

Figure S1

A bootstrapped maximum likelihood tree for different genomes-sequenced Chlorobia/Ignavibacteria and related species/strains based on concatenated sequences of UvrD and PolA proteins. Statistical support values for different branches are indicated on the nodes. The tree was rooted using the sequences from the species *Fibrobacter succinogenes*. The type strains of different species are marked by the superscript "T". Some species clades observed in this tree are marked.

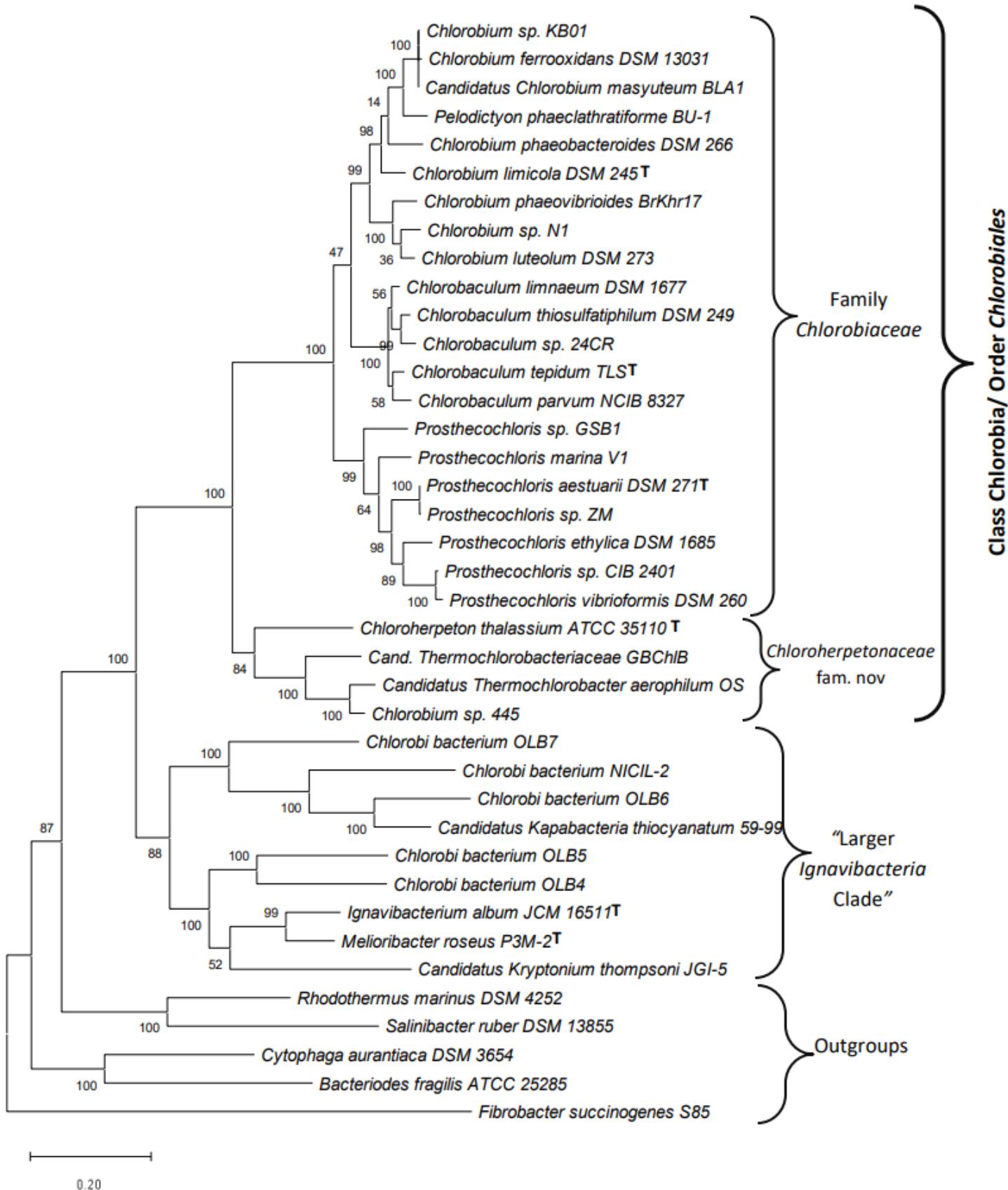


Figure S2

A bootstrapped maximum likelihood tree for different genomes-sequenced Chlorobia/Ignavibacteria and related species/strains based on concatenated sequences of GyrA and Gyr B proteins. Statistical support values for different branches are indicated on the nodes. The tree was rooted using the sequences from the species Fibrobacter succinogenes. The type strains of different species are marked by the superscript "T". Some species clades observed in this tree are marked.

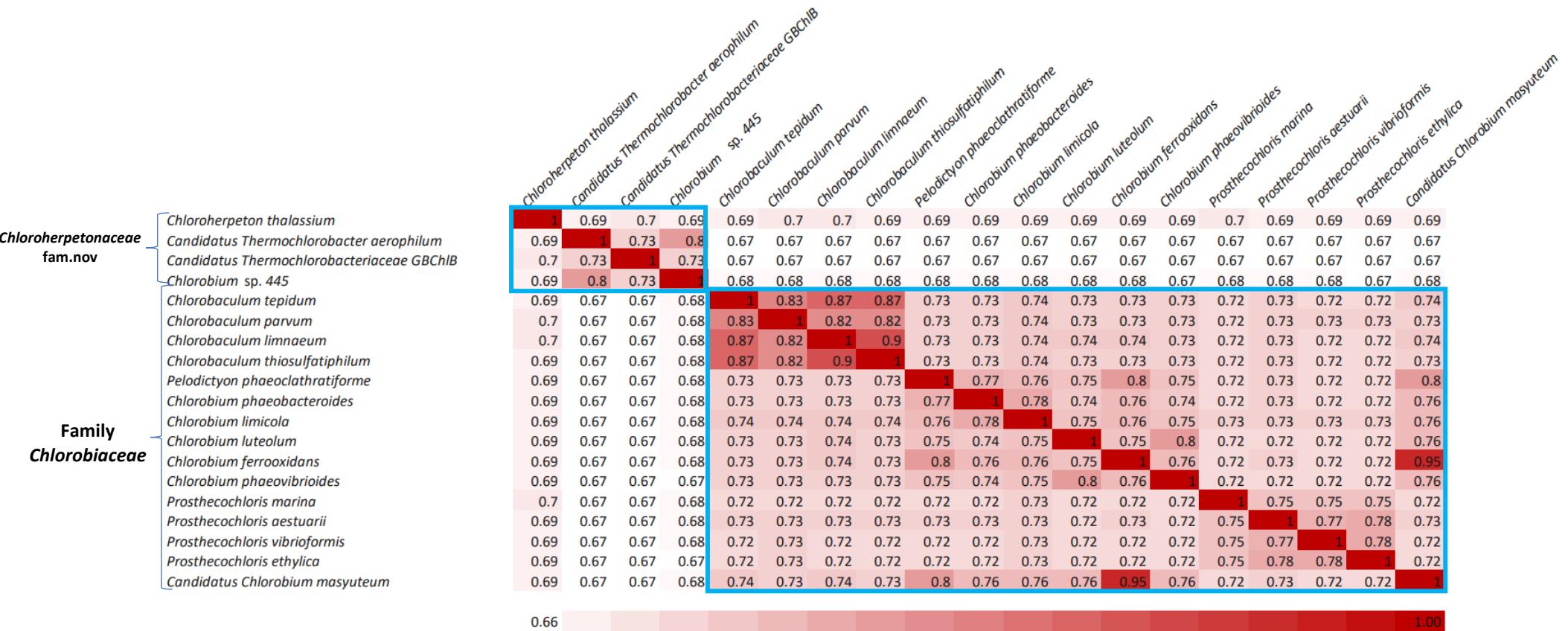


Figure S3

AAI matrix indicating the pairwise percentage average amino acid identities based on 355 core proteins for the type species of different genera within the order *Chlorobiales*. Genome pairs sharing higher amino acid identity are shaded more darkly (red). The regions of the matrix corresponding to species from different proposed families are labeled

| | | 248 | | |
|--------------------------------------|---|---|--|--|
| Order <i>Chlorobiales</i> | <i>Chlorobium luteolum</i> 78185909 <i>Chlorobium chlorochromatii</i> 78188020 <i>Chlorobium phaeovibrioides</i> 145218843 <i>Chlorobium phaeoclostriforme</i> 194335210 <i>Chlorobium phaeobacteroides</i> 119355895 <i>Chlorobium limicola</i> 189345581 <i>Chlorobaculum parvum</i> 193211698 <i>Chlorobaculum tepidum</i> 21675063 <i>Prosthecochloris aestuarii</i> 194332877 <i>Chlorobium ferrooxidans</i> 493410795 <i>Chloroherpeton thalassium</i> 193214675 <i>Chlorobaculum limnaeum</i> 2501895760 <i>Candidatus Chlorobium masyuteum</i> 166807360 <i>Chlorobaculum thiosulfatiphilum</i> 170181247 <i>Prosthecochloris marina</i> 161953525 <i>Candidatus Thermochlorobacter GBch1B</i> KER10651 <i>Candidatus Thermochlorobacter aerophilum</i> RFM23176 <i>Chlorobium sp. N1</i> 165498823 <i>Chlorobium sp. KB01</i> 076789630 <i>Chlorobium sp. 445</i> PI048809 <i>Prosthecochloris sp. HL-130-GSB</i> 085658738 <i>Chlorobaculum sp. 24CR</i> 167599212 <i>Prosthecochloris sp. ZM</i> 114617902 <i>Prosthecochloris sp. GSB1</i> 094081604 <i>Chlorobi bacterium OLB4</i> KXX01802 <i>Chlorobi bacterium OLB6</i> KXX33418 <i>Chlorobi bacterium OLB7</i> KXX53003 <i>Segetibacter koreensis</i> 517441118 <i>Salitalea canadensis</i> 387789634 <i>Hymenobacter norwichensis</i> 551197521 <i>Arcticibacter svalbardensis</i> 510820536 <i>Mucilaginibacter paludis</i> 495781065 <i>Flexithrix dorotheae</i> 522015193 <i>Joostella marina</i> 495886513 <i>Chitinophaga pinensis</i> 256419457 <i>Pedobacter heparinus</i> 255533134 <i>Porphyromonas endodontalis</i> 490463729 <i>Dyadobacter fermentans</i> 255038651 <i>Dyadobacter beijingensis</i> 518785017 <i>Anaerophaga thermohalophila</i> 515149603 <i>Flavobacterium columnare</i> 365960887 <i>Lewinella cohaerens</i> 522025975 <i>Paludibacter propionicigenes</i> 313204242 <i>Porphyromonas catoniae</i> 491610397 <i>Anaerophaga thermohalophila</i> 498104982 <i>Mariniradius saccharolyticus</i> 495906476 | EFEQMEMQYFVKPGTQAEAFEAWEERYQWY T RQLGIRPEKLHWYKHDKLAHY -----L-----FA- V NS---T-S----- -----V-----FN- - SS---S----- -----FA- - SA---L-K----- -----FA- - SA---NRD----- -----S-I-----FN- V TE---TQ----- -----L-----FR- S ET---MTK----- -----L-----FR- S ET---MSK----- -----K-T-----FN- K ER---LT-G----- -----KG-----FA- S TA---L-A----- -----M-E-----R-MS- ES---SK-----Q----- -----L-L-----FR- S ET---MTK----- -----K-----FA- S AT---L-A----- -----L-----FR- S ET---MTR----- -----G-S-----N- I EK---ER----- -----I-A-K-----SM-Q-K-FN- A DY---NT-N-V---Q----- -----R-E-MA-----M-Q-Q-MK- EC---FKK-N-V----- -----T-----G- - A---LS----- -----K-E-T-FA- S TT---L-A----- -----V-L-----M-Q-R-MK- A ER---LK---N-S----- -----L-T-----FN- V KS---S-D-----R----- -----L-----FR- S ET---MTR----- -----K-T-DA-----FN- K ER---LT-G----- -----A-E-----FN- V ER---GQ-----R----- -----F-DDS-W-Y-K-Q-I-F EKY-M-K---KF-E-E----- -----F-IA---D-W-Y-AA-WE-L VN---LT---N-RRKP-E----- -----F-ED-KWYDY---Q-WN- D---MQM-R-RMKP-E----- -----F-R-EG-WRYR-K-A-L-H LS---S-K-YRYHD-V----- -----F-R-EM-WNY-K-A-LK-H KA---TD---YRYHD-T----- -----F-R-EG-WY-H-KAT-RA-H EAM---PTN---RFHD----- -----F-R-EDKKW-DY-KIA-LK-H TA---TSS---YRFHD-V----- -----F-IR---EM-WY-Y-KAA-LK-H VA---TD-A-YQYHD-A----- -----F-R-SEM-WY-K-KAQ-MA-H TV---TD---RFHD----- -----F-I---K-WYDY-K-T-LK-H MS---MGE-NYRFHD-E----- -----F-R---K-W-AY-K---LK-H KS---D-D-YRFHV---F----- -----F-R-EDKKW-AY-K-A-LK-H LA---TPA---YRYHD-V----- -----F-A-EEMKW-Y-KKT-MAFH KS---LGDN-YRYHD-E----- -----F-R-EQ-WY-K-K-A-LKFH KAI-LPA---KYHD----- -----F-R-EE-WYNK-K-A-LKFH KAI-LPA---KYHD----- -----F-R-EEM-W-NY-KQV-MK-H KA---MGED-YRFHD----- -----F-EEMKW-Y-QKT-LK-H KS---LGA-NYRYHD-E----- -----F-IQ---KKWYDY-K-A-LK-H LA---TSAS-YRFHD-N----- -----F-R-EEMKW-H-KAF-LK-H KA---LGDQ-YRFHD----- -----F-R-EELKW-DF-KDL-LR-H KA---LGDK-YRFHD-E----- -----F-R-EEM-W-NY-KQV-MK-H KA---MGE-YRFHD----- -----F-R-SELDWYKE-AAT-M-H KA---PE---RRHD-E----- | | |

Figure S4

Partial sequence alignment of the protein Glycine-tRNA synthetase showing a 1 aa insertion (boxed) that is specific for the order *Chlorobiales*

| | | | YDMPVDTVEYKR | AKRALDKLIKGNLRFVVSVAK |
|---|--|------------|---------------------------------------|---------------------------------|
| Order <i>Chlorobiales</i> | <i>Chlorobaculum parvum</i> | 193212077 | EIGKYDLLTAEDEVKLTMAIKEG | |
| | <i>Chlorobium limicola</i> | 189345950 | - - - - - D - - - - - | - - - - - P - - - - - |
| | <i>Prosthecochloris aestuarii</i> | 194334634 | - - - - - E - - - - - | - - - - - F - - - - - |
| | <i>Chlorobium phaeoclathestratiforme</i> | 194337394 | - - - - - K - - - - - | F - - - - T - - - - - |
| | <i>Chlorobium ferrooxidans</i> | 493409341 | - - - - - K - - - - - | F - - - - T - - - - - |
| | <i>Chlorobium chlorochromatii</i> | 78188365 | - - - - - R - K - - - | F - - - - P - - - - - |
| | <i>Chlorobium luteolum</i> | 78186275 | - - - - - D - R - K - | F - - - - T - - - - - |
| | <i>Chlorobium phaeobacteroides</i> | 189500956 | - - - - - E - - - K - A | - - - - T - - - - - |
| | <i>Chlorobium phaeovibrioides</i> | 145219258 | - - - - - D - R - K - | F - - L - T - - - - - |
| | <i>Chlorobaculum tepidum</i> | 1519644 | - - - - - RRRGEAHEGH - | - - - - - - - - - - - |
| | <i>Chlorobaculum limnaeum</i> | 2501895477 | - - - - - K - - - - - | - - - - - - - - - - - |
| | <i>Prosthecochloris marina</i> | 110022354 | - - - - - E - - - - - | - - - - - P - - - - - |
| | <i>Chloroherpeton thalassium</i> | 193216316 | - - - F - - QE - IE - RQ - K | DGQ - I - SK - RE - R - E - A - |
| | <i>Chlorobium sp. N1</i> | 131356747 | - - - D - - R - K - | F - - T - - - - - - - |
| | <i>Chlorobium sp. 445</i> | PI048482 | - - - FE - - Q - IE - RK - R | EGKS - SK - RE - Q - E - A - |
| | <i>Cand. Thermochlorobacteria aerophilum</i> | KER10064 | - - - F - - Q - IT - - K - K | DKG - - SK - F - E GR - E - A - |
| | <i>Chlorobaculum sp. 24CR</i> | 129052373 | - - - R - - - - - - - | R - - - - - - - - - - - |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085660123 | - - - E - - - R - - - | - - - P - - - - - - - |
| | <i>Prosthecochloris sp. GSB1</i> | 094083210 | - - - E - - - K - - - | - - - P - - - - - - - |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobi bacterium OLB6</i> | KXK33417 | - - - RV - - GD - IT - AQ - - R | GFE GGL - ER - V - A - |
| | <i>Chlorobi bacterium OLB5</i> | KXK55587 | - - - VE - DV - E - ID - AKK - Y | DE - E KQK - E - T - A - |
| | <i>Chlorobi bacterium OLB7</i> | KXK54381 | - - - VE - - PDM - IE - AKR - R | GAE GE - EQ - V - A - |
| | <i>Chlorobi bacterium NICIL-2</i> | KXB98252 | - - - IE - SP - E - IE - AKR - RQ | GPE GEA - E - V - A - |
| | <i>Melioribacter roseus</i> | 397689183 | - - - VE - - P - - IE - AIK - K | DQK - E - V - A - |
| | <i>Ignavibacterium album</i> | 385811725 | - - - V - - PQ - ID - AIR - - K | DEK - KE - - A - |
| | <i>Gillisia marina</i> | 497916484 | - - - V - - I - - E - E - AQR - A | DD - - E - T - A - |
| | <i>Nafulsellla turpanensis</i> | 516339844 | - - - V - - PDE - E - AKR - R - | DQM - E - T - A - |
| | <i>Psychroflexus tropicus</i> | 517868227 | - - - V - - I - - E - E - AQR - A | DD - - E - T - A - - S - |
| | <i>Gramella forsetii</i> | 120434745 | - - - V - - I - - DE - E - AQR - A | DD - - E - T - A - |
| | <i>Fulvivirga imtechensis</i> | 497263748 | - - - V - - PDE - E - AQR - - | DQL - E - T - A - |
| | <i>Zhouia amyloolytica</i> | 568803407 | - - - V - - I - - E - E - AQR - A | DQ - - E - T - A - |
| | <i>Polaribacter porphyrae</i> | 105016810 | - - VS - I - - I - - DE - E - AVK - - | SQ - - - V - A - |
| | <i>Sediminibacterium ginsengisoli</i> | 217698805 | - - - V - - ISP - E - Q - S - R - R | CQ - S - R - S - |
| | <i>Tenuifilum thalassicum</i> | 173075478 | - - - V - - - E - T - ARK - - | DQE - E - V - A - |
| | <i>Saccharicrinis aurantiacus</i> | 075601683 | - - - VK - - - E - - AKR - D | DTV - - R - NA - |
| | <i>Labilibaculum manganireducens</i> | 101308830 | - - - V - - IS - - E - Q - ARE - K | N - K - R - C - - S - |
| | <i>Flavobacterium psychrophilum</i> | 123910415 | - - - V - - I - - DE - E - AQR - A | DQ - - E - T - A - |
| | <i>Myroides marinus</i> | 074744386 | - - - V - - I - - E - E - AQR - A | DQ - - E - T - A - |
| | <i>Aureitalea marina</i> | 104811626 | - - - V - - I - - E - E - AQR - A | DQ - - E - T - A - |
| | <i>Planktosalinus lacus</i> | 188440396 | - - - V - - I - - DE - E - AQR - K | DQ - - E - T - A - |
| | <i>Tenacibaculum skagerrakense</i> | 132792729 | D - - I - - I - - DE - E - SQQ - K | NQ - - E - VNA - |
| | <i>Psychroflexus salis</i> | 188406779 | - - - V - - I - - E - E - AQR - A | DDL - E - T - A - - S - |
| | <i>Massilibacteroides vaginae</i> | 085537662 | - - - RE - - I - V - E - E - AQR - R | DR - - E - TRA - |
| | <i>Parabacteroides distasonis</i> | 121736482 | - - - RE - - I - V - E - E - AQR - R | DR - - E - TRA - |
| | <i>Salmonella enterica</i> | MBH0619490 | - - - V - - PDE - - T - AQR - RD | DQK - E - T - A - |
| | <i>Chryseolinea serpens</i> | SHG77818 | - - - RV - - I - - DE - - QR - A | DQA - E - MV - A - |
| | <i>Pukyongia salina</i> | 105216487 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Flavobacterium haorani</i> | 072781367 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Marinirhabdus gelatinilytica</i> | 115124369 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Catalinimonas alkaloideigena</i> | 089683757 | - - - V - - PDE - - E - AKR - R | DQ - - E - T - A - |
| | <i>Ancylomarina salipaludis</i> | 129253240 | - - - V - - V - - E - A - ARQ - K | N - T - NR - A - - S - |
| | <i>Imtechella halotolerans</i> | 008238527 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Dokdonia pacifica</i> | 089373128 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Ginsengibacter hankyongi</i> | 150415004 | - - - V - - I - P - E - - ALR - Q | DQQ - - - T - A - |
| | <i>Pustulibacterium marinum</i> | 093022816 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Robertkochia marina</i> | 136335350 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Tenacibaculum adriaticum</i> | 148869371 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Polaribacter porphyrae</i> | 105017184 | - - - V - - I - - DE - - E - AQL - A | DQ - - E - T - A - |
| | <i>Tenacibaculum dicentrarchi</i> | 101902490 | - - - V - - I - - DE - - E - AQR - A | DQ - - E - T - A - |
| | <i>Hymenobacter norwichensis</i> | 551195676 | - - - V - - PDE - - T - AQR - - | DQQ - - E - T - A - |
| | <i>Cesiribacter andamanensis</i> | 496485220 | - - - V - - PDE - - E - AKR - RQ | DQM - E - T - A - |
| | <i>Porphyromonas macacae</i> | 517172454 | - - - RE - - I - V - E - E - AQR - R | DR - - E - TRA - |
| | <i>Rhodoneillum psychrophilum</i> | 518430040 | - - - V - - - DE - - V - AKR - R - | DQL - E - T - A - |
| | <i>Belliella baltica</i> | 390944276 | - - - V - - - DE - - V - AKR - R - | DQL - E - T - A - |
| | <i>Microscilla marina</i> | 488786349 | - - - V - - - DE - - E - AKR - R - | DQV - E - T - A - |
| | <i>Echinicola vietnamensis</i> | 431798761 | - - - V - - - DE - - V - AKR - R - | DQL - E - T - A - |

Figure S5

Partial sequence alignment of the protein RNA polymerase sigma factor RpoD/SigA showing a 12 aa insertion (boxed) that is exclusive to all members belonging to the order *Chlorobiales*

333

| | | | |
|--|--|--|--------------------------|
| | | 284 | |
| | | QGNLDPTVLYGTPEKIKSEAALKQFGQ | HTE SSGHVFNGLGHGILPDVDP |
| | | - - - - - DR - A - S - I - - S - | - - H - - - - M - |
| | | - - - - - AL - - S - I - - S - | - - H - - - - M - |
| | | - - - M - - - - - I - - - | - - D C - - - |
| | | - - - M - - - - - D - R - - I - - | - - N D H - - - |
| | | - - - M - - - - - A - - - R - - | - - A T - - - |
| | | - - - M - - - - - A - G - I - - | - - A Q - - - |
| | | - - - M - - - - - HD - A - - I - - | - - A N - - - |
| | | - - - M - - - - - HD - RA - - G - I - ER - | - - A E - - - |
| | | - - - M - - - - - HD - RA - - G - I - ER - | - - S C - - - |
| | | - - - M - - - - - ND H - - - | - - N D H - - - |
| | | - - - M - - - - - I - - - HDR - A - - I - - | - - Q K - - - |
| | | - - - M - - - - - AS - R - RE - G - I - AK - | - - K - - - - N - |
| | | - - - M - - - - - Q - R - I - G - I - S - | - - NH H - - - M - |
| | | - - - M - - - - - HD - RA - - I - ERF - K | - - N - - - |
| | | - - - M - - - - - I - - - APHDV - RK - - I - QRF - E | - - D T - - - IE - |
| | | - - - M - - - - - AL - A - SKI - SF - | - - TH - H - - - M - |
| | | - - - M - - - - - R - - | - - ND H - - - |
| | | - - - M - - - - - A - - I - - | - - A Q - - - |
| | | - - - M - - - - - I - - - HDR - A - - I - - | - - Q K - - - |
| | | - - - M - - - - - M - - H - R - RA - - I - Q - | - - K - - - |
| | | - - - M - - - - - M - - H - R - RA - - I - R - | - - G N - - - |
| | | - - - M - - - - - ANKNY - Q - VIS - QS - E | - - G - I - - V - - |
| | | - - - M - - - - - AGKD - FE - TVRT - NSY - K | - - GP - - - I - - |
| | | - - - M - - - - - S - - AS - R - RQ - VETI - QA - H | - - G - - - T - K - |
| | | - - - M - - - - - S - - AS - R - RL - V - R - - QD - N | - - G - - - T - G - N - |
| | | - - - M - - - - - M - - AK - A - RQ - V - RI - ASY - | - - GT - - - T - E - |
| | | - - - M - - - - - S - - L - S - R - RQ - VQSI - DSY - | - - G - - - T - - |
| | | - - - M - - - - - I - - A - - - AG - RH - V - R - - GE - H | - - G - - - Y - IN - |
| | | - - - M - - - - - M - - SM - AP - R - RQ - V - DI - SRY - E | - - G - - I - - T - - |
| | | - - - M - - - - - M - - AR - A - RA - V - RI - ASY - E | - - G - - - T - E - |
| | | - - - M - - - - - M - - AM - APKAR - RQ - V - DI - - RY - S | - - G - - - T - - |
| | | - - - M - - - - - M - - AN - QA - RA - VGRI - ERY - K | - - G - - - T - E - |
| | | - - - M - - - - - M - - S - - AS - DR - RA - V - RI - SE - R | - - GN - - - T - E - |
| | | - - - M - - - - - M - - AM - APKAR - RQ - V - DI - - RY - S | - - G - - - T - - |
| | | - - - M - - - - - M - - SI - LAN - R - RE - V - SI - ASY - R | - - G - - I - - VT - K - |
| | | - - - M - - - - - M - - M - - I - - AS - R - RE - VGRI - SSY - S | - - G - - I - - T - E - |
| | | - - - M - - - - - M - - S - - AN - AA - RA - V - RI - AAY - E | - - GT - - - T - E - |
| | | - - - M - - - - - C - - AS - - Q - RA - V - R - - ESY - R | - - GH - - - T - - |
| | | - - - M - - - - - M - - I - - AS - DR - RE - VGRI - - SY - A | - - G - - - T - E - |
| | | - - - M - - - - - M - - SI - - AS - R - RQ - V - TI - EKY - K | - - G - - - H - E - |
| | | - - - M - - - - - M - - I - - AS - R - RT - VGDI - QAY - K | - - G - - - T - E - |
| | | - - - M - - - - - M - - M - - AK - A - RN - V - RI - ASY - K | - - GT - - - T - E - |
| | | - - - M - - - - - M - - AI - L - S - R - RQ - VQMI - DG - | - - G - - - T - - |
| | | - - - M - - - - - M - - S - - AK - AR - EE - VQSI - QSY - S | - - GT - - - H - E - |
| | | - - - M - - - - - M - - M - - AR - A - RQ - V - RI - ASY - S | - - GT - - - T - E - |
| | | - - - M - - - - - C - - AS - R - RA - V - R - - ESY - R | - - GH - - - T - - |
| | | - - - M - - - - - A - - AS - R - RA - V - A - - ES - S | - - G - - - T - K - |
| | | - - - M - - - - - M - - S - - L - - R - RQ - VQTI - DG - | - - GT - - I - - T - - |
| | | - - - M - - - - - M - - I - - AS - R - RE - VGRI - SSY - S | - - G - - I - - T - E - |

Figure S6

Partial sequence alignment of the protein uroporphyrinogen decarboxylase, showing a 3 aa insertion (boxed) that is exclusive to all members belonging to the order *Chlorobiales*

| | | 211 | | 250 |
|--------------------------------------|--|---|--|-----|
| Order <i>Chlorobiales</i> | <i>Chlorobium phaeoclathratiforme</i> 194336061 <i>Prosthecochloris vibrioformis</i> 139626275 <i>Chlorobium chlorochromatii</i> 78189476 <i>Chlorobium limicola</i> 189346423 <i>Chlorobium luteolum</i> 78187285 <i>Chlorobium phaeobacteroides</i> 189499890 <i>Chlorobaculum tepidum</i> 21674247 <i>Chlorobaculum parvum</i> 193212402 <i>Chlorobium ferrooxidans</i> 493411236 <i>Prosthecochloris aestuarii</i> 194334345 <i>Chlorobium phaeovibrioides</i> 145220049 <i>Chlorobium phaeobacteroides</i> 119357592 <i>Chloroherpeton thalassium</i> 193215988 <i>Chlorobaculum limnaeum</i> 501894998 <i>Prosthecochloris vibrioformis</i> 139626275 <i>Chlorobaculum parvum</i> 012501988 <i>Chlorobaculum thiosulfatiphilum</i> 139457331 <i>Chlorobium limicola</i> 012465852 <i>Prosthecochloris marina</i> 110023205 <i>Candidatus Chlorobium masyuteum</i> 166808915 <i>Cand. Thermochlorobacter aerophilum</i> RFM23265 <i>Chlorobium sp. N1</i> 131353836 <i>Chlorobium sp. KB01</i> 076790786 <i>Chlorobaculum sp. 24CR</i> 129052243 <i>Prosthecochloris sp. CIB 2401</i> 068866687 <i>Prosthecochloris sp. HL-130-GSB</i> 085659333 <i>Prosthecochloris sp. GSB1</i> 094082178 | ITNRILSKAETLAEELGTNKV L PFETFREHLHEFDIIITA ----I-----Q-----D----- ----I-----A-----Q-----Y-SYK----- ----A-----G-----SYK-Y----- ----Q-----A-----R-----SYK----- ----A-----Q-----R-----Y-SYK----- ----Q-----A-----R-----Y-SYK----- ----A-----A-----S-----Y-SYK----- ----M-----A-----Q-----DN----- ----R-----S-A-----Q-----Y-SYK----- ----A-----A-----K-----SYKDY----- ----YE---L---Q---G-I-----L-EMT-R-----VV----- ----A-----R-----Y-SYK----- ----Q-----Q-----D----- ----Q-----A-----R-----Y-SYK----- ----Q-----A-----R-----Y-SYK----- ----A-----G-----SYK-Y----- ----A-----A-----Q-----Y-SYK----- ----A-----A-----S-----Y-SYK----- ----PEH--A--RKI--SNI I T-DY---R-----V-S----- ----Q-----A-----R-----SYK----- ----A-----A-----Q-----Y-SYK----- ----A-----A-----S-----Y-SYK----- ----Q-----A-----R-----Y-YK----- ----I-----Q-----D----- ----M-----A-----Q-----D----- ----A-----A-----Q-----SYK----- L----FE---K---T---N-AVF --D---K---K-----S----- -A---MER--K---KVNGGVI --PSLK---NY---S----- V----M---K---C---V-GDTL --SY---S-EKY---V-SS----- V----F-R---I----INGEAI --DN-YNK-N-V---V-SS----- V----Y-R---K---Q-INGIP D--R-T-Y-KKT---V-SS----- V----FER-LQ---K-VNGEA -I-K-V-Y-K-V---V-SS----- V----FE---LK---FSGDA T-DR-AQK-P-I---SS----- L----FQR--EI-K-FNGVA --GNLK-Q-VKT---V-CS----- V----FER-QR---F-GEAI --DELFL---KA---VL-S----- V----FAR---K---T-FKGRA A--N-QDO---QA---VLSS----- V----FAR---K---S-FSGQAI L--N-HD---QQV---LLSS----- V----FARGQA---QFNGEAI --DQLF-Q-EKV---SS----- V----FER-VK---FDGKA NY-ELFD---KA---VLSS----- V----FA-G-R---A-F-GEAI --ELFQE---RA---VLSS----- V----YER-VK---FEGKP S-DDLFQ---KA---VLSS----- V----YOR-KE---A-FKGEA ---HLF---ADV---SS----- V----YER---K---FDAKP H--ALM-T-PKA---LSS----- V----ER-VH---QFRGEA --LLH-R---V---SS----- V----FER-VK---FDGKA NY-DLFDQ---KA---LSS----- V----YE---K---K---GMAI K-DKLE-A-RYA---V-S----- -A---YER---C---R---RGIAI H-DKL---A-KYA---V-S----- ----Q---QI---A---FEV --NC---AMN-A-V-VCS----- ----F---S---A-C-LKTI --QTF-VMKD---V-SS----- ----T---QH---C---LEVL --DMVQG-K-A-VV-SS----- ----A---VP---C---MEVL --DIVQG-K-A-V---SS----- V----ER-VK---FNGRAL --D-TH---QQV---L-S----- LI---EG---QA---LHTA --ML-YQIR-C---V----- VA---QR-QG---F-AQVM TL-AIP---A---A-V-SS----- VA---YER---R---N---KGMA H-DKLN-A-NFS---C----- -S---YER---R---K---KGIAI N-DKLK-AINYNS---C----- -A---FE---QA---AG---YSVI --DEVN-AMAAA-VVVSS----- ----FE---KE---D---FEVI --RCV-AMKDA-VVVSS----- -C---A---K---A-C-AKVI --NV-QAIL-A---V-SS----- -A---ERG-K---AQF-AGTM RLADLP-----Y-AV-S----- VAS---YER---AG---KT---NGSAL G-DE-K-E-YRA---VVSS----- V----F-R-VD---QQFDGHAF -I-ALA-N---RA---V-SS----- VA---RER---KFST-F-AEVI GL-QIP---RA---V-SS----- VA---QR-QG---F-AQVM TL-AIP---A---A-V-SS----- VA---RER---GI-R-F-AEVI SL-EIP---RA---V-SS----- VA---VAR---A---T-DASVF TLSQVP---KD---V-SS----- -A---ERG-K---TRF-GEVM RLADLPD-----AV-S----- | | |
| Other Bacteria | <i>Ignavibacterium album</i> 385809127 <i>Melioribacter roseus</i> 397689614 <i>Denitrovibrio acetiphilus</i> 291288797 <i>Deferribacter desulfuricans</i> 291279208 <i>Flexistipes sinusarabi</i> 336323776 <i>Calditerrivibrio nitroreducens</i> 313673929 <i>Mucispirillum schaedleri</i> 555554431 <i>Thermodesulfovibrio yellowstonii</i> 206890822 <i>Geobacter lovleyi</i> 189423574 <i>Geopsychrobacter electrodiphilus</i> 522166020 <i>Desulfuromonas acetoxidans</i> 492848965 <i>Desulfovibrio desulfuricans</i> 518845794 <i>Geobacter uraniireducens</i> 148262443 <i>Pelobacter propionicus</i> 118581777 <i>Geobacter metallireducens</i> 404498082 <i>Bilophila wadsworthia</i> 491168655 <i>Geobacter bemandjiensis</i> 197116805 <i>Desulfovibrio gigas</i> 545642716 <i>Geobacter daltonii</i> 222054399 <i>Methanocaldococcus jannaschii</i> 15668315 <i>Methanotorris formiciclus</i> 494103966 <i>Algorphagus aestuariicola</i> 206567720 <i>Xanthovirga aplysinae</i> 155206362 <i>Pontibacter roseus</i> 018476482 <i>Botryobacter ruber</i> 114777573 <i>Desulfuromonas soudanensis</i> 053551860 <i>Taibaiella koreensis</i> 118973184 <i>Aeromonas diversa</i> 005351703 <i>Methanothermococcus okinawensis</i> 336121948 <i>Methanococcus voltae</i> 297619964 <i>Nitritalea halalkaliphila</i> 496347363 <i>Mariniradius saccharolyticus</i> 495899215 <i>Microscilla marina</i> 488784857 <i>Chryseobacterium taeanense</i> 515974948 <i>Nitrospina gracilis</i> 491151936 <i>Magnetococcus marinus</i> 117926918 <i>Photobacterium damsela</i> 491440282 <i>Aeromonas diversa</i> 491493979 <i>Photobacterium leiognathi</i> 496274273 <i>Alteromonas macleodii</i> 406596336 <i>Acidovorax ebreus</i> 222109996 | | | |

