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Cloning, Sequencing, and Expression of a Gene from *Campylobacter jejuni* Encoding a Protein (Omp18) with Similarity to Peptidoglycan-Associated Lipoproteins

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A *Campylobacter jejuni* genomic plasmid library was screened with antiserum generated against whole *C. jejuni*, revealing two immunoreactive clones. Sequence analysis of the recombinant plasmids revealed a common open reading frame of 498 nucleotides encoding a protein of 165 amino acids with a calculated molecular mass of 18,018 Da. The recombinant product partitioned to the outer membrane fractions of *Escherichia coli* transformants and has been designated Omp18. The deduced amino acid sequence of the cloned *C. jejuni* gene exhibits considerable similarity to peptidoglycan-associated lipoproteins from other gram-negative bacteria.

Campylobacter jejuni, a gram-negative bacterium, is a common cause of gastrointestinal illness in humans (2). Experimental evidence suggests that protective immunity against *C. jejuni* is developed with recurrent exposure (1). Adults in developing countries and chronic drinkers of raw milk in the United States exhibit an age-related decrease in attack rate for diarrheal illness caused by *C. jejuni*. Accordingly, there is interest in identifying *C. jejuni* proteins which may be useful in the development of diagnostic assays or vaccine formulations (21). A nontoxic, effective vaccine would be desirable in developing countries where *C. jejuni* infections are hyperendemic. Because of the antigenic heterogeneity of heat-labile antigens, a multisubunit vaccine would likely be most effective in protecting individuals from infection with *C. jejuni.*

A genomic plasmid library of *C. jejuni* M275 was constructed with chromosomal DNA that was partially digested with *Sau*3AI and ligated into *Bam*HI-digested pBluescriptII SK+ (pBSKII+; Stratagene, La Jolla, Calif.). *Escherichia coli* XL1-Blue (Stratagene) was transformed with the plasmid library, and the resultant transformants were screened with an anti-*C. jejuni* serum (11, 23). Screening resulted in the identification of two recombinant *E. coli* clones that expressed products that were specifically reactive with the antiserum. Restriction endonuclease mapping of the two recombinant plasmids, designated pMEK10 and pMEK14, revealed that they contained overlapping inserts of 3.6 and 4.7 kb, respectively (Fig. 1). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12) and immunoblot (26) analyses of whole-cell extracts of *E. coli* transformants containing pMEK10 or pMEK14, but not pBSKII+, revealed the appearance of an immunoreactive protein with an apparent molecular mass of 18 kDa when probed with anti-*C. jejuni* serum (data not shown).

Outer membrane protein preparations of *E. coli* transformants were analyzed by SDS-PAGE and immunoblotting. The 18-kDa immunoreactive band was observed in the outer membrane protein extracts of *E. coli* transformants harboring either the pMEK10 or pMEK14 recombinant plasmid but not in outer membrane protein extracts of transformants harboring the parental pBSKII+ plasmid (Fig. 2). This finding suggested that the 18-kDa product was located in the outer membrane of *E. coli*. Accordingly, the protein was designated Omp18 on the basis of the assumption that it is similarly located in *C. jejuni.*

To more precisely locate the gene encoding Omp18 and facilitate nucleotide sequence analysis, pMEK10 was digested with *Pst*I and *Sac*I and the resultant 2.3-kb fragment was ligated into pBSKII+ to yield plasmid pMEK10-25 (Fig. 1). *E. coli* transformed with pMEK10-25 was found to produce Omp18 as judged by immunoblotting (data not shown). Sequence analysis of the pMEK10-25 plasmid subclone revealed a single open reading frame approximately 400 bases from the T7 primer sequence and in an orientation opposite that of the *lac* promoter, indicating that the expression of the gene encoding Omp18 was driven by a functional *C. jejuni* promoter. The open reading frame is 498 nucleotides in length and is capable of encoding a protein of 165 amino acids with a calculated molecular mass of 18,018 Da (Fig. 3). The open reading frame begins with an ATG codon at nucleotides 129 to 131 and is terminated by an ochre (TAA) codon at nucleotides 624 to 626. Five bases upstream of the proposed methionine initiation codon is a potential ribosomal binding site (AGGT). Putative promoter elements, TTGGTT-18 bp-TATAAT, are found approximately 30 bases upstream of the methionine start codon. The G+C content of the cloned gene (33.5 mol%) compares favorably with the known base composition of *C. jejuni* genomic DNA (20). Southern hybridization analysis (15) with nick-translated pMEK10-25 and *C. jejuni* M275 chromosomal DNA digested with *Hae*II, *Hin*dII, and *Sau*3AI indicated that there is a single copy of the gene encoding Omp18 in the *C. jejuni* genome.

