

P167

Expression of Carboxymethylcellulase Gene of *Bacillus amyoliquefaciens* DL-3 in *E. coli* and its Production

You-Jung Lee², Bo-Kyung Kim¹, Bo-Hwa Lee¹, Hye-Jin Kim², Gongyuan Wei³,
Chung-Han Chung^{1,3} and Jin-Woo Lee^{1,3*}

¹Division of Applied Biotechnology, ²Department of Medical Bioscience, Graduate School of Dong-A University,
³BK21 Bio-Silver Group, Dong-A University, Busan 604-714, Korea

The microorganism isolated from soil was identified as *Bacillus amyoliquefaciens* by morphological and biochemical analyses and was named *B. amyoliquefaciens* DL-3. It was cultivated in the medium containing 2.0% (w/v) glucose, 0.25% yeast extract, 0.5% K₂HPO₄, 1% NaCl, 0.02% MgSO₄·7H₂O and 0.06% (NH₄)₂SO₄ at 37°C for 72 hr. The gene coding carboxymethylcellulase (CMCase) of *B. amyoliquefaciens* DL-3 was isolated and cloned. The deduced amino acid sequence of the CMCase showed high identity to the cellulase from other *Bacillus* species. *Escherichia coli* JM109 was used as a host strain for cloning and maintenance of plasmid and the transformant were grown at in Luria-Bertani(LB) broth containing 100µg/ml ampicillin. The best carbon source for production of the CMCase by a transformant, the *E. coli* JM109 having gene coding for CMCase of *B. amyoliquefaciens* DL-3, was found to be rice bran and production of the CMCase from 2.0% (w/v) rice bran was 1,950 u/ml.

Key words: *Bacillus amyoliquefaciens*, cloning, sequencing, cellulase

P168

Production of Cellulases by *Bacillus licheniformis* LBH-52 Isolated from the Seashore of the Kyungsang Province in Korea

Hye-Jin Kim², Bo-Hwa Lee¹, Bo-Kyung Kim¹, You-Jung Lee², Gongyuan Wei³,
Chung-Han Chung^{1,3} and Jin-Woo Lee^{1,3*}

¹Division of Applied Biotechnology, ²Department of Medical Bioscience, Graduate School of Dong-A University,
³BK21 Bio-Silver Group, Dong-A University, Busan 604-714, Korea

The best carbon source and nitrogen sources for the production of cellulases by *Bacillus licheniformis* LBH-52 were found to be rice hull and ammonium nitrate. Optimal conditions for production of cellulase such as concentrations of carbon and nitrogen sources, initial pH of medium and temperature were investigated using L₉(3⁴)-orthogonal array method with three distinct levels. The highest production of cellulases with 107.9 u/ml of CMCase, 37.4 u/ml of avicelase and 19.2 u/ml of filter paperase was obtained when concentrations of rice hull and ammonium nitrate were 5.0% (w/v) and 0.05% (w/v), respectively. Optimal initial pH of medium and temperature for production of cellulase by *B. licheniformis* LBH-52 were 7.0 and 35°C.

Key words: *Bacillus licheniformis*, cellulases, orthogonal array