e-ISSN:2320-3528
p-ISSN:2347-2286

Research & Reviews: Journal of Microbiology and Biotechnology

Isolation, Identification and Characterization of Cellulose-Degradation Bacteria from Fresh Cow Dung and Fermentation Biogas Slurry

Zhou Hong-li[1], Yang Xiao[2], Xiong Dong-mei[3], Zeng Lu[3], Tian Kai-zhong[3], Xiong Xing-yao[3,4], Liu Yun[2], Su Xiao-jun[*1,3,5]

[1]College of Food Science and Technology, Hunan Agricultural University, Changsha, PR China

[2]Beijing Key Laboratory of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, PR China

[3]Hunan Provincial Key Laboratory of Crop Germplasm Innovation and Utilization, Hunan Agricultural University, Changsha, PR China

[4]The Institute of Vegetables and Flowers Chinese Academy of Agricultural Sciences, Beijing, PR China

[5]Hunan Collaborative Innovation for Utilization of Botanical Functional Ingredients, Hunan Agricultural University, Changsha, PR China

Research Article

Received date: 26/06/2015
Accepted date: 01/08/2015
Published date: 07/08/2015

***For Correspondence**

Xiaojun Su, College of Food Science and Technology, Hunan Agricultural University, Changsha, PR China.

E-mail: suxiaojun5606@163.com

Keywords: Screening, Identification, Characteristics, *Stenotrophomonas, Bacillus cereus,* Cellulase.

ABSTRACT

Two new isolates designated strain MY6 and strains FY2 were isolated from the mixture of fresh cow dung and fermentation biogas slurry, which were found to be potential cellulase producers. On the basis of cellular morphology, physiological and chemotaxonomic characteristics and similarity of 16S rDNA gene sequences, the former was confirmed as *Stenotrophomonas* sp., while the latter was identified as *Bacillus cereus* sp. Key fermentation factors including culture time, initial pH and culture temperature for cellulase production were optimized using single factor experiments for the two strains. The endoglucanase (CMCase) activity of MY6 was increased by 200.74% and reached 137.36 U/ml under the optimized conditions (cultured for 48 h at pH7.0 and 40°C with shaking at 160 rmp); while the activity of FY2 was enhanced by 150.61% and achieved 177.58 U/ml under the optimized conditions (cultured for 48 h at pH7.0 and 45°C with shaking at 160 rmp). The effects of four native lignocellulosic feed stocks on CMCase activities were compared. The results showed that cellulase from strains MY6 and FY2 were similar in cellulose degradation and the activity was strongest for filter paper, then for degreased cotton, straw powder and sawdust as substrates. These findings indicated that high cellulose concentration used as carbon sources could promote the generation of CMCase.

INTRODUCTION

Cellulose is the most abundant renewable natural resources, which can be employed to produce biofuels and bio-chemicals. Recently, increasing attentions have been paid on biofuels from cellulose resources throughout the world, owning to security of oil-based fuels and environmental pollution [1,2]. Cellulose biofuels by enzymatic catalysis is a potential promising approach in the past few years. However, cost-efficient of enzymatic catalysis hinders the progress of this technology [3,4]. Therefore, it is vital to screen new cellulase and cellulase-producing strain in nature. For instance, Saddler et al. addressed an isolate which belonged to *Aspergillus flavus Linn* with CMCase activity of 0.0731 IU/ml [5]. Goldbeck et al. reported cellulase producing yeast-like microorganisms, which cellobiase activity was 0.038U/ml [6]. Ruegger and Tauk demonstrated a cellulase-producing microorganism

Penicillium purpurogenum with the activity of CMCase 0.034 U/ml and FPase 0.016 U/ml [7]. Deswal et al. optimized the cellulase-producing process from *Fomitopsis* sp. RCK2010 under solid fermentation, which produced CMCase to be 1.832 IU/g under the optimized condition [8].

From the above-mentioned consideration, it has been found that most researches are focusing on cellulase-producing fungus. However, few detailed information on cellulase-producing bacteria is available up to date although some cellulase-producing bacteria are reported, such as *Cytophaga* sp.LX-7 [9], *Bacteroidaceae* [10] and thermostable *Escherichia coli* [11]. Compared with fungi, bacteria have four obvious advantages: (1) bacteria can usually ferment under facultative anaerobic conditions; (2) they could grow in a wide pH range (pH 2.2-8.0); (3) strong stress resistance; (4) shorter culture time. Even under their optimal conditions, almost all fungi need a relatively long culture period (about 4-8 days) to grow and accumulate metabolites before cellulase can be obtained, while bacteria including strain MY2 and strain FY6,generally need a shorter culture time (about 2-5 days). Therefore, more and more interesting have triggered on for biofuel biomass.

