Supplemental Material to

Evolved Cobalamin-Independent Methionine Synthase (MetE) Improves the Acetate and Thermal Tolerance of *Escherichia coli*

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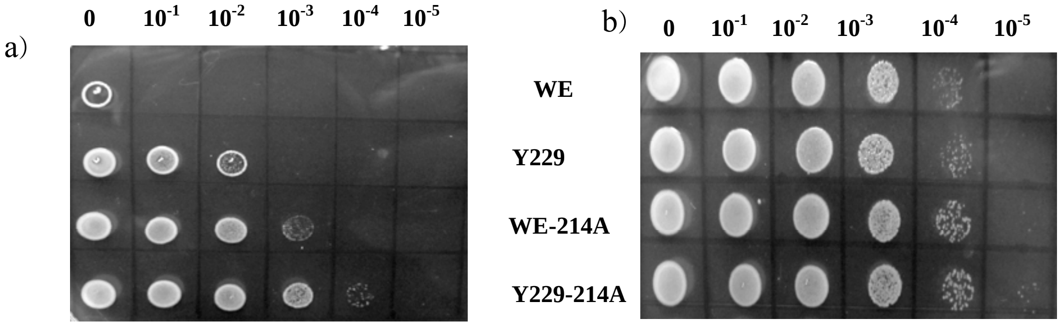

-methionine, 45^{0}C

+methionine, 45^{0}C

Fig. S1 Synergic effect of the stabilized MetA/MetE enzymes on *E. coli* viability at elevated temperature.

Serial dilutions of cultures of WE, Y229, WE-214A and Y229-214A growing logarithmically at 37°C in M9 glucose medium (OD_{600} of 0.5) were spotted onto M9 glucose (A) or M9 glucose L-methionine (50 µg ml^{-1}) (B) agar plates. The cells were then incubated for 24 h at 45°C.

