Adaptive Mechanisms of Listeria monocytogenes to Stressors: An overview

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Abstract
Listeria monocytogenes is a food borne pathogen which usually infects individuals with impaired cellular immunity and the healthy. Gastrointestinal tract (GIT) of the humans has lots of defensive mechanisms placed to prevent pathogens from establishing themselves and cause infectious diseases. Survival depends on the pathogen's ability to overcome such preventive mechanism of the host. *Listeria monocytogenes* exhibits array of mechanisms that ensure its survival against these stressor. These stressors include gastric acid, bile salt, low oxygen tension, antimicrobial peptides e.t.c. Acid tolerance system (ATR), glutamate decarboxylase system (GAD), BilE system, MVs, oxygen sensors are used by *Listeria monocytogenes* to enhance its chances of survival within host. Our interest here is to look at such adaptive mesures with respect to the stressors encountered.
Key words: Glutamate decarboxylase system (GAD), Bile Expulsion (BilE), Membrane Vesicles (MVs), Acid tolerance system (ATR), Listeria monocytogenes, Stressors,
Bile salt hydrolase (BSH).

Introduction
Listeria monocytogenes is a Gram-positive, facultative intracellular pathogen that infects human and animals through consumption of contaminated food (Schlech 2000). This organism can invade intestinal epithelium and gain access to the lymphatic system and blood stream, eventually resulting in dissemination to the liver, spleen, and central nervous system (Gahan,and Hill 2005). It is one of the most virulent food borne pathogens and has high mortality rate especially among the immunocompromised and in those with impaired cell-mediated immunity (neonates, pregnant woman, elderly persons) causing septicemia, meningoencephalitis, still brith. L. monocytogenes can also induce febrile gastroenteritis in healthy individuals if it is ingested at high doses (Dalton et al.1997).
Despite its low incidence, listeriosis has a high mortality rate (30%), making it the most deadly foodborne disease in the United Kingdom and the United States, as it claims more lives than any other foodborne pathogen (Mook 2019). In Nigeria, few studies done regarding this pathogen especially in humans, there is inadequacy of data regarding listeriosis**.** It has developed many mechanisms that enable it to thrive and survive within GIT, multiplying and getting access to the human system. Adaptation to the GIT conditions such as acidity, osmolarity, oxygen tension, or the challenging effects of antimicrobial peptides and bile is critical in order to survive. Interestingly, the more it is exposed to those challenges, the more it adapts to the environment which is achieved through expression of certain genes. The finding that the bacteria are able to colonize and persist in the gallbladder (Begley et al. 2009) suggests the occurrence of long-term and chronic infections and demonstrates the ability of pathogenic Listeria to survive within the various microenvironments of the gastrointestinal tract. The aim of this review is to discuss on the mechanisms employed by L. monocytogenes to cope with the harsh environment of the gastrointestinal tract.

Response to Acids

Gastric acid represents the first stressor encountered during passage of Listeria monocytogenes in the GIT. The main constituent of gastric acid is hydrochloric acid produced by parietal cells (also called oxyntic cells) creating a pH of 1.5 to 3.5. In order to cause an infection, L. monocytogenes requires robust acid resistance mechanisms to overcome the acidic stress presented by fermented foods, gastric juice, volatile fatty acids in the intestine, and even the low pH of the macrophage phagosome (Jeroen et al. 2018).

Higuchi et al. 1997 and Paul et al. 2005 showed that the GAD system plays a vital role in adaptation to acidic environment in L. monocytogenes. The GAD system classically involves two proteins, a glutamate decarboxylase enzyme coupled to a glutamate/gamma-aminobutyrate antiporter, which results in the consumption of an intracellular proton for each glutamate entering the system. Some strains of L. monocytogenes (LO28) have three decarboxylases genes (gadD1, gadD2, and gadD3) with two antiporters (gadT1 and gadT2). These are organized in two pairs (gadD1T1 and gadD2T2) and a distinct gadD3. Now, to further elucidate the role of the system, Paul et al. 2005 analysed 15 isogenic mutants and found that GadD2/T2 are primarily responsible for surviving severe acid challenge (pH 2.8) thus confirming previous observations from other researchers. They also established that GadD1 plays a major role in growth at mildly acidic pHs (pH 5.1) but failed to state the role of gadD3.

