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The *ompH* Gene of *Yersinia enterocolitica*: Cloning, Sequencing, Expression, and Comparison with Known Enterobacterial *ompH* Sequences

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We have recently described a previously uncharacterized outer membrane protein of *Salmonella typhimurium* and *Escherichia coli* and cloned and sequenced the corresponding gene, the *ompH* gene, of *S. typhimurium* (P. Koski, M. Rhen, J. Kantele, and M. Vaara, J. Biol. Chem. 264:18973–18980, 1989). We report here the cloning, sequencing, and expression of the corresponding gene of *Yersinia enterocolitica*. It is significantly homologous to the *ompH* genes of *E. coli* and *S. typhimurium* (homology percentages, 65 and 64%, respectively), has a promoter region strongly homologous to the *E. coli* 17-bp class consensus promoter, and encodes a protein consisting of 165 amino acids (22 of which form the signal sequence). The plasmid-borne *Y. enterocolitica ompH* was found to be expressed both in the *E. coli* host and in minicells. The isolated outer membrane of *Y. enterocolitica* was shown to contain OmpH. The homology of the *Y. enterocolitica* OmpH protein is 66% with *E. coli* OmpH and 64% with *S. typhimurium* OmpH. All OmpH proteins have almost identical hydrophobic profiles, charge distributions, and predicted secondary structures. Because yersiniae are considered rather distant relatives of *E. coli* and *S. typhimurium* in the *Enterobacteriaceae* family, these results might indicate that most or all strains of the family *Enterobacteriaceae* have OmpH proteins remarkably homologous to those now sequenced.

We have recently reported that the isolated outer membranes (OMs) of *Escherichia coli* and *Salmonella typhimurium* contain a cationic (pI above 10.0) 16-kDa protein (24). Furthermore, we have cloned and sequenced the gene encoding this protein in *S. typhimurium* and named it the *ompH* gene (23, 24). The mature OmpH protein of *S. typhimurium* consists of 141 amino acids, possesses regions rich in basic amino acids, and has a molecular mass of 15,862 Da. In addition, the OmpH preprotein contains a cleavable, 20-amino-acid signal sequence. The structural *ompH* gene is preceded by a putative promoter region which has strong homology to the enterobacterial 17-bp class promoter consensus sequence. In amino acid composition, the OmpH protein closely resembles the 15-kDa lipopolysaccharide (LPS)-binding outer membrane protein, preliminarily characterized by Geyer et al. (12) in 1979. Furthermore, we have shown (16) that the histonelike protein HLP-I of *E. coli* is the homolog of OmpH of *S. typhimurium* (91% identical amino acid residues). This was a surprising finding because HLP-I is believed to be a cytoplasmic protein, a constituent of bacterial chromatin (18, 19). The presence of signal sequences in the OmpH and HLP-I preproteins and the fact that those sequences are not present in isolated or minicell-made mature OmpH and HLP-I give further credence to our concept that those proteins are secreted to the OM.

The function of *S. typhimurium* and *E. coli* OmpH in the OM has not yet been elucidated. Is it a crucially needed component? Does it occur commonly in strains of the family *Enterobacteriaceae*, or is its presence more limited? If OmpH is a ubiquitous enterobacterial OM protein, how conserved is its structure? Does OmpH have conserved regions and variable regions as do two other OM proteins of enteric bacteria, OmpA (6) and PhoE (38)? To partially answer these questions, we started to search for the *ompH* gene of *Yersinia enterocolitica*. We chose *Y. enterocolitica* because it is believed to be phylogenetically a rather distant relative of *E. coli* and *S. typhimurium*, to which other genera such as *Klebsiella*, *Enterobacter*, and *Citrobacter* are more closely related (2, 7, 8, 20, 31). In this paper, we show that *Y. enterocolitica* has the OmpH protein and that this protein is remarkably homologous to the OmpH proteins of *E. coli* and *S. typhimurium*. This could indicate that most or all *Enterobacteriaceae* strains have OmpH proteins remarkably homologous to those now sequenced.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. *Y. enterocolitica* EH902 was used (43); it is a rough, plasmidless derivative of a clinical *Y. enterocolitica* 03 isolate, EH822 (42). The cloning vector was pUC19 (Pharmacia, Uppsala, Sweden), and its host was *E. coli* JM109 (30). Bacteria were grown to exponential growth phase (unless stated otherwise) in LB broth, and 100 μg of ampicillin per ml was added when necessary (30).

