Deoxyribonucleic Acid Sequence Relatedness Between Thermophilic Members of the Genus *Campylobacter*

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The genomic relatedness among 28 catalase-positive *Campylobacter* strains was assessed by determination of DNA base composition, by DNA :DNA hybridization followed by S1 endonuclease digestion of single-stranded DNA, and by determining the thermal stability of homologous and heterologous double-stranded DNA. The catalase-positive *Campylobacter* strains were shown to comprise 3 species, *C. coli*, *C. fetus* and *C. jejuni*; each included the type strain (respectively, CIP 7080, CIP 5396 and CIP 702).

INTRODUCTION

The genus *Campylobacter* contains Gram-negative curved or spiral bacteria which are microaerophilic. The catalase-positive members of the genus cause abortion and infertility in cattle, abortion in sheep, and are associated with dysentery in swine (Smibert, 1978). Certain members of the genus have recently been recognized as a common source of acute enteritis in man (Butzler *et al.*, 1973; Skirrow, 1977).

Almost all the strains associated with acute enteritis are able to grow at 42–43 °C (Butzler & Skirrow, 1979). These were first described by King (1957) who called them 'related vibrios'. The taxonomy of these thermophilic campylobacters has been based on a limited number of phenotypic characters and has always been controversial. Véron & Chatelain (1973) considered them as belonging to two species, *Campylobacter jejuni* and *Campylobacter coli*. In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, Smibert (1974) included these strains in a single subspecies, *Campylobacter fetus* subsp. *jejuni*. The Approved Lists of Bacterial Names (Skerman *et al.*, 1980), adopted the nomenclature of Véron & Chatelain (1973). Most recently Skirrow & Benjamin (1980, 1982) proposed that the group be divided into *C. jejuni* biotype 1, *C. jejuni* biotype 2, *C. coli* and nalidixic acid-resistant thermophilic campylobacters (NARTC) according to nalidixic acid sensitivity, hippurate hydrolysis, and H₂S production in an iron-containing medium. *Campylobacter jejuni* biotype 1 is the type most often isolated from patients suffering from acute enterocolitis, but *C. jejuni* biotype 2 and *C. coli* are also found. NARTC strains are rarely isolated from man and so far are not associated with illness (Skirrow & Benjamin, 1982).

While these phenotypic groupings may be most useful practically, the precise definition of the taxonomic relationships between related organisms requires a knowledge of DNA sequence homologies. Because of the controversy surrounding the taxonomic relationships within the genus *Campylobacter*, we have determined the DNA homologies between type, reference and wild-type strains of various species of *Campylobacter* from human and animal sources.

METHODS

Bacterial strains. The strains of *Campylobacter* examined in this study are listed in Table 1. *Vibrio anguillarum* ATCC 14181 (NCMB 829, National Collection of Marine Bacteria, Aberdeen, Scotland) and *Escherichia coli* B ATCC 11303 were also used.

Abbreviation: NARTC, nalidixic acid-resistant thermophilic campylobacters

Table 1. *Strains of Campylobacter used in this study*

Species	Strain number*	Source†
<i>Campylobacter coli</i>	CIP 7080 (NCTC 11366)‡	Pig faeces, CIP
	Z2	Human faeces, Butzler
	Soton 11	Human faeces, Pearson
	Soton 12	Human faeces, Pearson
	8466	Human blood, Skirrow
	2555	Pig faeces, Skirrow
	C-602	Human faeces, Lior
	Pen 5	Human faeces, Penner
	Pen 30	Human faeces, Penner
	Pen 46	Human faeces, Penner
	Pen 48	Human faeces, Penner
<i>Campylobacter fetus</i>	NCTC 10348	Vaginal mucus of heifer, NCTC
	CIP 6829 (NCTC 10354, ATCC 19438) subsp. <i>venerealis</i> ‡	Vaginal mucus of heifer, CIP
	CIP 5396 (NCTC 10842, ATCC 27374) subsp. <i>fetus</i> ‡§	Sheep foetus brain, CIP
	VC 75	Human blood, this laboratory
<i>Campylobacter jejuni</i>	CIP 702 (NCTC 11351) biotype 1‡	Human faeces, CIP
	ATCC 29428 biotype 1	Human faeces, ATCC
	NCTC 11168 biotype 1	Human faeces, NCTC
	C-130 biotype 1	Human faeces, Lior
	1123-47578 biotype 1	Human faeces, McDermott
	1125-47614 biotype 1	Human faeces, McDermott
	79-159 biotype 1	Dog faeces, Blaser
	VDP-3 biotype 1	Human blood, Butzler
	Soton 3 biotype 1	Human faeces, Pearson
	Soton 5 biotype 1	Human faeces, Pearson
	VC 74 biotype 1	Human faeces, this laboratory
<i>Campylobacter sputorum</i>	NCTC 11392 biotype 2	Human faeces, NCTC
	NCTC 11352 NARTC	Herring gull, NCTC
	NCTC 10355 subsp. <i>bubulus</i>	Bovine semen

