Identification and Characterization of an Anaerobic Ethanol-Producing Cellulolytic Bacterial Consortium from Great Basin Hot Springs with Agricultural Residues and Energy Crops

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Introduction

The world economy has been largely depending on fossil energy resources such as oil, coal, and natural gas. There has been significant research on renewable sources of liquid fuels to replace fossil fuels, to improve energy security and reduce greenhouse emissions. Nowadays, bioethanol is gaining worldwide acceptance, essentially to overcome problems associated with exploitation and depletion of fossil fuels and environmental pollution [23]. Bioethanol has been receiving widespread interest and the global bioethanol market has entered the rapid growth phase [30]. Bioethanol produced from renewable biomass, such as agricultural residues, energy crops, or cellulosic materials, is one of the alternative energy resources that are both renewable and environmentally friendly [20, 39]. It is indigenous and can therefore contribute to reducing dependency on oil imports and increasing the security of supply.

Ethanol production using easily available substrates and microbes that could convert them to ethanol could be very useful. The debate of potential conflict between food and fuel will continue unless the feedstock for the biofuel shifts from major crops to production of second generation biofuels, which uses waste materials and nonfood crops that can be grown on marginal land as feedstocks [52].
Agricultural processes yield by-products and waste streams collectively known as residues, such as sugarcane bagasse and spent mushroom substrate, which yield significant energy potential and are relatively conducive to utilization because they have already been collected [13]. Moreover, perennial herbaceous crops are a huge potential resource for producing fuel ethanol. The term energy crop is used to principally describe a crop grown primarily to provide a natural cellulosic feedstock for biofuels such as ethanol.

Cellulosic biomass, available in enormous quantities, is the feasible replacement and a renewable resource with great potential for bioconversion to value-added by-products [10, 17]. However, the biorefining process remains economically unfeasible owing to a lack of biocatalysts that can overcome costly hurdles, such as cooling from high temperature, pumping of oxygen/stirring, and neutralization from acidic or basic pH [33]. The bottleneck of cellulosic ethanol production is how to convert cellulose into glucose effectively [3]. Highly efficient cellulase is a key factor for cellulosic biomass conversion into biofuel and for biorefinery. Screening and isolation of microbes from the thermophilic environment help to overcome these challenges [7, 36]. Many cellulose-decomposing strains were isolated based on cultivation and pure culture. However, the isolated bacterium achieved by high dilution principle cannot represent the natural bacterial consortium, and most of the microbial species in nature cannot be cultured. The functions of uncultured bacteria would be neglected. Therefore, the bacterial consortia rather than cultured bacteria have provided more information about how bacteria play their roles. The exploitation of a bacterial consortium in the search for improved strategies provides a means to upgrade the feasibility for biomass conversion [33].

In this study, an anaerobic ethanol-producing cellulolytic bacterial consortium, named SV79, was isolated from Great Basin hot spring (Nevada, USA). Its morphological characterization and microbial structure were studied. Structure dynamics of the consortium was analyzed by denaturing gradient gel electrophoresis (DGGE) to assess its stability during the cellulose degradation procedure, and the microbial diversity was checked by construction of a 16S rRNA gene library. The enzyme fermentation characteristics of SV79 and the ability of the consortium in using different agricultural residues and energy crops were also determined.

