Identification of *Campylobacter* spp. and discrimination from *Helicobacter* and *Arcobacter* spp. by direct sequencing of PCR-amplified *cpn60* sequences and comparison to cpnDB, a chaperonin reference sequence database

Janet E. Hill,^{1†} Ana Paccagnella,² Kee Law,² Pasquale L. Melito,³ David L. Woodward,³ Lawrence Price,³ Amy H. Leung,⁴ Lai-King Ng,³ Sean M. Hemmingsen¹ and Swee Han Goh^{2,4,5}

Correspondence

Swee Han Goh
shgoh@interchange.ubc.ca

¹National Research Council Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada

²British Columbia Centre for Disease Control, 655W 12th Ave, Vancouver, BC, V5Z 4R4, Canada

³Bacteriology and Enteric Diseases Program, National Microbiology Laboratory, Winnipeg, Manitoba, Canada

^{4,5}Dept of Pathology and Laboratory Medicine⁴, and UBC CDC⁵, University of British Columbia, BC, Canada

A robust method for the identification of *Campylobacter* spp. based on direct sequencing of PCR-amplified partial *cpn60* sequences and comparison of these to a reference database of *cpn60* sequences is reported. A total of 53 *Campylobacter* isolates, representing 15 species, were identified and distinguished from phenotypically similar *Helicobacter* and *Arcobacter* strains. Pairwise *cpn60* sequence identities between *Campylobacter* spp. ranged from 71 to 92 %, with most between 71 and 79 %, making discrimination of these species obvious. The method described overcomes limitations of existing PCR-based methods, which require time-consuming and complex post-amplification steps such as the cloning of amplification products. The results of this study demonstrate the potential for use of the reference chaperonin sequence database, cpnDB, as a tool for identification of bacterial isolates based on *cpn60* sequences amplified with universal primers.

Received 4 August 2005
Accepted 6 December 2005

INTRODUCTION

Although >95 % of human *Campylobacter* infections are caused by *Campylobacter jejuni* and *Campylobacter coli*, numerous other species of *Campylobacter*, including *Campylobacter upsaliensis*, *C. jejuni* subsp. *doylei*, *Campylobacter fetus* subsp. *fetus*, *Campylobacter concisus*, *Campylobacter lari*, *Campylobacter sputorum*, *Campylobacter mucosalis*, *Campylobacter rectus* and *Campylobacter hyointestinalis*, have been shown to cause disease in humans (Butzler, 2004). Twenty-five species of *Campylobacter* from human and other animal sources have been defined (Euzéby, 1997),

although the clinical significance of some species is not known, and identification and discrimination of clinically significant strains remains challenging. A panel of phenotypic tests have been used for *Campylobacter* species identification based on biochemical profiling (On, 1996), but interpretation of the results can be problematic due to inconsistencies in phenotype within species. For example, an important tool for the discrimination of *C. coli* and *C. jejuni* is the hippurate hydrolysis assay in which *C. jejuni* isolates are hippurate-positive; however, hippurate-negative *C. jejuni* strains have been observed (Totten *et al.*, 1987), and different assay methods have been found to give variable results (Morris *et al.*, 1985). Identification of non-*C. jejuni*/*C. coli* *Campylobacter* species by biochemical profiling is also challenging due to the inconsistency of the phenotypic profiles observed among strains within these species (On, 1996). Thus, robust molecular tests are required for differentiating between various *Campylobacter* species, especially for those known to commonly cause disease in humans and

†Present address: Dept of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada.

Abbreviations: BCCDC, British Columbia Centre for Disease Control; NML, National Microbiology Laboratory.

The GenBank/EMBL/DBJ accession numbers for the *cpn60* sequences determined in this study are DQ059425–DQ059481.

animals (Nachamkin, 2003). Identification is further complicated by the similar phenotypic and biochemical profiles of other related Gram-negative curved rods, *Arcobacter* and *Helicobacter*.

