

SUPPLEMENTARY INFORMATION

An allosteric pocket for inhibition of bacterial Enzyme I identified by NMR-based fragment screening

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E. coli	1	MISGILASPGIAFGKALLLKEDEIVIDRKKISADQVDQEVERFLSGRAKASQALETIKTK	60
C. freundii	1	MISGILASPGIAFGKALLLKEDEIVIDRKKISADKVDQEVERFLSGRAKASQALEAIKTK	60
K. pneumoniae	1	MISGILASPGIAFGKALLLKEDEIVIDRKKISADKVDQEVERFLSGRAKASQALEVIKTK	60
S. aureus	4	LIKGIAASDGVAIKAYLLVPEPDLTFDKNEKVD--VEGEVAKFNSAIEASKVELTKIRNN	62
E. faecalis	4	MLKGIASDGVAVAKAYLLVQPDLSFN--KTSVEDTDAEATRLDDALAKSTEELQAIRDK	61
N. gonorrhoeae	4	VLHGVAAGKGIAGVCAHLIARGTEEVPQYDVAQADTDAEAERFDAAVKATRKELEQLRSA	63
E. coli	61	AGETFGEKEEAI FEGHIMLLEDEELEQE I IALIKDKHMTADAAAHEVIEGQASALEELDD	120
C. freundii	61	AGETFGEKEEAI FEGHIMLLEDEELEQE I IALIKDKHMTADAAAHEVIEGQATALEELDD	120
K. pneumoniae	61	AGETFGEKEEAI FEGHIMLLEDEELEQE I IALIKDKHMTADAAAHEVIDGQATALEELDD	120
S. aureus	63	AEVQLGADKAAIFDAHLLVLDDELPDIQPIQDKIKNENANAATALTDTVTTQFVTIFESMDN	122
E. faecalis	62	AAQSLGEAEAVFADAHMLVSDPEMVGQIKQNIQDNKNVAEALKEVTDYIMGFEMDD	121
N. gonorrhoeae	64	IPENAPTELGAFISLHMLLTDVTLRSREPV DILREQKINAEWALKQQSDKLAQFDNMDD	123
E. coli	121	E-YLKERAADV RDIGKRLRLN ILG----LKI IDLSAIQDEVILVAADLTPSETAQLNLKK	175
C. freundii	121	E-YLKERAADV RDIGKRLRLN ILG----LAI IDLSAIQEEVILVAADLTPSETAQLNLNK	175
K. pneumoniae	121	E-YLKERAADV RDIGKRLRLN ILG----LAI IDLSAIQDEVILVAADLTPSETAQLNLKK	175
S. aureus	123	E-YMKERAADIR DVSKRVL SHILG----VELPNPSMIDESVIVGNDLTPSDTAQLNKEF	177
E. faecalis	122	NAYMQERAADIR DVAKRI LAHLLG----VTLPNPSMINEEVIVVAHDLTPSDTAQLDRTY	177
N. gonorrhoeae	124	A-YLRERKQDMLQVVRRIHNNLIGQGNELEVAD--NLFDETVLIANDLS PADTVLFKEQR	180
E. coli	176	VLGFITDAGGRS HTS IMARSLELPAIVGTGSVTSQVKNDY LILDVAVNNQVYVNPTEV	235
C. freundii	176	VLGFITDAGGRS HTS IMARSLELPAIVGTGSVTSQVKNDY LILDVAVNNQVYVNPTEV	235
K. pneumoniae	176	VLGFITDAGGRS HTS IMARSLELPAIVGTGSITAQVKNQDYLILDVAVNNQVLINPSNEQ	235
S. aureus	178	VLGFATNIGGRS HSAIMARSLEIPAI VGTKSITQEVKQGDMIIVDGLNGDVIVNPTEDE	237
E. faecalis	178	VKAFVTDIGGRS HSAIMARSLEIPAI VGTKEITDKVKAGDILAVNGIIGDVIIDPTDAE	237
N. gonorrhoeae	181	IAAFVTDAGGPTG HTAILGRSLDIPSVVGLHNARKLITEGETVIVDGINGVLIISPDES	240
E. coli	236	IDKMRVQEQVASEKAELAKLKDLPAILTLDGHQVEVCANIGTVRD VEGAERNGAEGVGLY	295
C. freundii	236	IEQLRAVQEQVATEKAELAKLKDLPAILTLDGHQVEVCANIGTVRD VEGAERNGAEGVGLY	295
K. pneumoniae	236	IEALRSLQAQVAEKAELAKLKDLPAILTLDGHQVEVCANIGTVRD VEGAERNGAEGVGLY	295
S. aureus	238	LIAYQDKRERYFADKKEQLKLRDADTVTV DGVHAEALANIGTPNDLPGVIEAQAQGI GLY	397
E. faecalis	238	LSEFEAEAKAYADQKAEDWLKNAETVTADGKHVELAANIGTPKDLEGVHKNGEAVGLY	297
N. gonorrhoeae	241	KNEYRRAREYRSHKRDLNKLKTAATAADGVCIELVGNIESAEDVKPLHNLGADGIGLF	300
E. coli	296	RTEFLFMDRDALPTEEEQFAAYKAVAEACGSQAVIVRTMDIGGDKELPYMNFPEE----	351
C. freundii	296	RTEFLFMDRESLPTREEQFAAYKAVAEACGSQAVIVRTMDIGGDKELPYMNFPEE----	351
