Journal of Integrative Agriculture 2016, 15(10): 2369–2379

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RESEARCH ARTICLE

Screening of a microbial consortium with efficient corn stover degradation ability at low temperature

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Abstract

To speed up the degradation of corn stover directly returned to soil at low temperature, the corn stover-degrading microbial consortium GF-20, acclimated to biological decomposition in the frigid region, was successfully constructed under a long-term limiting substrate. To evaluate its potential in accelerating the decomposition of un-pretreated corn stover, the decomposing property, fermentation dynamic and the microbial diversity were analyzed. GF-20 degraded corn stover by 32% after 15-day fermentation at 10°C. Peak activities of filter paperlyase (FPA), β-glucosidases (CB), endoglucanases (Cx), and cellobiohydrolases (C1) were 1.15, 1.67, 1.73, and 1.42 U mL^{-1}, appearing at the 6th, 3rd, 11th, and 9th d, respectively. The pH averaged at 6.73–8.42, and the optical density (OD) value peaked at 1.87 at the 120 h of the degradation process. Cellulase, hemicellulase and lignin in corn stover were persistently degraded by 44.85, 43.85 and 25.29% at the end of incubation. Result of denaturing gradient gel electrophoresis (DGGE) profiles demonstrated that GF-20 had a stable component structure under switching the temperature and pH. The composition of the GF-20 was also analyzed by constructing bacterial 16S rDNA clone library and fungal 18SrDNA-PCR-DGGE. Twenty-two bacterial clones and four fungal bands were detected and identified dominant bacteria represented by *Cellvibrio mixtus* subsp., *Azospira oryzae*, *Arcobacter defluyii*, and *Clostridium populeti* and the fungi were mainly identified as related to *Trichosporon* sp.

Keywords: corn stover, degradation, microbial consortium, low temperature

1. Introduction

Cellulosic biomass has been recently recognized as a source for renewable energy (Sukumaran *et al*. 2009; Honeina *et al.* 2012). Transformation of corn stover to nutrient substance considerably reduces waste accumulation in the environment, thereby serving as a means to produce value-added products. In China, the total annual production of crop straw was estimated to be 700 million t (Bi *et al*. 2011), 221.56 million t of which was corn stover. However, only a small portion of stovers were used to produce animal feed or organic fertilizer, most stovers were burnt (Wang *et al*. 2011). Corn stover returned on field (38.99 million t) accounts for only 17.6% of the corn residues (Lü *et al*. 2013). The northeast region, where the harvest is 54.79 million t of corn, with 68.49 million t of stover, accounting for 31% of the total in the country, is the largest corn belt in China.

Received 24 July, 2015 Accepted 11 January, 2016
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doi: 10.1016/S2095-3119(15)61272-2

However, less than 10% stover returned on field because of lower temperature, drought windy and dusty weather (Lü *et al*. 2013), and unsatisfactory decomposition impacts planting and seedling emergence in the following year, and aggravates plant diseases and insect pests.

Therefore, acceleration of stover biological decay in the field is badly in needed in northeastern China. Many microorganisms which are characterized by multicomponent, cellulolytic enzyme systems have been reported with cellulosic activities. Cellulolytic bacterial species include *Fibrobacters*, *Actinomycetes*, *Cellulomonas*, *Cellvibrio*, *Cytophaga*, *Bacillus*, *Paenibacillus* sp., *Clostridium thermocellum*, *Caldicellulosiruptor ohsidiansis*, *Methanobrevibacter ruminantium*, etc. (Freier *et al*. 1988; Kirclman *et al*. 2002; Zhang *et al*. 2008; Lü *et al*. 2011; Maki *et al*. 2011; Miranda *et al*. 2011; Wu and He 2015), fungal species include *Aspergillus niger*, *Trichoderma reesei*, *Trichoderma viride*, *Penicillium*, *Trichoderma* sp., etc. (Li *et al*. 2013; Okeke *et al*. 2014; Strakowska *et al*. 2014; Guerfali *et al*. 2015). However, it has been reported that the mixture microbial composite showed rather higher effective decomposition capacity and cellulase activity compared with any single strains (Haruta *et al*. 2002; Guo *et al*. 2008). But previous investigations on microbial communities were conducted at medium-high temperature (Guo *et al*. 2008; Li *et al*. 2011), not appropriate for low temperature. There are still no reports on microbial communities which can efficiently hydrolyze un-pretreated natural lignocellulases at low temperature. In order to accelerate the decomposition of corn stover, a mixture of microbes, which had a higher enzyme activity at low temperature, was specifically grown and selected in a limiting substrate of corn stover.

2. Materials and methods

2.1. Experimental materials

Inoculums sources of soil and cow dung were collected from 31 different sites in the cold regions of Northern China (Table 1). Each sample was placed in a sterile plastic bag, sealed and transported to the laboratory on ice and then stored in the dark at 4°C. Post-harvest corn stover was obtained from the experimental farm of Inner Mongolia Agricultural University. The stover, without any diseases and contaminating insects, was air-dried, washed with water, and then oven-dried at 80°C. For submerged fermentation, dried stover was cut into scraps approximately 1 cm in length and mixed thoroughly with the medium. The filter paper (10 cm×1 cm in length and width, about 0.1 g) used in this study was Whatman No. 1 (Japan).

The culture media were consisted of enrichment medium No. 1 peptone cellulase solution (PCS) medium (Lü *et al*. 2012), the corn stover as the carbon source (2% w/v), enrichment medium No. 2 (2 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2 g $CaCO_3$, 2 g NaCl, filter paper, and 1 000 mL distilled water (pH=7.5±0.1)). The basal medium used in this study was the same as enrichment medium No. 2. All media were sterilized at 121°C for 20 min.

2.2. Enrichment cultivation for corn stover degrading microbial consortium

The collected samples were inoculated in an enrichment medium No. 1 and enrichment medium No. 2 for the enriching of cellulolytic microbial consortium. These cultures were incubated (100 r min^{-1}) for 2 d in a shaker incubator at 28°C and then incubated for 13 d in a static condition.

Enrichment cultivation experiment No. 1: corn stover with 10 g inoculants were inoculated in 250-mL Erlenmeyer flasks containing 100 mL autoclaved enrichment medium No. 1. Corn stover (2% (w/v)) was served as the carbon source. Filter paper with 1 g inoculants was inoculated in 25-mL test tube containing 10 mL autoclaved enrichment medium No. 2. Filter paper (10 cm×1 cm, about 0.1 g) was the carbon source. Each treatment was repeated 3 times. When the filter paper was decomposed into pieces, 10%

Table 1 Sample collecting sites and characteristics

Sampling position	Sample	Longitude and latitude	Altitude (m)	The annual average temperature (°C)
Inner Mongolia	Meadow soil, forest soil , cornfield soil, rotten straw, corn stover returning soil, cattle and sheep manure	40.33–50.39°N 108.65–122.32°E	300–2 200	–6.4–6
Heilongjiang	Corn stover returning soil	45.50–50.25°N 127.52–130.32°E	100–200	–1.3–3
Jilin	Corn stover returning soil	43.82°N, 125.32°E	250	4.8
Shanxi	Corn stover returning soil	39.52°N,112.82°E	1 100	3.6–7.3
Tibet	Rotten straw, cattle manure, cow dung	29.12–31.37°N 89.27–91.15°E	3 680–4 772	–0.3–7.5
Mongolia	Cornfield soil	46.20°N,102.95°E	1 700	–1.3