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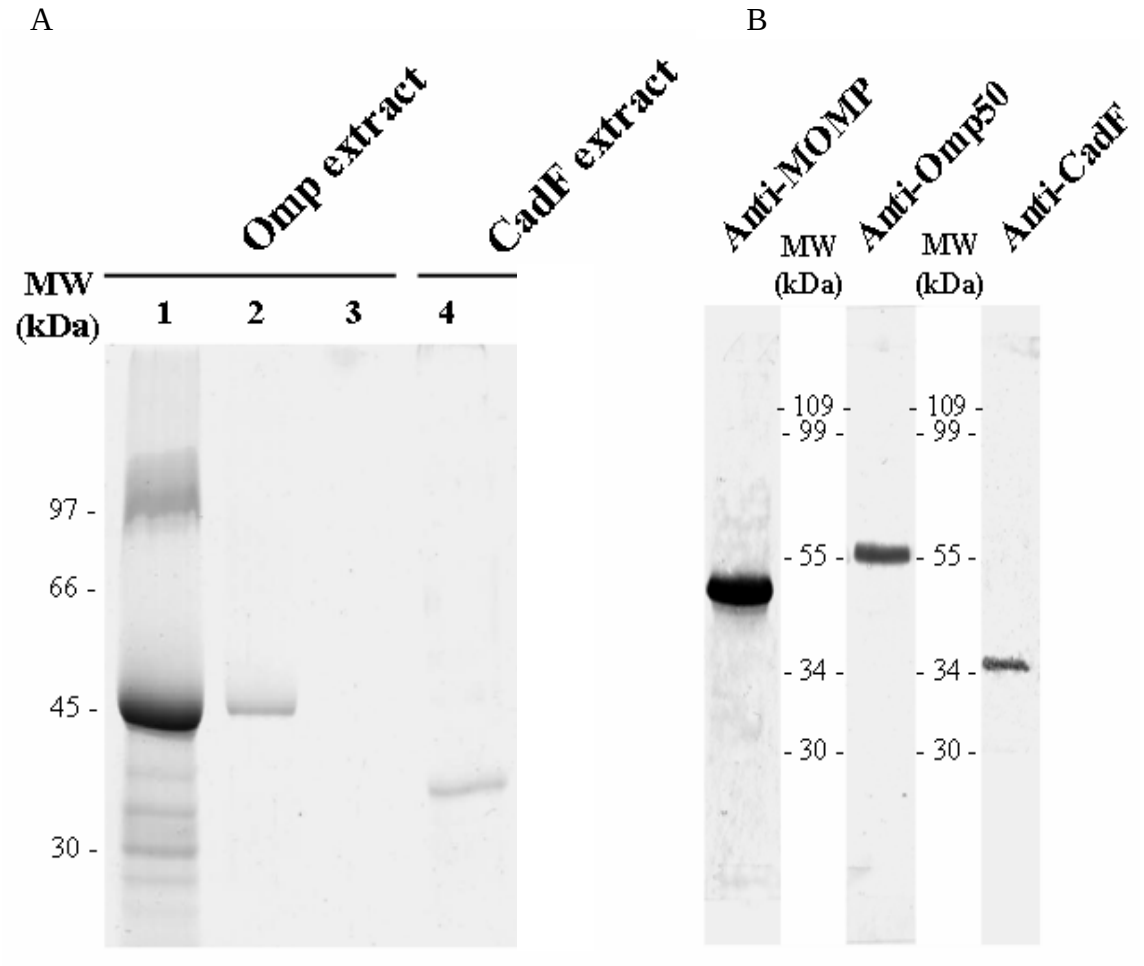
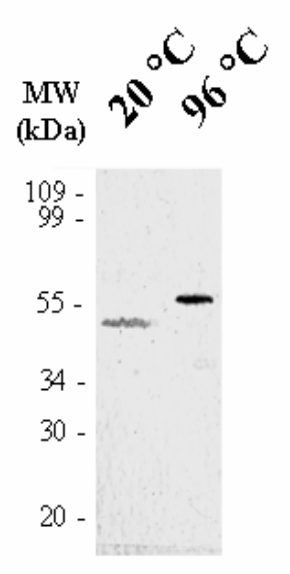
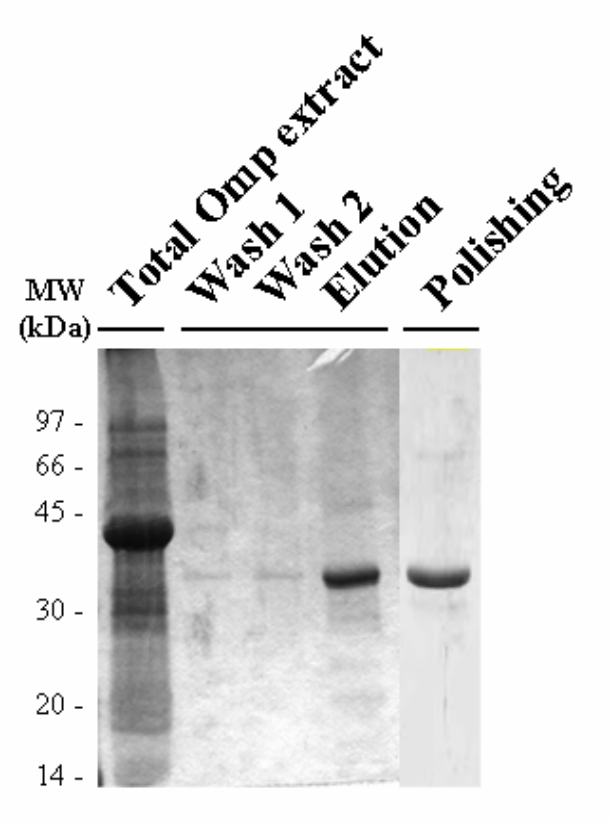
Chromosomal His-tagging: An alternative approach to membrane protein purification



