.1	1.1	7.9	*Bacillus sp*
	CTS1B	1.2	0.7	2.9	*Bacillus sp*
	CDS2B	3.3	1.0	10.9	*Bacillus sp*
	CDS1B	2.5	0.7	12.7	*Aeromonas sp*
	AS3	3.1	1.9	2.7	*Arthrobacter sp*
	AS2B	3.3	0.9	13.5	*Exiguobacterium sp*
	CDS2A	3.4	1.2	8	*Chryseobacterium sp*
	Xylanilytica[a]	3.0	0.8	14.0	*Xylanilytica*
	E.coliBL21[b]	–	0.7	–	*Escherichiacoli*

Notes: [a]a positive control: *C. xylanilytica*; [b]a negative control: *Escherichiacoli*BL21.

was then sealed and boiled for 5 min to maintain incubation above 95 °C. After this, 36 μl of the solution was removed from each well and added to 160 μl of double distilled water (ddH_2O) in a new microtitre plate. Finally, the $OD_{540\ nm}$ was measured by an xMark spectrophotometric plate reader (BioRad Laboratories, Canada). All measurements were done in triplicate and the results were averaged.

2.4. Determination of Ethanol Produced from Agave Degradation

Three bacteria (*Duganella*55S2, *Bacillus*65S3 and *Pseudomo*nasCDS3), along with two controls were grown for 7 days in 50 ml of minimal salts medium with 5% agave at 30 °C, shaking at 200 rpm. Consecutively, 1 ml of the cultured bacteria was centrifuged for 1 min at 17000 g. The supernatant was used for bioethanol and HPLC tests. The ethanol concentration produced by bacterial cultures was determined using EnzyChromTM Ethanol Assay Kit (ECET-100), purchased from Thermo Fisher Scientific Inc, USA, following the manufacturer's instructions. A blank control was prepared following the same procedure; however, in exception the working solution was replaced by water. Each sample was measured in triplicate and the results were averaged. The linearity of the calibration curves was excellent with a correlation coefficient of ($r^{(2)} > 0.99$); the sample ethanol concentration was calculated as follows, $[\text{Ethanol}] = (OD_{SAMPLE} - OD_{BLANK})/9.6683(\%) \times 50$, where OD_{SAMPLE} and OD_{BLANK} are the $OD_{565\ nm}$ values of the sample and blank, respectively. Moreover, 9.6683 is the slope, while 50 is the dilution factor of the samples. The instruction provided by the supplier shows 1% (v/v) ethanol equals 170 mM.

2.5. Determination of Xylitol Produced from Agave Degradation

During 7 days of incubation, 1 ml samples were obtained and centrifuged every day. The supernatants were kept at 4 °C for the determination of xylitol from the agave degraded by three bacteria (55S2, 65S3 and CDS3). Five hundred microlitres of supernatant was taken and then filtrated using a membrane RC 4 filter (size of 0.2 μm). Clear solution was obtained and further diluted a dilution factor of 5000. After this, internal standard (L)-fructose, with an equal molar of reactant, was added. Subsequently, the sample underwent HPLC analysis on High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) using a Dionex ICS3000 system (Dionex, Sunnyvale, USA) with the CarboPac PA20 carbohydrate column (3×150 mm) (Dionex, Sunnyvale, USA). The analysis condition was performed as follows: mobile phase consisting of 2 mM NaOH solution; a flow rate of 0.5 mL/min^{-1}; an injection volume of 25 μl; and column temperature of 25 °C.

2.6. Morphology of Agave

The morphology of agave fiber was observed using a scanning electron microscope (SEM). Samples were collected from the raw agave control and bacterial treated agave after 14 and 28 days incubation on 50 ml minimal salts medium. The same medium without bacteria (untreated agave) was used as a control. Firstly, each sample was immersed in 2% glutaraldehyde buffered with 0.1 M phosphate buffer solution (pH = 7.2), for 2 h at 4 °C. Then, the tissue was rinsed with the same phosphate buffer (3 times for 10 min each wash) and dehydrated in a graded ethanol solution in water –50%, 70%, 80%, 90%, and 100% for 10 min