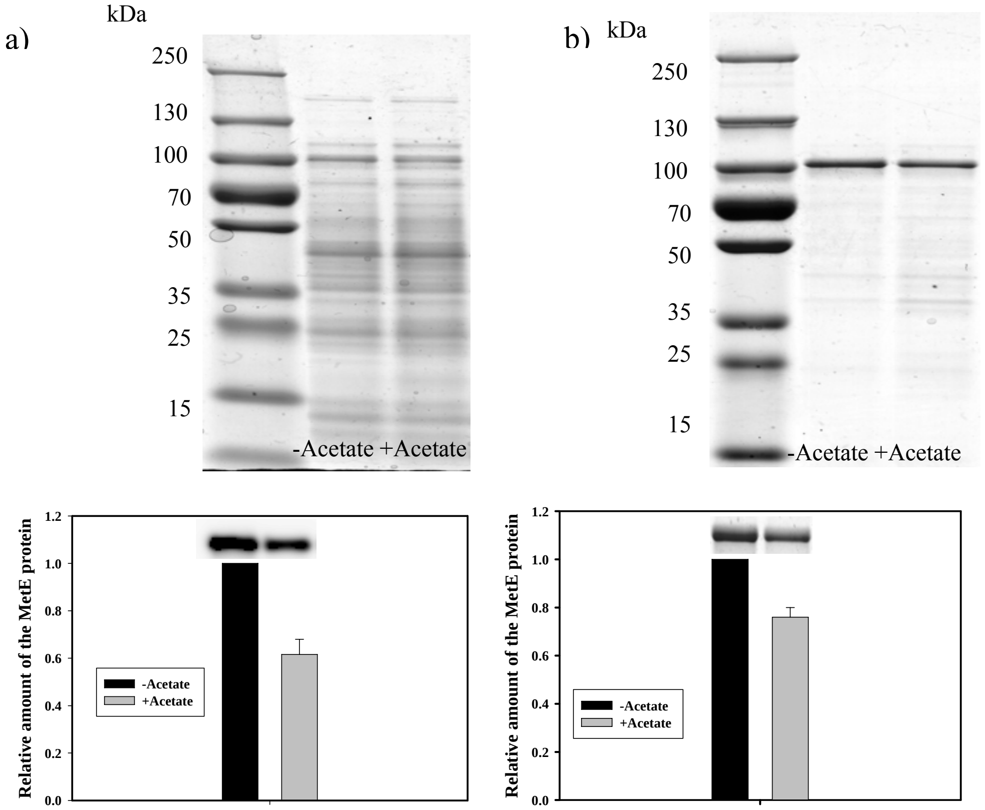

Fig.S2 **Effect of acetate stress on the native MetE protein solubility.**

(A) The WE strain was grown in 50 ml of M9 glucose medium (pH6.0) to exponential phase (approximately OD_{600} = 0.3) at 37^0C. (B) The strain BL21(DE3) harboring the plasmid pET22b/MetE was cultured in 50 ml of the $LBAmp_{100}$ medium to an OD_{600} of 0.5 and induced with 1 mM of IPTG for 1 h. Twenty-five milliliters of each culture were treated with 20 mM sodium acetate for 2h. The remaining 25 ml were used as a control. Soluble protein fractions were purified as previously described (1,2) in the presence of EDTA-free Halt protease inhibitor cocktail (Pierce, Rockford, USA). Three (A) and two (B) micrograms of total protein from the soluble fractions were subjected to 12% SDS-PAGE, followed by Western blotting using rabbit anti-MetE antibody. The MetE in the samples was quantified through densitometry using WCIF ImageJ software. The MetE amount from the untreated cells was equal to 1. The error bars represent the standard deviations of triplicate independent cultures.

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CLUSTAL 2.1 multiple sequence alignment


E.coli                 ---------MTILNHTLGFPRVGLRRELKKAQESYWAGNSTREELLAVGRELRARHWDQQ 51
Salmonella             ---------MTILTHTLGFPRVGLRRELKKAQESYWAGNATREELLAVGRELRARHWQQQ 51
Yersinia               ----MGNKTMTILNHTLGFPRVGLKRELKKAQESYWAGNSTQEELLNVGRELRARHWQQQ 56
Vibrio                 ----------MTTTHILGYPRIGEKRELKFALEKYWRGEIDQAALKQVGSQIRQKNWALQ 50
Acidithiobacillus      ----------MTSVHSLGFPRIGHKRELKKALESFWSREIDEQELQSRAAQLRDRHWRIQ 50
Acidobacterium         MATDEASTSLSLKTANLGFPRMGQQRELKFALEDYWTGKCTEEQLQNVAGQLRQSHWELQ 60
Acidiphilium           ---------MSIATS-LGFPRIGRRRELKSALEAHWAGELSEAGLQEAARLLRAESHSLQ 50
Thermotoga             -----------MKAYAFGFPKIGEKREFKKALEDFWKGKITEEQFEEEMNKLRMYMVENY 49
Streptococcus          ----------MTKVSSLGYPRLGENREWKKLIEAYWAGKVSKNDLFAGAKELRLDFLKKQ 50
                                       :*:*::* .** *   * .*  :  .  : V39A :* R46C

E.coli                 KQAGIDLLPVGDFAWYDHVLTTSLLLGNVPARHQNKDGSVDIDTLFRIGRGRAP----TG 107
Salmonella             KQAGIDLLPVGDFAWYDHVLTTSLLLGNVPARHQNNDGSVDIDTLFRIGRGRAP----TG 107
Yersinia               QQAGVDLVPVGDFAWYDHVLTTSLLLGNVPERHQNADGSIDIDTLFRIGRGRAP----TG 112
Vibrio                 KEAGLDFVTAGDFAWYDHVLTTTLLLGHVPKRHS--HGFPDLDTLFRVGRGQSQNACGCG 108
Acidithiobacillus      QTCGMDLVPVGDFSLYDHMLDMSCTLGAIPPRYGFAGGQVGLDTFFAMARGSAT------ 104
Acidobacterium         KTAGIDFIPSNDFSLYDQVLDMLVLLGATPERFGT--DTVTLPRYFAMARNS-------- 110
Acidiphilium           QGLGIGHIPSNDFALYDHVLDTACMVGAIPPGYGWRGGEVTLPTYFALARGTGGG--DAA 108
Thermotoga             RKN-VDVIPSNELSYYDFVLDTAVMVGAVPERFG---EYRGLSTYFDMARGGK------- 98
Streptococcus          LNAGLDLIPVGDFSLYDHILDLSVQFNIIPKRFAK--EPIDIDLYFAIARGNKE------ 102
                           :. :. .::: ** :*     ..  *  .        :   * :.*.      T106I

