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Stability of the genotype of *Campylobacter jejuni* under controlled culture conditions

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Abstract

Campylobacter (C.) jejuni is identified as the major cause of bacterial gastro-enteritis in the Netherlands. Although poultry is considered as the main source of *C. jejuni*, many strains found in poultry (identified by various genotyping techniques) cannot be traced in the human population and in the Netherlands, of all human isolates, only 30% has been detected in poultry. Variations in genotype due to mutations or exchange of DNA might underlie these observations. This study was undertaken to monitor changes in genotype of *C. jejuni.*

We cultured *C. jejuni* for approximately 150 generations in nutrient rich medium in the absence of exchangeable DNA under various microaerobic conditions. Alterations in genotype were studied by pulsed field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) and the multi locus sequence typing (MLST) technique.

No rearrangements, inserts or deletions of large DNA fragments were detected. PFGE and AFLP patterns of cultures at start were indistinguishable from cultures 150 generations later. No mutations were observed in any of the restriction sites and no detectable mutations had occurred in the loci subjected to MLST analysis. In the absence of exchangeable DNA under constant culture conditions the genotype of *C. jejuni* appeared to be stable.

Contents

Samenvatting

Campylobacter (C.) jejuni is de meest frequente veroorzaker van gastro-enteritis in Nederland. Onderzoek met verschillende genetische typeringstechnieken laat zien dat er zeer veel campylobactertypen bestaan. Pluimvee wordt beschouwd als een belangrijke bron *van C. jejuni,* maar veel van de stammen die worden geïsoleerd uit pluimvee worden niet teruggevonden in de humane populatie, terwijl van de stammen die zijn geïsoleerd uit humane patiënten slechts 30% wordt teruggevonden bij pluimvee. Hieraan kunnen verschillende oorzaken ten grondslag liggen:

(1) Mogelijk bestaan er nog andere bronnen van *C. jejuni*. Risicofactoren voor het oplopen van een infectie met *Campylobacter* zijn het houden van (jonge) huisdieren en het drinken van ongepasteuriseerde melk. Opvallend genoeg wordt het eten van kip niet altijd als risicofactor gezien; (2) Groei van campylobacters lijkt beperkt te zijn tot het maagdarmstelsel van warmbloedig dieren. Onder omstandigheden waar groei niet mogelijk is, blijft *Campylobacter* weliswaar vitaal, maar neemt de kweekbaarheid, en daarmee de aantoonbaarheid, af. Mogelijk is *Campylobacter* in deze vitale, maar niet meer kweekbare vorm toch nog in staat om een infectie te veroorzaken; (3) Pluimvee is besmet met verschillende typen *C. jejuni* waarvan er een of enkele domineren en dus worden aangetoond. Bij een voedselinfectie worden alle typen overgedragen, maar omdat in de mens andere typen gaan domineren, worden in de mens ook andere typen aangetoond; (4) Mogelijk treden er veranderingen op in het genotype van *Campylobacter*, waardoor het slechts lijkt alsof er sprake is van niet-verwante typen.

In deze studie is *C. jejuni* gedurende 150 generaties gekweekt onder gecontroleerde omstandigheden en is van op geregelde tijdstippen genomen monsters met behulp van verschillende genetische technieken het genotype bepaald. Er zijn in deze periode en onder de gebruikte omstandigheden geen veranderingen in genotype waargenomen.

Summary

Campylobacter (C.) jejuni is identified as the major cause of bacterial gastro-enteritis in the Netherlands. The *C. jejuni* population seems to consist of many types as identified by various genotyping techniques. Although poultry is considered as the main source of *C. jejuni*, many strains found in poultry cannot be traced in the human population and in the Netherlands, of all human isolates, only 30% has been detected in poultry. Various reasons can be thought of explaining this seeming discrepancy:

(1) Poultry is not the only source of *C. jejuni*. Identified risk factors for an infection with *Campylobacter* are keeping pet puppies and drinking non-pasteurised milk. Interestingly, consumption of chicken is not always identified as a risk factor; (2) Growth of *Campylobacter* seems to be restricted to the gastrointestinal tract of warm-blooded animals. In an environment where not all growth conditions are met, *Campylobacter* remains viable, but looses its culturability. Possibly, this viable but non-culturable form of *Campylobacter* is still capable of causing disease; (3) Both chickens and humans harbour a population of genotypically different campylobacters, but the dominating, and thus most likely isolated genotype from poultry differs from the dominating genotype in human patients; (4) The genotype of *Campylobacter* is not a constant, but changes.

