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# Lysyl-tRNA Synthetase Gene of *Campylobacter jejuni*

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**We report the cloning and complete nucleotide sequence of the *Campylobacter jejuni* lysyl-tRNA synthetase gene (*lysS*). The *C. jejuni lysS* gene sequence shows high homology to the two *Escherichia coli* lysyl-tRNA synthetase genes, *lysS* and *lysU*. The *Campylobacter* lysyl-tRNA synthetase protein (LysRS) shows 47.9 and 46.6% sequence identity to the *E. coli* enzymes encoded by the *lysS* and *lysU* genes, respectively. The LysRS encoded by the *C. jejuni* gene is a polypeptide of 501 amino acids with a deduced molecular weight of 57,867. The enzyme is active in *E. coli*. The gene is expressed from its own promoter, and the transcription start site has been mapped. The carboxyl-terminal codon of the *C. jejuni lysS* gene overlaps by 1 bp with the Met initiation codon of the *glyA* gene, which has been shown to have a promoter which is functional in *E. coli* (V. L. Chan and H. L. Bingham, Gene 101:51–58, 1991). *C. jejuni*, unlike *E. coli*, has only one lysyl-tRNA synthetase gene.**

Aminoacyl-tRNA synthetases play a key role in protein synthesis by catalyzing the covalent attachment of amino acids to the 3′ end of their cognate tRNAs. In *Escherichia coli*, with the exception of lysine, each amino acid is catalyzed by a single aminoacyl-tRNA synthetase (10). There are two distinct lysyl-tRNA synthetases in *E. coli* encoded by two widely separated genes, *lysS* (32) and *lysU* (8). These two genes have been cloned and sequenced recently and were shown to be highly homologous, with 86% of their amino acid sequences identical (6, 21). The *lysS* gene is expressed constitutively (14, 15), while *lysU* can be induced by culturing cells under various selective conditions, including growth in the presence of L-leucine, L-alanine, or glycyl-L-leucine, growth with D-fructose as the carbon source, and growth at high temperature (14, 16).

*Campylobacter jejuni* is now recognized worldwide as a major causative agent of bacterial diarrhea and enteritis in children and adults. The mechanism(s) of pathogenesis of this organism is still to be resolved. The genomes of two different strains of *C. jejuni* were recently analyzed by field inversion gel electrophoresis or pulsed-field gel electrophoresis and were shown to be about 1,900 kb (18) and 1,700 kb (5, 26). To date, only a few *C. jejuni* chromosomal genes have been cloned (4, 17, 20, 25), and even fewer have been sequenced (3, 27).

In this report, we describe the cloning and the complete nucleotide sequence of the lysyl-tRNA synthetase (*lysS*) gene of *C. jejuni*. This study also demonstrates that the *C. jejuni* chromosome, unlike that of *E. coli*, contains a single copy of the lysyl-tRNA synthetase gene.

## MATERIALS AND METHODS

**Materials.** Restriction endonucleases were purchased from Boehringer, Mannheim, Germany; GIBCO-Bethesda Research Laboratories, Burlington, Ontario, Canada; and Pharmacia, Uppsala, Sweden. [$^{35}$S]methionine was from ICN Biomedicals, Irvine, Calif. [$^{3}$H]lysine was from Amersham, Oakville, Ontario, Canada, and [$^{32}$P]dATP was from Dupont-NEN, Mississauga, Canada. Amino acids, vitamins, *E. coli* strain W transfer RNA, glycyl-DL-leucine, and ATP were obtained from Sigma. Sequencing was done with the Sequenase DNA sequencing kit from U.S. Biochemical, Cleveland, Ohio.

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. Transformation of bacteria with plasmids was by the $CaCl_2$-RbCl procedure (22). A GIBCO-Bethesda Research Laboratories electroporator was used to introduce pK0-1 and pK0-1L into MH318 by using the manufacturer's standard protocol.

**Media.** *E. coli* and *C. jejuni* strains were grown and maintained as described by Chan et al. (4). For the enzyme assay, *E. coli* strains were grown in minimal medium supplemented with amino acids, vitamins, and bases (SMM) (11). L-Leucine, a requirement for C600, was added at a concentration of 40 μg/ml, and glucose (0.036%) was used as the carbon source. Glycyl-DL-leucine (3 mM) was added when necessary for the induction of lysyl-tRNA synthetase. The antibiotics ampicillin and chloramphenicol were added to the media, when necessary, at a concentration of 100 and 30 μg/ml, respectively. *C. jejuni* was grown in campylobacter defined medium (CDM) (31).

