SUPPLEMENTARY INFORMATION

High-resolution crystal structure of the eukaryotic HMP-P synthase (THIC) from *Arabidopsis thaliana*.

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Supplementary materiels and methods:

HPLC conditions for THIC activity assays:

An Agilent 1260 HPLC was used for detection and separation of the reaction intermediates and products employing a LC-18 column (Supelcosil[TM], 25 cm x 10 mm, 5 µm). The buffers employed were Buffer A, water; Buffer B, 100 mM potassium phosphate, pH 6.6; Buffer C, methanol and were used under the following gradient: 0 min - 100% buffer B; 7 mins - 10% buffer A, 90% buffer B; 12 min - 25% buffer A, 60% buffer B, 15% buffer C; 17 min - 25% buffer A, 10% buffer B, 65% buffer C; 19 min - 100% buffer B, 29 min - 100% buffer A.

Liquid Chromatography Mass Spectrometry (LC-MS) parameters for 5'-deoxyadenosine and HMP-P characterization:

Liquid Chromatography - ElectroSpray Ionization - Time Of Flight - Mass Spectrometry (LC-ESI-TOF-MS) analysis was performed using an Agilent 1260 HPLC system equipped with a binary pump, an autosampler and a 1200 diode array detector upstream of a MicroToF-Q II mass spectrometer (Bruker Daltonics) using the ESI source in positive ionization mode.

The buffers used were Buffer A, 20 mM *N,N*-dimethylhexylamine and 10 mM ammonium acetate, pH 6.4; Buffer B, 75% methanol and 25% water. The column used was an Agilent Poroshell 120 LC18-T (Supelcosil – 15 cm x 3 mm, 3 µm). The LC gradients used for the purification were the following: 0 min - 100% buffer A; 5 min - 100% buffer A; 15 min - 60% buffer A, 40% buffer B; 27 min - 10% buffer A, 90% buffer B; 36 min - 100% buffer A. The following parameters were used for the mass spectrometry measurements: Capillary, -4500 V; Capillary offset, -500 V; Nebulizer gas, 3.0 bar; Dry gas, 10.0 L/min; Dry gas temperature, 200^{o}C; Funnel 1 RF, 200.0 Vpp; Funnel 2 RF, 250.0 Vpp; ISCID, 0.0 eV; Hexapole RF, 150 Vpp; Quadrapole, Ion energy, 5.0 eV; Low mass, 80 *m/z*; Collision cell, collision energy, 10.0 eV; Collision RF, 150.0 Vpp, Transfer time, 100.0 µs; Prepulse storage, 5.0 µs. Data was processed with DataAnalysis ver. 4.0 SP4 (Bruker Daltonics).

Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) quantitation for magnesium, iron, nickel and zinc:
The concentrations of the elements Mg, Fe, Ni and Zn were determined on a 7700x inductively coupled plasma mass spectrometer (Agilent Technologies, France) in no gas, helium and/or high-energy helium mode(s). The purified protein samples at 0.3 mM concentration in 20 mM Hepes, pH 7.5, containing 150 mM NaCl, 1 mM β-mercaptoethanol and 10% (v/v) glycerol) were diluted 100 times in 2% suprapur nitric acid (VWR, Switzerland). Internal standardization was used to overcome concentration or matrix effects. External calibration was applied using standard solutions of single elements ranging from 1 to 500 µg/L (SCP Science, France). The protein sample was analyzed four times. The accuracy of the method was determined on the basis of the mean values obtained on certified reference materials SRM 1643e and LPCS-01R-5 (TechLab, France). The limits of detection (LOD) were calculated as three standard deviations added to the mean value of the background signal obtained on 10 Blank samples. The limits of quantitation (LOQ) were calculated by multiplying the LOD by a factor of 3.3.

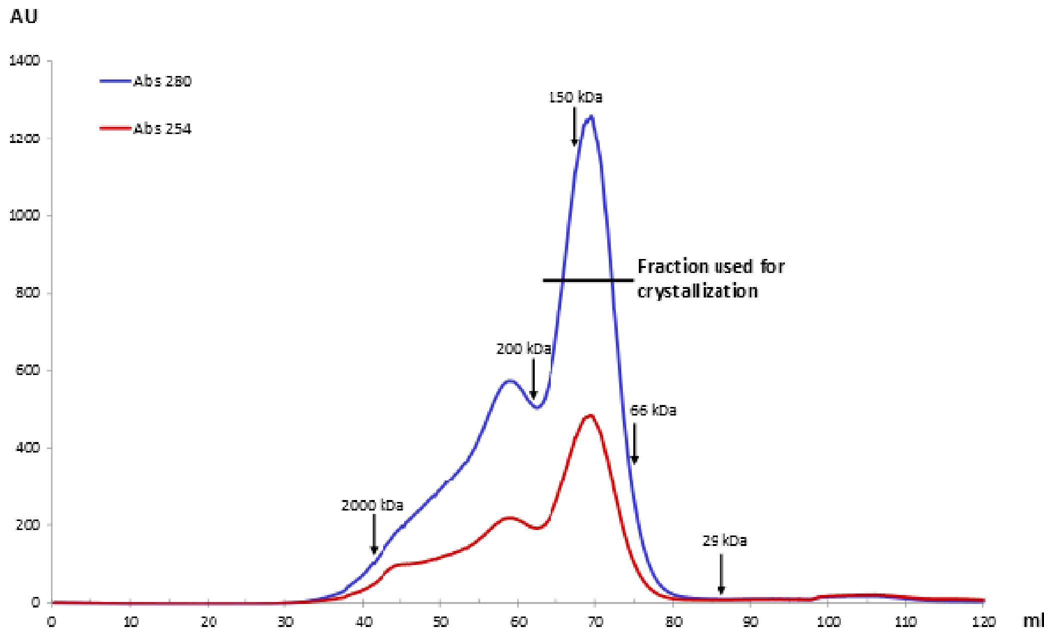
Supplementary Figure Legends:

Supplementary Figure 1: Purified ΔN71-AtTHIC is dimeric as determined by size exclusion chromatography. The purified protein was separated on a Superdex 200 column (GE-Healthcare) equilibrated in 20 mM Hepes, pH 7.5, containing 150 mM NaCl, 1 mM β-mercaptoethanol and 10% (v/v) glycerol. The blue trace represents the absorbance at 280 nm (protein) and the one in red the absorbance at 254 nm (nucleic acid). Protein molecular mass standards are indicated with arrows to illustrate the approximate mass of the ΔN71-AtTHIC-containing peak. The protein standards used were carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), β-amylase (200 kDa) and blue dextran (2000 kDa).