In this study *C. jejuni* was grown for approximately 150 generations under controlled conditions. The genotype was determined in samples taken at regular time intervals, using three different techniques. With these techniques, in this period of time and under the used conditions no changes in genotype were detected.

1. Introduction

Campylobacter (C.) jejuni is identified as the major cause of bacterial gastro-enteritis in the Netherlands (Wit et al., 2001). *C. jejuni* is a Gram-negative, motile microorganism, of which the morphology varies from spiral-shaped to coccoid. *C. jejuni* is primarily micro-aerophilic and grows well in environments containing 3-5% CO_2 and 3-15% O_2. The lack of tolerance towards atmospheric oxygen concentrations must place serious constraints upon their survival in foods and environments where they are likely to encounter oxidative stress. Yet they are able to survive in such environments, due to the presence of at least three enzymes: katalase ($H_2O_2 \rightarrow H_2O + O_2$; Grant and Park, 1995), superoxide dismutase (Pesci et al., 1994) and alkylhydroperoxide reductase (Baillon et al., 1999). *C. jejuni* grows within a short temperature range, being unable to multiply at temperatures either above 45 or below 30°C. In an environment where not all growth conditions are met, *Campylobacter* can remain viable for a long period of time, but will become non-culturable. This viable but non-culturable (VBNC) state might be considered as a dormant one, analogues to Gram-positive spores, but most investigators consider the VBNC state as a degenerated form of *Campylobacter*, preluding death (Federighi et al., 1998; Fearnly et al., 1996; Boucher et al., 1994; Beumer et al., 1992).

All required growth conditions are met in the gastrointestinal tract of warm-blooded animals. Poultry is considered to be a main source of *C. jejuni.* Pigs and cattle are a source of *C. coli*, but have not been identified as important reservoir for *C. jejuni* (Tauxe, 1992). Although poultry is considered the main source of *C. jejuni*, many strains found in poultry (identified by various genotyping techniques) cannot be traced in the human population and in the Netherlands, of all human isolates, only 30% has been detected in poultry (Duim et al., 1999). In Canada and the UK, similar findings have been reported (Nadeau et al., 2002; Kramer et al., 2000). Various reasons can be thought of explaining this discrepancy:

(1) In the Netherlands other sources and/or reservoirs exist, but have not been identified yet. In the UK, not poultry, but drinking water from private supplies and nonpasteurised milk are the most frequently reported vehicles (Frost, 2001). From a case control study in England (Rodriques et al., 2000), travel (OR 3.62), and consumption of chicken in a restaurant (OR 2.38) were recognised as risk factors, keeping pet puppies or consumption of chicken prepared at home were not. In Australia (Tenkate and Stafford, 2001), ownership of pet puppies (OR: 16.58) or pet chicken (OR: 11.80), and the consumption of mayonnaise (OR: 4.13) were identified as risk factors, but not travel and, again, not consumption of chicken at home. In Sweden (Studahl and Andersson, 2000) however, consumption of chicken increased the risk of infection (OR 2.29), like drinking nonpasteurised milk (OR:3.56), or eating pork (with bone: OR: 1.22; without bone: 1.83), barbecuing (OR: 1.98), living/working on a farm (OR: 3.06), being a chicken breeder (OR: 3.32), or having daily contact with chicken (OR: 11.83). In Japan, outbreaks were associated with the consumption of meat or poultry products, egg product, salad and (well) water, either inadequately heated, or cross contaminated (Michino and Otsuki, 2000);

