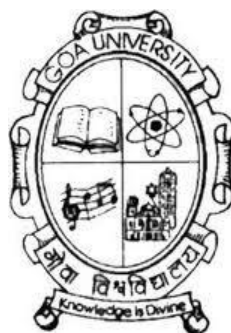# "Effect of Stress Factors on Survival and Adaptation of *Listeria monocytogenes*"

A Thesis Submitted to

GOA UNIVERSITY

for the award of the degree of

DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

by

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Certificate

Certified that the research work embodied in this thesis entitled "**Effect of Stress Factors on Survival and Adaptation of *Listeria monocytogenes***" submitted by **Mr. Satyajit Babasaheb Kale** for the award of **Doctor of Philosophy** degree in **Microbiology** at Goa University, Goa, is the original work carried out by the candidate himself under my supervision and guidance.

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Statement

As per the University ordinance O.D. 09, I hereby state that this thesis for the Ph.D. degree on "**Effect of Stress Factors on Survival and Adaptation of *Listeria monocytogenes*"** is my original contribution and that the thesis or any part of it has not been previously submitted for the award of any degree/diploma of any University or Institute. To the best of my knowledge, the present study is the first comprehensive work of its kind from this area. All the corrections suggested by the reviewers are included at respective pages.

<div align="right">

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Abbreviations

ABC: ATP-binding cassette

ATP: Adenosine triphosphate

a_w: Water activity

BAM: Bacteriological Analytical Manual

BF_3:   Boron trifluoride

BHI: Brain Heart infusion (broth)

BIS: Bureau of Indian Standards

Bp: Base pairs

BSA: Bovine serum albumin

CDC: Center of Disease Control

CDSs: Coding DNA Sequences

CFU: Colony Forming Unit

CHEF: Contour Clamped Homogeneous Electrophoresis

DNA: Deoxyribo nucleic acid

dNTP: Deoxynucleotide Triphosphates

EDTA: Ethylenediaminetetraacetic acid

FAME: Fatty acid methyl ester

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

FSIS: Food safety and inspection service

FSSAI: Food Safety and Standards Authority of India

GMP: Good Manufacturing Practices

GNAT: Gcn5-related N-acetyltransferases

HCl: Hydrochloric acid

ILCC:	Indian *Listeria* Culture Collection
ISO:	International Organization of Standardization
KEGG:	Kyoto Encyclopedia of Genes and Genomes
KOH:	Potassium hydroxide
MALDI:	Matrix Assisted Laser Desorption Ionization
mdrL:	Mutlidrug resistance (efflux pump) of *Listeria* species
MHFW:	Ministry of Health and Family Welfare
mRNA:	Messenger Ribonucleic acid
NaCl:	Sodium Chloride
NADPH:	Nicotinamide Adenine Dinucleotide Phosphate
NCDC:	National Centre for Disease Control
ORF:	Open reading frames
PALCAM:	Polymyxin Acriflavin Lithium-chloride Aesculin Mannitol (Agar)
PHAC:	Public Health Agency of Canada
PBS:	Phosphate Buffered Saline
PCR:	Polymerase chain reaction
PFGE:	Pulsed field gel electrophoresis
PTS:	Phosphotransferase system
qPCR:	Quantitative polymerase chain reaction
RNA:	Ribonucleic acid
RT:	Reverse Transcriptase
RTE:	Ready-To-Eat (Food)
SDS-PAGE:	Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis
SEM:	Scanning electron microscopy
Spp.:	Species