In this study, two cellulase-producing bacteria from fresh cow dung and fermentation biogas slurry are screened and identified on the basis of cellular morphology, physiological and chemotaxonomic characteristics and similarity of 16S rDNA gene sequences. Furthermore, the fermentation parameters influencing cellulase production are optimized using cellulose as sole carbon source. At the end, the effects of four different biomass raw materials as carbon sources are compared on CMCase activity. The findings in this work will provide new microorganisms of cellulase-producing bacteria, which is helpful to produce cellulase for cellulose ethanol production in the future.

MATERIALS AND METHODS

Medium: Enrichment medium contained (per L) 2.0 g of Na_2HPO_4, 1.2 g of K_2HPO_4, 0.1 g of resazurin, 0.5 g of cysteine-HCl, 0.5 g of yeast extract, 1.0 g of tryptone at pH 6.5-7.0. Filter paper strip (6 cm × 2 cm) 1 g were added as carbon source. Preliminary screening medium (per L) is consisted of 2.0 g of Na_2HPO_4, 1.2 g of K_2HPO_4, 0.5 g of cysteine-HCl, 0.5 g of yeast extract, 1.0 g of tryptone at pH 6.5-7.0. Filter paper strip (5 cm × 1 cm) 1 g was added as carbon source. Isolation medium (per L) is consisted of 5 g of CMC-Na, 0.5 g of cysteine-HCl, 1.0 g of yeast extract, 0.5 g of tryptone, 2.0 g of Na_2HPO_4, 1.2 g of K_2HPO_4, 0.05 g of $MgSO_4$, 0.5 g of NH_4Cl, 0.02 g of $FeSO_4$, 0.01 g of $CaCl_2$, 0.005 g of $MnSO_4$, 1.5 g pf Congo red, 15 g of agar at pH 6.5-7.0. Differential medium (per L) contained CMC-Na 5.0 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.5 g, $(NH_4)_2SO_4$ 2.0 g, NaCl 0.5 g, Congo red 1.5 g, agar 15 g at pH 6.5-7.0. Fermentation medium (per L) contained CMC-Na 1.0 g, yeast 1.0 g, peptone 1.0 g, Na_2HPO_4 1.0 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.1 g at pH 6.5-7.0. These chemicals were A.R. and purchased from Beijing Luqiao Technology Co Ltd.

Bacterium isolation and identification

Isolation: Strains designated MY6 and FY2 were isolated in our laboratory by the dilution agar planting method, from fresh cow dung(preserved in 4°C) and fermentation biogas slurry (preserved in 4°C) were inoculated in enrichment medium at about 30% v/v, and cultured at 37°C for 5-7 days. The microbes proliferated in the enrichment medium were used to inoculate in preliminary screening medium and cultured at 37°C. Bacteria's were then isolated and purified on isolation medium. The isolates obtained were subjected to a two-method secondary screening procedure [12]: They were cultured in differential medium at 37°C for 3 days to select the isolates that form shape and clearer transparent ring. The selected isolates were inoculated into preliminary screening medium and cultured at 37°C for 3 days. Weight loss rate of filter paper was measured and recorded. Alternatively, the isolates obtained from preliminary screening procedure were cultured in fermentation medium at 37°C for 3 days and assayed for carboxymethyl cellulase (CMCase) activity. Two strains MY6 and FY2 were obtained based on the data from the two methods and used for further study.

Morphological and culture characteristics: The morphological and cultural characteristics of MY6 and FY2 were determined as described [13-15].

DNA extraction, PCR amplification and sequence analysis: The total DNA was extracted with genomic DNA extracting reagent D305S (TAKARA) and was analysed as described (John et al). Evolutionary distance matrices were generated and a phylogenetic tree was constructed using the neighbour joining method.

Enzyme assays:

Pretreatment of enzyme: 50 ml fermentation medium was inoculated with 0.5 ml bacteria-culture fluid and incubated at 37°C for 48 h. Different fermentation treatments were conducted using the shake-flask method. The fermentation media were centrifuged at 4,000 rpm for 20 min and filtered to remove cell debris. The cell-free supernatants were used for the determination of cellulase activity.