(2) VBNC organisms from poultry or other sources and reservoirs are still infectious. There has been a lot of debate about this hypothesis. The results of some groups do not confirm this hypothesis (Beumer et al., 1992; Fearnley et al., 1996; Medema et al., 1992; Giessen et al., 1996), whereas other groups found that non-culturable suspensions of *Campylobacter* were still able to cause an infection in laboratory animals (Cappelier et al., 1999; Jones et al., 1991; Saha et al., 1991; Stern et al., 1994). Hazeleger et al. (1995) reported temperature-dependent physiological changes in non-culturable forms of campylobacters, those formed at low temperature (4^{o}C) being more similar to culturable forms of *Campylobacter*. Interestingly, groups who found colonisation by non-culturable

forms all prepared their suspensions at 4°C (Cappelier et al., 1999; Jones et al., 1991; Stern et al., 1994);

(3) Both chickens and humans harbour a population of genotypically different campylobacters, but the dominating, and thus most likely isolated genotype from poultry differs from the dominating genotype in human patients. In poultry, the *C. jejuni* population at flock level indeed can consist of a range of genotypes (Thomas et al., 1997; Petersen et al., 2001), and individual birds were found to be colonised by various genotypes (Camarda et al., 2000). In 4 out of 52 patients suffering from campylobacteriosis different genotypical patterns were observed (Steinbruecker et al., 2001). Additionally, isolation techniques for *C. jejuni* from poultry and humans might be different;

(4) The genotype of *Campylobacter* is not a constant, but changes. As already mentioned, in a flock, campylobacters with different genotypes have been isolated, isolates which might have originated from a single strain, since various groups found evidence for genetic instability in *Campylobacter* (On, 1998; Hänninen et al., 1999; Wassenaar et al., 1998; Thomas et al., 1997; and Guerry et al., 1988). In stools of 4 out of 52 human patients suffering from a *Campylobacter* infection, different PFGE pattern were observed during the infection episode (Steinbruecker et al, 2001).

C. jejuni is capable of natural transformation, is capable of taking up DNA from the environment and integrate it in its own genome (Wang and Taylor, 1990). In closely related *Helicobacter pylori* the frequency of mutation is higher than in any other organism (Wang et al., 1999), and both in *H. pylori* and *C. jejuni* DNA repair mechanisms present in *E. coli* seem to be absent (Wang et al., 1999; Parkhill et al., 2000). Hypervariable sequences have been demonstrated (Parkhill et al., 2000), and, interestingly, these hypervariable sequences

can be found in genes encoding for cell surface structures, among which flagella (Fischer and Nachamkin, 1991).

This study was undertaken to investigate the hypothesis that poultry is the source of infection of human patients, but that due to instability of the genome of *Campylobacter*, strains isolated from poultry and human patients differ in genotype.

We grew *C. jejuni* in nutrient rich medium in the presence of high or low concentrations of oxygen and studied genotypic stability using three generally applied techniques: pulsed field gel electrophoresis (PFGE; Boer et al., 2000), amplified fragment length polymorphism (AFLP; Duim et al., 1999) and the multi locus sequencing technique (MLST; Dingle et al., 2001). All studies were performed in continuous cultures for four weeks.

2. Materials and methods

2.1 Bacterial strains, and growth media

Poultry isolates *C. jejuni* B258 (this laboratory) and C356 (B. Duim; ID-Lelystad) were kept at –70^{o}C in a mixture containing 25% glycerol and 75% brain heart infusion broth (BHI). For continuous culturing we used medium containing 20 g l^{-1} BHI in an L&H 500 ml modular fermentor 500 Series II (LH Engineering Co. Ltd., Stoke Poges, Bucks, England). Prior to inoculation, one vial was taken from the –70^{o}C and transferred to approximately 100 ml fresh BHI in a 500 ml Erlenmeyer flask and cultured while shaken at 100 rpm for 24 hours in an atmosphere containing 10% oxygen and 5% CO_2 at 35^{o}C. Of this culture, 1 ml was used to inoculate the fermentor. The medium pump was switched on 24 hours after inoculation and the dilution rate was set on 0.16 +/- 2 h^{-1}. All experiments were performed at 35^{o}C and the impeller rotated at 400 rpm. The pH of the fermentor was monitored but not controlled and varied between 7 and 7.6. The fermentor was flushed with 60 l min^{-1} of a gas mixture containing either 3 % oxygen (9, 3, and 48 l h^{-1} of respectively air, carbon dioxide and nitrogen gas) or 13% oxygen (39, 3, 18 l h^{-1} of respectively air, carbon dioxide and nitrogen gas). The oxygen content of outcoming gas mixtures was measured on a Servomex 580A oxygen analyser.