**DNA sequence determination.** Sequence analysis was performed by the dideoxy-chain termination method (28, 30). The 1.6-kb *Hin*dIII fragment of pCP3b was cloned into pUC19 in both orientations (pUT1.6 and pUT1.6r) and sequenced from nested deletions obtained with exonuclease III and S1 nuclease (13).

**Analysis of plasmid-encoded proteins.** Minicell-producing *E. coli* DS410 was transformed with pK0-1 and pK0-1L. Minicells from the resulting strains together with minicells from the parent DS410 were purified, labelled, and analyzed as described in Chan and Bingham (3).

**Southern analysis of genomic DNA.** *C. jejuni* and *E. coli* DNA was digested to completion with restriction enzymes. The fragments were separated by gel electrophoresis, transferred to GeneScreen, and probed with a labelled probe by standard procedures (22). The restriction enzymes used were *Hin*dIII and *Eco*RV for *C. jejuni* and *Eco*RV for *E. coli*. The 1.6-kb *Hin*dIII fragment of pCP3b (Fig. 1) was labelled and used as the probe.

**Preparation of cell extracts.** Crude cell extracts were prepared as described by Chan et al. (4), with a few changes. The strains were grown in SMM supplemented with leucine and with the addition of ampicillin and glycyl-DL-leucine

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TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Genotype or relevant characteristic | Source or reference |
|---|---|---|
| Strains | | |
| *C. jejuni* TGH9011 | Serotype O:3 | J. L. Penner |
| *E. coli* | | |
| C600 | *supE44 hsdR thi-1 thr-1 leuB6* | This laboratory |
| JM101 | *supE thi* Δ(*lac-proAB*) F′ (*traD36 proA*$^+$ *B*$^+$ *lacI*$^q$*lacZ* Δ*M15*) | This laboratory |
| MH318 | *lysS*($K_m^{Lys}$) Δ(*adi mel lysU cadA*) Ap$^r$ Clr$^r$ | Hirshfield (11) |
| DS410 | *lac* Str$^r$ *minA minB* | 7 |
| Plasmids | | |
| pCP3b | pBR322 recombinant clone | 4 |
| pK0-1 | | 23 |
| pK0-1L | pK0-1 derivative containing *Rsa*I fragment of pCP3b | This work |
| pKK232-8 | pBR322 derivative containing promoterless CAT gene | 2 |
| pKK232-8L | pKK232-8 derivative containing *Rsa*I fragment of pCP3b | This work |
| pUC19 | Ap$^r$ *lac*α peptide multiple cloning site | 33 |
| pUT1.6 | pUC19 containing 1.6-kb *Hin*dIII fragment of pCP3b | This work |
| pUT1.6r | pUC19 containing 1.6-kb *Hin*dIII fragment of pCP3b in reverse orientation | This work |

where necessary. Overnight cultures were diluted 40-fold into fresh SMM or into SMM with 3 mM glycyl-DL-leucine and grown in a 37°C shaker bath for 5 h. Heat-induced cultures were grown under the same conditions for 3 h; the flasks were then shifted to a 46°C shaker bath for an additional 2 h. *C. jejuni* cultures were grown overnight in CDM at 37°C in an incubator with 5% $CO_2$. Cultures were then diluted threefold and grown for 5 h at 37°C in the $CO_2$ incubator, or, in the case of heat-induced cultures, shifted after 3 h to a 46°C $CO_2$ incubator. Cells were then pelleted, washed twice with 0.85% NaCl, and redispersed in 2 ml of standard assay buffer (SAB), consisting of 200 mM Tris HCl (pH 8.6) and 20 mM magnesium acetate. The cells were disrupted with a French press, and the resulting extract was centrifuged for 30 min at 4°C in an Eppendorf centrifuge. The supernatant was removed and used for enzyme assays and Bio-Rad protein determination.

**Lysyl-tRNA synthetase assay.** The activity of lysyl-tRNA synthetase was measured by its ability to charge tRNA with L-[$^3$H]lysine. The incubation mixture patterned after the assay of Hassani et al. (11), with changes, consisted of the following in a volume of 200 μl: 44 μM Tris-acetate (pH 8.6), 4.4 μM magnesium acetate, 0.64 μM ATP, 0.00005 μM L-[$^3$H]lysine (100 μCi/mol), 0.00005 μM L-lysine, 1.0 μM KCl, and 0.4 mg of *E. coli* tRNA. Routinely, 2 to 5 μg of crude extract was added, and the reaction was performed at 25°C for 15 min. Aliquots (100 μl) were spotted onto 3MM Whatman disks, washed three times with 10 ml of ice-cold 5% trichloroacetic acid–0.05% Casamino Acids and twice with 10 ml of 95% ethanol, dried, and counted in a liquid scintillation system.

