Plasmid profiles of urease-positive thermophilic campylobacter (UPTC) strains isolated in Europe and Asia (Japan)

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Urease-positive thermophilic campylobacter (UPTC), a microaerophilic and Gram-negative bacterium, is an organism only identified relatively recently in England.[1,2] After the original descriptions of UPTC appeared, isolates of UPTC were reported in France, Northern Ireland and The Netherlands, and UPTC strains have also recently been found in Japan, where they have been characterised both phenotypically and genotypically.[3-8]

So far, the detection, isolation and characterisation of plasmids of thermophilic *Campylobacter coli* and *C. jejuni*, which are the most representative thermophilic *Campylobacter* spp., and are frequently found to be causative agents of infection in humans and animals, have been reported.[9-15]

Cloning of *C. jejuni* DNA into *Escherichia coli* has achieved only limited success in the study of the gene expression and function of the pathogen, and genetic manipulation of *C. jejuni* DNA has relied primarily on just a few vectors.[15] To overcome these limitations, it appears that plasmid DNA from campylobacters, especially thermophilic ones, must be exploited for genetic manipulation.

To our knowledge, however, no reports of plasmid analysis of thermophilic strains of *C. lari*, including UPTC, have appeared. Therefore, the purpose of the present study is to detect and purify plasmid DNA in strains of UPTC and to perform molecular discrimination of the plasmid DNA, based on restriction enzyme digestion profiles.

In the present study, 47 UPTC strains were analysed (Table 1). These strains were isolated in Europe (Northern Ireland, England and France) and in East Asia (Japan). Cells were cultured on blood agar containing defibrinated horse blood (Nippon Bio-Test, Tokyo, Japan) and supplemented with campylobacter-selective medium (Institute Virion, Zurich) at 37°C for two days under microaerophilic conditions.

Plasmid DNA was isolated by a method described by Kado and Liu,[16] using a Wizard Plus SV Minipreps DNA purification system (Promega Corp., Tokyo, Japan), according to the manufacturer's instructions. Isolated plasmid DNA was electrophoresed in 0.7% agarose-ME (Nacalaitesque, Kyoto, Japan) gel using *Hin*d III digest of lambda DNA and *Eco*T 14 I digest of lambda DNA (Takara Biochems., Kyoto, Japan) as standard markers, and then purified with Geneclean II (Bio 101, CA, USA).

Purified DNA was digested with three restriction enzymes, *Alu* I, *Dra* I and *Ssp* I (Takara Biochems., Kyoto, Japan), according to the manufacturer's instructions, following a screening experiment with 26 distinct restriction enzymes (data not shown). Restricted plasmid DNA was

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Table 1. Strains of UPTC used in the present study and summary of plasmid profiles

Strain No.	Source	Country	Plasmid DNA (bp)
2	Oyster	N. Ireland	ND
11	Mussel	N. Ireland	ND
14	Mussel	N. Ireland	2300, 2700
15	Mussel	N. Ireland	ND
22	Mussel	N. Ireland	ND
23	Mussel	N. Ireland	ND
27	Mussel	N. Ireland	ND
87	Sea water	N. Ireland	5200
88	Sea water	N. Ireland	ND
98	Sea water	N. Ireland	2400
103	Sea water	N. Ireland	3800
107	Sea water	N. Ireland	ND
136	Scallop	N. Ireland	ND
142	Oyster	N. Ireland	2300, 2700
145	Mussel	N. Ireland	ND
150	Cockle	N. Ireland	ND
158	Mussel	N. Ireland	ND
161	Mussel	N. Ireland	ND
163	Mussel	N. Ireland	ND
182	Sea Water	N. Ireland	ND
237	Oyster	N. Ireland	2400
270	Mussel	N. Ireland	ND
412	Mussel	N. Ireland	2400
467	Mussel	N. Ireland	ND
472	Mussel	N. Ireland	ND
475	Mussel	N. Ireland	ND
476	Mussel	N. Ireland	ND
480	QC	N. Ireland	ND
482	Mussel	N. Ireland	ND
484	Mussel	N. Ireland	ND
487	Mussel	N. Ireland	ND
494	Unknown	N. Ireland	ND
497	Unknown	N. Ireland	ND
504	Mussel	N. Ireland	ND
505	Mussel	N. Ireland	ND
A1	Seagull	N. Ireland	ND
A2	Seagull	N. Ireland	ND
A3	Seagull	N. Ireland	ND
NCTCl2892	River water	England	2400
NCTC12893	River water	England	2400
NCTC12894	Sea water	England	2400
NCTC12895	Mussel	England	3700
NCTC12896	Mussel	England	2000, 3000
89049	Human	France	ND
92251	Human	France	ND
CF89-12	River water	Japan	ND
CF89-14	River water	Japan	ND