sRNA: Small Ribonucleic acid

TCA: Trichloroacetic acid

TE: Tris EDTA

TBE: Tris–borate EDTA buffer

tRNA: transfer Ribonucleic acid

UPGMA: Unweighted Pair Group Method with Arithmetic Mean

USDA: United States Department of Agriculture

UVM: University of Vermont media

WHO: World Health Organization

Units of measurement

μg: microgram

μm: micrometer

g: gravitational force

gm: grams

h: hour

kDa: Kilodaltons

M: Molar

mg: miligrams

ml: milliliter

mM: miliMolar

mm: millimeter

ng: nanogram

O.D.: Optical density

^{o}C: Degree Celsius

rpm: revolution per minute

μ: micron

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Chapter 1
Introduction

The foodborne illness is a problem faced by every country across the globe and major public health concern. Foodborne pathogens are the major cause of implications on health and economy domestically as well as internationally. In developed countries like the United States, 9.4 million deaths are from the foodborne illness and leading cause of deaths are *Salmonella* spp, *Toxoplasma gondii* and *Listeria monocytogenes* (Scallan *et al*., 2011). There are multiple factors responsible for foodborne illnesses, such as growing the transitory human population, changing food habits and increased trend and demand of ready-to-eat (RTE) foods (Newell *et al*., 2010). Safe and healthy food is a primary demand by the consumer, thus the food industries take constant efforts towards reducing the contamination and increase the shelf life of the food (Begley and Hill, 2015). Still considering the factors like the complexity of global food supply, food is traveling the longer distance than earlier, consumption pattern and emerging survival of foodborne pathogens; risk of foodborne illnesses found to be increasing (Chan, 2014). The failure of safety measures at any stage of the complex food chain from the environment via primary processing, final production, storage and transport to the consumer causes significant health and economy losses (WHO, 2009). The important factor behind this failure of safety measures is well known as stress resistance by foodborne pathogens. This stress tolerance not only raises the incidence of pathogens, it's also known to be altering their virulence properties and helping them to persist in the food related environment. There are pathogens like *Listeria monocytogenes* which adapt equally in the soil as well as inside the eukaryotic host. Being capable of resisting vast physical and physico-chemical stresses, they counter stress hurdles used in food industry; eating such contaminated food can result in life-threatening infection of immune-compromised individuals

(Durack *et al*., 2013). So the ways pathogenic microorganisms encounter different environmental stresses used in food processing, food preservation and/or similar stresses inside the host is a growing interest.

The stress universally can be referred as the agents, factors or treatments causing injury. This analogy can describe 'temporary or repairable damage' in case of higher organisms, but in terms of microorganisms, it is 'damage to the cell components, cell structures or loss of some cellular functions transiently or permanently' (Hurst, 1984; McMahon *et al*., 2007). Collectively these are effects of any detrimental factors, conditions or treatments adversely affecting survival and/or growth of microorganisms, used with the intention of prevention and control of pathogens or spoilage microorganisms in foods and food processing environments (Yousef and Courtney 2003; Wesche *et al*., 2009). Foodborne pathogens go through many stresses during their life cycle. The food itself contains many bacteriostatic conditions such as water activity (a_w), pH and oxidation-reduction potential. Foodborne pathogen particularly experiences many stresses during food production, food processing, storage and transport including osmotic shock; chemical treatments such as acids or detergents; freezing, thawing or combination of different stresses. So foodborne pathogens need to overcome many of such stress factors or conditions in succession. Exposure to the stresses causes injury to the bacterial cells by damaging cellular part/components. This damage obviously depends upon the degree and severity of the stresses. Researchers have found that foodborne pathogens sense the environmental stresses and act by modulating the gene expression assisting the survival of cells. These modulations in expression of genes results in the production of proteins, repairing damaged cell components, maintaining cell-homeostasis and/or facilitation of abolishing stress agents (Dahl *et*

