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Review

Effect of bile salts on the DNA and membrane integrity of enteric bacteria

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Enteric bacteria are able to resist the high concentrations of bile encountered throughout the gastrointestinal tract. Here we review the current mechanisms identified in the enteric bacteria *Salmonella*, *Escherichia coli*, *Bacillus cereus* and *Listeria monocytogenes* to resist the dangerous effects of bile. We describe the role of membrane transport systems, and their connection with DNA repair pathways, in conferring bile resistance to these enterics. We discuss the findings from recent investigations that indicate bile tolerance is dependent upon being able to resist the detergent properties of bile at both the membrane and DNA level.

Introduction

The digestive system typically combats potentially pathogenic microbes through the production of several bactericidal agents along the tract. Some of these bactericidal agents are gastric secretions, hydrochloric acid and bile. These agents have distinct roles in ensuring infections do not arise, but depending on the conditions they are not always effective in eliminating pathogens. Enterics have developed mechanisms that allow for their survival in the dangerous environments encountered in the gastrointestinal tract. This review will focus on several recently discovered mechanisms that allow for protection and continued proliferation in the stressful environment of high concentrations of bile salts for the Gram-negative enteric pathogenic bacteria *Escherichia coli* and *Salmonella enterica,* and the Gram-positive enteric pathogenic bacteria *Listeria monocytogenes* and *Bacillus cereus.* The effect of bile on the integrity of the membrane has been reviewed by others (Begley *et al.*, 2005a) and therefore will not be included in extensive detail in this review. The aim of this review is to aid in establishing a cohesive link between the effects of bile salts on bacteria and a common mechanism of resistance as it relates to protection of the DNA and the cell membrane among the enterics.

Background

Three of the main bactericidal agents produced by the gastrointestinal system are gastric secretions, hydrochloric acid and bile. Gastric secretions and hydrochloric acid together lower the pH of the stomach to approximately 3.0. This acidic environment destroys the majority of bacteria that enter the stomach. The importance of this acidic environment is evident in studies with patients with the disease hypochlorhydria. These patients produce less gastric juice, resulting in an increase in the number of bacteria that survive within the stomach. Since the bactericidal property of the stomach is weakened in these patients, potentially pathogenic microbes can then migrate to the small intestine and establish disease. This is evident by the fact that hypochlorhydria patients are more prone to infections by *Helicobacter pylori* and *Salmonella* spp. (McGowan *et al.*, 1996; Tennant *et al.*, 2008).

Bile is another bactericidal agent that is found in the digestive system. Bile is composed of a multitude of components, including proteins, ions, pigments, cholesterol and various bile salts. Of these components bile salts have been shown to provide protection against pathogenic bacteria. For instance, the small intestine, which contains a very high amount of bile acids, typically harbours very few bacteria (Inagaki *et al.*, 2006). If less bile is secreted, such as observed in patients with cirrhosis of the liver, bacterial overgrowth is observed in the small intestine (Ding *et al.*, 1993; Slocum *et al.*, 1992). This suggests that bile salts have bactericidal properties in addition to aiding in the digestion of fatty acids.

The primary bile acids cholic acid and chenodeoxycholic acid are synthesized in the liver from cholesterol (Hofmann, 1999; Okoli *et al.*, 2007). Further metabolism in the liver results in the formation of 'conjugated' bile salts through the attachment of either a glycine or taurine to the side chain of these various bile acids. These bile salts are then concentrated and stored in the gall bladder until the enterohepatic circulation is activated by the intake of food (Ridlon *et al.*, 2006) (Fig. 1). Once activated, cholecystokinin triggers the contraction of the gall bladder. This contraction leads to the release of bile into the intestines (Ridlon *et al.*, 2006). A small portion of the bile salts escape the enterohepatic circulation and are further metabolized into the secondary bile salts deoxycholic acid and lithocholic acid by bacterial 7-α-dehydroxylation found in the lumen of the intestine (Monte *et al.*, 2009; Ridlon *et al.*,