ND: not detected

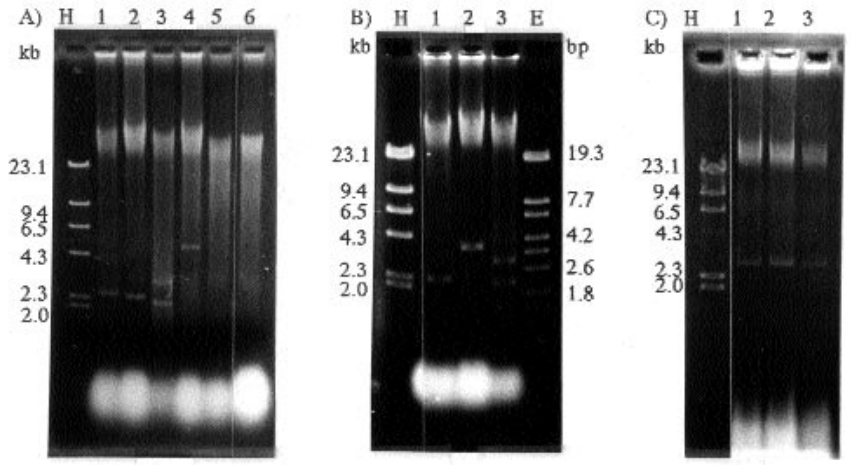

Fig. 1. Electrophoretic profiles of plasmid DNAs isolated from strains of UPTC using a Kado-Liu method. Lane H, *Hin*d III digest of lambda DNA; lane E, *Eco*T14 I digest of lambda DNA. (A) Lane 1, UPTC 237; lane 2, NCTC12894; lane 3, NCTC12896; lane 4, 87; lane 5, 27; lane 6, 476. (B) Lane 1, NCTC12894; lane 2, NCTC12895; lane 3, NCTC12896. (C) Lane 1, NCTC12892; lane 2, NCTC12893; lane 3, NCTC12894.

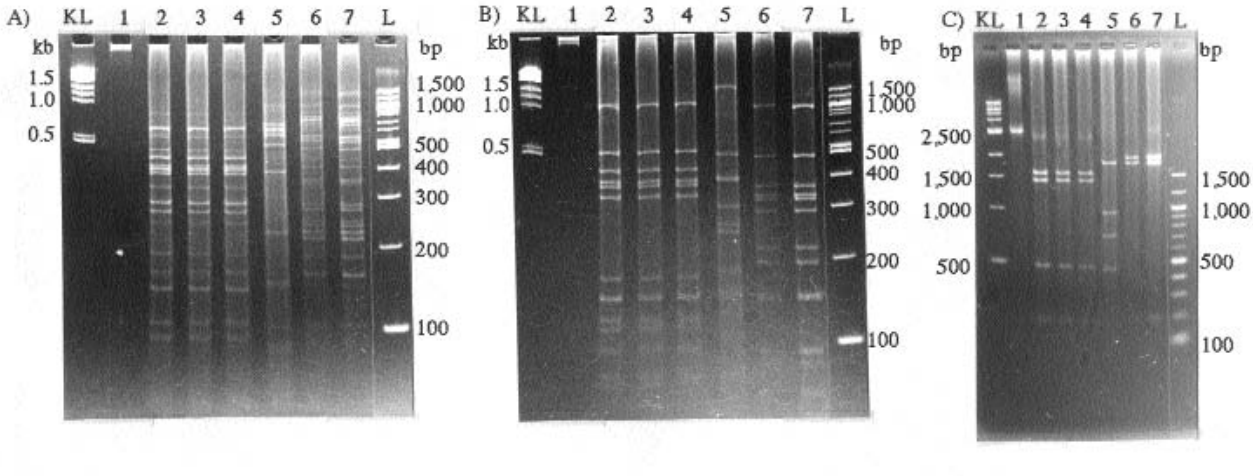

Fig. 2. Electrophoretic profiles of restricted plasmid DNAs after separate digestion with (A) *Alu* I (A), (B) *Dra* I and (C) *Ssp* I. For lanes H and E, see legend for Figure 1. Lane KL, 1 kb DNA ladder; lane L, 100 bp DNA Ladder. Lane 1, undigested plasmid DNA from UPTC NCTC12892; lane 2, NCTC12892 digested with the enzyme; lane 3, NCTC12893; lane 4, NCTC12894; lane 5, 98; lane 6, 237; lane 7, 412.

electrophoresed in 5% polyacrylamide gel for *Alu* I and *Dra* I digests and in 2% agarose-ME for *Ssp* I digest, using a 1 kb DNA ladder and a 100 bp DNA ladder (New England Biolabs., MA, USA), and were stained with ethidium bromide.

