Genetic relatedness within the genus *Campylobacter* inferred from *rpoB* sequences

Bożena M. Korczak,¹ Regina Stieber,¹ Stefan Emler,² André P. Burnens,^{1†} Joachim Frey¹ and Peter Kuhnert¹

Correspondence

Peter Kuhnert
peter.kuhnert@vbi.unibe.ch

¹Institute of Veterinary Bacteriology, University of Bern, CH-3012 Bern, Switzerland

²SmartGene, Zug, Switzerland

The genus *Campylobacter* comprises 17 species, some of which are important animal and human pathogens. To gain more insight into the genetic relatedness of this genus and to improve the molecular tools available for diagnosis, a universal sequencing approach was established for the gene encoding the beta-subunit of RNA polymerase (*rpoB*) for the genus *Campylobacter*. A total of 59 strains, including the type strains of currently recognized species as well as field isolates, were investigated in the study. A primer set specific for *Campylobacter* species enabled straightforward amplification and sequencing of a 530 bp fragment of the *rpoB* gene. The 16S rRNA gene sequences of all of the strains were determined in parallel. A good congruence was obtained between 16S rRNA and *rpoB* gene sequence-based trees within the genus *Campylobacter*. The branching of the *rpoB* tree was similar to that of the 16S rRNA gene tree, even though a few discrepancies were observed for certain species. The resolution of the *rpoB* gene within the genus *Campylobacter* was generally much higher than that of the 16S rRNA gene sequence, resulting in a clear separation of most species and even some subspecies. The universally applicable amplification and sequencing approach for partial *rpoB* gene sequence determination provides a powerful tool for DNA sequence-based discrimination of *Campylobacter* species.

The genus *Campylobacter* was first proposed by M. Sebald and M. Véron in 1963 and included only the type species *Campylobacter fetus* and *Campylobacter bubulus*, now known as *Campylobacter sputorum* (Sebald & Véron, 1963). These taxa had formerly been classified as *Vibrio* species. In 1973, M. Véron and R. Chatelain included several misclassified *Vibrio* in the distinct *Campylobacter* genus based on serological, biochemical and DNA–DNA hybridization investigations (On, 2001). Since then, the taxonomy of the genus has changed dramatically. At present, it comprises 17 species with validly published names and six recognized subspecies (On, 2001; Foster *et al.*, 2004). In addition, strains belonging to *C. sputorum* are divided into three biovars, *sputorum*, *fecalis* and *paraureolyticus*, on the

basis of their ability to produce catalase or urease (On *et al.*, 1998; Vandamme & On, 2001).

In general, members of the genus *Campylobacter* colonize the mucosal surfaces of the intestinal tract, oral cavity or urogenital tract of healthy, as well as diseased, humans and animals, especially birds. Several species may act as pathogens, causing disease in both human and animal hosts. Twelve of the 17 *Campylobacter* species are associated with human diseases. *Campylobacter jejuni* and *Campylobacter coli* are particularly frequent causative agents of human bacterial intestinal disorders worldwide (Skirrow, 1994). There have also been reported cases of diarrhoea in man caused by *Campylobacter upsaliensis* and *Campylobacter lari*, but the frequency of these infections is very low (Bourke *et al.*, 1998; Van Doorn *et al.*, 1998). Occasionally, *Campylobacter* species are implicated as causative agents of pericarditis, myocarditis and Guillain–Barré Syndrome (Kuwabara, 2004; Uzoigwe, 2005). The most important animal diseases are caused by *Campylobacter fetus* subsp. *fetus*, which is associated with abortion in cattle and sheep, and *Campylobacter fetus* subsp. *venerealis*, associated with infectious infertility in cattle (Skirrow, 1994).

Taxonomic classification and identification of *Campylobacter* species by phenotypic methods is often difficult and

Published online ahead of print on 23 December 2005 as DOI 10.1099/ijse.0.64109-0.

†Present address: MCL Medical Laboratories, CH-3186 Düringen, Switzerland.

