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RESPONSE OF *LISTERIA MONOCYTOGENES* TO BILE SALTS

By

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RESPONSE OF *LISTERIA MONOCYTOGENES* TO BILE SALT STRESS

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Listeria monocytogenes is a food-borne pathogen responsible for the disease listeriosis. The infectious process depends upon survival in high bile salt conditions encountered throughout the gastrointestinal tract, including the gallbladder. However, it is not clear how bile salt resistance mechanisms are induced, especially under physiologically relevant conditions. This study sought to determine how *L. monocytogenes* responds to bile salts under anaerobic conditions. The study found resistance to be strain specific and not dependent upon virulence. Changes in the expressed proteome were analyzed using multidimensional protein identification technology coupled with electrospray ionization tandem mass spectrometry. A general response among virulent and avirulent strains found significant alterations in intensity of cell wall associated proteins, DNA repair proteins, protein folding chaperones and oxidative response proteins. Strain viability was correlated with an initial osmotic stress response followed by strain specific proteins associated with biofilm formation in EGDe and a transmembrane efflux pump in F2365.

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CHAPTER I

LITERATURE REVIEW

1.1   Introduction

Listeria monocytogenes is a gram positive, facultative anaerobic bacterium that is morphologically bacillus. L. monocytogenes was originally isolated in 1924 (Murray E.G.D., R.A. Webb, and M.B.R. Swann 1926) and was later characterized as being the causative agent of food-borne disease listeriosis (Gellin and Broome 1989). L. monocytogenes is responsible for ~28% of all food-related deaths in the United States (Lynch et al. 2006, Mead et al. 1999). The immunocompromised, elderly, and pregnant women are most susceptible to infections with L. monocytogenes. Manifestations of listeriosis include: meningitis or meningoencephalitis and septicemia in immunocompromised adults and infection of the fetus in pregnant woman (Murray E. G. 1955). Listeriosis was documented as causing epidemic outbreaks in 1979 (Gellin and Broome 1989). This fueled research in the areas of transmission, prevention, and physiological characterization of the bacterium. It is thus necessary to characterize how L. monocytogenes colonizes humans and is transmitted.

Listeriosis is commonly contracted through consumption of contaminated food products such as soft cheeses, deli meats, and frankfurters (Swaminathan and Gerner-Smidt 2007). For successful transmission, L. monocytogenes must survive the harsh conditions of the stomach and gastrointestinal tract, including the acidic conditions of the stomach, the high osmolarity found in the small intestine as well as bile salts found in

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both the small intestine and in the gallbladder (Davis et al. 1996, Hardy et al. 2004, O'Driscoll et al. 1996). *L. monocytogenes* has been characterized as being able to grow in environments with pH ranging from 4.7-9.2 (Petran 1989). Furthermore, *L. monocytogenes* has the ability to live intracellulary by entering through enterocytes in the microvilli of intestinal mucosa or through the M-cells of Peyer's patches (Corr et al. 2006, Jensen et al. 1998). The intracellular pathogenesis of *L. monocytogenes* has been studied extensively, but the intestinal phase, which is critical to the pathogenic potential of *L. monocytogenes* as well as other enteric bacteria, has until recently been poorly investigated.

Hardy et al. (Hardy et al. 2004) have shown using bioluminescent technology that *L. monocytogenes* has the ability to survive the low pH found in the stomach, increased osmolarity found in the small intestine, and the stressful environment encountered in the gallbladder for successful extracellular colonization of the gallbladder lumen. Hardy et al. (Hardy et al. 2006) were also able to show the bacterium can be shed into the intestinal tract through biliary excretion after replication within the gallbladder, resulting in completion of the infectious pathway (Briones et al. 1992, Hof 2001). Although these studies provided information concerning growth within and release from the gallbladder, additional studies were needed to understand the pathway to gallbladder colonization.

Recent research has pieced together a modified murine oral route model of transmission in which *L. monocytogenes* enters the body by consumption of contaminated food products making its ways through the stomach and into the gastrointestinal (GI) tract. Stresses encountered during travel through the stomach and GI tract activate sigma factor B (SigB), which in turn activates the positive regulatory factor A gene (*prfA*) (Roche et al. 2005). SigB is a stress response regulator activated by heat, an increase in

osmolarity, increased or decreased pH and by oxidizing agents (Ferreira et al. 2001).
PrfA acts as a global regulator responsible for activation of several virulence associated
genes, including those involved with internalization and intracellular growth (*inlA*, *inlB*,
hly, *plcB* and *actA*) (Engelbrecht et al. 1996, Roche et al. 2005). Once in the
gastrointestinal tract, *L. monocytogenes* can penetrate intestinal mucosal epithelium using
internalin A (InlA) and internalin B (InlB) for invasion (Pentecost et al. 2010).
Internalization of *L. monocytogenes* requires a modified murine model as traditional
murine models lack E-cadherin required for adherence and invasion of intestinal
epithelium (Lecuit et al. 1999, Wollert et al. 2007). *L. monocytogenes* were shown to
invade intestinal epithelium through lymphoid follicles known as the Peyer's patches
(Jensen et al. 1998). The bacterium was then shown to disseminate through the body by
intracellular travel within the lymphatic cells (Jensen et al. 1998). Once in the liver, it is
hypothesized that *L. monocytogenes* pass into the gallbladder by use of the bile canaliculi
after transcytosis from Kupfer cells has occurred (Eimerman 2011). The exact
mechanism of travel from the liver to the gallbladder is not fully understood at this time.
If *L. monocytogenes* follow the suggested model for colonization of the gallbladder, then
the shedding through excretion from the biliary duct and the ability to grow
extracellularly within the gallbladder to high concentrations coupled with the recycling of
L. monocytogenes once the bacteria re-enter the GI tract could serve as a source of
constant shedding and thus completion of the infectious pathway.

This chapter will describe the current literature as it relates to three of the
common stressors encountered within the gastrointestinal tract by *L. monocytogenes*. The
first is the change in pH. The stomach, which serves as the first line of defense to food-
borne pathogens, is characterized by having a low pH while the lumen of the gallbladder

is characterized by having a high pH (6.5-9.0), indicating *L. monocytogenes* must be able to survive in both extreme acidic and basic environments. The second part of this chapter will explore the mechanisms involved in adaptation to high osmolarity conditions, such as those encountered in the lumen of the small intestine (salinity content of 0.3M) (Gupta and Chowdhury 1997). The last two parts of this chapter will focus on the last two environments encountered within the gallbladder: mixed atmospheric conditions and high concentrations of bile salts.

1.2   pH Induced Stress Response in *L. monocytogenes*

Several stress response genes have been identified in *L. monocytogenes* in association with conditions encountered during travel through the stomach to the gastrointestinal tract. Research of 30 clinical and 30 meat isolates showed all clinical strains to be resistant to acid stress, while 87% of meat isolates were resistant. The difference in resistance suggests that the low pH of the stomach serves as a defense barrier against food-borne listeriosis (Dykes and Moorhead 2000). *L. monocytogenes* has acquired several mechanisms of resistance to acidic conditions found in the stomach; the acid tolerance resistance (ATR) response allows resistance to lethal acidic conditions due to prior exposure to milder acidic conditions (Davis et al. 1996). The ATR response pathway also has an important role in the survival of *L. monocytogenes* in other environments by providing cross protection against oxidative, heat, and osmotic stress (Gahan et al. 1996, Lou and Yousef 1997, Marron et al. 1997, O'Driscoll et al. 1996). The cross protection provided by the ATR is thus vital for migration into the small intestine where an increase in salinity is also found. In addition to providing protection in the small

intestine, the ATR response may also assist in survival in the gallbladder where both osmotic and oxidative stresses are present as a result of stored concentrated bile.

A second system utilized by *L. monocytogenes* for resistance under reduced pH conditions is the glutamate decarboxylase system (GAD). Not all strains of *L. monocytogenes* are thought to possess a functional GAD system, but it has been proven to be necessary for the survival of the bacterium in gastric juices in order to regulate pH homeostasis under acidic conditions (Cotter et al. 2001). Interestingly the virulent strain EGD-e was shown to possess all *gad* genes, but has been shown to be acid-sensitive (Cotter et al. 2001). GAD makes the cell more alkaline by the removal of a proton from the cell through the conversion of extracellular glutamate into γ-aminobutyrate (GABA) (Cotter et al. 2001). Because GABA is more alkaline then glutamate, the exchange aids in alkanization of the cellular cytoplasm in addition to the removal of the proton (Small and Waterman 1998). The cell requires a constant cycle of alkalinization by the GAD system in order to balance the constant influx of protons from the extrcellular acidic environment. Removal of protons from the cell by GAD results in a neutral pH inside the cell. Several factors contribute to the expression of the GAD system, including changes in osmolarity associated with chloride ions, low pH, and anaerobiosis (Blankenhorn et al. 1999, De Biase et al. 1999, Sanders et al. 1998). Activation of the GAD system within the stomach due to low pH garners cross protection against the small intestine, where the bacterium is confronted by a shift in osmolarity and exposure to anaerobic environments. In addition to encountering a shift in osmolarity and an anaerobic environment in the small intestine, this system may help protect *L. monocytogenes* in the gallbladder as well, where the same conditions are encountered.

1.3   Osmolarity Induced Stress Response in *L. monocytogenes*

As *L. monocytogenes* enters the small intestine, a change in osmolarity is encountered. The lumen of the small intestine posses' a salinity content of 0.3M sodium chloride (Gupta and Chowdhury 1997). In order to survive high salinity environments *L. monocytogenes* imports osmolytes that are also known as compatible solutes. The compatible solutes are able to exist inside the cell in high concentrations in order to counteract osmotic stress encountered in foods and in passage through the gastrointestinal tract without disrupting cellular processes. Osmolytes act as osmoprotectants by stabilizing proteins and maintaining cell volume (Arakawa and Timasheff 1985). The osmoprotectants shown to be most effective in *L. monocytogenes* are betaine and carnitine (Bayles and Wilkinson 2000). Three independent osmoprotectant systems have been identified in *L. monocytogenes* for the uptake of betaine and carnitine; the expression of these systems is controlled by the general stress response regulator SigB (Fraser et al. 2000, Fraser et al. 2003). Two of the transporters dedicated to betaine transport are BetL (a sodium-dependent secondary transporter) (Sleator et al. 1999) and the ATP-binding cassette Gbu (Ko and Smith 1999). A substrate binding protein-dependent ABC transporter known as OpuC is the single system responsible for carnitine uptake used for osmoregulation (Fraser et al. 2000). Although Gbu was found to provide the highest tolerance to osmotic stress in brain heart infusion broth supplemented with 1.2M NaCl (Wemekamp-Kamphuis et al. 2002), OpuC is proposed to be the osmotic stress response utilized inside the host due to its transport of carnitine as an osmoprotectant and effect on virulence factors (Sleator et al. 2001).

Proteomic analysis of the salt stress response of *L. monocytogenes* revealed twelve proteins are upregulated in the presence of salt (Duche et al. 2002). Of these stress

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response proteins identified, two general stress response proteins were present: DnaK and Ctc. DnaK is a stress response protein responsible for the stabilization of cellular proteins (Hesterkamp and Bukau 1998). The exact function of the general stress response protein Ctc is unknown, but has been found to promote resistance to high salt conditions in the absence of an osmoprotectant (Gardan et al. 2003). Two other proteins identified were from the delayed response to salt stress known as CysK and Gap. The CysK protein is an O-acetylserine lyase A protein that is involved in cysteine biosynthesis (Duche et al. 2002). The Gap protein (glyceraldehyde-3-phosphate dehydrogenase associated protein) is associated with glycolysis and increases in expression upon cold stress response in *B. subtilis* (Graumann et al. 1996). It has been suggested by the expression of CysK and Gap that synthesis of amino acids and key components leading to the Kreb's cycle could be a crucial factor in alleviating salt stress in *L. monocytogenes* (Duche et al. 2002). The *relA* gene in *L. monocytogenes* is responsible for the production of ppGpp, which participates in peptidoglycan biosynthesis and accumulates in *L. monocytogenes* during amino acid depletion (Okada et al. 2002). The use of (p)ppGp as a regulator during amino acid starvation demonstrates the importance of amino acid biosynthesis under osmotically stressed conditions when coupled with the expression of CysK and Gap both of which are also responsible for amino acid biosynthesis.

1.4   Atmosphere Induced Stress Response in *L. monocytogenes*

As a facultative anaerobic bacterium, *L. monocytogenes* is well suited for the variety of atmospheres encountered throughout the gastrointestinal tract. Studies have shown that *L. monocytogenes* can survive microaerophilic to anaerobic environments quite well (King et al. 2003). Growth under anaerobic conditions has also been found to

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increase genes encoding for branched fatty acid production in the membranes and glutamate decarboxylase (Jydegaard-Axelsen et al. 2004). Low oxygen tension encountered during the gastrointestinal phase has been shown to promote adhesion to intestinal epithelial cells, which in turn promotes intracellular growth (Burkholder et al. 2009). It is important to understand the stress response mounted by *L. monocytogenes* under anaerobic conditions to fully understand the process required to survive in the gastrointestinal tract.

1.5   Bile Salt Induced Stress Response

Bile is composed of cholesterol, phospholipids, bilirubin, electrolytes, iron, copper, and bile salts (Evans et al. 1976, Mukhopadhyay 2004). Initially cholesterol is converted into unconjugated bile acids known as chenodeoxycholic acid and cholic acid (Vlahcevic et al. 1980). These unconjugated forms of bile acids are then conjugated with glycine or taurine to form glycocholate or taurocholate known as bile salts (Berg 2002). The bile salts are shuttled into the biliary canaliculus by transporters (Gerloff et al. 1998). From the biliary canaliculus the bile is transferred to the gallbladder, where it is stored in high concentrations. From the gallbladder bile enters the small intestine after consumption of a meal in reaction to cholecystokinin released from the duodenum (Wiener et al. 1981). Bile salts break down dietary lipids for absorption by the small intestine (Maldonado-Valderrama et al. 2011). In addition to the absorption of lipids, bile salts aid in the absorption of fat and fat-soluble vitamins (Garidel et al. 2007). Once bile salts enter the ileum they are deconjugated and bound to transporter proteins for recycling through passage of the enterocytes and transported to the liver by way of the portal vein as part of the enterohepatic circulation (Hofmann 1976). Bile is altered during passage

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through the intestine, resulting in a mixed population of conjugated and unconjugated bile acids. Several studies have been conducted to determine the difference in microbial interactions with both conjugated and unconjugated bile acids (Alvarez et al. 2003, Floch et al. 1970, Floch et al. 1971, Suskovic 2000).

Bile salts are the bactericidal agent of bile, demonstrating a detergent activity that results in damage to both the membrane and DNA (Begley et al. 2005, Merritt and Donaldson 2009, Prieto et al. 2004). Bile salts have been shown to disrupt the lipid bilayer, as well as induce protein misfolding, oxidative stress and damage to DNA (Bernstein et al. 1999, Prieto et al. 2006, Sanchez et al. 2005). The gallbladder has a high concentration of bile salts (15% or more) (Hardy et al. 2004), which contributes to its ability to remain a sterile environment. However, malnourishment and disorders with liver or biliary abnormalities result in decreased concentration of bile in the intestine, which results in an increase in bacterial growth (Lorenzo-Zuniga et al. 2003).