Enzyme assay: Unless otherwise noted, all experiments were run in quintuplicate and results were averaged. All microbes were incubated in flasks which were placed on a rotary shaker with speed 160 rpm.

The supernatants were diluted with sterile distilled water and cellulose sodium solution was heated in a water bath at 37°C for 30 min. The reaction mixtures containing 1ml each of the diluted supernatant and preheating cellulose sodium solution and

2 ml of DNS reagent were shaken vigorously by hand for several times and heated in a boiling water bath for 5 min. After cooling, sterile distilled water was added to give a final volume of 25 ml. One unit (U) of the activity was defined as the amount of enzyme releasing 1 µg reducing sugar from 0.8 g/ml CMC-Na solution per 1 min under the condition of 37 °C and pH 5.5, using glucose as a standard.

Optimization of culture condition for cellulase production

Culture time: Bacteria were incubated in fermentation medium and the CMCase activity was assayed at different times (24 h, 72 h, 96 h, 120 h, 144 h, 168 h and 216 h) at the standard screening temperature (37 °C) and pH (7.0).

pH: The effect of pH on cellulose production was evaluated by measuring the CMCase activity in fermentation medium at different initial pH (5.0, 6.0, 7.0, 8.0, 9.0, 10.0) and 37 °C for the optimized time (48 h).

Culture temperature: The effect of culture temperature on cellulase production was evaluated by determining the CMCase activity in fermentation medium at different temperatures (30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C) at the optimal initial pH 7 for 48 h.

Lignocellulosic sources: Four lignocellulosic feed stocks filter paper, degreased cotton, straw powder, sawdust were used to determine their effect on CMCase production. 10 ml bacterial solution was added into the peptone cellulose medium containing one of the feed stocks (0.5 g) and cultured under optimized static condition (strain MY6, cultured for 48 h at pH 7.0 and 40 °C; strain FY2: cultured for 48 h at pH 7.0 and 45 °C). Cellulase yield was determined based on the CMCase activity.

RESULTS AND DISCUSSION

Isolation of cellulase-producing bacteria

Congo red assay: A total of 15 isolates were isolated using the isolation medium whose sole carbon source is carboxymethyl. After Congo red staining, both the diameters of the colonies (d) and transparent zones (D) around the colonies were measured with a vernier caliper. The two diameters were used to calculate activity ratio R (D/d). Cellulase production and R value are positively correlated. Based on the R values, two isolates, named strain MY6 and strain FY2, were selected. Both of them produced larger and clearer transparent zones, suggesting that they have cellulase activity. For FY2, the diameters of transparent zones (D) and colony (d), and R values respectively were 15.2 mm, 2 mm and 7.6 mm. For MY6, the diameters of transparent zones (D) and colony (d), and R values respectively were 18.6 mm, 4 mm, and 4.56 mm **(Figure1)**.

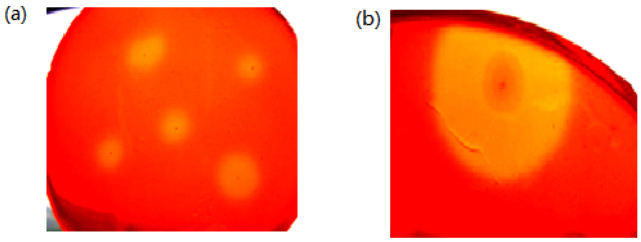

Figure 1. Assays for CMC-hydrolyzing activity using a Congo red assay for MY6 (a) and FY2 (b).

Filter paper degradation: Six isolates, MY1, MY2, MY3, MY5, MY6, FY2, were assayed for their ability to decompose filters paper and generated clear halos on the paper. They were selected for further screening. Similar to the plate screening, microanalysis of hydrolysis in filter paper generated from micro fermentations can also detect differences in the enzyme activity by observing the weight loss of the paper. The isolates were transferred to seed medium with an inoculating loop and incubated at 37 °C for 24-36h. Filter paper was then weighed after the enriched strains were inoculated into preliminary screening medium and incubated at 37 °C for 3 days. The decomposition ability to the filter paper was defined as weight loss rate and calculated according to the following equation:

$$\text{weightless ratio } (\%) = \frac{(W_0 - W_1)}{W_0}$$

Where W_0 is the original weight of filter paper and W_1 is the final weight of filter paper.

The weight loss rates of these isolates to decompose filter paper are shown in **Figure 2**, and ranged from 7.9% to 22.95%. MY6 showed a highest rate (22.95%), followed by MY1 (16.28%) and FY2 (16.21%), while the rests were lower (11.16%, 10.56%, 7.96%).

