# The function of the $Na^+$-driven flagellum of *Vibrio cholerae* is determined by osmolality and pH

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# Supporting Information

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## Table S1. Oligonucleotides used in this study.

| Oligonucleotide | Sequence (5’ – 3’) | Restriction sites |
|---|---|---|
| VC*motA*V-III | GCCGCGCGGCAGC*CATATG*GATTTAGCAAC | *Nde*I |
| VC*motB*R-III | GGTGGCTCCA*CTCGAG*TTGTTGTCCACCGC | *Xho*I |
| VC*motA*V-IV | GAAAGATCACACCGCCGTGGAAGAG | |
| VC*motB*R-IV | ACATTTCCCCGAAAAGTGCCACCTG | |
| VCb82V-D23N | GGCTG*GGTACC*TTTGCGAACTTGATGTCACTGC | *Kpn*I |
| VCb49R-D23N | GCAGTGACATCAAGTTCGCAAA*GGTACC*CAGCC | *Kpn*I |
| VCb82V-D23E | GGCTG*GGTACC*TTTGCGGAATTGATGTCACTGC | *Kpn*I |
| VCb49R-D23E | GCAGTGACATCAATTCCGCAAA*GGTACC*CAGCC | *Kpn*I |
| VCb90V-S26A | GGCTG*GGTACC*TTTGCGGACTTGATGGCACTGCTGATGTGC | *Kpn*I |
| VCb50R-S26A | GCACATCAGCAGTGCCATCAAGTCCGCAAA*GGTACC*CAGCC | *Kpn*I |
| VCb90V-S26T | GGCTG*GGTACC*TTTGCGGACTTGATGACACTGCTGATGTGC | *Kpn*I |
| VCb50R-S26T | GCACATCAGCAGTGTCATCAAGTCCGCAAA*GGTACC*CAGCC | *Kpn*I |
| *pomB*-D42N fwd | CGGAGATG*AACGTT*CTGAAATTTAAGCAGATCGCTGGC | *Acl*I |
| *pomB*-D42N rev | GCCAGCGATCTGCTTAAATTTCAG*AACGTT*CATCTCCG | *Acl*I |
| GFP-V | CTACTAGTATG*GCTAGC*AGCAAAGGAG | *Nhe*I |
| GFP-R | GCTCATTA*ACCGGT*TTTGTAGAGTTCATCC | *Age*I |
| VC*motB*V-*Nhe*I-*Age*I | CCGACTATG*GCTAGCACCGGT*ATGGATGACGAACAACAATG | *Nhe*I, *Age*I |
| VC*motB*R-*Nhe*I-*Age*I | GTTCGTCATCCAT*ACCGGTGCTAGC*CATAGTCGGCTCC | *Age*I, *Nhe*I |
| WS-013-GFPend-f | CCCAACGAAAAGCGTGACCACATGGTC | |

Introduced restriction sites are underlined and shown in italics.

**Table S2. Quantitative analysis of flagellation of *V. cholerae* Δ*pomAB* producing His-tagged PomA together with PomB variants.** Cells were grown in M9 minimal medium pH 8.0 without added sodium and inspected using Transmission Electron Microscopy (see Fig. S3). For each PomB variant, a total of 149 to 162 individual cells were inspected and categorized into flagellated cells (“+ flagellum”) or aflagellated cells (“– flagellum”).

| | **Number of cells** | | |
|---|---|---|---|
| **PomB variant** | **+ flagellum** | **– flagellum** | **Total number of cells inspected** |
| **WT** | 135 (83%) | 27 (17%) | 162 (100%) |
| **D23E** | 118 (75%) | 40 (25%) | 158 (100%) |
| **D23N** | 142 (95%) | 7 (5%) | 149 (100%) |
| **S26A** | 115 (76%) | 36 (24%) | 151 (100%) |
| **S26T** | 116 (75%) | 38 (25%) | 154 (100%) |
| **D42N** | 112 (74%) | 39 (26%) | 151 (100%) |

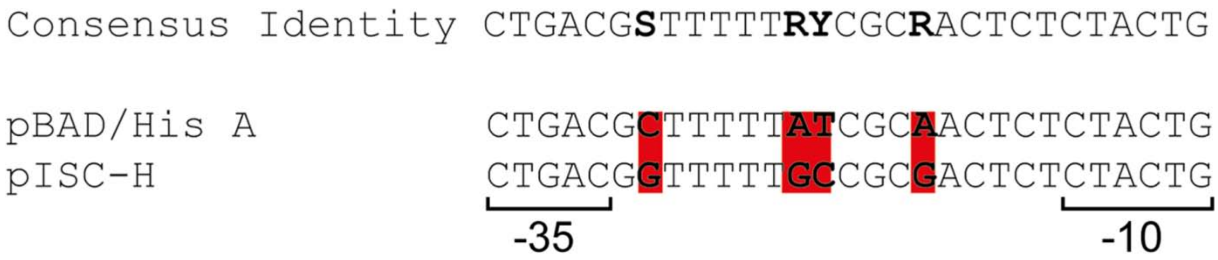


**Figure S1: Alignment of the arabinose promoters of pBAD/His (Invitrogen) and pISC-H.** Alignment was performed using Geneious Pro 5.4.6 (Biomatters, New Zealand). Identical and similar residues were identified using an identity and similarity threshold of 63%. -35 indicates the -35 regions, -10 the -10 regions of the promoters.

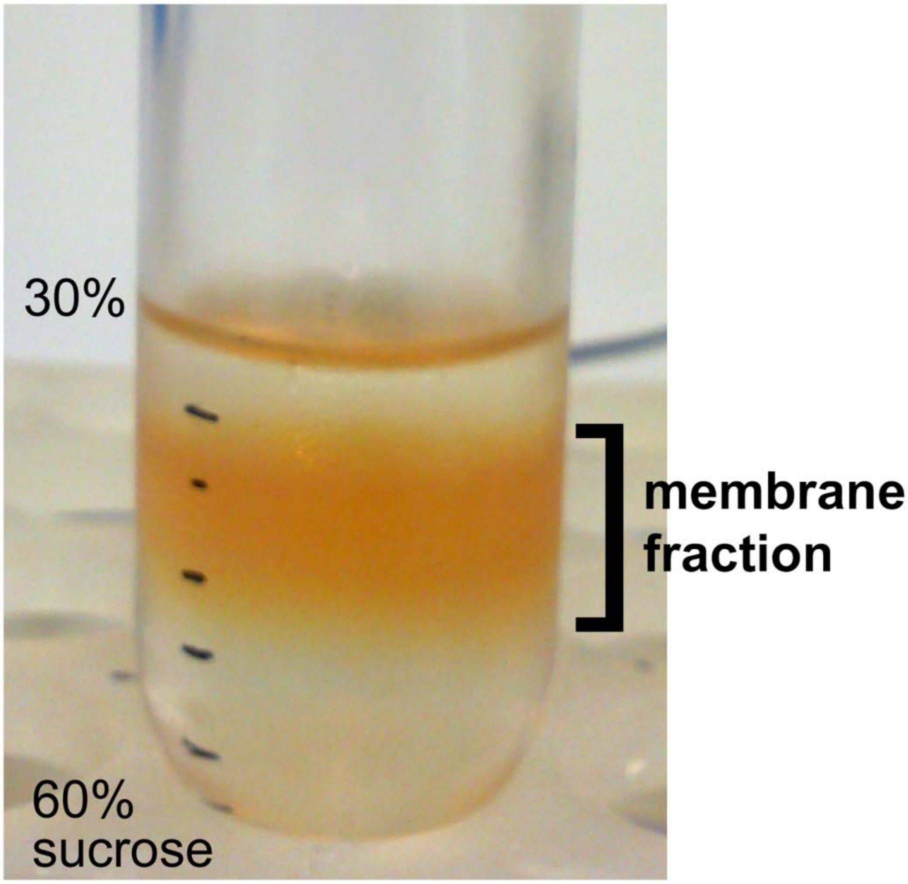


**Figure S2: Purification of isolated membranes using sucrose gradient centrifugation.** Gradient ranged from 30 to 60% sucrose (w/v). After centrifugation, a clear, brownish fraction was visible which represents the purified membranes. Protein concentration was determined using a BCA assay. The concentration of proteins in the upper and lower fraction was below the detection level. The purified membranes were used for further analysis (SDS-PAGE and western blot).

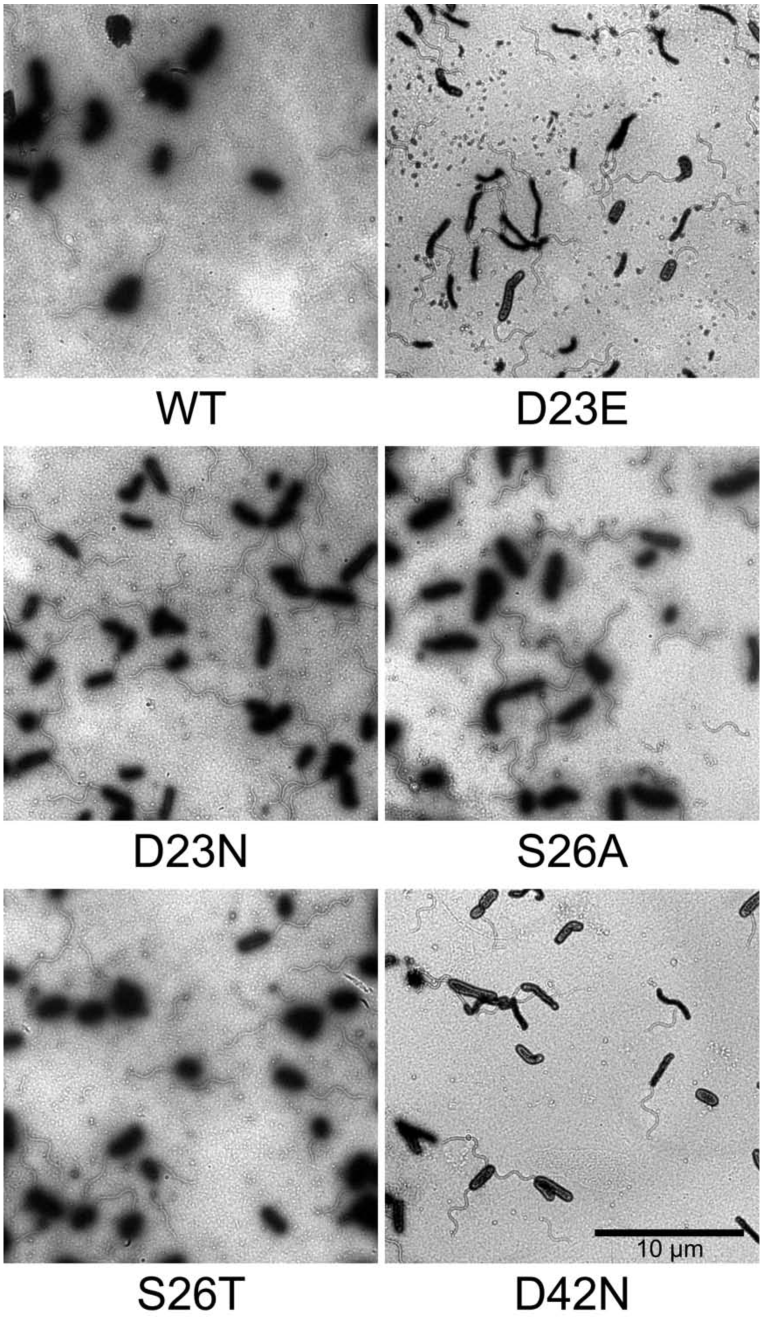
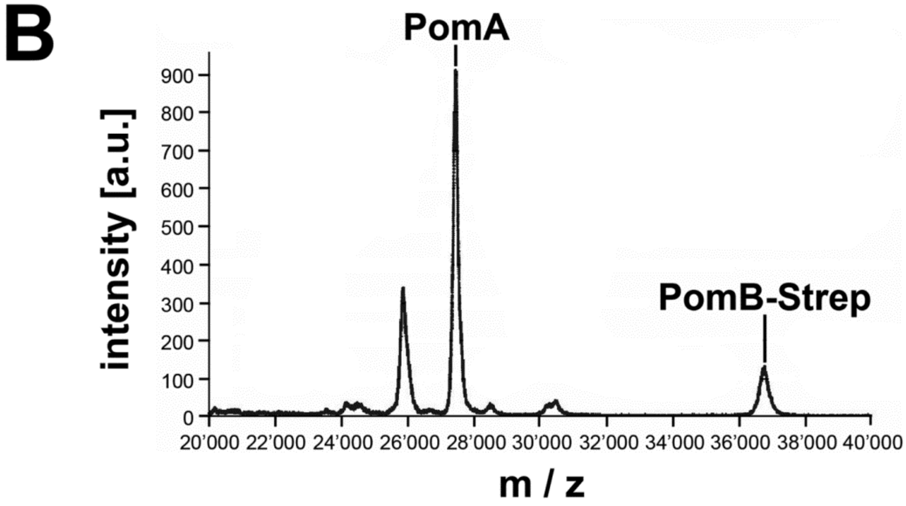


**Figure S3: Flagellation state of *V. cholerae* Δ*pomAB* complemented with plasmids pAB coding for His-PomA and PomB (wt and variants).** Cells were grown on sodium-depleted M9 minimal medium supplemented with 10 mM arabinose at pH 8.0. Aliquots of the bacterial cultures (10 µL) were adsorbed on Pioloform coated copper grids and stained with uranyl acetate as described in Materials and Methods. Pictures were recorded using a Transmission Electron Microscope (LEO 912AB, Zeiss, Germany) at 80kV and at a magnification of 630 x. Scale bar indicates 10 µm. See Table S2 for the relative abundance of flagella.