Abbreviation: MLST, multilocus sequence typing.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *rpoB* gene sequences of the *Campylobacter* species determined in this study are shown in Table 1.

Distance matrices for the 16S rRNA and *rpoB* gene sequences for all the strains investigated in this study are available as Supplementary Tables S1 and S2 in IJSEM Online.

time-consuming because of their fastidious growth, low biochemical activity and the variability of results (Moore & Madden, 2003). Therefore, several genotypic methods have been used for identification and taxonomic investigations within the genus *Campylobacter* (On, 1996). A large DNA–rRNA hybridization study on the *Campylobacter* complex was carried out by Vandamme *et al.* (1991) and provided a basis for the revision of its taxonomy. Numerical analysis of amplified fragment length polymorphism (AFLP) profiles has been used for the investigation of the genetic relationships within the genus *Campylobacter* (On & Harrington, 2000). In other studies, 16S rRNA and 23S rRNA gene sequence-based phylogenies within the genus were analysed (Van Camp *et al.*, 1993; Harrington & On, 1999; Gorkiewicz *et al.*, 2003). Kärenlampi *et al.* (2004) performed phylogenetic analysis within the genus *Campylobacter* using partial sequences of the heat shock protein gene *groEL*. Most recently, the genetic relatedness between *C. jejuni* and *C. coli* was investigated by multilocus sequence typing (MLST) of certain housekeeping genes (Dingle *et al.*, 2005; Miller *et al.*, 2005).

Analysis of the sequences of rRNA genes has deeply modified the taxonomy and identification of many groups of bacteria. However, this tool has limitations due to the low polymorphism of 16S rRNA gene sequence data and high intra-species diversity in some cases (Harrington & On, 1999; On, 2001). Therefore, additional genetic tools are needed to clarify the taxonomy of the genus *Campylobacter* and, at the same time, to enable the genetic identification of bacteria belonging to this genus. Analysis of the *rpoB* gene encoding the beta-subunit of RNA polymerase has greatly supported the identification and the elaboration of genetic relationships in several groups of bacteria (Mollet *et al.*, 1997; Kim *et al.*, 2003; Korczak *et al.*, 2004; Christensen *et al.*, 2004; Drancourt *et al.*, 2004). Recently, the *rpoB* gene has also been used to support the description of novel taxa (Angen *et al.*, 2003; Kuhnert *et al.*, 2004).

In this study, we used a universal amplification and sequencing approach for the *rpoB* gene to investigate genetic relatedness within the genus *Campylobacter*.

Sequence comparison analysis was used to select the primers CamrpoB-L, 5'-CCAATTTATGGATCAAAC-3', and RpoB-R, 5'-GTTGCATGTTNGNACCCAT-3', which enabled the amplification of a 524 bp fragment from the *rpoB* gene of most *Campylobacter* species. The PCR product corresponded to region 1536–2059 bp of the *Escherichia coli* *rpoB* gene. For *Campylobacter helveticus*, *Campylobacter curvus* and *Campylobacter lanienae*, the primer CamrpoB-L1, 5'-GGKCARCTYTCKCAATTYATGG-3', was required. In this case, the gene fragment generated (535 bp) corresponded to the 1525–2059 bp position of the *E. coli* *rpoB* gene. The three primers could be combined in the same PCR at an annealing temperature of 54 °C without interfering with each other. Amplification was carried out in 50 µl volumes containing 20 pmol of each primer, 1 mM dNTP, 1 × reaction buffer B (supplied with FIREPol DNA polymerase),

2.5 mM MgCl₂ and 2.5 U FIREPol polymerase (Solis BioDyne). Approximately 100 ng template was added as genomic DNA or as lysate. Cycling conditions and purification steps were performed as previously described (Korczak *et al.*, 2004). Finally, about 30 ng purified PCR product was used for sequencing with the BigDye Terminator cycle sequencing kit (Applied Biosystems) using either CamrpoB-L, CamrpoB-L1 or RpoB-R as sequencing primers. Sequences were analysed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and then edited using SEQUENCHER software (GeneCodes). The three primers used for amplifying and sequencing the partial *rpoB* gene of *Campylobacter* species were found to be universal within this genus, since all strains that have been analysed so far have yielded amplification products.