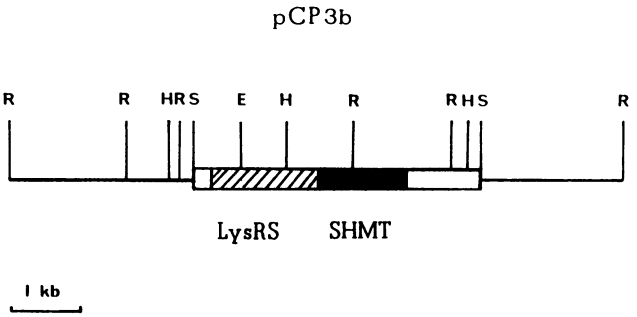


FIG. 1. Restriction map of recombinant plasmid pCP3b. Thin line indicates pBR322 DNA sequence. The rectangle represents *C. jejuni* DNA sequence, and the hatched and filled areas represent the encoding sequences of *lysS* and *glyA*, respectively. Abbreviations: E, *Eco*RV; R, *Rsa*I; H, *Hin*dIII; S, *Sal*I; SHMT, serine hydroxymethyltransferase; LysRS, lysyl-tRNA synthetase.

**Primer extension mapping of the transcription start site of the *lysS* mRNA.** RNA was isolated from late-exponential-phase cultures of JM101 and of JM101 containing pCP3b by the hot phenol method (1). Oligonucleotide lys-1, 5′ TTC TATTCTTTGCTGCTCTAAAATATTATC, is complementary to nucleotides 208 to 237, and its 3′ end is 6 nucleotides down from the start codon of the *lysS* gene. This oligonucleotide was end labelled and mixed with 25 μg of each RNA. The mixture was hybridized and extended as described by Chan and Bingham (3). For analysis, 2 μl of the newly synthesized DNA (previously suspended in 4 μl of $H_2O$ and 4 μl of formamide loading buffer) was loaded onto a 6% polyacrylamide sequencing gel alongside dideoxy sequencing ladders of the upstream flanking region of the *lysS* gene generated with the oligonucleotide lys-1 used as the primer.

**Nucleotide sequence accession number.** The nucleotide sequence data given in Fig. 2 are accessible in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under accession number M63448.

## RESULTS

**Nucleotide sequence of *lysS*.** The recombinant plasmid pCP3b (Fig. 1) was isolated from a genomic library of *C. jejuni* by its ability to complement an *E. coli glyA* mutant (4). The library was constructed with partial *Sau*3A fragments of *C. jejuni* inserted at the unique *Bam*HI site of pBR322. The *glyA* gene encodes serine hydroxymethyltransferase, and the complete encoding sequence was shown to be within the 2.3-kb *Hin*dIII fragment (Fig. 1). The start codon (Met) of the *glyA* gene overlaps a continuous open reading frame (ORF) by 1 base. With the aim of identifying the gene product of this ORF, we sequenced the adjacent 1.6-kb *Hin*dIII fragment 5′ upstream of the *glyA* gene. The complete ORF (1,503 nucleotides) with the deduced amino acid sequence, 201 bp of the 5′ flanking sequence, and 155 bp of the 3′ flanking sequence is shown in Fig. 2. The ORF encodes a polypeptide of 501 amino acids, initiated by a Met and terminated by two in-frame chain-terminating codons, TGA and TAG. Twelve nucleotides upstream from the proposed start codon, sequence AAAGG (nucleotides 186 to 190), which is identical to the typical Shine-Dalgarno se-