K. pneumoniae	296	RTEFLFMDRDALPTEEEQFAAYKAVAEACGSQAVIVRTMDIGGDKELPYMNFPEE----	351
S. aureus	298	RTEFLYMG RDQMPTEEEQFEAYKVEAMGGKRVVVRTLDIGGDKELSYLNLPEEM----	353
E. faecalis	298	RTEFLYMDSDFPTEEDQYQAYKAVLEGMGKPVVVRTMDIGGDKELPYLTLPHEM----	353
N. gonorrhoeae	301	RSEFLYLNRDTPSEDEQYEVYSAIVKMKGKSVTIRTVDLGVDKNPRW--FGKNSTPNG	358
E. coli	352	--NPFLGWRAIRIAMDR--REILRDQLRAILRASAFGKLRIMFPMIISVEEVRALRKEIE	407
C. freundii	352	--NPFLGWRAVRIAMDR--KEILRDQVRAILRASAFGKLRIMFPMIISVEEVRALRKEIE	407
K. pneumoniae	352	--NPFLGWRAVRIAMDR--KEILRDQVRAILRASAFGKLRIMFPMIISVEEVRALRKEIE	407
S. aureus	354	--NPFLGWRAIRLCLAQ--QDIFRPQLRALLRASVYGKLNIMFPMVATINEFREAKAILL	409
E. faecalis	354	--NPFLGWRAIRLISLSELGDMFRQTMRALLRASVHG NLRIMFPMVATLKEFRAAKAIFE	411
N. gonorrhoeae	359	SLNPALGM TGIRLCLAE--PVMFRQTMRRAILRAAVHGPVRMMWPMITSVSEVRQCLIHL	415
E. coli	408	IYKQELRDEGKAFDESIEIGVMVETPAAAT IARHLAKEVDFFSIGTNDLTQYTLAVDRGN	467
C. freundii	408	IYKQELRDEGKAFDESIEIGVMVETPAAAT IARHLAKEVDFFSIGTNDLTQYTLAVDRGN	467
K. pneumoniae	408	IYKQELRDEGKAFDESIEIGVMVETPAAAT IARHLAKEVDFFSIGTNDLTQYTLAVDRGN	467
S. aureus	410	EEKENLNKNEGHDISDIEELGIMVEIPATAALADVFAKEVDFFSIGTNDLIQYTLAADRMS	469
E. faecalis	412	DEKQKLVNEGVEVSNDIQVGMIEIPAAAVLADKFAKEVDFFSVGTNDLIQYTMAADRMN	471
N. gonorrhoeae	416	TAQRQLAERGD AFGV-GVICMIEIPSAALTVGSI LKLVDFISVGTNDLIQYILSVDRGD	475
E. coli	468	DMISHLYQPMSPSVNLRIKQVIDASHAEGKWTGMC GELAGDERATLLLLGMGLDEFMSA	527
C. freundii	468	DMISHLYQPMSPSVLTLIKQVIDASHAEGKWTGMC GELAGDERATLLLLGMGLDEFMSA	527
K. pneumoniae	468	DMISHLYQPMSPSVNLRIKQVIDASHAEGKWTGMC GELAGDERATLLLLGMGLDEFMSA	527
S. aureus	470	ERVSYLQYPYNPSILRLVKQVIEASHKEGKWTGMC GEMAGDETAIPLLLGLGLDEFMSA	529
E. faecalis	472	ERVSYLQYPYNPSILRLIKNVIDAAHAEGKWAGMCGEMAGDQTA VPLLLGMGLDEFMSA	531
N. gonorrhoeae	476	DSVSHLYQPGHPSVLKMLQHVIRTANRMDKDVSVCGEMAGDTAFTRVLLGMGLRRFSMNP	535
E. coli	528	ISIPRIKKIIRNTNFEDAKVLAEQALAQ-PTTDELMTLVNKFIEEKTIC	575
C. freundii	528	ISIPRIKKIIRNTNFEDAKVLAEQALAQ-PTTDELMTLVNKFIEEKTIC	575
K. pneumoniae	528	ISIPRIKKIIRNTNFEDAKVLAEQALAQ-PTTDELMTLVNKFIEEKTIC	575
S. aureus	530	TSILKARRQINGLSKNEMTELANRAVDC-ATQEEVIELVNNYVK	572
E. faecalis	522	TSILKTRSLMKRLDTTKMAELADRALKECDTMEEVFALVEEY	573
N. gonorrhoeae	536	NNILPVKNIIILHSN	549

Figure S1. EI sequence alignment. The sequence of the *E. coli* EI is aligned against EI from other, randomly selected, drug-resistant bacteria: *Citrobacter freundii* (identity 97%; similarity

99%; gaps 0%), *Klebsiella pneumoniae* (identity 96%; Similarity 98%; gaps 0%), *Staphylococcus aureus* (identity 51%; similarity 70%; gaps 0%), *Enterococcus faecalis* (identity 49%; similarity 68%; gaps 1%), and *Neisseria gonorrhoeae* (identity 36%; similarity 58%; gaps 2%). Residues forming the allosteric pocket are in red. Active site residues are in bold. Blue and red lines indicate the EIN and EIC domains, respectively.