Figure S7

Partial sequence alignment of the protein glutamyl-tRNA reductase, showing a 1 aa insertion (boxed) that is exclusive to all members belonging to the order *Chlorobiales*

| | | 60 | | 109 |
|--|--|------------|---|----------------------------------|
| | <i>Chlorobaculum tepidum</i> | 21674908 | GSWGPFI L GSMPHRTAAIEYTLR | N IGTSGFGTPIELEIEIAELLCKIVPS |
| | <i>Prosthecochloris vibrioformis</i> | 139626633 | -----I---L---H---T K | -----M-----TQ----- |
| | <i>Chlorobium ferrooxidans</i> | 493409722 | -----I---L-----K | -----M----- |
| | <i>Chlorobium phaeoclatratiforme</i> | 194337772 | -----I---L-----K | -----M----- |
| | <i>Chlorobium chlorochromatii</i> | 78189921 | -----I---L-----K | -----M-----Q----- |
| | <i>Chlorobium phaeobacteroides</i> | 119358325 | -----I---L-----K | -----M----- |
| | <i>Chlorobium limicola</i> | 189347866 | -----Q----- | -----S-----M-----SR----- |
| | <i>Prosthecochloris aestuarii</i> | 194333039 | -----I---L-H---T K | -----M-----Q----- |
| | <i>Chlorobium luteolum</i> | 78187880 | -----I---L-H---T K | -----M-----T----- |
| | <i>Chlorobium phaeobacteroides</i> | 189499186 | -----I---N---T K | -----M-----TQ----- |
| | <i>Chlorobium phaeovibrioides</i> | 145220484 | -----I---L-R---T | -----M-----VE----- |
| | <i>Chlorobaculum parvum</i> | 193211823 | -----L----- | -----I-----Q----- |
| | <i>Chloroherpeton thalassium</i> | 193213745 | -----HS---IE---R-MK | A H -----A-T-----L-----II-V----- |
| | <i>Chlorobaculum limnaeum</i> | 2501894144 | -----L----- | ----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139457606 | -----L----- | -----V----- |
| | <i>Pelodictyon phaeoclatratiforme</i> | 012509417 | -----I---L-----K | -----M----- |
| | <i>Prosthecochloris marina</i> | 110022620 | -----K---L-H---T K | V-----T----- |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM25453 | -----HS---V---H-K H | -----A-----L-----ISRL----- |
| | <i>Candidatus Chlorobium masyuteum</i> | 166807515 | -----I---L-----K | -----M----- |
| | <i>Chlorobium sp. 445</i> | PI047274 | -----HA---V---H-K H | V-----A-T-----QL-----ISTL----- |
| | <i>Chlorobium sp. N1</i> | 131355189 | -----I---L-F---T K | ----- |
| | <i>Prosthecochloris sp. GSB1</i> | 094081727 | -----I---L-H---T K | -----M-----Q----- |
| | <i>Prosthecochloris sp. ZM</i> | 114617802 | -----I---L-H---T K | -----M-----Q----- |
| | <i>Prosthecochloris sp. CIB 2401</i> | 068868140 | -----I---L-H---T K | -----M-----TQ----- |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085658843 | -----K---L-H---T K | -----M-----Q-----TQ----- |
| | <i>Chlorobaculum sp. 24CR</i> | 129052311 | -----L----- | -----Q----- |
| | <i>Melioribacter roseus</i> | 385811690 | -----HLF-HNP-FIKE-LLKA-E | D-----A-T-I-VKM---I-DL----- |
| | <i>Ignavibacterium album</i> | 397690482 | -----HLF-HNP-FIKK-LAEAFE | K-----A-T---VRM-Q-ITEL----- |
| | <i>Thermanaeromonas toyohensis</i> | 084665710 | -----L---HR---E-V---L-K---K E | M-----A-T---N-L-KMITQA----- |
| | <i>Pelotomaculum thermopropionicum</i> | 147677314 | C-----L---HR---VG-L-RC-N E | V-----A-T---NIL---MIVEA----- |
| | <i>Pelotomaculum propionicum</i> | 134213985 | -----L---HR---HAVE-LERC- D | V-----A-T-M-TKL-SMVAEA----- |
| | <i>Burkholderia phytopharmans</i> | 187925072 | -----M---HV---E-LE-VQRV-G | N-F---A-T-S-V---EI-L----- |
| | <i>Clostridium straminisolvens</i> | 575087331 | -----M---HS---LE-SK-MV | D-L---AAT---VQM---ILEL----- |
| | <i>Brevibacillus panacihumi</i> | 559035961 | -----L---HA---Q-L---TEVA | L-----A-T-R-T-M-K-V-QL----- |
| | <i>Bacillus cereus</i> | 507065470 | -----L---HA---E-VK---QEAAS | Q-----L-T-A-TKM---I-RSI----- |
| | <i>Histophilus somni</i> | 136121970 | -----M---HN---SILS-VLK-AE | N-L---TP---L---I-QL----- |
| | <i>Chloracidobacterium validum</i> | 211427812 | -----M---HA---E-L---RSVIE | R-----A-T---V-----IVS----- |
| | <i>Andrepreatovia chitinolytica</i> | 035054799 | -----L---HA---A-ID-V-RAA- | N-L---A-T-A---DL-D---ELL----- |
| | <i>Chitinolyticbacter meiyuanensis</i> | 148713814 | -----L---HA---A-E-V-R-I- | N-L---A-TAA---L-D---QQL----- |
| | <i>Nitrosobvrio tenuis</i> | 090829456 | -----L---HA---E-IE-V-AAAQ | N-LT---A-T-A-L-----L-S----- |
| | <i>Formivibrio citricus</i> | 091194206 | -----L---HA---A-VE-V-RAA- | N-L---A-T-A---DL-D---ELL----- |
| | <i>Gallibacterium salpingitidis</i> | 066111049 | -----MV---HN---TILO-QID-AK | N-L---A-T---KL---V-L----- |
| | <i>Azonexus hydrophilus</i> | 028993650 | -----M---HA---Q-I---VQEAVV | D-L---A-T-R-V---D---EM----- |
| | <i>Brevibacillus antibioticus</i> | 137029901 | -----L---HA---L---TEVA | L-----A-T-R-T-M-K-V-E----- |
| | <i>Nitrosospira multiformis</i> | 041352651 | -----L---HA---E-VD-VYAAAK | N-LT---A-T-A-L-----RL----- |
| | <i>Chloracidobacterium aggregatum</i> | 211426713 | -----M---HA---E-L---RSVIE | R-----A-T---V-----IVS----- |
| | <i>Caballeronia terrestris</i> | 087655126 | -----M---HV---E-LD-VQRV-G | N-F---A-T-A-----EI-L----- |
| | <i>Brevibacillus laterosporus</i> | 517502562 | -----L---HA---K-LQ---SE-AL | L-----A-T-R-TTM-K-V-D----- |
| | <i>Pasteurella multocida</i> | 378774174 | -----M---HN---TILD-VIS-AQ | N-L---A-TA---L---V-SL----- |
| | <i>Paenibacillus ginsengihumi</i> | 518363260 | -----L---V-HA---VE-VQRRAE | A-----A-T---T-M-K-VAER----- |
| | <i>Cohnella laeviribosi</i> | 517834161 | L-----L-A-HA---E-VE-KR-AE | K-----A-T---T-M---V-ER----- |
| | <i>Gallibacterium anatis</i> | 545092524 | -----MV---HN---SILN-VIE-AK | N-L---A-T-----L---V-RL----- |
| | <i>Haemophilus somnus</i> | 113461372 | -----M---HN---SILS-VLK-AE | N-L-----TP---L---I-QL----- |
| | <i>Gallibacterium anatis</i> | 517158368 | -----MV---HN---SILN-VIE-AK | N-L---A-T-----L---V-RL----- |
| | <i>Paraburkholderia silvatlantica</i> | 133790131 | -----M-V-HV---E-LD-VQRV-A | N-F---A-T-A-----EI----- |
| | <i>Aggregatibacter segnis</i> | 493770001 | -----M---HN---TILN-VMK-AQ | N-L---A-TP---L---V-ELI----- |
| | <i>Avibacterium paragallinarum</i> | 516416719 | -----M---HN---AILS-VQK-AE | K-L---A-TP---DL---V-QL----- |
| | <i>Pasteurella multocida</i> | 512748104 | -----M---HN---TILD-VIS-AQ | N-L---A-TA---L---V-SL----- |
| | <i>Aggregatibacter actinomycetemcomitans</i> | 491728551 | -----M---HN---SILN-VIK-AQ | N-L---A-TP---L---V-SL----- |
| | <i>Avibacterium paragallinarum</i> | 545595327 | -----M---HN---AILS-VQK-AE | K-L---A-TP---DL---V-QL----- |
| | <i>Planctomyces brasiliensis</i> | 325111308 | -----H---HQ---L---QEE-S | K-----A-TA---TDM---V-EC----- |
| | <i>Paenibacillus vortex</i> | 493234377 | -----L-M-HA---E-VD-LQE-AA | K-----A-TL---T-M-K-V-ER----- |
| | <i>Planctomyces limnophilus</i> | 296122991 | -----H---HR---V---HAAVD | R-----A-C-A-N-L-QMIEA----- |
| | <i>Desulfotomaculum kuznetsovii</i> | 333978951 | -----L---HR---E---LQECE-E | -----A-T-----L-RAIVEAL----- |
| | <i>Clostridium thermocellum</i> | 125975015 | -----M---HS---LE-VSK-MV | D-L---AAT---VQM---ITEL----- |
| | <i>Geobacter uraniireducens</i> | 148266255 | -----M---HC---Q-V---VKSADV | N-C---A-T---TL---MVIEA----- |
| | <i>Burkholderia kururiensis</i> | 516384471 | -----M-V-HV---E-LE-VQR-V | K-F---A-T-A-----VI-L----- |
| | <i>Haemophilus somnus</i> | 170719024 | -----M---HN---SILS-VLKIAE | N-L-----TP---L---I-QL----- |
| | <i>Burkholderia xenovorans</i> | 91784895 | -----M---HV---E-LE-VQRV-G | N-F---A-T-S-V---EI-L----- |
| | <i>Nitrosomonas europaea</i> | 30249394 | -----L---HA---D-VR-VQIAAG | H-----A-TAA-L-----RLL----- |

Figure S8

Partial sequence alignment of the protein glutamate-1-semialdehyde 2,1-aminomutase showing a 1 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*. The CSI is not present in any other bacterial species apart from the three exceptions shown above, in the top 500-BLASTp hits.

| | | | | | |
|--------------------------------------|--|------------|---|--|-----------|
| Order <i>Chlorobiales</i> | <i>Chlorobium chlorochromatii</i> | 78186883 | ----- | | -A- |
| | <i>Chlorobium phaeovibrioides</i> | 145219699 | ----- | | -Q- |
| | <i>Chlorobium chlorochromatii</i> | 78188704 | ----- | | -A- |
| | <i>Chlorobium ferrooxidans</i> | 493411307 | ----- | | -S--R- |
| | <i>Chlorobium phaeobacteroides</i> | 119357140 | ----- | | -T--R- |
| | <i>Chlorobium limicola</i> | 189346770 | ----- | | -S--Q-A- |
| | <i>Chlorobium phaeocloathratiforme</i> | 194336449 | ----- | | -T--H- |
| | <i>Prosthecochloris aestuarii</i> | 194333871 | -----A | | -H-S- |
| | <i>Chlorobaculum parvum</i> | 193212638 | -----A | | -H-A-A- |
| | <i>Prosthecochloris marina</i> | 110021934 | -----A-Y- | | -A----- |
| | <i>Chlorobaculum tepidum</i> | 21673879 | -----A | | -H-A-A-A- |
| | <i>Chlorobaculum limnaeum</i> | 2501894580 | -----A | | EHS-A-A- |
| | <i>Pelodictyon luteolum</i> | 011357757 | ----- | | -A----- |
| | <i>Prosthecochloris ethylica</i> | 175187116 | -----A-Y- | | -A-Q- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139457515 | -----A | | EHS-A-A- |
| | <i>Prosthecochloris sp. GS81</i> | 094082604 | ----- | | -Q- |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085659640 | -----A | | -A- |
| | <i>Chlorobium sp. N1</i> | 131353286 | ----- | | -A-A- |
| | <i>Prosthecochloris sp. ZM_2</i> | 114606996 | -----A-Y----- | | -A-Q- |
| <i>Ignavibacteriae</i> | <i>Chlorobaculum sp. 24CR</i> | 129051447 | -----A | | EHL-R-A- |
| | <i>Melioribacter roseus</i> | 397689677 | N-EQ CTRN EDGIL P-PL-F----- | | -A----- |
| | <i>Bacteroides pyogenes</i> | 545405147 | Y-T-EQ CHVDPATGRMV P-PLAF-V-DAVM-EL----- | | -A----- |
| | <i>Barnesiella viscericola</i> | 570742111 | Y-EQ CHVDPATGRLV P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Bacteroides salanitronis</i> | 325299001 | Y-EQ CHVDPKTGRLV P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Barnesiella intestinihominis</i> | 496138225 | Y-EQ CHVDPKTGRLV P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Parabacteroides distasonis</i> | 150008558 | Y-T-EQ CTIDPVTGKVM P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Bacteroides plebeius</i> | 494838222 | Y-EQ CHVDPATGRLV P-PLEFAV-DAVMEKL----- | | -A----- |
| | <i>Parabacteroides johnsonii</i> | 495425753 | Y-T-EQ CTIDPVTGKVM P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Bacteroides fragilis</i> | 492247874 | Y-TQEQ CHIDPKTGRMV P-PLAF-V-DGVM-EL----- | | -A----- |
| | <i>Prevotella saccharolytica</i> | 496453479 | Y-T-DQ CTRDPKTGRVL P-PLAF-V-DA-MEKL----- | | -A----- |
| | <i>Prevotella paludivivens</i> | 517276035 | Y-EQ CTRDPKTGRKL P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Fibrobacter succinogenes</i> | 261417271 | Y-EQ CTRN AQGKLV P-PLAF-V-HA-QKL----- | | -A----- |
| | <i>Treponema brennaborense</i> | 332297587 | RQ-EQ CTRD ADGVLP P-PLAF-V-A-KL----- | | -A----- |
| | <i>Megamonas hypermegale</i> | 479206841 | A-Y-T-DQ CTRN EQGILV P-PL-F-VA-L-N-A----- | | -A----- |
| Other Bacteria | <i>Barnesiella intestinihominis</i> | HAC12973 | Y-EQ CHVDPKTGRLV P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Phocaeicola coprophilus</i> | 204440964 | Y-EQ CHVDPATGRVL P-PLAF-V-DGVM-EL----- | | -A----- |
| | <i>Prevotella conceptionensis</i> | 044259939 | Y-T-EQ CTRDPKTGRVL P-PLAF-V-DA-M-KL----- | | -A----- |
| | <i>Pseudoprevotella muciniphila</i> | 111897780 | Y-EQ CTRDPKTGRKL P-PLAF-V-DA-MEKL----- | | -A----- |
| | <i>Parabacteroides distasonis</i> | 195473517 | Y-T-EQ CTIDPVTGKVM P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Bacteroides faecichinchii</i> | 073349791 | Y-T-EQ CHIDPVTGVM P-PLAFEV-DAVMEKL----- | | -A----- |
| | <i>Cand. Avibacter. excrementipullorum</i> | HIS09802 | Y-EQ CTVDPKTGRVL P-PLAF-V-DGVM-EL----- | | -A----- |
| | <i>Coprobacter secundus</i> | 021930517 | Y-EQ CTVDPKTGRVL P-PLAF-V-DGVM-EL----- | | -A----- |
| | <i>Parabacteroides johnsonii</i> | EEC95866 | Y-T-EQ CTIDPVTGKVM P-PLAF-V-DAVMEKL----- | | -A----- |
| | <i>Marseilla massiliensis</i> | 205111000 | Y-EQ CTRDPKTGRVL P-PLAF-V-D-MQKL----- | | -A----- |
| | <i>Cand. Prevotella stercoripullorum</i> | HIW44521 | Y-EQ CTRDPKTGRVL P-PLAF-V-D-MQKL----- | | -A----- |
| | <i>Cand. Cryptobacter. intestinipullorum</i> | HIS23577 | Y-EQ CTLDPKTGRVL P-PLAF-V-DGVM-EL----- | | -A----- |
| | <i>Prevotella buccae</i> | 048798599 | Y-T-EQ CTRDPKTGRVL P-PLAF-V-DA-MVQL----- | | -A----- |
| | <i>Brachyspira aalborgi</i> | 147526563 | Y-Q N-K-K----- | | -A----- |
| | <i>Streptobacillus notomytis</i> | 172793479 | H-D K-E----- | | -A----- |
| | <i>Fusobacterium necrophagense</i> | 115269440 | H-R----- | | -A----- |
| | <i>Sebaldella termtidis</i> | 012861482 | H-D N----- | | -A----- |
| | <i>Caproicibacterium amyloyticum</i> | 212507242 | Y-Q VS-ASSVP----- | | -A----- |

Figure S9

Partial sequence alignment of the protein class II fructose-1,6-bisphosphate aldolase showing a 11 aa deletion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | | | 325 | |
|---|---|-----------|------------------------|----------------------|
| Order <i>Chlorobiales</i> | <i>Chlorobaculum parvum</i> | 193212482 | SRSLMARLTPKEHRTEFFGFYD | G SFGKASAVIGPLVFGFVS |
| | <i>Chlorobium phaeovibrioides</i> | 145219537 | ---M-----PS-V- | T-----A-----L-- |
| | <i>Chlorobium tepidum</i> | 21674159 | -----V----- | -----I-----V-- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobium ferrooxidans</i> | 493410723 | ---M-----A-V- | T-----IA-----L-- |
| | <i>Pelodictyon phaeoclathestratiforme</i> | 194336684 | ---M-T---Q-V----- | T-----IA-----L-- |
| | <i>Prosthecochloris aestuarii</i> | 194334279 | ---M-----V----- | T-----IL-VI-V-- |
| Other Bacteria | <i>Chlorobium limicola</i> | 189347022 | ---M-K--R-VA- | T-----IV-----M-- |
| | <i>Chlorobium phaeobacteroides</i> | 119357455 | ---M-----Q-V----- | T-----IA-L-M-- |
| | <i>Chloroherpeton thalassium</i> | 193213931 | -----A-SA----- | -----IV-WI-LI- |
| Other Bacteria | <i>Chlorobium chlorochromatii</i> | 78188904 | ---M-T---Q-V----- | T-----IV-FL-VI- |
| | <i>Chlorobium luteolum</i> | 78187183 | ---M-----RA-LA- | T-----IA-----L-- |
| | <i>Prosthecochloris marina</i> | PWW81998 | ---M-----PK-V----- | T-----IA-VI-VI- |
| Other Bacteria | <i>Prosthecochloris ethyllica</i> | 194185816 | ---AM-----PK-V----- | T-----I-VF-MI- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139457788 | -----V----- | -----I-----I-- |
| | <i>Prosthecochloris vibrioformis</i> | TNJ37359 | ---M-----K-V----- | T-----IL-VL-I-- |
| Other Bacteria | <i>Candidatus Chlorobium masyuteu</i> | 208019732 | ---M-----A-V----- | T-----IA-----L-- |
| | <i>Chlorobium sp. N1</i> | 131353654 | ---M-----RA-LA- | T-----IA-----L-- |
| | <i>Chlorobium sp. KB01</i> | 076790259 | ---M-----A-V----- | T-----IA-----L-- |
| Other Bacteria | <i>Chlorobaculum sp. 24CR</i> | 129051526 | ---L-----V----- | -----I-----I-- |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | ARM31897 | ---M-K--V----- | T-----IV-----LI- |
| | <i>Prosthecochloris sp. ZM</i> | 114617037 | ---M-----V----- | T-----IL-VI-V-- |
| Other Bacteria | <i>Prosthecochloris sp. CIB 2401</i> | 083188230 | ---M-----K-V----- | T-----IL-VL-I-- |
| | <i>Prosthecochloris sp. GSB1</i> | 094082256 | ---M-K--A-V----- | T-----A-MI-LI- |
| | <i>Chlorobi bacterium OLB7</i> | KXK57455 | ---T-L---ADKK----- | F-----TL-IY- |
| Other Bacteria | <i>Ignavibacterium album JCM 1651</i> | 385810439 | ---SK---P-KK-----S | F-S-L-----L-- |
| | <i>Melioribacter roseus P3M-2</i> | 397689128 | ---SK---YDKK-----S | F-S-IL-----IT- |
| | <i>Candidatus Kryptonia bacterium</i> | HHD65179 | ---L-----V-AA----- | IA-N-----T-AI- |
| Other Bacteria | <i>Candidatus Kryptonium thompsonii</i> | 047133172 | ---SKII-P-KK-----S | F-S-IL----- |
| | <i>Weissella cibaria</i> | 498059571 | ---YLQQ-V---RSS---FN | I-F-I-AI-I-- |
| | <i>Paenibacillus larvae</i> | 558474612 | --T-IVQ-S-P-K-GQ---LFA | FS-V-SIV-LY-SIT |
| Other Bacteria | <i>Saccharibacillus kuerlensis</i> | 517806976 | --TMIVE-S-P-K-GQ---LFA | FS-L-S-L-Y-S-T |
| | <i>Listeria fleischmannii</i> | 494740195 | --FYGKII---RSN-Y-N | I-F-I-ALL-VIT |
| | <i>Lactobacillus oris</i> | 489811830 | --YFGQ-I---ASS---N | IL-F-IL-V-L-T |
| Other Bacteria | <i>Bacillus thuringiensis</i> | 489348371 | --YF-K-V---SAN---N | I-FA-IM-VLV-VTT |
| | <i>Lactobacillus oris</i> | 489810428 | --YFGQ-I---ASS---N | IL-F-IL-V-L-T |
| | <i>Bacillus anthracis</i> | 497564380 | --YF-K-V---SAN---N | I-FA-IM-VLV-VTT |
| Other Bacteria | <i>Bacillus cereus</i> | 488127310 | --YF-K-V---SAN---N | I-FA-IM-VLV-VTT |
| | <i>Weissella confusa</i> | 489703767 | --YLQII---RSS---FN | I-F-I-AI-I-- |
| | <i>Paenibacillus mucilaginosus</i> | 337750739 | --TMIVE---P-R-GQ---LFA | FS-V-SIV-LY-SIT |
| Other Bacteria | <i>Lactobacillus parabrevis</i> | 518933426 | --YFQ-V---RSS---N | IL-F-L-IL-VIT |
| | <i>Clostridium acetobutylicum</i> | 15894863 | --YLGK-V-NKAN-----N | I-F-IL-FMV-I-- |
| | <i>Caloramator australicus</i> | 496185404 | --YFGK-V---NSN---N | I-FA-IM-FLV-L-A |
| Other Bacteria | <i>Nevskia ramosa</i> | 551356333 | --YF---I-P-QAGQ---N | ML-FA-L-V-I-A |
| | <i>Marinitoga piezophila</i> | 374340297 | --YGK-I---KSA-----N | I-FA-I-FLV-F- |
| | <i>Caldithrix abyssi</i> | 493986443 | --A-GK-I-RGMEA-Y-A | LM-F-SIL-FT-L-- |
| Other Bacteria | <i>Flexistipes sinusarabici</i> | 336324118 | --FF---I-NDKSG-----N | ML-FA-L-FMM-L-A |
| | <i>Rhodospirillum centenum</i> | 209966532 | A-M---S-P-MQ---L-G | LT-R-IGF-AY-LAT |
| | <i>Syntrophus aciditrophicus</i> | 85858063 | --FFG---I---KSA-----N | I-FATIL-LMALA- |
| Other Bacteria | <i>Desulfohalobium retbaense</i> | 258405958 | --F-MV-E-KKA-----FS | ISA-FA-IV-F-AS-A |
| | <i>Candidatus Methylomirabilis</i> | 392374612 | --TF---S-I-GREG---C-A | LC-TASIL-A-- |
| | <i>Mariprofundus ferrooxydans</i> | 497536274 | --MY---MI-SHQS-----FN | ML-FA-L-MM-MA- |
| Other Bacteria | <i>Aminobacterium colombiense</i> | 294101413 | --Y-S-L-PSRSA----- | ISS-FAG-AI-LIT |
| | <i>Thermaanaerobacter acidaminovorans</i> | 269792021 | --FS-IV---RSA-L----- | LSS-FAG-L-MA-VI- |
| | <i>Thermaanaerobacter velox</i> | 493632134 | --FS-IV---DRSA-L----- | LSS-FAG-L-A-VI- |
| Other Bacteria | <i>Sphaerochaeta globosa</i> | 325970677 | --YYT---I-QRSA-----FN | ML-FA-I-LM-V-T |
| | <i>Candidatus Cloacimonas</i> | 218961816 | --TMLSL---RDRQA-----T | LT-RL-SI-ILY-WIA |
| | <i>Cand. Kryptobacter tengchongensis</i> | CUS92867 | --SKII-P-KK-----S | F-S-IL-L-- |
| Other Bacteria | <i>Cand. Thermokryptos mobilis</i> | 140944683 | --SKII-PSKK-----S | F-S-IL-T-- |
| | <i>Vagococcus salmoninarum</i> | 126781782 | --YF---II---RSN-----N | I-F-L-I-S-A-VMT |
| | <i>Marivibrio halodurans</i> | 210683211 | --HIA-P-L---L-A | LS-T-FM-IV-W-T |
| Other Bacteria | <i>Carnobacterium alterfunditum</i> | 143298371 | --FGQ-I-Q-AN-----N | I-FA-V-LV-II- |
| | <i>Ferruginivarius sediminum</i> | 114580575 | --A-E-VK-M-L-A | LS-MT-F-AIV-W- |
| | <i>Oenococcus kitaharae</i> | OEY83698 | --YFGK-L---AS-----FN | I-F-I-FL-- |
| Other Bacteria | <i>Leuconostoc suionicum</i> | 211636353 | --YFGQII-KYSG-----N | I-F-L-S-A |
| | <i>Xylanibacillus composti</i> | 213413321 | --YF---I---SN-----N | I-FASIM-LV- |
| | <i>Acidaminobacter hydrogenoformans</i> | 207646431 | --YFGK-V---QAN-----N | I-FA-IM-FLV-TT |
| Other Bacteria | <i>Bacillus mesophilus</i> | 163180499 | --YF-K-V-Q-SN-----N | I-FA-IM-LV-VTA |
| | <i>Psychrobacillus insolitus</i> | 111438262 | --YFGK-V---NAN-----N | I-FA-IM-IVA-T- |

Figure S10

Partial sequence alignment of the protein major facilitator superfamily transporter, showing a 1 aa insertion (boxed) that is specific for members of the order *Chlorobiales*. Except for an isolated exception shown here, the CSI was not found in any other bacterial species in the top 500-BLASTp hits.

| | | | | | |
|-------------------------------|--|--|-----------------------------|--|--|
| | | 294 | | 340 | |
| | | YLRHLTWEGAREKYG | DNPE | DGITPEDVKERIELELGVIEKMGFSSYF | |
| | <i>Chlorobium luteolum</i> | 78187124 | - - - Y - - KV - - A | Q - L - WQ - - EA - - R - - D - - TS - - - | |
| | <i>Prosthecochloris vibrioformis</i> | 139626774 | - - - Y - - KK - - A | HDS Q - LSW - KIE - - - K - - - IS - - - | |
| | <i>Chlorobium phaeobacteroides</i> | 642923410 | - - - - - A - - A | KSE - E - SQ - E - - - - - | |
| | <i>Chlorobium chlorochromatii</i> | 78188863 | - - - - - A - - A | SQA - - SS - - EA - - - - - | |
| | <i>Chlorobium phaeobacteroides</i> | 119357011 | - - - - - K - - A | GSEA - - E - - RA - - - - - | |
| | <i>Chlorobium limicola</i> | 189346342 | - - - - - A - - A | E - SK - E - S - - - - - | |
| | <i>Chlorobium ferrooxidans</i> | 493409850 | - - S - - - K - - A | GIS - EPSQ - E - RD - - - - - | |
| | <i>Chlorobium phaeovibrioides</i> | 145219820 | - - - A - - K - - P | - - - - - L - - K - - A | |
| | <i>Chlorobium phaeoclathestratiforme</i> | 194336619 | - - - - - L - - K - - A | - ADA - - SA - - RA - - - - - | |
| | <i>Prosthecochloris vibrioformis</i> | 139626774 | - - - Y - - KV - - A | PAS Q - L - WQ - - EA - - R - - D - - TS - - - | |
| | <i>Prosthecochloris marina</i> | 110023626 | - - - Y - - K - - A | PES Q - LSEKM - N - - - K - - ET - SS - - - | |
| | <i>Chloroherpeton thalassium</i> | 193215617 | - - - Y - - KK - - | LDA M - EKGAE - R - - - Y - SI - C - - YAT - - - | |
| | <i>Prosthecochloris aestuarii</i> | 194333751 | - - - Y - - - - A | PES Q - L - WQQ - ED - - K - - - IS - - - | |
| Order | <i>Chlorobiales</i> | TNJ36552 | - - - - - N - - | V - - AI - - E - - A - - - F - - - Y - A - - - | |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 21673669 | - - - - - K - - | AA - - TI - - E - - A - - - - - Y - A - - - | |
| | <i>Chlorobaculum tepidum</i> | 2501894946 | - - - - - | S - - AV - D - - A - V - - - - - Y - A - - - | |
| | <i>Chlorobaculum limnaeum</i> | 193212527 | - - - - - N - - | V - - AI - - E - - - - - Y - A - - - | |
| | <i>Chlorobaculum parvum</i> | 166808752 | - - S - - - K - - A | SEA E - SK - E - S - - - - - | |
| | <i>Candidatus Chlorobium masyuteum</i> | Cand. <i>Thermochlorobacter aerophilum</i> | RFM23112 | - - - Y - YQ - - LR - - | NLDA L - ERG - QIRN - - Y - NT - A - - - |
| | | 131353551 | - - - - - K - - | Q - S - - - K - - - - - | |
| | <i>Chlorobium sp. N1</i> | 076790384 | - - S - - - K - - A | SEA E - K - - - A - - - - - | |
| | <i>Chlorobium sp. KB01</i> | 068867018 | - - - Y - - KV - - A | PAS Q - L - WQ - - EA - - R - - D - - TS - - - | |
| | <i>Prosthecochloris sp. CIB 2401</i> | 094082691 | - - - Y - - - - A | PEA G - L - WKM - E - - - K - - - IS - - - | |
| | <i>Prosthecochloris sp. GS1</i> | 085659708 | - - - Y - - K - - A | PES Q - L - WQQ - ED - - K - - - TS - - - | |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 167399480 | - - - Y - - - - A | PES Q - L - WQQ - ED - - K - - - IS - - - | |
| | <i>Prosthecochloris sp. ZM</i> | 397689293 | - - TQ - AY - - LHRFRK | KVTREIED - MKY - D - I - - YAG - - - | |
| <i>Ignavibacteriae</i> | <i>Melioribacter roseus P3M-2</i> | 332299966 | - - - Y - - - - KKR - - | DPV - PE - Q - - DM - - - QN - - PG - - | |
| | <i>Porphyromonas asaccharolytica</i> | 521069907 | - - - K - - Y - - PR - K | EID - E - T - - - I - E - AG - - - | |
| | <i>Gracilimonas tropica</i> | 490463816 | - - Q - - Y - - KMR - - | DPI - - E - - - DF - - NT - KS - - PG - - | |
| | <i>Porphyromonas endodontalis</i> | 85818192 | F - - - VY - - KKR - - | EELS - E - T - - LDF - - D - - - T - YPG - - | |
| | <i>Dokdonia donghaensis</i> | 488757420 | - - - Y - - KR - - P | EITDE - R - - LDF - - SI - - - T - YPG - - | |
| | <i>Capnocytophaga sputigena</i> | 488750278 | - - - Y - - KR - - P | EITDE - R - - LDF - - SI - - - T - YPG - - | |
| | <i>Capnocytophaga ochracea</i> | 517457778 | - - - Y - - QIR - - | VM - DST - - DF - - FT - KT - - AG - - | |
| | <i>Niabella aurantiaca</i> | 489070762 | - - T - - Y - - KNR - - | EGETDEI - - LDF - - E - - ANT - YPG - - | |
| | <i>Chryseobacterium gleum</i> | 298207508 | F - K - - Y - - K - R - - | EITPEI - - LDF - - SI - - - T - YPG - - | |
| | <i>Croceibacter atlanticus</i> | 494155458 | - - - Y - - KKR - - | EITPEIE - - LDF - - T - - NT - YPG - - | |
| | <i>Kordia algicida</i> | 268317469 | - - - D - VF - - RR - - | DPL - HE - A - - L - H - AI - K - - AG - - | |
| | <i>Rhodothermus marinus</i> | 496475077 | - - - Y - - KKR - - P | VITDEI - - LDF - - ET - - RT - YPG - - | |
| | <i>Cecembia lonarensis</i> | 491609864 | - - - Y - - YM - - KKR - - | DELT - T - R - - DF - - ET - AN - PG - - | |
| | <i>Porphyromonas catoniae</i> | 518818409 | - - - K - - Y - - KKR - V | ELTDEI - - - DF - - AT - - T - YPG - - | |
| | <i>Empedobacter brevis</i> | 495857618 | - - - K - - Y - - QKR - - | SM - DST - - DF - - FT - KT - - AG - - | |
| | <i>Niabella soli</i> | 392390690 | - - - Y - - KQR - - | EITPEIT - - LDF - - AT - - NT - YPG - - | |
| | <i>Ornithobacterium rhinotracheale</i> | 340618163 | - - - I - Y - - KKR - - | EITPEID - - LDF - - K - - - T - YPG - - | |
| | <i>Zobellia galactanivorans</i> | 495915021 | F - - - YM - - KKR - - | EELT - EIT - - LDF - - S - - - NT - YPG - - | |
| | <i>Bizionia argentinensis</i> | 517772471 | F - K - - FV - - KKR - - | EIT - SI - - - LDF - - S - - - T - YPG - - | |
| | <i>Polaribacter franzmannii</i> | 488781602 | - - - Y - - TKR - - | EIT - EIR - - LDH - - NI - KS - - PG - - | |
| | <i>Microscilla marina</i> | 256423694 | - - - I - M - - HK - - A | EVTAE - E - - NF - S - - N - - AG - - | |
| | <i>Chitinophaga pinensis</i> | 496279563 | - - - K - - YQ - - KRR - A | EIT - EI - - LDF - - S - - - NT - YPG - - | |
| | <i>Galibbacter marinus</i> | 517866879 | - - - K - - F - - K - R - - | ELS - I - - L - F - - S - - - ANT - YPG - - | |
| | <i>Psychrophlexus tropicus</i> | 495886298 | - - - Y - - KRR - P | EIT - SI - - - LDF - - S - - - NT - YPG - - | |
| | <i>Joostella marina</i> | 522026781 | - - - Y - - KRR - D | TITPAI - - - LDF - - K - - - S - YPG - - | |
| | <i>Lewinella cohaerens</i> | 518500380 | - - - I - Y - - KKR - - | EITDEIR - - - DF - - KTV - NS - YPG - - | |
| | <i>Eudoraea adriatica</i> | 496382600 | F - - - Y - - K - E | EITDEIR - - LDF - - DI - A - T - YPG - - | |
| | <i>Elizabethkingia anophelis</i> | 496327208 | - - - Y - - KKR - K | EITDEI - - - LDF - - AT - - NT - YPG - - | |
| | <i>Indibacter alkaliphilus</i> | 544939559 | F - - - Y - - K - E | EITDEIR - - LDF - - DI - A - T - YPG - - | |
| | <i>Elizabethkingia meningoseptica</i> | 34539911 | - - - F - - Y - - R - - | SITPAI - - - LDF - - E - - RQS - YPG - - | |
| | <i>Saprosira grandis</i> | 379731533 | - - - Y - - KRR - - | EITDEIR - - LDF - - ET - - RT - YPG - - | |
| | <i>Cyclobacterium marinum</i> | 343084733 | F - - - Y - - KKR - - | EITPEID - - LDF - - K - - - T - YPG - - | |
| | <i>Cellulophaga lytica</i> | 325286607 | F - - - Y - - KIR - - | AITPEIQ - - LDF - - ET - A - T - YPG - - | |
| | <i>Cesiribacter andamanensis</i> | 496488129 | - - - Y - - KR - P | EDLSDEI - - - DF - - ET - KG - - PG - - | |
| | <i>Porphyromonas gingivalis</i> | 34539911 | - - - F - - Y - - R - - | HGL - KIRHLL - H - NL - - ELRYEP - - | |
| | <i>Planctomyces limnophilus</i> | 296121580 | - - Q - - - Q - - EQR - P | - GV - - K - I - TLRH - VTL - - DLQYEA - - | |
| | <i>Rhodopirellula europea</i> | 495942656 | H - KR - - - - GRWP | ENI - QEI - - - LSY - IS - - KQ - - G - - | |
| | <i>Dictyogloplomus turgidum</i> | 217967773 | - - FEK - C - - - KKRF - - | DPI - AAAR - - A - M - - - NR - - YDA - - | |
| | <i>Patulibacter medicamentivorans</i> | 494846815 | - - AQVE - - L - AR - - | | |

Figure S11

Partial sequence alignment of the protein DNA polymerase III subunit alpha, showing a 2-4 aa insert (boxed) that is specific for all members belonging to the order *Chlorobiales*

| | | 103 | DDKAIVGGFAILED | IPSGFS | QPVMIIGHQKGRDTK |
|--------------------------------------|--|------------|-----------------|---------------|-----------------|
| Order <i>Chlorobiales</i> | <i>Chlorobium phaeobacteroides</i> | 119356086 | -----RI- | SA----- | - |
| | <i>Chlorobium ferrooxidans</i> | 493410149 | -----R-- | RA--Y----- | V----- |
| | <i>Chlorobium luteolum</i> | 78187798 | -----R----- | RV----- | V----- |
| | <i>Chlorobium phaeovibrioides</i> | 145220402 | -----RI- | SAT----- | - |
| | <i>Chlorobium phaeoclathratiforme</i> | 194337654 | -----I---RI-E | ES----- | S-V----- |
| | <i>Chlorobaculum tepidum</i> | 21673002 | -----I---R-- | SA----- | T-M----- |
| | <i>Chlorobium limicola</i> | 189345751 | -----R-E | EAA----- | S-M----- |
| | <i>Chlorobaculum parvum</i> | 193213516 | -----I---L-R- | KD----- | S-V----- |
| | <i>Prosthecochloris aestuariai</i> | 194333138 | -----RI- | SEAS----- | T-V----- |
| | <i>Chlorobium phaeobacteroides</i> | 189499305 | -----LI---RI- | EERD----- | T----- |
| | <i>Chloroherpeton thalassium</i> | 193215260 | -----K-T | DEQR----- | KT----- |
| | <i>Chlorobaculum limnaeum</i> | 2501894381 | N-----I---RI- | EAT----- | S-V----- |
| | <i>Prosthecochloris marina</i> | 110022889 | -----RI-E | EE---K | T-V----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139457840 | -----I---R-A- | EAT----- | S-V----- |
| | <i>Candidatus Chlorobium masyuteum</i> | 166807605 | -----RI-E | PST----- | - |
| | <i>Candidatus Thermochlorobacteri GBCh1B</i> | KER09150 | -----I---K-S- | GQ----- | G-AV----- |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM23425 | -----RMI | EDGRA----- | T----- |
| | <i>Chlorobium sp. N1</i> | 131354938 | -----R- | RA--Y----- | V----- |
| | <i>Chlorobium sp. 445</i> | PT048713 | -----L-RMV- | DDHH----- | T----- |
| | <i>Chlorobium sp. KB01</i> | 076789707 | -----RI- | SA----- | R----- |
| | <i>Chlorobaculum sp. 24CR</i> | 129050980 | -----I---R- | EAT----- | S-V-----R |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085658914 | -----R- | RDLS----- | RT-V----- |
| | <i>Prosthecochloris sp. GSB1</i> | 094081818 | -----TIG | EADD-F | T-V----- |
| <i>Ignavibacteriae</i> | <i>Ignavibacterium album</i> | 78187798 | -----L-Q-GN | HK-V-M-Q----- | - |
| | <i>Melioribacter roseus</i> | 397689297 | -----K-DG | -K----- | - |
| | <i>Glaciecola polaris</i> | 494165670 | -----I---L-L-DE | --I-V----- | - |
| | <i>Salinimonas chungwhensis</i> | 517813267 | -----I---L-M-D- | --L----- | - |
| | <i>Glaciecola chattamensis</i> | 495264166 | -----I---L-M-DE | --I-V----- | - |
| | <i>Pseudoalteromonas atlantica</i> | 109897590 | -----I---L-M-DE | --I-V----- | - |
| | <i>Erwinia piriflorinigrans</i> | 562748821 | -----I-R--- | R-----E-- | - |
| | <i>Morganella morganii</i> | 571171262 | -----L-R-G | R--V----- | - |
| | <i>Erwinia pyrifoliae</i> | 259907558 | -----I-R-- | R-----E-- | - |
| | <i>Vibrio metschnikovii</i> | 490529482 | -----I-R-D | --E-R----- | - |
| | <i>Erwinia amylovora</i> | 312173371 | -----I-R-- | R-----E-- | - |
| | <i>Providencia sneebia</i> | 496196770 | -----I-R-G | --V-----E-- | - |
| | <i>Alteromonas macleodii</i> | 406595876 | -----P-M-D- | --N----- | - |
| | <i>Dickeya zeae</i> | 515508915 | -----I-R-D | R-----E-- | - |
| Other Bacteria | <i>Glaciecola arctica</i> | 494895163 | -----L-KIDG | -- | - |
| | <i>Dickeya dadantii</i> | 271499511 | -----I-R-D | R-----E-- | - |
| | <i>Nitrococcus mobilis</i> | 491140726 | -----P---V-R-G | --V----- | - |
| | <i>Salmonella enterica</i> | 487762226 | -----I-R-G | R-----E-- | - |
| | <i>Yersinia mollarettii</i> | 491012799 | -----I-R-G | R-----E-- | - |
| | <i>Shigella flexneri</i> | 491248512 | -----I-R-DG | R-----E-- | - |
| | <i>Yersinia frederiksenii</i> | 490844621 | -----I-R-DG | R-----E-- | - |
| | <i>Yersinia ruckeri</i> | 490857600 | -----I-R-G | R-----E-- | - |
| | <i>Geobacter daltonii</i> | 222056374 | -----H---L-R-DG | E--V----- | - |
| | <i>Geobacter uraniireducens</i> | 148264551 | -----H---L-R-DG | V--V----- | - |
| | <i>Solitalea canadensis</i> | 387792181 | -----TIDG | --KN----- | - |
| | <i>Thermotomaculum hydrothermale</i> | 201328645 | -----F-D- | I-C---Q----- | - |
| | <i>Alteromonas macleodii</i> | HBI76693 | -----P-M-D- | --N----- | - |
| | <i>Salinimonas lutimaris</i> | 137167805 | -----L---L-M-D- | --I-L----- | - |
| | <i>Morganella morganii</i> | HBH7053723 | -----I---L-R-G | R--V----- | - |
| | <i>Vibrio metschnikovii</i> | 217523606 | -----I-R-D- | --E-R----- | - |
| | <i>Bowmanella denitrificans</i> | 102796617 | -----L-R-D- | -- | - |
| | <i>Klebsiella pneumoniae</i> | NGE24729 | -----L-R-G | R--V----- | - |
| | <i>Providencia sneebia</i> | 008916607 | -----I-R-G | --V-----E-- | - |