E.coli                 EPAAAAEMTKWFNTNYHYMVPEFVKGQQFKLTWTQLLDEVDEALAL-GHKVKPVLLGPVT 166
Salmonella             EPAAAAEMTKWFNTNYHYMVPEFSKGQQFRLTWTQLLEEVDEALAL-GHKIKPVLLGPVT 166
Yersinia               KPAAAAEMTKWFNTNYHYMVPEFQQGQQFKLGWTQLLDEVDEALAL-GHKIKPVLLGPIT 171
Vibrio                 TGSAASDMTKWFNTNYHYIVPEFSSNDTFNVSWPQLFEEVNEALQA-GHDVKPVLLGPIS 167
Acidithiobacillus      --QPAMEMTKWFDTNYHFIVPEFHEGMDFRLSSERLFDQVKEVQAL-GLKAKPVLVGPIT 161
Acidobacterium         REQTAMEMTKWFDTNYHYLVPEWSEGLSFKINSAKLLGEVREARAL-GIETRPVLIGPLT 169
Acidiphilium           AGLPALEMTKWFDTNYHYLVPRLAAGQHFAVTANRPLALFREALAR-HRRTRPVLLGPVS 167
Thermotoga             ----ALEMTKFFNTNYHYLVPEIET-EEFYLLENKPLEDYLFFKSK-GIETAPWVIGPFT 152
Streptococcus          --NVASSMKKWFNTNYHYIVPEWSK-QRPKLNNNRLLDLYLEAREVVGDKAKPVITGPIT 159
                           * .*.*:*:****::**.        :   : :               * : **.:

E.coli                 WLWLGKVK---------------------------GEQFDRLSLLNDILPVYQQVLAELA 199
Salmonella             YLWLGKVK---------------------------GEPFDRLTLLKDILPVYQHVLAELA 199
Yersinia               YLWLGKVK---------------------------GEQFDRLSLLNDILPVYQQVLAELA 204
Vibrio                 YLYLGKEV---------------------------EEGFDRLTLLPRLLTAYQAILSKLA 200
Acidithiobacillus      YLWLGKEKDLNAEAHHDAQHHHDDSACHGHGAPIGAACFDRLTLLPKVLPVYAEILARLA 221
Acidobacterium         LLLLGKGV----------------------------DAFDPFTVTDRLIPAYQAVLAELA 201
Acidiphilium           FLLLAKTD----------------------------DGSDPLDLLDRLLPCYAQVLAELA 199
Thermotoga             FLYLSKRN-----------------------GEWIRRPNQMEKLLESLVSVYKEVFEKLV 189
Streptococcus          YVALSTG-----------------------------VEDFTAAVKSLLPLYKQVFTELV 189
                        : *..                                 :       ::. *  :: .*.

