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Genetic relatedness and identification of clinical strains of genus *Campylobacter* based on *dnaJ*, 16S rRNA, *groEL*, and *rpoB* gene sequences

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The range of divergence of members of genus *Campylobacter* was analyzed by sequencing housekeeping genes. The utility of the *dnaJ* gene for identifying *Campylobacter* species was investigated by analyzing sequences of 34 reference strains, representing 16 species and three subspecies. The mean sequence similarity value of the *dnaJ* gene (66.98%) was significantly smaller than that of the 16S rRNA, *rpoB* and *groEL* genes (93.55%, 78.84% and 80.05%, respectively), indicating a high discriminatory power of the *dnaJ* gene. The *dnaJ* sequence variation within major independent pathogenic species of the genus *Campylobacter* was less than 3.8%. The application of the selected area of the *dnaJ* sequences for the species identification was confirmed by assigning 66 clinical isolates to the correct species of the genus *Campylobacter*. Our data indicates that simple analysis of *dnaJ* sequence is a new valuable tool for correct and rapid identification of *Campylobacter* species.

Key words: *Campylobacter*, *dnaJ* gene sequence, identification

INTRODUCTION

Campylobacter species are a major cause of bacterial gastroenteritis in many industrialized countries (Frost *et al*., 2002; Friedman *et al*., 2000; Rautelin & Hanninen, 2000). At present, it comprises 17 species and 6 subspecies (Foster *et al*., 2004; On, 2001). More than 95% of human and animal *Campylobacter* infections are caused by *C. jejuni*, *C. coli* and *C. fetus* (Goossens *et al*., 1995; Lastovica & Skirrow, 2000; Skirrow, 1994), although the other species such as *C. lari*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. upsaliensis*, *C. concisus*, *C. rectus*, and *C. showae* have also been isolated from individuals with diarrheal illness or periodontal disease (Bourke *et al*., 1998; Goossens *et al*., 1995; Skirrow, 1994; van Doorm *et al*., 1998). It is often difficult and time-consuming to phenotypic identify of *Campylobacter* species, due to fastidious growth requirements and low biochemical activity (Moore & Madden, 2003; Morris *et al*., 1985; Nicholson & Patton, 1995; On & Harrington, 2000). Various molecular DNA-based methods for identification of *Campylobacter* species have been developed. These methods typically require the use of DNA-DNA hybridization (Vandemme *et al*., 1991), numerical analysis of amplified fragment length polymorphism (AFLP) fingerprinting (Duim *et al*., 2001; On & Harrington, 2000), species-specific PCR primer or RFLPs of amplified 16S rRNA sequences (Marshall *et al*., 1999), multilocus sequence typing (MLST) (Dingle *et al*., 2005; Miller *et al*., 2005; van Bergen *et al*., 2005). However, these methods are limited by their complexity or their inability to distinguish between closely related species such as *C. coli* and *C. jejuni*. Moreover, housekeeping genes such as 16S rRNA and 23S rRNA (van Camp *et al*., 1993), *rpoB* (Korczak *et al*., 2006), *groEL* (Hill *et al*., 2006; Kärenlampi *et al*., 2004), *gyrB* (Kawasaki *et al*., 2008), and *omp50* (Dedieu *et al*., 2004) were used in the phylogenetic analyses and species identification of *Campylobacter*. However, information of these genes is often limited to a limited subset of species or *omp50* gene was found to be absent from over 90% of *C. coli* strains or information regarding strain variation within a species is not available. Among these methods, MLST analysis is becoming more important for epidemiological analysis of strains

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within a species. However, seven primers (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, and *uncA*) were used for MSTL analysis of *C. jejuni* (Dingle *et al*., 2001), and are applicable only for the amplification of strains within *C. jejuni* and *C. coli*. The MSLT primers set often fail to amplify similar genes of closely related other species within the genus *Campylobacter*. Among the 30 established species in the genus *Campylobacter*, whole genome sequences of only seven species (*C. jejuni*, *C. coli*, *C. hominis*, *C. concisus*, *C. lari*, *C. curvus*, and *C. fetus*) are currently available. To use available MLST gene set for the taxonomy of the genus *Campylobacter*, whole genome information of all members of the genus should be accumulated in future.

DnaJ is a member of the Hsp40 family, co-regulates the active of heat shock sigma factor 32 (Ang *et al*., 1991). *dnaJ* gene approximately 1100-bp in length and is highly conserved among bacterial genera (Konkel *et al*., 1998). The *dnaJ* gene has been available used to identify *Legionella* spp. (Liu *et al*., 2003), *Streptococcus* spp. (Itoh *et al*., 2006),

Table 1 Origin of strains and Gene bank accession numbers of *dnaJ* sequences for the *Campylobacter* species used in this study