The *ompH* probe. The *ompH*-specific gene probe (*ompH* probe) was derived from the 537-bp *Pst*I-*Eco*RV fragment of *S. typhimurium ompH*. This fragment represents 96% of the total *ompH* structural gene but also has a 70-bp 3′-flanking sequence. To prepare this probe, pUCHS14 (24) was digested with *Bam*HI and *Eco*RV, and the smaller (0.55 kb) of the two fragments was isolated by 0.8% agarose electrophoresis. It was radiolabeled by nick translation (29) by using the Nick Translation kit and [α-^{35}S]dATP (specific activity, > 1,000 Ci/mmol), both from Amersham. The resulting probe contained approximately 10^8 cpm/μg of DNA and included, besides the sequences described above, a 13-bp pUC19-derived sequence.

DNA manipulations. The general DNA techniques (restric-

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tion digests, agarose electrophoresis, plasmid isolations, and transformations) were carried out as described by Maniatis et al. (28). The chromosomal DNA was prepared as described previously (24). In Southern hybridization (16, 24), the hybridization was done at 37°C overnight in 5× SSC (1× SSC is 0.15 NaCl plus 0.015 M sodium citrate) containing 0.5% sodium dodecyl sulfate (SDS), 5× Denhardt's solution, and 20% formamide. The hybridized filter was washed at 37°C in 6× SSC–0.5% SDS for 1 h and in 2× SSC–0.5% SDS for 30 min. Autoradiography was carried out at room temperature for 1 to 5 days. Appropriate restriction fragments were cut off from the agarose gel. The gel slice was cut into small pieces which were covered with phenol and kept at −70°C for 10 min. Phenolic and aqueous phases were separated in an Eppendorf minicentrifuge (at room temperature). The supernatant was washed twice with phenol and once with chloroform, and the DNA was precipitated with ethanol.

DNA sequencing. DNA sequencing was done directly from denatured plasmid (15) by the method of Sanger et al. (36) by using Sequenase (United States Biochemical, Cleveland, Ohio). The primers were pUC-M13 forward and reverse primers (Promega) and four 17-mer oligonucleotide primers synthesized on the basis of the determined sequence. These oligonucleotides were 5'>AATCAGCCAGTCGAATT<3', 5'>TTTCACTATAAACTCCT<3', 5'>CTCATATGCTAA ACCGG<3', and 5'>TTCAAGGTGTCTCTGAC<3'.

Minicell isolation and labeling. PK251 (27) minicells were isolated by the method of Dougan and Kehoe (10), and proteins were labeled with [^{35}S]methionine (Amersham; specific activity, 1 Ci/mol) under conditions described earlier (23). Before SDS-polyacrylamide gel electrophoresis (PAGE), samples were boiled for 10 min in the sample buffer containing 3.5% SDS and 10% mercaptoethanol.

SDS-PAGE. SDS-PAGE was carried out in 15% polyacrylamide by the method of Laemmli (26). Gels were stained with Coomassie blue and soaked in Amplify solution (Amersham) for 30 min. Dried gels were autoradiographed for 3 days at room temperature.

Isolation of the outer membrane. Cells were grown overnight at 37 (*E. coli* strains) or 30 (the *Y. enterocolitica* strain) °C in 100 ml of LB broth, washed with 10 mM HEPES buffer (*N*-2-hy 'roxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4), resuspended in 5 ml of the same buffer, and sonicated in an ice water bath 10 times for 30 s each time under the microtip of a Soniprep 150 sonicator (Measuring and Scientific Equipments, Crawley, Sussex, United Kingdom) by using the amplitude of 12 μm. Unbroken cells were removed by centrifugation at 1,500 × *g*, and the sonicate was treated with 1% (vol/vol) Triton X-100 for 20 min at 37°C. The outer membrane material was then pelleted by centrifugation at 30,000 × *g*.

