MICROBIOLOGY AND FOOD SAFETY

Identification of common highly expressed genes of *Salmonella* Enteritidis by *in silico* prediction of gene expression and *in vitro* transcriptomic analysis

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ABSTRACT Chickens are the reservoir host of *Salmonella* Enteritidis. *Salmonella* Enteritidis colonizes the gastro-intestinal tract of chickens and replicates within macrophages without causing clinically discernable illness. Persistence of *S.* Enteritidis in the hostile environments of intestinal tract and macrophages allows it to disseminate extra-intestinally to liver, spleen, and reproductive tract. Extra-intestinal dissemination into reproductive tract leads to contamination of internal contents of eggs, which is a major risk factor for human infection. Understanding the genes that contribute to *S.* Enteritidis persistence in the chicken host is central to elucidate the genetic basis of the unique pathobiology of this public health pathogen. The aim of this study was to identify a succinct set of genes associated with infection-relevant *in vitro* environments to provide a rational foundation for subsequent biologically-relevant research. We used *in silico* prediction of gene expression and RNA-seq technology to identify a core set of 73 *S.* Enteritidis genes that are consistently highly expressed in multiple *S.* Enteritidis strains cultured at avian physiologic temperature under conditions that represent intestinal and intracellular environments. These common highly expressed (CHX) genes encode proteins involved in bacterial metabolism, protein synthesis, cell-envelope biogenesis, stress response, and a few proteins with uncharacterized functions. Further studies are needed to dissect the contribution of these CHX genes to the pathobiology of *S.* Enteritidis in the avian host. Several of the CHX genes could serve as promising targets for studies towards the development of immunoprophylactic and novel therapeutic strategies to prevent colonization of chickens and their environment with *S.* Enteritidis.

Key words: *Salmonella*, transcriptome, common highly expressed genes, chicken, environment

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INTRODUCTION

Salmonella enterica sub sp. *enterica* serovar Enteritidis (*S.* Enteritidis) is a leading foodborne pathogen worldwide. *S.* Enteritidis causes persistent infection in chickens without inducing clinically discernible illness and has a unique pathobiology that allows it to contaminate internal contents of eggs (Denagamage et al., 2017; Gast et al., 2017). *S.* Enteritidis infection in chickens is relevant for public health because human infections are associated with consumption of contaminated eggs and meat [(Shah et al., 2017b; Goncalves-Tenorio et al., 2018) and reviewed in Chousalkar et al. (2018)]. Incidence of *S.* Enteritidis has increased steadily since the early 2000s, in both humans and chickens, according to laboratory-confirmed human cases (FoodNet and CDC Laboratory-based Enteric Diseases Surveillance or LEDS) and broiler chicken rinsate surveys (VetNet). In the last decade, at least 2 large multistate outbreaks reported in the US were attributed to shell eggs contaminated with *S.* Enteritidis, resulting in approximately 3,578 human illnesses and a nationwide voluntary egg recall from suspected suppliers (Centers for Disease Control and Prevention, 2010; Chai et al., 2012; Boore et al., 2015). Thus, *S.* Enteritidis poses a major challenge to poultry production, food-safety, and public health.

S. Enteritidis infects and persists in poultry largely because of its ability to survive and replicate in the harsh environments encountered in the gastrointestinal tract and systemic sites of the avian host (Shah et al., 2017a). Interestingly, wild-type strains of *S.* Enteritidis exhibit extensive phenotypic diversity, including dissimilar ability to infect chickens (Yim et al., 2010; Shah et al., 2011a; Gast et al., 2018). For instance, *S.* Enteritidis strains better able to tolerate acid and oxidative stress are also better able to colonize the avian gastrointestinal tract, internal organs and survive within the internal content of eggs (Shah et al., 2012; Baron

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et al., 2017). Phenotypic factors such as presence of high-molecular-mass LPS, high cell density growth and motility also correlate with the ability of certain *S.* Enteritidis strains to infect chickens, contaminate eggs, and cause human infection (Guard-Petter, 2001; Yim et al., 2011; Shah et al., 2011b). Studies using negative selection screens of mutant libraries and *in vivo* expression technology have identified genes that contribute to intestinal colonization and systemic dissemination in chickens and survival of *S.* Enteritidis within the internal contents of eggs (Silva et al., 2012; Raspoet et al., 2014a; Raspoet et al., 2014b). The genes identified by these studies, however, are generally present in all the *S.* Enteritidis strains due to the extensive within serovar genetic homogeneity at the gene content level (Shah et al., 2012). Moreover, epidemiologically distinct strains of *S.* Enteritidis show similar gene content that contrasts their phenotypic diversity and differential pathobiology in chickens (Allard et al., 2013). This poses significant challenges in defining gene function in the context of infection. Newer approaches are, therefore, needed to dissect the genetic mechanisms underlying phenotypic diversity and differential ability of *S.* Enteritidis strains to cause infection in chickens.

Recent studies show that the differential pathogenicity of genetically related *S.* Enteritidis strains might be driven at the transcriptional level. For instance, *S.* Enteritidis strains with high colonizing ability in chickens display distinct transcriptional profiles relative to the strains that are not as efficient in their colonization capabilities (Shah, 2014). This raises the possibility that strains with high colonizing ability may exhibit common transcriptional signatures that enable them to persist in the avian host. In this context, our hypothesis is that *S.* Enteritidis strains that successfully persist in the avian host consistently express a common set of genes that may play an important role in persistence of this organism. Consequently, we aimed to identify such common set of genes that are consistently highly expressed among multiple pathogenic *S.* Enteritidis strains, independently of the surrounding host microenvironment. To accomplish this, we coupled *in silico* prediction of gene expression (Karlin and Mrazek, 2000) with global *in vitro* transcriptome analysis of 3 highly pathogenic *S.* Enteritidis strains cultured under conditions that resemble the gastrointestinal and intra-macrophage compartments within the avian host. With this dual approach, we show that the genes predicted to be highly expressed *in silico* correlate with the experimental global transcriptomes generated from these pathogenic *S.* Enteritidis strains. Our results also show that a relatively small set of genes (n = 73) are consistently highly expressed in *S.* Enteritidis independently of strain or surrounding microenvironment. These genes encode proteins that are involved in protein synthesis, stress response, cell-envelope, or membrane biogenesis, bacterial metabolism, and a few proteins with poorly characterized functions. We discuss the functions of these genes in *Salmonella* or other related organisms and show that several of these genes likely play an important role in the pathobiology of *S.* Enteritidis. This study provides foundation for further investigations needed to dissect the contributions of the highly expressed genes in biologically-relevant systems and their potential application in the development of new immunoprophylactic and therapeutic measures to control this public health pathogen.

MATERIALS AND METHODS

In Silico Prediction of Gene Expression Levels

The complete genome sequence of *S.* Enteritidis str. P125109 (UK, phage type 4, NCBI accession number NC_011294) was analyzed *in silico* using the software package GEMBASSY-gphx (Itaya et al., 2013). For comparison, complete genome sequences of the genetically related *S.* Typhimurium LT2 (NC_003197) and other Gram-negative bacterial strains such as *E. coli* K12, *C. jejuni* NCTC 11168 (NC_002163), *Deinococcus radiodurans*, and Gram-positive bacterium such as *B. subtilis* were also analyzed (Table 1). GEMBASSY-gphx predicts highly expressed (**PHX**) genes based on their predicted general expression level [E(g)] (Karlin and Mrazek, 2000; Karlin et al., 2003). Prediction of expression level takes into account the difference in codon usage **(B)** between each specific protein-coding gene of interest **(g)** relative to all other protein-coding genes within the genome (**C**). The difference in codon usage is also calculated between the gene of interest **(g)** and three reference gene classes known to be highly expressed across different microorganisms. These gene classes include (i) **RP** = ribosomal protein genes, (ii) **CH** = chaperone genes, and (iii) **TF** = translation/transcription associated genes. The predicted expression level of a gene of interest relative to each of the reference family classes is calculated as follows:

$$\mathrm{ERP\ (g)} = \frac{\mathrm{B}\,(g|\mathrm{C})}{\mathrm{B}\,(g|\mathrm{RP})}\,,\mathrm{ECH\ (g)} = \frac{\mathrm{B}\,(g|\mathrm{C})}{\mathrm{B}\,(g|\mathrm{CH})}\,,\ \mathrm{ETF\,(g)} = \frac{\mathrm{B}\,(g|\mathrm{C})}{\mathrm{B}\,(g|\mathrm{TF})},$$

