Regulation of Quinone Oxidoreductase by the Redox-sensing Transcriptional Regulator QorR in Corynebacterium glutamicum

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Corynebacterium glutamicum cgR_1435 (cg1552) encodes a protein of the DUF24 protein family, which is a novel family of transcriptional regulators. CgR1435 (QorR) is a negative regulator of cgR_1436 (qor2), which is located upstream of cgR_1435 (qorR) in the opposite orientation, and its structural gene. QorR binds to the intergenic region between qor2 and qorR to repress their expression, which is induced by the thiol-specific oxidant diamide. The DNA-binding activity of QorR is impaired by oxidants such as diamide, H$_2$O$_2$, and cumene hydroperoxide in vitro, and its lone cysteine residue (Cys-17) is essential for redox-responsive regulation of QorR activity both in vivo and in vitro. Moreover, a disruptant of qor2, which is a homologue of the ytfG gene of Escherichia coli encoding quinone oxidoreductase, shows increased sensitivity to diamide. It is concluded that the redox-sensing transcriptional regulator QorR is involved in disulfide stress response of C. glutamicum by regulating qor2 expression.

Proteins that belong to the DUF24 protein family of wing-helix DNA-binding proteins are widely distributed among prokaryotes. However, their biochemical characterization as transcriptional regulators has so far been limited to only two proteins, HxlR and YodB, of Bacillus subtilis. HxlR is a positive regulator of hxlAB, an operon that is involved in formaldehyde fixation (1). Although HxlR is necessary for formaldehyde-induced expression of the operon, the finer details as to how formaldehyde modulates hxlAB expression are still not clear. YodB negatively controls expression of spx, aozR1, and yodC, genes involved in oxidative stress response (2, 3). The DNA-binding activity of YodB is inhibited by thiol-reactive compounds, and its cysteine residues function in redox sensing (2).

Transcriptional regulators involved in oxidative stress response sense cellular redox conditions via oxidation of their cysteine residues, which leads to disulfide bond formation or S-thiolation. For instance, OxyR of Escherichia coli is activated by H$_2$O$_2$ through the formation of an intramolecular disulfide bond between Cys-199 and Cys-208 and then induces the transcription of genes necessary for the bacterial defense against oxidative stress (4, 5). Likewise, the redox-regulated transcriptional regulator CprK of Desulfitobacterium dehalogenans controls expression of the cpr gene cluster involved in dehalorespiration (6). When CprK is inactivated by oxidation, itimerizes through a disulfide bond formation between Cys-11 and Cys-200 at the same time that Cys-105 and Cys-111 form an intramolecular disulfide bond (7). Recently, Cys-11 has been shown to play a dual role as a redox switch and in maintaining the correct tertiary structure that promotes DNA binding (8). In B. subtilis, the lone cysteine residue (Cys-15) of the organic peroxide sensor OhrR is essential for redox sensing (9). Oxidation of Cys-15 by organic peroxides does not lead to intermolecular disulfide bond formation but leads to a sulfenic acid-containing intermediate that retains DNA-binding activity, and then OhrR is inactivated by S-thiolation by cysteine, coenzyme A, or an unknown thiol with a molecular mass of 398 Da (10).

Corynebacterium glutamicum is a non-pathogenic, GC-rich, and Gram-positive bacterium that belongs to actinobacteria. It has been widely used for the industrial production of various amino acids and nucleic acids (11, 12). Meanwhile, the species is of increasing interest as a model organism for closely related pathogenic species such as Corynebacterium diphtheriae and Mycobacterium tuberculosis (13, 14). In C. glutamicum, the extracytoplasmic function sigma factor SigM is involved in response to disulfide stress, a subcategory of oxidative stress that causes the accumulation of non-native disulfide bonds in the cytoplasm (15). Induction of trxB1 and trxC encoding thiooredoxins and trxB encoding thioredoxin reductase by the thiol-specific oxidant diamide is abolished in a sigM disruptant. Disruption of sigM causes a reduction of cell viability after disulfide, heat, and cold stresses. Another extracytoplasmic function sigma factor, SigH, controls transcription of sigM. Disruption of sigH also makes cells sensitive to diamide and high temperature (16).