Supplementary Figure 2: Sequence alignment of THIC proteins from various species. The secondary structure of the *A. thaliana* THIC structure is indicated above the alignment (α-helices are shown as springs and β-sheets as arrows). Fully conserved residues are highlighted in red while almost conserved residues (50-95%) are colored pink. Green stars show the residues responsible for the association with the substrate AIR, the blue stars for the metal ion and the purple stars for the iron-sulfur cluster binding residues. Sequences are as follows: *Acinetobacter baumannii*: ref YP_001708358.1; *Agrobacterium tumefaciens*: gb EHJ96995.1; *Arabidopsis thaliana*: gb AEC08283.1; *Bacillus subtilis*: ref YP_004206889.1; *Burkholderia sp.*: ref YP_004228844.1; *Caulobacter crescentus*: gb ACL95574.1; *Escherichia coli*: ref YP_002410258.1; *Flavobacteriia bacterium*: emb CCG00800.1; *Haliangium ochraceum*: ref YP_003269601.1; *Halomonas boliviensis*: ref ZP_09186661.1; *Leptospira* *licerasiae*: ref ZP_09260399.1; *Lotus japonicus*: gb AFK38853.1; *Oryza sativa*: ref NP_001050897.1; *Phaeodactylum tricornutum*: ref XP_002182146.1; *Physcomitrella patens*: ref XP_001784371.1; *Populus trichocarpa*: ref XP_002314121.1; *Pseudomonas stutzeri*: ref YP_001174281.1; *Saccharomonospora viridis*: ref YP_003135247.1; *Salmonella enterica*: ref ZP_04657579.1; *Thellungiella halophila*: dbj BAJ34226.1; *Verrucomicrobium spinosum*: ref ZP_02928035.1; *Volvox carteri*: ref XP_002953184.1; *Zea mays*: ref NP_001151805.1.

Supplementary Table 1: Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) quantitation for magnesium, iron, nickel and zinc. Protein concentration was at 0.3 mM prior to its dilution for analysis. The buffer composition was 20 mM Hepes, pH 7.5, containing 150 mM NaCl, 1 mM β-mercaptoethanol and 10% (v/v) glycerol. Both samples were analyzed as indicated in the Materiel and Method section.

Supplementary Figure 1.



Supplementary Figure 2.

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A.thaliana
                  1         10         20         30         40         50         60
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T.halophila       ...MASVHSTLMSVVCNNKNHSAWPKLPNSSLLPGFDVSVVQAAAVRSKKETTS..TRATLTFDPPTTNS
P.trichocarpa     ..................................................MASG..ARATLTFDPPT...
O.sativa          ...MAALQPSFSSAMAMKS.....LKLPKTAVLPGFGGIARPQDVQDRSANFTCSRPRAASVTDQSTAEP
Z.mays            ...MAALQPSFSPAMTLKSSCSA.LKFPKTALLSGFGGIPRLQDAQDRNTSVAYLIPKAASVTDQSIAEP
L.japonicus       ......................................................................
P.patens          .........................................................MTATAPRPSSSSV
P.tricornutum     ...MAGCHS.....................SMVNAFRSVSRSLSIPSRLAVLKD....PQDLVTKEANVK
V.spinosum        ...............................MIATKNS.....FEPHSS...EQLPNSTRVYVEGQLHPD
B.subtilis        .................................MQNNS.....VQQANISIMSSFSGSKKVYVEGSSS.D
H.boliviensis     ............................MTKSVNNRTSHFLAETAQVDAAAIAPLPGSRKVYIEGS.RPD
Burkholderia.sp.  .................................MNANPKFLSADAHVDEAAVAPLPNSRKVYVTGS.RPD
P.stutzeri        ................................MSVQSNKNLSESAQVDQQSIQPFPRSQKIYVQGS.RPD
A.baumannii       ............................MNQLTNLSSAEISAQHEQDAKDLTRILPASKKVYIEGS.RPD
E.coli            ............................MSATKLTRREQRTQAQHFIDTLEGTAFPNSKRIYITGT.HPG
S.enterica        ............................MSTTTLTRREQRAKAQHFIDTLEGTAFPNSKRIYVTGS.QHD
F.bacterium       ............................MKTEKIPQQQ..........TISRSPFPNSEKIYVSGTLYPD
A.tumefaciens     ................................MNIVA......KNIPLVVTTGPHPASTKIHKTGILHPH
C.crescentus      ................................MNIQST....IKAVAETISTGPIPGSRKVYQAGELFPE
S.viridis         ................................MHGADR...ETSDVKPTVTTGPITGSRKVYLETE..SG
H.ochraceum       ...............MSTGNGAHGNGANGNGANDKHSLAQVGPAPVDGLTPIAGFPSSEKVYLERD...G
L.licerasiae      .............................................................MESNQTTTP
V.carteri         MQLCTRVRDSLTARPAGKARTAAVRSRSIILRVRAEAVSAPPAPAESDADAASEFARLQKGNAFEELKAL

                                                  α1        β1            β2
A.thaliana                                                                           T.T
                     70        80                 90        100        110       120
A.thaliana        ERAKQRKHTIDPSSP........DFQPIP..SFEECFPKSTKEHKEVVHEESGHVLKVPFRRVHLSG.GE
T.halophila       ERAKQRKHTIDPSSP........DFQPIP..SFEECFPKSTKEHSEIVHEETGHVLKVPFRRVHLSG.GE
P.trichocarpa     .RTNQKKHTVDPSSP........DFLPLP..SFEQCFPKSTKEYREVKHEESGHVLKVPFRRVHLSG.DE
O.sativa          AKPRQNKHTVDPSAP........EFLPLP..SFEECFPRSTKESREIVHEESGHVLKIPFRRIHLTG.DE
Z.mays            PKTKQNRHTVDPTAP........EFLPLP..SFEECFPRSTKESSEIIHEESGHVLKVPFRRVHLTG.DQ
L.japonicus       ......................................................................
P.patens          HSAGRTRPTKDPAAP........DFKPIP..SFEECFPSSTKEIKEVVHEPTGSVLQVPFRRIHLSG.DD
P.tricornutum     PAGTRTKPTVDPFNP........NFESIASVPYNTAFPSSTKEYKTVVHEATGHRLHVPFRRVHLEDPDQ
V.spinosum        VRVPMREIAVSPTKT....FSGKIEENAAVRVYDCSGPWGDPAFTGTAEEG.LPALRAKWIADRQDVEEY
B.subtilis        IQVPMREIALSPTTG....SFGE.EENAPVRVYDTSGPYTDPEVTINIQEG.LKPLRQKWITERGDVEEY
H.boliviensis     IRVPFREISLSPTKT.....TGIDEENPPLLVYDTSGPYTDPDAINDLRKG.LPALRRAWIEERDDTEFL
Burkholderia.sp.  IRVPMREITQADTPD.....SFGGEKNPPVYVYDTSGPYSDPDAKIDIRAG.LPALRQRWIEERGDTEAL
P.stutzeri        IRVPMREISLDVTPT.....DFGGEINAPVTVYDTSGPYTDPNVTIDVRKG.LADVRSAWIEDRGDTEKL
A.baumannii       IQVPMREISLTDTPT.....GLGGEHNPPIMVYDTSGVYTDPNVQIDLNKG.LPSVRQKWIEERNDTDVL
E.coli            VRVPMREIQLSPTLIGGSKEQPQFEENEAIPVYDTSGPYGDPQIAINVQQG.LAKLRQPWIDARGDTEEL
S.enterica        IRVPMREIQLSPTLIGGSKDNPQFEENEAVPVYDTSGPYGDPEVAINVQLG.LAKLRQPWIDARNDSEEL
F.bacterium       IKVPMRKINLADTVD...KFNGKVEKNEPVLVYDTSGPFTDAKISIDVKKG.LHPVRKQWIIDRKDTIQL
A.tumefaciens     IRVPMREIAVHPTAG...........EPPVTVYDSSGPYTDPLHNVLIEKG.LPRLRHDWVVARGDVAAY
C.crescentus      LRVPFREVAVHPSAN...........EPPVTIYDPSGPYSDPAIQIDIEKG.LPRTREALVVARGDVEEV
S.viridis         LRVPVRRIELSNGEH..............FDVYDTSGPYTDPQATIDVHKG.LPKLRAPWIEGR......
H.ochraceum       VRVPVRRIELSGGEP.............ALDVYDTSGPE.....NCDLHRG.LPKLRQPWIDAR......
L.licerasiae      FEIPRKEIRLSNGSV..............YNAYTTEGPWKDGWNPSDWKAG.IPKLRAEWIQRRVSG...
V.carteri         ASSPRQSVNRPQKND........DLTFRQAPTIEDCFPDSEKMYKAVQYDD.EVTLQVPFRRIHLSN..G

