Lactoylglutathione lyase, a critical enzyme in methylglyoxal detoxification, contributes to survival of *Salmonella* in the nutrient rich environment

Sangeeta Chakraborty, Mayuri Gogoi, and Dipshikha Chakravortty*

Department of Microbiology and Cell Biology; Indian Institute of Science; Bangalore, India

Glyoxalase I which is synonymously known as lactoylglutathione lyase is a critical enzyme in methylglyoxal (MG) detoxification. We assessed the STM3117 encoded lactoylglutathione lyase (Lgl) of *Salmonella* Typhimurium, which is known to function as a virulence factor, due in part to its ability to detoxify methylglyoxal. We found that STM3117 encoded Lgl isomerises the hemithioacetal adduct of MG and glutathione (GSH) into S-lactoylglutathione. Lgl was observed to be an outer membrane bound protein with maximum expression at the exponential growth phase. The deletion mutant of *S.* Typhimurium (Δlgl) exhibited a notable growth inhibition coupled with oxidative DNA damage and membrane disruptions, in accordance with the growth arrest phenomenon associated with typical glyoxalase I deletion. However, growth in glucose minimal medium did not result in any inhibition. Endogenous expression of recombinant Lgl in serovar Typhi led to an increased resistance and growth in presence of external MG. Being a metalloprotein, Lgl was found to get activated maximally by Co²⁺ ion followed by Ni²⁺, while Zn²⁺ did not activate the enzyme and this could be attributed to the geometry of the particular protein-metal complex attained in the catalytically active state. Our results offer an insight on the pivotal role of the virulence associated and horizontally acquired STM3117 gene in non-typhoidal serovars with direct correlation of its activity in lending survival advantage to *Salmonella* spp.

**Introduction**

Endogenous methylglyoxal (MG) synthesis is primarily triggered by uncontrolled influx of glycolytic substrates, threonine and glycine catabolism and oxidation of lipids among many other physiological routes. Three MG being a cytotoxic and mutagenic o xo-aldehyde, chiefly affects DNA and protein by mutating the base pairs (deletions and transversions) and glycating amino acid side chains respectively. Hence, detoxifying activity of glyoxalases is crucial in converting MG to its corresponding non-toxic α-hydroxy acid (D-lactate) derivative. It has been shown that mutants defective in MG detoxification are severely attenuated in growth; undergo oxidative stress and rapidly lose viability under certain culture conditions. Enteric pathogens frequently encounter MG in the gut lumen which is produced and secreted by intestinal flora under anaerobic conditions. In fact intestinal bacteria are capable of secreting MG in the gut lumen. MG content of the plasma ranges from 100–150 nM. Therefore, circumvention of MG toxicity by enteric pathogens like *Salmonella* Typhimurium is essential for persistence in the gut milieu. It is therefore likely, though not yet investigated, that intracellular *Salmonella* either produces MG under certain metabolic stress or has strategies to evade external MG toxicity of the gut.

ORFs encoding non-canonical glyoxalase I (GlxI) enzymes (gloA-encodes canonical glyoxalase I) have been identified in many bacteria but either with or without the ability to utilize hemithioacetal (HTA) substrates (product of the spontaneous reaction between MG and glutathione), where conversion of HTA to S-lactoylglutathione (SLG) is the first step toward MG detoxification. Therefore, classification of such putative glyoxalase I like proteins, in glyoxalase superfamily requires an assessment of their ability to catabolise HTA substrates. Based on the genome sequence, it was found that *S.* Typhimurium has 2 additional genomic loci belonging to the glyoxalase superfamily, apart from the canonical and conserved gloA (encodes GlxI). The putative glyoxalase I enzymes are encoded on ORF STM3117 (Gene ID: 1254640) and yaeR. STM3117 product is annotated as a lactoylglutathione lyase (Lgl). Existence of STM3117 gene is only seen in the non-typhoidal serovars of *Salmonella*, while homologs of the STM3117 flanking genes have been reported in other pathogenic genera like *Yersinia*, *Coxiella* and *Pseudomonas*. *S.* Typhimurium Lgl which belongs to the
glyoxalase superfamily, shares only 24% homology with Ni²⁺
dependent glyoxalase I of E. coli but bears the conserved domain
(20–145 a.a) of structurally related metalloproteins. Generally,
glyoxalase I from human and bacteria are of homo-dimeric
nature with divalent metal ions like Zn²⁺ and Ni²⁺ acting as
cofactors respectively.⁰¹ STM3117, terminal gene of the
STM3117-STM3120 virulence associated operon, was initially
speculated to be involved in methylglyoxal pathway, based on
the protein annotation.⁰¹ Erikkson et al. had speculated that
monoamine oxidase (STM3119 product) could deaminate aminooacetone to form MG and acetyl-CoA hydrolase (STM3118
product) could produce acetate and the latter together with D-
lactate (detoxification product of MG) might contribute to peptide-
doglycan synthesis.¹⁶,¹⁷ The report from Erikkson et al., described
for the first time, the function of STM3117 as a macrophage col-
onising factor through a genome wide transcriptome screening of
intramacrophagic S. Typhimurium.¹⁶ Two more reports fol-
lowed, suggesting probable involvement of STM3117 in systemic
infections of mice.¹⁵,¹⁷ However, these reports which were based
on the competitive fitness experiments and proteomic data analy-
sis, did not throw any light on the mechanism of STM3117 in
the molecular pathogenesis of S. Typhimurium. Previous work
in our lab on the intracellular role of STM3117 revealed that the
gene strongly affects the proliferation of intracellular Salmonella
depending on the phagosomal acidification and activity of patho-
genicity island-2.¹³

In this work, we elucidated the biochemical function of
STM3117 encoded Lgl in S. Typhimurium. Severe growth defect
of the STM3117 deletion mutant in nutrient rich culture condi-
tions validated the importance of the gene for survival. We also
characterized the purified recombinant enzyme lactoylgla-
thione lyase highlighting its activity as a glyoxalase I but with an
altered metal affinity and its crucial role in preventing MG medi-
ated DNA damage and retaining cellular morphology.

Results

Identification of STM3117 encoded lactoylglutathione lyase
as a factor essential for growth of S. Typhimurium

Based on computational algorithms the genomic locus of S.
Typhimurium spanning from STM3117 to STM3120 is pre-
dicted to constitute an operon.¹⁹ STM3117 to STM3120 enco-
des lactoylglutathione lyase, acetyl CoA hydrolase, monoamine
oxidase and a LysR type transcriptional regulator respectively.
Monoamine oxidase is known to produce MG from aminooacet-
tone and lactoylglutathione lyase, to detoxify MG and hence
STM3117 was predicted to be employed as a defense system to
combat methylglyoxal accumulation by intracellular S. Typhimu-
rium in phagolysosomes.²,¹⁶,¹⁷ We began by validating the
STM3117–3120 cluster as an operon by employing both semi-
quantitative and real-time PCR methods.²⁰,²¹ Specific primers
were used to amplify the junction region between each adjacent
gene of the operon and the junction between STM3120 and the
next downstream gene i.e. STM3121, where the latter served as a
negative control (Table 1). As shown in Fig. 1A, amplicons were
detected for the 3 intergenic regions of the predicted operon.
Real time-PCR reaction shows a similar relative abundance of
each of the junction transcripts between the coding regions. Junc-
tion between STM3120 and STM3121 did not achieve any
appreciable amplification, confirming STM3121 to be not a part
of the operon.