* NCTC, National Collection of Type Cultures, London, England; CIP, Collection de l'Institut Pasteur, Paris, France; ATCC, American Type Culture Collection, Maryland, U.S.A.

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‡ Type strain.

§ Type species of genus.

|| Recommended control strain (Skirrow & Benjamin, 1982).

Culture media and growth conditions. Mueller-Hinton agar (MH, Difco) was used. All strains were grown in anaerobic jars at 37 °C for 48 h in a 5% oxygen, 10% CO₂ atmosphere provided by a gas-generating kit for campylobacters (Oxoid). *Escherichia coli* and *Vibrio anguillarum* strains were cultured aerobically on Trypticase Soy Agar (TSA, BBL Microbiology Systems) at 37 °C and 30 °C, respectively.

Isolation of DNA. Cells were harvested into 50 ml saline/EDTA (0.15 M-NaCl, 0.1 M-EDTA, pH 8) and centrifuged for 20 min at 17 000 g. Sufficient numbers of culture plates to provide 2–3 g wet-packed cells were used. The cell pellet was resuspended and washed again in 50 ml saline/EDTA and the DNA extracted by the chloroform/isoamyl alcohol method of Marmur (1961). DNA purity was assessed from the 258:230 and 258:280 absorbance ratios, and pronase, RNAase, and chloroform/isoamyl alcohol extraction treatments were repeated until the absorbance ratios were greater than 2.0 and 1.8, respectively. DNA samples were then dialysed for 48 h against distilled water followed by dialysis for 48 h against 0.42 M-NaCl and then were stored at –80 °C.

Estimation of DNA base composition. The base composition of the DNA was estimated from the thermal denaturation temperature (T_m) according to Owen *et al.* (1969). Thermal denaturation was measured in 0.1 × SSC buffer (SSC is 0.15 M-NaCl buffered with 0.015 M-trisodium citrate at pH 7.0) in increments of 0.5 °C min⁻¹ in a Gilford 250 spectrophotometer equipped with a Gilford 2527 thermoprogrammer, 6046 analog multiplexer, and a 6051 recorder. Generally three samples were measured simultaneously with a fourth cuvette containing an adenine

blank to enable correction for solvent expansion. The DNA base composition was expressed as percentage guanine plus cytosine relative to the total base content (mol % G + C).

Radiolabelling of DNA. *In vitro* labelling of DNA prepared by the Marmur (1961) method was done using the 'nick-translation' procedure of Rigby *et al.* (1977) with slight modification. Cold DNA (2 µg) was nicked by DNAase I (100 ng ml⁻¹, Bethesda Research Laboratories, BRL) in the presence of 300 pmol (1.8 µM final concentrations) each of deoxyguanosine 5'-triphosphate, thymidine 5'-triphosphate, deoxy[5-³H]cytidine 5'-triphosphate [1.0 mCi ml⁻¹, 21 Ci mmol⁻¹ (777 GBq mmol⁻¹)] and deoxy[8-³H]adenosine 5'-triphosphate [1.0 mCi ml⁻¹, 19.2 Ci mmol⁻¹ (710 GBq mmol⁻¹)] (Amersham) at 15 °C in translation reaction buffer (0.05 M-Tris/HCl, pH 7.8, 0.005 M-MgCl₂, 0.01 M-2-mercaptoethanol, and 0.05 mg bovine serum albumin ml⁻¹). *Escherichia coli* DNA polymerase I (3 units, Boehringer Mannheim) was added and the reaction allowed to proceed for 1 h at 15 °C. The reaction was stopped by deproteinization with an equal volume of chloroform/isoamyl alcohol. Labelled DNA was separated from unreacted nucleoside triphosphates on a Sephadex G-50 column equilibrated with Rigby buffer (0.01 M-Tris/HCl, pH 8, 0.01 M-NaCl, 0.002 M-EDTA). Fractions containing labelled DNA were pooled and dialysed against distilled water then 0.42 M-NaCl as before. Specific activities of approximately 2.3×10^7 c.p.m. (µg DNA)⁻¹ were obtained.