al., 2015). It has been also postulated that with the exposure to minor/mild stress bacteria responds in an adaptive/protective manner accompanied by a temporary physiological change and makes it tolerant to a harsh level of the same type of stresses. This is called as stress adaptation of bacteria and has been observed previously in foodborne pathogens, including *L. monocytogenes*, *Escherichia coli*, and *Salmonella* (Sleator and Hill, 2002; Ryan *et al*., 2008; Soni *et al*., 2011; A'lvarez-Ordo'nez *et al*., 2011). The stress tolerance development may also occur through induction of proteins which repairs the damaged cellular parts or DNA or proteins, initiation of homeostatic systems and activation of enzymes for countering the stress (Dahl *et al*., 2015). This variation in adaptive tolerance and/or modulation of gene expressions could be because of presence or absence of some gene loci. The well-studied example of this phenomenon is acid stress tolerance by *L. monocytogenes* correlated with presence/absence of *gadD1T1* genes (Cotter *et al*., 2005; Van der Veen *et al*., 2008). The presence of such stable resistant variants has been exhibited in the case of *L. monocytogenes*. The work done on stable stress resistance of *L. monocytogenes* shows the large diversity within the variants (Van Boeijen *et al*., 2010). This diversity could be the one of the potential reason behind survival and adaptation of *L. monocytogenes* under diverse environmental conditions. Nevertheless, the exact mechanism, origin of such variants and its impact on food safety is yet to untangle. So investigation needs to be done towards the understanding of molecular basis of stress tolerance of *L. monocytogenes*; knowledge gained will help to design effective food processing strategies towards ensuring food safety.

L. monocytogenes is a zoonotic, foodborne pathogen with ubiquitous nature and the causative agent of listeriosis. It was recognized first time as a foodborne

pathogen in the early 1980s (Schlech *et al*., 1983). Being ubiquitous nature it has the extraordinary fitness to adapt diverse environmental conditions from soil to a eukaryotic host with the capacity to tolerate hardy conditions including low moisture content, higher salinity, extreme pH and colder temperature (Farber and Peterkin, 1991; Durack *et al*., 2013). This versatility comes from the ability of an organism to acquire information of external environment and process it accordingly towards successful survival and adaptation to these conditions. These abilities make *L. monocytogenes* one of the significant foodborne pathogens, which is difficult to control and major safety concern of food industries (Gandhi and Chikindas, 2007; Fox *et al*., 2011). Listeriosis has 20-30% case fatality rate, 50% neonatal death rate and 91% hospitalization rate (Farber and Peterkin, 1991; Sartor *et al*., 2015). Due to the severity of the infection, the occurrence of an even low number of *L. monocytogenes* has been cautioned in food as well as in food industrial premises (FSIS, 2014). According to US Economic Research Services, *Listeria* is third among 15 major foodborne pathogens responsible for foodborne illnesses-associated deaths imposing the total economic burden of $2.8 billion in a typical year (Hoffmann *et al*., 2015). Although infections can occur in healthy individuals, the target group under the risk of clinical listeriosis is the pregnant women, elderly people, unborn babies, neonates and people under immunosuppressive therapy (Lamont *et al*., 2011). This pathogen also causes the disease in domestic animals, including cattle and sheep, leading to spontaneous abortion in pregnant animals or circling disease in adult animals (Czuprynski, 2005). Generally, *L. monocytogenes* is widely present in a diverse environment including water, sludge, soil, plants, vegetation and also resides in a gastrointestinal track of many animal species (Liu, 2008; Esteban *et al*., 2009). The carriage of this pathogen by animals can lead to transmission of zoonotic

infection via milk or meat. The environmental contamination of raw foods going through further minimal processing is a likely great risk for human health (Swaminathan and Gerner-Smidt, 2007).