Various DNA-based methods have been developed, including those based on analysis of sequences or RFLPs of amplified rRNA sequences, amplified fragment length polymorphism fingerprinting or the application of species-specific PCR methods for the detection of individual species (Cardarelli-Leite *et al.*, 1996; Gorkiewicz *et al.*, 2003; Marshall *et al.*, 1999; Duim *et al.*, 2001; On & Harrington, 2000). Unfortunately, these methods are limited by their complexity or their inability to distinguish between closely related species such as *C. coli* and *C. jejuni*. Methods based on amplification or hybridization using targets such as *glyA*, *ceuE* or a putative GTPase-encoding gene (Van Doorn *et al.*, 1999; Al Rashid *et al.*, 2000; Gonzalez *et al.*, 1997) showed some promise, but the demonstrated applications showed discrimination amongst only a limited range of *Campylobacter* species, and studies often did not include potentially confounding *Arcobacter* and *Helicobacter* spp.

More recently, gene targets providing more discriminating information, such as *omp50* and *cpn60* (also known as the 60 kDa chaperonin, *groEL* or *hsp60*), have been found to provide the necessary inter-species discrimination within the genus *Campylobacter* (Dedieu *et al.*, 2004; Kärenlampi *et al.*, 2004). However, the *omp50* target was found to be absent from over 90 % of *C. coli* strains, requiring the application of additional methods for identification of these strains (Dedieu *et al.*, 2004). While offering excellent discriminating power, the *cpn60*-based method described by Kärenlampi *et al.* (2004) required RFLP analysis and/or cloning of PCR products prior to sequencing, adding time, cost and complexity, and limiting the utility of the method in a clinical laboratory.

The *cpn60* gene encodes the 60 kDa chaperonin protein that is found in virtually all eubacteria, some Archaea, and in the plastids and mitochondria of eukaryotes. The utility of this target for bacterial species identification, detection, quantification and phylogenetic analysis, and for complex microbial community profiling has been well established (Dumonceaux *et al.*, 2006; Eriks *et al.*, 1996; Goh *et al.*, 1997; Hill *et al.*, 2002, 2005; Jian *et al.*, 2001; Zeaiter *et al.*, 2002). Its use as a target for identification of bacterial isolates in a clinical laboratory setting is facilitated by the availability of degenerate, universal PCR primers for the amplification of the approximately 555 bp region of *cpn60* corresponding to nucleotides 274–828 of the *Escherichia coli* *cpn60* sequence (Goh *et al.*, 1996). More recently, a reference database of *cpn60* sequences, cpnDB, has been made freely available via a web interface (Hill *et al.*, 2004), permitting users to compare the *cpn60* sequence of an isolate to thousands of type strain, reference strain and clinical isolate sequences. Here we demonstrate how this resource can be exploited in the identification of *Campylobacter* spp. and

their distinction from phenotypically similar *Helicobacter* and *Arcobacter* spp.

METHODS

Bacterial strains. Strains used in this study are described in Table 1. Type strains were obtained from the American Type Culture Collection. *Campylobacter* strains provided by the National Microbiology Laboratory (NML) were cultured and identified to the species level using established procedures for biotyping (On, 1996), fatty acid profiling (Lambert *et al.*, 1987), 16S rRNA PCR–RFLP (Marshall *et al.*, 1999) and 16S rRNA gene sequencing (Edwards *et al.*, 1989). Clinical isolates from the British Columbia Centre for Disease Control (BCCDC) were identified to the genus level using standard phenotypic methods: evaluation of oxidase and catalase activity, resistance to nalidixic acid, resistance to cephalothin, hippurate hydrolysis and indoxyl acetate hydrolysis (Mills & Gherna, 1987; Morris *et al.*, 1985; Nachamkin, 2003; Versalovic & Fox, 2003).