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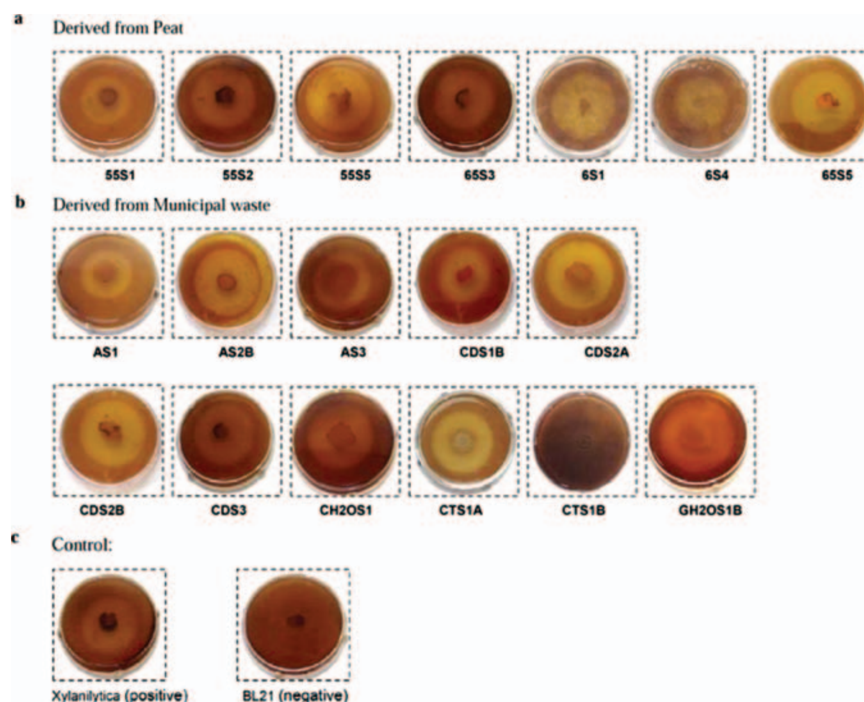

Fig. 1. Carboxymethyl cellulase activity analyses on eighteen different cellulase-producing bacteria (a) seven bacteria isolated from peat (b) eleven bacteria isolated from municipal wastes. (c) a positive and a negative control, *C. xylanilytica* and *Escherichia coli* BL21.

each.[21] Following this, samples were dried at room temperature and thereafter coated with gold for 45 s in a Denton-DeskII sputter coater (Denton Vacuum USA, Moorestown, NJ). The samples were examined on an SEM (Hitachi SU-70, Japan) at 5 kV.

3. RESULTS

3.1. Hydrolysis of Cellulose and Hemicellulose in Agave

Eighteen different cellulose-decomposing bacteria and two controls were tested on plates containing ground agave for cellulase and hemicellulase activity. Transparent zones representing cellulose and hemicellulose hydrolysis are referred to as halos. As shown in Figure 1, a total of 18 bacterial isolates exhibited activity towards the solubilized agave. The negative control (*Escherichia coli*BL21) displayed no halo. The diameter of halos (D) and colony (d) were measured using a standard ruler; thus, the relative hydrolysis activity could be calculated. As presented in Table III, the hydrolysis activity of the positive control is 14.0. Three bacteria (*Duganella*55S2, *Bacillus*65S3 and *Pseudomonas*CDS3) demonstrated greater relative hydrolysis activity than that of the positive control after 48 h of incubation on agave containing agar plates with an HC value (represents hydrolysis ability) of 15.0, 19.6 and 20.9, respectively. It is also evident that CDS3 *Pseudomonas* showed the highest rate of hydrolysis towards agave after 48 h incubation.

3.2. Reducing Sugars Produced by Degradation of Agave

After 7 days sampling at 24 h intervals, the reducing sugars were detected in three bacteria: *Duganella*55S2, *Bacillus*65S3, *Pseudomo*nas CDS3 and two controls using the microtitre plate method with 3,5-dinitrosalicylic acid (DNS) as substrate. No significant reducing sugars were detected.