E.coli                 KRGIEWVQIDEPALVLELPQAWLDAYKPAYD-ALQG--QVKLLLTTYFEGVTPNLDTITA 256
Salmonella             KRGIEWVQIDEPALVLELPQAWLDAFKLAYD-ALAG--QVKLLLTTYFEGVTPNLNTIIA 256
Yersinia               KRGIEWVQIDEPALVLELPQEWLDAYQPAYQ-ALQG--QVKLLLTTYFDSIGHNIDTIRA 261
Vibrio                 KQGVQWVQIDEPILALELEPRWQEAFKLAYQ-VIRG--DVKLLLTTYFDSVLDTLDKIVE 257
Acidithiobacillus      EMGVEWVQIDEPALALDLPQEWVEALESAYQ-TLRRDKTPKVLLATYFDSVADHAKALKA 280
Acidobacterium         AENVEWVQMDEPILATDLPEGAAEIFRKAYA-AFALSP-VKLMLTTYFERLGDNLPLAIA 259
Acidiphilium           AEGCAWVQMDEPVLALDLAPKARAALRHAYE-TLARGATPRLLLASYFAPIADNLPTALA 258
Thermotoga             ENGCKEILVNEPAFVCDLEKAHWDLILNVYR----ELSEFPLTVFTYYDSVSD-YEACVS 244
Streptococcus          KAGASYIQVDEPIFVTDEGKDYLQAAKAVYAYFAKEVPDAKFIFQTYFEGLID-SQVLSQ 248
                         .   : ::** :. :           .*           . . :*:  :


E.coli                 LPVQGLHVDLVHGKDDVAELHK--RLPSDWLLSAGLINGRNVWRADLTEKYAQIKDIVGK 314
Salmonella             LPVQGLHVDLIHGKDDVSELHQ--RLPADWLLSAGLINGRNVWRADLPEKYAQINDIVGK 314
Yersinia               LPVQGLHVDVVTGHDDLAVLNK--NLPKEWLLSLGVINGRNVWRADLSSWFERLQPLVNS 319
Vibrio                 LPVDGLHVDISAAPAQLETIVN--RLPSDWVLSAGVINGRNVWRADLSAILARLQPVKTL 315
Acidithiobacillus      LPVAGVHLDLRRAPQQLNSFLS--DYPADKVLSLGVVDGRNVWRADLDAALELLQPAHKQ 338
Acidobacterium         AHTAGLHVDAVRAPEQIEEVID--ALHPDQVLSVGCVDGRNIWLTDFSAADAMLRQAARK 317
Acidiphilium           LPVAGLHLDLVRGRDDLAPVLA--AIGPATWLSLGLVDGRNVWRADLRAALATAREAARA 316
Thermotoga             LPVKRLHFDFVS-NEENLKNLEKHGFPEDKKLVAGVINGRQPWKVDLRKVASLVEKLG-- 301
Streptococcus          LPVDAFGLDFVYGLEENLEAIKTGAFK-GKEIFAGVIDGRNIWSSDFVKTSALLETIEEQ 307
                         .  . .*      :               :  * ::**: *  *:       .

