Short Communication

A NEW SET OF PCR PRIMERS FOR SPECIFIC DETECTION OF THE GENE ENCODING THE PRINCIPAL SIGMA FACTOR IN CYANOBACTERIA

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(Received September 11, 1996; Accepted October 14, 1996)

In eubacteria, principal sigma factors (*rpoD* or *sigA* gene product) are responsible for the promoter recognition of housekeeping genes, and exhibit similarity to amino-acid sequences in 1, 2, 3, and 4 regions among them (4) (Fig. 1a). Especially, regions 2 and 4 are extensively conserved, and these regions have been further divided into subregions 2.1–2.4 and 4.1–4.2, respectively. A Southern blot analysis technique using “*rpoD* probe,” of which the sequence corresponds to consensus amino-acid residues (*rpoD* box: KFSTYATWWIRQA, Fig. 1a) that located in subregions 2.3–2.4 in the principal sigma factor, has been a powerful method for identifying and cloning the *rpoD* homologs (8). A cyanobacterium, which can perform photosynthesis, possesses multiple *rpoD* homologs encoding a principal sigma factor (1, 2, 7) and several alternative types. In this study, we developed a novel method of PCR for identifying the principal *rpoD* genes of cyanobacteria using a set of primers which can be used to amplify the nucleotide sequence corresponding to regions 2.1–4.2 in the principal sigma factors.

The nucleotide sequences of primers F2.1 and R4.2 used are shown in Fig. 1b. Each nucleotide sequence corresponds to the consensus amino-acid sequences IAKKYMN at subregion 2.1 and RERIRQIE at subregion 4.2, in the principal sigma factors of cyanobacteria *Microcystis aeruginosa* (*Synechocystis*) K-81 (1), *Synechococcus* sp. strain PCC 7942 (7), and *Anabaena* sp. strain PCC 7120 (2). PCR was carried out on a total volume of 10 μ l comprising the following contents:

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Fig. 1. PCR strategy.

(a) A principal sigma factor (RpoD1) of *Microcystis aeruginosa* K-81, and primers used in this study. The scale for the length of RpoD1 is shown at the top. The positions of consensus regions and the "rpoD box" are indicated by filled areas and a hatched small box, respectively. (b) The oligonucleotide sequences corresponding to amino-acid residues and a restriction enzyme. (c) The PCR products amplified from total DNAs of cyanobacteria: 7120, *Anabaena* sp. strain PCC 7120; 7942, *Synechococcus* sp. strain PCC 7942; 6803, *Synechocystis* sp. strain PCC 6803; K-81, *M. aeruginosa* K-81. The position of each PCR product (about 600-bp) is shown by an arrow. The positions of molecular size markers are also indicated as base pairs (bp) on the left.

0.1 μ g of isolated total DNA from cyanobacteria (5), 20 pmol of each primer, 0.2 mM dNTPs, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, and 1.4 U *Taq* DNA polymerase (Nippon Gene, Toyama, Japan). The reaction mixture was overlaid with a drop of mineral oil. For DNA amplification, a SYSTEM PC-700 controller (ASTEC, Tokyo, Japan) was run for 30 cycles after preincubation at 95°C for 2 min, with denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 65°C for 3 min. Then, 1 μ l from each reaction mixture was resolved by electrophoresis on a 2% agarose gel. The residual DNA fragments amplified in PCR were directly subcloned into pMOSBlue T-vector

(Amersham, Buckinghamshire, U.K.), and these inserts on the recombinant plasmid were sequenced by a method described previously (6).

When we performed PCR, about 600-bp of product was amplified from the total DNA of *M. aeruginosa* K-81, *Synechococcus* sp. strain PCC 7942, *Anabaena* sp. strain PCC 7120, and *Synechocystis* sp. strain PCC 6803, respectively (Fig. 1c). The size was in good agreement with the calculated value (Fig. 1a). The nucleotide sequences of PCR products, 12 samples each from *Synechococcus* sp. strain PCC 7942 and *Anabaena* sp. strain PCC 7120, completely matched those of the respective principal sigma factor genes, *rpoD1* and *sigA* (2, 7) (data not shown, see Fig. 2). Furthermore, the nucleotide sequences of all 24 clones from *M. aeruginosa* K-81 were also identical to those of the principal *rpoD1* gene (1). However, it was not possible to obtain any PCR product other than type *rpoD* genes encoding the alternative sigma factors from these cyanobacteria. These results indicate that the nucleotide sequence corresponding to regions 2.1–4.2 in these principal sigma factors were selectively amplified from unicellular and heterocystous cyanobacterial DNAs. Another unicellular cyanobacterium, *Synechocystis* sp. strain PCC 6803, also possesses multiple *rpoD* homologs (3, 7); however, a *rpoD* gene encoding the principal sigma factor has not been clarified. We therefore believe that the principal *rpoD* gene can be identified by this PCR approach. Results indicated that the nucleotide sequences of all six PCR products amplified from the PCC 6803 total DNA were identical to each other, and the sequence exhibited an extensive similarity to those of the principal *rpoD* genes found in other cyanobacteria. These