E = Expression relative to RP, CH, or TF. The codon usage difference between each test gene (g) and reference gene classes (RP, CH, or TF) is denoted by **B** (g | Reference Gene Class). A predicted highly expressed (PHX) gene displays a high ratio difference relative to the rest of protein-coding genes [B(g|C)] and a low ratio difference relative to the 3 representative gene classes [B(g/RP) or (g/CH) or (g/TF)]. Finally, the predicted general expression level [E(g)] is calculated by combining these 3 ratios, as follows:

$$\mathrm{E\,(g)} = \frac{\mathrm{B}\,(g|\mathrm{C})}{\frac{1}{2}\mathrm{B}\,(g|\mathrm{RP}) + \frac{1}{4}\mathrm{B}\,(g|\mathrm{CH}) + \frac{1}{4}\mathrm{B}\,(g|\mathrm{TF})}.$$

Table 1. General statistics for *in silico* predicted highly expressed (PHX) genes in selected bacterial genomes.

Genome	Genome size (kb)	Total CDS[1]	Total number of PHX[2] genes	% PHX genes from total CDS	CDS ≥ 100 codons[3]	PHX genes from CDS ≥ 100 codons	% PHX genes (CDS ≥ 100 codons)	Max E(g)[4]	Reference	NCBI accession
S. Enteritidis P125109	4,685	4206	161	3.8	3849	124	3.2	3.53	This study	NC_011294
S. Typhimurium LT2	4,857	4423	171	3.9	4009	130	3.2	3.75	This study	NC_003197
B. subtilis str.168	4,215	4174	145	3.5	3615(3612)	96(148)	2.7(4)	4.54 (2.34)	This study and (23)	NC_000964
E.coli K-12 substr.MG1655	4,641	4144	248	6.0	3800(3898)	204(306)	5.4(8)	3.71 (2.66)	This study and (23)	NC_000913
C. jejuni (NCTC 11168)	1,641	1622	129	8.0	1452	100	6.9	1.69	This study	NC_002163
D. radiodurans R1 (BAA-816)	3,284	2997	325	10.8	2828(2629)	310(362)	11(14)	2.94 (1.66)	This study and (37)	NC_001263 NC_001264

[1]CDS, coding sequences.
[2]PHX, Predicted Highly Expressed.
[3]Values in parenthesis have been reported previously by the studies cited in the Reference column.
[4]E(g), predicted general expression level.

The following 2 conditions must be met for a gene to be categorized as PHX: (i) the predicted general expression level [E(g)] must be ≥1; and (ii) at least 2 of the 3 values (E_{RP}, E_{CH}, or E_{TF}) must exceed 1.05 (Karlin and Mrazek, 2000; Karlin et al., 2003).

Transcriptome Analysis

S. Enteritidis UK (phage type 4), G1 (phage type 4), and BC8 (phage type 8) were cultured in LB-salt (300 mM NaCl) and LPM broth (5 mM KCl, 7.5 mM $(NH_4)_2SO_4$, 0.5 mM K_2SO_4, 38 mM glycerol, 0.1% casamino acids, 8μM $MgCl_2$, 337 μM $PO^{3-}{}_4$, pH 5.8) (Coombes et al., 2004) at avian physiologic temperature (42°C) until reaching exponential phase with constant agitation (200 RPM) as described previously (Shah, 2014). These conditions resemble the salt rich environment within the avian intestinal lumen (LB-salt) (Razdan et al., 1997; Huang et al., 2014) and the low pH, low-magnesium, low-phosphate conditions encountered within macrophages (LPM) (Coombes et al., 2004; Gibbons et al., 2005; Niemann et al., 2011; Heithoff et al., 2012). RNA extraction, mRNA enrichment, and RNA-Seq were performed as described previously (Shah, 2014). For comparison, we also included RNA-Seq data obtained from strains grown under nutrient rich conditions (LB) from a previously published report (Shah, 2014). Paired-end reads (75 bp) were trimmed and aligned against the *S.* Enteritidis str. P125109 reference genome using Geneious v 11.15 (Biomatters Ltd, New Zealand). Read counts were adjusted to Reads per kilobase per million (**RPKM**). Transcripts with RPKM values below 10 were removed from analysis and the remaining data was transformed to Log_2 units for further analysis. The transcriptome data have been deposited in NCBI's gene expression omnibus (Edgar et al., 2002) under the GEO series accession number GSE122177. In addition, data compiled for LB, LB-salt, and LPM media is included in Supplementary files 1, 2, and 3, respectively. The Pearson correlation between the transcriptome data (RPKM-log_2 transformed values) obtained from the 3 strains grown under each condition and *in silico* prediction of gene expression (Eg) was determined using NCSS 2007 version 07.1.19 (NCSS, USA). The most abundant transcripts from the experimental transcriptome analysis (top 10%) in all strains and all conditions that were concurrently identified as PHX were designated as common highly expressed (**CHX**) genes.

In Silico Determination of Binding Affinity Between Shine-Dalgarno (SD) Region and the Anti-Shine-Dalgarno Sequence (aSD)

The **SD** region was defined as 20 nucleotides upstream of the start codon of each gene (Nakagawa et al., 2017). The SD region was extracted from all coding sequences (CDs) annotated in *S.*

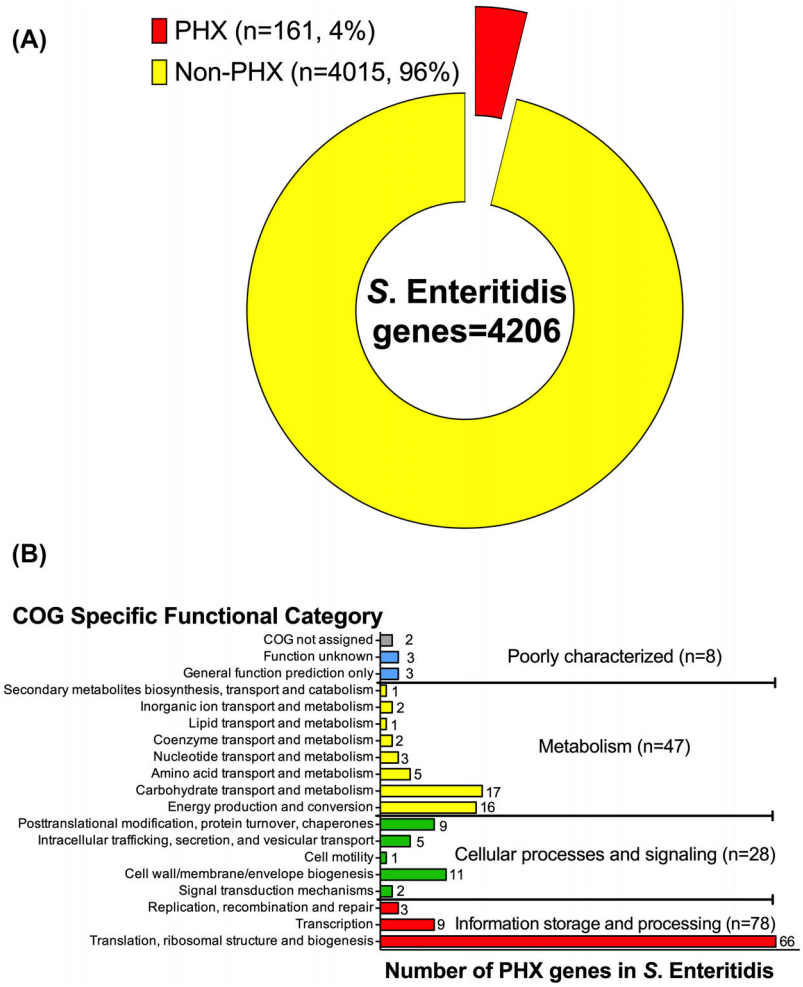


Figure 1. (A) Classification of *S.* Enteritidis str. P125109 genome into predicted highly expressed (PHX) genes according to GEMBASSY-gphx. (B) Classification of *S.* Enteritidis str. P125109 PHX genes according to cluster of orthologous groups (COG) specific functional categories.

