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https:FFdoi.orgF6A.6A@9FjacFd7adA;? Ad!ance Access pu&lication 6 1arch 8A89

Journal of Antimicrobial Chemotherapy

Antibiotic-induced degradation of antitoxin enhances the transcription of acetyltransferase-type toxin-antitoxin operon

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3eceive(14 5ctober !"!!; accepte(6 *ebr&ary !"!)

Bac(ground. Bacterial toxin-antitoxin (TA modules respond to !arious stress"ul conditions. The #cn$-related 0-acetyltrans"erase-type toxin (#%AT protein encoded &y the #%AT-' ((TA locus is in!ol!ed in the anti&iotic tolerance o" Klebsiella pne&moniae.

,bjecti)es. To in!estigate the transcriptional mechanism o" the #%AT-' ((operon 7ac+T under anti&iotic stress.

'ethods. The transcriptional le!el o" the 7ac+T operon o" K8 pne&moniae) as measured &y *uantitati!e real-time (*'T +C' assay. The degradation o" antitoxin ,acA) as examined &y) estern &lot and -uorescent protein. The ratio o" .,acA/:.,acT/) as calculated &y the -uorescence intensity o" ,acA-e#0+ and mCherry-,acT. 1athematical modelling predicted protein and transcript synthesis dynamics.

/esults. A meropenem-induced increase in transcript le!els o" 7ac+ and 7acT resulted "rom the relie" "rom transcriptional autoregulation o" the 7ac+T operon. 1eropenem induces the degradation o" ,acA through 2on protease3 resulting in a reduction in the ratio o" .,acA/:.,acT/. The decreased ratio causes the dissociation o" the ,acAT complex "rom its promoter region3) hich eliminates the repression o" 7ac+T transcription. 4n addition3 our dynamic model o" 7ac+T expression regulation *uantitati!ely reproduced the experimentally o&ser!ed reduction o" the .,acA/:.,acT/ ratio and a large increase in 7ac+T transcript le!els under the condition o" strong promoter autorepression &y the ,acAT complex.

"onclusions. 1eropenem promotes the degradation o" antitoxin &y enhancing the expression o" 2on protease. 5egradation o" antitoxin reduces the ratio o" intracellular .antitoxin/:.toxin/3 leading to detachment o" the TA complex "rom its promoter3 and releasing repression o" TA operon transcription. These results may pro!ide an important insight into the transcriptional mechanism o" #%AT-' ((TA modules under anti&iotic stress.

ntroduction

A"ter the disco!ery o" the toxin-antitoxin (TA modules on &acterial plasmids3[6] these TA modules) ere also "ound on pro7aryotic chromosomes.[8,9] 5epending on the nature o" the antitoxin and its interaction) ith the toxin3 TA modules ha!e &een recently di!ided into eight types (types 4–:444 .;[10] A typical type 44 TA module consists o" a sta&le toxin protein and a meta&olically unsta&le antitoxin protein3 "orming a non-toxic TA complex.[$3<] =ome toxins contain a #cn$-related 0-acetyltrans"erase (#%AT domain that can inhi&it protein translation &y acetylating aminoacyl t'%As3 such as AtaT3[>] AtaT8[?] and 4taT[@] "rom 9scherichia coli3 TacT3 TacT8 and TacT9 "rom Salmonella enterica[6AB68] and #m!T "rom Shigella :e/neri.[69] Their cognate antitoxin proteins possess a ri&&on-helix-helix ('((domain. Cur pre!ious study sho) ed that ,acAT is a typical #%AT-'((TA module present in the K8 pne&moniae clinical isolate (=668?<3) here ,acT can halt K8 pne&moniae gro) th and induce anti&iotic tolerance.[6;36$]

Because TA modules are usually transcriptionally up-regulated under stress"ul conditions3 many studies ha!e proposed them as stress-response elements.[6<–6?] The transcription o" type 44 TA operons is usually autoregulated &y the toxin-antitoxin complex.[;] 4n such TA modules3 toxin-antitoxin complexes) ith di""erent a"Dnities "or the operon region are "ormed due to di""erent ratios o"

antitoxin to the toxin3) ith antitoxin-saturated complexes sho) - ing a high a"Dnity "or their promoter.[6@–86] Ghen the ratio o" antitoxin to the toxin &ecomes smaller3 the repression o" TA complexes on their promoter is alle!iated3 the autoregulation o" TA operons is relie!ed and the translation o" TA modules is increased.[6<36@–86] Ge ha!e pre!iously conDrmed that anti&iotics can increase the transcription o" 7acT3 and the transcriptional le!el o" 7ac+T) as automatically regulated &y the , acA: , acT ratio.[6$386] (o) e!er3 the transcriptional mechanism and autoregulation o" the 7ac+T operon under anti&iotic conditions are still unclear.

The C-terminal domain or the entire antitoxin protein is o"ten irregular and highly sensiti!e to cellular protease. AT+-dependent proteases ha!e &een identiDed as the most important intracellular proteolytic enHymes3 including the 2on (2a and Clp+ protease "amilies.[6>38] Although proteases can degrade antitoxins3 e!idence also sho) s that once the antitoxin "orms a sta&le complex) ith its cognate toxin3 it) ill either not &e degraded or degraded !ery slo) ly.[893;8; ' ecently3 a study reported that the presence o" toxin protein IoeB and 1ps' enhances the sta&ility o" antitoxin Ie"1 and 1*sA under a heat-shoc7 condition.[6< (o) e!er3 no study has yet explored) hether , acA in the #%AT-' (("amily can &e degraded under the anti&iotic condition3 and) hether the degradation o" , acA is related to 7ac+T transcription.

4n this study3) e "ound that the increase in 7ac+T transcription induced &y the car&apenem anti&iotic meropenem resulted "rom the deregulation o" 7ac+T operon autoregulation. 5egradation o" , acA under the meropenem condition &y 2on protease reduced the ratio o" , acA to , acT3) hich caused the dissociation o" the , acAT complex "rom its promoter region. E!entually3 the repression o" 7ac+T transcription &y the , acAT complex) as relie!ed.

' aterials and 2 ethods

Strains and plasmids

5etails o" all the strains and plasmids used in this study are pro!ided in Ta&le =6 (a!aila&le as =upplementary data at J+$ Cnline 3 and all the oligonucleotides used in this study are listed in Ta&le =8.

Tolerance assay

The tolerance to meropenem) as tested &y the c"uFm2 count a"ter exposure to meropenem. C!ernight cultures o" K8 pne&moniae strains containing di""erent pBA599 deri!ati!es) ere diluted in "resh 2B medium at a ratio o" 6:6AA. Cells) ere incu&ated at 9>JC "or 6 h3 and A.8 K ara&inose) as added to the cultures to induce the expression o" the araB+D promoter. A"ter @A min o" incu&ation3 meropenem) as added to the cultures at $ µgFm2. The cultures) ere incu&ated "or another ; h at the 9>JC sha7er. To determine c"uFm23 ali*uots o" 6AA µ2 culture) ere serially diluted and spotted on the 2B solid plates to calculate the sur!i!ing cells. The sur!i!al rate) as calculated &y di!iding the c"uFm2 in the culture a"ter ; h o" incu&ation) ith the meropenem &y the c"uFm2 &e"ore adding the meropenem.[6$38$38<]

Western blot

The cells treated) ith meropenem or serine hydroxamic acid (=(L) ere collected and lysed &y sonication in lysis &u""er (8$ m1 Tris3 $AA m1 %aC43 $AA µ1 phenylmethylsul"onyl -uoride3 p(?.A . A"ter centri"ugation3 the cleared supernatant) as &oiled) ith a loading &u""er "or 6A min. As "or =5=-+A#E and immuno&lotting3 <A Mg protein) as loaded per lane and separated &y =5=-+A#E using 6A K polyacrylamide gels. A"ter trans"erring the separated protein to the poly!inylidene -uoride mem-&ranes (+:50E 1erc7 1illipore3 #ermany 3 the +:50 mem&rane) as &loc7ed) ith 8.$K B=A in TB=T (Tris-&u""ered saline) ith T) een-8A: 9A m1 Tris-&ase3 A.?K %aCl) F!3 A.6K T) een-8A3 p(>.$ "or 6 h at room temperature. Then3 the +:50 mem&rane) as) ashed three times) ith TB=T and incu&ated) ith <× (is primary anti&ody at ;JC "or a) hole night. 0ollo) ing incu&ation3 the +:50 mem&rane) as) ashed three times using TB=T and incu&ated) ith the corresponding second anti&ody at room temperature "or 6 h. 0inally3 the +:50 mem&rane) as) ashed) ith TB=T and !isualiHed &y an automatic chemiluminescence image analysis system (Tanon ;<AA=0E Tanon3 =hanghai3 China .

