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THE DNA-BINDING PROTEIN DPS FUNCTIONS AS A GLOBAL REGULATOR IN STARVED *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS DURING STARVATION.

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Abstract- The DNA binding protein of stationary phase cells (Dps)-first discovered in starved *Escherichia coli*-is capable of providing protection to cells during exposure to various environmental assaults. Its ability to do so is based on three intrinsic properties of the protein: DNA binding, iron sequestration, and its ferroxidase activity. Proteomic studies have lead to the inference of a regulatory role for Dps as well; however, the ability of Dps to serve as a global regulator during nutritional deprivation has yet to be directly examined. In this study, we utilized microarray analysis and quantitative real-time PCR to establish direct evidence for a regulatory role of Dps in starved *Salmonella enterica* serovar Enteritidis. The results of our microarray screening revealed over 150 genes significantly up or down regulated in starved cells lacking functional Dps protein. Also, we identified a small subset of genes regulated by Dps that are important for the induction of hydrogen peroxide, iron, and acid resistance. The fact that it positively regulates genes important for stress resistance further solidifies Dps as a virulence regulator in *S. enteritidis*; for resistance to such cytotoxic conditions is likely to translate into enhanced survivability and virulence within infected hosts.
Keywords: Dps, gene regulation, *Salmonella* Enteritidis

INTRODUCTION

Stress response proteins in *Salmonella* are often the product of *de novo* protein synthesis following the appropriate stimulus and ultimately help this pathogen to cope with fluctuating environments. One such stress-related protein, Dps (DNA binding protein in stationary phase cells), is particularly crucial for protection against highly stressful conditions in nutritionally deprived cells. Dps is initially produced in exponential phase-where it is expressed at low levels-and accumulates during stationary phase up to nearly 200,000 molecules per cell, at which time it is the most abundant protein in the cell [1]. Expression of Dps is regulated in an RpoS-dependent manner and is also regulated by OxyR and σ^s [2]. In addition to affording protection during nutritional deprivation, Dps has shown an ability to protect against other environmental stresses as well; including UV and gamma irradiation, metal toxicity, thermal stress, and acid/base shock. [3-8]. However, Dps has most notably been associated with protection against oxidative stress and is reported to contribute to oxidative stress resistance, survival in macrophages, and virulence in mice [6].

Although it has been suggested [1, 8-9], little evidence has been offered that demonstrates the participation of Dps in the direct and/or indirect regulation of genes expressed during starvation. However, the notion that Dps may play a role in regulation during stressful conditions is not unwarranted. In the initial study describing Dps [1], a dramatic difference in the proteomes of wild type and *dps* mutant strains, as well as the pleiotropic phenotype of the *dps* mutant strain was revealed by examining the patterns of proteins synthesized after three days of starvation via two-dimensional gel electrophoresis. Proteomic differences included both the absence of proteins in the mutant synthesized in the wild type after three days, as well as the appearance of proteins in the mutant that were not synthesized in the wild type. Furthermore, Dps shares several properties of histone-like proteins, such as the heterodimeric protein HU associated with the *E. coli* nucleoid and H-NS, the heat-stable nucleoid-structuring protein [10-11]. In addition to binding DNA in a highly stable manner, histone-like proteins can also act both as positive and negative effectors in different systems [10-11]. The concentration of the histone-like protein H-NS has been shown to increase slightly during stationary phase-as does that of Dps-and a highly similar pleiotropic phenotype with regard to gene expression is observed in cells lacking H-NS [12].

Although the aforementioned studies have lead to the inference of a regulatory role for Dps in the global regulation of protein expression following prolonged starvation, experimental evidence of the direct or indirect role of Dps in gene regulation during stationary phase has yet to materialize. Without direct evidence, a persuasive argument could be made that genes are not directly or indirectly regulated by Dps and that the observed pattern of protein expression occurs because DNA tightly bound to Dps is inaccessible to transcription factors that may positively or negatively regulate gene expression-and/or transcriptional machinery. Therefore, this work aims to establish direct evidence of the regulatory role of Dps in stationary phase (starved) *Salmonella* Enteritidis (*S. enteritidis*). We began by performing a microarray screen using*. S. enteritidis dps* and its parental wild type strain in order to identify genes that are differentially expressed in the absence of functional Dps protein. Following microarray screening, a subset of genes downregulated in the *dps* mutant were subjected to quantitative real-time PCR to verify the expression pattern obtained from microarray screening. We concluded this study by determining the level of importance of selected factors in hydrogen peroxide, iron, and acid resistance.

METHODS

Growth conditions and bacterial strains

The wild type strain *Salmonella enterica* sv. Enteritidis strain LK5 used in this study is a chicken isolate [13]. All bacteria were routinely propagated using Luria-Bertani (LB) media unless otherwise stated. Growth media was supplemented with kanamycin (50 μg/mL) when necessary. All plates and cultures were incubated at 37^{o}C at 225 rpm.