In order to confirm species identity and improve the available sequences (resolving unknown bases or generating longer sequence fragments), the 16S rRNA gene sequence of all the *Campylobacter* species was determined in parallel according to a previously described method (Kuhnert *et al.*, 2002). PCR and sequencing resulted in a 1.3–1.5 kb fragment, depending on the presence of an intervening sequence. All sequences obtained were deposited in databases for 16S rRNA and *rpoB* gene sequences using the SmartGene IDNS platform (www.idns-smartgene.com), which allows parallel sequence comparisons of both target genes. At the same time, all *rpoB* sequences and improved 16S rRNA gene sequences were deposited in GenBank. *Campylobacter* type strains and reference strains from bacterial culture collections were used in this study. For some species where only a few strains were available, some field strains were also included. These had been identified previously by the Swiss National Centre for Enteropathogenic Bacteria using standard phenotyping methods, and the original species designation was used in this study. The strains used in this study, their origin and the GenBank accession numbers of the corresponding *rpoB* and 16S rRNA gene sequences are given in Table 1. Distance matrices using Jukes–Cantor correction and phylogenetic analysis using neighbour-joining tree building were performed with the BioNumerics program version 4.0 (Applied Maths).

Congruence analysis in BioNumerics showed a good correlation between *rpoB* and 16S rRNA gene sequence-derived trees ($r=0.81$). Apart from a few incongruities, the trees showed a similar topology (Figs 1 and 2). Discrepancies in branching and clustering are most probably due to horizontal gene transfer, which is also known to occur with house-keeping genes such as 16S rRNA and *rpoB* (Ko *et al.*, 2002; Acinas *et al.*, 2004). The *rpoB* gene was found to provide a better resolution for *Campylobacter* species, with lower interspecies sequence similarities, ranging from 56.4 to 98.8%, compared with those for the 16S rRNA gene (86.8–100%). Thus, *Campylobacter* species can be better differentiated by using the *rpoB* gene than the 16S rRNA gene. This is also visible in Figs 1 and 2, which have the same scale bar, thereby showing clearly the higher resolution

Table 1. Origin of strains and GenBank accession numbers of 16S rRNA and *rpoB* gene sequences for the *Campylobacter* species used in this study

ATCC, American Type Culture Collection; CCUG, Culture Collection University of Goteborg; LMG, Bacteria Collection of the Belgian Co-ordinated Collections of Microorganisms; NCTC, National Collection of Type Cultures; Lio, Lior serogroup, NZ; E, Swiss National Centre for Enteropathogenic Bacteria; NA, not available.