LacZ activity assay

To construct the lacB reporter plasmid3 the 7ac+T promoter se*uence) as inserted upstream o" the lacB gene o" a promoterless plasmid3 p2ACN3 "orming the "usion plasmid p2ACN-+$_{7ac+T}$. 5i""erent com&inations o" p2ACN-+$_{7ac+T}$ and pBA599 plasmid) ere co-trans"ormed into ' '8 <7ac+T lacB= and ' '8 <lon 7ac+T lacB= cells. The trans"ormants) ere gro) n in an 2B &roth medium supplemented) ith A.8 K o" ara&inose "or 9 h3 then meropenem ($ µgFm2 and glucose (A.8 K) ere added. =amples "or enHymatic acti!ities) ere collected at the indicated time points (A3 6$3 9A and <A min . The β-galactosidase acti!ity) as measured according to the standard 1iller method using chloro"orm and =5= to permea&iliHe the cells.[8>]

Quantitative real-time (q-RT)-PCR experiments

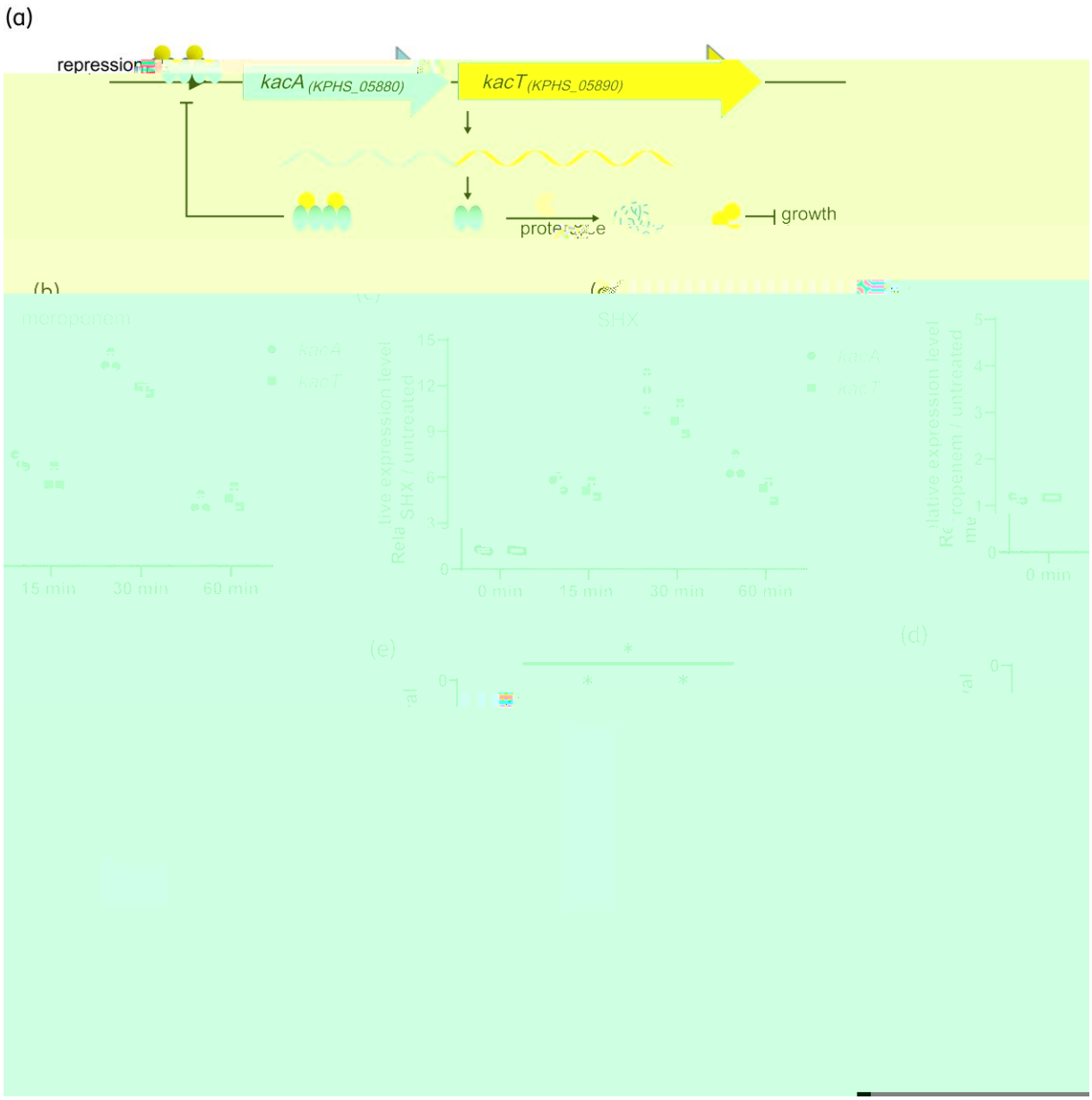
The total '%A o" cells) as extracted according to the manu"acturer's instructions using the '%easy , it (Oiagen3 #ermany . A"ter the digestion o" genomic 5%A using 5%ase 43 6AAA ng '%A) as con!erted to c5%A using +rime=cript™ 'T 'eagent , it (Ta7ara3 Papan . *+C' reaction mix (Beyo0ast™ =IB' #reen *+C' 1ix3 Category %o.: 5>8<A-6 m2) as purchased "rom Beyotime Biotechnology (=hanghai3 China 3 and the reactions) ere per"ormed on an AB4 >$AA instrument (Applied Biosystems . Each reaction) as per"ormed in triplicate simultaneously3 and the "old change o" gene expression) as calculated using the $8^{-\Delta\Delta CT}$ method.[8?] The house7eeping gene (glyceraldehyde-9-phosphate dehydrogenase3 K'-SC!"6"") as used to normaliHe the expression le!els o" the di""erent samples.

/esults and discussion

Meropenem induces the transcription of kacA and kacT

Ge Drst explored the gro) th state o" di""erent K8 pne&moniae strains under the meropenem condition. 0igure =6a sho) s that3 except "or the) ild-type K8 pne&moniae (=668?< containing the car&apenemase gene (bla$_{,+C-8}$ 3 the C5$_{<AA}$ o" bla$_{,+C-8}$ gene deletion strain (=668?<-' '8 and its deri!ed strains) as decreased gradually a"ter 6 h o" treatment3 meaning that cells &egan to die and lyse. Thus3) e treated the strains) ith meropenem "or A3 6$3 9A and <A min.

To see the e""ect o" meropenem on 7ac+T0s transcription le!el3) e examined the transcription o" 7ac+ and 7acT in K8 pne&moniae (=668?<-' '8 (re"erred to as ' '8 herea"ter under the exposure o" meropenem ($ µgFm2 . At the same time3 as a chemical that can stimulate a stringent response in &acteria3[8@] =(L (6AA µgF m2) as used to represent stress other than anti&iotic stress and) as used as a comparison) ith meropenem. As sho) n in 0igure 63 meropenem caused a signiDcant increase in 7acT0s transcriptional le!el3) hich is consistent) ith our pre!ious study.[6$] 1eropenem also increased the transcriptional le!el o" 7ac+



3igure 1⁞ 7ac+T is in!ol!ed in the response o" K⁞ pne&moniae to meropenem. (a =hematic o" acetyltrans"erase-type toxin-antitoxin pair⁝ 7ac+T. 7ac+ and 7acT are co-transcri&ed. T) o , acT molecules &ind) ith "our , acA molecules⁝ "orming a , acAT heterohexamer complex. The , acAT complex later &inds and represses the 7ac+T promoter. , acT independently halts the gro) th o" K⁞ pne&moniae⁝) hereas , acA can neutraliHe the toxicity o" , acT. +roteases such as 2on can degrade , acA. Changes in 7ac+ and 7acT transcriptional le!els responding to meropenem (& or =(L (c are depicted as measured &y *'T-+C'. (d The sur!i!al percentage o") ild-type ''8 or 7ac+T 7noc7out strain ''8 7ac+T⁝ treated &y meropenem ($ µgFm2 "or ; h. (e The sur!i!al percentage o" ''8 7ac+T strains har&ouring empty !ector pBA599⁝ , acT-expressing !ector (pBA599-7acT or , acAT-expressing !ector (pBA599-7ac+T a"ter exposure to $ µgFm2 meropenem "or ; h. The transcriptional le!els o" 7ac+ and 7acT genes) ere normaliHed using the house-7eeping gene⁝ gap+. The sur!i!al percentage) as calculated &y di!iding the c"uFm2 o" the meropenem-treated culture &y the c"uFm2 o" the culture &e"ore adding meropenem. =(L) as used to compare) ith meropenem. The &ar represents the mean o" three independent experiments⁝ and the error &ar indicates the =5 (*' !alue <A.A$. This Dgure appears in colour in the online !ersion o" J+$ and in &lac7 and) hite in the print !ersion o" J+$.