Examination of the amino terminus of the deduced amino acid sequence of Omp18 revealed a potential signal peptide 19 residues in length (22) (Fig. 3). The potential signal peptide is composed of two positively charged (Lys) residues at the second and third positions followed by a core of predominately hydrophobic amino acids. Inspection of the carboxyl terminus of the putative signal peptide revealed the sequence Ile-Ser-

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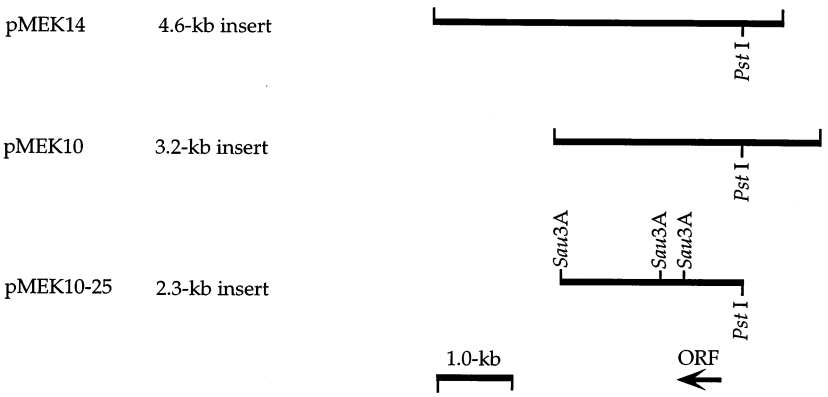

FIG. 1. Physical maps of pMEK14, pMEK10, and pMEK10-25. All three recombinant plasmids contained the gene for the 18-kDa recombinant product as judged by gel electrophoresis coupled with immunoblotting with anti-*C. jejuni* serum. The relative position of the open reading frame (ORF) is indicated by the arrow.

Gly-Cys, which is similar to the consensus tetrapeptide sequence Leu-X-Y-Cys exhibited by many bacterial lipoprotein precursor molecules (27). The X and Y residues are generally small amino acids such as alanine, glycine, and serine. This tetrapeptide sequence is believed be a substrate for diacylglyceryl transferase, which in turn confers sensitivity to subsequent proteolytic cleavage of the Gly-diacylglyceryl-Cys bond by signal peptidase II (24, 27). Signal peptidase II cleavage of this signal peptide in Omp18 would result in a mature protein of 147 amino acids with a calculated molecular mass of 16,140 Da. The apparent larger size of the recombinant product in *E. coli* (18 kDa) as determined by electrophoresis might, therefore, be explained by posttranslational modification, such as attachment of a fatty acid to an amino-terminal diacylglyceryl-Cys residue.

The deduced amino acid sequence of Omp18 exhibited similarity to certain bacterial outer membrane lipoproteins. The greatest degree of similarity was found with peptidoglycan-associated lipoproteins (PALs), including the PAL of *E. coli* (4, 13), the outer membrane protein P6 of *Haemophilus influenzae* (5, 18), PplA of *Legionella pneumophila* (6, 14), and Omp16 of *Brucella abortus* (25) (Fig. 4). Analysis of Omp18 and the aforementioned proteins demonstrated amino acid similarities ranging from 49 to 56% and identities ranging from 31 to 33%. On the basis of these findings and the presence of a consensus signal peptidase II site, we propose that Omp18 is a homolog or analog of PAL and have designated the cloned *C. jejuni* gene *pal.*