CMCase activity assay: In order to reproduce the 6 isolates obtained (MY1, MY2, MY3, MY5, MY6 and FY2), strains were incubated as previously described and then investigated for CMCase activity in the fermentation medium. The results are shown in **Figure 3**.The CMCase activities of MY6 and FY2 were higher than those of the others. Taken together the data from Congo red test and weight loss assay, MY6 and FY2 were chosen to study their morphological, physiological and biochemical characteristics and determine their CMCase activities.

e-ISSN:2320-3528
p-ISSN:2347-2286

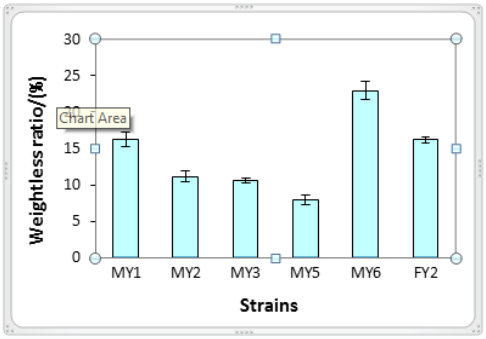

Figure 2. The weight loss rate of different strains following incubation on filter paper.

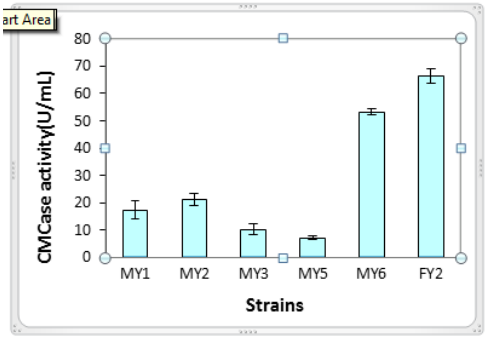

Figure 3. CMCase activities of different strains.

Identification of strain FY2 and strain MY6

Morphology: In order to observe the morphological characteristics of the two strains and their cultures, isolates were diluted, streak out on agar plates and incubated at 37°C for 48 h. Colonies of MY6 were 1-2 mm in diameter, round-shaped, lustrous, opaque wet with convex and neat center, neat edge, and smooth surface. They were opalescent or yellowish, while FY2 colonies were round with a diameter of 3-10 mm, irregular convex with undulated edge, lusterless, wet but rough surface, and were opaque milky in color. Cells of both strains were rod-shaped. After gram staining, cells were observed with a microscope (1000x) and photographed **(Figure 4)**.

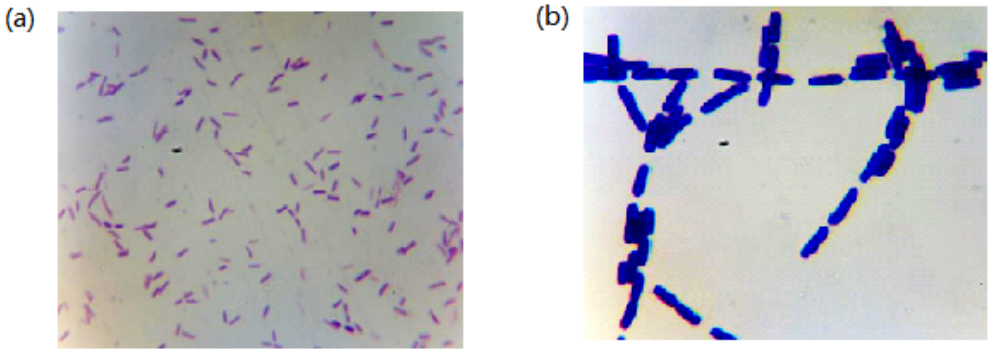

Figure 4. Bacterial morphology of strain MY6 (a) and strain FY2 (b).

Physiological and biochemical features: The physiological and biochemical characteristics of the two strains are listed in **Table 1**.

Strain MY6 was moving gram-negative rod with 1~1.5 × 0.4~0.5 μm in size. It was non-spore and could use glucose utilizing. The oxidase test and catalase test were positive, while FY2 was moving gram negative rod with near-center but non-bulgy thallus spore. It was positive for v-p test, lecithovitellin reaction and gelatin liquidizing and could also grow anaerobically. The bacterium was shown to hydrolyze tyrosine, ferment glucose and produce acids. Based mainly on the Bergey's Manual of Systematic Bacteriology [16] and Manual of Common Bacteria Identification [17], these physiological and biochemical analyses indicated that MY6 might belong to the genus Pseudomonas, while FY2 was almost similar to Bacillus cereus sp.