Enteritidis P125109 genome (NC_011294). Binding affinity (kcal/mol) between the SD region and the extended aSD (5′-CCUCCUUA-3′) (Wei et al., 2017) was calculated *in silico* using RNAcofold v2.4.9 with default parameters (Lorenz et al., 2011). Protein localization prediction was performed with PSORTb v3.0 (Yu et al., 2010).

RESULTS AND DISCUSSION

Identification of S. Enteritidis *PHX Genes Using GEMBASSY-gphx*

In this study, we first identified predicted highly expressed genes (**PHX**) *in silico* in the *S.* Enteritidis genome using GEMBASSY-gphx software tool. This *in silico* prediction is based on the difference in codon usage between a test gene and 3 different gene classes (RP, CH, and TF) (Karlin and Mrazek, 2000) known to be highly expressed as they encode highly abundant proteins relevant to bacterial physiology (VanBogelen et al., 1990; Ishihama et al., 2008; Maier et al., 2011). This *in silico* analysis of 4206 genes within the *S.* Enteritidis genome predicted a total of 161 PHX genes (3.8%) (Figure 1a), whose codon usage is similar to the reference highly expressed genes. The remaining 4015 genes were classified as non-PHX and will not be discussed in this study. The proportion and identity of PHX genes in the *S.* Typhimurium LT2 genome was similar to *S.* Enteritidis (Table 1). The proportion of PHX genes differed in other organisms including the Gram-positive *B. subtilis* (3.5%), the Gram-negatives *E. coli* K12 (6%) (Karlin and Mrazek, 2000), *Campylobacter jejuni* (8%), and the radiation-resistant *Deinococcus radiodurans* (10.8%) (Na Gao et al., 2009) (Table 1). The difference in proportion of PHX genes among these diverse bacterial genomes is expected *in lieu* of their individual genome content, niche-specificity and divergent evolution.

Functional Classes of S. Enteritidis *PHX Genes Identified Using GEMBASSY-gphx*

Classification of 161 PHX genes into functional categories based on cluster of orthologous genes (COG) revealed that the majority of PHX genes contribute

to information storage and processing (78/161, 48%) (Figure 1b). Of these 78 genes, 66 genes encode proteins involved in translation, ribosomal structure and biogenesis. The second most abundant functional category of PHX genes is related to bacterial metabolism (47/161 = 29%), wherein the majority of genes are involved in carbohydrate transport and metabolism (17/47, 36%). Identification of these functional classes is consistent with previous reports showing strong association between functional groups J (translation, ribosome structure, and biogenesis) and C (energy production and conversion) with highly expressed genes in bacteria (Ma et al., 2002; Rollenhagen and Bumann, 2006). Such association is not surprising given that genes within these functional categories are highly conserved and play critical roles in bacterial physiology (Nei and Kumar, 2000; Rocha and Danchin, 2004; Drummond et al., 2005). Indeed, the functional relevance and evolutionary selection pressure are so central for these PHX genes that their proteins are less prone to mutations that lead to amino acid substitutions (Drummond et al., 2005), allowing their efficient recognition by the most abundant tRNAs and translation from multiple tRNA isoacceptors to ensure appropriate protein synthesis rate (Abernathy et al., 2013). The third most abundant functional category of PHX genes is related to cellular processes and signaling functions (28/161 = 17%), where the majority of genes contribute to cell-envelope or membrane biogenesis (11/28). Finally, a small proportion of PHX genes (8/161 = 5%) with unknown or poorly characterized functions were also identified in this study (Figure 1b).

Identification of Highly Expressed Genes in pathogenic S. Enteritidis *Strains Using RNA-Seq*

We used RNA-Seq to determine transcriptomic profiles of 3 *S.* Enteritidis strains (UK, G1 and BC8) cultured at avian physiologic temperature under 2 different growth conditions that resemble the intestinal (LB-salt medium) and intramacrophage (LPM medium) microenvironments encountered in the chicken host. For comparison, we also included the transcriptomes of these *S.* Enteritidis strains cultured in nutrient rich LB medium from a previously published report (Shah, 2014). In general, Pearson correlation between RPKM-Log_2 values from RNA-seq analysis and *in silico* predicted general expression level (Eg) ranged between 0.42 and 0.52, with limited variation between strains cultured in each microenvironment (Table 2). In a previous study, the correlation between protein abundance and *in silico* prediction of expression levels in *E. coli* strain MC4100 grown to exponential phase at 37°C was 0.52, indicating that our results are in agreement with this report (Ishihama et al., 2008). Next, we selected genes that were concurrently predicted as PHX and that also produced the most abundant transcripts (top 10%) in each strain and under each culture condition (Figure 2). The number of such highly expressed genes between the test strains ranged from 92 to 104 (LB), 84 to 103 (LB-salt), and 92 to 121 (LPM) (Figure 2). Subsequently, we identified a core set of 73 highly expressed genes that were common across all strains and all microenvironments tested (Figure 3). We refer to these genes as common highly expressed genes (CHX) for the rest of this manuscript. The broad and specific functional classification of these genes according to cluster of orthologous genes (COG) is shown in Figure 3.

Table 2. Pearson correlation between RPKM-log_2 adjusted *in vitro* transcriptomic data and *in silico* predicted general expression levels E(g).

	S. Enteritidis strain		
Growth medium	G1	UK	BC8
LB[1]	0.44	0.45	0.47
LB-salt[2]	0.42	0.42	0.46
LPM[3]	0.51	0.50	0.52

[1]LB, Luria Bertani.
[2]LB-salt, Luria Bertani supplemented with 300 mM of sodium chloride.
[3]LPM, Low phosphate-low magnesium media.

CHX Genes Involved in Information Storage and Processing The majority of CHX genes (n = 45) identified in this study are involved in information storage and processing (Figure 3). Of these, 39 genes participate in translation, ribosomal structure, and biogenesis by encoding ribosomal proteins part of the large (50S) and small (30S) ribosomal subunits (Suppl. file 4). Most of these genes are identified as essential because of their indispensable role in assembly of the protein synthesis machinery and will not be discussed in detail here [reviewed in Kaczanowska and Ryden-Aulin (2007) and Shajani et al. (2011)]. In the following section, we focus our discussion on non-essential CHX genes that have been reported as dispensable in genetic screening studies conducted in either *E. coli*, *S.* Typhimurium, *S.* Typhi, or *S.* Enteritidis and, therefore, genetic manipulation is technically feasible in *S.* Enteritidis for follow-up investigations (Baba et al., 2006; Santiviago et al., 2009; Barquist et al., 2013; Porwollik et al., 2014). The 4 non-essential genes in this functional category include *SEN_RS19340* encoding transcription termination factor Rho and *cspA, cspC,* and *cspE* genes encoding cold-shock proteins (Baba et al., 2006; Barquist et al., 2013; Porwollik et al., 2014) (Table 3). Termination factor Rho plays a general regulatory function in bacterial transcription (Kriner and Groisman, 2017). Cold-shock proteins are known to function as transcription antiterminators to regulate expression of stress-responsive and virulence-associated genes in *E. coli* (Bae et al., 2000; Phadtare and Inouye, 2004) and in *S.* Typhimurium during intra-macrophage infection (Michaux et al., 2017). The CHX genes *hupA* and *hupB* encode the proteins HupAB, which bind to DNA and regulate various genes involved in ATP synthesis, glutathione metabolism, peptidoglycan

