Quinolone Induction of *qnrVS1* in *Vibrio splendidus* and Plasmid-Carried *qnrS1* in *Escherichia coli*, a Mechanism Independent of the SOS System[∇]

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Plasmid-carried *qnrS1* is derived from *Vibrio splendidus* chromosomal *qnrVS1*. *qnrVS1* transcripts increased 21- to 34-fold with subinhibitory concentrations of ciprofloxacin but much less with mitomycin. No LexA binding sites were upstream of *qnrS1* or *qnrVS1*, and similar induction levels were observed in *lexA*-positive and *lexA*-negative *Escherichia coli* strains with native *qnrS1* plasmid pMG306 but not with pUC18-cloned *qnrS1* or *qnrVS1*. Thus, *qnrS1* induction by quinolones is independent of the SOS system and requires sequence besides that of the structural gene.

Plasmid-encoded low-level quinolone resistance is mediated by *qnr* and other genes, is widespread, and promotes selection of higher levels of resistance (2, 10, 25, 27). The Qnr proteins are members of the pentapeptide repeat family of proteins that protect the quinolone target enzymes, DNA gyrase and DNA topoisomerase IV, from quinolone action (28–30), but since clinically used quinolones are synthetic agents, the natural functions of the Qnr proteins remain unknown.

Five different categories of plasmid-carried *qnr* genes, *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*, have been identified, with the largest number of alleles found for *qnrA*, *qnrB*, and *qnrS* (11, 12). Chromosomal *qnrA* of *Shewanella algae* (24), *qnrB* of *Citrobacter* spp. (13), and *qnrVS* of *Vibrio splendidus* (3) are sufficiently similar to their plasmid counterparts to be their likely sources. Conditions affecting the expression of chromosomal *qnr* genes may provide some insight into their native functions. Thus, we evaluated the induction of *qnrVS1* from *V. splendidus* and the closely related plasmid-carried *qnrS1* by quinolones,

other DNA-damaging agents, and other antimicrobials. We show that induction by subinhibitory concentrations of quinolones was stronger than that reported for the SOS-dependent induction of *qnrB* (31), greater than that produced by other DNA-damaging agents, and independent of the SOS system, suggesting a novel mechanism that was unexpectedly specific for quinolones.

To determine whether *qnrVS1* expression was affected by quinolone exposure and DNA damage, we measured by quantitative reverse transcription-PCR (qRT-PCR) as previously described (31) the effect of 30-min exposures to ciprofloxacin (CIP; LKT Laboratories, Minneapolis, MN) or mitomycin C (MMC; A.G. Scientific, San Diego, CA), each at one-quarter or one-half of its MIC (Table 1), on the expression levels of *qnrVS1* and *recA* (as a measure of the SOS response) (16) in *V. splendidus* 12B01 grown at 22°C in Trypticase soy broth (TSB; Difco, Sparks, MD) containing 2% sodium chloride or on Mueller-Hinton agar (MHA; Difco) with 0.5 M sodium chlo-

TABLE 1. Relative expression levels of *recA* and chromosomal *qnrVS1* in *V. splendidus*^a

Inducing agent	MIC (μg/ml)	Relative expression of <i>recA</i> at:		Relative expression of <i>qnrVS1</i> at:	
		0.25× MIC	0.5× MIC	0.25× MIC	0.5× MIC
Ciprofloxacin	0.5	1.71 (0.24)	2.85 (0.47)	21.45 (2.80)	33.92 (4.55)
Levofloxacin	0.5	2.84 (0.71)	3.04 (0.20)	27.47 (8.08)	30.77 (8.61)
Mitomycin C	0.06	2.21 (0.65)	2.57 (0.73)	0.80 (0.22)	0.61 (0.11)
Novobiocin	0.25	1.61 (0.13)	2.39 (0.79)	2.32 (0.39)	1.64 (0.44)
Coumermycin A1	0.5	1.58 (0.97)	1.68 (0.21)	2.19 (1.25)	2.40 (0.70)
5-Azacytidine	64	1.44 (0.27)	8.18 (4.32)	1.39 (0.19)	2.38 (1.38)
4-Nitroquinoline- <i>N</i> -oxide	0.25	1.41 (0.04)	0.67 (0.07)	1.50 (0.41)	0.67 (0.01)
Trimethoprim	8	0.88 (0.64)	0.88 (0.22)	0.87 (0.53)	0.99 (0.28)
Gentamicin	4	0.79 (0.07)	2.07 (0.82)	1.13 (0.34)	2.33 (1.33)
Ampicillin	8	0.93 (0.06)	0.57 (0.07)	1.51 (0.11)	1.00 (0.42)

^a Relative values are the transcript levels with exposure to the specified agent divided by transcript levels in parallel controls that were not exposed to the drug. Values represent the means for two or more replicate experiments, with standard errors of the means in parentheses.