C. glutamicum cgR_1435 (cg1552) encodes a protein of the DUF24 protein family. CgR1435 protein contains a lone cysteine residue, which is conserved among CgR1435 homologues, suggesting its involvement in oxidative stress response. In the present study, CgR1435 (QorR (quinone oxidoreductase regulator)) was shown to be a redox-sensing transcriptional regulator of qor2, encoding a quinone oxidoreductase, and its structural gene. This is the first report on a DUF24 family protein other than those of B. subtilis to our knowledge.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Culture Media, and Growth Conditions—C. glutamicum strain R (17) and its derivatives were grown at
33 °C in a medium with 4% (w/v) glucose as described previously (18). Disruptants of the qorR (cgR_1435) and qor2 (cgR_1436) genes were constructed by the transposon-mediated mutagenesis method as described previously (19). Disruption of the qorR and qor2 genes was confirmed by DNA sequencing of thermal asymmetric interlaced-PCR products of mutant cells. Transposon was inserted at 160 bases downstream of the 5' end of the qorR gene in Δ1435 and at 30 bases downstream of the 5' end of the qor2 gene in Δ1436.

A DNA fragment containing the promoter region of the bgf2 gene of C. glutamicum strain R was amplified by PCR using the primer pair PgblF2-F and PgblF2-R (supplemental Table S1) and was cloned between the EcoRI and SacI sites of a shuttle vector, pCRC500 (20), to construct pCRC531. DNA fragments containing the qorR coding region were excised from pCRD610 and pCRD611 (see below) with NdeI and HindIII and cloned between the NdeI and HindIII sites of pCRD531 to construct pCRD630 and pCRD631, respectively. The qorR gene is expressed from the bgf2 promoter on these plasmids. A cysteine codon of the qorR gene at position 17 is replaced with a codon for serine on pCRD631. pCRD630 and pCRD631 were used separately to transform E. coli BL21(DE3) cells harboring pCRD610 or pCRD611. E. coli BL21(DE3) cells harboring pCRD610 or pCRD611 were grown at 37 °C in 500 ml ofuria-Bertani medium supplemented with kanamycin (50 μg ml⁻¹). The recombinant gene was expressed in exponentially growing cells (absorbance at 600 nm of 0.6) by adding 1 mm isopropyl β-D-thiogalactopyranoside. After 1 h of incubation, the cells were harvested by centrifugation. Recombinant proteins were purified by using the Ni-NTA Fast Start kit (Qiagen). Elution fractions containing purified proteins were loaded onto a PD-10 column (GE Healthcare) equilibrated with buffer A (20 mM Tris-HCl (pH 8.0), 0.1 M NaCl, 10% glycerol), and the protein was eluted with buffer A.

Gel Mobility Shift Assay—His-QorR was incubated with Cy3-labeled probes (2 nm) in 20 μl of the binding buffer (20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 10 mM MgCl₂, 1 mM dithiothreitol (DTT), 10% glycerol) for 30 min at room temperature. Diamide, H₂O₂, and cumene hydroperoxide were added with the indicated concentrations. The mixtures were subjected to electrophoresis on a native 5% polyacrylamide gel, and Cy3-labeled probes were detected by the Typhoon Trio+™ variable mode imager (GE Healthcare).

RNA Isolation and DNA Microarray Analysis—Total RNA was extracted from C. glutamicum cells by using the RNeasy Mini kit (Qiagen) and was treated with DNase I (Takara Bio, Inc.) as described previously (18). Global gene expression analyses were performed with the C. glutamicum R DNA microarray as described previously (18). Microarray analyses were carried out using two sets of RNA samples isolated from independently grown cultures with different combinations of Cy dyes (a dye swap strategy). Because the C. glutamicum R DNA microarray contains two replicates per gene, a total of four replicates per gene were available to determine changes in gene expression. Genes with significantly differential transcription levels (p < 0.05 in Student’s t test) by at least a factor of two were determined.