Due to their bactericidal nature, enteric pathogens have established several mechanisms to endure the stress associated with bile salts in the gastrointestinal tract. Many gram negative bacteria have been found to adapt to bile salt induced stress by removing the bile salts that enter the cell through porins and the lipid bilayer of the outer membrane using a multi-drug efflux pump (Ma et al. 1994, Plesiat and Nikaido 1992, Thanassi et al. 1997). The multi-drug efflux systems are used by bacteria for transport of toxins, drugs, environmental compounds, and for the removal of bile salts (Hagman et al. 1995, Ma et al. 1995, Nikaido et al. 2008).

In *L. monocytogenes*, the systems responsible for bile salt resistance rely on several mechanisms. Some of these include the bile exclusion system (*bilE*) (Sleator et al. 2005), bile salt hydrolase (*bsh*) (Begley et al. 2005, Dussurget et al. 2002), and the

general stress response sigma factor (*sigB*) (Begley et al. 2005, Dowd et al. 2011) in *L. monocytogenes*. The bile exclusion system operates to prevent bile from entering the cell instead of controlling osmolyte uptake as was previously suggested (Sleator et al. 2005). Bile salt hydrolase is used for the detoxification of bile salts through the removal of glycine/taurine side chains (Dussurget et al. 2002). Sigma factor B (*sigB*) is an alternative sigma factor responsible for the direction of RNA polymerase to promoters of *bilE (Sleator et al. 2005)*and *bsh* (Zhang et al. 2011) for resistance against bile stress as well as a modulator of positive regulatory factor A (*prfA*) (Ollinger et al. 2009) active in promoting expression of virulence genes. Other systems associated with resistance to bile salt stress in *L. monocytogenes* include BetL, Gbu, and OpuC which are all involved in osmolyte stress (Angelidis and Smith 2003). A nucleotide excision repair protein known as UvrA has also been identified to play a role in bile salt stress response within *L. monocytogenes* (Kim et al. 2006, van der Veen and Abee 2011).

Extracellular growth within the gallbladder can be vital for shedding and thus efficient transmission of the bacterium. A previous study conducted by Hardy et al. (Hardy et al. 2004) found *L. monocytogenes* to grow in chain formation extracellularly in the gallbladder, suggesting active growth within the harsh gallbladder environment instead of migration to the organ after active replication. The ability to grow to such high concentrations within the gallbladder may also suggest that extracellular growth within this organ is a type of immune-privileged. The extracellular growth allows for quick transit of the bacterium from bile duct to the intestine within 5 minutes (Hardy et al. 2006). As indicated earlier several bile resistance genes exist in *L. monocytogenes*, but the specific mechanisms allowing for extracellular growth within the gallbladder have yet to be fully characterized.

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1.6   Conclusion

Many of the systems discussed in this review confer protection against multiple stressors, demonstrating how intricately woven these systems must be in order for *L. monocytogenes* to survive within the host. Numerous acid response systems, which also provide a level of osmotic relief and protection from oxidative damage, may play a role in survival in the intestinal tract and gallbladder lumen. Additionally, several studies have suggested bile salts lead to oxidative damage, which may be prevented by cross protection from previously activated stress response systems during travel through the GI tract.

Though much is known in regards to bile salt resistance, two major gaps exist in our knowledge related to bile resistance of *L. monocytogenes*. First, the mechanism of resistance to bile salts at a pH of 6.5-9.0, which is the pH normally found in the gallbladder (Crawford and Brooke 1955, Dowd et al. 2011), has not yet been described. Second, previous studies conducted on bile resistance of *L. monocytogenes* have focused upon aerobic conditions. Given that numerous obligate anaerobes and microaerophiles have been isolated from the gallbladder and biliary duct, the environment can be considered to be microaerophilic to anaerobic (Nielsen and Justesen 1976, Williams and Scobie 1976). Analysis of *L. monocytogenes* growth under bile salt stress in an aerobic environment does not accurately model the response due to the change in metabolic pathways being utilized, which may affect the proteome expressed (Covert et al. 2001, Marino et al. 2000, Starck et al. 2004). It has also been previously concluded that varied atmospheres have an effect on *L. monocytogenes'* ability to survive under bile stress (King et al. 2003) further enhancing the need to conduct additional analysis under anaerobic and microaerophilic environments. Thus, how *L. monocytogenes* are able to

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survive in high concentrations of bile salts at neutral pH under anaerobic conditions typical of the gallbladder remains elusive of the documented stress response mechanisms known to date.

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CHAPTER II

RESPONSE OF *LISTERIA MONOCYTOGENES* UNDER BILE SALT STRESS

2.1   Introduction

Listeria monocytogenes is a dangerous gram-positive food-borne pathogen responsible for nearly 28% of all food related deaths reported annually in the United States (Lynch et al. 2006, Mead et al. 1999). The immunocompromised, elderly, neonates and pregnant women are the most susceptible to infection. Listeriosis manifests as meningitis or meningoencephalitis and septicemia in immunocompromised adults and can also cause infections of the fetus in pregnant woman (Fayol et al. 2009, Thigpen et al. 2011).

L. monocytogenes endures a multitude of stressful environments throughout the infectious pathway, such as acidic conditions found in the stomach, osmolarity changes in the small intestine, and high concentrations of bile salts found in both the small intestine and the gallbladder (Davis et al. 1996, O'Driscoll et al. 1996). It has been suggested the ability to grow in bile salts is key to the pathogenic potential of *L. monocytogenes* as well as other enteric bacteria. Hardy et al. have shown *L. monocytogenes* can thrive extracellularly in the gallbladder, where bile salt concentrations are highest (Hardy et al. 2004). It has also been suggested that the mechanisms utilized by *L. monocytogenes* to resist the bactericidal effects of bile salts serve as virulence factors and are required for successful colonization within the gastrointestinal tract for both *L. monocytogenes* as well

as other enterics (Begley et al. 2002, Kus et al. 2011, Lin et al. 2003, Merritt and Donaldson 2009).

Bile salts have been found to alter the cell membrane of *L. monocytogenes* (Merritt et al. 2010) and introduce DNA damage in other enterics (Bernstein et al. 1999, Prieto A. I. et al. 2006b). In support of bile salts inflicting DNA damage in *L. monocytogenes*, several mechanisms devoted to DNA repair have been found to be involved in bile salt resistance, such as the nucleotide excision repair protein UvrA and the recombinational repair protein RecA (Kim S. H. et al. 2006, van der Veen and Abee 2011). Several mechanisms are also utilized by *L. monocytogenes* to resist the bactericidal properties of bile salts, including the bile exclusion system (bilE) (Sleator et al. 2005), the general stress response regulator sigma factor B (SigB) (Begley et al. 2005, Dowd et al. 2011), and bile salt hydrolase (Bsh) (Begley et al. 2005, Dussurget et al. 2002). Additional systems associated with the bile salt stress response are BetL, Gbu, and OpuC, which play a role in relieving osmotic stress encountered during passage through the gastrointestinal tract in route to fecal shedding or to the gallbladder through internalization at the Peyer's patches and dissemination to the liver (Jensen et al. 1998).

It has been suggested that variations exist in the ability of virulent and avirulent strains of *L. monocytogenes* to survive following exposure to bile salts (Merritt et al. 2010). Therefore, the aim of this study was to analyze the proteomes expressed by two virulent strains, EGDe and F2365 and the avirulent strain HCC23 in the presence of bile salts under slightly basic anaerobic conditions. The bile salt response was analyzed at a basic pH in order to mimic the conditions found in the gallbladder (Crawford and Brooke 1955).

2.2   Materials and methods

2.2.1   Bacterial strains and growth conditions

Strains of *L. monocytogenes* used in this study were the virulent strains EGDe (serovar 1/2a) and F2365 (serovar 4b) as well as the avirulent strain HCC23 (serovar 4a). Strains were routinely cultured in brain heart infusion (BHI) medium at 37°C. All anaerobic growth assays were conducted using sealed vials containing medium. Vials were acclimated to anaerobic conditions for 48 h in a Coy Laboratories vinyl anaerobic chamber using a gas mix of 10% H_2, 5% CO_2, and 85% N_2. Vials were plugged with rubber caps and sealed with aluminum seals prior to removal from the chamber.

2.2.2   Percent survival analysis

Cultures were grown overnight in BHI broth at 37°C and then subsequently diluted 1:100 in anaerobic vials containing 2 ml of fresh BHI broth using sterile syringes. To monitor oxygen levels within the vials, 5 μM resazurin was added to the sealed anaerobic vials. Inoculated vials were then allowed to grow at 37°C to mid-log phase ($OD_{600} = 0.4$). At mid-log 1 ml of cells was injected into a sealed anaerobic vial containing 0.2 g of bile salts from bovine and ovine (Sigma B8381) and a 1 ml aliquot of cells was injected into a sealed anaerobic vial without bile salts to serve as a control. Samples (0.2 ml) were removed via syringe hourly for 7 h post bile salt exposure, diluted in 1X PBS, and immediately plated on BHI agar. Plates were incubated under anaerobic conditions using the AnaeroPack System (Remel R681001) at 37°C for 24 h prior to viable plate count analysis. Three independent replicates were analyzed and $\log_{10}$ CFU/ml for each strain was determined. An additional set of vials was used to monitor the pH over the course of a six-hour period. EGDe, F2365, and HCC23 were inoculated

into anaerobic vials as described above with the exception of the addition of resazurin. Samples (0.1 ml) were acquired at 0, 2, 4, and 6 h post bile salt exposure and applied to EMD ColorpHast pH indicator strips. An additional survival analysis was conducted with control samples adjusted to a pH of 7.5, which was identical to the pH after the addition of bile salts. Survival under anaerobic conditions was monitored by viable plate counts as conducted.

Statistical analysis was conducted to determine significance for the percent change. A significant difference in growth between bile salt treated cells in comparison to non-treated cells was defined as $P<0.05$. To determine statistical significance within strains between bile salt treated and non-treated for each time point, a completely randomized design with repeated sampling using three independent replicates was analyzed with PROC MIX (SAS version 9.3, 2004). The GLM mix procedure was used to separate means when a level of significance ($P<0.05$) was identified.

2.2.3 Real-time RT-PCR

EGDe and F2365 were cultured under anaerobic conditions and treated with bile salts as described in section 2.2. Aliquots (0.4 ml) were removed 2 and 5 h post treatment with either 0 mg/ml or 0.2 mg/ml bile salts. Samples were centrifuged at 8,000 x g for 2 min prior to treatment with RNAprotect Bacteria Reagent (Qiagen) according to the manufacturer's protocol. Cell pellets were stored at -20°C overnight, and then RNA was isolated using the RNeasy kit (Qiagen) per manufacturer's suggested protocol. Briefly, cell pellets were thawed on ice, resuspended in 0.1 ml of TE buffer (30mM Tris-Cl, 1mM EDTA, pH 8.0) containing 15 mg/ml of lysozyme and RNase inhibitor (Applied Biosystems catalog #4368814). Samples were then treated with DNase I, washed on

columns, and then eluted in 0.05 ml of RNase-free water. RNA quality and quantity was analyzed using a Nanodrop ND-1000. Isolated RNA was converted to cDNA using the Applied Biosystems High Capacity cDNA Reverse Transcription Kit with RNase inhibitor (catalog #4374966) per manufacturer's protocol. A standard reverse transcriptase PCR protocol was followed: 25°C for 10 min, 37°C for 120 min, and 85°C for 5 sec. The quality and quantity of cDNA was determined using a Nanodrop ND-1000; cDNA was subsequently stored at -20°C.

Relative fold expression changes of *sigB* in relation to the expression of 16S rRNA were determined using an Applied Biosystems Step One Plus system. For *sigB*, the forward primer sequence was 5'–TGAAGCTGATTCGGATGGAAG–3', the reverse primer sequence was 5'–TTCTCGCTCATCTAAAACAGGG–3', and the probe sequence was 5'–TGATGTTGTTGGTGGTACGGATGATGG–3'. For 16S rRNA, the forward primer sequence was 5'–ACCCAACATCTCACGACAC–3', the reverse primer sequence was 5'–GTGGAGCATGTGGTTTAATTC G–3', and the probe sequence was 5'–CCACCTGTCACT TTGTCCCCGAA–3'. Both PrimeTime qPCR assay probes were 5' labeled with 6-carboxyfluorescein as the reporter dye and Iowa Black as the 3' quencher dye. A standard curve was generated for both genes, starting with 100 ng cDNA template (diluted 1:2) to verify primer efficiency. Each reaction received 1μl of 20X PrimeTime qPCR assay designed for either *sigB* or 16S rRNA (Integrated DNA Technologies), 10μl of 2X TaqMan Gene Expression Master Mix (Applied Biosystems) and 100 ng of template. The qPCR program was as follows: stage 1, 50°C for 2 min and 95°C for 10 min with a hold at each; stage 2, 95°C for 15 sec and 60°C for 1 min for 40 cycles. Fold changes were calculated for *sigB* based on expression levels of the 16S

rRNA using the formula derived for the comparative C_T method ($2^{-\Delta\Delta Ct}$) (Schmittgen and Livak 2008).

2.2.4   Protein purification, analysis and comparisons

EGDe, F2365, and HCC23 were grown overnight in BHI broth prior to a 1:100 dilution into 2 ml of BHI in sealed vials and incubated under anaerobic conditions at 37°C to mid-log ($OD_{600} = 0.4$). Samples (0.2 ml) were removed from the anaerobic vials via syringe at -10 min, 1 h and 5 h post bile salt exposure. Cells were pelleted by centrifugation at 4,800 x g, lysed by sonication and proteins were purified following procedures previously described by our laboratory (Donaldson et al. 2009, Donaldson et al. 2011). Purified proteins (50 μg) were treated with 2 μg trypsin overnight and desalted using a macrotrap (Michrom Bioresources, Inc.). Desalted peptides were re-suspended in 250 μl of 5mM monosodium phosphate in 25% acetonitrile, adjusted to a pH of 3 using formic acid and then processed using a strong cation exchange (SCX) macrotrap (Michrom Bioresources, Inc) according to the manufacturer's instructions. Cleaned samples were then dried and re-suspended in 40 μl of 2% acetonitrile (ACN), 0.1% formic acid (FA) and transferred to low retention HPLC vials for analysis. Proteins were isolated from EGDe, F2365, and HCC23 from three independent experiments.

Peptide mass spectrometry was accomplished using an EASY-nLC (Thermo Scientific) high performance liquid chromatography machine (HPLC) coupled with an LTQ Velos (Thermo Scientific) linear ion trap mass spectrometer. The Easy-nLC was configured for reverse phase chromatography using a Hypersil Gold KAPPA C18 column (Thermo #25005-150065) with a flow rate of 333 nl per minute. Peptides were separated for mass spectrometry analysis using an acetonitrile gradient starting at 2% ACN, 0.1%

FA and reaching 50% ACN, 0.1% FA in 120 min, followed by a 15 min wash of 95% ACN, 0.1% FA. Column equilibration was handled automatically using the EASY-nLC. The eluate from the HPLC was fed directly to the LTQ Velos for nanospray ionization followed by ms/ms analysis of detected peptides. The LTQ Velos was configured to perform 1 ms scan followed by 20 ms/ms scans of the 20 most intense peaks repeatedly over the 135 min duration of each HPLC run. Dynamic exclusion was enabled with a duration of 5 min, repeat count of 1, and a list length of 500. Raw spectral data from the LTQ Velos were converted to mzML format using the msConvert tool from the ProteoWizard software project (Kessner et al. 2008). The collected spectra were subsequently analyzed using the X!tandem (Craig and Beavis 2004) search algorithm using the appropriate strain protein database from the National Center for Biotechnology Information (NCBI). X!tandem was configured to use tryptic cleavage sites with up to two missed cleavages. Precursor and fragment mass tolerance were set to 1000 ppm and 500 ppm respectively. Amino acid modifications included in the database search were single and double oxidation of methionine and both carboxymethylation and carboxamidomethylation of cysteine. A decoy search was also performed using a randomized version of the target database with the same search parameters as above. The search results were filtered using the methods described by others (Filzmoser 2005, Rousseeuw 1998). A decoy score distribution was created and each match from the target database was evaluated as a possible outlier and assigned a probability of being correct. Peptides from the target database were accepted if the probability of being correct was 95% or higher.

