Results and Discussion

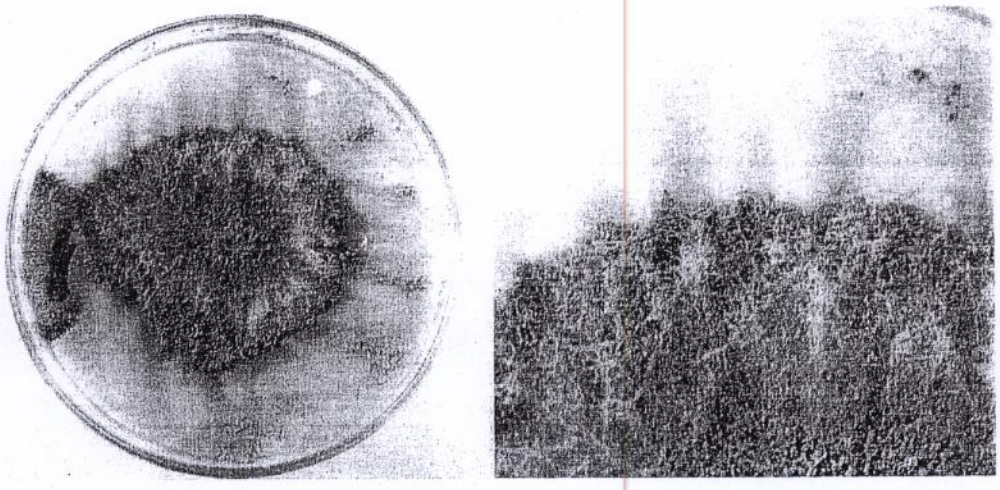

Figure 1 The characteristic of co-culture between fungus, *T. reesei* RT-P1 and yeast, *S. cerevisiae* RT-P2 on PDA after incubated at 37 °C for 5 days

The characteristic of co-culture on PDA after incubated at 37 °C for 5 days showed that white colonies of *S. cerevisiae* RT-P2 were covered by green *T. reesei* RT-P1 mycelium (Figure 1). According to the co-culture on LM mixed with cassava waste showed green hypha grew cover the dish. The result of morphological characteristics under a compound microscope (1000X) of the co-culture on PDA and LM mixed with cassava waste were shown that the fungal hyphae grew with yeast cells (Figure 2). Morphological characteristics under compound microscope of the co-culture were showed in Table 1. It was found that the conidiophore, phialide and spore size of co-culture on PDA (TY) were larger than on LM mixed with dried cassava waste (TY-S).

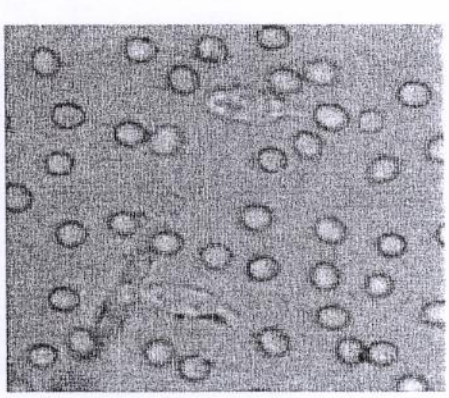

Figure 2 The characteristic of co-culture on PDA under a compound microscope (1000X)

Table 1 Morphological characteristics under compound microscope of the co-culture on PDA (TY) and LM mixed with dried cassava waste (TY-S).

Morphology		co-culture	
		TY	TY-S
Conidiophore (µm)	width	3.59 - 5.97	3.18 - 4.81
	length	25.60 - 30.29	18.39 -23.04
Phialide (µm)	width	3.03 - 3.07	2.05 - 3.07
	length	12.25 -14.59	10.29 -11.52
Spore (µm)	wide	2.88 - 5.23	2.73 - 3.75
	length	4.35 - 5.92	3.58 - 4.61

The scanning electron micrograph of fungus on PDA, *T. reesei* RT-P1 showing hypha (a), septum (b), phialide (c) and spore (d) (Figure 3). Oval cells and bud scar of *S. cerevisiae* RT-P2 on YMA was also showed in Figure 4. In addition, the transmission electron micrographs of *S. cerevisiae* RT-P2 on YMA was clearly different from the co-culture on PDA. The results showed that *S. cerevisiae* RT-P2 was covered by fungal hyphae. (Figure 5A and 5B).

