

Application of *Acinetobacter* sp. KKU44 for Cellulase Production from Agricultural Waste

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Abstract—Due to a high ethanol demand, the approach for effective ethanol production is important and has been developed rapidly worldwide. Several agricultural wastes are highly abundant in celluloses and the effective cellulase enzymes do exist widely among microorganisms. Accordingly, the cellulose degradation using microbial cellulase to produce a low-cost substrate for ethanol production has attracted more attention. In this study, the cellulase producing bacterial strain has been isolated from rich straw and identified by 16S rDNA sequence analysis as *Acinetobacter* sp. KKU44. This strain is able to grow and exhibit the cellulase activity. The optimal temperature for its growth and cellulase production is 37°C. The optimal temperature of bacterial cellulase activity is 60°C. The cellulase enzyme from *Acinetobacter* sp. KKU44 is heat-tolerant enzyme. The bacterial culture of 36h. showed highest cellulase activity at 120U/mL when grown in LB medium containing 2% (w/v). The capability of *Acinetobacter* sp. KKU44 to grow in cellulosic agricultural wastes as a sole carbon source and exhibiting the high cellulase activity at high temperature suggested that this strain could be potentially developed further as a cellulose degrading strain for a production of low-cost substrate used in ethanol production.

Keywords—*Acinetobacter* sp. KKU44, bagasse, cellulase enzyme, rice husk.

I. INTRODUCTION

THE world is currently encountering the fuel energy deficiency from a continuously increasing utilization rate. By 2030, the expected energy demand will increase 7-fold when compared in 2005 [1]. Other countries are seeking the alternative sources or renewable energy in various forms to provide a long-term energy supply. Furthermore, renewable energy is better than fossil energy in that it can reduce the CO₂ content which is a cause of global warming. Cellulose is the most common material which can be used as renewable energy. The main component of cellulose is polysaccharides and they are readily available mainly from agricultural wastes such as bagasse, rice straw, rice husk etc [2], [3]. The use of agricultural wastes can help to increase the value of these materials, which helps farmers indirectly. The production of ethanol from cellulose has three main steps including pre-treatment of cellulose, the enzymatic degradation and ethanol fermentation [4]. The Cellulase enzyme is the complex enzyme systems that

hydrolyze the β-1, 4 glycosidic bonds in the cellulose to release glucose units. The cellulosic enzymes required for the hydrolysis of cellulose include endoglucanases (CMCase), exoglucanases (FPase), and β-glucosidases (cellobiase) [5]. Many kinds of bacteria have been found to degrade cellulose such as *Bacillus*, *Cellulomonas* etc. In this study, we characterized and identified the isolated bacteria from rice straw for cellulase production. In addition, the suitable carbon source from agricultural wastes for bacterial growth and cellulase production was determined. The information obtained in this study will be useful for bioethanol production in the future.

II. MATERIALS AND METHODS

A. Microorganisms and Media

The cellulase producing bacteria has been isolated from rice straw. The bacterial strain was maintained in LB medium [1% (w/v) tryptone, 0.5% (w/v) yeast extract, 1% (w/v) NaCl]. The bacterial cell was grown in LB medium at 37°C, 150rpm for 24h to be used as inoculums. In order to find the suitable carbon source for cellulase production, the several agricultural wastes were used including bagasse, rice straw and rice husk. They are several cellulase producing media including bagass medium [BM: 1% (w/v) bagass, 0.1% (w/v) yeast extract, 0.1% (w/v) (NH₄)₂SO₄], rice straw medium [RSM: 1% (w/v) rice straw, 0.1% (w/v) yeast extract, 0.1% (w/v) (NH₄)₂SO₄], rice husk medium [RHM: 1% (w/v) rice husk, 0.1% (w/v) yeast extract, 0.1% (w/v) (NH₄)₂SO₄], LB medium, and LB medium containing 2% (w/v) of bagass, rice straw, or rice husk. A 10% (v/v) of bacterial inoculums were added into cellulose producing media and further grown at 37, 40, 45, or 50°C, 150rpm for 72h.

B. Bacteria Identification

The bacterial DNA was extracted by the GF-1 Bacterial DNA Extraction Kit (Vivantis). To identify the isolated bacteria, the sequence of 16S rDNA was amplified using 20F' primer (5'- GAGTTTGATCCTGGCTCAG - 3') and 1500R' primer (5'- GTTACCTTGTTACGACTT -3'). Then, the PCR product was sequenced and analyzed.

C. Pre-Treatment Agricultural Wastes

Each bagasse, rice straw and rice husk was chopped into small pieces, and then ground into powder. These materials were used in medium preparation for cellulase production.

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D. Enzyme Activity Assay

The bacterial cells were grown in LB at 37°C, 150rpm for 24h. Then, a turbidity of bacterial cultures was adjusted to McFarland standard 0.5 using as inoculums. A 1% (v/v) of bacterial inoculums was added into the BM, RSM, RHM, and LB medium containing 2% (w/v) of bagasse, and then further incubated at 37, 40, 45, or 50°C and 150 rpm for 72h. Each of bacterial cultures at 12, 24, 36, 48, 60, and 72h. was centrifuged at 8,000 rpm for 10 min to remove cells. Cellulase activity of cell-free supernatant was evaluated using a reducing sugar assay (1) which determined by the 3, 5-dinitrosalicylic acid (DNS) method [6]. To determine the optimal temperature, the enzyme activity was assayed at 50, 60, and 70°C.

$$\text{Enzyme activity (U/mL)} = \frac{[\text{reducing sugar}](1,000)(\text{dilution})}{(180)(10)(0.5)} \quad (1)$$

III. RESULTS AND DISCUSSION

The isolated bacterium was identified by 16S rDNA sequence analysis as *Acinetobacter* sp. (KKU44) (data not shown). The optimal temperature for bacterial growth and cellulase production was 37°C (data not show). The cellulase activity of *Acinetobacter* sp. KKU44 was determined when grown in LB medium at 37°C, 150 rpm for 72h. The bacterial culture of 36 h. showed the maximum of cellulase activity at 83, 93, and 46U/mL when determined at 50, 60, and 70°C, respectively (Fig. 1). The bacterial cellulase activity was tolerant to wide range of high temperatures similar to the previous study [7], [8].