                    β3                                    α2                 α3
A.thaliana
                    130                    140       150          160       170
A.thaliana        PAFDNYDTSG.............PQNVNAHIGLAKLRKEWIDRREKL...GTPRYTQMYYAKQGIITEEM
T.halophila       PAFDAYDTSG.............PQNVNPHVGLAKLRKEWIDRREKL...GTPRCTQMYYAKQGIITEEM
P.trichocarpa     PGFDNYDTSG.............PQNISPRVGLPKLRKEWVDRREKL...GTPRYTQMYYAKQGIITEEM
O.sativa          KHFDTYDTSG.............PQNISPRIGLPKIRKEWIDRREKL...GGPRYTQMYYAKQGIITEEM
Z.mays            KHFDTYDTSG.............PQNISPRIGLPKIRKEWIDRREKL...GSPRYTQMYYAKQGIVTEEM
L.japonicus       ......................................................................
P.patens          PHFDTYDTSG.............PQKVNPRYGLPKLRKEWIDRREAL...KDERFTQMYYAKKGIITEEM
P.tricornutum     LYLDLYDTSG.............PQGVDPKKGLAKLRQEWTDEREGK....YERYTQMHFAKQGIVTEEM
V.spinosum        DG.REVKPQDNGYLSGKHAEYASKAERNRLIEFPGLHGERRKPLRAS...AGHPVTQLWYARQGIITPEM
B.subtilis        EG.RAIKPEDNGYK.........KAKPN..VSYPGLK...RKPLRAK...AGQNVTQMHYAKKGIITPEM
H.boliviensis     EG.HTSEYGK.............RRANDPTLAQLRFD.LTRTPRRAK...AGKNVTQLYYARQGIITPEM
Burkholderia.sp.  TG.LTSDYGR.............ERAADPATAELRFQGLHRTPRRAL...PGKNVSQMHYAKKGIITPEM
P.stutzeri        PG.LTSEFGQ.............RRLNDAELSAMRFA.HVRNPRRAK...AEHNVSQMHYAKKGIITPEM
A.baumannii       SG.LTSKFGQ.............ERLKDIRTADIRFA.HIQNPRRAK...AGKNVTQMHYAKQGIITPEM
E.coli            TV.RSSDYTK.............ARLADDGLDELRFS.GLLTPKRAK...TGRRVTQLHYARQGIITPEM
S.enterica        DD.RSSAYTK.............ERLADDGLDDLRFT.GLLTPKRAK...AGKRVTQLHYARQGIVTPEM
F.bacterium       EG.LSSAYGR.............KQEQNAALDHLRFV.RNTNPLKAE...AGKNVTQMHYARLGIITPEM
A.tumefaciens     DG.RHVKPED.............NGFATGERLTPEFAVR.HQPLRAT...EGKAVTQLAYARAGIITPEM
C.crescentus      ADPRQVKPED.............NGFAQGKHLAPEFPDTGRKIYRAK...PGKLVTQLEYARAGIITAEM
S.viridis         ....................................................EHKTQLGWAKAGVITPEM
H.ochraceum       ............................................IAE...DDGNRTQMHYARRGLITEEM
L.licerasiae      .............................................PS...PVQNHSQMYFAKQGLITEEM
V.carteri         TDFDVYDTSG.............PRVADPRQGLPKLRQPWIARREAKGITAKAGVTQMALARAGIISEEM
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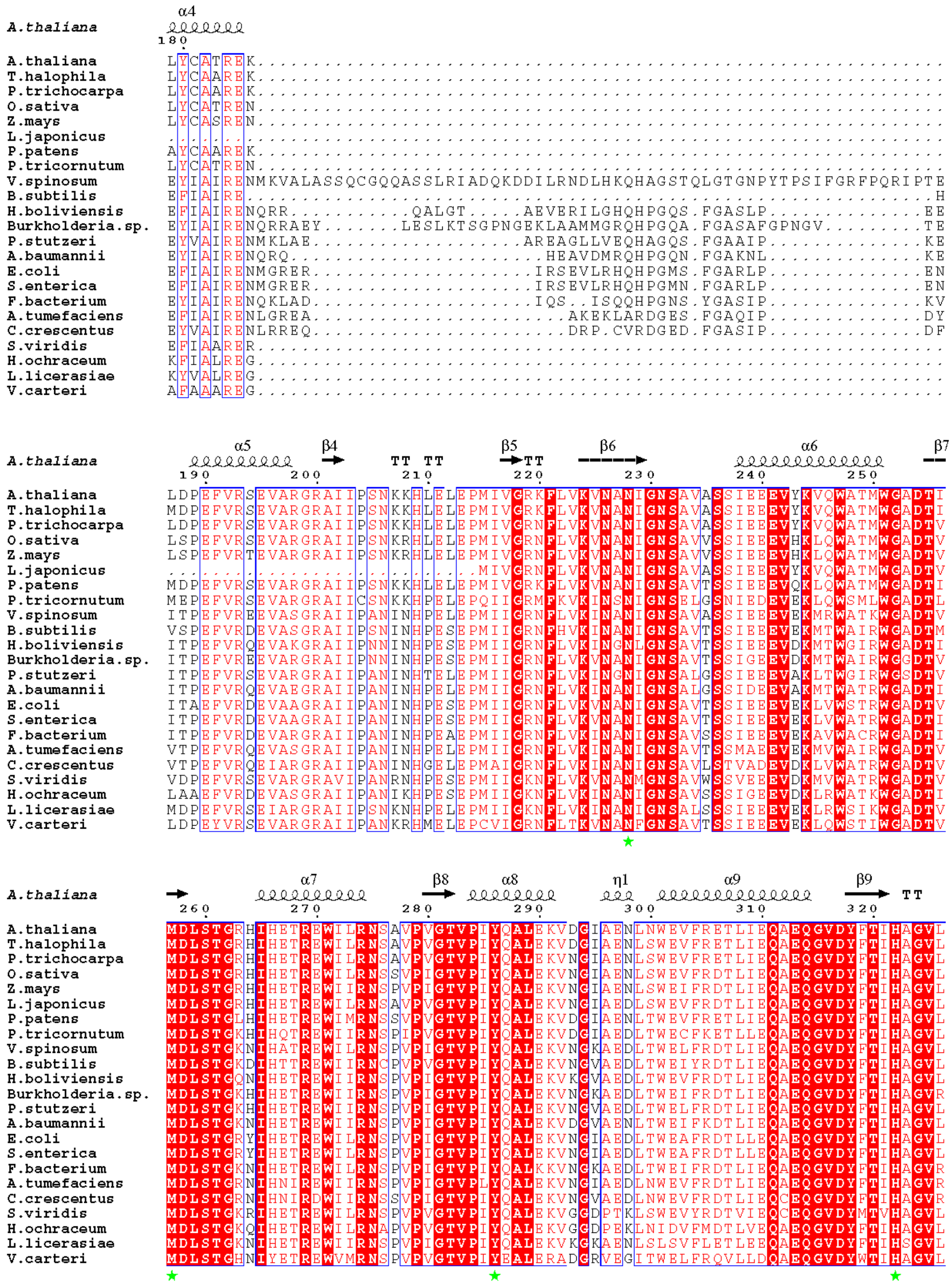
A.thaliana α4
180
A.thaliana LYCATREK...
T.halophila LYCAAREK...
