An isoleucyl-tRNA synthetase gene from *Campylobacter jejuni*

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A complete isoleucyl-tRNA synthetase gene (*ileS*) of *Campylobacter jejuni* was isolated from a *C. jejuni* TGH9011 genomic DNA library constructed in pBluescript. The complete coding sequence, flanking regions and transcription start point were determined. The deduced isoleucyl-tRNA synthetase (IleRS) had 917 amino acids with a molecular mass of 105399 Da, which was consistent with the observed size of 105 kDa in *Escherichia coli* maxicells. The *ileS* gene was mapped onto the physical map of the *C. jejuni* genome. Alignment of the *C. jejuni* IleRS sequence with six other bacterial IleRS sequences and two lower eukaryotic IleRS sequences identified seven conserved motifs, including the two signature sequences, HIGH and KMSKS, of class I aminoacyl-tRNA synthetases.

Keywords: isoleucyl-tRNA synthetase (IleRS), alignment of bacterial IleRS, conserved motifs

INTRODUCTION

The *ileS* gene encodes isoleucyl-tRNA synthetase (IleRS), an essential enzyme needed for the aminoacylation of isoleucyl tRNA. The 20 aminoacyl-tRNA synthetases (aaRSs) have been divided into two classes based on some conserved amino acid sequences and structural motifs (Webster *et al.*, 1984; Ludmerer & Schimmel, 1987; Schmidt & Schimmel, 1993; Eriani *et al.*, 1990; Cusack *et al.*, 1990; Burbaum & Schimmel, 1991a; Chalker *et al.*, 1994). IleRS is a class I aaRS enzyme that contains some well-conserved domains contributing to the catalytic core of the enzyme (Shiba & Schimmel, 1992; Burbaum & Schimmel, 1991b; Rould *et al.*, 1989). A conserved domain, the HIGH tetrapeptide, is located close to the N-terminus. A more conserved domain, KMSKS, is located around 600 amino acids away from the N-terminus (Moras, 1992; Shepard *et al.*, 1992).

ileS genes have been isolated and completely sequenced from *Escherichia coli* (Webster *et al.*, 1984), *Staphylococcus aureus* (Chalker *et al.*, 1994; Hodgson *et al.*, 1994), *Methanobacterium thermoautotrophicum* (Jenal *et al.*, 1991), *Saccharomyces cerevisiae* (Martindale *et al.*, 1989) and from a protozoan *Tetrahymena thermophila* (Csank & Martindale, 1992). Partial sequences of the same gene from *Pseudomonas fluorescens* (Isaki *et al.*, 1990a) and *Enterobacter aerogenes*

(Isaki *et al.*, 1990b) have also been published. *Campylobacter jejuni* is a major causative agent of bacterial diarrhoea in humans. Despite this, molecular biological understanding of *C. jejuni* is in its infancy. To date, only a handful of genes have been isolated, sequenced and characterized (Hani & Chan, 1994; Louie & Chan, 1993; Pei & Blaser, 1993; Labigne *et al.*, 1992; Chan & Bingham, 1991, 1992; Fisher & Nachamkin, 1991; Nuijten *et al.*, 1990; Kim & Chan, 1989).

In this study, we report the isolation, mapping and characterization of the *C. jejuni* *ileS* gene. The deduced IleRS sequence was compared to IleRS sequences of other organisms with the aim of identifying conserved motifs.

METHODS

Bacterial strains and other materials. *C. jejuni* TGH9011 (ATCC 43431, serotype reference strain for O:3) was obtained from J. L. Penner (University of Toronto, Toronto, Ontario, Canada). *E. coli* DH5 α (this laboratory) and *E. coli* DR1984 (Sancar *et al.*, 1979) were grown routinely in LB broth; LB and M9 minimal agar medium were supplemented with nutrients and/or antibiotics as required; when appropriate, ampicillin was added to a final concentration of 50 $\mu\text{g ml}^{-1}$. All restriction endonucleases and other enzymes were purchased from either Pharmacia or Boehringer Mannheim. [α - ^{32}P]ATP, [γ - ^{32}P]ATP and [^{35}S]methionine were from ICN Biomedicals. The sequencing kit was purchased from United States Biochemical.

***ileS* gene isolation.** The *C. jejuni* chromosomal DNA library used was constructed previously in pBluescript (H. L. Bingham & V. L. Chan, unpublished data). One particular *C. jejuni* DNA

Abbreviation: aaRSs, aminoacyl-tRNA synthetases.

The GenBank accession number for the nucleotide sequence reported in this paper is U15295.

clone containing a complete *ileS* gene, designated as E8-1 (8.85 kb), was isolated as a false positive clone in screening with a ^{32}P -labelled PCR fragment that was obtained from two degenerate primers designed for the isolation of the *dnaA* gene.

Restriction mapping and subcloning. The *Hind*III and *Eco*RV sites in the *C. jejuni* genomic DNA insert in E8-1 were mapped by the partial digestion method (Smith & Birnstiel, 1976). Three *Hind*III fragments (2 kb, 0.3 kb and 1 kb) at the 3' end of the E8-1 insert and two *Eco*RV fragments were subcloned individually into pUC19 and propagated in *E. coli* DH5 α .

Construction of deletion derivatives. A unidirectional DNA deletion with the Erase-a-Base system was accomplished essentially as described by Henikoff (1984). The above 2 kb and 1 kb *Hind*III subclones were digested with *Kpn*I and *Sal*I to generate a 3' and a 5' overhang, respectively. Approximately 2 μg of the linearized DNA was then digested with exonuclease III and S1 nuclease, ligated with T₄ DNA ligase and then transformed into *E. coli* DH5 α cells to generate the nested deletion clones.