Acid urea-PAGE. Before acid urea-PAGE, the outer membrane material was processed as previously described by us (24). Briefly, to the membrane suspension, Triton X-100 and $CaCl_2$ were added to final concentrations of 1% and 100 mM, respectively. The suspension was incubated for 10 min at 37°C and centrifuged at 8,000 × *g*. The supernatant was mixed 1:1 with the sample buffer (5% acetic acid, 15% glycerol, 0.1% methylene blue). Acid urea-PAGE was carried out as described earlier (24) by the method of Panyim and Chalkley (34). The separating gel was 15% polyacrylamide containing 2.5 M urea and 5% acetic acid. Preelectrophoresis and electrophoresis were carried out as described earlier (24).

Computer analyses. Analyses of the nucleotide sequence data and amino acid sequence data were performed by using the HIBIO DNASIS and PROSIS computer program packages (Pharmacia, Bromma, Sweden). The hydrophobicity profile was calculated by the method of Kyte and Doolittle (25) with the window of 6 amino acids. The secondary structure was predicted according to Chou and Fasman (9) and Rose (35) with the PROSIS program.

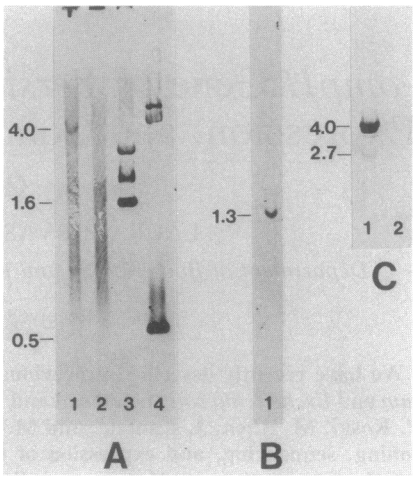

FIG. 1. Southern blot hybridizations. (A) Lanes 1 and 2, Hybridization of the *S. typhimurium*-derived *ompH* probe with the restriction enzyme-digested chromosomal DNA of *Y. enterocolitica*; lane 1, *Bam*HI-*Hin*dIII digest; lane 2, *Bam*HI-*Kpn*I digest; lanes 3 and 4, control hybridizations of the *ompH* probe with the *Pst*I (lane 3)- or *Bam*HI-*Eco*RV (lane 4)-digested pUCHS14 DNA (pUCHS14 contains the *ompH* gene of *S. typhimurium*). (B) *Pst*I digest of *E. coli* JM109 chromosomal DNA probed with the *ompH* probe. (C) Southern blots of two pUC19 recombinants that contain the approximately 4-kb *Hin*dIII-*Bam*HI *Y. enterocolitica* fragment. The plasmids were digested with *Hin*dIII-*Bam*HI before electrophoresis. The *Hin*dIII-*Bam*HI fragment of pUCHS115 strongly hybridized with the *ompH* probe (lane 1), whereas other recombinants did not hybridize (lane 2). Numbers on the left sides of the gels are molecular sizes in kilobases.

RESULTS

The search for the *ompH* gene of *Y. enterocolitica*. The *ompH* genes of *S. typhimurium* and *E. coli* share a high degree of homology (16). Therefore, we thought it might be possible to use the *ompH* gene of *S. typhimurium* as a hybridization probe in the search for *ompH*-related sequences in the *Y. enterocolitica* chromosome. This *S. typhimurium ompH* probe hybridized with a 4-kb *Hin*dIII-*Bam*HI fragment of *Y. enterocolitica* DNA (Fig. 1). The hybridization was, however, relatively weak under the conditions employed, compared with the strong hybridization with the homologous *S. typhimurium ompH* (Fig. 1, lanes 3 and 4) or compared with a fragment which contains 340 3' terminal bp of the *E. coli ompH* (Fig. 1B, lane 1).

The 4-kb *Hin*dIII-*Bam*HI DNA fragment of *Y. enterocolitica* (Fig. 1) was isolated, ligated to a pUC19 cloning vector, and used to transform competent *E. coli* JM109 cells (see Materials and Methods). Recombinant plasmids from 34 transformants were probed with the *ompH* probe in South-

Nucleotide sequence accession number. The GenBank accession number for the *ompH* gene of *Y. enterocolitica* is 1900 34854.