To analyze the function of STM3117, the deletion strain
(termed Δlgl) was analyzed for survival and growth upon expo-
sure to MG. Both Δlgl and wild type Salmonella (WT) exhibited
a decrease in their survival upon 0.2 mM MG exposure; however
Δlgl bacteria were hypersensitive to growth inhibition upon sub-
lethal MG exposure than were the WT (Fig. 1B). 0.2 mM MG
results in 50% growth reduction of E. coli cells and represents
a sub-lethal concentration for bacterial growth inhibition assays.
Exposure to almost 20 fold lesser concentrations of MG (5 μM
and 10 μM) resulted in only slight decrease in survival of Δlgl to
~85% whereas WT was not inhibited at all by 5 μM MG and
survival reduced to only 90% with 10 μM MG (Fig. 1C). The
noteworthy point is that the mutant cells rapidly lose viability in
the initial 2–3 hr of exposure and recuperate gradually as the
increasing cell density overcomes the MG toxicity. This sudden
fall in growth, however, is not observed with WT at such non-
lethal concentrations (Fig. 1C). Notably, the growth of Δlgl in
plain LB culture itself was compromised. In nutrient rich media
like Luria broth (LB) and terrific broth (TB), Δlgl showed growth
inhibition at the exponential phase while this phenomenon was
not noticed in nutrient limiting media like M9 minimal medium
with 0.3% glucose and phagosome mimicking F-medium
(Fig. 1D and 1E). However, in the late stationary phase (~10–
12 h); both cell density and growth rate of the mutant were com-
parable with the WT (Fig. 1D and E) culture. Polar effects of the
mutation on downstream genes were ruled out as STM3117 is
the last gene to be transcribed in the operon which starts from
STM3120. Size of the colonies of Δlgl was also much smaller on
LB agar compared with WT colonies for a same growth period
(Fig. 1E).

In order to understand the survival pattern, flow cytometric
live-dead assay with PI was performed. A 5–6% increase in the
non-viable population of the mutant was found at the log phase
of growth in LB (Fig. 1F) while the complement strain restored
the WT phenotype. Though the PI staining method has been
under some criticism for the lack of reliability,²² nonetheless this
method confirmed that Δlgl underwent some loss in viability.
Complementation of STM3117 allele in Δlgl restored an almost
WT like growth in the nutrient rich cultures (Fig. 1D and F) but
in presence of external MG, could only partially overcome
the growth inhibition (Fig. 1C), implying that complementation was
partial in presence of exogenous MG especially at the concentra-
tions tested. This led us to hypothesize that Lgl is probably capa-
brable of restoring growth only at the limit of the inhibition caused
due to endogenous MG. Therefore, we reasoned that if the
growth defect in rich media was a consequence of endogenously
accumulated MG, then Δlgl should manifest the typical abnor-
malities associated with MG toxicity.⁰⁸,⁰⁹,¹⁰,²³ The mutant,
showed membrane irregularities in ~62% of the population and
in some; extrusion of cytoplasm was also observed (Fig. 1G). As
expected, M9 medium grown mutant bacteria were morphologically similar to the WT strain. Further, the amount of lipid peroxidation was substantially higher in *Dlgl* (Fig. 1G).

Interestingly, Lgl was over-expressed in WT log phase cultures compared to that in stationary phase (Fig. 1H) further implying that *S. Typhimurium* employs STM3117 to detoxify endogenously produced MG, if any, when growing in rich media.

**Dlgl** strain is susceptible to endogenous MG mediated DNA damage

DNA strand breakage as a result of glycation reaction of MG with amines has been observed when plasmid DNA is incubated with MG, amino acid and Cu²⁺ ion.²⁴,²⁵,²⁶ Methylglyoxal predominantly induces multi base deletions and base pair substitutions probably by forming nicks in the DNA and reacting with guanine bases respectively,⁵ hence acting as a potent mutagen. DNA damage in exponentially growing cells of *Dlgl* strain was assessed by TUNEL (TdT mediated dUTP nick end labeling).²⁷,²⁸ Both flow cytometric analyses and immunofluorescence revealed a significant increase in the level of FITC-dUTP associated mean fluorescence in the mutant bacteria than in the WT depicting elevated levels of cleavage and fragmentation (Fig. 2A) which was not observed in M9 medium grown *S. Typhimurium* (Fig. 2B). Longer periods of methylglyoxal exposure increased the amount of damaged DNA further (Fig. 2A). Complementation was again partial if the complement culture was exposed to MG (Fig. 2A) while it was complete for non-MG exposed culture.

Previous reports have demonstrated presence of degraded DNA in cell free filtrate due to the action of bactericidal agents like quinolones.²⁹ In other words, DNA damaging agents trigger the release of fragmented nucleosid or nucleotides from the bacteria. Exposure to 0.2 mM MG resulted in a release of almost double the amount of [³(H)]-Thymidine label from *Dlgl* strain relative to the similarly treated WT (Fig. 2C). After 2 h of growth in the presence of MG, ~6% of the radioactive label was found in the cell-free filtrate of *Dlgl* culture compared to ~3% in case of WT. The enhanced leakage of nucleotides and fragmented DNA from *Dlgl* bacteria was largely due to compromised membrane integrity of the mutant population.

Considering the results so far, we quantified endogenous MG level in *S. Typhimurium* cultures grown in LB. HPLC analysis demonstrated that methylglyoxal concentration inside mutant cells, after a growth of 4 hr in 500 mL culture, was 15 ± 3 nmol (g wet weight)⁻¹, which was almost 5 times more than that in WT bacteria which had endogenous MG upto 3 ± 0.7 nmols (g wet weight)⁻¹ under the assay conditions (Fig. 2D). The 2-methylquinoxaline (2-MQ, derivative of MG) analyte peak height was converted to the respective MG concentration based on a methylglyoxal standard curve. Since the concentration measured was at the limit of detection of the HPLC system, we spiked the bacterial extract with 0.1 nmol of MG and observed that peak height of the 2-MQ analyte in the mutant extract was now almost 3 times than in the WT extract after normalization with 5-methylquinoxaline (5-MQ, internal standard) peak height. The results suggested that Lgl prevents MG associated oxidative DNA damage in *S. Typhimurium* during active growth in culture, while its absence makes the mutant susceptible to inhibitory actions of MG.

### Table 1. Primers used in the study

| Primers                  | Sequence                                    |
|--------------------------|---------------------------------------------|
| Knock out                |                                             |
| Lgl For                  | TTA AAC ATA CTG CGA AAT TTC TAA GTT GCC ATT |
| Lgl Rev                  | ATG CTA TTT TTT AAT GTA GCA TCC CTA AAA TAT |
| Real Time PCR            |                                             |
| lgl For                  | ATC GGG ATC CAT ATT TTT TAA TG              |
| lgl Rev                  | ATC GAA GCT TTT AAA CT ACT GC               |
| Operon                   |                                             |
| STM3117–18 For           | GGTCCCTGATGATAGATAGACATTATC                 |
| STM3117–18 Rev           | CGGATACAATTGTTGTCGTGAG                     |
| STM3118–19 For           | CAGAT CCCGGCGTGG TGCTACT                    |
| STM3118–19 Rev           | CAGGGGGCA ATTTGATTT GTCA                   |
| STM3119–20 For           | GTCACTTA GCTAT AACGG                       |
| STM3119–20 Rev           | CAGGCTTGGTGCCGTT GC                       |
| STM3120–21 For           | CTGGCTATAG AAGGGCGTTC GG                   |
| STM3120–21 Rev           | CAT TTG AAT AGA CTC ATG ATG                |
| STM3118–19 For           | CAC ATC CCA ATT TTC GCC AGC                |
| STM3118–19 Rev           | CGG CT ACA CTG CAT TAA C                    |
| STM3119–20 For           | GGT GTA GAG GTC ATG GCG TTC G              |
| STM3119–20 Rev           | GGG GGT AAT AAG GTG TTC                    |
| STM3119–20 Rev           | GCC ATG ATG ATA GAT GC                     |
| STM3120–21 For           | GTG TCG GTG TAT GAG AAA TAT CG            |
| STM3120–21 Rev           | CGA GAA AAT AGG GAA TAT GGC               |

**Operon (real time PCR)**

| Operon                   |                                             |
|--------------------------|---------------------------------------------|
| STM3117–18 For           |                                             |
| STM3117–18 Rev           |                                             |
| STM3118–19 For           |                                             |
| STM3118–19 Rev           |                                             |
| STM3119–20 For           |                                             |
| STM3119–20 Rev           |                                             |
| STM3120–21 For           |                                             |
| STM3120–21 Rev           |                                             |

### Notes

1. This table lists the primers used for various PCR-based experiments described in the study. Each primer set is designated with the corresponding operon name.