DNA sequencing analysis. Both strands of the *ileS* gene were sequenced using the dideoxy chain-termination method of Sanger *et al.* (1977) and the Sequenase Version 2.0 from United States Biochemical.

Primer extension analysis. A primer extension analysis was performed to locate the transcription start site. RNA was extracted from *C. jejuni* by the hot phenol method (Aiba *et al.*, 1981). Oligonucleotide 5' TCAAGTTCCGCTAAATTCG-CCCGC 3' is complementary to the coding strand of *ileS* and is located at nucleotides 48–71 downstream from ATG, the initiation Met codon of the *ileS* gene. This oligonucleotide was end-labelled with [γ - ^{32}P]ATP and polynucleotide kinase. Labelled oligonucleotide (30 ng) (approximately 1×10^7 c.p.m.) was mixed with 25 μg total RNA of *C. jejuni*. The mixture was hybridized and extended as described previously by Chan & Bingham (1992). For analysis, 4 μl of the newly synthesized DNA (previously suspended in 8 μl formamide loading buffer) was loaded onto a 6% (w/v) polyacrylamide sequencing gel alongside dideoxy sequencing ladders of the upstream flanking region of the *ileS* gene.

Analysis of plasmid-encoded protein. The *ileS* gene from *C. jejuni* was expressed in *E. coli* DR1984 by the maxicell protocol described by Sancar *et al.* (1979). E8-1-bearing DR1984 cells were grown in M9 medium plus 1% (w/v) Casamino acids and were UV-irradiated at a cell concentration of 2×10^8 ml $^{-1}$ for 30 or 40 s with a 15 W germicidal lamp set at a height of 50 cm. The survival was between 10^{-5} to 10^{-6} following a 15 h incubation in M9 medium containing 200 μg D-cycloserine ml $^{-1}$. Proteins encoded by the plasmid DNA were labelled with [^{35}S]methionine and resolved by SDS-PAGE (1%, w/v, SDS; 6%, w/v, polyacrylamide) (Laemmli, 1970) and visualized by autoradiography.

IleRS assay. In order to test the function of the IleRS expressed by the *ileS* gene, recombinant clone E8-1 was transformed into *E. coli* 331c $^{-}$ (Isaksson *et al.*, 1977), which is a temperature-sensitive strain due to a mutation of the chromosomal *ileS* gene (kindly provided by Dr H. C. Wu, Uniformed Service University of the Health Sciences, Bethesda, MD, USA). Both recipient *E. coli* 331c $^{-}$ and E8-1-bearing 331c $^{-}$ cells were grown in LB medium at 32 °C, 37 °C, 40 °C and 42 °C. The activity of IleRS was measured by its ability to charge tRNA with L-[^3H]isoleucine, as described by Chan & Bingham (1992) with minor modification. In a 100 μl reaction volume, 0.64 μM ATP, 40 μM Tris/HCl (pH 8.6), 4 μM magnesium acetate, 0.000044 μM L-[4,5- ^3H]isoleucine and 80 μg *E. coli* tRNA were

used. Crude cell extract (10 μg) from 331c $^{-}$ or E8-1-bearing *E. coli* 331c $^{-}$ was added and incubated at 25 °C for 15 min. The reactions were terminated by adding 20 μl 5% (v/v) trichloroacetic acid/0.05% Casamino acids and the complete reaction mixtures were spotted onto 2.4 cm 3 MM Whatman disks. The disks were washed three times with 10 ml ice-cold 5% trichloroacetic acid/0.05% Casamino acids, twice with 10 ml 95% (v/v) ethanol, dried and counted in a liquid scintillation system.

Mapping of the *ileS* gene in the *C. jejuni* genome. The *ileS* gene was mapped by Southern hybridization using a ^{32}P -labelled 2 kb *Hind*III fragment of E8-1 as the probe. The *C. jejuni* genome was digested with *Sma*I, *Sal*I and *Sac*II and fragments were resolved by PFGE as described by Kim *et al.* (1992).

RESULTS

Cloning of the *ileS* gene

Two 17-mer degenerate primers (GAG/AGAG/ATTATTACAC/TAC and GTG/ATGATCT/ACG/TNCCNCC) synthesized on the basis of two conserved motifs of the DnaA protein of eubacteria amplified a 600 bp fragment which was used to screen the pBluescript genomic library of *C. jejuni*. Clone E8-1 was one of the hybridizing clones and has an insert of about 6 kb. The *Hind*III and *Eco*RV sites in the E8-1 insert were mapped. Southern hybridization using the PCR-amplified fragment as a probe localized the hybridizing region to the 3' terminal *Hind*III fragment of the insert. This region was sequenced and shown to have high homology to the C-terminal IleRS sequence of various organisms as determined in a database search using the Blast program (Altschul *et al.*, 1990). The complete IleRS coding sequence was shown to be localized to three adjacent *Hind*III fragments (2 kb, 0.3 kb and 1 kb).

Nucleotide sequence of the *ileS* gene

The complete IleRS-coding sequence was determined to show a 2751 nucleotide ORF which encodes a 917 amino acid protein with a predicted molecular mass of 105399 Da. The N-terminal sequence of the *C. jejuni* IleRS and its 5'-flanking region are shown in Fig. 1, where the proposed start codon is indicated. Eleven nucleotides

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GATAAAGCAAATGTTTCATAAATTTTCCTTTTCTTATAATTATAGCC 50
                                     ^
AAAAACAAGTAGAAATTAAAGCTAATTGAGTAGAATTAAAGATTATTA 100
                               -35          PB
AAAATTAGGTGGAAAAATGGACTACAAAGAACTCTACTTTTGCCGAGC 150
                               SD          M D Y K E T L L L P S
ACTACTTTTGCTATGCGGGCGGAATTAGCGGAAGTGAACCGCAAGATT 200
T T F A M R A N L A E L E P Q R F

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Fig. 1. The N-terminus of the *C. jejuni* IleRS coding sequence and its flanking region. The transcription start site, residue A (located at nucleotide 94), is marked by an inverted solid triangle. The potential Shine–Dalgarno sequence (SD), Pribnow box (PB) and –35 consensus regions are indicated by solid underlining.