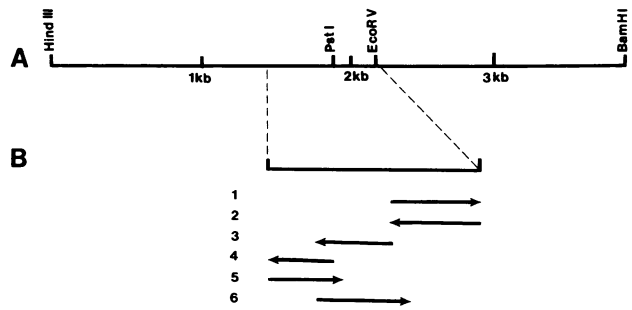

FIG. 2. Partial restriction endonuclease map of the 4-kb *Hin*dIII-*Bam*HI fragment of pUCHS115 (A) and the sequencing strategy (B). Sequencing was done directly from denatured plasmids. Primers were pUC-M13 forward (arrow 3) and reverse (arrow 1) primers and four synthetic oligonucleotides (arrows 2, 4, 5, 6).

ern blotting. One positive plasmid was identified (Fig. 1), named pUCHS115, and characterized further.

The nucleotide sequence and its comparison to the known *ompH* sequences. The restriction enzyme *Pst*I cuts the hybridizing 4-kb fragment into two pieces that both hybridize with the *ompH* probe (*Bam*HI-*Pst*I and *Pst*I-*Hin*dIII). For sequencing, these were cloned separately to pUC19. Nucleotide sequences were determined directly from plasmids by using M13-pUC19 forward and reverse primers. Also, four synthetic 17-mer oligonucleotide primers were made on the basis of the determined sequence (see Materials and Methods) (Fig. 2). A long open reading frame was found. It contains 495 bases from the initiation codon (GTG) to the stop codon TAA (Fig. 3). This corresponds to a protein with 165 amino acids. The open reading frame is preceded by a Shine-Dalgarno sequence (TAAGGAGTT) 5 nucleotides from the putative initiation codon. A putative promoter region was also found. The sequences GTGATA (nucleotides 120 to 125) and TTTAAC (nucleotides 143 to 148) have homology (4 of 6 bases each) to the canonical −35 region (TTGACA) and −10 region (TATAAT) of *E. coli* promoters (32). The spacing between these two regions (−35 and −10) is 17 nucleotides. The distance of the promoter region from the initiation codon is 177 nucleotides. The sequenced fragment is notably homologous with the *ompH* genes of *E. coli* and *S. typhimurium* (Fig. 3). The homology of the open reading frame with the structural *ompH* gene of *E. coli* is 65%, and with that of *S. typhimurium* it is 64%. The

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STMOMPH.DNA   G A  G        CG TC AG    C  C   A G  C  G   A C   T
ECOOMPH.DNA  ATA   G  C  T   G TC A        C   A G    G   A C   T
YECOMPH.DNA  TATTCCTGATTACAGTAAAGCCAGCAATATTCGTGTCTCTGCCGGTGTGGCACTACAATG      (60)

STMOMPH.DNA        -          G  G  C   T C  C  CC G   T     A G     T
ECOOMPH.DNA        C          G  G      T C  C  CC G   T  C  A G     T
YECOMPH.DNA  GATGTCTCCATTGGGGCCTTTAGTGTTCGCATATGCTAAACCGGTTAAAGATTACGAAGG      (120)

STMOMPH.DNA  A  C  AG C     G                        C        ------------
ECOOMPH.DNA  A  C   G    A  G                    C   C      G ------------
YECOMPH.DNA  TGATAAGTCAGAGCAATTCCAGTTTAACATTGGTAAAACTTGGTAATTGATTGGCAAAAG      (180)

STMOMPH.DNA  --------------------GTT     CA AGGA TGC TTGG  GT      A   --C
ECOOMPH.DNA  --------------------GTT T CACA AGGA TG  GTGG  GT      A   --C
YECOMPH.DNA  TAGTTGCAAATTTTAAAGACAGCACTGGTAGTTGAAATA-ACCTAAGGTAGCGCTGATGA      (239)

STMOMPH.DNA  T       A GCCCCCA GGA CGC   ---GCCAC   AAGAGCT  AC  TC GGTGC
ECOOMPH.DNA  T  A    A -CAA  TAAGA CGC G ---GCCAC   AAGA CT CAC CTCCGGTGC
YECOMPH.DNA  GTTTGGCGCTATTTTAGGCATTATTCAGTATTAAGTGCACTTCAAGGTGTCTCTGACACA      (299)

