Degenerate Polymerase Chain Reaction을 통한 [NiFe]-Hydrogenase의 탐색

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Search for [NiFe]-Hydrogenase using Degenerate Polymerase Chain Reaction

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Key words :

Abstract : 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 subunitsfor catalytic activity. It is known that only small subunit has signal peptide for periplasmic localization and large & small subunitscome 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

Introducti

Hydrogenases catalyze the reversible oxidation of molecular hydrogen (H2 ↔ 2H+ + 2e-) and play a central role in microbial energy metabolism. According to the composition of hydrogen-activating site, hydrogenases are classified as [NiFe]-hydrogenase, [Fe]-hydrog enase, and metal-free hydrogenase. The [NiFe] -hydrogenases contain nickel as well as iron and are a heterodimeric protein with relatively well conserved large subunits (about 1800 bp) and more diverse small subunits (about 1100 bp) containing one or more 4Fe4S clusters. It has been shown that hydrogen activation is mediated by the nickel center and the iron-sulfur clusters are probably involved in electron transfer between active site, molecular surface and redox partners of enzyme such as ferredoxins. However, a problem related to the utilization of [NiFe]-hydrogenases is that they are usually unstable and very sensitive to oxygen.

In this study, the degenerate primers were designed on the basis of several [NiFe]-hydrog enase sequences in the database. Using the constructed degenerate primers, we performed polymerase chain reaction to obtain the [NiFe] -hydrogenase gene from chromosome of *E. coli* and isolated marine bacterium as model

hydrogen-producing microorganisms.

Materials and methods

Bacterial strain, *Escherichia coli* and isolated marine bacterium were used in this study. Degenerate primers were designed from the conserved region of the [NiFe]-hydrogenases from 30 different organismsin the database and reports. The conserved region was chosen on the basis of the N-terminus of small subunits and the C-terminus of the large subunit of the [NiFe]-hydrogenases. The PCR amplification was performed using chromosomes of *E. coli* and isolated marine bacterium as templates. Reactions of PCR amplication were performed using denaturation for 5 min at 94 °C, then 40 cycles denaturation for 80 sec at 94°C, annealing for 30 sec at 55°C, and extension for 3 min at 72°C, followed by 5 min of extension at 72°C. PCR products were separated by 0.7% agarose gel with ethidium bromide, and size of PCR products were determined from 1 kb DNA size marker (Bioneer). PCR product obtained from chromosome of *E. coli* was ligated into pGEM-T Easy Vector (Promega), and then the size of ligation sample was confirmed by *Eco*RI digestion. The T-vector ligated with PCR product was sequenced with T7 primer and Sp6 primer (Genotech).

Result

The PCR amplification was performed with the designed degenerate primers. The PCR products amplified from chromosome of *E. coli* and isolated marine bacterium were checked in 0.7% agarose gel at about 2900 bp. After PCR amplification, PCR products were sequenced. Sequencing result of hydrogenase extracted from chromosome *E. coli* was accorded with the hydrogenase sequence of *E. coli* in database. As a consequence of this study, we ascertained possibility of these designed degenerate primers. In the future, more hydrogenase genes will be searched with the designed degenerate primers from lots of hydrogen-producing microorganism

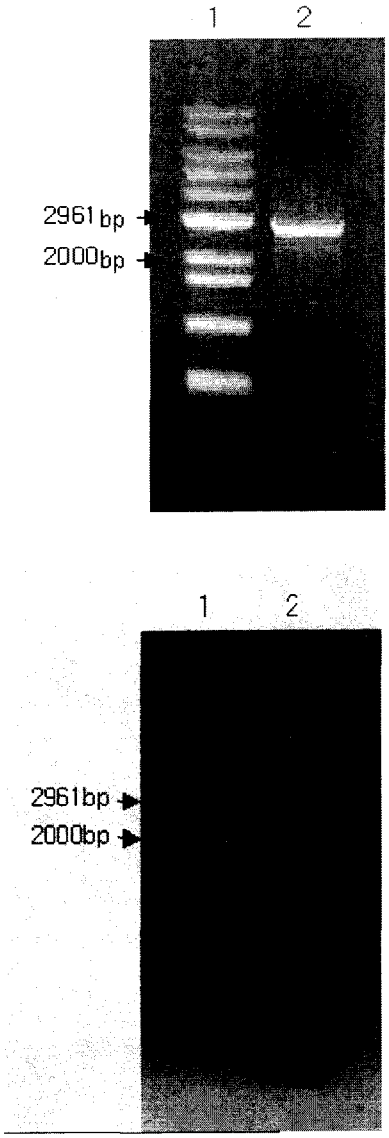

Figure 1. Amplification of a 2900 bp of PCR product with the designed degenerate primers using chromosome of (A) *E. coli* and (B) isolated marine bacterium as templates. Lane1: 1 kb size marker, lane2: PCR product.

References

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