(0igure 6& . Cn the other hand⁝ the transcriptional le!els o" 7ac+ and 7acT) ere also o&!iously enhanced &y =(L (0igure 6c . These results indicated that⁝ similar to other "amilies o" TA modules⁝ the #%AT-' ((type TA module⁝ , acAT⁝ also responded to di""erent stress conditions.

Overexpression of kacAT operon enhances the tolerance of K. pneumoniae to meropenem

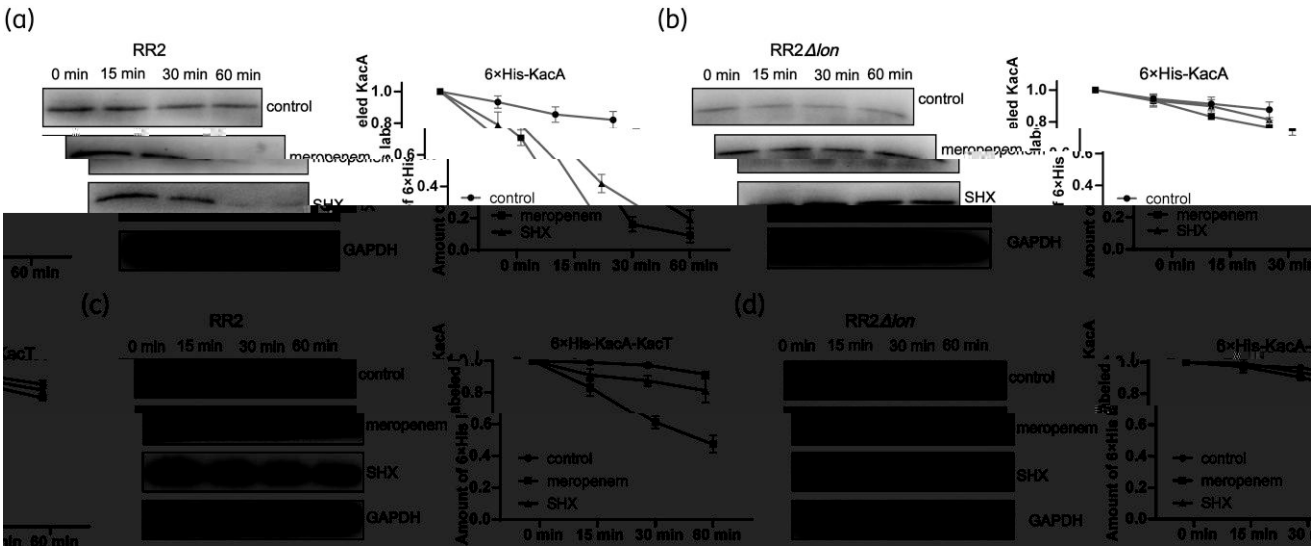
The expression o" the toxin gene 7acT signiDcantly inhi&ited the gro) th o" K⁞ pne&moniae⁝) hereas the expression o" 7ac+T or

empty pBA599 plasmid did not (0igure =6& and c . Additionally3)e pre!iously "ound that ,acT o!erexpression induced meropenem tolerance in K8 pne&moniae.[6$] (o)e!er3 the e""ect o" the 7ac+T operon on meropenem tolerance remains to &e elucidated. Ge examined)hether the 7ac+T operon a""ects meropenem tolerance in ' '8. As 0igure 6d sho)s3 the sur!i!a&ility o" ' '8 under meropenem exposure)as not a""ected3 disregarding the presence o" the 7ac+T operon. 4t is)orth noting that3 except "or ,acT3 o!erexpression o" ,acAT also induced meropenem tolerance in ' '8

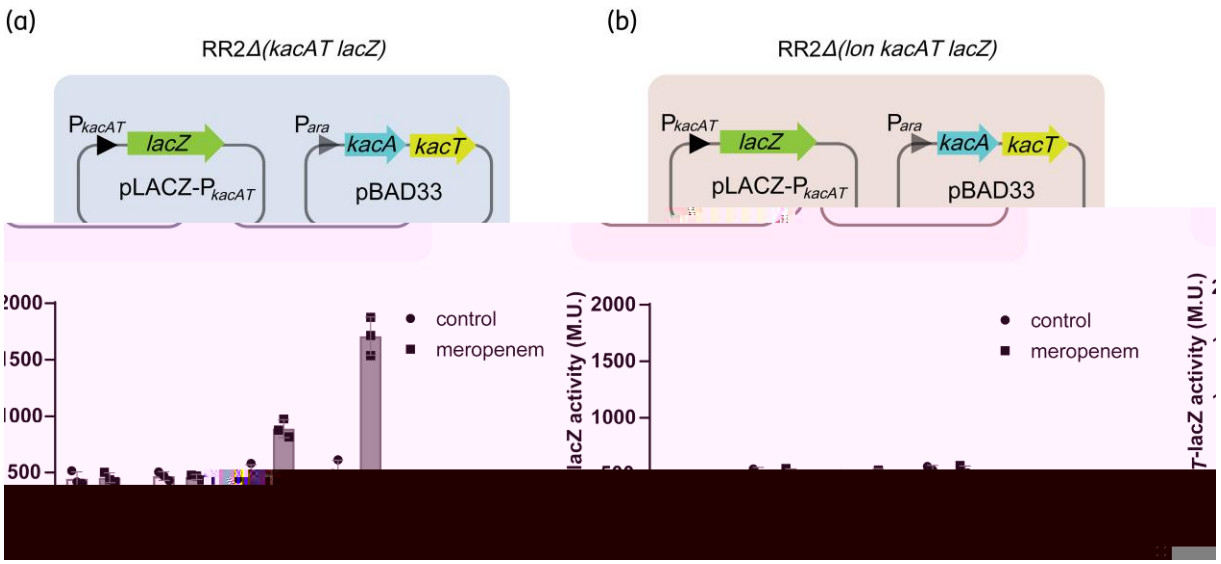
(0igure 9a and & . Rsing the) ild-type ' '8 and ' '8 lon strains3) e "urther examined the e""ect o" lon on 7ac+T\s transcription a"ter meropenem exposure. Cur results sho) ed that the transcription le!els o" 7ac+ and 7acT in the ' '8 lon strain) ere remar7a&ly lo) er than in the) ild-type ' '8 strain a"ter meropenem or = (L exposure (0igure 9c and d . These results suggest that lon is transcri&ed at a higher rate under meropenem exposure3 possi&ly translating more 2on protease that could a""ect 7ac+ and 7acT transcription.

