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Overexpression of NADH oxidase gene from *Deinococcus geothermalis* in *Escherichia coli*

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Abstract

When using stable enzyme genes from a thermophile to create a biosensor in *Escherichia coli*, it is vital that these genes be overexpressed in order to provide a sufficient supply of enzymes. In this study, overexpression of the NADH oxidase (Nox) gene from the thermophile *Deinococcus geothermalis* was successfully achieved with the aim of creating a stable biosensor active at room temperatures. To do so, modification of 10 nucleotides, GAAATTAACT, upstream of the start codon of the Nox gene was necessary.

Key words: biosensor; stable enzyme; *Deinococcus geothermalis*; overexpression

Introduction

Biosensors are widely used to measure concentrations of various substances. Creation of such biosensors requires a supply of enzymes, and in *Escherichia coli* this has been achieved through overproduction of a stable enzyme from a thermophile. We previously documented overexpression of the NADH oxidase (Nox) gene from an extreme thermophile, *Thermus thermophilus* HB8 (Suzuki et al., 2009). The obtained Nox was very stable and active at 80°C, but at room temperature it showed very low activity. We therefore attempted to use the Nox from another thermophile, *Deinococcus geothermalis*, which lives at temperatures of 40°C–50°C and was expected to have enzymes both stable and active at room temperature. Overexpression of the Nox gene in *E. coli* was again successfully achieved through modification of 10 nucleotides upstream of the start codon of the Nox gene (Suzuki et al., 2009).

1 Materials and methods

Genomic DNA of *D. geothermalis* was extracted using the Gentra Puregene Yeast/Bact. Kit (QIAGEM, Netherlands). The pKK223-3 plasmid was used for cloning and as an expression vector. The Nox gene of *D. geothermalis* was amplified and modified by polymerase chain reaction using primers purchased from Sigma-Aldrich, Japan. Cloning of the Nox gene was carried out as previously reported (Suzuki et al., 2009). *E. coli* JM109 harboring the appropriate plasmids were grown as reported previously (Kreutzer et al., 1992). The Nox gene modifications in the constructed expression vectors are shown in **Fig. 1**. Enzyme purification with His-Tag technology (Hengen, 1995) was performed using the HisLink TM Spin Protein Purification System (Promega, USA). Enzyme assay was carried out as previously reported (Suzuki et al., 2009).

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2 Results and discussion

The Nox gene from the *D. geothermalis* genome was cloned and overexpressed in the expression vector pKK223-3 as pDNoxP, with modification of 10 nucleotides upstream of the start codon (GAAATTAACT) as previously reported (Suzuki et al., 2009). *E. coli* JM109 was transformed by pDNoxP then cultured in 30 mL LB medium with 10 μmol/L IPTG (isopropyl-β-D-thiogalactopyranoside), the inducer of expression vector pKK223-3. The produced Nox was to be purified as a soluble protein after heat treatment of *E. coli* extracts at 70°C for 1 hr then centrifugation in order to remove any thermolabile proteins; however, Nox activity could not be detected after heat treatment, as a result of denaturation (data not shown). Heat treatment at various temperatures was examined, but proteins from *E. coli* could not be removed at temperatures below 65°C. Therefore, in order to purify Nox using His-Tag technology, the Nox gene was modified through the addition of 6 His codons at the 5′ and/or 3′ terminus of the gene, respectively, then both were employed as expression vectors (pDNoxP5′His,