Supplementary Figure 1. Production and purification of CadF. A. SDS-PAGE analysis of CadF extraction. Three successive extractions of the outer membrane with 0.5 % octyl-POE were necessary to remove the major outer membrane proteins (Omp extract, lanes 1, 2 and 3, respectively). CadF extraction was further performed for 1 h at 37°C in 1 % octyl-POE containing 1 M NaCl (lane 4). These conditions allowed the solubilization of 1 µg and 60 µg of CadF and MOMP, respectively, from 5 x 10^9 bacteria (1 UOD 600 nm) The samples were heated at 96°C prior to loading on the SDS-PAGE in order in to monitor the protein's folding. B. Immunoblots on total cell samples using rabbit anti-MOMP, anti-Omp50 and anti-CadF as indicated.



Supplementary Figure 2. Immunoblot with a rabbit anti-Omp50 serum on an outer membrane preparation of *E. coli* transformants harboring a recombinant plasmid containing the complete *omp50* gene. The Omp50 was produced and addressed to the outer membrane of *E. coli*.



Supplementary Figure 3. Monitoring of CadF6His purification as judged by SDS-PAGE analysis coupled with Coomassie blue staining. To perform the purification, cell membranes were treated under high salt concentration conditions as outlined earlier (see Supplementary Fig. 1 online). The solubilized outer membrane fraction collected from the LMCadF6his cells was diluted and loaded onto a HiTrap Chelating nickel-affinity column (Amersham Biosciences, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Succesive wash steps were performed with 10 and 50 mM imidazole, respectively. The first fractions eluted with 250 mM imidazole were loaded onto a Superose gel filtration column as a polishing step. Protein absorbance was monitored at 280 nm, resulting in a major peak that corresponded to the elution of the CadF6His protein. SDS-PAGE analysis showed that the CadF6His protein was homogenously purified.

Supplementary Methods:

Bacterial strains, plasmids and growth conditions

The strains and plasmids used in this work are listed in Supplementary Table 1 online. *E. coli* strains were grown at 37°C in Luria-Bertani (LB) broth or on solid LB agar. The *C. jejuni* strains were grown at 37°C on Columbia agar under microaerophilic conditions (CampyGen, OXOID Ltd, Hampshire, England). When indicated, ampicillin and tetracycline were added to the growth media at 100 μg/mL and 15 μg/mL final concentrations, respectively.