Meropenem leads to KacA degradation through Lon protease

The in vivo degradation rate o" , acA) as examined. Ge Drst used

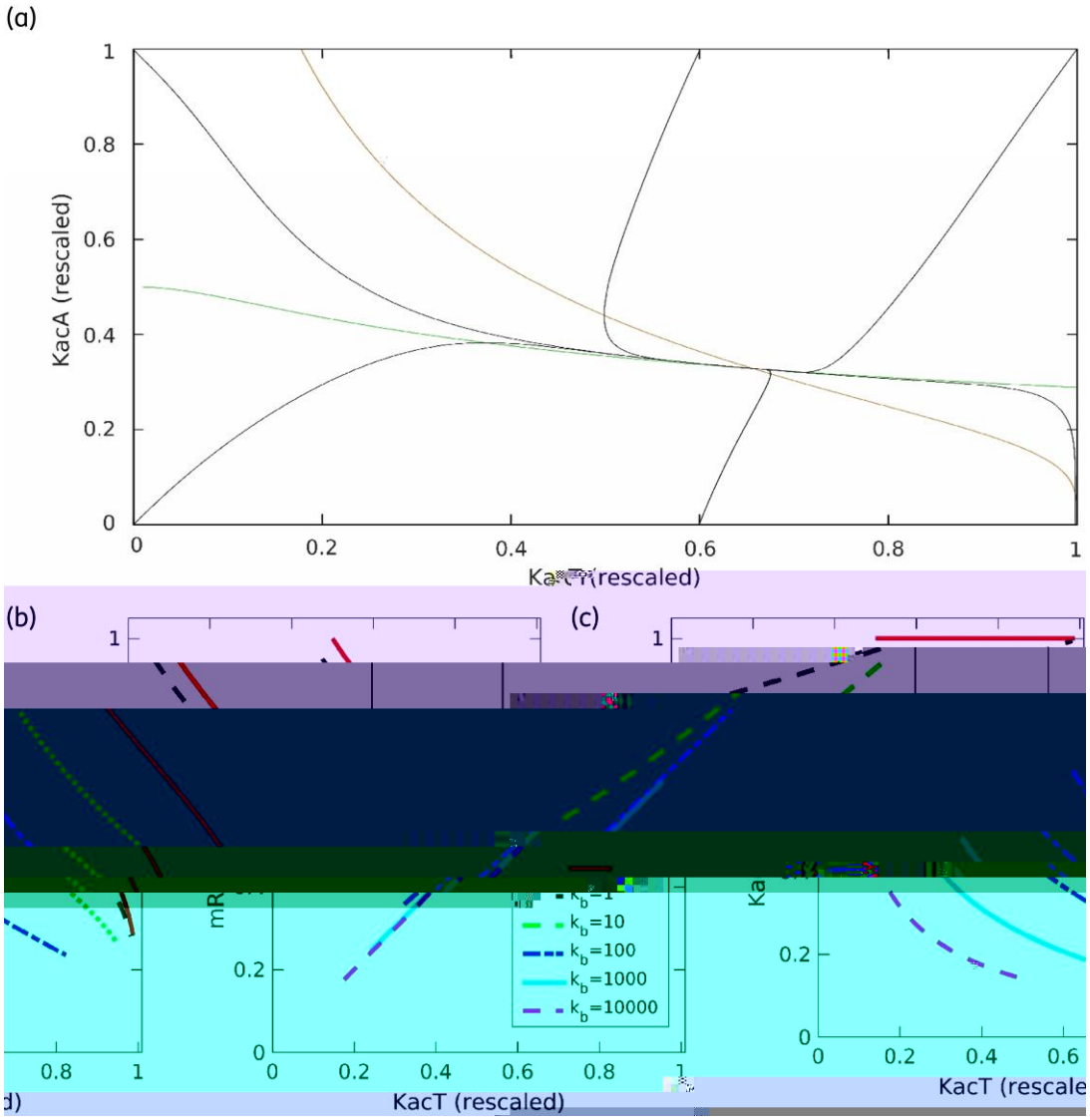


3igure *4 1eropenem induces ,acA degradation through 2on protease. Gild-type ' '8 and lon>deletion (' '8 lon strains har&ouring the pBA599 !ector that expresses (a3 & only <× (is-,acA and (c3 d) ith ,acT. The strains) ere gro) n in 2B medium at 9>JC3 C5$<AA=A.93) ith A.8K ( ) F! o" ara&inose. A"ter @A min o" induction3 A.8K ( ) F! o" glucose) as added to inhi&it ,acA expression together) ith meropenem. =amples "or) estern &lot) ere collected at the indicated time points (A3 6$3 9A and <A min . (a and (& sho) that the "ree ,acA is degraded &y 2on protease a"ter meropenem exposure. (c and (d sho) that ,acT could not sta&iliHe ,acA under meropenem exposure. =(L) as used to compare) ith meropenem. 5ata are presented as mean±=5 (error &ars E n=9. #A+5 (3 #lyceraldehyde-9-phosphate dehydrogenase.



3igure 54 1eropenem promotes the dissociation o" the ,acAT complex "rom its promoter. 7ac+T promoter (+7ac+T and the do) nstream lacB) ere cloned on the p2ACN-+7ac+T plasmid3) hereas 7ac+ and 7acT) ere on the pBA599 plasmid. p2ACN-+7ac+T and pBA599 in com&ination expressing ,acA and ,acT) ere co-trans"ormed into ' '8 <7ac+T lacB (a and ' '8 <lon 7ac+T lacB= (& cells. 1eropenem and A.8K glucose) ere added a"ter 9 h o" induction o" ,acA and ,acT &y ara&inose (A.8K . =amples "or enHymatic acti!ities) ere collected at the indicated timepoints (A3 6$3 9A and <A min . 1R3 miller unit. This Dgure appears in colour in the online !ersion o" J+$ and in &lac7 and) hite in the print !ersion o" J+$.

meropenem or =(L3 the -uorescence intensity o" ,acA-e#0+) as signiDcantly decreased in the) ild-type ' '8 compared) ith ' '8 lon cells (0igure =9& and c . 5espite the presence o" ,acT3 the -uorescence intensity o" ,acA-e#0+ in) ild-type ' '8) as still signiDcantly reduced a"ter meropenem treatment compared) ith the ' '8 lon strain (0igure =9d and e 3) hich is consistent) ith the results o") estern &lot. Besides3) e also "ound that imipenem caused reduced -uorescence intensity o" ,acA-e#0+ in) ild-type ' '8 compared) ith ' '8 lon cells3) hich implies that imipenem can also induce the degradation o" ,acA (0igure =; .



3igure 6⁴ ’esult o" modeling 7ac+T expression dynamics. +hase plane analysis o" the system dynamics "or the rescaled parameter !alues ˜ = 6⁞ $\tilde{K}_T$ = 6⁞ $\tilde{K}_B$ = 6AA⁞ Δ˜ = 9 (see 1aterials and methods . The orange and green cur!es correspond to ,acA and ,acT nullclines⁞ respecti!ely⁞) ith their intersection determining the system⁄s steady state. =olid &lac7 cur!es present trajectories "or di""erent system initial conditions (a . E*uili&rium !alues o" ,acA !ersus ,acT (& and 7ac+T m’%A !ersus ,acT (c . 5i""erent cur!es correspond to di""erent $\tilde{K}_B$!alues indicated in the legend. +oints on each cur!e correspond to changing Δ˜ "rom A (le"t edge to < (right edge ⁞ and the !alues on the axes are rescaled. This Dgure appears in colour in the online !ersion o" J+$ and in &lac7 and) hite in the print !ersion o" J+$.

Additionally⁞) e studied) hether meropenem could induce ,acT’s degradation. Rnder meropenem treatment⁞ the non-toxic ,acT$^{I6;\$0}$ in ’’8 or ’’8 lon did not degrade much (0igure =$a and &. 2i7e) ise⁞ the -uorescence intensity o" mCherry-,acT$^{I6;\$0}$ in) ild-type ’’8 did not change a"ter meropenem treatment (0igure =$c and d .

Meropenem promotes dissociation of the KacAT complex from its promoter

Because meropenem can promote the degradation o" ,acA &ut not ,acT⁞ meropenem li7ely alters the intracellular ratio o" .,acA/:.,acT/. To our 7no) ledge⁞ the change in antitoxin to toxin ratio has not &een success"ully in!estigated in vivo although some approaches ha!e &een tried such as the pulse-chase assay.[6<] Ge initially used) estern &lotting to explore changes in the .,acA/:.,acT/ ratio &ut also "ailed (data not sho) n . (ence⁞) e "used ,acA and ,acT) ith e#0+ and mCherry⁞ respecti!ely. The -uorescence intensity o" ,acA-e#0+ and mCherry-,acT⁞ under meropenem stress⁞) as measured &y a microplate reader. Cur results sho) ed that the ratio o" remaining ,acA-e#0+ to mCherry-,acT) as signiDcantly reduced in the) ild-type ’’8 under the meropenem condition (0igure =<a . 1ean) hile⁞ the ratio o" .,acA/:.,acT/ in the lon deletion strain remained unchanged (0igure =<& .

5ue to the reduced ratio o" .,acA/:.,acT/ caused &y meropenem3)e suggest that meropenem can promote the dissociation o" the ,acAT complex "rom its promoter region. Ge per"ormed a 2acN acti!ity experiment in the 7ac+T promoter ($+_{7ac+T}$. Cur results sho)ed that3)ith the prolongation o" meropenem treatment time3 2acN acti!ity in ''8 <7ac+T lacB= har&ouring ,acA and ,acT increased3)hereas the ''8 <lon 7ac+T lacB= did not (0igure $. Additionally3 in ''8 <7ac+T lacB= and ''8 <lon 7ac+T lacB= containing the empty pBA599 plasmid3 2acN acti!ity)as also unchanged under the meropenem conditions (0igure =>. A plausi&le explanation is that3 under meropenem conditions3 the ,acAT complex dissociates "rom its promoter $+_{7ac+T}$3 leading to the transcription o" 2acN.

A quantitative model of kacAT expression dynamics explains experimental observations

Based on the experimental results presented a&o!e3)e de!eloped a *uantitati!e model that can predict protein and transcript synthesis dynamics (see =upplemental methods. Ge aimed to achie!e the "ollo)ing through the model: (6 Chec7 i" and under)hat conditions (parameter range the model can explain the experimentally o&ser!ed system response to anti&iotic stress3 in particular3 the signiDcant increase in 7ac+T transcript amounts and the decrease in .,acA/:.,acT/ ratio. (8 +redict the dynamics o" ,acT under anti&iotic stress3 i.e. upon an increase in ,acA degradation. 4n particular3)e aimed to understand the some)hat perplexing o&ser!ation that ,acAT o!erexpression induces anti&iotic stress tolerance3)hereas 7ac+T deletion does not a""ect this tolerance. (9 4n"er general properties o" 7ac+T expression dynamics3 such as the steady state(s num&er and sta&ility3 and ho) the steady states change)ith changing parameter !alues ()hich is also related to the t)o pre!ious points.