RpoD Family	F2.1 →	<i>rpoD</i> box	← R4.2
E. co-RpoD*	IAKKYTN	KFSTYATWWIRQA	RERIRQIE
B. su-SigA*	IAKRYVG	KFSTYATWWIRQA	RERIRQIE
K-81-RpoD1*	IAKKYMN	KFSTYATWWIRQA	RERIRQIE
7942-RpoD1*	IAKKYMN	KFSTYATWWIRQA	RERIRQIE
6803-RpoDI*	IAKKYMN	KFSTYATWWIRQA	RERIRQIE
7120-SigA*	IAKKYMN	KFSTYATWWIRQA	RERIRQIE
K-81-RpoD2	IAKRYLN	KFSTYAYWWIRQA	RERVRIQI
7942-RpoD2	IAKKYQK	KFSTYAYWWIRQA	RERVRLKE
6803-S110306	IAKKYQK	KFSTYAYWWIRQA	RERVRLKE
6803-S110184	VAKKYQN	RSFTYSYWWIRQG	RERVRIQIE
7120-SigB	IAKKYQK	KFSTYAYWWIRQG	RERVRLQVE
7120-SigC	VAKKYQN	RFSTYAYWWIRQG	RERVRIQIE

Fig. 2. RpoD family and sequence alignment.

The consensus amino-acid sequences in regions 2.1, 4.2 and the *rpoD* box of RpoD proteins from *Escherichia coli* (E. co), *Bacillus subtilis* (B. su), and cyanobacteria (K-81, 6803, 7120, 7942) are shown as one-letter codes. These sequences were translated from the GenBank, DDBJ and CyanoBase (URL Address: <http://www.kazusa.or.jp/cyano>) entries given in parentheses as follows: E. co-RpoD (J01687), B. su-SigA (X03897); K-81-RpoD1 (D50318), -RpoD2 (D86575); 7942-RpoD1 (D21136), -RpoD2 (D78583); 7120-SigA (M60046), -SigB (M95760), -SigC (M95759); 6803-RpoDI (D86213), -S110306, -S110184. The identical amino-acid sequences are indicated by shadows. The principal sigma factors (6803-RpoDI is tentative) are represented by asterisks.

results show that we were also able to detect a part of the principal *rpoD* homolog (*rpoDI*) from *Synechocystis* sp. strain PCC 6803. Furthermore, we isolated the entire *rpoDI* gene-containing *Xba*I fragment from PCC 6803 genomic DNA, based upon hybridization using the PCR product as a probe, and sequenced it. The nucleotide sequence of *rpoDI* was identical to that of the PCC 6803 PCR product (data not shown).

We summarized these results and performed an amino-acid sequence alignment, which involves the *rpoD* box, for the RpoD family (Fig. 2). The amino-acid sequences corresponding to primers F2.1 and R4.2 were perfectly conserved in the principal sigma factor (or homolog in PCC 6803) among cyanobacteria, whereas not in the other six cyanobacterial RpoD proteins. When we carried out PCR with these primers, no PCR products could be amplified from *Escherichia coli* or *Bacillus subtilis* total DNAs as templates (data not shown), suggesting that the gene encoding the principal sigma factors of these bacteria could not be detected under the PCR conditions used in this study. The specificity of amplification in PCR depends on the design of the primer sequences. The selective amplifications of a principal *rpoD* gene under the PCR conditions could be caused by a primer-template combination for annealing. Especially, the ATG sequence for methionine codon in the F2.1 primer might be effective for the specific selection of the principal *rpoD* gene from cyanobacteria, because the substitution of a nucleotide sequence corresponding to the amino-acid residue M (methionine) to T (threonine) in *E. coli* RpoD (sigma 70, Fig. 2) resulted in no amplification of the principal sigma factor gene from this bacteria. However, use of the F2.1 and R4.2 primers is a powerful method for obtaining the PCR product corresponding to regions 2–4, which contain the complete *rpoD* box sequence, in the principal sigma factors from cyanobacteria. Considering this fact, it is also possible to characterize the *rpoD* box sequences between the principal *rpoD* gene and other alternate types in cyanobacteria. To date, the *rpoD* box sequence KFSTYATWWIRQA has been perfectly conserved in the principal sigma factors among not only cyanobacteria but also eubacteria (4). Interestingly, other alternative RpoD homologs completely possess a substituted Y (tyrosine) residue instead of T (threonine) in the *rpoD* box of cyanobacteria. This finding should be attributable to particular properties of the cyanobacterial RpoD family.

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