STMOMPH.DNA    - T GG               T              A TA  T   G
ECOOMPH.DNA    - T GG               T              A TA  T   G    C
YECOMPH.DNA  AACAGGTAATGGTAAGGAGTTTATAGTGAAAAAGTGGTTGTGTGCCGCAAGTCTTGGTTT      (359)

STMOMPH.DNA  G  GA   T A G  ------  CA  G  T  T          A  C  C   A GGG
ECOOMPH.DNA     C  CT A     ------T C   G  G  T          A  C  C   A GGG
YECOMPH.DNA  AGCATTGGCAGCTTCTGCCAGCGTTCAAGCCGCAGACAAAATTGCTATTGTTAACGTTTC      (419)

STMOMPH.DNA  T ATC G         GG TG GCA AAGACGGGT   T C  TACAC    A  C
ECOOMPH.DNA     C G     G  GG  G GCA AAAACCGGT  TT    CAC C    A     G
YECOMPH.DNA  CAGCATTTTCCAACAATTACCTGCGCGTGAAACCGTAGCTAAACAGTTGGAGAATGAATT      (479)

STMOMPH.DNA  T        T  GG T         AA      AAC          AT          CG
ECOOMPH.DNA           T  C G         GC T     AAC           G        A A
YECOMPH.DNA  CAAAGGCCGAGCAACCGAACTGCAAGGAATGGAGCGCGATCTGCAGACTAAAATGCAGAA      (539)

STMOMPH.DNA  T      ---------  C TG AAGC  GT  C        T  G        A
ECOOMPH.DNA  G     G---------  C TG AAGCG GC  C     C  T  G        A
YECOMPH.DNA  ACTGCAACGTGACGGTTCTACCATGAAAGCCAGTGATCGTACCAAACTGGAAAATGACGT      (599)

STMOMPH.DNA  G   TCT     CC G      G ACAA     G            A     CGCGCT
ECOOMPH.DNA  G   GCT     CC G     TG  CAG     G              G   CGCGCA
YECOMPH.DNA  AATGAAACAGCGTGAAACTTTCTCTACTAAAGCCCAGGCTTTTGAGCAAGATAATCGCCG      (659)

STMOMPH.DNA      TCC AC     A  C  C   C GG   CT     C   AC   G  G   AAA
ECOOMPH.DNA      TCC AC     A  CGGC   C GG T CT     C   AC        G    C
YECOMPH.DNA  TCGTCAGATGGAAGAGCGTAATAAAATCCTGAGCCGTATTCAGGATGCTGTTAAATCTGT      (719)

STMOMPH.DNA  G    A G CCAGA  ATC  TC     G A  C     CA C     T  CAACAGCAG
ECOOMPH.DNA  C    A  GCCAG A ATC  TC     TG           C  C     T  CAACAGCAG
YECOMPH.DNA  TGCTAGCAAAGGCGGTTATGACGTGGTGATTGATGCAAATGCTGTTGCATATGCAGATCC      (779)

STMOMPH.DNA  CGA GTG     C     C        A                  GTAATG
ECOOMPH.DNA  CGA GAG     C     TC       A                  GTAATG
YECOMPH.DNA  TTCT---AAAGATATCACTGCTGACGTGCTGAAACAGGTTAAATAAAAGTAC          (831)
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FIG. 3. *OmpH* nucleotide sequences of *S. typhimurium* (23, 24) (STMOMPH.DNA), *E. coli* (18, 16a, 36a) (ECOOMPH.DNA), and *Y. enterocolitica* (this study) (YECOMPH.DNA) The −35 and −10 putative promoter regions, the Shine-Dalgarno sequence, and the initiation codon (GTG) are underlined. The termination codon is shown by asterisks. Numbers on the right enumerate the nucleotide bases.