Figure S12

Partial sequence alignment of the protein acetyl-CoA carboxylase carboxyltransferase subunit alpha, showing a 6 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | | | | | |
|--|--|--|------------------------------|--------------|-----------------|
| | | | LHVHYAIPHAMSALLAKEMLKEKEDRH | QNF | KLVTTLHGTDITIVG |
| | | | V-A-----L--M--RQ--IED--CSEL | KC | -V-- |
| | | | V-A-----L--M--RQ--EDSCSEA | KC | -A-----V- |
| | | | V-A-----I--M--RQ--E--CPAA | EC | RI----- |
| | | | V-A-----M--RQ--EDRCPDA | SC | R-A-----V- |
| | | | V-A-----I--V--RQ--E--RCPES | -C | RIA----- |
| | | | V-A-----M--RQ--ED--CCGS | NC | -IA-----V- |
| | | | V-A-----L--M--RQ--ED--CAEA | RC | R-A-----V- |
| | | | V-A-----L--M--LQ--ED--CAAA | HC | -----V- |
| | | | V-A-----M--RQ--ED--CPQR | DC | R-A-----V- |
| | | | V-A-----L--M--RQ--E--SCSDA | RC | R-A-----V- |
| | | | V-A-----L--I--RQ--VED--CSKA | -C | RIA-----V- |
| | | | V-A-----I--M--RQ--E--CPAA | EC | RI----- |
| | | | V-A-----I--M--RQ--E--CPAS | EC | RI----- |
| | | | V-A-----M--RQ--ED--CSSS | KC | R-A-----V- |
| | | | V-A-----M--RQ--ED--CPER | GC | R-A-----V- |
| | | | V-A-----M--RQ--ED--CCGS | NC | -IA-----V- |
| | | | V-A-----L--I--RQ--VED--CSKA | -C | RIA-----V- |
| | | | V-A-----L--I--RQ--ED--CSES | RC | R-A-----V- |
| | | | V-A-----I--M--RQ--E--CPAS | EC | RIA----- |
| | | | V-A-----L--M--RQ--ADTCRDA | DC | RIA----- |
| | | | -----V-GY----QV-ARDGIQL | RV | --L----- |
| | | | V-----V-GF--SLI--D-YPF | --L----- | --II-----LI- |
| | | | -----S--YM--LI-N-QGRDI | --II-----LI- | --FI-----LM- |
| | | | M-----V--Y--QIR--KSNGDI | --FI----- | --FI----- |
| | | | V-----T--Y--KGYCDL | --II----- | --II----- |
| | | | -----S--Y--QI--SQGIHI | PFI----- | --L----- |
| | | | -----SA--YM--QI----NINI | PV----- | --L----- |
| | | | -----T--F--QI--QQEGLDI | PVI----- | --L----- |
| | | | -----ST--Y--QIVSKQGRIV | PFI----- | --L----- |
| | | | -----SA--YM--QI--TQGIDI | PV----- | --L----- |
| | | | -----SA--YM--QI--T--GINI | PF----- | --L----- |
| | | | -----YA--YI--QI--RGKDL | PII----- | --L----- |
| | | | -----SA--YM--QI--TQGIEI | PV----- | --L----- |
| | | | -----S--YM--QI--TEGIQI | PV----- | --L----- |
| | | | -----SA--Y--QI--RSYGIDI | PV----- | --L----- |
| | | | -----SA--YM--QI--EGIQI | PV----- | --L----- |
| | | | -----SA--YM--QI--TEGINI | PV----- | --L----- |
| | | | -----SA--YM--QI--SQGISI | PV----- | --L----- |
| | | | -----T--F--QI--CHGINI | PII----- | --L----- |
| | | | -----SA--YM--QI--KQGVII | PV----- | --L----- |
| | | | -----SA--YM--QI--KQGIVI | PV----- | --L----- |
| | | | -----SA--YM--QI--SEGISI | PVI----- | --L----- |
| | | | I--A-----YT--YM--QI--EKEGLDV | -II----- | --L----- |
| | | | -----SA--FM--QOI--AQGINI | PFI----- | --L----- |
| | | | -----SA--YM--QI--EGIHI | PV----- | --L----- |
| | | | -----SA--YM--KI--EKEGKHI | PVI----- | --L----- |
| | | | -----SA--YM--QI--EGIHI | PV----- | --L----- |
| | | | -----I--Y--Q--V--KDV | -II----- | VL-- |
| | | | -----SV----RQ--EA--GI--L | PFI----- | --L----- |
| | | | -----I--F--R--AKRGLHI | PI----- | --L----- |
| | | | -----V--Y--Q---GELEL | PVI----- | --L----- |
| | | | -----YA--YM--Q----GIDI | -V----- | --L----- |
| | | | -----SI--Y--RQ--A--RRRL | PF----- | --L----- |
| | | | -----YA--YM--Q----GIDI | -VI----- | --L----- |
| | | | -----YA--YM--Q----GLDV | RV----- | --L----- |
| | | | -----SA--YM--QI----NIKI | PV----- | --L----- |
| | | | -----YA--FM--Q----QGIDI | -V----- | --L----- |

Figure S13

Partial sequence alignment of the protein N-acetyl-alpha-D-glucosaminyl L-malate synthase BshA, showing a 3 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 228 | 278 |
|--------------------------------------|---|--|-----|
| Order <i>Chlorobiales</i> | <i>Chlorobium limicola</i> WP_041466021 <i>Chlorobium luteolum</i> WP_011358316 <i>Candidatus Chlorobium masyuteu</i> WP_166806951 <i>Chlorobium sp. KB01.</i> WP_076791726 <i>Chlorobium sp. N1</i> WP_131354081 <i>Chlorobium phaeobacteroides</i> WP_011744490 <i>Chlorobium phaeovibrioides</i> WP_011890615 <i>Pelodictyon phaeoclathestriforme</i> WP_012507517 <i>Chlorobium ferrooxidans</i> WP_006365450 <i>Chlorobaculum tepidum</i> WP_010933254 <i>Chlorobaculum thiosulfatiphilum</i> WP_139456325 <i>Chlorobaculum parvum</i> WP_012502749 <i>Chlorobaculum limnaeum</i> WP_069808880 <i>Prosthecochloris ethylica</i> WP_114608737 <i>Prosthecochloris vibrioformis</i> WP_139626252 <i>Prosthecochloris aestuarii</i> WP_012505177 <i>Prosthecochloris marina</i> WP_110024280 <i>Chloroherpeton thalassium</i> WP_012500494 <i>Cand. Thermochlorobacteriaceae GBCh1B</i> KER09762.1 <i>Chlorobium sp. 445</i> PI047396.1 <i>Chlorobi bacterium OLB7</i> KXK56915.1 <i>Chlorobi bacterium OLB4</i> KXK04106.1 <i>Chlorobi bacterium OLB5</i> KXK53076.1 <i>Chlorobi bacterium OLB6</i> KXK35191.1 <i>Ignavibacteriales bacterium UTCHB3</i> OQY75246.1 <i>Melioribacter roseus</i> WP_014854729 <i>Ignavibacterium album</i> WP_014560320 <i>Cand. Kryptonium thompsoni</i> WP_047133370 <i>Cand. Kapabacteria thiocyanatum</i> OJX61272.1 <i>Rhodothermus marinus</i> WP_012842647 <i>Bacteroides fragilis</i> WP_008768732 <i>Phocaeicola massiliensis</i> WP_117462813 <i>Salinibacter ruber</i> WP_118838368 <i>Cytophaga aurnatiaca</i> WP_018341732 <i>Sinomicrobiun oceanii</i> WP_072318454 <i>Thermaurantimonas aggregans</i> WP_124398693 <i>Seonamhaeicola marinus</i> WP_148541715 <i>Schleiferia thermophila</i> WP_037361247 <i>Snuelia sedimenti</i> WP_199115674 <i>Tamiana sedimentorum</i> WP_044632150 <i>Flavobacterium koreense</i> AFR46549 <i>Seonamhaeila aphaedonensis</i> WP_116039982 | KTLRGPRLGIMMMGSDFENPLGI TI KTKTGSRVKMMSEVMDAEVMPGIQGG -----K---M-----N-P----- -----M----- -----M----- -----R---M---V---N-P----- -----T----- -----K---M---N-Q----- -----M-----L----- -----M----- -----K-----L---N-N-----I----- -----K-----L---N-N-P----- -----K---M---V---N-N-P-----I----- -----DK----L---N-N-P----- -----Q---M---R-T---I---I----- -----K-----V---N-P-T---I---I----- -----E---M---A-R-T---I---I----- -----Q-----V---K-V-T---I---I----- -----L-K---M---KA---E-I---V---LL-M----- -----L-K---F---KL---E-L-L---L-T---V----- -----L-K---F---KV---E-L---L-T---V----- -----V-L---F---VAPKSG-T---II-SW----- -----I-L---K---TF---VA-S---T---LI-SM----- -----L-A---F-L---TAPKSG---LI-SM-I---V----- -----L-LL-K-V---F-V---AP-S---LI-SM----- -----V-L---F---TVPKTG-A---LI-STI----- -----V-L---F-----VK-D-L-L---IF-GM----- -----I-LI-K-K---W-L---T-PK-D---LI-GM----- -----L-L-HK-R---F---PK-D-LR---II-GT----- -----L-I---KNY---F-V---AP-S---T-L-G-LI-SM----- -----LI-R---D-F---TA-S---I-K---LL-SA-F---T----- -----V---K---P---W-K---KPK-EI---QLL-SA-F----- -----V---K---P---W-K---PK-EI---QLL-SA-F----- -----LL-D-Y---M-K---TARKSG-T---LL-SA-F---T----- -----V---K---F-L---PK-E-R---N-L-MG-F---T----- -----K---F---LKNGKPR---SLL-SA-F---N----- -----L-L---K---F-K---PK-EIR---SLI---A-F---V----- -----Q---F---LKSGKLRK---SLL-SA-F---N----- -----L-L---N---F-K---PK-EIR---SLI---A-F---V----- -----E---D-F---LKSGKLRK---SLL-SA-F---N----- -----K---F---LKNSLRK---SLL-SA-F---N----- -----K---W-L---PK-EIR---H-L-MS-F---N----- -----L---K---F---LKSGKLRK---SLL-SA-F---N----- | |

Figure S14

Partial sequence alignment of the protein Serine hydroxymethyltransferase, showing a 2 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | | 94 | | |
|---|--|-----------|--------------------------|---|
| Order <i>Chlorobiales</i> | <i>Chloroherpeton thalassium</i> | 193215546 | ALNGAPGVSYARYAATPQKEKPS | YAD NVDKLLSDMQNLTNRTARFRFTVIAL |
| | <i>Chlorobium phaeobacteroides</i> | 189500796 | G-----F-PV-EGTS-T | -D - NH - KE - E - V - D - K - T - - - V - |
| | <i>Chlorobium ferrooxidans</i> | 493409268 | --G-----F-PM-EGKS- | -Q - - RH - - AS - AGCS - - K - L - - - |
| | <i>Prosthecochloris aestuarii</i> | 194334496 | --K-----F-PM-EGRE- | -E - - QH - - TT - - HTLE - S - T - - - I |
| | <i>Chlorobium phaeovibrioides</i> | 145219308 | --G-----PVANG---T | -R - - TH - - NE - EGEKD - S - - - |
| | <i>Chlorobium phaeoclatratiforme</i> | 194337267 | G-D-----F-PV-DGQS-T | -K - - AH - - HC - KGIA - E - - - |
| | <i>Chlorobaculum parvum</i> | 193213344 | ----E-----F-PM-PGQS-T | - - - RH - - DR - TGK - D - S - - - M |
| | <i>Chlorobaculum tepidum</i> | 21673169 | --G-----PV-EGVART | -E - - RH - - E - RGKSK - - - M |
| | <i>Chlorobium chlorochromatii</i> | 78188461 | --D-----P-ADGTA-T | -S - - NH - - KN - AGKEE - S - C - SL - |
| | <i>Chlorobium luteolum</i> | 78186333 | Q-G-R-----F-PR-EG- | -S - - RH - - QE - EGKVE - S - - - |
| | <i>Chlorobium limicola</i> | 189347225 | S-G-E---L---F-PVKTGM-T | -E - - I - H - - LR - NGIKE - N - - - |
| | <i>Prosthecochloris ethylica</i> | 175187634 | --D-----F-PM-EGQL-A | -E - - RH - - KV - - HHS - - N - T - - - I |
| | <i>Prosthecochloris marina</i> | 110024145 | G-----F-PM-GGKP-T | -D - - NH - - KM - ESI - D - K - L - - - |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139457588 | --G-----PV-EGVART | - - - RH - - E - RGKSE - - - M |
| | <i>Candidatus Chlorobium masyuteum</i> | 166807860 | --D-----F-PM-EGKS- | -Q - - RH - - AN - AGCS - - N - Q - - - |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM23469 | --G-E---H---H---AELGRQ-T | -SE - - A - - RK - - IRQ - Q - V - - - MAL |
| | <i>Cand. Thermochlorobacteriaceae GBCh1B</i> | KER10877 | --G-----TAEGLR-T | - - - E - - R - LSGASS - A - - - LS - |
| | <i>Prosthecochloris sp. ZM</i> | 114616877 | --K-----F-PM-EGRE- | -E - - QH - - TT - - HTLE - S - T - - - I |
| | <i>Chlorobaculum sp. 24CR</i> | 129051383 | --G-----PV-EGATRT | - - - RH - - E - RGKSE - - - M |
| | <i>Chlorobium sp. KB01</i> | 076792242 | --G-----F-PM-EGKS- | -Q - - RH - - AS - AGCS - - K - L - - - |
| | <i>Chlorobium sp. 445</i> | PI048559 | --G-----H-----EREGLR- | -SE - - A - - RELKGV - H - E - K - - - |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085660539 | -----F-PM-EGTS-T | -E - - RH - - GT - KGHS - N - T - - - M |
| | <i>Prosthecochloris sp. GSB1</i> | 094082037 | --D-----PVNDERS-T | -E - - RH - - AM - R - ER - - E - S - - - M |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobi bacterium OLB7</i> | KKK51637 | --E-----GPDATYAD | - - - CR - - IDTLADQE - - R - Q - - LC |
| | <i>Ignavibacterium album</i> | 385810929 | Q---E - I-----GESADDL | -NE - - IKAI - KFPEPHRAKFCV - S |
| | <i>Bacteroides stercoris</i> | 491891338 | -----GDHNSEA | -MQ - - Q - EGME - K - - - F - |
| | <i>Bacteroides clarus</i> | 496413113 | -----GDHNSEA | -MK - - Q - EGIE - K - Q - - F - |
| | <i>Bacteroides gallinarum</i> | 517495029 | --G-----GNAHDEA | -MK - - Q - EGIE - K - - - F - |
| | <i>Porphyromonas levii</i> | 517170024 | --G-----GPKCNP-D | -IE - - GEL - GI - K - - - |
| | <i>Bacteroides massiliensis</i> | 492742940 | --E-----GGEGHNAE | A - MQ - - QN - - GVQ - - K - Q - - C - |
| | <i>Owenweeksia hongkongensis</i> | 375012035 | -----GEGANSEN | -M - - TELDGKE - - K - Q - - Y |
| | <i>Echinicola vietnamensis</i> | 431796557 | --D-----G - GA - RSDDR | - - - EN - EGV - D - K - Q - - |
| | <i>Gracilimonas tropica</i> | 521070784 | --D-----F-----GEDASY-D | - LE - - E - EGK - V - S - Q - - V - |
| | <i>Parabacteroides distasonis</i> | 492483186 | V - N-----G - GHDEA | - MN - - E - NHKE - K - - - |
| | <i>Prevotella maculosa</i> | 518812447 | --G-----EQDDHNSE | A - MA - - HN - ADI - C - N - - - S - |
| | <i>Porphyromonas somerae</i> | 516661827 | --R-----GLECRAED | -IQ - - GEL - GIQ - S - - - V - |
| | <i>Etmicicia oligotrophica</i> | 408673533 | --E - F-----GEHGNHAK | -IE - - QTLEGVE - G - Q - S - T - |
| | <i>Prevotella veroralis</i> | 490517531 | --E - H-----EGTDHDSE | A - MR - - K - S - VKD - - - S - |
| | <i>Cecembia lonarensis</i> | 496476136 | --D-----G - GE - RSDER | -IN - - KE - EGK - K - - - |
| | <i>Parabacteroides goldsteinii</i> | 497546258 | --N-----GPGDHSEA | -MK - - RE - EGME - K - - - |
| | <i>Photobacterium angustum</i> | 491513700 | Y-----I - F - GEGASDAD | -I - - AE - KDVPAEQRTA - FHCV - |
| | <i>Pseudoalteromonas spongiae</i> | 498247569 | --G-----I - F - GVANDSD | -I - - ELGDPN - - WC - LV - |
| | <i>Vibrio cholerae</i> | 446647661 | Y-----I - GEHASDGD | -LN - - AA - - VP - DQRSA - FHCV - |
| | <i>Staphylococcus warneri</i> | 488381256 | --E-----GLDKNDQD | -IN - - KN - E - ISD - N - Q - VC - SM |
| | <i>Staphylococcus epidermidis</i> | 488407224 | --E-----GLGKNDED | -IE - - TNLEDVQD - R - Q - VC - SM |
| | <i>Prevotella bergensis</i> | 494310511 | --G-----H - EGTDHDE | A - MA - - HELEGKE - - - S - |
| | <i>Echinicola vietnamensis</i> | 041739508 | --D-----G - GA - RSDD | R - - - EN - EGV - D - K - Q - - |
| | <i>Flavobacterium cerinum</i> | 128389128 | --E-----GEQKSAE | - - M - - KALEDS - - N - K - - |
| | <i>Enterococcus faecalis</i> | RXF53884 | --G-----GENKSFD | - - IE - - RE - NGAND - N - - - |
| | <i>Niabella yanshanensis</i> | 114790679 | --E - K - DNEPEFA | - - I - - NK - K - QG - N - - - S - |
| | <i>Hymenobacter busanensis</i> | 151078078 | -----GPQRDA | - - IQ - - LEL - - Q - D - A - Q - - V - |
| | <i>Porphyromonas levii</i> | 134849553 | --G-----GPKCNP - | - - IE - - GEL - GI - K - - - |
| | <i>Chitinophaga silvisoli</i> | 116851431 | -----GEHKSEE | - - I - A - - E - KGK - D - R - Q - - S - |

Figure S15

Partial sequence alignment of the protein deoxyribonucleotide triphosphate pyrophosphatase, showing a 3 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | | 37 | 69 |
|---|---|------------|--|
| Order <i>Chlorobiales</i> | <i>Chlorobium limicola</i> | 189346136 | LGDTMVLATVSSKKTPP P NQDYFPLQVEYREK |
| | <i>Chlorobium phaeoclathratiforme</i> | 194335788 | Q-----I----- |
| | <i>Chlorobium ferrooxidans</i> | 493409513 | ----- |
| | <i>Chlorobium chlorochromatii</i> | 78189646 | ----- |
| | <i>Chlorobium phaeobacteroides</i> | 119356388 | -----TP-A |
| | <i>Chlorobium phaeovibrioides</i> | 145220228 | M-----I-----S-----F----- |
| | <i>Chlorobaculum parvum</i> | 193212209 | -N-----TP-S-----S-F----- |
| | <i>Prosthecochloris aestuarii</i> | 194333349 | -N-----TP-S-----SF----- |
| | <i>Chlorobium luteolum</i> | 78187489 | M-----I-----R-----S V-----F----- |
| | <i>Chlorobaculum tepidum</i> | 21674467 | MN-----TP-S-----F----- |
| | <i>Chlorobium phaeobacteroides</i> | 189499525 | M-----I-----RTP-S-----F-----M----- |
| | <i>Chloroherpeton thalassium</i> | 193214790 | -EE-----K-DA-A-----NF----- |
| | <i>Chlorobaculum limnaeum</i> | 2501894253 | MN-----TRTP-S-----F----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139456275 | -----T-TP-L-----F-----M----- |
| | <i>Prosthecochloris marina</i> | 110023770 | M-----TP-----F----- |
| | <i>Cand. Thermocholorobacter aerophilum</i> | RFM23016 | -AE-----ARKDD-V-----G-FI-----F----- |
| | <i>Candidatus Chlorobium masyuteum</i> | 166806995 | ----- |
| | <i>Chlorobium sp. N1</i> | 131354161 | M-----I-----R-A-S V-----F----- |
| | <i>Chlorobium sp. KB01</i> | 076791792 | ----- |
| | <i>Chlorobaculum sp. 24CR</i> | 129050239 | -N-----P-S-----F----- |
| | <i>Chlorobium sp. N1</i> | 131354161 | M-----I-----R-A-S V-----F----- |
| | <i>Prosthecochloris sp. ZM</i> | 114618152 | -N-----TP-S-----SF----- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobi bacterium OLB5</i> | KXK53677 | ---I-C-GADE-K E GM-----F----- |
| | <i>Chlorobi bacterium OLB4</i> | KXK02521 | V--NV-C-A-TA-EE-A -----G-F-----S-D----- |
| | <i>Chlorobi bacterium OLB7</i> | KXK53815 | -----VA-AAG---R E GI-FL-----F----- |
| | <i>Ignavibacterium album</i> | 385809228 | Y-----V-A-EDVKD D-----F----- |
| | <i>Melioribacter roseus</i> | 397690716 | Y-----V-A-AGE VRD DI-F-----S----- |
| | <i>Marinimicrobia bacterium</i> | 551209032 | Y-----V-A-ANYFSKS E-----F-----R----- |
| | <i>Kiloniella laminariae</i> | 522079354 | Y---V-C-A-GARDVK-G-----F-----T-N-Q----- |
| | <i>Magnetococcus marinus</i> | 117926996 | Y---V-V-A-AETSMRA G-----F-----S-H-Q----- |
| | <i>Acetobacter pasteurianus</i> | 517918340 | Y-E-V-C-A-GA-DVK-G-----F-----T-N-Q----- |
| | <i>Gluconobacter oxydans</i> | 414341474 | Y---V-C-A-GA-SVK-G-----F-----T-N-Q----- |
| | <i>Oceanicaulis alexandrii</i> | 550952395 | Y-E-T-----AQ-DVK-GM-----F-----T-N-Q----- |
| | <i>Gluconobacter frateurii</i> | 565862949 | Y---V-C-A-GA-SVK-G-----F-----MT-N-Q----- |
| | <i>Gluconobacter thailandicus</i> | 494494374 | Y---V-C-A-GA-SVK-G-----F-----MT-N-Q----- |
| | <i>Acetobacter pomorum</i> | 493059067 | Y-E-V-C-A-GA-EVK-G-----F-----T-N-Q----- |
| | <i>Saccharibacter floricola</i> | 517809778 | Y-E-V-C-A-GA-TVKA G-----F-----T-N-Q----- |
| | <i>Gluconacetobacter hansenii</i> | 489712894 | Y---V-C-A-GA-SVK-G-----F-----T-N-Q----- |
| | <i>Gluconacetobacter diazotrophic</i> | 162147166 | Y---V-C-A-GARSVK-G-----F-----T-N-Q----- |
| | <i>Elioraea tepidiphila</i> | 517846087 | Y---V-----GA-T-K G-----F-----S-H-Q----- |
| | <i>Bartonella schoenbuchensis</i> | 498389360 | Y-E-I-----A-NPK D-----F-----T-N-Q----- |
| | <i>alpha proteobacterium</i> | 496820156 | W---VIIS-A-A----N DT-----F-----T-N-Q----- |
| | <i>Xanthobacteraceae</i> | 517199826 | Y---T-----A-A-SAK G-----F-----T-N-Q----- |
| | <i>Bartonella bovis</i> | 498386729 | Y-E-I-----A-NPK D-----F-----T-N-Q----- |
| | <i>Acetobacter tropicalis</i> | 493605112 | Y-E-V-C-A-GA-NVKA G-----F-----T-N-Q----- |
| | <i>Azospirillum lipoferum</i> | 374293408 | Y---T-C-A-AA-APK GV-----F-----T-N-Q----- |
| | <i>Bartonella melophagi</i> | 494740673 | Y-E-I-----A-NPK D-----F-----T-N-Q----- |
| | <i>Azospirillum amazonense</i> | 490393941 | Y---V-C-A-GA-SVK G-----F-----T-N-Q----- |
| | <i>Agrobacterium tumefaciens</i> | 523694192 | Y-E-----A-SPK G-----F-----T-N-Q----- |
| | <i>Methylovorus glucosetrophus</i> | 253997787 | Y---V-V-GKTDVK G-----F-----T-D-Q-R----- |
| | <i>Methylobacillus flagellatus</i> | 91774427 | Y---V-VS-GKREVKE G-----F-----T-D-Q----- |
| | <i>Thiobacillus denitrificans</i> | 74316711 | MD-----V-AKNEVK G-----F-----T-D-Q----- |
| | <i>Leeia oryzae</i> | 516890550 | M---V-VS-AATNVK G-----F-----T-D-Q-R----- |
| | <i>Selenomonas bovis</i> | 518371421 | Y---V-V-ATA-EPRE GV-----F-----T-D-E----- |
| | <i>Mitsuokella multacidica</i> | 492423460 | Y---V-V-ATA-AEPRGV-----F-----T-D-E----- |
| | <i>Selenomonas flueggei</i> | 493740328 | Y---V-V-ATA-APRE GV-----F-----T-D-E----- |
| | <i>Caldithrix abyssi</i> | 493987866 | Y-----V-ANDEMTE QR-FM-----T----- |
| | <i>Rhodothermus marinus</i> | 268316873 | Q-----C-A-LADEPRE G-SF-----T----- |
| | <i>Enterococcus faecalis</i> | 514895238 | Y---V-SAA-A-EAKD T-F-----TIN-E----- |
| | <i>Haemophilus influenzae</i> | 270315521 | MD-T-FV-----AK-DVKE G-----F-----T-N-Q-R----- |
| | <i>Pasteurella multocida</i> | 401694220 | MD-T-FV-----AK-DVKE G-----F-----T-N-Q-R----- |
| | <i>Megamonas funiformis</i> | 547819988 | Y-E-V-V-ATA-AEPRGV-----F-----T-D-E----- |
| | <i>Acidithiobacillus ferrovorans</i> | 344200724 | S---V-V-A-ARREMKG-----F-----T-N-Q----- |
| | <i>Stenotrophomonas maltophilia</i> | 491555837 | M---V-V-A-G-RAKD GL-F-----T-----R----- |
| | <i>Fangia hongkongensis</i> | 517111971 | A---V-V-A-AK-EVKD GA-F-----T-N-Q----- |
| | <i>Roseiflexus castenholzii</i> | 156743526 | Y---L---A-A-EPRT DT-F-----T-D-E----- |
| | <i>Thermovibrio ammonificans</i> | 319789227 | Q---I-V-A-M-DEPRE DV-F-----L-----R----- |

Figure S16

Partial sequence alignment of the protein polynucleotide phosphorylase/polyadenylase, showing a 1 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

**Order
Chlorobiales**

Ignavibacteriae

Other Bacteria

| | | | | |
|---|------------|------------------------------|--------------------------------|--------------------------------|
| <i>Chlorobaculum parvum</i> | 193212209 | KEERSDRTKAIYKETIAKTLHEHFSS | ITAEELIAANPEKALCQS | EHMIDECIHDVEKKVMRHMILDDSKRLDGR |
| <i>Chloroherpeton thalassium</i> | 193214790 | ---A-K-AEV-ADAS-AV-RKYKAE | --D-IL-E----IYLN | -KA-NDY---I-H--E--A-A--- |
| <i>Prosthecochloris aestuarii</i> | 194333349 | ---AEK-AE---NAK-Q--QRYQQE | --P-V---E---YLN | -QI-GDA-SI--Q--E---A--- |
| <i>Chlorobium phaeobacteroides</i> | 189499525 | ---AEK-T-V--SITEQI-GRYKNE | --D---D-S---YLN | -QI-SGH-AI--Q-----A- |
| <i>Chlorobium phaeoclatratiforme</i> | 194335788 | ---A-Q-A---H-I-ES-I---KAT | CSS-FSTD-A---IN | P-I---Q-M--R-----A- |
| <i>Chlorobium luteolum</i> | 78187489 | ---V-A-E----S--VK-AI---AA | FGP-AL--D-S---LN | --I-E-E-SF--N---R-----G- |
| <i>Chlorobium phaeovibrioides</i> | 145220228 | ---AE---E--G--LKSAV---AA | FSS-DL--D-S---TN | --I-E-E-S---D--R-----A- |
| <i>Chlorobium chlorochromatii</i> | 78189646 | ---AE---A---N-L-A---RAT | SS--AQ-DTA---LN | --I-EDQ-A--E-----A- |
| <i>Chlorobium ferrooxidans</i> | 493409513 | --D-A-Q-AH-R--QAIV-K-K-S | -SS-D---D-A---LN | --I-E-E-S----- |
| <i>Chlorobium limicola</i> | 189346136 | --D-AEQ-A---R-I-QS-V--KAA | -SP---Q-D-S---LN | --I---Q-A-----A- |
| <i>Chlorobaculum tepidum</i> | 21674467 | ---AEK----TQ---R---T---TDR | VGPDQ-E-D-TS-F-LN | --E-----A-----G- |
| <i>Chlorobaculum limnaeum</i> | 2501894253 | ---AEK----TQ-LR---T---TDR | VGPDQ-E-D-SS-F-LN | --E-----S----- |
| <i>Chlorobaculum thiosulfatiphilum</i> | 139456275 | ---AEK----TQ-LR---T---TDR | VGPDQ-E-D-SS-F-LN | --E-----A----- |
| <i>Candidatus Chlorobium masyuteum</i> | 166806995 | --D-A-Q-AH-R--QAIV-KFK-- | -SS-D---A---LN | --I-E-E-S----- |
| <i>cand. Thermochochlorobacter aerophilum</i> | RFM23016 | -DV-AEK----D-L-AV--KYKAE | --P-VL-S---YLN | --KL-RQV--C-RDA--QT--N-RV-- |
| <i>Chlorobium sp. N1</i> | 131354161 | ---A-E----G--VQQ-I---AA | L---L---DSS---LN | --I-E-E-SI-----R-----G- |
| <i>Chlorobium sp. KB01</i> | 076791792 | --D-A-Q-AH-R---QTIV-KF-- | -SS-D---D-A---LN | --I-E-E-S----- |
| <i>Prosthecochloris marina</i> | 110023770 | ---AEK-SE---SVTQ-I-ARYKEE | -S-DD---D-A---YLN | --QI-SGH--I-----A- |
| <i>Prosthecochloris sp. ZM</i> | 114618152 | ---AEK-AE---NAK-Q--QRYQQE | -P-V---E---YLN | --QI-GDA-SI--Q--E-----A- |
| <i>Prosthecochloris sp. GSB1</i> | 094083107 | --K-AEK-SE-TSSAFD---RYRNE | --D-ALR-D-SR-IYLN | --QI-S-Q--KI--Q-----E----- |
| <i>Melioribacter roseus</i> | 397690716 | ---QKN-ELEE-VVEQLA-K-PEQ | KV-KTIL--M--EL--QR--SEGL-- | |
| <i>Ignavibacterium album</i> | 385809228 | ---AKN-ELAEFVKTSLA-KYPEQ | -KV-A-LL--M--EL--QR--TEGI-- | |
| <i>Gordonia polyisoprenivorans</i> | 378717577 | -Q---D-K-DELKEQILTQLA-Q-EGR | -KE-GAAFRS-T--LV-QR--T-HF-I-- | |
| <i>Gordonia paraffinivorans</i> | 493955506 | -Q---D-K-DELKADILEQLADK-EGR | -KE-GGAYRALT--LV-QR--T-HF-I-- | |
| <i>Gordonia bronchialis</i> | 262202098 | -Q---DEK-DELKDDIL-QLG-Q-EGR | -KE-GAAYRS-T--LV-QR--T-HF-I-- | |
| <i>Gordonia araii</i> | 494531914 | -Q---DEK-DELKA-LLEQLGGQ-EGR | -KE-GAAYRSLT--LV-QR--T-HF-I-- | |
| <i>Mycobacterium gilvum</i> | 145224603 | --A-D--NE-KA-VVERLA-QYAGR | -RE-RAAHN--T-TIV-DR--TEGF-I-- | |
| <i>Corynebacterium bovis</i> | 497951292 | -Q---DEAL-A-NLD-VVETLAGQ-PDR | -GE-RAAHN--T-QLV-QR--EEGF-I-- | |
| <i>Corynebacterium resistens</i> | 336325779 | -Q---D-ALAENMQA-VDEL--E-PER | -KELGAAFRS-T--LV-QR--T-GF-I-- | |
| <i>Nocardia farcinica</i> | 54025857 | -Q---EEKIDE-KLAVLDRLA-Q-AGR | -KE-GAAFRSLT--LV-QR-VT-HF-I-- | |
| <i>Mycobacterium liflandii</i> | 443490346 | -A--NO--EE-KA-VLERLADTYEGR | -KE-GAAFRSLT--LV-QR--R-QF-I-- | |
| <i>Rhodococcus coprophilus</i> | 555592905 | -Q---D--DE-KV-VLEQIAPK-EGR | -KE-GGAYRSLT--LV-KR--T-HF-I-- | |
| <i>Gordonia terrae</i> | 490121385 | -Q---D-K-DELKADIL-QLG-Q-EGR | -KE-GAAYRSLT--LV-QR--T-HF-I-- | |
| <i>Gordonia soli</i> | 494899217 | -Q---DSK-DELKADVLLEQLAGQ-EGR | -KELSAAYRS-Q--LI-QR--T-GF-I-- | |
| <i>Cellulomonas fimi</i> | 332670053 | -QT-EN-LDE-KA-VVGQLA-Q-EGR | -KE-GAAFRSLT--LV-QR--R-QF-I-- | |
| <i>Rhodococcus ruber</i> | 490033249 | -Q---DE--DE-KV-VLEQVAPN-EGR | -LT-KGILDSIQ-EIV-QK--KEGI-I-- | |
| <i>Carboxydotothermus hydrogenoformans</i> | 78044607 | -Q---DEYLENLK--LTT-F--TYPEE | -NK-KDA-YKL---V-EY--KEG--V-- | |
| <i>Clostridium papyrosolvens</i> | 490755727 | -QD-DAKVELTTE--Q-ALA-QYPEM | -TLLKNAFD-IVREI--S---QE-V-I-- | |
| <i>Candidatus Latescibacter</i> | 551163936 | --T--NATLAT-FEKA-E-FE-KYPES | -ND-A-VL--IL-EEV-SA--NEGI-P-- | |
| <i>Alicyclobacillus acidoterrestris</i> | 544884231 | -LA-EAIISEVNE-VQEHFA-Q-PEM | -AE-A-IM--IL-EEV-RA--QEGI-P-- | |
| <i>Alicyclobacillus hesperidum</i> | 493492321 | -LA-EEALSNLNE-VQ-HFA-A-PER | -AAD-A-VL--IL-EEV-SA--NEGM-P-- | |
| <i>Kyridia tusciae</i> | 295696164 | -LA-E-AIR-VEE--QEHFA-Q-PEG | | |

Figure S17

Partial sequence alignment of the protein polynucleotide phosphorylase/polyadenylase, showing a 17 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | | 119 | | 164 |
|-------------------------------------|---|---|---|--|
| Order Chlorobiales | <i>Chlorobium phaeobacteroides</i> <i>Chlorobium limicola</i> <i>Chlorobium chlorochromatii</i> <i>Chlorobium phaeocladathratiforme</i> <i>Chlorobium ferrooxidans</i> <i>Chlorobium phaeovibrioides</i> <i>Chlorobium phaeobacteroides</i> <i>Chlorobium luteolum</i> <i>Chloroherpeton thalassium</i> <i>Prosthecochloris aestuaria</i> <i>Prosthecochloris ethylica</i> <i>Prosthecochloris marina</i> <i>Chlorobaculum thiosulfatiphilum</i> <i>Chlorobaculum parvum</i> <i>Chlorobaculum tepidum</i> <i>Candidatus Chlorobium masyuteum</i> <i>Chlorobium sp. N1</i> <i>Chlorobium sp. KB01</i> | 500067752 189347255 78188483 194337298 493409287 145219296 189500807 78186318 193215257 194334507 175187643 110023272 139457424 193213357 21673157 166807843 TCD47685 076792264 385808941 397689554 517434067 253997441 543962518 82703824 254000097 490464234 544831712 517818178 297539673 91776688 291612993 517331384 372487166 56476931 71909659 490513686 498060970 344208553 MBU2824517 WP_162050708 WP_041100787 WP_205203486 MBW9249360 BA031070 AFE10952 493497825 515684535 516030927 346725939 495083596 488692977 442317511 383452467 328953829 | DLKPPSSGVERCNCPEINTLA ---A-----NK-N-Q--E-- ---A-----A-K-N-A--R-- DQ-N-----E-- -----T-SDK-N---A-- -----ESGK--M----- ---A----ESG-I-TS--E-- -----RDKE--M---DI- -I-T---EMS-W--YR-LE-S -----SSSN-HET--RI- -I-----SQN--TQ--L-- ---A----ES-K--LK--EYV -I-A----AAE--AT-FA-- -I-T----A-Q--MD--P-- -I-A----M-H--AA-F-- -----T-SDK-N---A-- -----CA-E--M--VE-- -----T-SDK-N---A-- -----C----MMKK-LYD--YFL | LHAGKKL SQTFEFKIVVASREDYDW MRS--AE ISS-----D---L- -E-APEE QRR---M-I-N---E- -A-VLPH R-N-----E- -KQKDHA NRLI-L-----D---L- FK---E QKS---M---D-N---L- VS-AEP KKS---LLL----- -N--EGE RKRY-----A-D---L- IAEA-SG VLKT-----LS-EG--R- -K-PPAE QAL-----I-GK---E- -Q-PPEQ RH-W-----I-D-N---N- IASSVLQ KK-----LII---Q---E- -DT PER-----E- -AS PKR-----E---L- -NE PER-----EA---L- -KQKDHA NRSI-L---I-D---L- -A-VTAE RKR-----A-D-R---L- -KQKDHA NRLI-L-----D---L- KP-D-V-F-IGT----- KPGD-V-F-IGD-N---E- RASD-L--IISDEQ--L- KKAD-V-F-LC--A---E- T P-D -V-F-LCGE--Q- TSHD-V-F-LCDED--R- KAQD-V-F-LCDHA--L- TPHD-I---L-GEA--E- RAQD-V-M-L-DEA--- AAHD-I---L-DEA--A- KSSD-V-F-LC---Q- KPND-V---LC--A---E- GRHD-I-F-LCGEA--Q- TSRD-I-F-L-N-A---E- TPHD-L-L-L-D-A--- NERD-V---L-D-L---E- TPRD-I---I---G--E- NARD-L-F-L-DAA--A- TARDQI-F-IC--A---E- TARDQI-F-ICN----- NPQDQI-F-LC--A--- R---D-V---I---A--- TAHD-L-F-L----- KAGD-I---II-D-A--- NPQDQI-F-LC--A--- TAHD-L-F-L----- NAND-L---IG--V--E- TARDQI-F-IC--A---E- TARDQI-F-IC--A---E- LPHDQV---I---A---E- TARDQI-F-IC--A---E- LPHDQI---I---E---E- NARD-M-F-IG-----E- NARD-L-F-IG-----E- NAND-L---IG--V--E- TEKD-L-F--SN-R-F-- |
| Other Bacteria | <i>Ignavibacterium album</i> <i>Melioribacter roseus</i> <i>Uliginobacter gangwonense</i> <i>Methylotenera mobilis</i> <i>Sulfuricella denitrificans</i> <i>Nitrosospira multiformis</i> <i>Methylovorus glucosetrophus</i> <i>Thauera linaloolentis</i> <i>Thauera terpenica</i> <i>Azoarcus tolulaelasticus</i> <i>Methylophilus versatilis</i> <i>Methylbacillus flagellatus</i> <i>Sideroxydans lithotrophicus</i> <i>Thiobacillus thioparus</i> <i>Dechlorosoma suillum</i> <i>Aromatoleum aromaticum</i> <i>Dechloromonas aromatica</i> <i>Thauera phenylacetica</i> <i>Xanthomonas campestris</i> <i>Stenotrophomonas maltophilia</i> <i>Acidithiobacillus ferrooxidans</i> <i>Fluviibacter phosphoraccumulans</i> <i>Sulfuritalea hydrogenivorans</i> <i>Azonexus hydrophilus</i> <i>Acidithiobacillus ferriphilus</i> <i>Sulfuritalea hydrogenivorans</i> <i>Corallococcus coralloides</i> <i>Xanthomonas gardneri</i> <i>Xanthomonas vasicola</i> <i>Dyeila ginsengisoli</i> <i>Xanthomonas axonopodis</i> <i>Rhodanobacter spathiphylli</i> <i>Stigmatella aurantiaca</i> <i>Myxococcus stipitatus</i> <i>Corallococcus coralloides</i> <i>Desulfovobacca acetoxidans</i> | -----C----MMKK-LYD--YFL -----C----KMEKK-RY---EFL -----A-G--EVSK-RL---P-L -V-T-D--ELKN-LWG-LAHL -I-T-G--EA-K-RWK-LE-L -----T-G--EV-K-LWS-VQYL -----T-G--EV-K-LWS-VQYL -----I-T-G--EL-K-LWG-LAHL -----A-G--EV---LYA--A-L -----A-G--EL---L-A--P-L -----A-G--EVA--RW--LAHL -----V-T-D--ELKN-VWS-LDHL -----I-T-D--EVAK-HWD-LSRL -----I-T-G--EVQK-LWG-LQHL -----I---E--EEA--RW--VAHL -----A-G--EV--LWS-LEYL -----A-G--EVA--RWD-LARL -----A-D-AESA--LW--L-VL -----A-G--ELA--RL--LA-L -----I-T-A--EEA--RW--LP-L -----I-T-G--EAEEA--RL--LP-L -----S-D--E---LW--LP-L -----A-G--EEAK--W--G-L -----S-D--E---LW--LP-L -----A-G--EEAK--W--G-L -----A-G--EEAK--W--G-L -----M-T----E-D--DYR-L-SM -----I-T-A--EEQ--RW--LP-L -----I-T-A--EEA--RW--LP-L -----A-D--ESA--LWA-LAHL -----I-T-A--EEA--RW--LP-L -----A-G--ESA--LWS-LDHL -----M-T----ESD--DVR-FSSM -----M-T----ESS--DLR-FASM -----M-T----E-D--DYR-L-SM -----C-G--MAQH-YLK-LDYL | -----C----MMKK-LYD--YFL -----C----KMEKK-RY---EFL -----A-G--EVSK-RL---P-L -V-T-D--ELKN-LWG-LAHL -I-T-G--EA-K-RWK-LE-L -----T-G--EV-K-LWS-VQYL -----T-G--EV-K-LWS-VQYL -----I-T-G--EL-K-LWG-LAHL -----A-G--EV---LYA--A-L -----A-G--EL---L-A--P-L -----A-G--EVA--RW--LAHL -----V-T-D--ELKN-VWS-LDHL -----I-T-D--EVAK-HWD-LSRL -----I-T-G--EVQK-LWG-LQHL -----I---E--EEA--RW--VAHL -----A-G--EV--LWS-LEYL -----A-G--EVA--RWD-LARL -----A-D-AESA--LW--L-VL -----A-G--ELA--RL--LA-L -----I-T-A--EEA--RW--LP-L -----I-T-G--EAEEA--RL--LP-L -----S-D--E---LW--LP-L -----A-G--EEAK--W--G-L -----S-D--E---LW--LP-L -----A-G--EEAK--W--G-L -----A-G--EEAK--W--G-L -----M-T----E-D--DYR-L-SM -----I-T-A--EEQ--RW--LP-L -----I-T-A--EEA--RW--LP-L -----A-D--ESA--LWA-LAHL -----I-T-A--EEA--RW--LP-L -----A-G--ESA--LWS-LDHL -----M-T----ESD--DVR-FSSM -----M-T----ESS--DLR-FASM -----M-T----E-D--DYR-L-SM -----C-G--MAQH-YLK-LDYL | |