Real-time qRT-PCR—A one-step real-time quantitative reverse transcription-PCR (qRT-PCR)² was performed with the Power SYBR® Green PCR Master Mix (Applied Biosystems) and a pair of gene-specific primers (supplemental Table S1) by using the 7500 Fast Real-time PCR system (Applied Biosystems) as described previously (18). Relative ratios were normalized with the value for 16 S rRNA.

Mapping of Transcription Initiation Sites by Rapid Amplification of cDNA Ends (RACE)-PCR—Transcription initiation sites were determined by using the SMART™ RACE cDNA amplification kit (Clontech). 5' RACE-PCRs were carried out as recommended by the supplier with 1 μg of total RNA and gene-specific primers (supplemental Table S1). Resulting PCR products were cloned into a pGEM®-T Easy vector (Promega Corporation). At least 10 clones for each 5' RACE-PCR product were sequenced.

² The abbreviations used are: qRT-PCR, quantitative reverse transcription-PCR; RACE, rapid amplification of cDNA ends; DTT, dithiothreitol; QBS, QorR-binding site.
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**TABLE 1**

| Gene       | Δ1435/WT | Δ1435[cont]/WT[cont] | Δ1435[1435]/WT[cont] |
|------------|----------|----------------------|----------------------|
| cgR_1435   | 4.7 ± 1.8 | ND                   | ND                   |
| cgR_1436   | 522.5 ± 224.5 | 322.2 ± 53.1       | 1.0 ± 0.1            |

polyacrylamide gels by the method of Laemmli (21) using 20 μg of protein. Separated proteins were blotted to polyvinylidene difluoride membranes (Immobilon-P, Millipore) and probed with polyclonal antibodies to His-QorR proteins. The bound antibodies were detected with goat anti-rabbit IgG secondary antibodies conjugated to alkaline phosphatase (Sigma). Chemiluminescence reactions were done using the CDP-∗Star detection reagent (GE Healthcare), and the signal was scanned by a luminescent image analyzer (LAS-3000, FUJIFILM). Protein concentration was determined by using a protein assay (Bio-Rad) with bovine serum albumin as the standard.

**Disc Diffusion Assays**—Approximately 2 × 107 cells of *C. glutamicum* strains were uniformly spread onto plates of A medium with 4% glucose, and a paper disc impregnated with 10 μl of 1 mM diamide solution was placed onto the lawn. The susceptibility of *C. glutamicum* strains was determined by measuring the size of a cleared zone of growth inhibition after 22 h of growth at 33 °C using ImageJ software (National Institutes of Health).

**RESULTS**

*CgR1435 (QorR) Negatively Regulates Expression of *qorR and *qor2*—To ascertain the physiological role of the DUF24 family protein encoded by cgR_1435 as a transcriptional regulator in *C. glutamicum*, gene expression profiles during exponential growth were compared between the wild type and a cgR_1435 disruptant (Δ1435) using a DNA microarray, and differential expression was confirmed by qRT-PCR analysis. Expression of only cgR_1436, which is located upstream of cgR_1435 in the opposite orientation (see Fig. 3), was affected by the disruption of cgR_1435. The transcript level of cgR_1436 was >500-fold higher in Δ1435 (Table 1). Because promoters of cgR_1435 and cgR_1436 are likely to overlap each other, the transcript level of cgR_1435 was determined by qRT-PCR using the primer pair designed upstream of the transposon insertion site of Δ1435. The transcript level of cgR_1435 in Δ1435 was about five times higher than that of the wild type (Table 1). To confirm that the observed changes in the transcript levels were due to the disruption of cgR_1435, the plasmid pCRD630 carrying the coding region of cgR_1435 and the control plasmid pCRC531 were used separately to transform Δ1435 cells. Whereas the transcript level of cgR_1436 in the resultant strain complemented with cgR_1435 was comparable with that of the wild type, the control plasmid did not effect any measurable changes in the transcript level of cgR_1436 (Table 1). These results indicate that CgR1435 negatively controls expression of cgR_1436 and its structural gene.