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STMOMPH  .....L..G....MVT..--..........MGNL...AAQKTG.SNT.........A....
ECOOMPH  .....L..G......T..--..........MG.L...VAQKTG.SNT.........S....
YECOMPH  MKKWLCAASLGLALAASASVQAADKIAIVNVSSIFQQLPARETVAKQLENEFKGRATELQ

STMOMPH  K..T...S...R...---.MKAG.......K...S..Q..AQ......K.RA..SN.....
ECOOMPH  R..T...A..K...---.MKAG.......K...A..Q..AQ........RA..SN...G.
YECOMPH  GMERDLQTKMQKLQRDGSTMKASDRTKLENDVMKQRETFSTKAQAFEQDNRRRQMEERNK

STMOMPH  LVT...T...K..NDQSI.L.V...T...NSSDV............
ECOOMPH  LVT...T......NSQDI.L.V.......NSSDV............
YECOMPH  ILSRIQDAVKSVASKGGYDVVIDANAVAYADPS-KDITADVLKQVK
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FIG. 4. Comparison of the OmpH proteins of *S. typhimurium* (STMOMPH), *E. coli* (ECOOMPH), and *Y. enterocolitica* (YECOMPH). The solid rectangles indicate amino acids identical to those of *Y. enterocolitica*. The deletions are marked with −.

Shine-Dalgarno sequences of the *ompH* genes of the three bacteria are identical. Clear homology also exists in the promoter regions (nucleotides 120 to 167; homology with *E. coli* sequence, 83%) and in the 119-bp region immediately upstream of the −35 region of the promoter (homology with *E. coli*, 74%). On the other hand, while the region between the promoter and the Shine-Dalgarno sequence is very homologous in *E. coli* and *S. typhimurium*, the corresponding region of the *Y. enterocolitica* gene is remarkably different and is 36 bp longer than that of *S. typhimurium*.

The deduced primary structure of the OmpH protein of *Y. enterocolitica*. The deduced amino acid sequence of the OmpH protein of *Y. enterocolitica* is shown in Fig. 4. Figure 4 also shows the amino acid sequence alignment of the OmpH proteins of *S. typhimurium* and *E. coli*. The mature OmpH protein of *Y. enterocolitica* contains 143 amino acids and has the calculated molecular mass of 16,086 Da. Its precursor has a 22-amino-acid signal sequence and a calculated molecular mass of 18,179 Da. The homology between *Y. enterocolitica* and *E. coli* is 66%, and between *Y. enterocolitica* and *S. typhimurium* it is 64%. The signal sequence of *Y. enterocolitica* is two amino acids longer than those in the two other bacteria. *Y. enterocolitica* also has three additional amino acids (residues 75 to 77) and one amino acid deletion (residue 154). Homology is found throughout the protein; no part is notably more homologous than the others. Of the amino acid residues, 63% are identical in all three of these OmpH proteins. Most of the amino acid substitutions are conservative; we observed 100 direct matches, 37 conservative substitutions, and only 19 mismatches between *E. coli* and *Y. enterocolitica* OmpHs.

Figure 5 shows the hydrophobicity profiles of the OmpH proteins. They are very similar. Both the NH_2- and the COOH-terminal ends are hydrophobic, whereas the middles of all three proteins are notably hydrophilic. Furthermore, all three proteins have almost identical charge distributions and predicted secondary structures (data not shown).

Expression of the *ompH* gene of *Y. enterocolitica*. The expression of the *ompH* gene of *Y. enterocolitica* in the recombinant plasmid pUCHS115 was studied in metabolically labeled minicells of *E. coli* PK251 (Fig. 6). The plasmid encoded two proteins besides β-lactamase (30 kDa; precursor, 32 kDa) and a 19-kDa protein. Their apparent molecular sizes, 16 and 18 kDa, corresponded well to the sizes expected for the mature OmpH protein and its precursor, respectively. Furthermore, the 18-kDa protein predominanted when the labeling was performed in the presence of 9% ethanol (which inhibits the processing of signal sequences), while the 16-kDa protein prevailed in the absence of ethanol. We have previously demonstrated this effect for the OmpH protein of *S. typhimurium* (23).