Species	Strain	Source	GenBank accession no.*	
			16S rRNA gene	<i>rpoB</i> gene
<i>C. fetus</i> subsp. <i>fetus</i>	ATCC 27374 ^T	Sheep fetus brain	DQ174127	DQ174184
	CCUG 32676	NA	DQ174128	DQ174185
	CCUG 32677	NA	DQ174129	DQ174186
<i>C. fetus</i> subsp. <i>venerealis</i>	CCUG 32114	Human wound abscess	DQ174130	DQ174187
	NCTC 10354 ^T	Heifer vaginal mucus	DQ174131	DQ174188
	CCUG 24260	Bovine	DQ174132	DQ174189
	CCUG 33902	Bull prepucce	DQ174133	DQ174190
	CCUG 33871	NA	DQ174134	DQ174191
<i>C. coli</i>	LMG 6440 ^T	Pig faeces	AF372092	AF372098
	Lio8 (=CCUG 12068)	Human	DQ174135	DQ174192
	NZ 1905-94	Chicken	DQ174136	DQ174193
	NZ 2695-96	Water	DQ174137	DQ174194
	NZ 899-00-1	Human faeces	DQ174138	DQ174195
	NZ 900-95	Human	DQ174139	DQ174196
	NZ 4812-94	Human	DQ174140	DQ174197
<i>C. jejuni</i> subsp. <i>jejuni</i>	NCTC 11351 ^T	Bovine faeces	AF372091	AF372097
	CCUG 10937	Human faeces	DQ174141	DQ174198
	ATCC 29428	Child diarrhoeic stool	DQ174142	DQ174199
	Lio 6 (=CCUG 12066)	Human	DQ174143	DQ174200
<i>C. jejuni</i> subsp. <i>doylei</i>	LMG 8843 ^T	Child diarrhoeic stool	DQ174144	DQ174201
<i>C. lari</i>	NCTC 11352 ^T	Herring gull cloacal swab	DQ174145	DQ174202
	NZ 145-95	Human faeces	DQ174146	DQ174203
	NZ 3541-96	Water	DQ174147	DQ174204
	NZ 3663-96	Water	DQ174148	DQ174205
<i>C. sputorum</i>	LMG 7795 ^T	Human oral cavity	DQ174149	DQ174206
	NCTC 11415	Sheep faeces	DQ174150	DQ174207
	NCTC 11367	Bull sperm	DQ174151	DQ174208
	CCUG 886	Bull sperm	DQ174152	DQ174209
	CCUG 24261	Sheep stillborn	DQ174153	DQ174210
	NZ 504-95	Human wound abscess	DQ174154	DQ174211
<i>C. showae</i>	CCUG 30254 ^T	Human gingival crevice	DQ174155	DQ174212
	CCUG 11641	Human dental root canal	DQ174156	DQ174213
<i>C. upsaliensis</i>	CCUG 14913 ^T	Dog faeces	DQ174157	DQ174214
	NZ 4310-94	Human faeces	DQ174158	DQ174215
	NZ 5564-94	Human faeces	DQ174159	DQ174216
	NZ 1209-95	Human faeces	DQ174160	DQ174217
<i>C. helveticus</i>	NCTC 12470 ^T	Cat faeces	DQ174161	DQ174218
	LMG 12639	Cat faeces	DQ174162	DQ174219
	NCTC 12472	Cat	DQ174163	DQ174220
	E 504-90	Dog faeces	DQ174164	DQ174221
<i>C. curvus</i>	ATCC 35224 ^T	Human alveolar (or jaw) abscess	DQ174165	DQ174222
<i>C. concisus</i>	ATCC 33237 ^T	Human gingival sulcus	DQ174166	DQ174223
	NZ 539-95	Human	DQ174167	DQ174224
<i>C. gracilis</i>	ATCC 33236 ^T	Human gingival sulcus	DQ174168	DQ174225
<i>C. rectus</i>	CCUG 20446 B ^T	Human periodontal pocket	DQ174169	DQ174226
	CCUG 11640	Human apical periodontitis	DQ174170	DQ174227
	CCUG 11643	Human apical periodontitis	DQ174171	DQ174228
	CCUG 27948	Human pleura	DQ174172	DQ174229

Table 1. cont.

Species	Strain	Source	GenBank accession no.*	
			16S rRNA gene	<i>rpoB</i> gene
<i>C. mucosalis</i>	ATCC 43264 ^T	Pig small intestine	DQ174173	DQ174230
	CCUG 10771	Pig small intestine	DQ174174	DQ174231
	CCUG 21559	Pig necrotic colitis	DQ174175	DQ174232
	CCUG 21560	Pig necrotic colitis, colon	DQ174176	DQ174233
<i>C. hyointestinalis</i> subsp. <i>hyointestinalis</i>	LMG 7817 ^T	Pig intestine	DQ174177	DQ174234
	NZ 701-94	Calf faeces	DQ174178	DQ174235
	NZ 3637-94	Human faeces	DQ174179	DQ174236
	NZ 5171-94	Calf faeces	DQ174180	DQ174237
<i>C. hyointestinalis</i> subsp. <i>lawsonii</i>	CCUG 34538 ^T	Pig stomach	DQ195237	DQ195238
<i>C. lanienae</i>	NCTC 13004 ^T	Human	DQ174181	DQ174238
<i>C. hominis</i>	NCTC 13146 ^T	Human	DQ174182	DQ174239
<i>C. insulaenigrae</i>	NCTC 12927 ^T	Marine mammal	DQ174183	DQ174240