**A**

| | Amino acid position / identified amino acid | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| **PomA** | M | D | L | A | T | L | V | G | L | I | G | – | – | A | – | – |
| **PomB-Strep** | M | D | D | E | Q | Q | C | K | C | P | P | P | G | L | P | A |



| | calculated mass [Da] | measured mass [Da] | calculated – measured mass [Da] |
|---|---|---|---|
| **PomA** | 27'420 | 27'448 | - 28 (- 0.10%) |
| **PomB-Strep** | 36'676 | 36'779 | - 103 (- 0.28%) |

**Figure S4: Edman sequencing and MALDI-MS analysis of *V. cholerae* PomAB. (A)** For N-terminal sequencing, PomAB was expressed in *E. coli* C41 (DE3) from plasmid pET18ABS and enriched as described in the main text. After separation by SDS-PAGE, the proteins were transferred to polyvinylidene fluoride (PVDF) membrane following the semi-dry protocol described by the manufacturer of the blotting apparatus (Amersham Biosciences Europe GmbH, Germany). Blot staining was performed with 0.1% Coomassie-G250, and membrane pieces containing bands of PomA or PomB were used for N-terminal sequence analysis using a protein sequencer (Applied Biosystems 470/A) with on-line phenylthiohydantoine amino acid detection by HPLC (Applied Biosystems 120). **(B)** The molecular masses of PomA and PomB were determined on a Perseptive Biosystems Voyager Elite system, a MALDI time-of-flight instrument with reflector. The measurements were recorded in the linear positive mode with an instrument accuracy of 0.3%. A layer of sinapinic acid was deposited on the target from a saturated stock solution in acetonitrile/ddH$_2$O (2:1, v/v), containing 0.1% trifluoroacetic acid. Immediately before analysis, dried protein samples were dissolved in a buffer containing 20 mM Tris-HCl pH 7.5 and 1% octylglucoside and placed onto the layer of sinapinic acid.

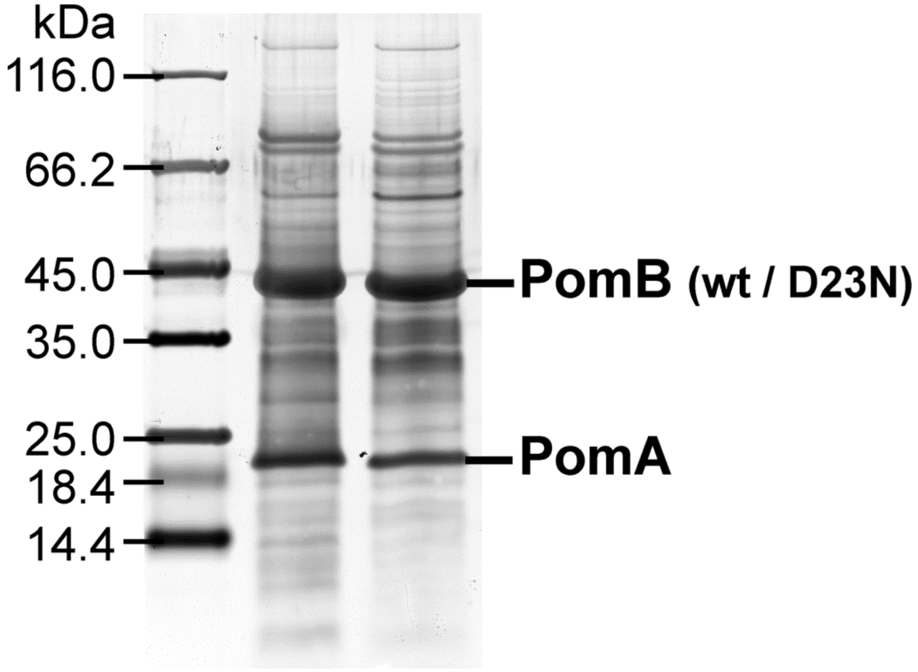
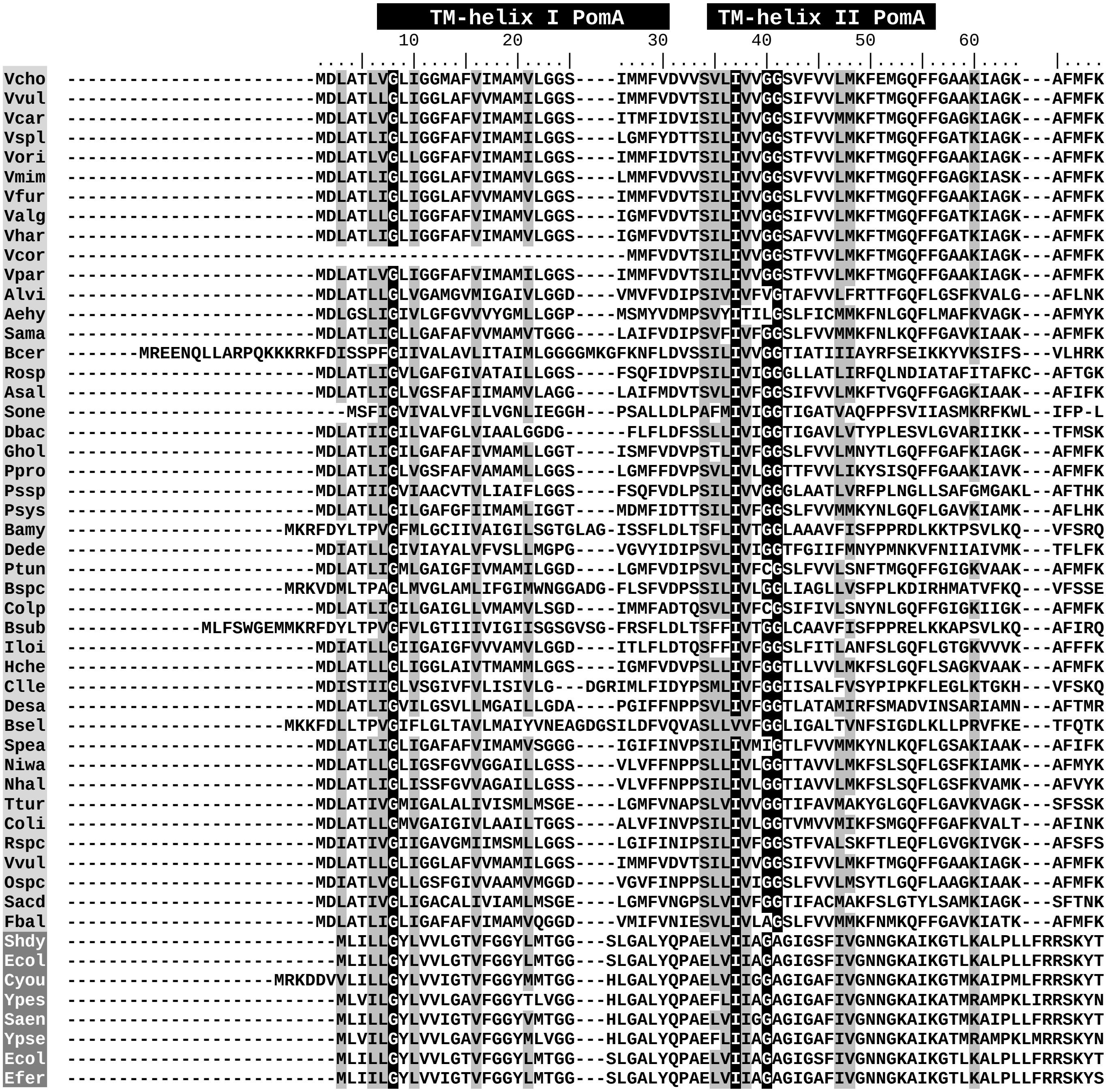


**Figure S5: SDS-PAGE of PomA in complex with PomB or with the PomB variant carrying the D23N substitution.** PomAB containing membranes were solubilised with Cymal-5 as described in Materials and methods. PomB was bound to Strep-Tactin *via* its C-terminally fused Strep-tag, PomA was co-purified by its interaction with PomB. Left: wild-type PomA co-purified with wild-type PomB (2.2 µg); right: wildtype-PomA co-purified with the D23N variant of PomB-D23N (2.8 µg). The gel was stained with silver.



