

Two Strains Co-Culture of *Trichoderma Reesei* RT-P1 and *Saccharomyces Cerevisiae* RT-P2 and Its Production of Ethanol from Pineapple Peel Waste

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Abstract— The production of ethanol from pineapple peel waste in submerge-state fermentation by using co-culture starter of TY obtained from *Trichoderma reesei* RT-P1 and *Saccharomyces cerevisiae* RT-P2 has been studied. *T. reesei* RT-P2 and *S. cerevisiae* RT-P2 were cultured on a same PDA Petri dish for 5 days to produced plate starter co-culture, TY-P. The fresh starter co-culture, TY-FS was performed in solid-state fermentation using 200 g cassava waste mixing with 200 mL liquid medium pH 5 and 2×10^{10} cell of TY-P at room temperature 26°C for 5 days. The dry powder starter co-culture, TY-DP was obtained by drying TY-FS at 50°C for 2 hours in hot air oven. The ethanol fermentation was carried out in 250 mL shake flasks with pineapple peel, 100 mL liquid medium pH 5 and TY-DP. The effects of condition factors on co-culture TY-DP concentration were investigated using orthogonal experiments method. The optimal conditions were 60 g/L TY-DP, 4 days, 30 g/L coconut-palm sugar and 80 g/L pineapple peel at room temperature (32°C). Ethanol obtained from these conditions was 42 g/L or 52.5% dry weight of pineapple skin.

Keywords— *Trichoderma reesei*, *Saccharomyces cerevisiae*, co-culture, ethanol

1. INTRODUCTION

Pineapple peel waste is a suitable lignocellulosic material for bio-ethanol production which decrease air pollution and diminish atmospheric carbon dioxide accumulation [1]. A cannery factory produced this solid waste about 100-150 ton/day in Thailand [2] which chemical compositions in pineapple peel are 0.7% protein, 0.5% fat, 2% fiber, 15% carbohydrate and 1% ash [3]. Lignocellulose bioconversion requires the action of multiple enzymes containing cellobiohydrolase, endoglucanase and β -glucosidase [4]. These enzymes are produced from monoculture, co-culture and/or mutants of bacteria and fungi in solid-state [5-8] or submerge-state fermentation [9]. The fermentation procedure of starter co-culture or mixed culture was performed by each of monoculture strain inoculated in a specified production medium and then mixing together in the submerge-state fermentation [10-11]. The method of co-culture preparation between two strains which were TY-DP of *Trichoderma reesei* RT-P1 (T) and *Saccharomyces cerevisiae* RT-P2 (Y) was investigated in the present study. The obtained starter co-culture from cassava

waste solid-state fermentation in specified liquid medium was used to produce ethanol from pineapple peel in submerge-state fermentation. The optimal condition of ethanol production was verified by using orthogonal experiments method. Ethanol fermentation of pineapple peel at the optimal condition with TY-DP co-culture was compared with monoculture of *Trichoderma reesei* RT-P1 (T-DP).

2. METHODOLOGY

2.1 Substrate

Raw pineapple peel was kindly provided by Siam Winery Co.Ltd., Thailand which used sun dried and milled with particle size range 1-5 mm. Dry powder cassava waste with particle size 80-100 mesh was used as a substrate without any pretreatment in solid-state fermentation for cellulase production of TY-DP co-culture strains.

2.2 Microorganism and inoculum

This work used two strains of a fungi *Trichoderma reesei* RT-P1 (T) and a yeast *Saccharomyces cerevisiae* RT-P2 (Y). Both strains were preserved in our laboratory. Stock cultures of *T. reesei* RT-P2 and *S. cerevisiae* RT-P2 were maintained on potato dextrose agar (PDA) and yeast-malt extract agar (YMA) slants, respectively. Initially yeast, Y was streaked on a PDA Petri dish and then transfer fungi, T with inoculating needle tip in the middle of the same PDA dish. The inoculated dish of TY-P was incubated at 30°C for 5 days.

2.3 Liquid medium pH 5 (LM-pH 5)

The liquid medium pH 5 was composed of: 1 g/L CaHPO₄, 1 g/L MgSO₄·7H₂O, 8 g/L urea (46%

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