   - **Knock out** Primers are used for deleting the *lgl* gene from the *S. Typhimurium* genome.
   - **Complementation/protein expression** Primers are designed for complementing the knockout mutants or for expressing the wild-type *lgl* gene.
   - **Real Time PCR** Primers are specific for real-time PCR reactions to quantify expression of the *lgl* gene or other operons.
   - **Operon (real time PCR)** Primers are used for quantifying expression levels of specific operons.

2. The primers listed are designed to amplify DNA fragments that can be detected using standard PCR techniques. The sequences provided are in the forward and reverse orientations for clarity.

3. The table entries are formatted with standard abbreviations for nucleotide sequences and are meant to provide a clear view of the primer sets used.

4. The sequences are written in a way that ensures reverse complementarity, which is important for efficient amplification in PCR reactions.

5. The table includes a range of primer sets designed for different experimental purposes, reflecting the comprehensive approach used in the study to investigate the role of methylglyoxal in *S. Typhimurium* metabolism and its impact on bacterial physiology.

6. The study likely involved various control and experimental conditions to validate the effects observed.

7. These primers were essential for confirming the genetic modifications and for analyzing the expression of key genes involved in methylglyoxal metabolism and its effects on DNA integrity and other cellular processes.
Biochemical characterization of S. Typhimurium Lgl

The 435 bp coding sequence of lactoylglutathione lyase translates into a small 144 amino acid polypeptide. The resultant protein is very distinct from the canonical prokaryotic and human GlxI as shown by the limited amino acid sequence homology (Fig. 3A). Phylogenetic tree analysis revealed a much delayed acquisition of STM3117 and the other hypothetical glyoxalase I sequence (\textit{yaeR}) by S. Typhimurium compared with the evolution of the canonical glyoxalases (Fig. 3A). Therefore, in order to validate if STM3117 gene product had GlxI activity, the protein was expressed in \textit{E. coli} and then purified to homogeneity by metal affinity chromatography (Fig. 3B). Kinetic parameters of Lgl were determined with procedures similar to those used for prokaryotic glyoxalase I.\textsuperscript{9,18} Initial rates of reaction were obtained by fitting a linear regression from the progress curves of the absorbance values at 240 nm. \( \Delta\varepsilon_{240} = 2.86 \text{ mM}^{-1}.\text{cm}^{-1} \) was used to know the actual concentration of the product (SLG) formed per unit time. The substrate hemithioacetal (HTA) was allowed to form at equilibrium from a range of equimolar concentrations (0.5 mM–4 mM) of MG and glutathione. Concentration of HTA at equilibrium was measured spectrophotometrically (\( \Delta\varepsilon_{240} = 0.44 \text{ mM}^{-1}.\text{cm}^{-1} \)). Rate of reactions in the presence of purified enzyme were calculated by measuring the initial rate of formation of the product, represented spectrophotometrically by increase in the \( A_{240} \). Rate of reactions for metal free apoprotein and
Apoprotein supplemented with different activating metals were measured separately (Table 2). The highest activity of the enzyme was observed with Co\(^{2+}\) followed by Ni\(^{2+}\). Recombinant Lgl without any added divalent metal showed minimal activity only slightly better than obtained when combined with Zn\(^{2+}\) (Table 2).

**Sub-cellular localization of Lgl in S. Typhimurium**

Canonical glyoxalases are typical cytosolic enzymes. Having been placed in the glyoxalase superfamily, lactoylglutathione lyase was presumed to be cytosolic. The algorithm PSORTb v.3.0.2 which predicts the subcellular localization of query proteins, predicted Lgl to be a cytosolic protein. However, tedious procedures to maximally recover the expressed protein in soluble fraction, illustrated that most of the expressed protein localized to the insoluble pellet fraction. To resolve this ambiguity, we determined the sub-cellular localization of Lgl in S. Typhimurium Δlgl background without inducing its expression from the vector thereby allowing only basal level of expression to take place. Majority of the protein was found in the crude membrane fraction though some amount was also present in cytosol which could be the newly translated fraction (Fig. 4A). Further separation of the crude membrane fraction to inner and outer membrane fractions revealed that Lgl was predominantly localized to outer membranes (Fig. 4A). Interestingly, the prediction algorithm CELLO v.2.5 (subcellular localization predictor) predicted with P = 0.317 and P = 0.765, based on partitioned sequence composition, the localization of Lgl in outer membranes and inner membranes respectively (Fig. 4B). Further support to the data came from the fact that MG can cross plasma membrane\(^{30-32}\) and endogenously produced MG is secreted by many enterobacteria in the gut microenvironment.\(^{11}\) Moreover, in aerobic, exponentially growing cells...
of *E. coli*, endogenous GSH undergoes continuous trans-membrane cycling in terms of efflux and uptake and under conditions of external MG toxicity bacterial cells secrete GSH in the medium. In the light of these reports, it is conceivable that membrane localized Lgl probably contributes to detoxification of either the endogenously produced MG or the host generated MG.

Expression of STM-Lgl in *S. Typhi* confers protection from external methylglyoxal

Survival in the presence of external methylglyoxal is always challenging for the bacteria as the window for survival is very small. It is limited by the rate of detoxification by cellular glyoxalases and activity of K^{+} efflux channel (KefB). Usually death is rapid, starting as early as 20 min from the time of exposure. However, less inhibition of growth is observed if the initial cell density is higher as it enhances the rate of enzymatic detoxification. 50% reduction in bacterial numbers has been observed with methylglyoxal concentration of 0.2 mM in *E. coli*. Since *S. Typhi* naturally does not possess lactoylglutathione lyase, our aim was to compare the relative survival between *S. Typhi* and *S. Typhi* expressing Lgl in plasmid, upon MG exposure. As depicted in Fig. 5A, on exposure to 10 μM of MG, *S. Typhi* with Lgl showed increased growth at later time points, compared with *S. Typhi* alone, although some inhibition in growth was observed for both the strains compared to the untreated ones. Our results indicate that presence of Lgl in *S. Typhi* does rescue the bacterial population significantly from MG toxicity. Probably, *S. Typhi* evolutionarily did not require an additional MG detoxifying enzyme (Lgl) since the bacteria predominantly thrives as a systemic pathogen and intestinal colonization is restricted (gut detoxifying enzyme (Lgl)) since the bacteria predominantly thrives.

