Construction, expression and characterisation of recombinant molecules of the urease gene operon from a urease-positive thermophilic *Campylobacter* (UPTC) isolate

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ABSTRACT

A recombinant molecule of the full-length urease gene operon was constructed *in vitro* from the Japanese urease-positive thermophilic *Campylobacter* (UPTC) CF89-12 isolate and expressed in *Escherichia coli* cells. Several large deletion recombinant variants of urease subunit genes were also constructed and expressed in *E. coli* cells. A positive urease reaction with the log-phase cultured *E. coli* JM109 cells in the $NiCl_2$-containing medium transformed with pGEM-T vector carrying the recombinant molecule of the full-length operon was detected with isopropyl-β-D-thiogalactoside. Among the several deletion recombinant variants, each *ureA*-, *ureB*-, *ureE*-, *ureF*-, *ureG*- and *ureH*-large deficient, only *ureE*-large deletion variant (63% deficient) showed a positive urease reaction (approximately 15-fold). In addition, a *ureE*-complete deletion recombinant variant (100% deficient) constructed also showed a positive reaction of urease (approximately 18-fold). Recombinant urease subunits A and B were immunologically identified by Western blot analysis with anti-urease α (A) and β (B) raised against *Helicobacter pylori*.

Key words: Campylobacter.
Genes, recombinant.
Urease.

Introduction

Campylobacter lari was first recognised as a nalidixic acid-resistant thermophilic *Campylobacter*.[1] In 1985, an atypical and unusual organism of urease-positive and nalidixic acid-sensitive thermophilic *Campylobacter* (UPTC) was isolated from the natural environment in England.[2] Thereafter, the characterisation of UPTC as a variant or biovar of *C. lari* has been described.[3,4] After the original description of UPTC appeared, isolates of UPTC were reported in France,[3,5] Northern Ireland,[6–9] The Netherlands,[10] England,[11] Japan,[12,13] and, more recently, Sweden.[14]

Many species of bacteria produce urease (urea amidohydrolase; EC3.5.1.5), a nickel-containing metalloenzyme that hydrolyses urea to ammonia and carbamate.[15] This organism, UPTC, is an atypical taxon within the genus *Campylobacter* that produces urease,[16] as well as *C. sputorum* biovar paraureolyticus.[17] Most recently, a reclassification of *Bacteroides ureolyticus* as *Campylobacter ureolyticus* com. nov., the third taxon of urease-producing *Campylobacter*, was also described.[18,19]

The authors have already demonstrated cloning, sequencing and characterisation of a urease gene operon consisting of two putative promoter structures, at the -35- and -10-like regions, six closely spaced and putative open reading frames (ORFs) of two structural (*ureA* and *ureB*) and four accessory (*ureE*, *ureF*, *ureG* and *ureH*) genes, probable ribosome-binding sites (RBSs) for each ORFs and a putative ρ-independent transcriptional terminator region from a genomic DNA library constructed with the Japanese UPTC CF89-12 isolate cells.[20] This urease gene operon was approximately 5.1 kbp in length and showed high nucleotide sequence identities to those of some *Helicobacter* organisms.[20] In addition, most recently, the authors have also described molecular analysis and characterisation of a urease gene operon from *C. sputorum* biovar paraureolyticus.[21] However, they have not attempted to construct *in vitro* an approximately 5.1 kbp recombinant DNA molecule which can express catalytically active urease enzyme in *Escherichia coli* by amplifying the urease gene operon from the UPTC CF89-12 isolate.

Therefore, the aim of the present study is to construct *in vitro* a recombinant molecule of the UPTC CF89-12 full-length urease gene operon and express the recombinant urease molecule in *E. coli* cells. It also aims to construct *in vitro* several deletion recombinant variants of urease subunit genes and to express and characterise those in *E. coli* cells, in order to clarify the roles of the accessory gene products in UPTC urease activation.