Ge start)ith (9 a&o!e3)ith 0igure <a presenting the phase space analysis o" the system dynamics. The system has one steady state corresponding to the intersection o" the t)o nullclines (the orange and the green cur!es. 2inear sta&ility analysis leads to t)o negati!e real eigen!alues "or this steady state3 corresponding to a sta&le node. 0igure <a sho)s that trajectories)ith di""erent initial conditions con!erge to this sta&le node. As the system parameters are changed3 the phase space topology does not change3 &ut the position o" the steady state changes its location in the phase space (not sho)n in 0igure <a.

Ge next analyse ho) the steady state changes as $\tilde{\Delta}$ (scaled degradation rate o" ,acA and $\tilde{K}_B$ (scaled &inding a"Dnity o" ,acAT complex to the promoter are changed. $\tilde{\Delta}$ is !aria&le as the experimental analysis "ound that3 !ia this parameter3 the anti&iotic stress in-uences the system dynamics3)here $\tilde{\Delta}$ is changed "rom A (the a&sence o" anti&iotic stress to the relati!ely high !alue o" $\tilde{\Delta}$ =<.4t is also clear that $\tilde{K}_B$ is a crucial parameter controlling system &eha!iour3 gi!en the reported derepression o" the promoter upon anti&iotic stress. $\tilde{K}_B$ = A corresponds to a constituti!e (unregulated promoter3 allo)ing in!estigation o" the system's &eha!iour during o!erexpression experiments. =imilarly3 high !a-

j/T1 4 1 4199 3 0.iBi75ark&r0.3 (*3i!Ê8 ¤(Ay3)-4i)-28ja9899 ¤(p)j6.3 0 0 6.3 427.8

right edge to $\Delta\tilde{}$ = <3 and di""erent cur!es correspond to di""erent $\tilde{K}_B$!alues (see the legend . 4n 0igure <&3) e see that irrespecti!e o" $\tilde{K}_B$3 the ratio . , acA/:. , acT/ decreases as $\Delta\tilde{}$ increases3 consistent) ith experimental o&ser!ations. 1oreo!er3) e o&tain a ro&ust (independent o" parameter !alues prediction that anti&iotic stress leads to decreased , acA and increased , acT. This prediction is non-tri!ial &ecause the decrease o" the . , acA/:. , acT/ ratio can also &e reali#ed through other scenarios3 e.g. i" , acT remains constant or e!en decreases3 accompanied &y a "aster decline o" , acA.

0igure <c sho) s 7ac+T m ' %A !ersus , acT steady-state !alues. 5i""erent lines correspond to di""erent $\tilde{K}_B$!alues3 and points on each line correspond to increasing ("rom le"t to right along the lines $\Delta\tilde{}$!alues. The hori#ontal (topmost line corresponds to the constituti!e promoter ($\tilde{K}_B$=A 3 i.e. to the conditions o" the o!erexpression experiment. The 0gure sho) s that smaller $\tilde{K}_B$!alues do not lead to a signi0cant increase in the transcript amounts3 contrary to) hat) as experimentally o&ser!ed. Conse*uently3 the strong &inding o" the complex to the promoter (high $\tilde{K}_B$!alues is consistent) ith the experimental results. 4nterestingly3 "or high $\tilde{K}_B$!alues (see the &ottommost line corresponding to $\tilde{K}_B = 6A^{\cdot}$ 3 the highest !alue o" , acT (the right edge o" the line3 o&tained "or the highest !alue o" $\Delta\tilde{}$ is still smaller than the lo) est , acT !alue (the le"t line edge corresponding to $\Delta\tilde{} = A$ in the constituti!e case. This prediction might explain the nai!ely surprising result that the o!erexpression experiment led to anti&iotic stress tolerance3) hich is not the case "or the nati!e (autoregulated system. That is3 due to the strong &inding a"Dnity o" the repression complex to promoter 5%A3 e!en a signi0cant increase in , acA degradation rate might not &e enough to achie!e large enough , acT le!els necessary to o&ser!e anti&iotic tolerance.

Transcriptional mechanism of the kacAT operon under meropenem stress

Based on the a&o!e3) e propose a putati!e model that explains the transcriptional mechanism o" the 7ac+T operon under the meropenem condition (0igure > . 4n normal circumstances3 the relati!ely lo) er translation e"0ciency o" , acT ensures the amount o" , acA molecules is more than that o" , acT. , acA molecules counteract all , acT molecules to "orm the , acAT complex) ithout releasing the toxicity o" , acT. The , acAT complex can &ind to its promoter 5%A region and &loc7 the transcription o" 7ac+T. Cnce the li!ing conditions are changed3 such as in meropenem stress3 the transcriptional le!el o" the 2on protease gene is increased3 resulting in the degradation o" unsta&le , acA. 5ue to the degradation o" , acA3 the ratio o" . , acA/:. , acT/ &ecomes <63 and the , acAT complex su&se*uently dissociates "rom the promotor region o" the 7ac+T operon3 there&y relie!ing repression o" 7ac+T transcription.

3unding

This) or7) as supported &y the %ational %atural =cience 0oundation o" China (#rant no. 98A>A$>8 3 the =cience and Technology Commission o" =hanghai 1unicipality (#rant no. 6@ ; 9A>$A<AA 3 the 1edical Engineering Cross ' esearch 0und o" =hanghai Piao Tong Rni!ersity (I #8A6@N5A6 ; 3 the %ational %atural =cience 0oundation o" China (#rant no. 98A>A$>8 3 the 1edical Excellence A) ard 0unded &y the Creati!e ' esearch 5e!elopment #rant "rom the 0irst A"Dliated (ospital o" #uangxi 1edical Rni!ersity (L , 8A6@A8$ and The =cience 0und o" the ' epu&lic o" =er&ia (#rant no. >>$A8@ ; 3 *-&ioB5= .

#ransparency declarations

All authors: none to declare.

7upple 2 entary data

0igures =6 to =>3 Ta&les =6 and =83 and =upplemental methods are a!aila&le as =upplementary data at J+$ Cnline.

/ eferences

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&9 Nhou L3 Ec7art 1'3 =hapiro 2. A &acterial toxin pertur&s intracellular amino no(amino nothiJi/T1 1277 (& (p022o -1.24T1 1 28 ff5.999826.5 (n026)]TJm3 -

Supplemental methods

26

29

20

21

22 *K. pneumoniae* HS11286- HS11286- *TV4 G*

23 *TV4 G*

24 *TV4 G*

25

24

27 *TV4* **Figure 2A**

36 *TV5*

39

30 *TV5*

31 *TV4*

32

33

34

35 *TV4* *TVG*

34

37 *TV4 : 9C 6 TVG 8 ! V*

46 *

49

40

41

42

43

44 *TV4 TVG*

45

44 ℃

47

56

59

50

51

52 m φ

53 λ_m

54
$$\frac{\mathrm{d}m}{\mathrm{d}t} = \varphi - \lambda_m m. \qquad \textbf{(1.1)}$$

55

54 $\mathrm{d}m / \mathrm{d}t = 0$

57
$$m = \frac{\varphi}{\lambda_m}. \qquad \textbf{(1.2)}$$

46

49 φ_0

40
$$m = \frac{\varphi_0}{\lambda_m}. \qquad \textbf{(1.3)}$$

41 *' 4 G 4*

42 G

43

44
$$\varphi = \frac{\varphi_0}{1 + \frac{[4A2T]}{K_D}}, \qquad \textbf{(1.4)}$$

45 φ_0 $[4A2T]$

44 K_D

47

76

79
$$\frac{\mathrm{d}A}{\mathrm{d}t} = K_A m - (\lambda_C + \Delta\lambda)A - 4\lambda_C[4A2T] \qquad \textbf{(1.5)}$$

70
$$\frac{\mathrm{d}T}{\mathrm{d}t} = K_T m - \lambda_C T - 2\lambda_C[4A2T], \qquad \textbf{(1.6)}$$

71 K_A K_T λ_C

72 $\Delta\lambda$

73

74

75

74 A

77 A G

966

969

960 $4A2T$

961

962 $$4A + 2T \underset{K_-}{\overset{K_+}{\rightleftarrows}} [4A2T] \xrightarrow{\lambda_C} 0, \qquad \textbf{(1.7)}$$

963 $\mathrm{d}[4A2T]/\mathrm{dt} = 0$

964 $$K_+ A^4 T^2 - K_-[4A2T] - \lambda_C[4A2T] = 0. \qquad \textbf{(1.8)}$$

965

964

967 $$[4\,\mathrm{A}\,2\,\mathrm{T}] = \frac{K_+}{K_-} A^4 T^2 = \frac{A^4 T^2}{K^5}, \qquad \textbf{(1.9)}$$

996 $K^5 \equiv K_- / K_+$ K

999

990 $\mathrm{d}A/\mathrm{d}t$

991 $\mathrm{d}T/\mathrm{d}t$ A T

992 $K_A = 2K_T$ A T $4A2T$

993

994 *4* *G*

995

994 $$A^* = \frac{2\lambda_C}{\lambda_C + \Delta\lambda} T^*. \qquad \textbf{(1.10)}$$