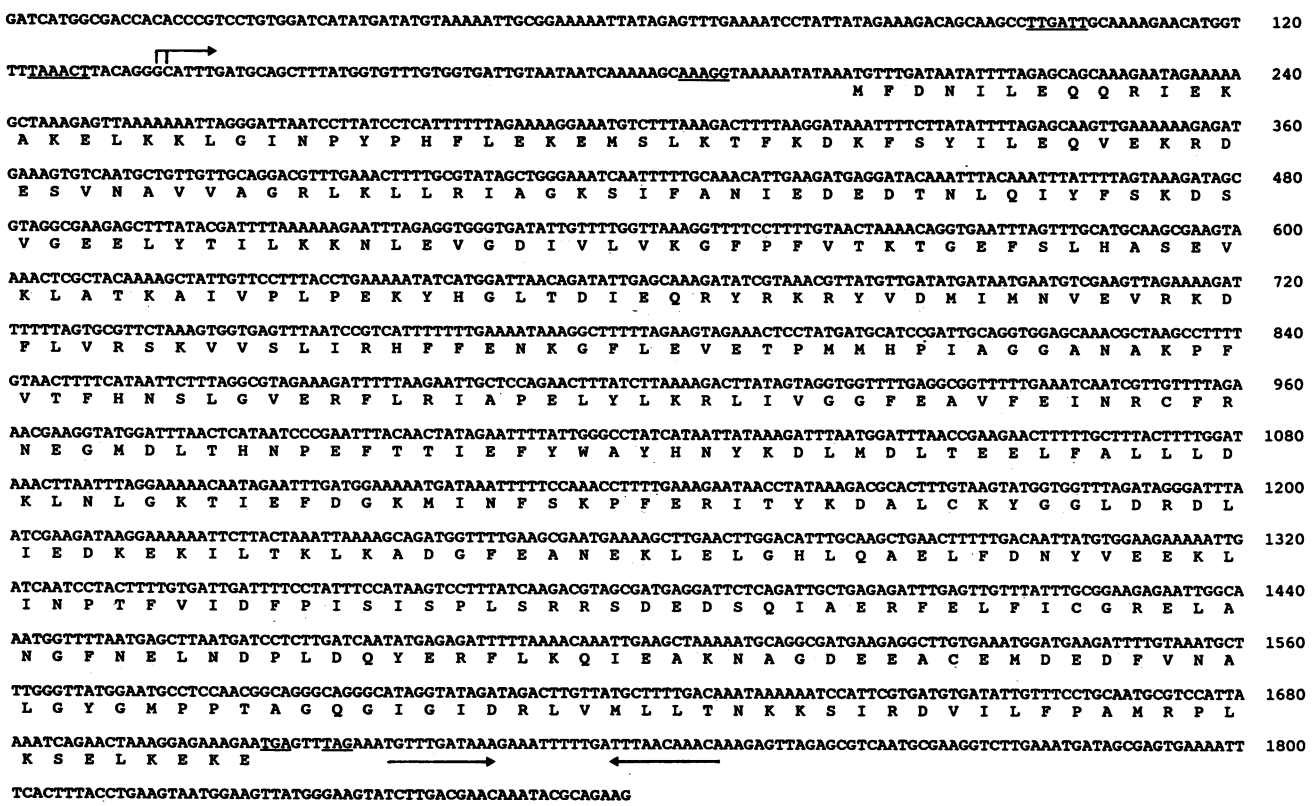


FIG. 2. Nucleotide sequence of the *C. jejuni lysS* gene and deduced amino acid sequence. The putative ribosome-binding sequence (Shine-Dalgarno sequence), −10 and −35 consensus sequences, and stop codons are underlined. Inverted repeats are indicated by arrows, and the transcription start sites are marked with a bent arrow. The amino acid sequence, as deduced from the nucleotide sequence of the *lysS* gene, is given below the nucleotide sequence in single-letter code.

quence (AGGAGG) at 3 of the 6 nucleotides, was observed. At nucleotides 123 to 128, a sequence (TAAACT) which matches 4 of the 6 nucleotides of the Pribnow box (TATAAT) can be observed. Seventeen nucleotides upstream of the putative Pribnow box is TTGATT (nucleotides 100 to 105), which shares 4 of 6 nucleotides with the −35 consensus sequence (TTGACA) of the *E. coli* and *Bacillus subtilis* housekeeping genes (12).

**Comparison of LysRS of *E. coli*, *Saccharomyces cerevisiae*, and *C. jejuni*.** The LysRS amino acid sequence, encoded by the *E. coli lysS* gene, shows 47.9% identity to the translated product of the ORF immediately upstream of the *glyA* gene (Fig. 2). This *C. jejuni* ORF amino acid sequence also shows homology to the *E. coli lysU* gene product, with 46.6% identity. LysRS belongs to the class II aminoacyl-tRNA synthetases, which are characterized by the presence of three conserved motifs (9). The three motifs (amino acids 184 to 202 [I], 267 to 276 [II], and 456 to 488 [III]) can be seen with the LysRS of *C. jejuni* (Fig. 3). The characteristic signature HIGH sequence of class I aminoacyl-tRNA synthetases (10) is absent. These observations suggest that the ORF upstream of the *glyA* of *C. jejuni* encodes LysRS, and the gene is therefore designated *lysS*. The alignment of the four LysRS sequences also identifies two other conserved regions besides the three motifs that are found in all class II aminoacyl-tRNA synthetases. One of these sequences is located upstream of motif I at amino acids 153 to 166 of the *C. jejuni* LysRS, and the other highly conserved sequence is located between motifs I and II at amino acids 206 to 242. The codon usage of the *lysS* gene is similar to that of the *glyA* gene and shows distinct preference for synonymous codons that are rich in A or T (Table 1). The predicted unmodified molecular weight of the *C. jejuni* LysRS is 57,867, and it has a pI of pH 6.04.

