Supplementary Information for

# Kinetic and Inhibition Studies of Dihydroxybenzoate-AMP Ligase (EntE) from *Escherichia coli*[†]

Alison L. Sikora[1], Daniel J. Wilson[2], Courtney C. Aldrich[2], and John S. Blanchard[1]*

[1]Albert Einstein College of Medicine, Department of Biochemistry

1300 Morris Park Avenue, Bronx, NY 10461

[2]Center for Drug Design, Academic Health Center, University of Minnesota,

Minneapolis, MN 55455

```
EntE    1  ---------- -----MSIPF TRWPEEFARR YREKGYWQDL PLTDILTRHA AS--DSIAVI   43
DhbE    1  ---------- -----MLKGF TPWPDELAET YRKNGCWAGE TFGDLLRDRA AKYGDRIAIT   45
VibE    1  ---------- -----MTTDF TPWPEALAAQ YRQLGYWQDK TLLDYLQQSA ERTPNALALV   45
YbtE    1  ---------- -----MNSSF ----ESLIEQ YP-------L PIAEQLRHWA ARYASRIAVV   34
MbtA    1  MPPKAADGRR PSPDGGLGGF VPFPADRAAS YRAAGYWSGR TLDTVLSDAA RRWPDRLAVA   60

EntE   44  DG-----ERQ LSYRELNQAA DNLACSLRRQ GIKPGETALV QLGNVAELYI TFFALLKLGV   98
DhbE   46  CG-----NTH WSYRELDTRA DRLAAGFQKL GIQQKDRVVV QLPNIKEFFE VIFALFRLGA  100
VibE   46  GD-----NQQ WRYQAMLERI EQLAAGFTEL GLGCGDNVVL QLGNVAEFYL CFFALLRQGI  100
YbtE   35  DA-----KGS LTYSALDAQV DELAAGLSSL GLRSGEHVIV QLPNDNAFVT LLFALLRLGV   89
MbtA   61  DAGDRPGHGG LSYAELDQRA DRAAAALHGL GITPGDRVLL QLPNGCQFAV ALFALLRAGA  120

EntE   99  APVLALFSHQ RSELNAYASQ IEPALLIADR QHALFSGDDF LNTFVTEHSS IRVVQLHNDS  158
DhbE  101  LPVFALPSHR SSEITYFCEF AEAAAYIIPD AYSGFDYRSL ARQVQSKLPT LKNIIVAGEA  160
VibE  101  RPILALPAHR LAEIRYFCQH SQAKAYLIDG AQRPFDYQAL AQELLACCPT LQTVIVRGQT  160
YbtE   90  IPVLAMPSQR ALDIDALIEL AQPVAYVIHG EN----HAEL ARQMAHKHAC LRHVLVAGET  145
MbtA  121  IPVMCLPGHR AAELGHFAAV SAATGLVVAD VASGFDYRPM ARELVADHPT LRHVIVDGDP  180

EntE  159  GEHN---LQD AINHPAEDFT ATPSPADEVA YFQLSGGTTG TPKLIPRTHN DYYYSVRRSV  215
DhbE  161  EEFLP--LED LHTEPVKLPE VKSSD---VA FLQLSGGSTG LSKLIPRTHD DYIYSLKRSV  215
VibE  161  RVTDPKFIEL ASCYSASSCQ ANADP-NQIA FFQLSGGTTD TPKLIPRTHN DYAYSVTASV  219
YbtE  146  VSDDFTPLFS LHGERQAWPQ PDVSA---TA LLLLSGGTTG TPKLIPRRHA DYSYNFSASA  202
MbtA  181  GPFVS--WAQ LCAQAGTGSP APPADPGSPA LLLVSGGTTG MPKLIPRTHD DYVFNATASA  238

                                    *          *
EntE  216  EICQFTQQTR YLCAIPAAHN YAMSSPGSLG VFLAGGTVVL AADPSATLCF PLIEKHQVNV  275
DhbE  216  EVCWLDHSTV YLAALPMAHN YPLSSPGVLG VLYAGGRVVL SPSPSPEIAF PLIEREKVTI  275
VibE  220  EICRFDQHTR YLCVLPAAHN FPLSSPGALG VFWAGGCVVL SQDASPQHAF KLIEQHKITV  279
YbtE  203  ELCGISQQSV YLAVLPVAHN FPLACPGILG TLACGGKVVL TDSASCDEVM PLIAQERVTH  262