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Materials and methods

Bacterial isolate and culture condition

The Japanese isolate UPTC CF89-1212 was used in the present study. The cells were cultured as described previously.[22,23]

PCR amplification of the UPTC urease gene cluster

For the amplification of the approximately 5.1 kbp urease gene operon from UPTC CF89-12 consisting of the promoter region and six urease genes, nucleotide position (np) 389 through 5396 bp (DDBJ/EMBL/GenBank accession number AB201709), a polymerase chain reaction (PCR) primer pair was designed for UPTC ureP-f (5′-AAGACTATGAAACTGAATTA-3′, np 389–408 bp) and ureH-r (5′-TTATAATCCTTAGTTTGTT-3′; np 5378-5396) (Fig. 1). PCR amplification and product purification were carried out as described by Sambrook and Russell.[24] TA cloning of the amplified urease gene operon using the pGEM-T vector and *E. coli* JM109 cells was also carried out, as described by Sambrook and Russell.[24]

In the present study, the authors attempted to construct several large deletion recombinant variants of UPTC urease subunit genes by using their specific PCR primer pairs and inverse (I)-PCR procedures with TA-cloned full-length UPTC urease gene operon. The primer pairs shown in Figure 1 for the I-PCR were designed based on the nucleotide sequence data of the approximately 5.1 kbp full-length urease gene operon from the UPTC CF89-12 isolate (AB201709).

The PCR mixture contained 1x iProof HF buffer, 200 µmol/L each dNTP, 0.5 µmol/L each primer, one unit iProof DNA polymerase (Bio-Rad Laboratories, Tokyo, Japan) and 100 ng template DNA. The PCR reaction was performed in 50 µL reaction volumes at 98°C for 30 sec, with 35 cycle at 98°C for 5 sec, 50°C for 10 sec, and 72°C for 10 sec to 3 min, followed by a final extension at 72°C for 5 min.

Amplified PCR products were separated by 0.7% (w/v) agarose gel electrophoresis in 0.5x TBE at 100 V and detected by staining with ethidium bromide. The PCR products amplified by the newly constructed primer pairs for the urease gene operon were purified using a QIAEXII gel extraction kit (Qiagen, Tokyo, Japan). The purified amplicons were then subjected to cycle sequencing with BigDye Terminator (Applied Biosystems, Tokyo, Japan), with the PCR primers or the I-PCR primers and other sequence primers constructed by primer walking procedures, if necessary. The reaction products were separated and detected on an ABI PRISM 3100 genetic analyser (Applied Biosystems).

Urease activity measurement of recombinant UPTC urease gene operon

Urease activities of the recombinant molecule of the full-length UPTC urease gene operon and several deletion recombinant variants of urease subunit genes (each *ureA*-, *ureB*-, *ureE*-, *ureF*-, *ureG*- and *ureH*-large deficient) constructed *in vitro* were determined, with log-phase cultured *E. coli* JM109 cells, transformed with pGEM-T vector carrying the TA-cloned full-length UPTC urease gene cluster and deletion recombinant variants with isopropyl-β-D-thiogalactoside (IPTG) (0.1 mmol/L) and without IPTG. Recombinant *E. coli* JM109 cells were cultured in L Broth medium containing 750 µmol/L $NiCl_2$ at 37°C.[25]

The *E. coli* cells containing the recombinant UPTC urease gene operon were pelleted by centrifugation (7000 x*g*) at 4°C for 20 min. The cells were washed with phosphate-buffered saline (PBS) and resuspended in the same buffer. After the cells were disrupted by sonication, cell lysate extracts were collected by centrifugation at 27,000 x*g* for 30 min at 4°C. Protein concentration determination of the extracts was conducted using the DC Protein Assay kit (Bio-Rad Laboratories). Quantitative detection of urease activity was achieved using the indophenol method (µmol/min/mg protein)[26] following the urease reaction of the fresh extract (4 µg) after adding to 50 mmol/L urea in PBS at 37°C for 4 h.