**Minicell analyses.** Minicells were used to determine whether the putative *C. jejuni lysS* gene can be expressed in *E. coli*. The 2.1-kb *Rsa*I fragment of pCP3b (Fig. 1), which contains the complete LysRS-encoding region and the 5′ and 3′ flanking sequences, was cloned into the *Sma*I site of pK0-1 (22) to produce pK0-1L. These plasmids were introduced into the *E. coli* minicell-producing strain DS410 (7) by transformation. As shown in Fig. 4, minicells containing pK0-1 synthesized two polypeptides of 42 and 31 kDa. In contrast, minicells containing pK0-1L produced three polypeptides, two identical to that of pK0-1 plus a new polypeptide of about 56.5 kDa. The 31-kDa protein is the unprocessed β-lactamase, and the 42-kDa polypeptide is probably the galactose kinase. The 42-kDa polypeptide was synthesized at a very low level in pK0-1 minicells compared with that in pK0-1L. These findings suggest the presence of an active promoter(s) in the 2.1-kb *Rsa*I *C. jejuni* DNA fragment that functions in the *E. coli* cells. This notion was further examined by inserting this fragment immediately upstream of the chloramphenicol acetyltransferase (CAT)-encoding sequence in pKK 232-8 (2). The CAT gene was activated in this recombinant plasmid (pKK 232-8L) because JM101 cells transformed by pKK232-8L were resistant to chloramphenicol and ampicillin while the cells transformed