Identification of 16SrRNA from strains MY6 and FY2

Results of the total DNA extraction and PCR amplification: Genomic DNA was extracted from the two strains using the reagent purchased (TAKARA company).DNA yield and purity were checked by agarose gel electrophoresis **(Figure 5A)**. The DNA was pure enough for PCR amplification as template and amplified using 16S rDNA-specific primers. A band of about 1500 bp was amplified **(Figure 5B)**.

e-ISSN:2320-3528
p-ISSN:2347-2286

Table 1. Physiological and biochemical features of MY6 and FY2[a].

Physiological and biochemical features	Strain MY6	Strain FY2
Size (length × width, μm)	1-1.5 × 0.4-0.5	2.5-3.5 × 1-1.25
Shape	Rod-shaped	Rod-shaped
Spore	NG[b]	G/NC[b]
Gram reaction	-	+
Ability of motion	+	+
Hydrolysis of starch	-	+
Catalase	+	+
Glucose fermentation (acid-producing)	+/W	+
Glucose fermentation (gas-producing)	-	-
V-P test[c]	-	+
MR test[c]	-	+
Hydrolysis of tyrosine	-	+
Indole test	-	-
Citrate utilization test	-	-
Oxidase test	+	+
Lecithovitellin reaction	-	+
PAL test[c]	-	-

a: + , ≥ 90% cell positive ; - , ≥ 90% cell negative.
b: G: Grow; NG: Not Grow; NC: Near Center.
c:V-P: Voges-Proskauer; MR: Methyl-red; PAL: Phenylalanine Ammonia Lyase.

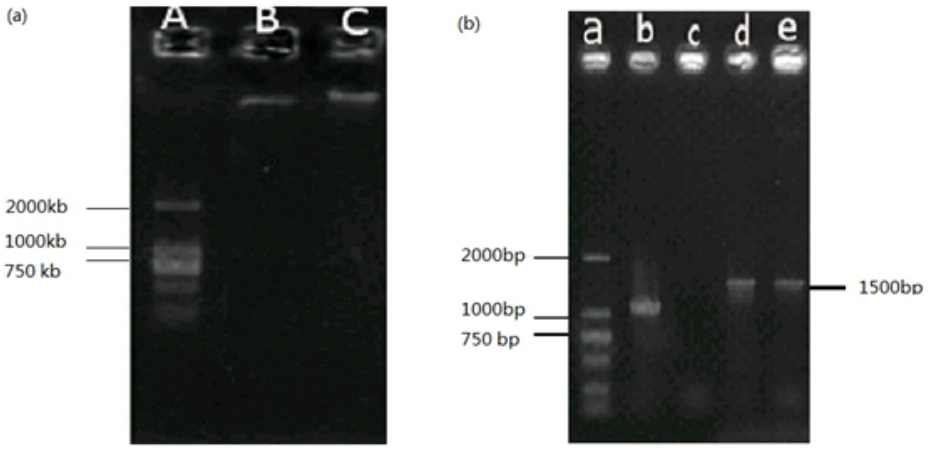

Figure 5. (a) Gel electrophoresis of genomic DNA and PCR product. A, DNA molecular weight marker (TAKARA, DL2000 DNA marker); B and C, genomic DNA of MY6 and FY2, respectively.
(b): a- DNA molecular weight marker (TAKARA, DL2,000 DNA marker); b- positive control; c- negative control; d and e- PCR product of MY6 and FY2, respectively.

Analysis of 16SrDNA sequence and its phylogeny: The amplified products of 16SrDNA were sequenced and gave rise to two fragments of 1537 pb (from MY6) and 1544 bp (from FY2) long. The sequences were compared with the sequences of reference species contained in GenBank database. The phylogenetic trees were constructed using the neighbour joining method **(Figure 6)**.

On the basis of the morphological and culture characteristics, chemotaxonomic and physiological analyses, strain MY6 was identified as the *Pseudomonas sp*. However, by 16S rRNA analysis, it was confirmed as *Stenotrophomonas* sp. From **Figure 7A**, it can be seen that the similarity ranged from 99% to 100% within the *Stenotrophomonas* species and strain MY6 formed a common cluster with *S. acidaminiphila* st31 (GenBank Accession No.FJ544377.1) and *S. acidaminiphila* (AMX 19 ATCC 700916). Meanwhile, strain FY2 was proved to be *B. cereus* sp. **(Figure 7B)**, which is in accordance with the morphological and culture characteristics and chemotaxonomic and physiological analyses. It formed a common cluster with *B. cereus* strain NA10 (GenBank accession no. FJ462699.1).

