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ALİ OSMAN BELDÜZ

ZİHNİ DEMİRBAĞ

SABRİYE DÜLGER

ALİ OSMAN KILIÇ

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Determination of the Presence of *crp* genes in *Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus delbrueckii* and *Corynebacterium verasus*

Ali Osman BELDÜZ, Zihni DEMİRBAĞ, Sabriye DÜLGER
Karadeniz Teknik Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, 61080, Trabzon–TURKEY

Ali Osman KILIÇ
Karadeniz Teknik Üniversitesi, Tıp Fakültesi, 61080, Trabzon–TURKEY

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Abstract:Polymerase chain reaction (PCR) was employed to detect the presence of cyclic AMP receptor protein (CPR) in a number of diverse organisms. In PCR, two primers specific to the *crp* gene of *Escherichia coli* were used. *Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus delbrueckii* and *Corynebacterium verasus* all showed the same size of PCR fragments (708 bp) and same restriction fragment length polymorphizm (RFLP).

Key Words: *crp, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus delbrueckii* and *Corynebacterium verasus.*

Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus delbrueckii ve Corynebacterium verasus'da *crp* Geninin Belirlenmesi

Özet: Polimeraz zincir reaksiyonu (PCR), birkaç değişik organizmadaki siklik AMP reseptör proteininin (CRP) varlığının belirlenmesinde kullanıldı. PCR'nda *Escherichia coli*'nin *crp* geni için spesifik olan iki tane primer kullanıldı. Çalışmada, *Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus delbrueckii* ve *Corynebacterium verasus*'un benzer PCR fragmentlerine (708 bp) ve benzer restriksiyon fragmenti uzunluk polimorfizmi (RFLP)'ne sahip oldukları görüldü.

Anahtar Sözcükler: *crp, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus delbrueckii* ve *Corynebacterium verasus.*

Introduction

The cyclic AMP receptor protein (CRP or CAP) is a positive regulator of gene expression. When complexed with cyclic AMP (cAMP), it binds specifically in the promotor region of the operons which it regulates (1, 2). The sequence of the *E. coli* K–12 *crp* gene coding for CRP (3, 4) and the structure of the CRP:cAMP complex were determined. *In vivo* and *in vitro* studies have shown that *crp* is autoregulated (5, 6). The transcription start site has been idendified, and different regulatory elements, such as the binding sites for CRP and for RNA polymerase, have been located (5).

CRP is a dimer with a molecular weight of 45 000 and 209 amino acid residues in each subunit. Each subunit of the CRP dimer folds into two domains. The two subunits have different conformations. In the subunit in the "closed" conformation, the carboxy–terminal and amino–terminal domains lie closer together than in the "open" conformation, with a larger amino–terminal domain that extends from amino acid 1 to 129 and a smaller carboxy–terminal domain that continues from amino acid 139 to 209. The two domains are connected by a "hinge" region of residues 130 to 138, which has a very different conformation in the two subunits (7).

The secondary structure of CRP consists of six α–helices (αA, αB, αC, αD, αE, and αF), eight strands forming β–roll (β1, β2, β3, β4, β5, β6, β7 and β8) and four β–sheet in a small domain of CRP (β9, β10, β11, and β12) (7).

Earlier, the presence of *crp* genes was shown in *E. coli* K–12, *Shigella flexneri* 2B, *Salmonella typhimirium* (8) and *Klebsiella aerogenes* (9). Anderson and Pastan (10) have shown the presence of a similar protein in *Photobacterium fisheri, Aerobacter aerogenes* and *Proteus mirabilis*. Some other papers have indicated the presence of *crp* genes in a number of diverse organisms: *Vibrio fischeri* (11), *Salmonella choleraesuis* (12), *Bradyrhizobium japonium* (13), *Haemophilius influenzae* and (14) *Salmonella typhi* (15). This is the first study to determine the presence of *crp* genes in the indicated bacteria.

Our interest in elucidating the role of the structure in CRP function prompted us to search for the presence of the CRP gene and to analyze the structure of this gene in different bacteria. By using polymerase chain reaction (PCR) amplification, we amplified and cloned the *crp* genes of *Lactobacillus delbruckii, Staphylococcus epidermidis, Staphylococcus aureus* and *Corynebacterium verasus*.