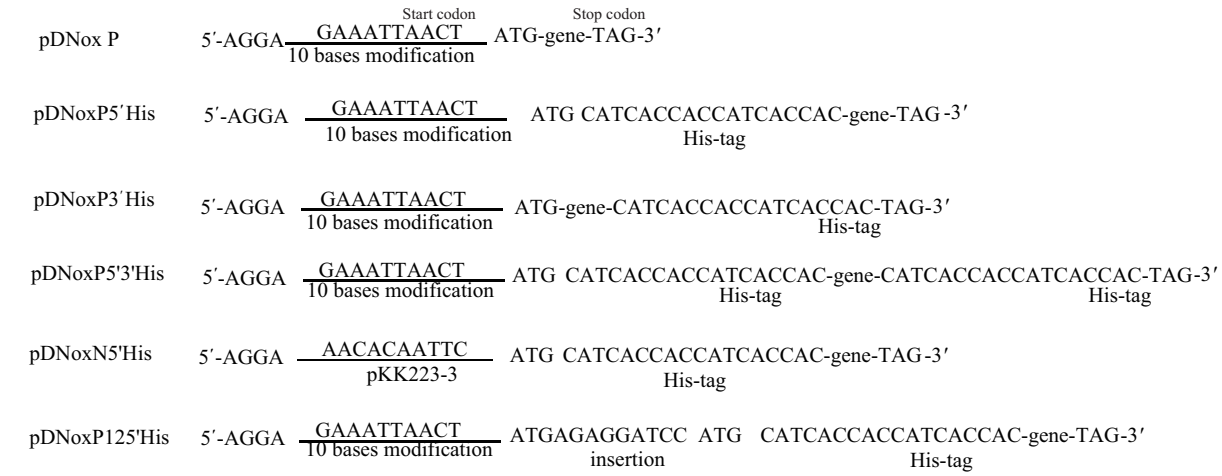

Fig. 1 Modification of sequence of Nox gene in variants of the expression vectors.

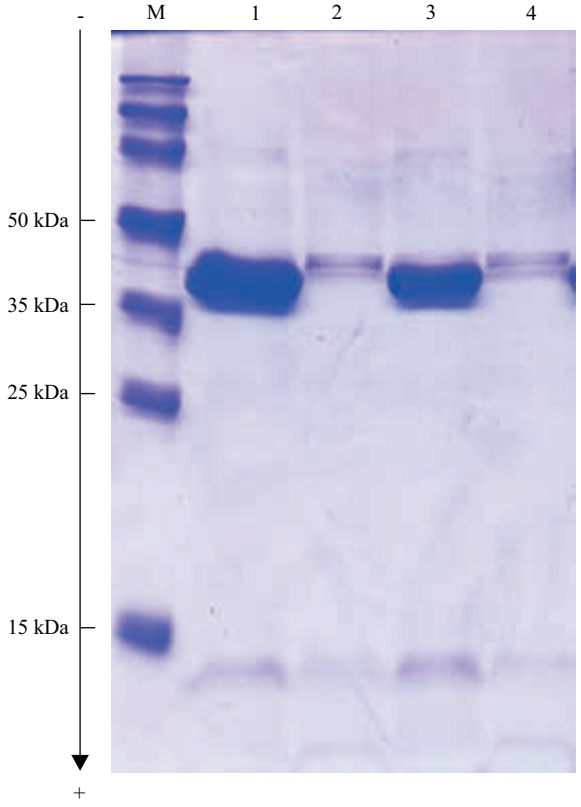

Fig. 2 SDS-PAGE of purified Nox obtained from the variants using His-Tag technology. Lane M: size marker, lane 1: pDNoxP5′His, lane 2: pDNoxP3′His, lane 3: pDNoxP5′3′His, lane 4: pDNoxP.

