# Degenerate Polymerase Chain Reaction을 통한 [NiFe]-Hydrogenase의 탐색

정희정, 차형준*
포항공과대학교
(hjcha@postech.ac.kr*)

For biohydrogen production, hydrogenase is a key enzyme. In the present work, we performed search of [NiFe]-hydrogenases from hydrogen producing microorganisms using degenerate polymerase chain reaction (PCR) strategy. Degenerate primers were designed from the conserved region of [NiFe]-hydrogenase group I especially on structural genes encoding for catalytic subunit of [NiFe]-hydrogenase from bacteria producing hydrogen. Most of [NiFe]-hydrogenase (group I) are expressed via complex mechanism with aid of auxiliary protein and localized through twin-arginine translocation pathway. [NiFe]-hydrogenase is composed of large and small subunits for catalytic activity. It is known that only small subunit has signal peptide for periplasmic localization and large & small subunits come together before localization. During this process, large subunit is treated by endopeptidase for maturation. Based on these information, we used signal peptide sequence and C-terminal of large subunit by recognized by endopeptidase as templates for degenerate primers. About 2,900 bp of PCR products were successfully amplified using the designed degenerate primers from genomic DNAs of several microorganisms. The amplified PCR products were inserted into T-vector and then sequenced to confirm.