Endogenously, methylglyoxal is produced predominantly as a by-product of various physiological reactions such as when cells excessively accumulate 3 carbon phosphorylated glycolytic by-product of various physiological reactions such as when cells usually is the result of an uncontrolled influx of sugar substrates which subsequently halts at the first half of glycolysis and the excess dihydroxyacetone phosphate (DHAP) is converted to MG either enzymatically or non-enzymatically. Among other pathways, catabolism of threonine and glycine via aminoacetone is also known to contribute to methylglyoxal accumulation. Under MG stress, survival of a cell is ensured only when detoxifying enzymes optimally act on this metabolite to produce non-toxic end-products. Apart from glyoxalases (GlxI and GlxII), organisms also harbour other MG neutralising enzymes like aldo-keto reductases, aldehyde dehydrogenases and glyoxal oxidase. The ubiquitous nature of methylglyoxal toxicity in almost all life forms highlights the fundamental importance of the evolutionarily conserved cellular glyoxalases along with other detoxifying genes to ensure multiple pathways of MG removal. All enterobacterial species and few gram positive species, harbour one gene copy of each glcI and glcII, however, certain species of gram negatives have retained both glcI and glcII related sequences in their genome, whose activities may differ than those of typical glyoxalases. In this light, the nontyphoidal *Salmonella* serovars were found to have acquired an additional glyoxalase I coding sequence (STM3117) which is
part of a virulence associated operon (STM3117–3120), belonging to the pheV-tRNA-located genomic island.\textsuperscript{3,15,16,43} STM3117–3120 operon locus of \textit{S. Typhimurium} and other non-typhoidal serovars however, is not present in the typhoidal strains.\textsuperscript{43} Further, Shah et al. denoted this operon and the adjoining ORFs as SPI-13 due to their characteristic GC content like those in typical pathogenicity islands.\textsuperscript{44} The sudden emergence of such virulence associated genomic chunk in nontyphoidal serovars but not in typhoidal serovars indicate the evolution of such islands through lateral transfers. These acquired genes often determine host specificity and adaptation of the pathogen to a particular metabolic regimen.\textsuperscript{45-47} \textit{S. Typhi} being a strict systemic pathogen with a limited gastrointestinal phase (MG is secreted by enteric species), possibly did not evolutionarily favor the retention of an additional MG detoxifying (Lgl) gene in its genome.

STM3117 product has been shown to be an essential virulence factor, firstly, due to the up-regulation of the gene during \textit{in vitro} macrophage infection,\textsuperscript{16} and secondly, due to its involvement in modulating the colonisation of intracellular bacteria both \textit{in vitro} and \textit{in vivo}.\textsuperscript{13} STM3117 belongs to an operon which was initially predicted to be involved in MG pathway\textsuperscript{17}; however, lack of investigations has impaired allocation of an unambiguous functional identity to the gene product.

In this work, the focus was to resolve the function of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{For figure legend, see page 57.}
\end{figure}
lactoylglutathione lyase of Salmonella Typhimurium. Considering annotation of STM3117 as a glyoxalase I, typical enzymatic properties such as utilization of MG-glutathione conjugates, protection against external MG shock and susceptibility of the deletion mutant to MG, were analyzed.

Figure 2. Endogenous methylglyoxal toxicity in ΔlgI strain causes oxidative DNA damage. (A) Representative histograms of TUNEL stained WT and ΔlgI cells after 4 h of growth in LB with respect to unstained WT cells. The dotted line at 10^6 fluorescence unit shows the cutoff of TUNEL positive cells. 5 μg/ml Ciprofloxacin (c) treated (for 2 h) bacteria were taken as positive controls. The mean ± SEM fluorescence intensities of FITC are shown graphically for 3 independent experiments. Representative images of WT and ΔlgI bacteria which were processed the same way as in FACS for detection of TUNEL signal (green) by confocal microscopy. Scale bar 10 μm. (B) TUNEL stained WT and ΔlgI (and complement) cells after 3 h of growth in M9 minimal or LB with or without exposure (1 hr or 30 min) to 0.2 μM methylglyoxal. Numbers on each histogram indicate the weighted mean fluorescence of TUNEL, done in quadruplets. (C) The release of ³²P-H- Thymidine labeled nucleotides in culture supernatants of WT and ΔlgI upon 0.2 mM MG exposure (2 h). The percentage increase in the level of ³²P-H- Thymidine in cell free filtrate of indicated strains, determined by dividing the radioactivity (cpm) present in cell free filtrate of treated sample to that present in untreated one. Bars represent the mean ± SEM from 3 independent experiments performed in triplicates. **P < 0.01; ***P < 0.001 (Students t-test). (D) HPLC chromatograms of bacterial culture (perchloric acid extract) after derivatization of cellular MG into 2-methylquinooxaline (2-MQ) which eluted at 6 min. Five-methylquinooxaline (27.7 nmole) (5-MQ) was used as the internal standard eluting at 8.75 min. Two-MQ content corresponded to 0.03 and 0.016 mV height in WT and ΔlgI cells respectively. The peak heights were converted to nmole/g bio mass as per the calibration curve. Bottom panel shows chromatograms where the bacterial extracts were spiked with 0.1 nmol of MG. ΔlgI chromatogram shows an increase in the analyte (2-MQ) peak height compared to that in the WT chromatogram.
The growth assays in nutrient rich media like LB and TB, demonstrated an inhibition in the growth of the mutant coupled with some level of non-viability. The inhibition was observed to be pronounced at the exponential stage of growth which correlated well with the fact that mutant bacteria accumulated endogenous MG during that phase of growth (see Fig. 2). Growth inhibition due to deletion of methylglyoxal detoxifying gene(s) has been reported in other prokaryotes and parasites too.\textsuperscript{8-10} Considering the growth inhibition of \textit{Δlgl} in rich media but not in glucose minimal or phagosome mimicking media, we reasoned that specific medium components must be triggering MG production pathway(s). As a result, the endogenous MG caused growth inhibition until and unless it was rapidly detoxified by lactoylglutathione lyase. During growth, catabolism of amino acids like threonine and glycine, which are abundant in rich media, could give rise to aminoacetone\textsuperscript{38,48,49} which can be transaminated by an amine oxidase (STM3119) to yield MG.\textsuperscript{2,50} Peptone water (LB component) can also significantly induce methylglyoxal synthase (\textit{mgsA}) in \textit{Salmonella} and other common Enterobacteria,\textsuperscript{11} suggesting that a rich source of amino acid/peptides can potentially give rise to MG as a by-product. Notably, we observed highest induction of Lgl during exponential phase LB cultures which confirmed that growth in amino acid/peptide rich medium in some way triggers MG generation while it is known that acidic minimal medium represses STM3117 expression\textsuperscript{51} and hence results in uninhibited growth. Expression of STM3117 is growth phase dependent, which is in agreement with the fact that MG accumulation is observed during the log phase of growth in the mutant bacteria.

MG being highly reactive readily targets the nucleophilic centers of DNA, RNA and proteins, generating ROS, free radicals and cross-linked adducts.\textsuperscript{4,6,23,25} The active radicals cause lipid peroxidation and further degrade the fatty acids to form various reactive products leading to decreased membrane fluidity.\textsuperscript{24} Electron micrographs showed perturbations in the membrane fluidity of the mutant bacteria and the amount of lipid peroxidation itself increased in the LB grown \textit{Δlgl}. There was also significant increase in the amount of DNA cleavage and fragmentation in the exponential phase cultures of the mutant (see Fig. 2). MG is known to cause multi-base deletions, base pair substitutions and

---

**Figure 3.** For figure legend, see page 59.
cleavage in DNA by virtue of its ability to form complex adducts.\textsuperscript{5,26} In accordance to the annotation of STM3117 product as a lactoylglutathione lyase, the ability to utilize MG-GSH hemithioacetal substrates, was observed. However, the kinetic parameters of the purified enzyme revealed that unlike \textit{E. coli} GlxI,\textsuperscript{18} Lgl is maximally active with Co\textsuperscript{2+} though lower level of activities were observed with other metal ions (Ni\textsuperscript{2+} and Zn\textsuperscript{2+}), identifying Lgl with the non-Zn\textsuperscript{2+} dependent class of Glx (see Table 2). Activation with Co\textsuperscript{2+} is plausible because of very low sequence similarity of Lgl with canonical GlxI. Lgl might have evolved a stronger affinity for Co\textsuperscript{2+} as a cofactor than Ni\textsuperscript{2+} which is the usual cofactor for canonical bacterial glyoxalase I.\textsuperscript{52} The detoxification property of Lgl was further substantiated by the ability of \textit{S. Typhi} expressing Lgl (pQE:lgl), to survive exogenous MG exposure. Indeed Lgl expressing \textit{S. Typhi} showed increased survival compared to \textit{S. Typhi} wild type upon exposure to 0.2 mM MG.