Chemicals

Sodium lauryl sarcosinate, imidazole, and antibiotics were purchased from Sigma-Aldrich (St Louis, USA), and n-octylpolyoxyethylene (octyl-POE) from Bachem-AG (Weil and Rhein, Germany).

Bio-informatics

The *cadF* gene was recovered from the genome of *C. jejuni* NCTC 11168 (Sanger centre, http://www.sanger.ac.uk/Project/C_jejuni/) [1]. Studies on the translated CadF sequence were carried out by PSI-Blast search [2] performed on the SWISS-PROT database [3] using the PAM30 score matrix. Sequence alignment was perform using CLUSTALW [4].

DNA techniques

DNA restriction and modification enzymes were purchased from New England Biolabs (Beverly, MA, USA), and were used as recommended by the manufacturer. The genes were amplified using PCR (Taq DNA Polymerase, Invitrogen, Carlsbad, CA, USA) from NCTC 11168 genomic DNA and ligated into pGEM-T Vector System (Promega, Madison, WI, USA). Plasmid DNAs were purified using a Wizard Plus Minipreps DNA Purification System kit (Promega). DNA fragments were sequenced by standard methods (Eurogentec, Seraing, Belgium). DNA fragments were gel-extracted by using a Concert Rapid Extraction kit (Invitrogen).

Primers and PCR conditions

The upstream primer was 5'-tcatgaaaaaaatattcttatgtttaggtttggcaagtg-3' (start codon was underlined), and the downstream primer was 5'-gagctcttaatgatgatgatgatgtcttaaaataaatttagcatcc-3' and containing a hexa-histidine sequence in frame with the *cadF* gene (underlined). The reaction was 30 cycles of 30 sec at 96°C, 60 s at 54°C, and 90 s at 72°C.

Campylobacter electro-transformation

Electro-competent cells of *C. jejuni* F38011 were transformed by electroporation with a Bio-Rad Laboratories Gene Pulser II Apparatus (Hercules, CA, USA), in a 0.2 cm cuvette, at 2 kV, 25 µF, and 300 Ohm with pLMsuCadF (see Supplementary Table 1 online) and plated onto Mueller-Hinton (MH) supplemented with 5 % sheep blood. After 6 h of incubation on nonselective media under microaerophilic conditions at 37°C, cells were plated onto Columbia agar supplemented with 15 µg/mL tetracycline.

SDS-PAGE and immunoblotting

The method of Laemmli [5] was used to perform SDS-PAGE analysis (MiniProtean II, BioRad Laboratories, Marnes-la-Coquette, France). The migration was performed at 160 V for 1 h, and proteins were stained with Coomassie brilliant blue (0.1 % w/v). Western immunoblots were performed using a polyclonal rabbit antiserum raised against CadF [6]. After one hour incubation, the membrane was reacted with goat anti-rabbit antibodies coupled to alkaline phosphatase. The nitrocellulose membrane was stained with NBT/BCIP color reagent.

Purification of wild-type CadF

Prior to chromatography, the following 10 steps were performed: Step 1, *C. jejuni* NCTC 11168 bacterial cells were harvested and washed in 10 mM Tris-HCl, 1 mM EDTA, pH 7.2; Step 2, the pellet was washed once in 100 mM glycine-HCl, pH 2.2 and twice in 100 mM Tris-HCl, pH 7.2; Step 3, the cells were then sonicated in 10 mM Tris-HCl, pH 7.2 and cell debris were removed by centrifugation at 10 000 x *g* for 20 min at 4°C; Step 4, the total membrane fraction was pelleted by ultracentrifugation at 100 000 x *g* for 1 h at 4°C; Step 5, the membrane pellet was ressuspended in 10 mM Tris-HCl (pH 7.6) containing 0.1 % sodium lauryl sarcosinate (w/v), and outer membrane proteins were recovered by ultracentrifugation at 100 000 x *g* for 1 h at 4°C; Step 6, 7, and 8, the outer membrane protein pellet was homogenized in 50 mM sodium phosphate buffer (NaPi, pH 7.6) containing octyl-POE at a final concentration of 0.5 % (v/v), and insoluble materiel was harvested by ultracentrifugation at 100 000 x *g* for 1 h at 4°C (This step was repeated three times in order to eliminate the major outer membrane protein and other contaminants); Step 9, the CadF protein was extracted from the pellet by incubation with the same buffer containing 3 % octyl-POE and 1