**FIGURE 1. Changes in the transcript levels of *qor2* and *qorR* in response to diamide.** The relative transcript levels of *qor2* (A) and *qorR* (B) before (0 min) and at 5, 10, 15, 20, and 30 min after addition of 3 mM diamide were determined by qRT-PCR. The transcript levels were determined in triplicate using two independently grown cultures. The transcript level at 0 min was taken as 1.

*cgR_1436* is a homologue of the *yfgG* gene of *E. coli* with 54% amino acid identity. Given that *yfgG* codes for a novel type of NADPH-dependent quinone oxidoreductase (QOR2) (22), we designated cgR_1436 as *qor2* and cgR_1435 as *qorR* for quinone oxidoreductase regulator.

Expression of *qor2* and *qorR* Is Induced by Diamide—As quinone oxidoreductase plays a protective role against oxidative stress (23, 24), changes in expression of *qor2* and *qorR* upon oxidative stress were examined. The transcript levels of *qor2* and *qorR* in exponentially growing cells were determined by qRT-PCR before and after treatment of cells with diamide and H2O2 (Fig. 1). The results showed that expression of *qorR* was induced within 5 min after addition of diamide, with the *qorR* transcript level increasing about 8-fold after 10 min, before gradually decreasing (Fig. 1A). Expression of *qor2* was also induced by diamide, with drastic increases in the transcript level of ~200-fold after 5 min of diamide treatment (Fig. 1B). There was no increase observed in *qor2* or *qorR* transcripts in response to the oxidative stress caused by H2O2 (data not shown). Expression of *qor2* and *qorR* was thus induced only by disulfide stress.

**QorR Binds to Promoter Regions of *qor2* and *qorR*—** Gel mobility shift assays were carried out with purified His-QorR and a DNA probe F1 of the intergenic region between *qor2* and *qorR* (Fig. 2, A and C). His-QorR reduced the electrophoretic mobility of probe F1, and the amount of the QorR-F1 complex formed increased in proportion to the concentration of His-QorR (Fig. 2A, lanes 1–4). Three forms of the QorR-F1 complex with different electrophoretic mobility (C1, C2, and C3) were detected. The band intensity of the QorR-F1 complex was reduced upon addition of a nonlabeled F1 fragment (Fig. 2A, lanes 5 and 6). However, addition of a fragment F2 that contains...
the oxidative stress-responsive promoters of cgR_2930 and cgR_2931\(^3\) did not affect the amount of the QorR-F1 complex formed (Fig. 2A, lanes 7 and 8). These observations lead to the conclusion that His-QorR binds to the qor2-qorR intergenic region in a sequence-specific manner.

The genetic organization of qor2 and qorR is conserved among many bacterial species belonging to actinobacteria, prokaryotes, and cyanobacteria, based on data from the KEGG Sequence Similarity Database. For example, the ytfH gene encoding a transcriptional regulator of the DUF24 protein family, which is the sole gene of E. coli belonging to this family, is located upstream of the ytfG gene in the opposite orientation on the genome of E. coli. This suggests that regulatory sequences may also be conserved among these bacteria. Searches for conserved DNA motifs within the intergenic regions between qorR and qor2 homologues were performed using the BioProspector program (25). The sequence QBS1 was identified as a conserved sequence (Fig. 3A). In addition, similar sequences were found upstream of QBS1 in the opposite orientation (QBS2) and downstream of QBS1 (QBS3) (Fig. 3A). Interactions between His-QorR and the QBS sequences were elucidated by gel mobility shift assays (Fig. 2, B and C). Deletion of QBS1 abolished the interaction between His-QorR and the qor2-qorR intergenic region (Fig. 2B, lanes 1–4). His-QorR bound to QBS2- and QBS3-deficient probes, but the complex C3 was not observed with either probe (Fig. 2B, lanes 7–12). The inferences that QBS1 is indispensable for QorR binding to the qor2-qorR intergenic region and that QorR also binds to QBS2 and QBS3 follow. The putative QorR recognition sequence contains an inverted repeat sequence, ctTac\(^{17}\)N\(^{13}\)-5Gttag (Fig. 3B). The QBS1 sequence, A\(^{14}\)CTTACT\(^{30}\)GATAGT, was replaced with AGGCCGT\(^{5}\)CGGCTT to generate probe M1. No interaction between His-QorR and the probe M1 was observed (Fig. 2B, lanes 5 and 6), confirming that the inverted repeat sequence is recognized by QorR.