Karatzas et al. 2010 and Wemekamp-Kamphuis et al. 2004b hypothesised that GadD3 plays an important role in acid resistance by mediating the conversion of glutamate into GABAi with concomitant consumption (removal) of protons in the cytoplasm.

Ma´ire et al. 2010 stated that several studies uncovered a link between the acid stress response of L. monocytogenes and nisin (bacteriocin) resistance (Bonnet et al. 2006; McEntire 2004 and Van et al. 1999). GAD system seems to provide innate protection against nisin which is commonly seen among GAD Gram-positive microorganisms. In silico analyses reveal that GAD genes are present in the genomes of a variety of bacterial genera, including the Gram-positive food pathogens.

In addition, there are other mechanisms used by L. monocytogenes to cope with stress from acidic environments. Regulation of protons movement across the cell membranes results pH homeostasis. In aerobic organisms it is coupled with electron transport chains while in anaerobic bacteria with H^+-ATPase molecules, using energy generated from the hydrolysis of ATP molecules. L. monocytogenes being a facultative anaerobe is able to utilize both processes of pH homeostasis as demonstrated by Shabala et al. 2002.

Interestingly, exposure to acidic stress confers resistant to various other stresses encountered by L. monocytogenes. Phan-Thanh and Mahouin, in 1999 showed that under acidic stress, various transcriptional regulators, GroEL protein, and ATP synthase are expressed in L. monocytogenes. Furthermore, Gahan et al. 1996 demonstrated that adaptation to acid offers cross protection against heat, ethanol, oxidative, osmotic stresses and the bacteriocin nisin. And later in 2000, Phan-Thanh et al. agreed to most of the findings of Gahan et al.

Ferreira et al. 2003 and Paul et, al. 2005 observed that survival of L. monocytogenes in the gastric fluid is partially due to the stress sigma factor σB. According to Kazmierczak et al. 2003, sigma factor σB regulates the expression of the gadB and OpuC genes that encode glutamate decarboxylase and carnitine transporter respectively helping survival in acidic pH.

Lastly, histidine kinase and a response regulator that were identified in 1999 by Cotter et al. were encoded by lisK and lisR genes respectively. Histidine kinase associated with membrane senses environmental changes, (low pH, oxidative stress e.t.c) and the alteration of gene expression is

enabled by the response regulator according to Meenakshi et al. 2018. Low pH not only stimulates stress responses but also expression of virulence genes such as prfA. In 2013 Neuhaus et al. showed that acid shock at low temperatures of 25 °C may also induce prfA .

Response to Bile

Bile or gall is a dark green to yellowish brown fluid, produced by the liver of most vertebrates that aids the digestion of lipids in the small intestine. Bile acids (BA), major components of bile, are synthesized from cholesterol and conjugated to either glycine or taurine in the liver, stored in gallbladder before secretion into the duodenum via the common bile duct (Li & Chiang, 2015). BA is biological detergents that facilitate the emulsification and solubilization of dietary lipids and fat-soluble vitamins, favouring its absorption by enterocytes. Although the major fraction of BAs are actively reabsorbed by enterocytes and sent back to the liver (enterohepatic circulation), a small fraction is modified by the indigenous microbiota (Chand et al. 2017). The composition of hepatic bile is 97% water, 0.7% bile salts, (Barrett and Kim 2012) 0.2% bilirubin, 0.51% fats (cholesterol, fatty acids, and lecithin), and 200 meq/l inorganic salts (Guyton and Hall 2011). The salts are amphipathic molecules that have been shown to possess antimicrobial properties; bile salts have been shown to degrade viral and bacterial membranes containing lipids and also induce DNA damage (Gunn 2000 and Bernstein et al. 1999).

L. monocytogenes possesses numerous mechanisms which allow for resistance against bile; the bile salt hydrolase bsh (Begley 2005 and Dussurget et al. 2002), the general stress response sigma factor sigB (Dowd 2011 and Begley 2005), the bile exclusion system bilE (Sleater et al. 2005), and virulence regulator prfA (Dussurget et al. 2002).