3.3. Ethanol Produced During Degradation of Agave

It was observed that the natural fibers of *agave americana* become susceptible to hydrolysis by cellulases produced by three bacteria: *Duganella*55S2, *Bacillus*65S3, *Pseudomo*nas CDS3 and positive control *C. xylanilytica*, from 2 to 7 days. The negative control was found unable to degrade fibers of agave within 7 days of incubation. Therefore, three bacteria (*Duganella*55S2, *Bacillus*65S3, *Pseudomo*nasCDS3) and two controls (*C. xylanilytica* and *E. coli* BL21) were used to test ethanol production after growth with agave. The ethanol production of the three bacteria and two controls is described in Table II. As displayed, a maximum ethanol yield of 0.18 g/g was obtained by *Duganella*55S2 at 4 days incubation. In addition, a maximum yield of 0.92 g/g, 0.30 g/g and 0.27 g/g was obtained by *Bacillus65S3*, *Pseudomonas*CDS3 and positive control using agave at 5 days incubation, respectively. It should be noted that on day 5, the ethanol produced by *Bacillus65S3* and *Pseudomonas*CDS3 measured higher than the positive control; particularly, *Bacillus*65S3 exhibited the highest production

Table II. Yields of ethanol from agave degradation by three bacteria and 2 controls.

Time (days)	Y_{55S2}(g/g)	Y_{65S3}(g/g)	Y_{CDS3}(g/g)	Y+(g/g)
2	0	0.06	0.07	0.10
3	0.02	0.10	0.19	0.12
4	0.18	0.25	0.20	0.15
5	0.17	0.92	0.30	0.27
6	0.05	0.56	0.11	0.12
7	0	0.13	0	0.01

Note: $Y_{Ethanol}$ = Ethanol(g/L)/(50 g agave fiber per liter).

rate, which was 3.41 times that produced by positive control.

3.4. Xylitol Produced During Degradation of Agave

Xylitol yield by three bacteria (55S2, 65S3 and CDS3) using agave as feedstock from 3 to 7 days was examined. The chromatograms of the samples were similar and typical chromatograms are shown in Figure 2. The baseline is plain and the resolution of the xylitol is good. As shown in Table III, after 3 days of incubation,0.05 g/g, 0.14 g/g, and 0.02 g/g of xylitol was produced by *Pseudomonas*CDS3*,* *Bacillus*65*S3,* and *Duganella*55S2, respectively. The maximum xylitol produced after 5 days incubation was 0.82 g/g by *Pseudomonas*CDS3, 0.98 g/g by *Bacillus*65S3 and 0.25 g/g by *Duganella*55S2. Bacteria*Pseudomonas*CDS3 produced more xylitol than the others, and the highest yield of 0.98 g/g was the achieved at 5-day incubation. Table III shows the xylitol yield variation during 7-days incubation by the three bacteria. It is observed that in all cases, the concentration of xylitol first increased to a maximum value on the 5th day and then decreased over time. Thus, 5 days could be determined as an optimal incubation time for the production of xylitol for the three bacteria.

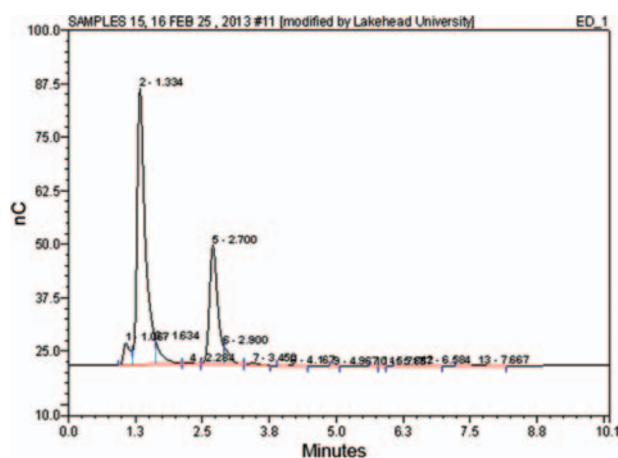

Fig. 2. Typical chromatogram of standard and xylitol showing their retention times (RT) in minutes. RT1.334, (L)-fucose; RT2.700, xylitol.

Table III. Xylitol production from agave degradation by three bacteria.

Time (days)	Y_{55S2} (g/g)	Y_{65S3} (g/g)	Y_{CDS3} (g/g)
3	0.02	0.14	0.05
4	0.08	0.60	0.42
5	0.25	0.98	0.82
6	0.22	0.44	0.16
7	0.14	0.15	0.05

Note: $Y_{Xylitol}$ = Xylitol (g/L)/(50 g agave fiber per liter).