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Vpar ------------------------MDLATLVGLIGGFAFVIMAMILGGS----IMMFVDVTSILIVVGGSTFVVLMKFTMGQFFGAAKIAGK---AFMFK
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Sama ------------------------MDLATLIGLLGAFAFVVMAMVTGGG----LAIFVDIPSVFIVFGGSLFVVMMKFNLKQFFGAVKIAAK---AFMFK
Bcer -------MREENQLLARPQKKKRKFDISSPFGIIVALAVLITAIMLGGGGMKGFKNFLDVSSILIVVGGTIATIIIAYRFSEIKKYVKSIFS---VLHRK
Rosp ------------------------MDLATLIGVLGAFGIVATAILLGGS----FSQFIDVPSILIVIGGGLLATLIRFQLNDIATAFITAFKC--AFTGK
Asal ------------------------MDLATLIGLVGSFAFIIMAMVLAGG----LAIFMDVTSVLIVFGGSIFVVLMKFTVGQFFGAGKIAAK---AFIFK
Sone ---------------------------MSFIGVIVALVFILVGNLIEGGH---PSALLDLPAFMIVIGGTIGATVAQFPFSVIIASMKRFKWL--IFP-L
Dbac ------------------------MDLATIIGILVAFGLVIAALGGDG------FLFLDFSSLLIVIGGTIGAVLVTYPLESVLGVARIIKK---TFMSK
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Ppro ------------------------MDLATLIGLVGSFAFVAMAMLLGGS----LGMFFDVPSVLIVLGGTTFVVLIKYSISQFFGAAKIAVK---AFMFK
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Clle ------------------------MDISTIIGLVSGIVFVLISIVLG---DGRIMLFIDYPSMLIVFGGIISALFVSYPIPKFLEGLKTGKH---VFSKQ
Desa ------------------------MDLATLIGVILGSVLLMGAILLGDA----PGIFFNPPSVLIVFGGTLATAMIRFSMADVINSARIAMN---AFTMR
Bsel ---------------------MKKFDLLTPVGIFLGLTAVLMAIYVNEAGDGSILDFVQVASLLVVFGGLIGALTVNFSIGDLKLLPRVFKE---TFQTK
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      70        80        90                         100       110        120       130       140
      |....|....|....|....|....|....                   |....|....|....|  ....|....|....|....|....|....|....
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Alvi  STAPTALIEEAVALANIARREGLLALEGKQ-------------------ISHPFLQTGISLCIDG-HPPEVVQKVLSKDINLAIQRQEAGYSVFKATAEV
Aehy  GDKLDDLIAKAVELADAARKGGFLALEAAE-------------------IPNTFMKKGIDMLVDG-HDADVVRAVMEKDIELTSERHVIAVKVFRSLGDL
Sama  IDKPEELIEQSVAMADAARKGGFLALEEAQ-------------------ISNSFMQKAVDMLVDG-HDGDVVREALEKDIALTEERHRTGIGIFRAFGDV
Bcer  EEDLEQLTEMFVEFSKKSKKYGLLSLEADGEQ-----------------IDNAFIQKGIRLMLSG-YDEDELREILTKDIETEVYELKKGAILLDKIGDF
Rosp  AANPRDLIAEITNLGDIVRKSGPLGLENVD-------------------ISDPVLAKGVQYIADG-YETEFIKDSMERERDLNLTRLSEGKRVMKALGDS
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Dede  SEDPSKLIEQLVNFAVRARRDGILALESAEGE-----------------ISDEFLKKGIRLAVDG-TEPEVIKSILETELSYMEERHKEGVGILESIASF
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Hche  LDKPEELIEQVVELADAARKGGLLSLEGKE-------------------IPNAFLSKGIQLLIDG-HDGDVVKALLTKDRNLTVDRHKQGSSVFGAMGDV
Clle  EIDPTEVISKINELALSARKEGLLALEEIAQG-----------------MDDPFLQKGILLIVDG-TDAELLRSILETEIAFVENRHKDNQKFWDSIAEL
Desa  VNNPQEVIAEIVNLAHVARKNGLIVLEQQP-------------------ITDPFLKKAIMYCVDG-HEAEFIEEVLQKEVELTQARHLVGNQVFNGMGTS
Bsel  ENDLEELIDTFVDLSTRARREGLLALEAGLED-----------------VDDPFIQKGVLLAVDG-IEPDIIKDIMMAEVVAMEERHRKGRSIVEKAGEY
Spea  LDKPEDLIEQSISMADAARKGGFLALEEAE-------------------ISNSFMQKAVDMLVDG-HDGDVVRDALEKDIALTEERHRTGIGIFKSIGDV
Niwa  TEKPEGIIKQAVEMAGTARKEGLLALEGWE-------------------IKNEFMKKGVTLLVDG-HDPEMVRRVLMTDLRQTLSRHELGQKIFKAIGDV
Nhal  AEKPEEIIKQALEMANTARKDGLLALESWD-------------------IKNEFMKKGVTLLVDG-HEPEMVRRVLMTELRQTLERHDLGQKIFRAIGDV
Ttur  LPDPNMLIDEIVALADEARKGGLLSLEGKE-------------------VSSDFLQRGIQLLVDG-HDPDVVKTLLSIDKNKAMERHTVGASIFAAMAEM
Coli  SSDPEELIEKIVELANIARKEGMLALENQE-------------------IDNDFLDEGVKMLIDG-NSREVVSTVLSKDMQQTIERHSWGAKVFSATADV
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Sacd  LPDPNALIEEVVALADEARKGGLLSLEGKE-------------------VSSDFLQRGIQLLVDG-HDPDVVKALLSKDKNQAVERHNVGATIFASMADM
Fbal  LDKPEELIDQSVTMADAARKGGFLALEEAE-------------------VNNSFMKKAVDLLVDG-HDAEVVRDALAKDIGLTAERHAAGIDIFRKLGEV
Shdy  KAMYMDLLALLYRLMAKSRQMGMFSLERDIENPRESEIFASYPRIIADSVMLDFIVDYLRLIISGHMNTFEIEALMDEEIEPHESEAEVPANSLALVGDS
Ecol  KAMYMDLLALLYRLMAKSRQMGMFSLERDIENPRESEIFASYPRILADSVMLDFIVDYLRLIISGHMNTFEIEALMDEEIETHESEAEVPANSLALVGDS
Cyou  KAMYMDLLALLYRLMAKSRQQGMFSLERDIENPKESEIFASYPRILADAVMLDFIVDYLRLIISGNMNTFEIEALMDEEIETHESEAEVPANSLAMVGDS
Ypes  KALYMDLMALLYRLLAKSRQQGMLSLERDIENPLESEIFSNYPRIMADKTLVEFITDYLRLIVSGNMNAFEIEALMDEEIETHEQESEVPAGSLAMVGDS
Saen  KTMYMDLLALLYRLMAKSRQQGMFSLERDIENPKESEIFASYPRILADAVMLDFIVDYLRLIISGNMNTFEIEALMDEEIETHESESEVPANSLAMVGDS
Ypse  KALYMDLMALLYRLLAKSRQQGMLSLERDIENPLESEIFSNYPRIMADKTLVEFITDYLRLIVSGNMNAFEIEALMDEEIETHEQESEVPAGSLAMVGDS
Ecol  KAMYMDLLALLYRLMAKSRQMGMFSLERDIENPRESEIFASYPRILADSVMLDFIVDYLRLIISGHMNTFEIEALMDEEIETHESEAEVPANSLALVGDS
Efer  KAMYMDLLALLYRLMAKSRQQGMFSLERDIENPQESEIFASYPRILADSVMLEFIVDYLRLIISGHMNSFEIEALMDEEIETHESEAEVPANSLAIVGDS
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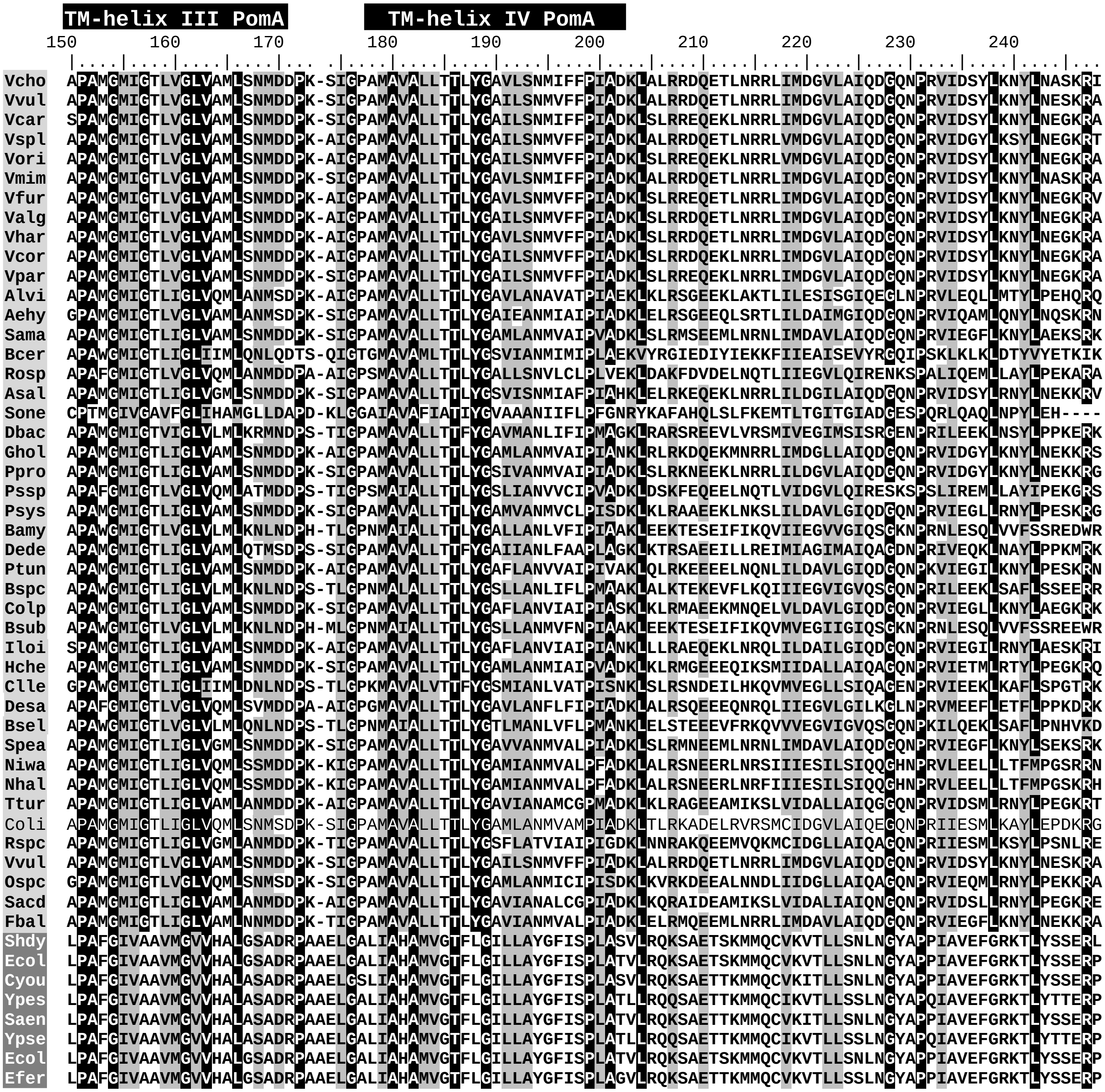