In order to find suitable substrate (carbon source) from agricultural wastes, *Acinetobacter* spp. KKU44 was grown in BM, RSM, and RHM at 37°C, 150rpm for 72h. For BM culture, the bacterial culture of 36 h. showed the highest cellulase activity at 83, 93, and 56U/mL when determined at 50, 60, and 70°C, respectively. For RSM culture, the bacterial culture of 36h. showed the highest cellulase activity at 74, 83, and 56U/mL when determined at 50, 60, and 70°C, respectively. For RHM culture, the bacterial culture of 36h. showed the highest cellulase activity at 74, 83, and 46U/mL when determined at 50, 60, and 70°C, respectively (Fig. 2). The results showed that bagasse is suitable for using as a substrate in cellulase production.

In order to optimize cellulase production, *Acinetobacter* spp. KKU44 was grown in LB medium containing 2% (w/v) bagasse at 37°C, 150rpm for 72h. The bacterial culture of 36h. showed cellulase activity at 102, 120, and 102 U/mL determined at 50, 60, and 70°C, respectively. The result showed that the optimized cultured medium able to enhance cellulase production by 29% (from 93 up to 120 U/mL) (Fig. 3). The information obtained in this study will be useful for application in bioethanol production in the further.

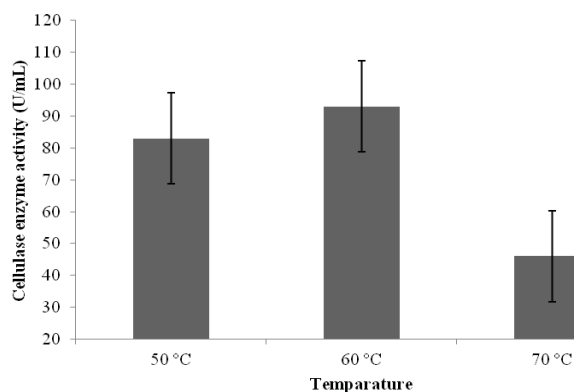


Fig. 1 Bacterial cellulase activity (U/mL) determined at 50, 60 and 70°C when grown in LB medium. Data shown are the mean \pm S.D. of three separated experiments

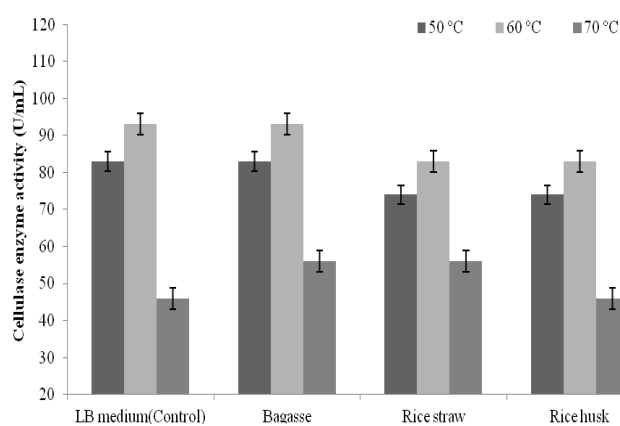


Fig. 2 Bacterial cellulase enzyme activity (U/mL) determined at 50, 60 and 70°C when grown in different carbon source media. Data shown are the mean \pm S.D. of three separated experiments

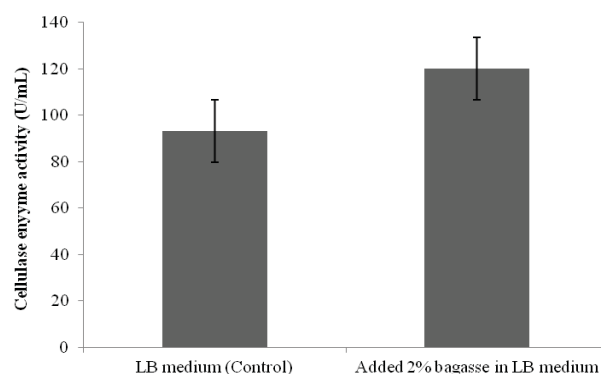


Fig. 3 Bacterial cellulase enzyme activity (U/mL) when grown in LB medium and LB medium containing 2% (w/v) bagasse at 60°C. Data shown are the mean \pm SD of three separated experiments

IV. CONCLUSIONS

The cellulase enzyme producing bacterial strain has been isolated from rice straw and identified by 16S rDNA sequence analysis as *Acinetobacter* spp. KKU44. The KKU44 strain was able to grow and exhibit high cellulase enzyme activity at high temperature. The optimal

temperature for its growth and cellulose production was 37°C. The optimal temperature of cellulase activity was 60°C. The highest cellulase enzyme activity was 120U/mL at 36 h when grown in LB medium containing 2% (w/v) bagasse at 37°C, 150rpm.

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