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Supporting information for article:

Structure of shikimate kinase, an *in vivo* essential metabolic enzyme in the nosocomial pathogen *Acinetobacter baumannii*, in complex with shikimate

Kristin A. Sutton, Jennifer Breen, Ulrike MacDonald, Janet M. Beanan, Ruth Olson, Thomas A. Russo, L. Wayne Schultz and Timothy C. Umland

Supplementary Material:

Crystal structure of shikimate kinase in complex with shikimate, an *in vivo* essential metabolic enzyme in the nosocomial pathogen *Acinetobacter baumannii*

Kristin A. Sutton, Jennifer Breen, Ulrike MacDonald, Janet M. Beanan, Ruth Olson,

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Supplementary Table S1

abSK prokaryotic homologues annotated as essential in the DEG database*.

Essential SK Homologue	Non-Essential SK Homologue**	
<i>Acinetobacter baylyi</i> ADP1	<i>Bacillus subtilis</i> 168	<i>Pseudomonas aeruginosa</i> PAO1
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> NCTC 11168	<i>Bacteroides fragilis</i> 638R	<i>Pseudomonas aeruginosa</i> PAO1
<i>Caulobacter crescentus</i>	<i>Bacteroides thetaiotaomicron</i> VPI-5482	<i>Salmonella enterica</i> serovar Typhi
<i>Escherichia coli</i> MG1655 I	<i>Burkholderia pseudomallei</i> K96243	<i>Salmonella enterica</i> serovar Typhi Ty2
<i>Francisella novicida</i> U112	<i>Burkholderia thailandensis</i> E264	<i>Salmonella enterica</i> serovar Typhimurium SL1344
<i>Haemophilus influenzae</i> Rd KW20	<i>Escherichia coli</i> MG1655 II	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium str. 14028S
<i>Helicobacter pylori</i> 26695	<i>Mycobacterium tuberculosis</i> H37Rv II	<i>Salmonella typhimurium</i> LT2
<i>Mycobacterium tuberculosis</i> H37Rv	<i>Mycobacterium tuberculosis</i> H37Rv III	<i>Staphylococcus aureus</i> N315
<i>Pseudomonas aeruginosa</i> UCBPP-PA14	<i>Mycoplasma genitalium</i> G37	<i>Staphylococcus aureus</i> NCTC 8325
<i>Shewanella oneidensis</i> MR-1	<i>Mycoplasma pulmonis</i> UAB CTIP	<i>Streptococcus pneumoniae</i>
<i>Sphingomonas wittichii</i> RW1	<i>Porphyromonas gingivalis</i> ATCC 33277	<i>Streptococcus sanguinis</i>
		<i>Vibrio cholerae</i> N16961

* DEG database v.11.0 (<http://tubic.tju.edu.cn/deg>); the majority of the compiled essentiality screens were conducted on rich laboratory media and not *in vivo*.

** Several species are present in both the Essential SK and Non-Essential SK Homologue categories due to discrepancies between independent essentiality screens.

AB307_0294

η1 β1
 $\underbrace{\quad\quad\quad}_{\eta1}$ $\underbrace{\quad\quad\quad}_{\beta1}$

AB307_0294	1MKAIEIGGALPSKAFETLPNIY
E_coli	1MAEKRNIF
C_burnetii	1MKKNLTNIY
D_chrysanthemi	1MTEPIF
A_aeolicus	1MRIY
M_tuberculosis	1MAPKAV
B_thetaiotaomicron	1MVRIF
A_thaliana	52	RDHRLRSVSDKNSSALLETGSLHSPFDEEQILKKKAEVVKPYLNGRSMY
H_pylori	1MQHLV
C_jejuni	1MKAKNIV