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150       160       170          180       190       200       210       220       230       240
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Vcho  APAMGMIGTLVGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAVLSNMIFFPIADKLALRRDQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNASKRI
Vvul  APAMGMIGTLVGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAILSNMVFFPIADKLALRRDQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNESKRA
Vcar  SPAMGMIGTLVGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAILSNMIFFPIADKLSLRREQEKLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNEGKRA
Vspl  APAMGMIGTLVGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAILSNMVFFPIADKLALRRDQETLNRRLVMDGVLAIQDGQNPRVIDGYLKSYLNEGKRT
Vori  APAMGMIGTLVGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAILSNMVFFPIADKLSLRREQEKLNRRLVMDGVLAIQDGQNPRVIDSYLKNYLNEGKRA
Vmim  APAMGMIGTLVGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAVLSNMIFFPIADKLALRRDQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNASKRA
Vfur  APAMGMIGTLVGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAVLSNMVFFPIADKLSLRREQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNEGKRV
Valg  APAMGMIGTLVGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAILSNMVFFPIADKLSLRRDQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNEGKRA
Vhar  APAMGMIGTLVGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAVLSNMVFFPIADKLSLRRDQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNEGKRA
Vcor  APAMGMIGTLVGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAILSNMVFFPIADKLSLRRDQEKLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNEGKRA
Vpar  APAMGMIGTLVGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAILSNMVFFPIADKLSLRREQEKLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNEGKRA
Alvi  APAMGMIGTLIGLVQMLANMSDPK-AIGPAMAVALLTTLYGAVLANAVATPIAEKLKLRSGEEKLAKTLILESISGIQEGLNPRVLEQLLMTYLPEHQRQ
Aehy  GPAMGMIGTLVGLVAMLANMSDPK-SIGPAMAVALLTTLYGAIEANMIAIPIADKLELRSGEEQLSRTLILDAIMGIQDGQNPRVIQAMLQNYLNQSKRN
Sama  APAMGMIGTLIGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAMLANMVAIPVADKLSLRMSEEMLNRNLIMDAVLAIQDGQNPRVIEGFLKNYLAEKSRK
Bcer  APAWGMIGTLIGLIIMLQNLQDTS-QIGTGMAVAMLTTLYGSVIANMIMIPLAEKVYRGIEDIYIEKKFIIEAISEVYRGQIPSKLKLKLDTYVYETKIK
Rosp  APAFGMIGTLVGLVQMLANMDDPA-AIGPSMAVALLTTLYGALLSNVLCLPLVEKLDAKFDVDELNQTLIIEGVLQIRENKSPALIQEMLLAYLPEKARA
Asal  APAMGMIGTLIGLVGMLSNMDDPK-SIGPAMAVALLTTLYGSVISNMIAFPIAHKLELRKEQEKLNRRLILDGILAIQDGQNPRVIDSYLRNYLNEKKRV
Sone  CPTMGIVGAVFGLIHAMGLLDAPD-KLGGAIAVAFIATIYGVAAANIIFLPFGNRYKAFAHQLSLFKEMTLTGITGIADGESPQRLQAQLNPYLEH----
Dbac  APAMGMIGTVIGLVLMLKRMNDPS-TIGPAMAVALLTTFYGAVMANLIFIPMAGKLRARSREEVLVRSMIVEGIMSISRGENPRILEEKLNSYLPPKERK
Ghol  APAMGMIGTLIGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAMLANMVAIPIANKLRLRKDQEKMNRRLIMDGLLAIQDGQNPRVIDGYLKNYLNEKKRS
Ppro  APAMGMIGTLIGLVAMLSNMDDPK-SIGPAMAVALLTTLYGSIVANMVAIPIADKLSLRKNEEKLNRRLILDGVLAIQDGQNPRVIDGYLKNYLNEKKRG
Pssp  APAFGMIGTLVGLVQMLATMDDPS-TIGPSMAIALLTTLYGSLIANVVCIPVADKLDSKFEQEELNQTLVIDGVLQIRESKSPSLIREMLLAYIPEKGRS
Psys  APAMGMIGTLIGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAMVANMVCLPISDKLKLRAAEEKLNKSLILDAVLGIQDGQNPRVIEGLLRNYLPESKRG
Bamy  APAWGMIGTLVGLVLMLKNLNDPH-TLGPNMAIALLTTLYGALLANLVFIPIAAKLEEKTESEIFIKQVIIEGVVGIQSGKNPRNLESQLVVFSSREDWR
Dede  APAMGMIGTLIGLVAMLQTMSDPS-SIGPAMAVALLTTFYGAIIANLFAAPLAGKLKTRSAEEILLREIMIAGIMAIQAGDNPRIVEQKLNAYLPPKMRK
Ptun  APAMGMIGTLIGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAFLANVVAIPIVAKLQLRKEEEELNQNLILDAVLGIQDGQNPKVIEGILKNYLPESKRN
Bspc  APAWGMIGTLIGLVLMLKNLNDPS-TLGPNMALALLTTLYGSLLANLIFLPMAAKLALKTEKEVFLKQIIIEGVIGVQSGQNPRILEEKLSAFLSSEERR
Colp  APAMGMIGTLIGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAFLANVIAIPIASKLKLRMAEEKMNQELVLDAVLGIQDGQNPRVIEGLLKNYLAEGKRK
Bsub  APAWGMIGTLVGLVLMLKNLNDPH-MLGPNMAIALLTTLYGSLLANMVFNPIAAKLEEKTESEIFIKQVMVEGIIGIQSGKNPRNLESQLVVFSSREEWR
Iloi  SPAMGMIGTLIGLVAMLSNMDDPK-AIGPAMAVALLTTLYGAFLANVIAIPIANKLLLRAEQEKLNRQLILDAILGIQDGQNPRVIEGILRNYLAESKRI
Hche  APAMGMIGTLIGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAMLANMIAIPVADKLKLRMGEEEQIKSMIIDALLAIQAGQNPRVIETMLRTYLPEGKRQ
Clle  GPAWGMIGTLIGLIIMLDNLNDPS-TLGPKMAVALVTTFYGSMIANLVATPISNKLSLRSNDEILHKQVMVEGLLSIQAGENPRVIEEKLKAFLSPGTRK
Desa  APAFGMIGTLVGLVQMLSVMDDPA-AIGPGMAVALLTTLYGAVLANFLFIPIADKLALRSQEEEQNRQLIIEGVLGILKGLNPRVMEEFLETFLPPKDRK
Bsel  APAWGMIGTLVGLVLMLQNLNDPS-TLGPNMAIALLTTLYGTLMANLVFLPMANKLELSTEEEVFRKQVVVEGVIGVQSGQNPKILQEKLSAFLPNHVKD
Spea  APAMGMIGTLIGLVGMLSNMDDPK-SIGPAMAVALLTTLYGAVVANMVALPIADKLSLRMNEEMLNRNLIMDAVLAIQDGQNPRVIEGFLKNYLSEKSRK
Niwa  APAMGMIGTLIGLVQMLSSMDDPK-KIGPAMAVALLTTLYGAMIANMVALPFADKLALRSNEERLNRSIIIESILSIQQGHNPRVLEELLLTFMPGSRRN
Nhal  APAMGMIGTLIGLVQMLSSMDDPK-KIGPAMAVALLTTLYGAMIANMVALPFADKLALRSNEERLNRFIIIESILSIQQGHNPRVLEELLLTFMPGSKRH
Ttur  APAMGMIGTLIGLVAMLANMDDPK-AIGPAMAVALLTTLYGAVIANAMCGPMADKLKLRAGEEAMIKSLVIDALLAIQGGQNPRVIDSMLRNYLPEGKRT
Coli  APAMGMIGTLIGLVQMLSNMSDPK-SIGPAMAVALLTTLYGAMLANMVAMPIADKLTLRKADELRVRSMCIDGVLAIQEGQNPRIIESMLKAYLEPDKRG
Rspc  APAMGMIGTLIGLVGMLANMDDPK-TIGPAMAVALLTTLYGSFLATVIAIPIGDKLNNRAKQEEMVQKMCIDGLLAIQAGQNPRIIESMLKSYLPSNLRE
Vvul  APAMGMIGTLVGLVAMLSNMDDPK-SIGPAMAVALLTTLYGAILSNMVFFPIADKLALRRDQETLNRRLIMDGVLAIQDGQNPRVIDSYLKNYLNESKRA
Ospc  GPAMGMIGTLVGLVQMLSNMSDPK-SIGPAMAVALLTTLYGAMLANMICIPISDKLKVRKDEEALNNDLIIDGLLAIQAGQNPRVIEQMLRNYLPEKKRA
Sacd  APAMGMIGTLIGLVAMLANMDDPK-AIGPAMAVALLTTLYGAVIANALCGPIADKLKQRAIDEAMIKSLVIDALIAIQNGQNPRVIDSLLRNYLPEGKRE
Fbal  APAMGMIGTLIGLVAMLNNMDDPK-TIGPAMAVALLTTLYGAVIANMVALPIADKLELRMQEEMLNRRLIMDAVLAIQDGQNPRVIEGFLKNYLNEKKRA
Shdy  LPAFGIVAAVMGVVHALGSADRPAAELGALIAHAMVGTFLGILLAYGFISPLASVLRQKSAETSKMMQCVKVTLLSNLNGYAPPIAVEFGRKTLYSSERL
Ecol  LPAFGIVAAVMGVVHALGSADRPAAELGALIAHAMVGTFLGILLAYGFISPLATVLRQKSAETSKMMQCVKVTLLSNLNGYAPPIAVEFGRKTLYSSERP
Cyou  LPAFGIVAAVMGVVHALASADRPAAELGSLIAHAMVGTFLGILLAYGFISPLASVLRQKSAETTKMMQCVKITLLSNLNGYAPPIAVEFGRKTLYSSERP
Ypes  LPAFGIVAAVMGVVHALASADRPAAELGALIAHAMVGTFLGILLAYGFISPLATLLRQQSAETTKMMQCIKVTLLSSLNGYAPQIAVEFGRKTLYTTERP
Saen  LPAFGIVAAVMGVVHALASADRPAAELGALIAHAMVGTFLGILLAYGFISPLATVLRQKSAETTKMMQCVKITLLSNLNGYAPPIAVEFGRKTLYSSERP
Ypse  LPAFGIVAAVMGVVHALASADRPAAELGALIAHAMVGTFLGILLAYGFISPLATLLRQQSAETTKMMQCIKVTLLSSLNGYAPQIAVEFGRKTLYTTERP
Ecol  LPAFGIVAAVMGVVHALGSADRPAAELGALIAHAMVGTFLGILLAYGFISPLATVLRQKSAETSKMMQCVKVTLLSNLNGYAPPIAVEFGRKTLYSSERP
Efer  LPAFGIVAAVMGVVHALGSADRPAAELGALIAHAMVGTFLGILLAYGFISPLAGVLRQKSAETTKMMQCVKVTLLSSLNGYAPPIAVEFGRKTLYSSERP
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     250
      .|....
Vcho  LDVDKE-------------------
Vvul  LDIND--------------------
Vcar  LDVDNE-------------------
Vspl  IDGEPA-------------------
Vori  LDVDNE-------------------
Vmim  LDVDKE-------------------
Vfur  LDVDNE-------------------
Valg  LEIDE--------------------
Vhar  LEIDE--------------------
Vcor  LDVDNE-------------------
Vpar  LDVDNE-------------------
Alvi  SSKD---------------------
Aehy  NGTE---------------------
Sama  IDTTDGE------------------
Bcer  KEKRAA-------------------
Rosp  EFAEAA-------------------
Asal  VDTGEE-------------------
Sone  -------------------------
Dbac  VRS----------------------
Ghol  VDVDG--------------------
Ppro  VDVDG--------------------
Pssp  DMIEAAA------------------
Psys  GGSTEDGAA----------------
Bamy  KG-QPSNKKKGAVHEA---------
Dede  SQFE---------------------
Ptun  VDTEG--------------------
Bspc  DLDKAVNEEEALDNEA---------
Colp  VDTTDE-------------------
Bsub  KQPNQVKTKKGSVHEA---------
Iloi  GNEDEDE------------------
Hche  TEEA---------------------
Clle  VFDVKNKDE----------------
Desa  NSMANR-------------------
Bsel  KKDEEPEETEENE------------
Spea  IDTLEGA------------------
Niwa  NGYGEKGR-----------------
Nhal  NNNGEK-------------------
Ttur  VEAAE--------------------
Coli  GDDG---------------------
Rspc  GA-----------------------
Vvul  LDIND--------------------
Ospc  AAEG---------------------
Sacd  VADAD--------------------
Fbal  IDTTED-------------------
Shdy  SFIELEEHVRAVKNPQQQTTTEEA-
Ecol  SFIELEEHVRAVKNPQQQTTTEEA-
Cyou  SFIELEEHVRAVRNPAAQQTTEDA-
Ypes  SFIELEEHVRRVKAPASQATEEDA-
Saen  SFIELEEHVRAVRNPNQQQTTEEA-
Ypse  SFIELEEHVRRVKAPASQATEEDA-
Ecol  SFIELEEHVRAVKNPQQQTTTEEA-
Efer  SFVELEEHVRAVKNPNQQATTEEV-
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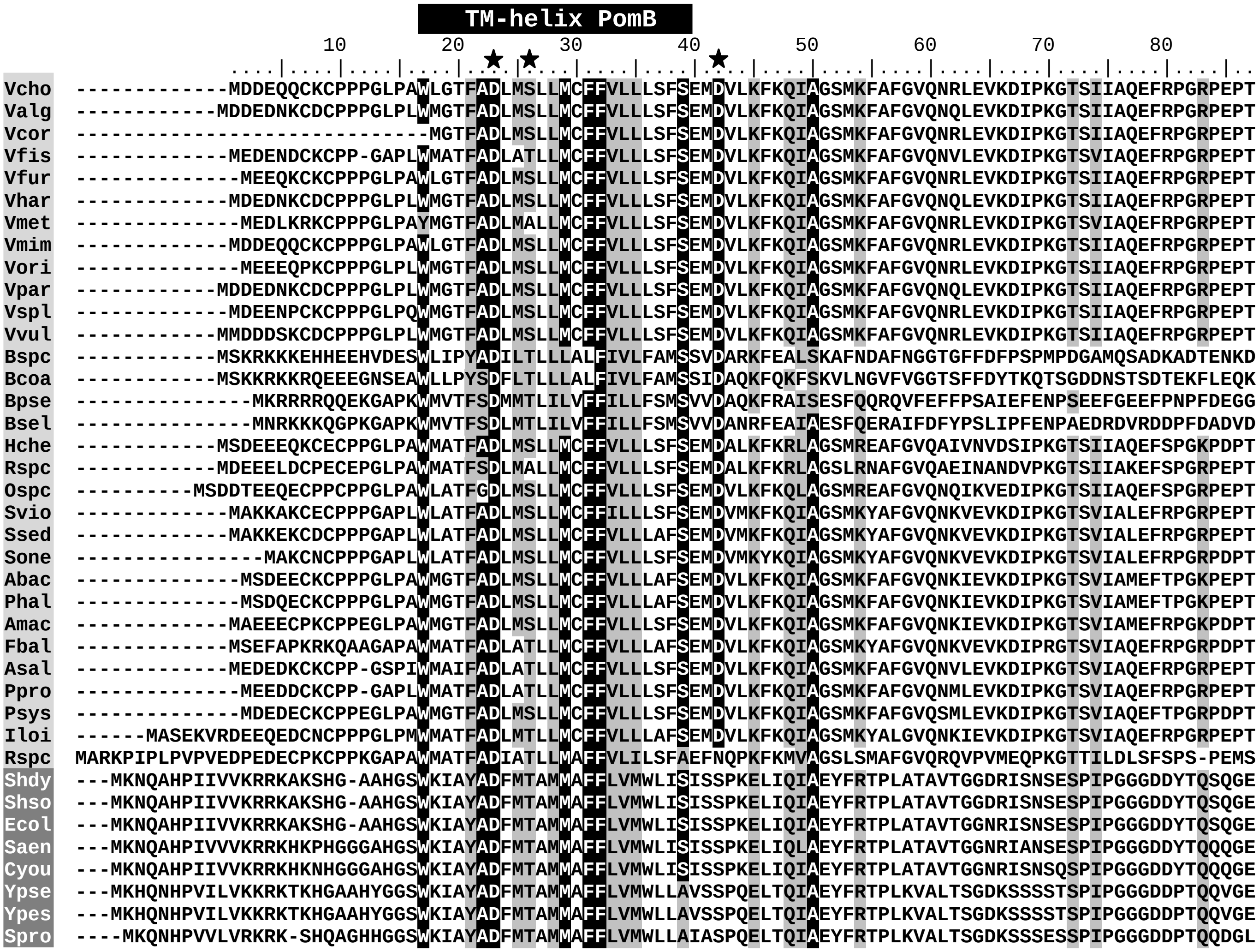
**Figure S6: Sequence alignment of PomA homologues.** Amino acid sequences of PomA homologues were obtained from NCBI BLAST®, algorithm blastp (protein-protein BLAST) with query sequence gi|51241595 PomA *V. cholerae*. A multiple sequence alignment of the 53 sequences was performed using the ClustalW algorithm (1) implemented in the BioEdit Sequence Alignment Editor (Version 7.0.5.3, (2)) with default settings. Identical and similar residues were identified with BioEdit using an identity and similarity threshold of 83% and BLOSUM62 (3) scoring matrix. Bacteria which are predicted to operate a $Na^+$-dependent flagellar motor are shaded in light grey, bacteria which are predicted to operate a $H^+$-dependent flagellar motor are shaded in dark grey. Vcho *Vibrio cholerae* (gi|51241595); Vvul *Vibrio vulnificus* (gi|27363787); Vcar *Vibrio caribbenthicus* (gi|312884944); Vspl *Vibrio splendidus* (gi|84394352); Vori *Vibrio orientalis* (gi|261252276); Vmim *Vibrio mimicus* (gi|258621631); Vfur *Vibrio furnissii* (gi|260767265); Valg *Vibrio alginolyticus* (gi|91225059); Vhar *Vibrio harveyi* (gi|153835782); Vcor *Vibrio coralliilyticus* (gi|260776527); Vpar *Vibrio parahaemolyticus* (gi|254509022); Alvi *Allochromatium vinosum* (gi|288942519); Aehy *Aeromonas hydrophila* (gi|117620588); Sama *Shewanella amazonensis* (gi|119775567); Bcer *Bacillus cereus* (gi|152975139); Rosp *Roseibium sp.* (gi|307944456); Asal *Aliivibrio salmonicida* (gi|209694504); Sone *Shewanella oneidensis* (gi|24375769); Dbac *Desulfomicrobium baculatum* (gi|256830502); Ghol *Grimontia hollisae* (gi|262274768); Ppro *Photobacterium profundum* (gi|90410907); Pssp *Pseudovibrio sp.* (gi|254473242); Psys *Psychromonas sp.* (gi|90408166); Bamy *Bacillus amyloiquefaciens* (gi|308174664); Dede *Deferribacter desulfuricans* (gi|291280095); Ptun *Pseudoalteromonas tunicata* (gi|88859187); Bspc *Bacillus sp.* (gi|319651876); Colp *Colwellia psychrerythraea* (gi|71279500); Bsub *Bacillus subtilis* (gi|296332049); Iloi *Idiomarina loihiensis* (gi|56461235); Hche *Hahella chejuensis* (gi|83648503); Clle *Clostridium lentocellum* (gi|296440180); Desa *Desulfurivibrio alkaliphilus* (gi|297569176); Bsel *Bacillus selenitrireducens* (gi|297583573); Spea *Shewanella pealeana* (gi|157962806); Niwa *Nitrosococcus watsoni* (gi|300114850); Nhal *Nitrosococcus halophilus* (gi|292492680); Ttur *Teredinibacter turnerae* (gi|254787124); Coli *Congregibacter litoralis* (gi|88704265); Rspc *Reinekea sp.* (gi|88799580); Ospc *Oceanospirillum sp.* (gi|89093475); Sacd *Saccharophagus degradans* (gi|90022861); Fbal *Ferrimonas balearica* (gi|308048594); Shdy *Shigella dysenteriae* (gi|82776485); Ecol *Escherichia coli* (gi|89108730); Nimo *Nitrococcus mobilis* (gi|88812419); Cyou *Citrobacter youngae* (gi|291085222); Ypes *Yersinia pestis* (gi|145599065); Saen *Salmonella enterica* (gi|161502955); Ypse *Yersinia pseudotuberculosis* (gi|51596730); Efer *Escherichia fergusonii* (gi|218548502).


