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Screening, isolation and characterization of cellulose biotransformation bacteria from specific soils

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Abstract. The objective of this study was to isolate the indigenous cellulose-degrading bacteria for bioethanol production. Additionally, the characteristics of these bacteria were investigated after isolation by culture enrichment and strains acclimation. For this study, three soil samples were gathered and which were from hot spring, Yang-ming-shan soil and gas station drainage soil. The strains were isolated through ten subcultures and separation using washed cellulose as the sole carbon source. The 16S rDNA sequence of the strains were identified and shown those five degrading bacteria were *Sphingomonas* sp., *Pseudomonas* sp.M1, *Achromobacter* sp., *Pseudomonas* sp. M2, and *Stenotrophomonas* sp.. The morphological observation found the strains were all gram's negative. The μ_{max} of the *Pseudomonas* sp. M1 was 0.439hr^{-1}, which was the optimal rate among those five strains with the saturation coefficient K_s of 776 mg/L. The highest concentration of the reduction sugar produced per gram of CMC is by *Pseudomonas* sp. M1 in 8 g/L of CMC and produce 51.36 mg of reduction sugar.

Keywords: bacteria, cellulose, microbial identification, growth kinetics, biodegradation

1. Introduction

Major developed countries around the world begin to develop the biomass ethanol as an emerging energy to replace the depleting oil. However, to using food crops as raw materials for bioethanol need higher production cost and indirectly enhance the global food crop prices. And thus seriously affect the survival of people in developing countries. Additionally, a large number of fertilizer and water consumption resulted negative environmental impacts. The crops planted also causes soil erosion, reduction of biodiversity, create high volatile organic compounds (VOC), and nitrogen oxides (NO_x) pollution [1]. Cellulose is the most abundant material on the planet and the most diverse sources of the biological material. In which, the various types of forestry and agricultural residues can be more used for environmental improvement and energy use. It is high value for development of energy, and reduce load of waste and greenhouse gas to the environment [2]. Cellulosic ethanol is therefore the most important role of the second-generation bioethanol development [3]. Nevertheless, the decomposition of the cellulose still need more study to achieve the higher efficiency for using as the material of bioenergy [4][5]. According to these aspects, this study was using agricultural waste biomass materials as the material for the production of ethanol which would not have to compete with the food situation. Also, reduce the traditional agricultural waste manner for not only reduces emissions of air pollutants but also contribute to energy production. The waste sugar cane bagasse was used in this study to as the raw material for isolate the cellulose biotransformation bateria and hope the new species of native cellulose decomposing bacteria can screening and identified for the application of the bioethanol. The experiment was trying to screening the cellulose biotransformation strains. After that, the bacteria were acclimated in the environment with abundant cellulose. Finally, the characteristics of the strains were

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analyzed. And wish the research conduction could enhance the effectiveness of the biomass ethanol development.

2. Materials and Methods

2.1 Source of the soils

The source of the strains were the soil from: (1) Yangmingshan National Park, the volcanic soil material.

The soil is soft and light with organic matter and mostly as small granular; (2) Hot spring soil taken from the Beitou Hot Spring Valley, The hot spring temperature is about 90 ℃ ~ 100 ℃, that is a hydrochloric acid spring water with pH of 1.6; (3) The soil beside the gas station, content much organic material with black outlook.

2.2 Carboxymethyl cellulose (CMC)

CMC is a cellulose derivative, the polymer retains the structure of cellulose material which purchase from the Merck company (Sigma-Aldrich, USA, 2010)

2.3 Isolation process for the bacteria

The screening method is using bagasse as the carbon source for microbial growth. The initial step was taken 3g of bacteria sources soil and 5g of bagasse by adding 150ml of minimal salt medium (MSB) for shaking incubation. The shaking speed is 150rpm, the temperature is 30℃ for 60 days. After 60 days acclimation, the strain can using bagasse as the sole carbon source. The bacteria were empolyed to CMC liquid culture to confirm that the bacteria can survive in liquid CMC. The survival bacteria then cultured to solid-state CMC. After screening the subculture of bacteria, The growth of the bacteria was testing and strain identification was followed.