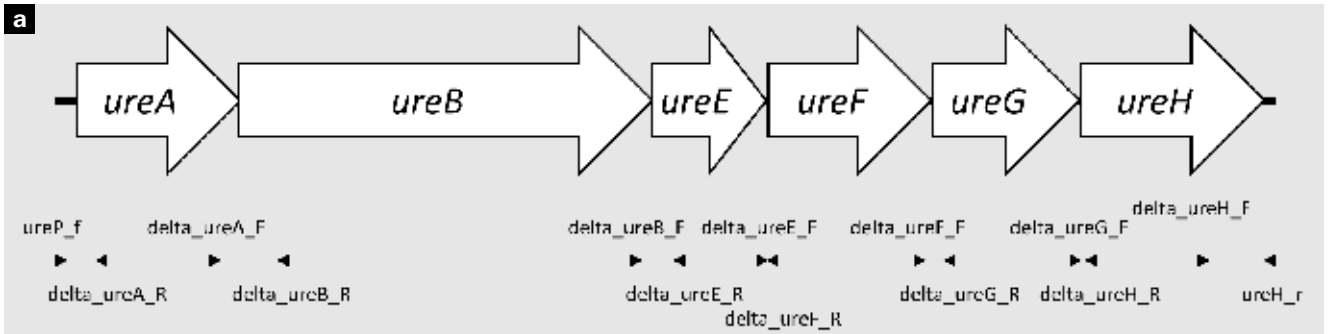
Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Western blotting

Soluble extracts were analysed by polyacrylamide gel electrophoresis (PAGE) on a mini-slab gel comprising 1% (w/v) SDS, 12% (w/v) PAG with Tris-glycine buffer at 24 mA for 2 h. Proteins were transferred to a PVDF membrane (Immobilon, Millipore, USA) at 90 V for 2 h with cooling. The PVDF membrane was then blocked with 5% (w/v) non-fat dry milk in basis buffer, as described by Sambrook and Russell,[24] at room temperature for 30 min. The membrane was then examined for reactivity with rabbit polyclonal anti-*H. pylori* urease α (A) and β (B) subunit

Table 1. Details of the large deletion recombinant variants of UPTC CF89-12 urease subunit genes.

Large deletion recombinant variant	Deletion			Notes (mutation etc.)
	Nucleotide position (AB201709)	aar position	% deletion (remaining aar/ putative ORF aar)	
ureA-deletion variant (ΔA)	603 ... 1025	41 ... 181	63 (82/223)	
ureB-deletion variant (ΔB)	1348 ... 2754	67 ... 535	83 (96/565)	
ureE-deletion variant (ΔE)	2977 ... 3270	44 ... 141	63 (57/155)	T3273C
ureF-deletion variant (ΔF)	3369 ... 3905	18 ... 196	81 (43/223)	T3368A
ureG-deletion variant (ΔG)	4084 ... 4551	30 ... 185	78 (43/199)	A4554G T4083C
ureH-deletion variant (ΔH)	4670 ... 5080	25 ... 161	55 (113/250)	
ureE,F,G,H-deletion variant (ΔE–H)	2977 ... 5080	UreE 44 ... UreH 161	16 (132/827)	*ureE* 1–43 and *ureH* 162–250 remaining

aar: amino acid residues



b

Primer	Nucleotide sequence (5'→3')	Nucleotide position
ureP-f	AAGACTATGAAACTGAATTA	389...408
ureH-r	TATAATCCTTAGTTTGTT	5378...5396
ure_lac_F_SacII	AACAACCGCGGACAGGAAACAGCTATGACC	
ure lac R ApaI	TTGGAGGGCCCGCTTGAGTATTCTATAGTG	
delta_ureA_F	GAATGTACAGTAAGTTTAATAGAATTTGG	1020...1048
delta_ureA_R	TTCTGTACATATATAAGCTAAAGCTTC	582...608
delta_ureB_F	AGAAGTGGATCCACAAACTTATGAAGTA	2746...2773
delta_ureB_R	ACTGGATCCGGAAAGTCTCCCTCACTC	1327...1353
delta_ureE_F	GATATGGCTAGCTTTATCCAAGTTGATCC	3262...3290
delta_ureE_R	TTTTGCTAGCGACATCTAGTCCTTTTAAAGTCG	2951...2983
delta_ureF_F	AAAACCATGGTGTGAAAATCACAATATC	3899...3926
delta_ureF_R	CTCCCCATGGAAAAGAAGAATCACTAATTTG	3345...3375
delta_ureG_F	GAAGGTTTACTCGAGATCATTACTTGGAT	4540...4568
delta_ureG_R	GCCAACTCGAGCTCATCTTTTAAAGCTTGAC	4061...4091
delta ureH F	GAGTTTAACTCAAATTTCTAGAAATGATACT	5062...5092
delta ureH R	AGGTGTGAATCTAGAATTTTTTATGATAGT	4652...4681

