PCR Detection and Typing of *Campylobacter jejuni* and *Campylobacter coli* in Poultry Meat

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Introduction

Over the last ten years the polymerase chain reaction (PCR) has become an important analytical tool. *Campylobacter (C.) spp.* have been known to be a major cause of gastrointestinal disease in humans for about twenty years. *Campylobacters* can be isolated from more stool samples of patients with diarrhea than *Salmonella*, and the organisms are isolated from important food sources such as milk, water, poultry or pork (HUTCHINSON et al., 1985; PHILIPPS, 1995)
Understanding the epidemiology depends on two factors: first, the organisms must be reliably detected in contaminated food or environmental samples; second, methods that can discriminate between species isolated from different sources must be available. Traditional detection of *Campylobacter jejuni* and *C. coli* relies on culture. An intensive interlaboratory study revealed significant problems with the sensitive detection of the bacteria by culture methods and the most reliable method required one week or more (SKIRROW et al., 1993). In contrast, a rapid and reliable detection of *Campylobacter spp.* in food and environmental samples can be achieved using polymerase chain reaction (PCR) or isothermal RNA amplification. Since these methods do not yield isolated strains, combination with typing systems that are based on the availability of pure bacteria is not possible.
There is a vast number of typing schemes available for *Campylobacters* which fall into two categories: phenotypic methods, that is, serotyping according to PENNER, HENNESSY and LIOR, biotyping, phage typing, and fatty acid analysis; and genotypic methods, that is, multilocus enzyme electrophoresis (MEE), restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), and plasmid analysis.

Material and Methods

Samples

282 samples of poultry meat (skin, breast muscle and hind leg) were examined for the occurrence of *Campylobacter* using cultural methods, RFLP and RAPD-PCR.

Detection by cultural methods

The existence of *Campylobacter* was proven by means of method I and II according to ISO 1989 as well as the following procedure: Preliminary accumulation – test samples and *Campylobacter* accumulation broth No. 2 (Oxoid) were incubated at 37°C for 24 hours and at 42°C for 24 hours. Affirmation was carried out on CCDA selective supplement Preston (Oxoide) and *Campylobacter* agar base (Oxoid). Incubation was carried out under microaerophilic conditions (8% O_2, 5-8% CO_2) at 37°C for 24 hours and at 42°C for 42 hours. Parallel to classical microbiological methods the incubated *Campylobacter* accumulation broth was used for RFLP-PCR.
A suspicious colony was taken from overgrown plates and examined for mobility at 37°C (+), Gram stain (-), oxidase (+), nitrate reduction (+), katalase (+), indole (-).

Direct detection by PCR

DNA extraction. 10 g of meat were mixed with PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 9) to a final volume of 40 ml, incubated at 45°C for 1 hour to release bacteria adhering to meat particles and centrifuged afterwards at 200 g for 10 minutes to pellet the meat. Then, the supernatant was filtered through a AP25 pre-filter (Millipore, Bedford, MA, USA) to remove remaining meat particles. The filtered samples were centrifuged at 4200 g for 10 minutes to pellet the bacteria. The supernatant was discarded and the pellet resuspended in 200 µl of PCR buffer. The samples were transferred to micro-tubes and the cells were lysed by incubation at 95°C for 15 minutes. Cell debris was removed by centrifugation in a Biofuge (Heraeus, South Plainfield, NY, USA) at 14000 g for 10 minutes. 5 µl of the undiluted or fivefold diluted supernatant were used for PCR analysis.
Amplification. A semi-nested PCR system was applied detecting both *C. jejuni* and *C. coli* (WEGMUELLER et al., 1993). Primers CF03 and CF04 were used in the first PCR (25 cycles) and primers CF02 and CF03 in the second PCR (30 cycles). 2 µl of the first PCR reaction were subjected to the second PCR. PCR conditions were as described above. 20 µl of PCR products were analyzed by agarose gel-electrophoresis.
Controls for sample preparation and PCR. 10 g of meat spiked with approximately 10^8 cells (positive control) were included in all experiments and treated as described above. In each semi-nested PCR negative (PCR buffer only) and positive control (extracted *C. jejuni* DNA of approximately 10^7 cells/ml) were included.

RFLP analysis

20 µl of the PCR product (generated using the primers CF02 and CF03) were digested with 5 units of the restriction endonuclease *Dra*I (Gibco BRL, Germany) at 37°C for 3 hours. Digested products were separated on 2% (w/v) MS agarose gels (Boehringer Mannheim, Germany).

RAPD typing

RAPD typing was performed exactly as described previously (OWEN and HERNANDEZ, 1993) with a 10-mer primer (5`-CCTGTTAGCC-3`) being allowed to target the whole genome, but with no prior knowledge of the binding sites being required. The resulting mixture of amplicons is again separated on the basis of molecular weight. The result is a much more complex pattern of bands than with PCR/RFLP but is used to type the isolates in the same manner.

Results

Using classical microbiological culture methods *Campylobacter* could be found in 37% (104 samples) of the examined probes, whereas by RFLP-PCR 118 samples (41,8%) could be identified as positive. From two samples the detection of *Campylobacters* was only possible via culture methods and RFLP-PCR showed negative results.

No satisfying data were obtained using the RAPD technique for the direct detection of these organisms from food. By this method only *Campylobacters* that have already been cultivated by cultural methods can be examined and classified more accurately.

The fragment length of the CF-system (RFLP) varied between 181 and 209 bp. By this means *C. jejuni, C. coli* and *C. upsaliensis* could be identified.

After colonisation (culture method) suspicious colonies were examined more closely and differentiation of the colonies was done biochemically. *C. jejuni, C. coli, C. fetus subsp. fetus, C. sputorum fecalis* and *Helicobacter cinaedi* wereconfirmed.

Discussion

Usually microbiological diagnostic in the field of food- and meat hygiene is carried out using classical culture methods. However, this is a very time-consuming process, results are often obtained after six or more days, and often differentiation of the bacterial strains is not performed. Our results show that RFLP-PCR is a powerful and fast tool for the detection of *Campylobacter* from food. In our study *Campylobacter* was detected in two samples by cultural methods whereas RFLP-PCR showed a negative result. We therefore suggest that at the moment a combination of classical cultural methods and modern molecularbiological methods should be applied for routine diagnostics. The fast detection of the organisms by RFLP-PCR is an important means to quickly identify contaminated food and thus allow immediate action to ensure the consumer`s health.

RAPD should not be used for the detection of *Campylobacters* directly from food, but is useful for further investigation and differentiation after cultivation.

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