TM-helix PomB
10 20 30 40 50 60 70 80
★ ★ ★
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Valg ------------MDDEDNKCDCPPPGLPLWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNQLEVKDIPKGTSIIAQEFRPGRPEPT
Vcor ------------------------------MGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSIIAQEFRPGRPEPT
Vfis -------------MEDENDCKCPP-GAPLWMATFADLATLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNVLEVKDIPKGTSVIAQEFRPGRPEPT
Vfur --------------MEEQKCKCPPPGLPAWLGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSIIAQEFRPGRPEPT
Vhar -------------MDEDNKCDCPPPGLPLWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNQLEVKDIPKGTSIIAQEFRPGRPEPT
Vmet --------------MEDLKRKCPPPGLPAYMGTFADLMALLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSVIAQEFRPGRPEPT
Vmim -------------MDDEQQCKCPPPGLPAWLGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSIIAQEFRPGRPEPT
Vori --------------MEEEQPKCPPPGLPLWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSIIAQEFRPGRPEPT
Vpar ------------MDDEDNKCDCPPPGLPLWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNQLEVKDIPKGTSIIAQEFRPGRPEPT
Vspl -------------MDEENPCKCPPPGLPQWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSIIAQEFRPGRPEPT
Vvul ------------MMDDDSKCDCPPPGLPLWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNRLEVKDIPKGTSIIAQEFRPGRPEPT
Bspc ------------MSKRKKKEHHEEHVDESWLIPYADILTLLLALFIVLFAMSSVDARKFEALSKAFNDAFNGGTGFFDFPSPMPDGAMQSADKADTENKD
Bcoa ------------MSKKRKKRQEEEGNSEAWLLPYSDFLTLLLALFIVLFAMSSIDAQKFQKFSKVLNGVFVGGTSFFDYTKQTSGDDNSTSDTEKFLEQK
Bpse ---------------MKRRRRQQEKGAPKWMVTFSDMMTLILVFFILLFSMSVVDAQKFRAISESFQQRQVFEFFPSAIEFENPSEEFGEEFPNPFDEGG
Bsel ---------------MNRKKKQGPKGAPKWMVTFSDLMTLILVFFILLFSMSVVDANRFEAIAESFQERAIFDFYPSLIPFENPAEDRDVRDDPFDADVD
Hche ------------MSDEEEQKCECPPGLPAWMATFADLMSLLMCFFVLLLSFSEMDALKFKRLAGSMREAFGVQAIVNVDSIPKGTSIIAQEFSPGKPDPT
Rspc ------------MDEEELDCPECEPGLPAWMATFSDLMALLMCFFVLLLSFSEMDALKFKRLAGSLRNAFGVQAEINANDVPKGTSIIAKEFSPGRPEPT
Ospc ----------MSDDTEEQECPPCPPGLPAWLATFGDLMSLLMCFFVLLLSFSEMDVLKFKQLAGSMREAFGVQNQIKVEDIPKGTSIIAQEFSPGRPEPT
Svio -------------MAKKAKCECPPPGAPLWLATFADLMSLLMCFFILLLSFSEMDVMKFKQIAGSMKYAFGVQNKVEVKDIPKGTSVIALEFRPGRPEPT
Ssed -------------MAKKEKCDCPPPGAPLWLATFADLMSLLMCFFVLLLAFSEMDVMKFKQIAGSMKYAFGVQNKVEVKDIPKGTSVIALEFRPGRPEPT
Sone ----------------MAKCNCPPPGAPLWLATFADLMSLLMCFFVLLLSFSEMDVMKYKQIAGSMKYAFGVQNKVEVKDIPKGTSVIALEFRPGRPDPT
Abac --------------MSDEECKCPPPGLPAWMGTFADLMSLLMCFFVLLLAFSEMDVLKFKQIAGSMKFAFGVQNKIEVKDIPKGTSVIAMEFTPGKPEPT
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Fbal -------------MSEFAPKRKQAAGAPAWMATFADLATLLMCFFVLLLAFSEMDVLKFKQIAGSMKYAFGVQNKVEVKDIPRGTSVIAQEFRPGRPDPT
Asal -------------MEDEDKCKCPP-GSPIWMAIFADLATLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNVLEVKDIPKGTSVIAQEFRPGRPEPT
Ppro --------------MEEDDCKCPP-GAPLWMATFADLATLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQNMLEVKDIPKGTSVIAQEFRPGRPEPT
Psys --------------MDEDECKCPPEGLPAWMGTFADLMSLLMCFFVLLLSFSEMDVLKFKQIAGSMKFAFGVQSMLEVKDIPKGTSVIAQEFTPGRPDPT
Iloi ------MASEKVRDEEQEDCNCPPPGLPMWMATFADLMTLLMCFFVLLLAFSEMDVLKFKQIAGSMKYALGVQNKIEVKDIPKGTSVIAQEFRPGRPEPT
Rspc MARKPIPLPVPVEDPEDECPKCPPKGAPAWMATFADIATLLMAFFVLILSFAEFNQPKFKMVAGSLSMAFGVQRQVPVMEQPKGTTILDLSFSPS-PEMS
Shdy ---MKNQAHPIIVVKRRKAKSHG-AAHGSWKIAYADFMTAMMAFFLVMWLISISSPKELIQIAEYFRTPLATAVTGGDRISNSESPIPGGGDDYTQSQGE
Shso ---MKNQAHPIIVVKRRKAKSHG-AAHGSWKIAYADFMTAMMAFFLVMWLISISSPKELIQIAEYFRTPLATAVTGGDRISNSESPIPGGGDDYTQSQGE
Ecol ---MKNQAHPIIVVKRRKAKSHG-AAHGSWKIAYADFMTAMMAFFLVMWLISISSPKELIQIAEYFRTPLATAVTGGNRISNSESPIPGGGDDYTQSQGE
Saen ---MKNQAHPIVVVKRRKHKPHGGGAHGSWKIAYADFMTAMMAFFLVMWLISISSPKELIQLAEYFRTPLATAVTGGNRIANSESPIPGGGDDYTQQQGE
Cyou ---MKNQAHPIIVVKRRKHKNHGGGAHGSWKIAYADFMTAMMAFFLVMWLISISSPKELIQIAEYFRTPLATAVTGGNRISNSQSPIPGGGDDYTQQQGE
Ypse ---MKHQNHPVILVKKRKTKHGAAHYGGSWKIAYADFMTAMMAFFLVMWLLAVSSPQELTQIAEYFRTPLKVALTSGDKSSSSTSPIPGGGDDPTQQVGE
Ypes ---MKHQNHPVILVKKRKTKHGAAHYGGSWKIAYADFMTAMMAFFLVMWLLAVSSPQELTQIAEYFRTPLKVALTSGDKSSSSTSPIPGGGDDPTQQVGE
Spro ----MKQNHPVVLVRKRK-SHQAGHHGGSWKIAYADFMTAMMAFFLVMWLLAIASPQELTQIAEYFRTPLKVALTSGDKSSSESSPIPGGGDDPTQQDGL