After major outbreak in 1981 (Schlech *et al*., 1983) from coleslaw (a regional salad dish in US), many researchers explored different types of food products and isolated *Listeria* from milk and milk products (D'Costa *et al*., 2012), meat and meat products (Derra *et al*., 2013), seafood (Parihar *et al*., 2008) and raw vegetables (Ananchaipattana *et al*., 2012). Of these, industrially processed and refrigerated food revealed to be frequently linked to *L. monocytogenes* outbreaks than raw foods (Gianfranceschi *et al*., 2002). Though, several efforts have been taken to avoid bacterial contamination in food industries, biofilm formation, tolerance and an adaptation capability of *L. monocytogenes* allows persistence in foods and food processing industries. In addition, methods used for food preservation or to increase shelf-life of food such as high salt concentration, storage at low temperature, lowering the pH of food, addition of food preservatives has found ineffective in case of *L. monocytogenes*. Following the food related stresses to establish an infection *L. monocytogen*es must pull through the stressful conditions inside the human host including the acidic pH within the stomach, followed by immensely different stress conditions during intestinal passage and/or infection (Sleator *et al*., 2005). It is also resistant to detergents and disinfectants used for sanitizing the processing in food industry environments. These abilities may reason behind the survival and persistence of *L. monocytogenes* in the food industry and increase its occurrences in the foods (Gandhi and Chikindas, 2007). However, such persistence may cause contamination of food being processed. Further, such contaminated food that stored at refrigerator exclusively enriches the growth of *L. monocytogenes* (Martins and

Germano, 2011; Kramarenko *et al*., 2013). The 99% of the infections of *L. monocytogenes* are thought to be foodborne (Swaminathan and Gerner-Smidt, 2007). The worldwide studies confirm 20-25% prevalence of *L. monocytogenes* in foods and food processing environments related to meat, milk, fish and other food products (Lianou and Sofos, 2007). The high mortality rate and its prevalence in different environments led governments as well as food safety agencies make policies to reduce the occurrence of *L. monocytogenes* in foods. The United States has adopted a zero-tolerance policy in case of *L. monocytogenes*. The detectable presence of this pathogen costs recalls of RTE foods and it has big economic implications on food industries. The disease also found to responsible health and productivity loss including high medical costs every year (Ivanek *et al*., 2005; Hoffman *et al*., 2015). Effective control strategies are highly demanded by food industries. While to device the comprehensive approach, the central mechanism lying behind the persistence of the *L. monocytogenes* needs to be understood. The current research is frequently concluding that stress tolerance abilities of *L. monocytogenes* allow the survival and persistence in food industrial premises. Earlier studies have observed large variation in stress tolerance of *L. monocytogenes* under different conditions of high salt stress, acidic and/or alkaline pH stress and low-temperature stress (De Jesus and Whiting, 2003; Van der Veen *et al*., 2008; Valero *et al*., 2014). The limited studies are available attempting relation between stress tolerance and serotype or origin of isolation. Numerous investigations are based on a physiological basis of stress tolerance, but most of these studies are available with limited numbers of strains (Vialette *et al*., 2003; Liu *et al*., 2005; Lianou *et al*., 2006; Vermeulen *et al*. 2007). This approach limits investigation from giving a full picture of the physiology of stress tolerance and adaptation. In order to

control the spread, the stress tolerance mechanisms of *L. monocytogenes* have been a prime area of present research worldwide. Several universal stress mechanisms have been identified in *L. monocytogenes*, which help cells to get easily adapted to low-level stresses inducing tolerance capabilities (Begley and Hill, 2015). Nevertheless, mutants prepared to eliminate or control these stress mechanisms from *L. monocytogenes* could only partially explain the stress tolerance, suggesting that there are still unknown mechanisms exist for stress tolerance. In-depth cognizance of molecular mechanism behind stress tolerance of *L. monocytogenes* is, therefore, vital for devising the effective control strategies and consequently to control the incidence of foodborne listeriosis. It is also important to understand the innate stress tolerance capability of *L. monocytogenes* for different stresses. Considering these factors a study was designed to understand survival, adaption and innate stress response of *L. monocytogenes* for different food processing and/or food storage related stresses. This study also plotted with the aim, to perceive qualitative, quantitative and mechanistic knowledge on the stress response of *L. monocytogenes* for important hurdles used in food processing or preservation, namely high salt concentration, low temperature, acidic and alkaline pH. The research was conducted using *L. monocytogenes* strains isolated from diverse sources; broadly food (raw, processed and RTE), environmental (natural and food processing) and clinical (human and animal) with the following objectives-

1. To screen *Listeria monocytogenes* strains from diverse sources for their ability to tolerate salt, pH and growth at low temperature.

