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The *aroA* gene of *Campylobacter jejuni*

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Abstract

The gene for 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (*aroA*) cloned from *Campylobacter jejuni* (*Cj*) strain 81116 was identified by complementation of an *Escherichia coli* (*Ec*) auxotrophic *aroA* mutant. The *Cj aroA* gene has been sequenced. It encodes an enzyme of 428 amino acids (aa), that is homologous to other bacterial EPSP synthases, especially that of *Bacillus subtilis* with which it has a 39% aa identity. The transcriptional start point was mapped. It is present in an upstream open reading frame (ORF) that has a strong homology to the gene encoding phenylalanine tRNA synthetase (*pheS*). Downstream from *aroA* another ORF is present which is homologous to the *lytB* gene of *Ec*. The stop codon of the *aroA* gene overlaps the start codon of *lytB*.

Keywords: 5-Enolpyruvylshikimate-3-phosphate synthase; *lytB* gene; *pheS* gene; Complementation; Primer extension

1. Introduction

The enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19) is present in bacteria, yeast and plants where it has a function in the biosynthesis of aromatic aa (Pittard, 1987). Disruption of the *aroA* gene encoding this enzyme leads to an auxotrophy of the organisms for 4-aminobenzoic acid, 2,3-dihydroxybenzoic acid and aromatic aa. In addition, the synthesis of enterobactin, which is important for the binding of iron by a number of intestinal bacteria, is inhibited (Stocker, 1988).

Campylobacter jejuni (*Cj*) has been recognized as one of the most important causes of enteritis in humans (Wallis, 1994) with symptoms varying from mild watery diarrhoea to a painful inflammatory enterocolitis (Wallis, 1994). In addition to being a pathogen for humans, *Cj* is carried as a commensal in the gastrointestinal tracts of a variety of wild and domestic animals, which function as reservoirs of *Cj*. Particularly poultry is a source of infection of man by *Cj* (Shane, 1992). The gene products involved in pathogenesis and colonization are poorly understood. However, as in other enteric bacteria the *aroA* gene product may be expected to be essential for growth and thereby for the expression of virulence and colonization factors.

In this report, we describe the cloning and characterization of the *Cj aroA* gene and compare the deduced aa sequence with that of other bacterial *aroA* genes.

2. Experimental and discussion

2.1. Cloning and sequencing of the Cj 81116 aroA gene

By complementation of the auxotrophic *Ec* mutant AB2829, lacking a functional *aroA* gene, we isolated four clones pAROA2, pAROA2A1, pAROA3A1 and pAROA5A1 (Fig. 1). The growth of *Ec* AB2829 on minimal medium was fully complemented in 48 h by pAROA2A1, in 72 h by pAROA3A1 and pAROA5A1, while pAROA2 complemented growth only after 5 days. Without a recombinant plasmid the AB2829 strain was

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Abbreviations: aa, amino acid(s); *aroA*, gene encoding EPSP synthase; *Bs*, *Bacillus subtilis*; bp, base pair(s); *Cj*, *Campylobacter jejuni*; EPSP, 5-enolpyruvylshikimate-3-phosphate; *Ec*, *Escherichia coli*; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; ORF, open reading frame; *pheS*, gene encoding phenylalanine tRNA synthetase; RBS, ribosome-binding site(s); *tsp*, transcription start point(s).

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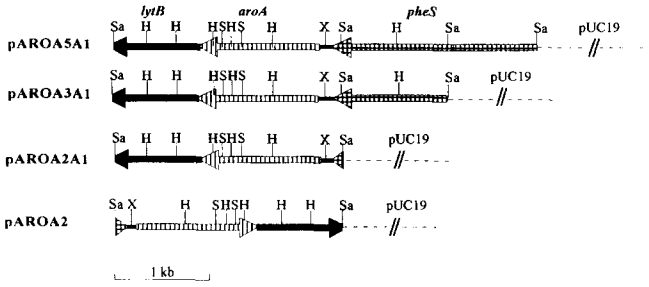