Materials and Methods

Samples and Medium

The water and sludge sediment samples were collected from Great Basin hot springs in Nevada, USA (41D 31'57.2"N, 120D 04'15.7"W- 41D 32'03.4"N, 120D 04'23.9"W). The water temperatures of the hot springs were between 40°C and 78°C. The Z-AECC medium for enrichment and subcultivation of the anaerobic cellulolytic consortium was composed of the following (per liter): KH$_2$PO$_4$ 0.75 g, K$_2$HPO$_4$ 1.5 g, MgCl$_2$ 6H$_2$O 0.4 g, NH$_4$Cl 0.9 g, NaCl 0.9 g, yeast extract 0.6 g, peptone 2.0 g, l-cysteine 0.5 g, carbon source 10.0 g, 10% FeSO$_4$ 0.3 ml, 1% Resazurin 1 ml, Wolfe's mineral solution 9 ml, and Wolfe's vitamin solution 1 ml [51]. The agricultural residues (sugarcane bagasse and spent mushroom substrate) and energy crops (Spartina anglica, Miscanthus floridulus and Pennisetum sinese Roxb) were used as the carbon sources. The whole operation for enrichment and subcultivation of microbial consortia was performed under anoxic conditions in a Forma 1029 Anaerobic Chamber (Thermo Scientific, Marietta, USA) with the gas phase N$_2$/CO$_2$/H$_2$ = 85:5:10. All culturing manipulations were performed as described by Hungate [24].

Consortium Enrichment and Characteristics of the Bacterial Consortium

For isolation, approximately 5.0 g of the environmental sample at each site was transferred to fresh 100 ml of the Z-AECC medium containing filter paper as the sole carbon source in 125 ml serum bottles, for incubation at 40–78°C for 24–72 h without shaking. Five milliliters of the suspension was inoculated into 100 ml of Z-AECC medium and incubated statically for one week. A volume of 0.5 ml of each positive culture, in which filter paper was completely decomposed, was subinoculated into 5 ml of Z-AECC medium in Hungate’s tubes. Positive samples were continuously subinoculated using fresh Z-AECC medium in order to construct stable cellulolytic consortia. A stable cellulolytic bacterial consortium, SV79, was enriched after subcultivation for seven times. For evaluation of the cellulose degradation efficiency of the consortium, the culture of SV79 was inoculated into seven Hungate’s tubes with Z-AECC medium. Time-course degradation dynamics of filter paper were observed on each day. The reducing sugars were liberated in the reaction mixture. Fermentation characteristics of the reducing sugar production of the microbial consortium were determined. The effect of carbon source, time, temperature, and initial pH values on the reducing sugar production of the bacterial consortium was studied. The temperature ranges were determined from 30°C to 70°C with 5°C interval. The pH dependence was determined from pH 4.0 to 11.0. The reducing sugar yield in the cultural process was determined with the 3,5-dinitrosalicylic acid colorimetric method [35]. Reducing sugar yield (%) was represented as the gram amount of reducing sugars per 10.0 g of carbon substrates. Furthermore, cell morphologies of the species from consortium SV79 were also examined by transmission electron microscopy (JEM-1230, JEOL, Peabody, MA, USA) after cultivation under the optimal temperature and pH value [39].

Structure Dynamics of the Microbial Consortium

Total genomic DNA of the consortium from the one-week-
incubation cultures was extracted by the SDS-CTAB method as previously described [50]. PCR primers GC341F (5’-CGGCCGCCGCCCCGCCGCCCCGCCCTACAGGG AGGCAGCAG-3’; containing 40 bp of GC clamp) and 534R (5’-ATTACCGCGCTGCTGG-3’) were used to amplify the variable V3 region of bacterial 16S rRNA [47]. The PCR procedure included an initial cycle of 5 min at 94°C, followed by 20 cycles of 1 min at 94°C, 45 sec at 65°C, and 1 min at 72°C, with a touchdown of 0.5°C per cycle, followed by 16 cycles of 1 min at 94°C, 45 sec at 55°C, and 1 min at 72°C; for the final extension, the reaction was held at 72°C for 10 min. The PCR products of the consortium were loaded on the same gel for DGGE analysis with a DCode Universal Mutation Detection System (Bio-Rad, USA). Electrophoresis was performed at 60°C in an 8% (w/v) polyacrylamide gel with a denaturant gradient ranging from 30% to 60% for 15 min at 30 V, and then 4.5 h at 130 V in 1× Tris-acetate-EDTA buffer. After electrophoresis, the gel was stained with ethidium bromide for 15 min, and patterns were visualized and photographed using the Gel Doc XR system (Bio-Rad, USA). The band patterns on gels were checked to analyze the dynamics of the microbial consortium structures in the incubation process of one week and in the procedure of subcultivation. The dynamics of the microbial structure of consortium SV79 in different biomass substrates were also analyzed by DGGE gels.

