

**MULTIPLEX AMPLIFICATION REACTION
METHOD FOR DETERMINATION OF
CAMPYLOBACTER JEJUNI
PENNER/CAPSULE TYPE**

CROSS-REFERENCES TO RELATED
APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/307,632, filed 24 Feb. 2010, which is incorporated by reference, herein.

BACKGROUND OF INVENTION

1. Field of Invention

The inventive subject matter relates to a molecular method for determining *Campylobacter jejuni* capsule/Penner types.

2. Background

Campylobacter is a major cause of human bacterial diarrheal disease worldwide, with *C. jejuni*, and to a lesser extent *C. coli*, the most important pathogenic *Campylobacter* species. *Campylobacteriosis* symptoms range from asymptomatic infection to bloody diarrhea associated with abdominal pain and fever. The major source of human infection is through consumption of uncooked poultry, which is commonly colonized by *C. jejuni*. Post infectious sequelae associated with *C. jejuni* include reactive arthritis, Guillain-Barré syndrome and irritable bowel syndrome.

The molecular pathogenesis of *C. jejuni* is not well understood, but a polysaccharide capsule (CPS) is one of the few recognized virulence determinants of this pathogen. The capsular polysaccharide undergoes a reversible phase variation in expression (Bacon, et al., *Mol. Microbiol.* 40:769-777 (2001)). The capsule contributes to serum resistance of *C. jejuni*, the ability of *C. jejuni* to invade intestinal epithelial cells in vitro, and, in a ferret model, is required for virulence (Bacon, et al., *Mol. Microbiol.* 40:769-777 (2001)). More recently, polysaccharide capsule conjugated to a protein carrier has been shown to protect non-human primates against diarrheal disease Monteiro, et al., *Infect Imm.* 77(3): 1128-36 (2009). Differentiation of *Campylobacter jejuni* strains is typically conducted through the use of Penner serotyping.

The Penner or "heat stable" serotyping scheme is a passive slide hemagglutination assay for both *C. jejuni* and *C. coli* that includes 47 *C. jejuni* serotypes. Rabbit polyclonal antibodies are generated against whole cells of each of the 47 type strains. Antigens are extracted from *C. jejuni* strains to be tested by heating bacterial suspensions in saline at 100° C. These "heat-stable" antigens are used to sensitize sheep erythrocytes, which are used in a passive slide hemagglutination assay with the specific polyclonal antisera. Genetic studies indicate that CPS is the major serodeterminant of the Penner scheme. Thus, mutation of genes required for CPS biogenesis rendered many strains untypable in the Penner scheme.

However, other surface heat stable surface structures such as lipooligosaccharides (LOS) may also contribute to serospecificity of some Penner types. The capsular polysaccharides of *C. jejuni* are known to be structurally diverse (Karlyshev et al., *Molecular Microbiology* 55:90-103). This structural diversity is consistent with the variability observed in the genes encoding the capsule in *C. jejuni*. The capsule locus of *C. jejuni* includes both highly conserved genes involved in capsule synthesis and highly variable loci that encode genes involved in synthesis of specific sugars and specific glycosyl transferases required to link the sugars together. The variable CPS locus located between two con-

served genes, *kpsC* and *kpsF*, and the variable genes can range from 15 to 34 kb (FIG. 1). Variable genes also encode synthesis and transfer of modifications to the sugars, such as methyl phosphormidate (MeOPN) (Karlyshev et al., *Molecular Microbiology* 55:90-103).

Penner serotyping is technically difficult to perform and expensive to produce the type antisera. As a result, only a handful of reference laboratories routinely perform Penner typing. Moreover, many serotypes fall into Penner "complexes". The significance of these complexes is not totally understood in most cases, but they appear to include capsules with related structures (Aspinall et al. *Carbohydr Res.* 231: 13-30 (1992)).

Others have tried to replace the laborious Penner serotyping using a molecular typing approach involving restriction fragment length polymorphism (RFLP) analysis of PCR amplified lipooligosaccharide (LOS) loci (Shi et al. *J Clin Microbiol.* 40(5):1791-7 (2002); Nakari et al., *J Clin Microbiol.* 43(3):1166-70 (2005)). However, these RFLP methods have not been widely used and have not replaced Penner serotyping as the typing method of choice. This may be due in part to the RFLP method requiring amplification of a 9.6 kb fragment. Using PCR to generate such large amplicons is difficult and can place special requirements on the PCR conditions and reagents used, as demonstrated by Nakari et al., who were unable to generate amplified fragments using the amplification conditions described by Shi et al. These RFLP methods are also limited because they are based on the amplification of the LOS locus. At the time of the Shi et al. study, it was known that both the LOS and CPS structure were part of the Heat Stable antigen (HS) recognized through the Penner serotyping method. However, in 2005, CPS was demonstrated to be the major serodeterminant of the Penner method (Karlyshev, et al., *Mol. Micro.* 55: 90-103 (2005)). This helps explain why Shi et al. and Nakari et al. found only partial correlation between the Penner serotypes and RFLP groups. Penner serotyping distinguishes strains that cannot be distinguished by this RFLP method. For example, the most common RFLP type, Hh1Dd1, contained strains belonging to several HS serotypes, including HS 6,7, HS12, HS 27, HS 55, HS 21, HS10, HS 57, HS 6, HS 15, HS 23,36,53, and HS 27+HS 31 (Nakari et al., *J Clin Microbiol.* 43(3):1166-70 (2005)). And some serotypes, such as HS 2, HS 3, HS 4 complex, HS 8, HS10, HS11, HS12, HS15, HS19, HS 31, HS 32, HS 41, HS 57, and HS 23,36,53 include more than one RFLP (Nakari et al., *J Clin Microbiol.* 43(3):1166-70 (2005)).

SUMMARY OF THE INVENTION

The current invention relates to reagents and method to identify *Campylobacter jejuni* Capsule/Penner types via molecular, rather than serological, methods.

Therefore, an object of the invention is a panel of multiplex DNA primers for identification of *C. jejuni* Capsule/Penner types by polymerase chain reaction (PCR).

Several important advantages of amplification reactions over serological determination are evident. First, it is technically difficult to perform and expensive to produce type antisera. As a result, few reference laboratories are capable of routine Penner typing. Additionally, many serotypes fall into Penner "complexes."

Amplification methods, unlike typing sera methods, are relatively available to research and reference laboratories. Furthermore, no expression of capsule is needed. Therefore, there are no affects due to phase variation in capsule expression, as is possible with serotyping. Multiplexing reduces the number of reactions to be performed per samples. Addition-