P.trichocarpa LYCAAREK...
O.sativa LYCATREN...
Z.mays LYCASREN...
L.japonicus ...
P.patens AYCAAREK...
P.tricornutum LYCATREN...
V.spinosum EYIAIRENMKVALASSQCGQQASSLRIADQKDDILRNDLHKQHAGSTQLGTGNPYTPSIFGRFPQRIPTE
B.subtilis EFIAIRE...H
H.boliviensis EFIAIRENQRR...QALGT...AEVERILGHQHPGQS.FGASLP...EE
Burkholderia.sp. EYIAIRENQRRAEY...LESLKTSGPNGEKLAAMMGRQHPGQA.FGASAFGPNGV...TE
P.stutzeri EYVAIRENMKLAE...AREAGLLVEQHAGQS.FGAAIP...KE
A.baumannii EYIAIRENQRQ...HEAVDMRQHPGQN.FGAKNL...KE
E.coli EFIAIRENMGRER...IRSEVLRHQHPGMS.FGARLP...EN
S.enterica EFIAIRENMGRER...IRSEVLRHQHPGMN.FGARLP...EN
F.bacterium EYIAIRENQKLAD...IQS..ISQQHPGNS.YGASIP...KV
A.tumefaciens EFIAIRENLGREA...AKEKLARDGES.FGAQIP...DY
C.crescentus EYVAIRENLRREQ...DRP.CVRDGED.FGASIP...DF
S.viridis EFIAARER...
H.ochraceum KFIALREG...
L.licerasiae KYVALREG...
V.carteri AFAAAREG...
A.thaliana α5 β4 TT TT β5 TT β6 α6 β7
190 200 210 220 230 240 250
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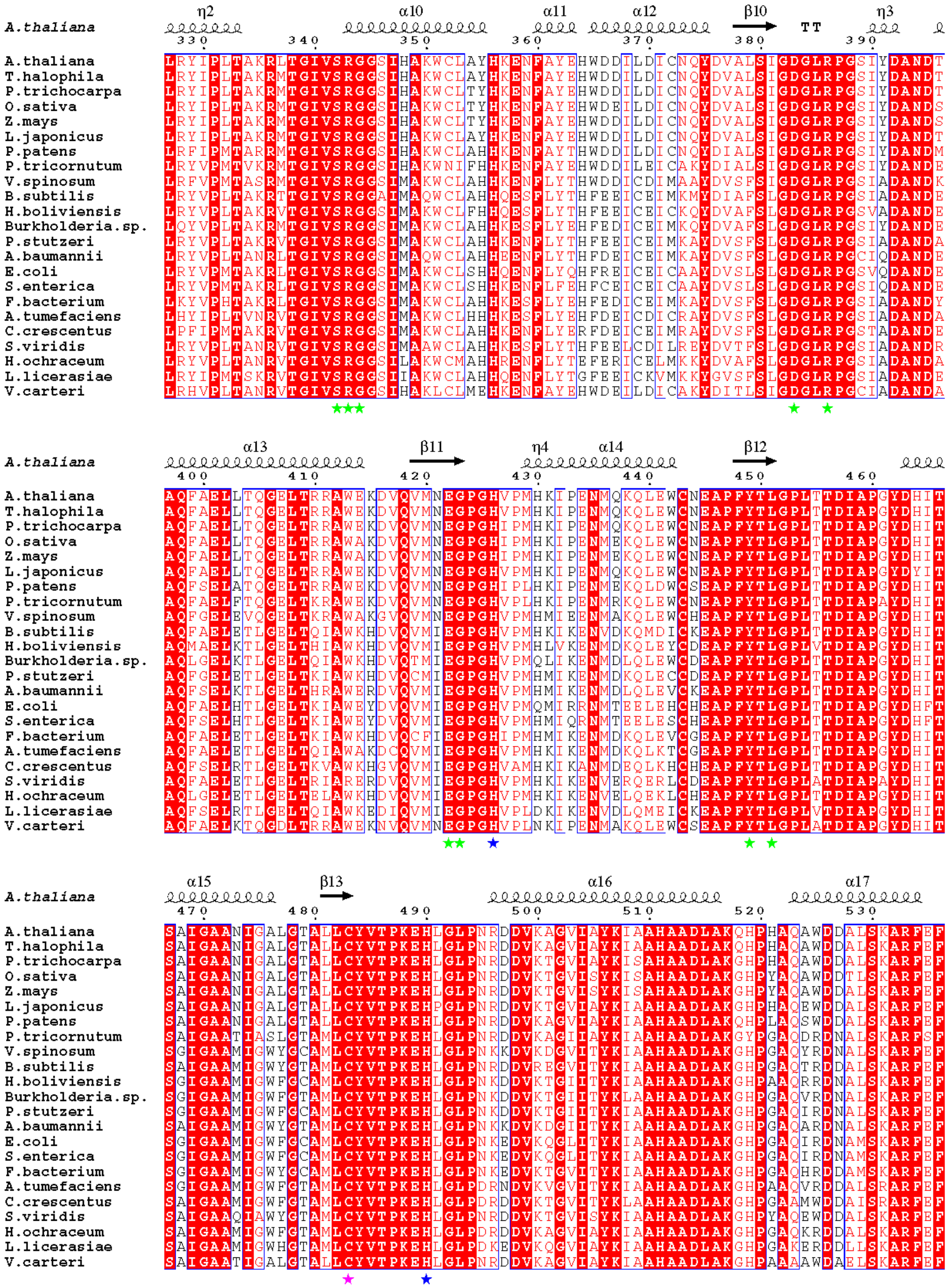
T.halophila MDPEFVRSEVARGRAIIPSNKKHLELEPMIVGRKFLVKVNANIGNSAVASSIEEEVYKVQWATMWGADTI
P.trichocarpa LDPEFVRSEVARGRAIIPSNKKHLELEPMIVGRNFLVKVNANIGNSAVASSIEEEVYKVQWATMWGADTV
O.sativa LSPEFVRSEVARGRAIIPSNKRHLELEPMIVGRNFLVKVNANIGNSAVVSSIEEEVHKLQWATMWGADTV
Z.mays LSPEFVRTEVARGRAIIPSNKRHLELEPMIVGRNFLVKVNANIGNSAVVSSIEEEVHKLQWATMWGADTV
L.japonicus ...MIVGRNFLVKVNANIGNSAVASSIEEEVYKVQWATMWGADTV
P.patens MDPEFVRSEVARGRAIIPSNKKHLELEPMIVGRKFLVKVNANIGNSAVTSSIEEEVQKLQWATMWGADTI
P.tricornutum MEPEFVRSEVARGRAIICSNKKHPELEPQIIGRMFKVKINSNIGNSELGSNIEDEVEKLQWSMLWGADTL
V.spinosum ITPEFVREEVASGRAIIPANINHPELEPMIIGRNFLVKINANIGNSAVASSIEEEVEKMRWATKWGADTV
B.subtilis VSPEFVRDEVASGRAIIPSNINHPESEPMIIGRNFHVKINANIGNSAVTSSIEEEVEKMTWAIRWGADTM
H.boliviensis ITPEFVRQEVAKGRAIIPNNINHPESEPMIIGRNFLVKINGNLGNSAVTSSIEEEVDKMTWGIRWGADTI
Burkholderia.sp. ITPEFVREEVARGRAIIPNNINHPESEPMIIGRNFLVKVNANIGNSAVTSSIGEEVDKMTWAIRWGGDTV
P.stutzeri ITPEFVRSEVARGRAIIPANINHTELEPMIIGRNFLVKINGNIGNSALGSSIEEEVAKLTWGIRWGSDTV
A.baumannii ITPEFVRQEVAEGRAIIPANINHPELEPMIIGRNFLVKVNANIGNSALGSSIDEEVAKMTWATRWGADTI
E.coli ITAEFVRDEVAAGRAIIPANINHPESEPMIIGRNFLVKVNANIGNSAVTSSIEEEVEKLVWSTRWGADTV
S.enterica ITPEFVRDEVAAGRAIIPANINHPESEPMIIGRNFLVKVNANIGNSAVTSSIEEEVEKLVWATRWGADTI
F.bacterium ITPEFVRDEVARGRAIIPANINHPEAEPMIIGRNFLVKINANIGNSAVSSSIEEEVEKAVWACRWGADTI
A.tumefaciens VTPEFVRQEVASGRAIIPANINHPELEPMIIGRNFLVKINANIGNSAVTSSMAEEVEKMVWAIRWGADTV
