Review Article

Adaptive Mechanisms of Listeria monocytogenes to Stressors: An overview

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 defends 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 stressor include gastric acid, bile salt, low oxygen tension, antimicrobial peptides e.t.c. Acid tolerance system (ATR), glutamate decarboxylase system (GAD), BilE system, 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.

Introduction

Listeria monocytogenes is a species of pathogenic bacteria implicated in a disease condition listeriosis. It survives in the presence or absence of oxygen hence facultative in nature. 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 and gastroenteritis in healthy individual.

Listeriosis is relatively rare and annual incidence is decreasing; in the United States from 7.7 cases per million population in 1990 to 3.1 cases per million population in 2003. In France, the incidence of listeriosis declined from 4.5 cases per million population in 1999–2000 to approximately 3.4 in 2002–2003 (Liu, 2008). Although the incidence is low, the high mortality rates (about 30%) associated with listeriosis make *L. monocytogenes* one of the most deadly human food borne pathogens. 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 systems especially in those with impaired cell-mediated immunity. 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 by 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.

This review focuses on the mechanisms employed by *L. monocytogenes* to cope with the harsh environment of the gastrointestinal tract.

Response to Acidic environment

The main constituent of gastric acid is hydrochloric acid which is produced by parietal cells (also called oxyntic cells) in the gastric glandsin the stomach. Its secretion is a complex and relatively

energetically expensive process. Parietal cells contain an extensive secretory network (called canaliculi) from which the hydrochloric acid is secreted into the lumen of the stomach. The pH of gastric acid is 1.5 to 3.5 (Marieb and Hoeh 2010) in the human stomach lumen, the acidity being maintained by the proton pump H^+/K^+ ATPase.

The first stressor encountered by L. monocytogenes after ingestion is the acidic nature of the stomach. However, there are several mechanisms through which *L. monocytogenes* acquire resistance to acid stress.

Upon ingestion, *L. monocytogenes* encounter the stressor of acidic conditions within the stomach as first physiologic barrier to bacterial invasion. Adaptation to such stressors is the key in the survival and invasion of this pathogen. Several mechanisms have been acquired in order to overcome this environment, including the acid tolerance response (ATR) and the glutamate decarboxylase system (GAD**)** Morgan *et al.,* 2019. Pre-exposure of *L. monocytogenes* to milder acidic conditions enables enhanced resistance to lethal acid exposure due to its adaptive acid tolerance response (ATR). Acid adaptation offers cross protection against heat, ethanol, oxidative, and osmotic stresses and against the bacteriocin nisin (Gahan *et al.,* 1996). Adaptive ATR cross protection may also enhance the ability of *L. monocytogenes* to cause illness by contributing to bacterial survival of a variety of challenges imposed by a host. Such host challenges include exposure to gastric fluid, bile, and competitive intestinal flora; the presence of organic acids found in the small intestine; and the oxidative products in the phagosome (Vázquez-Boland *et al.,* 2001). This mechanism enhances the survival chance of *L. monocytogenes* within the entire GIT leading to development of listeriosis. The GAD system confers resistance to more severe acidic conditions (pH < 4.5; Karatzas *et al.,* 2012). It is comprised of two proteins, a cytoplasmic glutamate decarboxylase (GadA or GadB) and a glutamate/GABA antiporter (GadC) located in the cytoplasmic membrane (Cotter *et al.,* 2005). The role of the GAD system is to increase pH_i by converting extracellular glutamate to γ - aminobutyrate (GABA) in an enzymatic reaction that reduces the intracellular proton concentration (Cotter *et al.,* 2001). Furthermore, the resistance to acid is not only through the systems alone. The ADI and AgDI systems are both involved in the response of *L. monocytogenes* to extreme acidity (Ryan *et al.,* 2009). ADI imports arginine molecules from the extracellular environment, converting them to ornithine, CO_2, ammonia (NH_3), and ATP. NH_3 is then protonated to ammonium (NH_4), which increases pH_i(Cotter and Hill, 2003). The same is true for AgDI, which converts agmatine into putrescine and NH_3 (Chen *et al.,* 2011). Not all *L. monocytogenes* strains possess the GAD system; it has been shown to be required by certain strains for maintaining homeostasis within gastric juices (Cotter *et al.,* 2001). However, recent studies have shown that acid shock at low temperatures of 25 °C may induce *prfA* (Neuhaus *et al.,* 2013). Therefore, low pH could serve as a trigger for the expression of virulence and stress response. These mechanisms enhance the survival chance of *L. monocytogenes* within the entire GIT leading to development of listeriosis.

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. In humans, bile is produced continuously by the liver (liver bile), and stored and concentrated in the gallbladder. Human bile is composed of individual conjugated and unconjugated bile acids that are present in the small intestine at average concentrations of 5 mM (De Smet, *et al.,* 1995)**.** 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). Bile salts are metabolized by conjugation to glycine or taurine, which decreases their pK_a value to about 5 (Vlahcevic 1996). 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). Survival of enteric pathogens such as listeria monocytogenes depends solely on its ability to resist the antimicrobial effects of bile salts.