3.5. Morphology Observation of Agave Using Scanning Electron Microscopy

Figure 3 shows the SEM images of the untreated and bacteria treated agave leaf structures during an incubation period of 14 days (Figs. 3(a)–(d)) and 28 days (Figs. 3(a')–(d')) by the three strains of bacteria. The untreated agave leaf structure (control) was presented in Figures 3(a) and (a'). It clearly shows a smooth, flat surface after 14 days

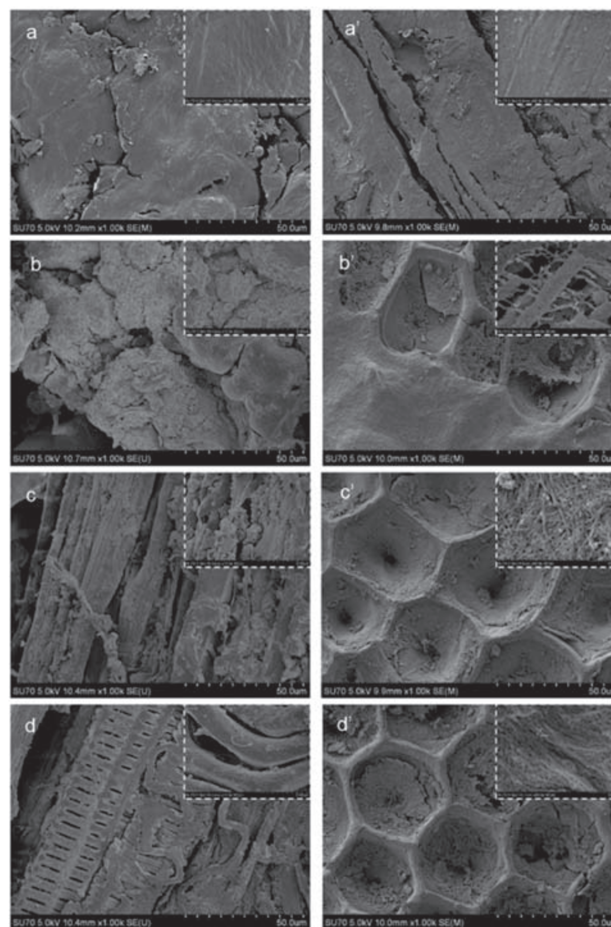

Fig. 3. Scanning electron microscopy(SEM) images of agave fibers.(a)the untreated agave leaf structure (control)during an incubation period of 14 days. (a')control during an incubation period of 28 days. (b)-(d)bacteria (*Pseudomonas*CDS3,*Bacillus*65S3*and Duganella*55S2) treated agave leaf structures after 14 days. (b')-(d')bacteria(*Pseudomonas* CDS3,*Bacillus*65S3*and Duganella*55S2)treated agave leaf structures after 28 days treatment, showing degraded cell walls and eroded fibers.

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(Fig. 3(a)) and 28 days (Fig. 3(a')) of incubation, indicating no degradation occurred during this period of time. As expected, after degradation by the bacteria the fiber structure was significantly different from that of the untreated fiber. As shown in Figures 3(b)–(d), after 14 days' treatment with bacteria (*Pseudomonas*CDS3, *Bacillus*65S3 and *Duganella*55S2), the cell walls of the agave leaf were broken; hence the surface of agave is rough and a large number of crevasses appear. The structure change of the agave leaf caused by *Duganella*55S2 (Figs. 3(b) and (b')) is less significant compared with that caused by the other two strains. The bacterial degraded agave fibers with open cell walls allow increased enzyme access. Then after 28 days of incubation, the agave fiber was highly degraded. For instance, as shown in Figure 3(b'), after 28-day treatment by *Duganella*55S2, some cell walls of the agave were destroyed while some still existed. However, for the case of the agave cells treated by *Pseudomonas*CDS3 and *Bacillus* 65S3 after 28 days of incubation (Fig. 3(c') and (d')), it was observed that fine fibrillar structures with diameters in the nanometer range as well as larger microfibrillated cellulose aggregates were present. The microfibers on the surface of these celluloses (2 and 40 nm) are interwoven into a complex network structure. It has been reported that the typical diameter of the microfibers in cellulose range from 2 to 40 nm.[22,23]

4. DISCUSSION

We previously reported that the eighteen bacterial isolates hold good potential for degrading lignocellulosic biomass;[17] however, different types of lignocellulose have significant differences in chemical composition and structure.[24] In this study, we first determined those bacteria capable of degrading agave fibers; the cellulase and hemicellulase activities were detected in all of eighteen bacterial isolates. The hydrolysis ability data (Table I) confirmed that all of the bacteria could degrade agave fiber. Among them, three bacteria (*Duganella*55S2, *Bacillus*65S3, and *Pseudomo*nasCDS3) displayed the greatest potential for the decomposition of agave. Similarly,[17,25] reported the production of extracellular cellulase by a *Duganella* sp. And a *Pseudomo*nas sp.. while another research group reported that a *Bacillus* sp. exhibited degradation ability towards cellulose and xylan.[26] Considering the known cellulase and hemicellulase activities in these strains and due to the composition (mainly cellulose and hemicellulose), there is potential to produce environmentally-friendly industrial products with agave.