C.crescentus VTPEFVRQEIARGRAIIPANINHGELEPMAIGRNFLVKINANIGNSAVLSTVADEVDKLVWATRWGADTV
S.viridis VDPEFVRSEVARGRAVIPARNNHPESEPMIIGKNFLVKVNANMGNSAVWSSVEEEVDKMVWATRWGADTV
H.ochraceum LAAEFVRDEVASGRAIIPANIKHPESEPMIIGKNFLVKINANIGNSAVSSSIGEEVDKLRWATKWGADTI
L.licerasiae MDPEFVRSEIARGRAIIPSNKNHPELEPMIIGKNFLVKINANIGNSALSSSIEEEVEKLRWSIKWGADTV
V.carteri LDPEYVRSEVARGRAIIPANKRHMELEPCVIGRNFLTKVNANFGNSAVTSSIEEEVEKLQWSTIWGADTV
A.thaliana α7 β8 α8 η1 α9 β9 TT
260 270 280 290 300 310 320
A.thaliana MDLSTGRHIHETREWILRNSAVPVGTVPIYQALEKVDGIAENLNWEVFRETLIEQAEQGVDYFTIHAGVL
T.halophila MDLSTGRHIHETREWILRNSAVPVGTVPIYQALEKVDGIAENLSWEVFRETLIEQAEQGVDYFTIHAGVL
P.trichocarpa MDLSTGRHIHETREWILRNSAVPVGTVPIYQALEKVNGIAENLSWEVFRETLIEQAEQGVDYFTIHAGVL
O.sativa MDLSTGRHIHETREWILRNSSVPIGTVPIYQALEKVNGIAENLSWEIFRDTLIEQAEQGVDYFTIHAGVL
Z.mays MDLSTGRHIHETREWILRNSPVPIGTVPIYQALEKVNGIAENLSWEIFRDTLIEQAEQGVDYFTIHAGVL
L.japonicus MDLSTGRHIHETREWILRNSAVPVGTVPIYQALEKVNGIAEDLSWEVFRDTLIEQAEQGVDYFTIHAGVL
P.patens MDLSTGLHIHETREWIMRNSSVPVGTVPIYQALEKVDGIAENLTWEVFRETLIEQAEQGVDYFTIHAGVL
P.tricornutum MDLSTGKHIHQTREWIIRNSPIPVGTVPIYQALEKVDGIAEDLTWECFKETLLEQAEQGVDYFTIHAGVL
V.spinosum MDLSTGKNIHATREWILRNSPVPIGTVPIYQALEKVNGKAEDLTWELFRDTLIEQAEQGVDYFTIHAGVL
B.subtilis MDLSTGKDIHTTREWIIRNCPVPVGTVPIYQALEKVNGVAEDLTWEIYRDTLIEQAEQGVDYFTIHAGVL
H.boliviensis MDLSTGQNIHETREWILRNSPVPIGTVPIYQALEKVKGVAEDLTWEVFRDTLIEQAEQGVDYFTIHAGVL
Burkholderia.sp. MDLSTGKHIHETREWIIRNSPVPIGTVPIYQALEKVNGKAEDLTWEIFRDTLIEQAEQGVDYFTIHAGVR
P.stutzeri MDLSTGKHIHETREWIIRNSPVPIGTVPIYQALEKVNGVAEDLTWELFRDTLIEQAEQGVDYFTIHAGVL
A.baumannii MDLSTGKNIHETREWIIRNSPVPIGTVPIYQALEKVDGVAENLTWEIFKDTLIEQAEQGVDYFTIHAGVL
E.coli MDLSTGRYIHETREWILRNSPVPIGTVPIYQALEKVNGIAEDLTWEAFRDTLLEQAEQGVDYFTIHAGVL
S.enterica MDLSTGRYIHETREWILRNSPVPIGTVPIYQALEKVNGIAEDLTWEAFRDTLLEQAEQGVDYFTIHAGVL
F.bacterium MDLSTGKNIHETREWILRNSPVPIGTVPIYQALKKVNGKAEDLTWEIFRDTLIEQAEQGVDYFTIHAGVR
A.tumefaciens MDLSTGRNIHNIREWIIRNSPVPIGTVPLYQALEKVNGIAEDLNWEVFRDTLIEQAEQGVDYFTIHAGVR
C.crescentus MDLSTGRNIHNIRDWIIRNSSVPIGTVPIYQALEKVNGVAEDLNWEVFRDTLIEQCEQGVDYFTIHAGVR
S.viridis MDLSTGKRIHETREWILRNSPVPVGTVPIYQALEKVGGDPTKLSWEVYRDTVIEQCEQGVDYMTVHAGVL
H.ochraceum MDLSTGKQIHETREWILRNAPVPVGTVPIYQALEKVGGDPEKLNIDVFMDTLVEQAEQGVDYFTIHAGVL
L.licerasiae MDLSTGKNIHETREWILRNSPVPVGTVPIYQALEKVKGKAENLSLSVFLETLEEQAEQGVDYFTIHSGVL
V.carteri MDLSTGHNIYETREWVMRNSPVPVGTVPIYEALERADGRVEGITWELFRQVLLDQAEQGVDYWTIHAGVL