Fig. 1. Schematic representation of the genetic organisation of the urease gene operon from UPTC CF89-12 and the primer sites for the amplification of the operon **(a)** and primers **(b)**.

antibodies (Santa Cruz Biotech, USA) diluted in 0.5% (v/v) blocking buffer at 4°C overnight. Immunoreactants were then detected using the ECL Western blotting detection system with horseradish peroxidase-labelled secondary antibody (GE Healthcare Life Science, Tokyo, Japan).

Results

Construction of* in vitro *recombinant molecule of full-length urease gene operon from the UPTC CF89-12 and its expression

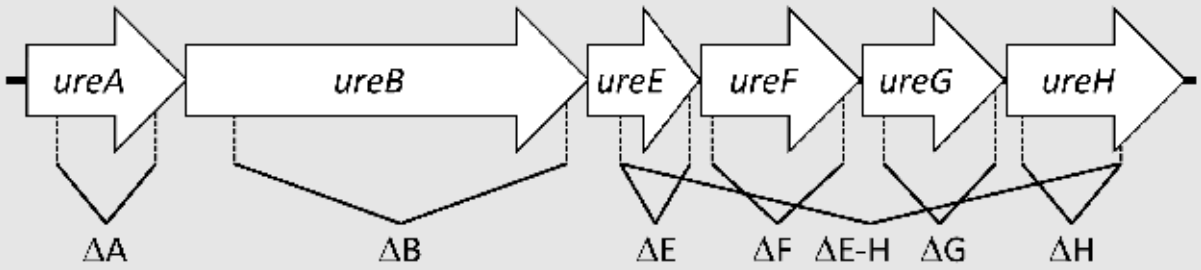
In the present study, the authors first constructed an *in vitro* recombinant molecule of the full-length urease gene operon from the UPTC CF89-12 isolate represented schematically in Figure 1 and expressed the recombinant urease in *E. coli* JM109 cells. As shown in Figure 2B, the recombinant UPTC urease genes including the two full-length structural (*ureA* and *ureB*) and four accessory (*ureE*, *ureF*, *ureG* and *ureH*) genes expressed catalytically active urease in *E. coli*.

Thus, urease enzyme activity of the recombinant full-length molecule of UPTC CF89-12 isolate urease gene operon was detected with the transformed and log-phase cultured *E. coli* JM109 cells using the indophenol method. However, no activity was detected with the cultured *E. coli* JM109 not carrying the recombinant urease gene operon analysed as a negative control (Fig. 2B).

Construction of large deletion variants of UPTC CF89-12 urease subunit genes and their expression

In addition, large deletion variants of UPTC CF89-12 urease subunit genes were constructed, as shown schematically in Figure 2A. The details of the large deletion are shown in Table 1. Consequently, among seven large deletion variants of UPTC CF89-12 urease subunit genes, *ureA*-(ΔA), *ureB*-(ΔB), *ureE*-(ΔE), *ureF*-(ΔF), *ureG*-(ΔG) and *ureH*-(ΔH) and *ureE*-H (ΔE–H)-deficient recombinant variants represented schematically in Figure 2A and summarised in Table 1, only the *ureE*-deficient recombinant variant (remaining amino acid residues of the positions 1–43 and 142– 155, namely from amino acid position 44 to 141 deleted UreE, 63% deficient; 98 amino acids residues deletion out of the UreE 155 amino acids of the full length; Table 1) showed a change in the colour of the assay to red, judged as a positive reaction for urease with IPTG. Moreover, the urease activity in the *ureE* 63% deficient recombinant variant fraction was accelerated more than 10-fold in the recombinant full-length UPTC urease gene operon in *E. coli* cells. This urease activity value is almost equivalent to that in the UPTC CF89-12 cell lysate (Fig. 2B). Regarding the seventh large deletion variant, ΔE-H, shown in Figure 2 and Table 1, the variant would generate a partial UreE (aar 1–43) and partial UreH (aar 162–250) fusion protein, which lacks the partial UreE (aar 44–155), complete UreF (aar 1–223), complete UreG (aar 1–199) and partial UreH (aar 1–161).