2.2 Genomic DNA isolation

Genomic DNA's were extracted using a Wizard genomic DNA purification kit (Promega, Madison, Wis.) according to the manufactor's instructions. Concentrations were measured in a GeneQuant II RNA/DNA calculator (Pharmacia/Biotech). If necessary, DNA solutions were diluted in such a manner that the final concentration was 4 ng μl^{-1}.

2.3 Pulsed Field Gel Electrophoresis: PFGE

PFGE was performed essentially according to Boer et al (2000), using SmaI as restriction enzyme. For electrophoresis, we used a Chef-DRIII system (Bio-Rad), programmed at 6 V/cm, the included angle was 120 degrees and ramp times were 5 to 10 seconds for 4 hours, 10 to 40 seconds over14 hours, and 50 to 60 seconds over 4 hours. After electrophoresis, gels were stained in a 1 mg ml^{-1} ethidium bromide solution and destained in electrophoresis buffer and bands were visualised under UV light.

2.4 Amplified Fragment Length Polymorphism: AFLP

AFLP analysis was performed as described by Duim et al. (1999). Briefly, genomic DNAs were digested with *HindIII* and *HhaI*. Simultaneously, site-specific adapters were ligated to the restriction fragments. A preselective PCR amplification was followed by a selective PCR using a labelled *HindIII* primer containing a selective A nucleotide and a *HhaI* primer containing a selective A nucleotide. Used primers and adapters are shown in Table 1. Final products were analysed on a ABI 3700 DNA sequencer. Data analysis were performed using Genescan 3.5 (PE Biosystems) and BioNumerics (Applied Maths).

Table 1: Primers and adapters used in the AFLP analysis.

Primer/Adapter	**Oligo-sequence**	**Remark**
*Hind*III restriction site-specific adapter	5'-CTC GTA GAC TGC GTA CC-3' 5'-AGC TGG TAC GCA GTC-3'	Forward Reverse
*Hha*I restriction site-specific adapter	5'-GAC GAT GAG TCC TGA TCG-3' 5'-ATC AGG ACT CAT CG-3'	Forward Reverse
*Hind*III preselective primer	5'-GAC TGC GTA CCA GCT T-3'	
*Hha*I preselective primer	5'-GAT GAG TCC TGA TCG C-3'	
*Hind*III selective primer	5'-GAC TGC GTA CCA GCT T**A**-3'	FAM labelled
*Hha*I selective primer	5'-GAT GAG TCC TGA TCG C**A**-3'	FAM labelled

2.5 Multi Locus Sequence Typing: MLST

MLST was performed as described by Dingle et al (2001). Briefly, genes encoding for three housekeeping enzymes plus the katA, AhpC, SodB and FlaA gene were amplified by PCR (2 µl DNA, 20 pmol forward primer, 20 pmol reverse primer, 12,5 µl Taq PCR master mix (Qiagen); 15 min 95°C, followed by 20 cycles of 30 sec 94°C, 1 min 50°C, and 1 min 72°C, and finally 10 min at 72°C) in a PCR express (Hybaid). Used primer combinations are presented in Table 2. Amplified product was purified with Qiaquick PCR purification Kit (Qiagen). Prior to sequencing, 7 µl sequence buffer (200 mM Tris pH 9, 5 mM $MgCl_2$), 1 µl Big Dye Terminator reaction kit (PE-Biosystems), 5 pmol primer and 10-40 ng purified DNA (final volume: 20 µl) were mixed and amplified by PCR (25 cycles of 30 sec 95°C, 15 sec 50°C and 4 min 60°C) in a Hybaid PCRexpress. Sequence analysis was performed in an ABI 3700 DNA sequencer; data analysis with 'Sequence analysis 3.6 (PE Biosystems) and Dnastar seqman (Haaxman, Wastie and Partners).

Table 2. Used primers for MLST.