Species	Strains	Source	dnaJ accession No[a]
C. jejuni subsp. *jejuni*	GTC 8783 (=ATCC 29428)	Child diarrhoeic stool	**AB543241**
C. jejuni subsp. *jejuni*	GTC 259^T (=NCTC 11351^T)	Bovine faeces	AB542732
C. jejuni subsp. *jejuni*	NCTC 11168	Human	**AL111168**
C. jejuni subsp. *jejuni*	81116	Human	CP000814
C. jejuni subsp. *jejuni*	81-176	Human	CP000538
C. jejuni subsp. *jejuni*	RM 1221	Chicken	CP000025
C. jejuni subsp. *doylei*	269.97	Human blood	CP000768
C. coli	GTC 258^T (=LMG 6440^T)	Pig feces	**AB542729**
C. coli	GTC 8762 (=NCTC 11350)	Human	**AB542738**
C. coli	RM2228	Chicken	AAFL01000001
C. heveticus	NCTC 12470^T	Cat feces	**AB543240**
C. insulaenigrae	DSM 17739^T	Marine mammal	**AB543242**
C. lari	RM 2100	Human	CP000932
C. canadensis	CCUG 54429^T	Animal	**AB542737**
C. hyointestinalis subsp. *hyointestinalis*	NCTC 11608^T	Pig intestine	**AB542731**
C. hyointestinalis subsp. *lawsonii*	Clinical strain	Human	**AB613223**
C. fetus subsp. *fetus*	GTC 260^T (=ATCC 27374^T)	Sheep fetus brain	**AB542724**
C. fetus subsp. *fetus*	82-40	Human blood	CP000487
C. fetus subsp. *venerealis*	GTC 256^T (=NCTC 10354^T)	Heifer vaginal mucus	**AB542730**
C. fetus subsp. *venerealis*	GTC 8739 (=CCUG 33900)	Bull prepuce	**AB542733**
C. sputorum subsp. *sputorum*	DSM 10535^T	Human oral cavity	**AB542734**
C. concisus	13826	Human feces	CP000792
C. concisus	DSM 9716^T	Human gingival sulcus	**AB542725**
C. curvus	525.92	Human feces	CP000767
C. curvus	DSM 6644^T	Human alveolar abscess	**AB543243**
C. showae	CCUG 30254^T	Human gingival crevice	**AB542726**
C. rectus	ATCC 33238^T	Human periodontal pocket	**AB542735**
C. lanienae	NCTC 13004^T	Human	**AB542727**
C. hominis	ATCC BAA-381	Human gastrointestinal	CP000776
C. hominis	NCTC 13146^T	Human	**AB542736**
C. upsaliensis	RM3195	Human	AAFJ01000006
H. pylori (Out group)	26695	Human	AE000511

ATCC, American Type Culture Collection, Manassas; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany; NCTC, National Collection of Type Cultures, London NW9 5HT, UK; LMG, Bacteria Collection of Belgian Coordinated Collection of Microorgamisms; CCUG, Culture Collection, University of Göteborg, Sweden; GTC, Gifu Type Culture Collection, Japan; [a]Sequences obtained in this study are indicated in boldface.

Staphylococcus spp. (Shah *et al*., 2007), *Vibrio* spp. (Nhung *et al*., 2007a), *Aeromonas* spp. (Nhung *et al*., 2007c), *Enterobacteriaceae* family (Nhung *et al*., 2007b) and *Mycobacterium* spp. (Yamada *et al*., 2007).

In the present study, partial *dnaJ* sequences of 34 strains representing 16 *Campylobacter* species, three subspecies and 66 clinical isolates were determined. The sequence similarity, divergence and phylogenetic analysis based *dnaJ* sequences were compared with those of the 16S rRNA gene and other housekeeping gene sequences (*rpoB*, *groEL*, and *gyrB*) available in public databases to evaluate the usefulness of the *dnaJ* gene as a phylogenetic marker for the identification and differentiation *Campylobacter* species.

MATERIALS AND METHODS

Bacterial strains and cultivation

The bacterial strains used in this study are listed in Table 1. All *Campylobacter* strains were grown on *Campylobacter* agar supplemented with 5 or 10% (v/v) defibrinated sheep blood (*Nippon Becton Dickinson Co., LTD. Japan*). Strains were incubated at 37℃ in microaerophilic atmosphere of 8% O_2, 7% CO_2, and 85% N_2 for 48h, except *C. cuvus*, *C. concisus*, and *C. rectus* species, which were grown under anaerobic atmosphere of approximately 10% H_2, 7% CO_2, and 85% N_2.

Isolation of genomic DNA

Genomic DNAs were extracted by quick heat lyses. One colony was suspended in 100 *μl* distilled water and boiled for 5 min. The supernatant was obtained by centrifugation (about 10,000 rpm for 3 min) at room temperature and treated as a template.

Primer design for determination of *dnaJ* sequences

The *dnaJ* sequences of *C. jejuni*, *C. fetus*, and *C. lari*, (GenBank accession number AL111168, CP000487 and CP000932, respectively) were aligned to determine primers to be used for PCR and sequencing. Additional primers were selected during ongoing base sequence determination. All primers used in these studies are summarized in Table 2. Species-specific primers were used to amplify each species. Finally, DNC-F & DNC-R1 primers were designed to amplify most *Campylobacter* species except *C. showae*, and *C. sputorum*. These two species need DNC-F & DNC-R2 primers.

