Effect of Culture Conditions on Morphology, Polysaccharide Production and Bioactivity of Tremella mesenterica in Submergence Culture

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ABSTRACT

Tremella mesenterica Retz.: Fr., belongs to the so-called 'Jelly Mushrooms' group, has pharmacological activities, including cytokine-stimulating, anti-diabetic, anti-inflammatory, vascular-stimulating, hypocholesterolemic, anti-allergic and hepatoprotective effects. Submerged fermentation has the potential for higher mycelial production in a compact space, stable product composition under controlled environments and in shorter time with fewer chances for contamination. Culture medium and process is important for the yield of cultivation products, culture metabolic activity and its bioactivity. Therefore, the optimization of medium including both carbon source and nitrogen source might improve the production of various substances of nutraceutical, pharmaceutical and functional foods. Cell morphology is strongly related to culture metabolic activity, the application of image analysis has been suggested as a monitoring tool. Some polysaccharides or polysaccharide–protein complexes from mushrooms are able to stimulate the non-specific immune system, to activate effector cells to secrete cytokines and to exert antitumor activity through the stimulation of the host's defence mechanism. Therefore, the objectives of this study were to investigate the effect of culture conditions on the changes in cell morphology, the polysaccharide production rate, and the bioactivities of Tremella mesenterica. There are three parts included in this study: (1) The effect of different culture conditions on morphological characterization of T. mesenterica and the relationship between polysaccharide production rate and cell morphology bry image analysis; (2) Effects on cytokine-stimulating activities of extracellular polysaccharides (EPS) from T. mesenterica with various carbon sources; and (3) Induction of apoptosis in human lung carcinoma A549 epithelial cells with an ethanol extract of T mesenterica. Results show that (1) the morphological characterization (including cell roundness, elongation, area and budding ratio) changes of T. mesenterica cells depend on the culture conditions such as pH, temperature, nitrogen sources and C/N ratio. There appeared to be no causal relationship between those morphological characterizations and polysaccharides production rate. The morphological form of T. mesenterica cells also change during cultivated in batch fermentation experiments. However, the changes in biomass or EPS production rate of T. mesenterica have been found to correlate with the changes in ratio of elongation or budding cells in the culture. In batch cultures it was therefore possible to control fermentation process by monitoring the changes in ratio of elongation or budding cells in the culture; (2) the predominant sugars in EPS identified in this study were glucose, mannose, xylose, and galactose. The component sugar and uronic acids within the EPS vary with the different carbon sources, a variation which also affects cytokine (interlukin-6 and tumor necrosis factor-α) and nitric oxide production in RAW 264.7 macrophage cells. Xylose and glucose were better carbon sources from the viewpoint of immunomodulatory activity due to the relatively high mannose content in EPS; (3) The EE (ethanol extract) of T. mesenterica, but not the EPS or the IPS (intracellular polysaccharides), almost completely inhibited the growth of A549 cells. The results of Annexin V-FITC/PI staining and flow cytometric analysis indicated that the percentage of Annexin V+/PI- cells in EE-treated cells increased to 32.8%. The results of further investigation showed a disruption of mitochondrial transmembrane potential, the production of reactive oxygen species (ROS), and the activation of caspase-3 protein in EE-treated cells. These findings indicate that EE can decrease cell viability and induce apoptosis in A549 cell lines by activating a mitochondrial pathway. Taken together, these results suggest that intake of EPS or EE from T. mesenterica could contribute to the antitumor effect occur by activating immune responses in the host and/or inducing apoptosis in cancer cell.

Keywords: Tremella species, Tremella mesenterica, fermentation, morphology, image analysis, extracellular polysaccharide, cytokine, apoptosis
2.6.5 碳源（Carbon source）

2.6.6 碳/氮比（C/N ratio）

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2.7.2 脂多醣體

2.7.3 細胞激素

2.7.3.1 腫瘤壞死因子（TNF）

2.7.3.2 介白質6號（IL-6）

2.7.3.3 一氧化氮（NO）

2.8 細胞凋亡

2.8.1 細胞凋亡路徑

2.8.2 粒線體與細胞凋亡

2.8.3 活性氧與細胞凋亡

2.8.4 凋亡蛋白（Caspases）

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