Figure S18

Partial sequence alignment of the protein 7-carboxy-7-deazaguanine synthase QueE, showing a 3-7 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*. While other *Chlorobiales* species contain a 7 aa insertion, *Chlorobaculum* species have a 3 aa insert in this position

| | |
|--|--|
| Order <i>Chlorobiales</i> <i>Ignavibacteriae</i> Other Bacteria | 47 RGVGKTTAARILAKGLNCEKILKD EA YRETVE PCGECESCRDFDAG -----VF---A---Q-MME- AE -LQQ-T- ----- -----VF---AV---QRMME- PV -LQE-T- -----S- -----F-RA---Q-LID- AD -LQQ-T- ----- -----VF---AV---Q-MID- PV --QE-T- -----L- -----VF---AV---QRMIE- PK -LKE-T- ----- -----VF---AV---QR-ID- PE -LRK-T- ----- -----VF---AV---I-MTE- KD -LQH-T- -----E- -----VF---A---QRMID- AD WLRD-T- -----ET- -----VF---A---QRMIE- AD WLRD-T- ----- -----VF---AV---QRMID- PQ -LKE-T- -----V- -----VF---AV---SRMIE- PV -LKE-T- -----EQ- -----VF---AV---RMID- PK -LKE-T- ----- -----F-AV---HRMIE- -T --QE-T- -----E- -----VF---AV---RMID- PK -LKE-T- ----- -----S-A---V-T- D --RD-A- --V-D- -----VF---AV---RMIE- PK -LKE-T- ----- -----T-A---R-T- --HE-A- --M- -----VF---A---QRMIE- PD WLQN-T- -----S- -----F-AV---RMIA- -T --HD-T- -----S- -----F-AV---HRMIE- -T --QE-T- -----E- -----VF---AV---SRMIE- PV -LKD-T- -----S- -----T-A---LOPVD F --NK-DM--AINEM -----T-V---LNP-D G --N--M-KS-HNS -----C-TI---QNL-S- T --N----KS-N-S -----C-F-TI---LNLPN A --N----KA-N- -----C-F-TI---QNLQPN A --N----KA-NT- -----C-TI---N-SS T- A ----KS-AG- -----CS-TI---LN-TPE T- --D-S-KS-N- -----L-AI---QTP-EE --DHA- --RR--A-VA-EE- -----C-TI---QNLQAE GGP-DI- --N----KS-NTS -----C-F-TI---MNLQPN A- --N----KA-ND- -----AI---TPREE --DGA- --C-D-ES-E-- -----C-TI---QNVSA- T- --N-T-KN-AQN -----C-TI---QN-TPE V- A-N----S-NSN -----C-TI---NRQD- Q- A----VS-KNN -----C-TI---N-SP- G- A-NH-H--VS-NE- -----C-TI---NLQP- G- A-NQ-H--TS-NE- -----N-TI---QSLTDE V- A----VA-QNN -----C-TI---QNLTE- IS --N----KS-NNN -----N-TI---TN-TA- T- A-DQ----KS-NNN -----N-TI---TN-TA- I- A-D----KS-NNN -----C-TI---HSTA- G- A-N-P--IS-NQ- -----C-TI---QNLGD- V- A----VS-QNN -----C-TI---NLSAE T- A--V-P--A-LN- -----C-TI---H-TSE V- A--V-D--KS-QN- -----C-TI---QNLTA- F- A-N-D--KA-NNN -----C-TI---TSLTAE Q- A-T----VS-QT- -----C-V---TI---QPTA- F- A----LS-KH- -----C-TI---HLTE- F- A-NQ-D--KA-NQN -----C-TI---QNLGD- V- A----VS-QNN -----C-TI---HATPE G- A-N-N-IS-NL- -----C-F-AI---LAPNGA - A-N----S-NE- -----C-TI---HLTA- H- A-NK-D--LA-NNN -----C-L---NPTS- T- --D-P--TA-REN -----SC-F-RAI---LER-P- G- A-R----KA-EQ -----C-F-AI---NPTQ- G- A-N----A-NE- -----I-SV---F-AIR-SR-E- -N A-T----Q--SA -----C-F-AI---LSPNGA - A-N----S-NE- -----I-F-S---GQSS D -----ST---I--- -----I-F-S---QGTSA D ---Q-AA-L-I--- -----I-F-S---RGTGA - -----NA-V-I--- -----I-F-S---QGTSA D ---Q-AA-L-I--- -----I-F-S---QGTSA D ---Q-PA-L-I--- |
|--|--|

Figure S19

Partial sequence alignment of the protein DNA polymerase III subunits gamma and tau, showing a 2 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | | 220 | GSMRDAQSILDQVIAFA | IDSE | GERAIRYDKVS DLLSYIDDEHFFMVTDA |
|---|--|-----------|-------------------|-------------------|---|
| Order <i>Chlorobiales</i> | <i>Chlorobaculum parvum</i> | 193212992 | - | - | -E----- |
| | <i>Chlorobaculum tepidum</i> | 21674147 | - | S AQ-G | -DG--S-QN-AE--N----QL-A--- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobium luteolum</i> | 78187181 | - | - | VEQD D---S-E--AE--N----D--A- |
| | <i>Chlorobium phaeobacteroides</i> | 189499967 | - | - | AEND H-GS-S-QS-AE--N----M-G- |
| | <i>Chlorobium ferrooxidans</i> | 493410721 | - | T | AEND HTGS-T-SG-AE--N----L-E-- |
| | <i>Chlorobium phaeoclatrathriforme</i> | 194336682 | - | S | SENA L-GS-T-RG-A--N----DTM-A-- |
| | <i>Chlorobium chlorochromatii</i> | 78188902 | - | L-N | L-N-----E-----DH---- |
| | <i>Chlorobaculum limnaeum</i> | 069809131 | - | S F-R | S F-R SDG--S-A--AE--N----D--A- |
| | <i>Chlorobium limicola</i> | 059138195 | - | S VGND | S VGND A-KV-A-QG-AE--N----DQ--D-S- |
| | <i>Prosthecochloris aestuarii</i> | 194334272 | - | L | L-----V-----E----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139457273 | - | -EQD | -EQD E--S-H--AE--N----DQ--E--A- |
| | <i>Prosthecochloris marina</i> | 110023130 | - | S | S SGEGL-QG-RE--N----QL-A-- |
| | <i>Chlorobium phaeovibrioides</i> | 145219538 | - | -A-----L-----S-F | -A-----L-----S-F L-T ENEK-E-TN-AA--GD-----A-S |
| | <i>Chloroherpeton thalassium</i> | 193213861 | - | -GEN | -GEN H-EG-S-QR-A--N-V----A-- |
| | <i>Prosthecochloris ethylica</i> | 175186743 | - | -T E | -T E KGK-E-AA-A--NI--R--D-S-- |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM23307 | - | -L-----C | -L-----C |
| | <i>Chlorobaculum sp. 24CR</i> | 129051538 | - | -L-----C | -L-----C |
| | <i>Prosthecochloris sp. GSB1</i> | 094082258 | - | -ER | -ER -D-T--ET-AE--N----DH--A-- |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085659383 | - | -RT | -RT VS-EN--G-N-----A-S-- |
| | <i>Prosthecochloris sp. ZM_2</i> | 114608329 | - | -S | -S -RT VS-EN--G-N-----A-S-- |
| Other Bacteria | <i>Chlorobium sp. N1</i> | 131353650 | - | -T AEGD | -T AEGD H-EG-S-QR-A--N-V----A-- |
| | <i>Candidatus Thermochlorobacteri GBCh1B</i> | KER09939 | - | -A-----L-----S-C | -A-----L-----S-C TQD -A-----L-----S-C |
| | <i>Chlorobium sp. 445</i> | PI047289 | - | -A-----L-----C | -A-----L-----C TEN -KGK-E-VA--E--NV--E-RL-D-S-- |
| | <i>Chlorobi bacterium OLB7</i> | KXK57074 | - | -F-----T-C | -F-----T-C GTT-T-HQAN-A-NL--QD-L-R-- |
| | <i>Ignavibacterium album</i> | 385809646 | - | -G-----YF-----C | -G-----YF-----C GENVDSET--K1-NL--E-TY-NIS-- |
| | <i>Melioribacter roseus</i> | 397690783 | - | -AL---E-LF--I-SYS | -AL---E-LF--I-SYS GKKVNSEEI-QM-NL--QDIY-RIS-- |
| | <i>Rhodothermus marinus</i> | 345304445 | - | -AL---L-AF--AVSLC | -AL---L-AF--AVSLC GTTL--AELAQ-A-GVV-LDLY-A--H |
| | <i>Parabacteroides distasonis</i> | 150008698 | - | -G-----L-F--VS-T | -G-----L-F--VS-T NGN-T-QA-I-N-NVL-Y-YY-RL-- |
| | <i>Parabacteroides johnsonii</i> | 495429919 | - | -G-----L-F--VS-T | -G-----L-F--VS-T NGN-T-QA-I-N-NVL-Y-YY-RL-- |
| | <i>Fibrisoma limi</i> | 496578583 | - | -GL---L-MF-LNVT-S | -GL---L-MF-LNVT-S AD-T---KE-L-N-HIL-YDYY-KL--Q |
| | <i>Parabacteroides merdae</i> | 547920597 | - | -G-----L-F--VS-T | -G-----L-F--VS-T NGN-T-QA-I-N-NVL-Y-YY-RL-- |
| | <i>Capnocytophaga gingivalis</i> | 488745336 | - | -A-----L-F-R-VS-C | -A-----L-F-R-VS-C GKT-TROQA--EI-NVL-YDTY-K--L |
| | <i>Spirosoma luteum</i> | 517446762 | - | -GL---L-MF-LNVT- | -GL---L-MF-LNVT- AD-V---KE-L-N-HIL-YDYY-KL--L |
| | <i>Thermophagus xiamensis</i> | 498213992 | - | -A-----L-F--IV--S | -A-----L-F--IV--S GKR-S-QQ-IEN-NVL-YDYY-KLV-- |
| | <i>Spirosoma linguale</i> | 284034966 | - | -GL---L-MF-LNVT-- | -GL---L-MF-LNVT-- AD-I---KE-L-N-HIL-YDYY-KL--L |
| | <i>Clostridium botulinum</i> | 153941061 | - | -A-----L-----STG | -A-----L-----STG DGKVE--Q-L-M-GLVTN-NLLRI--S |
| | <i>Clostridium sporogenes</i> | 489578394 | - | -A-----L-----STG | -A-----L-----STG DGKVE--Q-L-M-GLVTN-NLLII-NS |
| | <i>Candidatus Symbiobacter</i> | 550990475 | - | --L---L-LT--A--G | --L---L-LT--A--G GG-VQTPS-REM-GCV-RS-VYALL-- |
| | <i>Verminephrobacter eiseniae</i> | 121607506 | - | --L-LT--A--G | --L-LT--A--G SGQLQEAA-RQM-GAV-RSYV-RLI-- |
| | <i>Aromatoleum aromaticum</i> | 56476074 | - | --L-L---A--HG | --L-L---A--HG SGRVEEEQ--HM-GTVG-D-LYA-L-- |
| | <i>Nitrosospira multiformis</i> | 82702979 | - | --L-----A--G | --L-----A--G -GK-EEAG-R-M-GAV-QGYL-DLLE-- |
| | <i>Cupriavidus taiwanensis</i> | 516633018 | - | --L-LT--A--YS | --L-LT--A--YS AGQVSEEA-RGM-GA--QGYLVQLL-- |
| | <i>Methylibium petroleiphilum</i> | 124267720 | - | --L-LT--A--G | --L-LT--A--G AGTLQEAG-RQMIGAVGRS-VYALI-- |
| | <i>Cupriavidus necator</i> | 339326421 | - | --L-LT--A--YS | --L-LT--A--YS AGQVSE-A-RGM-GA--QGYLVQLL-- |
| | <i>Ralstonia picketii</i> | 549447903 | - | --L-LT--A--YS | --L-LT--A--YS AGQVSEEA-RGM-GA--QGYLVQLL-- |
| | <i>Caldithrix abyssi</i> | 493984161 | - | --S-----L-S-S | --S-----L-S-S GNE-KFED-AKA-GV-HQDE--HL--Y |
| | <i>Geobacter metallireducens</i> | 404498270 | - | --SL-V----L--C | --SL-V----L--C GDQV-DEE-VS--GVV-RRLLLDA-A- |
| | <i>Desulfococcus multivorans</i> | 527022898 | - | --L-L----MTCT | --L-L----MTCT --GE-THEG-LN--GAA-R-VL-RI-A- |
| | <i>Geobacter sulfurreducens</i> | 39995205 | - | --SL-V----L--C | --SL-V----L--C GETVND-D-VS--GVV-RRLLEASA- |
| | <i>Bdellovibrio bacteriovorus</i> | 426405587 | - | --S-L----T-- | --S-L----T-- NGPLTRAS-VEI-GLT-RALL-ETLN- |
| | <i>Mobiluncus curtisi</i> | 490107874 | - | --V--SL----L--GE | --V--SL----L--GE A-PKHL--RAVS--G-T-SGLGGMV-- |
| | <i>Hyphomicrobium denitrificans</i> | 488578002 | - | --V--GL----A--MG | --V--GL----A--MG -GHVTAMS-R-M-GLA-RARI-DLAE- |

Figure S20

Partial sequence alignment of the protein DNA polymerase III subunits gamma and tau, showing a 4 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

Figure S21

Partial sequence alignment of the protein cytidylate kinase, showing a 4 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | | |
|--|---|----------------------------|--|
| Order <i>Chlorobiales</i> | | 264 | |
| | | LFNICKASNPRTYFAEDEKDIQDEWL | |
| | <i>Prosthecochloris aestuarii</i> | 194333263 | -LEN R QPVQSAGVCGATSTPMWL |
| | <i>Chlorobium phaeobacteroides</i> | 189499431 | -----RE---S--IA-QTGL----F YD- G R--G-V----- |
| | <i>Chlorobium limicola</i> | 189345888 | --G--RSA---S--I--IE-LE-S-F RD-EG S--E-I-I----- |
| | <i>Chlorobium ferrooxidans</i> | 493410283 | -Y--RDA---S--I--VEE--P-- SLNG SG-A--I----- |
| | <i>Chlorobium luteolum</i> | 8187590 | K-ANG S--G-V-I----- |
| | <i>Chlorobaculum parvum</i> | 193213390 | -YS---DA--HS--I--VDE--P-- VGENG K--E-V-I----- |
| | <i>Chlorobium chlorochromatii</i> | 78188557 | -----A---F-I--IEE-NP--F AAHEG KA-E-V-I-----H |
| | <i>Chlorobaculum tepidum</i> | 21673122 | -YS---DA--HS--I--VDE-RP--F VGENG K--E-V-I----- |
| | <i>Chlorobium phaeovibrioides</i> | 145220322 | -YA--EA--ESH-I--VEEL-S-F TTTOG KT-E-I----- |
| | <i>Chlorobium phaeoclatheatiforme</i> | 194335597 | -YH--EA--S--I--EIEE-EER-- RNSDG RA-ATV-----H |
| | <i>Chloroherpeton thalassium</i> | 193213833 | -EV--DA-SN-H-I-NQSELERA-F -RPDD SL-E-V-----V |
| | <i>Prosthecochloris ethylica</i> | NUK47677 | -Y---S---S--V-N-E--DRD-- MRN G S--DNV----- |
| | <i>Prosthecochloris marina</i> | 110022730 | -----Q--S-S--I-N-N-L---F SDN G ST-ENV----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139456775 | -YS--RDA--KS--I--VDE--P--F VGENG K--E-V-I----- |
| | <i>Chlorobaculum limnaeum</i> | 2501895126 | -YS--RDA--KS--I--VE--LP--F VGDDG K----V-I----- |
| | <i>Prosthecochloris aestuarii</i> | 012505013 | ----- |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM22955 | ----- |
| | <i>Chlorobium sp. 445</i> | PIO48543 | --GV--A-----H-DEL-AD-F MREDG TL-E-V----- |
| | <i>Chlorobium sp. KB01</i> | 076792162 | --GV--A-----H-GEL-A-F QRSDG L--E-V----- |
| | <i>Prosthecochloris sp. ZM</i> | 114617735 | -Y--RDA--S--I--VEE--P--F -SLND SS-A--I----- |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085660515 | ----- |
| | <i>Prosthecochloris sp. ZM_2</i> | 114607604 | -Y---E--S-S--V--H--R-- I-K G --DNT----- |
| | <i>Chlorobaculum sp. 24CR</i> | 129050439 | -Y---S---S--V-N-E--DRD-- MR- G S--DNV----- |
| | <i>Chlorobium sp. N1</i> | 131354410 | -YS--RDA--HS--I--VEE-LP--F VGDDG K----V-I----- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobi bacterium NICIL-2</i> | KXB97964 | -Y--REA--ESH-I--VEE-DP--F RLKDGTAE-V----- |
| | <i>Chlorobi bacterium OLB6</i> | KXK35371 | --DVARSE-----I--VSEL-S--F EGAE-V-IT-----Q- |
| | <i>Pontibacter roseus</i> | 517290586 | --EVSRA--A-H-I-EIDE--P--F TN-NTV-IS-----Q- |
| | <i>Nitritalea halalkaliphila</i> | 496346574 | -YSV--QH--NS--V-N-EEL-K-- ENA--V-I----- |
| | <i>Cyclobacterium marinum</i> | 343084681 | -Y-V--ER--N--H-VNSNAQV--S-F G-G--V-I----- |
| | <i>Cyclobacteriaceae bacterium</i> | 499125948 | -YQV--HI--S--V-N-TE-NSD-- N-ADKI-I----- |
| | <i>Rhodonellum psychrophilum</i> | 518425835 | -Y-V--ER--N--VSNPEEVSDA-F S-N-TV----- |
| | <i>Cand. Azobacteroides pseudotrichonymphae</i> | 212550484 | --V--ER-QN--VSNSEQVDK--F LEG-TI-I----- |
| | <i>Echinicola vietnamensis</i> | 431797370 | --E--RRA-LN--LIS-----S-F NGIK-V-I-----K- |
| | <i>Echinicola pacifica</i> | 517284044 | -YQV--Q--S--V-N-GE-DPD-F ANGDVK-I----- |
| | <i>Cesiribacter andamanensis</i> | 496487984 | -YQV--E--S--V-N-NELDPK-F D-QDKV-I----- |
| | <i>Hymenobacter norwichensis</i> | 551196148 | -YAV--HH--QS--I-N-SE-DPA-- EGK--V-I----- |
| | <i>Mariniradius saccharolyticus</i> | 495907062 | --SVVNKT--S--V-N-QE-DE--F HGAE-V-I----- |
| | <i>Cecembia lonarensis</i> | 496476169 | -QV--LSE--S--V-N-TE-DPS-F T-QDKV-I----- |
| | <i>Marivirga tractuosa</i> | 313675572 | -Y-V--EK--N--VSNPDQVLSD-F VSNETV-I----- |
| | <i>Flexithrix dorotheae</i> | 522016070 | -Y-V--DK--N--ISNTTELDKS-F END-V-I----- |
| | <i>Cyclobacterium qasimii</i> | 527058107 | -Y-V--KF--S--I-N-SE-AS--F S-NDKV-IS----- |
| | <i>Thermonema lapsum</i> | 166917974 | --V--EK--Q--VSCPEEVDAK-F KAGE-V-I----- |
| | <i>Chitinophagales bacterium</i> | MBX7109056 | --EV--RSE--HS--I--AE--E-- EGAR-V-I----- |
| | <i>Hymenobacter wooponensis</i> | 135529319 | --V--A--NS--VSE-A-L-Q--F RSA--V-I-----L- |
| | <i>Candidatus Kapabacteria bacterium</i> | TAE32473 | --SVVNKT--S--V-N-QEL---F GMV--V-I----- |
| | <i>Cyclobacterium plantarum</i> | 166150147 | -Y-VA-----I--VQE----- EG-KTV-IS-----Q- |
| | <i>Raineya orbicola</i> | 101358740 | --QV--GV--S--V-N-Q--NPD-V R-ED-V-I----- |
| | <i>Algoriphagus aquimarinus</i> | 146915010 | --AV-QKA--S--V----EL-P--F ENCE-I-I----- |
| | <i>Cyclobacterium lianum</i> | 073090759 | --V--DT--N-F--VSNSEE--ESD-F IENDRV-I----- |
| | <i>Pontibacter koreiensis</i> | 046308989 | --V--REK----VVSPEEVDA--F AEGE-V-I----- |
| | <i>Nitritalea halalkaliphila</i> | 009055752 | -YSV--QH--NS--V-N-EEL-R--F AE-G-V-I----- |
| | <i>Catalinimonas alkaloidigena</i> | 089684683 | -Y-V--ER--N--H-VNSNAQV--S-F G-G--V-I----- |
| | <i>Cyclobacterium plantarum</i> | 166146163 | -Y-V--ET--H--VSTRSEV-SA-F --HDTV-I----- |
| | <i>Solirubrum puertoricensis</i> | 059067372 | --V--EK--Q--VSSPEEVNPD-F SEG--V-I----- |
| | <i>Marivirga sericea</i> | 085516784 | --GVVNHE--S--V-N-QEL---F DDAA-V-I----- |
| | <i>Penaeicola halotolerans</i> | 226390616 | -YQV--Q--NS--I-N--EVPL-F EQQKTV-I----- |
| | <i>Siccationidurans arizonensis</i> | SFP99765 | -YQV--LQE--S--V-N-TE-SN-- EKSDKV-I----- |
| | <i>Cecembia rubra</i> | 211299928 | -YQV--LET--Q-H-ISNVAE--P--F G-G--V-I----- |
| | <i>Sporocytophaga myxococcoides</i> | 028981416 | -Y-V--DK--N--VSNPEQVNLD-F SANE-V-I----- |
| | <i>Siccationidurans arizonensis</i> | 092675538 | -YSV--E--EKS--V-N-LEDPN-F K-SY-V-I----- |
| | <i>Aquiflexum balticum</i> | 084119878 | --SVNV--S--I-N-AEDEVDE-- AGAEKV-I-----L- |
| | <i>Mucilaginibacter pankratovii</i> | 191189761 | -YQV--NI--KS--V-N-TE-DLS-I N-QDKV-I----- |
| | <i>Algoriphagus machiponganensis</i> | 495474052 | -YQV--REA--HC--VSGTN--AG-F S-G-KV-I----- |
| | <i>Cytophaga hutchinsonii</i> | 110636514 | -YQV--LGQ--S--I-N-TELDP--F KSNDKV----- |
| | <i>Indibacter alkaliphilus</i> | 496324935 | -YGV--QE-E-S--V-N-TE-EPS-I RATDNV-I----- |
| | <i>Alistipes indistinctus</i> | 496424989 | -HQV--GV-E-S--V-N-TE-DPR-- MHAD-V-I----- |
| | <i>Cytophaga aurantiaca</i> | 517152557 | -YQT--L--H--S-TV--TEL-PA-F DNCR-V-I-----K- |
| | <i>Hymenobacter aerophilus</i> | 518788809 | -YGV--QE-E-S--V-N-NE-EES-I RSTDKI-I----- |
| | <i>Alistipes putredinis</i> | 548242045 | --SVVNQT--S--V-N-QELDEA-F HGAD-V-I----- |
| | <i>Hymenobacter aerophilus</i> | 518790560 | --EV--RRA--S-NV-EASELRP--F EGAE-V-I-----K- |
| | <i>Pedobacter heparinus</i> | 255533499 | -YQV--DT--A-H-ISKID-LDPA-F --G--V-I-----Q- |
| | <i>Nafulsella turpanensis</i> | 516343586 | -YDV--KY-DNS--ISNTEE-DK--F S-GDKV-I----- |
| | | | -YSV--RQQ-EKS--I-N-SELEPS-F NGTK-V-I----- |

Figure S22

Partial sequence alignment of the protein 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, showing a 2-5 aa insertion (boxed) that is found in all members belonging to the order *Chlorobiales*

| | 70 | |
|--|----------------------------|-----------------------------|
| | KRFMGRTQDEVPEETKMPVYQIVS | EGNQARVKIGDKTHSPQEISAMILOK |
| | -----RY---N-K-IA--KVIN | ANGE---QV--NY-----V-- |
| | -----KY---N-K-A--E-IN | -SGE---N-IY---V----- |
| | -----KY---IND-K-LA--E-IN | --GE----N-IY---V----- |
| | -----KY---N-K-LAS-DV-N | --GY-K-----Y----- |
| | -----KF---N-K-IA--KV-N | INGE---E-D--NY----- |
| | -----KY---N-K-LAS-DV-N | --GY-K-----Y----- |
| | -----I-KF---N-K-IA--EV-N | --GE----N-Y---V----- |
| | -----V-KF---N-K-IA--AV-N | --GE----N-Y---V----- |
| | -----KY---N-K-FAS-DV-N | --GD-----E-SY----- |
| | -----V-KF---N-K-LA--D-N | --GE----N-AY----- |
| | -----KY---N-K-LAS-DVIN | --GE----N-IY---V----- |
| | -----RF---G-K-A--ELIN | DSGE---N-VY-----V----- |
| | -----KY---N-K-FAS-DV-N | --GD-K---A-KSY----- |
| | -----KY---N-K-LAS-DV-N | --GD-K-----SY----- |
| | -----KF---N-K-IA--KV-N | VNGD---E-----Y-----V----- |
| | -----KF---N-K-IA--KV-N | TNGE---E-D--Y----- |
| | -----V-KF---N-K-IA--EV-N | --GE----N-Y----- |
| | -----KY---N-K-A--E-IN | -SGE---N-IY---V----- |
| | -----GYA--T-I---EVIN | --GQ---R-SG-VY----- |
| | -----KY---N-K-FAS-DV-N | --GD-K-----Y----- |
| | -----KF---N-K-IA--KV-N | VNGD---E-----Y-----V----- |
| | -----KF---N-K-IA--KV-N | VNGD---E-----Y-----V----- |
| | -----RY---N-K-IA--KVIN | ANGE---EV--NY-----V----- |
| | -----KY---N-K-IA--KV-N | VNGE---ADV-G-VY----- |
| | -----SYS--TS-M-L--EV-- | GN-SVT-RADG-EFT----- |
| | -----KY---N-K-A--E-IN | -SGE----N-IY---V----- |
| | -----SYA--T-I---EVIN | --NG---R-A-KVY----- |
| | -----QMN-ISD-S-S--K-A G | DN-T---D-DGRHY--P----- |
| | -----FY--ER-RSE--EV-R G | -N-TV-----RLY--P----- |
| | -----R1---QQ-I-E--EV-G S | DNGS-----RLY--P----- |
| | -----RYA--QQ-I---KVID | N--DV--EA-G-LY-----F----- |
| | -----E-Y-QCKK-AER--FKV-N | --GYP--Q-D-RKY----- |
| | -----E-Y-Q-SK-INR--KVIR G | N--TP--E--RKY----- |
| | -----E-Y-Q-QK-ISR--SV-R G | DN--TP--D-EGRLYT-----V----- |
| | -----E-Y-Q-QK-ISR--KV-R G | DN--TP--D-EGRLYT-----V----- |
| | -----E-Y-Q-SR-VDR--FKV-R G | DN--TP--D-DSRLYT----- |
| | -----A-W--KD-VSR--EV-K G | D--TP--D--MYT----- |
| | -----LHS--G--E-L--HV-D G | NNGA-A--D--YT-P----- |
| | -----FY--I--ARR--KV-R G | PN--T--Q--RVY--P----- |
| | -----FY--I--ARR--KV-R G | PN--T--Q--RVY--P----- |
| | -----MF--KD--Q-S-E--K G | DD--T--E-RMYA-----V----- |
| | -----E-Y-Q-AK-INR--KV-R G | N--TP--E--RKY----- |
| | -----MYS--S--L-NT--K-IK G | GN--TP--S--NRLY----- |
| | -----YY--ES-RNT-Q-EV-R G | SS--D-----VY-----K----- |
| | -----NFS--T--L-NI--K-IK G | NDTP--S--N-LYT----- |
| | -----E-Y-Q-DK-INR--KV-R G | N--TP--E--RKY----- |
| | -----NYS--A--M----KV-E G | PH--SV--VV--QYA-E--R----- |
| | -----NY-DLG--LDE--EV-R G | D--GTP--L--REYT----- |
| | -----E-Y-Q-SK-INR--KVIR G | N--TP--E--RKY----- |
| | -----KFK--S--K-I-S---E G | NN--TV--R--RLFT----- |
| | -----E-F-Q-QK-INR--KV-R G | DN--TP--D-DGRLYT----- |
| | -----KY--KD-LSHIS-KV-R G | DNDTI-ID-DGRLYT----- |
| | -----E-Y-Q-SR-VER--FKV-R G | DN--TP--D-DGRLYT----- |
| | -----E-Y-Q-QK-INR--KV-R G | DN--TP--D-EGRLYT----- |
| | -----E-Y-Q-QK-ISR--VAR G | N--TP--D-DGRLYT----- |
| | -----E-Y-Q-QK-INR--KV-R G | DN--TP--D-EGRLYT-----V----- |
| | -----E-Y-Q-QK-ISR--SV-R G | DN--TP--D-DGRLYT----- |
| | -----E-Y-Q-QK-IAR--SV-K G | DN--TP--D-EGHLYT----- |
| | -----TMY--I-N-V-R--KV-K G | DN--TP--Q-D-RQYT----- |
| | -----NSY--SAK-AGR--KV-K G | DN--TP--E-D-RKY----- |
| | -----KY--QN-ISE--KVMK G | NNET--S--EY--P--FV----- |
| | -----SYA--S--M----KV-K G | PK--DV--IEVQG-QY--P----- |
| | -----RY--N--M----KV-R G | NGD--D-MG-KY--P-----T----- |
| | -----FYN--EK-I-E--KVER G | NN--VVV-EVMG-KYT--P----- |

Figure S23

Partial sequence alignment of the protein molecular chaperone DnaK, showing a 1 aa deletion (boxed) that is found in all members belonging to the order *Chlorobiales*. Except for an isolated exception shown here, the CSI was not found in any other bacterial species in the top 500-BLASTp hits.

| | | | | | |
|--------------------------------------|--|------------|-------------------------|---------------|----------------|
| Order <i>Chlorobiales</i> | <i>Chlorobium chlorochromatii</i> | 78188056 | LHLGHYTGALENWVAQQNQCSA | DGNRAYDT | YFLIADYHTLTTSL |
| | <i>Prosthecochloris vibrioformis</i> | 139626009 | -----ER--ETL- | --S---E- | --V---S---- |
| | <i>Chlorobium phaeobacteroides</i> | 189501390 | -----F-----R-ELD- | --Q---FE- | F-----N---- |
| | <i>Prosthecochloris aestuarii</i> | 194335025 | -----R-----ETGP | -----V--- | F-----N---- |
| | <i>Chlorobium phaeoclathratiforme</i> | 194337843 | -----F-----S-E---LDE | T-A-V-E- | C-----N---- |
| | <i>Chlorobaculum parvum</i> | 193213678 | -----S-----I-----LLQP | --S---E- | C-----S---- |
| | <i>Chlorobium luteolum</i> | 162139381 | -----IE---LRAP | --S-L-E- | C-----S---- |
| | <i>Chlorobium limicola</i> | 189347951 | -----H-----LLAE | --S-V-E- | C-----I---- |
| | <i>Chlorobium phaeovibrioides</i> | 145220533 | -----V-----LTNP | --T-GCE- | C-----N---- |
| | <i>Chlorobium ferrooxidans</i> | 493409794 | -----S-E---RNE | A-E-V-E- | C-----N---- |
| | <i>Chlorobaculum tepidum</i> | 21672854 | -----I-----LLHP | --S---E- | C-----S---- |
| | <i>Chlorobaculum limnaeum</i> | 2501894004 | -----I-----LLHP | --S---E- | C-----S---- |
| | <i>Pelodictyon phaeoclathratiforme</i> | 012509488 | -----F-----S-E---LDE | T-A-V-E- | C-----N---- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139456449 | -----I-----LLHP | --S---E- | C-----S---- |
| | <i>Prosthecochloris marina</i> | 110024017 | -----R-----R-DELDT | G-K-VFE- | -----S---- |
| | <i>Candidatus Chlorobium masyuteum</i> | 166807408 | -----S-E---RNE | A-E-V-E- | -----CN---- |
| | <i>Cand. Thermochlorobacter aerophilum</i> | KER10736 | -----V-----HL---ERAQ | N-AKT-QN | FH-----A--N |
| | <i>Chlorobium sp. KB01</i> | 076791230 | -----W-E---RNE | A-E-V-E- | C-----N---- |
| | <i>Chlorobium sp. N1</i> | 131355342 | -----IE---LTAQ | --T-V-E- | C-----N---- |
| | <i>Prosthecochloris sp. ZM</i> | 114616499 | -----R-----ETDP | --V--- | F-----N---- |
| | <i>Prosthecochloris sp. GSB1</i> | 094083507 | -----R-----ELD- | Q-----E- | W-----S---- |
| | <i>Prosthecochloris sp. CIB 2401</i> | 068868322 | -----ER---ETL | --S---E- | --V---S---- |
| | <i>Chlorobaculum sp. 24CR</i> | 129051262 | -----I-----LLHP | --S---Q- | C-----S---- |
| | <i>Cand. Thermochlorobacteriaceae GBCh1B</i> | KER10736 | -----V-----HL---ERAQ | N-AKT-QN | FH-----A--N |
| <i>Ignavibacteriae</i> | <i>Ignavibacterium album</i> | 385810136 | --I---V-----KL-H-YQS | FY-----V--N- | |
| | <i>Melioribacter roseus</i> | 397691543 | --V-----IDL---YDS | -H-----V--D- | |
| | <i>Terriglobus saanensis</i> | 320108440 | --N-M---Y---RL-DEYC | --F---I-A--DY | |
| | <i>Acidobacterium capsulatum</i> | 225874157 | --N-M---A---RL-HHEYEC | --F---W-A--DY | |
| | <i>Granulicella mallensis</i> | 374313410 | --N-M---Y---RL-HHEYDC | --F---L-A--DY | |
| | <i>Rubrivivax benzoatilyticus</i> | 497542852 | -----H---K---RL-D-YDC | FYFV--W-A--HY | |
| | <i>Rubrivivax gelatinosus</i> | 383759274 | -----H---K---RL-D-YDC | F-FV--W-A--HY | |
| | <i>Pseudogulbenkiania ferrooxidans</i> | 545114536 | -----H-VIK---EL-HSHEC | F-MV--W-A--NF | |
| | <i>Chromobacterium violaceum</i> | 34497152 | -----H-VIK---EL-HSHEC | F-MV--W-A--NF | |
| | <i>Sulfuricella denitrificans</i> | 543963298 | -----H-V-K---IKL-HHEYEC | L-FV--W-A--HY | |
| | <i>Methylotenera versatilis</i> | 297538009 | -----N-V-K---IKL-HEHEC | L-FV--W-A--HY | |
| | <i>Methyloversatilis universalis</i> | 519008995 | -----H-V-K---KL-HEYPC | L-FV--W-A--NY | |
| | <i>Uliginosibacterium gangwonense</i> | 517434921 | --I---H---K---RL-EEKEC | F-FV--W-A--AY | |
| | <i>Ralstonia pickettii</i> | 241662685 | -----H-V-K---RL-AEYPC | F-FV--W-A--HY | |
| | <i>Thiobacillus denitrificans</i> | 516742345 | -----H-V-K---RL---EYQC | L-FV--W-A--HY | |
| | <i>Sideroxydans lithotrophicus</i> | 291614089 | -----H-V-K---QM-H-PEC | L-FV--W-A--HY | |
| | <i>Methylotenera mobilis</i> | 518739869 | -----N-V-K---IRL-HEHEC | L-FV--W-A--HY | |
| | <i>Methylovorus glucosetrophus</i> | 253998633 | -----H-V-K---TRL-HEHEC | L-FV--W-A--HY | |
| | <i>Ralstonia syzygii</i> | 344174282 | -----H-V-K---RL-AEYPC | F-FV--W-A--HY | |
| | <i>Ralstonia solanacearum</i> | 489361767 | -----H-V-K---RL-AEYPC | F-FV--W-A--HY | |
| | <i>Nitrosospira multiformis</i> | 82703434 | -----H-V-K---IEL-H-FEC | L-FV--W-A--HY | |
| | <i>Thauera phenylacetica</i> | 490504997 | -----H-V-K---KL-EEFPC | L-FV--W-A--HY | |
| | <i>Aquaspirillum serpens</i> | 550892077 | -----H---K---KL-SEHEC | F-FV--W-A--Y | |
| | <i>Thiobacillus thioparus</i> | 517333907 | -----H-V-K---LRL---EYQC | L-FV--W-A--HY | |
| | <i>Aromatoleum aromaticum</i> | 56478939 | -----H-V-K---KL-AEYPC | L-FV--W-A--AY | |
| | <i>Marinobacter manganoxydans</i> | 495449703 | -----H-V-K---KL-HEFEC | F-FV--W-A--NY | |
| | <i>Bermanella marisrubri</i> | 494075287 | -----H-V-K---KL-HHEYEC | F-FV--W-A--TY | |
| | <i>Marinobacter nankaiticus</i> | 490719494 | -----H-V-K---KL-HEFEC | F-FV--W-A--NY | |
| | <i>Marinobacter hydrocarbonoclasticus</i> | 387814531 | -----H-V-K---KL-HHEYEC | F-FV--W-A--QY | |
| | <i>Hahella chejuensis</i> | 83635470 | -----H-V-K---QL-HEYEC | F-FV--W-A--HY | |
| | <i>Methylophaga lonarensis</i> | 497412846 | -----H-V-K---IKL-HEFDC | F-FV--W-A--HY | |
| | <i>Flexistipes sinusarabici</i> | 336323462 | --I---F---Q---SL-DKYEC | FYFV--W-A--GY | |
| | <i>Deferribacter desulfuricans</i> | 291279394 | --I---F---K---KL-E-YEC | FYFV--W-A--Y | |
| | <i>Denitrovibrio acetiphilus</i> | 291287275 | --I---F---K---DL-DKYDC | NYFV--W-A--NY | |
| | <i>Calditerrivibrio nitroreducens</i> | 313673527 | --I---F---K---KL-DNYDC | -YF--W-A--NF | |
| | <i>Desulfurella acetivorans</i> | 573478392 | --NLY---Q---INL-DKYKC | -YFV--W-A--GF | |
| | <i>Pelobacter propionicus</i> | 118580108 | --I---FH-V---IRI-D-MEC | F-FV--W-S--EY | |
| | <i>Syntrophobacter fumaroxidans</i> | 116748129 | --NLH---D---IQL-Q-YEC | F-FV--W-A--EY | |
| | <i>Geobacter sulfurreducens</i> | 39996932 | --FH-V-A---REL-EEYEC | F-FA--W-S--EY | |
| | <i>Desulfurobacterium thermolithotrophum</i> | 325295466 | I---N-L---KT-LEL-DKAEC | F-F---W-AI--Y | |
| | <i>Thermovibrio ammonificans</i> | 319790140 | I---N-F---KA-IEL-DRAES | F-F---W-AI--Y | |

