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Screening of Genes Related to Methylglyoxal Susceptibility

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Methylglyoxal (MG) is a reactive metabolite known to accumulate in certain physiological conditions. We attempted to isolate genes associated with this metabolite by genome-wide mutagenesis with Tn*phoA* derivative. After screening on methylglyoxal-containing plate, we obtained insertions in three different genes, *ydbD*, *yjjQ*, and *yqiI*, which gave rise to reproducible MG-sensitive phenotypes in glyoxalase-deficient strain. In addition to its MG sensitivity, the insertion in *yqiI* exhibited an impaired motility resulting from a reduced flagellar expression.

Keywords: genome screening, methylglyoxal, dihydroxyacetone, glyoxalase

Methylglyoxal (MG) is a reactive α-oxoaldehyde that is produced by intracellular metabolism including glycolysis and amino acid degradation (Kim *et al.*, 2004). The major pathway in *Escherichia coli* is the conversion from dihydroxyacetone phosphate, which is mediated by methylglyoxal synthetase [Mgs, (Totemeyer *et al.*, 1998)]. MG is also generated from aminoacetone, an intermediate in threonine degradation (Kim *et al.*, 2004). In some physiological conditions, e.g. an altered glycerol metabolism (Freedberg *et al.*, 1971) and facilitated ribose uptake (Kim *et al.*, 2004), uncontrolled production of MG occurs, leading to a cell death. However, it remained unclear how the production of this toxic metabolite is intracellularly regulated. Dihydroxyacetone (DHA) is also a reactive intracellular metabolite, whose role is recently illuminated by the presence of devoted PTS systems, consisting of DhaKLM and DhaR in *E. coli* (Bachler *et al.*, 2005). Although it was initially proposed as an intermediate from glycerol assimilation, an exact role of this compound is still obscure (Kim *et al.*, 2004).

An *E. coli* cell produces various types of aldehyde compounds intracellularly as metabolic intermediates or upon oxidative stress. Among these, short chain carbohydrates are particularly toxic due to their inability to form hemiacetal, e.g. methylglyoxal. The well-known system to remove these compounds is the glyoxalase pathway involving glutathione (GSH) as a cofactor (Clugston *et al.*, 2004). For example, the glyoxalase I (GloA) converts MG into S-lactoylglutathione, which is further metabolized into D-lactate by glyoxalase II. Although *E. coli* was shown to have glyoxalase III activity independent of GSH, its genetic identity has not yet been characterized (Misra *et al.*, 1995). In this study, we attempted to screen *E. coli* genes associated with MG detoxification by random transposon insertions into the chromosome and further characterized their functions through genetic and biochemical analyses.

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Materials and Methods

Bacterial strains and growth conditions

All strains used were derivatives of *E. coli* K-12. We introduced *gloA::kan* allele (MJF388) into the wild-type strain (MG1655) to construct glyoxalase-deficient strain that was used for gene disruption. M9 minimal medium supplemented with 0.4% (wt/vol) glycerol as a carbon source was used. M9 plate was prepared by an addition of Bacto Agar (Difco, France) to a final concentration of 1.5%. Luria-Bertani (LB) medium (Difco, France) was prepared as described previously. Unless otherwise specified, cells were grown at 37°C in broth containing appropriate antibiotics.

Mutagenesis with TnphoA and screening of methylglyoxal-sensitive derivatives

A library of random transposon insertions derived from strain MG1655 *gloA::kan* was made by Tn*phoA*-132 (Tc-resistance) mutagenesis as previously described (Wilmes-Riesenberg and Wanner, 1992). To isolate mutants with transposon insertions, stationary-phase cells at $O.D._{600}$ 1.0 were collected by centrifugation, resuspended in 0.1 ml of 10 mM $MgSO_4$, and then infected with 5 μl of a phage lysate (c.a. 10^{11} pfu/ml). Appropriate dilutions of the mutant library were plated onto LB agar plates containing 17 μg of Tc per mililiter and incubated for 15 h at 42°C. To screen for methylglyoxal (MG, Sigma, USA)-sensitive mutants, Tc^R colonies were replica plated onto LB plate with 0.5 mM MG, and colonies were selected.