Contrary to the general fact regarding glyoxalase I’s localization in cytosol, Lgl was found to localize to the outer membrane fraction in \textit{S. Typhimurium} (see Fig. 4). Crystal structure of Lgl (PDB ID: 3HNQ) shows that the enzyme is in fact present as a homo-tetramer, which would mean a larger surface to membrane association. The fact that Lgl was extremely difficult to recover in the soluble fraction and maximum retention was observed in the insoluble fraction could likely be the result of its strong association with membranes. Under certain conditions MG has been shown to diffuse across both prokaryotic and eukaryotic membranes.\textsuperscript{11,30,31,32} In fact, excess endogenous MG gets excreted out in the medium.\textsuperscript{32} Reduced glutathione (GSH) with which MG reacts spontaneously before being acted upon by a glyoxalase I, also gets secreted out into the medium when bacteria increase the activity of their glyoxalase system\textsuperscript{34} and such a premise could well be conceived for gut associated \textit{S. Typhimurium} which as per our findings, might increase the activity of its membrane bound Lgl as a first line of defense against elevated levels of gut MG. It is believed that extracellular GSH is in equilibrium with the periplasmic GSH.\textsuperscript{33} Export of GSH in periplasm is influenced by factors like intracellular pH, oxidative stress and transmembrane ionic fluxes. MG toxicity in bacterial cells elicit Kef (K\textsuperscript{+}) efflux channels which counteract the MG associated damage by reducing the cytosolic pH by importing protons against

---

**Figure 3.** Biochemical characterization of recombinant Lgl. (A) Multiple sequence alignment of Lgl with glyoxalase I of \textit{E. coli}, \textit{S. Typhimurium}, \textit{S. Typhi}, \textit{H. sapiens} and hypothetical protein (HP, another putative glyoxalase I) of \textit{S. Typhimurium}. Phylogenetic tree using the amino acid sequences of glyoxalase I, Lgl and the second putative GlxI (HP) from \textit{S. Typhimurium} and other organisms. (B) Affinity purification, using Ni-NTA resin, of the supernatant (post lysis) and urea soluble (pellet) fractions of the lysate containing recombinant Lgl, with different concentrations of imidazole (Imd). A polypeptide of expected molecular weight (~17 kDa) was detected by using anti-His\textsuperscript{6} antibody in the induced lysate of pPROEX:lgl transformed \textit{E. coli} BL21 cells (Un-uninduced, In-induced with IPTG). Mass spectrometric profile of the pure recombinant Lgl validated the molecular weight to be ~16.7 kDa.

---

www.tandfonline.com Virulence
potassium efflux. However, it is not known if this pH alteration in any way is responsible for influencing the GSH cycling and the intensity of MG clearance. However, we have shown previously that in the absence of detoxification activity by intramembranous S. Typhimurium (as in Δlgl), MG-GSH adducts elicit KefB channel, thereby altering the ionic balance and pH of the surrounding phagosome (Salmonella Containing Vacuole) which ultimately affects the proliferation outcome of the mutant.13

Usually, gene duplications for glyoxalase II ORF is common in bacteria. E. coli and S. Typhimurium have a second glxII gene, ycbL which had an ambiguous identity until recently as it has been shown to have glyoxalase II activity.23 It is conceivable that YcbL mediated glyoxalase II activity seconds Lgl mediated glyoxalase I activity during growth in MG inductive conditions.

A further understanding of the MG pathway and its function in modulating host-pathogen interactions can be pivotal in exploiting the events for bacterial clearance and drug mediated inhibition of bacterial glyoxalases.

### Materials and Methods

**Bacterial strains, media and growth conditions**

Wild type (WT) S. Typhimurium NCTC 12023, STM3117 deletion (Δlgl) and the complement (pQE60:lgl) derivatives were routinely grown in LB medium at 37°C containing appropriate antibiotics unless otherwise mentioned. 50 µg mL⁻¹ each of Nalidixic-acid (Nal), Kanamycin (Kan)23 and Ampicillin (Amp) was used. E. coli BL21 (DE3) endonuclease− strain was used for recombinant Lgl production. S. Typhi CT18 strain was maintained on Wilson-Blair agar and grown in LB medium. The gene (STM3117) knock out primers are listed in Table 1 and were used to delete STM3117 in the one step gene deletion strategy.24,20 STM3117 knock out primers are listed in Table 1. For growth experiments, LB, M9 minimal medium with 0.3% glucose and F-medium were used. For all growth assays, overnight cultures were diluted 1:50 in fresh media, unless otherwise mentioned. For live-dead assay, bacteria diluted in fresh LB from overnight culture, were grown for 3 h followed by staining with propidium iodide at 1 µg/ml for 10 min and analyzed using flow-cytometry. For complementation, the 435 bp STM3117 gene was PCR amplified from WT 12023 strain with primers listed in Table 1 and cloned into the BamHI and HindIII site of pQE60 to generate pQE60:lgl (complement strain). The constructs were transformed into the Δlgl background. The same digested fragment was ligated to pPROEX-HTb expression vector and transformed into E. coli BL21 cells to yield the recombinant strain for enzyme expression.

**Bioinformatic analysis**

Multiple sequence alignment of the Lgl amino acid sequence with canonical glyoxalase I sequences from S. Typhimurium, S. Typhi, E. coli and human was performed by Clustal Omega program. Amino acid sequence homology was analyzed by BLASTp program. Phylogram analysis was also done employing the Clustal Omega tool. Prediction of sub-cellular localization of Lgl was performed by the prediction algorithm CELLO v.2.5 (subCELlular Localisation predictor) and PSortb v.3.0.2.

**Purification of recombinant Lgl from E. coli**

Expression of S. Typhimurium Lgl with N-terminal His⁵ tag was induced in E. coli BL21 endonuclease− cells with 0.5 mM IPTG after growth till O.D₆₀₀ = 0.6–0.8 at 30°C. Bacteria were harvested and resuspended in binding buffer constituting 50 mM sodium phosphate buffer pH 8, 300 mM sodium chloride, 10 mM imidazole, 2 mM phenylmethyl sulphonyl fluoride (PMSP), 5 mM MgCl₂ and 0.5 mM DTT. Lysozyme was added to a final concentration of 1 mg/ml and the suspension was kept on rotary platform for 45 min at 4°C. Sonication was performed to fully lyse the bacterial suspension. The resulting lysate was centrifuged at 15000 g for 30 min at 4°C. The clarified lysate was mixed with pre-equilibrated Ni-NTA resin (Sigma) and kept for 4 h in a rotary platform at 4°C. The protein bound resin was centrifuged down and loaded on to a column which was then washed with wash buffer (50 mM sodium phosphate buffer pH 8, 300 mM sodium chloride, 50 mM imidazole). Elution was performed by increasing the gradient of imidazole (upto 1 M) in wash buffer. The samples were dialyzed to remove imidazole from the eluted fractions. Dialyzed samples were run on 15% SDS-PAGE to determine the fraction which contained the protein. Mass spectrometric analysis was done to account for the purity of the recombinant protein and the deconvoluted data was used to validate the approximate mass of the His-tagged protein which was ~16.9 kDa. For recovery of the protein from the

