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REVIEW

Acid stress response in environmental and clinical strains of enteric bacteria

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Abstract **The success of many enteric bacteria is hinged on the ability to tolerate environmental stress such as extreme acidity. The acid stress response (ASR) has been investigated in many enteric bacteria and has been shown to involve variable expression of a broad spectrum of genes involved in transcriptional regulation, metabolism, colonization and virulence; representing a linkage between acid tolerance and pathogenicity. Though the majority of ASR studies have been conducted in laboratory conditions and from the perspective of pathogenicity, the role of environmental reservoirs on acid adaptation has recently emerged as an important aspect of pathogenic microbial ecology. This mini-review profiles ASR in three opportunistic enteric pathogens and synthesizes recent work pertaining to the study of this dynamic response.**

Keywords acid stress response, enteric bacteria, microbial ecology, transcriptional regulation, virulence

Introduction

In the past 30 years there has been more than a 6-fold increase in the frequency of food outbreaks in the United States resulting from contaminated produce and prompting investigations into the role of environmental reservoirs and food processing techniques on stress conditioning and pathogenicity of infectious bacteria (Doyle and Erickson, 2008; Capozzi et al., 2009). Opportunistic enteric pathogens occupy unique and austere niches and must maintain the capacity to react to mercurial environmental conditions and the caustic and highly variable acidic conditions of the gastrointestinal milieu. Environmental strains of enteric bacteria may also respond to acid stress in the form of industrial waste, decomposition of organic matter and the chemical constituents of food preservation. As such, robust response to acid stress is central to survivability in the environment and the host. A key adaptive feature of enteric bacteria involves the rapid induction of physiological and biochemical changes upon exposure to extreme acidity (pH 1–4) after sub-lethal (pH 4–6) preconditioning. This mechanism is referred to as acid habituation (Goodson and Rowbury, 1989), acid tolerance (Foster and Hall, 1990) and the acid stress response (ASR); it varies by organism, acid classification (organic vs. inorganic), phase of bacterial growth (logarithmic vs. stationary) and growth media as well as other environmental factors (Foster, 1991; Rowbury, 1995; Lin et al., 1996; Foster, 1999; Koutsoumanis et al., 2003). In addition to protection from lethal acidity, ASR has been shown to provide cross protection to other forms of stress (Rallu et al., 2000; Frees et al., 2003; Xie et al., 2004) and may be elicited upon exposure to various environmental cues (Flahaut et al., 1996; Frees et al., 2001; De Angelis and Gobetti, 2004). Further, induction of ASR has been demonstrated to activate additional adaptive behavior phenomena including virulence, biofilm formation, chemotaxis and antibiotic resistance (Leyer and Johnson, 1992; Merrell and Camilli, 2002; Polen et al., 2003; Maurer et al., 2005; Butler et al., 2006; Hayes et al., 2006; Merrell et al., 2001).

The physiological and biochemical mechanics of acid stress response have frequently been investigated in food borne pathogens such as enterohemorragic *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium (*S. typhimurium*). Recently, *Vibrio cholerae*, the waterborne pathogen responsible for Cholera, has emerged as capable of robust ASR. Several studies have investigated ASR in Gram-positive, industrially relevant bacteria, primary pathogens and

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Archaea (van de Guchte et al., 2002; Merrell et al., 2003; Cotter and Hill, 2003; Beales, 2004; Xie et al., 2004; Ciaramella et al., 2005; Padan et al., 2005; Baker-Austin and Dopson, 2007; Mols et al., 2010); however, reports contextualizing ASR in environmental and clinically derived strains of enteric bacteria are limited. This review will discuss reports on the study of acid stress response and synthesize recent and transformative molecular microbiological investigations of ASR in *E. coli*, *S. typhimurium* and *V. cholerae* in laboratory and environmental conditions and the agricultural phenomena eliciting this response.

Escherichia coli

Escherichia coli is an ubiquitous Gram-negative and rod-shaped bacteria, commonly found in the gastrointestinal tract of warm-blooded animals. Though harmless strains exist, enteric *E. coli* is commonly implicated as a food and water borne pathogen. Demonstrating three genetically and physiologically distinct AR strategies, the glucose-repressible oxidative pathway (Lin et al., 1995; Lin et al., 1996), glutamate-dependent acid resistance (GDAR; Hersh et al., 1996) and arginine-dependent ASR (Lin et al., 1996), this opportunistic pathogen is arguably the "gold-standard" in the study of enteric ASR. In the past 20 years, *E. coli* has emerged as an infectious agent when associated with minimally processed foods, thus garnering investigations on stress response and pathogenicity when associated with produce and during food processing (Capozzi et al., 2009). Arnold et al. (2001) investigated the impact of the food preservative acetate on expression of ASR genes in enterohemorragic *E. coli* (EHEC) O157:H7 reporting > twofold reduction in more than 60 genes; 48 of which are involved in transcription and translational regulation. By contrast, approximately 25 genes were shown have > twofold increased expression; most noteworthy being five *rpoS* sigma factors which have been identified as central for response to acid and peroxide stress as well as heat and osmotic shock (Cheville et al., 1996; Price et al., 2000; Nyström et al., 2004). Utilization of alternative sigma factors (i.e. Rpo family) have also been shown to regulate expression of virulence factors as well as genes that enhance both pathogenicity and environmental viability such as colonization and biofilm formation for *E. coli* and other enterotypes (Joelsson et al., 2007; Dong and Schellhorn, 2010).

Long-term survival in lethal pH is a hallmark of *E. coli* ASR with two separate mechanisms that involve amino acid antiporters. The first employs glutamate antiporters and several key decarboxylase enzymes, which maintain internal pH (pH_i; Hersh et al., 1996). It has been reported that all of these metabolic enzymes must be synthesized for extensive ASR at pH 2; however, either decarboxylase coupled with the glutamate antiporter will provide long-term resistance at pH > 2.5 (Foster, 1999, 2004). Prolonged survival to lethal pH is also facilitated by alteration in membrane phospholipid composition (Chang and Cronan, 1999) and the subsequent variance in membrane potential ($\Delta\Psi$) and increased pH_i (Richard and Foster; 2004). This durational response to extreme pH also involves synthesis of acid shock proteins that vary temporally during pH exposure (Foster, 2004) and sequestration of DNA promoter regions (Choi et al., 2000). Further, it was recently reported that osmolytes such as NaCl, KCl, proline and sucrose facilitate rapid pH_i homeostasis and therefore hasten the recovery of *E. coli* from acid shock (Kitko et al., 2010). The second long-term survival mechanism is linked to arginine-agmatine amino acid antiporters, which participate in extreme acid response (XAR) and are highly conserved phylogenetically (Iyer et al., 2003). Sun et al. (2011) recently investigated the role of ATP dependent DNA repair machinery in *E. coli* XAR citing marked reduction in cell viability at extreme pH when genes coding for the ATP biosynthesis enzymes (i.e. *purA, purB* and *adk*) were knocked out. These results suggest that ATP dependent metabolic processes such as select DNA repair systems, contribute to long-term survival at extreme pH. The phenomenon of DNA repair during stress induced mutagenesis was reviewed by Foster (2007) who reports that in response to environmental cues such as low pH, many alternative sigma factors (i.e. RpoS) moderate increased expression of error prone polymerases (i.e. Pol IV) and downregulate error-correcting enzymes (i.e. RecO/A) to increase mutations that vary progeny genotypes and facilitate survival.