2. To determine the change in morphological and physico-chemical parameters of the stress tolerant strains

3. To determine the genetic basis for tolerance.

Chapter 2

Screening of *Listeria monocytogenes* strains from diverse sources for their tolerance against different stresses

2.1 Introduction

The frequency of foodborne outbreaks and illnesses has been increased in recent years and found to be imposing a considerable economic burden (DeWaal *et al*., 2012; de Noordhout *et al*., 2014). These foodborne outbreaks are also responsible for socio-economic impact due to illnesses, productivity loss and deaths, as well as loss of food industries due to food recalls and shut downs. The changing life-style and food habits have increased the demand for RTE Foods; which has made the food industries, a part of daily life and hence problems associated with food industries have become evident. The primary challenge in food processing is a successful removal of naturally present microflora from food, as it has a greater impact on the shelf-life of foods (Bhunia 2008; Quigley *et al*. 2013). Also, this flora may contain pathogens of major public health concern. Studies so far have revealed that pathogens can enter in food processing environment from the natural environment and/or raw material being processed and finally in foods. Once entered in food processing environment they establish themselves in there, in a small niche (Carpentier and Cerf, 2011; Asselt *et al*., 2017; Netzeler and White, 2017). This makes *L. monocytogenes* a significant cause of public health concern. Besides being of important public health hazard, *L. monocytogenes* also has economic importance as the detectable presence of this pathogen costs recalls of RTE foods and it has big economic implications on food industries (Hoffman *et al*., 2015). Since it is mandatory to the food industries to deliver the 'pathogen free' food (especially, RTE food industries) till it reaches to table of consumer, all the food industries need to be sure of absence of such pathogens in the foods being processed and to be sold (Robach, 2012). Hence to control the spread of such pathogens, government bodies have specified certain rules and regulations for the food industries (FDA, 2012;

FDA-BAM 2013). In India, since August 2011, Food Safety and Standards Authority of India (FSSAI) have included *L. monocytogenes* pathogen in the regulations and have made it mandatory for the absence of *L. monocytogenes* in foods (MHFW, 2011).

The competence of *L. monocytogenes* to accommodate, endure and proliferate at a broad range of environmental stresses appears to be associated with foodborne transmission. In food processing environments, *L. monocytogenes* is known to survive and persist for longer periods of time; but, many times source of contamination is unknown (Ferreira *et al*., 2014). Since last two decade, the incidences of listeriosis have been increasing which can be traced to increased use of RTE and industrially processed food (Garrido *et al*., 2010). Food industries use several food preservation methods to inhibit the bacterial growth and increase in shelf-life of the product. Reduction of temperature (freezing), reduction of water activity (a_w) (by the addition of salt) and alteration of pH (by addition of acids or by fermentation) are common techniques used by different food industries for prevention and elimination of pathogen (Jay, 2000).

A number of *Listeria* strains have been isolated from clinical, food, and environmental samples by many researchers all over the world. In India, *L. monocytogenes* strains have been isolated from diverse foods like meat, dairy products and environmental sources such as piggery environments and different food processing plants (Barbuddhe *et al*., 2000 ; Barbuddhe *et al*., 2002; Doijad *et al*., 2011; Raorane *et al*., 2014). It has been also isolated from fish and fishery products (Karunasagar and Karunasagar, 2000; Parihar *et al*., 2008; Gawade *et al*., 2010; Rodrigues *et al*., 2015). The advancement in understanding of stress response in *L. monocytogenes* is required to limit and/or prevent the occurrences of *L.*

monocytogenes in foods and/or food related environments. Although there is no sufficient information available regarding health risks as strain specific, there is virulence heterogeneity among the strains of *L. monocytogenes* (Barbour *et al*., 2001). This heterogeneity could partially explain the distribution of serotypes. To date, variability in stress responses of *L. monocytogenes* is generally poorly characterized with few exceptions (Shabala *et al*., 2008). Hence, it is required to characterize a large number of *L. monocytogenes* strains of different origins representing the epidemiologically important serotypes for their physiological properties and phenotypic characters. This characterization includes growth behavior of such strains under different environmental conditions or resistance to various stresses. This can help to obtain better insights of the virulence, distribution, and epidemiology of this organism with respect to serogroups and source of isolation.