Diversity Analysis of the Microbial Consortium

The 16S rRNA library of the microbial consortium was constructed [34]. Nearly complete 16S rRNA gene sequences were amplified by PCR from genomic DNA of the microbial consortium using universal primers 27F and 1492R [48]. They were purified and ligated into pMD18-T (TaKaRa, Japan). The ligation mixture was transformed into E. coli DH5α (TaKaRa, Japan) to generate a gene library. More than 300 clones were selected randomly and checked for correct insert size via PCR and agarose gel electrophoresis, and then the inserted fragments were sequenced. Pairwise alignments were carried out using the EzTaxon-e program [26]. To test the evolutionary relationships, phylogenetic analysis was performed with the program MEGA 4.0 [43]. Multiple alignments of the sequences were performed using CLUSTAL W [44]. Distance matrices were calculated according to the Kimura’s two-parameter correction model [27]. Phylogenetic trees were inferred using neighbor joining. Bootstrap values were determined based on 1,000 replications.

Cellulase and Ethanol Production Abilities of the Microbial Consortium

The consortium SV79 was grown in the Z-AECC medium under the optimum fermentation condition, in which the carbon source was replaced by agricultural residues (sugarcane bagasse and spent mushroom substrate) and energy crops (S. anglica, M. floridulus, and P. sinensis Roxb). The enzymatic activities were assayed by filter paper cellulase (FPase) and carboxymethylcellulase (CMCase) methods and determined using the IUPAC standard procedure [21]. Hemicellulose is the second most abundant renewable polysaccharide after cellulose. Xylan is the main component of hemicellulose. The assay to measure xylanase activity was also performed according to the method of Liu et al. [31]. One unit (U) of enzyme activity was defined as the amount of enzyme releasing 1 µg reducing sugar per 1 min under the assay conditions using glucose as a standard. Ethanol production was analyzed with gas chromatography (Agilent 6820) equipped with a flame ionization detector (FID) and HP-INNOWAX columns (0.25 mm × 30 m × 0.25 µm; Agilent).

Results

Isolation and Analysis of the Anaerobic Cellulolytic Consortium SV79

After incubation in Z-AECC medium for one week under anaerobic static conditions, five cultures showed the capability of decomposing filter paper. These positive cultures were chosen for successive subcultivation using the same medium. After subcultivation for seven times, consortium SV79, which exhibited the highest efficiency on filter paper degradation, was chosen for further study. Time-course dynamics of cellulose degradation by consortium SV79 was determined using filter paper as the substrate (Z-AECC medium) (Fig. 1A). After a 2–3 day incubation period in the medium, it was observed that small visible spots started to appear on the filter paper and the whole filter paper shattered. The filter paper was obviously decomposed after incubation for 4 days, and almost completely degraded after 7 days, which implied SV79’s high efficiency for cellulose degradation. Filter paper could be degraded efficiently even after successive subcultivation for seven times, but more than one week was needed for complete decomposition. Moreover, the different strains that developed in the medium were observed. The bacteria group consisted of both gram-positive and gram-negative bacteria. Differences in shapes were observed. The electron microscopy results revealed three forms of bacteria, including rod, spherical, and arc shapes, in consortium SV79 (Fig. 1B). The flagella were also seen on the electron micrographs of the cells a, b, and d.

Four factors (carbon source, time, temperature, and initial pH) that are known to influence fermentation processes were assayed by measuring the amount of reducing sugars released by the bacterial consortium. The fermentation conditions of the conversion from cellulose to sugar were determined. The optimal fermentation time, fermentation temperature, and pH were 7 days, 42.5°C, and 8.0, respectively (Fig. 2). The growth curve of bacterial consortium SV79 was not identical to the standard growth curve, in which...
no obvious death phase of bacterial consortium SV79 was observed (data not shown).