Microarray analysis

Microarray analysis was performed according to the protocol provided by The Pathogen Functional Genomics Resource Center (Rockville, Maryland). *S. enteritidis* Δ*dps* and its parental strain were grown to late stationary phase (18 hours). Total RNAs were isolated from starved cultures using an RNeasy® mini kit (QIAGEN) and treated with DNAse. For each sample (mutant and wild type), ten micrograms of total RNA was incubated at 42°C overnight to generate cDNA in the following reaction mixture: 0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP, 0.2 mM dTTP, 1 mM amino allyl-modified dUTP (Sigma), 0.01 M dithiothreitol (DTT), 40 U RNaseOut™ (Invitrogen), 400 U SuperScript™ III reverse transcriptase (Invitrogen) and 1X First Strand buffer (Invitrogen). The cDNA solution was hydrolyzed by the addition of 50 mM EDTA and 100 mM NaOH and then incubation for 15 min at 65°C. To neutralize the reaction mixture, 1 M Tris, pH 7.0 was added to each sample. The cDNA was then washed, concentrated using a MinElute PCR purification kit (QIAGEN), and coupled to Cy3 or Cy5 dye (Amersham Biosciences) according to the manufacturer's instructions. The Cy3- and Cy5-labeled cDNA was then washed and eluted. The concentration of the labeled cDNA and dye incorporation into the cDNA was determined using a spectrophotometer NanoDrop, ND-1000 (NanoDrop Technologies). Differentially labeled cDNAs were then combined and dried using a speed vacuum. The mixture was resuspended in 1X hybridization buffer (5X SSC, 0.1% sodium dodecyl sulfate (SDS), 40% formamide, 0.6 μg/mL salmon sperm DNA (Ambion) and 0.1 mM DTT). Microarray slides were incubated in pre-hybridization solution (5X SSC, 0.1% SDS, 1% BSA) for two hours at 42°C and dried by centrifugation prior to hybridization. The labeled cDNA mixture was hybridized to the microarray slide for 16 hours at 42°C. Following hybridization, the microarray slide was washed in three different wash solutions; low stringency solution (2X SSC, 0.1% SDS and 0.1 mM DTT), medium stringency solution (0.1X SSC, 0.1% SDS and 0.1 mM DTT) and high stringency solution (0.1X SSC and 0.1 mM DTT). Finally, the rinsed slide was dried by centrifugation and scanned using a GenePix 4000 Array Scanner. Spot data were normalized using locally weighted scatterplot smoothing (Lowess) algorithm and analyzed using GenePix 5.0 and. Proper controls were included in the microarray analysis.

Quantitative Real Time PCR

Three genes significantly repressed in the *S. enteritidis dps* deletion mutant (as determined by microarray analysis) were selected for further study. Genetic repression at the mRNA level was further verified with quantitative real-time PCR (qRT-PCR). Total RNA isolation of *S. enteritidis* Δ*dps* and its parental strain was achieved using Qiagen's RNeasy® Mini Kit following the manufacturer's instructions. (Enzymatic lysis of cell wall material was performed beforehand by incubating freshly harvested cells in TE buffer containing 1mg/mL lysozyme for five minutes at room temperature.) Following DNAse treatment, cDNA was synthesized (QuantiTect® Reverse Transcrition Kit, Qiagen) and subsequently diluted ten-fold in preparation for PCR. Primers utilized in this assay (listed in Table 1) were designed using gene specific sequences from *S.* Typhimurium LT2 obtained from *coli*BASE, an online database (http://xbase.bham.ac.uk/colibase/), and were designed to amplify ~100-base pair internal fragments from each gene. All primers were validated and deemed suitable for RT-PCR beforehand using PCR analysis. qRT PCR was performed as a relative quantification run using the StepOne™ Real-Time PCR System (Applied Biosystems) and SYBR green reagents for detection.

Reaction mixtures were prepared as follows: 10 μL 2X SYBR Green PCR master mix (Applied Biosystems), 10 pmol/μL forward primer, 10 pmol/μL reverse primer, cDNA template (~10 ng), and nuclease-free water up to 20 μL. Thermocycling conditions were as follows: 95^{o}C for 10 min, followed by 40 cycles of 95^{o}C for 15 sec, 60^{o}C for one min. Runs for each target included a negative control lacking target cDNA . The 16s RNA gene was used for normalization of all data. qRT-PCR data was analyzed using StepOne™ software (Applied

Biosystems). The reproducibility of each the RT-PCR reactions was confirmed by running independent reactions on different days. All runs were performed five times and the results for each gene were averaged.

Deletion mutant construction

Knockout *S. enteritidis* mutants harboring deletions in either *dps*, STM0410, *galR*, or STY4154 were made using an overlapping PCR extension protocol previously described [14] and the λ Red recombination system [15]. Primers used to create deletion cassettes are listed in Table 3. For deletion mutant construction, the immediate up and downstream regions (300-500 bp) of the gene targeted for deletion, as well as the kanamycin resistance cassette from pKD4 were amplified in separate PCR reactions.

A final overlapping extension PCR reaction (using the three aforementioned PCR products) was performed to create a single linear deletion cassette (total length 2.2 – 2.3 kb). The deletion cassette was incorporated into the genome of *S. enteritidis* via the λ Red recombinase system previously described [15]. Deletion of the target genes was initially confirmed by colony PCR and ultimately by sequencing. Finally, isogenic strains were constructed in a fresh background for each knock-out strain by P22 HT *int* mediated transduction of the mutations into wild type *S. enteritidis*.

Stress assays

Starved cultures were subjected to oxidative stress, acid stress, and iron stress. Cultures of the parental *S. enteritidis* strain and all derived mutant strains (harboring mutations in *dps*, STM0410, *galR*, or STY4154) were grown for eighteen hours in M9 glucose minimal medium without antibiotics. One milliliter samples were washed twice and resuspended in PBS. Stressors for the hydrogen peroxide and iron stress assays were added at the following concentrations: 20mM H_2O_2 and 100mM $FeSO_4$, respectively. For the acid stress assay, a one milliliter sample of the starved culture was washed twice and resuspended in PBS (pH 2.0).

Challenge cultures were then grown for one hour with samples removed at 0, 20, 40, and 60 minutes, then serial diluted, and plated. The CFU/mL of each challenge culture was calculated and the percent survival for the culture was determined after each sampling time point using the following formula

$$\%\ \text{survival at time}_X = (\text{CFU per mL at time}_X / \text{CFU per mL at time}_0) \times 100$$

All challenge assays were performed in triplicate and the presented results represent the average for each strain.

Statistical methods

The data reported for resistance studies are the average values from three independent trials. Data reported for qRT-PCR runs were the average of five independent trials. All data was analyzed using the Student's *t*-test and *P* values <0.05 were considered to be significant.