a



b

Deletion recombinant variant	μmol/min/mg protein	% activity of full-length wild type recombinant
E. coli cell lysate without urease gene	0.002	4.1
E. coli cell lysate with urease gene	0.049	100
E. coli cell lysate with urease gene (ΔA)	0.002	4.1
E. coli cell lysate with urease gene (ΔB)	<0.002	0
E. coli cell lysate with urease gene (ΔE)	0.723	1,476
E. coli cell lysate with urease gene (ΔF)	<0.002	0
E. coli cell lysate with urease gene (ΔG)	0.003	6.1
E. coli cell lysate with urease gene (ΔH)	0.003	6.1
E. coli cell lysate with urease gene (ΔE-H)	<0.002	0
UPTC CF89-12 cell lysate	2.403	4,904
UN*C. lari* 299 cell lysate	<0.002	0

Fig. 2. Schematic representation of the genetic organisation of the recombinant full-length urease gene operon molecule from UPTC CF89-12 and several urease subunit genes as large deletion recombinant variants **(a)**. Urease activity determination was carried out using the indophenol method **(b)**. ΔA, ΔB, ΔE, ΔE–H, ΔG and ΔH show the deficient regions schematically within the urease subunit gene large deletion recombinant variants **(a)**.

Urease gene recombinant variants containing 100% deletion of the UPTC* ureE *subunit gene

In Figure 3, deduced amino acid sequence alignment analysis of the putative ORFs of the full-length and large deletion recombinant variant of the UPTC *ureE* (63% deletion) was carried out. As, in the present study, urease enzyme activity of the *ureE* deletion recombinant variant (63% deficient of the full-length UreE subunit) showed an accelerated urease activity to approximately 15-fold (Fig. 2B), it would be interesting to see if urease activity is detected within the urease gene recombinant variant containing 100% deletion of the UPTC *ureE* subunit gene. The authors then constructed a 100% *ureE* complete deletion variant (155 amino acid residue-deficient) of the UPTC CF89-12 full-length urease gene operon and examined the urease enzyme activity. The urease activity in the 100% *ureE* deletion recombinant variant constructed also showed a positive acceleration (approximately 18-fold) similar to that of the 63% *ureE* deletion variant described above (Table 2). In addition, 100% *ureE* and *ureG* deletion recombinant variants were constructed. When the recombinant variant was examined, no urease activity was detected (Table 2).

Effects of $NiCl_2$ on urease activity

Effect of $NiCl_2$ (750 μmol/L) in the *E. coli* culture medium on urease activity of the full-length recombinant UPTC urease

Table 2. Urease activities in the 100% *ureE* and 100% *ureE–G* deletion recombinant variants of the UPTC CF89-12 full-length urease gene operon.

Deletion recombinant variant	mol/min/mg protein	% activity of full-length wild-type recombinant
E. coli cell lysate without urease gene	0.004	5.7
E. coli cell lysate with full-length urease gene operon	0.070	100
E. coli cell lysate with urease gene (63% E)	1.176	1680
E. coli cell lysate with urease gene (100% G, E)	0.003	4.3
E. coli cell lysate with urease gene (100% G)	0.002	2.9
E. coli cell lysate with urease gene (100% E)	1.258	1797