The presence of *Y. enterocolitica* OmpH in the OM. We have previously described a method by which one can relatively easily analyze isolated OMs for the presence of

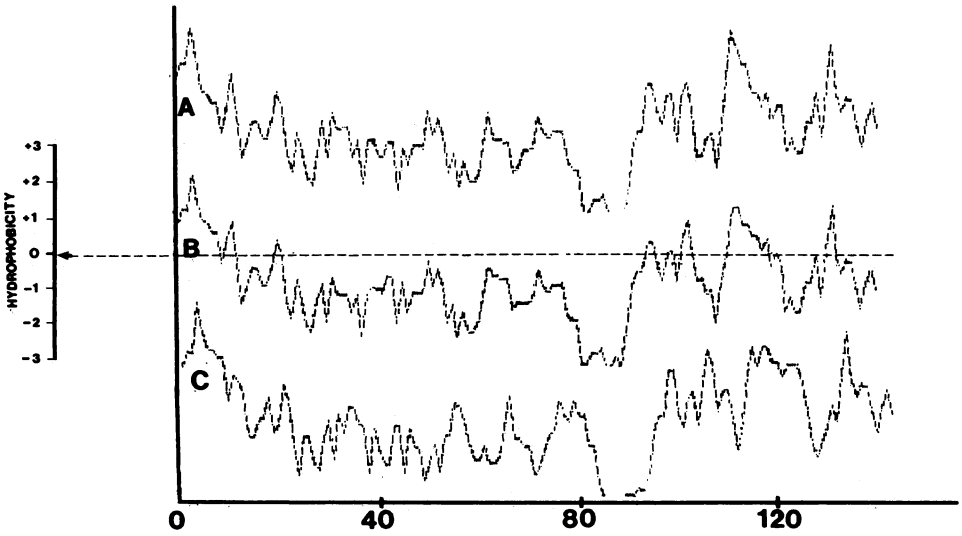

FIG. 5. Hydrophobicity profiles of the mature OmpH proteins of *S. typhimurium* (A), *E. coli* (B), and *Y. enterocolitica* (C).

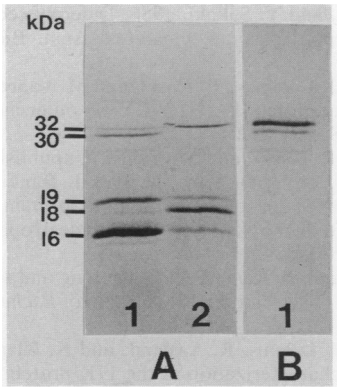

FIG. 6. Autoradiography of proteins synthesized in *E. coli* minicells. (A) Minicells harboring the plasmid pUCHS115 and labeled in the absence of ethanol (lane 1) or in the presence of 9% ethanol (lane 2). (B) Minicells harboring the control plasmid pUC19 and labeled in the presence of ethanol. Apparent molecular masses (in kilodaltons) are indicated.

OmpH (24). This method employs extraction of OmpH with 0.1 M $CaCl_2$–1% Triton X-100 and analysis of the extract by acid urea-PAGE; it enabled us to detect OmpH in all strains of *Salmonella* spp. and *E. coli* we studied and formed the basis for isolation and purification of OmpH (24). With this method, a protein probably corresponding to OmpH can be found in the OM of *Y. enterocolitica* (Fig. 7, lane 1). That protein was very abundant in the membranes of the *E. coli* strain which harbors the *Y. enterocolitica ompH* in the multicopy plasmid pUCHS115 (Fig. 7, lane 3).

DISCUSSION

In this paper we have shown that *Y. enterocolitica* possesses a gene which is homologous to the *ompH* genes of *E. coli* and *S. typhimurium* (homology percentages, 65 and 64%, respectively). This *ompH* gene of *Y. enterocolitica* encodes a cationic protein which has, in its mature form, the calculated molecular mass of 16,086 Da. The preprotein has a 22-amino-acid typical signal sequence. The homology of this protein is 66% with *E. coli* OmpH and 64% with *S. typhimurium* OmpH. Furthermore, most (66%) of the amino acid substitutions are conservative. Homology exists throughout the protein, and large regions of very strong or less strong homology were not found.