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TABLE 2. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics or phenotype	Source and/or reference
Strains		
<i>V. splendidus</i> 12B01	Obtained from the Plum Island Sound Estuary, northeastern Massachusetts	Martin Polz (20)
<i>E. coli</i> J53 Azi ^r	<i>met pro</i> ; azide resistant	14
<i>E. coli</i> DH5 α	F ⁻ ϕ 80dlacZ Δ M15 (<i>lacZYA-argF</i>)U169 <i>deoR recA1 endA1 phoA</i> <i>hsdR17</i> (r _K ⁻ m _K ⁻) <i>supE44 thi-1 gyrA96 relA1</i>	New England Biolabs (Ipswich, MA)
<i>E. coli</i> AB1157 <i>lexA</i> [Def]	AB1157 <i>lexA</i> ; SOS induction deficient	23
<i>E. coli</i> DM49 <i>lexA3</i> [Ind ⁻]	<i>thr-1 araC14 leuB6</i> Δ (<i>gpt-proA</i>) <i>lacY1 tsx-33 qsr'-0 glnV44 galK2 LAM</i> ⁻ <i>Rac-0 hisG4 rfbC1 mgl-5 rpsL31 kdgK51 xylA5 mtl-1 argE3 thi-1</i> <i>lexA3</i> [Ind ⁻]	23
<i>E. coli</i> 3-48	Clinical isolate carrying plasmid with <i>qnrS1</i>	18
<i>E. coli</i> 6-74	Clinical isolate carrying plasmid with <i>qnrS1</i>	18
<i>K. pneumoniae</i> 6-39	Clinical isolate carrying plasmid with <i>qnrS1</i>	18
<i>K. pneumoniae</i> 6-65	Clinical isolate carrying plasmid with <i>qnrS1</i>	18
Plasmids		
pUC18	Cloning vector, Amp ^r , 2.7 kb	GenScript (Piscataway, NJ)
pUC18- <i>qnrVS1</i>	<i>qnrVS1</i> gene and its potential promoter sequence cloned into pUC18	This study
pMG306	10-kb <i>qnrS1</i> plasmid with Tn7 insertion providing a selectable chloramphenicol resistance marker	8
pUC18- <i>qnrS1</i>	<i>qnrS1</i> and its potential promoter sequence cloned into pUC18	This study

ride (1, 20). Relative expression levels of *qnrVS1* and *qnrS1* were calculated using the $\Delta\Delta CT$ method, with the expression levels of *pvuA* and *mdh* used as internal controls, respectively (6, 31). The expression levels of *pvuA* and *mdh* genes did not change in response to ciprofloxacin, mitomycin C, or other DNA-damaging agents. *qnrS* clinical isolates *Escherichia coli* 3-48 and 6-74 and *K. pneumoniae* 6-39 and 6-65 (Table 2) were used as *qnrS1*-positive strains (18). As expected, treatment of

V. splendidus with either CIP or MMC produced increased levels of *recA* transcripts between 1.7- and 2.9-fold (Table 1). Unexpectedly, the expression of *qnrVS1* increased between 21- and 34-fold following exposure to CIP, while no induction was seen after exposure to MMC (Table 1). Similar results were seen with levofloxacin (27- to 31-fold). In contrast, novobiocin and coumermycin A1, which are DNA gyrase ATPase inhibitors, and other DNA-damaging and antimicrobial agents with

TABLE 3. Relative expression levels of *recA* and *qnrS* alleles in selected SOS mutants and clinical isolates^a