E.coli                 R----DLWVASSCSLLHSPIDLSVETRLDAEVKSWFAFALQKCHELALLRDALNS---GD 367
Salmonella             R----ALWVSSSCSLLHSPIDLSVETRLDAEVKSWFAFALQKCGELALLRDALNS---GD 367
Yersinia               R----PLWLGSSCSLLHSPIDLNEETRLDAEVKSWFAFALQKCAELALLTQALNAPNDAK 375
Vibrio                 LG--ERLWVASSCSLLHSPVDLDLEGDLSAETRSWFAFAKQKVTEVALLGRALEG-DAAA 372
Acidithiobacillus      LG--DRLWVAPSCSLLHTPVDLEQETELDAELKSWLSFSVQKLDEVAIIGRALKEGVESV 396
Acidobacterium         LG-QARVIAAPSCSLLHVPHTLRGETQLPARMRGWLRFAEEKLAELTALAT------GDP 370
Acidiphilium           LGGSERLMIAPSCSLLHVPVDLAQEDRLDPAIRPWLAFATQKLAEVATIARGLDEGEGAI 376
Thermotoga             -----ASAISNSCPLFHLPVTLELENNLPGGLKEKLAFAKEKLEELKMLKDFLEGK---T 353
Streptococcus          SA---ALTIQPSCSLLHVPVTTKNETDLDPVLRNGLAFADEKLTEVKRLAEHLDGREDPA 364
                                  **.*:* *     *  *    :  : *: :*  *:  :
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E.coli               TAALAEWSAPIQARRHSTRVHNPAVEKRLAAITAQDSQRANVYEVRAEAQRARFKLPAWP 427
Salmonella           TAAVTEWSAPIQARRHSTRVHNVGVEKRLAAITAQDSQRENPYEVRAEAQRARFKLPAWP 427
Yersinia             LAELAAYSAPIRARRSSSRVHNAQVEQRLAAITSQDIERQLPYEARAETQRKRFNLPAWP 435
Vibrio               ILACDTYSQPIVARKSSHIVNKASVQTRINNITAALAERSAPYIERAHHQAEVLGLPFLP 432
Acidithiobacillus    AQELAAARAAVASRKSSPRIHNPAVAQRLEGLGQDDGRRKSPFPIREAAQRARFKLPAFP 456
Acidobacterium       AAAAEN-AAAIQDRAAAESSTNAAVRAQLHALKASDFTRSSAYPERARVQRDQLRLPLFP 429
Acidiphilium         AEALEASDAALRTRRDSARVHRTDVAARLLGATPEMERRPAPHAARRARQRQRLPLPAFP 436
Thermotoga           FDLPNVSFEDFAVDLQA--------VERVRNLPEDSFRREKEYTERDRIQRERLNLPLFP 405
Streptococcus        YDLHIAHFDALQAADFR--------NVKLEDLSRVATKRPSDFAKRRDIQQEKLHLPLLP 416
                               .                ::         *   .  *   *   : **  *

E.coli               TTTIGSFPQTTEIRTLRLDFKKGNLDANNYRTGIAEHIKQAIVEQERLGLDVLVHGEAER 487
Salmonella           TTTIGSFPQTTEIRGLRLDFKKGNLDANHYRTGIAEHIRQAIIEQERLGLDVLVHGEAER 487
Yersinia             TTTIGSFPQTTEIRGLRLDFKQGRLDGKNYRTGISEHIKHAIAEQERLGLDVLVHGEAER 495
Vibrio               TTTIGSFPQTGEIRTERSAYRQGKLSEQEYVQALKGHIADAVKRQEALGLDVLVHGEAER 492
Acidithiobacillus    TTSIGSFPQTPEIRKARLQNRKGELSNADYQKAMEAEIALVVKEQERLGIDVPVHGEPER 516
Acidobacterium       TTTIGSFPQTSEVRKHRAAHKHGHETAGQYETFLREATAQCIRQQEAIGLDVLVHGEFER 489
Acidiphilium         TTTIGSLPQTSGVRRTRAALARGEIGAAEYDEAIATWTEDAIRLQERIGLDVLVHGEFER 496
Thermotoga           TTTIGSFPQTPEVRKMRSKYRKGEISKEEYEAFIKEQIKKAIELQEEIGLDVLVHGEFER 465
Streptococcus        TTTIGSFPQSREIRRTRLAWKRGDISDAEYKQFIQAEIERWIRIQEDLDLDVLVHGEFER 476
                     **:***:**:  :*  *    :*     .*   :       :  ** :.:** **** **