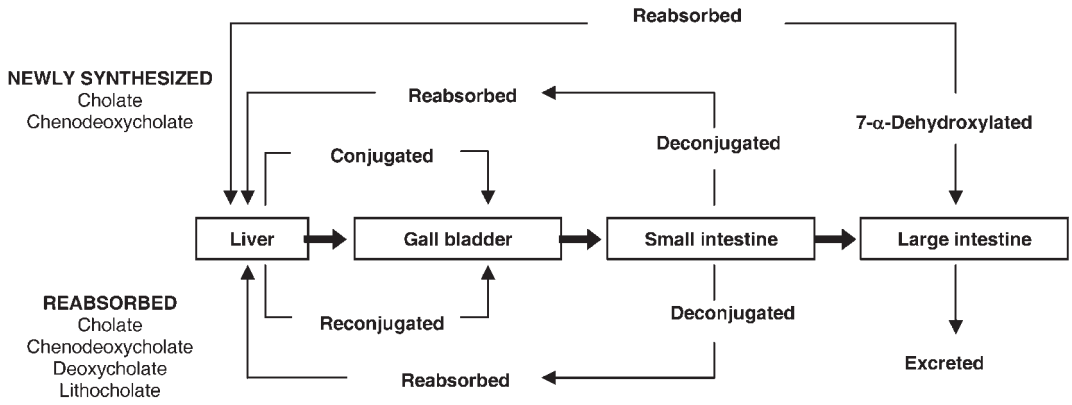

Fig. 1. Bile salt synthesis, processing and cycling through the human gastrointestinal system. Bile salts are conjugated with either glycine or taurine before passing to the gall bladder. From here, they are circulated throughout the enterohepatic cycle. Bile salts are either excreted or reabsorbed by the liver.

2006). Additionally, intestinal bacteria further metabolize lithocholic acid into the tertiary bile salt ursodeoxycholic acid (Hay & Carey, 1990). Since the composition of bile, especially in regards to the type of bile salts present, may change as it passes through the gastrointestinal tract, understanding the differences in the antimicrobial properties of both conjugated and unconjugated forms of bile salts is of great importance in combating bile-salt-resistant pathogenic bacteria. As a result many studies elucidating the bactericidal role of bile salts have been conducted on bile mixtures that contain both conjugated and unconjugated forms of salts, such as bile from bovine and ovine gall bladder (oxgall) (Ding & Shah, 2007; Kheadr *et al.*, 2007; Van der Aa Kuhle *et al.*, 2005), bovine bile (Paterson *et al.*, 2009) and human bile (Alvarez *et al.*, 2003).

Even though the gastric microbial barriers of the stomach and small intestine decrease the chance of colonization by pathogenic bacteria, they do not provide protection against bacteria that have adapted to survive within these extremely harsh conditions. The enteric bacteria are one class of bacteria that have mechanisms that allow for survival and proliferation within the human gut. Several of these bacteria invade the gall bladder, including *Listeria monocytogenes*, *S. enterica*, intestinal colonizer enteroaggregative *Escherichia coli* and faeces-present *Bacillus cereus* (Crawford *et al.*, 2008; Hardy *et al.*, 2004; Joo *et al.*, 2007; Kristoffersen *et al.*, 2007). It is possible that the ability of these microbes to survive in the presence of large quantities of bile salts is directly related to their ability to establish invasive infections.

In recent years, much work has been dedicated to understanding the role of bile salts in the resistance of bacteria, especially the enterics. It has been speculated that the pathogenic potential of an enteric bacterium is directly related to its ability to grow in the presence of bile salts. However, to determine if this hypothesis is true the mechanisms by which bacteria are able to grow in bile salt environments need to be determined. To date, the mechanisms by which bile induces cell death are poorly understood; it has not been determined whether cell death results from damage at the membrane and/or DNA level. It is possible that the antimicrobial effects of bile salts elicit various mechanisms of resistance, including the activation of several different stress-response genes involved in membrane synthesis and protection, as well as in DNA repair (Bernstein *et al.*, 1999; Kristoffersen *et al.*, 2007; Prieto *et al.*, 2006).