*Sequences starting with DQ were all determined in this study.

obtained with *rpoB* gene sequences. The distance matrices of the 16S rRNA and *rpoB* gene sequences for all the strains investigated in this study are available as Supplementary Tables S1 and S2 in IJSEM Online.

Differentiation between *C. jejuni* and *C. coli*, two genetically and phylogenetically closely related species, is difficult. Moreover, a few studies have shown that the hippurate hydrolysis test, which is critical for discrimination of these species, can sometimes give a false negative result for *C. jejuni*, leading to misidentification (Morris *et al.*, 1985; Nicholson & Patton, 1995). Many molecular tests that are useful for the differentiation of these two species have been developed, e.g. multiplex PCR or MLST (Cloak & Fratamico, 2002; Dedieu *et al.*, 2004; Dingle *et al.*, 2005; Nayak *et al.*, 2005; Miller *et al.*, 2005). In this study, it was impossible to separate *C. jejuni* and *C. coli* strains based on the 16S rRNA gene sequence (Fig. 1). Several *C. jejuni* and *C. coli* strains shared identical 16S rRNA gene sequences and nearly all the investigated strains of these taxa were mixed in a common cluster. Only the type strain of *C. coli*, LMG 6440^T, formed a separate branch (Fig. 1) as previously observed by Gorkiewicz *et al.* (2003). In contrast, the *rpoB* gene is strongly conserved within most *C. coli* strains, which shared 100% sequence identity with *C. coli* LMG 6440^T. The location of the *C. coli* type strain in the *rpoB* tree on the same branch together with other *C. coli* strains is also seen with a tree based on *groEL* gene sequences (Kärenlampi *et al.*, 2004). However, the still low *rpoB* sequence divergence between *C. coli* and *C. jejuni* strains, ranging from only 1.2% to 3.8%, makes it difficult to discriminate between the two species. A further discrepancy in the *C. coli* cluster in the 16S rRNA gene-based tree was found for strains NZ 2695-95 and NZ 1905-94, which formed a distinct cluster of their own in the *rpoB*-based tree (Fig. 2). Pairwise sequence comparison of these strains with *C. coli* LMG 6440^T revealed *rpoB* gene sequence similarities of only 83.6% and 82.8%,

respectively. This suggests that strains NZ 2695-95 and NZ 1905-94 may be genetically atypical *C. coli*-like strains or represent a novel species distinct from *C. coli*. Between these two strains, the *rpoB* sequence divergence was 6.2%, indicating further heterogeneity within putative *C. coli*-like strains. Further investigation of the relatedness of these two strains, including genotypic and phenotypic analysis, is necessary.

The type strain of *C. lari*, NCTC 11352^T, and the recently described novel species *Campylobacter insulaenigrae* displayed high 16S rRNA gene sequence similarity and both species are located within the *C. jejuni/C. coli* cluster. In contrast, in the *rpoB* tree, *C. lari* and *C. insulaenigrae* are clearly separated from other members of the genus *Campylobacter* and are also clearly separated from each other. This clustering based on *rpoB* sequences is supported by DNA–DNA hybridization results, which showed a close genetic relationship between *C. insulaenigrae* and *C. lari* and a significantly lower genetic relationship between *C. insulaenigrae* and *C. jejuni* (Foster *et al.*, 2004).