DNA : DNA hybridization. Prior to hybridization, the DNA concentration was adjusted to approximately 70 µg ml⁻¹, and solutions were sonicated for three periods of 1 min at 4 °C at a frequency of 20–25 kHz and an amplitude of 2–5 µm in a 150 W ultrasonic disintegrator (Branson Sonifier model 185E). Fragment sizes were estimated by agarose (1.2%, w/v) gel electrophoresis with bacteriophage λDNA *Hind*III fragments (BRL) as standard. The sonication procedure yielded fragments with an approximate molecular mass of 2.5×10^5 .

Hybridization experiments were carried out for 20 h in 0.42 M-NaCl at 55 °C (optimal reassociation temperature) and 70 °C (stringent reassociation temperature) as described previously (MacInnes *et al.*, 1979). These temperatures were chosen after establishing that the thermal denaturation temperatures in 0.42 M-NaCl were between 84 and 86 °C for reference *C. coli* and *C. jejuni* DNA samples. The optimal reassociation temperature was taken as 85 – 30 °C (Wetmur & Davidson, 1968) and was confirmed by hybridization experiments. The stringent reassociation temperature was taken as $T_m - 15$ °C (Wetmur & Davidson, 1968). The extent of polynucleotide sequence homology was estimated by calculating the ratio of the amount of radiolabelled probe present in the S1-treated and untreated samples following subtraction of the S1-resistant 'core' material ($8.1 \pm 1.7\%$ at 55 °C and $9.7 \pm 1.9\%$ at 70 °C). Zero-time binding values did not differ significantly from these values. Results were then normalized to the homologous reassociation values (62–80%) after correction for self-reassociation of labelled DNA (<3%).

Estimation of sequence divergence. The susceptibility of homologous and heterologous double-stranded DNA to S1 endonuclease as a function of temperature was determined according to Crosa *et al.* (1973). The temperature at which 50% of the labelled DNA was rendered TCA soluble by S1 endonuclease was the melting temperature $T_{m(e)}$ of the duplex. The difference between the $T_{m(e)}$ for homologous and heterologous double-stranded DNA is defined as $\Delta T_{m(e)}$. A decrease in $T_{m(e)}$ of 1.0 °C was taken to correspond to approximately 1% base mismatching or base sequence divergence (McConaughy & McCarthy, 1970). The $\Delta T_{m(e)}$ values were determined for double-stranded DNA reassociated at 55 °C.

RESULTS

Mean DNA base compositions

The DNA base compositions of the 20 *Campylobacter* strains examined (Table 2) were broadly distributed in the range 29.0 to 35.4% GC, within which were two narrower ranges. The 33.6 to 35.4% GC range comprised the 3 strains of *C. fetus*, while the 29.0 to 32.9% GC range comprised the 16 thermophilic campylobacters and a representative strain of *C. sputorum* subsp. *bubulus*. These base composition results were in general agreement with the previously published data for members of the genus *Campylobacter* also given in Table 2 (Neill *et al.*, 1979; Véron & Chatelain, 1973; Owen & Leaper, 1981).

DNA–DNA hybridization

Tables 3 and 4 list the percentage homologies obtained in reassociation experiments and $\Delta T_{m(e)}$ values for selected strains. Each $\Delta T_{m(e)}$ value was determined at least twice and the standard deviation did not exceed ± 0.5 °C. The standard deviation for triplicate determinations of percentage homology did not exceed $\pm 2.5\%$ at the 55 °C reassociation temperature or $\pm 4\%$ at 70 °C. Reciprocal reassociation reactions performed at 55 °C with four reference DNAs