Fig. 2. Physical map of *C. jejuni* TGH9011 (Kim *et al.*, 1993). The location of the *ileS* gene was identified by hybridization of the 2 kb *HindIII* fragments of E8-1 to *SalI* A, *SacII* A and *SmaI* A bands of *C. jejuni* DNA resolved in agarose using PFGE.

upstream from the start codon, a sequence, AGGTGG, matches five of the six nucleotides of the Shine–Dalgarno sequence.

The 5' end of the *ileS* mRNA was located by primer extension at nucleotide 94, residue A in the sequence (see Fig. 1). Eleven nucleotides upstream of the transcription start point is a sequence, TAGAAT, which matches five of the six nucleotides of the Pribnow box and 33 nucleotides upstream is a sequence, TAGAAA, which matches four of the six nucleotides of the consensus –35 sequences.

Maxicell analysis

Proteins encoded by the 8.8 kb E8-1 clone were determined using the maxicell system. pBluescript DNA encoded 31 kDa and 28 kDa proteins representing precursor and mature β -lactamase, respectively. An extra polypeptide of 105 kDa was encoded by the E8-1 clone which likely represents the IleRS protein as it conforms to the size predicted from the gene sequence.

Temperature complementation test and enzyme assay

Plasmid E8-1 containing the complete *ileS* gene was unable to restore growth of *E. coli* 331c[–] at 42 °C. However, the enzyme assay revealed an approximately threefold increase in IleRS activity after *E. coli* 331c[–] was transformed with E8-1. The IleRS activities in extracts of E8-1 transformed and nontransformed *E. coli* 331c[–] cells were completely inactivated after 5 min incubation at 50 °C (data not shown), suggesting thermal sensitivity of the *C. jejuni* TGH9011 IleRS proteins. IleRS thermal

sensitivity may have contributed to the failure of complementation of the *E. coli* temperature sensitive mutant.

Mapping of the *ileS* gene in *C. jejuni* genomic DNA

The genomic size of *C. jejuni* TGH9011 is approximately 1812 kb (Kim *et al.*, 1993). The mapping of the *ileS* gene in the *C. jejuni* genome showed that the *ileS* gene probe hybridized to the PFGE *SmaI* A, *SalI* A and *SacII* A fragments. Thus, the *ileS* gene is located in the 1360–1812 kb region of the genomic map of *C. jejuni* TGH9011 (Fig. 2).

Comparison of the *ileS* gene from different organisms

The amino acid sequence encoded by the *C. jejuni* *ileS* gene showed a high level of homology to the *ileS* gene product of *E. coli* (Webster *et al.*, 1984; Yu *et al.*, 1984), *Staph. aureus* (Chalker *et al.*, 1994; Hodgson *et al.*, 1994), *M. thermoautotrophicum* Marburg (Jenal *et al.*, 1991), *Sac. cerevisiae* (Martindale *et al.*, 1989) and *T. thermophila* (Csank & Martindale, 1992) as shown in Fig. 3.

Two signature sequences, HIGH and KMSKS, of class I aaRS can be seen in Fig. 3 at amino acids 66–69 and 610–614 in IleRS. Five other conserved motifs can also be observed at amino acids 95–104, 235–242, 319–333, 465–477 and 569–578 in IleRS.

DISCUSSION

A *C. jejuni* pBluescript recombinant clone, E8-1, was shown to contain the *ileS* gene. This gene is 2751 bp in size and the sequence predicts an IleRS protein of 917 amino acids with a molecular mass of 105 399 Da. Maxicell analysis provided support for a molecular mass of 105 kDa. The size of the *C. jejuni* IleRS enzyme is similar to that of the other bacteria. For instance, the IleRS enzymes of *E. coli* K12 and *Staph. aureus* are 939 amino acids (Webster *et al.*, 1984) and 918 amino acids (Chalker *et al.*, 1994), respectively. On the other hand, the IleRS protein of *M. thermoautotrophicum* (1045 amino acids) and a number of eukaryotic IleRS proteins including *Sac. cerevisiae* (1072 amino acids) (Martindale *et al.*, 1989) and *T. thermophila* (1081 amino acids) (Csank & Martindale, 1992) are somewhat longer.

Despite differences in the size of IleRS enzymes among different species, their sequences show a high degree of homology (Fig. 3). Multiple alignment analysis of all the IleRS sequences reported so far (Webster *et al.*, 1984; Chalker *et al.*, 1994; Hodgson *et al.*, 1994; Jenal *et al.*, 1991; Martindale *et al.*, 1989; Csank & Martindale, 1992; Isaki *et al.*, 1990b; gene accession number S40549 in GenBank) identified seven conserved motifs. Two domains among the motifs, the 'HIGH' in motif I and 'KMSKS' of motif VII, are conserved in almost all class I aaRSs. Among the four residues HIGH in motif I, only the first histidine and the third glycine were almost invariant and were involved in ATP binding (Moras, 1992). Our alignment analysis proved that the H and G