2.4 The identification of the strains

The method is based on the isolation of 16S rDNA nucleotide sequences and compared to the sequences in NCBI database. First of it was extracted the chromosome DNA form the cell and purified [6]. The primers used in this study were F8 and R1510. The F8 is 5'-AGAGTTTGATCCTG-GCTCAG-3', and R1510 is 5'-GGTTACCTTGTTACGACTT-3'. Finally, it confirmed by agarose gel electrophoresis and then sent the DNA fragments to MingShin company for the identification of the sequence. The NCBI database matching is available on the website using NCBI BLAST program.

2.5 The growth kinetic analysis of the strains

Microbial growth kinetics is an important indicator of microbial activity. To further understand the decomposition of substrate, the substrate concentration and time period corresponding to bacteria growth was need to conduct. This study was use the isolated strain of *Sphingomonas* sp., *Pseudomonas* sp.M1, *Achromobacter* sp., *Pseudomonas* sp.M2, and *Stenotrophomonas* sp. for bacteria growth kinetics analysis. The analysis was using the model of a single carbon substrate (by Monod eqation) and high concentrations inhibited (by Haldane-Andrews model). The experiment is based on CMC and the MSB liquid medium for bacteria culturing. The strains were incubated in 150 rpm, 30℃ temperature with shaking, and every four hours to measure the culture turbidity using spectrophotometer with OD_{600} absorbance.

2.6 The measurement of the reduction sugar concentration

The determination of reduction sugar concentration is refered to DNS method. DNS reagent (Dinitrosalicyclic acid reagent) in the culture medium was restored and then quantitative evaluate by spectrophotometer[7]. The DNS reagent preparation is as follows (100 ml): first, a small amount of deionized water, 1.6g NaOH solution adjusted to alkaline, add 30g potassium sodium titrate, then add 1g 3,5-dinitro-water Yang acid and quantitatively to the 100ml, stored in non-illumination at 4℃. To consider the hydrolysis, the cellulose as substrate intermediate is mainly with glucose. The samples were collected with approximately 2ml culture medium and centrifuged (6,000 rpm×5min). 1ml supernatant obtained by adding an equal volume of DNS reagent mix to screw cap in the glass tube, placed in boiling water bath for heating 10 min, rapid cooling to room temperature, the water was then empolyed to the visible light spectrophotometer at 540 nm and the absorbance was measured.

3. Result and Discussion

3.1 The isolation and identification of the strains

As shown in table 1, all those five strains which can using CMC as the solo carbon source were isolated from the culture contain CMC and using MSB as the basic salts nutrients. After 10 sub-cultures, the strains were identified by 16S rDNA sequence alignment. The strains are *Sphingomonas* sp., *Pseudomonas* sp. M1, *Achromobacter* sp., *Pseudomonas* sp. M2, and *Stenotrophomonas* sp.. Compare the isolation and identification results to the literatures. The strains of *Pseudomonas* are most ever appeared in the references. The other four strains in this study were not reported yet in the literature. So, the strains of *Sphingomonas* sp., *Achromobacter* sp., and *Stenotrophomonas* sp. maybe the novel discovery strains by this study. Thus, it need more explore for the characteristics for cellulose biotransformation

Table 1 Identification of micro-organisms isolated from samples by NCBI

Source of strains	Carbon source and inorganic salts	Number of sub-cultures	Alignment results by BLAST to NCBI database	Length (μm)	Wide (μm)	Appearance	Gram staining
S1	CMC+MSB agar solid medium	10	*Sphingomonas* sp.	1	1	bacillus	negative
M1	CMC+MSB agar solid medium	10	*Pseudomonas* sp. M1	3	1	bacillus	negative
S2	CMC+MSB agar solid medium	10	*Achromobacter* sp.	1	1	bacillus	negative
S3	CMC+MSB agar solid medium	10	*Pseudomonas* sp. M2	2	1	bacillus	negative
M2	CMC+MSB agar solid medium	10	*Stenotrophomonas* sp.	2	1	bacillus	negative

The exterior of the five strains can also be observed from figure 1 and recognized all those five strain are gram negative. The morphological observation using optical electron microscope in 1000 multiple found the strains were with length of 3μm and width of 1μm for *Pseudomonas* sp.M1, 2μm and 1μm for *Sphingomonas* sp. and *Achromobacter* sp., 1μm and 1μm for *Sphingomonas* sp. and *Achromobacter* sp., respectively.