**Microbial Diversity of Consortium SV79**

The time-course dynamics of the microbial structure of consortium SV79 were analyzed by DGGE (Fig. 3). More than 20 bands were found on the gels through the whole cultivation procedure, indicating a complex microbial consortium. Moreover, the DGGE patterns of each gel lane corresponding to each-day samples of the 7th subcultivation were not similar and differences were found. Several weak bands among these samples were changing. However, the main bands of each sample, which stood for the dominant members of the consortium, were almost the same. The

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**Fig. 1.** Characteristics of the anaerobic cellulolytic consortium SV79.

(A) Time-course degradation of filter paper by consortium SV79. ck, the control Z-AECC medium that was not inoculated with consortium SV79; 1–7 d, the Z-AECC media incubated for 1–7 days after being inoculated with consortium SV79. (B) Scanning electron micrographs of consortium SV79. B-a and B-d show an arc with the flagellum; B-b had a flagellum, of length about 5 µm; B-c was about 0.5 µm in diameter size, of globular shape; B-e and B-f show rod bacteria, with no flagellum. The black line for length indicates 1 µm for reference.

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**Fig. 2.** Effects of carbon substrates (A), time (B), temperature (C), and initial pH values (D) on the fermentation of consortium SV79. Reducing sugar yields (%) from 1.0% carbon substrates were tested at pH 7.0, 40°C for 7 days (A). FP-filter paper, ScB-sugarcane bagasse, SMS-spent mushroom substrate, SA-Spartina anglica, MF-Miscanthus floridulus, and PSR-Pennisetum sinese Roxb. Reducing sugar yields (%) from 1.0% filter paper were tested at pH 7.0, 40°C for varying lengths of time (B), at various temperatures ranging from 30°C to 70°C at pH 7.0 for 7 days (C), and in different pH buffers at 40°C for 7 days (D).
dynamics of the microbial structure of consortium SV79 in five different biomass substrates were analyzed by DGGE. The proportion of bacteria in the consortium was a little different by each biomass substrate. However, the main bands were bands 1–5 and remained stable (Fig. S1). Even for a stable community, the proportion of bacteria was also changed in different stages and medium composition. The 16S rRNA clone library of bacterial consortium SV79 was constructed. Subsequently, the different isolates of the 16S rRNA library were subjected to DNA sequencing. The nucleotide sequence data reported in this work will appear in the GenBank nucleotide database under accession numbers JQ316682 to JQ316694 and JQ694703 to JQ694710 (Table 1). As their closest taxonomic relatives were almost <97%, all the clones likely represented new bacterial species. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolates belonged to four different families: Ruminococcaceae, Clostridiaceae, Lachnospiraceae, and Veillonellaceae. Among the detected bacteria in the consortium SV79, the following genera occurred: Acetanaerobacterium, Acetivibrio, Anaerotruncus, Bacteroides, Cellulosilyticum, Clostridium, Ruminococcus, and Sporomusa (Fig. 4). Among the 21 clones, six clones belonged to

Table 1. Bacterial isolates from consortium SV79 enriched with cellulosic biomass.