Table 2. *Base composition of genome DNA from strains of Campylobacter*

DNA source	Mean T_m^* (°C)	G + C (mol %)	Published G + C values (mol %)
<i>C. coli</i>			
CIP 7080	66.2	31.3	32.5 (Véron & Chatelain, 1973); 32.3 and 31.2 (Owen & Leaper, 1981)
Z2	65.6	30.0	
Soton 11	66.0	30.9	
Soton 12	67.0	32.9	
2555	66.6	32.1	
Species mean $\pm$ S.D.		31.4 $\pm$ 1.1	33.3 (Véron & Chatelain, 1973); 32.6 and 31.4 (Owen & Leaper, 1981)
<i>C. fetus</i>			
NCTC 10348	67.5	34.0	34.3 (Neill <i>et al.</i> , 1979); 36.7 (Owen & Leaper, 1981)
CIP 6829 subsp. <i>venerealis</i>	68.2	35.4	35.7 (Neill <i>et al.</i> , 1979); 36.3 and 36.5 (Owen & Leaper, 1981)
CIP 5396 subsp. <i>fetus</i>	67.3	33.6	33.1 (Véron & Chatelain, 1973); 36.3 and 35.5 (Owen & Leaper, 1981)
Species mean $\pm$ S.D.		34.3 $\pm$ 0.95	35.4 (Véron & Chatelain, 1973); 36.3 and 36.2 (Owen & Leaper, 1981)
<i>C. jejuni</i>			
CIP 702 biotype 1	66.0	30.9	31.5 (Véron & Chatelain, 1973); 31.6 and 30.1 (Owen & Leaper, 1981)
ATCC 29428	65.7	30.2	
NCTC 11168 biotype 1	65.3	29.4	31.6 and 33.4 (Owen & Leaper, 1981)
C-130	65.8	30.4	
1123-47578	65.8	30.4	
1125-47614	65.8	30.4	
79-159	65.2	29.0	
VDP-3	66.0	30.9	
Soton 3	65.6	30.0	
NCTC 11394 biotype 2	66.6	32.1	32.3 and 33.0 (Owen & Leaper, 1981)
NCTC 11352 NARTC	66.7	32.3	32.6 and 32.5 (Owen & Leaper, 1981)
Species mean $\pm$ S.D.		30.6 $\pm$ 1.0	31.6 (Véron & Chatelain, 1973); 31.8 and 32.3 (Owen & Leaper, 1981)
<i>C. sputorum</i> subsp. <i>bubulus</i>			
NCTC 10355	65.9	30.6	32.2 (Owen & Leaper, 1981)
Taxon mean $\pm$ S.D.		31.3 $\pm$ 1.6	32.1 (Owen & Leaper, 1981)
<i>E. coli</i>			
ATCC 11303	75.3	50.3	50 (Marmur & Doty, 1962); 50.9 (DeLey, 1970)

* Standard deviations for triplicate determinations were all less than ± 0.1 °C.

gave homology values which differed by less than 3%, suggesting that the genome sizes were not significantly different in the reference strains used.

The data show that the 28 strains of catalase-positive *Campylobacter* belonged to three discrete DNA homology groups. The first group (Table 3) contained 10 strains with high homology to *C. coli* CIP 7080 (>79% at 55 °C), intermediate homology to *C. jejuni* CIP 702 (32–48% at 55 °C) and low homology to *C. fetus* CIP 5396 (<19% at 55 °C). The second group (Table 3) contained 3 strains with high homology to *C. fetus* CIP 5396 (>97% at 55 °C) and low homology to both *C. coli* CIP 7080 and *C. jejuni* CIP 702 (<18% at 55 °C). The third group (Table 4) contained 11 strains with high homology (>80% at 55 °C) to *C. jejuni* CIP 702 and *C. jejuni* ATCC 29428, intermediate homology (30–50% at 55 °C) to *C. coli* CIP 7080, and low homology (<21% at 55 °C) to *C. fetus* CIP 5396. Included in this DNA homology group were the recommended control strains for *C. jejuni* biotype 2 (NCTC 11392) and the NARTC group (NCTC 11352). Phenotypically, *C. jejuni* biotype 2 differs from *C. jejuni* biotype 1 by producing

Table 3. DNA homologies (%) at reassociation temperatures of 55 °C and 70 °C, and sequence divergence [$\Delta T_{m(e)}$] between reference strains *C. fetus* CIP 5396, *C. jejuni* CIP 702 and *C. coli* CIP 7080, and strains of *C. coli*, *C. fetus*, and *C. sputorum* subsp. *bubulus*