RESULTS

Microarray screening

We initially performed a microarray analysis to monitor changes in gene expression in a *S. enteritidis dps* deletion mutant grown to late stationary phase. The mRNA levels for over sixty genes were significantly reduced in the *dps* deletion mutant (Supplementary Table 1) and were significantly elevated for over 130 genes (Supplementary Table 2). The threshold for significance in this study was at least a four-fold increase or decrease in expression (M=2.0). Although all genes differentially expressed in the *dps* mutant were prospective targets for further investigation into the regulatory role of Dps, we focused on three genes (each coding for a known or putative regulatory element in *Salmonella*) showing significant downregulation in the *dps* mutant: STM0410, *galR* (STM3011), and STY4154. (STM0410 and STY4154 are locus tags of two previously uncharacterized genes in *Salmonella* Typhimurium LT2.) These three genes were chosen because of their known (or putative) regulatory ability. By selecting these genes we hoped to show that Dps' elevated position within the regulation hierarchy in starved *S. enteritidis* and would therefore have a profound effect on the expression of regulatory elements lower in the cascade. The *S. enteritidis dps* mutant to wild type ratios of mRNAs for STM0410, *galR*, and STY4154 (obtained via microarray) are depicted in Figure 1a. A ratio of less than one is indicative of excess target mRNA in the parental strain when compared to that in the *dps* mutant.

Quantitative Real-time PCR

Quantitative real-time PCR was performed to verify that STM0410, *galR*, STY4154 mRNA transcripts are truly diminished in the *dps* mutant. The expression of each gene in parental strain was taken to be the basal level of expression for that particular gene (for the growth conditions used in this study) to which the expression in the *dps* deletion mutant was compared. The relative quantification of each target gene (Figure 2) was calculated from the RT-PCR data obtained using the comparative ΔC_T ($\Delta\Delta C_T$) method. When compared to parental cultures, all three targets with reduced mRNA levels in *S. enteritidis* Δ*dps* (as determined via microarray) showed reduced expression at the transcriptional level. STM0410, *galR*, and STY4154 showed a 82%, 31%, and a 62% decrease in expression (respectively) in the *dps* mutant. The combined results of the microarray screen and qRT-PCR experiments suggest that STM0410, *galR*, and STY4154 are indeed underexpressed at the transcriptional level in the *S. enteritidis dps* deletion mutant.

Stress assays

Deletion mutants harboring mutations in three genes verified as being significantly underexpressed in the *dps* deletion mutant (STM0410, *galR*, and STY4154) were constructed (as described in experimental procedures) and subjected to oxidative, iron, and acid stress to determine the relevance of each gene in stress

resistance in starved *S. enteritidis*. We set out to determine if the genes we have identified as being regulated(directly or indirectly) by Dps were also important for hydrogen peroxide, iron, and acid resistance.

Stress assays were carried out as described in the experimental procedures section. Briefly, starved *S. enteritidis* Δ*dps*, Δ*STM0410*, Δ *galR*, Δ*STY4154* and wild type cultures were exposed to 20mM H_2O_2, 100mM $FeSO_4$, and pH 2.0 for one hour. When subjected to H_2O_2, the parental strain demonstrated a significant level of resistance, with a percent survival of 86% after 20 minutes and 20% after one hour. Conversely, all four mutant strains were hypersensitive to hydrogen peroxide when compared to parental *S. enteritidis* (Figure2a). The STM0410, *galR*, and STY4154 deletion mutants performed slightly better than the *dps* mutant after 20 minutes, but showed similar survivability after 40 minutes exposure. With the exception of *S. enteritidis* Δ*STM0410* (which had a percent survival of 64%), the percent survival of each mutant dropped below fifty percent within 20 minutes of exposure. After one hour of exposure however, all mutant cultures were completely killed, except for the STM0410 deletion mutant, in which one percent of the culture remained viable.

The iron and acid stress assays produced results comparable to those of the hydrogen peroxide stress assay. In these assays, the deletion mutants were also more susceptible to the aforementioned stresses than the wild type. When exposed to 100mM $FeSO_4$, the STM0410, *galR*, and STY4154 mutants performed similar to the *dps* mutant at 20, 40, and 60 minutes (Figure2b). Less than 45% of all mutants remained viable after 20 minutes exposure to $FeSO_4$, while 88% of the wild type culture remained at this time point. After 60 minutes exposure to $FeSO_4$, less than 1% of each mutant culture remained viable while nearly 15% of the parental culture remained viable. All deletion mutants examined were also hypersensitive to acid stress (Figure2c). With the exception of the STM0410 deletion mutant culture (which had a percent survival of 44%) less than 30% of all mutants remained viable after 20 minutes exposure to pH 2.0. At this time point, the parental strain had a percent survival of 69%. The STM0410 mutant was slightly less sensitive to extreme acidity than the STY4154, *dps* and *galR* mutants. After 40 minutes, this mutant showed a survival rate similar to the wild type (17% and 23% respectively). As in the H_2O_2 sensitivity assay, the acid resistant phenotype of the STM0410 mutant seemed to be between that of the wild type and *dps* mutant. Yet, the acid resistance observed in the STY4154 mutant and the *galR* mutant were very similar to that observed in the *dps* mutant.

