Supplementary Material

In *Campylobacter jejuni* a new type of chaperone receives heme from ferrochelatase

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# Supplementary Figures

|  |  |
| --- | --- |
| **A** | **B** |

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**Supplementary Figure 1. Functional complementation by *C. jejuni* UroD and PpfC enzymes**. **A.** *Escherichia coli* wild type, *uroD*, aqnd *ppfC* strains containing the empty vector (pØ) or plasmid encoding the corresponding *C. jejuni* homologous gene (P*uroDC.jejuni* or P*pfCC.jejuni*) streaked on LB-agar medium with no hemin. **B.** UV-Visible spectrum of the oxidized form of the reaction product of *C. jejuni* UroD.

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| --- | --- | --- |
| **A** | **B** | **C** |
|  | Uma imagem com gráfico  Descrição gerada automaticamente |  |

**Supplementary Figure 2. UV-Visible spectra of *C. jejuni* CgdH proteins.** **A**. CgdH1 (50 µM) spectrum exhibits two broad bands centered around 330 nm and 420 nm characteristic of a [4Fe-4S]2+ cluster. **B**. *C. jejuni* CgdH2 (20 µM) spectrum showing the presence of heme and Fe-S cofactors. **C.** *C. jejuni* CgdH2 containing only the Fe-S center.



**655 m/z coproporphyrin**

**Supplementary Figure 3. HPLC chromatogram of copro’gen III.** The product of the control reaction obtained in the absence of *C. jejuni* Cj0992c, with peak at m/z of 655 corresponding to coproporphyrin III (oxidized form of copro’gen III).

# Supplementary Tables

**Supplementary Table 1.** Strains used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Strain** | **Genotype** | **Resistance** | **Reference** |
| *C. jejuni* | ATCC 700819  NCTC 1168 |  | - | NCTC collection\* |
| *E. coli* | BL21(DE3) pLysS | F-*ompT* *hsdS*B(rB- mB-) *gal dcm* (DE3) pLysS | CmR |  |
| *E. coli* | BW25113 |  | - | Baba et al 2006 |
| *E. coli* | IH71 | F+ Δ*visA*(=Δ*ppfC*) HfrC *lac*am125 *trp*am *su*O | - | NBRP-*E. coli* at NIG\*\* |
| *E. coli* | IH81 | Δ*hemE(=*Δ*uroD*)*::cat* | CmR | NBRP-*E. coli* at NIG\*\* |

\* National Collection of Type Cultures collection

\*\* National BioResource Project *E. coli* strain at National Institute of Genetics, Microbial Physiology Laboratory, 1111 Yata, Mishima, Shizuoka, 411-8540 Japan

CmR – chloramphenicol resistance (*cat* gene)

**Supplementary Table 2.** Primers used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primer Name** | **Plasmid** | **Restriction sites** | **Gene** | **Sequence 5´-3 ´** |
| Forward *uroD* | pPR-IBA 2 | KpnI | *uroD* | GGCCGGTACCCATGATTTTTATCGATGCTTGC |
| Reverse *uroD* | pPR-IBA 2 | AvrII, BamHI | *uroD* | CCGGGGATCCTTAACCTAGGTCATTTAGCTGAACTTTCTT |
| Forward *ppfC* | pPR-IBA 2 | EcoRI | *ppfC* | CCGGGAATTCGGTGAAATTAGTTTTATTTTT |
| Reverse *ppfC* | pPR-IBA 2 | AvrII, BamHI | *ppfC* | CCGGGGATCCTAACCTAGGTTAGTTAAGATCAGATAAAT |
| CgdH1\_pET23b\_Fw\_NdeI | pET23b | NdeI | *cgdH1* | CCGGCATATGAGAGATTATAAAGCTTTTG |
| CgdH1\_pET23b\_Rv\_XhoI | pET23b | XhoI | *cgdH1* | CCGGCTCGAGCACCGTTTTTGAGAATAC |
| CgdH2\_pET23b\_Fw\_NdeI | pET23b | NdeI | *cgdH2* | CCGGCATATGAATTTATTTCAAAATTTAGC |
| CgdH2\_pET23b\_Rv\_XhoI | pET23b | XhoI | *cgdH2* | CCGGCTCGAGGTTTGTCTCCGCTTTAAATT |
| CgdH3\_pET23b\_Fw\_NdeI | pET23b | NdeI | *cgdH3* | CCGGCATATGCATTTTTATATCCATATTCC |
| CgdH3\_pET23b\_Rv\_XhoI | pET23b | XhoI | *cgdH3* | CCGGCTCGAGAGATGAGAGATATAAAGCAA |