Recent molecular advancements provide for wholesale screening of microbial transcriptomes enabling nuanced understanding of the diverse factors expressed during enteric ASR and the environmental conditions eliciting this response. Maurer et al. (2005) observed expression variation of approximately 760 genes in response to pH, identifying genes involved in flagellar motility and chemotaxis, oxidative stress and catabolism being variably moderated in response to pH. Further, this study demonstrated the induction of regulons involved in heat shock and oxidative stress response upon exposure to low pH. In an expansive survey of the *E. coli* genome, Kang et al. (2005) suggest a role for the DNA binding protein, FNR, in regulation of nearly 300 genes, including 189 operons, 5% of which are expressed during acid stress under anaerobic conditions. By contrast, Hayes et al. (2006) report on the impact of oxygen which moderates expression of > 260 genes upon exposure to extreme acid with aeration; though core constituents of these enzymes were expressed independent of oxygen. These ASR factors include hydrogenases and enzymes involved in sugar fermentation, antibiotic resistance and cell membrane composition.

In a microarray survey, Tucker et al. (2002) identified 28 novel genes expressed during GDAR representing functional groups involved in metabolism, cell envelope composition and modification, chaperoning and transcriptional regulation. These findings suggest clustering of nine acid-inducible

genes in the *gadA* region involved in GDAR. Though the exact role of each of these genes during ASR is unclear, mutations to these factors reduced acid tolerance. Using molecular techniques and bioinformatics, Hommais et al. (2004) report clustering of *gadE* into chromosomal fitness islands which are highly conserved across multiple *E. coli* strains. The *in silico* analysis suggested preferential denaturing of promoters required for pH homeostasis during acid adaptation. These findings suggest a highly conserved fitness island for ASR in the *E. coli* chromosome. This study is complimented by identifying the role of *gadW* in activation of *gadE* promoters under acidic conditions which resulted in improved colonization capabilities in the infant mouse model (Tucker et al., 2003). Ma et al. (2004) characterized the GDAR pathway and identified the novel regulatory factors, EvgA and YdeO, during *gadA* activation in exponential and stationary ASR. These factors were identified across several ASR pathways in *E. coli* and involve multiple EvgA interactions along inverted repeat promoters in response to acid stress (Masuda and Church, 2003). Additionally, Ma et al. (2003) reported on the regulatory role of acid induced GadE/X and W during ASR as they govern expression of glutamine decarboxylases encoded by *gadA* and *gadB* and the glutamate: γ-aminobutyric acid (GABA) antiporter, GadC. These results suggest that GadE is central to expression of these factors regardless of media or growth conditions, illustrating the complexity of this stress response cascade which involves five regulatory proteins, two sigma factors and multiple feedback loops.

Molecular investigations have resulted in the discovery of additional key factors that directly govern regulatory cascades during *E. coli* ASR. Krin et al. (2010a) identified H-NS as being near the top of the governance hierarchy during glutamate-, arginine- and lysine-dependent acid resistance. Krin et al. (2010b) also reported that the director of these three ASR strategies is moderated by RcsB. This is facilitated by RcsB-P/GadE complexes that interact with at least one other alternative regulator (i.e. H-NS, HdfR, CadC or AdiY) and subsequently regulate overlapping ASR pathways. It was later shown that RcsB is conserved and required for ASR in *E. coli* K12 and O157:H7 and interactions with GadE occur when cells are in logarithmic phase. In stationary phase however, ASR is controlled by RcsB interactions with promoters that are not GadE regulated (Johnson et al., 2011). The elaborate interactions governing enteric ASR were further elucidated by Zwir et al. (2005) in a study characterizing the complex regulatory cascade of promoter interactions for the alternative regulatory proteins, PhoP/PhoQ. Using the Gene Promoter Scan technique, this study identified members of the PhoP regulon and characterized previously unknown regulatory interactions, thus mapping the complex network of the transcriptional regulatory cascade involved in moderating ASR. Very recent work has employed systems biology approaches combining molecular and phenotypic analysis combined with computational modeling to identify the transcriptional networks underlying *E. coli* ASR; this resulted in identification of the outer membrane family of proteins (Omp) as central to robust response to mild and extreme acid in aerobic and anaerobic conditions (Stincone et al., 2011).

Studies to assess the role of fermentation and food production on ASR elicitation have been conducted. In one such study, Polen et al. (2003) employed molecular techniques to investigate the impact of exposing *E. coli* to the short chain fatty acids propionate and acetate. This study reports increased expression of families of genes involved in flagellar function and biosynthesis (*fli*, *fth* and *flg*), antibiotic resistance (*mar*), chemotaxis (*che*) and fimbriae morphology (*fim*) as well as the reduced expression of carbon uptake and utilization genes (*cst*, *gal*, *srl*, *ebg*, *mal* and *mgl*) based on short-term exposure to propionate and/or acetate. A related study investigates the role of fermentation byproducts (formate and acetate) on *E. coli* ASR (Kirkpatrick et al., 2001) citing increased expression of RpoS and periplasmic transporters in the presence of acetate but reduced expression of this regulatory factor when exposed to formate. A recent investigation of *E. coli* K12 and O157:H7 exposure to complex acid cocktails revealed a more robust response on the part of the enterohemorragic strain involving molecular mechanisms not identified in the laboratory strain, thus suggesting improved ASR capability in conditions mimicking those that occur during digestion which subsequently aid in survival in the host GI tract (King et al., 2010). Bergholz et al. (2009) identified more than 330 genes, 104 of which are specific to O157:H7, that are upregulated during cultivation of *E. coli* in apple juice (pH 3.5), including genes involved in osmotic, acid and oxidative stress. Price et al. (2000) identified the diet of cattle and the fermentative conditions therein, which subsequently induce RpoS, as central to shedding of viable, acid-adapted *E. coli* into the environment thus implicating ASR in carriage and cell survival in the bovine GI tract. Later, Price et al. (2004) were the first to report on the utilization of select acid response systems based on the chemical profile of the various acidic environments encountered by the pathogen. They demonstrate the crucial role of RpoS for survival in apple cider (pH 3.5) and in cattle intestines, thus prompting efforts to target this regulatory protein in calves colonized by *E. coli* O157:H7. Additionally, RpoS has been implicated in the release and survival of other acid adapted enteric pathogens into aquatic environments (Merrell and Camilli, 2000) and has been shown to regulate multiple stress response pathways of environmentally derived pathogens (Small et al., 1994; Cheville et al., 1996; Nyström, 2004; Bhagwat et al., 2008). Further, results of a genome-wide expression study suggest that nearly 10% of all *E. coli* genes are under the direct or indirect regulatory control of RpoS which governs all cell physiology under non-optimal growth conditions (Weber et al., 2005).

In an effort to assess the acid adaptive capability of environmentally derived enterohemorragic *E.coli*, Bhagwat et

al. (2005) surveyed 82 isolates from 35 countries to examine their GDAR response through traditional laboratory techniques and molecular characterization. Their findings reveal that nearly 40% of the isolates were defective in inducing GDAR under aerobic conditions while approximately 5% of the isolates were defective under both aerobic and fermentative conditions. However, introduction of *rpoS* on a low-copy-number plasmid resulted in the restoration of GDAR under aerobic growth in nearly 80% of acid sensitive isolates, emphasizing the significance of this regulatory agent in *E. coli* ASR. Further, environments where GDAR positive EHEC isolates originated may represent conditioning reservoirs for acid resistance and pathogenicity.