Figure S24

Partial sequence alignment of the protein tryptophanyl-tRNA synthetase, showing a 8 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 153 | QHPIL | GEVFPPEPAPKITFKSRLPGLDG | 204 |
|------------------------------|---|------------|-----------------------------|--------------------------|-------------------|
| Order <i>Chlorobiales</i> | <i>Chlorobium ferrooxidans</i> | 493409794 | -I----- | -GT | -S-E-E-R-V- |
| | <i>Prosthecochloris vibrioformis</i> | 139626009 | -Q----- | -VF | -D-E-E-A- |
| Order <i>Chlorobiales</i> | <i>Chlorobium phaeobacteroides</i> | 189501390 | -Q----- | -LY | -N-A-E-A- |
| | <i>Chlorobium chlorochromatii</i> | 78188056 | -I-----K-HF | P-LY | -L-E-V- |
| | <i>Chlorobium luteolum</i> | 78167834 | -I-----K-H | P-TK | -D-A-V- |
| | <i>Chlorobaculum parvum</i> | 193213678 | -I-----K-SH | -E | -D-A-V- |
| | <i>Chlorobium phaeoclathratiforme</i> | 194337843 | -I-----V-K-HF | P-L | -A-E-V- |
| | <i>Chlorobaculum tepidum</i> | 21672854 | -I-----K-SH | -E | -D-A-V- |
| | <i>Chlorobium limicola</i> | 189347951 | -I-----K-FH | P-ET | -N-E-V- |
| | <i>Chlorobium phaeovibrioides</i> | 145220533 | -I-----K-SH | P-QS | -P-V-V- |
| | <i>Prosthecochloris aestuarii</i> | 194335025 | -I-----KH | LL-DG | VA-D-E-M- |
| | <i>Prosthecochloris marina</i> | 110024017 | -KQ----- | -E | -D-E-E-A- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139456449 | -I-----K-SH | -E | -D-S-A-V- |
| | <i>Prosthecochloris aestuarii</i> | 012506768 | -I-----KH | LL-D | VA-D-E-M- |
| | <i>Chlorobaculum limnaeum</i> | 2501894004 | -I-----K-SH | -T | -D-A-V- |
| | <i>Candidatus Chlorobium masyuteum</i> | 166807408 | -L-----AT | PK-DG | NG-EG-V- |
| | <i>Candidatus Thermochlorobacteri GBCh1B</i> | KER10736 | -I-----K-H | P-TK | -V-E-V- |
| | <i>Chlorobium sp. N1</i> | 131355342 | -I-----K-SH | -L | -L- |
| | <i>Chlorobium sp. KB01</i> | 076791230 | -I-----K-SH | -A | -D-S-A-V- |
| | <i>Chlorobaculum sp. 24CR</i> | 129051262 | -I-----Q | -GT | -S-E-G-R-V- |
| | <i>Prosthecochloris sp. CIB 2401</i> | ANT65961 | -L-----L-HF | P-AT | G-E-E-R-A- |
| | <i>Prosthecochloris sp. GSB1</i> | 094083507 | -I-----KH | LL-DG | VA-D-E-M- |
| | <i>Prosthecochloris sp. ZM</i> | 114616499 | -I-----QQ | PK-NG | QA-EGRL-A-V- |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM23396 | -V-I-S-----Q | -E | LL-A-Q- |
| <i>Ignavibacteriae</i> | <i>Ignavibacterium album</i> | 385810136 | -V-----Q | -E | LL-S-A- |
| | <i>Meliorbacter roseus</i> | 397691543 | -V-----Q | -S | QALLS-P-I- |
| | <i>Paenibacillus terrigena</i> | 517587309 | I-----L-SS-Y-V | E-- | KP-LV-K-LVGDVP-V- |
| | <i>Paenibacillus alvei</i> | 544846112 | V-I-Q-K-V-----Q | AP-----E-LL-E-A- | |
| | <i>Candidatus Kryptonium thompsoni</i> | CUT00468 | -L-----D-----QT | K-----D-IV-RIPK-T- | |
| | <i>Marinitoga litoralis</i> | 205100913 | -Y-I-LS----- | AP-----E-LL-E-A- | |
| | <i>Candidatus Chrysopegis kryptomonas</i> | 092346731 | -D-K-----QV | D-----EA-LG-VTK-L- | |
| | <i>Orenia marismortui</i> | 134118996 | -I-----Y | Q-IL-AKV-T- | |
| | <i>Acetivibrio cellulolyticus</i> | 026073659 | -L-L-----FF | AP-----E-LL-E-A- | |
| | <i>Candidatus Kryptobacter tengchongensis</i> | CUU06683 | -L-----D-K-----QV | K-I-----KAML-EEAK-L-I- | |
| | <i>Hydrogenothermus marinus</i> | 170145601 | -I-L----- | EP-----QALL-EEAK-I- | |
| | <i>Persephonella atlantica</i> | 200673337 | -I-L----- | K-----D-IV-RIPK-T- | |
| | <i>Acetivibrio saccincola</i> | 101301108 | -L-L-----HM | AP-----E-LL-E-A- | |
| | <i>Persephonella atlantica</i> | MBK331940 | -I-L----- | D-----EA-LG-VTK-L- | |
| | <i>Sporohalobacter salinus</i> | 204989152 | -I-----Y | Q-IL-AKV-T- | |
| | <i>Pelosinus propionicus</i> | 090937075 | I-----L-LS-----V | EP-----QALL-EEAK-I- | |
| | <i>Oceanidesulfovibrio indonesiensis</i> | 144303480 | Q-----L-L-----H | E-----E-LGEVPK-L- | |
| | <i>Halanaerobium hydrogeniformans</i> | 312143949 | I-----I-Q-K-V-K | -PI-----K-SLS-SAI-I- | |
| | <i>Halanaerobium saccharolyticum</i> | 491631366 | I-----I-Q-K-V-K-----M | T-L-----QA-L-EQAK- | |
| | <i>Acetivibrio cellulolyticus</i> | 497929432 | -L-L-----FF | -----KAL-SD-P-M- | |
| | <i>Acetohalobium arabaticum</i> | 302392435 | I-----I-----H | IL-----EAL-SD-P-M- | |
| | <i>Clostridium phytofermentans</i> | 160881602 | M-M-----K-V-----D | Q-IL-----AKV-T- | |
| | <i>Halobacteroides halobius</i> | 435853355 | I-----D-----Y | T-----E-LGEVPK-L- | |
| | <i>Clostridium clariflavum</i> | 374295865 | -L-L-----F-F | -----TLH-KA-LSSC-V- | |
| | <i>Halothermothrix orenii</i> | 220931740 | I-----Q-R-V-K | E-----ES-LA-VP- | |
| | <i>Caldicellulosiruptor saccharolyticus</i> | 146295971 | -L-L-----Y | -----QAIL-AKV-T- | |
| | <i>Persephonella marina</i> | 225850029 | I-----L----- | AP-----EALVSDIP-V- | |
| | <i>Sulfurihydrogenibium azorens</i> | 225849351 | I----- | -QI-----Q-ILNTVKV-I-T- | |
| Other Bacteria | <i>Thermocrinis albus</i> | 289548704 | I-----L-----H | DQI-----EALL-EE-K-I- | |
| | <i>Aquifex aeolicus</i> | 15606299 | I-----LS-----R | KP-----V-KALL-EA-K-L-T- | |
| | <i>Sulfurihydrogenibium yellowstonense</i> | 494812488 | I----- | --T-----QALL-ETP-V-T- | |
| | <i>Spirochaeta thermophila</i> | 307719108 | K-NV-----L-----F | -KI-----KALL-ETPKI-T- | |
| | <i>Geobacter daltonii</i> | 222056098 | Q-----I-L-----Y | SPI-K-----QALL-ES-K-L-I- | |
| | <i>Geobacter bemandjiensis</i> | 197118679 | Q-----I-L-----Y | -----V-D-LGEVPK-V-I- | |
| | <i>Pelobacter propionicus</i> | 118580108 | Q-----L-----H | -----SALL-ETPKVV- | |
| | <i>Desulfovibrio desulfuricans</i> | 376296380 | I-----I-Q-----H | -Q-----ALL-ETPK-L- | |
| | <i>Anaeromyxobacter dehalogenans</i> | 86158110 | V-----V-L-----LS-----V-K-FH | -----I-EYRVS-A-P- | |
| | <i>Mucispirillum schaedleri</i> | 555550898 | V-----V-L-----LA-----V | -----Q-LL-AAPKIL-T- | |
| | <i>Trichoplax adhaerens</i> | 196018955 | I-----Q-R-V-K | -----I-V-ESML-NVPK- | |
| | <i>Mesotoga prima</i> | 389844710 | IY-----L-----K-----QF | AP-----EALVSDIP-V- | |
| | <i>Roseiflexus castenholzii</i> | 156742130 | K-----AS-L-V-----T | A-----E-ILA-VPK-T- | |
| | <i>Dethiosulfovibrio peptidovorans</i> | 491903638 | SA-----L-----F | -----L-ETL-GDVPK-V-I- | |
| | <i>Slackia exigua</i> | 493405898 | K-----I-V-Q-----KR | -----DILL-PTPKV-T- | |
| | <i>Acetotetraena longum</i> | 490199933 | D-----M-QA-K-V-----D | -K-----EGILSDAIEI- | |
| | <i>Dehalogenimonas lykanthroporepellens</i> | 300088125 | I-----L-LA-----V | -----TLKV-KELLSATP- | |
| | | | DHF | -----P-QG-L-N-PMVV- | |

Figure S25

Partial sequence alignment of the protein tryptophanyl-tRNA synthetase, showing a 5 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | | IREVPIF | NTVKDAVKKAEANTSIVFVPAFA |
|--------------------------------------|--|--|---------|-------------------------|
| Order <i>Chlorobiales</i> | <i>Chloroherpeton thalassium</i> 193216377 <i>Chlorobium phaeovibrioides</i> 145220333 <i>Chlorobium chlorochromatii</i> 78188444 <i>Chlorobium limicola</i> 189345876 <i>Chlorobium luteolum</i> 78187602 <i>Chlorobium phaeobacteroides</i> 119356239 <i>Chlorobium phaeoclathratiforme</i> 194335584 <i>Chlorobaculum parvum</i> 193213404 <i>Chlorobaculum thiosulfatiphilum</i> 139456761 <i>Prosthecochloris marina</i> 110022769 <i>Prosthecochloris aestuarii</i> 194333247 <i>Chlorobaculum limnaeum</i> 2501893444 <i>Chlorobium ferrooxidans</i> 493411426 <i>Chlorobaculum tepidum</i> 21673108 <i>Prosthecochloris ethylica</i> 175187487 <i>Candidatus Chlorobium masyuteum</i> 166807707 <i>Cand. Thermochlorobacter aerophilum</i> RFM23093 <i>Chlorobium sp. 445</i> PI048084 <i>Chlorobium sp. N1</i> 131354450 <i>Chlorobium sp. KB01</i> 076791543 <i>Prosthecochloris sp. ZM_2</i> 114608810 <i>Prosthecochloris sp. HL-130-GSB</i> 085659004 <i>Chlorobaculum sp. 24CR</i> 129050450 <i>Prosthecochloris sp. GSB1</i> 094081941 <i>Chlorobi bacterium OLB6</i> KXK34392 <i>Chlorobi bacterium OLB7</i> KXK56074 <i>Chlorobi bacterium OLB5</i> KXK40992 <i>Ignavibacterium album</i> 385810678 <i>Melioribacter roseus</i> 397691702 <i>Crocobacter atlanticus HTCC25</i> 298208207 <i>Rhodothermus marinus</i> 268318044 <i>Flavobacterium limnosediminis</i> 559216499 <i>Riemerella anatipestifer</i> 313206103 <i>Myroides injenensis</i> 497939601 <i>Riemerella anatipestifer</i> 386321909 <i>Flavobacterium cauense</i> 559194736 <i>Elizabethkingia meningoseptica</i> 510824792 <i>Echinicola vietnamensis</i> 431795425 <i>Leadbetterella byssophila</i> 312130295 <i>Cecembia lonarensis</i> 496475462 <i>Myroides odoratimimus</i> 493301313 <i>Chryseobacterium indologenes</i> 573452557 <i>Bergeyella zoohelcum</i> 488739944 <i>Chryseobacterium gleum</i> 489067148 <i>Marivirga tractuosa</i> 313675255 <i>Pontibacter roseus</i> 517290593 <i>Riemerella columbina</i> 517505678 <i>Fluviicola taffensis</i> 327405863 <i>Saprositria grandis</i> 488735375 <i>Polaribacter franzmannii</i> 517773502 <i>Spirosoma panaciterrae</i> 522085613 <i>Fibrisoma limi</i> 496584386 <i>Fibrella aestuarina</i> 436835319 <i>Cyclobacterium marinum</i> 343082734 <i>Spirosoma linguale</i> 284038109 <i>Gracilimonas tropica</i> 521069933 <i>Muricauda ruestringensis</i> 344204408 <i>Spirosoma spitsbergense</i> 522091410 <i>Empedobacter brevis</i> 518819263 <i>Desulfobacca acetoxidans</i> 328953555 <i>Bacillus cereus</i> 446037332 <i>Thermocrinis albus</i> 289548927 <i>Archaeoglobus fulgidus</i> 11499767 <i>Arenimonas oryziterrae</i> 488601692 <i>Clostridium ultunense</i> 551350688 491759884 | <i>YGTNVVAGVTPGKGGMQYNGNEKDPF</i> -----VL----DR-S-----C-P--V-----E-E-----T-----P -----TL-H-D-K-----C-P--V-----R-E-T-----P -----IL-H-D-R-----C-P--V-----E-E-T-----P -----VL-H-DR-K-----C-P--V-----E-----AT-----P -----TI-H--R-H-----C-P--V-----D-----AT-----P -----IL-H----K-----C-P--V-----RE--Q-----AT-----P -----IL-----Q-----C-P--V-----D-RE--E-----AT-----P -----IL-----Q-----C-P--V-----D-Q---D-----AT-----P -----L-T-DR-T-----TSS--VY-----EAT--A-----P -----L-----A-----C-P--V-----EE-G-A-----P -----IL-----Q-----C-P--V-----D-Q---D-----AT-----P -----VL-H----K-----C-P--V-----R-E-----AT-----P -----E-----R-----C-P--V-----RE--EE-G-----L-----P -----VL-H----K-----C-PV-V-----R-E-----AT-----P -----L-----H-A-----R-----Y-----RE--AAQRV--I-Y-----P -----L-----Y-A-----R-----VY-----RE--AAQKV--I-Y-----P -----VM-----DR-K-----C-P--V-----RE--L-----AT-----P -----VL-H----K-----C-P--V-----RD--E-----AT-----P -----E-----R-----C-P--V-----RE--EE-G-----L-----P -----E-T---R-S-----C-P--V-----D-R---EQ-----A-----P -----IL-----Q-----C-P--V-----D-QE--D-----AT-----P -----L-G-DR-G-----C-P--VY-----E-EE-G-A-----P -----S-----SM-S-KGD-T-----V-PL-VY-----RE--TATG--V-I-----P -----TE-HPA-FIRA-----V-----SE--AATG--A-----P -----TLFDEKIPV-----C-E--E-G-----P -----TKFE-IPIF-----EE--EQK-----P -----QEFDTGIP-----E---ETK-TA-A-----P -----QTHLDKPVF-----DE--E-K-D--I-----P -----QKHLRDPVF-----AE--E-EG-----I-----P -----T-HLDRPVF-----EQ-G-D--I-----P -----TEHL-KPVF-----A--E-D-V-----P -----TMHLRDPVF-----AE--E-G-D--I-----P -----QTHLDKPVF-----A--E-G-V-----P -----T-HLDRPVF-----DQ-G-D--I-----P -----SEH-L-KPVF-----AE--E-G-V-----P -----STHL-KPVF-----S-AE--AT--D--I-----P -----QTHLDKPVF-----EE--ETG-DV-----PG -----TKHLEKPVF-----S-EE--A-TG-----I-----P -----TTHLDRPVF-----SE--E-G-D--I-----P -----SEH-L-KPVF-----A--E-G-V-----P -----QKHLRDPVF-----A--Q-G-DV-----P -----SEH-L-KPVF-----A--E-G-V-----P -----QKHLRDPVF-----A--E-G-V-----P -----NNHLDLPVF-----EE--RTG-DV-----P -----SEH-L-KPVF-----AE--E-G-V-----P -----SSHLDRPVF-----A--A-A-TG-DV-----P -----TSHLRDPVF-----E--ETG-DV-----P -----QEHL-KPVF-----VES-E-VG-D-I-----P -----QTHLDRPVF-----QE--D-G--V-----P -----QTHLDRPVF-----QQ--DE-G-DV-----P -----QTHLDKPVF-----YE--E-G-D--I-----P -----STHL-KPVF-----E--TKG-D-TI-----P -----QTHLDRPVF-----QQ--DQ-G--V-----P -----QTHLDRPVF-----A--EVQ-----P -----QEHL-KPVF-----E--EVG-D-TI-----P -----QTHLDRPVF-----QQ--DQ-G--V-----P -----QTHL-LPIF-----DQ-G-DV-----P -----QKMDDPVPF-----E-AA-G--L-----P -----TEIE-VPVF-----E--ATG--A--VY-----P -----QKVEDIPVF-----DE--ETQ--C-L-----P -----TEVL-VPVF-----DS--E--RE-D--A-----P -----EVH-IPVF-----E--EETS--A-----P -----TTHI-LPVF-----A--NETG-DA--Y--PP-----P -----TELE-IPVF-----D--AE--A-TG--A-I-Y-----P | | |

Figure S26

Partial sequence alignment of the protein succinyl-CoA synthetase subunit alpha, showing a 7 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 2 | AEEA | SDVEFSGVFDLNPERLQEIGKK |
|--|--|------------|--|---|
| Order <i>Chlorobiales</i> | <i>Chlorobaculum tepidum</i> | 21673819 | RIGVAGVGKLGEGHTNLLKQI | - - - N P - - L - - - |
| | <i>Chlorobaculum parvum</i> | 193212872 | - - - - - | - Q - R P - - HCA - L - - DSN - AE - MAR - |
| | <i>Chlorobium phaeoclathratiforme</i> | 194336309 | K - - - I - - - - - K - TE - | - QNR H - T - LA - I - - RI - AE - - H - |
| | <i>Chlorobium phaeobacteroides</i> | 119356931 | - - - I - T - - - - - K - GE - | - N - Q PA - CA - - - TA - AE - MAA - |
| | <i>Chlorobium phaeovibrioides</i> | 145219610 | - - - II - - - - - K - AE - | - Q - T PG - TCA - - - D - A - AR - - AS - |
| | <i>Chlorobium luteolum</i> | 78187012 | - - - I - - - - - K - AE - | - L - S P - - HLA - IY - KSSL - AE - AH - |
| | <i>Chlorobium limicola</i> | 189346519 | - - - I - T - - - - - K - RD - | - H - R T - LHVA - I - - TQ - AE - MAQ - |
| | <i>Chlorobium chlorochromatii</i> | 78188699 | K - - - I - - - - - K - TEL | - G - S P - - - - - |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139455688 | - - - - - | - R - L P - - TCA - I - - DRN - AD - MAAR - |
| | <i>Chlorobium ferrooxidans</i> | 493411370 | Q - - - I - - - - - K - TE - | C - - Q KN - HLT - - - QT - AA - A - - |
| | <i>Chloroherpeton thalassium</i> | 193216278 | - - - A - I - - - - - K - E - | - - - S Q - - - A - - - |
| | <i>Chlorobaculum limnaeum</i> | 2501894514 | - - - - - | - R - L P - - TCA - I - - DRN - AD - MAAR - |
| | <i>Candidatus Chlorobium masyuteum</i> | 166808114 | Q - - - I - - - - - K - TE - | SL - R Q - TA - I - - - I - A - - CA - VA - |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM23578 | - - - IV - - - - - L - AK - - SEL | VR - R N - AR - V - - - I - GA - AD - - ARR - |
| | <i>Candidatus Thermochlorobacteri GBChIB</i> | KER10002 | - - - LA - V - - - - - L - AK - - SEL | - R - L P - - TCT - I - - DRN - AD - MAAR - |
| | <i>Chlorobium sp. KB01</i> | 076791136 | Q - - - I - - - - - K - TE - | SL - R K - I - V - - - CKET - CKDVAR - |
| | <i>Chlorobium sp. 445</i> | PIO49003 | - - - IV - - - - - L - AK - - REL | - R - Q PA - TCA - - - D |
| | <i>Chlorobium sp. N1</i> | 131357401 | K - - - I - - - - - K - AE - | SN - R K - FSI - - - Y - IDN - K - SGIS - |
| | <i>Chlorobi bacterium OLB4</i> | KXK01619 | K - SII - L - H - - KI - LKI - SEL | ENC - LI - - Y - A - EQTAKSVAQE |
| Larger <i>Ignavibacteriae</i> Clade | <i>Ignavibacterium album</i> | 385809634 | KV - - - I - - - H - - KI - - KMFS - | DN - - LC - - Y - KDF - KSKNVSEE |
| | <i>Melioribacter roseus</i> | 397691384 | K - - - I - T - H - - RI - VK - - EL | - - - - - QI - LT - I - - V - LA - A - - SER |
| | <i>Candidatus Hydrothermae bacter</i> | MBP7702301 | KF - IV - - - - - I - VRN - S - | A - A - LI - I - - A - L - ATQ - AE - |
| | <i>Leptospira terpstrae</i> | 489062375 | - - - L - I - T - HM - QY - V - VA - L | - - - A - LI - I - - SDS - - ATQ - AE - |
| | <i>Leptospira wolffii</i> | 514354638 | K - - - I - T - HM - QY - V - VA - TL | NSINLK - IY - ADENH - N - VSN - |
| | <i>Brachyspira pilosicoli</i> | 404475256 | - - - SLI - - RM - Q - - L - VIN - | NOINL - - IY - ADENH - N - S - |
| | <i>Brachyspira hampsonii</i> | 495999332 | - - - SLI - - RM - Q - - L - VVS - | PN - - - A - I - ETSDA - AA - CA - |
| | <i>Opitutus terrae</i> | 182413903 | - - - C - - - S - - QH - ARIYSTL | R - IKLTHIA - V - ED - CR - - SNR |
| | <i>Nitrospina gracilis</i> | 491147715 | - - - A - - - I - - M - QY - VGV - SEM | KN - N - I - IY - TDMN - AKK - S - A |
| | <i>Ureibacillus thermosphaericus</i> | 515282761 | - - - V - V - T - - M - F - CSK - - M | S DQC - VV - IY - V - ER - AS - LAS - |
| | <i>Bacillus massiliensis</i> | 517982370 | KV - - - I - T - - M - - N - LKNYIE - | SNLQ - - - QD - - - AAAVAAE |
| | <i>Acidithiobacillus caldus</i> | 340781596 | - - - TA - V - - - H - - R - - AQKYAAC | - KY - LI - FY - A - - YA - K - V - E |
| | <i>Kordia algicida</i> | 494153791 | KA - - L - A - H - - KI - LR - - Q - S | K - T - LV - - A - I - V - - GLDTAS - |
| | <i>Trichodesmium erythraeum</i> | 113475581 | - - - V - - - I - - NM - QH - - RV - SLL | K - - - LV - - S - I - V - - GIDVAS - |
| | <i>Oscillatoria formosa</i> | 518317616 | - - - - - I - - - NM - QH - - RV - SLL | K - I - LV - - A - I - V - - GIDVAS - |
| | <i>Microcoleus vaginatus</i> | 493685732 | - - - - - I - - - NM - QH - - RV - SLL | K - - - LY - - A - I - VT - GLD - AS - |
| | <i>Dactylococcopsis salina</i> | 428779731 | - - - V - - - I - - - NM - QH - - RV - GLL | K - - - LV - - S - I - V - - GL - TAS - |
| | <i>Cylindrospermum stagnale</i> | 434404788 | - - - - - I - - - NM - QH - - RV - SSM | K - - - LV - - S - I - V - - GL - TAS - |
| | <i>Anabaena variabilis</i> | 75908580 | - - - V - - - I - - - NM - QH - - RV - SSM | K - - - LV - - S - I - V - - GL - TAS - |
| | <i>Raphidiopsis brookii</i> | 496702689 | - - - M - - - I - - - NM - QH - AR - - SSM | K - - - LV - - S - I - V - - GI - TASR |
| | <i>Scytoneema hofmanni</i> | 516355105 | - - - - - I - - - NM - QH - ARI - SSM | K - - - LV - - A - I - V - - GL - TAS - |
| Other Bacteria | <i>Peribacillus alkalitolerans</i> | 163100509 | - - - - - V - T - - M - TY - CSK - - M | KN - D - V - - - KDLK - AE - - SR - |
| | <i>Candidatus Nitronauta litoralis</i> | QPJ61148 | - - - - - I - - - M - - Y - VGV - SEQ | - AA - LTL - A - N - EQ - A - - - R |
| | <i>Leptoneima illini</i> | KAB2929387 | KV - - - I - T - HM - QY - V - VIVN - | AQA - VT - C - - AD - N - A - - S - R |
| | <i>Pelagicoccus mobilis</i> | 200355414 | K C - - - Y - - QH - ARIYSEL | EN - LA - I ET - T - AA - SE - |
| | <i>Ruminiclostridium cellobioparum</i> | 004630087 | KAAII - F - GM - S - - AQQI - - | NSI - IT - - Y - I - - QKV - - GV - |
| | <i>Brachyspira alvinipulli</i> | 028330838 | - - - SLI - - RM - Q - - L - VIN - | NQINL - - IY - E - KNH - D - - SQ - |
| | <i>Calothrix brevissima</i> | 096642204 | R - - - I - - - NM - QH - - RV - SSM | K - - - LV - - S - I - V - - GL - TAS - |
| | <i>Anabaenopsis elenkinii</i> | 200988229 | R V - - - I - - - NM - QH - ARI - SSM | K - - - V - - S - I - V - - GL - TAS - |
| | <i>Nitrospina gracilis</i> | 005006113 | - - - A - - - I - - M - QY - VGV - SEM | R - IKLTHIA - V - ED - CR - - SNR |
| | <i>Thermodesulfobacterium commune</i> | 038060646 | - - - V - - - II - - I - H - - R - - AEK - A - - | DKADLVALV - I - - - V - - AV - - |
| | <i>Leptospira ryugenii</i> | 108977756 | - - - L - - - I - T - HM - QY - V - VA - ML | - - - ATLE - I - AST - - ASQ - AE - |
| | <i>Cuspidothrix issatschenkoi</i> | 104386150 | R - - - I - - - NM - QH - - RV - SSM | K - - - LV - - S - I - V - - GL - TAS - |
| | <i>Cylindrospermum stagnale</i> | 015208246 | R - - - I - - - NM - QH - - RV - SSM | K - - - LV - - S - I - V - - GL - TAS - |
| | <i>Brachyspira hampsonii</i> | 008728832 | - - - SLI - - RM - Q - - L - VVS - | NQINL - - IY - ADENH - N - S - |

Figure S27

Partial sequence alignment of the protein Gfo/Idh/MocA family oxidoreductase, showing a 4 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | |
|---|---|--|
| <p>Order <i>Chlorobiales</i></p> <p>Larger <i>Ignavibacteriae</i> Clade</p> <p>Other Bacteria</p> | <p><i>Chlorobaculum tepidum</i> 21673819 <i>Chlorobaculum parvum</i> 193212872 <i>Chlorobium chlorochromatii</i> 78188699 <i>Chlorobium ferrooxidans</i> 493411370 <i>Chlorobium limicola</i> 189346519 <i>Chlorobium phaeoclathestratiforme</i> 194336309 <i>Chlorobium phaeobacteroides</i> 119356931 <i>Chlorobium phaeovibrioides</i> 145219610 <i>Chlorobium luteolum</i> 78187012 <i>Chloroherpeton thalassium</i> 193216278 <i>Chlorobaculum limnaeum</i> 2501894514 <i>Chlorobaculum thiosulfatiphilum</i> 139455688 <i>Candidatus Chlorobium masyuteum</i> 166808114 <i>Cand. Thermochlorobacter aerophilum</i> RFM23578 <i>Cand. Thermochlorobacteriaceae GBCh1B</i> KER10002 <i>Chlorobium sp. 445</i> PI049003 <i>Chlorobaculum sp. 24CR</i> 129051696 <i>Chlorobium sp. KB01</i> 076791136 <i>Chlorobium sp. N1</i> 131357401 <i>Chlorobi bacterium OLB4</i> KXX01619 <i>Chlorobi bacterium OLB7</i> KXX54677 <i>Chlorobi bacterium NICIL-2</i> KXB98010 <i>Chlorobi bacterium OLB6</i> KXX35631 <i>Ignavibacterium album</i> 385809634 <i>Melioribacter roseus</i> 397691384 <i>Niastella koreensis</i> 375147951 <i>Empedobacter brevis</i> 518819185 <i>Haliscomenobacter hydrossis</i> 332663296 <i>Lewinella persica</i> 522055502 <i>Gramella forsetii</i> 120435026 <i>Leeuwenhoekiella blandensis</i> 497466201 <i>Formosa agariphila</i> 537742421 <i>Chitinophaga pinensis</i> 256420576 <i>Weeksella virosa</i> 325954811 <i>Flavobacterium rivuli</i> 519055416 <i>Candidatus Chrysopegis kryptomonas</i> 092348819 <i>Candidatus Kryptobacter tengchongensis</i> 072149809 <i>Candidatus Kryptonium thompsonii</i> 047133254 <i>Caldithrix abyssi</i> HED11325 <i>Caldithrix abyssi</i> HHJ53862 <i>Nonlabens xiamenensis</i> 124980683 <i>Leeuwenhoekiella blandensis</i> 009780399 <i>Leeuwenhoekiella nankaiensis</i> 099646996 <i>Taibaiella lutea</i> 150030949 <i>Gemmimonas groenlandica</i> 171224539 <i>Luteitalea pratensis</i> AMY11198 <i>Owenweeksia hongkongensis</i> 375011569 <i>Zunongwangia profunda</i> 295135065 <i>Capnocytophaga cynodegmi</i> 517090214 <i>Psychroflexus tropicus</i> 517867825 <i>Bizionia argentinensis</i> 495914711 <i>Flavobacterium frigoris</i> 494229777 <i>Lewinella cohaerens</i> 522022900 <i>Niabella soli</i> 495859222 <i>Zhouia amylolytica</i> 568804158 <i>Bergeyella zoohelcum</i> 488740381 <i>Elizabethkingia meningoseptica</i> 510825000 <i>Nonlabens dokdonensis</i> 443244512 <i>Polaribacter franzmannii</i> 517771826 <i>Croceibacter atlanticus</i> 298208473 <i>Riemerella anatipestifer</i> 407452326 <i>Ornithobacterium rhinotracheale</i> 392389643 <i>Caldithrix abyssi</i> 493985549 <i>Singulisphaera acidiphila</i> 430746299 <i>Marinimicrobia bacterium</i> 551209323 <i>Nitrosococcus oceanii</i> 77165232 <i>Thioalkalivibrio thiocyanodenitrificans</i> 517042302 <i>Nitrosococcus halophilus</i> 292491337 </p> | <p>209</p> <p>ANVTASRLSRSLRKLKMRFFTRN PK SYASLDFTSGKSEVFLR</p> <p>-----L---S-K -----N-----</p> <p>-----M-L---CTE -----L---IY-----</p> <p>-----RM-L---GT -----L-T-----</p> <p>-----NRY-----CNE -----L-T-----</p> <p>-----NRM-L---CND -----L-T-----</p> <p>-----KSM-L---CND -----L-T-----</p> <p>-----CRQ-L---GTD -----L-T-----</p> <p>-----RS-L---GTA -----L-T-----</p> <p>-----S-I-T-S-V-SK -N-----L-N---I-I-----</p> <p>-----S-----S-----</p> <p>-----RM-L---GT -----L-T-----</p> <p>-T-----I-TRV-----CK -P-----LM-----</p> <p>-T-----I-T-V---L-CAD -N-----L-T-A-----</p> <p>-T-----I-TRV-----CK -Q-----LIT-----</p> <p>-----S-K-----</p> <p>-----RM-L---GT -----L-T-----</p> <p>-----RS-L---GT -----L-T-----</p> <p>-----I-Q-M---I-QK- A-I-I--VDNST-I-S-----</p> <p>--I---I-QRPM-L-M-Q-D --I---AKPSV-I-I-----</p> <p>--L---TPKPL-L-L-QPM A-M---SVPDL-Y-I-----</p> <p>--L---I-MKQM---V-Q-D A-I---LEKNAQ-V-----</p> <p>--L---I-LKNM---V-L-QPD A-I---LLEKE-QIV-----</p> <p>--L---I-MKNM---S-Q-D A-I-V--LEK-C--VKM-----</p> <p>--L---I-MK-M---A-Q-D A-I-V--LEK-C--V-M-----</p> <p>--L---I-LKSM---T-Q-D A-I-V--LEK-C--VKM-----</p> <p>--L-S-I-LK-M---L-QKD A-IGI--LDK-T-II-----</p> <p>V-IST-M-LKNM---V-Q-- A-I-I--LNK-T-IIK-----</p> <p>--L---I-MKNM---S-Q-D A-I-V--LDKVC--VKM-----</p> <p>--I-KNE---I-QK- A-I-I--LQ-IA-I-----</p> <p>--I-KNE---I-Q-D A-I-I--LQ-VA-I-----</p> <p>--I---I-KNE---I-QKD A-I-I--LQ-IT-I-----</p> <p>--I-AK-M---V-QK- A-I-F--SD-EA-A-----</p> <p>--L---I-AK-M---L-QKD A-I-M--ND-L---I-Y-----</p> <p>--L---I-LK-M---A-Q-D A-I-V--L-K-V--V-M-----</p> <p>--L---I-MK-M---A-Q-D A-I-V--LEK-C--V-M-----</p> <p>--L---I-MK-M---A-Q-D A-I-V--LEK-C--V-M-----</p> <p>--L---I-LK-M---L-Q-D A-IGI--LDK-T-II-----</p> <p>--I---V-ER---L-I-Q-S G-I---LAA-TG-F-----</p> <p>--I-DRV---V-IEPA A-V-V-YA-REL--W-----</p> <p>--L---I-LKNM---T-I-Q-D A-VTI--LEKS--I-M-----</p> <p>--L---I-MKNM---S-Q-D A-I-V--LEK-C--VKM-----</p> <p>--L---I-LKNM---S-QKD A-I-V--LYK-V--V-M-----</p> <p>--L---I-LKNM---S-Q-D A-V-V--LEKTH--VKM-----</p> <p>--L---I-LKNM---T-Q-D A-I-V--LEK-C--VKM-----</p> <p>--L---I-MKAM---S-QKD A-I-V--LEK-C--VKM-----</p> <p>--L---I-MKNM---T-QKD A-I-V--LEK-C--VKM-----</p> <p>--L---I-LKQM---V-QKD A-I---LLEKQAQLI-----</p> <p>--L-S-I-MK-M---L-QKD A-IGI--LNK-T-IIK-----</p> <p>--L---I-LKNM---S-Q-D A-I-I--LEK-V--V-M-----</p> <p>--L-T-I-MKAM---S-QKD A-I-V--LEK-C--V-M-----</p> <p>--L-T-I-MKAM---S-QKD A-I-I--LEK-A--I-M-----</p> <p>--L---I-LK-M---A-QKD A-I-V--LTK-V--V-M-----</p> <p>--L---I-MKNM---T-QKD A-I-VN-L-KE--V-M-----</p> <p>--L---I-LKNM---A-QKD A-I-V--LTK-C--VKM-----</p> <p>--L-T-I-MKAM---S-QKD A-I-V--LEK-A--I-M-----</p> <p>--I-T-I-MKNM---I-QKD A-I-V--LEK--II-M-----</p> <p>--L---I-AK-M---I-QKD A-I-M--NE-Y--I-Y-----</p> <p>--L---A-FQAM---LWSAE G-M---MTKQGTLV-K-----</p> <p>--L---V-AKQM-R-I-Q-D A-F-I--LNKST-IY-----</p> <p>--V-MKAQ---V-QQD A-I-I--QDKVLS-Y-K-----</p> <p>--S-V-MKTE---I-QQ- A-I-V--QRGLA-H-V-----</p> <p>--V-MKAQ---I-QQD A-I-I--QDKILSIY-K-----</p> |
|---|---|--|