E.coli               NDMVEYFGEHLDGFVFTQNGWVQSYGSRCVKPPIVIGDISRPAPITVEWAKYAQSLTDKP 547
Salmonella           NDMVEYFGEHLDGFVFTQNGWVQSYGSRCVKPPVVIGDISRPAPITVEWAKYAQSLTDKP 547
Yersinia             NDMVEYFGEHLDGFVFTQNGWVQSYGSRCVKPPVIIGDISRPEAITVEWAKYAQSLTEKP 555
Vibrio               NDMVEYFAENLAGFQTTQFGWVQSYGSRCVKPAIVVADIEREKPITVEWSTYAQSLTSKQ 552
Acidithiobacillus    NDMVEYFGEQLAGFAFTRHGWVQSYGSRYVKPPLIFGDVSRPTPMTVAWSKYAQSLTQRP 576
Acidobacterium       NDMVEYFAEFLEGFAFTQNGWVQSYGSRCVKPPVIFGDVSRPRAMTVEWSRYAQSLTERP 549
Acidiphilium         NDMVKYFGEQLDGFAFTRHGWVQSYGSRCVAPPIIWGDVARPRPMTVRWARHAQSLTARP 556
Thermotoga           TDMVEFFAEKLNGIATTQNGWVLSYGSRCYRPPIIYGTVTRPEPMTLKEITYAQSLTEKP 525
Streptococcus        VDMVEFFGQKLAGFTTTKFGWVQSYGSRAVKPPIIYGDVQHLEPITVEETVYAQSLTDRP 536
                      ***::*.: * *:  *: *** *****   *.:: . : :  .:*:    :***** :

E.coli               VKGMLTGPVTILCWSFPREDVSRETIAKQIALALRDEVADLEAAGIGIIQIDEPALREGL 607
Salmonella           VKGMLTGPVTILCWSFPREDVTRETIAKQIALALRDEVADLEAAGIGIIQIDEPALREGL 607
Yersinia             VKGMLTGPVTILCWSFPREDVSRETIAKQIALALRDEVEDLEKAGIGIIQIDEPALREGL 615
Vibrio               MKGMLTGPVTILCWTFPREDISRQEIAQQLALALRDEVADLQDAGINIIQIDEPAIREGL 612
Acidithiobacillus    MKGMLTGPVTILQWSFVRDDQPRERTALQIALAIRDEVRDLIDAGIGIIQIDEPAYREGL 636
Acidobacterium       VKGMLTGPITILQWSFPRNDIPRQQIAWQIALALRDEVQDLEAAGIRIIQVDEPALREGL 609
Acidiphilium         VKGMLTGPVTMLQWSFVRDDLPRETVCRQIALALRDEVADLEAAGLAIIQVDEPAFREGL 616
Thermotoga           VKGMLTGPVTIMSWSYYREDIPEREIAYQIALAINEEVKDLEEAGIKIVQIDEPAFREKA 585
Streptococcus        VKGMLTGPITITNWSFERTDIPRDQLFNQIGLAIKDEIKLLENAGIAIIQVDEAALREGL 596
                     :*******:*:  *:: * * ..     *:.**:.:*:  *  **: *:*:**.* **

E.coli               PLRRSDWDAYLQWGVEAFRINAAVAKDDTQIHTHMCYCEFNDIMDSIAALDADVITIETS 667
Salmonella           PLRRSDWDAYLAWGVEAFRINAAAAKDETQIHTHMCYCEFNDIMDSIAALDADVITIETS 667
Yersinia             PLRRADWQAYLQWAVDAFKLNAAVAQNDTQIHTHMCYCEFNDIMDSIAALDADVITIETS 675
Vibrio               PLKKRDHQTYLDWAVQAFKISAGSARPETQIHTHMCYSEFNEIIESVAALDADVITIETS 672


Acidithiobacillus    PLKRKDWGHYLEWASRAFRISAQIAPDDVQIHTHMCYSEFNDILPAIAAMDADVITIETS 696
Acidobacterium       PLRRADWAAYLEWAVQAFRLATSGVRDETQIHTHMCYCEFEDVLPSIAALDADVISMEAA 669
Acidiphilium         PLRTADREAYLRWAVSCFRLATAVVRDDTAIHTHMCYAEFQDIMPAIATMDADAISIETA 676
Thermotoga           PIKKSKWPEYFEWAINAFNLAAN-ARPETQIHAHMCYSDFNEIIEYIHQLEFDVISIEAS 644
Streptococcus        PLRKSKQKAYLDDAVHAFHIATSSVKDETQIHTHMCYSKFDEIIDAIRALDADVISIETS 656
                     *::  .   *:  .  .*.: :  .  :. **:****  *::::  :  :: *.*::*::
                                                      C645A