L. monocytogenes possesses numerous mechanisms to allow for resistance against bile, including 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 is catalyzed by bile salt hydrolase (BSH) enzymes (EC 3.5.1.24), 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.

The general stress response sigma factor sigB is involved in regulating the expression of osmolyte transporters, such as OpuC, and is also involved in regulating processes needed for survival during oxidative stress, reduced pH, and starvation. The sigB transcription factor also serves as a positive regulator of factor A (PrfA), thus leading to the activation and regulation of major virulence factors (Ferreira *et al.,* 2003).
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. However, L. monocytogenes overcome the toxicity of the gall badder and extracellularly grow. Expression of internalins were found to increase in avirulent strain HCC23 (internalin A) but decrease in virulent strains (Payne *et al.,* 2013). A recent analysis of the transcriptomic response demonstrated that bile exposure regulates many virulence factors in L. monocytogenes. In particular the work identified a TetR-type regulator [renamed bile-regulated transcription factor A (BrtA)] that senses bile (in particular the bile acid cholic acid) and regulates expression of two multidrug resistance (MDR) efflux pumps (MdrM and MdrT) that mediate bile tolerance and liver/gall bladder colonization (Quillin *et al.,* 2011). 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 pathogen (Crimmins *et al.,* 2008). This shows that bile plays important role by differentially regulating the invasive nature of *L. monocytogenes.*

Response to Anaerobic Condition

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

In an in vitro experiment conducted by Stefanie *et al.,* 2014 which showed none of the anaerobically transcribed genes is essential for anaerobic survival, it is meaningful to explain that adaptation to anaerobiosis could enhanced the fitness of bacteria in naturally low-oxygen environments. Anaerobiosis might be an environmental signal, which triggers the first colonisation of *Listeria 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.

It has already been shown that anaerobic pre-culture of *Listeria monocytogenes* enhances adhesion in *in vitro* cell culture assays and virulence *in vivo* in the guinea pig model (Bo Andersen *et al.,* 2007). Anaerobic induction of InlB (*inlB, lmo0433,* $\log_2$RTL 1.3), involved in adherence to (Lindén *et al.*, 2008) and invasion in (Pentecost *et al.*, 2010) intestinal tissue, and LAP (a bifunctional enzyme, also with metabolic capability, *lmo1634,* described previously in the text as *adh*, $\log_2$RTL 1.4), involved in adherence (Burkholder *et al.*, 2009) and paracellular translocation (Burkholder & Bhunia, 2010), was already described previously (Burkholder *et al.,* 2009 ; Stritzker *et al.,* 2004 , 2005). In addition, an upregulation of *lmo0971–lmo0973,* encoding the Dlt proteins involved in D-alanine esterification of lipoteichoic acid and wall teichoic acid (*dltD*, *dltC* and *dltB,* $\log_2$ 1.2, 1.3 and 1.5) were also anaerobically observed. The D-alanine esterification has been shown to contribute to adhesion to host cells and to virulence in *Listeria monocytogenes* (Abachin *et al.,* 2002). Furthermore, *lmo2467,* which encodes a protein similar to chitinase and chitin-binding protein, was much more anaerobically transcribed than aerobically. It has already been shown that this gene also contributes to virulence in *Listeria monocytogenes* (Chaudhuri *et al.,* 2010). Studies have demonstrated that the activity of the bile salt hydrolase increases under anaerobic conditions (Dussurget *et al.,* 2007). These enhanced and synergistic mechanisms occur during anaerobic growth could enhance the initial colonization of the intestine by *Listeria monocytogenes in vivo*.

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). Even though little research has been conducted on *L. monocytogenes* in order to analyze the connection between anaerobiosis and increased survival in the presence of stressors, much is known about other 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 a.,*l 2013). However, this activation is in conjunction with multiple two-component systems (Michel *et al.,* 2006).

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 Holc *et al.,* 2013) . This suggests that there is a link in similarity between virulence, stress response and two-component signal transduction systems affecting 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.

Conclusion

Listeria monocytogenes infection is rare but it is the most dangerous foodborne disease due to its high mortality rate. In addition, its unique way of adapting to environmental stressor, gene expression and the inter-relationship between will make it the most deadly foodborne pathogen in near future if attentive and preventive measures not taken.
Several studies that have analyzed and characterized the bile resistance mechanisms of *L. monocytogenes* were conducted under aerobic conditions. The conditions of the gallbladder and small intestine, where bile salt concentrations are at its highest, is an environment ranging from microaerophilic to anaerobic (Crawford, 1955) hence not accurately modeling the physiological conditions within the human gastrointestinal tract.
Recently, researchers began to show a connection between oxygen availability and the stress response in Gram-positive bacteria but further studies needed to identify the potential oxygen sensor for detecting oxygen availability especially in *Listeria monocytogenes*. Drugs to target this sensor will hopely reduce and prevent infection with *L. monocytogenes*.

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