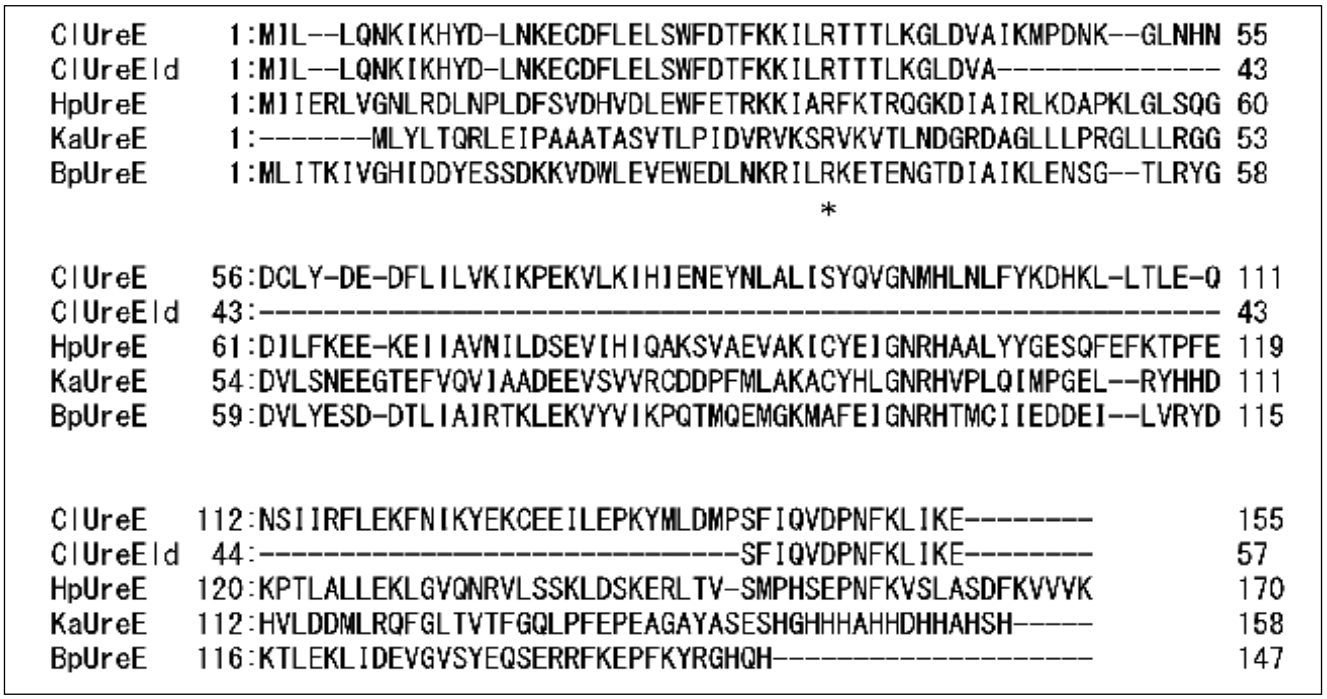


Fig. 3. Deduced amino acid sequence alignment analysis of the putative ORFs of the full-length UPTC *ureE* (ClUreE) and UPTC *ureE* large deletion (63%) variants (ClUreEld), as well as the putative ORFs of the full-length urease gene *ureE* from *H. pylori* ATCC43504 (HpUreE), *K. aerogenes* (KaUreE) and *B. pasteurii* (BpUreE).

gene operon and the recombinant UPTC urease gene variants of the large *ureE* deletion (63% segment-deficient) and 100% *ureE* deletion were examined. Urease activity was positively affected by $NiCl_2$, as shown in Table 3. Consequently, the recombinant molecule of the full-length urease gene operon and a recombinant variant containing a large deletion of the *ureE* subunit from UPTC CF89-12 showed positive urease activities when cultured in the medium only containing $NiCl_2$.

Western blot analysis

As shown in Figure 4, no A and B subunits in the large deletion variants of UPTC CF89-12 urease genes *ureA* (ΔA) and *ureB* (ΔB) were identified immunoreactively by the anti-*H. pylori* urease α (A) and β (B) subunit antibodies, respectively. In addition, anti-*H. pylori* urease α (A) and β (B) subunit antibodies identified immunoreactive bands at approximately 59 kDa and 26 kDa, respectively, in the UPTC cells (CF89-12) and *E. coli* JM109 cells transformed with pGEM-T vector ligating the recombinant full-length UPTC urease gene operon of about 5.1 kbp (WT), and the five *ureE* (ΔE)-, *ureF* (ΔF)-, *ureG* (ΔG)-, *ureH* (ΔH)- and *ureE–H* (ΔE–H)-large deficient recombinant variants. However, no band was identified for *E. coli* JM109 transformed with pGEM-T vector only (T-v in Fig. 4).

