SB-1

Detection of *Campylobacter jejuni* in food and poultry visera using immunomagnetic separation and microtitre hybridization

Ronald, E. SIMARD
Department of Food Science & Technology, Laval University, Quebec, CANAD.

Abstract

Campylobacter jejuni is most frequently identified cause of cause of acute diarrhoeal infections in developeed countries, exceeding rates of illness caused by both *salmonella* and *shigilla*(Skirrow, 1990 ; Lior 1994). Previous studies on *campylobacter jejuni* contamination of commercial broiler carcasses in u.s.(Stern, 1992). Most cases of the disease result from indirect transmission of *Campylobactor* from animals via milk, water and meat. In addition to *Campylobactor jejuni*, the closely relates species *Campylobactor coli* and *Campylobactor lari* have also been implicated as agents of gastroenteritis in humans. *Campylobactor coli* represented only approximately 3% of the *Campylobactor* isolates from patients with *Campylobactor* enteritis(Griffiths and Park, 1990) whereas *Campylobactor coli* is mainly isolated from pork(Lmmerding et al., 1988). Campylobactor jejuni has also been isolated from cases of bacteremia, appendicitis and, recently, has been associated with Guillai-Barré syndrome(Allos and Blaser, 1994; von Wulffen et al., 1994; Phillips, 1995). Studies in volunteers indicated that the infectious dose for *Campylobactor jejuni* is low(about 500 organisms)(Robinson, 1981). The methods traditionally used to detect *Campylobactor ssp.* in food require at least two days of incubation in an enrichment broth followed by plating and two days of incubation on complex culture media containing many antibiotics(Goossens and Butzler, 1992). Finnaly, several biochemical tests must be done to confirm the indentification at the species level. Therfore, sensitive and specific methods for the detection of small numbers of *Campylobactor* cells in food are needed. Polymerase chain reaction(PCR) assays targeting specific DNA sequences have been developed for the detection of *Campylobactor*(Giesendorf and Quint, 1995; Hemandex et al., 1995; Winter and Slavidk, 1995). In most cases, a short enrichment step is needed to enhance the sensitivity of the assay prior to detection by PCR as the number of bacteria in the food products is low in comparison with those found in clinical samples, and because the complex composition of food matrices can hinder the PCR and lower

its sensitivity. However, these PCR systems are technically demanding to carry out and cumbersome when processing a large number of samples simutaneously.

In this paper, an immunomagnetic method to concentrate *Campylobactor* cells present in food or clinical samples after an enrichment step is described. To detect specifically the thermophilic *Campylobactor*, a monoclonal antibody was adsorbed on the surface of the magnetic beads which react against a major porin of 45kDa present on the surface of the cells(Huyer et al., 1986). After this partial purification and concentration step, detection of bound cells was achieved using a simple, inexpensive microtitre plate-based hybridization system. We examined two alternative detection systems, one specific for thermophilic *Campylobactor* based on the detection of 23S rRNA using an immobilized DNA probe. The second system is less specific but more sensitive because of the high copy number of the rRNA present in bacterial cell(10^3-10^4). By using specific immunomagnetic beads against thermophilic *Campylobactor*, it was possible to concentrate these cells from a heterogeneous media and obtain highly specific hybridization reactions with good sensitivity. There are several advantages in using microtitre plates instead of filter membranes or other matrices for hybridization techniques. Microtitre plates are much easier to handle than filter membranes during the adsorption, washing, hybridization and detection steps, and their use faciilitates the simultanuous analysis of multiple sample. Here we report on the use of a very simple detection procedure based on a monoclonal anti-RNA-DNA hybrid antibody(Fliss et al., 1993) for detection of the RNA-DNA hybrids formed in the wells.

Reference

Allos, B. M and Blaser, M. J. (1994) *Campylobactor jejuni* infection and the Guillain-Barre syndrome : Mechanism and Implications, *Zentralblat für Bakuriologic*, 281, 544-548

Fliss, I., St-Laurent, M., Emond, E. et al. (1993) Production and characterization of anti-DNA-RNA monoclonal antibodies and their application in Listeria detection. Applied and Environmental Microbiology, 59, 2698-2705

Giescndorf, B. A. J. and Quint, W. G. V.(1995) Detection and identification of *Campylobactor spp.* using the polymerase chain reaction *Cellular and Molecular Biology*, 625-638

Goossens, H. and Butzler, J. P. (1992) Isolation and identification of *Campylobactor spp.* In *Campylobactor jejuni, current status and Future Trends.* Nachamlisn, M. J.., Blaster, L. S. and Tompkins. Washington DC : American Society for Microbiology

Griffiths, P. L. and Park R. W. A. (1990) Campylobactor associated with human diarrhocal disease. *Journal of Applied Bacteriology* 69, 281-301

Hernandez, J., Alonso, J. L., Fayos, A., Amoros, I. and Owen, R. J. (1995) Development of a PCR assay combined with a short enrichment culture for detection of *Campylobactor jejuni* in estu-urine surface waters. *FEMS Microbiology letters* 127, 201-206

Huyer, M., Parr, T. R., Hancock, R. E. W. and Page, W. J. (1986) Outer membrane porin protein of *Campylobactor jejuni. FEMS Microbiology letters 37, 247-250*

Lammerding, A. M., Garda, M. M., E. D. et al., (1988) Prevalence of *Salmonella* and thermophilic *Campylobactor* in fresh pork, beef, veal and poultry in Canada. *Journal of food Protection* 51, 47-52

Lior, H. (1994) *Campylobactor*-epidemiological markers. *Dairy food and Environmental Sanitation* 14, 317-324

Philips, C. A. (1995) Incidence, epidemiology and prevention of foodborne *Campylobactor* species. *Trends in Food Science Technology* 6-83-87

Robinson, D. A. (1981) Infective dose of *Campylobactor jejuni* in milk. *British Medical Journal* 282, 1584

Skirrow, M. B. (1990) *Campylobactor. Lancet* 339, 921-923

Stern, N. J. (1992) Reservoirs for *Campylobactor jejuni* and approach for intervention in poultry. In *Campylobactor jejuni : Current Status and Future Trends ed.* Nachamlisn, M. J., Blaster and Tompkins, L. S. pp 49-60Washington DC : American Society for Microbiology

Wnter, D. K. and Slavik, M. F. (1995) Evaluation of a PCR based assay for specific detection of *Campylobactor jejuni* in chicken washes. *Molecular and Cellular probes* 9, 307-310

von Wulffen, H., Harward, C. and Scharcin, E. (1994) Seroreactivity to *Campylobactor jejuni* and gangliosides in patientwith Guillain barré syndrome. *Journal of Diseases* 170, 828-833