PCR amplification and DNA sequencing

Amplification reactions contained 1×PCR buffer, 0.2 mM of each dNTP, 0.1 U Taq polymerase (*Takara Shuzo, Otsu, Shiga, Japan*), 0.4 *μ*M of each primer, and 2 *μl* of the template in a final reaction volume of 20 *μl*. PCR amplification was performed in a thermal cycler (*GeneAmp® PCR system 9700*; *Applied Biosystems, Foster City, CA, USA*) as follows: 3-min at 95℃ for the initial denaturation step, followed by 40 cycles of 95℃ for 1 min, 56℃ for 1 min, and 74℃ for 1 min, with a final extension step of 7 min at 72℃ . Amplified products were examined by 1.5% agarose gel electrophoresis and ethidium bromide staining. Purified PCR products were sequenced with the use of a BigDye ™ Terminator v3.1 Cycle Sequencing Ready Reaction Kit (*Applied Biosystems*) in an ABI Prism 3130xl Genetic

Table 2 *dnaJ* primer used for detection and sequencing in this study

Primers name	Target species	Sequence (5'-3')	Position
DNC.homi-F & DNC.homi-R	*C. hominis*	5-GTAACTCTTTTTCGCCGTGC	131
		5-CTCAAATACCATCCCGACC	999
DNC.con-F & DNC.con-R	*C. concisus*	5-GACTTGCCTGAAACTATGCC	79
		5-GCGACGAGATAAAAAAAGCC	1094
DNC.rec-F & DNC.rec-R	*C. rectus*	5-CTGCTGCTTGTCCTTTGCTC	265
		5-TAGCCCTAAAATACCATCCC	839
DNC.cur-F & DNC.cur-R	*C. curvus*	5-GGCGATGAGATAAAAAAGGC	49
		5-GTGACGGACAAAATACTCG	771
DNC-F & DNC-R1	All *Campylobacter* spp.	5-AGTTCTTTTTGYTCATCRKT	98[a]
		5-CATCCTGATAGAAAYCAAG	1029[a]
DNC-F & DNC-R2	*C. showae, C. sputorum*	5-CWKTATCYACRCCTTCTGG	658[a]

[a]Position relative to the *C. jejuni dnaJ* sequence. F; forward, R; reverse

Analyzer (*Applied Biosystems, Hitachi, Japan*) according to the manufacturer's instructions.

Phylogenetic data analysis

dnaJ fragments of approximately 750-bp (600-bp for *C. showae* and *C. sputorum*) were sequenced and 16S rRNA, *groEL*, *rpoB*, and *gyrB* gene sequences obtained from GenBank were aligned with the Clustal W program 1.83 (Perriere & Gouy, 1996; Thompson *et al*., 1994). Phylogenetic trees were constructed by the neighbor-joining method (Saitou & Nei, 1987) and drawn with NJPLOT. Bootstrap values (1000 replicates) were calculated to estimate the reliabilities of nodes of the phylogenetic trees obtained. Maximum-likelihood trees were also generated by the DNAML program in the PHYLIP software package and they were drawn with Tree View (Page, 1996).

***dnaJ* sequence-based identification**

dnaJ sequences of all clinical strains listed in Table 3 were selected from position 98 to 1029 according to the *dnaJ* sequence of *C. jejuni* (GenBank accession number AL111168) and aligned with sequences of type strains of the genus *Campylobacter*.

Nine non-*Campylobacter* species (*Helicobacter pylori*, *Helicobacter cinaedi*, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Vibrio cholerae*, *Vibrio hepatarious*, *Enterobacter intermedium*, *Salmolella enterica subsp. enterica* and *Escherichia coli*) were used as negative controls. Because sequence variation among each type strain was smaller than 3.8%, clinical strains were considered to be identified to the closest type strain when the sequence variation between a clinical strain and the type strain was smaller than 3.8%. At this condition, all clinical strains were correctly assigned to each type strain.

Nucleotide sequence accession number

The partial *dnaJ* and 16S rRNA gene sequences obtained in this study were deposited in DDBJ/EMBL/Genbank databases. The accession numbers are shown in Table 1. For comparison, published *dnaJ*, 16S rRNA, *rpoB*, and *groEL* gene sequences were downloaded from GenBank (Table 1 and Fig 3).