Genomic DNA extraction and *cpn60* PCR. Extraction of crude DNA from bacterial cultures by the Instagene method (Bio-Rad) was performed according to the manufacturer's protocol. PCR reactions contained 5 µl Instagene extract, 2.5 U *Taq* DNA polymerase (Invitrogen), 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris/HCl pH 8.3, 250 µM each of the dNTPs and 20 pmol each of degenerate primers H729 (5'-CGC CAG GGT TTT CCC AGT CAC GAC GAI III GCI GGI GAY GGI ACI ACI AC-3') and H730 (5'-AGC GGA TAA CAA TTT CAC ACA GGA YKI YKI TCI CCR AAI CCI GGI GCY TT-3'), which include landing sites for sequencing primers M13(–40)F and M13(48)R (underlined). PCR reactions were conducted in two different laboratories, using different PCR machines. Temperature cycling was performed on a Bio-Rad iCycler (95 °C for 2 min, followed by 40 cycles of 30 s at 95 °C, 30 s at 46 °C, 30 s at 72 °C, and a final extension at 72 °C for 5 min) or a Stratagene Robocycler (95 °C for 3 min, followed by 40 cycles of 1 min at 94 °C, 2 min at 37 °C and 5 min at 72 °C, and a final extension at 72 °C for 10 min). Optimal PCR parameters for a given amplification will vary with the thermocycler used (due to variations in factors such as sample temperature ramp time). Both of the protocols described here efficiently produced PCR product for sequencing. PCR products were purified using the QIAquick Spin PCR purification kit (Qiagen) and sequenced directly using primers M13(–40)F and M13(48)R. The 555 bp *cpn60* sequences of clinical isolates were compared to cpnDB (<http://cpndb.cbr.nrc.ca>) using FASTA (Hill *et al.*, 2004; Pearson & Lipman, 1988). Phylogenetic analysis was conducted using the PHYLIP software package (Felsenstein, 1989).

RESULTS AND DISCUSSION

Sequences of *cpn60* for each of the strains listed in Table 1 were determined and have been deposited in GenBank with the accession numbers listed. The primers employed for PCR amplification of *cpn60* sequences include landing sites for standard sequencing primers, permitting the direct sequencing of PCR products and the elimination of the time-consuming cloning steps required by other methods for *Campylobacter* identification using this gene target (Kärenlampi *et al.*, 2004). Direct sequencing also virtually eliminates the impact of *Taq* DNA polymerase-induced sequence artefacts that confound methods based on cloned PCR products where each clone originates from a single, potentially error-containing PCR amplicon.