**Supplementary Table 3.** Plasmids used in this study.

|  |  |
| --- | --- |
| **Plasmid\*** | **Plasmid description** |
| pPR-IBA2-*uroD* | *C. jejuni uroD* fused to Strep tag at N-terminus |
| pPR-IBA2-*ppfC* | *C. jejuni ppfC* fused to Strep tag at N-terminus |
| pPR-IBA2-*pgdH2* | *C. jejuni pgdH2* fused to Strep tag at N-terminus |
| pET-23b-*cgdH1* | *C. jejuni cgdH1* fused to Strep tag at N-terminus |
| pET-23b-*cgdH2* | *C. jejuni cgdH2* fused to His tag at C-terminus |
| pET-23b-*cgdH3* | *C. jejuni cgdH3* fused to His tag at C-terminus |
| pET-23b-cgdH2Y66L | *C. jejuni cgdH2*Y66Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*Y46L | *C. jejuni cgdH2*Y46Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*H48L | *C. jejuni cgdH2*H48Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*M44L | *C. jejuni cgdH2*M44Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*Y244L | *C. jejuni cgdH2*Y244Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*H62L | *C. jejuni cgdH2*H62Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*H53L | *C. jejuni cgdH2*H53Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*H285L | *C. jejuni cgdH2*H285L(fused to His tag at C-terminus |
| pET-23b-*cgdH2*H133L | *C. jejuni cgdH2*H133Lfused to His tag at C-terminus |
| pET-23b-*cgdH2*M227L | *C. jejuni cgdH2*M227Lfused to His tag at C-terminus |

\*All plasmids mediate resistance to ampicillin.