DISCUSSION

The regulatory role of the ferritin-like protein Dps was investigated herein for the first time utilizing analytical techniques designed to monitor changes in gene expression. The possibility of a regulatory function for Dps was initially suggested [1] due to the pleiotrophic phenotype of *E. coli dps* (with respect to protein expression) revealed via 2D gel electrophoresis. To elucidate this function, we monitored gene expression within *S. enteritidis dps* utilizing in depth genetic expression analysis to establish concrete evidence of the regulatory role of Dps. Microarray screening performed in this work revealed the differential expression of hundreds of functionally distinct genes in starved *S. enteritidis dps*. Furthermore, we were able to show that the putative regulators encoded from STM0410 and STY4154 and the galactose repressor protein *galR* are indeed underexpressed in starved *S. enteritidis dps.* Cumulatively, these results strongly suggest that Dps functions as a global regulator in *S. enteritidis* during conditions of nutrient deprivation and that the protein positively regulates the expression of *galR,* STM0410 and STY4154 under said conditions. Although our results are indicative of Dps' regulatory role, we did not ascertain if Dps directly or indirectly regulates the aforementioned target genes in this particular study. Direct regulation of targets by Dps would imply the protein was able to recognize specific DNA sequences or promoters and bind accordingly. Such a regulatory phenomenon would best be proven utilizing an *in vitro* DNA binding assay employing specific DNA sequences or promoters as a binding targets and purified Dps protein.

However, a known binding property of Dps vital for its ability to sequester and protect chromosomal DNA during stress is its capacity to bind DNA in a sequence independent manner. Dps may have an ability to bind with sequence specificity depending on the nature of its activity, i.e. Dps could recognize DNA with sequence specificity when functioning in a regulatory capacity and non-specifically when functioning as a sequestering and protective agent. As previously mentioned, Dps has been labeled a histone-like protein due its many similarities with the eukaryotic histone. These proteins are known to perform discriminately in differing physiological conditions with regards to gene regulation [10-11]. Such discriminate recognition of DNA is therefore plausible for Dps, as there is no direct evidence that Dps is incapable of binding DNA in a sequence specific manner under certain circumstances.

In the process of elucidating the regulatory role for Dps, we identified *galR* as an important element for the induction of resistance in starved *S. enteritidis*; specifically resistance to hydrogen peroxide, iron, and acid. The gene product of *galR* is the DNA-binding transcriptional regulator of the galactose operon GalR. This genetic repressor is a member of the LacI/GalR family; a family consisting of numerous transcriptional regulators involved in metabolic regulation. The expression of *galR* is controlled (at least partially) by Dps in starved *S. enteritidis* (as shown in this study). However, the expression of *galR* is controlled by the global regulator, RegR in *Streptococcus pneumonia* [16]. RegR is important for the adaptive response of *Streptococcus pneumonia* and serves as a virulence regulator in this organism as well [16]. These stress and

virulence-related characteristics of RegR are strikingly similar to those of Dps; as Dps is also a known virulence regulator and is involved in the adaptive stress response in *Salmonella* [1, 4-8]. Taking into account that *galR* is regulated by the virulence regulator RegR in *Streptococcus pneumonia*, it is not surprising that *galR* is subject to regulation by Dps-a virulence regulator- in *S. enteritidis*. Consequently, GalR may also have a role in the virulence of *S. enteritidis*.

The genetic elements represented by locus tags STM0410 and STY4154 were uncharacterized prior to this study. NCBI's Entrez Gene (www.ncbi.nlm.nih.gov/gene) currently describes both simply as "predicted" regulatory elements. Although we did not attempt to assign a definite regulatory role for these proteins or attempt to identify their possible regulatory targets at this time, we were able to demonstrate importance of these proteins in induction of stress resistance in starved *S. enteritidis*; particularly to oxidative stress, acid stress, and iron stress. Dps is crucial for hydrogen peroxide and iron detoxification and for acid resistance [5, 17-18]. Because they are (directly or indirectly) regulated by Dps, it is not completely unexpected that these genes would be important for protection against these cytotoxic elements as well. STM0410 and STY4154 may themselves mediate stress resistance or positively and/or negatively regulate proteins necessary for/or deleterious to the induction of stress resistance in starved *S. enteritidis*. It should be mentioned however, that results of the acid resistance assay may suggest that STM0410 plays a less significant role in the induction of acid resistance than *galR* and STY4154. The STM0410 deletion mutant was less sensitive to the extreme acid conditions of the assay, resulting in a resistant phenotype similar to that of the wild type. This trend is most obvious after 40 minutes of exposure to pH 2.0 when the percent survivability of the two cultures is separated by less than 10 percentage points. Although this study is the first to provide evidence for a possible role for these proteins in *S. enteritidis* or any other organism, more characterization studies are needed to further elucidate the functions and possible roles for the gene products of STM0410 and STY4154.

In conclusion, we have identified a supplementary role for the DNA binding protein, Dps in *S. enteritidis*. In addition to functioning as a ferritin and a protective element for DNA under stressed conditions, Dps also performs as a global regulator in starved *S. enteritidis*. Also, we revealed three genes regulated by Dps and verified their importance for hydrogen peroxide and iron resistance (possibly as detoxification agents), as well as for the induction of acid resistance. Although did not explore whether Dps regulates gene expression in starved *S. enteritidis* via a direct or indirect mechanism, we believe that both mechanisms are feasible and have proposed to investigate the precise mechanism of regulation in future studies. The significance of this work lies in the fact that it demonstrates the absence of functional Dps protein results in the significant under or overexpression of over 150 genes in starved *S. enteritidis*. These genes represent potential targets of regulation that could indeed be the subject of future studies focused on Dps regulation under varying physiological conditions. Furthermore, stress resistance and/or virulence genes regulated by Dps in *S. enteritidis* could theoretically be simultaneously repressed by targeting Dps for deletion; making this pathogen significantly more susceptible to stress conditions with a single gene mutation.