Differential expression of proteins between bile salt treated and non-treated samples was evaluated based on peptide spectral intensity. The raw spectral data were

converted to the MS1 tab delimited format (McDonald et al. 2004) using the MakeMS2 tool available from the MacCoss laboratory at the University of Washington (https://proteome.gs.washington.edu/software/hardklor/programs.html) (Hoopmann 2007). The intensities for each peptide elution peak were pulled from its associated MS1 file using the Perl scripting language and summed. For each identified protein, the peptide intensities were combined and organized by experimental replicate. Differential expression was evaluated using confidence interval and Monte Carlo resampling techniques to compare the replicate intensities between treatments. Each comparison used 1 million iterations and was assigned a p-value based on the number of times each test favored one treatment over another. A protein was accepted as significantly differentially expressed if the treatment intensity distribution was within the 95% confidence interval computed from the resampling results, with the direction of protein expression reflecting the favored treatment. Functional classifications of significantly changed proteins were identified using ListiList (http://genolist.pasteur.fr/ListiList/). Fold changes were calculated by normalizing intensity values by the addition of 1 to all values. The $\log_{10}$ of all intensities were then calculated and used to measure change in protein expression. Supplementary proteomic data may be found in the appendix.

2.3   Results

2.3.1   Viability in bile salts varies between strains

To determine whether variations existed between the virulent strains F2365 and EGDe and the avirulent strain HCC23 in viability when exposed to bile salt stress under anaerobic conditions, viability was assessed for a period of 7 h post bile exposure. F2365 displayed no significant difference (P = 0.08) in viability between bile salt treated and

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non-treated groups. Both EGDe and HCC23 displayed a significant difference (P = 0.03 and 0.02, respectively) in viability between bile treated and non-treated groups. The avirulent strain HCC23 decreased in viability under bile salt stress throughout the 7 h time period examined. There was a significant difference (P = 0.04) in viability of bile salt treatment groups between the aviruelnt strain HCC23 and the virulent strain F2365. A within strain comparison of virulent strain EGDe was found to have a significant difference (P = 0.0003) in growth under anaerobic conditions without bile salt treatment over the 7 h period examined.

The pH of bile salt treated and non-treated samples was monitored; the pH of control samples remained 6.0, while bile salt treated samples was 7.5 for the six hour period analyzed. Therefore, to determine whether differences in pH between bile salt treated and non-treated samples accounted for the variation in growth, the pH of control samples was adjusted to 7.5. Survival curve analysis of samples at a pH of 7.5 were similar to those obtained for samples at a pH of 6.0 (data not shown), therefore indicating that the alteration in pH was not a contributing factor to the variation in viability exhibited in the presence of bile salts.

The survival analysis of EGDe and F2365 suggested that within 5 h post bile salt treatment, these bacteria were transitioning out of stress response to bile salts (Table 2.1). The virulent strains exhibit a significant decrease in viability under bile salt stress at 2 h for EGDe and F2365 (P=0.02 and 0.01, respectively). EGDe also exhibited a significant decrease in viability under bile salt stress at 5 h post bile salt exposure (P= <0.001). Comparison of viability within EGDe under bile salt stress found a significant difference between viability at 5 h and 6 h (P=<0.001) where an increase in viability was exhibited. Given both EGDe and F2365 were able to increase in viability

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without a subsequent decrease by 5 h post exposure to bile salts, the 5 h time point could display proteins required for bile salt resistance. Real-time PCR was therefore used to analyze the expression of the general stress response regulator *sigB* at 2 h and 5 h post bile salt exposure. The fold change of *sigB* for F2365 was not found to be significant between 2 h and 5 h displaying a fold change from 1.42 to 0.046 suggesting that the response to this environment is activated before 5 h post exposure. The fold change of *sigB* expression in EGDe increased from 66.72 at 2 h post exposure, to 187.14 by 5 h post exposure, suggesting 5 h post exposure is a point where both virulent strains have mounted a stress response to bile salt exposure. The avirulent strain was not included in the quantitative PCR study since HCC23 did not recover from the bile salt stress (Table 2.1). Extended survival analysis for strain comparisons can be found in Appendix A.

Table 2.1   Percent Change of virulent and avirulent strains during bile salt stress

hr	HCC23	Bile salts–HCC23	P-value	EGD	Bile salts–EGD	P-value	F2364	Bile Salts–F2365	P-value
0	0	0	-	0	0	-	0	0	-
1	0.02	-4.56	**0.16**	-1.14	-4.66	**0.28**	4.38	-0.24	**0.16**
2	-0.42	-7.81	***0.03**	-0.02	-7.97	***0.02**	5.56	-2.68	***0.01**
3	0.78	-8.38	***0.006**	1.71	-4.67	**0.05**	3.18	-1.99	**0.12**
4	0.63	-7.64	***0.01**	2.01	-4.11	**0.07**	3.92	-1.79	**0.08**
5	-0.05	-8.04	***0.02**	2.18	-13.53	***<0.001**	4.24	-1.31	**0.09**
6	0.97	-9.64	***0.002**	5.86	-1.62	***0.02**	4.57	-0.95	**0.09**
7	1.50	-5.74	***0.03**	7.29	3.70	**0.27**	6.76	3.13	**0.27**

P-values represent comparisons made at each time point between bile-treatment and control groups for each strain. Significance was determine by $P<0.05$ and denoted (*).

2.3.2   Proteins associated with cell envelope and cellular processes are differentially expressed in EGDe, F2365, and HCC23

Comparisons within bile salt treated and non-treated strains revealed a significant change in the levels of peptidolgycan bound internalins expressed for all three strains.

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Virulent strains EGDe and F2365 had a decrease in expression level s of separate putative peptidoglycan bound internalins (GI#16802378 and GI#46906910, respectively) (Table 2.2). Internalin A increased in expression level for EGDe after 5 h of bile salt exposure. There was a significant decrease 1 h post bile salt exposure in the expression level of the invasion protein P60 (Kohler et al. 1990) (GI#16802625) in EGDe. A protein similar to internalin B (GI# 46908437) was decreased in expression level at the 5 h time point in F2365. Interestingly HCC23 increased expression level of an internalin protein (GI# 217965399) at both 1 h and 5 h post bile salt.

A transmembrane efflux protein (GI# 46909007) was found to increase in expression level for F2365 after 5 h of bile treatment. Other membrane proteins of interest were a lipoprotein (GI# 46906526), which was decreased in F2365 when exposed to bile salt stress. An autolysin (GI# 46906369) was increased in F2365 upon initial exposure to bile salts and a peptidoglycan-synthesizing protein (GI# 46908271) was decreased in expression level at each comparison made.

The expression of lipoprotein (GI# 217966164) was decreased in expression level in HCC23 upon initial exposure to bile salts (1 h). The cell division initiation protein FtsZ (GI# 217963823) was decreased in HCC23 after 5 h of exposure compared to the 1 h exposure to bile salts. Additionally, the membrane export protein SecDF (GI# 217964326) was decreased in expression level after 5 h of exposure compared to the non-treated control (-10 min) in HCC23 (Table 2.2).

Table 2.2 Cell envelope associated proteins (ListiList category 1) with a significant change in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGDe								
16802625	P60 extracellular protein, invasion associated protein Iap	1.1	6.6888	6.1572	-	down	-	-
16802845	similar to putative Na+/H+ antiporter	1.2	0.0000	6.7551	-	up	-	-
16802250	actin-assembly inducing protein precursor	1.8	0.0000	-	6.9124	-	-	up
16802378	similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)	1.8	-	7.2792	6.5863	up	down	-
16802477	Internalin A	1.8	0.0000	0.0000	7.9704	-	up	up
16802877	putative peptidoglycan bound protein (LPXTG motif)	1.8	6.3495	0.0000	7.1911	-	up	up
F2365								
46906369	similar to autolysin: N-acetylmuramoyl-L-alanine amidase	1.1	-	5.1977	6.8117	-	up	-
46908271	similar to peptidoglycan synthesis enzymes, putative phospho-N-acetylmuramoyl-pentapeptide-transferase	1.1	6.5136	6.9599	5.6439	up	down	down
46906526	putative lipoprotein	1.2	7.4726	6.4354	0.0000	down	down	down
46909007	similar to transmembrane efflux protein	1.2	0.0000	0.0000	7.7297	-	up	up
46906395	putative peptidoglycan bound protein (LPXTG motif)	1.8	8.2475	6.8638	7.0153	up	down	down
46906910	similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)	1.8	6.2004	0.0000	-	down	-	-
46908437	similar to N-acetylmuramoyl-L-alanine amidase and to internalin B	1.8	7.0009	-	0.0000	-	-	down
HCC23								
217964326	similar to protein-export membrane protein SecDF	1.6	7.2049	-	5.4954	-	-	down
217963823	similar to cell-division initiation protein FtsZ	1.7	-	7.5557	6.2771	-	down	-
217965399	similar to internalin protein, putative peptidoglycan bound protein (LPXTG motif)	1.8	0.0000	7.4355	6.5847	up	down	up
217965750	putative peptidoglycan bound protein (LPXTG motif)	1.8	0.0000	0.0000	6.8358	-	up	up
217966164	conserved lipoprotein	1.8	8.5897	7.9079	-	down	-	-

2.3.3 Metabolism associated proteins are differentially expressed upon exposure to bile salts

Metabolic proteins associated with osmotic stress, biofilm formation, and vacuole lysis were found to be differentially expressed among the three strains studied. Several osmotic stress response proteins were differentially expressed, but strain specific differences in expression were observed. The only osmotic stress response protein found to be differentially expressed in all three strains was the cysteine synthase (CysK) (Duche et al. 2002), which was decreased in expression level at the 5 h time point for both EGDe (GI#16802269) and F2365 (GI#46906455) but increased in expression level in the avirulent strain HCC23 (GI#217965691) at 5 h. The osmotic stability protein glutamate dehydrogenase (GAD) (Cotter et al. 2001) of EGDe (GI#16804401) decreased in expression level by 5 h. The protein (p)ppGpp synthetase, which is associated with amino acid synthesis and is also part of the osmotic stress response (Okada et al. 2002), decreased in expression level by the 5 h time point for both EGDe (GI#16803563) and F2365 (GI#46907751). Alanine dehydrogenase, a non-osmolyte osmotic stress response protein (Duche et al. 2002), increased in expression level at 1 h and then subsequently decreased in expression level by 5 h for F2365 (GI#46907810). The protein 1-pyrroline-5-carboxylate reductase (ProC), which is associated with proline synthesis (Sleator et al. 2001) and acts as an osmoprotectant in osmotic stress response (Beumer et al. 1994), was found to increase in expression level by 5 h post exposure in HCC23 (GI#217965514) (Table 2.3).

The metabolic proteins 6-phophofructokinase (GI#16803611) and pyruvate dehydrogenase (GI#16803094) were increased in expression level at 1 h post bile salt exposure in EGDe. A protein responsible for listeriolysin-O independent lysis of epithelial cell vacuole lysis, Zinc metalloproteinase precursor (GI#217965711), was

found to be increased at 1 h and decreased in expression level at the 5 h time point in

HCC23 (Table 2.3).

Table 2.3   Intermediary metabolism associated proteins (ListiList category 2) with
significant changes in expression levels during bile salt stress

			Intensity			Expression		
GI#	Protein	ListiList	0h	1h	5h	0-1h	1-5h	0-5h
EGDe								
16802269	similar to cysteine synthase	2.2	7.8370	6.1658	-	down	-	-
16803611	similar to 6-phosphofructokinase	2.1.1	5.6525	7.1303	-	up	-	-
16803094	similar to pyruvate dehydrogenase (dihydrolipoamide acetyltransferase E2 subunit)	2.1.2	5.0832	6.2789	-	up	-	-
16804401	similar to glutamate decarboxylase	2.2	-	6.9576	0.0000	-	down	-
16803563	similar to (p)ppGpp synthetase	2.3	-	6.7714	0.0000	-	down	-
F2365								
46906455	similar to cysteine synthase	2.2	8.2415	-	7.2933	-	-	down
46907810	similar to alanine dehydrogenase	2.2	6.3497	8.4927	5.8728	up	down	down
46907751	similar to (p)ppGpp synthetase	2.3	-	6.8110	0.0000	-	down	-
HCC23								
217965514	similar to 1-pyrroline-5-carboxylate reductase (ProC)	2.2	-	0.0000	7.0523	-	up	-
217965691	similar to cysteine synthase	2.2	-	6.7372	7.4123	-	up	-
217965711	Zinc metalloproteinase precursor	2.2	6.4973	7.5446	0.0000	up	down	down

**2.3.4   Stress response and repair proteins are differentially expressed upon
exposure to bile salts**

Several significant differences were found between the three strains analyzed that

were related to stress response and DNA repair mechanisms. For instance, all three

strains had a significant increase in the expression level of catalase; both F2365

(GI#46908975) and HCC23 (GI#217966008) increased in expression level within 1 h and

EGDe (GI#16804822) increased by 5 h post bile salt exposure. The virulent strains EGDe

and F2365 exhibited an increase in excinuclease ABC proteins (UvrABC), which are

responsible for recognizing and processing DNA lesions. UvrA (GI#16804526) and a

protein similar to UvrA (GI#16804089) in EGDe as well as UvrB (GI#46908661) and a protein similar to UvrA (GI#46908285) in F2365 all increased due to bile salt exposure. Interestingly, the expression of listeriolysin O precursor (GI# 16802248) decreased in EGDe following bile salt exposure, which was normal given the extracellular growth characteristic of *L. monocytogenes* in the gallbladder (Hardy et al. 2004) would not require the expression of a protein utilized for escape from the vacuole during intracellular growth.

EGDe expressed a protein similar to the ATP-dependent dsDNA exonuclease SbcC (GI# 16803685) by 5 h post bile salt exposure. The virulent strain F2365 had an increased in expression level of the DNA repair protein Sms (GI# 46906466) and a protein similar to the DNA polymerase beta protein YshC (GI#46907450) upon initial exposure to bile salts, which later decreased in expression level by 5 h. Furthermore, the recombinational repair protein RecA (GI# 46907626) in F2365 decreased in expression upon initial exposure to bile salt stress. Two DNA mismatch repair proteins (GI# 217964449 and GI# 21764450) increased in HCC23 upon exposure to bile salt stress, but subsequently decreased by 5 h post bile salt exposure.

In EGDe class I heat-shock proteins DnaK (GI# 16803513) and GroEL (GI# 16804107) were both initially decreased in expression level upon initial exposure to bile salt stress, but GroEL subsequently increased in expression level by 5 h post exposure. A third heat-shock protein DnaJ (GI# 16803512) was also identified to be decreased in expression level upon initial exposure followed by an increase in expression level 5 h post bile exposure. A trigger factor protein (GI# 16803307) was found to be increased in expression level for all time point comparisons rendered.