AB307_0294

→ TT α1 β2 α2 α3

AB307_0294	23	LVGPMGAGKTVGRHLAELLGREFLDSDEHEIERK.TGATIPWIFEFKEGEVGV
E_coli	9	LVGPMGAGKSTIGRQLAQQLNMEFYDSDEHEIERK.TGADVGVVFDLEGEEG
C_burnetii	10	LIGLMGAGKTSVGSQAKLTKRILYDSDEHEIERK.TGADIAWIFEMEGEAG
D_chrysanthemi	7	MVGARGCGKTVGRELARALGYEFVDTDFMQHT.SGMTVADVVAEEGWPG
A_aeolicus	5	LIGFMCSGKSTVGSLSRSLNIPFYDVEEVQKR.EGLSIPQIFEFKGEAY
M_tuberculosis	7	LVGLPGSGKSTIGRRLAKALGVGLLDTDVAIEQR.TGRSIADIFATDGEQE
B_thetaiotaomicron	6	LTGYMGAGKTLGKAFARKLVNPFIDLWYIEER.FHKTVGELFETERGEAG
A_thaliana	103	LVGMCSGKTVGKIMARSLGYTFDFCDTLEQAMKGTVAEIEFHFGEVSV
H_pylori	6	LIGFMCSGKSSLAQELGLALKLEVLDTDMIISER.VGLSVREIFEEELGEDN
C_jejuni	8	EIGFMCSGKSTLARA LAKDLVFLVDSDFLIEQK.FNQKVSEIFEQKRENF

AB307_0294

α4 β3 η2 α5 β4

AB307_0294	73	FRTRRETIVLNELTS.RKALVLA TGGGAITQAPNREFLKQRGIVVYLYTVE
E_coli	59	FRDREEKVINELTE.KQGI VLA TGGGSVKSRETRNRLSARGVVVYLETTIE
C_burnetii	60	FRRREREMIEALCK.LDNIILA TGGGVVLDKRNQOISETGVVIYLTASID
D_chrysanthemi	57	FRRRESEALQAVA..TPNRVVA TGGGMVLEQNRQFMRAHGTVVYLFAPAE
A_aeolicus	55	FRKLEFEVLKDLSE.KENNVIS TGGGLGANEALNFMKSRGTTVFIDIPFE
M_tuberculosis	57	FRRIEEDVRAALA.DHDG VLS TGGGAVTSPGVRAALAG.HTVVYLEISAA
B_thetaiotaomicron	56	FRLEERNMLHEVAE.FENVVIS TGGGAPCFYDNMFMNRTGKTVFLNVHPD
A_thaliana	154	FRKEKTEALKKLSLMYHQVVVST TGGCAVIRPINWKYMHK.GISIWLDVPLE
H_pylori	56	FRMFEEKNLIDELKTLKTPHVIS TGGGI VMH...ENLKG LGTTFY LKMDFE
C_jejuni	58	FRREQEQKMAFFSS.CEKACIA TGGGFVNV...SNLEKAGFCIY LKADFE

AB307_0294

α6 TT η3 α7

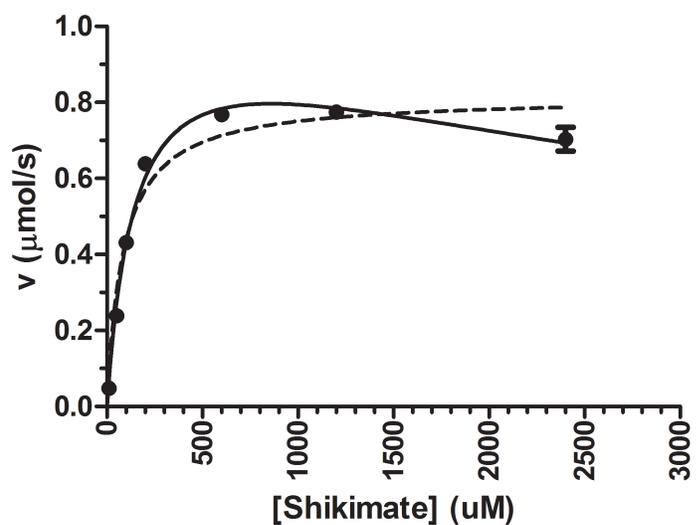
AB307_0294	123	LQLQR TYRD...KNR P L L QVENPE.....QK.LRDLLKIR D P L YREVAVH
E_coli	109	KQLARTQRD...KKR P L L HVETPP.....REVLEALANER N P L YEEIADV
C_burnetii	110	TQLKRIGQK.GEMRR P L L .FIKNS.....KEKLQQLNEIR K P L YQAMADL
D_chrysanthemi	106	ELALR LQAS PQAHQ R P L L TG.RPI.....AEEMEAVLRE R EAL YQDVAVH
A_aeolicus	105	VFLE RCKDS...KER P L L KR..P.....LDEIKNLFEE R R K I Y S K A . D I
M_tuberculosis	106	EGVRR TGGN...TVR P L L AG.PDR.....AEKYRALMAK R A P L Y R R V A T M
B_thetaiotaomicron	106	VLFR R L R I A . K Q Q R P L L Q G K E D D E L . . . M D F I I Q A L E K R A P F Y T Q A . Q Y
A_thaliana	204	ALAH R I A A V . G T G S R P L L H D D E S G D T Y T A A L N R L S T I W D A R G E A Y T K A S A F
H_pylori	103	TLIK R L N Q K . E R E K R P L L N N L T Q A K E L F E K R Q A L Y E K N A S F
C_jejuni	104	YLKK R L D K D . E I S K R P L L F Y D E I K A K K L Y N E R L S K Y E Q K A N F