It is known that *C. lari* is a phenotypically and genetically heterogeneous species. In a recent study by Duim *et al.* (2004), several groups were discerned within this species. A previous study based on numerical analysis of AFLP profiles demonstrated that classical urease-negative and urease-positive strains form closely related, but distinct, clusters (On & Harrington, 2000). Our results confirm that *C. lari* is a heterogeneous group as demonstrated in both the *rpoB* and 16S rRNA gene-derived trees. The *C. lari* cluster is split into two branches. The first branch is formed by the urease-negative and nalidixic acid-resistant type strain NCTC 11352^T and the clinical isolate NZ 145-95. The second branch includes the two urease-positive and nalidixic acid-sensitive strains (NZ 3541-96 and NZ 3663-96) which share 100% sequence similarity within the *rpoB* and 16S rRNA

Fig. 1. Neighbour-joining phylogenetic tree of the genus *Campylobacter* based on 16S rRNA gene sequences. *E. coli* was used as an outgroup. Bootstrap values of 500 simulations are indicated at major branches. The GenBank accession numbers for the strains are shown in Table 1. Bar, 2% sequence divergence.

gene fragments. Their high *rpoB* sequence divergence from the type strain of *C. lari* (11.8%) once more confirms the genetic diversity of this species. Sequencing of the *rpoB* gene therefore appears to be a promising tool for the differentiation of *C. lari* strains.

In both the *rpoB* and 16S rRNA gene-derived trees, *C. upsaliensis* and *C. helveticus* clustered in a similar way to that described for the phylogeny of the genus based on *groEL* gene sequences (Kärenlampi *et al.*, 2004). A common cluster can be observed which consists of two clearly separated branches formed by *C. upsaliensis* and *C. helveticus* strains. It confirms their notable DNA similarity (Stanley *et al.*, 1992). Moreover, *C. helveticus* appears to be a very genetically homogeneous group, reflected in a 100% sequence match in *rpoB* genes between all investigated strains.

C. fetus and *Campylobacter hyointestinalis* subsp. *hyointestinalis* formed one cluster in both the *rpoB* tree and the 16S rRNA gene tree. The DNA–DNA hybridization study performed by Roop *et al.* (1984) also revealed some DNA similarity between these species. The two species are phenotypically and genotypically similar, but associated with different diseases (Skirrow, 1994; On, 2001). In the 16S rRNA gene sequence-based tree, *C. hyointestinalis* subsp. *lawsonii* clustered with *C. lanienae* and was clearly distinct from *C. hyointestinalis* subsp. *hyointestinalis*. Harrington *et al.* (1999) have already described wide 16S rRNA gene sequence diversity (1.0–4.3%) between members of the two *C. hyointestinalis* subspecies. In contrast, in the *rpoB* gene based tree, both subspecies of *C. hyointestinalis* belong to the same subcluster, which is coupled to the branch formed by *C. fetus* strains. This location in the *rpoB* tree confirms the

Fig. 2. Neighbour-joining phylogenetic tree of the genus *Campylobacter* based on partial *rpoB* gene sequences. *E. coli* was used as an outgroup. Bootstrap values of 500 simulations are indicated at major branches. The GenBank accession numbers for the strains are shown in Table 1. Bar, 2% sequence divergence.

close phylogenetic relationship between these two subspecies and strongly supports the DNA–DNA hybridization results and phylogeny based on the *groEL* gene (On *et al.*, 1995; Kärenlampi *et al.*, 2004). In general, the *rpoB* and *groEL* genes possess similar characteristics concerning genetic

relatedness within the genus *Campylobacter*. Both targets achieved nearly the same level of resolution of *Campylobacter* species. Moreover, a comparison of the tree topologies found with both gene targets shows similar clustering of species within the genus *Campylobacter*. However, the

groEL gene tree is less extensive as it does not show all the recognized species and analyses fewer representative strains for the species that are included.

C. lanienae settles on a distinct branch, joined to the *C. fetus*/*C. hyointestinalis* cluster, in both the *rpoB* and 16S rRNA gene trees.