Table 3 Clinical strains and their sources

Species	Clinical strains	Source
C. jejuni subsp. *jejuni*	GTC 3270, GTC 3264, GTC 3275, GTC 10845, GTC 8799, GTC 3280, GTC 8798, GTC 8798, GTC 3272, GTC 3278, GTC 10847, GTC 10843, GTC 10750, GTC 3266, GTC 3282, GTC 3284, GTC 3281, GTC 3279, GTC 3283, GTC 3273, GTC 3269, GTC 3277, GTC 8797, GTC 3267, GTC 8784, GTC 8768, GTC 8796, GTC 8428, GTC 3274, GTC 3263, GTC 10844, GTC 3271, GTC 1247, GTC 3286	Human diarrhea
C. jejuni subsp. *doylei*	GTC 8785	Human diarrhea
C. coli	GTC 10849, GTC 10850, GTC 3264, GTC 8760, GTC 8761, GTC 8764, GTC 8765, GTC 3285, GTC 8800, GTC 3901, GTC 10928	Human diarrhea
C. lari	GTC 3901, GTC 10928	Human blood
C. fetus subsp. *fetus*	GTC 3268, GTC 3732, GTC 8727, GTC 3276	Human stool
	GTC 12267, GTC 12526, GTC 12131, GTC 11237, GTC 11236, GTC 11235, GTC 11234, GTC 9812, GTC 8741, GTC 8740, GTC 8731	Spinal fluid
	GTC 9811, GTC 10384, GTC 9810	Human blood
	GTC 3918	Human stomack
C. fetus subsp. *venerealis*	GTC 8743	Animal

GTC=Gifu Type Culture Collection, supported National Bioresource Project (NBRP) of Japan

RESULTS AND DISCUSSION

dnaJ gene sequences analysis for identification of *Campylobacter* species and subspecies

An analysis of the sequence similarity of four conserved genes, *dnaJ*, 16S rRNA, *rpoB* and *groEL* is shown in Fig. 1. The *dnaJ* gene sequences similarity (range, 52.3% to 90.2%; mean 67.8%) are more discriminative than the sequences similarity of 16S rRNA (range, 88.7% to 99.8%; mean 93.55%), *rpoB* (range, 68% to 98.8%: mean 78.84%), *groEL* (range, 66.1% to 94%; mean 80.05%), and *gyrB* (range, 59% to 89.2%; mean 74.1%) (Kawasaki *et al*., 2008). Our results indicated that *dnaJ* was advantageous for discriminating among *Campylobacter* species.

Moreover, in terms of interspecies sequence similarities, *dnaJ* appeared to be the most discriminatory gene (range, 52.3% to 90.2%). The greater divergence of *dnaJ* sequences was particularly evident for species not well differentiated by other gene analyses. For example, two pairs *C. coli* and *C. jejuni* or *C. fetus* and *C. hyointestinalis*, which are the most closely related species and could not be clearly differentiated by phenotypic methods (Morris *et al*., 1985; Nicholson & Patton., 1995; Steinhouserova *et al*., 2001). However, their *dnaJ* sequence similarity of their ranged from (80.2% to 81.8%) or (74.4%) was lower than that of MLST (86.5% between *C. coli* and *C. jejuni*) (Miller *et al*., 2005), 16S rRNA (98.1% to 98.8%; 98%), *rpoB* (96.2% to 98.8%; 86.9%), *groEL* (91.1% to 99.8%; 86.7% to 87.2%), and *gyrB* (89.2%; 84%). Addition, *C. lari*, *C. insulaenigrae*, *C. heveticus*, *C. upsaliensis*, *C. lanienae*, *C. fetus*, *C. hyointestinalis*, *C. showae*, and *C. rectus* shared more than 97% 16S rRNA, 81% to 92% *rpoB* , 70% to 91% *groEL*, and 68.7 % to 85.6% *gyrB* sequence similarity, but only 59.5% to 74.4% *dnaJ* sequence similarity (with the exception of 90.2% *dnaJ* sequence similarity between *C. showae* and *C. rectus*).

The intraspecies, *dnaJ* sequence similarities for *C. coli* ranged from 97.8% to 98.5% and those for *C. lari* ranged 99.3% to 99.7%. The intraspecies sequence similarities between two strains of *C. curvus*, *C. concisus* and *C. homonis* were 95%, 93.5%, and 99.7%. However, when attempting to distinguish *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* poses a special problem for veterinary laboratories, although several phenotypic and genotypic methods are useful for discriminating these two subspecies (Gorkiewicz *et al*., 2003; Hum *et al*., 1997, van Bergen *et al*., 2005), the final determination is based mainly on the different pathogenic association of the subspecies. *C. fetus* subsp. *fetus* causes abortion in the cattle and sheep, whereas *C. fetus* subsp. *venerealis* causes infectious infertility in cattle (Skirrow, 1994). Unfortunately, sequence similarities of (*dnaJ*, 16S rRNA, *groEL*, and *rpoB*) in these studies were greater than 99%, indicating that complete discrimination of these subspecies was not possible.