Lazzaroni and Portalier (13) have reported that the carboxyl terminus of *E. coli* PAL is responsible for the noncovalent association with the peptidoglycan, as judged by compartmentalization analysis of PAL-PhoA fusion proteins. Koebnik (10) has recently identified a peptidoglycan-associated alpha-helical consensus motif in the carboxyl termini of a number of bacterial cell surface proteins. The $NX_2LSX_2RAX_2VX_3L$ motif, where X represents a variable amino acid, is proposed to be directly involved in the noncovalent association of a protein with peptidoglycan. The sequence NQALGLKRAKAVKEAL is found at the carboxyl terminus of the Omp18 sequence between residues 108 to 123 and matches the reported consensus sequence with the exception of one amino acid, a Ser residue at the fifth position of the 16-amino-acid sequence. Interestingly, of the 26 proteins in which the alpha-helical consensus motif has been identified, a Gly residue is found to be substituted for the Ser residue in the fifth position in 5 of the proteins. These five proteins represent the PALs of *E. coli* and *L. pneumophila*, P6 (or PAL) of *H. influenzae* and *Haemophilus parainfluenzae*, and Omp16 (or PAL) of *B. abortus*. This observation further supports the proposed relationship between Omp18 and the previously identified PALs.

PALs have been demonstrated to be highly immunogenic and conserved among different strains of various gram-negative bacteria (5–7, 9, 16–19). Several studies have indicated that PALs might be useful as immunogens to protect humans and animals from disease caused by gram-negative pathogens. For example, antibody in pooled human sera against a 16-kDa *H. influenzae* PAL (P6) is bactericidal (7, 17), and rabbit anti-P6 antibodies passively protect infant rats from *H. influenzae* type b-induced meningitis (16). It has also been proposed that PALs may be useful as diagnostic antigens (9, 19, 25). Kasten et al. (9) found that a P6-like (PAL) protein is expressed by all 16 somatic serotypes of *Pasteurella multocida*, and Nelson et al. (18, 19) reported that all strains of *H. influenzae*, both typeable and nontypeable, expressed the P6 protein. Examination of the distribution of the *C. jejuni* PAL-like protein, Omp18, among various isolates and strains will require the production of monospecific anti-Omp18 antibodies.

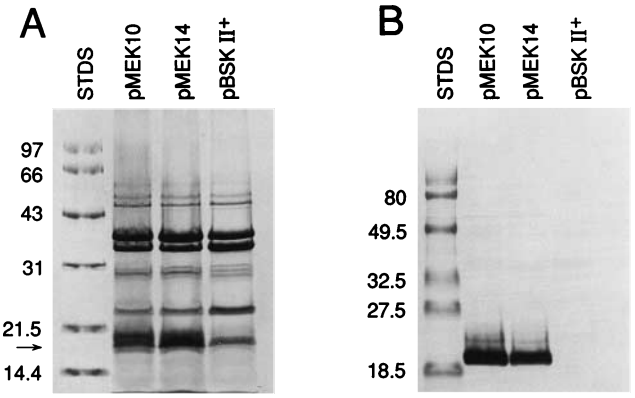

FIG. 2. Electrophoretic and immunoblot analysis of outer membrane proteins from *E. coli* transformants harboring the pMEK10, pMEK14, or pBSKII+ plasmid. Bacterial outer membrane protein extracts (Sarkosyl-insoluble fractions; 25 μg per lane) were separated in SDS–12.5% polyacrylamide gels and either stained with Coomassie brilliant blue R-250 (A) or transferred to polyvinylidene fluoride membranes and reacted with a 1:250 dilution of *C. jejuni* antiserum (B). The 18-kDa protein expressed by *E. coli* transformants harboring the pMEK10 or pMEK14 recombinant plasmid is indicated by an arrow. Molecular mass standards (STDS; in kilodaltons) are on the left.