Figure S28

Partial sequence alignment of the protein Gfo/Idh/MocA family oxidoreductase, showing a 2 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 176 | YVSKQSP | HQRCEGLIVNVVSRKLTIVGT |
|--------------------------------------|--|--|--|-----------------------|
| Order <i>Chlorobiales</i> | <i>Chlorobaculum parvum</i> 193213033 <i>Chlorobium tepidum</i> 21673344 <i>Chlorobium phaeobacteroides</i> 119357755 <i>Prosthecochloris aestuarii</i> 194333503 <i>Chlorobium limicola</i> 189346025 <i>Chlorobium phaeovibrioides</i> 145219389 <i>Chlorobium luteolum</i> 78186405 <i>Pelodictyon phaeoclathratiforme</i> 194336956 <i>Chlorobium ferrooxidans</i> 493409164 <i>Chlorobium chlorochromatii</i> 78188726 <i>Chloroherpeton thalassium</i> 193216324 <i>Chlorobaculum limnaeum</i> 2501894184 <i>Prosthecochloris marina</i> 110024153 <i>Prosthecochloris ethylica</i> 198911867 <i>Chlorobaculum thiosulfatiphilum</i> 170181222 <i>Candidatus Chlorobium masyuteum</i> 166807966 <i>Chlorobium sp. N1</i> 131358104 <i>Chlorobium sp. KB01</i> 076791152 <i>Chlorobaculum sp. 24CR</i> 167599335 <i>Prosthecochloris sp. ZM</i> 114617576 <i>Prosthecochloris sp. ZM_2</i> 114608494 <i>Prosthecochloris sp. HL-130-GSB</i> 085659969 <i>Prosthecochloris sp. GSB1</i> 094082062 <i>Ignavibacterium album</i> 385809169 <i>Coprococcus comes</i> 547810973 <i>Dorea formicigenerans</i> 491476797 <i>Ruminococcus gnavus</i> 547306355 <i>Ruminococcus lactaris</i> 491807899 <i>Brevibacillus borstelensis</i> 489487643 <i>Bacillus methanolicus</i> 489446679 <i>Brevibacillus brevis</i> 515820898 <i>Capnocytophaga granulosa</i> 512458271 <i>Gillisia limnaea</i> 494046916 <i>Salinibacter ruber</i> 83815922 <i>Capnocytophaga gingivalis</i> 488743213 <i>Pedobacter heparinus</i> 255531532 <i>Mucilaginibacter palidis</i> 495785702 <i>Joostella marina</i> 495886911 <i>Muricauda ruestringensis</i> 344202636 <i>Veillonella parvula</i> 544718202 <i>Dorea formicigenerans</i> 117980906 <i>Candidatus Blautia avicola</i> HJD28867 <i>Veillonella dispar</i> MBF1741846 <i>Blautia faecis</i> 148463538 <i>Hespellia stercorisuis</i> 084533899 <i>Zhouia amyloytica</i> 038268184 <i>Veillonella dispar</i> 566235315 | GQSLHGDEVEVLVTKVPE ----L-Q---Q---S NS-M-----Q----- SDAI---D-----Q NSAI---I---V----- NSAI-----A---SS NSAI-----LT----K -L-I-----S---D NF-I-----D----- -AA-----SST----- NGA-D---I---R-S-S -EA---L----- -DAI-----K AI-R---D-----P -A-L-QI----- -I-----D AI-----LT----K -I-----D -G-V-----V-T-K -TAFD-K---ILFAKQKG NGAMQ-----VI-A-G -GA-D-T---II---ASDR NGAFQ-----F-I-S-G NGAFQ-----CII-GS-SG NGA---T-L-RME-ISGG NNAM---T-L-RISTESSG NGA---T-F-R-E-EAGG NKA---L---Y-FPRRRRE NKA-N---IYIYNKRNK -EA---L-R-A-AARAPE NKA---V---Y-FPRRRRE HNA---K-K-YIYAKKSG RNA---R-K-Y-YAKSKG NKA---L---Y-FPRRRRK NRAF---T---FIKPKRKS -TAM-N-K-R-RIVPSDY- -GA-D---T---II---ASD -GAF---Q-QI-LK-EER -TAM-N-K-R-R-IPSNY -TAM-Q-K-R-II---E-Q -AFO---K---I---S-- KA-----Y-FPRRR -TAM-N-K-R-RIVPSDY- | -YVSKQSP -A-R----- -A-ST----- -QTQ----- -SA-ST----- -S-RST----- -S-RST----- -S-ST----- -S-ST----- -TA-A----- -S-RST----- -R----- -Q-Q-T----- -Q-Q----- -R----- -D----- -STRST----- -S-ST----- -V----- -Q-TQ----- -VTA-RQ-N-SVI----- -VTA-IE-RIQS----- -V-K-N-S-HSV----- RNL---Q---L---RKEY---I K-R---K-TKI-Q-GTQR---Y DGK-R---K---KI-E-G---V-L R-K---K---R---HGT-KVI-L R-Q---K---GII-HQV-KV--L N-A---R-IR---E-GI-EV--- --R---TV-R-IE-GTQQ--- N-L---Q-IRI-E-GIKQV--- KL---E-TQ-IE---R-F---L RKS---EV-KIIK---R-EF---I DKKR---CEVLE---E-RC-EV--- KL---E-TQIIE---R-F---V RKN---EV-EIIE-SK-DFI-V K-K---EVIEIIK-AKMEFT-I GKKI---E-TKILE---K-F---I KKM---EVSK-LE---K-SY---I KHKR---I---D-IE-ANE----- RDGK-R---K---KI-E-G---V--- -GK-R---RV-KILE-GMEE--- TKHKR---I---IE-ANE-V--- EGK-R---IV-K-LE-GM-Q--- ER-K---K---KILE-GT---L--- GSK-L---E-TK-IN---R-F--- KHKR---I---D-IE-ANE----- | |
| Other Bacteria | | | | |

Figure S29

Partial sequence alignment of the protein ribonuclease R, showing a 7-8 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 558 | KVAILANFKKIGYTLQLTGKG | KNGAP | VVKAKALRDLKKQVHGTNVEFLVN | 608 |
|------------------------------|--|------------|---|---------|---|-----|
| Order <i>Chlorobiales</i> | <i>Prosthecochloris aestuarii</i> | 194333503 | - - - T - RR - - - K - D - A | - G - - | K-SSQ - - N - - - - - | |
| | <i>Chlorobium phaeobacteroides</i> | 189499728 | R - QV - - - R - - FD - K - DR | - DS A | T - SSR - - E - - QK - - - | |
| | <i>Chlorobaculum parvum</i> | 193213033 | - - QV - S - R - - FD - K - DR | - DS A | T - SS - - E - - QK - R - - | |
| | <i>Chlorobaculum tepidum</i> | 21673344 | RIVQ - SE - - T - L - FK - E - KKNA | L - N | T - SS - G - S - E - - K - S - - I - - | |
| | <i>Chloroherpeton thalassium</i> | 193216324 | R - I - - - - - D - KIN - - | - D - | I - S - S - E - - QK - - - | |
| | <i>Chlorobium luteolum</i> | 78186405 | R - T - - - - M - E - K - N - - | - D - | I - S - - E - - QK - R - S - | |
| | <i>Chlorobium phaeovibrioides</i> | 145219389 | R - LV - - - R - - - KIN RE - | - T - | I - S - - Q - Q - - S - - - | |
| | <i>Chlorobium limicola</i> | 189346025 | - - I - S - - RF - FD - K - N R - | - E - | I - T - - - Q - Q - - K - - - S | |
| | <i>Chlorobium ferrooxidans</i> | 493409164 | - - G - - - R - - S - - N - - | - G - V | KISS - - - - - M - - - | |
| | <i>Prosthecochloris marina</i> | 110024153 | R - TV - - - R - - FD - K - DR - | - ES - | T - S - - E - - QK - - - | |
| | <i>Chlorobaculum thiosulfatiphilum</i> | TNJ39330 | R - G - - - R - - Q - - D - S - - | - - - | N - TS - - QG - - - S - - - | |
| | <i>Prosthecochloris ethylica</i> | 198911867 | - - L - - - RL - FD - K - N R - | - E - | I - S - S - - Q - Q - - K - S - I - - S | |
| | <i>Chlorobium phaeoclathratiforme</i> | 194336956 | - - VMV - - - RL - D - KIN RS - | - E - I | I - S - QS - - Q - Q - - K - - - S | |
| | <i>Chlorobium chlorochromati</i> | 78188726 | R - TV - - - R - - FE - K - DR - | - ES - | T - SS - S - - E - - QK - - - | |
| | <i>Chlorobaculum limnaeum</i> | 2501894184 | - - IKT - A - - RL - E - E - KKNA | F - N E | TATSQ - - - K - AD - K - S - - Y - S | |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM24073 | R - IV - - - - - E - K - NKGK | EGP | I - T - - - E - - QK - - - | |
| | <i>Chlorobium sp. N1</i> | 131358104 | - - - - - - - - - - - - - - - - | - | - - - - - - - - - - - - - - - - | |
| | <i>Prosthecochloris sp. ZM</i> | 114617576 | - - G - - - R - - N - - D - S - | - K - G | T - TS - - - - - | |
| | <i>Prosthecochloris sp. GSB1</i> | 094082062 | - - G - - - R - - Q - - D - S - | - - - | N - TS - - QG - - - S - - - | |
| | <i>Prosthecochloris sp. ZM_2</i> | 114608494 | R - QV - - - - - FD - K - DR - | - ES - | T - SS - - E - - QK - - - | |
| | <i>Chlorobaculum parvum</i> | HHE33015 | R - QV - S - - R - - FD - K - DRR - | - ES - | T - SS - - E - - QK - R - - - | |
| | <i>Chlorobaculum sp. 24CR</i> | RXK82182 | - - I - S - - RF - FD - K - NRGK | EG P | I - T - - - E - - QK - - - | |
| | <i>Chlorobium sp. KB01</i> | 076791152 | - - IMEFVR - - SL - - QVSQNLIK | - | - - - - - - - - - - - - - - - - | |
| <i>Ignavibacteriae</i> | <i>Ignavibacterium album</i> | 385809169 | - - INEF - R - - SL - - SDFDNAAN | - | - TSEFQK - - D - - K - KEE - P - I - | |
| | <i>Melioribacter roseus</i> | 397690865 | - - IRO - - QY - RVF - - H - E - - ED - | - | - PMEFQK - - DAAK - SEE - AVI - | |
| | <i>Rhodothermus marinus</i> | 345303465 | RLGTF - - - A - - F - HEVKIDDEQ | - | T - SS - - NE - - QH - K - PE - PVIE | |
| | <i>Rhodonellum psychrophilum</i> | 518430380 | - - L - D - - E - - GRL - - K - RT - - TP | - | KVS - - - KK - - DEIQ - KPEQNVL | |
| | <i>Porphyromonas levii</i> | 517169631 | - - YESFSK - - R - F - FEAAPKQSE | - | EMI - - - N - - EK - - - KPEAE - IE | |
| | <i>Marinilabilia salmonicolor</i> | 498351409 | - - MEN - SE - IRRF - - KIKVVD - SK | - | TISSS - NRV - GE - Q - KKQQNI - E | |
| | <i>Dysgonomonas gadei</i> | 493853178 | - - MKN - SE - IRRF - - KIKVVD - TK | - | TNVS - SINH - - DE - S - KPE - N - IS | |
| | <i>Dysgonomonas mossii</i> | 493896130 | - - L - GI - EQ - - P - IKFDVKEKNI | - | TNVS - - INH - - DE - S - KPE - N - IS | |
| | <i>Gracilimonas tropica</i> | 521070004 | - - IKN - KQL - RSF - - K - NIKRIN | - | SP - KIN - - GK - EN - SL - YI - - | |
| | <i>Candidatus Azobacteroides</i> | 212550560 | RLETFS - - A - - F - HEIKVGEGN | - | NETS - NISS - F - - - Q - - REQN - IE | |
| | <i>Belliella baltica</i> | 390945305 | - - LML - QGVIS - F - - SIDMKS - K | - | RIS - - - NQ - MDEIQ - KPEQNVL | |
| | <i>Joostella marina</i> | 495886911 | - - FETFSR - - R - F - LEAKPK - QE | - | AVTSS - NA - - EE - Q - KEQN - D | |
| | <i>Thermophagus xiamenensis</i> | 498212010 | RLETF - T - A - - F - HD - KISEGA | - | TISHS - NR - - DK - Q - QKHQNI - E | |
| | <i>Mariniradius saccharolyticus</i> | 495907092 | - - RLETFS - - A - - F - HEVSI - EAT | - | KIS - - - NQ - MDEIV - KPEQNVL | |
| Other Bacteria | <i>Echinicola vietnamensis</i> | 431796052 | - - LQNF - - AGRF - FSIRTDSDK | - | RVSAT - NK - MGEIE - KPEQN - LE | |
| | <i>Emticicia oligotrophica</i> | 408673906 | - - YETFSK - - R - L - FEAMPK - RE | - | SLSNSNMNKMMS - E - - P - QNVLE | |
| | <i>Anaerophaga thermohalophila</i> | 515148300 | - - L - S - KQ - - IHQF - - K - E - GDRK | - | TISHS - NR - - DE - Q - KKQQNI - E | |
| | <i>Ornithobacterium rhinotracheale</i> | 392390324 | - - L - Q - RS - - MRF - - QMKSDAV - | - | KTTASINQ - - HD - K - KGE - NMIE | |
| | <i>Alistipes indistinctus</i> | 496424899 | RLQNF - EY - GRL - - KFIY - - DA | - | KAL - - DINK - MTN - - KQE - N - IS | |
| | <i>Porphyromonas bennonis</i> | 516799870 | RLMD - S - - - QF - - QVDTKNRN | - | QSISDN - NK - - EEIK - KPE - NVIQ | |
| | <i>Owenweeksia hongkongensis</i> | 375012907 | RLQTF - - - A - L - LK - EVEEES | - | GISRS - NKK - SD - K - KGEANMIE | |
| | <i>Dyadobacter beijingensis</i> | 518787359 | - - L - GI - GQ - APL - IKFEVRD - I | - | KI - - SMNAM - AK - E - KPEQN - IE | |
| | <i>Balneola vulgaris</i> | 516842897 | - - LMA - NGVISRF - HSIN - DOPK | - | TP - YINN - - QK - KE - P - MI - - | |
| | <i>Muricauda ruestringensis</i> | 344202636 | RMIQFKE - IHNF - - E - KGKIN - | - | SING - NK - - ED - K - KKEQN - D | |
| | <i>Halanaerobium hydrogeniformans</i> | 312143213 | - - IRA - - T - INNF - FSHMIGANE | - | - HPRS - QAI - EE - R - SKE - KIIIE | |
| | <i>Dorea formicigenerans</i> | 491479248 | - - ISE - NSYLRLP - - VINYDE - V | - | - RP - EIQQ - - GK - E - - PQ - A - IS | |
| | <i>Peptoniphilus rhinitidis</i> | 497928569 | - - LQV - KT - - ANF - LN - P - KNGE | - | EPVVDVQKV - EKAK - - KE - MFIS | |
| | <i>Stomatobaculum longum</i> | 497218087 | RLEA - RS - LQT - NLN - PTGALR | - | - TPREIQK - - LR - E - - ET - D - S | |
| | <i>Novispirillum itersonii</i> | 518473365 | - - QE - - R - LQSL - IS - SKSAEL | - | PQNFNTVISK - R - - SQ - H - - | |
| | <i>Bartonella elizabethae</i> | 492187130 | - - QE - - RT - LQSL - IS - SKSAEL | - | TSAR - NRI - EK - AH - EQQE - - | |
| | <i>Bartonella tribocorum</i> | 163868292 | - - QE - - RT - LQSL - IS - SKSAEL | - | TSAR - NKI - EK - AH - EQQE - - | |

Figure S30

Partial sequence alignment of the protein ribonuclease R, showing a 4-5 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 640 | HFTSPIRYPDLVIHLIF | ETTR | KKRRKVTPERITEISATITVECQITNEREKIAEARE | 720 |
|---|---|------------|-------------------|--------------------|---|---|
| Order <i>Chlorobiales</i> | <i>Chlorobaculum parvum</i> | 193213033 | - - - - - | IV | - - - - - | SQ-T - - - - - |
| | <i>Chlorobaculum tepidum</i> | 21673344 | - - - - - | IV - M - - | AF | - - - I - A - A - LTQK - ET - R - S - - - V - - - |
| | <i>Chlorobium phaeovibrioides</i> | 145219389 | - - - - - | IV - L - - | GL | - - - I - SA - LA - LTDK - QT - - - S - - - S - V - - - |
| | <i>Chlorobium ferrooxidans</i> | 493409164 | - - - - - | IV - M - - | NL | - - - ISAT - LA - LTDK - QT - - - S - - - S - V - - - |
| | <i>Chlorobium phaeoclathestriforme</i> | 194336956 | - - - - - | IV - L - - | AF | - - - MPEA - A - LKQK - DT - R - S - - - V - V - - - |
| | <i>Chlorobium luteolum</i> | 78186405 | - - - - - | L - - M - - | RM | - - - MSET - LG - LET - MQR - - - S - - - S - A - - - |
| | <i>Chlorobium chlorochromatii</i> | 78188726 | - - - - - | V - L - - | SL | - - - S - K - EPLR - LK - ESK - V - I - K - A - - - S - V - - - |
| | <i>Prosthecochloris aestuarii</i> | 194333503 | - - - - - | V - L - - | ML | - - - NRKK - I - DK - LA - L - EK - RVTSR - S - - - S - V - - - |
| | <i>Chlorobium limicola</i> | 189346025 | - - - - - | IV - L - - | SF | - - - Q - K - PAK - LK - GGV - D - T - A - - - S - V - - - |
| | <i>Prosthecochloris ethylica</i> | NUK48115 | - - - - - | IV - L - - | AS | - - - R - K - LSAK - LA - - TK - EQI - KNS - - - S - M - - - |
| | <i>Chlorobium phaeobacteroides</i> | 189499728 | - - - - - | MV - L - - | NR | - - - S - K - PPK - A - LRSK - PL - EQASAQ - QN - A - - - D |
| | <i>Chloroherpeton thalassium</i> | 193216324 | - - - - - | IV - L - Y - | GA | - - - K - IS - - SA - TGMLGS - - - S - - - |
| | <i>Chlorobaculum limnaeum</i> | 2501894184 | - - - - - | IV - L - - | AY | - - - S - - IPSK - L - D - - K - EQ - VA - - - S - M - - - |
| | <i>Prosthecochloris marina</i> | 110024153 | - - - - - | V - L - - | AY | - - - S - - IPSK - L - D - - K - EQ - VA - - - S - M - - - |
| | <i>Chlorobaculum thiosulfatiphilum</i> | TNJ39330 | - - - - - | IV - L - - | GA | - - - K - IS - - SA - TGMLGS - - - S - - - |
| | <i>Cand. Thermocholorobacter aerophilum</i> | RFM24073 | - - - - - | T - L - Y - | L | - - - QSQ - LSKP - LRKLN - L - PAI - EHAS - Q - RNA - - - D |
| | <i>Candidatus Chlorobium masyuteum</i> | 166807966 | - - - - - | IV - L - - | GL | - - - I - SA - LA - LTDK - QT - - - S - - - S - V - - - |
| | <i>Chlorobium sp. N1</i> | 131358104 | - - - - - | LV - M - - | AF | - - - RMPEA - N - LKGKLEA - R - S - - - V - V - - - |
| | <i>Chlorobium sp. KB01</i> | 076791152 | - - - - - | IV - L - - | GL | - - - ITSA - LA - LTDK - QT - - - S - - - S - V - - - |
| | <i>Chlorobaculum sp. 24CR</i> | RXK82182 | - - - - - | - - - - - | - | - |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085659969 | - - - - - | IV - L - - | AS | - - - Q - K - A - K - LKS - GGL - D - - R - S - - - S - V - - - |
| | <i>Prosthecochloris sp. GSB1</i> | 094082062 | - - - - - | S - V - L - - | HL | - - - MS - G - LAGI - SR - D - - A - - - S - V - - - |
| | <i>Prosthecochloris sp. ZM_2</i> | 114608494 | - - - - - | IV - L - - | SF | - - - Q - K - PAK - LK - GGV - D - - I - A - - - S - V - - - |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobi bacterium NICIL-2</i> | KXB97643 | - - - - - | V - L - K - | SLG-P - Q - AHLRDRLEPIAEH - S - - - RH - V - | - |
| | <i>Chlorobi bacterium OLB7</i> | KXK55686 | - - - - - | IV - M - A - - | EAGMPVRQRN - YA - - LGPIADQCS - - RA - V - D - | - |
| | <i>Chlorobi bacterium OLB5</i> | KXK52504 | - - - - - | IV - - - - - | LNGLNAK - AQHYHKOLPDI - KQST - T - IN - Q - - - | - |
| | <i>Ignavibacterium album</i> | 385809169 | - - - - - | S - LV - L - K - | IEN - QLPGYTLEELE - I - EHISAC - RT - M - - - Y | - |
| | <i>Dorea longicatena</i> | 548195207 | - - - - - | Q - - - - - | L - GRMNESK - EHYQSILP - - AKRSS - M - RR - D - - - | - |
| | <i>Bacteroides dorei</i> | 547774176 | - - - - - | F - MMV - L - TR - | LAGGR - VQETKYE - L - DHSS - M - Q - - AN - A - A | - |
| | <i>Megamonas hypermegale</i> | 479207615 | - - - - - | IV - L - K - Q | LNG - M - A - - QEKLKSRLP - - ATH - SKQ - R - - - | - |
| | <i>Tepidanaerobacter syntrophicus</i> | 059033581 | - - - - - | A - - - - - | QEN - LDLK - QEKL - K - VEK - AK - SS - - R - D - - - | - |
| | <i>Megamonas funiformis</i> | 225091553 | - - - - - | IV - L - K - Q | LNG - M - A - - QEKLKSRLP - - ATH - SKQ - R - - - | - |
| | <i>Moorella mulderi</i> | KYH31529 | - - - - - | V - - - R - T | WSPGGL - Q - MA - LE - FVALAAQAS - - - V - E - | - |
| | <i>Biomaibacter acetigenes</i> | 122015104 | - - - - - | M - - - IR - H | LEGRDAK - QEKL - KIVAR - AK - SS - - R - D - - - | - |
| | <i>Aneurinibacillus aneurinilyticus</i> | ERI11065 | - - - - - | IV - MIR - S | L - KGGF - Q - ME - WY - KMPQ - AEQSS - - RN - VD - - - | - |
| | <i>Megamonas hypermegale</i> | CBL07268 | - - - - - | IV - L - K - Q | LNG - M - A - - QEKLKSRLP - - ATH - SKQ - R - - - | - |
| | <i>Alicyclobacillus mali</i> | 206885292 | - - - - - | A - - - IR - V | LT - GSLS - K - EA - LREFVA - ASRQSS - - R - Q - - - | - |
| | <i>Thermovenabulum gondwanense</i> | 068747371 | - - - - - | I - - - R - Q | LEGGI - EK - QEQL - KILGK - AKLSS - - R - DD - - - | - |
| | <i>Moorella stamsii</i> | PRR71716 | - - - - - | V - - - R - T | WSPGGL - Q - MA - LD - FVALAAQAS - - - V - E - | - |
| | <i>Alicyclobacillus mali</i> | 206881148 | - - - - - | A - - - IR - V | LT - GSLS - - EAQLREFVADASRQSS - - R - Q - - - | - |
| | <i>Peptococcaceae bacterium</i> | MBU7006244 | - - - - - | IK - N | LAKGRL - EK - K - KLASFVADAAIQSS - - R - - - | - |
| | <i>Paludifilum halophilum</i> | OYD08178 | - - - - - | I - - - IW - V | W ENGGTLSR - - KN - LDEWLP - A - KHAS - - RL - ID - - | - |
| | <i>Aneurinibacillus migulanus</i> | KIV50047 | - - - - - | IV - MIR - S | L - KGGF - Q - E - WY - KMPQ - AEQSS - - RN - VD - - - | - |
| | <i>Laceyella tengchongensis</i> | 154987632 | - - - - - | L - - - VIR - I | VEKGAL - V - - D - LN - YL - DAA - QSSI - - RV - VD - - - | - |
| | <i>Ruminiclostridium cellobioparum</i> | 200859494 | - - - - - | I - - - MKQ - | LKGEM - EQ - - GQLEGMLP - IAKQCS - - RA - D - - - | - |
| | <i>Brevibacillus fulvus</i> | 204519821 | - - - - - | IV - LIR - W | VE - GEM - G - - MSYW - EKLP - IADHSSQ - - RL - V - - - | - |
| | <i>Candidatus Kryptonium thompsonii</i> | CUS84555 | - - - - - | V - L - Y - | TEKG - DDK - LR - - AQLPQI - KHSS - M - IR - M - V - - - | - |
| | <i>Effusibacillus pohliae</i> | 018132105 | - - - - - | IR - A | I EHGGTL - - DQLR - KLP - IA - HSS - - RL - VD - - - | - |
| | <i>Candidatus Thermokryptitus mobilis</i> | HGD82170 | - - - - - | V - L - Y - | TEGR - DDK - LN - - AEKLPHI - KHSS - M - IK - - - | - |
| | <i>Megamonas funiformis</i> | 495813334 | - - - - - | IV - L - K - Q | LNG - M - A - - QEKLKSRLP - - ATH - SKQ - R - - - | - |
| | <i>Megamonas rupellensis</i> | 517829942 | - - - - - | IV - L - K - Q | LNG - M - A - - QEKLKSRLP - - ATH - SKQ - R - - - | - |
| | <i>Selenomonas artemidis</i> | 496716638 | - - - - - | MV - L - R - T | FATGHISA - - REKLR - ILP - TAEHSSA - - R - V - - - | - |
| | <i>Mitsuokella multacida</i> | 492427663 | - - - - - | IV - L - R - T | FATGSIPA - - OAKLRTMLP - IADHAS - - R - I - - - | - |
| | <i>Ruminococcus lactaris</i> | 491807899 | - - - - - | Q - - - IK - N | L - GRL - G - - MEHYRKIL - - ASQCSQ - - RT - E - S - - - | - |
| | <i>Dorea formicigenerans</i> | 491479248 | - - - - - | Q - - - IKDN | L - GRMN - - MEHYRKILE - - TKHAS - T - RR - D - - - | - |
| | <i>Clostridium termtidis</i> | 490767815 | - - - - - | I - - - MKQ - | LKGEM - EQ - - GQLEGMLP - IAKQCS - - RA - D - - - | - |
| | <i>Moorella thermoacetica</i> | 83589142 | - - - - - | V - - - R - T | WSPAGFSA - - MAQL - - FVALAAVQAS - - - L - E - - - | - |
| | <i>Eubacterium ramulus</i> | 545613973 | - - - - - | Q - - - IKDN | L - GRMNEK - - EHYWKIL - - A - Q - SQL - RR - D - - - | - |
| | <i>Dorea longicatena</i> | 493473057 | - - - - - | Q - - - IKDN | L - GRMNEK - - EHYQSILP - - AKRSS - M - RR - D - - - | - |
| | <i>Alicyclobacillus acidocaldarius</i> | 258510746 | - - - - - | A - - - IR - V | LT - GSLS - Q - EAHLREFVA - ASRQSS - - R - Q - - - | - |

Figure S31

Partial sequence alignment of the protein ribonuclease R, showing a 4 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 128 | KDVGDFHPENLGRVLVMGH | LD | KCFVSVTPYQILEL | 161 |
|---|---|------------|---------------------|---------------|-------------------|--------------|
| Order <i>Chlorobiales</i> | <i>Chlorobium luteolum</i> | 78186789 | - | - | - | - |
| | <i>Chlorobium phaeovibrioides</i> | 145219770 | - | - | - | - |
| | <i>Chlorobium limicola</i> | 189346631 | - | - | - | - |
| | <i>Chlorobium chlorochromatii</i> | 78188750 | - | - | - | - |
| | <i>Chlorobium phaeoclathestratiforme</i> | 194336300 | - | - | - | - |
| | <i>Chlorobium phaeobacteroides</i> | 119356824 | - | - | - | - |
| | <i>Chlorobium ferrooxidans</i> | 493409873 | - | -E- | - | - |
| | <i>Chloroherpeton thalassium</i> | 193214168 | - | -V--L- | - | -F-I-I |
| | <i>Prosthecochloris aestuariai</i> | 194333994 | - | -V-QM-L-N | -I--- | -F----- |
| | <i>Prosthecochloris vibrioformis</i> | 139626438 | - | -V-QM-L-N | -YI | - |
| | <i>Prosthecochloris aestuariai</i> | 012505742 | - | -V-QM-L-N | -I--- | -F----- |
| | <i>Prosthecochloris marina</i> | 110022066 | - | -V-QM-L-N | -N | -L----- |
| | <i>Candidatus Chlorobium masyuteu</i> | 166808243 | - | -E | - | - |
| | <i>Cand. Thermocholorobacter aerophilum</i> | RFM24899 | - | -V--L-R | -E | -E-----V |
| | <i>Cand. Thermocholorobacteriaceae GBChIB</i> | KER10279 | - | -V---MI-R | -E | -E-----F-VV- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobium sp. 445</i> | PI047889 | - | -V---I-R | -N | -D-----L-V- |
| | <i>Prosthecochloris sp. CIB 2401</i> | 068867189 | - | -V-QM-L-N | -YI | - |
| | <i>Prosthecochloris sp. GSB1</i> | 094083698 | - | -V-QM-L-N | -YI | - |
| | <i>Prosthecochloris sp. ZM</i> | 114617229 | - | -V-QM-L-N | -I | -F----- |
| | <i>Chlorobium sp. N1</i> | 131353427 | - | -K---E | - | - |
| | <i>Chlorobi bacterium OLB5</i> | KXK44039 | - | -V-A---MI-E | -TP | -A-H- |
| | <i>Chlorobi bacterium OLB6</i> | KXK33887 | - | -M-A---LI-Q | -G-AP | -A-M-M |
| | <i>Chlorobi bacterium NICIL-2</i> | KXK98178 | - | -V-V---V-L | PALLP | -A-V- |
| | <i>Melioribacter roseus P3M-2</i> | 397690900 | - | -Q-V-N---I-K | DT-YP | -V-I |
| | <i>Ignavibacterium album JCM 16511</i> | 385810387 | - | -MSV-N---I-K | ET | -A-Q-- |
| Other Bacteria | <i>Thermoflexus hugenholtzii</i> | 088572012 | - | -A---GLKG K | P--IP | -L--AM |
| | <i>Roseburia inulinivorans</i> | 547180385 | - | -Q-V-K---I-E | EG | -A-VIQ- |
| | <i>Parabacteroides goldsteinii</i> | 494928053 | - | -I-V---MSI-L | P-----A-A- | |
| | <i>Bacteroides pyogenes</i> | 567224504 | - | -I-V---MSI-L | P-Y-A-N----- | |
| | <i>Barnesiella viscericola</i> | 570742677 | - | -V-V---MSI-L | P----A-A-M-- | |
| | <i>Caldimicrobium thiodismutans</i> | 068516299 | - | -V-V-K---I-E | P--IP-----V-M- | |
| | <i>Thermodesulfobacterium geofontis</i> | HHH86329 | - | -I-V-KM---E | P--LP-----V- | |
| | <i>Dissulfurirhabdus thermomarina</i> | 163298669 | - | -V-V-KM---I-E | Q--LP-----M | |
| | <i>Cyprideis torosa</i> | CAD7233470 | - | -V-I---M-L-E | Q--LP-----H-V- | |
| | <i>Thermodesulfobacterium hveragerdense</i> | 028841618 | - | -F----- | PT-IP-----V- | |
| | <i>Massilibacterium senegalense</i> | 062198499 | - | -M-V-KMLL-E | E--I-----F--M | |
| | <i>Thermodesulfobacterium commune</i> | 038060483 | - | -F----- | PT-IP-----F- | |
| | <i>Sporosarcina psychrophila</i> | HJF33052 | - | -V-KMII-Q | -S-L-----IK- | |
| | <i>Vagococcus vulgaris</i> | 125984437 | - | -M-M-K-L-E | PDKIA-----I- | |
| | <i>Lachnobacterium bovis</i> | 027421766 | - | -V-A---I-Q | -G-----F-IQ- | |
| | <i>Fictibacillus gelatinii</i> | 026676258 | - | -V-V-KMML-Q | E--I----- | |
| | <i>Candidatus Electrothrix aarhusiensis</i> | RWX44970 | - | -V-V-KM-V-E | Q--LP-----H-V- | |
| | <i>Desulfovulbus propionicus</i> | 043770998 | - | -V-V-KMML-E | R--LP-----H----- | |
| | <i>Desulfocapsa sulfexigens</i> | HIP82974 | - | -V-V-KM-L-E | ---LP-----M | |
| | <i>Dissulfuribacter thermophilus</i> | 067616757 | - | -V-V-M-I-E | R--LP-----H-----M | |
| | <i>Bacteroides ovatus</i> | 490428650 | - | -I-V---MSI-L | P-Y-A-N----- | |
| | <i>Parabacteroides distasonis</i> | 150006885 | - | -I-V---MSI-L | P----A-A----- | |
| | <i>Pontibacter roseus</i> | 517289359 | - | -A-V---M-A-L | SS-LPA-----Q- | |
| | <i>Roseburia hominis</i> | 347531843 | - | -V-K---I-E | PG-----A-IQ- | |
| | <i>Ruminococcus lactaris</i> | 565839991 | - | -SV---WI-E | -G-L-----A-VIQ- | |
| | <i>Desulfocapsa sulfexigens</i> | 451948750 | - | -V-V-KM-L-E | Q--LP-----H----- | |
| | <i>Desulfurivibrio alkaliphilus</i> | 297568807 | - | -V-V-KM-L-E | Q--LP-----H----- | |
| | <i>Sphaerochaeta pleomorpha</i> | 374314810 | - | -I-V-K-LI-L | P-----K-V--M | |
| | <i>Sporosarcina newyorkensis</i> | 497174567 | - | -M-V-KMLI-Q | QT-LP-----MQ- | |

Figure S32

Partial sequence alignment of the protein bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase, showing a 2 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*. The CSI is not present in any other bacterial species except for one other bacterium as indicated above, in the top 500-BLASTp hits.

**Order
*Chlorobiales***

Ignavibacteriae

Other Bacteria

| | | | |
|--|------------|-------------------------|-----------------------|
| <i>Chlorobium luteolum</i> | 78187149 | IDHIAIAVSNLDEAVRTYMNVLG | CGADAIRIEEVPSSEQVRVA |
| <i>Chlorobium phaeoclathratiforme</i> | 194336268 | -----Q--ES-LE-FR- | --ARE-----N--- |
| <i>Chlorobium limicola</i> | 189346400 | -----Q--ES-LE-F-- | --APGD-T-----K--- |
| <i>Chlorobium phaeovibrioides</i> | 145219844 | -----T--D-LK-FTSL | --SRENEV-V---T-G-- |
| <i>Chlorobium ferrooxidans</i> | 493411298 | -----Q--EVSL-EFL-- | --DPKSVT-----N--- |
| <i>Chlorobaculum parvum</i> | 193212493 | -----QD-NS--D-FVA- | --ERSVT-H---A-K--- |
| <i>Chlorobium phaeobacteroides</i> | 119356984 | -----E--ER-IE-FKS- | --ASET-I---A-N--- |
| <i>Chloroherpeton thalassium</i> | 193214308 | --V---NSI-ATLA-FKQL | VD E-VAS-SY---AQK-K-- |
| <i>Prosthecochloris aestuariae</i> | 194333709 | -----ND--S-IQQF SAL | T- IDPESVH-Q---K--- |
| <i>Chlorobium chlorochromatii</i> | 78189093 | -----Q--N-LD-FCTI | --VDRQSVH---A-K--G |
| <i>Chlorobaculum limnaeum</i> | 2501895295 | -----E--S-ID-WI-I | --DRS-VT-H---K-A- |
| <i>Prosthecochloris marina</i> | 110023485 | -----HD-ENSLEQFKIL | TD ATDAQ-----K-- |
| <i>Chloroherpeton thalassium</i> | 012499144 | --V---NSI-ATLA-FKQL | VD E-VAS-SY---AQK-K-- |
| <i>Chlorobaculum thiosulfatiphilum</i> | 139457284 | -----EH--S-ID-WT-L | --DRS-VT-H---K-A- |
| <i>Candidatus Chlorobium masyuteum</i> | 166808062 | -----Q--EVSL-EFL-- | --DPKSVT-----N--- |
| <i>Cand. Thermochlorobacter aerophilum</i> | RFM24960 | -----V--RSI-A-LA-FSQL | --TEVLR-T---L-QHH-- |
| <i>Chlorobium sp. 445</i> | PI047471 | -----HS--A-LE-FSKL | -S GSTMH-T---L-AHH-I- |
| <i>Chlorobaculum sp. 24CR</i> | 129050599 | -----E--N-ID-WTKL | --SRS-VT-H---K-A- |
| <i>Prosthecochloris sp. GS1</i> | 094082721 | -----D ES-LERF-AL | T- IAPGN-----K-- |
| <i>Prosthecochloris sp. ZM</i> | 114618132 | -----ND--S-IQQF SAL | T- IDPESVH-----K-- |
| <i>Prosthecochloris sp. HL-130-GSB</i> | 085660616 | -----RD--S-IER-IAL | T- ISRESVH---A-H-- |
| <i>Ignavibacterium album</i> | 385810015 | -E--G--K--H--IKF-EE- | F-LKCYAV---ADQK-T- |
| <i>Melioribacter roseus</i> | 397689645 | -E--G--K--EQ-IKY-EE- | L-LKCYS---IRTOKT-T- |
| <i>Calditerrivibrio nitroreducens</i> | 313673009 | -----G--KS---LIF-KSI | -VSPYHF---E-QK-K-- |
| <i>Deferribacter desulfuricans</i> | 291280307 | -----G--K--I---LSL-KTL | -ITPYHF---E-QK-KT- |
| <i>Denitrovibrio acetiphilus</i> | 291288330 | -----G--K--E--TAF-KTM | -IEPYHF---I-QR-K-- |
| <i>Mucispirillum schaedleri</i> | 555549200 | -N--GV--KSIEA-LPF-KAM | -----INH-----QK-KT- |
| <i>Flexistipes sinusarabici</i> | 336322433 | -----G--KS-E---F-ETL | -VSVESY---T-QK-K-- |
| <i>Geobacillus thermolevorans</i> | 375009421 | V----G---RSIEK-LPF-TD- | L-LPFLG---E---K-- |
| <i>Geobacillus kaustophilus</i> | 519662137 | V----G---RSIEK-LPF-TD- | L-LPFLG---E---K-- |
| <i>Anoxybacillus kamchatkensis</i> | 518246766 | V----G---KS---LPF-TDT | L-LSCVG---T-E----- |
| <i>Akkermansia muciniphila</i> | BBP47461 | -----G--KS--AT-PY-RDA | L-LGEPH---TQK-- |
| <i>Nitrososphaeraceae archaeon</i> | MBD0360489 | -----KSV--KD-QS- | LNI-QLEF-V--N-K-K-- |
| <i>Caldithrix abyssi</i> | HHJ53415 | -N-----ES---IPY-RD- | L-LEL-AT---DKO----- |
| <i>Chrysogenes arsenatis</i> | 027389778 | -----GV--RS-----GF-RAM | --EPYHF-----QK-- |
| <i>Acinetobacter baumannii</i> | 171253172 | -E--G--K-----QF-TQI | L-LKL-GF-T-E-K-- |
| <i>Fictibacillus phosphorivorans</i> | 153237885 | -----G--KS---LPF-V-- | L-LTLEA-----QK-K-- |
| <i>Ureibacillus manganicus</i> | 036186694 | -----G--N---RITY-TET | L-LKLLK-----QN-- |
| <i>Lysinibacillus halotolerans</i> | 122973306 | V----G--K---RITY-TET | L-LTLLK-----QH-- |
| <i>Psychrobacillus glaciei</i> | 151700256 | V----G---KSIE--LPY-IET | L-LKL-H-----K-- |
| <i>Candidatus Nitrosotalea okcheo</i> | 157927039 | L--V----N---A-KQ-KE- | L-VGDVEF-T-E-G-- |
| <i>Ktedonosporobacter rubrisoli</i> | 207229296 | ---V--I-K-IEQ-LSF-RD- | L-ITPSA-K---T---I- |
| <i>Candidatus Nitrososphaera garginis</i> | 015019962 | V-----K-VE--LKN-QKI | LNV-HLE-----N-K-K-- |
| <i>Paenisporosarcina indica</i> | 075618245 | V----G--K---TIPY-TET | L-LTLLK---A-S----- |
| <i>Kurthia huakuii</i> | 029499089 | V----G--K-I--RIAY-TD | L-MNLEKV---E---K-- |
| <i>Psychrobacillus soli</i> | 142606884 | V----G---K-IEDSLPY-TET | L-LKL-H-----K-- |
| <i>Lysinibacillus timonensis</i> | 106779771 | V----G---R--N-RITF-TET | L-LKLLK-----TQH-- |
| <i>Kurthia zopfii</i> | 109350013 | V----G---KD--ARITY-T-- | L-MKLLNV---Q----- |
| <i>Paenactinomyces guangxiensis</i> | 181752266 | -S--G--Q---SS--W-QE- | L-LPFEGL---A-E----- |
| <i>Rummeliibacillus stabekisii</i> | 066786744 | -----G--K---RTTY-T-- | L-MKLLN---E-G----- |
| <i>Planococcus chinensis</i> | MBM7531103 | V----G--R--E-VIPY-TET | L-CPLLK---A-QK----- |
| <i>Ureibacillus sinduriensis</i> | 036202110 | V----G---KD--KQ-TY-TET | I-LKLLK-----Q----- |
| <i>Lysinibacillus fusiformis</i> | 205444228 | V----G--R---RITY-TEI | L-LKLMKV---E-Q----- |
| <i>Nitrosopumilales archaeon</i> | RPJ30166 | V----V---NSIS--LKN-EKI | LKT-K-N---I-T-R-K-- |
| <i>Rummeliibacillus stabekisii</i> | 147193591 | -----G--K---RTTY-T-- | L-MKLLN---E-G----- |
| <i>Ureibacillus massiliensis</i> | 036173336 | V----G-S-R--E-QITY-TE- | L-LKLLK-----QN-- |
| <i>Planomicrobium mcmeekinii</i> | MBP1899710 | V----G---RK--DVIPY-TET | L-CPLLK---E-R----- |
| <i>Anoxybacillus flavithermus</i> | 491027944 | V----G--KS---LPF-TDT | L-LSCVGM-T-E----- |
| <i>Bacillus methanolicus</i> | 490570485 | V----G--KS--KTSF-TE- | LRLELLG---E-K-K-- |
| <i>Geobacillus thermodenitrificans</i> | 138895934 | V----G--RSIEK-LPF-TD- | LRLPFLG---E---K-- |
| <i>Lysinibacillus sphæericus</i> | 499131666 | V----G--R--R---RITY-TEI | L-LKLLKV---E-Q----- |
| <i>Bacillus massiliensenegalensis</i> | 517984218 | V----G--KSME--LPF-TD- | L-LEVQA---T-E-K-K-- |
| <i>Geobacter bemandjiensis</i> | 197116956 | -N--VGV--AS-E--LPF-RDS | L-MTFSGT-----QL-- |
| <i>Geopsychrobacter electrodiphilus</i> | 522166183 | -N--G--K--ET--PF-RDT | L-MTFEGT---TEQK-- |
| <i>Desulfurispora thermophila</i> | 516757206 | -----G--K--QQ-IEF-ETT | L-LKVAG-----EQK-- |
| <i>Halanaerobium saccharolyticum</i> | 491632004 | -E--G--Q--E-SLFQ-QDI | L-LEFLKQ-T-E-QG-KI- |
| <i>Coraliomargarita akajimensis</i> | 294054336 | -----G--KS--SIEY-EKA | L-LHCHGK---E-QK-KT- |
| <i>Butyricimonas synergistica</i> | 517147053 | -E--G--AS---IPY-E-- | L-LKCYA---KDOQ-KT- |
| <i>Porphyromonas gingivalis</i> | 34541263 | -E--LG---KSIE--LPY-E-- | L-LKCYS-----DQK-T- |
| <i>Archaeoglobus sulfaticallidus</i> | 488600058 | -----G--K---EL-KKL | -FEVKG---EEQK-K-- |
| <i>Ferroglobus placidus</i> | 288932190 | -----GV--K--E--IEL-KKL | -FEVKG---KDOQK--- |

