

S37-011

Cloning and sequencing of the genes encoding the subunits of bidirectional hydrogenase of *Anabaena variabilis* IAM M58

K Nakamura¹, H Masukawa¹, M Mochimaru², S Iwata¹, H Sakurai¹

¹*Department of Biology, School of Education and Division of Pure and Applied Physics, Graduate School of Science and Engineering, Waseda University, Nishiwaseda, Shinjuku, Tokyo 169-8050, Japan. Fax: +81-3-3207-9694. sakurai@mn.waseda.ac.jp*

²*Natural Science Faculty, Faculty of Letters, Komazawa University, 1-23-1 Komazawa, Shinjuku, Tokyo 154-8525, Japan. Fax: +81-3-3418-9324. mari@komazawa-u.ac.jp*

Keyword: hydrogenase, nitrogenase, hydrogen production, cyanobacteria

Introduction

Photobiological H₂ production by cyanobacteria is expected to become an environmentally acceptable future alternative to fossil fuel resources, because heterocystous N₂-fixing cyanobacteria have the unique ability of sustained H₂ production simultaneously with photosynthetic O₂ evolution without anaerobic treatments of the cells. In addition, they are able to produce H₂ using sunlight and water with the simplest nutritional requirements.

Previously, we reported the rates of H₂ production of 12 heterocystous N₂-fixing cyanobacterial strains and *Anabaena variabilis* IAM M58 being most active in H₂ production. In this study, we have sequenced *hoxFUY* genes, a partial *hoxH* gene and two unidentified ORFs in the genome of *A. variabilis* IAM M58 by a PCR-based strategy. The sequences of the *hox* genes showed high homologies with those of other cyanobacteria.

Materials and methods

Anabaena variabilis IAM M58 was obtained from IAM culture collection, University of Tokyo. The cyanobacteria were grown in BG11 medium with continuously stirring and bubbled with air at 26 °C, under continuous illumination with white fluorescent light at 80 μE m⁻² s⁻¹ PAR. *Escherichia coli* strain JM109 was grown in LB medium at 37°C. The concentrations of antibiotics were 100 μg/ml for ampicillin.

Genomic DNA was extracted from the cyanobacterial cells by the glass beads method. PCR amplification was carried out using a PCR Thermal Cycler PERSONAL2 (Takara) and *Ex-Taq* polymerase (Takara). Oligonucleotides used as PCR primers were designed on the basis of highly conserved sequences identified by aligning the *hox* DNA sequences from *A. nidulans* PCC 6301 (Boison et al. 1996, 1998), *Synechocystis* PCC 6803 (Kaneko et al. 1996), *A. variabilis* ATCC 29413 (Schmitz et al. 1995) and *Anabaena* PCC 7120 (Table 1). The *Anabaena* PCC 7120 *hox* genes have been identified in ongoing genome project of *Anabaena* PCC 7120 (<http://www.kazusa.or.jp/cyano/anabaena/>). The amplified PCR products were purified by using Quantum Prep Freeze 'N Squeeze DNA Extraction Spin Columns (Bio-Rad) after electrophoresis on 1% agarose gels and then cloned into pGEM-T

Easy Vector (Promega). Nucleotide sequencing was performed by the dideoxy-chain termination method using SequiTherm EXCEL 11 DNA Sequencing Kit-LC and fluorescent dye-labeled M13 primer (Epicentre Technologies). The DNA sequences of the hydrogenase *hoxF*, *hoxU* and *hoxY* genes and ORFs have been deposited to the DDBJ/EMBL/GenBank databases (accession no. AB057405).