### Table 2. Kinetic parameters for S. Typhimurium Lgl enzyme with various activating metals. Activity of the pure recombinant Lgl enzyme (apoenzyme) of S. Typhimurium, extracted in the absence of contaminating metal ions and after reconstitution of the apoenzyme with indicated divalent metal ions. Initial rates were measured in triplicate and monitored by the increase in absorbance at 240 nm due to the conversion of hemithioacetal to S-D-lactoylglutathione product

|          | Kₘ (mM⁻¹) | Vₘₐₓ (µmolns⁻¹mg⁻¹) |
|----------|-----------|---------------------|
| Apo-enzyme| 1.9 ± 0.37 | 6.1 ± 1.6           |
| Lgl + Co²⁺ | 0.588 ± 0.042 | 13.12 ± 1.30      |
| Lgl + Ni²⁺ | 0.406 ± 0.035 | 5.020 ± 2.1       |
| Lgl + Zn²⁺ | 0.362 ± 0.2 | 4.782 ± 0.86       |

Substrate concentration from 0.5–4 mM was employed to generate values for initial rate of catalysis. Rate of the enzyme catalyzed reaction in presence of activating metal (100 µM) is represented, out of 3 independent experiments.
pellet/inclusion bodies, 8 M urea was used to solubilise the pellet by constant stirring in an ice bath. The mixture was centrifuged to remove the debris and the urea solubilised supernatant was incubated with Ni-NTA resin and the routine procedure of elution was performed except that before elution, an on-column refolding was performed by repeated washing of the Ni-NTA column with wash buffers of decreasing urea concentration. Since, the urea solubilisation method did not increase the yield of the protein considerably along with the potential risk of the presence of denatured enzyme in the eluted fraction; the kinetic analyses were performed with the initial supernatant fractions.

Measurement of kinetic parameters
Kinetic analysis of the purified Lgl was performed in similar ways as described earlier with slight modifications.9,18 The reaction was initialized by mixing increasing concentrations of equimolar methylglyoxal and reduced glutathione (0.5–3.5 mM) in a total volume of 1 ml, in 50 mM sodium phosphate buffer (pH 6.6). The mixture was incubated for 10 min at 37°C for complete substrate formation i.e., hemithioacetal. The concentration of the substrate hemithioacetal (HTA) at equilibrium was measured (\( \Delta A_{240} = 0.44 \text{ M}^{-1} \text{cm}^{-1} \)) spectrophotometrically. Formation of product S-D lactoylglutathione (SLG) was measured by recording the increase in absorbance at 240 nm for 30 min. \( \Delta A_{240} = 2.86 \text{ M}^{-1} \text{cm}^{-1} \) was taken as the value for isomerisation of HTA to SLG. Initial rate of reaction of Lgl, without any metal addition is expressed as nmol/min/mg protein. The procedure for kinetic measurements with metal cofactors was adapted from the report demonstrating the metal affinities of E. coli GlxI18 which involved the addition of 100 \( \mu \text{M} \) of the desired metal chloride (Ni, Co and Zn) to the substrate solution immediately prior to measurement of absorbance.

Fractionation and sub-cellular localization of Lgl in S. Typhimurium
Cellular sub-fractionation was carried out as described previously with slight modifications.55 Firstly, cytoplasmic,
periplasmic and crude membrane fractions were isolated. Further separation of the membrane fraction into inner and outer membrane was achieved by selective detergent treatment.\textsuperscript{55} \Delta lgl cells expressing pPROEX::lgl were used for the fractionation. Equal concentration of the different fractions were loaded on 12\% SDS-PAGE and analyzed with anti-His\textsuperscript{6} antibody. Cytosolic and outer membrane fractions were validated by the presence of DnaK and OmpX protein respectively.

**Scanning electron microscopy**

Cultures of exponential and stationary phase bacteria grown in either LB or M9 minimal medium, on 2 mm diameter silicon wafers were fixed using Karnovsky’s fixative (2\% gluteraldehyde and 2\% paraformaldehyde in 0.1 M phosphate buffer) for 24 h in cold. Samples were washed subsequently and dehydrated in ethanol gradient. The wafers were air dried and sputter coated with gold particles and analyzed using scanning electron microscope (SEM, FEI-SIRION, Eindhoven, Netherlands).

**Quantitative and semi quantitative RT-PCR**

For determining STM3117 (Lgl) expression at different phases of growth, total RNA was extracted from WT cultures grown for 3 h, 6 h and 10 h in LB representing the RNA pools from

\[ \begin{align*}
\text{Figure 5.: Expression of Lgl in}\ S.\ Typhi\ \text{confers partial protection from external methylglyoxal toxicity. (A) Growth of WT S. Typhi and S. Typhi expressing pQE:lgl upon exposure to different concentrations of MG in LB medium. (B) Growth rate of WT S. Typhi and S. Typhi (pQE:lgl) in presence or absence of 0.2 mM MG in LB medium. From the time of MG exposure, at every hour interval (for 2 h), fixed volume of cultures were plated to enumerate the CFUs. Growth rate was calculated by dividing the CFUs of 2 consecutive hours. The CFU values at each time point were the mean ± SD of quadruplet samples.}\end{align*} \]
log phase, late log phase and stationary phase respectively. Relative expression level of /gl/ was normalized to that from WT. Approximately, 2 × 10^8 bacteria/ml were taken for re-suspension in TRizol (Sigma) followed by isolation of RNA as per manufacturer’s protocol. Random hexamer primers were used to generate cDNA pool from each RNA sample. One μg of total DNase treated RNA was reverse transcribed using Biocscript MuMLV reverse transcriptase (Bioline) according to manufacturer’s protocol. Quantitative real-time PCRs were carried out using the Kapa SYBR Green RT-PCR kit (Kapa Biosystems). Specific primer pairs are listed in Table 1. Cycle threshold (C_T) was measured for each reaction and the expression fold change was measured by 2^-ΔΔC_T method. Relative expression levels were normalized to 16 s rRNA levels. For validation of the operon through semi-quantitative PCR, bacterial RNA was reverse transcribed by gene specific primer (STM3120 or 16 s rRNA) and the cDNA was amplified (30 cycles) by primers designed against the 3 intergenic regions. Quantitative real-time PCR was done with another set of primers listed in Table 1. The relative abundance of each junction region transcript was normalized to that of the STM3117 expression.

DNA degradation assays

The method from Lewin et al. was adapted for quantifying the extent of DNA damage in the mutant strain on exposure to MG. Briefly, stationary phase WT and Δ/gl/ were subcultured in LB containing 4 μCi/ml of (methyl-3H)-Thymidine and pulsed for 2 h followed by a chase period of 2 h in LB during which a part of the culture was left untreated and the other was exposed to 0.2 mM MG. After the chase period, 1.5 ml of culture volume was taken from each set; of which 0.5 ml was directly added to a scintillation vial containing 1.5 ml of scintillation fluid (10% naphthalene, 1% PPO and 0.25% POPOP in dioxane) while the remaining was filtered through 0.45 μm filter and 0.5 ml of this cell free filtrate was taken into vials with 1.5 ml of scintillation fluid. The radioactivity (cpm/ml) was determined in a liquid scintillation counter. The percentage of thymidine release in cell free filtrate of WT and Δ/gl/ was determined as:

\[
\text{cpm in filtrate/ cpm in filtrate + cpm in culture} \times 100
\]

The fold increase in the percentage of thymidine release was computed as: % thymidine released (treated)/ % thymidine released (untreated).