Figure S33

Partial sequence alignment of the protein lactoylglutathione lyase, showing a 2 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | | |
|----|---------------------------|--------------------------|----------------------------|
| 55 | LVTVALRRFNREQLEDDLFGPLSQL | E | GITLMPNTSGASTAAEAVKAHISRE |
| | -E- | P | I--N-- |
| | -KE- | S | R---A-- |
| | -I-----A-----AI | -V----- | IR---A-- |
| | -A-----EI | -L----- | A--K-- |
| | -A-----A-----EI | -AV----- | IR---A-Q |
| | -IA-----A-----Q | -L----- | A--K--I---A-- |
| | -PG-----T-----TSI | P | R--IH--A-- |
| | -A-----AI | K | Q--R-A-- |
| | M-----I--MDLKSDAENIMKFLLD | K | KYF-L---A-CY--K---LT-ELA-- |
| | -V-H--AA--TI | -L----- | N-Q--R-- |
| | -A-----EI | -L----- | A--S-- |
| | -V-H--AA--TI | -L----- | N-Q--R----- |
| | P----- | P | R---A-- |
| | -KE- | S | R---A-- |
| | -E----- | -L----- | |
| | -A-----EI | -L----- | A--K-- |
| | --M-K-VDCGGQQ--ILT--RAM | P | HRLWL--RD-K--IL--LA-- |
| | --M-IK-LEPGKVH--ILS--L-- | -VK-L----- | K-----F--LA-- |
| | --M-K-VDLAERD--ILA--IEA | -H-L----- | K--RD--F--LA-- |
| | --M-K-VDIQNR--ILA--IAS | -N-L----- | K--F--RLA-- |
| | --M-K-V-IAERD--ILA--I-A | -VN-L----- | KN-K--IF--QLA-- |
| | --M-K-V-IAERD--ILA--I-A | -VN-L----- | KN-K--IF--QLA-- |
| | --M-IK-LEPGKRH--ILS--LK- | -VK-L----- | K-----F--LA-- |
| | MA-M-K-VDLN--N--IL--IE- | -VH-L----- | KN-KD-IF--LA-- |
| | --M-K-VDVQNR--ILA--IAA | NLN-L----- | K--K--IF--RLA-- |
| | --M---VDVHATQ--ILT--RE- | -VR-L----- | KN-Q--F--RLA-- |
| | --M-K-IDLA-RD--ILA--LDM | KMN-L----- | KN--D--Y--QLA-- |
| | --M-K-VELGKKY--ILS--K-- | -VA-L----- | K--E--IF--RLA-- |
| | --M-IK-LEPGKRH--ILS--LA- | -VK-L----- | K-----F--LA-- |
| | --M-K-VDLQHR--ILA--IAA | -VQ-L----- | K--R--IY--LA-- |
| | --L-IK-IDIR-ST-N-MA--S- | -VK-L----- | RN-Q--IF--QLA-- |
| | --M-IK-LEPGKAH--ILS--L-- | -VK-L----- | K-----F--LA-- |
| | -I---K-VDL-CES--LSY--HD | N-S-L----- | VR--K--F--QL-- |
| | 319954194 | IDI-GQ--ILKW-KKG | NFN-L-----RN-K--F--TLA-- |
| | 517154023 | IDIIDGQ--ILKW-KKG | NFN-L-----RN-K--F--TLA-- |
| | 110636668 | -I---K-VDVQNN--ILRH-KHA | H-N-L-----VR--K--F--EL-- |
| | 527071521 | -----K-VDVQNN--ILRH-KHA | H--L-----VR--K--F--EL-- |
| | 517147441 | -----K-VDL-SK--ILAH-KAP | H--L-----VR--K--F--EL-- |
| | 451947508 | M-----V-LDNP--IMKA--R | DY-FL-----RN-N--IRL--LA-A |
| | 167573688 | --M-IK-LEPGKAH--ILS--L-- | -VK-L-----K-----F--LA-- |
| | 206819215 | --M-IK-LEPGKAH--ILS--L-- | -VK-L-----K-----F--LA-- |
| | EFZ8856520 | --L-MK-V-LR-HN--ILA--IAE | V--L-----K--E--IF--LA-- |
| | 149451965 | --L-MK-V-LR-HN--ILA--IAE | V--L-----K--E--IF--LA-- |
| | 211826209 | --MK-VDLQ-Q--IL--Q-A | C DVR-L-----R--E--F--KLA-- |
| | 052882252 | -I-----KT--YA--AS- | --A-----RD-D--CR--RLG-- |
| | VG015799 | --D-A--Y----- | NVR-----MN-K--R--MLG-- |

Order
Chlorobiales

Other Bacteria

| | |
|--|------------|
| <i>Chlorobium luteolum</i> | 78187419 |
| <i>Chlorobium phaeovibrioides</i> | 145220164 |
| <i>Chlorobium ferrooxidans</i> | 493409422 |
| <i>Chlorobium phaeoclathratiforme</i> | 194337038 |
| <i>Chlorobaculum parvum</i> | 193212337 |
| <i>Chlorobaculum chlorochromatii</i> | 78189233 |
| <i>Chlorobaculum tepidum</i> | 21673528 |
| <i>Chlorobacteroides</i> | 119356564 |
| <i>Chlorobium limicola</i> | 189346198 |
| <i>Chlorobaculum limnaeum</i> | 2501893292 |
| <i>Chloroherpeton thalassium</i> | 193215746 |
| <i>Prosthecochloris marina</i> | 110022181 |
| <i>Chlorobaculum thiosulfatiphilum</i> | 139456668 |
| <i>Prosthecochloris marina</i> | 110022181 |
| <i>Candidatus Chlorobium masyuteum</i> | 166807938 |
| <i>Chlorobium sp. KB01</i> | 076790525 |
| <i>Chlorobium sp. N1</i> | 131354040 |
| <i>Chlorobaculum sp. 24CR</i> | 129050323 |
| <i>Plesiomonas shigelloides</i> | 499149739 |
| <i>Aeromonas veronii</i> | 330831321 |
| <i>Vibrio fluvialis</i> | 521102759 |
| <i>Grimontia holliiae</i> | 491643491 |
| <i>Photobacterium leiognathi</i> | 565848455 |
| <i>Photobacterium angustum</i> | 491514775 |
| <i>Aeromonas media</i> | 491473250 |
| <i>Vibrio ordalii</i> | 498005262 |
| <i>Enterovibrio norvegicus</i> | 515572621 |
| <i>Salinivibrio costicola</i> | 543216350 |
| <i>Vibrio mimicus</i> | 446854660 |
| <i>Photobacterium damselae</i> | 491444567 |
| <i>Aeromonas molluscorum</i> | 492609492 |
| <i>Vibrio metschnikovii</i> | 490528628 |
| <i>Glaciecola punicea</i> | 492851889 |
| <i>Enterococcus gallinarum</i> | 537098851 |
| <i>Cellulophaga algicola</i> | 319954194 |
| <i>Cytophaga aurantiaca</i> | 517154023 |
| <i>Cytophaga hutchinsonii</i> | 110636668 |
| <i>Winogradskyella psychrotolerans</i> | 527071521 |
| <i>Butyrivibronas synergistica</i> | 517147441 |
| <i>Desulfovibrio sulfexigens</i> | 451947508 |
| <i>Aeromonas veronii</i> | 167573688 |
| <i>Aeromonas hydrophila</i> | 206819215 |
| <i>Shigella flexneri</i> | EFZ8856520 |
| <i>Escherichia albertii</i> | 149451965 |
| <i>Kistimonas asteriae</i> | 211826209 |
| <i>Kiritimatiella glycovorans</i> | 052882252 |
| <i>Pontiella desulfatans</i> | VG015799 |

Figure S34

Partial sequence alignment of the protein thiazole synthase, showing a 1 aa insert (boxed) that is found in all members belonging to the order *Chlorobiales*.

| | | 599 | 644 |
|------------------------------------|--|---|---|
| Order <i>Chlorobiales</i> | <i>Chloroherpeton thalassium</i> | 193213813 | GRHPVLEKIMPIDRKYVPNDCRDLDVE |
| | <i>Prosthecochloris vibrioformis</i> | 139626255 | -----R-LGV-EA-A-SF-EQ |
| | <i>Chlorobium phaeoclathratiforme</i> | 194336140 | -----RM-SAEDP-----HF-EK |
| | <i>Chlorobium ferrooxidans</i> | 493410219 | -----SD-EP-Q-LF-EK |
| | <i>Chlorobium chlorochromatii</i> | 78189282 | -----RLLGAES-I-HF-DK |
| | <i>Chlorobium phaeovibrioides</i> | 145220133 | -----R-KP-EPF-K-HF-GQ |
| | <i>Chlorobaculum parvum</i> | 193213235 | -----R-LGA-EP-A-VGSD |
| | <i>Chlorobium phaeobacteroides</i> | 189499799 | -----R-LHV-EP-FF-KK |
| | <i>Chlorobaculum tepidum</i> | 21674323 | -----R-LGA-EP-A-QVGS- |
| | <i>Prosthecochloris aestuarii</i> | 194334404 | -----R-VDV-EP-S-LF-ER |
| | <i>Chlorobium limicola</i> | 189347201 | -----R-L-A-EP-IA-ALF-DR |
| | <i>Prosthecochloris ethylica</i> | 175186793 | -----R-LGV-EP-A-TF-D- |
| | <i>Chlorobaculum limnaeum</i> | 2501895063 | -----R-LGA-EP-S-VGAS |
| | <i>Chlorobium luteolum</i> | 78186335 | -Y-W-L-SHR-EVL-L-LE-GLN |
| | <i>Prosthecochloris sp. CIB 2401</i> | 068866586 | -----R-LGV-EA-A-SF-EQ |
| | <i>Prosthecochloris sp. ZM_2</i> | 114607669 | -----R-LGV-EP-A-TF-D- |
| | <i>Prosthecochloris marina</i> | 167392938 | -----R-LGV-EP-A-FF-RK |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | 085659292 | -----R-LGV-EP-A-VF-AQ |
| | <i>Prosthecochloris sp. GSB1</i> | 094082121 | -----R-LDVGEP-E-NDR |
| | <i>Chlorobium sp. KB01</i> | 076791947 | -----R-SD-EP-Q-LF-E- |
| | <i>Candidatus Chlorobium masyuteum</i> | 166806887 | -----R-SD-EP-Q-LF-EK |
| | <i>Chlorobium sp. N1</i> | 131353967 | -----RLLSPEDPF-S-LF-EQ |
| | <i>Prosthecochloris sp. ZM</i> | 114618085 | -----R-VDV-EP-S-LF-ER |
| | <i>Chlorobaculum sp. 24CR</i> | 129051820 | -----R-LGA-EP-IS-VGAS |
| | <i>Chlorobi bacterium OLB7</i> | KXK55477 | -----V-TLL-PGER-TA-TA A D-IA-----Y |
| | <i>Chlorobi bacterium OLB6</i> | KXK35082 | A---I-RLL-AGTR---NIV-T T HQLMVL-----YL- |
| | <i>Melioribacter roseus</i> | 397690923 | -----V-Q-L-PGE-FT-S-SSS E DQII-L-A-VY- |
| | <i>Ignavibacterium album</i> | 385809871 | -----V-R-LSPGE-FT-K-NNS D QQIIL-A-VY- |
| <i>Chthonomonas calidirosea</i> | 512552186 | -----I-R-GLGGD-I-H-E- N-LL-----Y | |
| <i>Rubrobacter xylanophilus</i> | 108803143 | -----V-HA TETPF-A-I-GG S-L-----A-VY- | |
| <i>Taylorella asinigenitalis</i> | 479191464 | -----V-RTI E-FTA-F-NS- N-M-----G-TYM- | |
| <i>Desulfurispora thermophila</i> | 516757174 | -----V-LD-GSF-LT-KQ -LILL-----A-T-M- | |
| <i>Thermophagus xiamenensis</i> | 498212269 | -----I-TQL-AGEE-VY-S- T QQ-M-----A-AL- | |
| <i>Prevotella micans</i> | 494011157 | -----I-TQL-GEO-VL-S- H QQIM-----A-AL- | |
| <i>Anaerophaga thermohalophila</i> | 498107809 | -----I-QL-VGEE-VF-ND T QQIM-----A-AL- | |
| <i>Gracilimonas tropica</i> | 521070739 | -----V-SL-MGEPI-IK-N- N DQIL-----A-II- | |
| <i>Gillisia marina</i> | 497914865 | -----I-QL-HGEQ-T-VF-R- T QQ-IM-----AI- | |
| <i>Cytophaga hutchinsonii</i> | 110636573 | -----I-QL-LGES-I-Y- D- T QQ-M-----A-AL- | |
| <i>Nonlabens dokdonensis</i> | 443243179 | -----I-ML-A-QP-I-VQ-RD S QQIM-----A-II- | |
| <i>Porphyromonas catoniae</i> | 491610388 | -----I-QL-FGES-VH-QK E -QIMV-----AL- | |
| <i>Cytophaga aurantiaca</i> | 517154534 | -----I-QL-LGES-I-Y- D- N QQ-M-----A-AL- | |
| <i>Bacteroides ovatus</i> | 490426514 | -----I-QL-GE-IA-VM-SD T QQII-----A-AL- | |
| <i>Bacteroides vulgatus</i> | 511016684 | -----I-QL-VGE-IA-VF-S- T QQII-----A-AL- | |
| <i>Eudoraea adriatica</i> | 518500928 | -----I-QL-LGEA-I-LV-NR- S QQIM-----AI- | |
| <i>Candidatus Amoeobophilus</i> | 189502159 | -----I-QQLSV-VS-I-Y- N- T QQ-IV-----A-AL- | |
| <i>Prevotella marshii</i> | 494007441 | -----I-TQL-LGE-IF-S- R QQIM-----A-AL- | |
| <i>Prevotella nanceiensis</i> | 517174161 | -----I-MQL-GER-IL-S- K QQIMM-----A-AL- | |
| <i>Bacteroides xylinosolvens</i> | 495298839 | -----I-QL-GE-IA-VM-SN T QQII-----A-AL- | |
| <i>Kordia algicida</i> | 494155465 | -----I-QL-GES-IA-VY-RN T QQIM-----AI- | |
| <i>Halanaerobium praevalens</i> | 385799933 | -----V-MFT EQF-Y-QT E Q-FV-----TYM- | |
| <i>Butyrivibrio fibrisolvens</i> | 479189702 | -----V-QMINA-QFIS-Q-LD S RTIA-----A-TY- | |
| <i>Nematostella vectensis</i> | 156325874 | -----I-QL-GEE-IA-VV-NRN Q QQIM-----AI- | |
| <i>Caldithrix abyssi</i> | 493983722 | -----V-TL-PGEDFIA-AY-PD S EQIW-----A-T- | |

Figure S35

Partial sequence alignment of the protein DNA mismatch repair protein (MutS), showing a 1 aa deletion (boxed) that is found in all members belonging to the order *Chlorobiales*. The CSI is not present in any other bacterial species except for the four other bacteria as indicated above, in the top 500-BLASTp hits.

Figure S36

Partial sequence alignment of the protein peptide chain release factor 3, showing a 1 aa deletion (boxed) that is specific for members of the family *Chlorobiaceae*.

| | | 260 | PEYVHTATAAYRTAIDFCINRRNDP | QFREEY | SELTTLKLKEDLATVYNRGFSKGFYFG |
|--------|----------------------|--|--|--|---|
| Family | <i>Chlorobiaceae</i> | 493410285 194337501 119357950 189347571 78188525 145219174 78186182 189501046 2501893814 193211992 21674702 139457271 110022762 175186871 194334724 166807158 131356391 193214872 397690227 385811731 490483825 496098877 517091584 493902504 340620824 498212208 498104798 565855801 392389808 565858531 490466178 490179276 232308722 023940636 200356609 048123772 077094689 142532834 069974372 155283725 013044600 212923789 MBE6485441 097626982 097056891 HHT30867 MBE6504141 011025895 191615848 097552668 093113199 | -----T---SS---TT----- -----T---R---TSH-S- -----S---N---TTN-T- -----T-T---Q-LNM-MQQ-HQA ----QAT----K-----EE-SK- ----Q-V-S---R-L-C---SE-S- ----T-KC---K---YTLEHGHEK ----A-T---K---IAAH-G-G ----A-T---K---IAEH-D-E ----A-T---K---IAAH-E-E ----T-TC---K---YTLEHGHEK ----T-SI---S-F-YILQHQD- ----T-SC---Q---YIVAH-HET ----Q-V-R-----C-MEE-GK- EF---ASV-R---A-L-SYAKNPDTY ---I-KVVS---K---LYYENKLND ----AKVSV---N---LYFEGKLNT ----VVTC---E---ESVLDGTFTE ----VVSC-KE---ESVLDGTFTE ----A-VIKT---E---AYYEGTYSK ----Y-VVKC-KE---AAVLGDTFTE ----A-VIKT---E---AYYEGTYSK ----K-VVSC---E---NAVV-NTYSQ ----K-VVSC---E---NSVLDDTYSQ ----Y-VVK---E---AVV-GTYSE ----A-V-KC---E---SISEGTFTQ ----Y-VVK---E---AVV-GTYSE ----VVT-C-KE---EATL-GTYTE ----K-TVSC---K-L-AVYDGNY- -G-LEVVSRC---E---A-KEG-YTR ----Y-VVK---E---AVV-GTYSE ----S-V-----RGV-AIQSGTFTE -G-LEMVSRC---E---AF-EGNYT- ----Y-VCRC-KE---EA-C-GTY-E ----VVK---NA-LNAILDGSY- ----A-IVG---K---QFCEKGIO- ----A-VIKT---E---AYYEGTYSK ----KLV-R---S-VNAVFDGTYSR ----A-VIKT---E---AYYEGTYSK -D-S-V-NC-SD---KLYE-GLW-Q ----Y-VCRC-KE---EA-C-GTY-E ----A-V-K---E---SWYEGTYSE ----R-V---C-KE-VEAYC-NSFSQ -D-GAMV-SV---E---SYL-NPDEY ----ASVVK---D---SYYEE-AFD ----S-V-----RGV-AIQSDTF-Q ----Y-VCRC-KE---EA-S-GTY-E ----A-V-R---E---AWYEGTYSE | E---N--- E---T--- A-GDG- NA-SQ---K-L---E--- G---KRN G---AR- D---NR- D---A-T- N---D-A- D---D-A- D---A-A- D---S-QTF G---ADT- S---E-D- G---AR- EF---ASV-R---A-L-SYAKNPDTY ---I-KVVS---K---LYYENKLND ----AKVSV---N---LYFEGKLNT ----VVTC---E---ESVLDGTFTE ----VVSC-KE---ESVLDGTFTE ----A-VIKT---E---AYYEGTYSK ----Y-VVKC-KE---AAVLGDTFTE ----A-VIKT---E---AYYEGTYSK ----K-VVSC---E---NAVV-NTYSQ ----K-VVSC---E---NSVLDDTYSQ ----Y-VVK---E---AVV-GTYSE ----A-V-KC---E---SISEGTFTQ ----Y-VVK---E---AVV-GTYSE ----VVT-C-KE---EATL-GTYTE ----K-TVSC---K-L-AVYDGNY- -G-LEVVSRC---E---A-KEG-YTR ----Y-VVK---E---AVV-GTYSE ----S-V-----RGV-AIQSGTFTE -G-LEMVSRC---E---AF-EGNYT- ----Y-VCRC-KE---EA-C-GTY-E ----VVK---NA-LNAILDGSY- ----A-IVG---K---QFCEKGIO- ----A-VIKT---E---AYYEGTYSK ----KLV-R---S-VNAVFDGTYSR ----A-VIKT---E---AYYEGTYSK -D-S-V-NC-SD---KLYE-GLW-Q ----Y-VCRC-KE---EA-C-GTY-E ----A-V-K---E---SWYEGTYSE ----R-V---C-KE-VEAYC-NSFSQ -D-GAMV-SV---E---SYL-NPDEY ----ASVVK---D---SYYEE-AFD ----S-V-----RGV-AIQSDTF-Q ----Y-VCRC-KE---EA-S-GTY-E ----A-V-R---E---AWYEGTYSE | K---Q---E----- NS-SKQ---E-R-----E--- NA-SQ---K-L---E--- A---AS---H-----N--- E---IGN---E-L-----N--- A---AE---E-R-----D--- EA-AKE-ATE-HK-----H---L- GK-ARE-E-E-KK-----F--- RT---DG-QD---R-----E-F- RS---DR-QN---VR-----E-F- RS-ADG-E-K-KK-----E-F- DA---KE-MKE-E----- RRFAE-QA-E-R-----E-D--- QAVADN-TRQ-K-----E-S--- G---GR---GE-EK-----S--- TFDPAW---EAISH-LY-T-A- -IKSE---NK-TE-----A---L- -KKKEFLTE-E-----S--- -KKDLWDKR-S---F---WD-Y-Q- -KKD-WD-R-S---F---WD-Y-Q- -KVE-WM-A-S-----WS-Y-L- -KKDEWD-K---F---WD-Y-Q- -KVE-WM-A-S-----WS-Y-L- -KIEEWEQR-S-F---WD-Y-L- DKINHWE-S---S-F---WD-Y-L- DKIAVWD-Q---F---WD-Y-L- -KVAEWML-E-----WS-Y-L- DKIAVWD-Q---F---WD-Y-L- -KK-E-D-R-SR-F---WD-Y-Q- -AGE---K-KA-----Q---K- -KVETW-SE-S-----T--- DKIAVWD-Q---F---WD-Y-L- D-V-Q-ND---KK---H---H-S-Y-L- -KIETWRRE-S-----T--- -AIGRW-N-Q---F---WD-Y-L- KKIEDWNKE---F---WD-Y-L- K-DQR---I-V-QIF---K-T---YL-- -KVG-WM-A-S-----WS-Y-L- -FVET-M---IKK---L-D-Y-L- -KVE-W---T-S-----WS-Y-L- SSK---IP-W---E-KS-F---DT--- -AIGRW-D-Q---F---WD-Y-L- -KVADWM-R-S-----WS-Y-L- DKIELWN-K---F---WD-Y-L- -FNPQW---E-ES-F---DT--- S---AM-EM-K-F---T-YL- P-VAE-ND---KK---H---H-S-Y-L- -AIGRW-D-Q---F---WD-Y-L- -KVE-WMGE-S-----WS-Y-L- |

Figure S37

Partial sequence alignment of the protein U32 family peptidase, showing a 6 aa insertion (boxed) that specific for members of the family *Chlorobiaceae*.

| | | | LAREV | SCGERLAVAPQVASGRSAILKEIGERKAAE |
|--|---|---|---|---|
| Family <i>Chlorobiaceae</i> | <i>Chlorobium phaeovibrioides</i> <i>Chlorobium ferrooxidans</i> <i>Chlorobium phaeobacteroides</i> <i>Chlorobium luteolum</i> <i>Chlorobaculum parvum</i> <i>Chlorobium limicola</i> <i>Pelodictyon phaeoclathratiforme</i> <i>Prosthecochloris aestuarii</i> <i>Chlorobium tepidum</i> <i>Chlorobium chlorochromatii</i> <i>Chlorobaculum limnaeum</i> <i>Prosthecochloris ethylica</i> <i>Chlorobaculum thiosulfatiphilum</i> <i>Prosthecochloris marina</i> <i>Candidatus Chlorobium masyuteu</i> <i>Chlorobium sp. KB01</i> <i>Chlorobium sp. N1</i> <i>Cand. Thermochlorobacter aerophilum</i> <i>Melioribacter roseus</i> <i>Ignavibacterium album</i> <i>Caldithrix abyssi</i> <i>Thermovibrio ammonificans</i> <i>Syntrophus aciditrophicus</i> <i>Geobacter metallireducens</i> <i>Geobacter bimediensis</i> <i>Geobacter daltonii</i> <i>Syntrophobacter fumaroxidans</i> <i>Geobacter uranireducens</i> <i>Candidatus Nitrospira defluvii</i> <i>Thermodesulfovibrio yellowstonii</i> <i>Acetivibrio cellulolyticus</i> <i>Eubacterium eligens</i> <i>Clostridium clariflavum</i> <i>Nitrospina gracilis</i> <i>Thermodesulfovibacterium geofontis</i> <i>Thiothrix nivea</i> <i>Rudanella lutea</i> <i>Flexithrix dorotheae</i> <i>Runella slithyformis</i> <i>Marivirga tractuosa</i> <i>Cyclobacteriaceae bacterium</i> <i>Parabacteroides goldsteinii</i> <i>Segetibacter koreensis</i> | 145220314 493410273 119356260 78187582 193213382 189345895 194335608 194334590 66576257 78188544 2501894174 198911889 139457401 167392930 166807448 083694608 131354373 RFM24603 397690794 385809643 493988209 319789539 85860140 404495733 197118030 222055074 116747616 148263556 302037249 206890255 497937468 238917088 374295594 491153350 337288075 488795658 518831224 522017869 338213581 313674192 499123756 494928094 517440681 | -----L-----I----A --ISSL-----Q --S-L-----L --V--L-----R --I--L---T----- ---SL-----I----R ---L-I-T--I----A --V--L---T-P---K --S-L---L-H-L --V-L---K-P--R-S --I--L-I-T--L---A --V--L---K-P--R-R --A--L---T-L--E-- ----L-----I----A ----L-----I----A --S-L-----L --I-L---TF-K--N-Y --VS-L---TY-E--N-K --FS-L---TY-E--N-K -----TY-D-K--T ---G-L---PY-R-R--- --V--L---PY-----P --V-SL---PY-R-E--A ----L---PF-Q---P -----PF-P-S--P ---T-L---PF-P---P --V-----PF-P-T--P --FS-----Y-S---A IGFS-L---PY-K--F-K ISFSAM---KY-Q-K--P VHFYEM-I-KY-R-K-- INF-AM---KY-Q-K--P --FT-L---TY-P-K--T S--NWL-I-PF-L---F VGFGHL-I---Y---E--K --IS-L---TY-E--N-H --DIS-L---TY-E--N-E --VS-L---TY-E--N-L --VS-L---TY-E--N-D --VS-L---TY-E-AN-R --DISQL---Y-E----Q --DIS-L---TY-E-AN-L | LAREV ----QT --EQ- -G-Q- -S-I --AQL --LQI --Q --FA-I --FA-I --FA-I --FA-I --SKQI ----QT --QT --Q -AELL DR--WQP--DK-KQA--RK-H-LSFF-KQK- AEKMPEH-THEIRKK-NNM-RILS-K-RN-- AISLPKG-EVNERKK-NNM-RILSDK-KNA- -FKMKPK----KKL-NR-MI-M-Q--KFA- AARL-NS-T--EKRR-GEL-R-V-TE-SV-- AAAMPDQ-S-DEKKK-AE--RTL-T--REA- AARMEGQ-DGK-IR--AE--R-V----RRS- AATMTPQ-H-K-IKE-AEA-RVLS---KSDY AATMEGH-HSS-IKE-AKA-RKLS-E-KKS- AADMPGRIPGDETKK-ARL-QD--T--RRQ- AATMGDH-RSS-IKE-AKA-RKLS-E-KKAY AARLEDQIS-A-IRQ--KA-S-LSRT--LA- ASEMTEQIPENIKRQ-ADC-I-V-K--KL-Y AAEFSNOIS-KKKDE--EV-I-LSSK-TK-- ADKMKEQ--DT-K-E--V-LALEKAQSL-Y AANFENQ-S--KKEE--N--L-LSA-MTR-- AFAMKNN-PKTDKKE-NRR-T-L-RD--LT- AEKLNKSKIPQ-EIEN-KK--NLI-G-REK- AA-LPGQ--EP-KKQ--QT-H---LMKQ-W ALSI-PI-PGN-RAE--KM-HILSDK-RRA- AIELEGV-PMK-RNQ--KM-RGLSHK-KRA- ALEIKPV-PLSQRAE--KM-HILSDK-RRA- ADEMDEV-PMK-RNE--KM-RSLS-K-KRA- AVEMEVG-PMKKRNE--KM-RILS-K-RRQ- ALKIAHS-D-K-KHA--QQ-LD-SD--LHA- ATEMKEV-PVN-RHE-NKK-RNLSYM-MS-H |

Figure S38

Partial sequence alignment of the MiaB-like tRNA modifying protein, showing a 5 aa insertion (boxed) that is specific for members belonging to the family *Chlorobiaceae*.

| | | 262 | | 312 |
|--|--|--------------|----------------------------|------------------------|
| | <i>Chlorobium limicola</i> | WP_041465658 | EGMVSFKALLFLPKAPELLYRQSE | RGPAQLYVKVMIQSECRLLP |
| | <i>Chlorobium phaeovibrioides</i> | WP_126383222 | --A-----QN-M-----G- | K-----N----- |
| | <i>Chlorobium luteolum</i> | WP_011358007 | --A-----I-----M-----G- | K-----H----- |
| | <i>Candidatus Chlorobium masyuteum</i> | WP_166808743 | -----S-----G----- | -----L--N----- |
| | <i>Chlorobium ferrooxidans</i> | WP_040433047 | -----S-----S-----G----- | -----L--N----- |
| | <i>Chlorobaculum limnaeum</i> | WP_069809908 | --A-----I-S---M---N-GA | K-----L--N----- |
| | <i>Pelodictyon phaeoclathratiforme</i> | WP_012508305 | -----T-----M-----G----- | H-----L--N----- |
| | <i>Prosthecochloris vibrioformis</i> | WP_068867009 | --V-----I-Q---MDM-Q-GA | H-----L--N-K----- |
| | <i>Chlorobaculum tepidum</i> | WP_010932510 | --A-----I-S---M---N-GA | K-----L-H----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | WP_139457868 | --A-----I-S---MD---N-GA | K-----L-H----- |
| | <i>Chlorobaculum parvum</i> | WP_012502101 | --V-----I-S---M---Q-GA | K-----L-H----- |
| | <i>Prosthecochloris ethyllica</i> | WP_175187146 | --A-----MDMM-Q-GA | H-----AH---H-K----- |
| | <i>Prosthecochloris aestuarii</i> | WP_012505491 | --V-----MDM-Q-GA | H-----R-L--H-K----- |
| | <i>Chlorobacter phaeobacteroides</i> | WP_011745048 | --K-C-----E-----M-----GD | S-----L-Q----- |
| | <i>Prosthecochloris marina</i> | WP_110023524 | --V-N-----MDM-Q-GA | H-----R-H-K----- |
| | <i>Chlorobium sp. N1</i> | WP_131353572 | --A-----I-----M-----G----- | Q-----H----- |
| | <i>Chlorobium sp. KB01</i> | WP_076790368 | --S-----S-----G----- | L-N----- |
| | <i>Chloroherpeton thalassium</i> | WP_012501118 | --R----S-----S-MG--HLKD- | KSL----N-L--DD-K----- |
| | <i>Chlorobi bacterium OLB7</i> | KXK52223 | --TFEYF---I-SR--MD-FT-EGV | H-LN---R-F-MEDAK---T |
| | <i>Ignavibacterium album</i> | WP_014558883 | DAPIQ-N---I-RSYEFWRWSKDD | Y-LD---RR-L-HQNKT----- |
| | <i>Melioribacter roseus</i> | WP_041356047 | DAPIQ-N---I-KSDEFFWIDRN | Y-LD---R-L-HHKNK----- |
| | <i>Williamsia sterculiiae</i> | WP_076482150 | --SFE-Q---I-SQ--YD-FM-DRA | H-V----R-F-MDD-D-M-- |
| | <i>Mycobacterium neoaureum</i> | WP_019514778 | --TFEYQ---I-SQ--FD-FN-DGN | T-V----R-F-MGD-DE-V-- |
| | <i>Micrococcus paurometabola</i> | WP_013128275 | --TFEYQ---I-TMP-FDMF--NTK | P--A---R-F-MDH-EA---G |
| | <i>Maledivibacter halophilus</i> | WP_079492068 | D-L-RYN-I--I-ENM-FDYYTKEY- | K-LE--SNG-L-MNK-Q---D |
| | <i>Chryseobacterium mucoviscidosis</i> | WP_079350953 | D-A-VYN-I--I-ENT-FDYYTKEY- | K-LE--SNG-L-MDK-G---D |
| | <i>Pedobacter himalayensis</i> | 00K78077 | --KQEYTS--YI-AQ--WDMWN-DHK | H-LK---QR-F-MDDAEQFM-N |

Figure S39

Partial sequence alignment of the molecular chaperone HtpG protein, showing a 3 aa insertion (boxed) that is specific for members of the family *Chlorobiaceae*.

| | | 114 | |
|----------------------------|---|--------------------------|-----------------------|
| Family | | VEKAITPATKAVVAIPMWAPPK | MVELAAICDRHSIMLIEDAAQ |
| Chlorobiaceae | <i>Chlorobium limicola</i> | -----K-----A- | -D-I-E--E-RG-T---- |
| | <i>Chlorobaculum Limnaeum</i> | -----K-----A- | -D---E--E-RGVT---- |
| | <i>Chlorobaculum parvum</i> | -----K-----A- | -D-I-E--E-RGVT---- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | -----K-----A- | -D-I-E--E-RGVT---- |
| | <i>Chlorobaculum tepidum</i> | -----K-----A- | -D---E--R--NLT-V-- |
| | <i>Chlorobium luteolum</i> | -----K-----V-S- | -D-S-L-RERGLI---- |
| | <i>Pelodictyon phaeoclathratiforme</i> | -----S----- | -D-IS---RK-NLI---- |
| | <i>Chlorobium ferrooxidans</i> | -----S----- | -D-----QSNNLI---- |
| | <i>Chlorobium phaeobacteroides</i> | -----S----- | -K--QD--T-RG-I---- |
| | <i>Chlorobium phaeovibrioides</i> | -----S----- | -D--S-L---GLV---- |
| | <i>Prosthecochloris aestuarii</i> | --R-LSSK-----Q | -D--EV-R--ELV---- |
| | <i>Prosthecochloris ethyllica</i> | --R-V-GR-----R | -D--E--G--NLT-V-- |
| | <i>Chlorobium sp. N1</i> | --R----K-----V-S- | -D-IS---RK-NLI---- |
| | <i>Candidatus Chlorobium masyuteum</i> | --R----E-----S- | -D-IS---RK-NLI---- |
| | <i>Chlorobium sp. KB01</i> | --R----S----- | -D-IS---RK-NLI---- |
| Chloroherpetonaceae | <i>Chloroherpeton thalassium</i> | I-A----K--VI-PVHLYGQ-A A | LD-IHQ-AEK-GLEV---- |
| fam.nov | <i>Cand Thermochlorobacter aerophilum</i> | I-AE---Q---ILPVHLYGQAA E | IDKIVE-AT--G-AV--N-- |
| Larger | <i>Chlorobi bacterium OLB5</i> | ISRLV--K-R--VVHYGGIAC R | -D-IKQ-T--YGLY---- |
| <i>Ignavibacteriae</i> | <i>Chlorobi bacterium OLB4</i> | I--H---E--IIPVHLFGL-V A | -DIILE-AR-YKLKV--S- |
| Clade | <i>Ignavibacterium album</i> | --A---SR--I--VHLYGQ-A D | -DP-RE-SKAKN-H-V-C-- |
| | <i>Melioribacter roseus</i> | I--K---K--IIPVHLYQSA E | -D-IMEVARK-NLYV--G-- |
| | <i>Cand Kapabacteri thiocyanatum</i> | -RA---R-R-I-PVHLFGQSA D | -DA-V--ATEAG-PIV---- |
| | <i>Salinicoccus albus</i> | -----IIVNLYGQSA K | --VD--N-YGVPIV--E |
| | <i>Corynebacterium aquilae</i> | --A---DK---MPVHLYGL-A N | -PA-RE--KRGL--F-S-- |
| | <i>Nitrosospira briensis</i> | -RQ-L--Q---I-VHLFGE-V D | LAP-QQF-ES-GLS----- |
| | <i>Flavobacterium sylvaticum</i> | -K-----K-R--L-VHLYGRLA L | -D--D--KQNNLL----- |
| | <i>Confluentibacter lentinus</i> | -----K--MVVQ-CGSMG D | -DA-QS--KAYNVL--C- |
| | <i>Luteibaculum oceanii</i> | --SK---K--II-VHIYGLTS N | LKR-RE--KKG-I-----E |
| | <i>Confluentibacter flavum</i> | -----K--MVVQ-CGSMG N | -DA-QR--NA-NVF--C- |
| | <i>Muriicola jejuensis</i> | -R----K---I-PVHLFGQCA P | -D--LE-AEE-GLF--N-- |
| | <i>Pontibacter burrus</i> | --L--SK---I-VHLYGQAC E | -D-ILQL-E-Y-LK----- |
| | <i>Galbibacter marinus</i> | -----N--VIMPVH-CGSMA R | LK--K---K-GLL-L--C- |
| | <i>Mariniflexile fucanivorans</i> | -----K--IMVVH-CGSMA N | -DA-QN--SK--LL-----C- |
| | <i>Prolixibacter bellariivorans</i> | I-----R---LL-QVFGSMG R | -D-IVEV-RKNNLK----- |
| | <i>Fulvivirga imtechensis</i> | -KE----R---I-VHLYGL-C R | -D-IM--A--YN-KV--C-- |
| | <i>Chitinophaga pinensis</i> | I-----K---I-PVHIYGQCA D | -DAIM--AEK-GLHV--T- |

Figure S40

Partial sequence alignment of the DegT/DnrJ/EryC1/StrS family aminotransferase protein, showing a 1 aa deletion (boxed) that is specific for the family *Chlorobiaceae*.