<table>
<thead>
<tr>
<th>Strains (GenBank Accession No.)</th>
<th>Closest type strains in GenBank database (Accession No.)</th>
<th>Length of fragment (bp)</th>
<th>Similarity (%)</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV79-13 (JQ316694)</td>
<td>Acetanaerobacterium elongatum Z73 (AY487928)</td>
<td>1,502</td>
<td>91.245</td>
<td>Ruminococcaceae</td>
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<td>SV79-17 (JQ694706)</td>
<td>Acetivibrio cellulolyticus CD2 (AEDB01000143)</td>
<td>843</td>
<td>92.527</td>
<td>Ruminococcaceae</td>
</tr>
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<td>SV79-21 (JQ694710)</td>
<td>Acetivibrio cellulolyticus CD2 (AEDB01000143)</td>
<td>904</td>
<td>90.069</td>
<td>Ruminococcaceae</td>
</tr>
<tr>
<td>SV79-6 (JQ316687)</td>
<td>Acetivibrio cellulolyticus CD2 (AEDB01000143)</td>
<td>1,517</td>
<td>92.103</td>
<td>Ruminococcaceae</td>
</tr>
<tr>
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<td>Acetivibrio cellulolyticus CD2 (AEDB01000143)</td>
<td>894</td>
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<td>Ruminococcaceae</td>
</tr>
<tr>
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<td>1,516</td>
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<td>Ruminococcaceae</td>
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<td>90.833</td>
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</tr>
<tr>
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<td>88.480</td>
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<td>SV79-19 (JQ694708)</td>
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<tr>
<td>SV79-5 (JQ316686)</td>
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<td>SV79-1 (JQ316682)</td>
<td>Clostridium amygdalinum BR-10 (AY353957)</td>
<td>1,524</td>
<td>89.563</td>
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<td>SV79-15 (JQ694704)</td>
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<td>840</td>
<td>92.024</td>
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<td>SV79-16 (JQ694705)</td>
<td>Clostridium methylpentosum DSM 5476 (ACEC01000059)</td>
<td>1,045</td>
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<td>SV79-8 (JQ316689)</td>
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<td>89.857</td>
<td>Lachnospiraceae</td>
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<tr>
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<td>1,547</td>
<td>93.240</td>
<td>Veillonellaceae</td>
</tr>
</tbody>
</table>
Clostridium, and five clones belonged to Acetivibrio, while Cellulosilyticum and Ruminococcus had four and two clones, respectively. At the genus rank, Clostridium (70% of the consortium) was prevalent in this community, containing the abundant bacterial species C. straminisolvens. In addition, there were also the following genera: Ruminococcus (12%), Acetivibrio (10%), and Cellulosilyticum (4%).

Cellulase and Ethanol Production Ability of the Microbial Consortium
The abilities of consortium SV79 to utilize agricultural residues (sugarcane bagasse and spent mushroom substrate) and energy crops (S. anglica, M. floridulus, and P. sinense Roxb) were researched (Fig. 5A). The highest FPase activity, CMCase activity, and xylanase activity were 9.41, 6.35, and
4.28 U/ml, using sugarcane bagasse, spent mushroom substrate, and S. anglica as substrate, respectively. Meanwhile, the ethanol production by consortium SV79 was also analyzed in Z-AECC medium by gas chromatography (Fig. 6), in which different natural cellulose biomasses were the sole carbon source. As shown in Fig. 6, the retention time for ethanol in all tested materials was 1.70 min, approximately. The peaks at about 6.5, 8.0, and 8.7 min in the chromatogram corresponded to the peaks of acetic acid, propionic acid, and butyric acid (data not shown). Ethanol yields were 2.63 mM ethanol/g *M. floridulus*, which was the highest, and 2.13 mM ethanol/g spent mushroom substrate, which was the lowest ethanol yield (Fig. 5B).

**Discussion**

A bacterial consortium usually has advantage over a single bacterium in the biodegradation of carbohydrate and crude fiber. Enrichment of a microbial consortium in natural environments provides an approach to simulate and investigate cellulose degradation. Several cellulolytic consortia have been enriched from composts, soils, decayed straws, etc. [42, 46, 54]. These studies mainly focused on microbial communities in terrestrial ecosystems. However, in an actual production process, the high unit cost of cellulases has limited their application to ethanol production, stimulating a demand for more cost-effective enzymes that
would make bioethanol an economically feasible alternative to fossil fuels [1]. Thermostable cellulases offer several potential benefits to mitigate the high costs of enzymatic saccharification of lignocellulose. A number of studies have focused on the isolation and characterization of cellulolytic thermophiles as possible sources of cellulases for the biofuels industry [7]. Therefore, hot springs are a potential source for thermophilic cellulases and ethanol-producing microorganisms [28, 40].