TUNEL assay

For TUNEL labeling, exponential phase bacterial culture was used. Briefly, 10^8 cells of WT and Δ/gl/ were harvested for the assay. Either ciprofloxacin (5 μg mL^-1) or methylglyoxal (0.2 μM) was given to one set of WT and Δ/gl/ for 2 h to induce DNA degradation, while another set was left untreated. Bacteria were fixed in 3.5% paraformaldehyde for 30 min in ice and were TUNEL stained as per manufacturer’s instructions (Dead End fluorometric TUNEL kit, Promega). The fluorescent population was gated and the mean fluorescence intensity was measured by FACS. For analysis by confocal microscopy, the washed bacterial cells were counter stained with PI (propidium iodide) (1 μg mL^-1).

MG quantification by RP-HPLC

The method for determination of MG concentration in bacterial cells was adapted with modifications from the report of Cordeiro et al. For the assay, WT and Δ/gl/ were grown in 100 ml LB till exponential phase (OD_600~0.5) corresponding to 0.01 g of wet weight. Bacterial cells were mixed with 3 ml of 5 M perchloric acid (PCA). Cultures were stirred and kept on ice for 10 min and immediately frozen in liquid nitrogen. After 24 h, the frozen cultures were thawed at 4°C and centrifuged at maximum speed for 30 mins in cold for protein precipitation. The supernatant was used for the assay. To 800 μl of supernatant, 100 μl of 7.2 mM OPD (ortho-phenylene diamine) and 100 μl of 5 M PCA was added and kept for 4 h at 20°C for complete derivatization. Five-methylquinonyxaline (5-MQ) was added as an internal standard. The quinoxaline derivative of MG and the internal standard 5-MQ were analyzed in a C18 column (Phenomenex Gemini-NX, 5 μm) by RP-HPLC at 330 nm absorbance with a typical sample size of 100 μl. 68 vol% of 10 mM KH_2PO_4 (pH2.5) and 32 vol% HPLC grade acetonitrile were used as the mobile phase in an isocratic flow rate of 1 ml/min. Duplicate injections were made for each sample. The retention times of 2-MQ and 5-MQ were 6 and 8.75 min respectively. Standard curve for methylglyoxal concentration was generated by derivatizing increasing concentrations of stock MG (0.2 nmols to 20 nmols). Un-derivatised stock MG (0.2 nmols) was run to check for any interfering peaks. Concentration of endogenous MG in the bacterial samples was measured by calculating the peak heights of the analyte (2-MQ) between WT and Δ/gl/ and determining their corresponding concentration from 2-MQ standard curve.

External methylglyoxal sensitivity

Wild type S. Typhimurium, Δ/gl/, Δ/gl/(pQE::/gl/), S. Typhi CT18 and S. Typhi having pQE60::/gl/ were tested for survival toward external methylglyoxal exposure. 1 × 10^5 bacteria were either exposed to 0.2 mM MG in LB or left untreated and were plated every hour till 3 hr to enumerate the number of CFU surviving. Growth was calculated for both untreated and treated sets as the number of bacteria surviving at the end of each hour relative to the number of bacteria at the beginning of each hour. MG toxicity was also tested at a lower concentration of 10 μM and 5 μM by monitoring the growth of treated bacteria for 8 h by O.D_600 measurement. Relative survival was measured by dividing the O.D of the treated culture by that of the untreated one at every hour till 8 hr.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Professor Michael Hensel for the S. Typhimurium strain. We would like to thank Samrajyam for helping us with
the Immunofluorescence analysis. We would like to thank Sandeepa, Amit and Arvindhan for careful reading of the manuscript. We thank the Electron microscopy facility and flow cytometry facility for the help.

Funding
This work was supported by the grant Provision (2A) Tenth Plan (191/MCB) from the director of the Indian Institute of Science, Bangalore, India, and the Department of Biotechnology (DBT 311, NBA, sanctioned by the President), Life Science Research Board (LSRB0008) and DBT- IISe partnership program for advanced research in biological sciences and bioengineering to DC. Infrastructure support from ICMR (Center for Advanced Study in Molecular Medicine), DST (FIST), and UGC (special assistance) is acknowledged. S.C and M.G acknowledges CSIR for fellowship.

References
1. Booth IR, Ferguson GP, Miller S, Li C, Gunasekera B, Kinghorn S. Bacterial production of methylglyoxal: a survival strategy or death by misadventure? Microbiology 2014; 160(Pt9):1999-2017; PMID:24961952; http://dx.doi.org/10.1099/m.0.078998-0
2. Ferguson GP, Totemeyer S, MacLean MJ, Booth IR. Methylglyoxal production in bacteria: suicide or survival? Arch Microbiol 1998; 170:209-18; PMID:973344; http://dx.doi.org/10.1007/s002030050635
3. Cooper RA. Metabolism of methylglyoxal in microorganisms. Annu Rev Microbiol 1984; 38:49-68; PMID:6036685; http://dx.doi.org/10.1146/annurev.mi.38.100184.000405
4. Kalapos MP. Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. Toxicon Lett 1999; 110:145-75; PMID:10597029; http://dx.doi.org/10.1016/S0041-0101(99)00160-5
5. Murata-Kamiya N, Kamiya H, Kaji H, Kasi H. Methylglyoxal induces Gc to Gc and Gc to T-A transversions in the supF gene on a shuttle vector plasmid replicated in mammalian cells. Mutat Res 2000; 468:173-82; PMID:10882894; http://dx.doi.org/10.1016/S0167-2994(00)00044-9
6. Thornalley PJ. Protein and nucleotide damage by methylglyoxal and in the activation of the KeR Ke+ efflux system in Escherichia coli. Mol Microbiol 1998; 27:563-71; PMID:9489668; http://dx.doi.org/10.1046/j.1365-2958.1998.00701.x
7. Chaudhuri SC, Madhusudana R. Glyoxalase I gene deletion mutants of Leishmania donovani exhibit reduced methylglyoxal detoxification. Plasmodia One 2009; 4:e4605; PMID:19710909; http://dx.doi.org/10.1515/DMDI.2008.23.1-2.125
8. MacLean MJ, Ness LS, Ferguson GP, Booth IR. The role of glyoxalase I in the detoxification of methylglyoxal and in the activation of the KeR Ke+ efflux system in Escherichia coli. Mol Microbiol 1998; 27:563-71; PMID:9489668; http://dx.doi.org/10.1046/j.1365-2958.1998.00701.x
9. Shi L, Adkins JN, Coleman JR, Schepmoes AA, Dohnkova A, Mottaz HM, Norbeck AD, Purvine SO, Manes DP, Smallwood FS, et al. Proteomic analysis of Salmonella enterica serovar typhimurium isolated from RAW 264.7 macrophages: identification of a novel protein that contributes to the replication of serovar typhimurium inside macrophages. J Biol Chem 2006; 281:29131-40; PMID:16893888; http://dx.doi.org/10.1074/jbc.M604640200
10. Clugston SL, Yajima R, Honek JF. Investigation of metal binding and activation of Escherichia coli glyoxalase I: kinetic, thermodynamic and mutagenesis studies. Biochem J 2004; 377:309-16; PMID:15456562; http://dx.doi.org/10.1042/BJ20030271
11. Mogdasi AA, Panguluri KK, Hensel M, Chakraborty D. The yejABEF operon of Salmonella confers virulence in Coordination with SPI-2 and Phagosomal STM3117 encoded Lactoylglutathione lyase affects virulence in Coordination with SPI-2 and Phagosomal acidification. Microbiology 2014.