In the present study, we screened for the presence of plasmid DNA in a total of 47 UPTC strains, using the plasmid purification method developed by Kado and Liu,[16] which is a rapid procedure for the detection and isolation of both small and large plasmids (2.6-350 mDa). Screening results indicated the presence of plasmid DNA in 12 of the 47 strains, at a frequency of approximately 26% (Table 1). A selection of the electrophoresed plasmid DNA profiles are shown in Figure 1.

Plasmids have been found in both *C. jejuni* and *C. coli* strains;[10,14] however, we are unaware of any reports of plasmid DNAs isolated from strains of *C. lari.* The present results of the occurrence of plasmid DNAs in UPTC strains correspond to those obtained in *C. jejuni* and *C. coli* strains. Seven of the 12 UPTC strains were from Northern Ireland, and five were from England. Two plasmid DNAs were observed in three strains of UPTC (14, 142 and NCTC12896), while none were observed in the four strains from France and Japan (Table 1).

Nucleotide lengths of the plasmid DNAs then were estimated by comparing the mobilities of the plasmid DNAs with the molecular weight standard markers. Three UPTC strains (14, 142, and NCTC12896) had two plasmid DNAs (approximate lengths: 2.3 and 2.7 kb, 2.3 and 2.7 kb and 2.0 and 3.0 kb, respectively), while the other nine strains with plasmid DNAs had only one each (approximate length: 2.4 - 5.2 kb) (Table 1).

As six (NCTC12892, NCTC12893, NCTC12894, 98, 237 and 412) of the nine strains were found to carry plasmid DNAs of approximately the same length (2.4 kb), an attempt was made to digest the plasmid DNAs with three restriction enzymes (*Alu* I, *Dra* I and *Ssp* I), in order to discriminate these. Three plasmid DNAs from the NCTC12892, NCTC12893 and NCTC12894 strains gave almost identical restriction profiles, and strains 237 and 412 gave very similar profiles after separate digestion with the three restriction enzymes (Figure 2).

These data suggest that the former three plasmid DNAs had identical nucleotide sequences and that the latter two had very similar sequences. At present, however, the functions of all these plasmids are unknown. To determine these functions, nucleotide sequence information will be required.

Recently, On and colleagues analysed 29 strains of *C. lari* – a species that has proved both genotypically and phenotypically diverse – by extensive phenotypic characterisation, whole-cell protein profiling, amplified fragment length polymorphism analysis, 16S rDNA sequencing and DNA – DNA hybridisation.[17] Results led them to propose the existence of three *C. lari* subspecies, namely *C. lari* subsp. *lari* ('classical' strains), *C. lari* subsp. *ureasum* (UPTC strains) and *C. lari* subsp. *subantarcticus* (subantarctic animal isolates).[17]

It is anticipated that the present study will stimulate greater interest in UPTC as a *C. lari* subspecies, and that molecular characterisation, including nucleotide sequencing of the plasmid DNAs of the UPTC strains detected in the present study, may result in an understanding of the function of the plasmids. □

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Multilocus sequence typing and *porA* gene sequencing differentiates strains of *Neisseria meningitidis* during case clusters

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Notified cases of meningococcal disease (MD) in Scotland and many other countries within Europe have increased in recent years[1] and are often associated with epidemic strains such as C:2a:P1.5 from the ET-37 complex.[2] A number of case clusters due to these strains have occurred in schools, universities and other close-contact situations.[3-5] Characterisation of *Neisseria meningitidis* strains isolated during case clusters is important for public health management, and indicates the necessity for molecular techniques to differentiate strains.[6]

In Scotland, serogroup C disease became prevalent from the mid-1990s and was the predominate serogroup in 1998, accounting for 46% of all serogroupable invasive isolates.[1] Strains isolated during case clusters could not be differentiated if they were C:2a:P1.5 because there was no provision for genotyping. Therefore, multilocus sequence typing (MLST)[7] and *porA* gene sequencing[8] were introduced as a national service in 2000, in order to fully characterise all meningococcal isolates in Scotland.

Although MLST is a recently-described method, it has proved to be highly discriminatory for the differentiation of a number of bacterial pathogens.[9] Here, we describe the use of MLST and *porA* gene sequencing to differentiate *N. meningitidis* strains in two clusters.

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