Salmonella enterica Serovar Typhimurium

Salmonella enterica Serovar Typhimurium is a Gram-negative, neutralophile commonly found in the intestines of animals such as birds, reptiles and humans making it another model organism in the study of enteric ASR. It is also found in contaminated and polluted pond water and within macrophage phagolysozomes; and, it has been shown to colonize a broad varietal of fruits, vegetables, nuts and poultry products resulting in its distinction as the most common foodborne pathogen (Foster and Hall, 1990; Faucher et al., 2006; Hanning et al., 2009). The acid adaptive capabilities of *S. typhimurium* vary according to log or stationary growth and involve synthesis of early and late stage acid shock proteins (ASPs) which moderate cell surface features (i.e. hydrophobicity and outer membrane porins), cytoplasmic pH and macromolecular repair (Foster, 1991; Foster, 1993; Bearson et al., 1998). The various stages of *S. typhimurium* acid resistance are governed by the regulatory proteins RpoS, Fur and PhoP/PhoQ, with each responding to a wide array of environmental cues and regulating separate subsets of ASR (Foster, 1991; Foster, 1993; Bader et al., 2003). Collectively these regulatory factors provide cross protection to numerous forms of environmental stress such as temperature, oxidative damage, salinity and antimicrobial agents (Leyer and Johnson, 1993; Foster and Spector, 1995; Bader et al., 2003; Greenacre et al., 2006) and enhance the expression of multiple virulence and colonization factors (Foster and Hall, 1990; Wilmes-Riesenberg et al., 1997; Dong and Schellhorn, 2010).

S. typhimurium has been investigated with regards to ASR variability based on population growth phase and acid classification. During logarithmic growth, *S. typhimurium* ASR involves induction of the alternative sigma factor σ^s (*rpoS*) which moderate multiple ASPs in response to short chain fatty acids but play a reduced role during inorganic acid exposure (Bearson et al., 1998). During stationary phase, ASR is determined in an RpoS-independent manner, which involves induction of outer membrane porins (Omp) in response to environmental cues such as pH and salinity; *V. cholerae* has been shown to respond in a similar manner (Foster, 1999). As is reported in other enteric pathogens, this pathway involves the regulatory factors PhoP/PhoQ which differentiate between acids (inorganic vs. organic), Mg concentrations and enhance expression of virulence factors when exposed to moderate levels of organic acids and when cultivated in mammalian phagosomes (Bearson et al., 1998; Prost et al., 2007); phenomena conserved in *Salmonella enterica* Serovar Typhi in response to host immunity (Faucher et al., 2006).

Regarding the impact of food processing on elicitation of enteric ASR, adaptation in inorganic acid has been demonstrated as sufficient for *S. typhimurium* and several other *Salmonella* species to remain viable and virulent for up to two months in organically acidic cheeses (Leyer and Johnson, 1992). Further, *S. typhimurium* cultivated in acidic conditions typical of food processing displayed robust acid tolerance at a lower pH (pH 4.5) than was observed for *Listeria monocytogenes* (pH 5.5) or *E. coli* O157:H7 (pH 5.0), suggesting enhanced ASR induction capability, and reduced sensitivity to environmental pH, compared to the common food pathogens *L. monocytogenes* and *E. coli* (Koutsoumanis and Sofos, 2004). Acid habituation of *S. typhimurium* during food production has been shown to provide cross protection to environmental stress such as heat, salt and lactoperoxidases, and confers tolerance to cell wall destroying agents and antibiotics (Leyer and Johnson, 1992). However, short-term exposure of *S. typhimurium* to the food preservative lactic acid was recently shown to reduce resistance to hydrogen peroxide via downregulation of the OxyR regulon which imparts protection to oxidative stress (Greenacre et al., 2006).

The interactions between enteric pathogens and the organisms they colonize may play a significant role in elicitation of ASR and was recently investigated by Bhagwat (2006) in a report on the linkage between plant stress response and acid adaptation in *S. typhimurium*. In this study, *S. typhimurium* cultivated on cut or damaged plant displayed elevated percent population survival upon subsequent exposure to lethal pH in laboratory conditions (pH2, 37°C, 2 h). In addition to the mildly acidic conditions of these wounded fruits and vegetables, apoplastic fluid from decomposing plants colonized by soft rot pathogens may induce acid resistance of *S. typhimurium* (Nachin and Barras, 2000). The antimicrobial peptides that are part of a wounded plants immune response result in upregulation of ASR factors in *S. typhimurium*'s *sap* operon which enhance survival upon subsequent exposure to the mammalian gut (Parra-Lopez et al., 1993; López-Solanilla et al., 1998, 2001). This pathogen has been shown to respond to cues from bacterial community constituents on spoiled plant biomass via acyl-homoserine lactones (AHLs) produced by the bacterial community (Ahmer, 2004), prompting the hypothesis that this inter-cellular communication is upregulating determinants of human pathogen growth on plants during decom-

position (Brandl, 2006). Additionally, these interactions have been shown to enhance population densities of *S. typhimurium* in wounded fruits and vegetables when co-occurring with other bacterial species (Wade and Beuchat, 2003) or proteolytic yeasts (Wade et al., 2003); with the latter being implicated as a transmission vector of enterics into plant tissue (Richards and Beuchat, 2005). It was recently demonstrated that *S. typhimurium* associated with the food vacuoles of the protist *Tetrahymena*, alter the expression of approximately 1000 and 1200 genes compared to water and culture broth respectively, including enhanced expression of alternative electron acceptors and factors involved in anaerobic respiration (Rehfuss et al., 2011). Further, expression of arginine-dependent ASR factors (i.e. AdiA, AdiY) were increased in *S. typhimurium* cells within the food vacuole, suggesting that elicitation of ASR confers resistance to digestion from the protist, provides survival advantage upon egestion and may represent an ASR pre-conditioning reservoir in the contamination cycle of *S. typhimurium*.

The role of plants as disease vectors during outbreaks of Salmonellosis has been realized since the 1990s when the shift in food contamination became clearly linked to irrigation and plant health (Hanning et al., 2009). Since then, there has been renewed interest in the role of aquatic reservoirs and the bacterial load thereof, in conditioning enteric pathogens and displacing them to fruit and vegetable products in various agricultural settings. Freshwater systems have commonly been a source of irrigation (Assadian et al., 1999; Garcia et al., 2001) and assessing density, virulence and elicitation of stress response of *Salmonella* in these aquatic reservoirs has become essential to risk management and infection reduction (Baudart et al., 2000). In one such study employing general media and media selecting for, and differentiating between, enteric pathogens, *Salmonella* was shown to be proportionally dominant in samples taken from beaches and freshwater reservoirs in North-eastern Spain (Polo et al., 1998). It has also been demonstrated, via traditional culturing techniques, that *Salmonella* is a dominant component of the cultivable bacterial community in the water column and in sediment systems impacted by point and non-point sources of pollution in a coastal Mediterranean system; with residence in marine sediments being central to its long-term viability in these aquatic reservoirs (Baudart et al., 2000). Cultivation in environmental conditions has been shown to increase expression of key virulence factors, with variability in pH being cited as the environmental parameter regulating virulence in samples taken from regions along the Rio Grande in southern Texas (Nutt et al., 2003).

Vibrio cholerae

Vibrio cholerae is the highly motile, Gram-negative, curve-shaped bacteria responsible for the gastrointestinal disease Cholera. This microorganism follows the oral route of infection, survives the low pH conditions of the stomach (pH 1–3) and colonizes the small intestine (pH 6–8). Here, it produces the cholera toxin (CT) that results in the loss of copious volumes of fluid from the host, and returns virulent strains to the aquatic environment. The mystery of how this environmentally ubiquitous bacteria, which is categorized as acid-sensitive but is capable of causing gastrointestinal disease in epidemic proportions, has been deciphered by the discovery that *V. cholerae* mounts a robust ASR when pre-conditioned at sub-lethal pH (Merrell and Camilli, 2002). In general, *V. cholerae* ASR involves overlapping responses to inorganic and organic acids, increased expression of > 60 and decrease in > 50 protein species with functions including transcriptional regulation, cell wall maintenance, catabolism, colonization, DNA repair, Na^+ homeostasis and membrane transport; collectively resulting in reduced sensitivity to acidic conditions and a greater capacity to cause harm in the mammalian host (Merrell and Camilli, 1999, 2000, 2002; Peterson, 2002; Reidl and Klose, 2002). Acid adapted *V. cholerae* display increased colonization capabilities and competitive advantage; thus requiring a reduced infective dose compared to non-adapted cells (Merrell and Camilli, 1999). This enhanced pathogenicity results from greater reproductive potential within the host, as opposed to increased expression of virulence factors such as cholera toxin (*ctx*) or toxin co-regulated pili (*tcp*) (Angelichio et al., 2004).