Discussion

This is the first demonstration of the construction, expression and characterisation of the recombinant molecule of full-length UPTC urease gene operon from the UPTC CF89-12 isolate with transformed and log-phase cultured *E. coli* JM109 cells.

In the present study, the recombinant full-length UPTC urease gene cluster, two structural *ureA* and *ureB*, four accessory *ureE*, *ureF*, *ureG* and *ureH* genes constructed, and promoter structures, were identified to express urease enzyme activity in the transformed and log-phase *E. coli* cells. As described previously,[20] some accessory genes, such as *ureI*, other than the four *ureE*, *ureF*, *ureG* and *ureH(D)* genes, were undetectable in the UPTC CF89-12 urease gene

Table 3. Effects of $NiCl_2$ on the urease activity in the large *ureE* deletion recombinant variant and full-length urease gene operon of UPTC CF89-12.

Deletion recombinant variant	mol/min/mg protein	% wild type
E. coli cell (grown in LB without $NiCl_2$) lysate without urease gene	0.001	4.8
E. coli cell (grown in LB without $NiCl_2$) lysate with urease gene	0.000	0
E. coli cell (grown in LB without $NiCl_2$) lysate with urease gene (E)	0.001	4.8
E. coli cell (grown in LB containing $NiCl_2$) lysate without urease gene	0.002	9.5
E. coli cell (grown in LB containing $NiCl_2$) lysate with urease gene	0.021	100
E. coli cell (grown in LB containing $NiCl_2$) lysate with urease gene (E)	0.469	2233

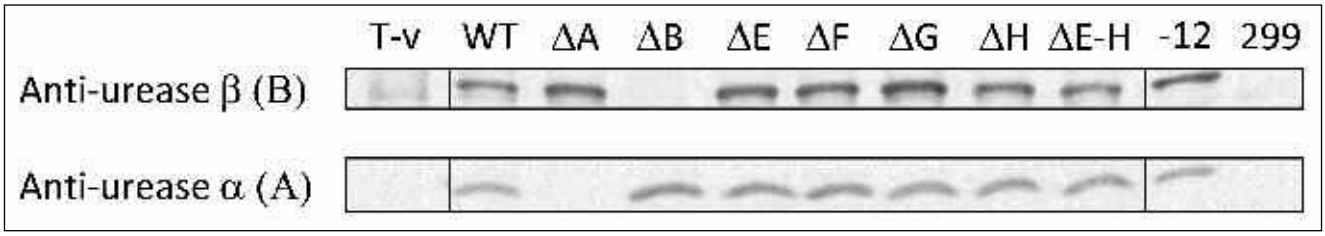


Fig. 4. Western blot analysis of the UPTC urease in the UPTC CF89-12 cells and the recombinant UPTC urease in *E. coli* cells using polyclonal anti-*H. pylori* urease α(A) and β(B) subunit antibodies. T-v: cell lysate of *E. coli* JM109 cells transformed with pGEM-T vector only; WT: cell lysate of *E. coli* JM109 cells transformed with pGEM-T vector ligating the recombinant full-length UPTC CF89-12 urease gene operon; ΔA–ΔE-H: cell lysates of *E. coli* JM109 cells transformed with pGEM-T vector ligating large deletion variants of UPTC CF89-12 urease subunit genes; CF89-12: UPTC CF89-12 cell lysate; 299: UN *C. lari* 299 cell lysate.

cluster operon. In addition, only the *ureE*-deficient recombinant large deletion variant showed a urease-positive reaction.