Deconjugation (bile salt) is catalyzed by bile salt hydrolase (BSH) enzymes, which hydrolyze the amide bond and liberate the glycine/taurine moiety from the steroid core. The resulting acids are termed unconjugated or deconjugated bile acids hence inactivating the potent salt. Deletion of the bsh gene invariably reduces the ability of L. monocytogenes to cause systemic infections as stated by Bergley, 2005 and Dussurget et al. 2002. Several previous works also suggest that BSH activity could play a role in bile tolerance in some Gram-positive bacteria and would be part of a cell detoxification strategy (Begley et al. 2006 and Grill et al. 2000). In fact, free BA produced by BSH activity have decreased solubility, precipitate at intestinal pH (decreased mainly by the activity of lactic acid bacteria) and leave the GIT in the faeces. In addition, the diminished detergent activity may be less toxic to bacteria in the intestine (Chand et al. 2017). In Listeria, BSH levels increased under certain conditions such as low oxygen tensions prevalent in the host during infection (Dussurget et al. 2002).

Also heat shock proteins DnaK, GroEL and GroES were seen to be responses related against bile and other several stimuli in many bacteria genera as shown by Ruiz et al. 2016; Siciliano & Mazzeo, 2012.

Ferreira et al. 2003 has shown that sigma factor sigB is involved in regulating gene expression for osmolyte transporters (OpuC) and regulates processes needed for survival in response to stresses of oxidation, reduced pH, and starvation. It also serve as a positive regulator of factor A which activates major virulence factors. A connection between sigB and the genes expression

related to bile resistance such as bilE and bsh have been shown by Sue, 2003. The bile exclusion system, (BilE) serves to prevent bile from entering the cell as bile is toxic to most pathogens.

Salt stress response related proteins such as RecA and UvrA were also identified in molecular vesicles (MVs) from salt stressed L. monocytogenes. RecA is an important factor in DNA repair. It contributes to acid and bile salt stress as well as adhesion and invasion of L. monocytogenes to Caco-2 intestinal epithelial cells (van der Veen and Abee 2011). Nucleotide-excision repair protein UvrA is also required for acid and bile resistance of L. monocytogenes (Kim et al. 2006). Another finding also shows that MVs contrary to the above function also confer and aid host cell survival. According to So-Hyun et al. 2019, MVs of L. monocytogenes can increase host cell viability, but not cell death, which may due to the fact that MVs secreted from extracellular L. monocytogenes can secure important niche for bacterial invasion.

It has been also described that multidrug resistance (MDR) transporters belonging to the ATP binding cassette or the major facilitator superfamily could play an important role in the bile resistance phenotypes in Gram-negative and Gram-positive bacteria Šárka et al. 2017. This is elucidated and expanciated in listeria monocytogenes by Quillin et al. 2011 which identified a TetR-type regulator [renamed bile-regulated transcription factor A (BrtA)] that senses bile and regulates expression of two multidrug resistance (MDR) efflux pumps (MdrM and MdrT) that mediate bile tolerance and liver/gall bladder colonization. This finding may be particularly relevant given the broader role of MdrM/T in mediating secretion of cyclic-di-AMP, a signaling molecule that triggers STING-dependent production of interferon-beta and promotes in vivo survival of the Listeria monocytogenes (Crimmins et al. 2008).

Response to Oxygen Tension

Carbon dioxide is known to inhibit the growth of most bacteria (Gill et al., 1980) and found as an acid reaction byproduct in the stomach with the amount produced differs from individual to individual.

Anaerobiosis might be an environmental signal, which triggers the first colonisation of L. monocytogenes within the intestine during in vivo growth. Furthermore, Jydegaard-Axelson and colleagues in 2004 observed an increased gene expression essential for survival in acidic conditions and also increased branch-chain fatty acids in the cell membrane when L. monocytogenes is cultured in elevated carbon dioxide and anaerobic conditions. It is obvious that gene expression changed for invasion-associated internalin proteins (InlA and LmaA) that are involved in attachment and invasion of the host cells in preference to escape the acidic environment.