Three heat shock proteins were differentially expressed in the virulent strain F2365 as well. Class I heat-shock protein DnaK (GI# 46907701) and GroEL (GI# 46908303) were decreased in expression level upon initial exposure to bile salts but increased in expression level by 5 h; however levels expressed by 5 h were lower than those prior to the treatment. The heat-shock protein HtrA serine protease (GI# 46906533) increased in expression level by 5 h post bile salt exposure. Only the trigger factor (GI# 217964590) was identified in the avirulent strain HCC23, which was decreased in expression level upon initial exposure to bile salts and increased in expression level by 5 h post bile salt exposure (Table 2.4).

Table 2.4   Information pathways associated proteins (ListiList category 3) with significant changes in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGDe								
16804089	similar to excinuclease ABC (subunit A)	3.2	6.4186	7.0296	-	up	-	-
16804526	excinuclease ABC (subunit A)	3.2	-	6.9792	7.6215	-	up	-
16804713	similar to UV-damage repair protein	3.2	6.2083	7.3874	6.5900	up	down	up
16803408	DNA repair and genetic recombination	3.3	0.0000	7.3560	0.0000	up	down	-
16803685	similar to ATP-dependent dsDNA exonuclease SbcC	3.3	-	0.0000	6.9806	-	-	-
16803307	trigger factor (prolyl isomerase)	3.9	0.0000	7.0102	7.6858	up	up	up
16803513	class I heat-shock protein (molecular chaperone) DnaK	3.9	7.0768	4.4469	-	down	-	-
16804107	class I heat-shock protein (chaperonin) GroEL	3.9	7.1684	6.6585	7.5920	down	up	up
16803512	heat shock protein DnaJ	4.1	8.3172	7.2852	7.2973	down	-	down
16804822	catalase	4.2	6.8721	0.0000	6.8622	down	up	-
16802248	listeriolysin O precursor	4.5	8.5253	6.6911	0.0000	down	down	down
F2365								
46906466	similar to DNA repair protein Sms	3.2	7.3560	8.3130	6.2723	up	down	down
46907450	similar to DNA polymerase beta, to B. subtilis YshC protein	3.2	6.2963	7.0393	5.2244	up	up	down
46908285	similar to excinuclease ABC (subunit A)	3.2	0.0000	7.7171	6.6456	up	up	up
46908660	excinuclease ABC (subunit A)	3.2	8.3152	7.4718	-	down	-	-
46908661	excinuclease ABC (subunit B)	3.2	0.0000	5.8013	6.8961	-	up	-
46907626	Recombination protein recA	3.3	6.1950	0.0000	-	down	-	-
46907874	similar to ATP-dependent dsDNA exonuclease SbcC	3.3	6.8480	8.3220	7.5935	up	down	up
46907701	class I heat-shock protein (molecular chaperone) DnaK	3.9	9.3159	7.0400	7.6381	down	up	down
46908303	class I heat-shock protein (chaperonin) GroEL	3.9	6.9716	0.0000	6.8403	down	up	down
46906533	similar to heat-shock protein htrA serine protease	4.1	-	0.0000	7.1363	-	up	-
46908975	catalase	4.2	4.6181	6.4091	-	up	-	-
HCC23								
217963803	similar to excinuclease ABC (subunit A)	3.2	7.3458	8.3471	7.0427	up	down	down
217964449	DNA mismatch repair protein	3.2	0.0000	7.0553	0.0000	up	down	down
217964450	DNA mismatch repair (recognition)	3.2	6.6055	7.4424	6.5693	up	down	-
217964590	trigger factor (prolyl isomerase)	3.9	7.4434	6.5446	7.6679	down	up	up
217966008	catalase	4.2	5.6313	7.2799	-	up	-	-

2.4   Discussion

Previous studies found the cell membrane is altered in virulent and avirulent strains of *L. monocytogenes* when exposed to bile salt stress under anaerobic conditions (Merritt et al. 2010). Bile salts have also been shown to induce DNA damage *in vivo* in some enterics, including *Salmonella enterica* and *Escherichia coli* (Kandell and Bernstein 1991, Prieto et al. 2006a). Therefore, the purpose of this study was to determine if virulent strains yielded a higher viability under concentrated bile salt exposure in anaerobic conditions than avirulent strains and what protein expression levels may be responsible for that survival. Interestingly, prolonged viability under bile salt stress was found to be strain specific within this study and not dependent upon strain virulence. Several proteins were identified as differentially expressed under the tested conditions giving rise to a number of possible pathways to be investigated in future studies for survival of bile salt stress within the gallbladder.

In this study prolonged viability in the presence of bile salts under anaerobic conditions at basic pH was shown to be strain specific and not dependent upon virulence as only F2365 did not displayed a significant difference in viability between bile salt treatment and non-treatment groups. Additionally, this study found a significant difference between the ability of virulent strain F2365 and avirulent strain HCC23 to survive bile salt stress suggesting the virulent strain may have mechanisms responsible for continued viability under bile salt stress not present in the avirulent strain. Differentially expressed proteins associated with continued viability under bile salt stress have been elucidated under acidic conditions, but not at a pH of 6.5-9.0, which is more

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similar to the resting state of the gallbladder (Crawford and Brooke 1955, Dowd et al. 2011).

Though *L. monocytogenes* possesses internalins that allow for entrance into non-phagocytic mammalian cells (Gaillard et al. 1991), this bacterium exists in an extracellular state in the lumen of the murine gallbladder (Dowd et al. 2011, Hardy et al. 2004). As stated in the results portion, conserved peptidoglycan bound internalins in EGDe and F2365 (GI# 16802378 and GI# 46906910, respectively) as well as internalin B in F2365 (GI# 46906919) were decreased in expression level in virulent strains supporting the report of extracellular growth observed in the gallbladder as virulent strains were not actively expressing proteins associated with intracellular growth. The avirulent strain, however, increased expression level of conserved peptidoglycan bound internalin protein (GI# 217963823) under bile salt stress, suggesting avirulent strains may prefer to grow intracellularly when exposed to bile salts. This study suggests virulent strains may alter protein expression for survival under bile salt stress in a manner not seen in the avirulent strain HCC23. The increase in expression level of Internalin A in EGDe can be contributed to the increased levels of stress response gene *sigB* indicated by the qPCR data (Kim et al. 2005, McGann et al. 2007, McGann et al. 2008). The connection between the differences exhibited in internalin expression by virulent and avirulent strains coupled with listeriolysin O needs to be further analyzed to determine if this results in a preferential intracellular v. extracellular growth *in vivo*. It is possible avirulent strains prefer to live intracellulary do to their inability to grow in the presence of bile salts under anaerobic conditions coupled with the increased expression of invasion proteins such as the conserved internalin and listeriolysin O.

Lipoproteins are another virulence factor in *L. monocytogenes* used for mammalian cell invasion and intracellular survival (Machata et al. 2008). Due to the extracellular nature of *L. monocytogenes* in the lumen of the gallbladder and the decrease in internalin expression levels in virulent strains, it makes sense that the lipoproteins would also be decreased in virulent strains. As would be expected due to the decrease in internalin expression levels, the virulent strain F2365 decreased expression level of lipoproteins at both the 1 h and 5 h time periods. Interestingly, even though internalins were increased in the avirulent strain HCC23, potentially promoting invasion of cells, the lipoproteins were decreased in expression upon exposure to bile salt stress. Metalloprotease is used for escape from the vacuole during the intracellular growth phase (Marquis et al. 1995) and was found to be increased in HCC23 upon exposure to bile salts, but decreased in expression level later at the 5 h time point. The decrease in metalloprotease expression level supports the extracellular growth phase recorded as characteristic of listerial growth within the gallbladder (Hardy et al. 2004) since metolloprotease would not be required for extracellular growth.

Biofilm formation has also been explored as a virulence factor employed during stress response in *L. monocytogenes* (Monk et al. 2004). The virulent strain EGDe was found to decrease expression level of invasion-associated protein (IAP P60) at 1 h post bile exposure. A decrease in expression of IAP P60 has been linked to rough colony formation leading to biofilm formation (Monk et al. 2004). From the data presented here and recent work by Begley et al. (Begley et al. 2009) concerning exposure to bile influencing biofilm formation in EGDe, biofilm formation may be responsible for the long-term survival of *L. monocytogenes* within the gallbladder.

Several proteins comprising efflux pumps have been found to remove metal ions, antibiotics, and bile for bacterial survival in a number of enteric bacteria, including *L. monocytogenes* (Godreuil et al. 2003, Sleator et al. 2005). The virulent strain F2365 was found to increase expression level of a transmembrane efflux protein within 5 h post bile salt exposure. The data presented support the transmembrane efflux protein is utilized for the removal of bile salts as it is increased in expression level, which corresponds to the increase in viability seen at 5 h.

Proteins associated with salt stress were differentially expressed for both EGDe and F2365 upon exposure to bile salts. Glutamate decarboxylase (GAD) associated with osmotolerance (Cotter et al. 2001) in *L. monocytogenes* was found to increase in expression in EGDe upon exposure to bile salts, but subsequently decreased in expression at the 5h time point. The decrease in expression by the 5 h time point may be due to the formation of a protective biofilm shielding *L. monocytogenes* from osmotic stress. A second osmotolerance system was differentially expressed in EGDe upon exposure to bile salt stress. The protein (p)ppGpp synthetase is a non osmolyte pathway utilized by *L. monocytogenes* during an osmotic stress response (Okada et al. 2002). The data indicate a decrease in (p)ppGpp expression level in EGDe at 5 h post bile exposure, supporting the theory that biofilm formation by EGDe protects the bacterium from osmotic stress. The protein (p)ppGpp was also found to be decreased in expression level in F2365 by 5 h. Alanine dehydrogenase is utilized during the salt stress response by *L. monocytogenes* (Duche et al. 2002) and was increased in expression level in F2365 within 1 h post bile exposure. Due to the increase in amino acid synthesis, which has been associated with the stress response of *L. monocytogenes* to osmotic stresses, alanine dehydrogenease can be thought of as a non-osmolyte response to osmotic stress (Duche et al. 2002). The ability

to survive osmotic stress is important to survival of *L. monocytogenes* not only in the GI tract, but also in the gallbladder during bile salt stress. The osmotic stress responses found to be differentially expressed may be part of the multifaceted mechanism utilized for cell viability in the gallbladder as more than one stress is encountered and mechanisms must be activated in response to each stress. In general, the osmotic stress response proteins found to be differentially expressed in virulent strains were not found to be differentially expressed in the aivrulent strain HCC23, suggesting the avirulent strain may be suppressed in viability by the osmotic stress encountered in the gallbladder.

When referring to the viability analysis the avirulent strain HCC23 was not able to adapt to the bile salts during the tested period (Table 2.1). Additionally the cell division initiation protein FtsZ (Gueiros-Filho and Losick 2002) was decreased in expression 5 h post bile salt exposure. Active replication of the avirulent strain appears to be completely halted by 5 h exposure to bile salts. The cease in growth directly contributes to the decline in viability, as cells damaged due to bile salt stress do not seem to have an active mechanism for repair.

Several studies have suggested bile salt stress results in oxidative damage to the DNA. The oxidative damaged characterized in the form of GC→AT transitions has been reported in *S. enterica* (Prieto et al. 2006a). Catalase is an enzyme used to remove reactive oxygen species responsible for oxidative damage, which has been correlated to lipid peroxidation damage(Meilhac et al. 2000, Wang et al. 2011), and was found to be increased in expression levels in all three strains upon exposure to bile salt stress. The increase in catalase expression levels supports previous findings indicating bile salt exposure damages the cell membrane, which was suggested to have arisen from oxidative damage (Merritt , Lawrence, and Donaldson 2010). Additionally, mismatch repair

proteins identified would be required to actively repair the DNA damage (Merino et al. 2002). Surprisingly, HCC23 did have an increase in the expression levels of these proteins, which indicates that this strain did attempt to repair DNA damage. Additionally, the response that was elicited within 1 h post exposure indicated that bile salt-induced DNA damage occurred upon exposure. Although excinuclease activity was found to be increased in expression level in virulent strains, studies have shown UvrABC to be a dispensable method of DNA repair of oxidative damage (Prieto et al. 2006b), suggesting that alternative methods of DNA repair may be utilized in the presence of bile-induced DNA damage. Other DNA repair proteins identified in virulent strains under bile salt stress were general stress response and recombinational repair proteins. Future studies will need to be conducted to determine the specific role that these DNA recombination proteins have in bile salt survival. In addition, the RecA protein in F2365 was decreased in expression level, but this finding does not exclude the possibility of recombination being key to survival under bile salt stress, as RecA-independent repair pathways do exist (Ozgenc et al. 2005). The DNA repair protein Sms is a recombination repair protein that increased in expression level in F2365 upon exposure to bile salt stress and has been suggested to be involved in repairing oxidative damage (Beam et al. 2002). The repair pathway of Sms is dependent upon expression of RecA (Diver et al. 1982) which most probably contributed to the decrease in Sms expression by 5 h post bile salt exposure due to the initial decrease in RecA expression. Both UvrA and RecA have been associated with repair of double stranded DNA breaks, the damage accrued from exposure to bile (Priet et al. 2006b, Thiagalingam and Grossman 1991). The increase in heat shock proteins levels for all strains upon exposure to bile salt stress in conjunction with the

activation of proteins related to repair of double stranded DNA breaks supports the oxidative damage theory suggested earlier.

In summary, prolonged viability under bile salt stress was found to be strain specific in this study and not dependent upon strain virulence. Several alterations in the expression levels of cell envelope associated proteins were identified, which may contribute to the ability of *L. monocytogenes* to survive in the gallbladder. Although a number of DNA repair proteins were identified for both virulent and avirulent strains, a universal mechanism for DNA repair could not be identified to be responsible for survival. The osmotic stress response proteins found in EGDe are also linked to proteins associated with biofilm formation. The initial stress response may be required for survival during early entry into the gallbladder and then abandoned once a biofilm has been established. F2365 may be able to survive in the gallbladder by utilizing the osmotic stress response pathways identified, coupled with the expression of an efflux pump. Interestingly proteins utilized for osmotic stress in *L. monocytogenes* have also been found to contribute cross-protection for the bacterium during anaerobiosis and alkaline stress. In conclusion, the ability of *L. monocytogenes* to survive in the gallbladder may be strain specific and dependent upon general stress response mechanisms active in DNA repair and osmotic stress response coupled with a transmembrane efflux pump or the ability to form biofilms.

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CHAPTER III

CONCLUSION

Bile serves as a natural barrier of defense against food borne pathogenic bacteria. The ability to survive bile salt stress, the main component and the bactericidal component of bile, is considered to be a virulence factor of *Listeria monocytogenes* as well as other enterics (Begley et al. 2002, Kus et al. 2011, Merritt and Donaldson 2009). *L. monocytogenes* contact bile salts during passage through the gastrointestinal tract and have developed several mechanisms to resist bile salt stress, such as the bile exclusion system (*bilE*) (Sleator et al. 2005), bile tolerance locus A (*btlA*) (Begley et al. 2003) and bile salt hydrolase (*bsh)* (Dussurget et al. 2002). Additionally, *L. monocytogenes* colonizes the lumen of the gallbladder during its infectious process where bile salts are highly concentrated, demonstrating the importance of bile salt resistance as a virulence factor (Hardy et al. 2004).

The literature review presented in Chapter I illustrated the multitude of stressful conditions that simultaneously affect *L. monocytogenes* during colonization of the gallbladder. The atmospheric conditions, alkaline pH, and concentrated bile salts are all antagonistic to the survival of *L. monocytogenes*. In response to each individual stress encountered, a number of mechanisms have been identified for survival/resistance, but several of the bile salt resistance mechanisms were shown to be active only under acidic conditions found in the intestinal tract (Dowd et al. 2011). A major gap in our knowledge

related to bile salt resistance is how *Listeria* responds to the gallbladder environment as a whole under anaerobic conditions.