Table 1. Strains included in sequence study

<i>cpn60</i> identification	Strain	Source*	Original identification†	GenBank accession no.
<i>Arcobacter butzleri</i>	ATCC49616 ^T	Type strain (human diarrhoeal stool, USA)	–	DQ059474
<i>Arcobacter nitrofigilis</i>	ATCC33309 ^T	Type strain (rhizosphere of <i>Spartina alterniflora</i>)	–	DQ059460
<i>Arcobacter skirrowii</i>	ATCC51132 ^T	Type strain (lamb faeces)	–	DQ059471
<i>Arcobacter cryaerophilus</i>	ATCC49615	Reference strain (human blood, Belgium)	–	DQ059481
<i>Campylobacter coli</i>	ATCC49941	Reference strain (quality control strain)	–	DQ059437
	ATCC43488	Reference strain (human faeces, Israel)	–	DQ059439
	ATCC33559 ^T	Type strain (pig faeces)	–	DQ059477
	NCTC11353	Reference strain	–	DQ059429
	ATCC43474	Reference strain (human faeces, Canada)	–	DQ059441
	ATCC43478	Reference strain (marmoset faeces)	–	DQ059438
	ATCC43473	Reference strain	–	DQ059440
	ATCC43472	Reference strain (sheep faeces, Canada)	–	DQ059442
	NML16631	Human, Canada	<i>C. coli</i>	DQ059449
	NML16657	Human, Canada	<i>C. coli</i>	DQ059452
	BCCDC3281	Human, Canada	<i>Campylobacter</i> sp.	DQ059430
	BCCDC3300	Human, Canada	<i>Campylobacter</i> sp.	DQ059476
	BCCDC 3358	Human, Canada	<i>Campylobacter</i> sp.	DQ059432
	BCCDC 3384	Human, Canada	<i>Campylobacter</i> sp.	DQ059433
	BCCDC 3596	Human, Canada	<i>Campylobacter</i> sp.	DQ059436
	BCCDC 3935	Human, Canada	<i>Campylobacter</i> sp.	DQ059466
	BCCDC 3915	Human, Canada	<i>Campylobacter</i> sp.	DQ059469
	BCCDC 3917	Human, Canada	<i>Campylobacter</i> sp.	DQ059470
<i>Campylobacter concisus</i>	ATCC33237 ^T	Type strain (human gingival sulcus)	–	DQ059465
<i>Campylobacter curvus</i>	ATCC35224 ^T	Type strain (human jaw abscess, USA)	–	DQ059457
<i>Campylobacter fetus</i>	Subsp. <i>venerealis</i>	Type strain	–	DQ059443
	ATCC19438 ^T			
	Subsp. <i>fetus</i>	Type strain	–	DQ059455
	ATCC27374 ^T			
	NML16995	Human, Canada	<i>C. fetus</i>	DQ059444
	NML17356	Human, Canada	<i>C. fetus</i>	DQ059454
<i>Campylobacter gracilis</i>	ATCC33236 ^T	Type strain (human)	–	DQ059467
<i>Campylobacter helveticus</i>	ATCC51209 ^T	Type strain (cat, Switzerland)	–	DQ059473
<i>Campylobacter hyoilei</i>	ATCC51729 ^T	Type strain (pig ileum, Australia)	–	AY789012
<i>Campylobacter hyointestinalis</i>	ATCC35217 ^T	Type strain (pig)	–	DQ059425
	NML16962	Human, Canada	<i>C. hyointestinalis</i>	DQ059451
	BCCDC 3911	Human, Canada	<i>Campylobacter</i> sp.	DQ059480
	BCCDC 3912	Human, Canada	<i>Campylobacter</i> sp.	DQ059479
<i>Campylobacter jejuni</i>	Subsp. <i>doylei</i>	Reference strain (human stomach, Germany)	–	DQ059458
	NCTC11847			
	Subsp. <i>jejuni</i>	Reference strain (human faeces)	–	AY789009
	NCTC11168			
	NML16758	Human, Canada	<i>C. jejuni</i>	DQ059446
	NML16814	Human, Canada	<i>C. jejuni</i>	DQ059447
	NML13371	Human, Canada	<i>C. jejuni</i>	DQ059448
	NML16625	Human, Canada	<i>C. jejuni</i>	DQ059453
<i>Campylobacter lari</i>	ATCC35221 ^T	Type strain	–	DQ059475
	BCCDC3301	Human, Canada	<i>Campylobacter</i> sp.	DQ059431
	BCCDC4315	Human, Canada	<i>Campylobacter</i> sp.	DQ059462
<i>Campylobacter mucosalis</i>	ATCC43264 ^T	Type strain (pig small intestine)	–	DQ059456
<i>Campylobacter rectus</i>	ATCC33238 ^T	Type strain (human periodontal pocket)	–	DQ059427
<i>Campylobacter showae</i>	ATCC51146 ^T	Type strain (human gingival crevice, Japan)	–	DQ059461
<i>Campylobacter sputorum</i>	Subsp. <i>sputorum</i>	Reference strain (human and cattle)	–	DQ059459
	ATCC35980			

Table 1. cont.