L. monocytogenes being a facultative anaerobe, capable to undergo aerobic respiration, fermentation, and anaerobic respiration, however, this is still dependent upon oxygen availability. This environmental sensing is typically controlled by a two-component signal transduction system which consists of a membrane bound sensor and a cytoplasmic response regulator (Stock et al. 2000). Few researches have been done to show the connection between anaerobiosis and increased survival in the presence of stressors in Listeria monocytogenes, but a lot is known about Gram-positive organisms.

In various Gram-positive bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium tuberculosis*, two-component systems have been shown to regulate metabolism and the expression of virulence factors in response to decreased oxygen

concentrations (Throup et al. 2001, Yarwood et al. 2001 and, Nakano et al. 1997). For instance, the SrrAB two-component system of *S. aureus* is involved in the activation of stress response proteins, specifically those involved in DNA repair, the oxidative stress response and the alternative sigma factor, SigB, in oxygen limited environments (Kinkel et al. 2013).

The two-component system ResDE of *B. subtilis,* homologous to SrrAB in *S. aureus,* has been shown to regulate virulence factors, sporulation, and fermentation in *B. subtilis* (Yarwood et al. 2001 and Nakano et al. 1997) A homolog to *resD* has been characterized in *L. monocytogenes* (Morgan et al. 2019). ResD was found to influence the activity of *prfA* in *L. monocytogenes,* which in turn alters the expression of several virulence genes, including *inlA* (Larsen et al. 2206). This point that ResD is an important element in the virulence factors regulation and stress responses under low-oxygen conditions.

A recent genomic study identified DosP in *L. monocytogenes,* which is similar to the histidine kinase found in *M. tuberculosis,* suggesting that *L. monocytogenes* belong to the category of Gram-positives that possess an oxygen sensor (Chiara et al. 2017 and Holch et al. 2013) . This suggests that there is a link in the organisms' ability to detect oxygen levels among Gram-positive bacteria.

Wright et al. 2016, recently showed a potential link between oxygen availability and bile resistance by observing several strains of *L. monocytogenes* growth in 0%, 1%, 5%, and 10% porcine bile. This shows that resistance to bile increases under anaerobic conditions as compared to aerobic for virulent strains F2365, 10403S and EGD-e but not for avirulent strain HCC23. A comprehensive total proteomic study to identify mechanisms (metabolism and stress response) found that proteins associated with the cell envelope, membrane bioenergetics, cell division, and dehydrogenases involved in NADH:NAD^+ alteration were increased under anaerobic conditions. It is possible that these proteins may play a role in bile resistance during anaerobic grow, despite oxygen sensor which may regulate these mechanisms has not been uncovered.

Vazquez-Boland et al. 2001 observed a significant difference in the bacterial loads in liver of the treated animals with high anaerobic dose of Listeria monocytogenes. The pathogen disseminates to the secondary target organs, including the brain and the uterus after invasion of the liver. In the treatment group, there were high loads of the pathogen in the GIT and feces, which may prime the bacteria to survive the GI tract stressors and cross to the liver. They further revealed that exposure of L. monocytogenes to anaerobic conditions prior to infection, such as what occurs within a food processing environment or packaging, enhances the probability of the pathogen to resist the stressors encountered within the GI tract.

Conclusion

Many mechanisms are used by L. monocytogenes to overcome stressors encountered within the GIT. As metioned in this paper; GAD system, protons movements across memebrane, BilE exclusion, BSH, MVs, anaerobiosis e.t.c are some of the mechanisms employed in adapting to environmental stresses. Extra-intestinal conditions such as pH and packaging of the food also aid in the adaptation to the GIT stressors. Listeriosis is few incases, but it high mortality rate (30%) makes it the most dangerous food borne pathogen. Employment of several adaptive mechanisms used for its survival present an additional challenge which may increase the cases and mortality rates in near future, if more effective measures are not taken.

There are need for further studies so as to determine if GAD genes contribute to the tolerance of nisin, whether MVs aid in host cell survival, to expanciate and confirm on the role of Gad3 and how SigB makes L. monocytogenes vulnerable to stresses under aerobic conditions. The fact that

prfA is stimulated by low pH and heat shock at low tempareture need more details from further experiments.

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