		I		II	
Cj	1	M-DYKETLLPSTTFAMRANLAELEPQRFKWFQNYAYEKKMKENRKNNAKSTFLHDGPPYANGHIHGHALNKILKETI IKTHYFKGESVRPTPGWDCH			
Sa1	1	M-DYKETLLMPKTDFFPMRGGLPNKEPQIQEKWDAEDQ-YHKALEKKN-GNETFILHDGPPYANGNLHMGHALNKILKDFIVRYKTMGGFYAPVPGWDTH			
Ec1	1	MSDYKSTLNLPEGTGPMRGDLAKREPGLMARWTDDDL-YGIIRAARK-GKKTFFILHDGPPYANGSIHIGHSVNKILKDIIVKSKGLSGYDSPVPGWDCH			
Ec2	1	MSDYKSTLNLPEGTGPMRGDLAKREPGLMARWTDDDL-YGIIRAARK-GKKTFFILHDGPPYANGSIHIGHSVNKILKDIIVKSKGLSGYDSPVPGWDCH			
Ec3	1	MSDYKSTLNLPEGTGPMRGDLAKREPGLMARWTDDDL-YGIIRAARK-GKKTFFILHDGPPYANGSIHIGHSVNKILKDIIVKSKGLSGYDSPVPGWDCH			
Sa2	1	LTKK-----YLTQNEISAFWNTQK-IFKKSIDNRKQGES-FVFYDGPPTANGLPHAGHVLGRVTKDLVARLKTQGGFYVERKAGWDTH			
Sc	1	MSESNAHF-----S-----FPKEEKVLSLWDEID-AFHTSLELTCKDPE-FSFFDGPFFATGTGPHYGHILASTIKDVVCRYASQNGKYVERRFPGWDTH			
Tt	1	MA-SKTTTF-----KDLQIPDFPKEENILKFWDEIN-AFKQOLEKTKDCPP-FTFYDGPFFATGLPHYGNLLAGTIKDVVCRYASQNGKYVERRFPGWDTH			
Mt	1	MPIQEAER-----SYKPHV-----IEEKVQSFWEERD-IYERVKELREEGPR-YSFLDGPFFYCSGRHILGTAMNKIMKDSYLRPKSMRGNVRRQPGWDCH			
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Cj	100	GLPIEQQVEVKLEKKK-----SLSKKEIREFCROHASEFVDIOREEFKNLGIADWDKPYLTMKF-EFEAAIYRTLCEIAKKGLLCERSKPFVWSWAAK			
Sa1	98	GLPIEQALTKK-GVDRK-----KMSTAEFRECKCEFALEQIELQKDFRRLGVGRGDFNDPYITLK-PEYEAQIRIFGEMADKGLIYKGGKPVVWSPSE			
Ec1	99	GLPIELKVEQEYKPGK-----KPTAAEFRAKCREYAAATQVDGQRKDFIRLGLVLDGWSHPYLTMDPKT-EANIIRALGKIIGNHGLHKGAKPVHWCDCR			
Ec2	99	GLPIELKVEQEYKPGK-----KPTAAEFRAKCREYAAATQVDGQRKDFIRLGLVLDGWSHPYLTMDPKT-EANIIRALGKIIGNHGLHKGAKPVHWCDCR			
Ec3	99	GLPIELKVEQEYKPGK-----KPTAAEFRAKCREYAAATQVDGQRKDFIRLGLVLDGWSHPYLTMDPKT-EANIIRALGKIIGNHGLHKGAKPVHWCDCR			
Sa2	83	GLPVELEVEKKIGIKGQDIE-KYGIENFINECKSVFNVEKWRDPSKDLGYWVDMDSPIYITL-NNYIESVWNILSTPHKKGLLYKGHVTPYCTHDQ			
Sc	88	GVPIEHIIIDKKLGITGKDDVF-KYGLENYNECRSIVMTYASDWRKTIGRLGRWIDFDNDYKTMV-PSFMESTWMAFKQLHEKGQVYRGFKVMPYSTGLT			
Tt	94	GLPVEYEIDKKLGITNRQEV-LKMGVDKYNACRSIVMRYAQEWSRVNRFGRWVDFDNDYKTL-DLKFMSVWVWFKQMFDKGLVYRGCKVMPYSNGCA			
Mt	90	GLPIEHKVEGLGVRSKKDIEDKIGIEEFVRKCREFAMENKAVMTSQFQRLGVWMDWDDPYVTFD-PAYMESCWTLKRAHEKDLLLRDLRVITWCPRCE			
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		III			
Cj	194	SALAAAEVEYQDKEDYSIFAVD-LDVKACK-KLGVSUSA-----VIWTTTTPWTLVANQAIALNPENYV-----ITKEGLIFASALLKSMVEK---GLT			
Sa1	192	SSLAAAEIEYHDKRSASIVAFNVKDDKG-----VDADAKFIWTTTTPWTLPSNVAITVHPELKYQ---YVNGEKYIIAEALSDAVAEALDWDKA			
Ec1	193	SALAAAEVEYDKTSPSIDVAFQAVDQDALKAKFAVSNVNGPISLVIWTTTTPWTLPANRAISIAPDFDYALV---QIDGQAVILAKDLVESVMQRIQ---VT			