AB307_0294

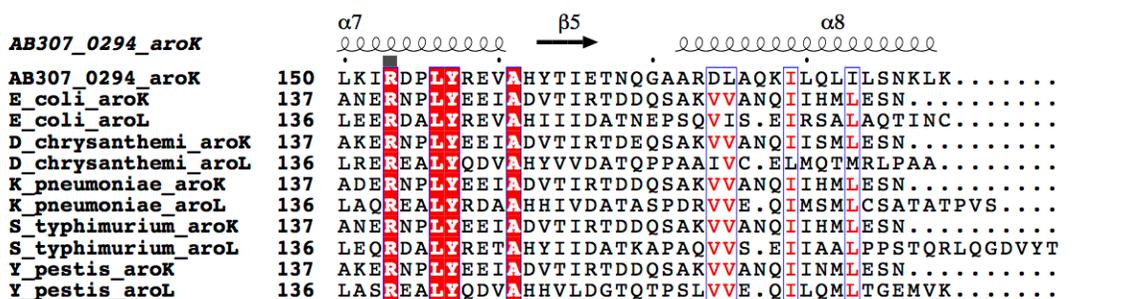
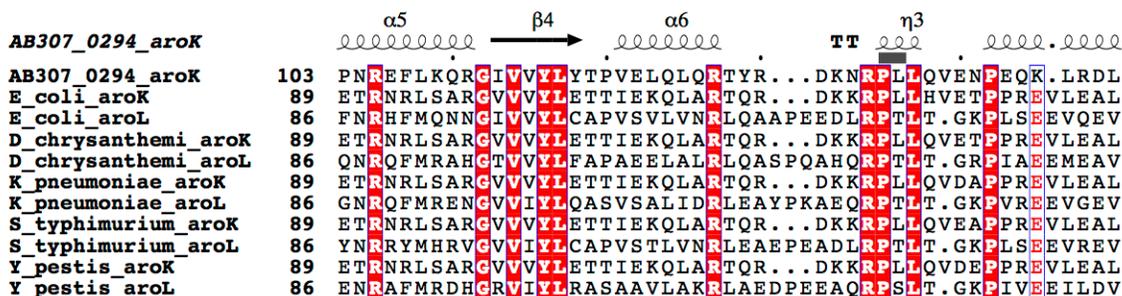
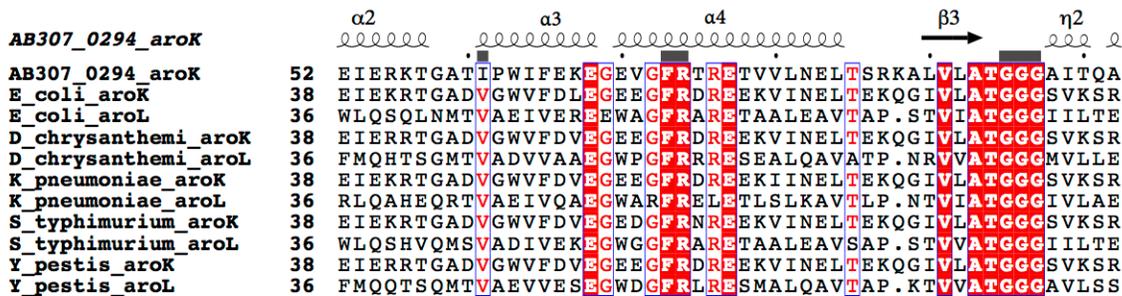
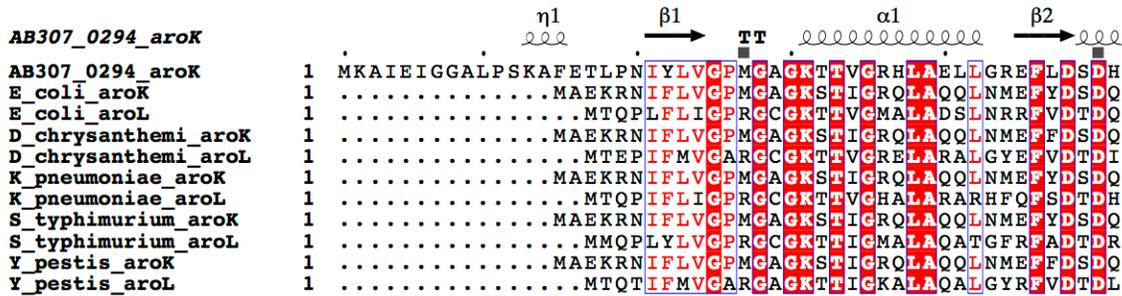
β5 α8