Source of unlabelled DNA	Reference DNA from strain:								
	<i>C. coli</i> CIP 7080			<i>C. fetus</i> CIP 5396			<i>C. jejuni</i> CIP 702		
	55 °C	70 °C	$\Delta T_{m(e)}$ (°C)	55 °C	70 °C	$\Delta T_{m(e)}$ (°C)	55 °C	70 °C	$\Delta T_{m(e)}$ (°C)
<i>C. coli</i>									
CIP 7080	100	100	—	14	9	11.2	32	19	6.2
Soton 11	100	100	0.5	16	11	12.6	48	23	5.8
2555	100	94		13	8		42	25	
Pen 5	100	100		19	14		44	20	
Z2	99	100		9	2		42	22	
C-602	98	90		8	6		39	30	
Pen 46	95	87		13	10		45	28	
8446	86	79		19	17		40	25	
Pen 30	83	73	2.3	16	12	12.1	46	31	5.4
Soton 12	81	88	1.2	7	4	13.8	32	20	7.1
Pen 48	79	62		14	10		41	29	
<i>C. fetus</i>									
CIP 5396 subsp. <i>fetus</i>	17	12	10.9	100	100	—	14	19	14.0
CIP 6829 subsp. <i>venerealis</i>	11	10		100	100	0.4	15	12	
VC 75	18	11		100	89		12	20	
NCTC 10354	14	13		97	90	0.7	18	15	
<i>C. sputorum</i> subsp. <i>bubulus</i>									
NCTC 10355	18	11		11	10		7	4	
<i>V. anguillarum</i>									
ATCC 14181	7			9			4		
<i>E. coli</i>									
ATCC 11303	2			7			3		

H₂S in an iron-containing medium, while NARTC strains, which also produce H₂S, differ from *C. jejuni* biotypes 1 and 2 in their ability to grow in the presence of nalidixic acid and inability to hydrolyse hippurate (Skirrow & Benjamin, 1980). The catalase-negative strain *C. sputorum* subsp. *bubulus* NCTC 10355 showed low homology to the reference DNAs as did the DNA of *V. anguillarum* ATCC 14181 and *E. coli* ATCC 11303.

Data obtained from reassociation at the stringent temperature of 70 °C confirmed these 3 DNA homology groups, as did the $\Delta T_{m(e)}$ values. Within each homology group, increasing the reassociation temperature from 55 °C to 70 °C did not significantly reduce the homology value obtained. Similarly, $\Delta T_{m(e)}$ values within each group were normally low (< 2 °C), while values between homology groups were higher (> 5 °C). There was one noticeable exception in the *C. jejuni* homology group. This was NCTC 11352, the recommended control NARTC strain. In this case the percentage homology with *C. jejuni* CIP 702 fell from 86 to 32 when the reassociation temperature was raised from 55 °C to 70 °C, while the percentage homology with *C. jejuni* ATCC 29428 fell from 92 to 48. Predictably, in the case of NCTC 11352 the $\Delta T_{m(e)}$ value of 4 °C obtained with both *C. jejuni* reference DNAs was significantly higher than other intra-group values.

DISCUSSION

Brenner *et al.* (1972) demonstrated that when the hydroxyapatite method was used to assess genomic homology among bacteria, strains belonging to a single species usually exhibited more than 70% homology at optimal temperature, a slight decrease in relatedness at stringent

Table 4. DNA homologies (%) at reassociation temperatures of 55 °C and 70 °C, and sequence divergence [$\Delta T_{m(e)}$] between reference strains *C. coli* CIP 7080, *C. fetus* CIP 5396, *C. jejuni* CIP 702 and ATCC 29428, and strains of *C. jejuni*

Unlabelled DNA from <i>C. jejuni</i> strain:	Reference DNA from strain:									
	<i>C. coli</i> CIP 7080			<i>C. fetus</i> CIP 5396			<i>C. jejuni</i> CIP 702			<i>C. jejuni</i> ATCC 29428
	55 °C	70 °C	$\Delta T_{m(e)}$ (°C)	55 °C	70 °C	$\Delta T_{m(e)}$ (°C)	55 °C	70 °C	$\Delta T_{m(e)}$ (°C)	$\Delta T_{m(e)}$ (°C)
CIP 702 biotype 1	30	15	6.0	13	7	14.4	100	100	—	—
C-130	48	21		9	10		94	90	1.0	86
VC 74	44	18		12	11		93	92		76
VDP-3	45	18		9	5		87	89		72
NCTC 11352 NARTC	29	12	8.2	21	18	9.9	86	32	4.3	4.0
Soton 3	33	10		7	5		86	80		86
Soton 5	37	17	7.3	9	5		84	86		85
79-159	42	21		10	6		84	76	1.8	84
ATCC 29428	50	31	5.1	9	4	15.0	84	87	1.8	100
1125-47614	37	22		10	11		81	75		81
Soton 5	36	20		8	7		81	80		98
NCTC 11168 biotype 1	48	25		8	9		81	74		69
NCTC 11392 biotype 2	32	21		7	6		80	76	1.4	88