Data presented in Chapter II demonstrated a strain specific prolongation of viability in bile salts under anaerobic conditions, instead of the suggested dependency upon strain virulence. The significant increase in viability in the virulent strain EGDe between 5 h and 6 h after bile salt treatment suggests a longer test period may support virulent strain resistance instead of strain specific resistance, as there was no significant difference between virulent strain F2365 treatment and control groups in viability over the 7 h test period. The effect from increased pH on viability of virulent and avirulent strains under anaerobic conditions was determined to be insignificant in this study suggesting depressions in viability observed were dependent upon exposure to bile salts instead of growth in alkaline conditions.

A proteomic analysis was conducted to identify differentially expressed protein levels in strains EGDe, F2365, and HCC23 related to viability in bile salts in anaerobic conditions. The data presented in Chapter II suggest that viability involves the efficient expression of a number of general stress response proteins, which included those associated with DNA repair and osmotic stress response. Specific expression of a protein associated with biofilm formation was observed in the virulent strain EGDe and a transmembrane efflux protein was found in the virulent strain F2365. Collectively, these data suggest the general stress response may be utilized for continued viability of virulent strains upon initial exposure to bile salt stress, but long-term viability may be strain specific. The avirulent strain HCC23 shared a protein associated with osmotic stress response, cysteine synthase (Duche et al. 2002), with virulent strains F2365 and EGD, but lacked differential expression of any other osmotic stress response proteins found in

virulent strains, possibly reflecting this as an inadequate means of osmotic stress resistance in concentrated bile salts. Additionally, the avirulent strain HCC23 increased expression level of an internalin-associated protein suggesting a preference for intracellular growth instead of extracellular growth when exposed to conditions similar to those found in the gallbladder.

This study provides essential information in the path to understanding how *L. monocytogenes* and potentially other enterics are able to survive in areas with high concentrations of bile salts such as the gallbladder. Several future studies are needed to confirm the findings reported. For instance, the role of biofilm formation in EGDe under anaerobic conditions and bile salt stress needs to be analyzed further. Studies have shown a connection between bile salt stress (Begley et al. 2009) and IAP expression (Monk et al. 2004) independently affecting biofilm formation. In this study EGDe decreased in IAP expression level suggesting biofilm formation under bile salt stress. Additionally, future studies should investigate the affect internalins have on biofilm formation during stress response as IAP (Kuhn and Goebel 1989) and internalins are both invasion associated proteins. Linking the expression of internalins may help determine when and how biofilm formation is triggered in the gallbladder. The ability of compatible solutes to provide cross protection for bile resistance has recently been established (Sleator and Hill 2010). Exposure to salt stress was shown to instill resistance against lethal concentrations of bile (Begley et al. 2002). Further studies considering non-compatible solute salt stress proteins in bile salt resistance should be conducted. Metabolic proteins active in osmotic stress response were increased in expression level in virulent strains, but not in the avirulent strain in this study. The ability to survive bile salt stress may be dependent upon the ability to survive osmotic stress within the gallbladder. Future studies should also be

conducted on the effects of the RecFOR pathway in bile salt resistance within *L. monocytogenes*. This study indicated an increase in expression of ATP-dependent dsDNA exonuclease SbcC in virulent strains EGDe and F2365, but found a decrease in expression level in avirulent strain HCC23. SbcC has been shown to prevent the RecBCD pathway resulting in activation of the RecF pathway instead for repair of DNA lesions (Zahradka et al. 2006). The ability to bypass the use of RecBCD for the RecFOR pathway may be a factor for survival within high concentrations of bile salts. Finally, the connection between potential intracellular growth by avirulent strains needs to be explored, as the data presented in Chapter II suggest that HCC23 may try to utilize invasiveness as means of survival *in vivo*. Expression of internalin A in HCC23 upon exposure to bile salt stress may result in internalization prior to entrance into the lumen of the gallbladder, which would explain the poor viability in bile salts reported in this study. Given the vast number of proteins differentially expressed for DNA repair, osmotic stress response, and invasion, the ability to survive under bile salt stress in the gallbladder may be dependent upon a combination of stress response pathways.

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APPENDIX A

SURVIVAL ANALYSIS DURING BILE SALT STRESS USING PROCMIX TO

DETERMINE SIGNIFICANCE FOR STRAIN COMPARISONS

Table A.1   Viability analysis of strain comparisons using ProcMix percent change data

Strain Comparisons	P-value
EGDe x Bile salt treated-EGDe	*0.03
F2365 x Bile salt treated-F2365	0.08
HCC23 x Bile salt treated-HCC23	*0.02
EGDe x HCC23	0.48
EGDe x F2365	0.47
HCC23 x F2365	0.17
Bile salt treated-EGDe x Bile salt treated-HCC23	0.36
Bile salt treated-EGDe x Bile salt treated-F2365	0.20
Bile salt treated-HCC23 x Bile salt treated-F2365	*0.04

P-values represent comparisons made at each time point between each strain.
Significance was determine by $P<0.05$ and denoted (*).

APPENDIX B

EXTENDED PROTEOMIC ANALYSIS FROM CHAPTER II CELL ENVELOP

ASSOCIATED PROTEINS (LISTILIST CATEGORY 1) WITH A

SIGNIFICANT CHANGE IN PROTEIN EXPRESSION

DURING BILE SALT STRESS

Table B.1   Cell envelope associated proteins (ListiList category 1) with significant change in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGD-e								
16802244	similar to UDP-N-acetylglucosamine pyrophosphorylase	1.1	5.5403	6.7426	-	up	-	-
16802625	P60 extracellular protein, invasion associated protein Iap	1.1	6.6888	6.1572	-	down	-	-
16802985	similar to C-terminal part of B. subtilis ComEC protein and to ComEA	1.1	0.0000	6.7266	0.0000	up	down	-
16803116	similar to autolysin (EC 3.5.1.28) (N-acetylmuramoyl-L-alanine amidase)	1.1	6.3537	7.4176	6.6345	up	down	up
16803120	similar to B. subtilis minor teichoic acids biosynthesis protein GgaB	1.1	7.6948	0.0000	7.0619	down	-	down
16803125	similar to teichoic acid biosynthesis protein B	1.1	0.0000	6.4828	7.3788	up	up	up
16803331	similar to acyltransferase (to B. subtilis YrhL_protein)	1.1	-	7.3715	6.4065	-	down	-
16803478	similar to penicillin-binding protein	1.1	7.2186	6.5579	-	down	-	-
16803931	similar to penicillin-binding protein 2A	1.1	7.5649	0.0000	7.5833	down	up	up
16804078	similar to penicillin-binding protein 2B	1.1	7.6337	6.6359	7.5565	down	up	down
16804564	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	1.1	-	6.4913	0.0000	-	down	-
16804590	similar to UDP-N-acetylglucosamine 1-carboxyvinyltransferase	1.1	0.0000	6.9965	8.4593	down	up	up
16802200	similar to oligopeptide ABC transporter-binding protein	1.2	-	6.9660	8.0074	-	up	-
16802324	similar to sugar ABC transporter, ATP-binding protein	1.2	6.5026	6.0308	7.1978	up	up	up
16802403	similar to PTS system, fructose-specific enzyme IIBC component	1.2	0.0000	6.8740	0.0000	up	down	-
16802584	similar to ABC transporter (binding protein)	1.2	0.0000	7.3242	6.0302	up	down	-
16802687	similar to amino acid transporter	1.2	0.0000	6.8663	-	up	-	-
16802786	similar to ABC transporter, ATP-binding protein	1.2	7.0812	7.9377	6.4534	up	down	down
16802829	similar to amino acid transporter	1.2	7.5970	-	6.9715	-	-	down
16802845	similar to putative Na+/H+ antiporter	1.2	0.0000	6.7551	-	up	-	-
16802860	similar to cation transporting ATPase	1.2	6.9182	0.0000	-	down	-	-
16802882	similar to cation (calcium) transporting ATPase	1.2	7.0211	6.3719	6.2498	down	down	down
16802964	similar to ABC transporter, ATP-binding protein (N-terminal part)	1.2	0.0000	6.6371	7.7172	up	up	up
16803102	similar to ABC transporters (permease protein)	1.2	6.7283	-	5.8639	-	-	down
16803104	similar to membrane and transport proteins	1.2	6.5625	0.0000	6.1616	down	up	down
16803171	similar to ABC transporters, ATP-binding proteins	1.2	-	7.8092	0.0000	-	down	-
16803429	similar to sugar ABC transporter, ATP-binding protein	1.2	-	6.8272	7.9231	-	down	-
16803461	similar to glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)	1.2	0.0000	6.8800	5.6416	up	down	up

Table B.1 (continued)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
16803579	similar to glycerol uptake facilitator	1.2	-	7.2984	0.0000	-	down	-
16803722	similar to transmembrane transport proteins	1.2	0.0000	6.9214	-	up	-	-
16803887	similar to adhesion binding proteins and lipoproteins with multiple specificity for metal cations (ABC transporter)	1.2	6.2281	7.1053	7.4290	up	up	up
16803893	similar to heavy metal-transporting ATPases	1.2	6.1635	-	7.0396	-	-	up

Table B.2 Cell envelope associated proteins (ListiList category 1) with significant change in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
F2365								
46906369	similar to autolysin: N-acetylmuramoyl-L-alanine amidase	1.1	-	5.1977	6.8117	-	up	-
46906695	similar to penicillin-binding protein (D-alanyl-D-alanine carboxypeptidase)	1.1	7.6565	7.3323	6.3852	up	down	down
46906761	similar to Bacillus anthracis encapsulation protein CapA	1.1	6.9164	5.9007	0.0000	down	down	down
46907087	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diamino pimelate-D-alanyl-D-alanyl ligase	1.1	-	0.0000	6.1367	-	up	-
46907177	similar to C-terminal part of B. subtilis ComEC protein and to ComEA	1.1	6.1426	-	6.8012	-	-	up
46907776	similar to cell-shape determining protein MreB	1.1	-	6.7663	0.0000	-	down	-
46908271	similar to peptidoglycan synthesis enzymes, putative phospho-N-acetylmuramoyl-pentapeptide-transferase	1.1	6.5136	6.9599	5.6439	up	down	down
46908723	similar to UDP-N-acetylglucosamine 1-carboxyvinyltransferase	1.1	-	0.0000	8.3491	-	up	-
46908941	similar to D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 5)	1.1	7.0532	8.4331	7.3832	up	down	up
46906335	similar to PTS system mannose-specific, factor IIAB	1.2	4.9922	6.1752	7.3118	up	up	up
46906388	similar to oligopeptide ABC transporter-binding protein	1.2	5.2283	-	8.4065	-	-	up
46906413	similar to sugar ABC transporter, sugar-binding protein	1.2	7.7386	-	7.1269	-	-	down
46906520	putative lipoprotein	1.2	7.4726	6.4354	0.0000	down	down	down
46906662	similar to PTS fructose-specific enzyme IIC component	1.2	0.0000	0.0000	6.4260	-	up	up
46906853	similar to ABC transporter, ATP-binding protein	1.2	7.3048	6.4958	0.0000	down	down	down
46906887	similar to heavy metal-transporting ATPase	1.2	6.5557	7.7859	6.3895	up	down	down

Table B.2 (continued)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
46906896	conserved membrane protein	1.2	8.5937	6.7256	7.2401	down	down	down
46906989	similar to ABC transporter, ATP-binding protein	1.2	9.3145	7.7901	6.1685	down	down	down
46907068	similar to ABC transporter (ATP binding protein)	1.2	8.6431	6.4050	5.9911	down	down	down
46907072	similar to cation (calcium) transporting ATPase	1.2	7.9493	-	6.6231	-	-	down
46907078	similar to Glutamine ABC transporter (binding and transport protein)	1.2	0.0000	6.9400	-	up	-	-
46907090	similar to putative sugar ABC transporter, periplasmic sugar-binding protein,	1.2	-	5.3832	7.0361	-	up	-
46907154	similar to ABC transporter ATP-binding protein (antibiotic resistance)	1.2	6.8685	6.4329	4.4783	up	down	down
46907219	similar to antibiotic ABC transporter, ATP-binding protein,	1.2	6.9629	5.9144	-	down	-	-
46907235	phosphotransferase system enzyme I	1.2	7.3829	6.1450	8.5245	up	up	down
46907320	similar to PTS system, cellobiose-specific IIB component (cel A)	1.2	7.5325	0.0000	6.8579	down	up	down
46907348	similar to ABC transporters, ATP-binding proteins	1.2	7.7260	6.3286	-	down	-	-
46907426	similar to cobalt transport ATP-binding protein CbiO	1.2	-	0.0000	7.4815	-	up	-
46907442	similar to ABC transporter, ATP-binding proteins	1.2	7.8507	-	5.2676	-	-	down
46907734	similar to transporter	1.2	-	6.1502	7.9054	-	up	-
46907960	similar to sugar ABC transporter binding protein	1.2	7.7272	-	6.4563	-	-	down
46908085	similar to heavy metal-transporting ATPases	1.2	6.9779	-	6.0396	-	-	down
46908194	similar to ferrichrome ABC transporter (ATP-binding protein)	1.2	7.0841	8.9152	5.5305	up	down	down
46908418	similar to ferrichrome ABC transporter (binding protein)	1.2	5.9251	7.6959	-	up	-	-
46908426	similar to oligopeptide ABC transporter (ATP-binding protein)	1.2	-	0.0000	6.4489	-	up	up
46908505	similar to phosphotransferase system (PTS) fructose-specific enzyme IIABC component	1.2	-	0.0000	7.4451	-	up	up
46908590	similar to ABC transporter (ATP-binding protein)	1.2	7.6535	0.0000	0.0000	-	-	up
46908667	similar to phosphate ABC transporter (ATP-binding protein)	1.2	0.0000	7.0857	-	up	-	-
46908670	similar to phosphate ABC transporter (permease protein)	1.2	5.3512	-	7.3530	-	-	up
46908741	similar to dipeptide ABC transporter (dipeptide-binding protein)	1.2	7.0940	-	6.2169	-	-	down
46908751	similar to ABC transporter, ATP-binding protein	1.2	6.2849	-	7.3268	-	up	-
46908965	hypothetical membrane protein	1.2	7.9283	0.0000	-	-	down	-
46908986	similar to phosphotransferase system mannitol-specific enzyme IIBC	1.2	0.0000	-	7.2870	-	-	up
46909007	similar to transmembrane efflux protein	1.2	0.0000	0.0000	7.7297	-	up	up
46908181	similar to two-component sensor histidine kinase (ResE)	1.3	5.8540	-	6.7731	-	-	up