<i>cpn60</i> identification	Strain	Source*	Original identification†	GenBank accession no.
<i>Campylobacter upsaliensis</i>	ATCC43954 ^T	Type strain (dog)	–	DQ059472
	NML17352	Human, Canada	<i>C. upsaliensis</i>	DQ059445
	NML17328	Human, Canada	<i>C. upsaliensis</i>	DQ059450
	NML17283	Human, Canada	<i>C. upsaliensis</i>	DQ059478
	BCCDC4318	Human, Canada	<i>Campylobacter</i> sp.	DQ059463
	BCCDC4340	Human, Canada	<i>Campylobacter</i> sp.	DQ059464
	BCCDC3518	Human, Canada	<i>Campylobacter</i> sp.	DQ059434
	BCCDC3532	Human, Canada	<i>Campylobacter</i> sp.	DQ059435
	BCCDC3913	Human, Canada	<i>Campylobacter</i> sp.	DQ059468
<i>Helicobacter acinonychis</i>	ATCC51101 ^T	Type strain (cheetah stomach)	–	AY789011
<i>Helicobacter canis</i>	ATCC51401 ^T	Type strain (dog, Switzerland)	–	AY787949
<i>Helicobacter fennelliae</i>	ATCC35684 ^T	Type strain (human rectum)	–	AY787947
<i>Helicobacter hepaticus</i>	ATCC51448 ^T	Type strain (mouse liver)	–	DQ059428
<i>Helicobacter muridarum</i>	ATCC49282 ^T	Type strain (rat ileum)	–	AY787951
<i>Helicobacter mustelae</i>	ATCC43772 ^T	Type strain (ferret stomach)	–	AY787942
<i>Helicobacter nemestrinae</i>	ATCC49396 ^T	Type strain (pig-tailed macaque stomach)	–	AY789010
<i>Helicobacter pametensis</i>	ATCC51478 ^T	Type strain (pig)	–	AY787945
<i>Helicobacter pullorum</i>	ATCC51801 ^T	Type strain (chicken)	–	DQ059426
<i>Helicobacter pylori</i>	ATCC43504 ^T	Type strain (human)	–	AY787944

*Sources for reference and type strains are according to the ATCC catalogue.

†Initial phenotypic and genotypic identification of NML isolates is described in the text. BCCDC isolates were initially identified to the genus level using assays described in the text and assigned to species in this table based on *cpn60* sequence analysis.

The phylogenetic relationships of the reference and clinical strains of *Campylobacter*, *Helicobacter* and *Arcobacter* are illustrated in Fig. 1. The *Campylobacter* species were clearly distinguished from each other, and from the *Arcobacter* and *Helicobacter* type and reference species examined. In addition, the NML clinical isolates were found to cluster with reference strains, in accordance with their initial identification.

Pairwise DNA identities between the 15 species of *Campylobacter* included in this study ranged from 71 to 92 % (excluding the identical pair of *C. coli* and *Campylobacter hyoilei* discussed below). Of the 105 pairwise identities, 78 were between 71 and 79 %, and 23 were between 80 and 89 %. Only *C. upsaliensis* and *Campylobacter helveticus* (92 % identical), and *C. coli* and *C. jejuni* (90 % identical) had identities ≥ 90 %. For species clusters containing multiple strains or isolates, with the exception of *C. coli*, the intra-species pairwise DNA identities were uniformly high: 96–100 % (*C. upsaliensis*), 95–98 % (*C. lari*), 97–100 % (*C. jejuni*), 100 % (*C. hyointestinalis*) and 99–100 % (*C. fetus*).

The eight reference strains of *C. coli*, including the type strain ATCC33559^T, were found to form a relatively broad cluster

with pairwise sequence identities ranging from 94–100 %, and the type strain of *C. hyoilei* (ATCC51729^T) was found to be identical to the *C. coli* type strain (ATCC33559^T) as reported previously (Kärenlampi *et al.*, 2004). Two distinct groups were evident within the *C. coli* cluster (Fig. 1). One group contains 13 strains, including the type strains of *C. hyoilei* and *C. coli*, and has inter-strain sequence identities of 96–100 %. Another separate and distinct group encompasses six *C. coli* reference and clinical strains with pairwise sequence identities of 99–100 %, and may represent the typical *C. coli* taxon. Sequence identities between the two *C. coli* groups are 94–96 %.