Determining the effect that bile salts have on the integrity of the bacterial membrane mainly has been investigated through molecular analyses studying the regulation of membrane proteins in the presence of bile salts (Ruiz *et al.*, 2009). Analysing the regulation of genes encoding outer-membrane proteins, efflux pumps and cell membrane biosynthesis enzymes has indicated that bile salts interact with bacterial cell membranes (Nikaido *et al.*, 2008; Rince *et al.*, 2003; Ruiz *et al.*, 2009). Efflux pumps have been shown in various pathogenic and commensal bacteria, such as *Escherichia coli*, *Vibrio cholerae* and *Campylobacter jejuni*, to expel bile salts from the cytoplasm after they have breached the cell membrane (Chatterjee *et al.*, 2004; Lin *et al.*, 2003; Thanassi *et al.*, 1997). The membrane damaging capability of bile salts has also been demonstrated by the fact that bile resistance requires the products of the *tol–pal* genes, which are essential for preserving the outer membrane of Gram-negative bacteria such as *Escherichia coli* and *Erwinia chrysanthemi* (Dubuisson *et al.*, 2005; Prouty *et al.*, 2002; Pucciarelli *et al.*, 2002; Ray *et al.*, 2000). Additionally, the enterobacterial common antigen, a glycolipid found in the outer membrane of *Enterobacteriaceae*, has also been found to be required for bile resistance in *S. enterica* (Ramos-Morales *et al.*, 2003). LPS (Froelich *et al.*, 2006; Picken & Beacham, 1977), PhoPQ (Van Velkinburgh & Gunn, 1999) and DNA adenine methyltransferase (Heithoff *et al.*, 2001; Pucciarelli *et al.*, 2002) have also been shown to be important for bile

resistance. These findings corroborate that the membrane and various components of the membrane are important for bacterial resistance to bile salts.

In addition to analysing the regulation of genes involved in cell membrane synthesis, studies have also investigated the interaction of bile with the bacterial cell membrane by analysing the composition of membrane grown in the presence of bile. Bile alters the fatty acid composition, as well as the ratio of membrane proteins to phospholipids, resulting in an altered cell surface structure in bacteria such as *Bifidobacterium animalis* and *Lactobacillus reuteri* (Ruiz *et al.*, 2007; Taranto *et al.*, 2003). Visual confirmation of cell surface deformities induced by bile salts has been accomplished by scanning electron microscopy and transmission electron microscopy (Breton *et al.*, 2002; Bron *et al.*, 2004; Ruiz *et al.*, 2007).

Bile-induced damage in Gram-negative bacteria

Escherichia coli

Escherichia coli is an enteric pathogen that has been extensively studied as a model organism for the effect of bile salts on Gram-negative bacteria. An initial study in 1991 by Kandell and Bernstein investigated whether bile salts could directly induce damage to the DNA of *Escherichia coli* using a modified SOS chromotest (Kandell & Bernstein, 1991). In the presence of chenodeoxycholic acid and sodium deoxycholate (NaDC), *Escherichia coli* showed an increased expression of the gene *sulA*. *sulA* is part of the SOS response system of bacteria, and acts to stall cell division through inhibiting the formation of the FtsZ ring, which is a critical step in the early stages of cell division (Jones & Holland, 1985). This result indicated that the SOS response is induced in the presence of bile salts. This also suggested that the activation of the SOS response is required for the survival of the bacterium in the presence of bile salts. The authors compared their results to those of SOS-deficient cells in the presence of mitomycin c, a known inducer of the SOS response. Both studies produced similar results, supporting the theory that bile salts induce DNA damage *in vivo* in bacteria and that exposure activates the SOS response.