Optimization of cellulose producing condition

Effect of culture time on cellulase activity: The cellulase enzyme from MY6 and FY2 both had an optimal culture time of 48h **(Figure 7)**. When fermentation was carried out at 37 °C and pH 7.0, extending the culture time resulted in an increase in the CMCase activity from approximate 106.48 U/ml to 144.07 U/ml when culture times were increased from 24 h to 48 h. However, further increase of culture time adversely affected CMCase activity, resulting in a gradual reduction to 58.27 U/ml. Similar results were observed in FY2, where the CMCase activity was elevated from 119.56 U/ml to186.57 U/ml when the culture time increased from 24 h to 48 h, whereas the cellulase-producing ability went down after 48h fermentation. The reason might be ascribed to the depletion of the nutrition's and the toxic action of metabolites from the cultures.

e-ISSN:2320-3528
p-ISSN:2347-2286

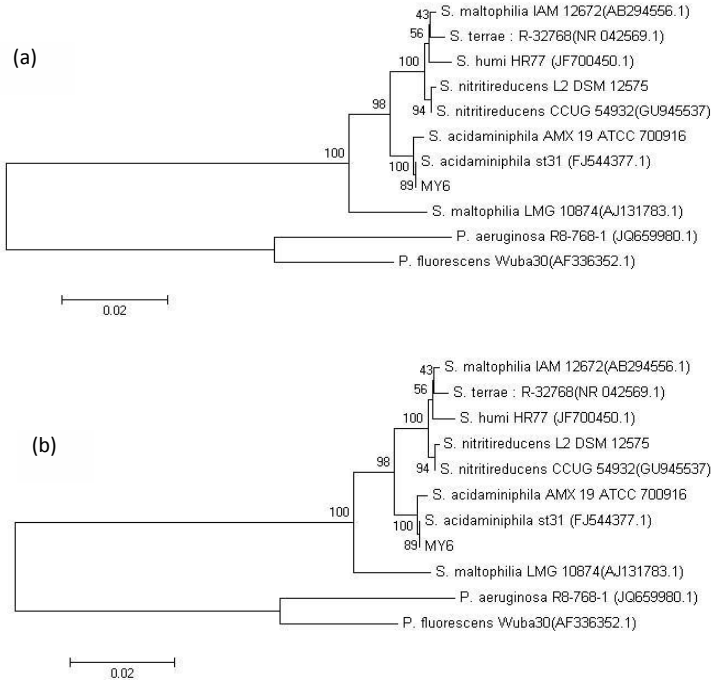

Figure 6. Phylogenetic trees of 16S rDNA gene sequences of strains MY6 (a) and FY2(b).

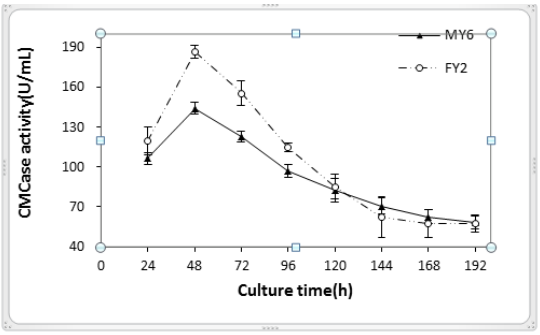

Figure 7. Effects of culture time on the CMCase activity in strains MY6 and FY2.

Effect of initial pH on cellulase activity

The impact of initial pH on the CMCase activity was investigated at a pH range of 5-10 using CMC-Na as substrate for the two strains. The results showed that there was marked impact of initial pH on the CMCase activity **(Figure 8)**. The CMCase activity of MY6 increased with increasing initial pH from 6.78 U/ml to 144.07 U/ml. But over pH7, the CMCase activity decreased. Under the same experimental conditions, CMCase of strain FY2 showed a similar response with the highest CMCase activity (186.57 U/ml) obtained at initial pH 7, which indicated that more cellulose could be decomposed in neutral environment (pH=7) by the two isolates. It has been demonstrated that the combination and catalysis between substrates and enzymes may depend on their dissociation degree, which is subjected to pH levels in reaction system.