Fig. 1. Restriction maps of the *aroA* clones. Overlapping *Cj* inserts present in pUC19 and pBluescript KS M13+ are shown. *Ec* strain AB2829 harbours a stable *aroA* mutation and was obtained from the *Ec* Genetic Stock Centre, Yale University, New Haven, CT. The direction of transcription is indicated by arrows. Restriction enzyme abbreviations: H, *Hin*dIII; Sa, *Sau*3AI; S, *Ssp*I; X, *Xba*I. **Methods:** *Cj* 81116 (Palmer et al., 1983) was routinely cultured at 42°C on saponin agar medium, under microaerophilic conditions (Nuijten et al., 1989). Chromosomal DNA, prepared as described previously (Wassenaar et al., 1991), was partially digested with *Sau*3AI. DNA fragments varying from 2.5 to 5.0 kb were separated by electrophoresis in a 2% agarose gel and purified using a Geneclean kit (BIO 101 Inc., La Jolla, CA). These fragments were ligated with *Bam*HI-digested, dephosphorylated pUC19 and transformed into *Ec* AB2829. This mutant is able to grow on 1 mM $MgSO_4$/0.1 mM $CaCl_2$/0.2% glucose (w/v)/10 μg thiamine/ml supplemented with 40 μg phenylalanine, tyrosine and tryptophan (Sigma, St. Louis, MO) per ml and 10 μg 4-aminobenzoic acid and 2,3-dihydroxybenzoic acid (Sigma, St. Louis, MO) per ml. Transformants containing an active *aroA* were selected on minimal medium with 100 μg Ap/ml.

not able to grow on minimal medium even after 8 days at 37°C. The long time needed for complementation by pAROA2 compared to the other clones can be explained by the fact that *aroA* is not efficiently transcribed from its own promoter. The other three clones may be more efficiently expressing *aroA* since they are transcribed from the *lacZ* promoter. Also the fact that the *Cj aroA* lacks a typical *Ec* RBS (see below) may explain the long time required for complementation of *Ec* AB2829. Restriction enzyme mapping of the four recombinant plasmids revealed that they contained overlapping inserts of about 2.2–4.6 kb. pAROA2 and pAROA2A1 contained the same 2.2-kb insert but positioned in the opposite orientation. Southern blot analysis with genomic *Cj* DNA digested with *Hin*fI, *Hin*dIII or *Xba*I and probed with complete pAROA2 showed that the *Cj* chromosome contains a single copy of the *aroA* gene (data not shown). The nt sequences of the *aroA* and part of the adjacent genes present on plasmids pAROA2, pAROA2A1, pAROA3A1 were determined. The complete *aroA* nt sequence is shown in Fig. 2. One ORF for a 428-aa protein homologous to other EPSP synthases was found. Upstream from *aroA* there is another ORF, of which the sequenced part showed significant identity at aa level to phe-tRNA synthase from a number of organisms. Downstream from *aroA* there is a third ORF which is homologous to the *Ec lytB* (Fig. 2). The *aroA* ORF overlaps the start codon TTG of *lytB* by 8 bp. Overlapping genes have been described before in *Cj* (Chan and Bingham, 1991, 1992; Guerry et al., 1994). They may need to overlap because of the small genome size (1700 kb; Nuijten et al., 1990) of *Cj*. A putative RBS is situated 6 bp upstream from the proposed start codon TTG of *lytB* while no RBS was found upstream from *aroA* (Fig. 2).

2.2. *Identification of the* tsp *of the* aroA *mRNA*

The 5′ end of the *aroA* mRNA was determined by primer extension as indicated in Fig. 3. The mRNA of the *aroA* gene starts with a G located at nt 159, 222 nt upstream from the *aroA* ATG codon and inside *pheS* (Figs. 2 and 3).

2.3. *Comparison of the deduced aa sequences of AroA from* Cj *and other bacteria*

The evolutionary distance between the *Cj aroA* gene product and that of other bacteria was determined by construction of a phylogenetic tree (data not shown). The *Cj* sequence has 29.9% and 38.8% aa identity with the EPSP synthases from *Ec* and *Bs*, respectively. In general the *Cj* EPSP is more closely related to the same enzymes from $Gram^+$ bacteria (*Dichelobacter nodosus*, *Synechocystis* sp. PCC6803, *Lactococcus lactis* and *Bs*) than from $Gram^-$. The sequences of 16 *aroA* genes of $Gram^-$ bacterial species are presently known. Of these, five contain an upstream *serC* gene, encoding 3-phosphoserine aminotransferase, in the same operon. There is no upstream *serC* gene in *Pasteurella multocida*, *Bordetella pertussis* or *Haemophilus influenzae* (Maskell et al., 1988; Maskell, 1993; Homchampa et al., 1992). The *Cj aroA* gene also lacks a *serC* gene. The gene downstream from *aroA* varies among bacteria, but in no other species a *lytB* has been found. This is in accordance with the position of *Cj* as a member of a diverse genus with only distant relationships to other eubacteria (Vandamme et al., 1991).