Mapping transposon insertions

To locate chromosomal region that harbor Tn*phoA*-132 insertion, the Tn*phoA*-chrosomal junction was amplified by inverse PCR with Tn*phoA*-132 specific primers; 5'-ACA GGG CAA AAC GGG AAA GGT TCC G-3' and 5'-GGG CTG CTC AGG GCG ATA TTA CTG C-3'. The ampli-

fied DNA was gel-purified, and sequenced with a same primers. DNA sequencing was performed by ABI 3100 DNA sequencer (Perkin-Elmer, USA). The sequence obtained were analyzed by BLAST search.

Susceptibility of the mutants to methylglyoxal and dihydroxyacetone

Strains were cultured in M9 medium supplemented with 0.4% (wt/vol) glycerol to an $O.D._{600}$ of 1.0. The cells were diluted to a fresh M9 medium as indicated, and 4 μl of the cells was spotted onto M9 plates containing 0.05 mM MG or 2 mM dihydroxyacetone (DHA, MP biomedical Co., USA). The spots were allowed to dry and incubated for 20 h at 37°C.

Cell preparation for NMR analysis

To analyze metabolites and their conversions from wild-type and mutant strains, cells were cultured overnight in LB medium. Cells were then harvested by centrifugation, washed twice with 100 mM potassium phosphate buffer (pH 7.0), and resuspended in the same buffer. The cells were disrupted by sonication, and debris was removed by centrifugation at 15,000×g for 30 min. The resulting supernatants were dialyzed for 15 h against three consecutive changes of 100 mM potassium phosphate buffer (pH 7.0). The supernatants were stored at -70°C until use.

NMR analyses of metabolites

The Bruker AVANCE-400 NMR spectrometer equipped with a temperature controller was used for NMR experiments. The sample was kept at 28°C during the measurement. All measurements were carried out in 5 mm NMR tube with 600 ml of solution using 10% D_2O as a locking substance. For characterization of metabolites from enzymatic reactions of MG with crude extracts, NMR measurement was carried out for the mixture of crude proteins (1 mg), MG (3 mM),

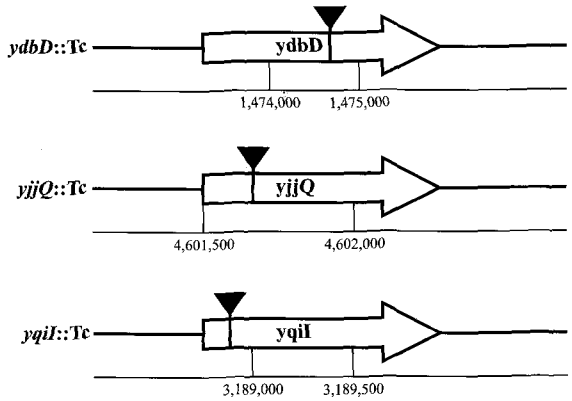

Fig. 1. *E. coli* genes identified to confer MG and DHA resistances. A library of transposon (Tn*phoA*-132, Tc-resistance) insertions obtained from MG1655Δ*gloA* were screened on LB medium with 0.5 mM MG, and sites of insertions on the chromosome were sequenced after inverse PCR. The insertion sites found in genes shown are marked.