Distinguishing between *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 (Hum *et al.*, 1997; On & Harrington, 2001), the final determination is based mainly on the different pathogenic associations of the subspecies. *C. fetus* subsp. *fetus* causes abortion in cattle and sheep, whereas *C. fetus* subsp. *venerealis* causes infectious infertility in cattle (Skirrow, 1994). Unfortunately, the resolution of the *rpoB* gene, as is the case for the 16S rRNA gene, does not allow the differentiation of *C. fetus* subsp. *fetus* from *C. fetus* subsp. *venerealis*.

The species *C. sputorum* and *Campylobacter hominis* clustered together in the 16S rRNA gene-based tree, but formed two clearly distant branches. This topology is similar to that found for the *rpoB* gene tree in this study, but is different from that shown in the 16S rRNA gene tree of the genus as described by Gorkiewicz *et al.* (2003).

In both the *rpoB* and 16S rRNA gene-derived trees, *Campylobacter mucosalis* and *Campylobacter concisus* showed some genetic relationship, in agreement with DNA hybridization results (Roop *et al.*, 1985).

In the 16S rRNA gene tree, *C. curvus* clustered with *C. concisus*, whereas in the *rpoB* gene tree *C. curvus* is on a separate branch close to *Campylobacter showae*, *Campylobacter gracilis* and *Campylobacter rectus*. All four species have been isolated from the gingival crevices of healthy individuals and patients with periodontal diseases. In the 16S rRNA gene tree of Gorkiewicz *et al.* (2003), *C. curvus* showed the same phylogenetic position as observed in the *rpoB* tree. The similar position and clustering of *C. rectus* and *C. showae* in both trees demonstrates a close phylogenetic relationship that has been observed previously (Gorkiewicz *et al.*, 2003). However, the heterogeneity of the *rpoB* sequences between the two *C. showae* strains is remarkable. The two strains showed a close relationship in their 16S rRNA gene sequences, sharing 99.8% sequence identity, but their *rpoB* gene sequence similarity was only 92%.

In certain *Campylobacter* species, intervening sequences (IVS) in the 16S rRNA gene have been described, particularly for *C. sputorum*, *C. curvus*, *C. rectus*, *C. helveticus* and *C. hyointestinalis* (Linton *et al.*, 1994; Etoh *et al.*, 1998). When compared with the 16S rRNA gene sequence of the type species *C. fetus* (GenBank accession no. DQ174127), all *C. sputorum* strains analysed in this study showed an IVS of 227–229 bp. In the *C. curvus* type strain, an IVS of 136 bp

was present. Two of the four *C. rectus* strains investigated, CCUG 11640 and CCUG 27948, contained an IVS of 189 bp when compared with the other two *C. rectus* strains.

In conclusion, the *rpoB* gene has greater resolution than the 16S rRNA gene sequence for the genus *Campylobacter*. The *rpoB* resolution is sufficient for the differentiation of very closely related species as well as of most subspecies. However, it is probably not possible to separate *C. coli* and *C. jejuni* based on *rpoB* gene sequences. A separate study including a large number of *C. coli* and *C. jejuni* strains could help to clarify whether the high *rpoB* gene sequence conservation seen with *C. coli* strains might be useful for the differentiation of this species from *C. jejuni*. Generally, the *rpoB* gene sequence better reflects DNA–DNA hybridization results than the 16S rRNA gene sequence, as previously observed for the family *Pasteurellaceae* (Korczak *et al.*, 2004). Therefore, the *rpoB* gene might be less prone to horizontal gene transfer and the partial *rpoB* gene sequence of 530 bp may better reflect the phylogeny of the genus *Campylobacter* (and eventually others) than does the 16S rRNA gene sequence. The *rpoB* gene sequence might be particularly useful for diagnostic purposes when sequencing a short fragment could give a clear-cut result.

The universally applicable amplification and sequencing approach for partial *rpoB* gene sequence determination that was devised in this study provides a powerful tool for DNA sequence-based identification of members of the genus *Campylobacter*.

Acknowledgements

This work was supported by a grant from the KTI (no. 6041.1 KTS). We thank G. Foster (Drummondhill, UK) for sending us the *C. insulaenigrae* type strain and Isabelle Brodard for technical help.

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