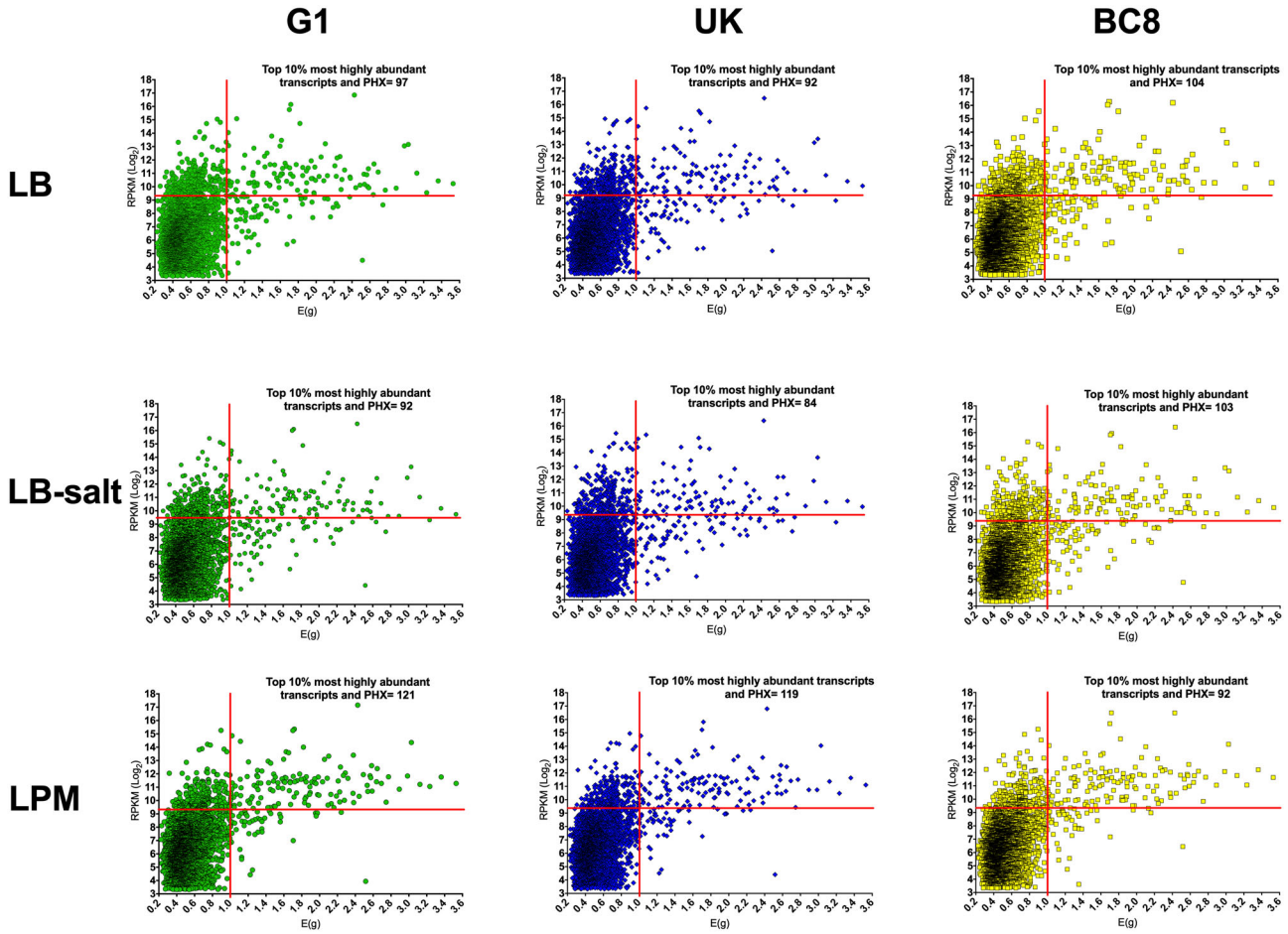


Figure 2. *In silico* predicted levels of gene expression (Eg) and *in vitro* transcript abundance in RPKM-Log2 adjusted values in *S.* Enteritidis strains G1, UK, and BC8 cultured in nutrient rich (LB), LB-salt, and LPM media at avian physiologic temperature (42°C). The top right quadrant of each chart corresponds to genes simultaneously predicted to be highly expressed (PHX) and among the top 10% most abundant transcripts. The genes common to these quadrants are considered as common highly expressed (CHX) genes.

biosynthesis and protein export. HupAB also controls expression of virulence related genes involved in motility (*motA* and *motB*), flagellar structure, and assembly (*flgE, fliA, fljK*) and SPI-2 genes (Guo and Adhya, 2007; Mangan et al., 2011). *Salmonella* Typhimurium *hupA* and *hupB* mutants are attenuated in chickens, pigs, and cattle (Chaudhuri et al., 2013). Taken together, these data suggest that CHX genes belonging to this functional group are involved in controlling or regulating expression of genes relevant for bacterial physiology and virulence. Therefore, it can be expected that the absence of these genes may negatively impact *S.* Enteritidis infection and persistence in chickens.

CHX Genes Involved in Cellular Processes and Signaling A total of 12 CHX genes are involved in cellular processing and signaling (Figure 3). Among these, *groES, groEL,* and *SEN˙RS01975* are involved in post-translational modification, protein turnover, and chaperone function (Table 3). Whereas, *groES* is considered an essential gene, the essentiality of *groEL* appears ambiguous (Baba et al., 2006; Barquist et al., 2013). These genes encode the protein complex GroES-GroEL that prevents aggregation of misfolded proteins in the bacterial cytoplasm and thus contribute to overall bacterial health [reviewed by Hayer-Hartl et al. (2016)]. The CHX gene *SEN_RS01975* encodes peroxiredoxin, or alkyl hydroperoxide reductase, a member of the AhpC-TSA superfamily that reduces reactive oxygen species to alcohols to protect bacteria against oxidative stress. In *S.* Typhimurium, *tsa* is highly expressed in murine macrophages and contributes to improve survival during oxidative stress in the absence of other catalases (Hebrard et al., 2009). TSA is also an immunodominant antigen in *H. pylory*, inducing early humoral responses in patients infected with this microorganism (Kimmel et al., 2000; Nurgalieva et al., 2005). The CHX gene *uspA* encodes the universal stress protein A, whose transcription is induced in *S.* Typhimurium upon exposure to oxidative, nutritional, or temperature stress (Liu et al., 2007; Karatzas et al., 2008), disinfectants (Karatzas et al., 2008), and bile salts (Hernandez et al., 2012). Upregulation of *uspA* in presence of bile salts may contribute to adaptation to bile and aid colonization and persistence in liver (Hernandez et al., 2012). *S.* Typhimurium *uspA* mutants are attenuated in orally infected mice (Liu et al., 2007),