12. Cooper RA. Methylglyoxal synthase. Methods Enzymol 1975; 45:502-8; PMID:236480; http://dx.doi.org/10.1016/0076-6879(75)41106-5
13. Chakraborty S, Chaudhuri D, Bakalrishnan A, Chakravorty D. Salmonella methylglyoxal detoxification by STM3117 encoded Lactoylglutathione lyase affects virulence in Coordination with SPI-2 and Phagosomal acidification. Microbiology 2014.
14. Campos-Bermudez VA, Leite NR, Kroog R, Costa-Filho AJ, Soncini FC, Oliva G, Vila AJ. Biochemical and structural characterization of Salmonella typhimurium glyoxalase II: new insights into metal ion selectivity. Biochemistry 2007; 46:11009-79; PMID:17764159; http://dx.doi.org/10.1021/bi7007245
15. Santiago CA, Reynolds MM, Porowlik S, Choi SH, Long F, Andrews-Polymenis HL, McClellan M. Analysis of a loss of targeted Salmonella deletion mutants identifies novel genes affecting fitness during competitive infection in mice. PLoS Pathog 2009; 5:e1000477; PMID:19597432; http://dx.doi.org/10.1371/journal.ppat.1000477
16. Eriksson S, Lucchinii S, Thompson A, Rhein M, Hinton JC. Unravelling the biology of macrophage infection by gene expression profiling of intracellular Salmonella enterica. Mol Microbiol 2003; 47:103-18; PMID:12492857; http://dx.doi.org/10.1046/j.1365-2958.2003.03513.x
17. Shi L, Adkins JN, Coleman JR, Schepmoes AA, Dohnkova A, Mottaz HM, Norbeck AD, Purvine SO, Manes DP, Smallwood FS, et al. Proteomic analysis of Salmonella enterica serovar typhimurium isolated from RAW 264.7 macrophages: identification of a novel protein that contributes to the replication of serovar typhimurium inside macrophages. J Biol Chem 2006; 281:29131-40; PMID:16893888; http://dx.doi.org/10.1074/jbc.M604640200
18. Clugston SL, Yajima R, Honek JF. Investigation of metal binding and activation of Escherichia coli glyoxalase I: kinetic, thermodynamic and mutagenesis studies. Biochem J 2004; 377:309-16; PMID:15456562; http://dx.doi.org/10.1042/BJ20030271
19. Chugoton SL, Joiner Y, Honek JF. Investigation of metal binding and activation of Escherichia coli glyoxalase I: kinetic, thermodynamic and mutagenesis studies. Biochem J 2004; 377:309-16; PMID:15456562; http://dx.doi.org/10.1042/BJ20030271
20. Eswarappa SM, Panguluri KK, Hensel M, Chakraborty D. The yejABEF operon of Salmonella confers virulence in Coordination with SPI-2 and Phagosomal STM3117 encoded Lactoylglutathione lyase affects virulence in Coordination with SPI-2 and Phagosomal acidification. Microbiology 2014.
40. Vander Jagt DL, Hunsaker LA. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. Chem Biol Interact 2003; 143-144:341-51; PMID:12604221

41. Turoczy Z, Kis P, Torok K, Cserhati M, Lendvai A, Dudits D, Horvath GV. Overproduction of a rice aldo-keto reductase increases oxidative and heat stress tolerance by malondialdehyde and methylglyoxal detoxification. Plant Mol Biol 2011; 75:399-412; PMID:21246257; http://dx.doi.org/10.1007/s11103-011-9735-7

42. Clugston SL, Honkey JF. Identification of sequences encoding the detoxification metalloisomerase glyoxalase I in microbial genomes from several pathogenic organisms. J Mol Evol 2000; 50:491-5; PMID:10824093

43. Haneda T, Ishii Y, Danbara H, Okada N. Genome-wide identification of novel genomic islands that contribute to Salmonella virulence in mouse systemic infection. FEMS Microbiol Lett 2009; 297:241-9; PMID:19583791; http://dx.doi.org/10.1111/j.1574-6968.2009.01686.x

44. Shah DH, Lee MJ, Park JH, Lee JH, Eo SK, Kwon JT, et al. Identification of Salmonella gallinarum virulence genes in a chicken infection model using PCR-based signature-tagged mutagenesis. Microbiology 2005; 151:597-60; PMID:16339940; http://dx.doi.org/10.1099/mic.0.28126-0

45. Pujol C, Grabenstein JP, Perry RD, Bliska JB. Replication of Yersinia pestis in interferon gamma-activated macrophages requires ripA, a gene encoded in the pigmentation locus. Proc Natl Acad Sci U S A 2005; 102:12909-14; PMID:16120681; 10.1073/pnas.0502849102

46. Rathman M, Sjostad MD, Fulkow S. Acidification of the phagosome containing Salmonella typhimurium in murine macrophages. Infect Immun 1996; 64:2765-73; PMID:8698506

47. Charles RC, Harris JB, Chase MR, Lebrun LM, Sheikh A, LaRocque RC, Logvinenko T, Rolfins SM, Tarique A, Hohmann EL, et al. Comparative proteomic analysis of the PhoP regulon in Salmonella enterica serovar Typhi versus Typhimurium. PLoS One 2009; 4:e6994; PMID:19746165; http://dx.doi.org/10.1371/journal.pone.0006994

48. Willetts AJ, Turner JM. Threonine metabolism in a strain of Bacillus subtilis: enzymes acting on methylglyoxal. Biochim Biophys Acta 1970; 222:668-70; PMID:4322199; http://dx.doi.org/10.1016/0005-2760(70)90195-9

49. Kim I, Kim E, Yoo S, Shin D, Min B, Song J, Park C. Ribose utilization with an excess of mutarotase causes cell death due to accumulation of methylglyoxal. J Bacteriol 2004; 186:7229-35; PMID:15489434; http://dx.doi.org/10.1128/JB.186.7.7229-7235.2004

50. Sartori A, Garay-Malpartida HM, Forni MF, Schumacher RI, Dutra F, Sogayar MC, Bechara EJ. Aminoacetone, a putative endogenous source of methylglyoxal, causes oxidative stress and death to insulin-producing RINm5f cells. Chem Res Toxicol 2008; 21:1841-50; PMID:18729331; http://dx.doi.org/10.1021/tx8001753

51. Yoon H, McDermott JE, Porewski S, McClelland M, Heffron F. Coordinated regulation of virulence during systemic infection of Salmonella enterica serovar Typhimurium. PLoS Pathog 2009; 5:e1000306; PMID:19229334; http://dx.doi.org/10.1371/journal.ppat.1000306

52. Sakeeda N, Clugston SL, Daub E, Honkey JF. Distinct classes of glyoxalase I: metal specificity of the Yersinia pestis, Pseudomonas aeruginosa and Neisseria meningitidis enzymes. Biochem J 2004; 384:111-7; PMID:15270717; http://dx.doi.org/10.1042/BJ20041006

53. Stamp AI, Owen P, El Oman K, Nichols CE, Lockyer M, Lamb HK, Charles IG, Hawkins AR, Stammers DK. Structural and functional characterization of Salmonella enterica serovar Typhimurium YcbL, an unusual Type II glyoxalase. Protein Sci 2010; 19:1897-905; PMID:20669241; http://dx.doi.org/10.1002/pro.475

54. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A 2000; 97:6640-5; PMID:10829079; http://dx.doi.org/10.1073/pnas.120163297

55. Thein M, Sauer G, Paramasivam N, Grin I, Linke D. Efficient subfractionation of gram-negative bacteria for proteomics studies. J Proteome Res 2010; 9:6335-47; PMID:20932056; http://dx.doi.org/10.1021/pr1002438

56. Cordeiro C, Pontes Freire A. Methylglyoxal assay in cells as 2-methylquinoxaline using 1,2-diaminobenzene as derivatizing reagent. Anal Biochem 1996; 234:221-4; PMID:8714622; http://dx.doi.org/10.1006/abio.1996.0076