Ec2	193	SALAAAEVEYDKTSPSIDVAFQAVDQDALKAKFAVSNVNGPISLVIWTTTTPWTLPANRAISIAPDFDYALV---QIDGQAVILAKDLVESVMQRIQ---VT			
Ec3	194	SALAKKISITIKLLRPSTLLSRAVDQDALKAKFAVSNVNGPISLVIWTTTTPWTLPANRAISIAPDFDYALV---QIDGQAVILAKDLVESVMQRIQ---VT			
Sa2	181	TALSSSEVA-----CGYKNVLDLAVVFKLTN-SKDTYFSLSWTTTTPWTLPANVALINKDLNYSKI---RVENEYIILATDLINSIT-----E			
Sc	186	TPLSNFEAQ-----QNYKDNDPAVTIGFNVIG-QEKTQLVAVTTTTPWTLPSNLSLCNADFEYVKIYDETRDRYFILLLESILIKLYKKPK--DG			
Tt	192	TVLNFEQ-----QNYKEVDDPSLFIAPKTA-EPTKTFIAWTTTTPWTLPSNLALVINKDFDYKVLDAKTQEHYILAECLRLPELYKKDK--NG			
Mt	189	TALALAEID-----YHEKEDPSIYKFPVSG-D---TYILVWTTTTPWTLPANMAVAVHPDFDYAHT---RLDGETYIMAEALVEKVL-----GE			
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		IV			
Cj	280	SGEIQKELNAKEFEKLEAINPL-----NGRSVLIMGEHVLMDGSGSLVHTAPGHGEDDYACLYGI-----EVLMPVDDSGCYDETIRAKRLPLSH			
Sa1	282	SKLEKEYTGKLEYVVAQHFF-----LDRESLVINGDHVTTDAGTCVHTAPGHGEDDYIVGQKYEL-----FVISPIDDKGVF-----TEEG			
Ec1	289	DYTLGTGKADVELLRFTHPF-----MGFDVPAILGDHVTLDAGTGAHVHTAPGHGPDYVIGQKYGL-----ETANPVGPDGTY-----LPGT			
Ec2	289	DYTLGTGKADVELLRFTHPF-----MGFDVPAILGDHVTLDAGTGAHVHTAPGHGPDYVIGQKYGL-----ETANPVGPDGTY-----LPGT			
Ec3	290	D-SRHGKRCGAGAAA-----FTHPF-----MGFDVPAILGDHVTLDAGTGAHVHTAPGHGPDYVIGQKYGL-----ETANPVGPDGTY-----LPGT			
Sa2	262	KYELIDTFSGSNLNLKYIP-----PFESDGLVNAYY-VVDGEFVTSNBTGIVHIAHAGHEDDYQLVLERDL-----DFLNVITREGVYNDRF-----			
Sc	273	KYKIVEIKIGSDLVGLKYEPFPYFAEQPHETAFRVISDDYVTSDDSGTGIVHNAAPAFGEEDNAAELKNGVISEDVLPNAIDLGFTKDV-----			
Tt	279	--YKILEKFGSELVGREYEPFPYFLSRKQDGFRLAGDFTADAGTGVHCAFGDGDYKVSANNIIPKDDP-PVPVDENGHTNVV-----			
Mt	266	EAEI IKTVRGSELEGLTYRHPDLDEVPCHRDMEHRVILGDHVTLDAGTGAHVHTAPGHGPDYVIGQKYGL-----PVFCVDEAGVFTEDA-----			
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Cj	368	LLEEFIGLHIFKANKEILELGEK--LLHSSKFISHPFCWRTHKPVIRATKQWFIILMDEPKLQKGTILRECAKEQLLKTTFYQSGV-KRIGSMVENRP			
Sa1	361	--GQFEGMFPYDKANKAVTDLLEKQALLKLDFTTHSYPHDWRKTKPVIFRATPQWFAISK--VRQDILDAIENNTN-----FKVNWGK-TRIYNMVRDRG			
Ec1	368	Y-PTLDGVNVFKANDIVVALLQEKGALLHVEKMQHSYPCWCRHKPTIIFRATPQWFSMDQKGLRAQSLKEIKGVQ-----WIPDWGQ-ARIESMVANRP			
Ec2	368	Y-PTLDGVNVFKANDIVVALLQEKGALLHVEKMQHSYPCWCRHKPTIIFRATPQWFSMDQKGLRAQSLKEIKGVQ-----WIPDWGQ-ARIESMVANRP			
Ec3	367	Y-PTLDGVNVFKANDIVVALLQEKGALLHVEKMQHSYPCWCRHKPTIIFRATPQWFSMDQKGLRAQSLKEIKGVQ-----WIPDWGQ-ARIESMVANRP			
Sa2	345	--PELVGNKAKNSDIEIKLLSKQLLYKQYEHNYPHCRGCPNPLIYAMEGFWIKTIN--FKNEIINNINIE--WPFSSHKEGRMGFNLENMV			