997 A^* T^*

906

909

900

901

902

903

904 $\tilde{m} = m / K \quad \tilde{A} = A / K \qquad \tilde{T} = T / K$

905 *TV4 G*

904 $\tau = \lambda_C t$

907 $\lambda_C \quad \tilde{K}_T = K_T / \lambda_C \qquad \Delta\tilde{\lambda} = \Delta\lambda / \lambda_C \qquad \tilde{K}_B = K / K_D$

916 $4A2T$ *A4* $\tilde{\varphi} = \varphi_0 / (\lambda_m \mathrm{K})$

919

910

911 $$\frac{\mathrm{d}\tilde{A}}{\mathrm{d}\tau} = 2\tilde{K}_T\tilde{m} - (1+\Delta\tilde{\lambda})\tilde{A} - 4\tilde{A}^4\tilde{T}^2 \qquad \textbf{(1.11)}$$

912 $$\frac{\mathrm{d}\tilde{T}}{\mathrm{d}\tau} = \tilde{K}_T\tilde{m} - \tilde{T} - 2A^4T^2. \qquad \textbf{(1.12)}$$

913

914

915

914

917

926

929

920 *TV4 G*

921 $\tilde{K}_B$ $4A2T$ $\Delta\tilde{\lambda}$

922

923 $\tilde{T}^*$

924

925 $$0 = K_T \frac{\varphi}{1 \quad K_B \quad \frac{2}{1 \quad \lambda} \quad ^4 \quad T^{*6}} \quad T^* \quad 2\left(\frac{2}{1 \quad \lambda} \right)^4 T^{*6}. \qquad \textbf{(1.13)}$$

924

927 $\tilde{T}^*$ $\tilde{A}^*$ $\tilde{m}^*$

936 $$\tilde{A}^* = \frac{2}{1+\Delta\tilde{\lambda}} \tilde{T}^*, \qquad \textbf{(1.14)}$$

939

930 $$\tilde{m}^* = \frac{\tilde{\varphi}}{1+\tilde{K}_B \left(\frac{2}{1+\Delta\tilde{\lambda}} \right)^4 \tilde{T}^{*6}}, \qquad \textbf{(1.15)}$$

931 $\tilde{K}_B = 0$

932

933 $\tilde{A}^*$ $\tilde{T}^*$ $\tilde{m}^*$ $\tilde{K}_B$ $\Delta\tilde{\lambda}$

934 **Statistical analysis**

935

934

937 *C*

946

949
940

941 **Table S1.**

Strain/plasmid	Description	Source or Reference
Strain		
K. pneumoniae:		
	T C FR (C FR T	[4]
		This study
TV4 G	*TV4 G*	This study
TV4 G TVM	*TV4 G TVM*	This study
TV4 G TVM	*TV4 G T TVM*	This study
8 ! V -		
8 ! V	*G T V F5*	Novagen
Plasmid		
pBAD33	p15A ori; araC; P*ara* promoter, Cml^R	[5]
pBAD33+*kacT*	pBAD33 bearing *kacT* and its SD sequence as an *Sac*I-*Hind*III insert	This study
pBAD33+*kacAT*	pBAD33 bearing *kacAT* and its SD sequence as an *Sac*I-*Hind*III insert	This study
pBAD33+6×His-*kacA*	pBAD33 bearing 6×His labeled *kacA* and its SD sequence as an *Sac*I-*Hind*III insert	This study
pBAD33+6×His-*kacA-kacT*	pBAD33 bearing 6×His labeled *kacA* with *kacT* and its SD sequence as an *Sac*I-*Hind*III insert	This study
pBAD33+Myc-$kacT^{Y145F}$	pBAD33 bearing Myc labeled $kacT^{Y145F}$ and its SD sequence as an *Sac*I-*Hind*III insert	This study
pBAD33+*kacT-mCherry*	pBAD33 bearing *kacT-mCherry* as an *Sac*I-*Hind*III insert	This study
pBAD33+*mCherry-kacT*	pBAD33 bearing *mCherry-kacT* as an *Sac*I-*Hind*III insert	This study
pBAD33+*kacT*+*eGFP-kacA*	pBAD33 bearing *kacT* with *eGFP-kacA* as an *Sac*I-*Hind*III insert	This study
pBAD33+*kacT*+*kacA-eGFP*	pBAD33 bearing *kacT* with *kacA-eGFP* as an *Sac*I-*Hind*III insert	This study
pBAD33+*mCherry-kacT*+*kacA*	pBAD33 bearing *mCherry-kacT* with *kacA* as an *Sac*I-*Hind*III insert	This study
pBAD33+*mCherry-kacT*+*eGFP-kacA*	pBAD33 bearing *mCherry-kacT* with *eGFP-kacA* as an *Sac*I-*Hind*III insert	This study
pBAD33+*mCherry-kacT*+*kacA-eGFP*	pBAD33 bearing *mCherry-kacT* with *kacA-eGFP* as an *Sac*I-*Hind*III insert	This study
pBAD33+*kacA-eGFP*	pBAD33 bearing *kacA-eGFP* as an *Sac*I-*Hind*III insert	This study
pBAD33+*kacA-eGFP-kacT*	pBAD33 bearing *kacA-eGFP* with *kacT* as an *Sac*I-*Hind*III insert	This study
pCD (pCDFDuet)	T7 promoter, KanaR	Novagen
pCD+*mCherry-kacT*+*kacA-eGFP*	pCD bearing *mCherry-kacT* with *kacA-eGFP* as an *Sac*I-*Hind*III insert	This study
pLacZ-P_{kacAT}	pLACZ derivative with promoter of *kacAT* operon inserted upstream of *lacZ*	This study

942

Table S2.

Name
KacTF
KacTR
KacATF
^{Y145F}F
^{Y145F}R
RTkacTF
RTkacTR
RTkacAF
RTkacAR
RTgapAF
RTgapAR
KacAEF
KacAER
KacTEF
KacTER
mkacTF
mkacTR
KacTmF
KacTmR
ekacAF
ekacAR
NkacAEF
NkacAER
CkacAEF
CkacAER
NkacTMF
NkacTMR
CkacTMF
CkacTMR