MbtA  239  ALCRLSADDV YLVVLAAGHN FPLACPGLLG AMTVGATAVF APDPSPEAAF AAIERHGVTV  298

EntE  276  TALVPPAVSL WLQALTEGES RAQLASLKLL QVGGARLSAT LAARIPAEIG CQLQQVFGMA  335
DhbE  276  TALVPPLAMV WMDAASS--R RDDLSSLQVL QVGGAKFSAE AARRVKAVFG CTLQQVFGMA  333
VibE  280  TALVPPLALL WMDHAEK--S TYDLSSLHFV QVGGAKFSEA AARRLPKALG CQLQQVFGMA  337
YbtE  263  VALVPALAQL WVQAREW--E DSDLSSLRVI QAGGARLDPT LAEQVIATFD CTLQQVFGMA  320
MbtA  299  TALVPALAKL WAQSCEW--E PVTPKSLRLL QVGGSKLEPE DARRVRTALT PGLQQVFGMA  356

EntE  336  EGLVNYTRLD DSAEKIIHTQ GYPMCPDDEV WVADAEGNPL PQGEVGRLMT RGPYTFRGYY  395
DhbE  334  EGLVNYTRLD DPEEIIVNTQ GKPMSPYDEV RVWDDHDRDV KPGETGHLLT RGPYTIRGYY  393
VibE  338  EGLVNYTRLD DSAELIATTQ GRPISAHDQL LVVDEQGQPV ASGEEGYLLT QGPYTIRGYY  397
YbtE  321  EGLLCFTRLD DPHATILHSQ GRPLSPLDEI RIVDQDENDV APGETGQLLT RGPYTISGYY  380
MbtA  357  EGLLNFTRIG DPPEVVEHTQ GRPLCPADEL RIVNADGEPV GPGEEGELLV RGPYTLNGYF  416

EntE  396  KSPQHNASAF DANGFYCSGD LISIDPEGYI TVQGREKDQI NRGGEKIAAE EIENLLLRHP  455
DhbE  394  KAEEHNAASF TEDGFYRTGD IVRLTRDGYI VVEGRAKDQI NRGGEKVAAE EVENHLLAHP  453
VibE  398  RADQHNQRAF NAQGFYITGD KVKLSSEGYV IVTGRAKDQI NRGGEKIAAE EVENQLLHHP  457
YbtE  381  RAPAHNAQAF TAQGFYRTGD NVRLDEVGNL HVEGRIKEQI NRAGEKIAAE EVESALLRLA  440
MbtA  417  AAERDNERCF DPDGFYRSGD LVRRRDDGNL VVTGRVKDVI CRAGETIAAS DLEEQLLSHP  476

EntE  456  AVIYAALVSM EDELMGEKSC AYLVVK--EP LRAVQVRRFL REQGIAEFKL PDRVECVDSL  513
DhbE  454  AVHDAAMVSM PDQFLGERSC VFIIPR-DEA PKAAELKAFL RERGLAAYKI PDRVEFVESF  512
VibE  458  AVHDAALIAI SDEYLGERSC AVIVLKPEQS VNTIQLKRFL HQAGLADYKI PDQIQFIDQL  517
YbtE  441  EVQDCAVVAA PDTLLGERIC AFIIAQ-QVP TDYQQLRQQL TRMGLSAWKI PDQIEFLDHW  499
MbtA  477  AIFSAAAVGL PDQYLGEKIC AAVVFA-GAP ITLAELNGYL DRRGVAAHTR PDQLVAMPAL  535

EntE  514  PLTAVGKVDK KQLRQWLASR ASA-------  536
DhbE  513  PQTGVGKVSK KALREAISEK LLAGFKK---  539
VibE  518  PKTSVGKIDK NALRRRFDTL GLALMS----  543
YbtE  500  PLTAVGKIDK KRLTALAVDR YRHSAQ----  525
MbtA  536  PTTPIGKIDK RAIVRQLGIA TGPVTTQRCH  565
```

**Figure S1**.   Sequence conservation of aryl acid adenylating enzymes (AAAE) in siderophore biosynthetic pathways.   Sequence alignment of adenylate-forming enzymes from *Escherichia coli* (gi:284822083) and homologues from *Bacillus subtilis* (gi:23306686), *Vibrio cholerae* (gi:9837411), *Yersinia pestis* (gi:3818608), and *Mycobacterium tuberculosis* (gi:3261670).   Identical and similar residues are highlighted in green and yellow shading, respectively.   Residues N235 and S240 are indicated by red asterisks.