In this report, hot spring samples were collected from Great Basin in Nevada, USA, and a stable anaerobic cellulolytic consortium, SV79, was enriched. The consortium showed high efficiency for cellulose degradation and maintained the cellulolytic ability after seven successive subcultivations. 16S rRNA sequencing was a preferable method, which can be used to detect each kind of bacterium in a consortium and directly show the proportion of different bacteria in the consortium [56]. Sequence alignments and phylogenetic analyses performed with type species of validly published genera showed that most of the 16S rRNA sequences of bacterium consortium SV79 had a lower similarity with uncultured microorganisms. A large number of novel microbial strains were contained in the bacterium consortium SV79. The phylogenic tree revealed that the bacterium consortium SV79 consisted of the Ruminococaceae, Clostridiaceae, Lachnospiraceae, and Veillonellaceae families. These four families had a relationship with lignocellular biomass degradation and ethanol production. Moreover, PCR-DGGE analysis further revealed the changes of its microbial community structure in the incubation process of 7 days. The result implied that the strains of consortium SV79 performed different functions at different stages and coexisted and took effect in degrading cellulose.

So far, a variety of microorganisms have been found to produce biofuels from fermentation of an organic complex, including obligate anaerobes such as Acetanaerobacterium, Acetivibrio, Bacteroides, Clostridium, Cellulosilyticum, Ruminococcus, and Sporomusa species. The strains from Acetanaerobacterium produced ethanol, H2 gas, and acetic acid from glucose fermentation [12]. Cellulolytic strains of Bacteroides were relatively slow in attacking easily degraded cellulose [6]. However, the strains of Anaerotruncus colihominis would be unable to degrade cellulose [29]. Acetivibrio cellulolyticus demonstrated its cellulose-degrading activities [16]. Another related genus, Ruminococcus, was the dominant rumen bacteria [38, 55]. Bacteria in the genus Clostridium were important members of cellulytic microorganisms. Clostridium was well known as a typical anaerobic cellulytic species. Meanwhile, Clostridium was accompanied by the formation of cellulosic ethanol in the process of degradation [25] and became the predominant bacteria with the extension of fermentation. At least two species, Clostridium thermocellum and C. acetobutylicum, have demonstrated the ability of fermenting sugars. C. thermocellum made use of crystalline cellulose to produce sugar and then as its carbon source [22]. A thermophilic anaerobic cellulose-degrading bacterium, C. straminisolve was also isolated and had the ability of converting cellulose to produce ethanol under high temperature conditions [41]. Cellulosilyticum ruminicola was a novel strain and able to take advantage of a variety of lignocellulosic growth, with the degradation of hemicellulose and lignin [8]. Among consortium SV79 bacteria, the Sporomusa also could contribute to produce ethanol through degrading by-products of cellulose conversion. Balk et al. [5] isolated Sporomusa sp. An4 from the underground gas storage reservoir, which was later identified as a mesophelic bacterium and had the ability to convert various alcohols. Hence, the various strains of consortium SV79 were suitable for the production of hydrolytic enzymes for biomass material degradation.

The negative consequences of existing biofuel technologies have stimulated interest in the development of so-called "second-generation" biofuel technologies, which derive fermentable sugars from crops or lignocellulosic waste produced by agriculture, forestry, and other industries. However, only limited information is available concerning agricultural residues and energy crops degradation [9]. Agricultural residues, which are primarily composed of stalks, leaves, and straws, contain approximately 10–20% lignin, 40–50% glucan, and 15–35% xylan, making them good candidates for bioethanol production through proper pretreatment, hydrolysis, and fermentation. The cellulose-rich sugarcane bagasse and spent mushroom substrate have been widely investigated for their potential for producing commercial quantities of cellulosic ethanol [2, 10, 18].