M NaCl for 1 h at 37°C, followed by ultra-centrifugation at 100 000 x *g* for 1 h at 4°C to remove insoluble contaminants; and Step 10, the soluble material was extensively dialyzed against 50 mM NaPi, pH 7.6, 0.3 % octyl-POE. Classical anion exchange chromatography was then performed with a flow rate of 2 mL/min. Conductivity and OD 280 nm were checked during all the chromatography steps. A MonoQ HR (Amersham Biosciences, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) ion exchange column was equilibrated with buffer [50 mM NaPi containing 0.3 % octyl-POE (pH 7.6)]. Each sample was thawed and centrifuged at 10 000 x *g* for 20 min before loading onto the column. After loading and extensive column washing with the same buffer, a linear gradient was performed from 0 M NaCl to 1 M NaCl. Fractions corresponding to the CadF protein were pooled and frozen.

Peptidoglycan binding

Peptidoglycan was prepared according to the method of Leduc *et al.*[7]. Detection of peptidoglycan binding to CadF6His was performed according to the method described for the peptidoglycan-associated lipoprotein (PAL) [8]. Seven micrograms of CadF6His was incubated with 50 µL of purified peptidoglycan, in 10 mM NaPi pH 8 containing 150 mM NaCl, for one hour at room temperature. The mixture was centrifuged for 2 hours at 250000 x *g*, which sediments the peptidoglycan and any associated proteins. The supernatant was recovered, and the pellet was washed in 200 µL of 10 mM NaPi pH 8 containing 500 mM NaCl. After centrifugation as above, the wash fraction was collected and the pellet was resuspended in Laemmli buffer [5]. The supernatant, wash and pellet fractions were analyzed by Western blotting, probed with anti-CadF and anti-Omp50 as indicated.

Reconstitution in planar lipid bilayers

The method was previously described by Dé *et al.* [9]. Briefly, solvent-free planar lipid bilayers were formed by the Montal and Mueller technique [10] modified by Saint *et al.* [11]. The current fluctuations were recorded using a BLM 120 amplifier (Biologic Science Instruments, Claix, France) and stored on a digital tape recorder DTR 1202 (Biologic Science Instruments). Currents and amplitude histograms were obtained from the stored signals using the Satori software from Intracell (Royston, Herts., U.K.). Diphytanoylphosphatidylcholine (DPhPC, Avanti Polar Lipids, Birmingham, AL, U.S.A.) were used as lipids. The protein was added to the measurement compartments containing 4 mL of electrolyte solution (1 M KCl, 10 mM HEPES, pH 7.4) at concentrations ranging from 0.25 to 0.5 ng/mL. Bilayer formation was monitored by the capacitance response and the voltage and current sign conventions are the usual ones.

References

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Supplementary Table 1

Table 1. Bacterial strains and plasmids used in this study.

	Relevant features	Source or reference
Strains		
NCTC 11168	Reference strain of *C. jejuni*	[1]
F38011	*C. jejuni*	[2]
LMCadF6his	*C. jejuni* strain harboring the NCTC 11168 *cadF* gene with a C-terminal hexa-histidine	This study
Plasmids		
pLMpromCadF	pGEM-T vector containing the *cadF6his* gene without promoter region	This study
pUOA15	Vector containing the *tetO* resistance gene	[3]
pLMsuCadF	pLMpromCadF containing the *tetO* gene, this vector is suicide in *Campylobacter*	This study

References

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