C. hyoilei was originally described by Alderton *et al.* (1995) and strains of this organism were implicated in causing proliferative enteritis in pigs. Vandamme *et al.* (1997) provided evidence that *C. hyoilei* (Alderton *et al.*, 1995) and *C. coli* (Véron & Chatelain, 1973) were subjective synonyms and it has been suggested that *C. hyoilei* is a variant of *C. coli* specialized for the porcine gastrointestinal tract (Vandamme & On, 2001). However, Dep *et al.* (2001) have provided molecular and phenotypic evidence that *C. hyoilei* and *C. coli* type strains are distinct *Campylobacter* species. Using hybridization methods, Metherell *et al.* (1999) found

Fig. 1. Inferred phylogenetic relationships of *Campylobacter*, *Helicobacter* and *Arcobacter* strains based on partial *cpn60* sequences. The tree is a consensus of 500 neighbour-joined trees. Bootstrap values (out of 500) are indicated at branch-points. The sources of the strains are described in Table 1.

that the *C. coli* type strain, NCTC 11366 (ATCC33559^T), was atypical for *C. coli*. Our data, based on the *cpn60* sequences from the type strains, other ATCC *C. coli* reference strains and clinical isolates, suggest that *C. hyoilei* may indeed be a valid species since the *C. coli* type strain is identical in sequence to it and is considered an atypical *C. coli*. Interestingly, human and animal isolates are found in both clusters.

The strain identification method demonstrated here offers time and cost advantages over previously described DNA-based methods for identification and discrimination of *Campylobacter* species (Cardarelli-Leite *et al.*, 1996; Dedieu *et al.*, 2004; Duim *et al.*, 2001; Gorkiewicz *et al.*, 2003; Kärenlampi *et al.*, 2004; Marshall *et al.*, 1999; On & Harrington, 2000) in that it involves direct sequencing of PCR-amplified *cpn60* sequences with no requirement for the cloning of PCR products, a method that could easily be adapted to a high-throughput mode. We have applied the universal, degenerate primers described here to the amplification and sequencing of hundreds of reference and type strain organisms, including human and animal pathogens (Brousseau *et al.*, 2001; Goh *et al.*, 1997, 1998, 2000), incorporating this data into the cpnDB database of *cpn60* sequences (Hill *et al.*, 2004). This data is intensely curated, containing only complete, unambiguous *cpn60* target sequences (amplified by primers H729 and H730) from identified sources (reference strains, type strains, clinical isolates). The database webpage provides users with free, on-line analysis tools for sequence identification, and has greatly enhanced the functionality of this diagnostic method, facilitating its application in phylogenetic studies, and the identification of established and emerging pathogens.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Jennifer Town for excellent technical assistance.