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Table 1- Primers used in this study

Primer name	Sequence (5'→3')	Amplification target
STM0410-F	GGCCATTTTGCCGGAAGAGAGCCGCCG	STM0410; RT-PCR
STM0410-R	GATGGATAAGATCAAACGCGCTGCGCG	STM0410; RT-PCR
STY4154-F	CCGAATTTGAACAGTTACGGAGAGGAACGG	STY4154; RT-PCR
STY4154-R	CGCGTTTCGATTCCCATTCCTGAACC	STY4154; RT-PCR
STM3011-F	GGCACAGCAGGCAACGGAAACCCTCGG	*galR*; RT-PCR
STM3011-R	CGGTGTGATACGCCACCTGTTCAACGGC	*galR*; RT-PCR
STM0410-P1	AAATGTAGAGAGTCAATATGACCAGACGCGCTGACCTGTGTA GGCTGGAGCTGCTT	Km^R gene from pKD4;deletion mutant construction
STM0410-P2	AGCGTTAGAGGTATCGCTATCACATCTTACGGCCATATGAAT ATCCTCCTTAG	Km^R gene from pKD4;deletion mutant construction
STM0410-UpF	CCCACACTCGCAGGAGGATG	Upstream region of STM0410; deletion mutant construction
STM0410-UpR	GGTCAGCGCGTCTGGTCATATTGACTC	Upstream region of STM0410; deletion mutant construction
STM0410-DwnF	GCCGTAAGATGTGATAGCGATACC	Downstream region of STM0410; deletion mutant construction
STM0410-DwnR	CGAACCGTACTGATCGGCTTCGG	Downstream region of STM0410; deletion mutant construction
STY4154-P1	CGCAAGTTTCCCGACTATTCTTAAGAGGCTTCGATGCTGT GTAGGCTGGAGCTGCTT	Km^R gene from pKD4;deletion mutant construction
STY4154-P2	TTTATTTTACTTTATTTCTCCAGGCCAGTAATCGGGCAT ATGAATATCCTCCTTAG	Km^R gene from pKD4;deletion mutant construction
STY4154-UpF	GCGCAAACCAGCGAGAGCAGG	Upstream region of STY4154; deletion mutant construction
STY4154-UpR	GCATCGAAGCCTCTTAAGAATAGTCGGG	Upstream region of STY4154; deletion mutant construction
STY4154-DwnF	CCCGATTACTGGCCTGGAGAAATAAAG	Downstream region of STY4154; deletion mutant construction
STY4514-DwnR	GGCCTTTAGCGCCGCTTTCGATGG	Downstream region of STY4154; deletion mutant construction
STM3011-P1	AAGCGTTTACCCACTATAGGTATTATCATGGCGACCTGTGTA GGCTGGAGCTGCTT	Km^R gene from pKD4;deletion mutant construction
STM3011-P2	CCATCATGCTACCCTGATTAATCGGTTGTCGACAGGCAT ATGAATATCCTCCTTAG	Km^R gene from pKD4;deletion mutant construction
STM3011-UpF	CCGTTACTGTTACAGGTTCAGGCG	Upstream region of *galR*; deletion mutant construction
STM3011-UpR	GGTCGCCATGATAATACCTATAGTGGG	Upstream region of *galR*; deletion mutant construction
STM3011-DwnF	CCTGTCGACAACCGATTAATCAGGG	Downstream region of *galR*; deletion mutant construction
STM3011-DwnR	GCTCTCGTTCCGGCGTATCGTGACC	Downstream region of *galR*; deletion mutant construction

Supplementary Table 1- Genes significantly downregulated in dps deletion mutant

Gene	Locus Tag	Description
safA	STY0332	Lipoprotein
	STM2609	DNA packaging like protein
oadG	STM3353	oxaloacetate decarboxylase subunit gamma
pepE	STM4190	Peptidase E
prlC	STM3594	Oligopeptidase A (heat shock protein)
hycE	STY2849	hydrogenase 3 large subunit
ilvM	STM3902	Acetolactate synthase 2 regulatory subunit
hiss	STM2522	Histidyl-tRNA synthetase
stfF	STM0199	Putative minor fimbrial subunit
	STM4080	Epimerase
	STM2630	HP
phoN	STM4319	Non-specific acid phosphatase
Glpc	STM2286	sn-glycerol-3-phosphate dehydrogenase subunit C
dppD	STM3627	Dipeptide transporter ATP-binding subunit
ybcI	STM0540	Putative membrane-bound metal-dependent hydrolase
ydiL	STM1362	Putative cytoplasmic protein
avtA	STM3665	Valine—pyruvate transaminase
pmrF	STM2298	Undecaprenyl phosphate 4-deoxy-4-formamido-L-arabinose transferase
murI	STM4131	Glutamate racemase
rfbA	STM2095	dTDP-glucose pyrophosphorylase
faeA	STY4842	Regulatory protein
ycjG	STM1681	Putative chloromuconate cycloisomerase
	STM0410	Putative regulatory protein
	STM2728	HP
	STY4675	HP
dmsC	STM1496	Putative dimethylsulfoxide reductase
	STM1673	Putative outer membrane lipoprotein
yqjA	STM3226	HP
rnpA	STM3840	Ribonuclease P
sitA	STM2861	Putative perplasmic binding protein
	STY3669	HP
yigF	STM3953	Putative inner membrane protein
fruK	STM2205	1-phosphofructokinase
uvrD	STY4664	Putative DNA helicase
eutP	STM2469	Putative ethanolamine utilization protein
lysR	STM3141	Molybdate ABC transporter periplasmic molybdate-binding protein
galR	GALR	DNA-binding transcriptional regulator
relB	STM4449	Bifunctional antitoxin/transcriptional repressor
	STY1361	HP
rhaB	STM4047	Rhamnulokinase
	STY2214	HP
	STM0761	Fumarate hydratase
nudE	STM3494	ADP-ribose diphosphatase
entE	STM0596	Enterobactin synthase subunit E
	STY1612	HP
	STY2059	Putative bacteriophage protein
	STM2629	HP
	STY0343	Pseudo
iap	STM2936	Alkaline phosphatase isozyme conversion amino peptidase

tmk	STM1200	Thymidylate
	STM3350	Putative inner membrane protein
pduJ	STM2045	Polyhedral body protein
sdhc	STM0732	Succinate dehydrogenase cytochrome b556 large membrane subunit
	STM2289	Putative aldolase
	STM2731	HP
	STM3126	Putative amino acid transporter
yiiF	STY3845	CopG family DNA-binding protein
yohI	STM2174	tRNA-dihydrouridine synthase C
tviC	STY4660	VI polysaccharide biosynthesis protein epimerase
	STM4104	Putative 5'-nucleotidase/2',3'-cyclic phospodiesterase
ispA	STM0423	Geranyltranstransferase
	STM2628	Regulatory protein
	STY1366	DNA invertase
	STY4154	Putative transcriptional regulator