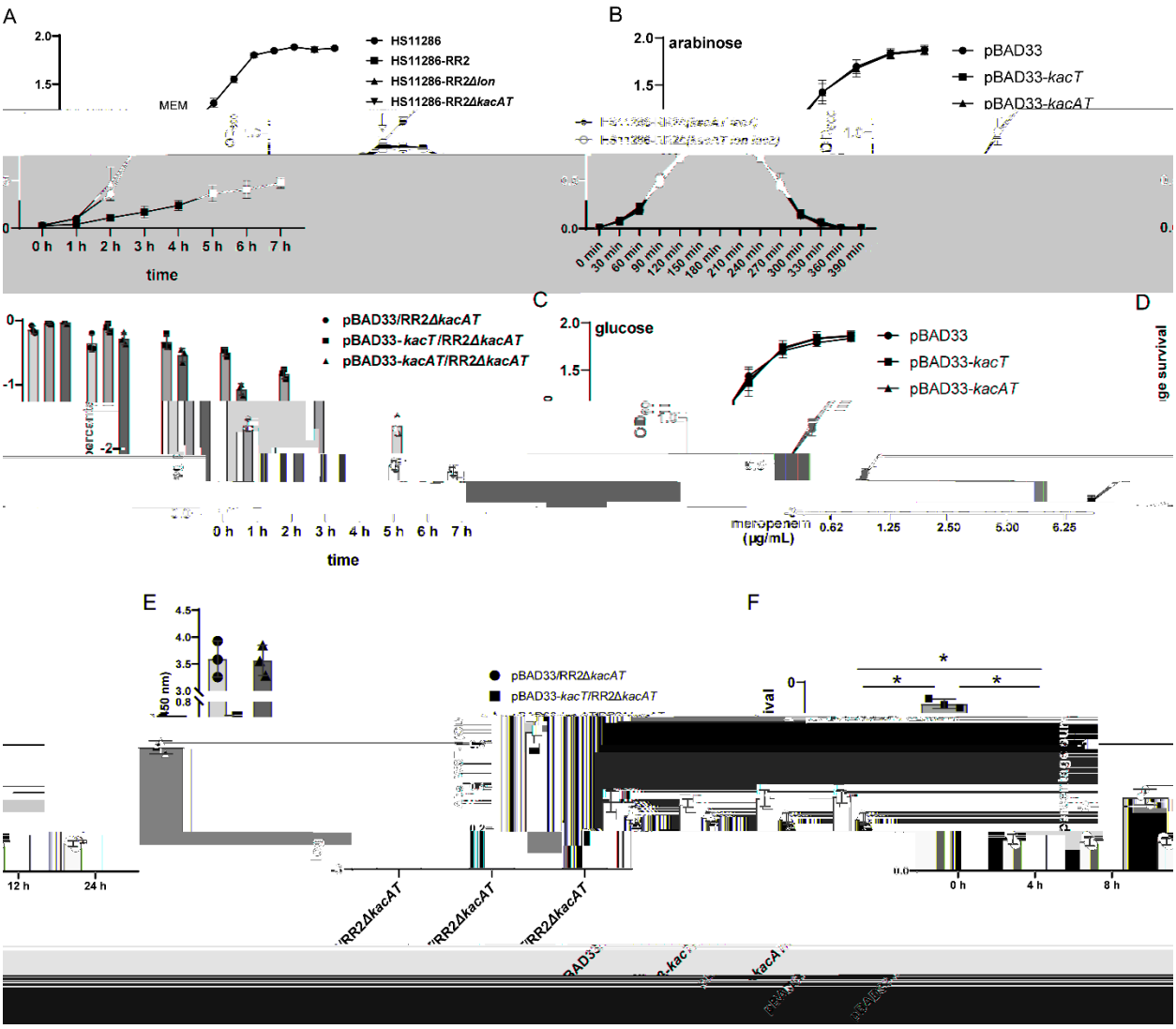


Figure S1. *!* *T*

A *!* *T*

B-C *!* *T* *TV4 G*

D *TV4 G*

TV4 G

953 *TVG* *TV4 G*

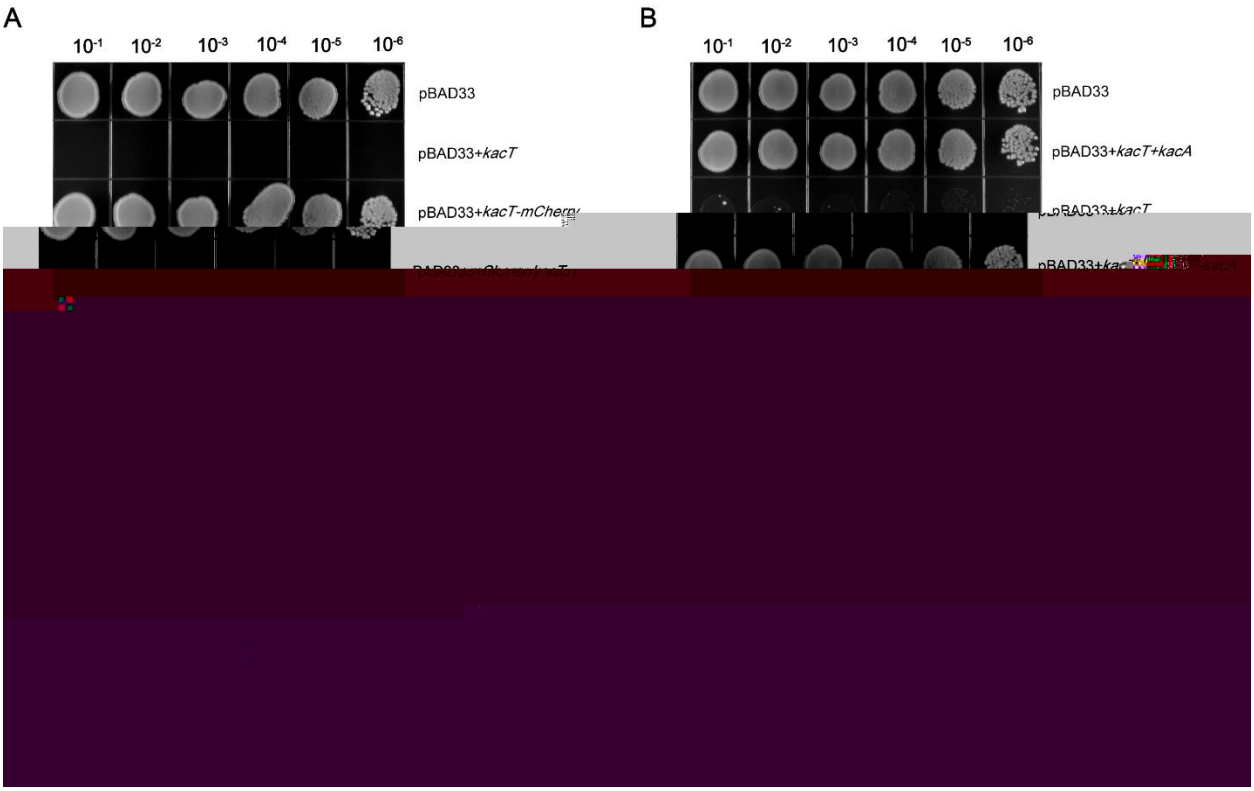
954 **E**

955 *!* *T* *TV4 G*

954

957 **F**

TV4 G *TVG P PTTT* *T*



943

944 **Figure S2.** **A-C**

945 *!* *T* *TV4 G*

944

947 **D** *!* *T*

976 *TV4 G TVM* *TV4 G* *TVM*

979

970 *TV4* *TVG*

971

972

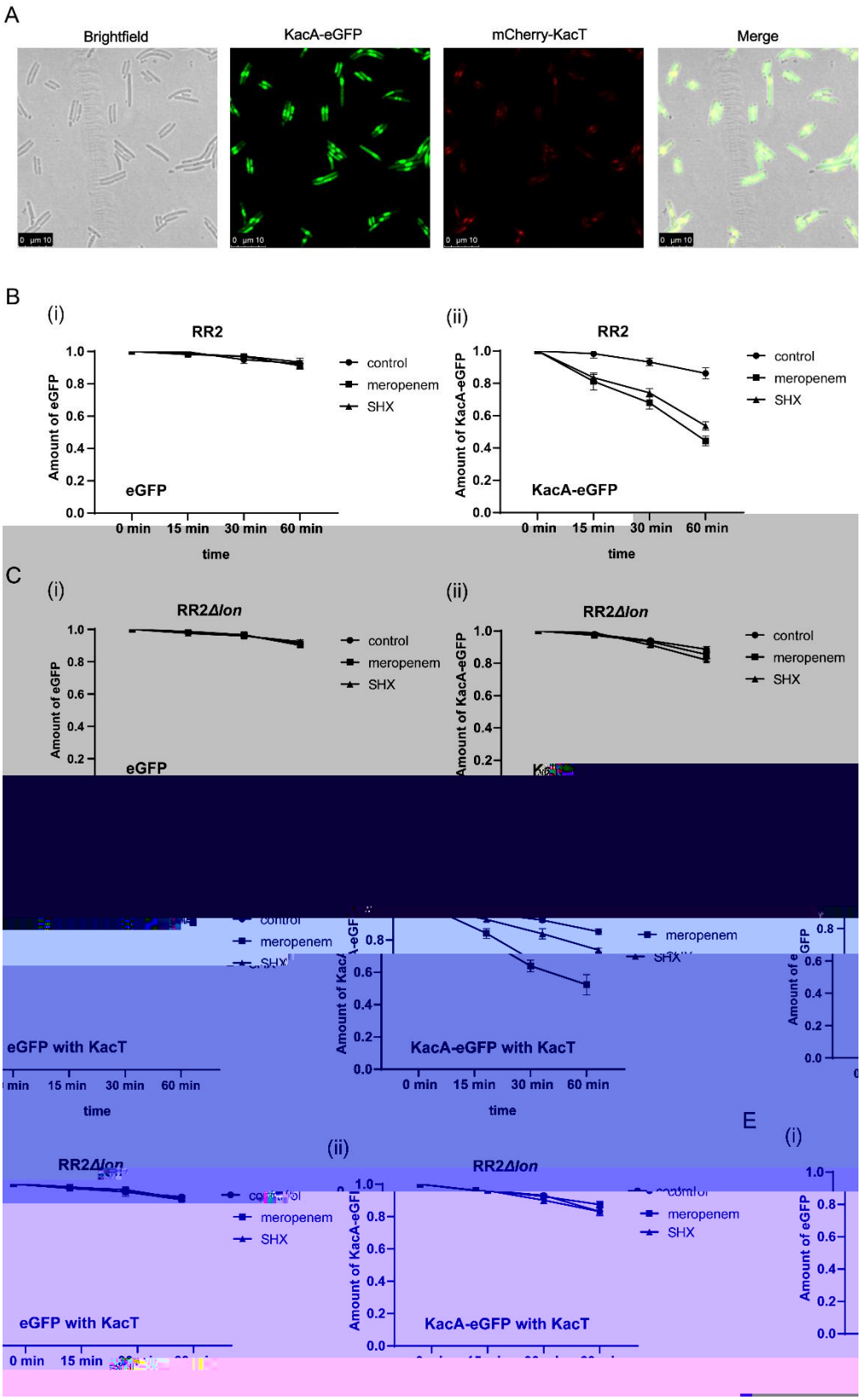

A
Brightfield
KacA-eGFP
mCherry-KacT
Merge
B
(i)
RR2
Amount of eGFP
eGFP
control
meropenem
SHX
0 min
15 min
30 min
60 min
time
(ii)
RR2
Amount of KacA-eGFP
KacA-eGFP
C
(i)
RR2Δlon
(ii)
RR2Δlon
eGFP with KacT
KacA-eGFP with KacT
E
(i)
(ii)
RR2Δlon
eGFP with KacT
KacA-eGFP with KacT