Expanding upon these results, Bernstein *et al.* (1999) (Table 1) investigated the stress response of *Escherichia coli* to bile salts. They tested the effect that the bile salts sodium deoxycholate (NaDC), sodium chenodeoxycholate (NaCDC), sodium ursodeoxycholate (NaUDC) and sodium glycocholate (NaGC) had on 13 specific *Escherichia coli* stress response genes (*osmY*, *recA*, *umuDC*, *micF*, *clpB*, *dinD*, *zwf*, *soi28*, *nfo*, *katG*, *uspA*, *merR*, *ada*). Using a similar technique as the Kandell & Bernstein (1991) study, the promoters of each gene were fused with a *lacZ* reporter gene, allowing for detection of activity by measuring the level of β-galactosidase. The results of the study indicated that the promoters *dinD*, *micF* and *osmY* were significantly activated by all four bile salts. *dinD* is well known for being induced in the presence of DNA damage (Kenyon & Walker, 1980; Lundegaard & Jensen, 1994; Ohmori *et al.*, 1995; Weel-Sneve *et al.*, 2008), but its function remains unknown. The increased expression of *dinD* suggests that the SOS response is a possible mechanism elicited in response to bile salts. *osmY* encodes a periplasmic protein of unknown function commonly involved in osmotic stress and *micF* is a negative regulator for the outer-membrane porin protein OmpF (Oh *et al.*, 2000). The increased transcription levels of *osmY* and *micF* genes suggest that oxidative damage could occur following exposure to bile (Chou *et al.*, 1993; Oh *et al.*, 2000) (Table 1). Together, these results suggest that bile salts could potentially induce DNA damage through oxidative stress.

Recently a study of enteroaggregative *Escherichia coli* showed that bile salts induce error-prone DNA repair in strains containing an *imp*-positive locus (Joo *et al.*, 2007). Error-prone repair involves the polymerases Pol IV (*dinB*) and Pol V (*umuDC*). This mechanism allows for cells to continue to replicate in the presence of DNA damage, although it also leads to an increase in spontaneous mutations (Foster, 2007). In the study the expression pattern of the LexA-repressed gene *impB*, which is involved in error-prone DNA repair and a known homologue of *umuC*, was analysed following treatment with either UV irradiation or bile salts. Following treatment the SOS response gene *lexA* was derepressed and the *impB* gene was upregulated. Thus, it was proposed that SOS is induced to allow for repair of the damaged DNA and continued survival. In support of this hypothesis, treatment of the *Escherichia coli impB* mutants with 1 % NaDC led to a significant decrease in cell survival. This study provided further evidence that bile salts damage both the membrane and the DNA of bacteria.

Salmonella spp.

Salmonella typhimurium is an important enteric pathogen and is associated with diseases such as gastroenteritis. It is also a chronic colonizer of the gastrointestinal system (Ohl & Miller, 2001). A recent study investigated the role of the drug-resistance operon *marRAB* in conferring bile resistance to *S. typhimurium* (Prouty *et al.*, 2004). The *marRAB* operon, a regulator of multiple antibiotic resistance, consists of a repressor (*marR*) and a positive transcriptional regulator (*marA*) of antibiotic-resistance genes, such as the efflux genes *acrA* and *acrB* (Sulavik *et al.*, 1997). Using microarray analyses, β-galactosidase activity assays, gel electrophoretic mobility shift assays and bile resistance assays, this study demonstrated that the *marRAB* operon is activated in the presence of bile. It was also suggested that resistance to bile and to antibiotics is interconnected to the survival of *S. typhimurium* within a host. A model was proposed in which bile salts enter the bacterium and then the bile salts bind with MarR, resulting in increased transcription of the *mar* operon. This regulation would in turn affect unknown genes required for survival within the host. The *acrAB* efflux pump, which was also found to be