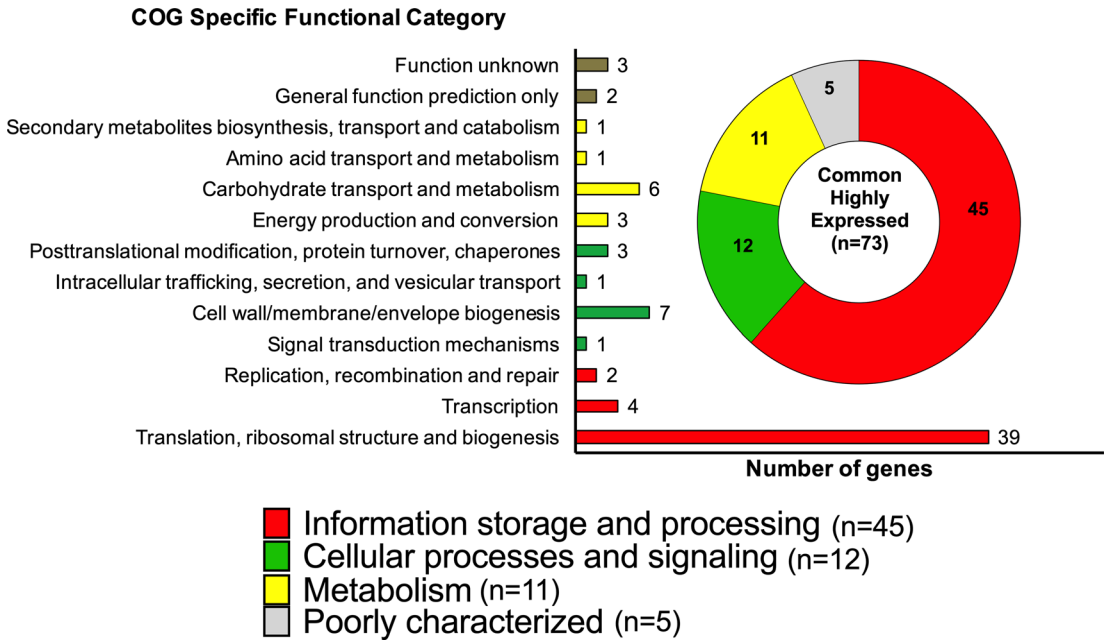


Figure 3. Specific functional classification of common highly expressed (CHX) genes in *S.* Enteritidis according to cluster of orthologous genes (COG). The inset corresponds to broad functional classification according to COG.

however *uspA* mutants do not colonize oviduct tissues efficiently and expression of *uspA* is induced in *S.* Enteritidis exposed to egg white, suggesting that *uspA* likely supports invasion, colonization, or persistence of *S.* Enteritidis in egg contents (Gantois et al., 2008; Raspoet et al., 2011). The CHX gene *tatE* encodes TatE, an active component of the twin-arginine translocation system that exports fully folded proteins across the bacterial inner membrane (Patel et al., 2014; Eimer et al., 2015). Although the function of *tatE* is not well characterized, mutations in Tat components induce failure to translocate Tat substrates resulting in cell-envelope defects, sensitivity to bile salts and virulence attenuation in *S.* Typhimurium (Reynolds et al., 2011; Craig et al., 2013). This potential role in cell-envelope fitness may be related to the survival of *S.* Enteritidis in egg contents as a recent proteomic survey shows that TatE abundance increases in *S.* Enteritidis exposed to egg white (Qin et al., 2018).

The CHX genes *ompA*, *ompC, ompD* (*nmpC)*, *ompX*, *lpp,* and *pal* encode outer membrane proteins (OMPs), whereas *hlpA* (*skp* in *E.coli*) encodes a periplasmic chaperone (Table 3). OmpA is one of the most abundant structural proteins of the bacterial OM (reviewed in Silhavy et al. (2010)). Expression of *ompA* increases upon exposure to antimicrobial compounds such as fluoroquinolones (Coldham et al., 2006) and chlorine in *S.* Typhimurium and *S.* Enteritidis (Wang et al., 2010). OmpA protein levels decrease after treatment with common disinfectants in *S.* Typhimurium (Karatzas et al., 2008) and organic acids in *S.* Enteritidis (de Almeida et al., 2017). Mutants of *ompA* in *S.* Enteritidis do not colonize avian tissues efficiently (Zhou et al., 2016), whereas OmpA abundance is increased in *S.* Enteritidis strains hypersensitive to human serum (Dudek et al., 2016), suggesting that OmpA is critical for cell-membrane homeostasis during infection *in vivo.* The porins OmpC and OmpD allow passive diffusion of small nutrients across the OM and adjust the OM permeability in response to stress such as high osmolarity, low pH, and toxic compounds (Santiviago et al., 2002; Hernandez et al., 2012; van der Heijden et al., 2016). Reduced expression or mutation of *ompC* and *ompD* favors resistance against cephalosporins (Hu et al., 2005) and beta-lactamases in *S.* Typhimurium (Sun et al., 2009). Moreover, *S.* Typhimurium *ompC* mutants are attenuated in orally infected mice, chickens, and pigs (Chatfield et al., 1991; Chaudhuri et al., 2013) and *ompC* and *ompX* are required for growth of *S.* Typhi in presence of bile salts (Langridge et al., 2009). Vaccination and passive transfer of antibodies against OmpD confer protection against *S.* Paratyphi A in mice (Yang et al., 2012). The extracellular L3 loop of OmpX binds to host complement system and cell membrane proteins, whereas its L1 loop contains an antibody binding domain (Vogt and Schulz, 1999). Taken together, CHX genes encoding these OMPs appear to play important functions in maintenance, response, and adaptation of the OM to the host environment and thereby may contribute to pathogenicity and antigenicity of *S.* Enteritidis in the chicken host.

The genes *lpp*, *pal,* and *hlpA* encode proteins central to biogenesis and stabilization of the OM. Lpp or Braun lipoprotein links the OM to muropeptides in the adjacent peptidoglycan layer (Braun, 1975). Deletion of *lpp* induces virulence attenuation and triggers protective immune responses in mice challenged with *S.* Typhimurium (Fadl et al., 2005; Erova et al., 2016). Reduced levels of Lpp in response to bile salts leads to cell-envelope reorganization in *S.* Typhimurium

Table 3. Non-ribosomal common highly expressed (CHX) genes in three pathogenic *S.* Enteritidis strains cultured under distinct environmental conditions (n = 34).