Sc	364	--PDFEGVVKADKLI I KYLTNTGNLLASQIRHSYPPCWSRDTPLLYRSVPWFVVRKN--IVPQMLDSVMKSH--WVPNTIKEKRFANWIANAR			
Tt	368	--SDFAGVYIKEADKLIIRKNLKERGLLLVDSSEFKHNYPPCWSRDTPLIYKAVHCWFVKTAA--LKDDLANNKAY--WVPKFAQEGRFNNWLNQVS			
Mt	352	--GKYRGLFKDADSDIIDLRSKNLLLRAETISHRYGFCWRCKPTIYILATEQWFLKITE--IKDKMLSELDRVQ-----WIPSWAGESRFNNWIANAR			
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		V			
Cj	465	DWCISRQRWGTPIAIFFRDKNTKEVI-FDD-ELFDFVAAIFEKHGADAWWEFEIKDLIP---TNSKYNAENLEKQVYDILDVWFDGSGSTFNVLNSGLYD-			
Sa1	451	EWVISRQRWGVPLPVFYAENGIEIM-TK--ETVNVHADLFAEHGNSIWFEREAKDLLPEGFTHPGSPNGTFTKETDMDVWFDGSGSHRGVLETRP-E-			
Ec1	461	DWCISRQRWGVPLPVFVHKDTEELH-PTLELMEEVAKRVEVDGQIQAOWDLDAKEIL-----GDEADQYVYKVPDITLDVWFDGSGSTHSSVVDVRP-E-			
Ec2	461	DWCISRQRWGVPLPVFVHKDTEELH-PTLELMEEVAKRVEVDGQIQAOWDLDAKEIL-----GDEADQYVYKVPDITLDVWFDGSGSTHSSVVDVRP-E-			
Ec3	460	DWCISRQRWGVPLPVFVHKDTEELH-PTLELMEEVAKRVEVDGQIQAOWDLDAKEIL-----GDEADQYVYKVPDITLDVWFDGSGSTHSSVVDVRP-E-			
Sa2	436	DWNIENRNRWGTPLNVVINCNDCHYEAPSSIKDLQNNINKIDEDIELHRPVDNITLS-----CPKNGKMSRVEEVDVWFDGSGSMPPAQHHY-PFD-			
Sc	455	DWNVSRNRWGTPIPLWVSDDFEVVCVGSIELEE--LTGVNRTDLHRVDIDKLTIP-----SKQKGDLKRIEVEFDCWFESGSMPPAQHHY-PFE-			
Tt	459	DWCFSRSRWGNPIPLWVSDDFEVVCVGSIELEE--LTGATEITDLHRVDIDKLTIP-----SKQKGDLKRIEVEFDCWFESGSMPPAQHHY-PFSM-			
Mt	443	DWTISRQRWGTPIPLWVSDDFEVVCVGSIELEE--LTGATEITDLHRVDIDKLTIP-----CGRCGRMRKPTDVLVDVWFDGSGVAGWAAHY-P--R			
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		VI		VII	
Cj	559	-----AGEKRASMYL-EGSDQHRGWFOSSLLVGTAINESTPYESILTHGFTTDEKGQKMSKSGNVIAPEYVAKTYGVEILRLWILLSDYS--SDLKISDN			
Sa1	546	-----LS-FPADMYL-EGSDQYRGWFNSITTSVATRGVSPYKFLLSHGFMVDGEGKKMSKSLGNVIVPDQVVKQKADIRLWVSSDYD--ADVRISDE			
Ec1	551	-----FAGHAADMYL-EGSDQHRGWFMSSSLMISTAMKGKAPYQVLTGHTFVDGQGRKMSKSGNTVSPQDVMMKLGADILRLWVASTDYT--GQMAVSDE			
Ec2	551	-----FAGHAADMYL-EGSDQHRGWFMSSSLMISTAMKGKAPYQVLTGHTFVDGQGRKMSKSGNTVSPQDVMMKLGADILRLWVASTDYT--GQMAVSDE			
Ec3	550	-----FAGHAADMYL-EGSDQHRGWFMSSSLMISTAMKGKAPYQVLTGHTFVDGQGRKMSKSGNTVSPQDVMMKLGADILRLWVASTDYT--GQMAVSDE			
Sa2	529	NQKIFNQHPFADFLAEGVDQTRGWFYLSLVTILKGSKSSYKRALSHGLHLDNGKMSKSGNVINPTLEINKYGADSLRWALISDAPMNNK-RFSEN			
Sc	546	NEKTFDRVFNANFISEGLDQTRGWFYLTAVLSTHFGSVFYKKNVIVSGLVLAADGKMSKSGNVIPDPSIVLNKYGADALRLYLINSFVLKAEKLFKEE			
Tt	551	NEEFKSRFPADFIIGEGIDQTRGWFYLTAVLSTALRNSNPKYKNIIVNGIVLAADGKMSKSGNVIPDPSIVLNKYGADALRLYLINSFVLKAEKLFKEE			
Mt	535	EKELFSEWFPYDFITGHDQTRGWFYSLQCGVIALDETPYRVLVLMHGFTLDEBGRKMSKSGNVPEPVDIEKYGADVLRFLYLWANKPW-EDLKFPWD			
*** ** *					