Table 1. Genes upregulated in bile-treated Gram-negative bacteria

Gene	Bile salt	Strain	Reference
Adaptation to atypical conditions			
clpB	NaUDC, NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
dps	NaDC	*S. typhimurium*	Prieto *et al.* (2006)
uspA	NaGC, NaDC	*E. coli*	Bernstein *et al.* (1999)
Cell wall			
osmY	NaGC, NaUDC, NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
Detoxification			
micF	NaGC, NaUDC, NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
katG	NaGC	*E. coli*	Bernstein *et al.* (1999)
soi28 (*sox*)	NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
DNA repair and recombination			
dinB	NaDC, bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
dinD	NaGC, NaUDC, NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
impB	NaDC	*E. coli*	Joo *et al.* (2007)
nfo	NaGC, NaUDC, NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
	NaDC, ox bile	*S. typhimurium*	Prieto *et al.* (2006)
recA	NaGC, NaCDC	*E. coli*	Bernstein *et al.* (1999)
	NaDC, NaGC, NaTC, NaGCDC, ox bile	*S. typhimurium*	Prieto *et al.* (2006)
recB	NaDC, bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
recC	NaDC, bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
recD	NaDC, bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
recJ	NaDC, bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
sbcB	NaDC, bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
sulA	NaDC, NaCDC	*E. coli*	Kandell & Bernstein (1991)
umuDC	NaUDC	*E. coli*	Bernstein *et al.* (1999)
xthA	Bovine bile	*S. typhimurium*	Prieto *et al.* (2006)
Metabolism			
fumC	NaDC	*S. typhimurium*	Prieto *et al.* (2006)
zwf	NaGC, NaCDC, NaDC	*E. coli*	Bernstein *et al.* (1999)
Transcriptional regulation			
acrAB	NaC	*S. typhimurium*	Prouty *et al.* (2004)
ada	NaUDC	*E. coli*	Bernstein *et al.* (1999)
marRAB	NaDC	*S. typhimurium*	Prouty *et al.* (2004)
merR	NaUDC, NaCDC	*E. coli*	Bernstein *et al.* (1999)

E., *Escherichia*; NaGCDC, sodium glycochenodeoxycholate; NaTC, sodium taurocholate; *S.*, *Salmonella*.

necessary for bile resistance, is transcribed in tandem to allow for the excretion of bile salts from inside the bacterium. Based on their model, activation of *marRAB*, which acts to reduce transcription of the *ompF* porin (Alekshun & Levy, 1997), would reduce the influx of bile salts into the cell, while *acrAB* would promote the efflux of bile salts out of the cell, thus creating a mechanism for resisting the damaging effect of bile salts.

The DNA damaging effect of bile salts and the bacterial response mechanisms utilized during exposure to bile have also been analysed in *S. enterica* using the bile sensitive DNA adenine methyltransferase (*dam*) mutant SV4392 (Prieto *et al.*, 2004) (Table 1). Using a random insertion to desensitize the strain to bile, they discovered that the mismatch repair proteins MutH, MutL and MutS confer bile sensitivity to *dam* mutants. RecA, a recombination protein, is a well known indicator of SOS response and is important to several repair processes in bacteria (Pierré & Paoletti, 1983). A β-galactosidase activity assay demonstrated that the SOS response was induced in the presence of NaDC and bovine bile only when a functional RecA protein was present. Reversions were detected in three alleles: *hisC3072* (a +1 frameshift), *hisG46* (a nucleotide substitution causing a missense mutation) and *leuA414* (a nucleotide substitution resulting in an amber codon). This work provided evidence that bile increases the frequency of nucleotide substitutions, frameshifts and chromosomal rearrangements, further supporting the idea that bile is a DNA damaging agent and possibly produces double-strand DNA breaks.

Another study by Prieto *et al.* (2006) provided evidence that the SOS response and homologous recombination are used as repair mechanisms in the presence of bile salts. This study indicated that RecA, RecBCD and PolV are required

for survival in the presence of bile salts (Prieto *et al.*, 2006). The RecBCD pathway is a recombination repair process activated in the presence of double-strand breaks and has been shown to be essential to the virulence of *S. enterica* (Cano *et al.*, 2002). To determine whether bile induces oxidizing or alkylating DNA damage, various assays were performed using strains deficient in genes involved in oxidative repair or alkylation DNA repair. Bile was found to act more as an oxidizing agent rather than an alkylating agent based on the MICs against these pathway-specific mutants. The study also indicated a role for base-excision repair in the presence of bile-induced damage. The investigators proposed a model for DNA repair in response to bile-induced damage: initial lesions produced by bile salts are repaired by Dam-directed mismatch repair and by base-excision repair, which in turn induce the SOS response and possibly impair DNA replication. DinB and RecBCD would then be required to repair the damaged DNA and aid in restarting replication. This study was essential in supporting the theory that bile salts act as DNA damaging agents and that DNA repair in virulent bacteria allows for survival and proliferation within the host's digestive system.