Locus[1]	Gene	Product	COG[2] specific functional category	Localization[3]	E(g)[4]	Hybridization energy (ΔG) in kcal/mol[5]	Protein abundance in *S.* Typhimurium[6]
SEN_RS19340	*rho*	Transcription termination factor Rho	Transcription	Cytoplasmic	1.42	-1.56	Top 10 to 25%
SEN_RS18060	*cspA*	Cold-shock protein	Transcription	Cytoplasmic	1.72	-3.34	Top 30 to 40%
SEN_RS03055	*cspE*	Cold-shock protein	Transcription	Cytoplasmic	1.70	-3.50	Top 5 to 10%
SEN_RS06220	*cspC*	Cold-shock protein CspC	Transcription	Cytoplasmic	1.82	-6.00	Top 5 to 10%
SEN_RS20610	*hupA*	DNA-binding protein HU-alpha CDS	Replication, recombination, and repair	Cytoplasmic	2.05	-5.70	Top 5%
SEN_RS02220	*hupB*	DNA-binding protein HU-beta	Replication, recombination, and repair	Cytoplasmic	1.45	-6.88	Top 5%
SEN_RS21340	*groEL*	Molecular chaperone GroEL	Post-translational modification, protein turnover, and chaperones	Cytoplasmic	2.38	-6.07	Top 5%
SEN_RS21335	*groES*	molecular chaperone groES	Post-translational modification, protein turnover, and chaperones	Cytoplasmic	1.65	-5.12	Top 5%
SEN_RS01975	*Peroxiredoxin*	Peroxiredoxin	Post-translational modification, protein turnover, and chaperones	Cytoplasmic	1.67	-7.45	Top 5 to 10%
SEN_RS17750	*uspA*	Universal stress protein A	Signal transduction mechanisms	Cytoplasmic	1.54	-7.89	Top 5%
SEN_RS03070	*tatE*	tatE	Intracellular trafficking, secretion, and vesicular transport	Cytoplasmic membrane	1.12	-4.30	25 to 75%
SEN_RS04830	*ompA*	Outer membrane protein A	Cell wall/membrane/envelope biogenesis	Outer membrane	3.03	-5.59	Top 5%
SEN_RS11705	*ompC*	Phosphoporin PhoE	Cell wall/membrane/envelope biogenesis	Outer membrane	1.63	-5.44	Top 5%
SEN_RS07685	*nmpC*	Phosphoporin PhoE (ompD)	Cell wall/membrane/envelope biogenesis	Outer membrane	2.99	-6.09	Top 5 to 25%
SEN_RS04025	*ompX*	ompX	Cell wall/membrane/envelope biogenesis	Outer membrane	2.65	-5.24	Top 10%
SEN_RS08655	*lpp*	Major outer membrane lipoprotein	Cell wall/membrane/envelope biogenesis	Outer membrane	2.43	-5.81	Top 5%
SEN_RS03605	*pal*	Peptidoglycan-associated lipoprotein Pal	Cell wall/membrane/envelope biogenesis	Outer membrane	2.49	-5.71	Top 5 to 10%
SEN_RS01165	*hlpA*	Chaperone protein Skp	Cell wall/membrane/envelope biogenesis	Periplasmic	1.58	-8.42	Top 5%
SEN_RS09130	*gapA*	Aldehyde dehydrogenase	Carbohydrate transport and metabolism	Cytoplasmic	2.58	-4.83	Top 5%
SEN_RS14530	*eno*	Enolase	Carbohydrate transport and metabolism	Cytoplasmic	2.57	-5.07	Top 5%
SEN_RS15150	*fba*	Class II fructose-bisphosphate aldolase	Carbohydrate transport and metabolism	Cytoplasmic	2.89	-4.13	Top 5 to 10%
SEN_RS12575	*ptsI*	Phosphoenolpyruvate–protein phosphotransferase	Carbohydrate transport and metabolism	Cytoplasmic	1.25	-8.63	Top 10 to 25%
SEN_RS12570	*ptsH*	Phosphocarrier protein HPr	Carbohydrate transport and metabolism	Cytoplasmic	1.29	-5.81	Top 5%
SEN_RS12580	*crr*	Glucose-specific phosphotransferase enzyme IIA component	Carbohydrate transport and metabolism	Cytoplasmic	1.94	-6.87	Top 5%
SEN_RS19120	*atpE*	ATP synthase subunit C	Energy production and conversion	Cytoplasmic membrane	1.61	-6.07	NA[7]
SEN_RS19115	*atpF*	ATP synthase subunit B	Energy production and conversion	Cytoplasmic membrane	1.45	-3.44	Top 5 to 10%
SEN_RS04545	*pflB*	Formate C-acetyltransferase	Energy production and conversion	Cytoplasmic	3.36	-7.11	Top 10%
SEN_RS21315	*aspA*	Aspartate ammonia-lyase	Amino acid transport and metabolism	Cytoplasmic	1.61	-3.26	Top 10%

Table 3. *continued*

Locus[1]	Gene	Product	COG[2] specific functional category	Localization[3]	E(g)[4]	Hybridization energy (ΔG) in kcal/mol[5]	Protein abundance in *S.* Typhimurium[6]
SEN_RS09630	*acpP*	Acyl carrier protein	Secondary metabolites biosynthesis, transport and catabolism	Cytoplasmic	1.60	-4.26	Top 10 to 25%
SEN_RS10710	*yeeX*	DUF496 domain-containing protein	Unknown	Cytoplasmic	1.27	-4.56	Top 5%
SEN_RS20875	*yjbJ*	CsbD family protein—stress response	Unknown	Unknown	1.50	-6.09	Top 5%
SEN_RS21370	*ecnB*	Entericidin B lipoprotein	Unknown	Cytoplasmic membrane	1.31	-6.00	Top 10 to 25%
SEN_RS06655	*hns*	DNA-binding protein H-NS	General function prediction only	Cytoplasmic	1.42	-2.73	Top 5%
SEN_RS13375	*yfiD*	Autonomous glycil radical cofactor GrcA	General function prediction only	Cytoplasmic	1.49	-3.76	Top 5 to 10%

[1]Locus in reference sequence *S.* Enteritidis P125109 (NCBI Accession NC_011294).
[2]COG, cluster of orthologous groups.
[3]Localization according to PSORTb v3.0 (Yu et al., 2010).
[4]E(g), predicted general expression level ≥ 1.05 indicates predicted highly expressed gene (PHX) based on codon usage similar to reference highly expressed gene classes.
[5]Hybridization energy (ΔG) according to RNAcofold V2.4.9 with default parameters (Lorenz et al., 2011).
[6]Protein abundance averaged by PaxDb protein abundance database version 4.1 (https://pax-db.org/) based on *S.* Typhimurium strain LT2 cultured under nutrient rich media (LB) at log and stationary phase, and low-magnesium/low-pH media (Adkins et al., 2006; Wang et al., 2015).
[7]NA, Data Not Available.

(Hernandez et al., 2015), a phenotype that is likely associated with reduced pathogenicity and altered antigenicity in *Salmonella*. Peptidoglycan associated lipoprotein Pal exists in complex with TolB and facilitates its interaction with OmpA and Lpp to maintain OM integrity (Lloubes et al., 2001). Indeed, *pal* mutants in *S.* Typhimurium display cell-envelope aberrations and are impaired in their survival within murine macrophages and colonization of extra-intestinal tissues in mice (Masilamani et al., 2018). The *hlpA* gene encodes the periplasmic chaperone HlpA (a.k.a. OmpH), a member of the Skp family that prevents non-specific auto aggregation of OM proteins (Burmann et al., 2013). Moreover, *hlpA* mutants in *S.* Typhimurium display competitive disadvantage in colonization of extra-intestinal tissues in mice (Rowley et al., 2011). Expression of *hlpA* is also downregulated in piglets orally infected with *Shigella dysenteriae* (Kuntumalla et al., 2011). It appears that CHX genes involved in cellular processes and signaling participate in cell-envelope fitness which is intimately coupled to stress response in bacteria. Functions encoded by these CHX genes may enable *S.* Enteritidis to withstand hazardous antimicrobial compounds and harsh environments encountered in the chicken host with repercussions in pathogenicity and immunogenicity of *S.* Enteritidis.

CHX Genes Involved in Salmonella Metabolism

A total of 11 CHX genes hold functions in metabolism (Figure 3). Six of these genes (*gapA*, *eno*, *fba*, *ptsI*, *ptsH,* and *crr*) are involved in carbohydrate transport and metabolism (Table 3). The genes *gapA, eno, and fba* are potentially essential, or at a minimum their essentiality is ambiguous in *Salmonella* (Hartman et al., 2014; Porwollik et al., 2014). The *gapA* gene encodes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that catalyzes oxidation and phosphorylation of glyceraldehyde-3-phosphate (GAP) to 1,3-biphosphoglycerate in the glycolysis pathway. GAPDH from *Lactobacillus plantarum* can bind to mucin, a major component of the extracellular matrix protecting the intestinal epithelium layer (Kinoshita et al., 2008), whereas GAPDH from *E. coli* can bind directly to the surface of human enteric epithelial cells (Egea et al., 2007). It is likely that GAPDH aids *S.* Enteritidis in gaining a foothold in the chicken intestinal tract via direct interactions with chicken enteric epithelial cells. The CHX gene *eno* encodes Eno protein that catalyzes the conversion of 2-phosphoglycerate into phosphoenolpyruvate in the glycolysis pathway. As a part of the RNaseE/degradosome complex, Eno also influences bacterial morphology during anaerobiosis (Murashko and Lin-Chao, 2017). The CHX gene *fba* encodes fructose 1,6-biphosphate aldolase, which performs reversible aldol condensation of dihydroxyacetone with GAP during glycolysis (reviewed in (Shams et al., 2014)). Although its function in *Salmonella* is not fully understood, Fba contributes indirectly to virulence via its role in glycolysis and gluconeogenesis and is important for intracellular growth and survival of microbes such as *Mycobacterium tuberculosis* (Puckett et al., 2014), *Toxoplasma gondii* (Blume et al., 2015), and *Francisella novicida* (Ziveri et al., 2017). Fba is also a promising target to develop antimicrobial compounds that can interrupt central glycolysis (Puckett et al., 2014). The genes *ptsH*, *ptsI,* and *crr* are located in the *ptsHI-crr* operon that encodes the phosphotransferase HPr, phosphotransferase enzyme I, and the glucose-specific phosphotransferase enzyme

IIA, respectively (De Reuse and Danchin, 1988). These genes participate in uptake and phosphorylation of glucose and catabolite repression via the PTS^{Glc} system for carbon metabolism (De Reuse and Danchin, 1988). In *S.* Typhimurium, *ptsH*, *ptsI*, and *crr* mutants do not replicate efficiently within cultured murine macrophages (Kok et al., 2003; Bowden et al., 2014) and *ptsI* mutants are attenuated in intraperitoneally challenged mice (Kok et al., 2003). Failure in transport and metabolism of glucose within the *Salmonella* containing vacuole may contribute to these phenotypes (Kok et al., 2003).