Enzyme (*gene*)	Primer	Function	Sequence	Reference
Aspartase (*aspA*)	Asp-A1F*	Amplification	5'-AAA GCT GCA GCT ATG GC-3'	Dingle et al., 2001.
	Asp-A2R*		5'-TTC CAG TGT TCA TTG CCT C-3'	
	Asp-S3F	Sequencing	5'-CCA ACT GCA AGA TGC TGT ACC-3'	
	Asp-S6R		5'-TCA ATT TGC GGT AAT ACC ATC-3'	
Phosphoglucomutase (*pgm*)	Pgm-A1F	Amplification	5'-TTG GAA CTG ATG GAG TTC G-3'	Dingle et al., 2001.
	Pgm-A2R		5'-AAG AGC TTA ATA TCT CTG GCT TCT AG-3'	
	Pgm-S3F	Sequencing	5'-GCT TAT AAG GTA GCA CCT ACT G-3'	
	Pgm-S2R		5'-TCC AGA ATA GCG AAA TAA GG-3'	
ATP syntase Subunit α (*uncA*)	Unc-A7F	Amplification	5'-ATG GAC TTA AGA ATA TTA TGG C-3'	Dingle et al., 2001.
	Unc-A8R		5'-ATA AAT TCC ATC TTC AAA TTC C-3'	
	Unc S3F	Sequencing	5'-AAA GTA CAG TGG	

	Unc-S4R		CAC AAG TGG-3’ 5’-TGC CTC ATC TAA ATC ACT AGC-3’	
Flagellin A (*flaA*)	Fla-A4F Fla-A1728R Fla-S442F Fla-S630R Fla-S845AF Fla-S1532R	Amplification Sequencing	5’-CGA TTT CGT ATT AAC ACA AAT GGT GC-3’ 5’-CTG TAG TAA TCT TAA AAC ATT TTGA-3’ 5’-CAA ATC GGC GCA AGT TC-3’ 5’-GCTCCA AGT CCT GTT CC-3’ 5’-AAG ATA CCA CAG GTG TTG AAG C-3’ 5’- ATG TCG GCT CTG ATT TGA TC-3’	Meinersmann et al., 1997
Alkyl hydroperoxidase reductase (*ahpC*)	AhpC-ASF AhpC-ASR	Amplification/ Sequencing	5’-CTC CAG CAG TAT TGG GAA-3’ 5’-AAG ATA TTC AGC CAC GCC-3’	Baillon et al., 1999
Superoxide dismutase (*sodB*)	SodB-ASF SodB-ASR	Amplification/ Sequencing	5’-TAA GCC AAG TAC AGG CTG TG-3’ 5’-CTA ACT CCC ATG CCT TC-3’	Pesci et al., 1994.
Katalase (*katA*)	Kata-AS1F Kata-S1R Kata-AS2R Kata-S2F	Amplification/ Sequencing Sequencing Amplification/ Sequencing Sequencing	5’-GCT AAG GGA AGT GGA GCT TA-3’ 5’-GGA CTA AAG GCA GCT TGT TC-3’ 5’-AAC TCC CTC CAT AGA AGT GG-3’ 5’-GAA CAA GCT GCC TTT AGT CC-3’	Grant and Park., 1995

3. Results

C. jejuni was grown in chemostat culture in nutrient rich medium for four weeks at a growth rate (μ) of 0.16 h^{-1}, or doubling time (T_d; $T_d = \ln 2 / \mu$) of 4.6 h, resulting in approximately 146 generations after 4 weeks. Both strains tested, *C. jejuni* B258 and C356, were grown in the presence of either high (approximately 13%) or low (approximately 3%) concentrations of oxygen. Samples were taken twice a week for approximately four weeks.

3.1 PFGE

Isolated genomic DNA was restricted using SmaI. Resulting fragments were separated on an agarose gel and results are shown in Figure 1 (*C. jejuni* B258) and Figure 2 (*C. jejuni* C356).

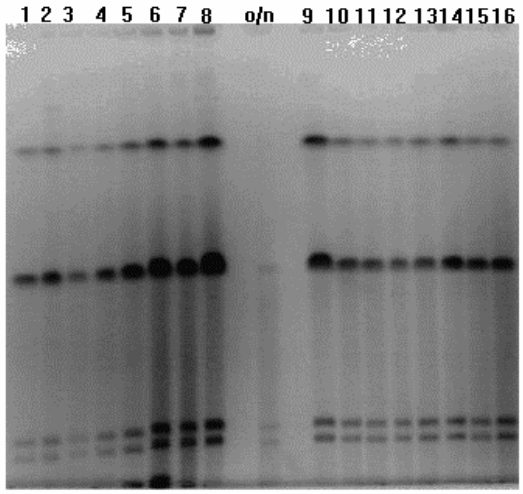

Figure 1. Results after PFGE of the genomic DNA of C. jejuni B258.