Fig. 3 For legend see facing page.

are completely conserved in all the IleRSs, including in those from Archaea and Eukarya species. Moreover, based on the consensus sequence in class I aaRSs, an 11-mer

peptide that ends in HIGH is highly conserved and is considered a 'signature sequence' for class I aaRSs (Burbbaum & Schimmel, 1991b). Among the IleRSs, this

Cj	652	ILKQV-GEQYRKIRNTIRFLLANTN-----DLKD-----LEVKEFSFIDKWILSRASKVFKAASKEAFFAYEFAKGSFLLLN-FLSADLSGIYLDIS
Sa1	638	ILKQT-SDVYRKIRNTIRFLMLGNIN-----DFNPDTDSIPESSELEVDYRLLNRLREFASTINNYENFDYLNIVQEVQN-FINVELSNFYLDYG
Ec1	644	ILKRA-ADSYRRIRNTARFLLANLN-----GFDPAKDMVKPEEMVVLDRWAVGCAKAAQEDILKAYEAYDFHEVVQRLLMR-FCSVEMVSFYLDII
Ec2	644	ILKRA-ADSYRRIRNTARFLLANLN-----GFDPAKDMVKPEEMVVLDRWAVGCAKAAQEDILKAYEAYDFHEVVQRLLMR-FCSVEMVSFYLDII
Ec3	643	ILKRA-ADTYRRIRNTARFLLANLN-----GFDPAKDMVKREEMVVLDRWAVVVRKRHRKTSRRRTKHTISTKWKYKRLMR-FCSVEMGSFYLDII
Sa2	628	IVAQTQSKFIDTLDNIYKF-----YNNYKID-HYNPNNEITKSR--NTLDNVALSRLNTLIKESNIYVNNYD-FTSAARLINEYT-NTISNWIYIGDS
Sc	646	GVKEVSVKVLPMWNSKFLDGGQIALLKMSNIDFQYDSDSVKSDNVH-----DRWILASMQSLVQFIHEEMGQYKLYTVVPKLLN-FI-DELTNWYIRFN
Tt	651	GVFAVKKIDIFLPWYNAYKFLIQSITRWELATGKDYMFNEQLSVDTTKLTNPNTDRWIIISQCNLINYVRIEMEKYHLNVPRLIH-FL-ENLTNWYIRLN
Mt	634	ELKNV-NKMFNILNWNVVFATT-----YMSLD-RFQPGDHELEDLHFRDEDRWIIISRVNSVALKVTEALDNLH-FHRATREIHDFIVEDLSRWYIRLI
		* * *
Cj	736	KDRLYCDSENAQRKSAQVAMALIAKELLNLLASNLSSVSDEAL-----EHANVLIGD-AKDV-FDLSTLQDFDYDFGIDDTFL-----
Sa1	726	KDILYIEQRDSHIRRSMQTVLVQILVDMTKLLAPILVHTAAEVSHTP-----HVKEESVH-----L-ADMPKVVEVDQALLDKWRTF-----
Ec1	732	KDRQYTPKRTVWARRSCQTALYHIAEALVRWMAPILSPTADEVWGYP-----GEREKYVLTGEWYEG-LGLADSEAMNDAF-----WDEL-----
Ec2	732	KDRQYTPKRTVWARRSCQTALYHIAEALVRWMAPILSPTADEVWGYP-----GEREKYVLTGEWYEG-LGLADSEAMNDAF-----WDEL-----
Ec3	731	KDRQYVAKGHSVARRSCQTALYHIAEALVRWMAPILSPTADEVWGYP-----GEREKYVLTGEWYEG-LGLADSEAMNDAF-----WDEL-----
Sa2	716	RGRFW-EQGISNKKDAYNTLYEILTSLRLVAPFPFISEKIH-----YNLTKSVHLQDYPQYKESFINQALEDEMHTVIKIVELSRQA
Sc	739	RRRLK-GENGVEDCLKALNSLFDALPTFRAMAPPTPFLSESIYLRKLEYIPEAVLAKYKDGGRSVHFLSYPVVKKEYFDEAETAVSRMQSVIDLGRNI
Tt	749	RRRLK-GDYGLEEQETSLNVLNVLNLTILMSPLVFFITESFYQNLKRVIPKG--SSYLEE--SIHFLRIPTPKQELLDKEIRNFERNQNIINFARTL
Mt	724	RSRTW-IERDDPKLAAHYSLYTALKTLTIVLTSPIAPHVCEDIYQNL-----VRGAEPDSPESHIMLDW-MVSEDAVDGKLEAEMDIVREIIEACARA
		..
Cj	814	----MSVREKFFFEQI-----DILKKDKIKSTLELNINISFNKFPNEELADWFMVSQISNE-----
Sa1	803	----MNLRDDVNRAL-----ETARNEKVIKGSLEAKVTI--ASNDKFNASEFLTSTF-DALHQL-----
Ec1	813	----LKVRGEVNKVI-----EQARADNKVGGSLAAAVTL--YAEPELSAK--LTALGDELRFV-----
Ec2	813	----LKVRGEVNKVI-----EQARADNKVGGSLAAAVTL--YAEPELSAK--LTALGDELRFV-----
Ec3	812	----LKVRGEVNKVI-----EQARADNKVGGSLAAAVTL--YAEPELSAK--LTALGDELRFV-----
Sa2	801	RKNADLKIKQPLSKMVKPNSQLNLSFLPNYYSIKDELNIKNIELTDINDY-ITYELKLPSSVGPGLGNK-TKNIQTLDLSLEYDRKSLIESNNF-
Sc	838	REKTTISLKTPLKTLVILHSDSEYLDKVEALKNYIIEELNVRDVVITSEAKYGVYKAVADVPVLGKKLKKD-AKKVKDALPSVTSEQVREYLESGL-
Tt	844	REKRKVSLLKQPIMSLTVINQDQEFHDSLKDYIQYIEDEINTPSILHEINTANY-VDLKAI PNHKLGLGQKLKEYNLDLKAAGNLAKDIETLKTTSI-
Mt	815	RDTARYKLRWPFVREIVVSVEDDKVLEAAESLKGVIABEQANAKSIRTSTEPFD--MRIIAKPNPATLGPQLRQDMPFVMRKLEADGAEVKAALESGSFR
		..
Cj	866	-----NEEILAEFEV-----ENEKFKITRASLC-----KCPRCWKL-----QSKDEETP-----CLRCSEVLKGIQ---
Sa1	854	-----FIVSQVKVVDKLLDDQATAYE-----HGDIV-----IEHADGKERCRCWNYSEDLAGVDELTHL-----CPRCQVQV-----KS
Ec1	863	-----LLTSGAT-VADYNDAPADAQ-----QSEVLKGLKVALSKAEGEKCPRCWHYTQDVGVKVAEHAET-----CGRCVSNVAGDGEKRR
Ec2	863	-----LLTSDRRYVADYNDAPADAQ-----QSEVLKGLKVALSKAEGEKCPRCWHYTQDVGVKVAEHAET-----CGRCVSNVAGDGEKRR
Ec3	862	-----LLTSGAT-VADYNDAPADAQ-----QSEVLKGLKVALSKAEGEKCPRCWHYTQDVGVKVAEHAET-----CGRCVSNVAGDGEKRR
Sa2	898	-KSLSSDAELTKDDF-I IKTLPKD---SYQLSEDNDCVILLDNKLSPELIRE-GHARELIRLIQQLRKKKNLPINQRIDIYIGVTGELLE-----SIKTNK
Sc	936	-EVAGI--ELVKGDLNARIGLPESAQAGQETDQDVLIMDTNYSILKSE-GLARELVNRIQKLKKCGLEATDDVLVEYELVKDTIDFEAIVKEHF
Tt	942	-DLVGK--KLLEDFTITQNYKKEYSSGDLGEGEVILLNLAQDEKLSK-GLVREFTSNVQRTKKKTGLKVDNDIVIYVDVTKAP-KLNAAIQSDSL
Mt	913	VLDLGRIIIVLEPDDIIFETELPENIVNAQFEGGS-----VFVDTELTPEIMSE-AMARELVRRIQDMRKDLDDVEARIEV---SVKCSPEFRELTEPQR
		..
Cj	917	-----C 917
Sa1	917	-----LV 918
Ec1	937	-----FA 938
Ec2	938	-----FA 939
Ec3	935	-----FA 936
Sa2	988	NMF-----KENFV-----IKNIHLNVIDEYENTIHFNKKEIKISLL-----Y- 1024
Sc	1032	DMLSKTRCSIAKYDGSKTDPIGDEEQS-----INDTIFKLKV-----KL 1072
Tt	1037	EAVQKVLKPLVPL-SEKNAALKIVENSLLTFQVDEESVTIEIA---YA 1081
Mt	1004	EFVENEVRASHLSFDYTEL-----EYTKEWKISDENLIISIKPSDKV 1045