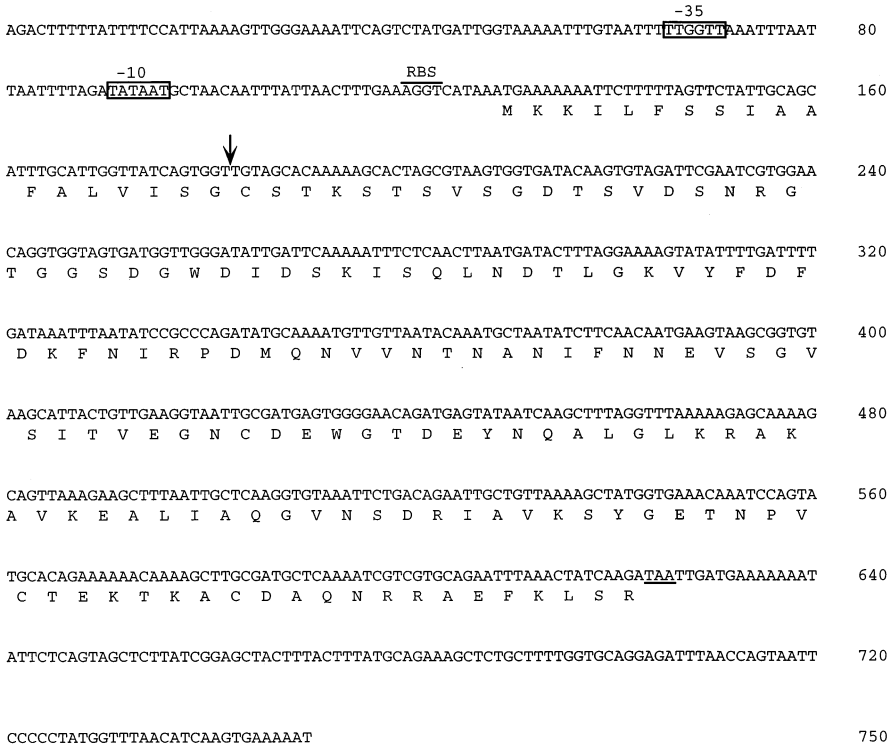

FIG. 3. Nucleotide sequence of *C. jejuni pal* and deduced amino acid sequence of Omp18. The proposed ribosomal binding site (RBS) is overlined. Potential −10 and −35 promoter elements are boxed. The ochre codon is underlined. The proposed signal peptidase II cleavage site is indicated by an arrow.

A number of antigenic lipoproteins are present in the cell envelope of *E. coli* (8). The most abundant membrane protein is Braun's (murein) lipoprotein, which is covalently linked to peptidoglycan and is presumed to be involved in maintenance of the cell envelope by bridging the outer membrane and peptidoglycan (3). PAL is less abundant and has the unusual property of being found in close association with but not covalently linked to the peptidoglycan. Functionally, PAL may serve as a bridge between the peptidoglycan and the outer membrane and, like Braun's lipoprotein, may participate in maintaining the integrity of the bacterial cell. *E. coli* strains possessing mutations that reduce the expression of PAL exhibit increased sensitivity to deoxycholate and EDTA and leak periplasmic constituents (13). These findings suggest that PAL function may be important to survival. Accordingly, it may not be possible to produce viable, isogenic strains of *C. jejuni* lacking a functional *pal* gene for studies of virulence and pathogenicity. Future studies will be designed to determine the immunogenic role of Omp18 in the resolution of *C. jejuni*-mediated enteritis by using monospecific antisera and purified Omp18.

Nucleotide sequence accession number. The nucleotide sequence of *pal* from *C. jejuni* has been deposited in GenBank and given the accession number U47617.

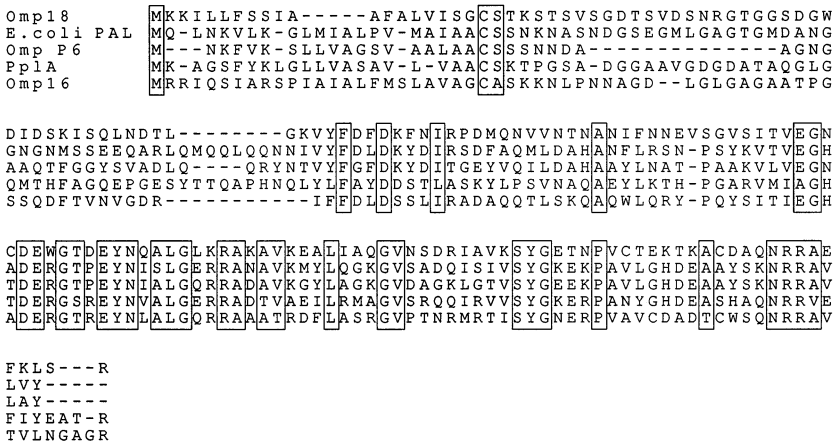

FIG. 4. Amino acid alignment of Omp18 from *C. jejuni*, PAL from *E. coli*, outer membrane protein P6 (PAL) from *H. influenzae*, PplA from *L. pneumophila*, and Omp16 from *B. abortus*. The references for the sequences are given in the text. Gaps, indicated by dashes, were introduced to obtain maximal alignment. Boxes identify amino acid residues conserved between Omp18 and at least three of the other four proteins.

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