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         90       100       110       120       130       140
      ....|....|....|....|....|....|....|....|....|....|....|....|
Vcho  PIDVIMQQTIDITQQTLEFQEGESDRAGGNQRDSGQLTGGQSAETSVQDSQNTQSEMQQQ----------------------------------------
Valg  PIDVIMQQTMDITQQTLEFHEGESDRAGGTKRDEGKLTGGQSPETSTQNNESAEADMQQQ----------------------------------------
Vcor  PIDVIMQQTIDITQQTLEFHEGESDRAGGTQRDKGKQTGGKSPDTSTQDSQNSESDSEQQ----------------------------------------
Vfis  PIEVIMQQTIDIPQKTLDFHDGESDRAGGTKREAGKQTGGESPSNSTQTSAQS-------N---------------------------------------
Vfur  PIDVIMQQTMDITQQTLEFHEGDADRAGGTQRDAGQLTGGQSPETATQDNQNTETEMQQQQ---------------------------------------
Vhar  PIDVIMQQTMDITQQTLEFHEGESDRAGGTKRDQGKLTGGQSPETSTHNNQSAESDMQQQ----------------------------------------
Vmet  PIDVIMQQTIDITQQTLEFHEGDSDRAGGNQRDDGQLTGGESPETSTEDSQDNEDAEQEQE---------------------------------------
Vmim  PIDVIMQQTIDITQQTLDFQEGDSDRAGGNQRDSGKLTGGQSAESSTQDNQNTQSEMQQQ----------------------------------------
Vori  PIDVIMQQTIDITQQTLEFHEGESDRAGGTQRDQGKLTGGQSPDTSTQNNQNSESDSQQQ----------------------------------------
Vpar  PIDVIMQQTMDITQQTLEFHEGESDRAGGTKRDEGKLTGGQSPETSTQSNQSAESDMQQQ----------------------------------------
Vspl  PIDVIMQQTIDITQQTLEFHEGESERAGGTMRDQGKMTGGKSPEVSTHDNQNSESDQQQQ----------------------------------------
Vvul  PIDVIMQQTIDITQQTLEFHEGESDRAGGTQRDQGKMTGGQSPETSTQTNQNTEQQQQQQ----------------------------------------
Bspc  EVEKASAAGTLEEGQNDMEAEGKIQKLTDK----------------------------------------------------------------------
Bcoa  KNDAQKNGDSTTNGSKSNSTTDASNGQGD-----------------------------------------------------------------------
Bpse  FSEEGSNTD-------------------------------------------------------------------------------------------
Bsel  PFESHMEMD-------------------------------------------------------------------------------------------
Hche  PLQTIMQQTEMDLPN-LEQLCEQQVADALQEECPKVQGEELSDIVLEKIK--------------------------------------------------
Rspc  PLNVVRQDTVADLKDSLEILCQDTFTMQEEAQGDQGQ------MTRQVIISD------------------------------------------------
Ospc  PLNEVRQMTVNNDMNTLDIRSKEGDSDSPDQE-----------AGEQQELQK------------------------------------------------
Svio  PIEIINQQTNEMTEPILEYQAGDDDSAGGVQQQRGEQRGGEASATAQEQDEAKAESKAESESKAKA----------------------------------
Ssed  PIEIINQQTNEMTEPVLEYQAGDDDSSGGVQQQRGEQRGGEASATAQEEAESKSESKSEAEAKAKS----------------------------------
Sone  PIEIINQQTNEMTEPVLDLQAGEDDSSGGTQQQNGSQRGGEASATAQETEDAVK----------------------------------------------
Abac  PIETIQQQTVEMTQQMLEFQAGDESSAGGRQEQRGDKRGGESRSTSQEQ---------------------------------------------------
Phal  PIETIQQQTVEMTQQMLEFQAGDESSAGGRQEQRGDKRGGESRSTSKEQ---------------------------------------------------
Amac  PIESIQQQTNEMTQQMLEFQAGNEDSAGGRQKQRGEQRGGQSQQTATQS---------------------------------------------------
Fbal  PIEVIQQQTVEFTRDMLNYQPGESDQAGGQQNQSGNSRGGTSSQTANQPRAEQSN---------------------------------------------
Asal  PIEVIMQQTIDIPQKTLDFHDGESDRAGGSKREAGNLTGGESSSTSTQTNAQS-------D---------------------------------------
Ppro  PIEVIMQQTIDMTQRTLDFHDGESDRAGGTKRDAGKLTGGQSADVATQMNQNN-------Q---------------------------------------
Psys  PIEVIMQKTIEMTEAKLDFSEGDSSRADGEEDSKEDIPEEGNQGN-------------------------------------------------------
Iloi  PIETIQQQTVEMTQQMLEFQEGESDFAGGQQTERGGQESDSADDQKEAEAEN------------------------------------------------
Rspc  VTEQMTQDTTETEQPEIQRPVTEEDGKGAAEGEGADAPGQSEAREMAEALQEALQSGQVKVESREGEVVMTFDAPDAQSLPGQLTEAAEALQQAAEATGQ
Shdy  VNKQPNIEELKKRMEQSRLR--------------------------------------------------------------------------------
Shso  VNKQPNIEDLKKRMEQSRLR--------------------------------------------------------------------------------
Ecol  VNKQPNIEELKKRMEQSRLR--------------------------------------------------------------------------------
Saen  VEKQPNIDELKKRMEQSRLN--------------------------------------------------------------------------------
Cyou  VNKQPNIDDLRKRMEQSRLS--------------------------------------------------------------------------------
Ypse  VRKQINSEESRQ--EIHRLN--------------------------------------------------------------------------------
Ypes  VRKQINSEESRQ--EIHRLN--------------------------------------------------------------------------------
Spro  VKRQIDTLEKRD--EELRLN--------------------------------------------------------------------------------
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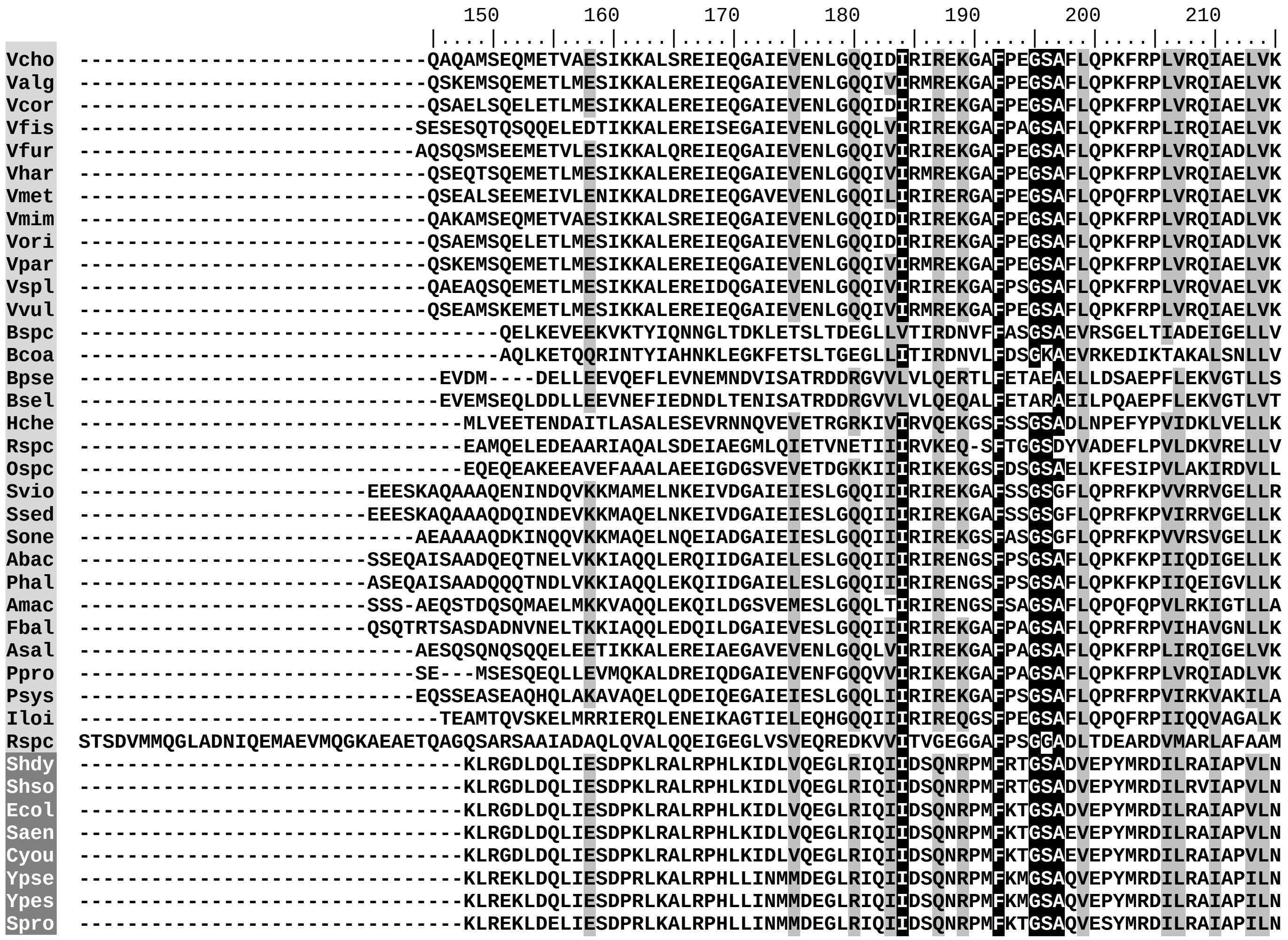