Results and Discussion

Nine PCR fragments generated by using primer pairs shown in Table 1 turned out to be parts of *hox* genes. By arranging these fragments, complete sequences of *hoxF*, *hoxU*, *hoxY* gene and two ORFs and partial sequence of *hoxH* gene were determined. The organization of *hox* genes of *A. variabilis* IAM M58 shown in Figure 1 is the same as those of *Anabaena* PCC 7120, *A. variabilis* ATCC 29413, *Synechocystis* PCC 6803 and *A. nidulans* PCC 6301 except that the genes are interrupted by different ORFs at different positions. An ORF (ORF8) located between *hoxU* and *hoxY* is also identified in the same position as in *A. variabilis* ATCC 29413 and *Anabaena* PCC 7120, but an unidentified ORF between *hoxF* and *hoxU*, that gave no significant homologies in the available databases, is missing in any of the other strains. In *Anabaena* PCC 7120, an additional ORF (*hoxE*) is located upstream of *hoxF*, but we could not identify it by PCR amplifications with several primers that were based on *hoxE* sequence of *Anabaena* PCC 7120.

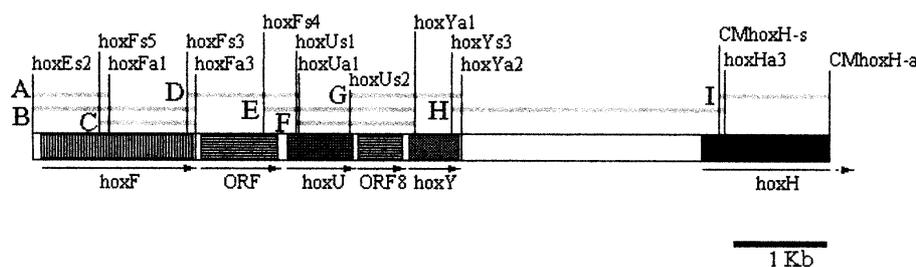


Fig. 1. Determination of *hox* genes of *Anabaena variabilis* IAM M58 by PCR. Positions of primers used for PCR amplification and the amplified fragments A-I are indicated above the gene cluster. Oligonucleotides used in the PCRs are shown in Table 1.

Table 1. Oligonucleotide primer pairs used in the PCRs.

PCR product Name	Sense primer Sequence 5'→3'	Antisense primer Name	Sequence 5'→3'	Fragment size (bp)
A	hoxEs2 GGTGCTTGTGGGATTGCTCC	hoxFa1	GCATAGGCAGCGATCGCCATT	804
B	hoxEs2 GGTGCTTGTGGGATTGCTCC	hoxFa3	TTGAGGTCAGGAATAGCGCAT	1729
C	hoxFs5 GTCACTCTGTAACGCCGATGAA	hoxFa3	TTGAGGTCAGGAATAGCGCAT	1032
D	hoxFs3 GTCTGCGCCTAATCCGGTATTT	hoxUa1	TCTAAATGGCACAGTGTCGGAAT	1180
E	hoxFs4 CAACACATCAGTGATGGTAGTT	hoxYa1	AAGGACATATGACAGCCAGAACA	1607
F	hoxUs1 ATTCCGACACTGTGCCATTTAGA	hoxYa1	AAGGACATATGACAGCCAGAACA	1259
G	hoxUs2 ATCGCGCAAACCTCGATTTTTAG	hoxYa2	TTAGCCAAATTTGATAAATCCCG	1178
H	hoxYs3 TCCGCCTAATGCAACTCAGAT	hoxHa3	AGGCTGGCGATCGCATTCTT	3.0 kb*
I	CMhoxH-s ATTTGTGGTATTTGTCCGGTGAGTC	CMhoxH-a	GAACAACCTAACAGGGGTCAAAGC	1187

*, fragment size of the PCR product I was calculated from electrophoresis.

The deduced amino acid sequences of the *hoxF*, *hoxU*, *hoxY* and *hoxH* genes showed 81, 89, 79 and 86% identities with the corresponding homologs of *A. variabilis* ATCC 29413, respectively. The products of the *hoxF*, *hoxU* and *hoxY* genes all share the characteristics that are conserved in the homologous gene products from other cyanobacteria, in particular, putative FeS-cluster binding motifs and NAD-, FMN-, Ni-binding regions (Fig. 3).