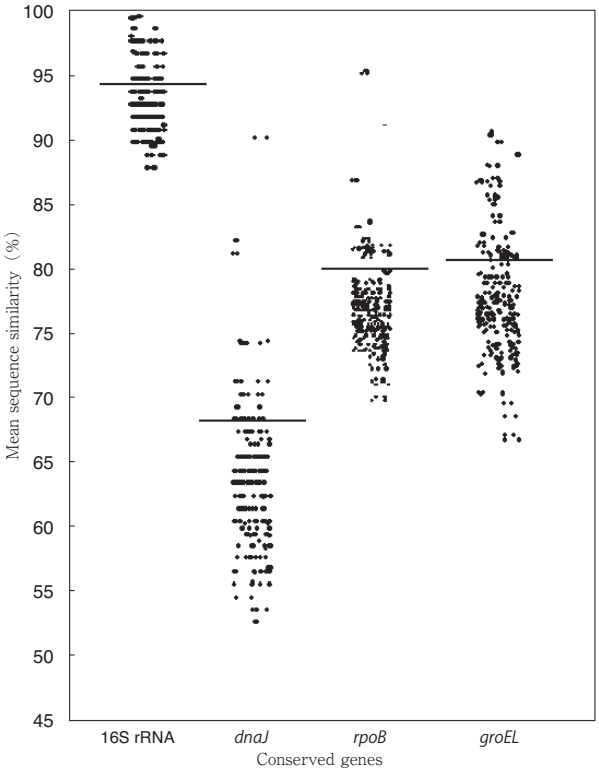

Fig. 1 *dnaJ* and other conserved genes sequence similarity. The horizontal lines indicate the mean sequence similarity value of the conserved genes.

The correlation coefficient between *dnaJ* and 16S rRNA sequence divergences (*r=0.778*) was statistically higher than the corresponding correlation coefficient between *rpoB* and 16S rRNA (*r=0.319*, *P<0.001*) or between *groEL* and 16S rRNA (*r=0.239*, *P<0.001*) (Fig. 2), indicating that the relationship between a pairs of *dnaJ* gene sequence taxa is more coherent than that of 16S rRNA and other genes. Our results suggesting that *dnaJ* gene is a promising phylogenetic marker in *Campylobacter*. The *dnaJ* nucleotide sequence variations ranged from 9.8% to 47.7% (mean 32.2%), which corresponding to 61-350 nucleotide differences; thus, *dnaJ* provided greater sequence variation than 16S rRNA

(mean, 5.49%), *rpoB* (mean, 21.16%) and *groEL* (mean, 19.95%). This greater variation of the *dnaJ* sequences was particularly evident for well-differentiated of species among genus *Campylobacter*.

Phylogenetic relationship analysis among *Campylobacter* species based on conserved genes

Phylogenetic trees based on *dnaJ*, 16S rRNA, *rpoB*, *groEL*, and *gyrB* (Kawasaki *et al*., 2008) sequences constructed by neighbour-joining and maximum-likelihood methods are shown in Fig. 3. In these trees, *Helicobacter pylori* species was used as an out group of the genus *Campylobacter*. Their allocations were stable and reliable, as supported by high bootstrap values. The major topology of the *dnaJ* tree was similar to the trees that are based on the 16S rRNA, *rpoB*, *groEL*, and *gyrB* gene sequences. There is a common cluster that consists of two clearly separated branches. However, there is a notable discrepancy between *dnaJ* and those trees. *C. coli*, *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei* formed a mix same cluster in both of 16S rRNA, *rpoB*, and *gyrB* trees. But in the *dnaJ* tree was discretely separated into two clusters, and each cluster formed a well-delineated branch with high bootstrap values. In 16S rRNA trees in the present study and study by Gorkiewicz *et al*., (2003), *C. lari* vs *C. insulaenigrae* are located within the *C. jejujni*/*C. coli* cluster, *C. lanienae* vs *C. hyointestinalis* subsp. *lawsonii*, *C. fetus* vs *C. hyointestinalis* subsp. *hyointestinalis* formed same cluster. These relations were in disagreement with the observations obtained from DNA-DNA hybridization study (Foster *et al*., 2004; van Doorm *et al*., 1998). In contrast, those species and both subspecies were distinctly clustered in the *dnaJ* tree.

The *dnaJ* and *groEL* tree topologies show similar clusters of species within genus *Campylobacter*, in *groEL* tree both *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei* formed the same cluster, but they are separated distinct into two subclusted in the *dnaJ* tree. In all trees based on (*dnaJ*, 16S rRNA, *rpoB*, and *groEL*), *C. showae* clustered with *C. rectus*, consistent with the previous reported of the close phy-