The CHX genes *atpE* and *atpF* encode ATP synthase subunits that belong to the ubiquitous F-type (F_1F_0) ATPase that produces ATP from ADP in a proton gradient mechanism [reviewed by Yoshida et al. (2001) and Ruhle and Leister (2015)]. Interestingly, *atpE* and *atpF* mutants of *S.* Typhimurium are attenuated in orally infected chicken, pigs, and cattle (Chaudhuri et al., 2013). The CHX gene *pflB* encodes formate-C-acetyltransferase-1 that converts pyruvate and Coenzyme A into formate and acetyl-CoA, and regulates anaerobic glucose metabolism in bacteria (Nnyepi et al., 2007). In *E. coli,* expression of *pflB* is induced by oxidative and osmotic stress after exposure to sodium salicylate (Pomposiello et al., 2001) and benzalkonium chloride (Moen et al., 2012), although a specific role in stress response has not been determined. PflB protein levels increase in *S.* Enteritidis exposed to egg white (Qin et al., 2018), suggesting that *pflB* may contribute to colonization and survival of *S.* Enteritidis in egg contents. The CHX gene *aspA* encodes aspartate ammonia-lyase that catalyzes conversion of aspartate to fumarate and is also involved in catabolism of the amino acids aspartate, glutamine, glutamate, proline, and asparagine as carbon sources. In *C. jejuni*, *aspA* mutants showed defects in caecal colonization in orally infected chickens (Guccione et al., 2008), and in *Y. pseudotuberculosis, aspA* contributes to acid stress tolerance by producing ammonia as byproduct of aspartate catabolism (Hu et al., 2010). The CHX gene *acpP* is an essential gene that encodes an acyl carrier protein involved in fatty acid biosynthesis and lipid metabolism (Baba et al., 2006; De Lay and Cronan, 2006; Byers and Gong, 2007; Porwollik et al., 2014). The antibiotic class pantothenamide inactivates AcpP by covalent modification, leading to accumulation of inactive AcpP and inhibition of fatty acid synthesis (Zhang et al., 2004). Several CHX genes encoding proteins involved in metabolic activities also have "moonlighting" abilities to perform additional roles in *Salmonella.* Their abundance is likely advantageous for acquisition of various carbon sources in nutrient-deprived microcompartments in the chicken host, thereby facilitating survival, persistence, and pathogenic potential of *S.* Enteritidis.

CHX Genes with Poorly Characterized or Unknown Functions A total of 5 CHX genes (*yeeX*, *yjbJ*, *ecnB*, *hns,* and *yfiD*) identified in this study encode poorly characterized proteins (Table 3). Expression of the CHX gene *yeeX* (SEN_RS10710) is upregulated in avian pathogenic *E. coli* (APEC) exposed to chicken serum (Li et al., 2011), suggesting that this gene is likely important for cecal colonization of *S.* Enteritidis in chickens. The CHX gene *yjbJ* is controlled by *rpoS* (Weber et al., 2005) and its expression is upregulated in response to cadmium (Worden et al., 2009) and osmotic stress in *E. coli* (Weber et al., 2006) and *S.* Typhimurium (Kroger et al., 2013). Thus, *yjbJ* potentially contributes to stress response in hostile environments such as intestinal lumen, host macrophages, reproductive tract and eggs of chickens infected with *S.* Enteritidis. The CHX gene *ecnB* encodes entericidin B membrane lipoprotein, which is the toxin component of the antitoxin/toxin pair *ecnAB* in *E. coli* (Bishop et al., 1998). Expression of *ecnAB* is also regulated by *rpoS* in response to osmoregulatory and starvation stress in *E. coli* to modulate competing bacterial populations (Bishop et al., 1998). EcnB promotes death of competing microbes by destabilizing cell membranes via a porin forming mechanism (Bishop et al., 1998). EcnB from *E. coli* and *Enterobacter* C6–6 inhibits *in vitro* growth of *Flavobacterium psychrophilum,* the causative agent of cold water disease in rainbow trout, implying potential antimicrobial applications (Schubiger et al., 2014). In *S.* Typhimurium, *encB* expression is upregulated in the presence of deoxicolate, suggesting that *encB* may play a role in adaptation to bile and potentially contribute to colonization of liver (Hernandez et al., 2012). Although the role of *encB* has not been investigated in *S.* Enteritidis, it would be interesting to determine if *ecnB* plays a role in inhibition of competing microorganisms during *S.* Enteritidis colonization of intestinal tissue in chickens.

The CHX gene *hns* encodes Hns protein, a master regulator of at least 60 different genes, including carbohydrate metabolism and osmotic regulation genes (Dorman, 2007). Hns binds to promoter regions and represses transcription of virulence genes from pathogenicity islands in *Shigella flexeneri*, enteroinvasive *E. coli* (Beloin and Dorman, 2003), *Vibrio cholerae* (Stonehouse et al., 2011), and *S.* Typhimurium (Dorman, 2007). Hns-deficient mutants in *E. coli* display increased sensitivity to low pH, high osmolarity, and bile (Erol et al., 2006), conditions that *S.* Enteritidis encounters in the chicken gut, macrophages, and liver. Hns also silences SPI-1 genes by repressing *hilA* transcription as well as various SPI-2, SPI-3, and SPI-5 genes in *S.* Typhimurium (Schechter et al., 2003; Navarre et al., 2006). In *S.* Enteritidis, expression of *hns* is downregulated when exposed to chlorine (Wang et al., 2010) and in biofilms exposed to benzalkonium chloride (Mangalappalli-Illathu and Korber, 2006). The CHX gene *yfiD* encodes the autonomous glycil radical cofactor GrcA, which promotes protection from oxidative stress by reactivating the oxygen sensitive PFL enzyme (*pflB*, see previous section) (Wagner et al., 2001). In *E. coli, yfiD* is strongly induced under aerobic

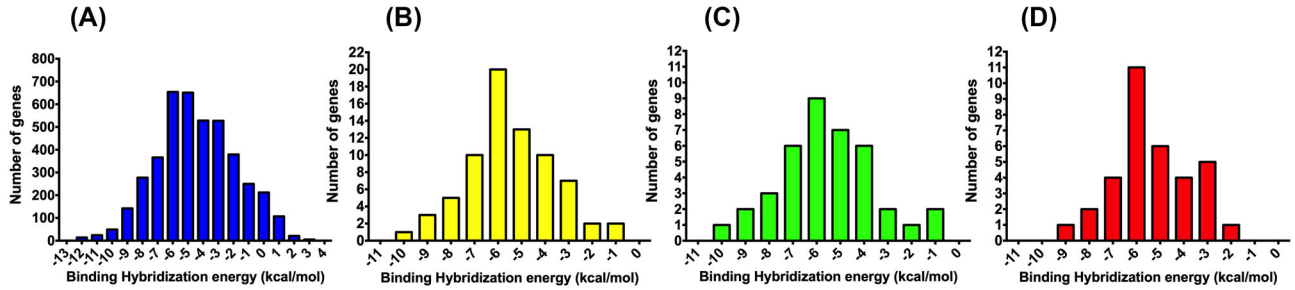
and anaerobic acidic conditions and upon exposure to sublethal doses of benzalkonium chloride (Blankenhorn et al., 1999; Wyborn et al., 2002; Moen et al., 2012). Although the functions of these CHX genes in *S.* Enteritidis remain poorly characterized, the potential role of these genes appears centered around stress responses or associated with expression of *Salmonella* pathogenicity genes. Therefore, it is of interest to define the roles of these genes and their products in pathogenicity and persistence of *S.* Enteritidis in the chicken host.

