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Purification and Characterization of Methenyltetrahydrofolate Synthetase from Pig Liver

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Methenyltetrahydrofolate synthetase extract was obtained from mouse liver and purified via 30~70% ammonium sulfate fractionation, Fast Q anion exchange and phenyl agarose chromatography. HPLC gel chromatography and SDS-polyacrylamide electrophoresis experiments showed that the enzyme is a monomer with molecular weight of 23 kDa. Optimum temperature and pH were 35°C and 6.5, respectively. The enzyme was chemically modified only by tetranitromethane and 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide (EDC), indicating that tyrosine and carboxylate are in the active site. pH studies showed that 2 tyrosines are involved in the binding of the substrates and a carboxylate in catalysis. Therefore, the chemical mechanism of the enzyme is likely that 2 tyrosines bind to ATP and 5-formylTHF and a carboxylate acts as a general base.

Key words: Methenyltetrahydrofolate synthetase; Folate metabolism; Chemical mechanism

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Optimization of Fermentation Conditions for Production of Pullulan by *Aureobasidium pullulans* HP-2001 with a Design of the Orthogonal Experiment

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Optimal conditions for cell growth and production of pullulan by *Aureobasidium pullulans* HP-2001 were investigated by the orthogonal experiment. Optimal temperatures for cell growth and production of pullulan by *A. pullulans* HP-2001 were 25°C and 30°C, respectively. Optimal conditions for production of pullulan were 100.0 g/L (w/v) glucose as a carbon source, 2.50 g/L (w/v) yeast extract as a nitrogen source, and the initial pH of 6.00, however those for cell growth were 100.0g/L glucose, 2.50 g/L yeast extract, and initial pH 7.00. Optimal concentrations of salts in medium for production of pullulan were 2.50 g/L K_2HPO_4, 0.25 g/L NaCl, 0.05 g/L $MgSO_4 \cdot 7H_2O$, and 0.30 g/L $(NH_4)_2SO_4$, however, those for cell growth were 7.50 g/L K_2HPO_4, 1.00 g/L NaCl, 0.10 g/L $MgSO_4 \cdot 7H_2O$, and 2.40 g/L $(NH_4)_2SO_4$.

Key words: *Aureobasidium pulluloans*, Pullulan production, Orthogonal experiment