The bidirectional hydrogenase is suggested to be a heterotetrameric enzyme consisting of a hydrogenase part (HoxYH) and a diaphorase part (HoxFU). The diaphorase part is proposed to have dual roles in both the bidirectional hydrogenase and respiratory complex I because of significant sequence similarities between the NADH dehydrogenase part of complex I from *E. coli* and the diaphorase part of the NAD⁺-reducing hydrogenase. However, *Nostoc* PCC 73102, which lacks not only *hoxYH* but also *hoxEFU*, respire at rates similar to those of other cyanobacteria and the respiration was unaffected by knocking out the diaphorase genes in *Synechocystis* PCC 6803 and *A. nidulans*. Though the physiological role of the bidirectional hydrogenase is still controversial, it has been so far assigned roles in H₂ uptake as a means of collecting additional reducing power during growth in anaerobic environments due to its capability of either consumption or production of hydrogen and to its low K_m for H₂. If so, disruption of *hox* genes may improve H₂ production.

References

- Boison G, Bothe H, Hansel A, Lindblad P (1999) *FEMS Microbiology Letters* **174**, 159-165.
- Boison G, Schmitz O, Mikheeva L, Shestakov S, Bothe H (1996) *FEBS Letters* **394**, 153-158.
- Boison G, Schmitz O, Schmitz B, Bothe H (1998) *Current Microbiology* **36**, 253-258.
- Hansel A, Lindblad P (1998) *Applied Microbiology and Biotechnology* **50**, 153-160.
- Houchins JP (1984) *Biochimica et Biophysica Acta* **768**, 227-255.
- Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y et al. (1996) *DNA Research* **3**, 109-136.
- Howitt CA, Vermaas WFJ (1999) p. 595-601. In: Peschek GA, Löffelhardt, Schmetterer G (ed.), *The Phototrophic Prokaryotes*. Kluwer Academic/Plenum Publishers, New York.
- Masukawa H, Nakamura K, Mochimaru M, Sakurai H (2001) p. 61-64. In Miyake J, Matunaga T, San Pietro A (ed.), *Biohydrogen II*. Elsevier Publishers.
- Schmitz O, Boison G, Hilscher R, Hundeshagen B, Zimmer W, Lottspeich F, Bothe H (1995) *European Journal of Biochemistry* **233**, 266-276.
- Tamagnini P, Troshina O, Oxelfelt F, Salema R, Lindblad P (1997) *Applied Environmental Microbiology* **63**, 1801-1807.

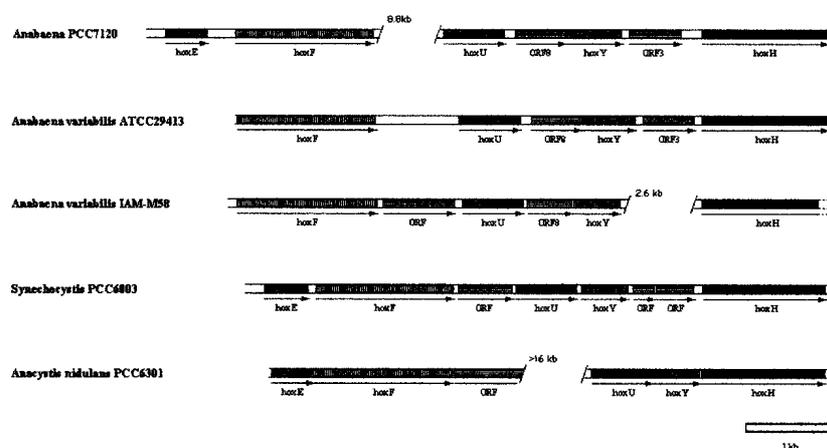


Fig. 2. Organization in the bidirectional hydrogenase genes from different cyanobacteria.