Bile-induced damage in Gram-positive bacteria

Bacillus cereus

Bacillus cereus is a common cause of food-borne acquired infections, making it an important bacterium to study in relation to its interaction with the host's gastrointestinal tract. The pathogenesis of this bacterium is not fully understood, especially in regards to its ability to colonize the human intestine. There are two proposed methods of infection: (1) infections are mediated by the production of a toxin, and (2) infections are mediated by the production of spores and the subsequent release of a toxin (Stenfors Arnesen *et al.*, 2008). In both cases either the cells or the endospores must resist the presence of bile salts to establish the infection. A study conducted on 40 strains of *Bacillus cereus* in the presence of bile salts showed that low levels of bile salts had a significant effect on survival. It was found that 100 genes were upregulated and 133 genes were downregulated (Kristoffersen *et al.*, 2007) (Table 2). Genes involved in general stress response, such as efflux pumps and transcriptional regulators (including MarR), were upregulated. Several genes associated with cell motility, cell wall and membrane synthesis, and DNA replication, recombination and repair were downregulated in the presence of bile. However, the ability of bile to induce oxidative damage was supported by the upregulation of genes involved in oxidative protection (superoxide dismutase and thioredoxins) and several chaperone-encoding genes. The motility genes *motA* and *cheY* were also upregulated, possibly indicating the cell's chemotactic response to bile salts. Additionally, the strains were only able to grow in the presence of low concentrations of bile salts [sodium cholate (NaC) : NaDC, 1 : 1]. The upregulation of genes encoding efflux pumps and other membrane components, as well as transcriptional regulators and chaperones, provide support that membrane and DNA protection mechanisms are utilized for the survival of *Bacillus cereus* in the presence of bile.

This study also tested the possibility that spore-production is essential for the pathogenesis of *Bacillus cereus* (Kristoffersen *et al.*, 2007). It was found that spores were able to tolerate high levels of bile, indicating that the spores are much more resistant to bile damage. This result suggests that *Bacillus cereus* endospore formation could be a preferred mechanism for establishing an enteric infection.

Listeria monocytogenes

Listeria monocytogenes is a food-borne pathogen that is responsible for nearly 28 % of food-related deaths each year (Mead *et al.*, 1999). This Gram-positive bacterium, like the Gram-negative *S. enterica*, can grow in stressful environments such as the gall bladder (Hardy *et al.*, 2004). Additionally, both *Listeria monocytogenes* and *S. enterica* respond to stress similarly (Gahan & Hill, 1999). Several genes have been identified to be important for bile resistance, including genes involved in the preservation of the cell envelope and in stress response (Begley *et al.*, 2002) (Table 2). Recently, it was found that *Listeria monocytogenes* contains genes required for bile resistance and these genes are regulated by the main virulence regulator *prfA* (Begley *et al.*, 2005b; Dussurget *et al.*, 2002) (Table 2). These genes are the *btlB* and *bsh* genes, and are involved in detoxifying bile salts that have been conjugated with either glycine or taurine (Begley *et al.*, 2005b). Another important discovery was the identification of a novel bile-exclusion system regulated by *prfA* that allows *Listeria monocytogenes* to survive high concentrations of bile salts (Sleator *et al.*, 2005). Additionally, it was found that the nucleotide-excision-repair protein UvrA is important for survival in bile salts (Kim *et al.*, 2006). The deletion of *uvrA* resulted in a significant impairment of the growth of *Listeria monocytogenes* in as little as 0.3 % bile salts.

The ability of *Listeria monocytogenes* to survive in the presence of bile salts was also found to be influenced by the growth atmosphere (aerobic or anaerobic), the growth phase (stationary or exponential) and strain specificity (King *et al.*, 2003). Four different strains isolated from food, the environment or clinical settings were subjected to both acid and bile under various atmospheric conditions. In all environments tested, stationary cells were much more resistant than exponential cells. In general the bile salt environment proved to be more difficult for the strains to resist. It was found that only the stationary bacterial cells grown in air and 100 % nitrogen survived after being exposed to the bile-salt environment. These results suggest that atmospheric conditions and strain specificity determine the microbe's ability to resist bile. These studies indicate that the pathogenic potential of *Listeria monocytogenes* is related to its ability to resist bile and possibly activate repair systems in the presence of bile.