The transcription initiation sites of qor2 and qorR were determined by RACE-PCR experiments, and putative −10 and −35 promoter regions, which correspond to those of the SigA-dependent promoter (18), were found upstream of the respective transcription initiation sites (Fig. 3A). Within the qor2 promoter region, QBS1 and QBS2 overlap with the −35 region, and QBS3 overlaps with the −10 region. QBS2 also overlaps with the −35 region of the qorR promoter.

DNA-binding Activity of QorR Is Regulated by Cys-17 Oxidation—The QorR protein contains a lone cysteine residue at position 17 from the N terminus (Cys-17). As this cysteine residue is conserved among QorR homologues (supplemental Fig. S1A), Cys-17 is likely to be involved in the redox-sensitive control of QorR activity. The effect of oxidants on DNA-bind-

\(^{3}\) S. Ehira, H. Teramoto, M. Inui, and H. Yukawa, unpublished data.
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FIGURE 4. Redox-sensitive control of the DNA-binding activity of QorR. A, His-QorR (100 nM) and probe F1 (2 nM) were incubated in the presence of 1 mM DTT (lanes 2–6) or 1 mM diamide (lanes 8–12) for 30 min, and then diamide (lanes 2–6) or DTT (lanes 8–12) was added in the amounts indicated above each lane. After 30 min, the mixtures were subjected to electrophoresis. Lanes 1 and 7, His-QorR was not added. B, purified His-QorR proteins (1 μg) incubated with 1 mM DTT (lane 2) or 1 mM diamide (lane 3) for 30 min were separated by nonreducing SDS-PAGE. Lane M, molecular mass standard marker; lane 1, His-QorR without additives. C, His-QorR (100 nM) and probe F1 (2 nM) were incubated in the presence of 1 mM DTT for 30 min, and then H$_2$O$_2$ (lanes 3–5) or cumene hydroperoxide (CHP) (lanes 6–8) was added in the amounts indicated above each lane. After 30 min, the mixtures were subjected to electrophoresis. Lane 1, His-QorR was not added.

FIGURE 5. Redox response of the QorRC17S protein. A, gel mobility shift assays with His-QorRC17S and the qor2-qorR intergenic region. Probe F1 (2 nM) was mixed with His-QorRC17S in the amounts indicated above each lane, and then the mixtures were subjected to electrophoresis. B, effects of diamide on the DNA-binding activity of QorRC17S. His-QorRC17S (300 nM) and probe F1 (2 nM) were incubated in the presence of 1 mM DTT for 30 min, and then diamide (lanes 3–5) was added in the amounts indicated above each lane. After 30 min, the mixtures were subjected to electrophoresis. Lane 1, His-QorRC17S was not added. C, purified His-QorRC17S proteins (1 μg) incubated with 1 mM DTT (lane 2) or 1 and 3 mM diamide (lanes 3 and 4) for 30 min were separated by nonreducing SDS-PAGE. Lane M, molecular mass standard marker; lane 1, His-QorRC17S was incubated without additives.