Table B.2 (continued)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
46908753	similar to two-component sensor histidine kinase	1.3	6.9959	0.0000	-	down	-	up
46906735	similar to NADH:flavin oxidoreductase	1.4	6.8338	6.6786	7.4455	up	up	up
46907060	similar to pyruvate-flavodoxin oxidoreductase	1.4	7.7715	7.2641	6.8684	down	down	down
46908469	similar to NADH oxidase	1.4	6.9565	6.3876	7.7465	up	up	up
46908701	similar to H+-transporting ATP synthase chain beta	1.4	5.6936	-	6.9763	-	-	up
46908810	similar to NADH dehydrogenase	1.4	8.2071	6.9923	6.5599	down	down	down
46906937	similar to motility protein (flagellar motor rotation) MotB	1.5	6.7089	5.3362	0.0000	down	down	down
46906956	similar to flagellar hook-associated protein FlgK	1.5	-	6.6765	5.2406	-	down	-
46906964	similar to flagellar basal-body M-ring protein fliF	1.5	7.3399	0.0000	-	down	-	-
46907755	similar to protein-export membrane protein SecDF	1.6	5.8453	-	0.0000	-	-	down
46908032	similar to signal recognition particle protein Ffh	1.6	7.3192	6.4480	7.9193	up	up	up
46906452	similar to cell division protein ftsH	1.7	7.8695	7.9643	6.7502	up	down	down
46907503	similar glucose inhibited division protein A	1.7	5.8779	8.0653	-	up	-	-
46907772	similar to cell division inhibitor (septum placement) protein MinD	1.7	5.9393	6.8089	5.7544	up	down	down
46908268	similar to cell-division initiation protein FtsZ	1.7	6.0562	8.2394	-	up	-	-
46908999	similar to GidA protein	1.7	6.7456	-	8.4110	-	-	Up
46906395	putative peptidoglycan bound protein (LPXTG motif)	1.8	8.2475	6.8638	7.0153	up	down	down
46906565	similar to cell surface proteins (LPXTG motif)	1.8	6.6889	-	7.3180	-	-	up
46906846	similar to cell surface protein	1.8	5.9581	5.9303	7.0544	up	up	up
46906908	similar to ORFA of Listeria seeligeri, (LPXTG motif)	1.8	8.3778	6.6490	-	down	-	-
46906910	similar to internalin proteins, putative peptidoglycan bound protein (LPXTG motif)	1.8	6.2004	0.0000	-	down	-	-
46907019	similar to cell surface proteins (LPXTG motif)	1.8	8.1909	-	0.0000	-	-	down
46907073	putative peptidoglycan bound protein (LPXTG motif)	1.8	8.3941	-	7.8021	-	-	down
46908178	putative peptidoglycan bound protein (LPXTG motif)	1.8	-	7.7818	6.9024	-	down	-
46908437	similar to N-acetylmuramoyl-L-alanine amidase and to internalin B	1.8	7.0009	-	0.0000	-	-	down
46908893	peptidoglycan anchored protein (LPXTG motif)	1.8	6.6521	0.0000	0.0000	down	-	down

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Table B.3 Cell envelope associated proteins (ListiList category 1) with significant change in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
HCC23								
217963345	similar to UDP-N-acetylglucosamine 1-carboxyvinyltransferase	1.1	0.0000	-	7.1720	-	-	up
217963814	similar to penicillin-binding protein 2B	1.1	4.6463	7.3105	-	up	down	-
217964137	similar to cell-shape determining proteins	1.1	6.5756	7.6748	-	up	down	-
217964332	similar to N-acetylmuramoyl-L-alanine amidase	1.1	6.8974	0.0000	0.0000	down	-	down
217964372	similar to putative integral membrane protein ComEC specifically required for DNA uptake but not for binding	1.1	8.0092	6.1060	7.3955	down	down	down
217964823	similar to teichoic acid biosynthesis protein B precursor	1.1	7.6899	0.0000	7.6518	down	-	down
217964828	similar to hypothetical protein 3 (capsulation locus) of Haemophilus influenzae	1.1	8.1115	-	7.4391	-	-	down
217964833	similar to autolysin (amidase)	1.1	7.4305	6.5327	-	down	-	-
217965458	similar to penicillin-binding protein (D-alanyl-D-alanine carboxypeptidase)	1.1	6.8774	6.4365	-	down	-	-
217966043	similar to D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 5)	1.1	0.0000	0.0000	8.7039	-	up	up
217966113	similar to autolysin, N-acetylmuramidase	1.1	7.5871	7.0190	4.2496	up	down	down
217963327	similar to dipeptide ABC transporter (dipeptide-binding protein)	1.2	7.7772	-	7.1227	-	-	down
217963399	similar to phosphate ABC transporter (binding protein)	1.2	-	6.8386	0.0000	-	down	-
217963435	similar to transport protein	1.2	6.6858	8.0741	7.3577	up	down	up
217963469	similar to B. subtilis ferrichrome ABC transporter fhuD precursor (ferrichrome-binding protein)	1.2	-	6.0700	7.4462	-	up	-
217963588	similar to amino acid ABC transporter, permease protein	1.2	-	0.0000	7.0954	-	up	-
217963703	similar to PTS system, fructose-specific enzyme IIB component	1.2	6.8130	0.0000	0.0000	down	-	down
217963725	similar to ABC transporter (ATP-binding protein)	1.2	0.0000	0.0000	7.3248	-	up	up
217964106	similar to ABC transporter (ATP-binding protein)	1.2	0.0000	6.5403	7.2511	up	up	up
217964123	similar to sugar ABC transporter binding protein	1.2	0.0000	-	6.9566	-	-	up
217964421	similar to ABC transporter (ATP-binding protein)	1.2	-	7.1034	5.8762	-	down	-
217964424	similar to glycine betaine/carnitine/choline ABC transporter (ATP-binding protein)	1.2	-	0.0000	7.3808	-	up	-
217964430	similar to glycine betaine/carnitine/choline ABC transporter (membrane protein)	1.2	5.8074	-	7.2624	-	-	up
217964708	similar to transporter, (to B. subtilis YdgH protein)	1.2	6.2018	6.6441	7.4645	up	up	up
217964803	similar to ABC transporters, ATP-binding proteins	1.2	-	0.0000	7.0889	-	up	-
217964837	similar to metal binding protein (ABC transporter)	1.2	0.0000	-	7.5694	-	-	up
217965087	similar to cation transporting ATPase	1.2	6.6259	7.5785	6.4825	up	down	down
217965238	similar to putative ABC transporter, permease protein	1.2	0.0000	0.0000	6.8217	-	up	up
217965258	conserved membrane protein	1.2	7.5871	6.8469	-	down	-	-

Table B.3 (continued)

HCC23

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
217965267	similar to heavy metal-transporting ATPase	1.2	0.0000	7.0966	0.0000	up	down	-
217965296	similar to ABC transporter, ATP-binding protein	1.2	0.0000	0.0000	7.1570	-	up	up
217965365	similar to ABC transporter (binding protein)	1.2	-	0.0000	7.0615	-	up	-
217965733	similar to sugar ABC transporter, sugar-binding protein	1.2	6.1227	-	7.0916	-	-	up
217965913	similar to PTS system, fructose-specific IIB component	1.2	0.0000	0.0000	7.3947	-	up	up
217966011	similar to PTS, cellobiose-specific IIB component	1.2	0.0000	5.7749	6.9898	up	up	up
217966069	similar to PTS system, fructose-specific IIABC component	1.2	0.0000	-	6.5775	-	-	up
217963398	two-component sensor histidine kinase	1.3	6.4333	7.2266	7.5091	up	down	up
217966124	similar to the two components sensor protein kdpD	1.3	8.6334	7.3350	0.0000	down	down	down
217963366	similar to H+-transporting ATP synthase chain alpha	1.4	7.4310	6.6674	-	down	-	-
217963420	thioredoxin reductase	1.4	6.4435	-	7.4079	-	-	up
217963603	similar to NADH oxidase	1.4	7.1966	6.6695	-	down	-	-
217965078	similar to pyruvate-flavodoxin oxidoreductase	1.4	8.9371	6.1089	6.7700	down	down	down
217965360	similar to putative NAD(P)-dependent oxidoreductase	1.4	7.8390	6.8337	7.0720	down	down	down
217965921	AA3-600 quinol oxidase subunit II	1.4	-	4.9001	7.6254	-	up	-
217966084	similar to cytochrome D ubiquinol oxidase subunit I	1.4	6.7938	-	0.0000	-	-	down
217965188	similar to flagellar motor switch protein fliG	1.5	0.0000	6.6297	7.3667	up	up	up
217965195	similar to flagellar hook-associated protein 2 FliD	1.5	8.5915	0.0000	6.9786	down	-	down
217964326	similar to protein-export membrane protein SecDF	1.6	7.2049	7.5557	5.4954	-	-	down
217963823	similar to cell-division initiation protein FtsZ	1.7	-	7.5557	6.2771	-	down	-
217964581	similar glucose inhibited division protein A	1.7	7.4818	6.6112	6.9048	down	down	down
217965983	similar to GidA protein	1.7	-	7.3007	8.1554	-	up	-
217963661	putative peptidoglycan bound protein (LPXTG motif)	1.8	-	7.8690	7.1756	-	down	-
217965168	putative peptidoglycan bound protein (LPXTG motif)	1.8	0.0000	6.2319	7.4879	up	up	up
217965399	similar to internalin protein, putative peptidoglycan bound protein (LPXTG motif)	1.8	0.0000	7.4355	6.5847	up	up	up
217965750	putative peptidoglycan bound protein (LPXTG motif)	1.8	0.0000	0.0000	6.8358	-	up	up
217965805	putative peptidoglycan bound protein (LPXTG motif)	1.8	8.9394	7.5385	7.3420	down	down	down
217966164	conserved lipoprotein	1.8	8.5897	7.9079	-	down	-	-

APPENDIX C

EXTENDED PROTEOMIC ANALYSIS FROM CHAPTER II INTERMEDIARY

METABOLISM ASSOCIATED PROTEINS (LISTILIST CATEGORY 2) WITH

SIGNIFICANT CHANGES DURING BILE SALT STRESS

Table C.1   Intermediary metabolism associated proteins (ListiList category 2) with significant changes in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGD-e								
16803126	similar to CDP-ribitol pyrophosphorylase	2.1	0.0000	6.7938	8.3962	up	up	up
16802389	similar to dehydrogenase/reductase	2.1.1	6.4192	6.9037	-	up	-	-
16802393	similar to dihydroxyacetone kinase	2.1.1	5.2343	6.7254	-	up	-	-
16802769	similar to L-glutamine-D-fructose-6-phosphate amidotransferase	2.1.1	7.9311	6.4686	-	down	-	-
16803040	similar to phytoene dehydrogenase	2.1.1	7.6811	6.4522	-	down	-	-
16803611	similar to 6-phosphofructokinase	2.1.1	5.6525	7.1303	-	up	-	-
16803674	similar to Alcohol-acetaldehyde dehydrogenase	2.1.1	7.2696	6.1791	-	down	-	-
16804147	similar to N-acetylglucosamine-6-phosphate deacetylase	2.1.1	7.7112	6.0468	-	down	-	-
16804700	similar to polyol dehydrogenase	2.1.1	0.0000	7.3713	-	up	-	-
16803094	similar to pyruvate dehydrogenase (dihydrolipoamide acetyltransferase E2 subunit)	2.1.2	5.0832	6.2789	-	up	-	-
16803095	similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	2.1.2	7.1434	4.5544	-	down	-	-
16803112	similar to pyruvate carboxylase	2.1.2	6.1273	7.3453	-	up	-	-
16802269	similar to cysteine synthase	2.2	7.8370	6.1658	-	down	-	-
16803390	similar to glycine dehydrogenase (decarboxylating) subunit 2	2.2	5.8741	6.9906	0.0000	up	down	down
16803477	similar to aspartate-semialdehyde dehydrogenase	2.2	-	5.5256	6.7115	-	up	-
16803630	similar to ornithine acetyltransferase and amino-acid acetyltransferases	2.2	0.0000	7.6830	-	up	-	-
16803668	similar to tryptophan synthase (beta subunit)	2.2	7.0052	-	5.9061	down	-	-
16803751	similar to aminopeptidases	2.2	6.4330	6.3588	7.0652	down	up	up
16803773	similar to glutamate synthase (small subunit)	2.2	8.5821	6.9820	7.1127	down	up	down
16803891	similar to carboxy-terminal processing proteinase	2.2	-	5.1988	6.5135	-	up	-
16803925	similar to probable thermostable carboxypeptidases	2.2	0.0000	-	7.0554	-	-	up
16804401	similar to glutamate decarboxylase	2.2	-	6.9576	0.0000	-	down	-
16804452	similar to aminotransferase	2.2	-	0.0000	7.0234	-	up	-
16804650	similar to glutamate decarboxylase	2.2	0.0000	6.9576	-	up	-	-
16804787	similar to para-aminobenzoate synthase component I	2.2	-	5.9908	7.1944	-	up	-
16804856	similar to carboxypeptidase	2.2	5.5805	6.4893	0.0000	up	down	down
16802103	similar to adenylosuccinate synthetase	2.3	0.0000	-	6.5524	-	-	up
16803136	GuaA similar to similar to GMP synthetase	2.3	6.2868	7.4428	7.6959	up	up	up

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Table C.1 (continued)

GI#	Protein	ListiList	Intensity			Regulation		
			0h	1h	5h	0-1h	1-5h	0-5h
EGD-e								
16803371	polynucleotide phosphorylase (PNPase)	2.3	7.6372	-	6.6758	-	-	down
16803563	similar to (p)ppGpp synthetase	2.3	-	6.7714	0.0000	-	down	-
16803564	similar to adenine phosphoribosyltransferase	2.3	0.0000	0.0000	6.8879	-	up	up
16803808	glutamine phosphoribosylpyrophosphate amidotransferase	2.3	0.0000	0.0000	6.4660	-	up	up
16803813	adenylosuccinate lyase	2.3	-	0.0000	7.6695	-	up	-
16803873	similar to dihydroorotase dehydrogenase	2.3	7.5088	7.0664	0.0000	down	down	down
16804032	similar to pyrimidine-nucleoside phosphorylase	2.3	-	7.1980	6.0537	-	down	-
16804576	similar to uracil phosphoribosyltransferase	2.3	7.0221	7.3311	7.7802	down	up	up
16803410	similar to branched-chain fatty-acid kinase	2.4	-	7.5256	0.0000	-	down	-
16803411	similar to branched-chain alpha-keto acid dehydrogenase E3 subunit	2.4	5.8683	-	7.3805	-	-	up
16803454	similar to Acetyl-CoA:acetyltransferase	2.4	6.4241	7.2781	-	up	-	-
16803613	similar to acetyl-CoA carboxylase beta subunit	2.4	5.7676	-	7.1151	-	-	up
16804241	similar to 3-oxoacyl- acyl-carrier protein synthase	2.4	0.0000	6.0883	7.0204	up	up	up
16802361	similar to hydroxyethylthiazole kinase (ThiM)	2.5	8.4811	-	6.9078	-	-	down
16802362	similar to phosphomethylpyrimidine kinase (ThiD)	2.5	0.0000	6.7794	-	up	-	-
16803089	similar to molybdopterin biosynthesis protein MoeB	2.5	7.1064	7.1764	0.0000	up	down	down
16803241	similar to uroporphyrinogen-III methyltransferase/uroporphyrinogen-III synthase	2.5	7.1497	5.6671	0.0000	down	down	down
16803248	similar to cobyric acid synthase CbiP	2.5	0.0000	5.9972	7.3492	up	up	up
16803405	similar to D-1-deoxyxylulose 5-phosphate synthase	2.5	0.0000	-	7.0187	-	-	up
16803632	similar to thiamin biosynthesis protein ThiI	2.5	7.0032	0.0000	-	down	-	-
16803713	similar to dihydroxynapthoic acid synthetase	2.5	0.0000	0.0000	7.3707	-	up	up
16803715	similar to 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase / 2-oxoglutarate decarboxylase	2.5	-	5.2871	7.2636	-	up	-
16803716	similar to menaquinone-specific isochorismate synthase	2.5	5.6364	6.4825	7.1805	up	up	up
16803941	similar to ketopantoate hydroxymethyltransferases	2.5	-	0.0000	7.5054	-	-	up
16804062	similar to L-aspartate oxidase	2.5	-	4.8190	6.7875	-	-	up
16804063	similar to nicotinate-nucleotide pyrophosphorylase	2.5	0.0000	-	6.8430	-	-	up
16804250	similar to ferrochelatase	2.5	-	5.7741	7.3141	-	up	-