Regarding the *ureE* from the UPTC, previous sequence analysis indicated that the putative *ureE* ORF was identified to be a 465 nucleotide sequence and it was predicted to encode a 155 amino acid residue with the calculated molecular weight (CMW) of 18,586.[20] Regarding the deduced amino acid sequence identities of the six ORFs of UPTC CF89-12 urease genes to those of *H. hepaticus*, *H. pylori*, *H. heilmannii* and *H. mustelae* (for the latter two, *ureA* and *ureB* only), the three accessory genes of *ureE*, *ureF* and *ureH* were characteristically demonstrated to give relatively lower identities (57.8–70.9%) among the three organisms, UPTC, *H. hepaticus* and *H. pylori*, when compared with the other three genes.[20] In addition, when a neighbour-joining tree was constructed based on the complete nucleotide sequence information of UPTC CF89-12 and both the urease structural and accessory gene sequences accessible in DDBJ/EMBL/GenBank, UPTC formed a cluster together with *H. pylori* and *H. hepaticus*, separate from the other urease-producing bacteria.[20]

In *Klebsiella aerogenes*, extensive biochemical studies show that three accessory subunits, UreD, UreF and UreG, are required *in vivo* for the assembly of the nickel metallocentre in urease.[27] UreE was shown to be a metallochaperone that delivers nickel to urease.[28] The *K. aerogenes* UreE contains a histidine-rich carboxyl terminus sequence in which 10 of the last 15 residues are histidine,[29] and is able to bind five to six Ni^{2+} ions per dimer.[28,29] Although the histidine-rich motif is thought to be essential to UreE function, not all UreE peptides possess a histidine-rich region.[30]

In the present study, the UPTC urease accessory *ureE* gene and its deduced amino acid sequence of the putative ORF sequenced and analysed was shown to lack the histidine-rich carboxyl terminus (Fig. 3). Thus, some bacterial UreEs did not possess a histidine-rich region.

Regarding the *ureE* gene of *H. pylori*, the *ureE* did not contain a histidine-rich motif; however, its presence (genetically-modified histidine-rich versions of UreE) resulted in a significant increase of urease activity.[31–33] In addition, Bellucci *et al.* recently described the metal-binding properties of the *H. pylori* UreE and its interaction with the related accessory subunit UreG, a GTPase involved in the assembly of the urease active site.[34] A possible alternative physiological role for UreE of *K. aerogenes* was suggested by the observation that the GTP concentration needed for optimal activation of urease *in vitro* is greatly reduced in the presence of UreE, compared with that required in its absence.[35]

In the present study, two types of *ureE* deletion recombinant variant, approximately 63% deficient and 100% deficient, showed accelerated urease activity to approximately 10-fold activity. In addition, a *ureE* and *ureG* deletion recombinant variant showed no urease activity (Table 2). Thus, the present study reveals that three (i.e., *ureF*, *ureG* and *ureH*) of the four accessory genes within the urease gene operon of UPTC CF89-12 may be necessary for urease expression. Whereas UreE may regulate activity and modulate level in the cells, long or complete *ureE* deletion recombinant variants of the gene may accelerate urease activity without possible regulation.

In addition, regarding the report by Bellucci *et al.*,[34] the results were discussed in relation to available evidence of a UreE–UreG functional interaction *in vivo*.[34] In the present study of *Campylobacter* UPTC urease, two *ureE* deletion recombinant variants accelerated the urease activity to approximately 10-fold, compared with a recombinant full-length urease gene operon, but the *ureE* and *ureG* deletion recombinant variants failed to generate urease enzyme activity. Thus, in the UPTC CF89-12 cells, UreE–UreG functional interaction may be absent.

This is the first report of the construction, expression and characterisation of a full-length recombinant urease gene operon and several large deletion recombinant variants of urease subunit genes from a UPTC organism. In the present study, the target DNA was transformed into the *E. coli* cell by employing the pGEM-T vector, and the urease gene operon (approximately 5.1 kbp) containing the promoter region and its UPTC urease activity was detected in *E. coli* cells. However, at present, it remains uncertain which promoter in the urease gene operon or in the pGEM-T vector was responsible. In addition to the expression of urease activity in the *E. coli* cells harbouring the recombinant UPTC operon, urease subunits A and B encoded on *ureA* and *ureB*, respectively, were confirmed immunologically by Western blot analysis with anti-urease α (A) and β (B) raised against *H. pylori*. This result strongly suggests that these subunits are immunologically reactive within UPTC and *H. pylori*. □

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