Materials and Methods

Materials

Luria–Bertani (LB) media have been described by Maniatis et al. (16). Restriction endonucleases were purchased from New England Biolabs and Promega. All enzymes were used according to the instructions of the suppliers.

Bacterial strains: For the amplification, the starting strain were *Staphylococcus aureus* RSKK 250, *Staphylococcus epidermidis* ATCC 146, *Lactobacillus delbrueckii* supsp. *lactic* ATCC 15808, *Corynebacterium verasus*. ATCC 6931, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 6464, *Neisseria flavescens* ATCC 13120 and *Proteus vulgaris* 6659

Methods

PCR amplification: Extraction and purification of genomic DNA were performed with approximately 0.25 mg (wet weight) of cells from fresh LB cultures according to Maniatis et al. (16). DNA pellets were resuspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8.0). The integrity of the DNA was checked by horizontal gel electrophoresis in 1% agarose. The *crp* gene was selectively amplified from purified genomic DNA with oligonucleotide primers designed to anneal to positions in the 3' and 5' regions of bacterial *crp* genes. The 5' primer is

5'–CCATATGGTGCTTGGC–3' and 3' primer is 5'–CGAGCTCAGTCTGCGCCAC–3'. Primers were supplied by GBF–Braunschweig, Germany.

The reaction conditions were as follows: 12 ng of template DNA, 5 µl of 10 x PCR buffer (100 mM Tris–HCl: pH 8.3, 500 mM KCl), 1.5 mM $MgCl_2$, 2 U of *Taq* DNA polymerase, 0.25 mM of each primer, and 170 µM dATP, 170 µM dCTP, 170 µM dGTP, 170 µM dTTP were combined in a total volume of 50 µl. Amplification was carried out in 500 µl tubes in a Techne Progene Fuse 230U TZA Thermal Cycler as follows: a preliminary denaturation step was done at 95°C for 2 min. followed by 36 cycles of 1 min at 94°C, 1 min at 56°C and 2 min at 72°C.

PCR products were electrophoresed at 10 V cm^{-1} in 1% agarose in TBE (0.089 M Tris–borate, 0.089 M boric acid and 0.002 M EDTA) buffer containing ethidium bromide (0.5 mg/ml).

Restriction enzyme digestion was performed with 6 µg of the PCR product at 37°C for 2 hrs, and electrophoresed in 1.5% agarose gel.

Results

We amplified the *crp* genes from *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Lactobacillus delbrueckii* and *Corynebacterium verasus* by PCR (Figure 1) and cloned 708 bp *crp* fragments into *Sma* I site of M13mp18. M13mp18*crp*WT was used as a positive control (17). There were no PCR products in the samples from the other species listed in Materials. Consistently, no fragments was determined by PCR in other species indicated in Materials (data not shown).

Restriction digestions of PCR products produced the expected fragment sizes (on the basis of *E. coli crp* gene results) 124, 444 and 440 bp long fragments with *Alw* NI; 39, 291 and 378 with *Taq* I; and 150, 224 and 334 with *Msp* I. The restriction maps of the *crp* regions of *E. coli* and others were very similar. *Alw* NI, *Taq* I and *Msp* I sites were at the same (or very close) locations in all organisms (Figure 2).

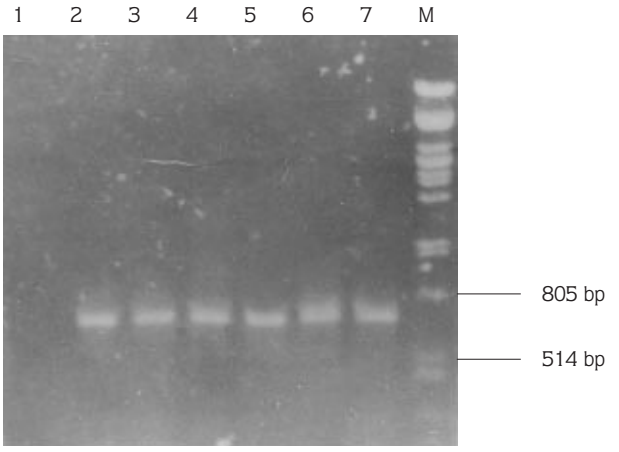

Figure 1. *crp* fragments from PCR. Lanes: (1), Negative control by using M13mp18; (2), Positive control, M13*crp* (Belduz et al, 1994); (3), *E. coli* B; (4), *Staphylococcus aureus;* (5), *Staphylococcus epidermidis;* (6), *Lactobacillus delbrueckii* supsp. *lactic*; (7), *Corynebacterium verasus.*