Table C.2   Intermediary metabolism associated proteins (ListiList category 2) with significant changes in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
F2365								
46907311	similar to CDP-ribitol pyrophosphorylase	2.1	-	5.9543	7.8894	-	up	-
46906455	similar to cysteine synthase	2.2	8.2415	-	7.2933	-	-	down
46906782	similar to N-carbamyl-L-amino acid amidohydrolase	2.2	0.0000	6.8120	7.5354	up	up	up
46906805	similar to NADP-specific glutamate dehydrogenase	2.2	5.8520	7.4383	6.3938	up	down	up
46906809	similar to phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	2.2	6.4876	0.0000		down	-	-
46907119	similar to alanine racemase	2.2	0.0000	6.5137	-	up	-	-
46907454	similar to aspartokinase II alpha subunit	2.2	6.9472	0.0000	6.7569	down	down	up
46907621	similar to putative protease	2.2	5.7254	5.7312	7.1634	up	up	up
46907665	similar to aspartate-semialdehyde dehydrogenase	2.2	0.0000	-	6.7424	-	-	up
46907721	similar to oligopeptidase	2.2	0.0000	0.0000	7.0252	-	up	up
46907810	similar to alanine dehydrogenase	2.2	6.3497	8.4927	5.8728	up	down	down
46907850	similar to Xaa-His dipeptidase	2.2	8.1822	6.5393	8.0560	down	up	down
46907858	similar to tryptophan synthase (beta subunit)	2.2	5.6679	-	6.9082	-	-	up
46907942	similar to aminopeptidases	2.2	0.0000	6.5127	7.5460	up	up	up
46907963	similar to glutamate synthase (small subunit)	2.2	6.7204	-	7.5384	-	-	up
46907964	similar to glutamate synthase (large subunit)	2.2	7.7608	8.8308	-	up	-	-
46908156	similar to 5-enolpyruvylshikimate-3-phosphate synthase	2.2	6.2138	5.2175	7.3922	down	up	up
46908311	similar to glycoprotein endopeptidase	2.2	-	7.8804	6.0683	-	down	-
46907751	similar to (p)ppGpp synthetase	2.3	-	6.8110	0.0000	-	down	-
46907771	similar to ribonuclease G	2.3	-	6.2988	0.0000	-	down	-
46907922	similar to deoxyuridine triphosphate nucleotidohydrolases	2.3	6.2049	0.0000	0.0000	down	-	down
46907994	phosphoribosylglycinamide synthetase	2.3	6.8466	7.8969	-	up	-	-
46907995	Bifunctional phosphoribosylaminoimidazole carboxy formyl formyltransferase and inosine-monophosphate cyclohydrolase	2.3	7.0981	5.8624	-	down	-	-
46907998	glutamine phosphoribosylpyrophosphate amidotransferase	2.3	7.2552	6.6059	7.9882	up	up	up
46908065	similar to dihydroorotase dehydrogenase	2.3	0.0000	6.2759	7.3043	up	up	up
46908066	similar to dihydroorotate dehydrogenase (electron transfer subunit)	2.3	6.4666	7.3925	0.0000	up	-	-
46908068	similar to carbamoyl-phosphate synthetase (glutaminase subunit)	2.3	6.9510	-	0.0000	-	-	down
46908188	similar to phosphopentomutase	2.3	-	6.0961	7.9558	-	-	up
46908710	similar to uracil phosphoribosyltransferase	2.3	7.5882	0.0000	6.3870	down	up	down

Table C.2 (continued)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
F2365								
46908730	similar to CTP synthases	2.3	5.7345	5.1643	7.1138	down	up	up
46908871	similar to thymidylate kinase	2.3	0.0000	0.0000	7.5523	-	up	up
46908945	similar to inosine-monophosphate dehydrogenase	2.3	7.3209	-	8.2932	-	-	up
46906236	similar to mevalonate diphosphate decarboxylase	2.4	6.6426	7.2573	-	up	-	-
46906237	similar to mevalonate kinases	2.4	6.9019	0.0000	5.3464	down	up	down
46907543	similar to deoxyxylulose 5-phosphate reductoisomerase	2.4	8.1007	7.3879	6.4773	down	down	down
46907583	acetyl-CoA carboxylase subunit (biotin carboxylase subunit)	2.4	7.2409	6.2887	-	down	-	-
46907600	similar to branched-chain alpha-keto acid dehydrogenase E2 subunit (lipoamide acyltransferase)	2.4	6.7113	0.0000	-	down	-	-
46907622	similar to 3-ketoacyl-acyl carrier protein reductase	2.4	7.7069	0.0000	0.0000	down	-	down
46908040	similar to malonyl CoA-acyl carrier protein transacylase	2.4	0.0000	0.0000	7.4454	-	up	up
46908041	similar to plsX protein involved in fatty acid/phospholipid synthesis	2.4	7.3560	6.2778	-	down	-	-
46906457	similar to dihydropteroate synthases	2.5	-	6.2482	7.8799	-	up	-
46906555	similar to phosphomethylpyrimidine kinase (ThiD)	2.5	-	6.1711	7.0567	-	up	-
46906556	similar to thiamin-phosphate pyrophosphorylase (ThiE)	2.5	0.0000	0.0000	7.1779	-	up	up
46907318	similar to NH(3)-dependent NAD(+) synthetases, nitrogen regulatory protein	2.5	0.0000	-	7.6137	-	-	up
46907410	similar to cobyrinic acid a,c-diamide synthase	2.5	7.1748	6.2009	6.2149	down	up	down
46907413	similar to cobalamin biosynthesis protein CbiD	2.5	7.8251	0.0000	7.2610	down	up	down
46907416	similar to precorrin-3 methylase	2.5	-	6.7189	0.0000	-	down	-
46907824	similar to iron-sulfur cofactor synthesis protein nifS	2.5	7.5088	-	6.9988	-	-	down
46907906	similar to 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase / 2-oxoglutarate decarboxylase	2.5	0.0000	6.8944	6.9709	up	up	up
46908057	similar to pantothenate metabolism flavoprotein homolog	2.5	6.3605	0.0000	-	down	-	-
46908336	similar to a protein required for pyridoxine synthesis	2.5	8.1916	-	6.5100	-	-	down
46908692	similar to B. subtilis O-succinylbenzoate-CoA synthase (MenC)	2.5	9.1893	7.9722	-	down	-	-

Table C.3   Intermediary metabolism associated proteins (ListiList category 2) with significant changes in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
HCC23								
217963486	similar to aminotransferase	2.2	-	6.3286	6.9949	-	up	-
217963773	similar to glycoprotease	2.2	-	0.0000	5.8038	-	up	-
217963862	similar to threonine dehydratase	2.2	6.8937	0.0000	7.5950	down	up	up
217963865	similar to 3-isopropylmalate dehydrogenase	2.2	0.0000	7.1708	-	up	-	-
217964072	similar to aminotripeptidase (peptidase T)	2.2	0.0000	6.3275	7.0900	up	up	up
217964139	similar to aminopeptidases	2.2	-	0.0000	7.0720	-	up	-
217964173	similar to 5-methyltetrahydrofolate-homocysteine methyltransferase (metH)	2.2	6.9662	5.3487	6.3599	down	up	down
217964227	similar to Xaa-His dipeptidase	2.2	-	7.3730	6.4941	-	down	-
217964504	similar to glycine dehydrogenase (decarboxylating) subunit 2	2.2	-	6.5236	0.0000	-	down	-
217964939	similar to proteases	2.2	4.9788	6.7772	5.4582	up	down	up
217965339	similar to histidinol dehydrogenases	2.2	6.4528	7.6489	5.8373	up	down	down
217965346	similar to NADP-specific glutamate dehydrogenase	2.2	-	6.3259	7.2104	-	up	-
217965420	similar to 3-dehydroquinate dehydratase	2.2	7.3339	6.4590	7.3420	down	up	-
217965514	similar to 1-pyrroline-5-carboxylate reductase (ProC)	2.2	-	0.0000	7.0523	-	up	-
217965691	similar to cysteine synthase	2.2	-	6.7372	7.4123	-	up	-
217965711	Zinc metalloproteinase precursor	2.2	6.4973	7.5446	0.0000	up	down	down
217965967	similar to phosphoserine aminotransferase	2.2	7.2971	-	0.0000	-	-	down
217965975	similar to peptidases	2.2	0.0000	0.0000	7.0248	-	up	up
217963684	similar to ribonucleoside-diphosphate reductase, subunit alpha	2.3	-	0.0000	6.9751	-	up	-
217964087	Bifunctional phosphoribosylaminoimidazole carboxy formyl formyltransferase and inosine-monophosphate cyclohydrolase	2.3	0.0000	-	6.5386	-	-	up
217965775	similar to inosine monophosphate dehydrogenase	2.3	-	0.0000	6.8931	-	up	-
217964039	similar to malonyl CoA-acyl carrier protein transacylase	2.4	-	0.0000	6.6733	-	up	-
217964437	similar to hydroxy-3-methylglutaryl coenzyme A synthase	2.4	7.6930	7.4968	5.1498	down	down	down
217964491	similar to geranyltranstransferase	2.4	-	0.0000	7.3806	-	-	up
217964536	similar to deoxyxylulose 5-phosphate reductoisomerase	2.4	5.9788	0.0000	7.3514	down	up	up
217963626	similar to uroporphyrinogen III decarboxylase	2.5	5.7154	-	7.3931	-	-	up
217963918	similar to heptaprenyl diphosphate synthase component II (menaquinone biosynthesis)	2.5	6.8983	0.0000	6.0969	down	up	down
217963946	similar to ketopantoate hydroxymethyltransferases	2.5	5.8681	7.1686	-	up	-	-
217963971	similar to formyl-tetrahydrofolate synthetase	2.5	5.2756	7.4664	-	up	-	-
217964022	similar to pantothenate metabolism flavoprotein homolog	2.5	7.3222	0.0000	-	down	-	-
217964175	similar to menaquinone-specific isochorismate synthase	2.5	0.0000	7.3176	0.0000	up	down	-

Table C.3 (continued)

GI#	Protein	ListiList	Intensity			Regulation		
			0h	1h	5h	0-1h	1-5h	0-5h
HCC23								
217964724	similar to cobyric acid synthase CbiP	2.5	7.0773	6.3057	-	down	-	-
217964731	similar uroporphyrinogen-III methyltransferase/uroporphyrinogen-III synthase	2.5	6.5433	7.2648	7.2300	up	-	up
217964738	similar to cobalamin biosynthesis protein CbiD	2.5	7.5931	0.0000	-	down	-	-
217965589	similar to phosphomethylpyrimidine kinase (ThiD)	2.5	6.7014	5.5369	5.8905	down	-	down

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APPENDIX D

EXTENDED PROTEOMIC ANALYSIS FROM CHAPTER II INFORMATION

PATHWAYS ASSOCIATED PROTEINS (LISTILIST CATEGORY 3) WITH

SIGNIFICANT CHANGES DURING BILE SALT STRESS

Table D.1   Information pathways associated proteins (Listilist category 3) with significant change in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGDe								
16803313	similar to ribonuclease H rnh	3.1	0.0000	-	7.8125	-	-	up
16803360	similar to DNA polymerase III (alpha subunit)	3.1	9.7137	7.1725	8.3015	down	up	down
16803605	DNA polymerase I	3.1	9.7137	-	7.9431	-	-	down
16803799	ATP-dependent DNA helicase	3.1	7.8520	7.0860	-	down	-	-
16802887	similar to excinuclease ABC, chain C (UvrC)	3.2	0.0000	7.0405	0.0000	down	down	-
16803489	similar to endonuclease IV	3.2	0.0000	5.6762	7.0554	up	up	up
16804089	similar to excinuclease ABC (subunit A)	3.2	6.4186	7.0296	-	up	-	-
16804526	excinuclease ABC (subunit A)	3.2	-	6.9792	7.6215	-	up	-
16804713	similar to UV-damage repair protein	3.2	6.2083	7.3874	6.5900	up	down	up
16803408	DNA repair and genetic recombination	3.3	0.0000	7.3560	0.0000	up	down	-
16803685	similar to ATP-dependent dsDNA exonuclease SbcC	3.3	-	0.0000	6.9806	-	up	-
16803981	similar to ATP-dependent DNA helicase	3.3	6.3707	6.6171	8.3205	up	up	up
16803315	similar to DNA topoisomerase I TopA	3.4	0.0000	7.0386	0.0000	up	down	-
16803646	similar to DNA translocase	3.4	5.9145	7.2013	0.0000	up	down	down
16803844	similar to Smc protein essential for chromosome condensation and partition	3.4	-	7.1419	7.5143	-	up	-
16802409	similar to transcription regulator	3.5.2	0.0000	6.4633	-	up	up	-
16802489	similar to transcription regulator	3.5.2	6.8873	0.0000	-	down	-	-
16802959	similar to transcription antiterminator BglG family	3.5.2	8.3543	7.3540	-	down	-	-
16803761	similar to transcriptional regulator (NifA/NtrC family)	3.5.2	0.0000	8.1410	-	up	-	-
16804781	weakly similar to transcription regulators CRP/FNR family	3.5.2	5.4908	6.5180	-	up	-	-
16802304	RNA polymerase (beta subunit)	3.5.3	8.4960	7.4707	-	down	-	-
16803362	similar to N utilization substance protein A (NusA protein)	3.5.4	0.0000	7.6060	-	up	-	-
16802907	similar to ATP-dependent RNA helicase	3.6	7.4403	7.5104	8.1434	up	up	up
16803762	similar to ATP-dependent RNA helicases	3.6	5.8441	-	7.2188	-	-	up
16804487	similar to exoribonuclease RNase-R	3.6	-	0.0000	7.1156	-	up	-
16804667	rplB 50S ribosomal protein L2	3.7.1	0.0000	7.0086	-	up	-	-
16804692	ribosomal protein S7	3.7.1	6.0833	6.8245	-	up	-	-
16803900	similar to peptidyl methionine sulfoxide reductases	3.8	-	5.5203	7.4137	-	up	-
16803307	trigger factor (prolyl isomerase)	3.9	0.0000	7.0102	7.6858	up	up	up
16803513	class I heat-shock protein (molecular chaperone) DnaK	3.9	7.0768	4.4469	-	down	-	-
16804107	class I heat-shock protein (chaperonin) GroEL	3.9	7.1684	6.6585	7.5920	down	up	up

Table D.1 (continued)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGDe								
16802947	similar to glutathione Reductase	4.1	7.2332	6.7850	7.3595	down	up	up
16802983	non-heme iron-binding ferritin	4.1	0.0000	6.7269	7.0165	up	up	up
16803473	similar to glutathione reductase	4.1	-	0.0000	7.1723	-	up	-
16803512	heat shock protein DnaJ	4.1	8.3172	7.2852	7.2973	down	-	down
16802536	similar to acylase	4.2	-	6.0535	7.5420	-	up	-
16803644	similar to 2-cys peroxiredoxin	4.2	0.0000	5.7611	7.2880	up	up	up
16804822	catalase	4.2	6.8721	0.0000	6.8622	down	up	-
16802248	listeriolysin O precursor	4.5	8.5253	6.6911	0.0000	down	down	down
16804848	similar to GTPase	4.5	-	5.5499	8.8399	-	up	-