Binding activity of QorR was thus examined by gel mobility shift assays (Fig. 4). Binding of QorR to probe F1 was prevented by addition of diamide (Fig. 4A, lanes 2–6). Addition of an excess of the reducing agent DTT restored DNA-binding activity of QorR that was inactivated by diamide (Fig. 4A, lanes 8–12), indicating that the effects of oxidation and reduction on DNA-binding activity of QorR are reversible. When the purified His-QorR protein was subjected to nonreducing SDS-PAGE, two bands of approximate molecular masses of 15 and 30 kDa were observed (Fig. 4B, lane 1). As the theoretical value of the molecular mass of His-QorR is 16.2 kDa, the 15 and 30 kDa bands are likely to correspond to the monomeric and dimeric forms of His-QorR, respectively. Treatment of QorR with DTT resulted in the loss of the 30-kDa band, whereas the intensity of the 30 kDa band was increased by diamide addition (Fig. 4B, lanes 2 and 3). The DNA-binding activity of QorR was also impaired by the other oxidants H$_2$O$_2$ or cumene hydroperoxide (Fig. 4C), and they enhanced dimerization of QorR (data not shown).

These results support the conclusion that QorR undergoes dimerization and loses the DNA-binding activity under oxidizing conditions.

To ascertain the role of Cys-17 in the redox-sensitive regulation of QorR activity, Cys-17 was replaced with serine to generate the His-QorRC17S protein. His-QorRC17S reduced the electrophoretic mobility of probe F1 (Fig. 5A) and required three times as much protein as His-QorR to bind all probes (see Fig. 2A). Unlike the His-QorR protein, binding of His-QorRC17S to probe F1 was not inhibited by addition of diamide (Fig. 5B). When His-QorRC17S was treated with diamide, it remained in the monomeric form (Fig. 5C). Thus, Cys-17 of QorR is required for dimerization of proteins and the redox-responsive control of DNA-binding activity.

**Cys-17 of QorR Is Required for Regulation of qor2 Expression in Response to Diamide**—To investigate the role of Cys-17 of QorR in regulation of qor2 expression, QorR and QorRC17S proteins were expressed from plasmids pCRD630 and pCRD631, respectively, in Δ1435. QorR and QorRC17S were produced at similar levels as shown by immunoblot analysis (Fig. 6A). In Δ1435-expressing QorR, expression of the qor2 gene was induced after treatment with diamide in a similar way to the wild type, whereas its induction level was 10 times lower than that of the wild type (Figs. 1B and 6B). The qor2 transcript level of the strain expressing QorRC17S was three times higher than that of the QorR-expressing strain in exponentially growing cells (time 0 in Fig. 6B). However, qor2 was not induced by diamide in the QorRC17S-expressing strain (Fig. 6B). Expression of the trxB1 gene was induced by diamide in the QorRC17S-expressing strain as shown previously by Nakunst et al. (15), indicating that cells were subjected to disulfide stress (Fig. 6C). These results confirm that Cys-17 of QorR is required for induction of qor2 in response to diamide.
Qor2 Plays a Protective Role against Diamide Stress—The role of qor2 in disulfide stress response was investigated by disc diffusion assays using a disruptant of the qor2 gene (Δ1436) (Fig. 7). A paper disc containing diamide was plated onto a lawn of C. glutamicum cells on an agar plate, and the size of a clear zone of growth inhibition surrounding the paper disc was measured. The clear zone of Δ1436 plates was 20% larger than that of the wild type plates, indicating that disruption of the qor2 gene resulted in increased susceptibility to diamide.

DISCUSSION

In the present study, we demonstrated that QorR binds to DNA in a sequence-specific manner and is involved in regulation of gene expression. A DUF24 protein family member was shown to function as a transcriptional regulator in the actinobacterium C. glutamicum, as was reported previously for the firmicute B. subtilis (1, 3). More than 1,000 proteins of 488 bacterial species are classified into the DUF24 protein family in the Pfam database (26), suggesting that the DUF24 protein family is a regulatory protein family widely spread among bacteria.