3. Conclusions

(*1*) This report shows that the *aroA* gene of *Cj* can complement *Ec aroA* mutations.

(*2*) The aa sequence derived from the *aroA* sequence is most closely related to EPSP of a number of $Gram^+$ bacteria.

(*3*) Two incomplete ORFs with similarity to *pheS* and *lytB* were identified in the upstream and downstream region of *aroA*, respectively.

(*4*) The *Cj aroA* gene overlaps *lytB*. The gene downstream from *aroA* varies, but in no other species a *lytB* gene has been found.

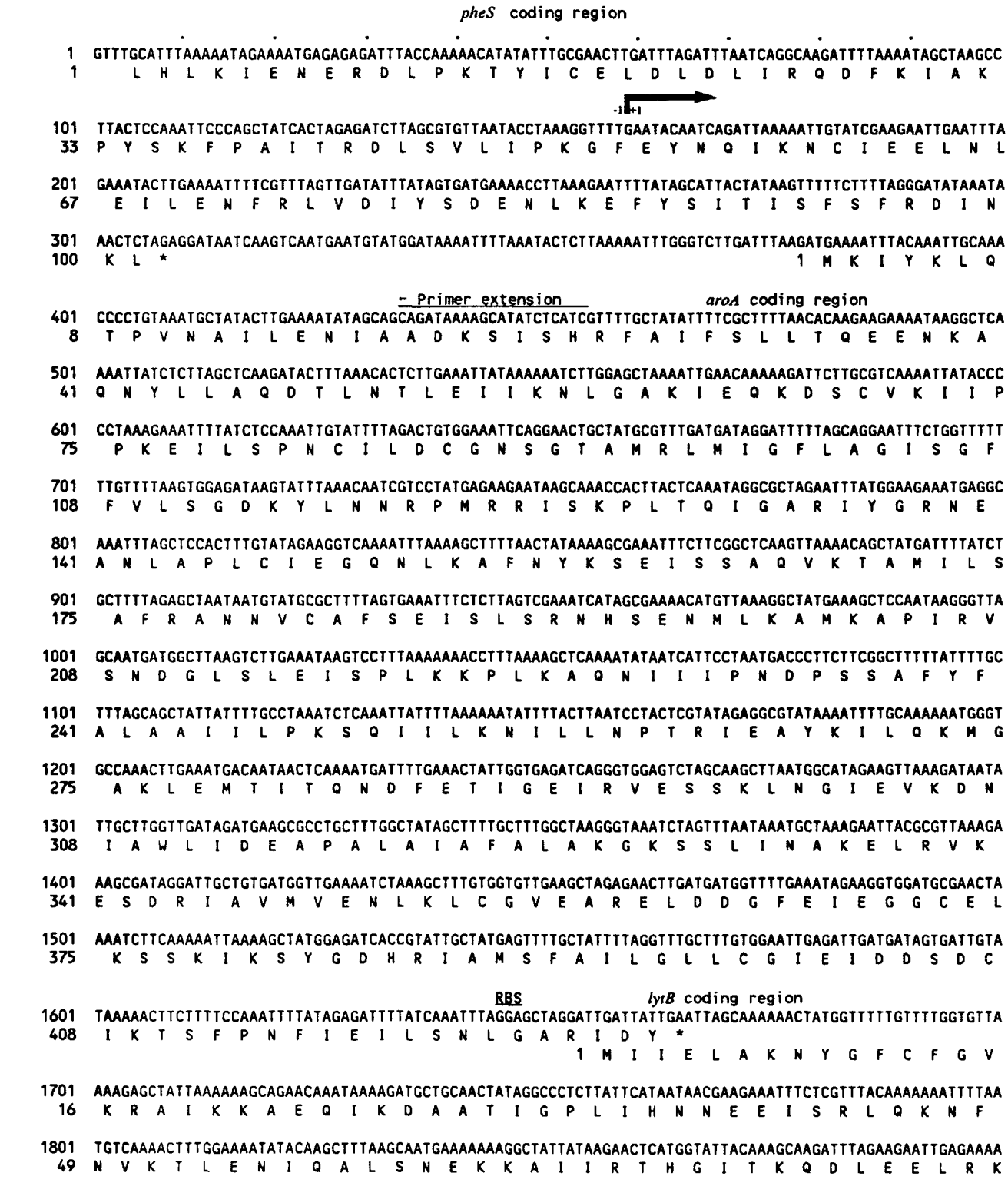

Fig. 2. Nucleotide and deduced aa sequence of the *Cj aroA* and regions of the adjacent *lytB* and *pheS*. The TTG start codon of *lytB* is preceded by a potential RBS. The location of the oligo primer used to determine the *tsp* is indicated. The *tsp* is marked with an arrow. This sequence has been deposited in GenBank/EMBL/DDBJ Databases under accession No. X89371. Plasmid DNA was isolated using the Qiagen plasmid kit (Qiagen, Inc., Chatsworth, CA). The sequence of the cloned DNA was determined by using the dideoxy chain termination method, with an Automated Laser Fluorescent DNA Sequencer (Pharmacia, Uppsala, Sweden) the autoread sequencing kit using T7 DNA polymerase (Pharmacia), and fluorescein labelled nt primers (Pharmacia). PC/Gene 6.70 was used to analyze nt and aa sequences.

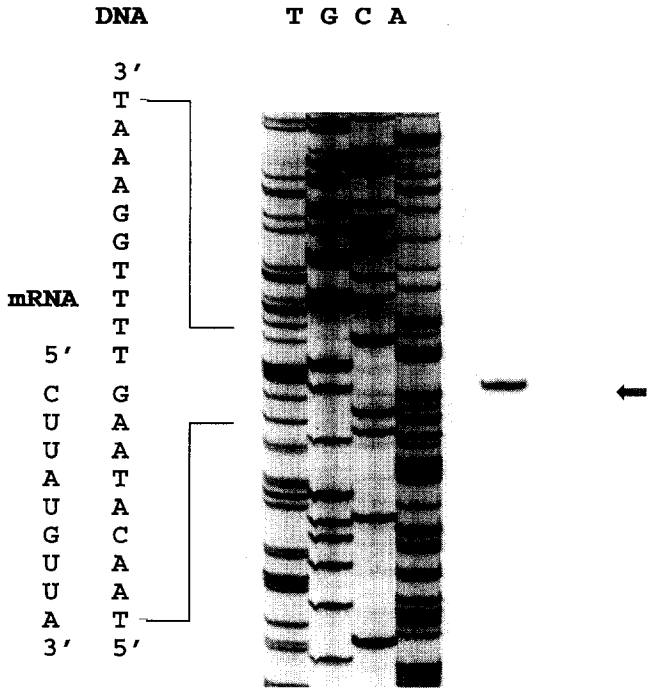