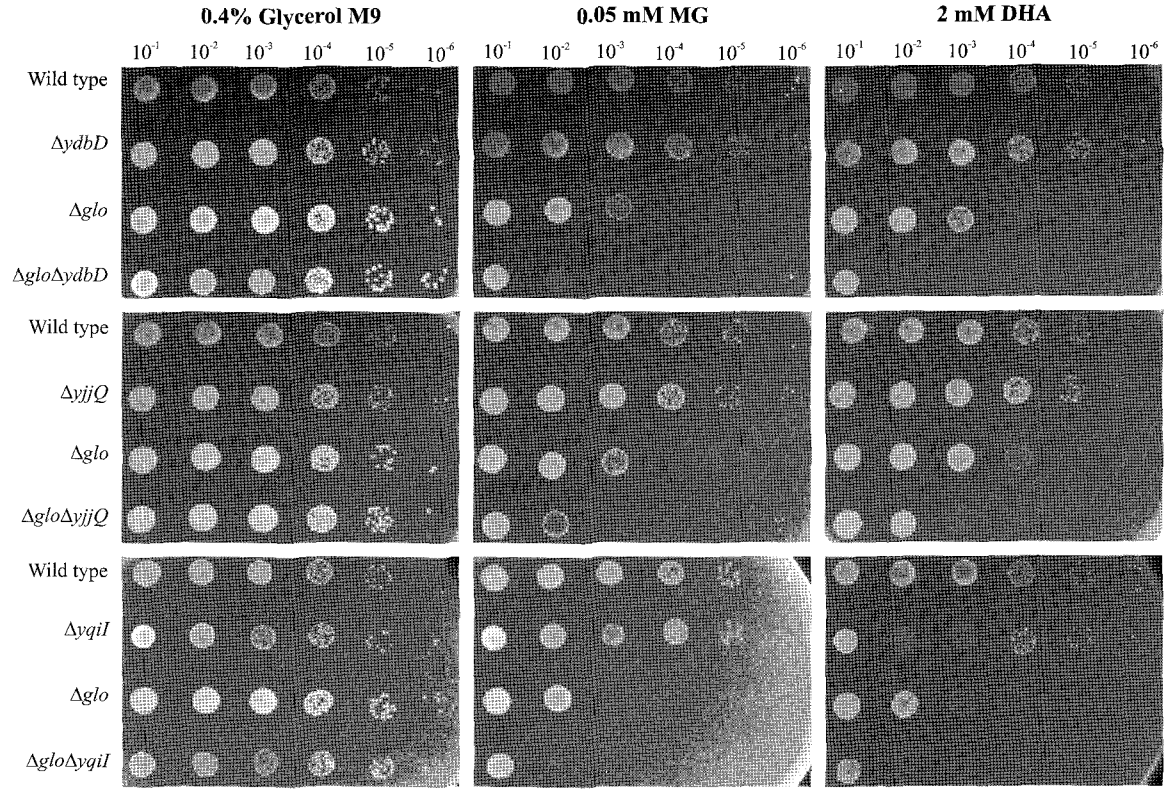

Fig. 2. Susceptibilities of mutants to MG and DHA. Strains with different genetic backgrounds were grown in 0.4% glycerol M9 medium to $O.D._{600}$ 1.0. The cells were diluted as the indicated, and spotted on 0.4% glycerol M9 medium containing 0.05 mM MG or 2 mM DHA.

and coenzymes (1 mM NADH, 1 mM NADPH) in buffer (100 mM potassium phosphate, pH 7.0) with D_2O. The NMR data were collected at indicated times.

Preparation and characterization of flagellum

The flagella of wild type strain and Δ*yqiI* mutant were isolated as previously described (Ko and Park, 2000). The strains were cultured in LB medium at 37°C to $O.D._{600}$ 1.0, collected by centrifugation, and washed three times with PBS. Subsequently, the flagellum was isolated by homogenization of the cells, followed by centrifugation to remove cell debris. The solubilized flagellar protein was dissolved by boiling in 5× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. After equal amount of extract were separated by SDS-12% PAGE, analysis for the wild-type protein that was absent in *yqiI* mutant was performed by MALDI-TOF (Genomine Inc., Korea).

Results

Screening and identification of E. coli genes related to MG susceptibility

In order to search for genes that are related to MG susceptibility, random transposon insertions using Tn*phoA*-132 (Wilmes-Riesenberg and Wanner, 1992) were carried out for the MG1655Δ*gloA* strain. The MG1655Δ*gloA* strain acquiring an additional transposon insertion that makes cells more sensitive to MG relative to *gloA* itself was isolated on LB medium containing 0.5 mM MG by replica plating. After screening more than 7,200 independent Tn insertions, three isolates showing reproducible MG sensitivity were obtained and sequenced, which were identified as *ydbD*, *yqiI*, and *yjjQ* by searching database with BLAST software. The positions of insertions in these genes are shown in Fig. 1. As a matter of fact, more than single insertion event was detected for *yqiI* and *yjjQ* genes (data not shown).