Fig. 3. Primary sequence homology alignment of IleRS from different organisms. Cj, *C. jejuni*; Sa1, *Staph. aureus* Oxford (Chalker et al., 1994); Sa2, *Staph. aureus* J2870, where the *ileS* gene conferred high-level resistance to mupirocin (Hodgson et al., 1994); Ec1, *E. coli* (Yu et al., 1984); Ec2, *E. coli* (Webster et al., 1984); Ec3, *E. coli* (GenBank accession no. S40549); Sc, *Sac. cerevisiae* (Martindale et al., 1989); Tt, *T. thermophila* (Csank & Martindale, 1992); and Mt, *M. thermoautotrophicum* Marburg (Jenal et al., 1991). Residues identical among all the organisms are indicated by asterisks under the sequence while residues with conserved changes are indicated by solid dots. The conserved sequences of motifs I-VII are overlined.

sequence extends to a 14 amino acid highly conserved motif, DGPPYANGHIHIGH (motif I in Fig. 3). This motif is involved in the formation of the catalytic core of the enzyme activity (Shiba & Schimmel, 1992). The KMSKS consensus motif serves as another signature sequence of class I aaRS, is perfectly conserved in all the listed IleRS sequences and is required for the binding of tRNA to the enzyme-bound isoleucyl adenylate during aminoacylation (Hountondji et al., 1990).

Approximately 25 amino acids downstream of the HIGH region is another strongly conserved region, motif II (Fig. 3). In this motif, a trimer peptide, GWD, is identical in all the sequences. This short, conserved peptide is believed

to be involved in isoleucine binding to the IleRS (Clarke et al., 1988).

Fig. 3 demonstrates another four highly conserved motifs III, IV, V and VI. Motif III and motif VI are located at strong homology regions in the alignment of *E. coli* IleRS and other aaRSs such as ValRS, LeuRS and MetRS (Heck & Hatfield, 1988). These regions are assumed to represent functionally related blocks although the specific function for these regions is not known. Motif IV is located in a region where sequences of different bacterial aaRSs show strong similarities (Shiba & Schimmel, 1992; Martindale et al., 1989), while motif V was noted to be conserved in class I aaRSs, specifically in Met, Ile, Val and Leu tRNA

synthetase (Ghosh *et al.*, 1991; Borgford *et al.*, 1987; Tzagoloff *et al.*, 1988). Motif V contains an Arg at residue 470 of *C. jejuni* IleRS; the corresponding residue of MetRS in *E. coli* is believed to play a critical role in activation of methionine to bind to the enzyme (Ghosh *et al.*, 1991).

Most of the conserved sequences are located close to the N-terminus, as shown by others (Webster *et al.*, 1984; Shiba & Schimmel, 1992; Jenal *et al.*, 1991; Martindale *et al.*, 1989). However, some conserved sequences are located close to the C-terminus. For instance, a zinc-finger-like metal-binding site, C₄ box in *C. jejuni* IleRS, composed of Cys⁸⁹⁰-X₂-Cys-X₁₁-Cys-X₂-Cys⁹⁰⁵ is similar to that of *E. coli* K12 IleRS in Cys⁹⁰²-X₂-Cys-X-His-X₁₄-Cys-X₂-Cys⁹²⁵ and that of *Staph. aureus* Oxford IleRS in Cys⁸⁸⁷-X₂-Cys-X₁₆-Cys-X₂-Cys⁹¹⁰ where X represents various amino acids (Cj, Ec2 and Sa1 in Fig. 3). This C₄ box is assumed to function in metal binding during tRNA aminoacylation (Miller & Schimmel, 1992). Another putative consensus at the C-terminus is a single amino acid K which is correspondingly located at amino acid 736 in *C. jejuni* IleRS, at amino acid 726 in *Staph. aureus* IleRS or at amino acid 732 in *E. coli* IleRS and is believed to interact with tRNA during aminoacylation (Shepard *et al.*, 1992).

Maxicell analysis and the enzyme assay provided evidence of *C. jejuni* *ileS* gene expression in *E. coli* cells. The IleRS activity in E8-1-transformed *E. coli* 331c⁻ was three times greater than in nontransformed *E. coli* 331c⁻.

The *ileS* gene was mapped to the 1360–1812 kb region of the *C. jejuni* genome (Fig. 2). It is noteworthy that unlike *E. coli*, *Enterobacter aerogenes* and *Pseudomonas fluorescens* (Innis *et al.*, 1984; Isaki *et al.*, 1990a, b), *C. jejuni* does not harbour the *lsp* gene (the gene encoding lipoprotein signal peptidase) immediately downstream of the *ileS* gene.

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