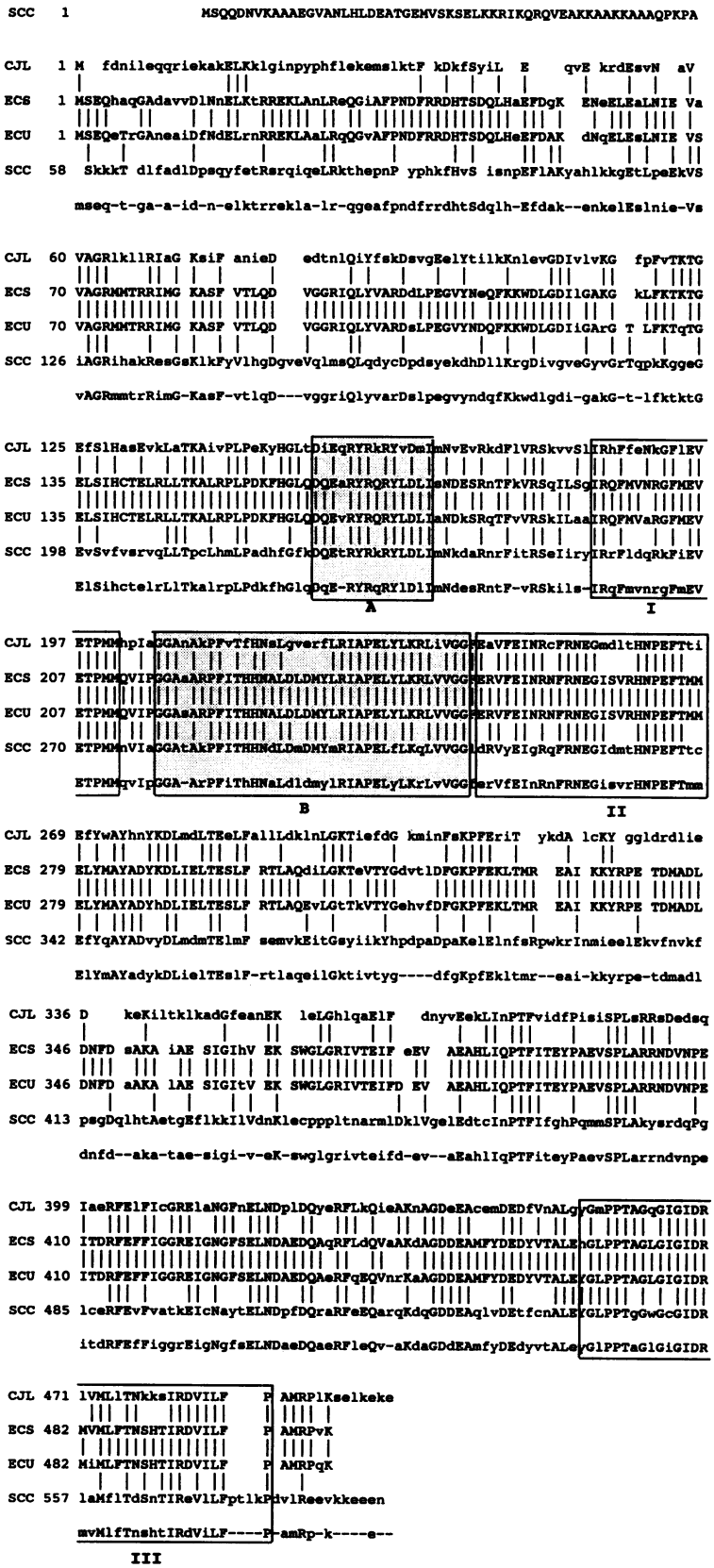


FIG. 3. Amino acid sequence homology between LysRS of *E. coli*, *S. cerevisiae*, and *C. jejuni*. The amino acid sequence is given in single-letter code. The *C. jejuni* LysRS (CJL) sequence is derived from the nucleotide sequence given in Fig. 2. The *E. coli* LysRS encoded by *lysS* (ECS) and *lysU* (ECU) are from Leveque et al. (21). The yeast LysRS sequence (SCC) is from Mirande and Waller (24). The three conserved motifs (I, II, and III) for class II aminoacyl-tRNA synthetase are shown as open boxes. Two highly conserved sequences (A and B) observed for lysyl-tRNA synthetases are shown as shaded boxes.

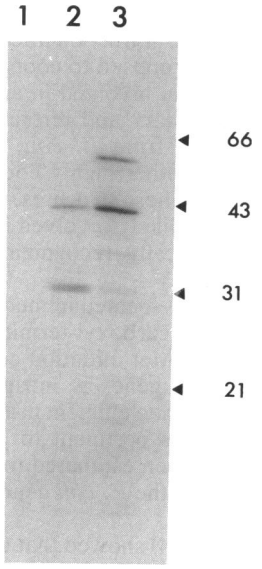


FIG. 4. Minicell analysis of plasmid-encoded proteins. Plasmid-encoded proteins in minicell-producing *E. coli* DS410 were labelled with [$^{35}$S]methionine and analyzed as described in our earlier study (3). The minicells were lysed, and proteins, denatured by boiling, were resolved in 11% polyacrylamide gels. After electrophoresis, the gels were strained, destained, dried, and subjected to autoradiography. Lanes: 1, labelled minicells without plasmid; 2, minicells with pK0-1; 3, minicells with pK0-1L. The apparent sizes of standard proteins (in kilodaltons) are indicated by arrowheads on the right margin.

by pKK232-8 were only resistant to ampicillin (data not shown). Similarly, the 1.6-kb *Hin*dIII fragment (Fig. 1), which contains the putative promoter of the *lysS* gene but not that of *glyA*, was able to activate the CAT gene (data not shown).

**Complementation of the *lys* mutations in MH318.** *E. coli* MH318 (11) is unable to grow in SMM without lysine because of a *lysU* deletion and a *lysS* mutation that alters the $K_m$ of the enzyme for lysine. This Lys auxotrophic lesion can be complemented by transformation with the *C. jejuni lysS*-containing plasmid pK0-1L. This finding again suggests that the *C. jejuni lysS* gene was expressed in *E. coli* and, furthermore, that the *Campylobacter* LysRS was able to synthesize the required levels of aminoacylated lysyl-tRNA for growth.

**Lysyl-tRNA synthetase activity.** *E. coli* MH318 has been shown to contain about a 1% level of wild-type LysRS activity (11), but in our assays the level was undetectable (Table 1). In our assays, *E. coli* C600, which was used as the wild-type cells, was shown to contain a specific activity of 0.11 U. When C600 was grown in the presence of 3 mM glycyl-DL-leucine, the specific activity was increased 2.4-fold, but LysRS activity was not elevated in heat-induced cells. MH318 cells transformed by pK0-1L were shown to have a specific activity of 0.38, which is 3.5-fold higher than that of C600. This finding supports the conclusion of the complementation experiment that the *Rsa*I fragment in pK0-1L contains a functional *lysS* gene. Because the *lys* gene of *C. jejuni* is unlike the *E. coli lysU* gene in that it was not inducible by glycyl-DL-leucine or by heat, it is designated *lysS*. The level of LysRS in the *C. jejuni* extract was only about 12% of that of *E. coli* C600 (see footnote *b* of Table 2).