η2 α10 α11 α12 β10 TT η3
A.thaliana
330 340 350 360 370 380 390
A.thaliana LRYIPLTAKRLTGIVSRGGSIHAKWCLAYHKENFAYEHWDDILDICNQYDVALSIGDGLRPGSIYDANDT
T.halophila LRYIPLTAKRMTGIVSRGGSIHAKWCLAYHKENFAYEHWDDILDICNQYDVALSIGDGLRPGSIYDANDT
P.trichocarpa LRYIPLTAKRMTGIVSRGGSIHAKWCLTYHKENFAYEHWDDILDICNQYDVALSIGDGLRPGSIYDANDT
O.sativa LRYIPLTAKRMTGIVSRGGSIHAKWCLTYHKENFAYEHWDEILDICNQYDVALSIGDGLRPGSIYDANDS
Z.mays LRYIPLTAKRMTGIVSRGGSIHAKWCLTYHKENFAYEHWDDILDICNQYDVALSIGDGLRPGSIYDANDS
L.japonicus LRYIPLTAKRMTGIVSRGGSIHAKWCLAYHKENFAYEHWDDILDICNQYDVALSIGDGLRPGSIYDANDT
P.patens LRFIPMTARRMTGIVSRGGSIHAKWCLAHHKENFAYTHWDDILDICNQYDIALSIGDGLRPGSIYDANDM
P.tricornutum LRYVPMTVKRMTGIVSRGGSIHAKWNIFHHKENFAYEHWDDILEICAKYDIALSIGDGLRPGSIYDANDE
V.spinosum LRFVPMTASRMTGIVSRGGSIMAKWCLAHHKENFLYTHWDDICDIMAAYDVSFSIGDGLRPGSIADANDK
B.subtilis LRYVPLTAKRTTGIVSRGGAIMAQWCLAHHQESFLYTHFEEICEIMKMYDIAFSLGDGLRPGSIADANDE
H.boliviensis LRYVPLTAKRVTGIVSRGGSIMAKWCLFHHQESFLYTHFEEICEICKQYDVAFSLGDGLRPGSVADANDE
Burkholderia.sp. LQYVPLTAKRMTGIVSRGGSIMAKWCLAHHKESFLYEHFEDICEIMKQYDVAFSLGDGLRPGSIYDANDE
P.stutzeri LRYVPLTAKRVTGIVSRGGSIMAKWCLAHHKENFLYTHFEEICEIMKAYDVSFSLGDGLRPGSIADANDA
A.baumannii LRYVPLTANRLTGIVSRGGSIMAQWCLAHHEENFLYTHFDEICEIMKAYDVSFSLGDGLRPGCIQDANDE
E.coli LRYVPMTAKRLTGIVSRGGSIMAKWCLSHHQENFLYQHFREICEICAAYDVSLSLGDGLRPGSVQDANDE
S.enterica LRYVPMTAKRLTGIVSRGGSIMAKWCLSHHKENFLFEHFCEICEICAAYDVSLSLGDGLRPGSIQDANDE
F.bacterium LKYVPHTAKRLTGIVSRGGSIMAKWCLAHHKESFLYEHFEDICEIMKAYDVSFSLGDGLRPGSIADANDY
A.tumefaciens LHYIPLTVNRVTGIVSRGGSIMAKWCLHHHKESFLYEHFDEICDICRAYDVSFSLGDGLRPGSIADANDA
C.crescentus LPFIPMTAKRVTGIVSRGGSIMAKWCLAHHKENFLYERFDEICEIMRAYDVSFSLGDGLRPGSTADANDE
S.viridis LRYVPLTANRVTGIVSRGGSIMAAWCLAHHKESFLYTHFEELCDILREYDVTFSLGDGLRPGSIADANDR
H.ochraceum LRYVPLTANRVTGIVSRGGSILAKWCMAHHRENFLYTEFERICELMKKYDVAFSLGDGLRPGSIADANDA
L.licerasiae LRYIPMTSKRVTGIVSRGGSIIAKWCLAHHQENFLYTGFEEICKVMKKYGVSFSLGDGLRPGSIADANDE
V.carteri LRHVPLTANRVTGIVSRGGSIHAKLCLMEHKENFAYEHWDEILDICAKYDITLSIGDGLRPGCIADANDA
α13 β11 η4 α14 β12
A.thaliana
400 410 420 430 440 450 460
A.thaliana AQFAELLTQGELTRRAWEKDVQVMNEGPGHVPMHKIPENMQKQLEWCNEAPFYTLGPLTTDIAPGYDHIT
T.halophila AQFAELLTQGELTRRAWEKDVQVMNEGPGHVPMHKIPENMQKQLEWCNEAPFYTLGPLTTDIAPGYDHIT
P.trichocarpa AQFAELLTQGELTRRAWEKDVQVMNEGPGHIPMHKIPENMQKQLEWCNEAPFYTLGPLTTDIAPGYDHIT
O.sativa AQFAELLTQGELTRRAWAKDVQVMNEGPGHIPMHKIPENMEKQLEWCNEAPFYTLGPLTTDIAPGYDHIT
Z.mays AQFAELLTQGELTRRAWAKDVQVMNEGPGHIPMHKIPENMEKQLEWCNEAPFYTLGPLTTDIAPGYDHIT
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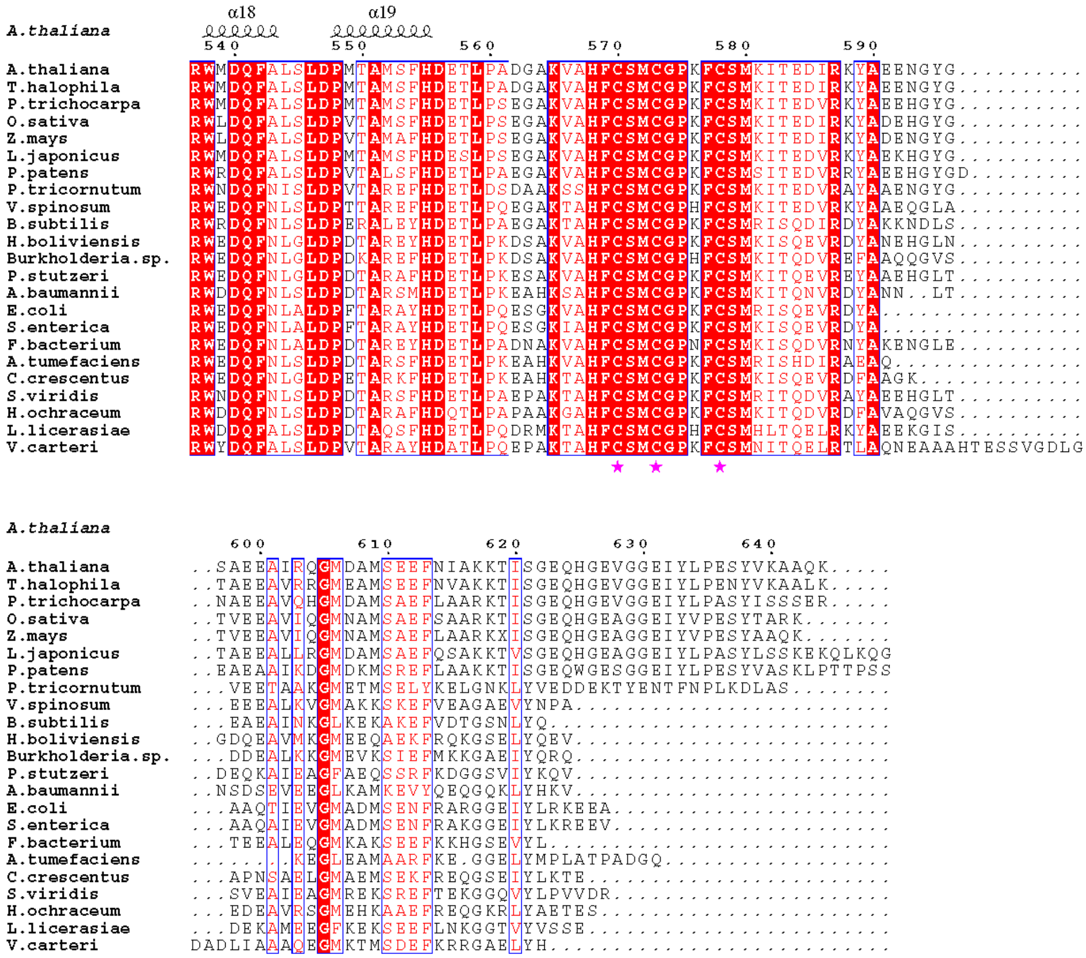
P.patens AQFSELATQGELTRRAWEKDVQVMNEGPGHIPLHKIPENMEKQLDWCSEAPFYTLGPLTTDIAPGYDHIT
P.tricornutum AQFAELFTQGELTKRAWEKDVQVMNEGPGHVPLHKIPENMRKQLEWCNEAPFYTLGPLTTDIAPAYDHIT
V.spinosum AQFGELEVQGELTKRAWAKGVQVMNEGPGHVPMHMIEENMAKQLEWCHEAPFYTLGPLTTDIAPGYDHIT
B.subtilis AQFAELETLGELTQIAWKHDVQVMIEGPGHVPMHKIKENVDKQMDICKEAPFYTLGPLTTDIAPGYDHIT
H.boliviensis AQMAELKTLGELTHIAWKHDVQVMIEGPGHVPMHLVKENMDKQLEYCDEAPFYTLGPLVTDIAPGYDHIT
