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Isolation of Cellulolytic Clostridia and their Performance for One-Step Butanol Production from Sugarcane Bagasse

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Abstract

The objective of this study was to screen cellulolytic clostridia from soil, compost, cow dung, buffalo dung and elephant dung in Thailand for one-step butanol production from cellulosic materials. Cellulolytic activity and butanol tolerance were used as criteria to isolate cellulolytic and butanol-tolerant bacteria using mineral salt medium supplemented with 20 g/L Avicel and 5 g/L butanol. It was found that two isolates from each source were obtained according to the isolation criteria. These isolates were gram positive, rod-shaped, endospore forming and sulfite-reducing, which corresponded to the *Clostridia* class. Then, a one-step butanol production from sugarcane bagasse by these isolates was investigated. Butanol production in a 5-L fermenter showed that the isolate E11-KKU from elephant dung exhibited the highest potential and achieved the highest butanol concentration of 3.17 g/L. Phylogenetic analysis of the 16S rDNA sequence showed that the isolate E11-KKU was identified as *Clostridium beijerinckii* with 99.99% identity.

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Keywords: Butanol; Clostridia; Cellulolytic activity; Sugarcane bagasse; 16S rDNA

1. Introduction

The rapid depletion of fossil fuel with continual rise in the consumption has made the search for alternative fuels a high importance. Cellulosic biomass is being considered as enormous renewable resources for biofuels production.

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The global biosynthesis of cellulose by both land plants and marine algae takes place at a rate of 8.5×10^{10} tonnes per year equivalent to more than four times the world's yearly total energy consumption [1, 2]. Recently, most research has focused on efficient processes for conversion of plant-derived biomass to biofuels. Isolation of cellulolytic strains probably is one of the procedures to develop a beneficial biofuel production process.

Butanol is a potential energy and being developed as a fuel for the substitution of fossil fuel due to its high energy content, miscibility with other fuels, octane enhancer, low volatility and other characteristics advantageous to combustion engines. Butanol can be produced from various types of agricultural biomass by solventogenic clostridia such as *Clostridium acetobutylicum* and *C. beijerinckii* in acetone–butanol–ethanol (ABE) fermentation. Clostridia are obligate anaerobic spore-forming solventogenic bacteria. Most cells are gram positive which has variable fermentative metabolism [3]. They are able to ferment several organic compounds. Their end products include butyric acid, acetic acid, butanol, ethanol, acetone, and large amounts of carbon dioxide and hydrogen gas.

Normally, the solventogenic clostridia lack cellulolytic activity. Fermentation of butanol from cellulosic materials usually requires substrate pretreatment resulting in high production cost. One-step butanol production process in which cellulose hydrolysis and butanol production can occur simultaneously has been developed to overcome this problem. The expenses of the fermentation not only reduce, but also time in the production is saved. Therefore, the objectives of this study were to isolate new cellulolyic clostridia and to study the performance of these isolates in order to produce butanol from sugarcane bagasse in one-step.

2. Materials and methods

2.1. Isolation of cellulolytic clostridia

The soil samples were collected from Khon Kaen and Udon Thani provinces, Thailand. The compost samples were collected from Khon Kaen and Nong Kai provinces, Thailand. The cow dung samples were collected from Khon Kaen and Nong Kai provinces, Thailand. The buffalo dung samples were collected from Khon Kaen, Nong Kai and Sakolnakorn provinces, Thailand. The elephant dung samples were collected from Khon Kaen, Nakornratchasima and Surin provinces, Thailand. Isolation of cellulolytic bacteria was conducted in a 100-mL serum bottle containing 30 mL of sterile modified mineral salt (MS) medium. MS medium contained: $MgSO_4$, 0.22 g/L; KH_2PO_4, 0.55 g/L; glacial acetic acid, 2.3 mL/L; $FeSO_4$, 0.011 g/L; Para-amino benzoic acid, 5 mL/L; Biotin, 4 ml/L; Resazurin, 1 ml/L; Avicel PH101, 20 g/L. The pH of MS medium was adjusted to 6.5. Three grams of each sample was inoculated into the MS medium. The medium was sparged with oxygen-free nitrogen (OFN) until achieving anaerobic condition. After inoculation, the serum bottles were heated at 80°C for 10 minutes in order to eliminate non spore-forming bacteria during isolation processes of spore-forming bacteria, such as *Clostridium* species and incubated at 37°C for 7 days under anaerobic condition. Serial dilution agar plating method was used to isolate cellulolytic bacteria. Selective medium was MS media agar containing Avicel as the carbon source. The plates were incubated at 37°C for 7 days. Morphologically different colonies of bacteria were picked and streaked on MS agar plate containing Avicel. The inoculum was repeatedly streaked on MS agar plates for three times until pure colonies were obtained. The purified colonies were stored at 4°C. To isolate butanol-tolerant anaerobic bacteria, 5 g/L of butanol was supplemented into MS medium. Morphological examination was investigated by a microscope using a Gram Stain.

2.2. Genus isolation for Clostridium identification

The cellulolytic bacteria were further subjected to the genus *Clostridium* identification e.g., gram staining, endospore staining using the malachite green technique [4] and sulfite-reducing ability test using differential reinforced clostridial media (DRCM) agar (Difco) [5].

2.3. Species identification

The PCR amplification and 16S rDNA sequences of the isolates which showed maximum butanol production performance from each source were analyzed for species identification by the Thailand Institute of Scientific and