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                                      150       160       170       180       190       200       210
                                   |....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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Valg  -----------------------------QSKEMSQEMETLMESIKKALEREIEQGAIEVENLGQQIVIRMREKGAFPEGSAFLQPKFRPLVRQIAELVK
Vcor  -----------------------------QSAELSQELETLMESIKKALEREIEQGAIEVENLGQQIDIRIREKGAFPEGSAFLQPKFRPLVRQIAELVK
Vfis  ----------------------------SESESQTQSQQELEDTIKKALEREISEGAIEVENLGQQLVIRIREKGAFPAGSAFLQPKFRPLIRQIAELVK
Vfur  ----------------------------AQSQSMSEEMETVLESIKKALQREIEQGAIEVENLGQQIVIRIREKGAFPEGSAFLQPKFRPLVRQIADLVK
Vhar  -----------------------------QSEQTSQEMETLMESIKKALEREIEQGAIEVENLGQQIVIRMREKGAFPEGSAFLQPKFRPLVRQIAELVK
Vmet  -----------------------------QSEALSEEMEIVLENIKKALDREIEQGAVEVENLGQQILIRIRERGAFPEGSAFLQPQFRPLVRQIAELVK
Vmim  -----------------------------QAKAMSEQMETVAESIKKALSREIEQGAIEVENLGQQIDIRIREKGAFPEGSAFLQPKFRPLVRQIADLVK
Vori  -----------------------------QSAEMSQELETLMESIKKALEREIEQGAIEVENLGQQIDIRIREKGAFPEGSAFLQPKFRPLVRQIADLVK
Vpar  -----------------------------QSKEMSQEMETLMESIKKALEREIEQGAIEVENLGQQIVIRMREKGAFPEGSAFLQPKFRPLVRQIAELVK
Vspl  -----------------------------QAEAQSQEMETLMESIKKALEREIDQGAIEVENLGQQIVIRIREKGAFPSGSAFLQPKFRPLVRQVAELVK
Vvul  -----------------------------QSEAMSKEMETLMESIKKALEREIEQGAIEVENLGQQIVIRMREKGAFPEGSAFLQPKFRPLVRQIAELVK
Bspc  -----------------------------------QELKEVEEKVKTYIQNNGLTDKLETSLTDEGLLVTIRDNVFFASGSAEVRSGELTIADEIGELLV
Bcoa  -----------------------------------AQLKETQQRINTYIAHNKLEGKFETSLTGEGLLITIRDNVLFDSGKAEVRKEDIKTAKALSNLLV
Bpse  ------------------------------EVDM----DELLEEVQEFLEVNEMNDVISATRDDRGVVLVLQERTLFETAEAELLDSAEPFLEKVGTLLS
Bsel  ------------------------------EVEMSEQLDDLLEEVNEFIEDNDLTENISATRDDRGVVLVLQEQALFETARAEILPQAEPFLEKVGTLVT
Hche  --------------------------------MLVEETENDAITLASALESEVRNNQVEVETRGRKIVIRVQEKGSFSSGSADLNPEFYPVIDKLVELLK
Rspc  --------------------------------EAMQELEDEAARIAQALSDEIAEGMLQIETVNETIIIRVKEQ-SFTGGSDYVADEFLPVLDKVRELLV
Ospc  --------------------------------EQEQEAKEEAVEFAAALAEEIGDGSVEVETDGKKIIIRIKEKGSFDSGSAELKFESIPVLAKIRDVLL
Svio  ------------------------EEESKAQAAAQENINDQVKKMAMELNKEIVDGAIEIESLGQQIIIRIREKGAFSSGSGFLQPRFKPVVRRVGELLR
Ssed  ------------------------EEESKAQAAAQDQINDEVKKMAQELNKEIVDGAIEIESLGQQIIIRIREKGAFSSGSGFLQPRFKPVIRRVGELLK
Sone  ----------------------------AEAAAAQDKINQQVKKMAQELNQEIADGAIEIESLGQQIIIRIREKGSFASGSGFLQPRFKPVVRSVGELLK
Abac  ------------------------SSEQAISAADQEQTNELVKKIAQQLERQIIDGAIELESLGQQIIIRIRENGSFPSGSAFLQPKFKPIIQDIGELLK
Phal  ------------------------ASEQAISAADQQQTNDLVKKIAQQLEKQIIDGAIELESLGQQIIIRIRENGSFPSGSAFLQPKFKPIIQEIGVLLK
Amac  ------------------------SSS-AEQSTDQSQMAELMKKVAQQLEKQILDGSVEMESLGQQLTIRIRENGSFSAGSAFLQPQFQPVLRKIGTLLA
Fbal  ------------------------QSQTRTSASDADNVNELTKKIAQQLEDQILDGAIEVESLGQQIIIRIREKGAFPAGSAFLQPRFRPVIHAVGNLLK
Asal  ----------------------------AESQSQNQSQQELEETIKKALEREIAEGAVEVENLGQQLVIRIREKGAFPAGSAFLQPKFRPLIRQIGELVK
Ppro  ----------------------------SE---MSESQEQLLEVMQKALDREIQDGAIEVENFGQQVVIRIKEKGAFPAGSAFLQPKFRPLVRQIADLVK
Psys  ----------------------------EQSSEASEAQHQLAKAVAQELQDEIQEGAIEIESLGQQLIIRIREKGAFPSGSAFLQPRFRPVIRKVAKILA
Iloi  ------------------------------TEAMTQVSKELMRRIERQLENEIKAGTIELEQHGQQIIIRIREQGSFPEGSAFLQPQFRPIIQQVAGALK
Rspc  STSDVMMQGLADNIQEMAEVMQGKAEAETQAGQSARSAAIADAQLQVALQQEIGEGLVSVEQREDKVVITVGEGGAFPSGGADLTDEARDVMARLAFAAM
Shdy  --------------------------------KLRGDLDQLIESDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFRTGSADVEPYMRDILRAIAPVLN
Shso  --------------------------------KLRGDLDQLIESDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFRTGSADVEPYMRDILRVIAPVLN
Ecol  --------------------------------KLRGDLDQLIESDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFKTGSADVEPYMRDILRAIAPVLN
Saen  --------------------------------KLRGDLDQLIESDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFKTGSAEVEPYMRDILRAIAPVLN
Cyou  --------------------------------KLRGDLDQLIESDPKLRALRPHLKIDLVQEGLRIQIIDSQNRPMFKTGSAEVEPYMRDILRAIAPVLN
Ypse  --------------------------------KLREKLDQLIESDPRLKALRPHLLINMMDEGLRIQIIDSQNRPMFKMGSAQVEPYMRDILRAIAPILN
Ypes  --------------------------------KLREKLDQLIESDPRLKALRPHLLINMMDEGLRIQIIDSQNRPMFKMGSAQVEPYMRDILRAIAPILN
Spro  --------------------------------KLREKLDELIESDPRLKALRPHLLINMMDEGLRIQIIDSQNRPMFKTGSAQVESYMRDILRAIAPILN
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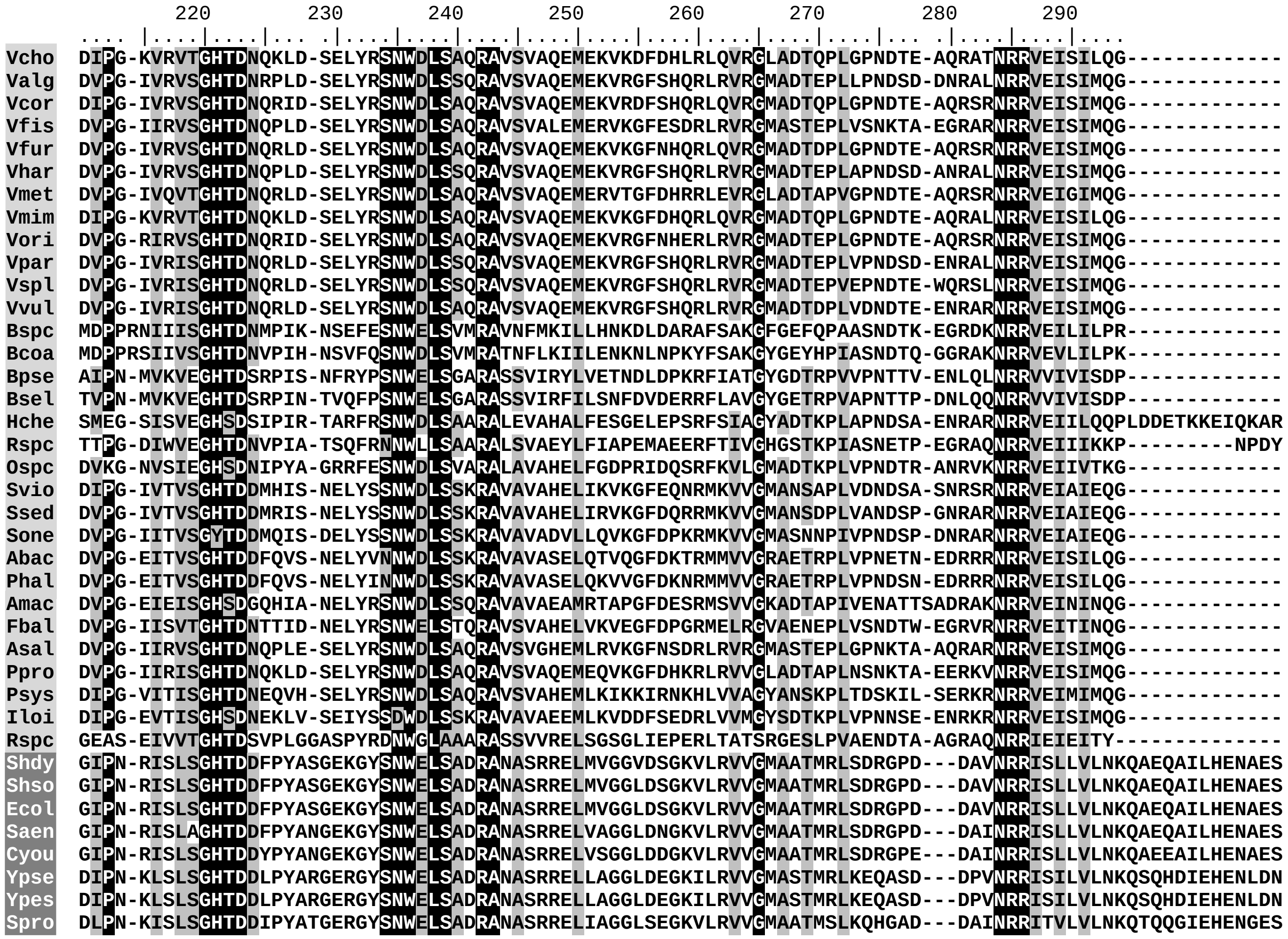

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              220       230      240       250       260       270       280       290
          .... |....|....|... .|....|....|....|....|....|....|....|....|... .|....|....|....
Vcho  DIPG-KVRVTGHTDNQKLD-SELYRSNWDLSAQRAVSVAQEMEKVKDFDHLRLQVRGLADTQPLGPNDTE-AQRATNRRVEISILQG-------------
Valg  DVPG-IVRVSGHTDNRPLD-SELYRSNWDLSSQRAVSVAQEMEKVRGFSHQRLRVRGMADTEPLLPNDSD-DNRALNRRVEISIMQG-------------
Vcor  DIPG-IVRVSGHTDNQRID-SELYRSNWDLSAQRAVSVAQEMEKVRDFSHQRLQVRGMADTQPLGPNDTE-AQRSRNRRVEISIMQG-------------
Vfis  DVPG-IIRVSGHTDNQPLD-SELYRSNWDLSAQRAVSVALEMERVKGFESDRLRVRGMASTEPLVSNKTA-EGRARNRRVEISIMQG-------------
Vfur  DVPG-IVRVSGHTDNQRLD-SELYRSNWDLSAQRAVSVAQEMEKVKGFNHQRLQVRGMADTDPLGPNDTE-AQRSRNRRVEISIMQG-------------
Vhar  DVPG-IVRVSGHTDNQPLD-SELYRSNWDLSSQRAVSVAQEMEKVRGFSHQRLRVRGMADTEPLAPNDSD-ANRALNRRVEISIMQG-------------
Vmet  DVPG-IVQVTGHTDNQRLD-SELYRSNWDLSAQRAVSVAQEMERVTGFDHRRLEVRGLADTAPVGPNDTE-AQRSRNRRVEIGIMQG-------------
Vmim  DIPG-KVRVTGHTDNQKLD-SELYRSNWDLSAQRAVSVAQEMEKVKGFDHQRLQVRGMADTQPLGPNDTE-AQRALNRRVEISILQG-------------
Vori  DVPG-RIRVSGHTDNQRID-SELYRSNWDLSAQRAVSVAQEMEKVRGFNHERLRVRGMADTEPLGPNDTE-AQRSRNRRVEISIMQG-------------
Vpar  DVPG-IVRISGHTDNQRLD-SELYRSNWDLSSQRAVSVAQEMEKVRGFSHQRLRVRGMADTEPLVPNDSD-ENRALNRRVEISIMQG-------------
Vspl  DVPG-IVRISGHTDNQRLD-SELYRSNWDLSSQRAVSVAQEMEKVRGFSHQRLRVRGMADTEPVEPNDTE-WQRSLNRRVEISIMQG-------------
Vvul  DVPG-IVRISGHTDNQRLD-SELYRSNWDLSAQRAVSVAQEMEKVRGFSHQRLRVRGMADTDPLVDNDTE-ENRARNRRVEISIMQG-------------
Bspc  MDPPRNIIISGHTDNMPIK-NSEFESNWELSVMRAVNFMKILLHNKDLDARAFSAKGFGEFQPAASNDTK-EGRDKNRRVEILILPR-------------
Bcoa  MDPPRSIIVSGHTDNVPIH-NSVFQSNWDLSVMRATNFLKIILENKNLNPKYFSAKGYGEYHPIASNDTQ-GGRAKNRRVEVLILPK-------------
Bpse  AIPN-MVKVEGHTDSRPIS-NFRYPSNWELSGARASSVIRYLVETNDLDPKRFIATGYGDTRPVVPNTTV-ENLQLNRRVVIVISDP-------------
Bsel  TVPN-MVKVEGHTDSRPIN-TVQFPSNWELSGARASSVIRFILSNFDVDERRFLAVGYGETRPVAPNTTP-DNLQQNRRVVIVISDP-------------
Hche  SMEG-SISVEGHSDSIPIR-TARFRSNWDLSAARALEVAHALFESGELEPSRFSIAGYADTKPLAPNDSA-ENRARNRRVEIILQQPLDDETKKEIQKAR
Rspc  TTPG-DIWVEGHTDNVPIA-TSQFRNNWLLSAARALSVAEYLFIAPEMAEERFTIVGHGSTKPIASNETP-EGRAQNRRVEIIIKKP---------NPDY
Ospc  DVKG-NVSIEGHSDNIPYA-GRRFESNWDLSVARALAVAHELFGDPRIDQSRFKVLGMADTKPLVPNDTR-ANRVKNRRVEIIVTKG-------------
Svio  DIPG-IVTVSGHTDDMHIS-NELYSSNWDLSSKRAVAVAHELIKVKGFEQNRMKVVGMANSAPLVDNDSA-SNRSRNRRVEIAIEQG-------------
Ssed  DVPG-IVTVSGHTDDMRIS-NELYSSNWDLSSKRAVAVAHELIRVKGFDQRRMKVVGMANSDPLVANDSP-GNRARNRRVEIAIEQG-------------
Sone  DVPG-IITVSGYTDDMQIS-DELYSSNWDLSSKRAVAVADVLLQVKGFDPKRMKVVGMASNNPIVPNDSP-DNRARNRRVEIAIEQG-------------
Abac  DVPG-EITVSGHTDDFQVS-NELYVNNWDLSSKRAVAVASELQTVQGFDKTRMMVVGRAETRPLVPNETN-EDRRRNRRVEISILQG-------------
Phal  DVPG-EITVSGHTDDFQVS-NELYINNWDLSSKRAVAVASELQKVVGFDKNRMMVVGRAETRPLVPNDSN-EDRRRNRRVEISILQG-------------
Amac  DVPG-EIEISGHSDGQHIA-NELYRSNWDLSSQRAVAVAEAMRTAPGFDESRMSVVGKADTAPIVENATTSADRAKNRRVEININQG-------------
Fbal  DVPG-IISVTGHTDNTTID-NELYRSNWELSTQRAVSVAHELVKVEGFDPGRMELRGVAENEPLVSNDTW-EGRVRNRRVEITINQG-------------
Asal  DVPG-IIRVSGHTDNQPLE-SELYRSNWDLSAQRAVSVGHEMLRVKGFNSDRLRVRGMASTEPLGPNKTA-AQRARNRRVEISIMQG-------------
Ppro  DVPG-IIRISGHTDNQKLD-SELYRSNWDLSAQRAVSVAQEMEQVKGFDHKRLRVVGLADTAPLNSNKTA-EERKVNRRVEISIMQG-------------
Psys  DIPG-VITISGHTDNEQVH-SELYRSNWDLSAQRAVSVAHEMLKIKKIRNKHLVVAGYANSKPLTDSKIL-SERKRNRRVEIMIMQG-------------
Iloi  DIPG-EVTISGHSDNEKLV-SEIYSSDWDLSSKRAVAVAEEMLKVDDFSEDRLVVMGYSDTKPLVPNNSE-ENRKRNRRVEISIMQG-------------
Rspc  GEAS-EIVVTGHTDSVPLGGASPYRDNWGLAAARASSVVRELSGSGLIEPERLTATSRGESLPVAENDTA-AGRAQNRRIEIEITY--------------
Shdy  GIPN-RISLSGHTDDFPYASGEKGYSNWELSADRANASRRELMVGGVDSGKVLRVVGMAATMRLSDRGPD---DAVNRRISLLVLNKQAEQAILHENAES
Shso  GIPN-RISLSGHTDDFPYASGEKGYSNWELSADRANASRRELMVGGLDSGKVLRVVGMAATMRLSDRGPD---DAVNRRISLLVLNKQAEQAILHENAES
Ecol  GIPN-RISLSGHTDDFPYASGEKGYSNWELSADRANASRRELMVGGLDSGKVLRVVGMAATMRLSDRGPD---DAVNRRISLLVLNKQAEQAILHENAES
Saen  GIPN-RISLAGHTDDFPYANGEKGYSNWELSADRANASRRELVAGGLDNGKVLRVVGMAATMRLSDRGPD---DAINRRISLLVLNKQAEQAILHENAES
Cyou  GIPN-RISLSGHTDDYPYANGEKGYSNWELSADRANASRRELVSGGLDDGKVLRVVGMAATMRLSDRGPE---DAINRRISLLVLNKQAEEAILHENAES
Ypse  DIPN-KLSLSGHTDDLPYARGERGYSNWELSADRANASRRELLAGGLDEGKILRVVGMASTMRLKEQASD---DPVNRRISILVLNKQSQHDIEHENLDN
Ypes  DIPN-KLSLSGHTDDLPYARGERGYSNWELSADRANASRRELLAGGLDEGKILRVVGMASTMRLKEQASD---DPVNRRISILVLNKQSQHDIEHENLDN
Spro  DLPN-KISLSGHTDDIPYATGERGYSNWELSADRANASRRELIAGGLSEGKVLRVVGMAATMSLKQHGAD---DAINRRITVLVLNKQTQQGIEHENGES
```