temperature, and less than 5% base sequence divergence. Grimont *et al.* (1980) recently demonstrated that the 70% homology value obtained with the hydroxyapatite method corresponded to 51–54% homology at optimal temperature and 64–66% homology at stringent temperature with the S1 endonuclease/TCA precipitation technique used in this study. These workers also demonstrated that percentage divergence figures obtained with the two methods were directly comparable. When we applied these criteria to the values obtained for catalase-positive campylobacters, three species were clearly distinguishable. These were *C. coli*, *C. fetus* and *C. jejuni*.

Strains of *C. fetus* were highly related. Indeed, although the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) contain two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, they could not be differentiated on the basis of DNA homology. The type strain of *C. fetus* subsp. *venerealis* CIP 6829 was 100% homologous with the type strain of *C. fetus* (Smith and Taylor) Sebald and Véron CIP 5396 even at the stringent reassociation temperature. Moreover, the base sequence divergence between the two strains was <1%. The species *C. fetus* was only distantly related to the thermophilic campylobacters. The mean DNA homology between strains of *C. fetus* and strains of *C. coli* in this study was only 14% at 55 °C and 10% at 70 °C, with a mean base sequence divergence of 12%. In the case of *C. jejuni* it was 11% at 55 °C, and 10% at 70 °C, with a mean base sequence divergence of 13%. *C. fetus* could also be differentiated from these thermophilic species on the basis of G + C content.

The species *C. coli* and *C. jejuni* appeared to be more closely related to each other than to *C. fetus*. They could not be distinguished by G + C content, and at 55 °C the mean DNA homology between these thermophilic species was 40%, with a mean base sequence divergence of 6%. However, at the stringent reassociation temperature the mean inter-species DNA homology was reduced to 22%, leaving no doubt that *C. coli* and *C. jejuni* are separate species. Within each species, strains displayed high DNA homology. In the case of *C. coli*, the mean DNA homology at the stringent reassociation temperature was 87%, while the mean base sequence divergence was only 1%. In the species *C. jejuni*, the mean DNA homology at the stringent reassociation temperature was 79%, and the mean base sequence divergence was 2%. Included among the strains belonging to this species were recommended control strains from the identification scheme recently proposed by Skirrow & Benjamin (1982), NCTC 11352 representing the NARTC group of organisms, and NCTC 11392 representing *C. jejuni* biotype 2. The >80% homology shown by these strains to both *C. jejuni* reference DNAs at the optimal reassociation temperature clearly places them in the species *C. jejuni* as do the base sequence divergence values of <5%. However, the NARTC strain was distinguishable from other *C. jejuni* by its reduced homology at the stringent reassociation temperature, and by its higher base sequence divergence values. Clearly more NARTC strains need to be examined to determine whether they form a distinct genetic group, and, if so, to determine the relatedness of the group to the thermophilic campylobacters pathogenic for man. For the present, NCTC 11352 should be included in the species *C. jejuni*.

In summary, this DNA homology study has shown that the catalase-positive campylobacters comprise three species, *C. coli*, *C. fetus*, and *C. jejuni*. This confirms the concepts of the speciation within the genus *Campylobacter* first proposed by Véron & Chatelain (1973). While DNA homology obviously provides the best basis for delineating bacterial species, the DNA homology grouping needs to be correlated with phenotypic characteristics to be useful for classification and identification. Although the identification and differentiation of campylobacters is based on a limited number of morphological and biochemical tests (Skirrow & Benjamin, 1980; Karmali *et al.*, 1981), two phenotypic characteristics appear to correlate with the differences in genomic homology. Phenotypically, *C. fetus* can be distinguished by its ability to grow at 25 °C and inability to grow at 43 °C, while *C. coli* and *C. jejuni*, which do grow in the temperature range 30.5–45.5 °C, can be differentiated by the inability of *C. coli* to hydrolyse hippurate (Skirrow & Benjamin, 1982). Within the species *C. jejuni*, a strain representing a phenotypic subgroup able to grow in the presence of nalidixic acid, while not clearly delineated genetically, was sufficiently different to suggest that some genetic drift may be occurring within the species.

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