**Supplementary Table 4.** List of proteins used for the phylogenetic tree analysis.

|  |  |  |
| --- | --- | --- |
| CgdH type | Accession No. | Strain |
| CgdH | AAN83245 | *Escherichia coli* |
| CgdH | WP\_028069898 | *Sphingobacterium thalpophilum* |
| CgdH | WP\_006365438 | *Chlorobium ferrooxidans* |
| CgdH | WP\_069810592 | *Chlorobaculum limnaeum* |
| CgdH | WP\_026853902 | *Geothrix fermentans* |
| CgdH | WP\_005034428 | *Holophaga foetida* |
| CgdH | AMW06315 | *Gemmatimonas phototrophica* |
| CgdH | BAH40890 | *Gemmatimonas aurantiaca* |
| CgdH | AFS53101 | *Leptospirillum ferriphilum* |
| CgdH | CUW40578 | *Magnetospirillum sp.* |
| CgdH | WP\_010883524 | *Chlamydia pneumoniae* |
| CgdH | WP\_021756895 | *Chlamydia pecorum* |
| CgdH | CAH07682 | *Bacteroides fragilis* |
| CgdH | ACB73891 | *Opitutus terrae* |
| CgdH | ACM93156 | *Nautilia profundicola* |
| CgdH | KIN92132 | *Thauera sp.* |
| CgdH | CUI82205 | *Achromobacter sp.* |
| CgdH | WP\_025305675 | *Thermocrinis ruber* |
| CgdH | WP\_012674071 | *Sulfurihydrogenibium azorense* |
| CgdH | EHC13895 | *Fischerella sp.* |
| CgdH | WP\_036489441 | *Myxosarcina sp.* |
| CgdH | AFZ05950 | *Oscillatoria nigro-viridis* |
| CgdH | P74132 | *Synechocystis* |
| ChuW | AIU70349 | *Thermococcus eurythermalis* |
| ChuW | WP\_051366200 | *Pseudothermotoga elfii* |
| ChuW | CDC62198 | *Clostridium sp.* |
| ChuW | WP\_020612921 | *Sediminispirochaeta bajacaliforniensis* |
| ChuW | EEF14474 | *Campylobacter rectus* |
| ChuW | WP\_013010134 | *Denitrovibrio acetiphilus* |
| ChuW | WP\_009353669 | *Veillonella sp.* |
| ChuW | WP\_051212161 | *Veillonella montpellierensis* |
| ChuW | EKX99441 | *Selenomonas sp.* |
| ChuW | WP\_019177805 | *Methanomassiliicoccus luminyensis* |
| ChuW | WP\_013506625 | *Desulfurispirillum indicum* |
| ChuW | EGW43871 | *Bilophila sp.* |
| ChuW | EIO71851 | *Escherichia coli* |
| ChuW | WP\_004344082 | *Thauera linaloolentis* |
| ChuW | WP\_008619339 | *Magnetospirillum caucaseum* |
| HutW | AIC83437 | *Vibrio alginolyticus* |
| Zbamorf26 | ACG60749 | *Streptomyces pilosus* |
| Blmorf8 | ABL74954 | *Streptoalloteichus hindustanus* |
| (continued) | | |
| Type of CgdH | **Accession\_No.** | **Strain** |
| Tlmorf1 | AAG02372 | *Streptomyces verticillus* |
| Class\_C\_RSM | EGG43722 | *Streptomyces griseoaurantiacus* |
| NosN | ADR01089 | *Nocardia* |
| PbtM3 | AGY49586 | *Planobispora rosea* |
| TpdU | ACS83765 | *Nonomuraea* |
| TbtI | YP\_003651165 | *Thermobispora bispora* |
| TpdL | ACS83777 | *Nonomuraea sp.* |
| Class\_C\_RSM | AGY49595 | *Planobispora rosea* |
| Tpdx2 | SEL93961 | *Nonomuraea pusilla* |
| Class\_C\_RSM | SDP98233 | *Lentzea jiangxiensis* |
| Class\_C\_RSM | OGO33555 | *Chloroflexi bacterium* |
| Class\_C\_RSM | WP\_067157183 | *Streptomyces sp.* |
| Jaw5 | BAO98806 | *Streptomyces roseoverticillatus* |
| Class\_C\_RSM | SEF54573 | *Streptomyces yanglinensis* |
| Class\_C\_RSM | WP\_011291186 | *Thermobifida fusca* |
| Class\_C\_RSM | GAD86125 | *Nocardia asteroides* |
| Class\_C\_RSM | WP\_026404953 | *Actinomadura rifamycini* |