Supplementary Table 2- Genes significantly upregulated in dps deletion mutant

Gene	Locus Tag	Description
ydiQ	STM1354	Putative electron transfer flavoprotein
wzxC	STM2102	Colonic acid exporter
cydC	STM0956	Cytosine/glutathione ABC transporter membrane/ATPbinding component
	STY4163	HP
wzzE	STM3919	Lipopolysaccharide biosynthesis protein
	STY2024	Putative bacteriophage protein
	STM2943	Putative cytoplasmic protein
yhjE	STM3609	Putative transport protein
gcvP	STY3209	Glycine dehyrogenase
ygeA	STM3015	Putative racemase
	STM3752	Putative cytoplasmic protein
	STY1238	HP
ptr	STM2995	Protease III
tyrR	STM1683	tyrR DNA-binding transcriptional regulator
	STM1934	Putative outer membrane lipoprotein
flhB	STM1914	Flagellar biosynthesis protein
	STY2355	HP
	STM2718	Head completion-like protein
	STM4520	Putative cytoplasmic protein
	STM2904	Putative ABC-type transporter
ybhS	STM0816	Putative transport protein
yahO	STM0366	HP
yjgA	STM4437	HP
	STY2062	Putative replication protein
	STM1266	Putative transcriptional regulator
rfbC	STM2094	dTDP-4 deoxyrhamnose 3, 5 epimerase
ddl	STM0380	D-alanyl-alanine synthetase A
	STM0567	Putative DNA repair ATPase
sefB	STY4837	Fimbrial chaperone protein
rspB	STM1506	Putative dehydrogenase
murA	STM3307	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
yhaK	STM3236	Putative cytoplasmic protein
gudT	STM2962	Putative D-glucarate permease
potC	STM1223	Spermidine/putrescine ABC transporter membrane protein

tcp	STM3577	Methyl-accepting transmembrane citrate/phenol chemoreceptor
	STM3261	Galactitol-1-phosphate dehydrogenase
yafE	STM0259	Putative methyltransferase
	STM3251	Putative sugar kinase
hsdS	STM4524	Type I restriction enzyme specificity protein
trpS2	STM4508	Tryptophanyl-tRNA synthetase III
	STM1585	Putative outer membrane lipoprotein
	STY4635	HP
	STY0386	HP
phnT	STM0428	2-aminoethyl phosphonate transporter
yheN	STM3451	Sulfur transfer complex subunit TusD
	STY1597	HP
	STM4428	Major facilitator superfamily transporter
malF	STM4228	Maltose transporter membrane protein
nagC	STM0682	N-acetylglucosamine operon transcriptional repressor
	STM2760	Putative intgrase
ftsE	STM3570	Cell division protein
	STY1638	Alternative bacteriophage tail fiber C-terminus
	STY0293	HP
yjbH	STM4225	Putative outer membrane lipoprotein
thiF	STY3723	Thiamine biosynthesis protein
yifE	STM3898	HP
rfbS	STY2299	Paratose synthase
	STM2712	Phage-like tail protein
ydcN	STM1605	Putative repressor
	STM4537	Putative PTS permease
	STM3082	Putative zinc-binding dehydrogenase
	STM3121	Putative transcriptional regulator
allA	STM0515	Ureidoglycolate hydrolase
tolB	STM0748	Transcriptional protein
yhfG	STM3471	HP
rfbI	STY2303	CDP-6-deoxy-delta-3, 4-glucoseen reductase
rfbG	STM2091	CDP glucose 4, 6 dehydratase
sdhB	STM0735	Succinate dehydrogenase iron-sulfur subunit
exbD	STM3158	Biopolymer transport protein
	STM1861	(Similar to *E. coli* prophage e14 integrase)
yraP	STM3267	HP
srfA	STM1593	Putative virulence protein
	STM2740	Integrase-like protein
ymdF	STM1121	Putative cytoplasmic protein
ygjP	STM3221	Putative metal-dependent hyrdolase
yegD	STM2125	Putative chaperone
	STM0099	Putative inner membrane protein
	STM3754	Putative cytoplasmic protein
	STM3071	Putative DNA-binding protein
	STM4421	Putative NAD-dependent aldehyde dehydrogenase
yhjV	STM3625	Putative transport protein
	STM2722	Terminase-like protein
crp	STM3466	CAMP-regulatory protein
yqaE	STM2796	Putative transport protein
yfeA	STM2410	HP
	STM3794	Putative regulatory protein
srlR	STM2837	DNA-binding transcriptional repressor
mutM	STM3726	Formamidopyrimidine-DNA glycosolase
yhjB	STM3606	Putative transcriptional regulator
osmE	STM1311	DNA-binding transcriptional activator
ycfH	STM1202	Putative metallodependent hydrolase