REFERENCES

- Alderton, M. R., Korolik, V., Coloe, P. J., Dewhirst, F. E. & Paster, B. J. (1995). *Campylobacter hyoilei* sp. nov., associated with porcine proliferative enteritis. *Int J Syst Bacteriol* **45**, 61–66.
- Al Rashid, S. T., Dakuna, I., Louie, H., Ng, D., Vandamme, P., Johnson, W. & Chan, V. L. (2000). Identification of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, *Arcobacter butzleri*, and *A. butzleri*-like species based on the *glyA* gene. *J Clin Microbiol* **38**, 1488–1494.
- Brousseau, R., Hill, J. E., Prefontaine, G., Goh, S. H., Harel, J. & Hemmingsen, S. M. (2001). *Streptococcus suis* serotypes characterized by analysis of chaperonin 60 gene sequences. *Appl Environ Microbiol* **67**, 4828–4833.
- Butzler, J. P. (2004). *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect* **10**, 868–876.
- Cardarelli-Leite, P., Blom, K., Patton, C. M., Nicholson, M. A., Steigerwalt, A. G., Hunter, S. B., Brenner, D. J., Barrett, T. J. & Swaminathan, B. (1996). Rapid identification of *Campylobacter* species by restriction fragment length polymorphism analysis of a PCR-amplified fragment of the gene coding for 16S rRNA. *J Clin Microbiol* **34**, 62–67.
- Dedieu, L., Pages, J. M. & Bolla, J. M. (2004). Use of the *omp50* gene for identification of *Campylobacter* species by PCR. *J Clin Microbiol* **42**, 2301–2305.
- Dep, M. S., Mendz, G. L., Trend, M. A., Coloe, P. J., Fry, B. N. & Korolik, V. (2001). Differentiation between *Campylobacter hyoilei* and *Campylobacter coli* using genotypic and phenotypic analyses. *Int J Syst Evol Microbiol* **51**, 819–826.
- Duim, B., Vandamme, P. A., Rigter, A., Laevens, S., Dijkstra, J. R. & Wagenaar, J. A. (2001). Differentiation of *Campylobacter* species by AFLP fingerprinting. *Microbiology* **147**, 2729–2737.
- Dumonceaux, T. J., Hill, J. E., Briggs, S. A., Amoako, K. K., Hemmingsen, S. M. & Van Kessel, A. G. (2006). Enumeration of specific bacterial populations in complex intestinal communities using quantitative PCR based on the *chaperonin-60* target. *J Microbiol Methods* **64**, 46–62.
- Edwards, U., Rogall, T., Blocker, H., Emde, M. & Bottger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17**, 7843–7853.
- Eriks, I. S., Munck, K. T., Besser, T. E., Cantor, G. H. & Kapur, V. (1996). Rapid differentiation of *Mycobacterium avium* and *M. paratuberculosis* by PCR and restriction enzyme analysis. *J Clin Microbiol* **34**, 734–737.
- Euzéby, J. P. (1997). List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590–592.
- Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Goh, S. H., Potter, S., Wood, J. O., Hemmingsen, S. M., Reynolds, R. P. & Chow, A. W. (1996). HSP60 gene sequences as universal targets for microbial species identification: studies with coagulase-negative staphylococci. *J Clin Microbiol* **34**, 818–823.
- Goh, S. H., Santucci, Z., Kloos, W. E., Faltyn, M., George, C. G., Driedger, D. & Hemmingsen, S. M. (1997). Identification of *Staphylococcus* species and subspecies by the chaperonin 60 gene identification method and reverse checkerboard hybridization. *J Clin Microbiol* **35**, 3116–3121.
- Goh, S. H., Driedger, D., Gillett, S. & 8 other authors (1998). *Streptococcus iniae*, a human and animal pathogen: specific identification by the chaperonin 60 gene identification method. *J Clin Microbiol* **36**, 2164–2166.
- Goh, S. H., Facklam, R. R., Chang, M. & 8 other authors (2000). Identification of *Enterococcus* species and phenotypically similar *Lactococcus* and *Vagococcus* species by reverse checkerboard hybridization to chaperonin 60 gene sequences. *J Clin Microbiol* **38**, 3953–3959.
- Gonzalez, I., Grant, K. A., Richardson, P. T., Park, S. F. & Collins, M. D. (1997). Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. *J Clin Microbiol* **35**, 759–763.
- Gorkiewicz, G., Feierl, G., Schober, C., Dieber, F., Kofer, J., Zechner, R. & Zechner, E. L. (2003). Species-specific identification of campylobacters by partial 16S rRNA gene sequencing. *J Clin Microbiol* **41**, 2537–2546.
- Hill, J. E., Seipp, R. P., Betts, M., Hawkins, L., Van Kessel, A. G., Crosby, W. L. & Hemmingsen, S. M. (2002). Extensive profiling of a complex microbial community by high-throughput sequencing. *Appl Environ Microbiol* **68**, 3055–3066.
- Hill, J. E., Penny, S. L., Crowell, K. G., Goh, S. H. & Hemmingsen, S. M. (2004). cpnDB: a chaperonin sequence database. *Genome Res* **14**, 1669–1675.