Figure S3. **(A)**

975

974 *8 ! V*

977 *8 ! V*

066

069

060 **(B-C)**

061 **(D-E)** ℃

062

063

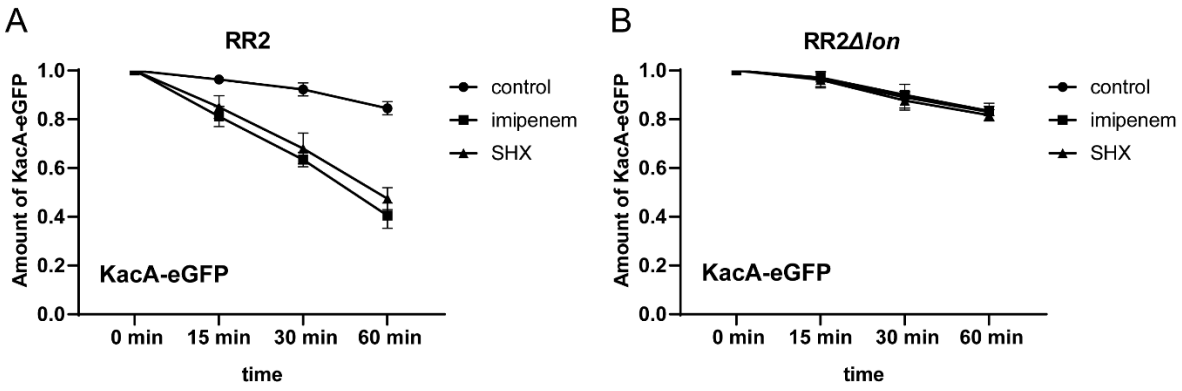
064

065

064

067

096



099

090 **Figure S4.**

091 **A** **B**

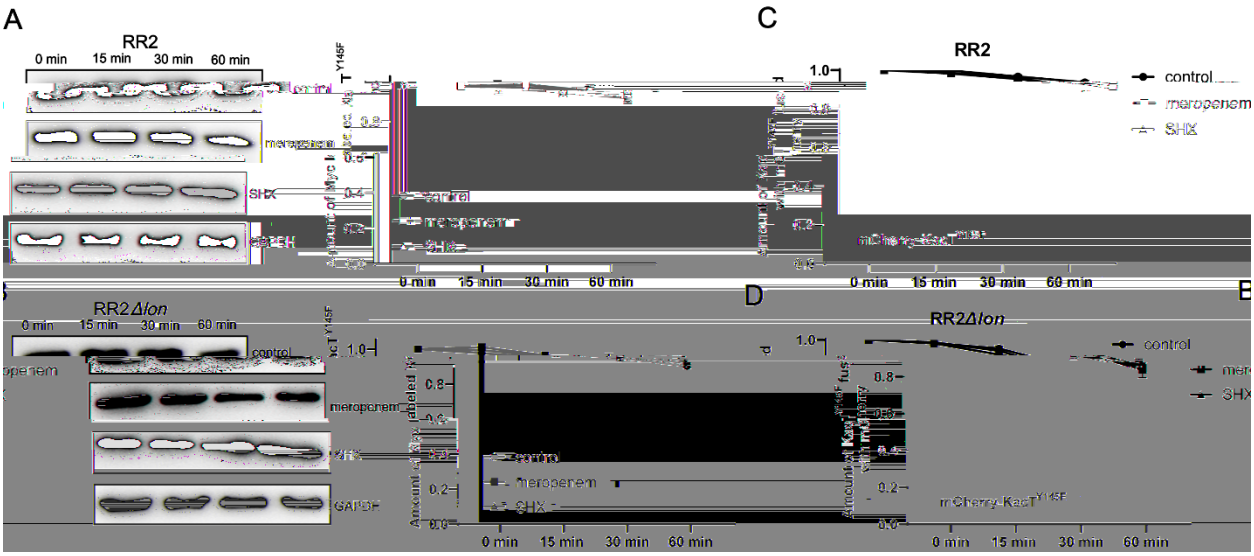
092 *!* *T*

093

094

095

094



097

006 **Figure S5.**

009

000 **(A-B)** **(C-D)**

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004

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004

007

016

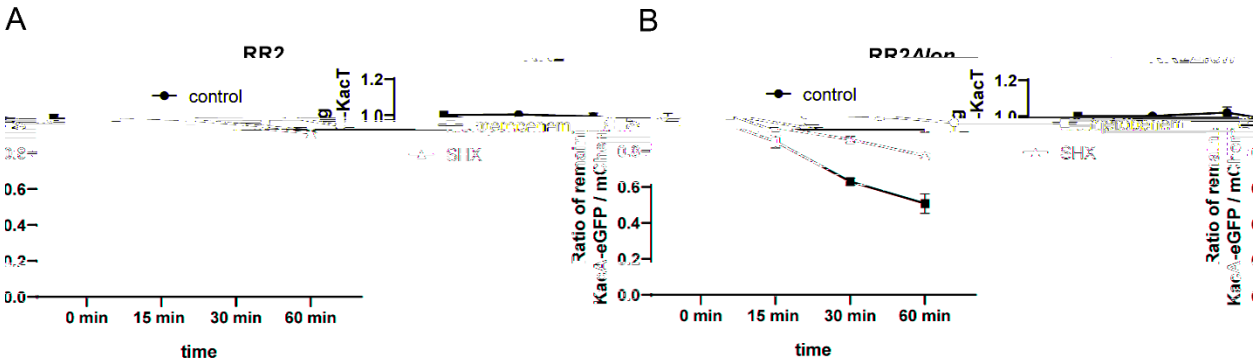

Figure S6.

(A) **(B)**

℃

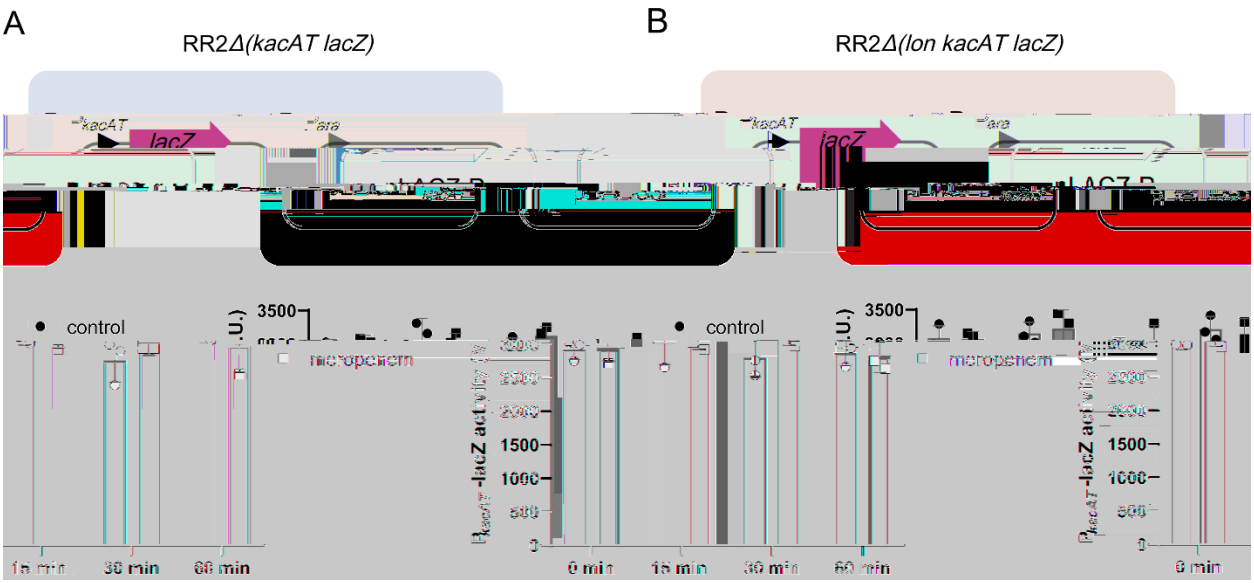

Figure S7. *TV4 G* *TVM* *TV4 G*

TV4 G *TVM* *TV4 G*

TV4 G

TV4 G TVM **(A)** *TV4 G TVM* **(B)**

References

1

4 *T* *A V V4V* *E*

28

2 *T 8 V V TV*

A V V4V *E* **46**

3

4 *V E* **56**

4 *T*

T *T*

4 *V* *6* **70**

5 *T* * *

5TV **177**