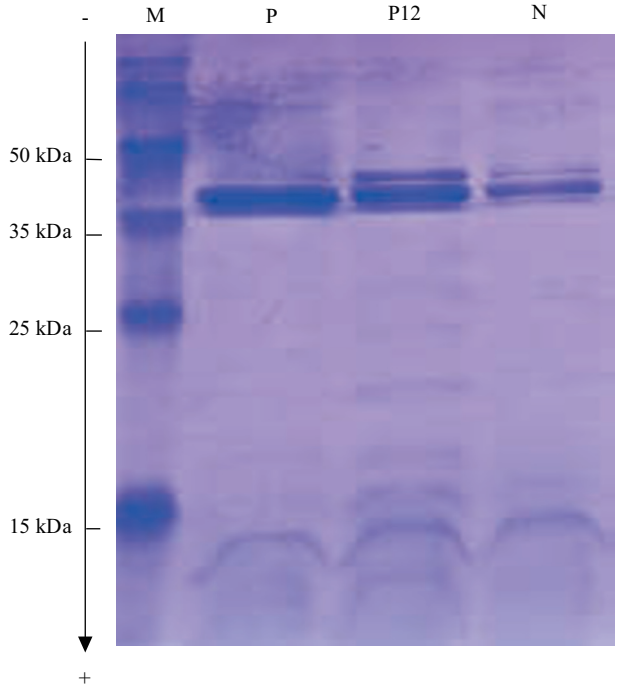

Fig. 3 SDS-PAGE of purified Nox obtained from the variants by His-Tag technology. Lane M: size marker, lane P: pDNoxP5′His, lane P12: pDNoxP125′His, lane N: pDNoxN5′His.

pDNoxP3′His and pDNoxP5′3′His, respectively). *E. coli* JM109 was then transformed using these expression vectors and cultured. The resulting extracts were purified using the HisLink TM Spin Protein Purification System, and the purified proteins analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as shown in **Fig. 2**. Nox protein were detected as a band at about 40 kDa in both pDNoxP5′His and pDNoxP5′3′His, respectively. This result shows that introduction of a His-Tag to the N-terminus of the Nox protein was effective in purifying Nox in extracts of *E. coli*. On the other hand, His-tag introduced to the C-terminus was not effective.

Next, in order to confirm that modification of 10 nucleotides (GAAATTAACT) was important for overexpression in *E. coli*, the Nox gene in DNoxP5′His was modified around the start codon. Twelve nucleotides, ATGAGAGGATCC, were inserted into pDNoxP125′His upstream of the start codon, and pDNoxN5′His was modi-

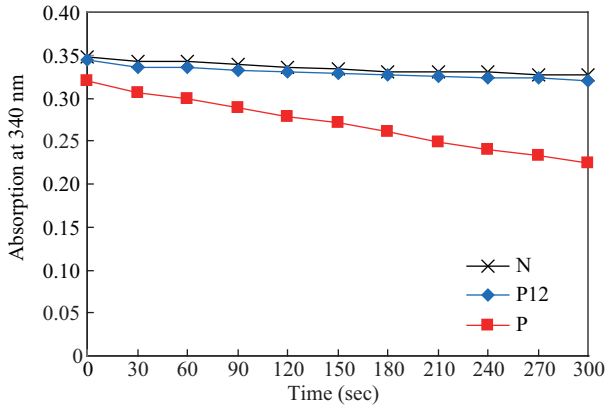

Fig. 4 Activity of purified Nox obtained from the variants by His-Tag technology. P: pDNoxP5′His, P12: pDNoxP125′His, N: pDNoxN5′His.

fied to the original sequence of pKK223-3, according to a previous report (Suzuki et al., 2009; Park et al., 1994). The level of expression was highest in *E. coli* transformed with pDNoxP5′His (40 kDa) compared with pDNoxP125′His and pDNoxN5′His. This result is similar to expression of the Nox gene from *T. thermophilus* HB8 in *E. coli*, thus confirming that the 10-nucleotide sequence, GAAATTAACT, upstream of the start codon is very important for overexpression of this gene.

Activity of the purified Nox obtained from each expression vector by His-Tag technology was measured by the decrease in absorption at 340 nm, λ_{max}, of NADH (**Fig. 4**). Activity of purified Nox from pDNoxP5'His was detected, confirming that the Nox of *D. geothermalis* was successfully expressed in *E. coli* transformed by pDNoxP5'His.

3 Conclusions

The Nox gene from *D. geothermalis* was successfully expressed in *E. coli* by modification of the same 10 nucleotides previously modified in the Nox gene from *T. thermophilus* HB8. The resulting Nox was easily purified from extracts of *E. coli* using His-Tag on the N-terminus.

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