| | | 238 | 279 |
|-----------------------------------|--|------------|---|
| Family | | | |
| <i>Chlorobiaceae</i> | <i>Chlorobium phaeoclathratiforme</i> | 194336576 | KKLKIITPHTVGSYELVG |
| | <i>Chlorobium ferrooxidans</i> | 493409890 | -RI--V----- |
| | <i>Chlorobaculum tepidum</i> | 21674004 | R-HV----K----- |
| | <i>Chlorobium chlorochromati</i> | 78188793 | QS-T----R----A- |
| | <i>Chlorobium phaeobacteroides</i> | 119357337 | -N---V----R----A- |
| | <i>Prosthecochloris aestuarii</i> | 194338827 | -N---V----TD---MI- |
| | <i>Chlorobium limicola</i> | 189346919 | QN-----T----A- |
| | <i>Chlorobaculum limnaeum</i> | 2501894719 | NR-H-V----K----- |
| | <i>Prosthecochloris marina</i> | 110021887 | -N-R----K----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | 139456507 | SR-H----K---T--- |
| | <i>Prosthecochloris vibrioformis</i> | 139626512 | -S-R-V----SID---IE |
| | <i>Chlorobium sp. KB01</i> | 076789444 | -RI--V----- |
| <i>Chloroherpetonaceae</i> | <i>Chloroherpeton thalassium</i> | 193216023 | QVELL-S-SKD---IKK D -ENA-V-QIK-AKE--K-AK--- |
| fam.nov. | <i>Porphyromonas guiae</i> | 517795839 | --ANVL-S-PAS----IR G TDKMLT-RIK-PQ--KN-KI-- |
| | <i>Porphyromonas gingivalis</i> | 334146329 | --ANVL-S-PAS----IR G TDKMLT-RIK-PQ--KN-KM-- |
| | <i>Alistipes shahii</i> | 479187696 | --ATLV-S-PE----T D ANKVVEK-IIIDPVRFWE-SKIL- |
| | <i>Odoribacter laneus</i> | 496428340 | --A-VLSV-PQD--A-EA G EDGMLS-T-K--NS--KQTKY--V |
| | <i>Bacteroides sp. HPS0048</i> | 488622481 | -RAELL-T-P-----K D D-GQLT-KITNPKE--SV--Y-- |
| | <i>Bacteroides massiliensis</i> | 511024358 | -RAELL-T-PA-----K D A-GQLT-KITNPTE--SV--Y-- |
| Other Bacteria | <i>Flexithrix dorotheae</i> | 522022264 | --AEL--S-PE---KIE- S D-KV-K-VIL-PEK--NS-KY--V |
| | <i>Bacteroides vulgatus</i> | 150004391 | -RAELL-T-PA-----K D A-GQLT-KITNPTE--SV--Y-- |
| | <i>Prevotella ruminicola</i> | 294674538 | -DA-VL-S-PAS--R-TA D ANKQLI-RIN-PI---ST-KY-- |
| | <i>Porphyromonas somerae</i> | 516662532 | -A-VL-E-PSS---IK D -NKMMI-KIK-VDS--SLG-N--V |
| | <i>Odoribacter splanchnicus</i> | 325281781 | --A-LLSV-PAD--T-ET G EDGNMT-KIN--NN--KQTKY--V |
| | <i>Coprobacter fastidiosus</i> | 550260015 | --A--L-N-PE---S--K N D-GLLT-VIS-PQN--SL-KY--V |
| | <i>Bacteroides barnesiae</i> | 517541810 | -RAELL-N-PS-----K D D-GQLT-KITNPKE--SV--Y-- |

Figure S41

Partial sequence alignment of the biogenesis of lysosome-related organelles complex 1 subunit 2 protein, showing a 1 aa deletion (boxed) that is specific for the family *Chlorobiaceae*.

| | | | | |
|--|--|---------------|------------------------|----------------------|
| | | GRGKVIRARAIVE | I | TPKNSRESIVVTELPYQVNK |
| | | -LI- | V | -Q--G----- |
| | | -V- | V | -Q--G---I----- |
| | | -V- | V | -Q--G----- |
| | | -L- | V | -Q--G---I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -R-----V | V | -Q--G---I----- |
| | | -V- | V | -Q--G---I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -L- | V | -Q--G-----I----- |
| | | -I-L--KVQ- | V | -G--II----- |
| | | -LT--KVNI- | V | N--D-T----- |
| | | -IT--VKI- | EN | -G-A----- |
| | | -IT--VKVI- | EN | -G----- |
| | | -II--K-GI- | VG | GD--II----- |
| | | -IT----NI- | -L | -D-N-I----- |
| | | -RILL--K-N- | -L | -D-N-II--I----- |
| | | -IY----E- | V | D--G-T-I-H-I----- |
| | | -RA----TM- | V | QKRSG--A----- |
| | | -Y----NI- | V | DD--G-T-I-N-I----- |
| | | -Y----E- | V | DE--G-T-I-H-I----- |
| | | -YL----E- | V | DE--G--I-H-I----- |
| | | --R--M--TL- | V | QKRSG--A----- |
| | | --S-TM----- | V | N-RTH-Q-LII----- |
| | | --IY----E- | V | D--G-T-I-H-I----- |
| | | --Y----E- | A | D-SG-T-I-H-I----- |
| | | --RI-M--K-EI- | SQS | E-K-I--I----- |
| | | --S-IM-G--TI- | PMRGD | -A-II--I----- |
| | | --ITL----NT- | ELRGG | -Q-I--I----- |
| | | --ITL----NT- | ELRGN | -Q-I--I----- |
| | | --R-MM-GV-EI- | -LP-D-R-I-S-I- | ----- |
| | | --RI----KTEI- | SD-DG-R--I--I-M- | ----- |
| | | --RI-M--K-HN- | EV-G-C-----I----- | ----- |
| | | --R-L--K-EI- | EV-GK-C-----I----- | ----- |
| | | --RI-M--K-RI- | EVKG-C-I--I----- | ----- |
| | | --R-V--K-H- | -LDSGK-Q--I--I-M- | ----- |
| | | --R-M--KSS- | EV-G-C-II--I----- | ----- |
| | | --RIL----IHE- | EIRPG-MAL-I--I----- | ----- |
| | | --RI-M--K-NF- | EVDG-----I----- | ----- |
| | | --RI----KSEI- | ADN-G-K--I--I-M- | ----- |
| | | --R-M--K-DI- | EIKG-CM-----I----- | ----- |
| | | --RI-M--K-NF- | EVDG-----I----- | ----- |
| | | --RI----KTEI- | -D-SG-R-II--I-M- | ----- |
| | | --R----K-LI- | -YGHDR--R--N-I--I----- | ----- |
| | | --RI-M--K-GF- | EV-G-----I----- | ----- |
| | | --R-M--KSS- | EV-GK--LII--I----- | ----- |
| | | --RI----KTEI- | -D-TG-R-II--I-M- | ----- |
| | | --R-M--K-TI- | EVQGK-----I----- | ----- |
| | | --RIL----IHE- | EIRPG-MAL-I--I----- | ----- |
| | | --R-M--K-TF- | EVQG-C-I--I----- | ----- |
| | | --R-M--K-TF- | EVQG-----I----- | ----- |
| | | --RI-V--KTSF- | EVDG-C-I--I----- | ----- |
| | | --RI-M--K--I- | EVKG-C-I--I----- | ----- |
| | | --R-M--TEI- | HTASG-C--I--I-M- | ----- |
| | | --S-TM-GKVA- | -TGRD--ALII--I----- | ----- |
| | | --SIMM--TI- | -VRKE-A-II--I----- | ----- |
| | | --S-IM-G--TI- | PMRGD--A-II--I----- | ----- |
| | | --SIPV--EI- | EIRKD-QA-II--I----- | ----- |
| | | --SI-M-GKVN- | -IRKD-A-I-S-I----- | ----- |
| | | --AII----E- | EIRKD-KA--I--I----- | ----- |
| | | --SIT--K-EI- | ETSSGK-R-I----- | ----- |
| | | --SIT--K-QI- | ETSSGKQV-II--I----- | ----- |
| | | --N-- | SH-D--II--F----- | ----- |

Figure S42

Partial sequence alignment of the protein DNA gyrase subunit A, showing a 1 aa insertion (boxed) that is specific for members belonging to the family *Chlorobiaceae*. The CSI is not present in any other bacterial species except for the nine other bacteria as indicated above, in the top 500-BLASTp hits.

| | | | |
|--|--|--------------|----------------------------------|
| <i>Chloroherpetonaceae</i> fam.nov. | <i>Chloroherpeton thalassium</i> | WP_012500714 | AIRASQLGYKTCIIEKE |
| | <i>Cand. Thermochlorobacter aerophil</i> | RFM24380 | Q TLGGVCLNWGCIPTKSL |
| | <i>Cand. Thermochlorobacter GBCh1B</i> | KER08989 | P----- |
| | <i>Chlorobium sp. 445</i> | PI048610 | P----- |
| | <i>Chlorobaculum tepidum</i> | WP_010932967 | --H-ARY-L---V--A V----V-----A- |
| | <i>Pelodictyon phaeoclathratiforme</i> | WP_012508516 | -L--AKA-M-V-L--G A----V-----A- |
| | <i>Chlorobium ferrooxidans</i> | WP_006366348 | -L-GAKA-M-V----A A----I-----A- |
| | <i>Chlorobium phaeobacteroides</i> | MBC8524564 | -VK-A-S-LQV--V--G AI----V-----A- |
| | <i>Prosthecochloris ethylica</i> | WP_175186716 | -L--AGE-LRV--V--G P----V-----A- |
| | <i>Chlorobaculum tepidum</i> | AAG12404 | --H-ARY-L---V--A V----V-----A- |
| | <i>Prosthecochloris aestuarii</i> | WP_012505992 | -V--ARK-LTV---RG A----V-----A- |
| | <i>Prosthecochloris vibrioformis</i> | CAB06298 | -LH-ARH-M-V-LV--R S----V-----A- |
| | <i>Chlorobaculum parvum</i> | WP_012501976 | -LH-ARH-M-V-LV--A S----V-----A- |
| | <i>Candidatus Chlorobium masyuteum</i> | WP_166808580 | -L-GAKA-M-V----A A----I-----A- |
| | <i>Chlorobi bacterium NICIL-2</i> | KXB98049 | --A--M--IC--RD R-----S-A- |
| | <i>Prosthecochloris sp. ZM</i> | WP_114617058 | -V--ARK-LTV---RG A----V-----A- |
| | <i>Chlorobium sp. KB01</i> | WP_076789414 | -L-GAKA-M-V----A A----I-----A- |
| | <i>Prosthecochloris sp. ZM_2</i> | WP_114607234 | -L--AGE-LRV--V--G P----V-----A- |
| | <i>Ignavibacteria bacterium</i> | MBK9333167 | -----F-A----D R--I-----A- |
| | <i>Chlorobi bacterium OLB7</i> | KXK57232 | -----L--A-V-RD R--I-----A- |
| | <i>Chlorobi bacterium OLB5</i> | KXK46273 | -----L--ALV-TA R--I-----S-- |
| | <i>Chlorobi bacterium OLB6</i> | KXK33210 | -----L-VACV-RD R-----S-- |
| | <i>Bizionia paragorgiae</i> | WP_092133119 | -----F-A----S-----A- |
| | <i>Chryseobacterium indologenes</i> | HA026821 | -----A--F-A----N--I-----A- |
| | <i>Salinimicrobium marinum</i> | WP_189603939 | -----F-A----S-----A- |
| | <i>Salegentibacter salinarum</i> | WP_079714361 | -----F-A----S-----A- |
| | <i>Muricauda aquimarina</i> | WP_127139765 | -----F-A----S-----A- |
| | <i>Stylophora pistillata</i> | PFX17179 | -----F-A----S-----A- |
| | <i>Aquimarina celericrescens</i> | MBQ0733288 | -----F-A----S-----A- |
| | <i>Salinimicrobium sediminis</i> | WP_097057020 | -----F-A----S-----A- |
| | <i>Flavobacterium johnsoniae</i> | WP_121360554 | -----F-A-V----N-----A- |
| | <i>Winogradskyella jejuensis</i> | WP_073086302 | -----F-A----S-----A- |
| | <i>Cellulophaga tyrosinoxydans</i> | WP_084061203 | -----F-A----S-----A- |
| | <i>Tenacibaculum dicentrarchi</i> | WP_101902757 | -----F-A----N--I-----A- |
| | <i>Bizionia myxarmorum</i> | WP_148403574 | -----F-A----S-----A- |

Figure S43

Partial sequence alignment of the protein hypoxanthine phosphoribosyltransferase protein showing a one amino acid insertion (boxed) that is exclusively shared by all members of the "*Chloroherpetonaceae*" clade.

| | | 30 | 64 |
|--|---|--|--|
| <i>Chloroherpetonaceae</i> fam.nov. | <i>Chloroherpeton thalassium</i> <i>Cand. Thermochlorobacter aerophilum</i> <i>Cand. Thermochlorobacter GBCh1B</i> <i>Chlorobium sp. 445</i> <i>Chlorobaculum tepidum</i> <i>Pelodictyon phaeoclathratiforme</i> <i>Chlorobium ferrooxidans</i> <i>Chlorobium phaeobacteroides</i> <i>Prosthecochloris ethyllica</i> <i>Chlorobaculum tepidum</i> <i>Prosthecochloris aestuarii</i> <i>Prosthecochloris vibrioformis</i> <i>Chlorobaculum parvum</i> <i>Candidatus Chlorobium masyuteum</i> <i>Chlorobi bacterium NICIL-2</i> <i>Prosthecochloris sp. ZM</i> <i>Chlorobium sp. KB01</i> <i>Prosthecochloris sp. ZM_2</i> <i>Chlorobi bacterium OLB7</i> <i>Chlorobi bacterium OLB5</i> <i>Chlorobi bacterium OLB6</i> <i>Ignavibacteria bacterium</i> <i>Flavobacterium columnare</i> <i>Bizionia paragorgiae</i> <i>Chryseobacterium indologenes</i> <i>Salinimicrobium marinum</i> <i>Salegentibacter salinarum</i> <i>Muricauda aquimarina</i> <i>Stylophora pistillata</i> <i>Aquimarina celericrescens</i> <i>Salinimicrobium sediminis</i> <i>Flavobacterium johnsoniae</i> <i>Winogradskyella jejuensis</i> <i>Cellulophaga tyrosinoxydans</i> <i>Tenacibaculum dicentrarchi</i> <i>Bizionia myxarmorum</i> | WP_012500714 RFM24380 KER08989 PIO48610 WP_010932967 WP_012508516 WP_006366348 MBC8524564 WP_175186716 AAG12404 WP_012505992 CAB06298 WP_012501976 WP_166808580 KXB98049 WP_114617058 WP_076789414 WP_114607234 KXK57232 KXK46273 KXK33210 MBK9333167 WP_213068832 WP_092133119 HA026821 WP_189603939 WP_079714361 WP_127139765 PFX17179 MBQ0733288 WP_097057020 WP_121360554 WP_073086302 WP_084061203 WP_101902757 WP_148403574 | Q TLGGVCLNWGCIPTKSL -----V-----P----- -----A-----F-----V-----P----- -----V-----P----- --H-ARY-L---V-A V-----V-----A- -L--AKA-M-V-L---G A-----V-----A- -L-GAKA-M-V---A A-----I-----A- -VK-A-S-LQV-V-G AI-----V-----A- -L-AGE-LRV-V-G P-----V-----A- --H-ARY-L---V-A V-----V-----A- -V-ARK-LTV---RG A-----V-----A- -LH-ARH-M-V-LV--R S-----V-----A- -LH-ARH-M-V-LV--A S-----V-----A- -L-GAKA-M-V---A A-----I-----A- ----A---M-IC--RD R-----S-A- -V-ARK-LTV---RG A-----V-----A- -L-GAKA-M-V---A A-----I-----A- -L-AGE-LRV-V-G P-----V-----A- -----L-A-V-RD R-----I-----A- -----L-ALV-TA R-----I-----S- -----L-VACV-RD R-----S- -----F-A---D R-----I-----A- -----F-VA---N S-----I-----A- -----F-A---S-----A- -----A-F-A---N-----I-----A- -----F-A---S-----A- -----F-A---S-----A- -----F-A---S-----A- -----F-A---S-----A- -----F-A---S-----A- -----F-A---S-----A- -----F-A---N-----I-----A- -----F-A---S-----A- |

Figure S44

Partial sequence alignment of the protein dihydrolipoyl dehydrogenase protein containing a 1 aa insertion (boxed) that is specific for members of the "*Chloroherpetonaceae*" clade.

| | | 236 | 280 | |
|---|---|--|---|--|
| <i>Chloroherpetonaceae</i> fam.nov. | <i>Chloroherpeton thalassium</i> <i>Cand. Thermochlorobacter aerophilum</i> <i>Chlorobium sp. 445</i> <i>Cand. Thermochlorobacteriaceae GBCh1B</i> | WP_012499003 RFM24898 PIO47888 KER10278 KXB98589 KXK55332 MBD1209187 NHZ74744 HEK45053 WP_101072079 MLB7976002 WP_094545675 WP_142714759 WP_028787551 WP_085767182 WP_026913634 WP_148897875 WP_151672747 WP_069132987 NGP89105 WP_101358019 WP_095511589 WP_011711129 WP_156277031 WP_103020725 WP_045114667 WP_103038273 GAK54610 WP_055143938 WP_127121542 WP_108170953 WP_213494753 WP_010047942 WP_052562761 | TEPVNIGNPDEITILDFAKEVQ H----L---S----- H-----S-----E----- V----L---V-V-EL--QIR ND-----L-M-EL-Q-IK D-----M-L---IL V-----S-----R-V VG-----E-S-E---II S----L-E-----II SY-----SM-L---II HD-----VS-E-Q-V D-----Q-E-R-II AM-----SL-----IR SD-----H-----IV SD-----II -----Q-S-E---II -H-----Q-II VY-----E-----II V-----E---II PY-----L-E---I- D---V---VS-KE-E-V S-----S-----D-II SD-----N-S-----R-II D-----QE-E-II AH-----S-K-E-II -D-----KE-E-II SD-----R-MS-Q-EKIK AQ-----N-A-E-II HL-----Q-E-II S-----N-S-----D-II H----L---V-E----L -G---V---M-E-EF-R HD---L---A-E-E-IK YD-----E-HQL---IL | TIVK A--R A--R -M- TIVK A--R A--R -F--REA-T-P-A-- D-V-SSSP-E-HP-E-- N---SSSP-E-RA-K-- -I-SKS-V-P-E-- R---SRS-V-P-Q-- ---SNS---P-A-- -I-SKS-VV-E-- ---TTSS-E-D-Q-- A---SSS-VH-A-- ---S-S-T-E-K-- Q-S-ERS-V-P-V-- K---T-QK-D-Q-- K---T-QK-V-D-Q-- D---S-SK-T-E-K-- K---T-QK-V---K-- DI---SNSK-V-E-- ---ES-S-T-E-K-- ---SKSK-V-P-K-- A---SSST-TYEP--- R---T-QK-V-E-K-- K---T-Q-V---Q-- -V-T-S-TYEP--- K---T-QK-Y-D-K-- -I-S-SP-TYE-K-- AI---TSS-V-EP-V-- K---T-QKV-Y-D-K-- K---T-QK---K-- K---T-QR-VY-Q-- -S-SKS-VY-P-Q-- K-G---SR-VHR-K-- K-A-SKS-V-P-Q-- A---SKSR-T-V-- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Chlorobi bacterium NICIL-2</i> <i>Chlorobi bacterium OLB7</i> <i>Ignavibacteria bacterium</i> <i>Rhodocaloribacter litoris</i> <i>Rhodothermus marinus</i> <i>Rhodohalobacter barkolensis</i> <i>Candidatus Kapabacteria bacterium</i> <i>Rubricoccus marinus</i> <i>Aliifodinibius sediminis</i> <i>Terrimonas ferruginea</i> <i>Nonlabens spongiae</i> <i>Gramella portivictoriae</i> <i>Fodinibius salinus</i> <i>Patirioscius marinus</i> <i>Rhodohalobacter halophilus</i> <i>Aliifodinibius halophilus</i> <i>Raineya orbicola</i> | | | |
| Other Bacteria | <i>Rubrivirga marina</i> <i>Gramella forsetii</i> <i>Gramella aestuarii</i> <i>Salinibacter altiplanensis</i> <i>Microscilla marina</i> <i>Salinivenus iranica</i> <i>Candidatus Moduliflexus flocculans</i> <i>Jiulongibacter sediminis</i> <i>Chryseotalea sanaruensis</i> <i>Gramella gaetbulicola</i> <i>Telmatocola sphagniphila</i> <i>Candidatus Fermentibacteria bacterium</i> <i>Gemmata obscuriglobus</i> <i>Candidatus Brocadia sinica</i> | | | |

Figure S45

Partial sequence alignment of the protein SDR family oxidoreductase, showing a 4 aa insertion (boxed) that is specific for members of the "*Chloroherpetonaceae*" clade

| | | | | | | |
|--|---|--------------|---------------------|-----------------------|------------|----------|
| <i>Chloroherpetonaceae</i> fam.nov. | <i>Chloroherpeton thalassium</i> | WP_012499407 | 164 | YDFRPSYQQIASQLKLIYPKT | RP | 203 |
| | <i>Cand. Thermochlorobacter aerophilum</i> | RFM23398 | ----- | K-TGIAQGLPK | -T----- | V-E-- |
| | <i>Chlorobium sp. 445</i> | PI048802 | ----- | K-TAG-AQGLPK | --T- | V-E-- |
| | <i>Chlorobium phaeobacteroides</i> | MBV5319837 | H----D | LSLSQLVT-FPDLP | VA-F----- | HQVQR-- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | WP_139456672 | H----D | LALS-IPEVLPGIP | VA-F----- | RVHQH-- |
| | <i>Prosthecochloris marina</i> | WP_110024258 | H----D | LSL-ALA-FPGTP | IA-F----- | HKVQQ-- |
| | <i>Chlorobi bacterium NICIL-2</i> | KXB98426 | H----D | RR-GELSGALGR-P | I----- | QOTRR-- |
| | <i>Prosthecochloris sp. HL-130-GSB</i> | WP_198166311 | H----D | RR-VRV-QA-PSNV | PV-T--- | NDRVHV-V |
| | <i>Fictibacillus encelensis</i> | WP_061970029 | H----D | LR-KDM-NNL--RP | -IV----- | K-VTE-- |
| | <i>Vagococcus silagei</i> | WP_136136718 | H----- | LKL-ETIQV-Q--P | TIV----- | P-VAQ-- |
| | <i>Bacillus marasmi</i> | WP_147534659 | H----- | RY-S-I-QH-HS-P | II----- | AQVRS-- |
| | <i>Candidatus Gallibacteroides avistercoris</i> | HIU54418 | H----- | L-QIRH-LPQVP | V----- | N-AI-- |
| | <i>Gracilimonas mengyeensis</i> | WP_142454845 | H----- | RE-R-S-ES-ADQ- | RW----- | P-VQ-- |
| | <i>Ornithinibacillus massiliensis</i> | WP_211741114 | H----- | RS-VPT-Q-RNIP | -YM----- | VIS-- |
| | <i>Oceanobacillus chungangensis</i> | WP_115748595 | H----- | RS-VPN-KLNNIP | -FV----- | VIN-- |
| | <i>Virgibacillus dokdonensis</i> | AUJ24938 | H----- | RS-IH-QQLPKRP | --M----- | T-VIE-- |
| | <i>Virgibacillus chiguensis</i> | SHH23913 | H----- | RS-IP-QQLPKRP | --M----- | T-VIE-- |
| | <i>Fictibacillus solisalsi</i> | WP_090233596 | H----- | LR-KDM-NNL--RP | -IV----- | K-VTE-- |
| | <i>Peptococcus niger</i> | WP_200781888 | H----- | R-AG-SALT-RP | IFA----- | PVQA-- |
| | <i>Bacillus amyloliquefaciens</i> | KJD52951 | H----- | RH-E-F-NSLGTRP | -V----- | P-VHE-- |
| | <i>Ornithinibacillus californiensis</i> | WP_047983848 | H----- | RS-VPN-R-GTIP | -IM----- | VIS-- |
| | <i>Ornithinibacillus caprae</i> | WP_155671718 | H----- | RS-VP-Q-RNIP | -YV----- | VIS-- |
| | <i>Virgibacillus subterraneus</i> | WP_092502198 | H----- | RS-V-N-RLSNIP | --IG----- | VIS-- |
| | <i>Virgibacillus salinus</i> | SDQ11862 | H----- | RS-V-N-RLSNIP | --IG----- | VIS-- |
| | <i>Spirochaetaceae bacterium</i> | MBP3450048 | H----- | E-RR-SEIRQIFPKAV | C----- | TVR-- |
| | <i>Enterococcus canintestini</i> | WP_095006432 | H----- | L-L-QEI-ALPKRP | AVI----- | PQVA-- |
| | <i>Alicyclobacillus ferrooxydans</i> | WP_206659046 | H----- | S-PF-EQLDERP | -VV----- | P-VTR-- |
| | <i>Litchfieldia alkalitelluris</i> | WP_078547561 | H----- | RN-K-MIDLKD-P | TIV----- | K-VIR-- |
| | <i>Oceanobacillus senegalensis</i> | WP_085993971 | H----- | RS-V-N-QFTNIP | -IV----- | VIN-- |
| | <i>Candidatus Kurthia intestingallinarum</i> | HIX42122 | F---E-L--EA-PKER-PI | ---S--- | NQ-MR-- | |
| | <i>Alkalihalobacillus bogoriensis</i> | WP_035177688 | H----- | MG-STF-SVLQ--P | -I----- | QVQA-- |
| | <i>Flavobacteriales bacterium</i> | NQY11258 | H----- | LN-SELR-FKTVP | VM----- | K-VV-- |
| | <i>Bacillus alkalicellulosilyticus</i> | WP_078554038 | H----- | RS-STYIDSQ--P | LI----- | QVQE-- |
| | <i>Xylanibacillus composti</i> | WP_213413892 | H----- | M-PKL-RQ-H-RP | -VA-F----- | DKVKE-- |
| | <i>Oceanobacillus caeni</i> | WP_204798664 | H----- | RS-V-T-Q-SNIP | -FV----- | VIH-- |
| | <i>Massilia timonae</i> | HAK90119 | H----- | A-IGL-AAIEA-GRPP | V----- | D-VIA-- |
| | <i>Gracilimonas amyloytica</i> | WP_103665834 | H----- | RE-R-S-ES-ADN- | RWI----- | P-VQ-- |
| | <i>Candidatus Tetragenococcus pullicola</i> | HYI56919 | H----- | LEL-ES-K-E-RP | AII----- | POVS-- |
| | <i>Bacillus paralicheniformis</i> | WP_145654026 | H----- | RH-E-F-NSLGTRP | -V----- | P-VHE-- |
| | <i>Bacillus licheniformis</i> | WP_031314645 | H----- | RH-EGF-NSLGTRP | -V----- | P-VHE-- |
| | <i>Oceanobacillus halophilus</i> | WP_121204935 | H----- | RS-V-N-QFSNIP | -FV----- | VIS-- |
| | <i>Enterococcus faecium</i> | WP_049143502 | H----- | L-M-N-DQLPNRP | -IV----- | VQVAA-- |
| | <i>Bacillus licheniformis CG-B52</i> | EQM27666 | H----- | RH-EGF-NSLGTRP | -V----- | P-VHE-- |
| | <i>Ornithinibacillus halotolerans</i> | WP_188383055 | H----- | RS-VPN-Q-GSIP | -VM----- | VIS-- |
| | <i>Enterococcus dispar</i> | WP_016173413 | H----- | L-L-QEI-ALPQRP | AVI----- | PQVA-- |
| | <i>Paenibacillus psychroresistens</i> | WP_155699081 | H----- | RS-STL-MLPERP | IVA-F----- | P-VVD-- |
| | <i>Lactobacillus paragasseri</i> | WP_113576156 | H----A-R-MDGVS-KSNP | NI----- | PSVQ-- | |
| | <i>Oceanobacillus limi</i> | WP_090872575 | H----- | RS-VP-QLNNIP | -YV----- | VIS-- |
| | <i>Virgibacillus alimentarius</i> | WP_226371238 | H----- | RS-VP-QLANIP | -FV----- | VIS-- |
| | <i>Priestia megaterium</i> | WP_026682619 | H----- | RS-IP-QLHHAP | IIM----- | VIF-- |
| | <i>Loigolactobacillus binensis</i> | WP_137638398 | H----- | L-VTAR-QELRT-P | -LV----- | RVAT-- |
| | <i>Halobacillus faecis</i> | WP_146815185 | H----- | RS-VPT-K-ANLP | -LM----- | Q-VI-- |
| | <i>Paenibacillus hemerocallicola</i> | WP_139601900 | H----- | MS-SRV-N-R-RP | -IA-F----- | DKVKE-- |
| | <i>Paenibacillus mesophilus</i> | WP_138881353 | H----- | MS-SRV-N-R-RP | -IA-F----- | DKVKE-- |
| | <i>Lactobacillus johnsonii</i> | WP_180872720 | H----A-R-MEGVS-KSQP | NI----- | PSVQ-- | |
| | <i>Halobacillus karajensis</i> | WP_074733221 | H----- | RS-VPT-E-PNLP | -LM----- | Q-VI-- |

Figure S46

Partial sequence alignment of the protein RecQ family ATP-dependent DNA helicase, showing a 2 aa insertion (boxed) that is found specific for members of the "*Chloroherpetonaceae*" clade

| | | 33 | 78 |
|--|---|-----------------------|-----------------------------|
| <i>Chloroherpetonaceae</i> fam.nov. | <i>Cand. Thermochlorobacter aerophilum</i> RFM24133 | IAIVIDQFRYDYLERYEAYY | LPATR |
| | <i>Chlorobium</i> sp. 445 | V-----L-----D-H- | VA--K -----IEQ---L----S |
| | <i>Chloroherpeton thalassium</i> | LT-----D---DMF | --VGK H---RK-R-Q---FMEKCF-D |
| <i>Ignavibacteriae</i> | <i>Ignavibacteriaceae bacterium</i> | V-V--M----QK-YPL- | GD-----M-D-FNC---V--N |
| | <i>Thermaurantimonas aggregans</i> | VG--V--MK-E----PL- | S KN---A---SE-FFYK---N |
| | <i>Schleiferia thermophila</i> | VG--V--MK-E----PLF | S D-----R-FT-Q-FN-K----N |
| | <i>Candidatus Kapabacteria bacteria</i> | VVVSF----G-PRQ---VF | P P-Q---R---M-E---T---SCR-E |
| | <i>Seonamhaeicola maritimus</i> | V-----M-----T---SK- | GE-----MIEE-FNCK-N-FN |
| | <i>Nonlabens agnitus</i> | VG--V--M-----T---YDR- | SN-----M-D-FSA---N--N |
| | <i>Echinicola rosea</i> | VG--V--M--E--HK-NDRF | -D-----MNE-FMMK---N |
| | <i>Cecembia rubra</i> | -G--V--M-QE-FYK---R- | SE-----M-D-FMMK---N |
| | <i>Echinicola vietnamensis</i> | VG--V--M--E--HK-NDRF | -D-----MND-FMMK---N |
| | <i>Flavobacteriaceae bacterium</i> | HAE71810 | SE-----V-E-FN-K-N--N |
| | <i>Echinicola soli</i> | VG--V--M--E--HK-NDRF | -D-----MND-FVMK---N |
| | <i>Echinicola shivajiensis</i> | WP_141613469 | -E-----MHE-FMMK-G--N |
| | <i>Nonlabens xylanidelens</i> | WP_215225323 | SD-----MNE-FNL--N--N |
| | <i>Ekhidna lutea</i> | WP_104515391 | ED-----IEE-F-YR---N |
| | <i>Cyclobacteriaceae bacterium</i> | WP_089358319 | GP-----MSD-FMLK---N |
| | <i>Arcticibacter tournebarensis</i> | NOT77289 | SE-----GQ-F---RDT--N |
| | <i>Cecembia lonarensis</i> | WP_161987516 | -E-----M-E-FMMK---N |
| | <i>Cecembia calidifontis</i> | WP_040400614 | SD-----M-E-FMMK---N |
| | <i>Algoriphagus marincola</i> | WP_130275039 | SE-----M-E-FMMK-G--N |
| | <i>Pontibacter actiniarum</i> | KPQ14508 | GND---K--SE-FS-R-T--N |
| | <i>Nonlabens dokdonensis</i> | WP_025608463 | -N---N---IND-YQL--N--N |
| | <i>Aureibaculum marinum</i> | WP_015360898 | GE-----NS-YN-E---N |
| | <i>Hymenobacter metallilatus</i> | WP_123896104 | G-----R---GE-FSYE---N |
| | <i>Algoriphagus antarcticus</i> | WP_125432143 | SD-----MM-D-FMMK-G--N |
| | <i>Hymenobacter woopenensis</i> | WP_086541318 | GN-----R---GE-FSYE---N |
| | <i>Pyrinomonadaceae bacterium</i> | WP_135532231 | APN-LRMM-RN--SW---TNFD |
| | <i>Seonamhaeicola algicola</i> | MBA202343 | GN-----MIRE-YNCK-H--N |
| | <i>Hymenobacter lapidiphilus</i> | WP_147135211 | G-----GE-FSYE-T--K |
| | <i>Gramella gaetbulicola</i> | WP_176909843 | EN-----VND-FT-K-T-FN |
| | <i>Kordia jejudonensis</i> | WP_108172269 | SE-----M-RE-FNCK-N--N |
| | <i>Mariniradius saccharolyticus</i> | WP_046756932 | -E-----MRE-FMM---G--N |
| | <i>Kordia antarctica</i> | WP_040480808 | SE-----M-NG-YNCK-N--N |
| | <i>Nibribacter ruber</i> | WP_160129351 | SND-----RE-FN-K-N--N |
| | <i>Aquimarea intermedia</i> | WP_160691664 | G-----IS-E-FQYK---FN |
| | <i>Leptobacterium flavescent</i> | WP_148782777 | GN-----INE-FNCK-N--N |
| | <i>Tenacibaculum jejuense</i> | WP_163608315 | GE-----I-D-FSLE---FN |
| | <i>Nonlabens mediterranea</i> | WP_095073954 | GD-----MND-FNL---N-FN |
| | <i>Psychroserpens damuponensis</i> | MBF4982940 | SD-----MINQ-FNCK-N-FN |
| | <i>Nonlabens xiamensis</i> | WP_040278820 | GE-----N-VRD-FNA---H--N |
| | <i>Hymenobacter gelipurpurascens</i> | WP_124979762 | GN-----R---GE-FSYE---N |
| | <i>Antarcticibacterium flavum</i> | WP_088845355 | EE-----VRN-FN-R---FN |
| | <i>Rufibacter radiotolerans</i> | WP_139067918 | -ND-----VNQ-FS-Q-N--N |
| | <i>Urechidicola croceus</i> | AKQ47831 | SE-----MNN-YNLK-T--N |
| | <i>Hymenobacter psychrophilus</i> | WP_070236445 | G-----GE-FSYE-T--N |
| | <i>Rufibacter glacialis</i> | SDY42225 | SAN-----AE-FN-R-N--N |
| | <i>Gramella sabulilitoris</i> | KAA6431211 | GQN-----VNN-FV-K-N-FN |
| | <i>Mangrovimonas yunxiaonensis</i> | WP_143411027 | GD-----N-MV-E-FNCK-N-FN |
| | <i>Belliella pelvolcani</i> | WP_036122168 | -E-----MND-FMM---G--N |
| | <i>Saprospiraceae bacterium</i> | WP_076500269 | GE-----AQ-FSC-E-T--N |
| | <i>Mesonia hippocampi</i> | MCB0575169 | QAD-----INE-FV-K-N-FN |
| | <i>Nonlabens marinus</i> | WP_183478300 | GN-----MNE-FNV--N--N |
| | | WP_041497300 | VG--V--M-----T---YSR- |

Figure S47

Partial sequence alignment of the protein alkaline phosphatase family protein, showing a 5 aa insertion (boxed) that is specific for members of the "*Chloroherpetonaceae*" clade

| | | 2 | KIGSIDIDRPVI | 30 |
|---|---|----------------|---------------|------------------|
| Order <i>Chlorobiales</i> | <i>Chlorobium limicola</i> | WP_012467025.1 | -----T-E----- | LAPMEDVTDRQFQLCK |
| | <i>Pelodictyon phaeoclathratiforme</i> | WP_012509195.1 | -----L----- | ----- |
| | <i>Chlorobium phaeovibrioides</i> | WP_126385139.1 | -----A----- | ----- |
| | <i>Chlorobium sp. KB01</i> | WP_076792104.1 | -----P----- | ----- |
| | <i>Candidatus Chlorobium masyuteu</i> | WP_166807179.1 | R--P----- | ----- |
| | <i>Chlorobium phaeobacteroides</i> | WP_011746071.1 | R--P----- | ----- |
| | <i>Chlorobium sp. N1</i> | WP_131356337.1 | ---AL----- | ----- |
| | <i>Chlorobium luteolum</i> | WP_011357039.1 | ---KL----- | ----- |
| | <i>Chlorobium ferrooxidans</i> | WP_006366291.1 | ---P-A----- | ----- |
| | <i>Chlorobaculum tepidum</i> | WP_164927287.1 | R--T-----I- | ----- |
| | <i>Chlorobaculum limnaeum</i> | WP_069810711.1 | R--T-E-----I- | ----- |
| | <i>Chlorobaculum parvum</i> | WP_012501542.1 | R--P-----I- | ----- |
| | <i>Chlorobaculum thiosulfatiphilum</i> | WP_139455934.1 | R--Q----- | ----- |
| | <i>Prosthecochloris vibrioformis</i> | WP_139626091.1 | Q---F-MA---M | S-K-- |
| | <i>Prosthecochloris marina</i> | WP_110022432.1 | R--KLH-E--- | S-KI-- |
| | <i>Prosthecochloris aestuarii</i> | HED30740.1 | -D-FEMQ---M | E-S-S--KI-- |
| | <i>Prosthecochloris ethylica</i> | WP_175186888.1 | --GF-MK---M | E-S-S--KI-- |
| | <i>Chloroherpeton thalassium</i> | WP_041468962.1 | --Q-E-EK--- | PS-R-- |
| | <i>Cand. Thermochlorobact. GBCh1B</i> | KER09073.1 | --T--EQ-IV | PS-R-- |
| | <i>Cand. Thermochlorobact. aerophilum</i> | RFM23383.1 | --H-----IV | PS-R-- |
| | <i>Chlorobium sp. 445</i> | PIO47072.1 | Q--K-H--K-IV | PS-R-- |
| Larger <i>Ignavibacteriae</i> Clade | <i>Melioribacter roseus</i> | WP_014857420.1 | --N-ELNK-LL | I--K-- |
| | <i>Ignavibacterium album</i> | HFI91396.1 | --V-N-E-NKA-L | LS-LI-- |
| | <i>Melioribacteraceae bacterium</i> | MCF8241219 | --N-E-NKALL | SIG-K-- |
| | <i>Ignavi. bacterium CG_4_9_14_3</i> | PJA95416 | V-K-S--KALL | IG-K-- |
| | <i>Ignavi. bacterium GWC2_38_9</i> | OGU63466 | --V-K-N--KALL | I-Y-R-- |
| | <i>Ignavi. bacterium HGW</i> | PKL82039 | K-N-EKALL | I-Y-K-- |
| | <i>Ignavi. bacterium CHB3</i> | MCE7857972 | --N-ELNKAIL | IS-- |
| Other Bacteria | <i>Prevotella copri</i> | WP_158463588 | --N-EFGNKPL F | IG--M-- |
| | <i>Alloprevotella tannerae</i> | MBF0970273 | --NV-LGKRPL L | IG--K-- |
| | <i>Prevotella marshii</i> | WP_006950262 | --N-LGSRPL L | IG--L-- |
| | <i>Marinilabilia rubra</i> | WP_109263596 | --NLELRKEPL L | QM-K-- |
| | <i>Moheibacter sediminis</i> | SMC71036 | --NELPKFPL L | S-PP--R-- |
| | <i>Alkaliflexus imshenetskii</i> | WP_044117808 | --N-ELRKPL L | HM-S-- |
| | <i>Cryomorphaceae bacterium</i> | HAP71276 | --D-E-GKFPL L | S-PP--K-- |
| | <i>Elizabethkingia meningoseptica</i> | WP_073552649 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Epilithonimonas caeni</i> | WP_027384989 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Epilithonimonas hungarica</i> | WP_089873071 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Elizabethkingia meningoseptica</i> | WP_077564192 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Muricauda aquimarina</i> | WP_127140348 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Muricauda oceanensis</i> | WP_127136015 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Tamana sedimentorum</i> | WP_044631505 | --N-ELPDFPL L | S-PP--K-- |
| | <i>Kaistella solincola</i> | WP_039343339 | --N-ELPEFPL L | S-PP--K-- |
| | <i>Chryseobacterium salivictor</i> | WP_133438697 | --N-ELPEFPL L | S-PP--K-- |
| | <i>Mesoflavibacter sabulilitoris</i> | WP_106680957 | --N-ELPEFPL L | S-PP--K-- |
| | <i>Prevotella melaninogenica</i> | WP_120174414 | --T--LGERPL F | IG--KM-- |
| | <i>Myroides profundi</i> | AJH16529 | --N-ELPDHPL L | S-PP--R-- |

Figure S48

Partial sequence alignment of the protein tRNA dihydrouridine synthase DusB, showing a 1 aa deletion (boxed) that is specific for members of the order *Chlorobiales* and the “Larger *Ignavibacteriae* Clade”.