- Hill, J. E., Hemmingsen, S. M., Goldade, B. G., Dumonceaux, T. J., Klassen, J., Zijlstra, R. T., Goh, S. H. & Van Kessel, A. G. (2005). Comparison of ileum microflora of pigs fed corn-, wheat-, or barley-based diets by chaperonin-60 sequencing and quantitative PCR. *Appl Environ Microbiol* **71**, 867–875.
- Jian, W., Zhu, L. & Dong, X. (2001). New approach to phylogenetic analysis of the genus *Bifidobacterium* based on partial HSP60 gene sequences. *Int J Syst Evol Microbiol* **51**, 1633–1638.
- Kärenlampi, R. I., Tolvanen, T. P. & Hänninen, M.-L. (2004). Phylogenetic analysis and PCR-restriction fragment length polymorphism identification of *Campylobacter* species based on partial *groEL* gene sequences. *J Clin Microbiol* **42**, 5731–5738.
- Lambert, M. A., Patton, C. M., Barrett, T. J. & Moss, C. W. (1987). Differentiation of *Campylobacter* and *Campylobacter*-like organisms by cellular fatty acid composition. *J Clin Microbiol* **25**, 706–713.
- Marshall, S. M., Melito, P. L., Woodward, D. L., Johnson, W. M., Rodgers, F. G. & Mulvey, M. R. (1999). Rapid identification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. *J Clin Microbiol* **37**, 4158–4160.
- Metherell, L. A., Logan, J. M. & Stanley, J. (1999). PCR-enzyme-linked immunosorbent assay for detection and identification of *Campylobacter* species: application to isolates and stool samples. *J Clin Microbiol* **37**, 433–435.
- Mills, C. K. & Gherna, R. L. (1987). Hydrolysis of indoxyl acetate by *Campylobacter* species. *J Clin Microbiol* **25**, 1560–1561.
- Morris, G. K., El Sherbeeny, M. R., Patton, C. M., Kodaka, H., Lombard, G. L., Edmonds, P., Hollis, D. G. & Brenner, D. J. (1985). Comparison of four hippurate hydrolysis methods for identification of thermophilic *Campylobacter* spp. *J Clin Microbiol* **22**, 714–718.
- Nachamkin, I. (2003). *Campylobacter* and *Arcobacter*. In *Manual of Clinical Microbiology*, pp. 902–914. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller & R. H. Tenover. Washington, DC: American Society for Microbiology.
- On, S. L. (1996). Identification methods for campylobacters, helicobacters, and related organisms. *Clin Microbiol Rev* **9**, 405–422.
- On, S. L. & Harrington, C. S. (2000). Identification of taxonomic and epidemiological relationships among *Campylobacter* species by numerical analysis of AFLP profiles. *FEMS Microbiol Lett* **193**, 161–169.
- Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.
- Totten, P. A., Patton, C. M., Tenover, F. C., Barrett, T. J., Stamm, W. E., Steigerwalt, A. G., Lin, J. Y., Holmes, K. K. & Brenner, D. J. (1987). Prevalence and characterization of hippurate-negative *Campylobacter jejuni* in King County, Washington. *J Clin Microbiol* **25**, 1747–1752.
- Vandamme, P. & On, S. L. (2001). Recommendations of the subcommittee on the taxonomy of *Campylobacter* and related bacteria. *Int J Syst Evol Microbiol* **51**, 719–721.
- Vandamme, P., Van Doorn, L.-J., Al Rashid, S. T., Quint, W. G. V., van der Plas, J., Chan, V. L. & On, S. L. W. (1997). *Campylobacter hyoilei* Alderton et al. 1995 and *Campylobacter coli* Véron and Chatelain 1973 are subjective synonyms. *Int J Syst Bacteriol* **47**, 1055–1060.
- Van Doorn, L.-J., Verschuuren-Van Haperen, A., Burnens, A., Huysmans, M., Vandamme, P., Giesendorf, B. A. J., Blaser, M. J. & Quint, W. G. V. (1999). Rapid identification of thermotolerant *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* from various geographic locations by a GTPase-based PCR-reverse hybridization assay. *J Clin Microbiol* **37**, 1790–1796.
- Véron, M. & Chatelain, R. (1973). Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. *Int J Syst Bacteriol* **23**, 122–134.
- Versalovic, J. & Fox, J. G. (2003). *Helicobacter*. In *Manual of Clinical Microbiology*, pp. 915–928. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller & R. H. Tenover. Washington, DC: American Society for Microbiology.
- Zeaiter, Z., Fournier, P. E., Ogata, H. & Raoult, D. (2002). Phylogenetic classification of *Bartonella* species by comparing *groEL* sequences. *Int J Syst Evol Microbiol* **52**, 165–171.