A PCR ASSAY FOR *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI*

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SUMMARY

We present the development and application of a PCR method for routine detection of *Campylobacter jejuni* and *Campylobacter coli* in meat and meat products. Selective enrichment and Polymerase Chain Reaction (PCR) were combined into a fast screening method. The performance of this method was evaluated in an implementation study with artificially contaminated and fresh swab samples from the meat production line. Correlation between the results of the PCR methods and the standard detection method was excellent. The total detection time of 28 hours might be further reduced with even shorter enrichment and/or Immunomagnetic separation (IMS) prior to PCR.

Introduction

Campylobacter enteritis has been recognized as a public health concern for more than a decade. Also the economic effects of Campylobacter infections are considerable, as the thermophilic Campylobacter species, particularly *Campylobacter jejuni* and the very similar organism *Campylobacter coli*, are among the most frequently isolated bacteria that cause diarrheal disease in humans. Campylobacters live as commensals in the intestinal tracts of a wide range of birds and mammals, including the domestic animals used for food production. They contaminate carcasses from intestinal contents during slaugther. As these fastidious, microaerophilic organisms are often difficult to culture and identify, the prevalent human pathogens *C. jejuni* and *C. coli* have been chosen as a target for DNA probe development and detection by in vitro amplification (PCR).

Probe and primer development

Probes specific for *C. jejuni* and *C. coli* have been selected from a library of randomly cloned Campylobacter DNA fragments by differential hybridization with chromosomal DNAs from several Campylobacters species. The complete nucleotide sequences of the specific probes has been determined and PCR primers were designed based on these sequences. The specificity of the two primersets, one for *C. jejuni* and another for *C. coli* was confirmed by testing in PCR on a large collection of eubacterial species. The two primersets were combined into one PCR assay. This "multiplex" PCR setting enables the simultaneous detection of both species. In this assay, the species classification can be inferred directly from the different sizes of the amplification products. Confirmation of the PCR results can be performed by hybridization with an internal fragment as hybridization probe.

Development and implementation of the PCR method

For implementation of the *C. jejuni / C. coli* multiplex assay we used 45 swabsamples from fresh pigcarcasses and slaughterhouse equipment. Simple enrichment and sample pretreatment steps have been combined with a *Campylobacter*-specific PCR. For the enrichment protocols we used an internationally recognized culture medium. The sample pretreatment before PCR consisted of simple dilution or centrifugation steps to remove PCR inhibiting components from meat and media. In this studie the results were visualized by agarose gel electrophoresis and ethidium bromide staining. Modification of the PCR will allow replacement by other visualization techniques in the near future, e.g. colorimetry.

The detection time for presence/absence testing of Campylobacter with this PCR assay was reduced to 28 hours. After 24 hours of selective enrichment in CCDB, inhibiting components from meat and medium were removed by centrifugation. PCR was carried out directly on the resuspended cells after boiling for 5 minutes, without the need