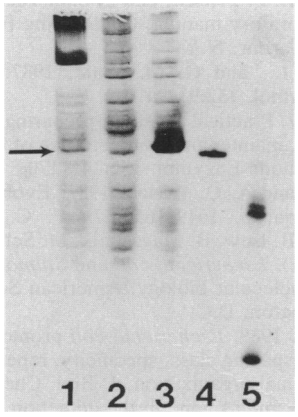

FIG. 7. Acid urea-PAGE analysis of Ca^{2+}-Triton X-100 extracts of the OM preparation of *Y. enterocolitica* EH902 (lane 1) and of the membranes of *E. coli* JM109 (lane 2) and JM109 harboring the plasmid pUCHS115 (lane 3). Lane 4, Purified OmpH protein of *S. typhimurium*; lane 5, basic protein standards, histone VII-S (upper band) and lysozyme (lower band). The arrow shows the *Y. enterocolitica* protein band in lane 1 which probably corresponds to OmpH.

We also showed in this paper that *Y. enterocolitica ompH* is expressed. The plasmid-borne *Y. enterocolitica ompH* is expressed both in the *E. coli* host and in minicells. The isolated OM of *Y. enterocolitica* was shown to contain OmpH.

Because yersiniae are considered rather distant phylogenetic relatives of *E. coli* in the *Enterobacteriaceae* family (while many other genera are closer to *E. coli*), as reviewed above, it is possible that most or all strains of the family *Enterobacteriaceae* have OmpH proteins remarkably homologous to those now sequenced. This is an important finding and is consistent with the early data of Geyer et al. (12), who had indirect, serological evidence that the LPS-binding protein or an antigenically very similar protein exists in all studied genera of *Enterobacteriaceae*. Even though the function of OmpH is not yet known, its universal presence could indicate that it has an important function.

On the other hand, if OmpH protein turns out to be a ubiquitous constituent of the OMs of enterobacteria, it would not be surprising that the OmpH proteins sequenced thus far are so homologous. Many of the OM proteins, such as the OmpA protein, the murein lipoprotein, and the porins, are structurally very conserved within members of the family *Enterobacteriaceae*. This was first demonstrated by showing that these proteins from various enterobacterial strains share serologically cross-reacting epitopes (17). It was also found that an *ompA*-specific gene probe derived from one species (*E. coli*) strongly hybridized with the chromosomal DNA from *S. typhimurium*, *Enterobacter cloacae*, *Y. enterocolitica*, *Serratia marcescens*, and *Proteus mirabilis* but not with the DNA from those gram-negative bacteria which are not members of the *Enterobacteriaceae* (33). As deduced from the nucleotide sequences, the homology of *E. coli* OmpA protein is 97% with *Shigella dysenteriae* OmpA (3, 4), 94% with *S. typhimurium* OmpA (11), 85% with *Enterobacter aerogenes* OmpA (5), and 77% with *Serratia marcescens* OmpA (6). Some parts of OmpA have been especially conserved during evolution; some carboxy-terminal regions of *E. coli* OmpA are highly homologous with the corresponding regions of *Pseudomonas aeruginosa* porin F (40), meningococcal OM protein class 4 (22), and gonococcal OM protein III (13). The most classic example of the homology is among the enterobacterial murein lipoproteins (21, 41). While *Morganella morganii* and *E. coli* are phylogenetically very distant within the family *Enterobacteriaceae*, their murein lipoproteins are 74% homologous. A further example is the PhoE porin; 81% of its amino acid residues are identical in *E. coli*, *Klebsiella pneumoniae*, and *E. cloacae* (38). The OmpC porin of *Salmonella typhi* is 77% homologous to the respective protein of *E. coli* (39). As was shown for OmpA, some regions of the *E. coli* porins are very conserved; the overall sequence homology of the *E. coli* OmpF porin is 23% with the *Haemophilus influenzae* porin P2 (14).

We are currently trying to clarify the function of OmpH. We are studying the OmpH protein of *S. typhimurium*. It binds to LPS, and measurements of its binding parameters are under way in our laboratory. On the other hand, as reviewed in reference 37, many other enterobacterial outer

membrane proteins also interact with LPS. As shown elsewhere (1, 16), the *ompH* gene is located close to the *lpxA* and *lpxB* genes involved in lipid A biosynthesis, and these genes might form a cistron. Evidence to be presented elsewhere appears to indicate that OmpH is normally not a major protein. According to our rough estimate, there are approximately 10^4 molecules of OmpH per cell. Is OmpH involved in translocation of LPS to the OM? Mutants or conditionally lethal mutants which produce defective OmpH will help remarkably in elucidating this and many other questions.

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