The first groundbreaking studies on *V. cholerae* ASR employed signature tagged mutagenesis (STM) to identify novel factors involved in acid adaptation (Chiang and Mekalanos, 1998; Merrell and Camilli, 1999, 2002). Merrell and Camilli (1999) were the first to identify the ASR factor CadA in *V. cholerae*, which moderates pH_i via lysine consumption in the bacterial cytoplasm. Since this study, the Cad family of proteins has been implicated in moderating pH_i during *S. typhimurium* ASR (Greenacre et al., 2006) and in the aquatic pathogen *V. vulnificus* (Rhee et al., 2004). Screening large pools of virulence attenuated *V. cholerae* resulted in identification of 9 novel ASR factors involved in sodium and potassium homeostasis, cell wall maintenance, DNA repair and protein synthesis (Merrell et al., 2002a). Acid-adapted *V. cholerae* with enhanced expression of these ASR factors were shown to be more competitive mutants and non-adapted cells when challenged to the suckling mouse model.

As is observed among enteric species, *V. cholerae* ASR involves activation of the Rpo family of transcriptional regulators which are central to quorum sensing (Joelsson et al., 2007), expression of virulence factors (Dong and Schellhorn, 2010) and secretion of outer membrane vesicles that improve colonization of the intestinal milieu (Song et al., 2008). Additionally, the Rpo family moderates biofilm formation and expression of the virulence pathogenicity island (VPI) during low and high cell density via the transcriptional regulators, AphA and HapR (Zhu and

Mekalanos, 2003; Kovacikova et al., 2010; Rutherford et al., 2011). These factors have been shown to govern ASR based on environmental conditions, with AphB moderating virulence in response to low pH and anaerobiosis (Kovacikova et al., 2010) and HapR governing protease production, motility, biofilm formation and expression of virulence factors *in vivo* (Zhu and Mekalanos, 2003).

Investigations into the role of the outer membrane porins (Omp) in *V. cholerae* ASR have revealed important interactions between this family of proteins and the intercellular regulatory agent's central to gene expression. Kovacikova and Skorupski (2002) report on the role of the Omp family in moderating the regulatory protein RpoE and enhancing infectivity and colonization capabilities of *V. cholerae* and *E. coli*. Mathur et al. (2007) demonstrate that OmpU acts as an environmental sensor in the signal transduction pathway which regulates the alternative sigma factor, σE and confers resistance to antibiotics such as polymixin B and human gut derived bacteriocidal proteins. Mathur and Waldor (2004) report on OmpU moderated ToxR expression as essential for antimicrobial resistance and the expression of virulence factors. Though not acid shock proteins per se, as Omp expression is not moderated exclusively by acid, this family of porins has been demonstrated as responsive to physiochemical signals such as bile and organic acids and responsible for regulating alternative sigma factors central to *V.cholerae* ASR (Li et al., 2000; Provenzano and Klose, 2000; Merrell and Camilli, 2000; Matson et al., 2007). Recent molecular investigations detecting genes involved in acid stress response (i.e. *ompU* and *toxR*), as well as other virulence and regulatory factors in *V. cholerae*, report a high degree of conservation of these factors among genetically diverse strains of *V. cholerae* isolated from aquatic environments within countries where it is endemic (Zo et al., 2009; Goel and Jiang, 2010). The conservation of such stress factors is imperative for environmental survival which is emphasized by the finding that *V. cholerae* from pH stressed environments is capable of growth at rates comparable to the natural assemblages in alkaline lake water (pH 7.8–9.1; Kirschner et al., 2008).

The Tox family (ToxT, ToxR and ToxS) has been shown central to transcriptional regulation for myriad factors involved in *V. cholerae* ASR. These regulators are moderated by the Omp family of proteins which stimulate transcription in response to environmental cues by binding *toxbox* regions upstream of promoter elements (Withey and DiRita, 2005, 2006). Subsequently, tox proteins govern a cascade of ASR factors involved in virulence (*ctx, tcp*), colonization (*msh*), antimicrobial resistance (*mex*), cellular metabolism (*gal*, *lac, mrs*) and DNA repair (*rec*); they are activated by an array of metabolic intermediates, regulatory messengers and environmental conditions such as temperature, salinity and pH (Merrell and Camilli, 2002; Peterson, 2002; Reidl and Klose, 2002; Tischler and Camilli, 2004; Abuaita and Withey, 2009).

Several studies have aimed to characterize the expression profile of ASR genes as *V. cholerae* transitions between the host GI tract and aquatic reservoirs. *In vivo* results suggest that *V. cholerae* that has passed through the host GI tract demonstrates enhanced virulence upon re-introduction to the host (Merrell and Camilli, 2002; Reidl and Klose, 2002). This increased infectivity is facilitated by reduced expression of chemotaxis factors such as CheW-1; a phenotype maintained in the aquatic environment and only elicited post-infection (Butler et al., 2006; Merrell et al., 2001). Schild et al. (2007) investigated *V. cholerae* in transit through the host and report increased expression of six unique genes in the latter stage of infection which play limited roles during infection, are critical for survival upon introduction to the aquatic reservoir and are expressed prior to exiting the host. In this study, viability of *V. cholerae* expressing late stage genes after transfer to various environments (i.e. rice water stools, pond water and nutrient broth) was monitored and resulted in identification of multiple factors functioning in transcriptional regulation, substrate level phosphorylation and nutrient transport; all of which improve the fitness of *V. cholerae* upon introduction to environmental reservoirs.

Investigations of *V. cholerae* transitioning between the aquatic environment and the mammalian host have revealed the role of quorum sensing induced biofilm formation as implicit in surviving the host defenses and inducing infection upon entering the GI tract (Kamruzzaman et al., 2010). Biofilm formation has been shown to involve activation of the regulatory protein, HapR upon exposure to bile acid and is central for *V. cholerae* survival in the aquatic environment (Zhu and Mekalanos, 2003). Further, Faruque et al. (2006) report that biofilm aggregated *V. cholerae* display enhanced viability in the environment after passage through the mammalian host, suggesting a role of ASR for survival in aquatic reservoirs. This study is complimented by the finding that biofilm formation in the environment results in a hyperinfective phenotype upon introduction to the host GI tract (Tamayo et al., 2010). Beyhan et al. (2006) report increased expression of genes involved in biofilm formation (*vps*), extracellular protein secretion (*eps*) and mannose-sensitive hemagglutinin (*msh*) as well as reduction of flagellar gene expression in response to variability in environmental concentrations of the second messenger 3′, 5′-cyclic diguanylic acid (c-di-GMP) which aids in the integration of environmental stimuli and effects cell physiology for many microorganisms.

The genetic predisposition of *V. cholerae* to transition between environmental and pathogenic lifestyles was recognized upon sequencing its two chromosomes (Heidelberg et al., 2000). Results indicate that the small chromosome has a greater proportion of hypothetical genes (59%) than the large chromosome (42%) and has more genes originating in plasmids and other γ-Proteobacteria. In addition to providing evidence that the small chromosome originated as a megaplasmid which was acquired by an ancestral *V. cholerae*, this study provides a foundation for deciphering how these

environmental bacteria emerged as enteric pathogens.