Strain	MIC (μ g/ml)		Relative expression of <i>recA</i> after exposure to drug at the indicated MIC:				Relative expression of <i>qnrVS</i> and <i>qnrS1</i> after exposure to drug at the indicated MIC:			
			CIP		MMC		CIP		MMC	
	CIP	MMC	0.25 $\times$ MIC	0.5 $\times$ MIC	0.25 $\times$ MIC	0.5 $\times$ MIC	0.25 $\times$ MIC	0.5 $\times$ MIC	0.25 $\times$ MIC	0.5 $\times$ MIC
<i>E. coli</i>										
J53 pUC18	0.030	0.50								
J53 pUC18- <i>qnrVS1</i>	0.500	2.00	1.90 (0.41)	3.61 (0.72)	20.01 (6.72)	21.59 (5.24)	1.12 (0.02)	1.22 (0.07)	0.93 (0.06)	0.74 (0.12)
DH5 α pUC18	0.030	0.06								
DH5 α pUC18- <i>qnrVS1</i>	0.500	0.06	1.04 (0.20)	1.19 (0.18)	1.26 (0.17)	1.11 (0.08)	1.27 (0.25)	1.38 (0.26)	1.26 (0.20)	1.31 (0.02)
J53	0.030	0.50								
J53 pMG306 (<i>qnrS1</i>)	1.000	0.50	1.91 (0.35)	2.14 (0.60)	3.28 (1.13)	6.37 (1.66)	18.66 (3.29)	15.63 (2.27)	0.79 (0.11)	1.14 (0.13)
J53 <i>gyrA</i> (S83L) pMG306 (<i>qnrS1</i>) ^b	4	1	1.79	2.98	12.60	17.35	5.80	8.91	1.98	0.86
J53 pUC18- <i>qnrS1</i>	0.25						1.06	1.09		
DH5 α	0.03	0.06								
DH5 α pMG306 (<i>qnrS1</i>)	0.500	0.06	1.28 (0.16)	1.46 (0.22)	1.33 (0.18)	1.39 (0.28)	10.02 (0.61)	11.67 (0.50)	1.27 (0.14)	1.07 (0.28)
AB1157 <i>lexA</i> [Def]	0.008	1.00								
AB1157 <i>lexA</i> [Def] pMG306 (<i>qnrS1</i>)	0.500	1.00	1.07 (0.32)	1.19 (0.23)	1.47 (0.47)	1.21 (0.45)	12.64 (1.27)	14.71 (1.66)	4.07 (0.23)	1.75 (0.64)
DM49 <i>lexA3</i> [Ind ⁻]	0.004	0.25								
DM49 <i>lexA3</i> [Ind ⁻] pMG306 (<i>qnrS1</i>)	0.500	0.25	1.06 (0.06)	0.96 (0.00)	1.02 (0.05)	1.25 (0.08)	8.41 (1.10)	9.05 (0.40)	1.21 (0.16)	1.05 (0.01)
Clinical isolate 3-48 (<i>qnrS1</i>)	1.000	1.00	2.16 (0.43)	3.82 (1.00)	10.97 (1.27)	13.42 (3.02)	15.38 (0.30)	19.78 (5.22)	1.10 (0.03)	1.65 (0.55)
Clinical isolate 6-74 (<i>qnrS1</i>)	4.000	0.50	4.17 (4.19)	5.34 (2.88)	6.16 (0.92)	10.21 (2.11)	9.36 (8.35)	10.14 (4.21)	0.41 (0.09)	0.46 (0.07)
<i>K. pneumoniae</i>										
Clinical isolate 6-39 (<i>qnrS1</i>)	16.000	4.00	2.57 (0.13)	2.37 (0.06)	4.62 (2.29)	8.53 (10.33)	23.94 (0.07)	21.74 (5.13)	1.09 (0.23)	1.37 (0.06)
Clinical isolate 6-65 (<i>qnrS1</i>)	2.000	1.00	3.11 (0.29)	3.48 (1.04)	5.74 (3.02)	11.20 (0.07)	17.28 (15.83)	17.60 (27.49)	0.57 (0.26)	0.66 (0.02)

^a Relative values are the transcript levels with exposure to the specified agent divided by transcript levels in parallel controls that were not exposed to the drug. Values represent the means for two or more replicate experiments, with standard errors of the means in parentheses. Those values without an accompanying value in parentheses were from an experiment performed once.