Burkholderia.sp. AQLGELKTLGELTQIAWKHDVQTMIEGPGHVPMQLIKENMDLQLEWCDEAPFYTLGPLTTDIAPGYDHIT
P.stutzeri AQFGELETLGELTKIAWKHDVQCMIEGPGHVPMHMIKENMDKQLECCDEAPFYTLGPLTTDIAPGYDHIT
A.baumannii AQFSELKTLGELTHRAWERDVQVMIEGPGHVPMHMIKENMDLQLEVCKEAPFYTLGPLTTDIAPGYDHIT
E.coli AQFAELHTLGELTKIAWEYDVQVMIEGPGHVPMQMIRRNMTEELEHCHEAPFYTLGPLTTDIAPGYDHFT
S.enterica AQFSELHTLGELTKIAWEYDVQVMIEGPGHVPMHMIQRNMTEELESCHEAPFYTLGPLTTDIAPGYDHFT
F.bacterium AQFAELETLGELTKIAWKHDVQCFIEGPGHIPMHMIKENMDKQLEVCGEAPFYTLGPLTTDIAPGYDHIT
A.tumefaciens AQFAELETLGELTQIAWAKDCQVMIEGPGHVPMHKIKENMDKQLKTCGEAPFYTLGPLTTDIAPGYDHIT
C.crescentus AQFSELRTLGELTKVAWKHGVQVMIEGPGHVAMHKIKANMDEQLKHCHEAPFYTLGPLTTDIAPGYDHIT
S.viridis AQFAELETLGELTRIARERDVQVMIEGPGHVPMHKIKENVERQERLCDEAPFYTLGPLATDIAPAYDHIT
H.ochraceum AQLGELETLGELTELAWKHDVQVMIEGPGHVPMHKIKENVELQEKLCHEAPFYTLGPLTTDIAPGYDHIT
L.licerasiae AQFSELRTLGELTQIAWKEDIQVMIEGPGHVPLDKIKENVDLQMEICKEAPFYTLGPLVTDIAPGYDHIT
V.carteri AQFAELKTQGDLTRRAWEKNVQVMNEGPGHVPLNKIPENMAKQLEWCSEAPFYTLGPLATDIAPGYDHIT
α15 β13 α16 α17
A.thaliana
470 480 490 500 510 520 530
A.thaliana SAIGAANIGALGTALLCYVTPKEHLGLPNRDDVKAGVIAYKIAAHAADLAKQHPHAQAWDDALSKARFEF
T.halophila SAIGAANIGALGTALLCYVTPKEHLGLPNRDDVKAGVIAYKIAAHAADLAKQHPHAQAWDDALSKARFEF
P.trichocarpa SAIGAANIGALGTALLCYVTPKEHLGLPNRDDVKTGVIAYKISAHAADLAKGHPHAQAWDDALSKARFEF
O.sativa SAIGAANIGALGTALLCYVTPKEHLGLPNRDDVKTGVISYKISAHAADLAKGHPYAQAWDDTLSKARFEF
Z.mays SAIGAANIGALGTALLCYVTPKEHLGLPNRDDVKTGVISYKISAHAADLAKGHPYAQAWDDALSKARFEF
L.japonicus SAIGAANIGALGTALLCYVTPKEHPGLPNRDDVKTGVIAYKIAAHAADLAKGHPHAQEWDDALSKARFEF
P.patens SAIGAANIGALGTALLCYVTPKEHLGLPNRDDVKAGVIAYKIAAHAADLAKQHPLAQSWDDALSKARFEF
P.tricornutum SAIGAATIASLGTAMLCYVTPKEHLGLPNRDDVKAGIIAYKIAAHAADLAKGYPGAQDRDNALSKARFSF
V.spinosum SGIGAAMIGWYGCAMLCYVTPKEHLGLPNKKDVKDGVITYKIAAHAADLAKGHPGAQYRDNALSKARFEF
B.subtilis SAIGAAMIGWYGTAMLCYVTPKEHLGLPNRDDVREGVITYKIAAHAADLAKGHPGAQTRDDALSKARFEF
H.boliviensis SGIGAAMIGWFGCAMLCYVTPKEHLGLPNKDDVKTGIITYKIAAHAADLAKGHPAAQRRDNALSKARFEF
Burkholderia.sp. SGIGAAMIGWFGTAMLCYVTPKEHLGLPNKDDVKTGIITYKLAAHAADLAKGHPGAQVRDNALSKARFEF
P.stutzeri SGIGAAMIGWFGCAMLCYVTPKEHLGLPNKDDVKTGVITYKIAAHAADLAKGHPGAQIRDNALSKARFEF
A.baumannii SAIGAAMIGWYGTAMLCYVTPKEHLGLPNKKDVKDGIITYKIAAHAADLAKGHPGAQARDNALSKARFEF
E.coli SGIGAAMIGWFGCAMLCYVTPKEHLGLPNKEDVKQGLITYKIAAHAADLAKGHPGAQIRDNAMSKARFEF
S.enterica SGIGAAMIGWFGCAMLCYVTPKEHLGLPNKEDVKQGLITYKIAAHAADLAKGHPGAQIRDNAMSKARFEF
F.bacterium SGIGAAMIGWYGCAMLCYVTPKEHLGLPNKEDVKTGVITYKIAAHAADLAKGHPGAQHRDDAMSKARFEF
A.tumefaciens SGIGAAMIGWFGTAMLCYVTPKEHLGLPDRNDVKVGVITYKIAAHAADLAKGHPAAQVRDDALSRARFEF
C.crescentus SAIGAAMIGWFGTAMLCYVTPKEHLGLPDRDDVKTGVITYKLAAHAADLAKGHPGAAMWDDAISRARFEF
S.viridis SAIGAAQIAWYGTAMLCYVTPKEHLGLPNRDDVKTGVISYKIAAHAADLAKGHPYAQEWDDALSKARFEF
H.ochraceum SAIGAAMIGWFGTAMLCYVTPKEHLGLPDRDDVKAGVIAYKIAAHAADLAKGHPGAQKRDDALSKARFEF
L.licerasiae SAIGAAMIGWHGTAMLCYVTPKEHLGLPDKEDVKQGVIAYKIAAHAADLAKGHPGAKERDDLLSKARFEF
V.carteri SAIGAATIGALGTALLCYVTPKEHLGLPNRDDVKAGVIAYKIAAHAADLAKGHPAAAAWDAELSKARFEF