Additional factors contributing to successful transition at the environment-host interface were investigated by Vezzulli et al. (2008) who identified dual role colonization factors (DRCF) such as the cholera toxin (*ctx*), toxin co-regulated pili (*tcp*), alternative sigma factor (*rpo*) and quorum sensing genes (*lux*) that are necessary for successful colonization of both the gastrointestinal epithelia and the chitinous exoskeleton of copepods in the aquatic environment. Further, Kirn et al. (2005) recently recognized a gene (*gpbA*) involved in chitin colonization in the aquatic environment that is also necessary for successful colonization of the epithelia in the mammalian host. Exoskeleton associations have been shown to be central for environmental survival, biofilm formation and intra-species gene exchange, as well as pathogenicity and pre-conditioning of ASR (Nalin, 1976; Nalin et al., 1979; Blokesch and Schoolnik, 2007; Pruzzo et al., 2008). The significance of these associations is further illustrated by the observation that Cholera epidemics are seasonal and strongly linked to algal blooms due to *V. cholerae* associations with phytoplankton and the chitinous exoskeleton of copepods within aquatic systems (Colwell, 1996; Hsieh et al., 2007).

Conclusions

Since the discovery of acid stress response and its characterization in *E. coli* and *S. typhimurium*, there has been a broad array of highly conserved acid stress response factors observed in Gram-negative and Gram-positive pathogens with new model organisms being reported. The use of large-scale, signature-tagged mutagenesis (STM), microarrays and metatranscriptomic analysis have led to rapid discovery of novel factors for acid resistance and provide insight into the mechanisms of this bacterial stress response. However, the role of various environmental reservoirs on acid adaptation and elicitation of the ASR in the context of pathogen ecology remains largely unexplored. Studies have focused upon bacterial pathogen response either within the mammalian host, in laboratory conditions mimicking the host or in industrially relevant food products during food preservation and processing. While understanding of ASR is immensely valuable and central to the control of microbial pathogens, gaining a deeper understanding of environmental reservoirs that condition for acid adaptation and the elicitation of stress response pathways will enable disruption of the fecal-environmental-oral cycling of these enteric pathogens. Such insights would aid in developing reliable models of pathogen distribution that effectively incorporate the functional niche of microorganisms and the environmental 'hot spots' of pathogenicity. Finally, characterizing these stress response pathways in environmental and clinical strains of enteric bacteria will provide a broader and more complete context of pathogenicity which could facilitate improved methods for bacterial control during food and water preparation and potentially aid in disease mitigation.

References

Abuaita B H, Withey J H (2009). Bicarbonate induces *Vibrio cholerae* virulence gene expression by enhancing ToxT activity. Infect Immun, 77(9): 4111–4120

Ahmer B M M (2004). Cell-to-cell signaling in *Escherichia coli* and *Salmonella enterica*. Mol Microbiol, 52(4): 933–945

Angelichio M J, Merrell D S, Camilli A (2004). Spatiotemporal analysis of acid adaptation-mediated *Vibrio cholerae* hyperinfectivity. Infect Immun, 72(4): 2405–2407

Arnold C N, McElhanon J, Lee A, Leonhart R, Siegele D A (2001). Global analysis of *Escherichia coli* gene expression during the acetate-induced acid tolerance response. J Bacteriol, 183(7): 2178–2186

Assadian N W, Fenn L B, Flores-Ortiz M A, Ali A S (1999). Spatial variability of solutes in a pecan orchard surface-irrigated with untreated effluents in the upper Rio Grande River basin. Agric Water Manag, 42(2): 143–156

Bader M W, Navarre W W, Shiau W, Nikaido H, Frye J G, McClelland M, Fang F C, Miller S I (2003). Regulation of *Salmonella typhimurium* virulence gene expression by cationic antimicrobial peptides. Mol Microbiol, 50(1): 219–230

Baker-Austin C, Dopson M (2007). Life in acid: pH homeostasis in acidophiles. Trends Microbiol, 15(4): 165–171

Baudart J, Grabulos J, Barusseau J P, Lebaron P (2000). *Salmonella* spp. and fecal coliform loads in coastal waters from a point vs. nonpoint source of pollution. J Environ Qual, 29(1): 241–250

Beales N (2004). Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: A review. Compr Rev Food Sci F, 3(1): 1–20

Bearson B L, Wilson L, Foster J W (1998). A low pH-Inducible, PhoPQ-Dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress (vol 180, pg 2409, 1998). J Bacteriol, 180(14): 3734–3734

Bergholz T M, Vanaja S K, Whittam T S (2009). Gene expression induced in *Escherichia coli* O157:H7 upon exposure to model apple juice. Appl Environ Microbiol, 75(11): 3542–3553

Beyhan S, Tischler A D, Camilli A, Yildiz F H (2006). Transcriptome and phenotypic responses of *Vibrio cholerae* to increased cyclic di-GMP level. J Bacteriol, 188(10): 3600–3613

Bhagwat A A (2006). Microbiological Safety of Fresh-cut Produce: Where Are We Now? American Society for Microbiology Press, 121–165

Bhagwat A A, Bhagwat M (2008). Methods and tools for comparative genomics of foodborne pathogens. Foodborne Pathog Dis, 5(4): 487–497

Bhagwat A A, Chan L, Han R, Tan J, Kothary M, Jean-Gilles J, Tall B D (2005). Characterization of enterohemorrhagic *Escherichia coli* strains based on acid resistance phenotypes. Infect Immun, 73(8): 4993–5003

Blokesch M, Schoolnik G K (2007). Serogroup conversion of *Vibrio cholerae* in aquatic reservoirs. PLoS Pathog, 3(6): e81

Brandl M T (2006). Fitness of human enteric pathogens on plants and implications for food safety. Annu Rev Phytopathol, 44(1): 367–392

Butler S M, Nelson E J, Chowdhury N, Faruque S M, Calderwood S B, Camilli A (2006). Cholera stool bacteria repress chemotaxis to increase infectivity. Mol Microbiol, 60(2): 417–426

Capozzi V, Fiocco D, Amodio M L, Gallone A, Spano G (2009). Bacterial stressors in minimally processed food. Int J Mol Sci, 10(7): 3076–3105

Chang Y Y, Cronan J E Jr (1999). Membrane cyclopropane fatty acid content is a major factor in acid resistance of *Escherichia coli*. Mol Microbiol, 33(2): 249–259

Cheville A M, Arnold K W, Buchrieser C, Cheng C M, Kaspar C W (1996). rpoS regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. Appl Environ Microbiol, 62(5): 1822–1824

Chiang S L, Mekalanos J J (1998). Use of signature-tagged transposon mutagenesis to identify *Vibrio cholerae* genes critical for colonization. Mol Microbiol, 27(4): 797–805

Choi S H, Baumler D J, Kaspar C W (2000). Contribution of dps to acid stress tolerance and oxidative stress tolerance in *Escherichia coli* O157:H7. Appl Environ Microbiol, 66(9): 3911–3916

Ciaramella M, Napoli A, Rossi M (2005). Another extreme genome: how to live at pH 0. Trends Microbiol, 13(2): 49–51

Colwell R R (1996). Global climate and infectious disease: the cholera paradigm. Science, 274(5295): 2025–2031

Cotter P D, Hill C (2003). Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiol Mol Biol Rev, 67(3): 429–453

De Angelis M, Gobbetti M (2004). Environmental stress responses in Lactobacillus: a review. Proteomics, 4(1): 106–122

Dong T, Schellhorn H E (2010). Role of RpoS in virulence of pathogens. Infect Immun, 78(3): 887–897

Doyle M P, Erickson M C (2008). Summer meeting 2007—the problems with fresh produce: an overview. J Appl Microbiol, 105(2): 317–330