3.2 The growth and kinetic analysis of the strains by using CMC as the carbon source

As shown in figure 2. The growths of the bacteria were highly depended on the concentration of CMC. When the CMC on the concentration of 1000 mg/L. most of the strains can not growth effectively. When the CMC increase to 5000 mg/L, the growth of the bacteria can obtain OD_{600} to 0.3 for the strain of *Pseudomonas* sp. M1, but the strain *Stenotrophomonas* sp still not growth appearently. After the CMC increase to 9000 mg/L, the growth amount of the bacteria can be attend to the optimal results. If still increase the CMC to 10000 mg/L, the growth of bacteria not obviously increase at that point. For those five strains, the greatest growth of the bacteria was happened by the strain of *Pseudomonas* sp. M1 with the OD_{600} greater than 0.5. The smallest growth was happened by the strain of *Stenotrophomonas* sp with the OD_{600} less than 0.3, and the growth start after 44 hours. The other three strains obtain the growth effect among them and the exponential growth all finish before 48 hours incubation. The μ_{max} of the *Pseudomonas* sp. M1 was 0.439hr^{-1}, which was the optimal rate among those five strains.

3.3 The generation of the reduction sugar

The ability for generation the reduction sugar represented the biotransformation ability of the strain. As shown in figure 3, the strain increase obtain the reduction sugar accompany with the increase of the CMC concentration. The reduction sugar generation rate is positive propositional to the CMC concentration for all those five strains. The strain of Pseudomoans sp. M1 obtained the higher amount of the reduction sugar at most of the CMC concentrations especially in high CMC concentration from 8 to 10 g/L. The second one is the strain of *Sphingomonas* sp. in most of the CMC concentration. When CMC was in the concentration of 1 g/L, the reduction sugar product from the *Sphingomonas* even higher than *Pseudomonas* sp. M1. The less

generation amount of the reduction sugar is happen from the strain of *Achromobacter* sp. It might be because the strain obtains less transformation enzyme during the processes of cell growth. In contrast, the highest concentration of reduction sugar produced per gram CMC is the *Pseudomonas* sp. M1 in the concentration of 8g / L CMC. Under the best yield per gram of CMC, it can produce 51.36 mg of reduction sugars.