α18
α19
A.thaliana
540 550 560 570 580 590
A.thaliana RWMDQFALSLDPMTAMSFHDETLPADGAKVAHFCSMCGPKFCSMKITEDIRKYAEENGYG..........
T.halophila RWMDQFALSLDPMTAMSFHDETLPADGAKVAHFCSMCGPKFCSMKITEDIRKYAEENGYG..........
P.trichocarpa RWMDQFALSLDPMTAMSFHDETLPSEGAKVAHFCSMCGPKFCSMKITEDVRKYAEEHGYG..........
O.sativa RWLDQFALSLDPVTAMSFHDETLPSEGAKVAHFCSMCGPKFCSMKITEDIRKYADEHGYG..........
Z.mays RWLDQFALSLDPVTAMAFHDETLPSEGAKVAHFCSMCGPKFCSMKITEDIRKYADENGYG..........
L.japonicus RWMDQFALSLDPMTAMSFHDESLPSEGAKVAHFCSMCGPKFCSMKITEDVRKYAEKHGYG..........
P.patens RWRDQFALSLDPVTALSFHDETLPAEGAKVAHFCSMCGPKFCSMSITEDVRRYAEEHGYGD.........
P.tricornutum RWNDQFNISLDPVTAREFHDETLDSDAAKSSHFCSMCGPKFCSMKITEDVRAYAAENGYG..........
V.spinosum RWEDQFNLSLDPTTAREFHDETLPQEGAKTAHFCSMCGPHFCSMKITEDVRKYAAEQGLA..........
B.subtilis RWRDQFNLSLDPERALEYHDETLPAEGAKTAHFCSMCGPKFCSMRISQDIRDYAKKNDLS..........
H.boliviensis RWEDQFNLGLDPDTAREYHDETLPKDSAKVAHFCSMCGPKFCSMKISQEVRDYANEHGLN..........
Burkholderia.sp. RWEDQFNLGLDPDKAREFHDETLPKDSAKVAHFCSMCGPHFCSMKITQDVREFAAQQGVS..........
P.stutzeri RWEDQFNLGLDPDTARAFHDETLPKESAKVAHFCSMCGPKFCSMKITQEVREYAAEHGLT..........
A.baumannii RWDDQFNLSLDPDTARSMHDETLPKEAHKSAHFCSMCGPKFCSMKITQNVRDYANN..LT..........
E.coli RWEDQFNLALDPFTARAYHDETLPQESGKVAHFCSMCGPKFCSMRISQEVRDYA................
S.enterica RWEDQFNLALDPFTARAYHDETLPQESGKIAHFCSMCGPKFCSMKISQEVRDYA................
F.bacterium RWEDQFNLALDPDTAREYHDETLPADNAKVAHFCSMCGPNFCSMKISQDVRNYAKENGLE..........
A.tumefaciens RWEDQFNLSLDPDTARSFHDETLPKEAHKVAHFCSMCGPKFCSMRISHDIRAEAQ...............
C.crescentus RWEDQFNLGLDPETARKFHDETLPKEAHKTAHFCSMCGPKFCSMKISQEVRDFAAGK.............
S.viridis RWNDQFNLSLDPDTARSFHDETLPAEPAKTAHFCSMCGPKFCSMRITQDVRAYAEEHGLT..........
H.ochraceum RWDDQFNLSLDPDTARAFHDQTLPAPAAKGAHFCSMCGPKFCSMKITQDVRDFAVAQGVS..........
L.licerasiae RWDDQFALSLDPDTAQSFHDETLPQDRMKTAHFCSMCGPHFCSMHLTQELRKYAEEKGIS..........
V.carteri RWYDQFALSLDPVTARAYHDATLPQEPAKTAHFCSMCGPKFCSMNITQELRTLAQNEAAAHTESSVGDLG
A.thaliana
600 610 620 630 640
A.thaliana ..SAEEAIRQGMDAMSEEFNIAKKTISGEQHGEVGGEIYLPESYVKAAQK.....
T.halophila ..TAEEAVRRGMEAMSEEFNVAKKTISGEQHGEVGGEIYLPENYVKAALK.....
P.trichocarpa ..NAEEAVQHGMDAMSAEFLAARKTISGEQHGEVGGEIYLPASYISSSER.....
O.sativa ..TVEEAVIQGMNAMSAEFSAARKTISGEQHGEAGGEIYVPESYTARK.......
Z.mays ..TVEEAVIQGMNAMSAEFLAARKXISGEQHGEAGGEIYVPESYAAQK.......
L.japonicus ..TAEEALLRGMDAMSAEFQSAKKTVSGEQHGEAGGEIYLPASYLSSKEKQLKQG
P.patens ..EAEAAIKDGMDKMSREFLAAKKTISGEQWGESGGEIYLPESYVASKLPTTPSS
P.tricornutum ...VEETAAKGMETMSELYKELGNKLYVEDDEKTYENTFNPLKDLAS........
V.spinosum ...EEEALKVGMAKKSKEFVEAGAEVYNPA.........................
B.subtilis ...EAEAINKGLKEKAKEFVDTGSNLYQ...........................
H.boliviensis ..GDQEAVMKGMEEQAEKFRQKGSELYQEV.........................
Burkholderia.sp. ...DDEALKKGMEVKSIEFMKKGAEIYQRQ.........................
P.stutzeri ..DEQKAIEAGFAEQSSRFKDGGSVIYKQV.........................
A.baumannii ..NSDSEVEEGLKAMKEVYQEQGQKLYHKV.........................
E.coli ...AAQTIEVGMADMSENFRARGGEIYLRKEEA......................
S.enterica ...AAQAIEVGMADMSENFRAKGGEIYLKREEV......................
F.bacterium ...TEEALEQGMKAKSEEFKKHGSEVYL...........................
A.tumefaciens ........KEGLEAMAARFKE.GGELYMPLATPADGQ..................
C.crescentus ...APNSAELGMAEMSEKFREQGSEIYLKTE........................
S.viridis ...SVEAIEAGMREKSREFTEKGGQVYLPVVDR......................
H.ochraceum ...EDEAVRSGMEHKAAEFREQGKRLYAETES.......................
L.licerasiae ...DEKAMEEGFKEKSEEFLNKGGTVYVSSE........................
V.carteri DADLIAAAQEGMKTMSDEFKRRGAELYH...........................

Supplementary Table 1.

ICP-MS Multi-element quantitation					
Sample	Concentration unit	Mg	Fe	Ni	Zn
ΔN71-AtTHIC	mg/L	< LOQ	17.91 ± 0.72	4.85 ± 0.10	4.17 ± 0.29
	mM	< LOQ	0.321 ± 0.01	0.083 ± 0.00	0.064 ± 0.00
Buffer	µg/L	92.70 ± 3.70	44.5 ± 8.60	6.10 ± 0.90	107.40 ± 17.60
	mM	0.004 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	0.002 ± 0.000