**Southern blot analysis.** To determine whether there is another LysRS-encoding gene in *C. jejuni*, as there is in *E. coli*, Southern blot analysis was performed, with the 1.6-kb *Hin*dIII fragment of pCP3b used as the *lysS* probe. *C. jejuni* DNA cleaved by *Eco*RV or *Hin*dIII and *E. coli* DNA digested by *Eco*RV were separated in an agarose gel and transferred onto a GeneScreen membrane. The *lysS* probe hybridized with two *C. jejuni Eco*RV fragments of 11.2 and 6.4 kb (Fig. 5). This result is to be expected if there is only a single copy of the *lysS* gene, which has a unique *Eco*RV site within the *lysS* probe. The 1.6-kb *Hin*dIII fragment of pCP3b contains 1.3 kb of *C. jejuni* DNA and 0.32 kb of pBR322 sequence. This probe hybridized with a single 1.4-kb *Hin*dIII fragment of *C. jejuni* DNA, thus again indicating a single gene copy of the *lysS* gene. Two *Eco*RV DNA fragments (7.5 and 5.1 kb) of *E. coli* weakly hybridized with the campylobacter *lysS* probe. Since the *E. coli lysS*

TABLE 2. Lysyl-tRNA synthetase activity

| Strain | Plasmid | Conditions[a] | Sp act (pmol/min/μg) | Relative activity[b] |
|---|---|---|---|---|
| C600 | | 37°C[c] | 0.11 ± 0.04 | 1 |
| C600 | | GLY-LEU[d] | 0.26 ± 0.06 | 2.4 |
| C600 | | 46°C[e] | 0.12 ± 0.04 | 1.1 |
| MH318 | | 37°C | 0 | |
| MH318 | | GLY-LEU | 0 | |
| MH318 | | 46°C | 0 | |
| MH318 | pK0-1L | 37°C | 0.38 ± 0.10 | 3.5 |
| MH318 | pK0-1L | GLY-LEU | 0.41 ± 0.07 | 3.7 |
| MH318 | pK0-1L | 46°C | 0.29 ± 0.04 | 2.6 |

[a] GLY-LEU, glycyl-DL-leucine.
[b] LysRS activities of extracts of *C. jejuni* grown at 37°C and at 37°C with 2 h at 46°C were 0.013 and 0.009, respectively.
[c] LysRS activity in SMM at 37°C.
[d] LysRS activity in SMM containing 3 mM glycyl-DL-leucine.
[e] LysRS activity in SMM at 46°C for 2 h.

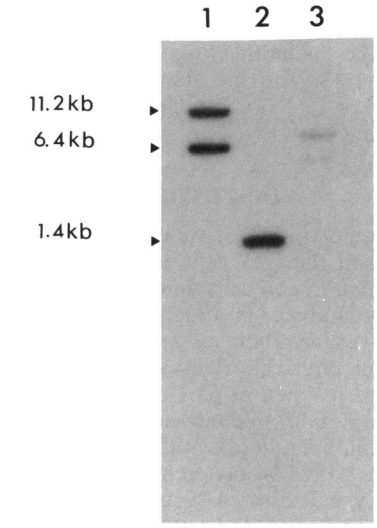


FIG. 5. Southern blot analysis of chromosomal DNA of *C. jejuni* (lanes 1 and 2) and *E. coli* (lane 3). The DNA was digested with *Eco*RV (lanes 1 and 3) or *Hin*dIII (lane 2). The fragments were separated by gel electrophoresis, transferred to GeneScreen, and probed with a radiolabelled 1.6-kb *Hin*dIII fragment of pCP3b (Fig. 1) as described by Maniatis et al. (22). The *C. jejuni* DNA fragments that hybridized with the probe are marked by arrowheads, and the sizes are given on the left margin.

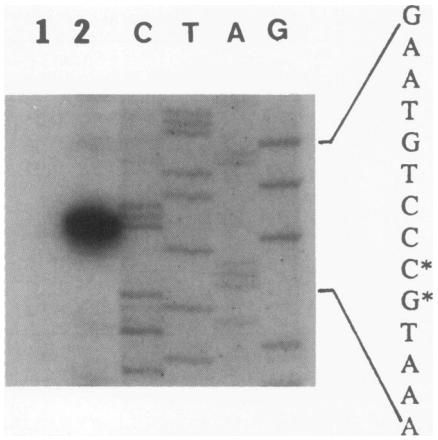


FIG. 6. Primer extension mapping of the transcription start site of the *lysS* mRNA. Lane 1 shows the JM101 mRNA extension, which is negative, and lane 2 shows the primer extension products of JM101 containing pCP3b. Lanes C, T, A, G are the chain-terminated products showing the complementary sequence of the transcription start site area. The nucleotides corresponding to the transcription start sites are indicated with asterisks.

and *lysU* genes do not contain an *Eco*RV site, the two *Eco*RV hybridizing bands likely represent these two genes.