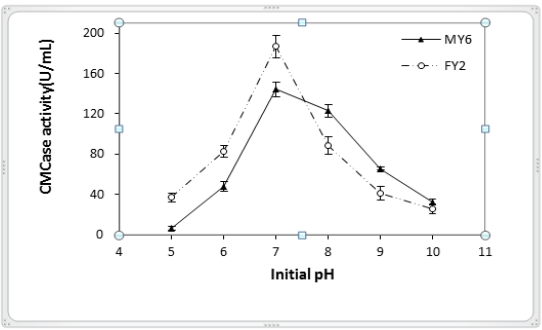

Figure 8. Effects of initial pH on the CMCase activity of strains MY6 and FY2.

Effect of temperature on cellulase activity

The highest activity for CMCase from strain MY6 was 150 U/ml obtained at 40°C **(Figure 9)**.

The CMCase varied from 90.96 U/ml at 35°C to 117.11 U/ml at 45°C. On the other hand, CMCase from FY2 was quite

stable between 40°C and 50°C with the maximum activity obtained at 45°C after incubation for 48h. It maintained approximately 94% and 91% of the maximal CMCase activity at 40°C and 50°C, respectively.

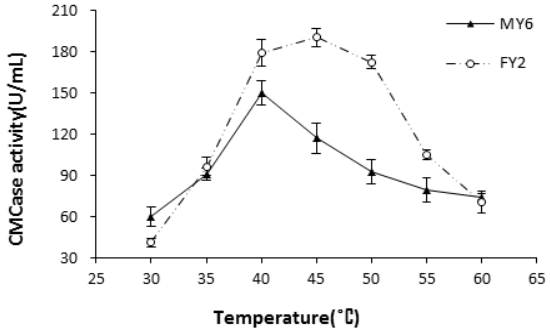

Figure 9. Effects of temperature on the CMCase activity of strains MY6 and FY2.

Effects of carbon source on cellulase activity

It has been reported that high concentration of cellulose used as carbon source could promote the synthesis of CMCase in specific studies including *Cellulomonas flavigena* on fruit fiber [18], *Neurospora crassa* on wheat straw [19] and *Trichoderma reeseion* dairy manure [20]. The results obtained in this work were similar with these reports. The CMCase activity of strain MY6 was found to be different with the different carbon sources in shaking bottle incubation. Use of filter paper resulted in the greatest enzymatic activities **(Figure 10)**.

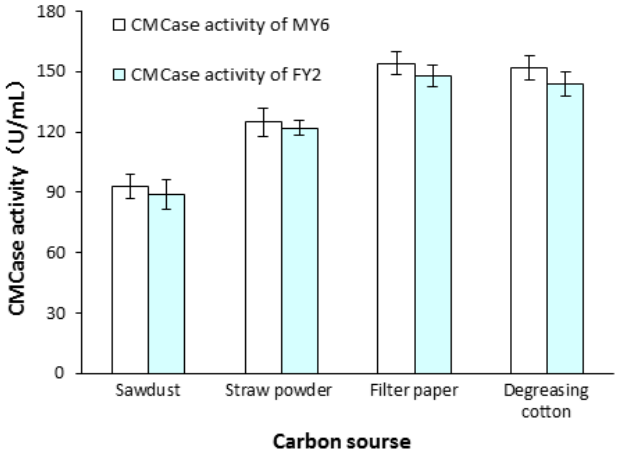

Figure 10. Effects of carbon source on the CMCase activity of strains MY6 and FY2.

Degreased cotton nearly had an equivalent influence on the CMCase activity of MY6 compared with filter paper. However, saw dust was not a good carbon source for producing CMCase. Straw powder was between the two. Similar results were observed in strain FY2 [21]. Compared with sawdust and filter paper, degreased cotton was much better as sole carbon source, followed by straw powder. The CMCase activity was improved from 98 U/ml to 148 U/ml when sawdust was replaced by filter paper, whose cellulose content was in the highest among the four feed stocks.