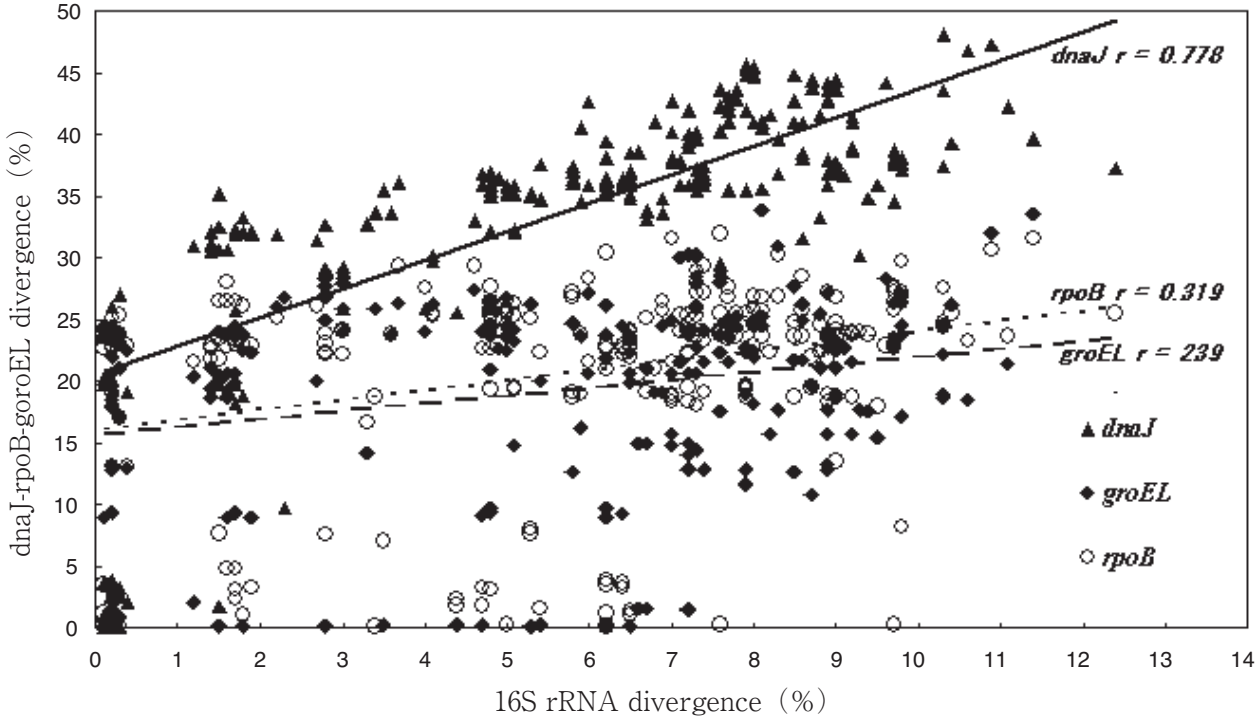

Fig. 2 Scatter plots of *dnaJ*, *rpoB* , *groEL* and 16S rDNA distances for Campylobacter reference and type strains. Each dot represents a pair of taxa, plotted according to their relative evolutionary distance to both genes. The regression line between *dnaJ* and 16S rDNA pairwise distances is shown by the dashed line.