Lane 1-8: day 1, 3, 8, 10, 15, 17, 20 and 22 from a culture grown at 13% oxygen; o/n: overnight culture; lane 9-16: day 1, 3, 8, 10, 15, 17, 20 and 22 from a culture grown at 3% oxygen.

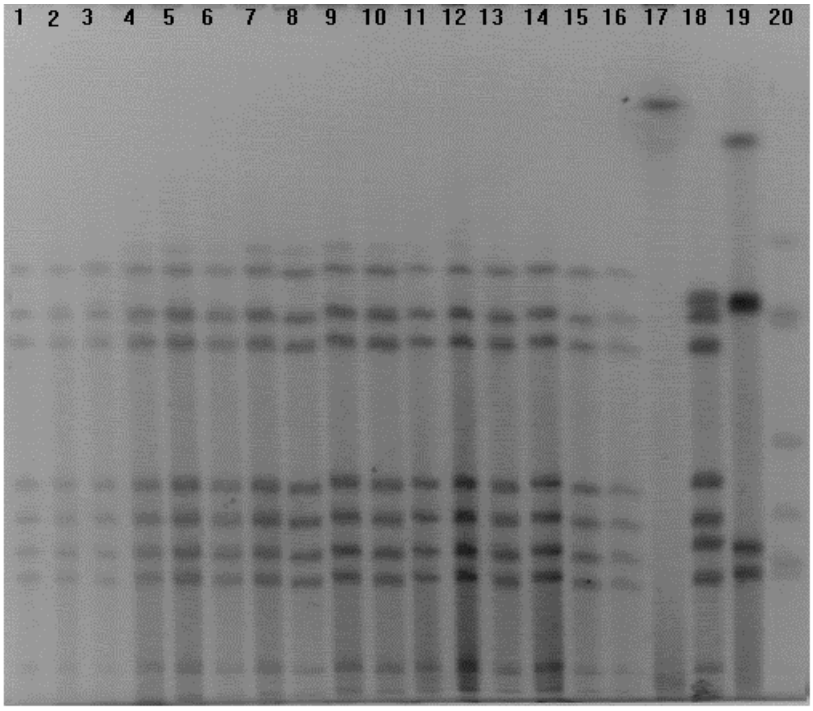

Figure 2. Results after PFGE of the genomic DNA of C. jejuni C356.

Lanes 1, 3, 5, 7, 9, 12 and 14: day 1, 3, 5, 10, 15, 22 and 24 of a culture grown at 13% oxygen; lanes 2, 4, 6, 8, 10, 11, 13 and 15: day 1, 3, 8, 10, 15, 17, 22 and 24 of a culture grown at 3% oxygen; lane 16-20: o/n cultures of *C. jejuni* strains C356, NCTC 11828, ATCC 11168, B258 respectively LB99hu.

The PFGE pattern of an overnight culture (lane o/n in Figure 1; lane 16 (C356) and 19 (B258) in Figure 2) was similar to that of a culture at the first day of sampling (lane 1 and 9 and lane 1 and 2 in Figure 1 respectively 2). During culturing under constant conditions, PFGE patterns of samples from both *C. jejuni* B258 and C356 had not changed after approximately 146 generations.

3.2 AFLP

The samples analysed with PFGE were also analysed using the AFLP technique. The results are shown in Figure 3.