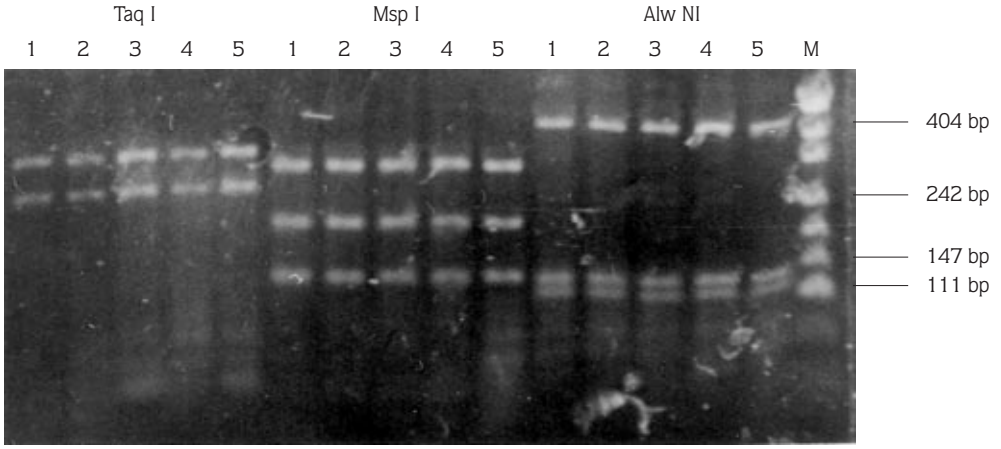

Figure 2. Restriction digestion of PCR products. Restriction endonucleases used are indicated on the top the figure. Lanes: (1) M13*crp* (19); (2). *E. coli* B; (3). *Staphylococcus aureus;* (4). *Staphlococcus epidermidis;* (5). *Lactobacillus delbrueckii* supsp. *lactic;* (6). *Corynebacterium verasus.*

Discussion

The role of cyclic AMP (cAMP) in mediating the glucose effect on the induction of catabolic enzymes in *E. coli* and its relatives is familiar. cAMP mediates its effect by binding to cyclic AMP receptor protein (CRP) in *E. coli* and its relatives. There are now many reports of cAMP in many nonenteric bacteria, but little is known about the role of the nucleotide in these bacteria. In many cases, cAMP appears not to mediate the glucose effect observed for induction of catabolic enzymes. Mutants lacking adenylate cyclase have not yet been isolated in these less familiar bacteria. In some cases, no cAMP dependent functions have been found, but no function for these proteins has been established (18).

At present, although only the presence of CRP in some enteric and nonenteric bacteria has been shown, the primary structures of CRP from three enteric bacteria other than *E. coli* have been determined, and the identity of *E. coli* CRP has been shown. In this study, the presence of *crp* genes was shown by PCR in bacteria other than enterics and identity of these genes to *E. coli crp* gene was indicated by digesting with restriction endonucleases.

Alw NI recognises positions 145, 147, which are involved in the αD, 193 and 195 which are between αF and β11, of CRP. One of *Taq* I recognition region corresponds to positions 11 and 12 in αA and the other corresponds to positions 137 and 138 of CRP, which are in the bridge region that connects the large and small domains of CRP. *Msp* I recognition regions involves the positions 110, 111, 160 and 161 of CRP. Corresponding structure in CRP for the position 110 is β9, β10, αB and αC which is responsible for intersubunit interaction. CRP from all strains did not show any difference in these positions.

Reason for detecting no fragment for *crp* gene in other species given in Materials can be either sequence differences of the gene in these species or absence of *crp* gene. Contrary to the result of immunological studies of Anderson and Pastan (10), we have been able to show the presence of *crp* gene is *Staphylococcus aureus* by PCR.

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