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Use of functional interactions with MarA to discover chromosomal genes affecting antibiotic susceptibility in *Escherichia coli*

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Sir,

Antibiotic action and cell responses to antibiotics are very complex and include not only the direct target of an antibiotic but also secondary targets, signalling effects and indirect effects such as antibiotic-induced generation of hydroxyl radicals or changes in gene expression and cell metabolism. Identifying genes important for antibiotic action in the cell or antibiotic resistance would uncover new targets for potentiating existing antibiotics or for discovering new ones. Several approaches have been used to identify these genes, including studying antibiotic-resistant strains of clinical origin or selected in the laboratory and, more recently, screening the entire Keio collection of 4000 single-gene knockouts of *Escherichia coli* for increased antibiotic susceptibility [1]. Such approaches have been successful but have limitations.

Here we propose a new approach to identify genes affecting antibiotic resistance or susceptibility based on screening genes that have functional interactions with other genes already known to be important for drug resistance. Such an approach was suggested by recent studies [2] with the multiple antibiotic resistance regulator MarA, a transcription factor in *E. coli* that upregulates multidrug efflux and downregulates membrane permeability [2]. The effect of inactivation of 24 unrelated chromosomal genes using the EZ-Tn5™ <Kan-2> transposome (Epicentre, Madison, WI) on antibiotic resistance mediated by MarA was studied [2]. Fifteen of these genes affected MarA-mediated antibiotic resistance, whereas nine of them did not. Here we examine whether these 24 inactivated genes affect antibiotic susceptibility per se in the absence of MarA.

Antibiotic susceptibility to cefoxitin, norfloxacin, chloramphenicol and minocycline of the parental strain CR2000 (*E. coli* BW25113 Δ marRA [2]) and of its 24 gene-inactivated mutant derivatives was determined by Etest as described previously [2]. Briefly, the different strains were grown overnight in Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/

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Competing interests

None declared.

Ethical approval

Not required.

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L yeast extract, 10 g/L NaCl) at 37 °C with agitation and their minimum inhibitory concentrations were determined using Mueller–Hinton plates and Etest strips (AB BIODISK, Solna, Sweden) according to the manufacturer's specifications following incubation for 20 h at 37 °C. The results for each mutant were compared with those of the parental strain by *t*-test (two independent samples with equal variance, two-tailed distribution, performed using Microsoft® Excel 2003 software) to determine statistically significant differences.

Nine (60%) of the fifteen genes functionally related to MarA-mediated antibiotic resistance also affected antibiotic susceptibility/resistance independently from MarA. Such a double role might help cells to have a more co-ordinated response to antibiotics. In contrast, only two (22%) of the nine genes that do not affect MarA-mediated antibiotic resistance affected antibiotic susceptibility/resistance (Table 1).

Those genes that affect both antibiotic resistance mediated by MarA and antibiotic susceptibility/resistance independently from MarA were as follows. First, *acrA*, *acrB* and *tolC*, whose inactivation strongly increased susceptibility to all the antibiotics tested (Table 1), as expected [1]. Second, *crp*, *cyaA*, *hns* and *degP*, whose inactivation increased resistance to cefoxitin (*crp* and *hns*) or norfloxacin (*crp* and *cyaA*), increased susceptibility to cefoxitin (*degP*) and strongly increased susceptibility to chloramphenicol and minocycline (*hns*) (Table 1). Inactivation of *crp* and *cyaA* was previously shown to produce resistance to some β -lactams [3] and fosfomycin [4]. Inactivation of *hns* or *degP* was found to increase susceptibility to rifampicin, sulphonamide and vancomycin (*hns*), or rifampicin and sulfamethoxazole (*degP*) in the Keio collection screen [1]. And finally, *pcnB* and *nikD*, two genes with no previous known role in antibiotic resistance whose inactivation increased resistance to cefoxitin (*pcnB*) or minocycline (*nikD*) (Table 1).

Only two genes that did not affect MarA-mediated antibiotic resistance (*ompR* and *metL*) affected antibiotic susceptibility/resistance (Table 1). Inactivation of *ompR* affected resistance to cefoxitin (in agreement with previous studies [3,5]) and norfloxacin. Inactivation of *metL*, a gene with no previous known role in antibiotic susceptibility, produced a small but statistically significant increase in susceptibility to cefoxitin (Table 1).

We have found that genes known to affect MarA-mediated antibiotic resistance were three times more likely to affect antibiotic susceptibility per se than genes that do not affect MarA-mediated antibiotic resistance. Moreover, these experiments have allowed us to confirm and expand the role of *crp*, *cyaA*, *hns*, *degP* and *ompR* in antibiotic susceptibility in *E. coli* and to identify three new genes (*pcnB*, *nikD* and *metL*) previously not known to affect antibiotic susceptibility in any bacterium. These findings support our proposed approach of studying genes that have functional interactions with other genes associated with drug resistance as a method to identify genes that independently affect antibiotic susceptibility.