The energy crops are the types of plants expected to be cultivated as raw materials for the production of the biofuels such as ethanol. Plants with a fast growing rate, such as grasses or shrubs, should represent the appropriate energy crops for the future. Grasses are targeted as potential energy crops because of their high productivity per hectare, abundance, availability, and utilization of the whole plant. Fibers and storage carbohydrates within some species of grass can be used as substrates to produce ethanol, whereas the species of grasses that contain high amounts of proteins can be used as nitrogenous waste for biorefineries [15]. Three species of energy crops (S. anglica, M. floridulus, and
P. sinense Roxb) with potential value to produce biofuels were selected [32]. They were the most suitable energy utilization index for fuel ethanol. So far, several Miscanthus species have been studied for possible production of second-generation biofuels in a number of laboratories [14, 53]. These plants are perennial crops, considered suitable as feedstock for lignocellulosic ethanol production because of high yields, low costs, fit for infertile land, and less environmental impacts [4].

Some studies showed FPase and CMCase played the major roles in crude fiber biodegradation [19]. From analyzing the enzymatic activity of FPase, it was found that the FPase activity obtained with the agricultural residues and energy crops was higher than the CMCase and xylanase activities obtained. The Z-AECC cultures with sugarcane bagasse had greater FPase activity than the other results had reported [9]. The FPase activities were most obvious in this study. Ethanol-producing bacteria have gained much attention in recent years because of increased interest in renewable energy sources. The highest ethanol yield reported is by the thermophilic bacterium T. ethanolicus, with 1.9 mol-EtOH mol-glucose⁻¹, but several strains are capable of yields reaching 1.5 mol-EtOH mol-glucose⁻¹ [49]. Several investigations on ethanol production by thermophilic bacteria from cellulosic biomass have been recently published. Clostridium thermocellum produced between 4.6 and 8.1 mM ethanol/g alkali-pretreated paddy straw, sorghum stover, and corn stubs [37]. The ethanol production using M. floridulus as substrate was up to 2.63 mM ethanol/g. The alkali treatments could be recommended for paddy straw for microcommunity-based conversions. After treatment, the net yields of reducing sugars were greatly enhanced [57]. Under pretreatment, the ethanol production of consortium SV79 would greatly increase.

Apart from ethanol, anaerobic ethanol-producing cellulolytic bacterial consortia may excrete a variety of metabolic products. These include polyalcohols, and monocarboxylic, dicarboxylic, and tricarboxylic acids [11]. In real applications, the analysis of such variety of reaction products by gas chromatography was difficult. During anaerobic fermentation by Clostridium, the production of ethanol or acetic acid was the major end products [45]. Detection of ethanol and acetic acid showed that Clostridium was the prevalent genus in this community. Although the productivity of ethanol by bacteria is still not better than fungus, bacteria possess other advantages, such as endurance of high temperature during the fermentation process, fast growth, and easy survival. The bacterial consortium SV79 has a good ability in a variety of cellulosic materials degradation and ethanol production with the cooperation of the four families.

In conclusion, a stable ethanol-producing cellulolytic anaerobic microbial consortium, SV79, was isolated using the anaerobic screening medium containing filter paper as the sole carbon source. The consortium could degrade cellulosic biomass efficiently under anaerobic condition. The novel new strains from consortium SV79 also provided a new insight into the microbial diversity of anaerobes in hot spring ecosystems. Acetivibrio, Cellulosilyticum, and Clostridium were detected as key degraders of agricultural residues and energy crops in the sediments of Great Basin hot springs. They and other potential degrading bacteria coexisted and took effect in degrading cellulosic biomass and reducing ethanol. This work would be useful for those involved in developing fuel ethanol production from lignocellulosic biomass. Consortium SV79 provides a candidate for investigating the mechanism of anaerobic cellulose degradation.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (31370146, 41306181 & 31071639), China Postdoctoral Science Foundation (2013M530303 & 2014T70602), Special Fund for Trial-Manufacture of Sanya City, China (2013XK08) and China National Engineering Research Center of Juncao Technology (JCGG1406).

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