yhhJ	STM3585	Putative ABC transport protein
katG	STM4106	Hydroperoxidase
	STM4039	Putative inner membrane lipoprotein
	STY2364	HP
ybjM	STM0871	Putative inner membrane protein
	STY4679	HP
fabH	STM1193	3-oxoacyl (acyl carrier protein) synthase III
	STM1671	Putative regulatory protein
fadI	STM2389	3-ketoacyl-CoA thiolase
	STM1808	Putative cytoplasmic protein
stcB	STM2151	Putative periplamsmic chaperone protein
yqfB	STM3050	HP
	STM3690	Putative inner membrane lipoprotein
yejM	STM2228	Putative hydrolase
	STM4032	Putative acetyl esterase
ybhF	STM0817	Putative ABC-type multidrug transport system ATPase component
	STY3683	HP
araC	STM0104	DNA-binding transcriptional regulator ABC
gcvP	STM3053	Glycine dehydrogenase
	STM1839	HP
elaC	STM2313	Ribonuclease Z
	STM0050	Putative nitrite reductase
	STM0572	Putative phosphosugar isomerase
	STY1874	Putative lipoprotein
flgG	STM1179	Flagellar basal body rod protein
nixA	STM2783	Putative nickel transporter
yfaX	STM2292	Putative transcriptional regulator
	STY3283	Bacteriocin immunity protein
	STY0326	HP
hisD	STM2072	Histodinol dehydrogenase
cutC	STY2115	Copper homeostasis protein
srfA	STY1472	Putative virulence effector protein
zntB	STM1656	Zinc transporter
sgbH	STY4121	3-keto-L-gulonate-6-phosphate decarboxylase

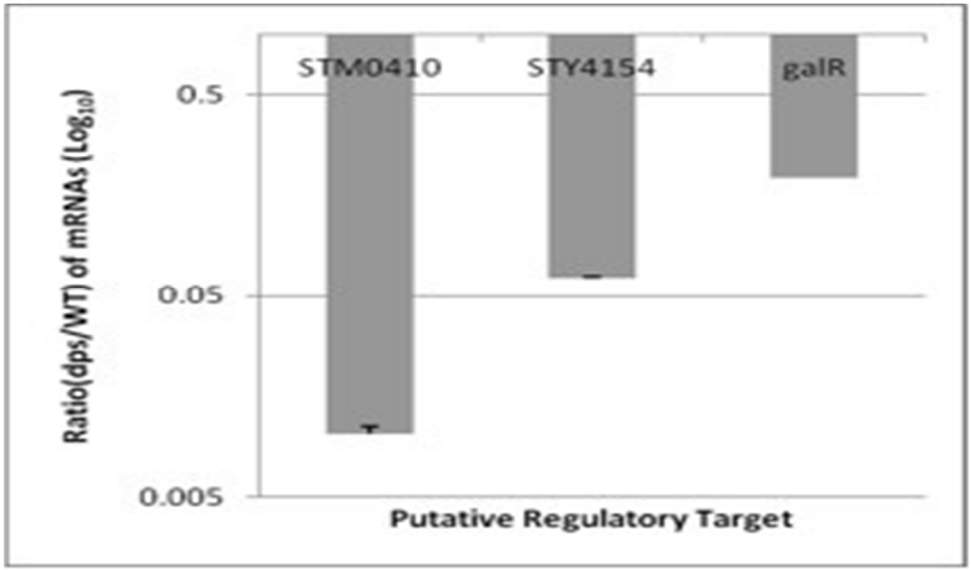

Fig. 1a- Differential expression of mRNAs. The *S. enteritidis dps* to wild type ratio of mRNAs for each target (as determined by microarray analysis) is graphically depicted. Ratios are derived from the M score of each given target utilizing the following equation: *dps* mutant/WT ratio of mRNAs=$2^{M\ score}$. Ratios of less than one indicate an abundance of target mRNA in the wild type; while ratios greater than one would be indicative of an excess of target mRNA in the *dps* mutant. Standard error is represented by error bars.

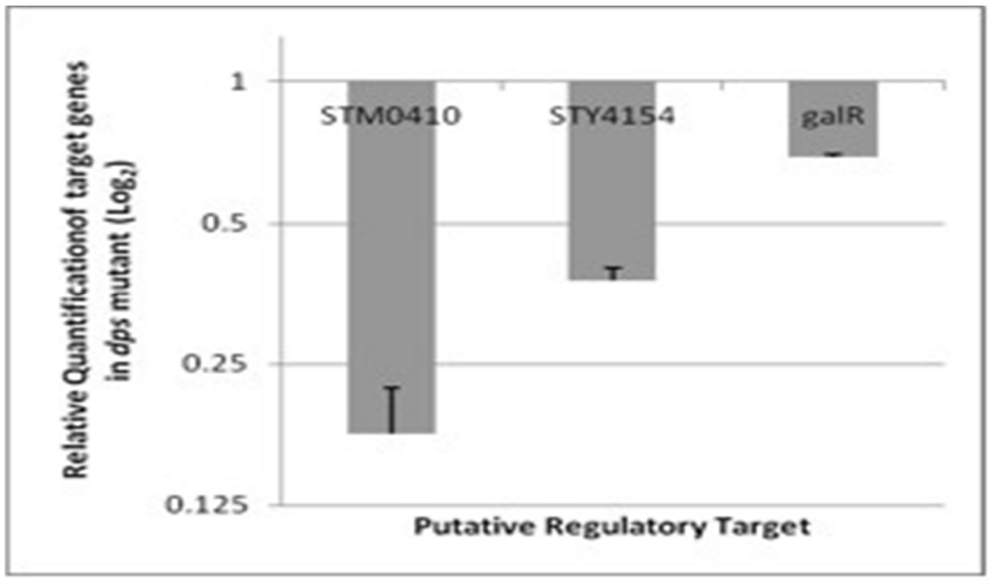

Fig. 1b- Relative quantification of target genes in the *dps* deletion mutant. The relative quantification of each target gene was obtained via qRT-PCR analysis and was calculated using the comparative $\Delta C_T(\Delta\Delta C_T)$ method. The level of expression of each target in *S. enteritidis* Δ*dps* mutant was compared to the level of expression of the identical target gene in the parental strain. Expression in the parental strain is taken to be the basal level of expression for each gene, to which the expression of the identical gene in the *dps* mutant is compared. The relative quantification of each target in the wild type is 1. The expression of each target in the *dps* mutant is compared to this number. All data obtained from RT-PCR experiments was normalized to the 16sRNA gene in *S. enteritidis*. Standard error is represented by error bars.