Change in MG and DHA sensitivities by the insertions

More extensive characterization of the insertion effects on MG and DHA susceptibilities was carried out. The concentrations of chemicals, 0.05 mM MG and 2 mM DHA on M9 medium with 0.4% glycerol, for assessing susceptibility were adopted from the previous study (Ko *et al.*, 2005). The single insertions in *ydbD*, *yqiI*, and *yjjQ* affect little on sensitivity to MG or DHA, which is far below the level of sensitivity to MG and DHA exerted by the *gloA* insertion (Fig. 2). However, when the insertions were introduced into the *gloA* mutant background, they became more sensitive to MG/DHA compared to that of *gloA* single insertion. These results indicate that *ydbD*, *yqiI*, and *yjjQ* play a role in detoxification of MG and DHA.

MG-converting activity of cell extracts in the mutants

In order to assess the role of *ydbD*, *yqiI*, and *yjjQ* in MG detoxification, cell extracts from these mutants were tested for their catalytic activity on MG converted into lactate and acetol. Crude extracts (1 mg) of the mutants were incubated with 3 mM MG in the presence of cofactors (1 mM NADH and NADPH) and analyzed with ^{1}H-NMR spectroscopy. Typical NMR spectra of MG conversions by the *gloA* and *gloA yqiI* mutants are shown in Fig. 3, in which the *gloA* single mutant eliminates MG more efficiently than the *gloA yqiI* double mutant, thereby producing more lactate and acetol. The result indicates that the *yqiI* gene is somehow involved in detoxification of MG in addition to *gloA*. Similarly, the *ydbD* and *yjjQ* mutants exhibited reductions in MG conversion, also demonstrated by NMR, which are summarized in Fig. 4. The reduced abilities to remove MG in the mutants roughly correlate with the MG susceptibilities shown in Fig. 2.

Impaired motility of yqiI mutant with reduced flagellar expression

Since the *yqiI* gene is predicted to encode a fimbriae-like

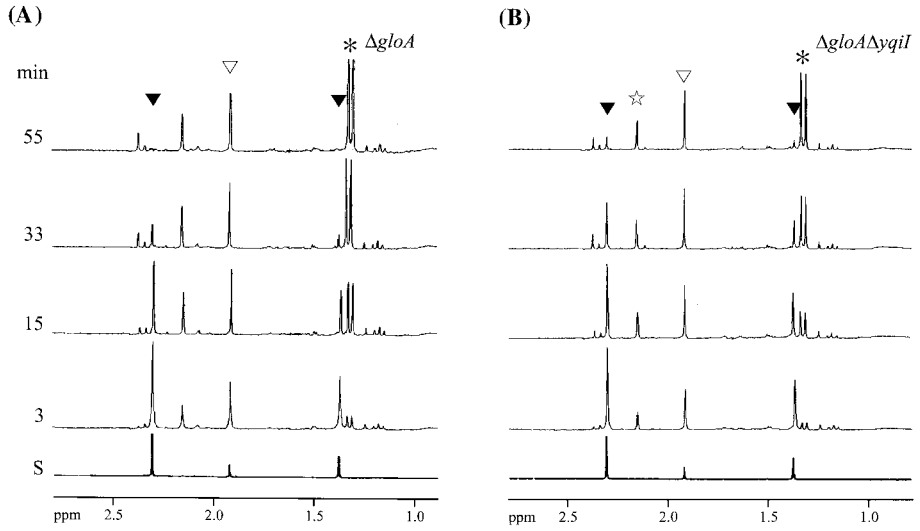

Fig. 3. Production of lactate and acetol from MG in Δ*gloA* single and Δ*gloA*Δ*yqiI* double mutants. Conversion of MG after reaction with 1 mg crude extracts, 3 mM MG and 1 mM NADPH in 100 mM photassium phosphate buffer (pH 7.0) was detected by ^{1}H-NMR spectroscopy. As a standard, NMR spectrum of commercially available MG is shown (▼, MG; ☆, acetol; ▽, acetate; ★, lactate)