CONCLUSIONS

Screening cellulose-decomposing microorganism has been considered to be a promising option for identifying new CMCase sources. In this study, strain MY6, has been identified to be *Stenotrophomonas* sp. based on the results of morphological, physicological and biochemical characterizations and 16S rDNA gene sequence analysis. Meanwhile, strain FY2, isolated from the mixture of fresh cow dung and fermentation biogas slurry, and has been identified as B. cereus sp. To our knowledge, this is the first reported *Stenotrophomonas* sp. that produces cellulases in experimental conditions. In order to obtain high cellulose production, three crucial factors (culture time, initial pH and culture temperature) affecting CMCase activity and yield were preliminarily optimized. Under the optimized conditions, the CMCase activity of strain MY6 increased by 150.61% compared with that using the original conditions; while that of strain FY2 increased by 200.74%. In addition, four native lignocellulosic feedstocks were compared to find out the relationship between cellulose content in the medium as sole carbon source and degrading ability of cellulose in this work. However, further studies are needed to study the interactions between these factors, to enhance the cellulase production and their stress tolerance before being used for large-scale of biodegradation. This study reveals that strains MY6 and FY2 are promising for cellulase enzyme production.

AKNOWLEDGEMENT

This study was supported by the National "863" Program of High Technology Research and Development of China

(2012AA101804), National Basic Research Program of China (973 Program) (2012CB723004), Fund of Key Laboratory for Crop Germplasm Innovation and Utilization of Hunan Province (12KFXM03), and National Natural Science Foundation of China (NSFC 21476016).

REFERENCES

1. Angenent LT, et al. Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol. 2004;22:477-485.
2. Percival Zhang YH, et al. Outlook for cellulase improvement:screening and selection strategies. Biotechnol Adv. 2006;24:452-481.
3. Charles E. Wyman. Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and power. Biotechnol Prog. 2003;19:254-262.
4. Galbe M and Zacchi G. A review of the production of ethanol from softwood. Appl Microbiol Biotechnol. 2002;59:618-628.
5. Ojumu TV, et al. Cellulase production by *Aspergillus flavus Linn* isolate NSPR 101 fermented in sawdust, bagasse and corncob. Afr J Biotechnol. 2003;2:150-152.
6. Goldbeck R, et al. Screening and identification of cellulase producing yeast-like microorganisms from Brazilian biomes. Afr J Biotechnol. 2012;53:11595-11603.
7. Ruegger MJS. Cellulase activity of fungi isolated from soil of the Ecological Station of Juréia-Itatins, São Paulo Paulo, Brazil. *Rev Bras Bot*. 2004;27:205-211.
8. Deswal D, et al. Optimization of cellulase production by a brown rot fungus *Fomitopsis* sp. RCK2010 under solid state fermentation. Bioresour Technol. 2011;102:6065-6072.
9. Li X and Gao P. Isolation and partial properties of cellulose-decomposing strain of *Cytophaga* sp.LX-7 from soil. J Appl Microbiol. 2008;82:73-80.
10. Saddler JN and Khan AW. Cellulose degradation by a new isolate from sewage sludge, a member of the bacteroidaceae family. Can J Microbiol. 1979;25:1427-1432.
11. Li XH, et al. Screening and identification of new isolate:thermostable *escherichia coli* with novel thermo alkalo tolerant cellulases. Curr Microbiol 2009;59:393-399.
12. Pangallo D, et al. Investigation of microbial community isolated from indoor artworks and air environment:identification, biodegradative abilities, and DNA typing. Can J Microbiol. 2009;55:277-287.
13. Bandounas L, et al. Isolation and characterization of novel bacterial strains exhibiting ligninolytic potential. BMC Biotechnol. 2011;11:94-98.
14. Chen HJ, et al. Screening,isolation and characterization of cellulose biotransformation bacteria from specific soils. International Conference on Environment & Industrial Innovation. 2011.
15. Lin CY and Hung WC. Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. Int J Hydrogen Energy. 2008;33:3660-3667.
16. Buchanan RE and Gibbons NE. Bergey's manual of determinative bacteriology. 8th Edition. Lippincott Williams & Wilkins. 1974.
17. Dong XZ and Cai MY. *Common bacteria system identification manual.* Science press, Beijing. 2001.
18. Torres N. Mendoza GD, et al. Effect of a fibrolytic enzymatic extract from Cellulomonas flavigena on *in vitro* degradation and *in vivo* digestibility and productive performance of lambs. Anim Nutr Feed Techn. 2013;13:583-592.
19. Romero MD, et al. Cellulase production by *Neurospora crassa* on wheat straw. Enzyme Microb Technol. 1999;25:244-250.
20. Wen Z, et al. Production of cellulase by *Trichoderma reesei* from dairy manure. Bioresour Technol. 2005;4:491-499.
21. Baker JO, et al. Catalytically enhanced endocellulase Cel5A from Acidothermus cellulolyticus. Appl Biochem Biotechnol. 2005;121:129-148.