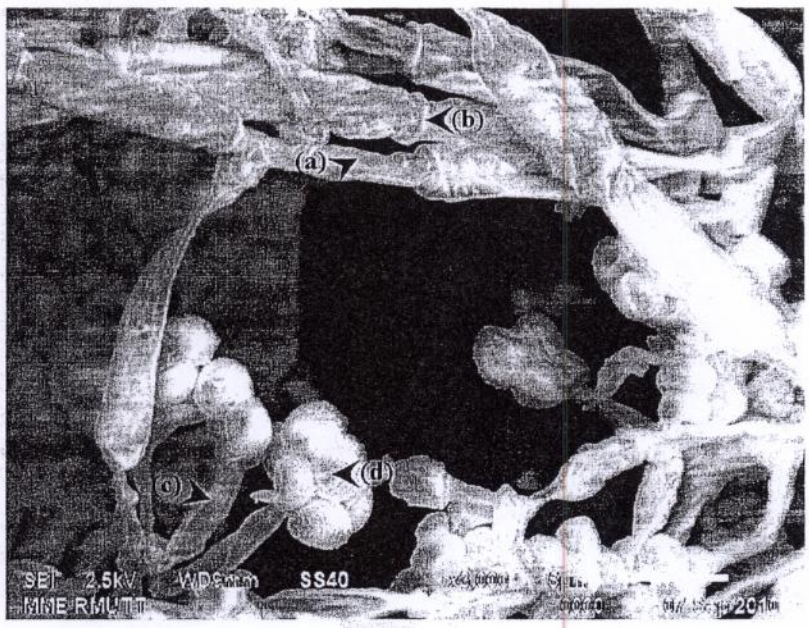

Figure 3 Scanning electron micrograph of *T. reesei* RT-P1 showing hypha (a), septum (b), phialide (c) and spore (d).

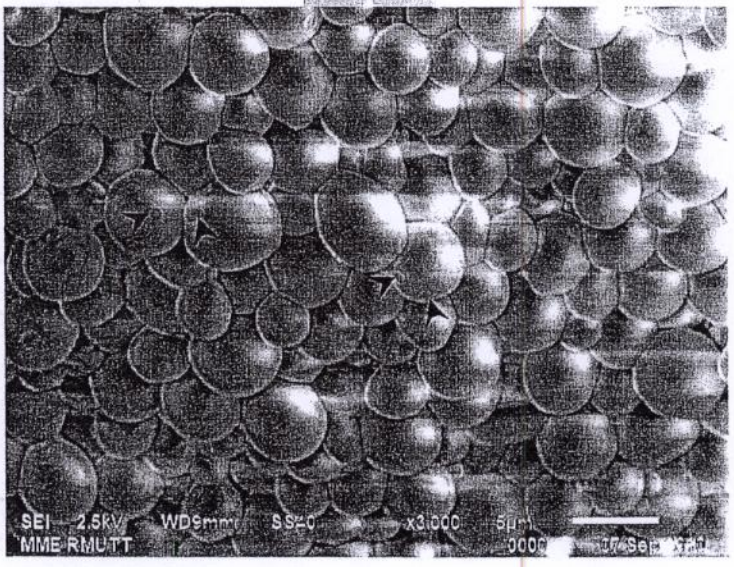

Figure 4 Scanning electron micrograph of yeast *S. cerevisiae* RT-P2 on YMA. Arrows indicated bud scars.

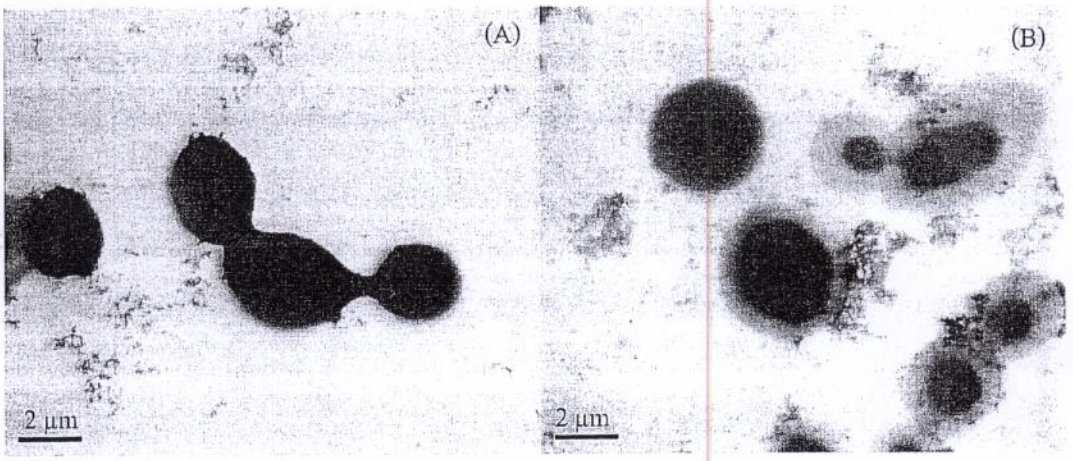

Figure 5 Transmission electron micrographs of *S. cerevisiae* RT-P2 on YMA (A) and the co-culture between *T. reesei* RT-P1 and *S. cerevisiae* RT-P2 on PDA (B) RT-P2. (A) (B) co-culture on PDA.