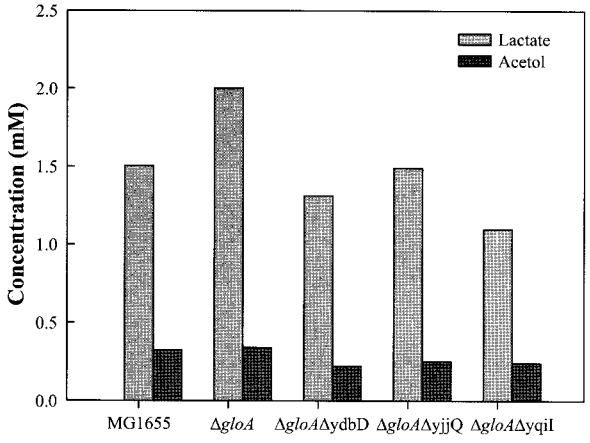

Fig. 4. Amount of lactate and acetol produced in the mutants. Productions of lactate and acetol after reaction with 1 mg crude extracts with 3 mM MG and 1 mM NADPH in 100 mM photassium phosphate buffer (pH 7.0) were detected by ^{1}H-NMR spectroscopy and plotted. Strains used are MG1655*ΔgloA*, MG1655*ΔgloAΔyjjQ*, MG1655*ΔgloAΔydbD*, and MG1655 *ΔgloAΔyqiI*.

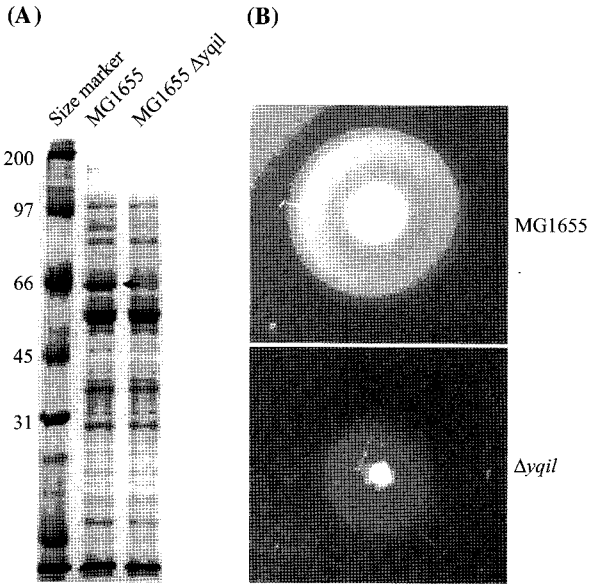

Fig. 5. Flagellar expression and motile phenotype of ΔyqiI muant. After SDS-PAGE, a protein at about 52-54 kDa in the wild type is shown to be absent in *yqiI* mutant (A), which was identified as flagellin by MALDI-TOF analysis. The motile defect of *yqiI* mutant is seen on tryptone swarm plate (B).

protein, we attempted to characterize its product by visualizing cellular proteins after homogenization and separation by 12% SDS-PAGE. However, we were unable to detect the corresponding protein from the soluble mixture. Instead, we found a single protein band of about 53 kDa missing in the *yqiI* mutant, which is present in the wild type (Fig. 5A). A MALDI-TOF analysis of that protein identified it as flagellin, which was confirmed by an impaired motility on tryptone swarm plate (Fig. 5B).

Disscussion

Except for GloI, II, and III, the intracellular machinery of *E. coli* for methylglyoxal susceptibility is largely unknown (Ko *et al.*, 2005). By employing the strategy using GloI-deficient cell as a background, we identified the genes involved in the intracellular detoxification of MG. Although molecular mechanisms underlying the MG susceptibilities need further characterizations involving these genes, it appears that effects of the genes are somehow associated with the metabolic removal of the chemical (Figs. 3 and 4).