**Identification of the transcription start site of the *lysS* mRNA.** The 5′ end of the *lysS* mRNA was determined by the primer extension method. A 30-mer oligonucleotide (lys-1) complementary to the LysRS RNA was end labelled and hybridized to RNA isolated from JM101 and from JM101 containing the plasmid pCP3b. The oligonucleotide was chosen from an area which displayed minimal homology with the *E. coli lysS* and *lysU* genes. The size of the extended product was determined on a sequencing gel. Sequencing ladders of pCP3b which were established by using the oligonucleotide lys-1 as a primer were used as size markers. As can be seen from Fig. 6, the extended product of JM101 containing pCP3b produced a doublet corresponding in size to the nucleotides marked with asterisks. Transcription of the *lysS* gene thus starts with a G or C located at nucleotides 135 or 136, 7 or 8 nucleotides downstream of the putative Pribnow box (Fig. 2).

## DISCUSSION

This study reports the identification, cloning, and complete sequence of the *lysS* gene of *C. jejuni*. Lysine is the only amino acid in *E. coli* cells that has two aminoacyl-tRNA synthetases to catalyze its attachment to the Lys transfer RNAs. These two synthetases are encoded by two highly homologous but physically well-separated genes, *lysS* and *lysU*, which are mapped at 62.1 min (8) and 93.5 min (32), respectively, in *E. coli*. In contrast, *C. jejuni lysS* appears to be the only gene encoding LysRS, giving a polypeptide of 501 amino acids with a predicted molecular weight of 57,867. The *C. jejuni* LysRS shows 47.9 and 46.6% identity to the *E. coli lysS*- and *lysU*-encoded synthetase, respectively. The high homology observed at the primary sequence level for the *C. jejuni* and *E. coli* LysRS proteins appears to be maintained at the quaternary structure level, since the *C. jejuni lysS* gene could complement the *E. coli lysS* and *lysU* mutations. Moreover, the *C. jejuni* LysRS was shown to be active in the in vitro assays that used *E. coli* Lys transfer RNA as a substrate.

The *C. jejuni* LysRS, like all the class II aminoacyl-tRNA synthetases, contains the three characteristic conserved motifs which have been proposed to cooperate in the formation of a functional domain involved in aminoacylation and in forming specific secondary and tertiary structure(s) (9). The alignment of LysRS from *E. coli*, *C. jejuni*, and *S. cerevisiae* identified two other highly conserved sequences besides the three motifs. These two sites, at amino acids 153 to 166 and 206 to 242, are likely involved in the formation of the domain(s) for specifically recognizing Lys and lysyl-tRNAs.

The *C. jejuni lysS* gene is located immediately upstream of the *glyA* gene, with the carboxyl-terminal codon of *lysS* overlapping 1 base of the Met initiation codon of *glyA*. The overlapping of these two genes is intriguing and may be selected for in evolution when efficient usage of limited DNA sequence is required. It is pertinent to point out that the genome of *C. jejuni* has been estimated to be about 1,700 to 1,900 kb (5, 18, 26), while the *E. coli* genome is about 4,550 kb (19, 29).

Our previous studies (3, 4) showed that the *glyA* gene of *C. jejuni* has a functional promoter in *E. coli* cells, and the transcription start site was mapped at an A nucleotide 35 residues upstream of the Met codon (ATG). The transcription start site of *glyA* is thus located within the encoding sequence of *lysS*. Upstream from the transcription start sites of the *lysS* gene, a putative Pribnow box, and a −35 consensus nucleotide sequence of the *E. coli* housekeeping genes (12) can be observed (Fig. 2). Unlike the *glyA* promoter, the −35 consensus sequence is located 17 nucleotides upstream from the Pribnow box, which is within the usual distance of 17 to 18 nucleotides in *E. coli* promoters. The expression of the *lysS* gene cloned into pK0-1 and the activation of the CAT gene by the 1.6-kb *Hin*dIII fragment cloned into pKK232-8 indicate the presence of an active promoter in the flanking sequence. The *lysS* and *glyA* genes could be potentially expressed independently or as a single transcription unit. Could the synthesis of LysRS be affected by the translation of the *glyA* gene? We are presently analyzing the regulation of expression of these two genes and examining the frequency of overlapping genes on the *C. jejuni* genome.

## ACKNOWLEDGMENTS

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