Detection and Identification of *Campylobacter coli* and *Campylobacter jejuni* by Two-Step Polymerase Chain Reaction

Giuseppe Comi,* Pierino Ferroni, Luca Cocolin, Carlo Cantoni, and Marisa Manzano

Abstract

Flagellin gene was used as target sequence to detect and distinguish *C. coli* and *C. jejuni* by a “nested PCR” technique. The method shows a high level of sensitivity and specificity. Application of this rapid diagnostic tool could provide further information about epidemiological and pathogenetic implications of each of these two microorganisms.

Index Entries: *Campylobacter*, polymerase chain reaction; flagellin gene.

Campylobacteriosis is a worldwide human acute gastroenteritis involving more than 2 million cases of infection in the United States yearly (1). The endemic level appears higher than salmonellosis or shigellosis both in developing or developed countries (2,3). The disease is characterized by such symptoms as fever, headache, muscular pain, nausea, vomiting, and diarrhea. The most frequent vehicles of transmission are untreated milk, raw or undercooked poultry meat, and untreated or improperly chlorinated water.

The accurate identification of *Campylobacter* in waterborne outbreaks as well as in sporadic cases was limited by nonoptimal growth conditions and by occurrence of viable but nonculturable forms of *Campylobacter* (4). For this reason, epidemiology of *Campylobacter* also is not well defined and does not provide the basis for a more specific control strategy. In addition, the difficulties to distinguish *C. coli* from *C. jejuni* do not allow a more accurate evaluation of epidemiological or pathogenetic implications of each of these two microorganisms.

We developed a rapid method for detection and identification of *C. coli* and *C. jejuni* by two-step polymerase chain reaction (“nested” PCR) amplifying nucleotide sequences of the subunit flaA of the flagellin gene. For the first PCR round two primers, suggested by Oyofo et al. (5) and located in highly conserved regions, were used (5): 5'-ATGGGATTTCGTATTAAC-3' (P1; outer sense, nt 319–336 for *C. coli* and nt 83–100 for *C. jejuni*) and 5'-GAACTACGAACCGATYrG-3' (P2; outer antisense, nt 760–776 for *C. coli* and nt 524–540 for *C. jejuni*). The *C. coli* and *C. jejuni* flagellin gene sequences were derived from those stored in the EMBL Data Library (Heidelberg, Germany), access numbers M64670 and M74578, respectively. A sequence complementary to P2 primer is also present in the subunit *FlaB* of *C. coli* (nt 2642–2658) and of *C. jejuni* (nt 2429–2445; the nucleotide numbers were derived from the sequence with code number J05635). Therefore, three amplimers, respectively of 458, 2340, and 2363 bp were expected; the first common for *C. coli* and *C. jejuni*, the other two for each of two microorganisms.

*Author to whom all correspondence and reprint requests should be addressed.: Department of Food Science, University of Udine, Via Marangoni, 97, 33100 Udine, Italy.

Molecular Biotechnology ©1995 Humana Press Inc. All rights of any nature whatsoever reserved. 1073-6085/1995/3:3/266-268/$4.60
For the second PCR round two primer pair within the nucleotide sequence of first amplimers were designed: 5'-ATATCTAATGGTAACGGC-3' (P3; inner sense, nt 511-530 for C. coli and nt 275-294 for C. jejuni) and 5'-ATTCTTGATTGGTAAAACCA-3' (P4; inner antisense nt 738-757 for C. coli). The P3 and P4 primers in presence of target DNA of C. coli flagellin gene generate a 228-bp fragment. The annealing of the P4 primer in the corresponding region of C. jejuni flagellin gene (nt 447--466) was avoided by the presence of the three different nucleotides in the 3' terminus. A site of annealing of P4 was present at the nucleotide interval 1245-1264 of flagellin sequence, which yielded an amplified product of 1044 bp.

The first PCR was performed in a 100-µL mixture containing 10 µL of the test sample, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 0.6 mM each deoxynucleoside triphosphate, 50 µM of each of the outer nested primers, and 2.5 U of Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). Forty heat cycles were employed, each consisting of denaturation for 90 s at 94°C, annealing for 80 s at 42°C, and extension for 120 s at 72°C. One microliter of the cocktail of the first reaction was then transferred to a second tube containing the same reaction mixture but with the inner pair of nested primers, and 35 additional heat cycles were carried out with the same program except the annealing temperature, which was raised to 52°C. A total of 10 µL of the product of the second reaction was subjected to electrophoresis on 2% agarose gel containing 0.5 µg/mL ethidium bromide and observed under UV light (Fig. 1).

We submitted to PCR the following microorganisms: C. jejuni (strains no. H1-H2-H3) isolated from patients with acute diarrhea; C. jejuni (strain no. C111) and C. coli (strains no. C113, C115-C117) isolated from poultry meat. We also examined strains obtained from National Collection of Type Cultures (London) as C. jejuni NCTC no. 12104, 12107, 12109 and C. coli NCTC no. 11350, 11353, 12143, 11366, and the C. jejuni strain no. 33291 from American Type Culture Collection (Rockville, MD). The strains were grown on brain heart infusion broth (Oxoid, Basingstoke, UK) at 37°C overnight under microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂). To establish specificity of PCR, C. laridis and other microorganisms as Escherichia coli, Helicobacter pylori, Listeria monocytogenes, Salmonella enteritidis, Yersinia enterocolitica were tested.

DNA was prepared by lysis of bacterial cells with lysozyme and proteinase K digestion and phenol-chloroform-isooamyl alcohol extraction according to standard procedure (6).

The "nested" PCR shows a high specificity since the primers failed to amplify bacterial DNA from non-Campylobacter strains. All C. coli examined shows a band of amplification costantly of 228 bp, whereas with the C. jejuni the 1044-bp band expected was inconstant; in the negative cases one band with molecular weight ranging from 800-900 bp was found. The presence of these amplimers were specifically associated to C. jejuni.