Table 2. Genes upregulated in bile-treated Gram-positive bacteria

Gene/protein	Bile salt	Strain	Reference
Adaptation to atypical conditions			
clpB	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
clpP	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
cspD	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
hsp20	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
terD	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Cell envelope			
capA	Bovine bile	*L. monocytogenes*	Begley *et al.* (2002)
Endopeptidase	NaC : NaDc	*B. cereus*	Kristoffersen *et al.* (2007)
hblA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
lytB	Bovine bile	*L. monocytogenes*	Begley *et al.* (2005b)
pva	Bovine bile	*L. monocytogenes*	Begley *et al.* (2005b)
txrA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
trxB	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
yku	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Detoxification			
baiE	Bovine bile	*L. monocytogenes*	Begley *et al.* (2005b)
bsh	Bovine bile	*L. monocytogenes*	Begley *et al.* (2005b)
msrA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
sodA1	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
DNA repair			
DNA/RNA helicase	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
res	NaC : NaDc	*B. cereus*	Kristoffersen *et al.* (2007)
uvrA	Porcine bile	*L. monocytogenes*	Kim *et al.* (2006)
xer	NaC : NaDc	*B. cereus*	Kristoffersen *et al.* (2007)
Metabolism			
bdhA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
carA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Fatty acid desaturase	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
fbp	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
fldA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
pgsA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
pykA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
spH	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
ytpA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
yugJ	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Mobility			
cheY	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
flaA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
motA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Other			
pfo	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Thiocillin	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Protein folding			
groEL	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
groES	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Protein synthesis			
miaA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
rplK2	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Transcription regulation			
Bm3R1	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
cheY	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
ctsR	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
gadE	Bovine bile	*L. monocytogenes*	Begley *et al.* (2002)
gntR	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
hrcA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
lytR	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)

Table 2. cont.

Gene/protein	Bile salt	Strain	Reference
marR	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
plcR	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
sigA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
tcdA-E operon negative regulator	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
tetR	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
ytsA	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
zurR	Bovine bile	*L. monocytogenes*	Begley *et al.* (2002)
Transport/binding proteins			
ABC transporter permease	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Bacitracin transport permease	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
crr	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Di- or tri-peptide transporter	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Lincomycin resistance	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
Multidrug-resistance proteins	NaC : NaDC	*B. cereus*	Kristoffersen *et al.* (2007)
yxiO	Bovine bile	*L. monocytogenes*	Begley *et al.* (2002)

B., *Bacillus*; *L.*, *Listeria*.

Concluding remarks

Bile is an important antimicrobial component of the human digestive system. The ways in which bacteria, both Gram-negative and Gram-positive, cope with its toxic effect differ in the exact mechanism, but a general theme can be determined. These bacterial models show that resistance is not exclusive to just overcoming damage to the membrane or the DNA, but rather is a result of a combination of defence and/or repair mechanisms. One mechanism several enteric bacteria possess is that of efflux pumps to remove bile salts from the cell, thus preventing potential damage to the membrane. If the membrane is compromised by bile salts, then the toxic effects could be conveyed to the DNA, leading to extensive damage in the form of reactive oxygen species. This would lead to the cessation of replication and eventually cell death. Many recent studies, as outlined above, have focused on determining the role that DNA repair has in the virulence capability of enterics. While the level of resistance seems to vary, the ability of bacteria to breach certain areas of the host's digestive system is contingent upon the ability to resist damage induced by bile salts. Bile salts have repeatedly been found to be oxidative agents with the ability to induce the SOS response in several bacteria. The identification of this mechanism of damage, as well as the resistance and repair of the bacterium, could aid in understanding its interaction with similar bactericidal agents and provide a better understanding of the role of the host's response in the enteric infection process. While bile salts do induce both DNA damage and membrane damage, the interaction between the two types of damage is still not understood. In particular research pertaining to the connection of the pathogenic potential of a bacterium to its ability to resist bile is still in its infancy.

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