Translation Initiation of CHX Genes is Energetically Favorable

The presence of SD sequences in the 5′ UTR of bacterial mRNA promotes expression of highly expressed genes. The SD sequence interacts with the aSD sequence in the 16S rRNA during recruitment of bacterial mRNA to the small ribosomal subunit 30S (Shine and Dalgarno, 1974). This interaction facilitates translation initiation and efficiency by aligning the ribosome and the mRNA start codon (Shine and Dalgarno, 1974; Vimberg et al., 2007). This SD:aSD pairing relies on complementarity between both sequences (Shine and Dalgarno, 1974) and inherent characteristics such as reduced secondary structures at the 5′UTR of mRNA (Keller et al., 2012; Scharff et al., 2017). The strength of this RNA-RNA pairing or binding can be determined *in silico* based on hybridization energy (ΔG) (Bernhart et al., 2006; Lorenz et al., 2011; de Almeida et al., 2017). The more negative ΔG values indicate more favorable SD:aSD binding affinity due to small energy requirements (Bernhart et al., 2006; Lorenz et al., 2011). We hypothesized that the CHX genes identified in this study display more favorable SD:aSD binding affinities relative to the entire *S.* Enteritidis genome. *In silico* ΔG analysis revealed that the entire protein-coding genome of *S.* Enteritidis displayed a relatively higher average ΔG (-4.46±0.04 kcal/mol) than the 73 CHX genes identified in this study (ΔG = -5.52±0.21 kcal/mol) (Figure 4). Among the CHX genes, the ΔG of ribosomal (-5.64±0.32 kcal/mol) and non-ribosomal genes (-5.37±0.28 kcal/mol) was lower than the average ΔG for the entire genome (Figure 4). Because ΔG for CHX genes is lower, the SD:aSD interaction is more energetically favorable for CHX genes. These CHX genes bear 2 key characteristics of highly expressed genes: (1) codon usage similar to highly expressed reference genes that results in predicted Eg ≥1.05, and (2) energetically favorable SD:aSD interactions (Table 3). Both key features can drive translation initiation and protein synthesis from CHX genes more efficiently, resulting in higher abundance of proteins encoded by CHX genes. Although no data is available for *S.* Enteritidis, comprehensive global proteomic analysis of the closely related serovar *S.* Typhimurium reveals that proteins encoded by CHX genes are highly abundant across various conditions including growth in nutrient rich media (logarithmic and stationary phase) and low magnesium/low pH media (Adkins et al., 2006; Wang et al., 2015). The functional abundance of these CHX-encoded proteins appears confined to the top 5 to 10% of the total proteome, suggesting that they are highly abundant regardless of the condition (Table 3). These findings partly corroborate the relationship between trancriptomic data, SD:aSD binding affinities and protein abundance previously demonstrated in the taxonomically related *E.coli* (Wei et al., 2017). Collectively, these data suggest that *S.* Enteritidis CHX genes identified in this study are not only highly expressed at the transcript level, but the proteins encoded by these CHX genes can also be expected to be highly abundant as well. The CHX genes identified in this study likely play important roles during *S.* Enteritidis infection and persistence in chickens and possibly the environment. Therefore, follow-up functional studies are warranted in biologically-relevant systems.

CONCLUSIONS

By combining *in silico* prediction of gene expression and *in vitro* transcriptomic analysis of pathogenic *S.* Enteritidis at chicken physiologic temperature (42°C) under microenvironments resembling those encountered in the chicken host, we identified a core of 73 common highly expressed genes (CHX). We show that a subset (n = 34) of these CHX genes encode proteins that participate in cell-envelope fitness, stress response, nutritional and metabolic fitness in *S.* Enteritidis. The genes encoding outer membrane proteins OmpA, OmpC, OmpD, and OmpX are particularly relevant for modulation of cell-envelope structure in response to stress, antimicrobials, disinfectants, and also induce immunogenic responses in the chicken host. The CHX genes *ptsH*, *ptsI,* and *crr* are of interest due to their role in metabolism of glucose, which serves as the source of energy in the nutrient-limited *Salmonella* containing vacuole within the host macrophage (Kok et al., 2003). CHX genes including *yjbJ* and *yfiD* are also of interest due to their role in stress responses, whereas *hns* is directly involved in control of *Salmonella* pathogenicity genes. The potential role of *encB* in promoting *S.* Enteritidis colonization of the chicken gastro-intestinal tract by inhibiting competing microbes is also intriguing. Given the potential role of these genes in infection, the current *in vitro* study provides a strong foundation to perform biologically-relevant follow-up investigations to clarify the role of this concise set of genes in *S.* Enteritidis pathobiology in the chicken host. Moreover, our *in silico* analysis shows that CHX genes display at least 2 features that cooperatively optimize their translation to favor protein abundance. First, the CHX genes display codon usage similar to reference highly expressed genes, resulting in high Eg (≥ 1.05) (Table 3). Second, CHX gene transcripts have energetically favorable



Parameter	Hybridization energy (ΔG)			
	A. Total Genome (n=4206)	B. Total CHX genes (n=73)	C. Ribosomal CHX genes (n=39)	D. Non-ribosomal CHX genes (n=34)
Average	-4.46	-5.52	-5.64	-5.37
SD	2.60	1.83	2.00	1.62
SEM	0.04	0.21	0.32	0.28
Min	-12.33	-10.38	-10.38	-8.63
Max	3.07	-1.26	-1.26	-1.56
Median	-4.67	-5.64	-5.64	-5.65
Mode	-5.29	-5.81	-6.22	-5.81

Figure 4. Hybridization energy (kcal/mol) between the Shine-Dalgarno (SD) region of *S.* Enteritidis mRNA and the anti-Shine-Dalgarno (aSD) region in *S.* Enteritidis 16S rRNA in (A) the entire protein-coding genome, (B) total common highly expressed (CHX) genes, (C) ribosomal CHX genes, and (D) non-ribosomal CHX genes. Hybridization energies were calculated using the software package RNAcofold v2.4.9 with default parameters.

SD:aSD binding affinities, which facilitates translation initiation (Table 3 and Figure 4). Therefore, proteins produced from CHX gene transcripts are expected to be abundant in *S.* Enteritidis irrespective of the surrounding environment. Indeed, the proteins encoded by all CHX genes identified in this study are among the most abundant proteins identified in *S.* Typhimurium in culture conditions similar to ours (Table 3) (Adkins et al., 2006). Finally, our transcriptomic compendiums obtained from distinct *S.* Enteritidis strains cultured under multiple growth conditions expand the number of publicly available tools to study this pathogen. Because such transcriptomic compendiums are already available for *S.* Typhimurium (Kroger et al., 2013; Srikumar et al., 2015; Li et al., 2018), the current study also allows meaningful comparisons to dissect gene function and relevance specific to *S.* Enteritidis infection in chickens. These efforts will aid in the development of new immunoprophylactic and therapeutic strategies to prevent *S.* Enteritidis infection in chickens, improve food safety, and prevent human infection with this pathogen.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

Supplementary File 4. Ribosomal Common highly expressed (CHX) genes in three *S.* Enteritidis strains under distinct environmental conditions (n=39).

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