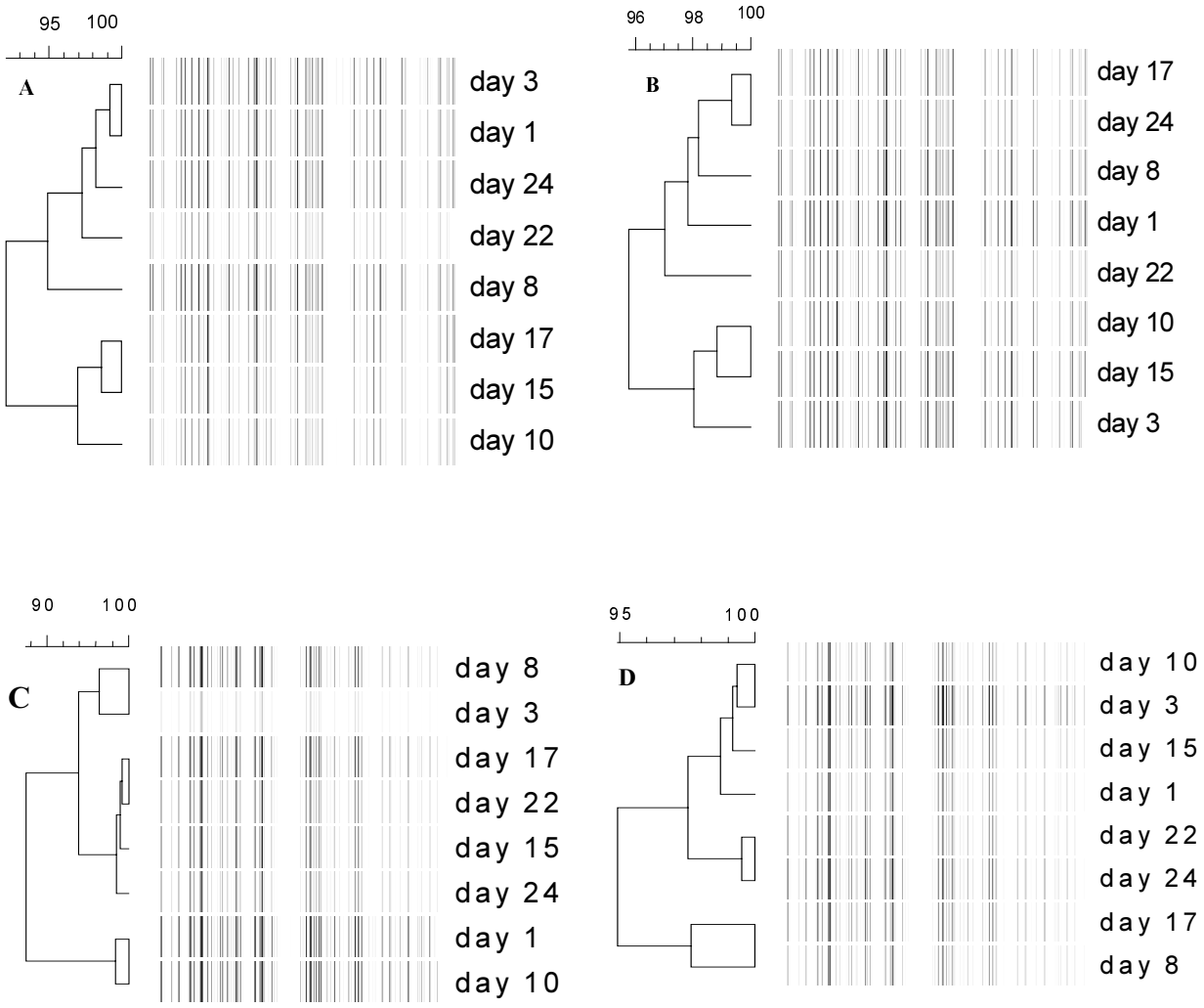

Figure 3. Pearson correlations and AFLP patterns of C. jejuni B258 (A and B) and C356 (C and D) grown for 24 days in continuous culture at 13% (A and C) or 3% (B and D) oxygen. Samples were taken twice a week at day 1, 3, 8, 10, 15, 17, 22 and 24.

After culturing *C. jejuni* B258 for 24 days, the similarity (calculated as the Pearson correlation) in AFLP pattern between samples taken on day 1 and day 24 was above 95%,

irrespective of the used oxygen concentration (3A and 3B). This was also found for *C. jejuni* C356 grown at 3% oxygen (Figure 3D); the AFLP pattern of samples of *C. jejuni* C356 grown in the presence of 13% oxygen at day 1 and 10 had a similarity lower than 95% compared to the other samples; the similarity between day 3 and 24 however was approximately 95% (Figure 3C).