The three genes discovered from this study have been poorly characterized with only predicted functions based on their sequence similarities. The *ydbD* gene does not have a similarity with genes of known function. Although the gene is found to be transcriptionally induced by nitric oxide (Justino *et al.*, 2005), the strain mutated on this gene behaved similarly in nitric oxide resistance. The *yjjQ* gene is located just upstream of *bglJ*, whose mutation was shown to allow an expression of the silent *bgl* operon (Giel *et al.*, 1996). Like *bglJ*, the YjjQ protein has the LuxR-type helix-turn-helix motif. Thus, YjjQ is likely to serve as a DNA-binding transcriptional regulator.

The *yqiI* gene encodes a fimbrial protein, which might be associated with various cellular functions including adhesion, pathogenecity, and even fimbriae-induced signal transduction (Schembri and Klemm, 2001). The serendipitous discovery of motile defect in *yqiI* mutant was made during the proteomic confirmation of missing cell-surface fimbrial protein. Although we failed to identify a protein product of *yqiI*, we instead found a reduction in the expression of flagella, i.e. flagellin. This effect is manifested as an inefficient motility on tryptone swarm plate (Fig. 5). The motile defect was also confirmed by an observation of free swimming cells under light microscope (data not shown). Since the study here characterized genes based on phenotypic screening, future research will be devoted to their molecular mechanisms underlying methylglyoxal susceptibility.

Acknowledgments

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References

Bachler, C., P. Schneider, P. Bahler, A. Lustiq, and B. Erni. 2005. *Escherichia coli* dihydroxyacetone kinase controls gene expression by binding to transcription factor DhaR. *Embo. J.* 24, 283-293.

Clugston, S.L., R. Yajima, and J.F. Honek. 2004. Investigation of metal binding and activation of *Escherichia coli* glyoxalase I: kinetic, thermodynamic and mutagenesis studies. *Biochem. J.* 377, 309-316.

Freedberg, W.B., W.S. Kistler, and E.C. Lin. 1971. Lethal synthesis of methylglyoxal by *Escherichia coli* during unregulated glycerol metabolism. *J. Bacteriol.* 108, 137-144.

Giel, M., M. Desnoyer, and J. Lopilato. 1996. A mutation in a new gene, *bglJ*, activates the bgl operon in *Escherichia coli* K-12.

Genetics 143, 627-635.

Justino, M.C., J.B. Vicente, M. Teixeira, and L.M. Saraiva. 2005. New genes implicated in the protection of anaerobically grown *Escherichia coli* against nitric oxide. *J. Biol. Chem.* 280, 2636-2643.

Kim, I., E. Kim, S. Yoo, D. Shin, B. Min, J. Song, and C. Park. 2004. Ribose utilization with an excess of mutarotase causes cell death due to accumulation of methylglyoxal. *J. Bacteriol.* 186, 7229-7235.

Ko, J., I. Kim, S. Yoo, B. Min, K. Kim, and C. Park. 2005. Conversion of methylglyoxal to acetol by *Escherichia coli* aldo-keto reductases. *J. Bacteriol.* 187, 5782-5789.

Ko, M. and C. Park. 2000. Two novel flagellar components and H-NS are involved in the motor function of *Escherichia coli*. *J. Mol. Biol.* 303, 371-382.

Misra, K., A.B. Banerjee, S. Ray, and M. Ray. 1995. Glyoxalase III from *Escherichia coli*: a single novel enzyme for the conversion of methylglyoxal into D-lactate without reduced glutathione. *Biochem. J.* 305, 999-1003.

Schembri, M.A. and P. Klemm. 2001. Coordinate gene regulation by fimbriae-induced signal transduction. *Embo. J.* 20, 3074-3081.

Totemeyer, S., N.A. Booth, W.W. Nichols, B. Dunbar, and I.R. Booth. 1998. From famine to feast: the role of methylglyoxal production in *Escherichia coli*. *Mol. Microbiol.* 27, 553-562.

Wilmes-Riesenberg, M.R. and B.L. Wanner. 1992. Tn*phoA* and Tn*phoA'* elements for making and switching fusions for study of transcription, translation, and cell surface localization. *J. Bacteriol.* 174, 4558-4575.