AB307_0294	164	TIETNQ.....GAARDLAQKILQLILSNKLLK.....
E_coli	151	TIRTD.....QSAKVVANQIIHMLSN.....
C_burnetii	153	VYPTDD.....LNPRQLATQILVDIKQTYSDL.....
D_chrysanthemi	150	VVDATQ.....PPAAIV.CELMQTMRLPAA.....
A_aeolicus	143	KVKGEK.....PPEEVVKEIILSLEGNALGG.....
M_tuberculosis	147	RVDTNR.....RNP GAVVRHILSRLQVPSPEAAT.....
B_thetaiotaomicron	150	IFNADE.....LEDRWQIESSVQRLQELL.EL.....
A_thaliana	254	.VSLNITLKLGYRSVSDLTPAEIAIEAFEQVQSYLEKEDGMARPDGL
H_pylori	143	IIDARG.....GLNNSLK.QVLQFIA.....
C_jejuni	144	ILNIEN.....KNIDELLSEIKKVIK.....

Supplementary Figure S1. Multiple sequence alignment of abSK and the nine SK homologues represented in the PDB. Bars under the sequences indicate abSK functional motifs, using the same color scheme as Fig. 2. Dark grey rectangles above the sequences denote abSK residues that contact shikimate. The N-terminal 51 residues of *A. thaliana* SK have been excluded. Solid boxes indicate fully conserved residues and outlined boxes indicate residues conserved at $\geq 80\%$. Secondary structure based on abSK crystal structure.



Supplementary Figure S2. abSK shikimate kinetics data analysis. Non-linear regression analyses of initial velocity versus shikimate concentration in an excess of ATP using a substrate inhibition model (solid line, $R^2=0.99$), and assuming conventional Michaelis-Menten kinetics (dashed line, $R^2=0.81$). The assay was conducted in triplicate.



Supplementary Figure S3. Multiple sequence alignment of abSK (*aroK*) and several bacterial pathogens that possess SK paralogues encoded by *aroK* and *aroL* genes. Bars under the sequences indicate abSK functional motifs, using the same color scheme as Fig. 2.

Dark grey rectangles above the sequence denote abSK residues that contact shikimate. Solid boxes indicate fully conserved residues and outlined boxes indicate residues conserved at $\geq 80\%$. Secondary structure based on abSK crystal structure.