Fig. 3. Primer extension mapping of the 5′ end of the *aroA* mRNA. The primer extension product is indicated by an arrow. Nt sequences were determined using the same primer. Nt and corresponding mRNA sequences are shown. **Methods:** Total RNA was isolated from overnight cultures of *Cj* by single-step extraction with acid guanidinium thiocyanate-phenol-chloroform (Chomczynski and Sacchi, 1987). To map the 5′ end of the *aroA* mRNA, a primer extension experiment was performed with an oligo complementary to the region indicated in Fig. 2. The primer was labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Labelled primer 150 ng; 500 cpm/ng was annealed to 10 μg of total *Cj*. RNA in 100 mM Tris-HCl pH 8.3, 140 mM KCl in a volume of 10 μl. The oligo was annealed to the RNA by incubation for 10 min at 55°C followed by an incubation of 15 min at 4°C. cDNA was synthesized for 30 min at 42°C, in a volume of 20 μl containing 50 mM Tris-HCl pH 8.3/75 mM KCl/3 mM $MgCl_2$/1 mM dithiothreitol/20 mM dNTPs/5 u of SuperScript™/RNaseH− reverse transcriptase (Gibco/BRL, Life Technologies Inc., Gaithersburg, USA). The reverse transcriptase reaction product was analysed by electrophoresis on a 6% polyacrylamide gel containing 8 M urea and compared to sequence ladders of pAROA2 obtained with the same oligo primer as used for the reverse transcriptase. Dideoxy chain termination sequence reactions were performed.

(5) The *tsp* of *aroA* is situated in *pheS*, but no typical *Ec* consensus promoter region could be identified.

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