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References

1. Tamae C, Liu A, Kim K, Sitz D, Hong J, Becket E, et al. Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. *J Bacteriol* 2008;190:5981–8. [PubMed: 18621901]
2. Ruiz C, Levy SB. Many chromosomal genes modulate MarA-mediated multidrug resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 2010;54:2125–34. [PubMed: 20211899]
3. Jaffé A, Chabbert YA, Derlot E. Selection and characterization of β -lactam-resistant *Escherichia coli* K-12 mutants. *Antimicrob Agents Chemother* 1983;23:622–5. [PubMed: 6344786]
4. Sakamoto Y, Furukawa S, Ogihara H, Yamasaki M. Fosmidomycin resistance in adenylate cyclase deficient (*cya*) mutants of *Escherichia coli*. *Biosci Biotechnol Biochem* 2003;67:2030–3. [PubMed: 14519998]
5. Hirakawa H, Nishino K, Yamada J, Hirata T, Yamaguchi A. β -Lactam resistance modulated by the overexpression of response regulators of two-component signal transduction systems in *Escherichia coli*. *J Antimicrob Chemother* 2003;52:576–82. [PubMed: 12951338]

Table 1

Effect of inactivation of chromosomal genes functionally related or unrelated to MarA-mediated antibiotic resistance on antibiotic susceptibility in *Escherichia coli*

Gene	Locus ^a	Functional category ^b	Protein/function	n-fold change in MIC compared with parental strain ^c			
				FOX	NOR	CHL	MIN
Genes whose inactivation affects MarA-mediated antibiotic resistance							
<i>crp</i>	b3357	R	Cyclic AMP receptor protein	1.3	1.4	1.2	0.7
<i>hns</i>	b1237	R	DNA-binding protein H-NS	1.4	1.2	0.3	0.2
<i>cyaA</i>	b3806	E	Adenylate cyclase	1.1	1.4	0.9	0.9
<i>degP</i>	b0161	E	Serine protease Do	0.7	0.8	1.1	0.8
<i>maoC</i>	b1387	E	Putative ring-cleavage enzyme of phenylacetate degradation	1.1	0.8	0.9	1.4
<i>pcnB</i>	b0143	E	Poly(A) polymerase I; RNA modification	1.7	1.0	1.2	1.3
<i>recD</i>	b2819	E	RecBCD DNA helicase/exonuclease	1.2	0.8	0.9	1.4
<i>treC</i>	b4239	E	Trehalose-6-phosphate hydrolase	1.1	1.2	0.9	1.2
<i>acrA</i>	b0463	T	AcrAB–TolC multidrug efflux system	0.4	0.4	0.1	0.2
<i>acrB</i>	b0462	T	AcrAB–TolC multidrug efflux system	0.5	0.4	0.1	0.2
<i>tolC</i>	b3035	T	TolC outer membrane channel	0.5	0.3	0.1	0.2
<i>nikD</i>	b3479	T	Subunit of nickel ABC transporter	1.1	1.0	0.9	1.8
<i>damX</i>	b3388	O	Predicted membrane-anchored protein	1.0	1.0	1.0	1.0
<i>yibL^d</i>	b3602	O	Conserved protein	1.0	0.8	0.9	1.2
<i>yniD^d</i>	b4535	O	Predicted unconserved protein	1.0	0.9	0.9	1.2
Genes whose inactivation does not affect MarA-mediated antibiotic resistance							
<i>cspG^d</i>	b0990	R	Cold-shock protein CspG	0.9	0.8	0.8	1.2
<i>ompR</i>	b3405	R	OmpR transcriptional dual regulator	5.8	1.6	1.2	1.3
<i>appA</i>	b0980	E	Acid phosphatase	1.0	1.0	0.9	1.1
<i>metL</i>	b3940	E	Aspartate kinase/homoserine dehydrogenase	0.7	1.0	1.0	1.3
<i>ynjE</i>	b1587	E	Oxidoreductase subunit paralogue of DmsA	1.0	1.0	0.9	1.1
<i>mhpT</i>	b0353	T	Putative propionic acid transporter	1.0	0.8	1.0	0.9
<i>alx</i>	b3088	O	Putative membrane protein	1.0	1.0	1.0	1.0
<i>metV</i>	b2816	O	tRNA–methionine	1.1	0.8	1.0	1.1
<i>yfiG</i>	b2350	O	CPS-53 (KpLE1) prophage; bactoprenol-linked glucose translocase (flippase)	1.0	1.0	0.9	1.3

MIC, minimum inhibitory concentration; FOX, cefoxitin; NOR, norfloxacin; CHL, chloramphenicol; MIN, minocycline.

^aThe locus tags refer to the accession number (<http://bioacyc.org/ecocyc/index.shtml>).

^bR, transcriptional regulator; E, enzyme; T, transport; O, other/unknown function.

^cThe MICs of the different strains are shown as the average (parental, $n = 14$; mutants, $n = 3$ 6) and normalised to (divided by) those of the parental strain CR2000 (cefoxitin MIC = 3.1 mg/L, norfloxacin MIC = 0.08 mg/L, chloramphenicol MIC = 7.7 mg/L and minocycline MIC = 2.5 mg/L); that is, they are shown as the n -fold change in MIC (n -fold = 1 corresponds to no difference in the MIC between a mutant and the parental strain). Values in bold indicate when inactivation of a gene significantly ($P < 0.01$) affected the MIC compared with the parental strain. The MICs obtained using bactericidal antibiotics (FOX and NOR) were less variable than those obtained using bacteriostatic antibiotics (CHL and MIN), which might be related to the different general mechanism of action of the two groups of antibiotics. This different variability explains why MIC differences of $\geq 30\%$ were significant for cefoxitin and norfloxacin, whereas only differences of $\geq 50\%$ were significant for chloramphenicol and minocycline.

^dInsertion of the transposome is in the promoter of the gene. All the other insertions are within the open-reading frame (ORF) of the genes [2].