^b Tested at a factor above the MIC of J53 pMG306 (*qnrS1*).

different mechanisms of action (Tables 1 and 3) had a modest effect or none at all. *recA* expression in *V. splendidus* was modestly induced by all agents tested, except 4-nitroquinoline-*N*-oxide, trimethoprim, and ampicillin. CIP exposure caused no change in the gene dosage of *qnrVS1*, as measured by PCR (data not shown). Upon DNA sequencing, we found no LexA binding site in the 320 bp upstream of *qnrVS1* (data not shown).

To determine whether *qnrVS1* could be induced in response to CIP and MMC in a foreign host (Table 3), we amplified by PCR (primer pairs in Table 4) and cloned a 1,011-bp BamHI-EcoRI fragment containing *qnrVS1*, 258 bp of upstream sequence, including the potential native promoter sequence (bacterial promoter prediction program from Softberry), and 85 bp of downstream sequence in the opposite direction of the *lac* promoter of pUC18 in *E. coli* DH10B (26). We then transformed pUC18-*qnrVS1* into *E. coli* J53 Azi^r, which resulted in a 16-fold increase in the CIP MIC (0.5 versus 0.03 µg/ml, as determined at 37°C in Mueller-Hinton broth or on Mueller-Hinton agar) (4). Upon exposure to CIP or MMC, induction of *recA* but not *qnrVS1* was observed (Table 3). Thus, the upstream promoter sequence of *qnrVS1* appeared to be insufficient for its induction in *E. coli*.

In contrast, *qnrS1* from native plasmid pMG306 was induced by exposure to CIP (14- to 16-fold) but not exposure to MMC in *E. coli* (Table 1), whereas a more modest induction of *recA* was seen with both drugs (2.3- to 2.8-fold and 5- to 8-fold, respectively). Notably, four other unrelated clinical isolates containing *qnrS1* plasmids, two *E. coli* and two *Klebsiella pneumoniae* strains from a prior study (18), exhibited similar induction levels with CIP but not with MMC (Table 3).

We then amplified by PCR (primer set in Table 2) and cloned a PstI fragment of *qnrS1* with 209 bp of upstream sequence and 73 bp of downstream sequence from pMG306 in the opposite direction of the *lac* promoter in pUC18; this plasmid was then digested with NdeI and religated to remove all but 46 bp upstream from *qnrS1*. No induction of *qnrS1* by CIP was seen in the latter plasmid (Table 3). Thus, pMG306 contains other elements necessary for the induction phenomenon. Sequences up to 209 bp upstream from *qnrS1* in pMG306, however, lacked a LexA box. Furthermore, CIP induction of *qnrS1* from pMG306 was maintained in the *E. coli* AB1157 *lexA*[Def] or DM49 *lexA*3[Ind⁻] (21) strain as well as in the DH5α *recA* strain, under conditions in which neither CIP nor MMC induced the expression of *recA*. Thus, CIP induction of *qnrS1* in plasmid pMG306 occurred independently of the SOS system.

To determine whether CIP induction of *qnrS1* was mediated by drug interaction with gyrase, we compared induction levels from pMG306 in J53 and in a J53 *gyrA* (Ser83Leu) resistant mutant. Notably, induction levels in J53 *gyrA* (0.25× and 0.5× MICs calculated based on the CIP MIC of both J53 pMG306 [1 µg/ml] and J53 *gyrA* pMG306 [4 µg/ml]) were reduced to about 30% of that in J53, indicating that induction was effected in part by CIP-gyrase interactions.

The induction of *qnrVS1* and *qnrS1* by quinolones was distinctive because of the magnitude of the increase above that of other DNA-damaging agents and because of its independence from the SOS system in *E. coli*. *qnrA* on plasmids or in its native organism, *Shewanella algae*, is not induced by quino-