| Class\_C\_RSM | WP\_030684647 | *Streptomyces sp.* |
| C10P | WP\_012324632 | *Shewanella* |
| Class\_C\_RSM | WP\_051685090 | *Clostridium sp.* |
| YtkT | WP\_055490826 | *Streptomyces sp.* |
| Class\_C\_RSM | WP\_014677041 | *Streptomyces* |
| HemZ | WP\_025117272 | *Lysinibacillus fusiformis* |
| HemZ | Q796V8 | *Bacillus subtilis* |
| HemZ | CON04352 | *Streptococcus pneumoniae* |
| HemZ | WP\_010233508 | *Clostridium arbusti* |
| HemZ | WP\_007286255 | *Intestinibacter bartlettii* |
| HemZ | WP\_039679463 | *Terrisporobacter othiniensis* |
| MenK | AFK50646 | *Thermogladius calderae* |
| MenK | ESQ25637 | *Acidilobus sp.* |
| MenK | AFZ70518 | *Caldisphaera lagunensis* |
| MenK | CCC80926 | *Thermoproteus tenax* |
| MenK | ABM80220 | *Hyperthermus butylicus* |
| MenK | WP\_055408506 | *Pyrodictium delaneyi* |
| MenK | AEM37888 | *Pyrolobus fumarii* |
| MenK | WP\_011998375 | *Ignicoccus hospitalis* |
| MenK | WP\_014025565 | *Pyrolobus fumarii* |
| MenK | WP\_011138079 | *Wolinella succinogenes* |
| MenK | WP\_028766718 | *Shewanella fidelis* |
| MenK | EGG57223 | *Parasutterella excrementihominis* |
| MenK | CBL03906 | *Gordonibacter pamelaeae* |
| (continued) | | |
| Type of CgdH | **Accession\_No.** | **Strain** |
| Plant | XP\_011398951 | *Auxenochlorella protothecoides* |
| Plant | XP\_005649356 | *Coccomyxa subellipsoidea* |
| Plant | XP\_001703480 | *Chlamydomonas reinhardtii* |
| Plant | XP\_002946784 | *Volvox carteri* |
| Plant | CAL50029 | *Ostreococcus tauri* |
| Plant | XP\_002507726 | *Micromonas commoda* |
| Plant | EDQ75811 | *Physcomitrium patens* |
| Plant | EFJ27703 | *Selaginella moellendorffii* |
| Plant | XP\_011627754 | *Amborella trichopoda* |
| Plant | ABI93924 | *Arabidopsis thaliana* |
| Plant | XP\_002262672 | *Vitis vinifera* |
| Plant | XP\_012487614 | *Gossypium raimondii* |
| Plant | XP\_003522422 | *Glycine max* |
| Plant | XP\_009616975 | *Nicotiana tomentosiformis* |
| Plant | XP\_010250874 | *Nelumbo nucifera* |
| Plant | BAF29564 | *Oryza sativa* |
| Plant | XP\_009415069 | *Musa acuminata* |
| Plant | XP\_010930027 | *Elaeis guineensis* |
| RSAD1 | EEC11362 | *Ixodes scapularis* |
| RSAD1 | ESA03321 | *Rhizophagus irregularis* |
| RSAD1 | CEP17932 | *Parasitella parasitica* |
| RSAD1 | CEG73035 | *Rhizopus microsporus* |
| RSAD1 | CDS13916 | *Lichtheimia ramosa* |
| RSAD1 | EDV19558 | *Trichoplax adhaerens* |
| RSAD1 | XP\_012557630 | *Hydra vulgaris* |
| RSAD1 | HXP\_001636936 | *Nematostella vectensis* |
| RSAD1 | EFX89896 | *Daphnia pulex* |
| RSAD1 | ELT99882 | *Capitella teleta* |
| RSAD1 | XP\_009064549 | *Lottia gigantea* |
| RSAD1 | XP\_005090240 | *Aplysia californica* |
| RSAD1 | XP\_012695163 | *Clupea harengus* |
| RSAD1 | NP\_001077026 | *Danio rerio* |
| RSAD1 | XP\_010862851 | *Esox lucius* |
| RSAD1 | XP\_007556327 | *Poecilia formosa* |
| RSAD1 | XP\_008327612 | *Cynoglossus semilaevis* |
| RSAD1 | XP\_005999660 | *Latimeria chalumnae* |
| RSAD1 | OCA21266 | *Xenopus tropicalis* |
| RSAD1 | XP\_007441654 | *Python bivittatus* |
| RSAD1 | XP\_008102717 | *Anolis carolinensis* |