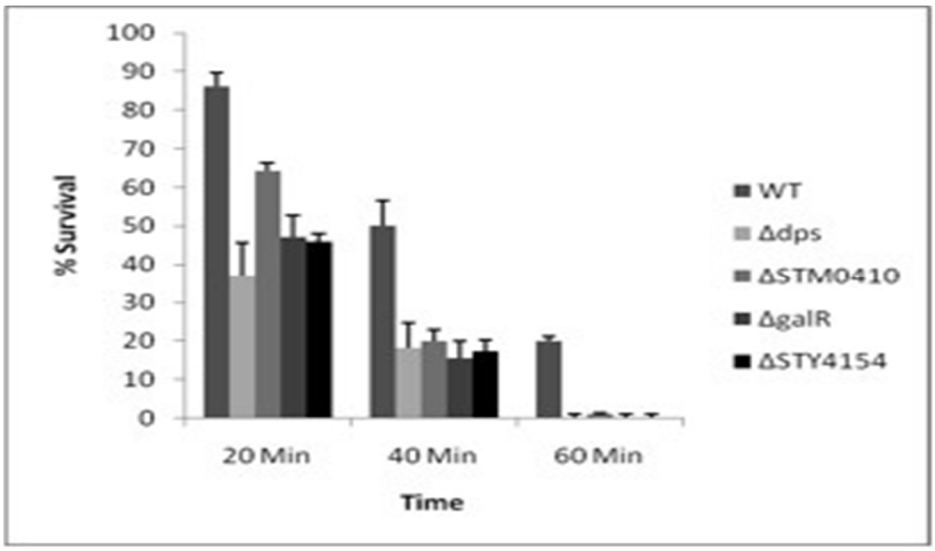

Figure 2a.

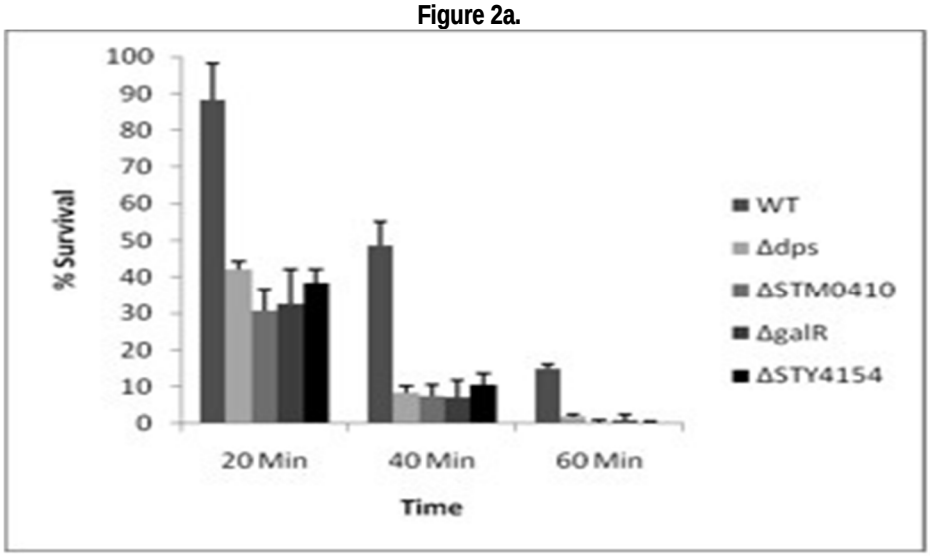

Figure 2b.

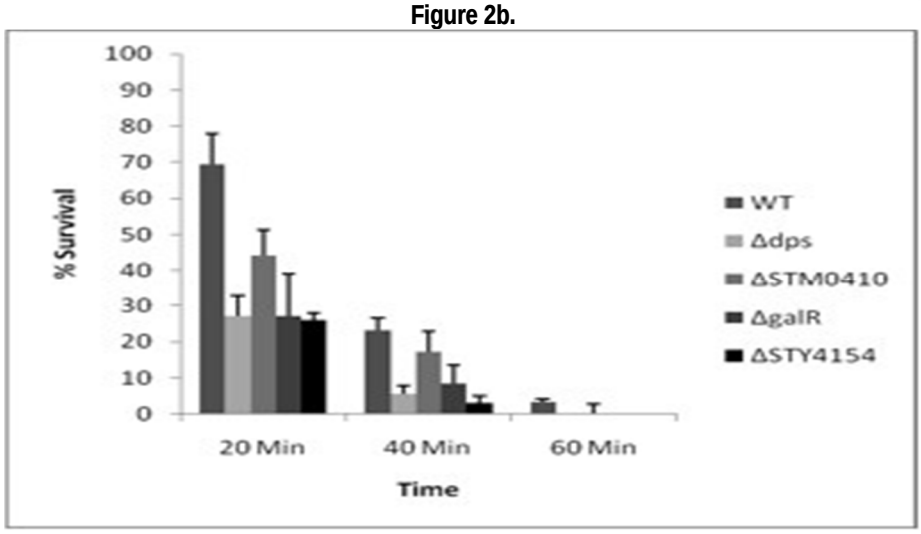

Figure 2c.

Fig. 2-Survival assays. *S. enteritidis* Δ*dps*, ΔSTM0410, Δ*galR*, ΔSTY4154, and their parental strain were subjected to three different stress assays to determine the significance of each deleted gene in stress resistance. Graphs illustrate the results of three separate resistance assays: hydrogen peroxide (a), iron (b), and acid (c) resistance. Stress was induced utilizing 20mM H_2O_2, 100mM $FeSO_4$, and pH 2.0 respectively. Resistance is measured by the percent survival of the challenge culture over one hour. Standard error is represented by error bars.