Table D.2   Information pathways associated proteins (Listilist category 3) with significant changes in protein expression levels during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
F2365								
46907989	ATP-dependent DNA helicase	3.1	8.3226	6.9907	7.5600	down	up	down
46908056	similar to primosomal replication factor Y	3.1	8.1042	7.0723	7.0495	down	down	down
46906446	transcription-repair coupling factor	3.2	8.7071	6.6162	-	down	-	-
46906466	similar to DNA repair protein Sms	3.2	7.3560	8.3130	6.2723	up	down	down
46907450	similar to DNA polymerase beta, to B. subtilis YshC protein	3.2	6.2963	7.0393	5.2244	up	up	down
46907451	similar to MutS protein (MutS2)	3.2	7.2516	6.0344	-	down	-	-
46908285	similar to excinuclease ABC (subunit A)	3.2	0.0000	7.7171	6.6456	up	up	up
46908660	excinuclease ABC (subunit A)	3.2	8.3152	7.4718	-	down	-	-
46908661	excinuclease ABC (subunit B)	3.2	0.0000	5.8013	6.8961	-	up	-
46907626	Recombination protein recA	3.3	6.1950	0.0000	-	down	-	-
46907874	similar to ATP-dependent dsDNA exonuclease SbcC	3.3	6.8480	8.3220	7.5935	up	down	up
46906231	DNA gyrase subunit A	3.4	7.9261	6.1112	-	down	-	-
46907098	similar to ATP-dependent RNA helicase	3.6	7.5195	-	6.5942	-	-	down
46907952	similar to ATP-dependent RNA helicases	3.6	-	0.0000	7.7939	-	up	-
46907003	similar to lipoate-protein ligase	3.8	0.0000	-	6.8878	-	-	up
46907493	trigger factor (prolyl isomerase)	3.9	7.3307	6.2488	-	down	-	-
46907701	class I heat-shock protein (molecular chaperone) DnaK	3.9	9.3159	7.0400	7.6381	down	up	down
46908303	class I heat-shock protein (chaperonin) GroEL	3.9	6.9716	0.0000	6.8403	down	up	down
46906443	similar to B. subtilis general stress protein	4.1	-	0.0000	6.4392	-	up	-
46906533	similar to heat-shock protein htrA serine protease	4.1	-	0.0000	7.1363	-	up	-
46907141	similar to glutathione Reductase	4.1	7.0610	8.1700	7.1563	up	down	up
46907195	similar to putative heat shock protein HtpX, Listeria epitope LemB	4.1	7.9454	6.6838	0.0000	down	down	down
46908975	catalase	4.2	4.6181	6.4091	-	up	-	-
46906361	similar to bacteriophage minor tail proteins	4.3	6.9853	5.3919	-	down	-	-
46908969	similar to probable GTP-binding protein	4.5	7.1343	5.6044	-	down	-	-

Table D.3 Information pathways associated proteins (Listilist category 3) with significant changes in protein expression level during bile salt stress

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
HCC23								
217964093	ATP-dependent DNA helicase	3.1	-	6.5627	7.6203	-	up	-
217964288	DNA polymerase I	3.1	8.2026	4.5075	6.7557	down	-	up
217964706	similar to B. subtilis ribonuclease HIII	3.1	6.4217	-	7.2935	-	-	up
217963803	similar to excinuclease ABC (subunit A)	3.2	7.3458	8.3471	7.0427	up	down	down
217964449	DNA mismatch repair protein	3.2	0.0000	7.0553	0.0000	up	down	-
217964450	DNA mismatch repair (recognition)	3.2	6.6055	7.4424	6.5693	up	down	-
217964703	similar to DNA polymerase beta, to B. subtilis YshC protein	3.2	6.3006	-	7.2676	-	-	up
217965753	similar to ATP dependent helicase	3.2	7.0728	0.0000	-	down	-	-
217963571	similar to ATP-dependent deoxyribonuclease (subunit B)	3.3	-	7.8113	7.0666	-	down	-
217964203	similar to ATP-dependent dsDNA exonuclease SbcC	3.3	7.8246	-	6.5567	-	-	down
217964328	similar to single-stranded-DNA-specific exonuclease (RecJ)	3.3	-	0.0000	6.9026	-	-	up
217964344	similar to exodeoxyribonuclease V	3.3	0.0000	6.3907	-	up	-	-
217964570	similar to DNA gyrase-like protein (subunit A)	3.4	6.1312	7.1962	-	up	-	-
217964025	similar to RNA-binding Sun protein	3.6	5.8658	7.1158	-	up	-	-
217964101	similar to hypothetical RNA methyltransferase	3.6	0.0000	6.0420	7.3961	up	up	up
217964323	similar to tRNA-guanine transglycosylase Tgt	3.6	0.0000	-	6.6669	-	-	up
217964341	similar to putative tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	3.6	6.6567	5.4955	-	down	-	-
217965039	similar to ATP-dependent RNA helicase	3.6	5.8417	7.0216	7.1863	up	up	up
217964141	similar to methionine aminopeptidases	3.8	0.0000	0.0000	6.8958	-	-	up
217964590	trigger factor (prolyl isomerase)	3.9	7.4434	6.5446	7.6679	down	up	up
217964419	similar to glutathione reductase	4.1	6.1917	6.6269	7.6697	-	up	up
217963559	similar to nitrilotriacetate monooxygenase	4.2	8.0131	6.9926	0.0000	down	down	down
217966008	catalase	4.2	5.6313	7.2799	-	up	-	-
217965143	similar to ATP/GTP-binding protein	4.5	0.0000	0.0000	7.0916	-	up	-

APPENDIX E

DIFFERENTIALLY EXPRESSED PROTEINS THAT ARE SIMILAR AND

DISSIMILAR TO UNKNOWN PROTEINS

Table E.1   Differentially expressed proteins that are similar to unknown proteins (Listilist Category 5) and Proteins with no similarity (Listilist Category 6)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
EGDe								
16802067	similar to unknown proteins	5.2	6.9224	-	5.9284	-	-	down
16802206	conserved hypothetical protein	5.2	0.0000	6.1094	7.1591	-	down	up
16802236	similar to B. subtilis YabH protein	5.2	0.0000	0.0000	7.5748	-	up	up
16802239	similar to unknown proteins	5.2	0.0000	6.7749	7.7129	up	up	up
16802313	similar to other proteins	5.2	0.0000	0.0000	7.6011	-	up	up
16802412	conserved hypothetical protein similar to B. subtilis YwbN protein	5.2	6.3019	7.1269	8.0141	up	up	up
16802577	similar to unknown proteins	5.2	7.2437	6.1941	0.0000	down	down	down
16802830	similar to unknown proteins	5.2	8.1527	-	7.6941	-	-	down
16802835	similar to conserved hypothetical protein	5.2	0.0000	6.8035	0.0000	up	down	-
16803044	conserved hypothetical protein	5.2	0.0000	5.9861	8.1297	up	up	up
16803132	similar to B. subtilis Yuek protein	5.2	0.0000	6.7550	0.0000	up	down	-
16803439	similar to phosphodiesterase	5.2	7.6775	6.4085	4.9786	down		down
16803735	similar to putative membrane proteins	5.2	7.7379	5.8077		down		
16803885	similar to conserved hypothetical proteins	5.2	-	6.5520	0.0000	-	down	-
16803957	similar to unknown proteins	5.2	0.0000	6.6742	7.1612	up	up	up
16803988	similar to unknown proteins	5.2	6.9482	0.0000	-	down	-	-
16804067	similar to unknown proteins	5.2	0.0000	6.6052	0.0000	up	down	-
16804260	similar to unknown proteins	5.2	0.0000	-	6.4932	-	-	up
16804263	similar to unknown proteins	5.2	0.0000	6.5973	-	up	-	-
16804398	transmembrane protein	5.2	-	7.2521	5.9098	-	-	down
16804823	similar to hypothetical protein	5.2	-	0.0000	7.3557	-	-	up
16804890	similar to B. subtilis Jag protein	5.2	0.0000	0.0000	6.9266	-	up	up
16802667	hypothetical protein	6	7.1332	-	0.0000	-	-	down
16802744	hypothetical protein	6	0.0000	-	6.9363	-	-	up
16803168	hypothetical protein	6	6.8643	-	7.6710	-	-	up
16803202	hypothetical protein	6	0.0000	0.0000	6.7566	-	up	up
16804143	hypothetical protein	6	7.8614	-	6.5037	-	-	down

Table E.2   Differentially expressed proteins that are similar to unknown proteins (Listilist Category 5) and no similarity (Listlist Category 6)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
F2365								
46906325	conserved hypothetical protein	5.1	7.0571	8.4769	8.5173	up	-	up
46906326	similar to other unknown proteins	5.1	6.9789	8.9143	8.2547	up	down	up
46906294	similar to B. subtilis YukA protein	5.2	7.3701	8.5700	7.5253	up	down	-
46906371	conserved hypothetical protein	5.2	6.0500	0.0000	0.0000	down	-	down
46906403	conserved hypothetical protein	5.2	6.1514	7.0921	-	up	-	-
46906418	similar to B. subtilis YabE protein	5.2	-	0.0000	6.7272	-	up	-
46906447	conserved membrane-spanning protein	5.2	-	6.1401	7.2665	-	up	-
46906507	similar to other proteins	5.2	0.0000	5.6275	7.4348	up	up	up
46906691	conserved hypothetical protein	5.2	-	0.0000	6.9975	-	up	-
46906707	similar to unknown proteins	5.2	-	6.7019	7.4398	-	up	-
46906775	hypothetical protein	5.2	7.4077	6.6589	6.4356	down	-	down
46906803	conserved hypothetical protein	5.2	6.8004	-	8.3521	-	-	down
46907022	hypothetical protein	5.2	5.6484	-	6.9426	-	-	up
46907257	hypothetical protein	5.2	6.7894	0.0000	-	down	-	-
46907443	similar to different proteins	5.2	7.4285	8.1559	-	up	-	-
46907560	similar to B. subtilis YqzD protein	5.2	-	7.8023	6.5083	-	down	-
46907563	similar to B. subtilis yqgP	5.2	0.0000	-	7.1592	-	up	-
46907719	similar to unknown proteins	5.2	7.4726	0.0000	6.6499	down	-	down
46907807	CBS domain-containing protein	5.2	7.7833	-	5.8290	-	-	down
46908046	similar to unknown proteins	5.2	8.1247	0.0000	6.7061	down	-	down
46908051	similar to unknown proteins	5.2	8.1651	6.4242	0.0000	down	-	down
46908152	similar to unknown proteins	5.2	7.5349	6.5135	-	down	-	-
46908366	similar to unknown protein	5.2	0.0000	6.7999	-	up	-	-
46908530	transmembrane protein	5.2	-	0.0000	6.9184	-	up	-
46908565	conserved hypothetical protein similar to B. subtilis YhfK protein	5.2	0.0000	0.0000	7.2443	-	up	up
46908582	similar to conserved hypothetical proteins	5.2	-	5.9197	7.3804	-	up	-
46908724	conserved hypothetical protein	5.2	0.0000	-	7.5494	-	-	up
46908762	similar to ATP binding proteins	5.2	6.9830	-	0.0000	-	-	down
46907307	hypothetical protein	6	0.0000	6.7151	-	up	-	-
46907982	hypothetical protein	6	6.8487	0.0000	0.0000	down	-	down

Table E.3   Differentially expressed proteins that are similar to unknown proteins (Listilist Category 5) and no similarity (Listilist Category 6)

GI#	Protein	ListiList	Intensity			Expression		
			0h	1h	5h	0-1h	1-5h	0-5h
HCC23								
217965765	hypothetical protein	5.1	0.0000	0.0000	6.8010	-	up	up
217963771	lipoprotein	5.1	7.2399	6.4504	6.2491	down	-	down
217963319	conserved hypothetical protein	5.2	0.0000	5.7803	8.5160	up	up	up
217963501	similar to B. subtilis YuzD protein	5.2	0.0000	0.0000	6.8296	-	up	up
217963670	enoyl-ACP reductase	5.2	7.1622	-	5.5804	-	-	down
217963812	S-adenosyl-methyltransferase MraW	5.2	-	0.0000	7.1863	-	up	-
217963824	pyridoxal phosphate enzyme, YggS family	5.2	6.0233	6.1720	7.2388	-	up	up
217963989	similar to dehydogenases and hypothetical proteins	5.2	-	7.4568	4.8269	-	down	-
217964156	similar to putative membrane proteins	5.2	6.0433	6.8360	-	up	-	-
217964318	similar to unknown proteins	5.2	0.0000	0.0000	6.3427	-	up	up
217964386	GatB/Yqey domain protein	5.2	6.5660	7.7644	0.0000	up	down	down
217964405	conserved hypothetical protein	5.2	0.0000	6.5459	-	up	-	-
217964520	similar to B. subtilis YqzC protein	5.2	0.0000	6.6415	5.9022	up	down	up
217964535	conserved hypothetical protein similar to B. subtilis YluC protein	5.2	7.0432	-	0.0000	-	-	down
217964542	hypothetical Protein	5.2	0.0000	6.8474	-	up	-	-
217964574	similar to Lactococcus lactis LacX protein	5.2	6.1604	7.0278	6.9932	up	down	down
217964834	hypothetical protein	5.2	0.0000	-	6.9200	-	-	up
217964950	hypothetical protein	5.2	7.3174	0.0000	4.9392	down	up	down
217964966	similar to B. subtilis YhbA protein	5.2	-	0.0000	6.9565	-	up	-
217965005	conserved hypothetical protein	5.2	-	8.8004	5.9251	-	down	-
217965122	CoA-substrate-specific enzyme activase	5.2	7.8977	8.4125	-	up	-	-
217965353	hypothetical Protein	5.2	6.6435	7.4466	6.7455	up	down	-
217965372	similar to unknown proteins	5.2	8.2150	7.4435	7.2705	down	-	down
217966197	hypothetical protein	5.2	0.0000	0.0000	7.3096	-	up	up
217966202	similar to B. subtilis YbaF protein	5.2	5.8231	-	0.0000	-	-	down

APPENDIX F

DIFFERENTIALLY EXPRESSED PROTEINS THAT ARE UNKNOWN

Table F.1   Unknown proteins differentially expressed (Listilist Category 7)

GI#	Protein	ListiList	Intensity			Regulation		
			0h	1h	5h	0-1h	1-5h	0-5h
HCC23								
217963619	unknown	7	-	7.1345	7.6679	-	up	-
217964644	unknown	7	5.7625	7.2505	5.9693	up	down	up
217964645	unknown	7	8.1115	7.5142	6.8958	down	down	down
217964644	unknown	7	-	7.2505	5.9693	-	down	-
217964834	unknown	7	-	0.0000	6.9200	-	up	-
217965416	unknown	7	6.6858	0.0000	-	down	-	-
217965530	hypothetical protein (EGD=87%)	7	6.4701	7.1177	0.0000	up	down	down
217965648	unknown	7	6.4078	0.0000	-	down	-	-
217966014	unknown	7	-	7.1245	0.0000	-	down	-
217966270	unknown	7	-	6.3563	5.7775	-	down	-
F2365								
46906306	unknown	7	7.5407	6.6954	-	down	-	-
46906902	unknown	7	6.9953	5.2544	-	down	-	-
46908103	unknown	7	7.9313	4.7715	-	down	-	-
46908582	unknown	7	6.8675	-	7.3804	-	-	up