Faruque S M, Biswas K, Udden S M N, Ahmad Q S, Sack D A, Nair G B, Mekalanos J J (2006). Transmissibility of cholera: *in vivo*-formed biofilms and their relationship to infectivity and persistence in the environment. Proc Natl Acad Sci USA, 103(16): 6350–6355

Faucher S P, Porwollik S, Dozois C M, McClelland M, Daigle F (2006). Transcriptome of *Salmonella enterica* Serovar Typhi within macrophages revealed through the selective capture of transcribed sequences. Proc Natl Acad Sci USA, 103(6): 1906–1911

Flahaut S, Hartke A, Giard J C, Benachour A, Boutibonnes P, Auffray Y (1996). Relationship between stress response toward bile salts, acid and heat treatment in *Enterococcus faecalis*. FEMS Microbiol Lett, 138(1): 49–54

Foster J W (1991). *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. J Bacteriol, 173(21): 6896–6902

Foster J W (1993). The acid tolerance response of *Salmonella typhimurium* involves transient synthesis of key acid shock proteins. J Bacteriol, 175(7): 1981–1987

Foster J W (1999). When protons attack: microbial strategies of acid adaptation. Curr Opin Microbiol, 2(2): 170–174

Foster J W (2004). Escherichia coli acid resistance: tales of an amateur acidophile. Nat Rev Microbiol, 2(11): 898–907

Foster J W, Hall H K (1990). Adaptive acidification tolerance response of *Salmonella typhimurium*. J Bacteriol, 172(2): 771–778

Foster J W, Spector M P (1995). How *Salmonella* survive against the odds. Annu Rev Microbiol, 49(1): 145–174

Foster P L (2007). Stress-induced mutagenesis in bacteria. Crit Rev Biochem Mol Biol, 42(5): 373–397

Frees D, Varmanen P, Ingmer H (2001). Inactivation of a gene that is highly conserved in Gram-positive bacteria stimulates degradation of non-native proteins and concomitantly increases stress tolerance in *Lactococcus lactis*. Mol Microbiol, 41(1): 93–103

Frees D, Vogensen F K, Ingmer H (2003). Identification of proteins induced at low pH in *Lactococcus lactis*. Int J Food Microbiol, 87(3): 293–300

Garcia S S, Ake C, Clement B, Huebner H J, Donnelly K C, Shalat S L (2001). Initial results of environmental monitoring in the Texas Rio Grande Valley. Environ Int, 26(7–8): 465–474

Goel A K, Jiang S C (2010). Genetic determinants of virulence, antibiogram and altered biotype among the *Vibrio cholerae* O1 isolates from different cholera outbreaks in India. Infect Genet Evol, 10(6): 814–819

Goodson M, Rowbury R J (1989). Resistance of acid-habituated *Escherichia coli* to organic acids and its medical and applied significance. Lett Appl Microbiol, 8(6): 211–214.

Greenacre E J, Lucchini S, Hinton J C D, Brocklehurst T F (2006). The lactic acid-induced acid tolerance response in *Salmonella enterica* Serovar Typhimurium induces sensitivity to hydrogen peroxide. Appl Environ Microbiol, 72(8): 5623–5625

Hanning I B, Nutt J D, Ricke S C (2009). Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathog Dis, 6(6): 635–648

Hayes E T, Wilks J C, Sanfilippo P, Yohannes E, Tate D P, Jones B D, Radmacher M D, BonDurant S S, Slonczewski J L (2006). Oxygen limitation modulates pH regulation of catabolism and hydrogenases, multidrug transporters, and envelope composition in *Escherichia coli* K-12. BMC Microbiol, 6(1): 89

Heidelberg J F, Eisen J A, Nelson W C, Clayton R A, Gwinn M L, Dodson R J, Haft D H, Hickey E K, Peterson J D, Umayam L, Gill S R, Nelson K E, Read T D, Tettelin H, Richardson D, Ermolaeva M D, Vamathevan J, Bass S, Qin H, Dragoi I, Sellers P, McDonald L, Utterback T, Fleishmann R D, Nierman W C, White O, Salzberg S L, Smith H O, Colwell R R, Mekalanos J J, Venter J C, Fraser C M (2000). DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. Nature, 406(6795): 477–483

Hersh B M, Farooq F T, Barstad D N, Blankenhorn D L, Slonczewski J L (1996). A glutamate-dependent acid resistance gene in *Escherichia coli*. J Bacteriol, 178(13): 3978–3981

Hommais F, Krin E, Coppée J Y, Lacroix C, Yeramian E, Danchin A, Bertin P (2004). GadE (YhiE): a novel activator involved in the response to acid environment in *Escherichia coli*. Microbiology, 150 (1): 61–72

Hsieh J L, Fries J S, Noble R T (2007). Vibrio and phytoplankton dynamics during the summer of 2004 in a eutrophying estuary. Ecol Appl, 17(5): S102–S109

Iyer R, Williams C, Miller C (2003). Arginine-agmatine antiporter in extreme acid resistance in *Escherichia coli*. J Bacteriol, 185(22): 6556–6561

Johnson M D, Burton N A, Gutierrez B, Painter K, Lund P A (2011). RcsB is required for inducible acid resistance in *E. coli* and acts at gadE dependent and independent promoters, J Bacteriol online

Jiang S C, Louis V, Choopun N, Sharma A, Huq A, Colwell R R (2000).

Genetic diversity of *Vibrio cholerae* in Chesapeake Bay determined by amplified fragment length polymorphism fingerprinting. Appl Environ Microbiol, 66(1): 140–147

Joelsson A, Kan B, Zhu J (2007). Quorum sensing enhances the stress response in *Vibrio cholerae*. Appl Environ Microbiol, 73(11): 3742–3746

Kamruzzaman M, Udden S M N, Cameron D E, Calderwood S B, Nair G B, Mekalanos J J, Faruque S M (2010). Quorum-regulated biofilms enhance the development of conditionally viable, environmental *Vibrio cholerae*. Proc Natl Acad Sci USA, 107(4): 1588–1593

Kang Y S, Weber K D, Qiu Y, Kiley P J, Blattner F R (2005). Genome-wide expression analysis indicates that FNR of *Escherichia coli* K-12 regulates a large number of genes of unknown function. J Bacteriol, 187(3): 1135–1160

King T, Lucchini S, Hinton J C D, Gobius K (2010). Transcriptomic analysis of *Escherichia coli* O157:H7 and K-12 cultures exposed to inorganic and organic acids in stationary phase reveals acidulant- and strain-specific acid tolerance responses App. Environ Microbiol, 76 (19): 6514–6528

Kirkpatrick C, Maurer L M, Oyelakin N E, Yoncheva Y N, Maurer R, Slonczewski J L (2001). Acetate and formate stress: opposite responses in the proteome of *Escherichia coli*. J Bacteriol, 183(21): 6466–6477

Kirn T J, Jude B A, Taylor R K (2005). A colonization factor links Vibrio cholerae environmental survival and human infection. Nature, 438 (7069): 863–866

Kirschner A K T, Schlesinger J, Farnleitner A H, Hornek R, Süss B, Golda B, Herzig A, Reitner B (2008). Rapid growth of planktonic *Vibrio cholerae* non-O1/non-O139 strains in a large alkaline lake in Austria: dependence on temperature and dissolved organic carbon quality. Appl Environ Microbiol, 74(7): 2004–2015

Kitko R D, Wilks J C, Garduque G M, Slonczewski J L (2010). Osmolytes contribute to pH homeostasis of *Escherichia coli*. PLoS ONE, 5(4): e10078

Koutsoumanis K P, Kendall P A, Sofos J N (2003). Effect of food processing-related stresses on acid tolerance of *Listeria monocytogenes*. Appl Environ Microbiol, 69(12): 7514–7516

