P103

## Purification and Characterization of Methenyltetrahydrofolate Synthetase from Pig Liver

## Yong Kweon Cho

Department of Biochemistry and Health Science, College of Natural Sciences, Changwon National University, Sarim-Dong, Changwon, Kyungnam 641-773, South Korea

Methenyltetrahydrofolate synthetase extract was obtained from mouse liver and purified via  $30 \sim 70\%$  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  $35^{\circ}$ 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

P104

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

Wa Gao<sup>1,3</sup>, Yi-Joon Kim<sup>1,3</sup>, Chung-Han Chung<sup>2,3</sup> and Jin-Woo Lee<sup>2,3</sup>

<sup>1</sup>Department of Medical Bioscience, Graduate School of Dong-A University, <sup>2</sup>Department of Biotechnology, and <sup>3</sup>BK21 Bio-Silver Group, Dong-A University, Hadan-2 Dong, Saha Gu, Busan, Korea. 604-714,

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<sub>2</sub>HPO<sub>4</sub>, 0.25 g/L NaCl, 0.05 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, and 0.30 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, however, those for cell growth were 7.50 g/L K<sub>2</sub>HPO<sub>4</sub>, 1.00 g/L NaCl, 0.10 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, and 2.40 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

Key words: Aureobasidium pulluloans, Pullulan production, Orthogonal experiment