| RSAD1 | XP\_005297689 | *Chrysemys picta* |
| RSAD1 | XP\_009562321 | *Cuculus canorus* |
| RSAD1 | KFV63667 | *Dryobates pubescens* |
| (continued) | | |
| Type of CgdH | **Accession\_No.** | **Strain** |
| RSAD1 | XP\_005144383 | *Melopsittacus undulatus* |
| RSAD1 | XP\_007505827 | *Monodelphis domestica* |
| RSAD1 | XP\_012405492 | *Sarcophilus harrisii* |
| RSAD1 | XP\_012581510 | *Condylura cristata* |
| RSAD1 | NP\_001013399 | *Mus musculus* |
| RSAD1 | XP\_008516193 | *Equus przewalskii* |
| RSAD1 | XP\_011228768 | *Ailuropoda melanoleuca* |
| RSAD1 | XP\_004446612 | *Dasypus novemcinctus* |
| RSAD1 | XP\_012518368 | *Propithecus coquereli* |
| RSAD1 | EAW94610 | *Homo sapiens* |
| RSAD1 | XP\_003414682 | *Loxodonta africana* |
| RSAD1 | XP\_007940360 | *Orycteropus afer* |
| HemW | ACD05922 | *Akkermansia muciniphila* |
| HemW | ALA58796 | *Nitrospira moscoviensis* |
| HemW | CUW28423 | *Streptomyces reticuli* |
| HemW | KOX89034 | *Thermus aquaticus* |
| HemW | WP\_052195205 | *Deinococcus radiopugnans* |
| HemW | ADI13526 | *Truepera radiovictrix* |
| HemW | WP\_062189862 | *Anaerolinea thermolimosa* |
| HemW | EFH90847 | *Ktedonobacter racemifer* |
| HemW | WP\_014433607 | *Caldilinea aerophila* |
| HemW | ACM05800 | *Thermomicrobium roseum* |
| HemW | WP\_039744988 | *Geobacter pickeringii* |
| HemW | ACU91423 | *Desulfomicrobium baculatum* |
| HemW | EFV43164 | *Bilophila wadsworthia* |
| HemW | WP\_015946007 | *Desulfovibrio vulgaris* |
| HemW | WP\_015898519 | *Acidobacterium capsulatum* |
| HemW | ACX75702 | *Fibrobacter succinogenes* |
| HemW | EDM26343 | *Lentisphaera araneosa* |
| HemW | WP\_000239943 | *Enterobacteriaceae* |
| Other proteins | WP\_013008727 | *Deferribacter desulfuricans* |
| Other proteins | ALD66217 | *Spiroplasma cantharicola* |
| Other proteins | AFG37251 | *Spirochaeta africana* |
| Other proteins | ACV39949 | *Leptotrichia buccalis* |
| Other proteins | ADO82048 | *Ilyobacter polytropus* |
| Other proteins | ERT66913 | *Cetobacterium somerae* |
| Other proteins | ACK42388 | *Dictyoglomus turgidum* |
| Other proteins | ACI19107 | *Dictyoglomus thermophilum* |
| Other proteins | EGG81213 | *Lachnospiraceae bacterium* |
| Other proteins | AGB18790 | *Thermoanaerobacterium thermosaccharolyticum* |
| Other proteins | WP\_036947005 | *Pseudobacteroides cellulosolvens* |
| Other proteins | YP\_001255455 | *Clostridium botulinum* |
| (continued) | | |
| Type of CgdH | **Accession\_No.** | **Strain** |
| Other proteins | CAB61616 | *Bacillus subtilis* |
| Other proteins | WP\_010735215 | *Enterococcus mundtii* |
| Other proteins | WP\_038057790 | *Thermodesulfobacterium hydrogeniphilum* |
| Other proteins | AIH03372 | *Thermodesulfobacterium commune* |
| Other proteins | WP\_009962340 | *Verrucomicrobium spinosum* |
| Other proteins | EAQ77478 | *Blastopirellula marina* |
| Other proteins | KLU03830 | *Rhodopirellula islandica* |
| Other proteins | ADB19145 | *Pirellula staleyi* |
| CgdH1 | YP\_002344387 | *Campylobacter jejuni* |
| CgdH2 | YP\_002343800 | *Campylobacter jejuni* |
| CgdH3 | YP\_002344010 | *Campylobacter jejuni* |