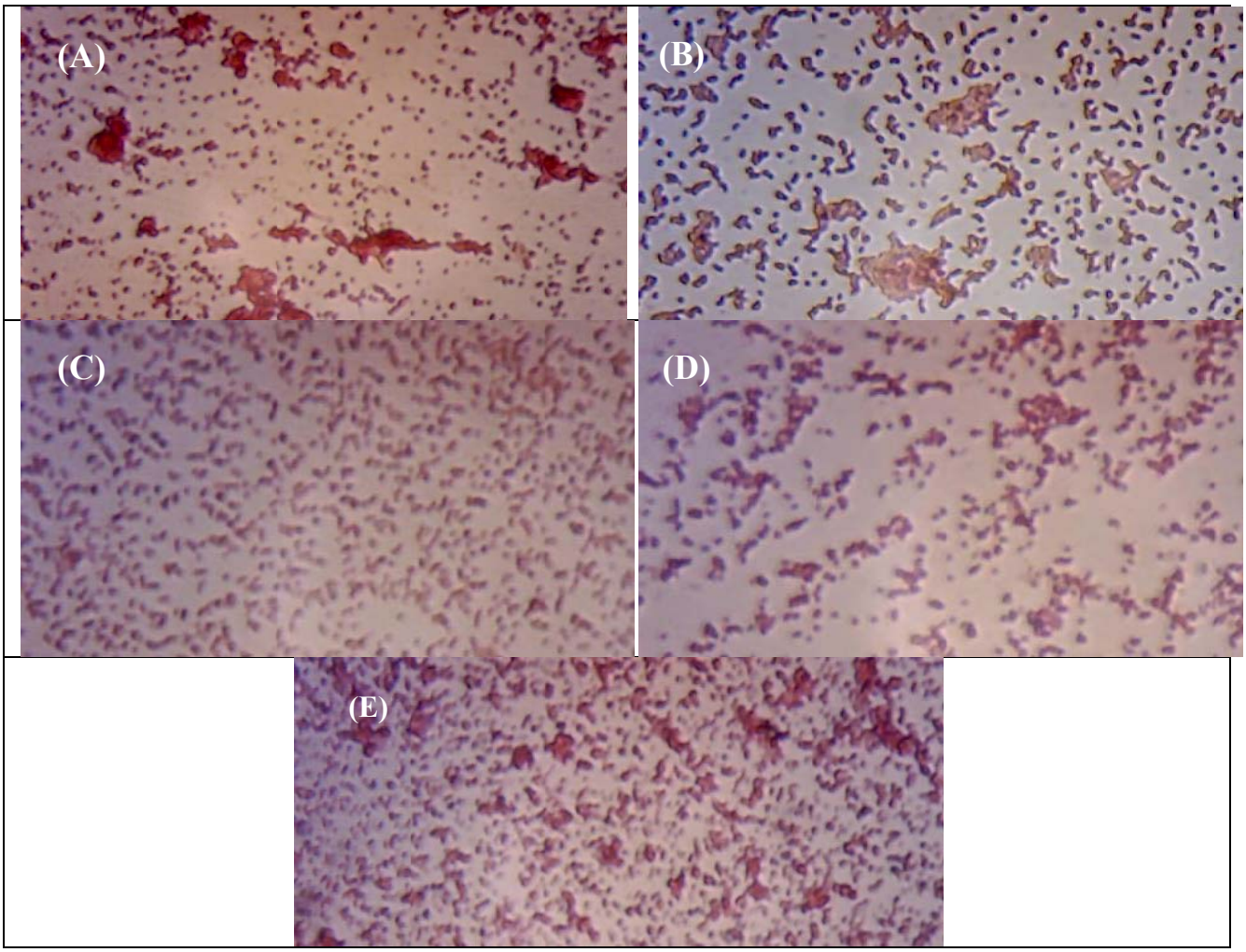

Figure 1 The morphology of the strains after gram staining, (A) *Sphingomonas* sp.(B) *Pseudomonas* sp. M1(C) *Achromobacter* sp.(D) *Pseudomonas* sp. M2 (E) *Stenotrophomonas* sp.

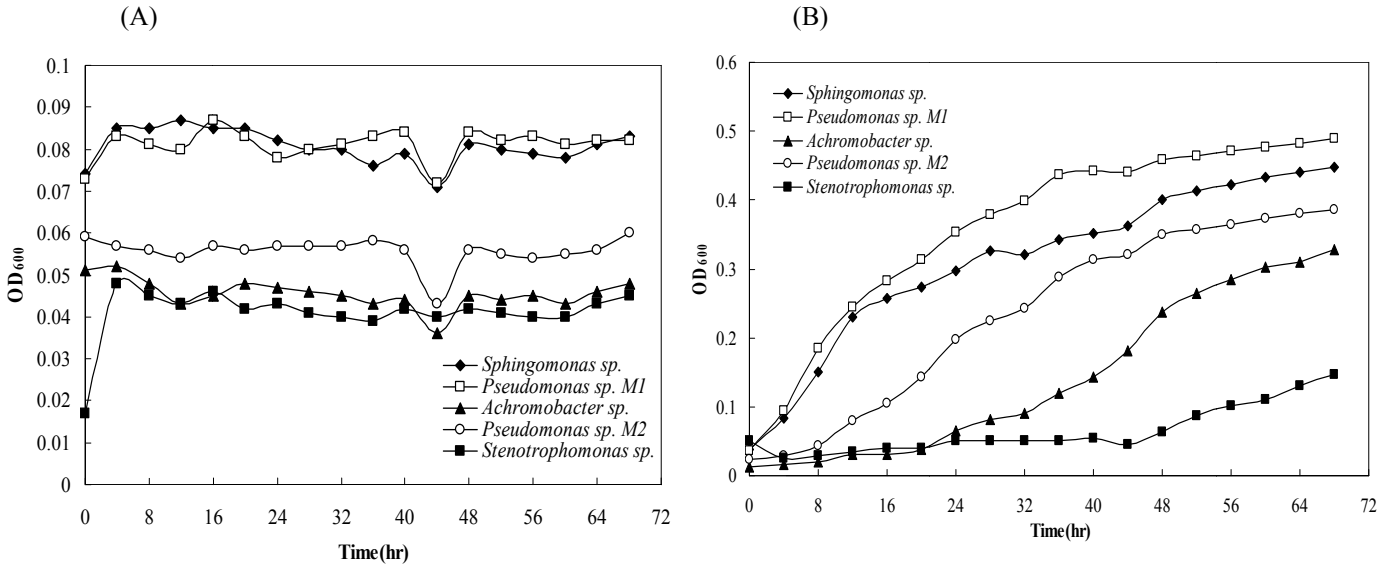

Figure 2 The growth of the strain in MSB broth containing (A)1000 mg/L Carboxymethyl cellulose, (B) 10000 mg/L