Koutsoumanis K P, Sofos J N (2004). Comparative acid stress response of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella Typhimurium* after habituation at different pH conditions. Lett Appl Microbiol, 38(4): 321–326

Kovacikova G, Lin W, Skorupski K (2010). The LysR-type virulence activator AphB regulates the expression of genes in *Vibrio cholerae* in response to low pH and anaerobiosis. J Bacteriol, 192(16): 4181–4191

Kovacikova G, Skorupski K (2002). Binding site requirements of the virulence gene regulator AphB: differential affinities for the *Vibrio cholerae* classical and El Tor tcpPH promoters. Mol Microbiol, 44 (2): 533–547

Krin E, Danchin A, Soutourina O (2010a). Decrypting the H-NS-dependent regulatory cascade of acid stress resistance in *Escherichia coli*. BMC Microbiol, 10(1): 273

Krin E, Danchin A, Soutourina O (2010b). RcsB plays a central role in H-NS-dependent regulation of motility and acid stress resistance in *Escherichia coli*. Res Microbiol, 161(5): 363–371

Leyer G J, Johnson E A (1992). Acid adaptation promotes survival of *Salmonella* spp. in cheese. Appl Environ Microbiol, 58(6): 2075–2080

Leyer G J, Johnson E A (1993). Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. Appl Environ Microbiol, 59(6): 1842–1847

Li C C, Crawford J A, DiRita V J, Kaper J B (2000). Molecular cloning and transcriptional regulation of ompT, a ToxR-repressed gene in *Vibrio cholerae*. Mol Microbiol, 35(1): 189–203

Lin J S, Lee I S, Frey J, Slonczewski J L, Foster J W (1995). Comparative analysis of extreme acid survival in *Salmonella typhimurium*, *Shigella flexneri*, and *Escherichia coli*. J Bacteriol, 177(14): 4097–4104

Lin J S, Smith M P, Chapin K C, Baik H S, Bennett G N, Foster J W (1996). Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. Appl Environ Microbiol, 62(9): 3094–3100

López-Solanilla E, García-Olmedo F, Rodríguez-Palenzuela P (1998). Inactivation of the sapA to sapF locus of *Erwinia chrysanthemi* reveals common features in plant and animal bacterial pathogenesis. Plant Cell, 10(6): 917–924

López-Solanilla E, Llama-Palacios A, Collmer A, García-Olmedo F, Rodríguez-Palenzuela P (2001). Relative effects on virulence of mutations in the sap, pel, and hrp loci of *Erwinia chrysanthemi*. Mol Plant Microbe Interact, 14(3): 386–393

Ma Z, Gong S M, Richard H, Tucker D L, Conway T, Foster J W (2003). GadE (YhiE) activates glutamate decarboxylase-dependent acid resistance in *Escherichia coli* K-12. Mol Microbiol, 49(5): 1309–1320

Ma Z, Masuda N, Foster J W (2004). Characterization of EvgAS-YdeO-GadE branched regulatory circuit governing glutamate-dependent acid resistance in *Escherichia coli*. J Bacteriol, 186(21): 7378–7389

Masuda N, Church G M (2003). Regulatory network of acid resistance genes in *Escherichia coli*. Mol Microbiol, 48(3): 699–712

Mathur J, Davis B M, Waldor M K (2007). Antimicrobial peptides activate the *Vibrio cholerae* sigmaE regulon through an OmpU-dependent signalling pathway. Mol Microbiol, 63(3): 848–858

Mathur J, Waldor M K (2004). The *Vibrio cholerae* ToxR-regulated porin OmpU confers resistance to antimicrobial peptides. Infect Immun, 72(6): 3577–3583

Matson J S, Withey J H, DiRita V J (2007). Regulatory networks controlling *Vibrio cholerae* virulence gene expression. Infect Immun, 75(12): 5542–5549

Maurer L M, Yohannes E, Bondurant S S, Radmacher M, Slonczewski J L (2005). pH regulates genes for flagellar motility, catabolism, and oxidative stress in *Escherichia coli* K-12. J Bacteriol, 187(1): 304–319

Merrell D S, Bailey C, Kaper J B, Camilli A (2001). The ToxR-mediated organic acid tolerance response of *Vibrio cholerae* requires OmpU. J Bacteriol, 183(9): 2746–2754

Merrell D S, Butler S M, Qadri F, Dolganov N A, Alam A, Cohen M B, Calderwood S B, Schoolnik G K, Camilli A (2002a). Host-induced epidemic spread of the cholera bacterium. Nature, 417(6889): 642–645

Merrell D S, Camilli A (1999). The cadA gene of *Vibrio cholerae* is induced during infection and plays a role in acid tolerance. Mol Microbiol, 34(4): 836–849

Merrell D S, Camilli A (2000). Regulation of *Vibrio cholerae* genes required for acid tolerance by a member of the "ToxR-like" family of transcriptional regulators. J Bacteriol, 182(19): 5342–5350

Merrell D S, Camilli A (2002). Acid tolerance of gastrointestinal pathogens. Curr Opin Microbiol, 5(1): 51–55

Merrell D S, Goodrich M L, Otto G, Tompkins L S, Falkow S (2003). pH-regulated gene expression of the gastric pathogen *Helicobacter pylori*. Infect Immun, 71(6): 3529–3539

Merrell D S, Hava D L, Camilli A (2002b). Identification of novel factors involved in colonization and acid tolerance of *Vibrio cholerae*. Mol Microbiol, 43(6): 1471–1491

Mols M, van Kranenburg R, Tempelaars M H, van Schaik W, Moezelaar R, Abee T (2010). Comparative analysis of transcriptional and physiological responses of *Bacillus cereus* to organic and inorganic acid shocks. Int J Food Microbiol, 137(1): 13–21

Nachin L, Barras F (2000). External pH: an environmental signal that helps to rationalize pel gene duplication in *Erwinia chrysanthemi*. Mol Plant Microbe Interact, 13(8): 882–886

Nalin D R (1976). Cholera, copepods, and chitinase. Lancet, 2(7992): 958–960

Nalin D R, Daya V, Reid A, Levine M M, Cisneros L (1979). Adsorption and growth of *Vibrio cholerae* on chitin. Infect Immun, 25(2): 768–770

Nutt J D, Pillai S D, Woodward C L, Sternes K L, Zabala-Díaz I B, Kwon Y M, Ricke S C (2003). Use of a *Salmonella typhimurium* hilA fusion strain to assess effects of environmental fresh water sources on virulence gene expression. Water Res, 37(14): 3319–3326

Nyström T (2004). Stationary-phase physiology. Annu Rev Microbiol, 58(1): 161–181

Padan E, Bibi E, Ito M, Krulwich T A (2005). Alkaline pH homeostasis in bacteria: new insights. Biochim Biophys Acta, 1717(2): 67–88

Parra-Lopez C, Baer M T, Groisman E A (1993). Molecular genetic analysis of a locus required for resistance to antimicrobial peptides in *Salmonella typhimurium*. EMBO J, 12(11): 4053–4062

Peterson K M (2002). Expression of Vibrio cholerae virulence genes in response to environmental signals. Curr Issues Intest Microbiol, 3(2): 29–38

Polen T, Rittmann D, Wendisch V F, Sahm H (2003). DNA microarray analyses of the long-term adaptive response of *Escherichia coli* to acetate and propionate. Appl Environ Microbiol, 69(3): 1759–1774

Polo F, Figueras M J, Inza I, Sala J, Fleisher J M, Guarro J (1998). Relationship between presence of *Salmonella* and indicators of faecal pollution in aquatic habitats. FEMS Microbiol Lett, 160 (2): 253–256