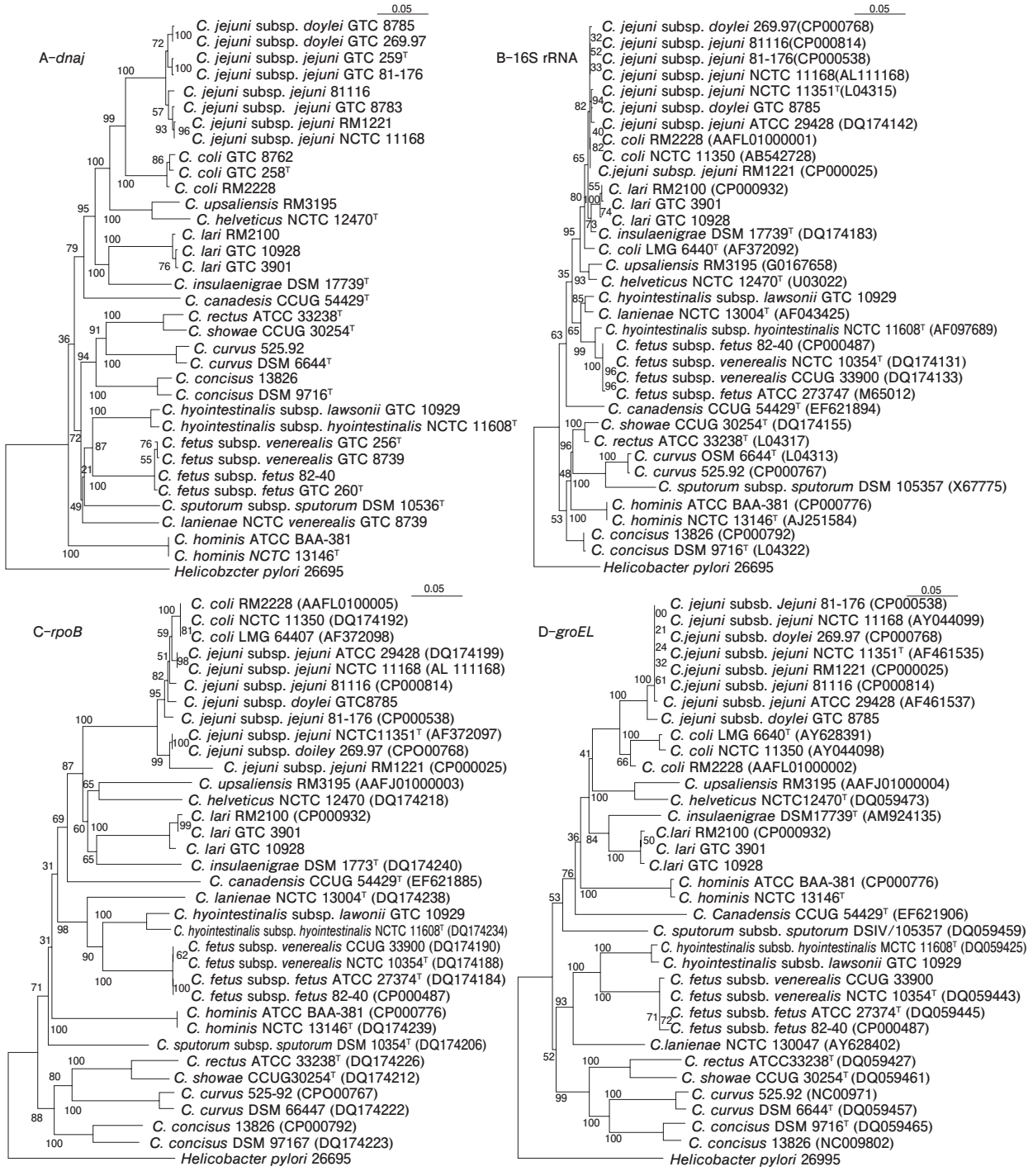

Fig. 3 Neighbour-joining phylogenetic trees based on partial *dnaJ* (~ 750-bp) and 16S rDNA (~ 1350-bp), *rpoB* (~ 480-bp) and *groEL* (~ 540 bp) gene sequences using 16 *Campylobacter* species. Bootstrap values (expressed as a percentage of 1000 replicates). The bar represents 5% sequence divergence.