Through evolution, plants have developed degradation-resisting cytoderms composed of cellulose, hemicellulose and lignin; the cytoderm is one of the most renewable resources in nature. Cellulose can be broken down into glucose, and hemicellulose can be decomposed into xylose, then xylose can be fermented into ethanol and xylitol—which can then be used as fuels and biological products. Lignin blocks the channel of absorbing cellulose and hemicellulose. In order to obtain the cellulose, a high-energy consumption way of deconstructing the cytoderm is applied, and it falls short of the cost benefit principle. In this study, the examination of agave fibers under the scanning electron microscope showed that these three strains of bacteria can damage the cytoderm of agave, thereby increasing the depolymerization of cellulose and hemicellulose. This study also proved that the three strains of bacteria can be more efficient, less energy-consuming and more environmentally-friendly for the degradation of agave. After 14 and 28 days' culture, the SEM showed the agave leaf changed the least in *Duganella*55S2 degradation with some residual cytoderm remaining after 28 days culture; while, after 28 days of culture with *Pseudomonas*CDS3 and *Bacillus*65S3, the cytoderm is destroyed and nucleus exposed. The possible explanation for this observation may be that the hydrolysis ability of *Pseudomonas*CDS3 and *Bacillus*65S3 is more intense than *Duganella*55S2, the results being consistent with the previously examined enzyme activity. As reported, *Pseudomonas* sp., *Bacillus* sp. and *Duganella*sp. have the ability to degrade cellulose, hemicellulose and lignin.[17,27,28] This explains reasonably the phenomenon observed under SEM.

Recently, researchers have also begun to focus on developing low-cost and high-efficiency methods or substrates for biomass-producing industries. The feasibility of using agave for alcohol production has been tested and confirmed in Mexico and Australia.[29] However, the conversion from lignocellulose to value-added chemicals and biofuels is limited by several factors such as the complexity in pretreatment, low conversion efficiency from pentose to ethanol, and high production cost of the enzyme. In this study, three cellulase-producing bacteria were used to degrade the untreated agave fiber and to produce bioethanol and xylitol. The results revealed that all three bacterial strains produced ethanol, yet no reducing sugars could simultaneously be detected. Nonetheless, the greatest yield of ethanol of 0.92 g/gwas obtained from *Bacillus* strain (65S3) at 5-day incubation. Using the enzyme-containing supernatant of *Pseudomonas* sp. CDS3 to ferment agave, the maximum productive rate of xylitol is 0.98 g/g at 5-day incubation. The yield of ethanol and xylitol decreased after five days of reaction, which is believed to be related to the half-life of the bacteria and the toxic effect of the products on the bacteria. Furthermore, no reducing sugars were detected during the production of ethanol and xylitol, which can be attributed to the fact that these end-products require consumption and conversion of sugars during the growth of these bacterial strains. Moreover, the bacterial consumption of reducing sugars such as glucose and cellobiose prevented the inhibition effect of glucose and cellobiose on enzymatic hydrolysis and ultimately end-product production, which permitted a good

ethanol and xylitol yield. This method avoids the complicated pretreatment process; reduces the generation of large amounts of wastewater; significantly lowers the cost for pollution control; and achieves the green production of ethanol and xylitol from cellulose. Therefore, this single-step process could eliminate the need for pretreatment of raw materials; combine the hydrolysis and fermentation into a single step; reduce the number of reactors; and ultimately, lower the cost.

The results of this study show that the *Bacillus* strain 65S3 and the *Pseudomonas* strain CDS3 are able to efficiently convert untreated agave to bio-ethanol and bio-xylitol. The *Bacillus* strain 65S3 and the *Pseudomonas* strain CDS3 can be deemed promising candidates for the production of environmentally friendly bioethanol and xylitol through agave degradation.

Competing Interests

The authors have declared that no competing interests exist.

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