Price S B, Cheng C M, Kaspar C W, Wright J C, DeGraves F J, Penfound T A, Castanie-Cornet M P, Foster J W (2000). Role of rpoS in acid resistance and fecal shedding of *Escherichia coli* O157:H7. Appl Environ Microbiol, 66(2): 632–637

Price S B, Wright J C, DeGraves F J, Castanie-Comet M P, Foster J W (2004). Acid resistance systems required for survival of *Escherichia coli* O157: H7 in the bovine gastrointestinal tract and in apple cider are different. Appl Environ Microbiol, 70(8): 4792–4799

Prost L R, Daley M E, Le Sage V, Bader M W, Le Moual H, Klevit R E, Miller S I (2007). Activation of the bacterial sensor kinase PhoQ by acidic pH. Mol Cell, 26(2): 165–174

Provenzano D, Klose K E (2000). Altered expression of the ToxR-regulated porins OmpU and OmpT diminishes Vibrio cholerae bile resistance, virulence factor expression, and intestinal colonization. Proc Natl Acad Sci USA, 97(18): 10220–10224

Pruzzo C, Vezzulli L, Colwell R R (2008). Global impact of *Vibrio cholerae* interactions with chitin. Environ Microbiol, 10(6): 1400–1410

Rallu F, Gruss A, Ehrlich S D, Maguin E (2000). Acid- and multistress-resistant mutants of *Lactococcus lactis*: identification of intracellular stress signals. Mol Microbiol, 35(3): 517–528

Rehfuss M Y M, Parker C T, Brandl M T (2011). *Salmonella* transcriptional signature in *Tetrahymena phagosomes* and role of acid tolerance in passage through the protist. ISME J, 5(2): 262–273

Reidl J, Klose K E (2002). *Vibrio cholerae* and cholera: out of the water and into the host. FEMS Microbiol Rev, 26(2): 125–139

Rhee J E, Ju H M, Park U, Park B C, Choi S H (2004). Identification of the *Vibrio vulnificus cadC* and Evaluation of Its Role in Acid Tolerance. J Microbiol Biotechnol, 14(5): 1093–1098

Richard H, Foster J W (2004). *Escherichia coli* glutamate- and arginine-dependent acid resistance systems increase internal pH and reverse transmembrane potential. J Bacteriol, 186(18): 6032–6041

Richards G M, Beuchat L R (2005). Infection of cantaloupe rind with *Cladosporium cladosporioides* and *Penicillium expansum*, and associated migration of *Salmonella poona* into edible tissues. Int J Food Microbiol, 103(1): 1–10

Rowbury R J (1995). An assessment of environmental factors influencing acid tolerance and sensitivity in *Escherichia coli*, *Salmonella* spp. and other enterobacteria. Lett Appl Microbiol, 20 (6): 333–337

Rutherford S T, van Kessel J C, Shao Y, Bassler B L (2011). AphA and LuxR/HapR reciprocally control quorum sensing in vibrios. Genes Dev, 25(4): 397–408

Schild S, Tamayo R, Nelson E J, Qadri F, Calderwood S B, Camilli A (2007). Genes induced late in infection increase fitness of *Vibrio cholerae* after release into the environment. Cell Host Microbe, 2(4): 264–277

Small P, Blankenhorn D, Welty D, Zinser E, Slonczewski J L (1994). Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of rpoS and growth pH. J Bacteriol, 176(6): 1729–1737

Song T, Mika F, Lindmark B, Liu Z, Schild S, Bishop A, Zhu J, Camilli A, Johansson J, Vogel J, Wai S N (2008). A new *Vibrio cholerae* sRNA modulates colonization and affects release of outer membrane vesicles. Mol Microbiol, 70(1): 100–111

Stincone A, Rahman A S, Antczak P, Henderson I, Cole J, Johnson M D, Lund P (2011). A systems biology approach sheds new light on *Escherichia coli* acid resistance. Nucl. Acids Res. 39(17): 7512–752

Sun Y R, Fukamachi T, Saito H, Kobayashi H (2011). ATP requirement for acidic resistance in *Escherichia coli*. J Bacteriol, 193(12): 3072–3077

Tamayo R, Patimalla B, Camilli A (2010). Growth in a biofilm induces a hyperinfectious phenotype in *Vibrio cholerae*. Infect Immun, 78(8): 3560–3569

Tischler A D, Camilli A (2004). Cyclic diguanylate (c-di-GMP) regulates *Vibrio cholerae* biofilm formation. Mol Microbiol, 53(3): 857–869

Tischler A D, Camilli A (2005). Cyclic diguanylate regulates *Vibrio cholerae* virulence gene expression. Infect Immun, 73(9): 5873–5882

Tucker D L, Tucker N, Conway T (2002). Gene expression profiling of the pH response in *Escherichia coli*. J Bacteriol, 184(23): 6551–6558

Tucker D L, Tucker N, Ma Z, Foster J W, Miranda R L, Cohen P S, Conway T (2003). Genes of the GadX-GadW regulon in *Escherichia coli*. J Bacteriol, 185(10): 3190–3201

van de Guchte M, Serror P, Chervaux C, Smokvina T, Ehrlich S D,

Maguin E (2002). Stress responses in lactic acid bacteria. Antonie van Leeuwenhoek, 82(1–4): 187–216

Vezzulli L, Guzmán C A, Colwell R R, Pruzzo C (2008). Dual role colonization factors connecting *Vibrio cholerae*'s lifestyles in human and aquatic environments open new perspectives for combating infectious diseases. Curr Opin Biotechnol, 19(3): 254–259

Wade W N, Beuchat L R (2003). Metabiosis of proteolytic moulds and *Salmonella* in raw, ripe tomatoes. J Appl Microbiol, 95(3): 437–450

Wade W N, Vasdinnyei R, Deak T, Beuchat L R (2003). Proteolytic yeasts isolated from raw, ripe tomatoes and metabiotic association of *Geotrichum candidum* with *Salmonella*. Int J Food Microbiol, 86(1–2): 101–111

Weber H, Polen T, Heuveling J, Wendisch V F, Hengge R (2005). Genome-wide analysis of the general stress response network in Escherichia coli: sigmaS-dependent genes, promoters, and σ factor selectivity. J Bacteriol, 187(5): 1591–1603

Wilmes-Riesenberg M R, Foster J W, Curtiss R 3rd (1997). An altered rpoS allele contributes to the avirulence of *Salmonella typhimurium* LT2. Infect Immun, 65(1): 203–210

Withey J H, DiRita V J (2005). Activation of both acfA and acfD transcription by *Vibrio cholerae* ToxT requires binding to two centrally located DNA sites in an inverted repeat conformation. Mol Microbiol, 56(4): 1062–1077

Withey J H, DiRita V J (2006). The toxbox: specific DNA sequence requirements for activation of *Vibrio cholerae* virulence genes by ToxT. Mol Microbiol, 59(6): 1779–1789

Xie Y, Chou L S, Cutler A, Weimer B (2004). DNA Macroarray profiling of *Lactococcus lactis* subsp. lactis IL1403 gene expression during environmental stresses. Appl Environ Microbiol, 70(11): 6738–6747

Zhu J, Mekalanos J J (2003). Quorum sensing-dependent biofilms enhance colonization in *Vibrio cholerae*. Dev Cell, 5(4): 647–656

Zo Y G, Chokesajjawatee N, Grim C, Arakawa E, Watanabe H, Colwell R R (2009). Diversity and seasonality of bioluminescent *Vibrio cholerae* populations in Chesapeake Bay. Appl Environ Microbiol, 75(1): 135–146

Zwir I, Shin D, Kato A, Nishino K, Latifi T, Solomon F, Hare J M, Huang H, Groisman E A (2005). Dissecting the PhoP regulatory network of *Escherichia coli* and *Salmonella enterica*. Proc Natl Acad Sci USA, 102(8): 2862–2867