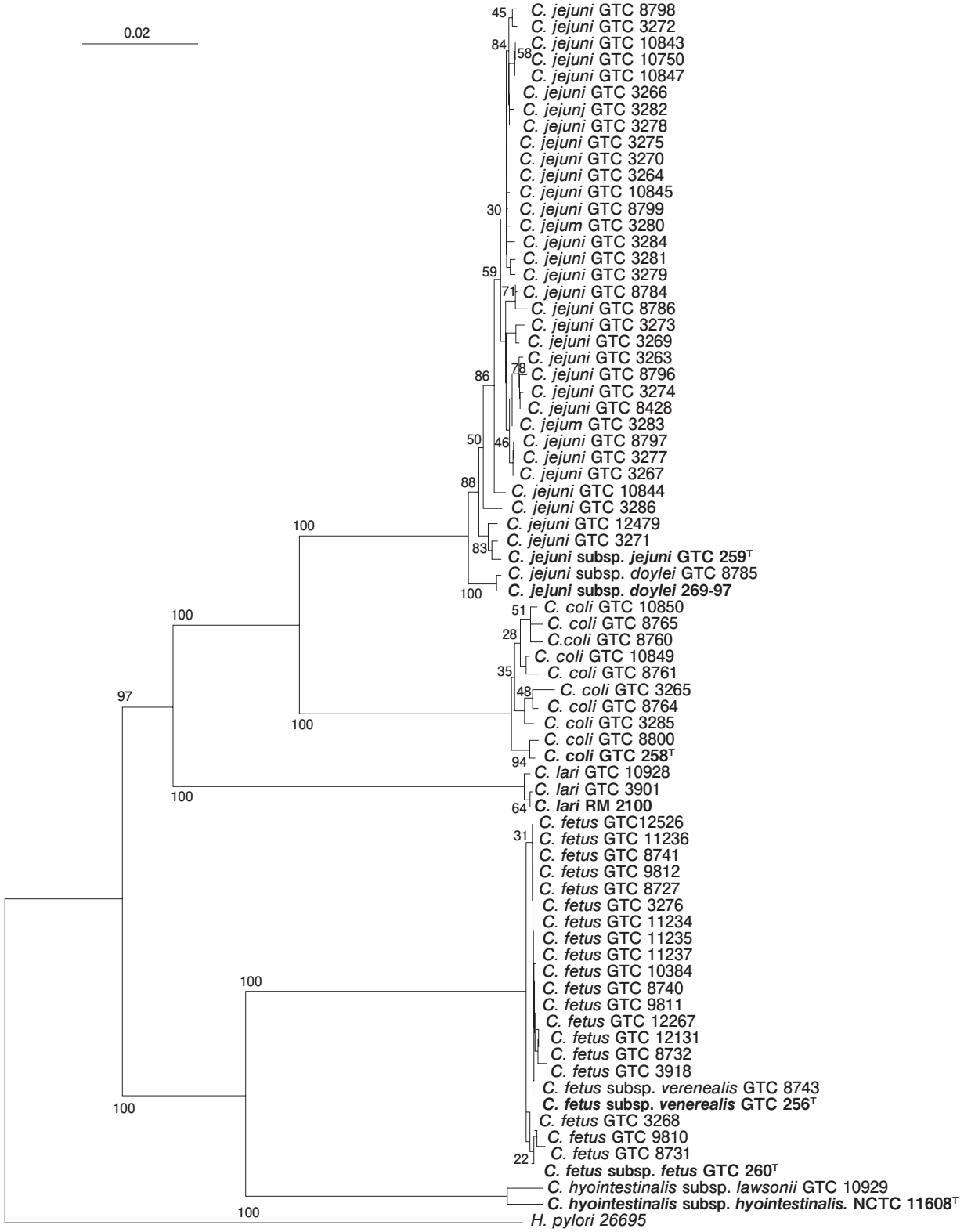

Fig. 4 Neighbour-joining phylogenetic trees based on partial *dnaJ* sequences of 66 clinical strains and 7 (reference and type strains). Bootstrap values (expressed as a percentage of 1000 replicates) are shown at tree nodes. *H. pylori* was used as an out group. The bar represents 2% sequence divergence.

Table 4 *dnaJ* sequence intraspecific variations and similarities among the 66 clinical isolates

Species	Source	Intraspecific variation (%)	Range of % similarity[a]	No. of strains
C. jejuni subsp. *jejuni*	Stool	0 – 3.8	96.5 – 98.2	34
C. coli	Stool, feces	0.5 – 2.9	97.0 – 99.5	9
C. fetus subsp. *fetus*	Stool, blood, meningitis, spinal fluid, and stomach	0 – 0.9	99.2 – 100	20
C. lari	Blood, stool	0.3 – 0.7	99.3 – 99.7	2
C. hyointestinalis subsp. *lawsonii*	Stool	0	96.1	1

[a]% Similarities between the clinical strains and type strains were calculated.

logenetic relationship (Gorkiewicz *et al*., 2003). Two strains showed a close relationship with 99.8% gene sequence similarity for 16S rRNA divergence among subspecies was 1.8% to 3.6% between *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*, and 3.9% between *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii*. Indicating that *dnaJ* sequence analyses would be useful in discrimination at subspecies level. However, the sequence divergence between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* was narrow (0% to 0.9%). This results was consistent with previous studies (Hum *et al*., 1997; Klein *et al*., 1986; Kawasaki *et al*., 2008).

dnaJ sequence-based identification of clinical strains

All clinical strains (Table 3) of genus *Campylobacter* were identified by conventional biochemical tests, yielded the expected amplicons. *Helicobacter* and *Arcobacter* and other non-*Campylobacter* strains used as controls did not yield amplicon, indicating that the designed primer were specific to *Campylobacter* species. All clinical *Campylobacter* strains were correctly identified based on *dnaJ* sequence analysis within the selected portion 98 to 1029 of *dnaJ* gene sequence of *C. jejuni* species. The phylogenetic tree based on the 66 clinical strains and seven reference and type strains are shown in Fig. 4. The seven pathogenic type strains formed separate clusters. All of the clinically identified strains representing five *Campylobacter* most associated with infectious disease (*C. jejuni*, *C. coli*, *C. lari*, *C. hyointestinalis* and *C. fetus*) strains matched perfectly with corresponding type strains in robust clusters. *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doyley*, which clearly appeared as two subclusters. But *C. fetus* subsp. *fetus* and *C. fetus* subsp. *verenealis* formed one cluster. This result was consistent with previous studies (Hum *et al*., 1997; Kärenlampi *et al*., 2004; Korczak *et al*., 2006; Kawasaki *et al*., 2008). To estimate intraspecies sequence divergences of all clinical strains ranged from 0% to 3.8% (Table 4), often showing values less than 3%, which were much less than the sequence divergence between species.

In conclusion, our finding show that *dnaJ* gene sequences offers advantageous to 16S rRNA and other housekeeping gene sequences in defining phylogenetic relations within genus *Campylobacter* strains at the species level with sequence variability less than 3.9%.

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Arcobacter 属細菌，*Campylobacter* 属細菌，*Helicobacter* 属細菌の分類・同定指標としての *dnaJ* 配列情報の解析

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Campylobacter 属の 16S rRNA，及び 3 つの housekeeping genes（*rpoB*，*groEL*，及び *dnaJ*）遺伝子配列を決定し，配列の多型を比較した．その結果，93.55%，78.84%　80.05%，及び 66.98% と housekeeping genes の多型の幅は遺伝子ごとに異なっており，*dnaJ* の多型が最も大きかった．また 16S rRNA との多型の相関をしらべた結果 *dnaJ* の相関が最も良かったので，この配列情報を使用し同定する方法を作成した．*Campylobacter* 属に共通のプライマーを作成し，臨床材料から出る *Campylobacter* を *dnaJ* 配列で同定を試みたところ，すべての菌株が基準株と 3.8%の多型の幅に収まり，正確に同定することができた．この情報は表現形で識別する特徴が少ない人病原性 *Campylobacter* の菌種の同定に有用であった．

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