E.coli               RSDMELLESFEEFD---YPNEIGPGVYDIHSPNVPSVEWIEALLKKAAKRIPAERLWVNP 724
Salmonella           RSDMELLESFEAFD---YPNEIGPGVYDIHSPNVPSVEWIEALLKKAAQRIPAQRLWVNP 724
Yersinia             RSDMELLESFEDFA---YPNEIGPGVYDIHSPNVPSVEWIEALLRKAAQRIPAERLWVNP 732
Vibrio               RSNMELLKAFEEFN---YPNEIGPGVYDIHSPNIPAQAWIEDLLRKAAEKIPAQRLWVNP 729
Acidithiobacillus    RSQMELLDAFATFN---YPNEIGPGVYDIHSPRVPSVEEMVGLMEKAVKVVPAERLWINP 753
Acidobacterium       RSRMELLDAFREHG---YPNEIGPGVYDIHSPRVPSVEEMHELLRLAARVLKPGQLWVNP 726
Acidiphilium         RSRMELLEAFAGHGPSAYPAEIGPGVWDIHSPRIPPEEEILALLRLARRKLADDQLWVNP 736
Thermotoga           RSKGEIISAFENFKG--WIKQIGVGVWDIHSPAVPSINEMREIVERVLRVLPKELIWINP 702
Streptococcus        RSHGDIIESFETAV---YPLGIGLGVYDIHSPRVPTKEEVVANIERPLRQLSPTQFWVNP 713
                     **  :::.:*       :   ** **:***** :*.   :   :.   . :    :*:**
                                                                 K713E

E.coli               DCGLKTRGWPETRAALANMVQAAQNLRRG----  753
Salmonella           DCGLKTRGWPETRAALANMVKAAHNLRQAE---  754
Yersinia             DCGLKTRGWPETRQALANMVLAAQRLREEQV--  763
Vibrio               DCGLKTRNWPEVEAALTNMVNAAKALRAEWQA-  761
Acidithiobacillus    DCGLKTRKWAEVTPALENMVEAARQVRARHG--  784
Acidobacterium       DCGLKTRKWPETIAALTNLCAAAERLREEAGA-  758
Acidiphilium         DCGLKTRNWREVIPALENLVGAARRLRAEAIPA  769
Thermotoga           DCGLKTRNWDEVIPSLRNMVALAKEMREKFES-  734
Streptococcus        DCGLKTRQEPETIAALKVLVAATKEVRQKLGN-  745
                     *******   *.  :*  :   :. :*
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Figure S3. ClustalW2 multiple sequence alignment of the MetE protein sequences. Amino acid substitutions in MetE$_{E.\ coli}$ -214A protein are indicated in the boxes. Abbreviations: *Salmonella* - *Salmonella enterica* Arizonae (YP_001572639.1)*; Yersinia* - *Yersinia pestis* Angola (NP_667780.1); *Vibrio*- *Vibrio cholerae* M662 (NP_231340.1)*; Acidithiobacillus* - *Acidithiobacillus ferrooxidans* ATCC 53993 (YP_002218926.1)*; Acidobacterium* - *Acidobacterium capsulatum* ATCC 51196 (YP_002755941.1); *Acidiphilium* - *Acidiphilium cryptum* JF-5 (P_001235325.1); *Thermotoga* - *Thermotoga maritima* MSB8 (NP_229090.1); *Streptococcus* - *Streptococcus mutans* UA159 (NP_721282.1)

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2. **Tomoyasu T, Mogk A, Langen H, Goloubinoff P, Bukau B.** 2001. Genetic dissection of the roles of chaperones and proteases in protein folding and degradation in the *Escherichia coli* cytosol. Mol Microbiol, **40**: 397-413.