3.3 MLST

Genes encoding three housekeeping enzymes, three enzymes involved in oxidative stress and flagellin were amplified and sequenced in the same samples used for PFGE- and AFLP analysis. No mutations were observed (not shown) during 24 days of culturing.

4. Discussion

C. jejuni has a relatively small genome (approximately 1641 kbp) and the results of many studies using various genetic tools revealed many genotypes. Other studies showed that most strains found in poultry are different from those present in human patients. It was hypothesised that the genotype of *C. jejuni* is not a constant, but changes. However, we cultured *C. jejuni* B258 and C356, both chicken isolates, in chemostat culture for roughly 150 generations, and no alterations were observed with any of the three used genetic techniques.

The mutation frequency in *Campylobacteriaceae* seems to be higher than in any other species tested (Wang et al., 1999), and horizontal genetic exchange has a major influence on *Campylobacter* populations (Dingle et al., 2001). PFGE is a technique in which the whole bacterial genome, including plasmids, is cleaved into a limited number of fragments. It is considered to be a high-resolution technique (Boer et al., 2000) which should be able to detect (large) chromosomal inserts and deletions. Small mutations are unlikely to be detected, unless present in a restriction site. We did not observe any change in PFGE patterns, which is not surprisingly, considering the low number of used restriction sites and absence of exchangeable DNA. Detectable intrachromosomal rearrangements had occurred neither. With the AFLP technique, more restriction sites are introduced, and created fragments are smaller than those created with PFGE, making small mutations more likely to be detected. On the other hand, part of the sensitivity is lost since not all created fragments are used for pattern building. Usually 80 to 100 fragments of 50 to 500 basepairs can be seen, thus maximally 50kbp of the complete chromosome is monitored. As with the PFGE technique however, no changes were detected, indicating again the absence of DNA exchange and detectable mutations.

Intrachromosomal rearrangements were not detected and *C. jejuni* was grown in the absence of exchangeable DNA making transformations unlikely. As a consequence, alterations in genotype, if any, most likely are the result of small mutations. In order to monitor mutations, it would be best to sequence the complete chromosome of *C. jejuni* during the experiment. For practical reasons we sequenced stretches of nucleotide of 50~500 bp from 7 genetic loci: three housekeeping loci, three loci involved in oxidative stress, and the FlaA locus. Mutations were expected to occur most likely under non-selective conditions. Non-selective conditions do not exist with respect to the housekeeping loci, since these loci encode enzymes required for growth and maintenance, and indeed no mutations were observed. Non-selective conditions are present at low oxygen concentrations for enzymes involved in oxidative stress. But also in katA, SodB and AphC, no mutations were detected.

The three housekeeping loci and three loci involved in oxidative stress represent only a very small part of the complete genome: approximately 3.5 of 1641 kbp and mutations in other parts might have occurred. However, in a chemostat, motility is not required since in a chemostat no gradient, neither attracting, nor repelling exists. Microscopical examination of campylobacters grown in chemostat culture (not shown) indeed showed non-motile bacteria. Mutations in the FlaA gene, resulting in impaired flagella would be without consequences, but even in the two hypervariable regions of the FlaA gene, no changes in nucleotide sequence were found.

All results presented strongly suggest that the genotypes of *C. jejuni* C356 and B258 are stable under the used conditions. How representative this is for other (varying) conditions and other *C. jejuni* isolates remains to be resolved.

5. Further research

Faecally contaminated foodproducts of animal origin have to be considered a source of infection of *C. jejuni* since *C. jejuni* is an inhabitant of the gastrointestinal tract of warm-blooded animals, a niche rich in biological building blocks, among which DNA. In the gastrointestinal tract and on the surface of faecally contaminated foodproducts *C. jejuni* experiences various concentrations of oxygen. Sofar we cultured two *C. jejuni* strains in the absence of exchangeable DNA under two different, but constant concentrations of oxygen and monitored changes in genomic patterns, and in sequences of various genes. In the near future we will study the genotypical stability of *C. jejuni* under changing oxygen concentrations and we aim to study genotypical stability in the presence of exchangeable DNA.

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Appendix 1 Mailing list

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