```
        300       310
          |....|....|....|
Vcho  ----DPVYSDEVPSLDGGQQ--------------------------------------------------------------------------------
Valg  ----EPLYSEEVPVIQ------------------------------------------------------------------------------------
Vcor  ----EPLYSDEVPSLPSTN---------------------------------------------------------------------------------
Vfis  ----KPHESDEVPSAQP-----------------------------------------------------------------------------------
Vfur  ----EPLYSDEVPSAQPTNAQ-------------------------------------------------------------------------------
Vhar  ----EPLYSEEVPVIQ------------------------------------------------------------------------------------
Vmet  ----KPLLSEEIPSLQ------------------------------------------------------------------------------------
Vmim  ----DPLYSDEVPSLGAPANQ-------------------------------------------------------------------------------
Vori  ----EALLSDEIPVNQ------------------------------------------------------------------------------------
Vpar  ----EPLYSEEVPVIQ------------------------------------------------------------------------------------
Vspl  ----EPLYSDEVPVIGQ-----------------------------------------------------------------------------------
Vvul  ----EPLYSDEIPAIPQDQ---------------------------------------------------------------------------------
Bspc  ----T-----------------------------------------------------------------------------------------------
Bcoa  ----NEKGNDW-----------------------------------------------------------------------------------------
Bpse  ----TYSEEDAF----------------------------------------------------------------------------------------
Bsel  ----SYDESELF----------------------------------------------------------------------------------------
Hche  DVAPGILPSDQSEEWQG------LAPDEIF----------------------------------------------------------------------
Rspc  VTPPDEIDEEPETVDPGDPSLFGLDPEEIF----------------------------------------------------------------------
Ospc  KDKESLEKIQARKEGDPDGQLINLQPDDIF----------------------------------------------------------------------
Svio  ----KPKYSDEILVGQ------------------------------------------------------------------------------------
Ssed  ----KPKESDEILVGQ------------------------------------------------------------------------------------
Sone  ----KAKESEEIQVDKPN----------------------------------------------------------------------------------
Abac  ----KAKESDPIDVR-------------------------------------------------------------------------------------
Phal  ----KAKESDPIDVR-------------------------------------------------------------------------------------
Amac  ----KPMISKPISVVDE-----------------------------------------------------------------------------------
Fbal  ----KAKESEEIRVLP------------------------------------------------------------------------------------
Asal  ----KPYESDEVPVAKN-----------------------------------------------------------------------------------
Ppro  ----KPHYSDEVTSADIQKSAE------------------------------------------------------------------------------
Psys  ----EATESGEIGIDK------------------------------------------------------------------------------------
Iloi  ----KAAESDPVDVID------------------------------------------------------------------------------------
Rspc  ----------------------------------------------------------------------------------------------------
Shdy  QNEPVSALEKPEVAPQVSVPTMPSAEPR------------------------------------------------------------------------
Shso  QNEPVSALEKPEVAPQVSVPTMPSAEPR------------------------------------------------------------------------
Ecol  QNEPVSALEKPEVAPQVSVPTMPSAEPR------------------------------------------------------------------------
Saen  QNEPVSVLQQPAAVPPASVPTSPQAEPR------------------------------------------------------------------------
Cyou  QNEPVSVLQRPAELPPVSVPTSPPANPR------------------------------------------------------------------------
Ypse  RALDIEKATGLKQIDTHGTVPVAAVTPSSAITQSSAITQSSAAIPSPAVIPSSVTTQSATTIRSATTIRSATTIQATATTQAVTTTRSATTTQASAVALS
Ypes  RALDIEKATGLKQIDTHGTVPVAAVTPSSAVTQSSAITQSSAITQSSAITPSSAAIPSPAVIPSS------------------VTTQSATTTQASAVALS
Spro  NSIEVAQPVELKSLAPAATAPATLIAPEQPAADPVPTNSDSQQR--------------------------------------------------------
```

```
             510       520       530       540       550       560       570
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|..
Vcho  ------------------------------------------------------------------------
Valg  ------------------------------------------------------------------------
Vcor  ------------------------------------------------------------------------
Vfis  ------------------------------------------------------------------------
Vfur  ------------------------------------------------------------------------
Vhar  ------------------------------------------------------------------------
Vmet  ------------------------------------------------------------------------
Vmim  ------------------------------------------------------------------------
Vori  ------------------------------------------------------------------------
Vpar  ------------------------------------------------------------------------
Vspl  ------------------------------------------------------------------------
Vvul  ------------------------------------------------------------------------
Bspc  ------------------------------------------------------------------------
Bcoa  ------------------------------------------------------------------------
Bpse  ------------------------------------------------------------------------
Bsel  ------------------------------------------------------------------------
Hche  ------------------------------------------------------------------------
Rspc  ------------------------------------------------------------------------
Ospc  ------------------------------------------------------------------------
Svio  ------------------------------------------------------------------------
Ssed  ------------------------------------------------------------------------
Sone  ------------------------------------------------------------------------
Abac  ------------------------------------------------------------------------
Phal  ------------------------------------------------------------------------
Amac  ------------------------------------------------------------------------
Fbal  ------------------------------------------------------------------------
Asal  ------------------------------------------------------------------------
Ppro  ------------------------------------------------------------------------
Psys  ------------------------------------------------------------------------
Iloi  ------------------------------------------------------------------------
Rspc  ------------------------------------------------------------------------
Shdy  ------------------------------------------------------------------------
Shso  ------------------------------------------------------------------------
Ecol  ------------------------------------------------------------------------
Saen  ------------------------------------------------------------------------
Cyou  ------------------------------------------------------------------------
Ypse  SAGVLPSDVTLPGTVALPAAEPVNTQPQPMSTTETQQSSTGNITSTANGPTTSLPAAPASNIPVSPTSRDAQ
Ypes  SAGVLPSDVTLPGTVALPAAEPVNMQPQPMSTTETQQSSTGNITSTANGPTTSLPAAPASNIPVSPTSRDAQ
Spro  ------------------------------------------------------------------------
```

**Figure S7: Sequence alignment of PomB homologues.** Amino acid sequences of PomB homologues were obtained from NCBI BLAST®, algorithm blastp (protein-protein BLAST) with query sequence gi|51241597 PomB *V. cholerae*. A multiple sequence alignment of the 41 sequences was performed using the ClustalW algorithm (1) implemented in the BioEdit Sequence Alignment Editor (Version 7.0.5.3, (2)) with default settings. Identical and similar residues were identified with BioEdit using an identity and similarity threshold of 83% and BLOSUM62 (3) scoring matrix. Bacteria which are predicted to operate a $Na^+$-dependent flagellar motor are shaded in light grey, bacteria which are predicted to operate a $H^+$-dependent flagellar motor are shaded in dark grey. Amino acids which were investigated in this work are highlighted with stars. Vcho *Vibrio cholerae* (gi|254225118); Valg *Vibrio alginolyticus* (gi|269965067); Vcor *Vibrio coralliilyticus* (gi|260776526); Vfis *Vibrio fischeri* (gi|59711322); Vfur *Vibrio furnissii* (gi|260767264); Vhar *Vibrio harveyi* (gi|156973487); Vmet *Vibrio metschnikovii* (gi|260773298); Vmim *Vibrio mimicus* (gi|258624381); Vori *Vibrio orientalis* (gi|261252277); Vpar *Vibrio parahaemolyticus* (gi|4322001); Vspl *Vibrio splendidus* (gi|84394351); Vvul *Vibrio vulnificus* (gi|37679056); Bspc *Bacillus sp.* (gi|89100891); Bcoa *Bacillus coagulans* (gi|229543005); Bpse *Bacillus* pseudofirmus (gi|288553786); Bsel *Bacillus selenitrireducens* (gi|297583574); Hche *Hahella chejuensis* (gi|83648502); Rspc *Reinekea sp.* (gi|88799579); Ospc *Oceanospirillum sp.* (gi|89093474); Svio *Shewanella violacea* (gi|294139895); Ssed *Shewanella sediminis* (gi|157376457); Sone *Shewanella oneidensis* (gi|24347292); Abac *Alteromonadales bacterium* (gi|119472095); Phal *Pseudoalteromonas haloplanktis* gi|77361278; Amac *Alteromonas macleodii* gi|196156245; Fbal *Ferrimonas balearica* (gi|308048595); Asal *Aliivibrio salmonicida* (gi|209694505); Ppro *Photobacterium profundum* (gi|90410908); Psys *Psychromonas sp.* (gi|90408165); Iloi *Idiomarina loihiensis* (gi|56461234); Rspc *Roseovarius sp.* (gi|149201621); Shdy *Shigella dysenteriae* (gi|194434087); Shso *Shigella sonnei* (gi|74311761); Ecol *Escherichia coli* (gi|293415204); Saen *Salmonella enterica* (gi|161502956); Cyou *Citrobacter youngae* (gi|237731926); Ypse *Yersinia pseudotuberculosis* (gi|170023989); Ypes *Yersinia pestis* (gi|45441604); Spro *Serratio proteamaculans* (gi|157371226).

## References

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2. **Hall TA.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. **41:**95-98.
3. **Henikoff S, Henikoff JG.** 1992. Amino acid substitution matrices from protein blocks. Proceedings of the National Academy of Sciences **89:**10915-10919.