QorR was shown to act as a transcriptional repressor of the qor2 and qorR genes (Table 1). Three forms of complexes of QorR and the qor2-qorR intergenic region (C1, C2, and C3) were observed (Fig. 2A), and three QorR-binding sites (QBS1–3) were identified within the qor2-qorR intergenic region (Fig. 3A). Overlapping of the QBSs with the qor2 and qorR promoter regions is coincident with the function of QorR as a transcriptional repressor. Deletion analyses of QBSs indicated that QorR hierarchically bound to these three sites. Deletion and mutation of QBS1 totally eliminated the binding of QorR to the intergenic region (Fig. 2B). Deletion of QBS2 or QBS3 resulted in loss of complex C3, indicating that QorR can bind to two different sites of the QBS2- or QBS3-deficient probe (Fig. 2B). These results suggest that QorR first binds to QBS1 and then to QBS2 and QBS3. It can be speculated that the QorR-QBS1 complex is responsible for recruiting the second and third QorRs to QBS2 and QBS3. Three QBSs overlap with the qor2 promoter region, whereas only QBS2 overlaps with the qorR promoter region (Fig. 3A). The drastic induction of the qor2 gene by diamide (~200-fold) is likely to reflect strict repression of the qor2 expression by QorR.

QorR homologues share the conserved cysteine residue as Cys-17 in C. glutamicum QorR (supplemental Fig. S1A). This conserved Cys-17 was shown to be essential for the redox-responsive regulation of QorR activity in vivo and in vitro. DNA-binding activity of QorRC17S proteins was not impaired by diamide, and dimerization of QorRC17S was not observed, even in the presence of excess diamide (Fig. 5). In addition, expression of the qor2 gene did not respond to diamide in the QorRC17S-expressing strain (Fig. 6). It is concluded that QorR activity is regulated by oxidation of Cys-17. Under oxidizing conditions, QorR becomes a dimeric and inactive form, probably through the formation of an intermolecular disulfide bond between Cys-17 of each subunit, though the possibility that QorR is inactivated by S-thiolation in vivo, as was reported for OhrR of B. subtilis (10), cannot be ruled out.

The DNA-binding activity of QorR was impaired by H2O2 in vitro (Fig. 4C), but the transcript levels of qor2 and qorR did not respond to H2O2 (data not shown). It has been shown that the sigM transcript level is also increased by diamide but not by H2O2 (15). As C. glutamicum is highly resistant to H2O2 (15), H2O2 is supposed to be unable to induce C. glutamicum oxidative stress response.

The GqR1999 (Gg2320) protein of C. glutamicum is another member of the DUF24 protein family. YodB, HxlR, and
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CgR1999 also contain cysteine residues in their N-terminal region, which correspond to Cys-17 of QorR (supplemental Fig. S1B). Cys-6 of YodB has been shown to play a role in DNA binding and response to thiol-reactive compounds (2). Thus, the cysteine residue of the N-terminal region of the DUF24 family proteins is likely to play a regulatory role.

The qor2 disruptant showed increased susceptibility to diamide (Fig. 7). It was shown previously that quinone oxidoreductase homologues of *Arabidopsis thaliana* confer tolerance toward diamide on yeasts (23). Quinone oxidoreductase catalyzes reduction of quinones, which generate reactive oxygen species as a result of redox cycling between quinones and semiquinone radicals (27). Although exactly how quinone oxidoreductase protects cells from disulfide stress has not been revealed, quinones have been shown to react with protein thiols leading to thiol-S-adduct formation, which causes disulfide stress (2). In the qor2 disruptant, disulfide stress caused by diamide could be reinforced by quinones.

The genetic organization of *qorR* and *qor2* homologues and the cysteine residue that is essential for the redox-responsive control of QorR activity are conserved among disparate bacteria (supplemental Fig. S1A). In addition, putative QorR recognition sequences are found within the intergenic regions between *qorR* and *qor2* homologues (supplemental Fig. S2). These observations strongly suggest the existence of a common regulatory mechanism of Qor2 expression in a wide variety of bacteria.

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