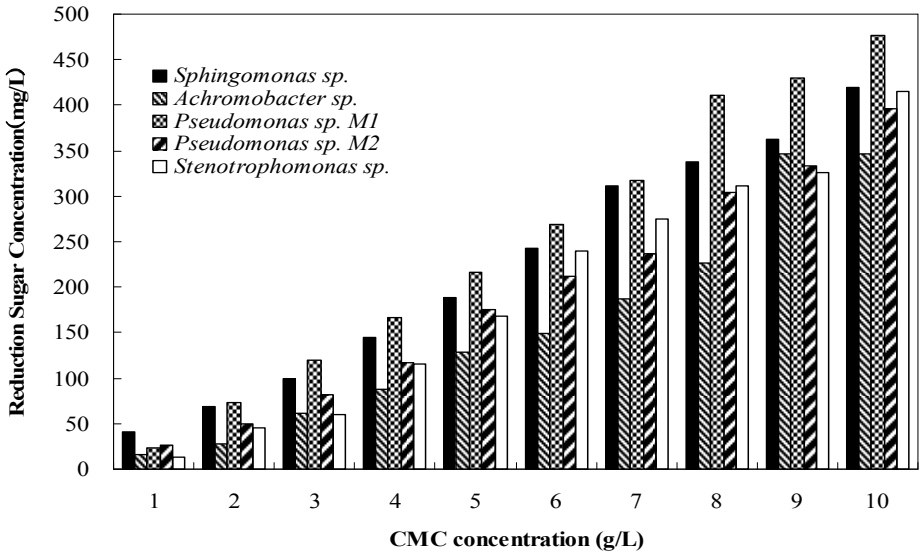

Figure 3 The strains produce reduction sugar in different concentration of Carboxymethyl cellulose

4. Conclusion

Five of the cellulosic bacteria were isolated from this study. The are *Sphingomonas* sp., *Pseudomonas* sp. M1, *Achromobacter* sp., *Pseudomonas* sp. M2, and *Stenotrophomonas* sp.. All of those five strains are bacillus and gram-negative, which can use CMC as the solo carbon source to generate the reduction sugar after the biotransformation process. The strain *Pseudomonas* sp. M1 is the greatest growth bacteria among those five strains and the growth amount was achieve to OD_{600} of 0.5. The growth kinetic analysis shown that the strain of *Pseudomomas* sp. M1 accessed the highest specific growth rate (μ_{max}) of 0.439 h^{-1} and inhibition coefficient of 36.8% medium culture. The highest concentration of reduction sugar produced per gram CMC is by the *Pseudomonas* sp. M1 in the concentration of 8g/L CMC and produce 51.36 mg of reduction sugar under the condition.

5. References

[1] Cheng, S. Y. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource technology. V83, 1-11.

[2] Chiu Yue, L., Wen Chin H. (2008). Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. Bioresource Technology.V33,3660-3667.

[3] Bernders, G., Azak, C., Kaberger, T., Abrahamson, D. (2001). The feasibility of large-scale lignocellulose-based bioenergy production. Biomass and Bioenergy. V20, 371-383.

[4] Galbe, M., Zacchi, G. (2002). A review of the production of ethanol from softwood. Appl Microbiol Biotechnol. V59, 618-628.

[5] Heinz, S., Adisa, A. (2009). Bioethanol from waste: Life cycle estimation of the greenhouse gas saving potential. Conservation and Recycling. V53, 624-630.

[6] Wright, R. M., Yablonsky, M. D., Shalita, Z. P., Goyal, A. K. and Eveleigh, D. E. (1992). Cloning, characterization, and nucleotide sequence of a gene encoding *Microbispora bispora* BglB, a thermostable *β*-glucosidase expressed in *Escherichia coli*. *Appl. Environ. Microbiol.* V58, 3455-3465.

[7] Gutierrez-Correa M., Portal, L., Moreno, P., Tengerdy R.P. (1999). Mixed culture solid substrate fermentation of *Trichoderma reesei* with Aspergillus niger on sugar can bagasse. Bioresource Technol. V68,173-178.