The changes of the co-culture morphology which can be used as a starter for ethanol production. According to Thalangkan and Nuinu [5] reported ethanol production from pineapple waste using the co-culture between *T. reesei RT*-P1 and *S. cerevisiae* RT-P2 on LM-pH5 medium mixed with cassava waste for starter. They found that the co-culture produced higher ethanol concentration than pure culture of *T. reesei* RT-P1. This result indicated that it can be considered as a starter in process of cellulose degradation. Similarity, Anjani Kumari and Panda [6] described an attempt to produce ethanol from a cellulosic substrate by a single-stage process using intergeneric hybrids obtained from *T. reesei* QM 9414 and *S. cerevisiae* NCIM 3288 fusants. Of the 201 fusants, 170 were found to be relatively stable. Two fusants (M14 and M62) showed the highest synthesis of ethanol from filter paper cellulose, producing 0.0149 and 0.0191 g/L, respectively.

Conclusion

The investigation on morphological characteristic of fungus, *T. reesei* RT-P1, and yeast, *S. cerevisiae* RT-P2. It was found that conidiophore, phialide and spore size of the co-culture on PDA (TY) and LM mixed with dried cassava waste (TY-S) were changed. Further work on molecular genetics study of the co-culture is interesting to develop an efficient species of fungi useful for ethanol production.

Acknowledgements

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Co-culture of *Trichoderma reesei* RT-P1 with *Saccharomyces cerevisiae* RT-P2 : Morphological Studies

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Abstract

This study aimed to investigate the morphology of fungus, *Trichoderma reesei* RT-P1 and yeast, *Saccharomyces cerevisiae* RT-P2 culturing in Potato dextrose agar that use to be the co-culture for ethanol production. It was found that *S. cerevisiae* RT-P2 was covered with the hyphae of *T.reesei* RT-P1. Morphological studies of the co-culture were investigated by compound microscope, scanning electron microscope and transmission electron microscope. The results showed that the sizes of conidiophore, phialide and spore were changed. The present study suggests that the morphology of the co-culture is useful technique to indicate the conversion of cellulose to ethanol production.

Background

Trichoderma reesei and *Saccharomyces cerevisiae* have been widely used in the fermentation technology for ethanol production. In the near future, alternative applications of enzyme from many microorganisms will be used in the production of fuel from biomass [1]. The fermentation procedure for ethanol production have many processes such as biomass preparation, enzyme separation and purification, catabolism of cellulose, saccharification of cellulosic substance, ethanol fermentation process. Each process is long and costly [2]. The cellulosic and hemicellulosic sugars obtained through enzymatic hydrolysis can efficiently be used for ethanol fermentation either using pure culture or using co-culture [3].

The utilization of two microorganism by single-stage direct bioconversion of cellulosic materials to ethanol was attempted by co-culture of *T. reesei* RT-P1 and *S. cerevisiae* RT-P2 to reduce processing time of ethanol production. The objective of this study was to investigate the morphology of co-culture between fungus, *T.reesei* RT-P1 and yeast, *S. cerevisiae* RT-P2.

Materials and Methods

Microorganisms

Two strains of *T. reesei* RT-P1 (T) were identified by http://nt.ars-grin.gov and a yeast *S. cerevisiae* RT-P2 (Y) was identified to species by 26S rRNA gene sequence.

Cultivation medium and culture

Both strains were preserved in our laboratory. Stock cultures of *T. reesei* RT-P1 and *S. cerevisiae* RT-P2 were maintained on Potato Dextrose Agar (PDA) and Yeast malt extract agar (YMA) slants, respectively. The co-culture was carried out initially with *S. cerevisiae* RT-P2 (Y), streaked on PDA and then *T. reesei* RT-P1(T) was transferred using inoculating needle tip in the middle of the same PDA dish. The inoculated dish (TY) was incubated at 30 °C for 5 days. The liquid medium pH 5.0 (LM) was consisted of 1 g/L $CaHPO_4$, 1 g/L $MgSO_4 \cdot 7H_2O$, 8 g/L urea (46% NH_4SO_4), 15 g/L mono potassium phosphate (NPK: 0-52-34), 30 g/L coconut-palm sugar and 1 L distilled water . The LM was mixed with dried cassava waste for preparation of the co-culture starter (TY-S). The inoculated dish of TY-S was incubated at 30°C for 5 days.

Morphological investigation

The conidiophore, phialide and spore size of co-culture on PDA and LM mixed with dried cassava waste were observed and measured with micrometer under compound microscope (Olympus CH30) by wet mount technique [4]. JEOL JSM – 6510 scanning electronmicroscope were used for observations. The samples were placed on aluminum stubs usingcarbon tape coated with gold, and examined under SEM. For TEM investigation, the samples were prepared by ionteching method and examined under TEM (JEOL JEM – 1230).