TABLE 4. Primers used in this study

Target gene or sequence	Primer	Primer sequence ^a	Purpose	Product size (bp)	Comment	GenBank accession no.
<i>qnrVS1</i>	<i>qnrVS1</i> -F	5'-GGGCAATTCAGTATGCAAGG-3'	qRT-PCR	150	Target gene	NZ_AAMR01000006
	<i>qnrVS1</i> -R	5'-TCAATCAGGCATCACCAAA-3'	qRT-PCR			
<i>V. splendidus recA</i>	<i>Vs_recA</i> -F	5'-AGTTCTACGCATCTGTTGCTTG-3'	qRT-PCR	135	Positive control	NZ_AAMR01000025
	<i>Vs_recA</i> -R	5'-GAGTTTCAGCTGTTTGAATGTTG-3'	qRT-PCR			
<i>V. splendidus pvuA</i>	<i>Vs_pvuA</i> -F	5'-GGGCTGGATAACGACCAA-3'	qRT-PCR	201	Internal control	NZ_AAMR01000001
	<i>Vs_pvuA</i> -R	5'-TGCGAATACAAATGCCGAAG-3'	qRT-PCR			
<i>qnrS1</i>	<i>qnrS1</i> -F	5'-TATCGAAGGCTGCCACTTTG-3'	qRT-PCR	122	Target gene	AB187515
	<i>qnrS1</i> -R	5'-AATCACACGACGGAATCT-3'	qRT-PCR			
<i>E. coli recA</i>	<i>Ec_recA</i> -F	5'-CGTCAGGGTAAAGCGAATG-3'	qRT-PCR	122	Positive control	V00328
	<i>Ec_recA</i> -R	5'-CAGAGAAATCCGGCTTGAG-3'	qRT-PCR			
<i>K. pneumoniae recA</i>	<i>Kp_recA</i> -F	5'-GGCGATTCTACATGGGTCT-3'	qRT-PCR	84	Positive control	NC_011283
	<i>Kp_recA</i> -R	5'-CGTGTGGACTCTCAGGT-3'	qRT-PCR			
<i>E. coli mdh</i>	<i>Ec_mdh</i> -F	5'-TGACCAACGATCCAGAAC-3'	qRT-PCR	118	Internal control	NC_000913
	<i>Ec_mdh</i> -R	5'-GGTCTGCTCTGGTTCGTGC-3'	qRT-PCR			
<i>K. pneumoniae mdh</i>	<i>Kp_mdh</i> -F	5'-TGAAGTCGTGGAAGCGAAAG-3'	qRT-PCR	88	Internal control	NC_011283
	<i>Kp_mdh</i> -R	5'-GCGAACCGAGAGACCAA-3'	qRT-PCR			
<i>qnrVS1</i> with its native promoter	<i>qnrVS1</i> -BamHI	5'-CAGGATCCCGCTTAGGACGGGTCAATAAT-3'	PCR/cloning	1,011		
	<i>qnrVS1</i> -EcoRI	5'-AAAGAATTCCAGCGCGAGCCAGTAGTTT-3'	PCR/cloning			
<i>qnrS1</i> with 209 bp of upstream sequence	<i>qnrS1</i> -PstI-F	5'-CCGGCTGACGACGGCTCTCTCTAACCTATC-3'	PCR/cloning	949		
	<i>qnrS1</i> -PstI-R	5'-GGGCTGCAGTCTCTCTATTGCAAGGTTGA-3'	PCR/cloning			

^a Underlining indicates engineered restriction site for restriction enzyme indicated by primer name.

lones but is increased selectively with cold shock (17, 31). Furthermore, although *qnrB* on plasmids is induced by quinolones, the magnitude of the increase is less than that of *qnrS*, is similar in magnitude for other DNA-damaging agents, and occurs in an SOS-dependent manner (7, 31).

Induction in *qnrS1* was selective for quinolones and was effected in part by interaction with DNA gyrase but was not seen with other gyrase inhibitors, such as novobiocin and coumermycin A1, or with other antimicrobials, such as trimethoprim, ampicillin, and gentamicin, which also activate the SOS response (19, 22).

Usually quinolones generate DNA damage that induces the SOS system (8, 21), but LexA-independent gene induction due to UV DNA damage or due to quinolone exposure has been reported in *E. coli*. In the case of quinolones, however, LexA-independent gene induction is small in relation to LexA-dependent gene induction (5, 9, 15). The genetic elements necessary for quinolone induction of *qnrS1* in the *E. coli* background appear to be present on plasmid pMG306, which lacks upstream LexA binding sites. Quinolone induction of *qnrS* also appears to be common among *qnrS*-carrying clinical isolates of *E. coli* and *K. pneumoniae*. Thus, the mechanism and the genetic elements that underlie quinolone induction of *qnrVS1* and *qnrS1* may be widespread but remain to be defined and are the subject of ongoing studies.

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