

## C426122 : MAJOR MICROBIOLOGY

KEYWORD: : Termitomyces sp./Volvariella volvacea/FUSANT-HYBRIDS

BOONLUCK CHERNSIRIDUMRONG : RELATIONSHIP BETWEEN TOTAL DND CONTENT AND COLONY CHARACTERISTIC OF FUSANT-HYBRIDS OF Termitomyces sp.

AND Volvariella volvacea. THESIS ADVISOR : ASSO. PROF. SUMALEE

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Protoplasts were obtained from Termitomyces sp. (T3) mycelium cultivated for 4 days. There were some lytic enzymes used to compared their activities of forming protoplasts. It was found that,  $7 \times 10^4$  cells/ml of protoplast were formed by using 20% Novozyme incubated for 4 hours. When mixing enzyme of 2 mg/ml Cellulase with 20% of Novozyme, the reaction showed increasing number of protoplasts. There were  $2.25 \times 10^5$  cells/ml of protoplast in this reaction at optimal conditions of  $30^\circ\text{C}$ , pH 7.5 using 0.6 M KCl as an osmotic stabilizer. Protoplasts of T3 were regenerated on 3 kinds of regenerating media : type I, II and III, the percentage of regenerated colonies were 0.53, 0.17 and 0.40, respectively. The protoplasts of Volvariella volvacea (V) were prepared by using mycelium cultivated for 4 days and lysed the cell wall by using mixed enzymes of 0.2 mg/ml Zymolase and 2 mg/ml Cellulase. After two strains of protoplast were obtained from T3 and V, the fusion were done in PEG(MW 8000) solution. The percentage of reversed protoplast cell on regenerating medium of type I, II and III were 0.0046, 0.0028 and 0.0033 respectively. Each of the colonies showed differences in their characters that could be arranged into 3 groups. Group I : mycelium was tiny, dense and convex (size 0.5-1 cm), Group II : mycelium was dense and size of colonies were 1-1.5 cm and Group III : mycelium light and fully. These fusant colonies, were detected total DNA and compared with parent strain. From this comparing, the fusant group I and II have more total DNA than two parent strains which show at 95% significant. But the fusant group III showed no significance. Each group was selected only few of them to be a representative for comparing the whole protein pattern separating by using DISC-gel electrophoresis. Most of representative fusant, showed the total protein bands similar to parent strains where as fusant al (represent group I) B8, B12 (represent group II) and C6, C30 (represent group III) showed few of uncommon band patterns.

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