2.2 Review of Literature

2.2.1 Brief History

Listeria was first isolated in 1926 by E.G.D. Murray from rabbits and named it *Bacterium monocytogenes* (Murray *et al*., 1926). Further, in 1927, Pirie isolated a bacterium from the liver of gerbils and named it *Listerella hepatolytica*. To honour pioneer of antiseptic surgery Joseph Lister, the genus was named eventually as 'Listeria' in 1940 due to taxonomic reasons (Pirie, 1940). The first listeric infection in a human was reported by Nyfeldt in 1929 (Nyfeldt, 1929). Since then over the years *Listeria* has been isolated from chicken, pig, cattle, buffaloes, goat, fish, diverse types of foods and different environments (Gray and Killinger, 1966; Swaminathan and Gerner-Smidt, 2007). In the 1980s, there were several outbreaks reported and source of one of the outbreaks found to be pasteurized milk. Then the

question arose about tolerance of *L. monocytogenes* to the pasteurization procedure (Fleming *et al*., 1985).

In India, listeriosis was first detected and described at Hyderabad in sheep in 1936-37 (Mahajan, 1936). The pathogen was isolated for the first time successfully in Madras from an infected sheep in 1950 (Vishwanatahan and Iyar, 1950). Krishna *et al*. (1966) reported the first case of human listeriosis. Following years there are reports of sporadic cases (Barbuddhe *et al*., 2012), but no surveillance data for listeriosis is available in India (NCDC, 2016). There is a lack of awareness about listeriosis in India. Since there is lack of mandatory notification and no organization keeps track of listeriosis; no data is available.

2.2.2 Genus *Listeria*

Listeria spp. are Gram positive, rod shaped, small size (0.5 X 1-2µm, diameter by length) facultative anaerobe, non-capsulated, non-endospore-forming, catalase positive and oxidase negative bacteria (Singleton, 1999). All species are motile at ambient temperature (20°C-25°C), but non-motile when cultured at 37°C (Glasworthy *et al*., 1990). The genus *Listeria* is classified under family *Listeriaceae* (Ryser and Marth, 1999). It is comprised of total 17 species namely, *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii, L. grayi, L. marthii, L. rocourtiae, L. weihenstephanensis* and *L. fleischmanii*. Recently, seven new species have been added, *L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis, L. booriae* and *L. newyorkensis* (den Bekker *et al*., 2014; Weller *et al*., 2015). Among 17 species only two are pathogenic; *L. monocytogenes* and *L. ivanovii*. The *L. monocytogens* is a major pathogen capable to develop the disease in

humans as well as in animals and *L. ivanovii* is predominantly pathogenic for ruminants and very rarely in humans (Guillet *et al.*, 2010).

2.2.3 *Listeria monocytogenes*

L. monocytogenes is a major pathogen of interest among the genus *Listeria* because of its capability to cause listeriosis in humans as well as animals. *L. monocytogenes* is also known to be ubiquitous in nature and capable to thrive diverse stressful environmental conditions; it can easily colonize in different ecological niches and can persist there for longer period of time. Another quirky character, this bacterium has both psychrophilic and mesophilic features. Being exclusively foodborne pathogen with the known to effectively survive and multiply in many different non-host environments, *L. monocytogenes* is one of the most important concerns of food industries. There are total 12 serotypes of *L. monocytogenes* namely 1/2a, 1/2c, 3a, 3c, 1/2b, 3b, 7, 4b, 4d, 4e, 4a and 4c. Although all the serotypes are pathogenic, 90% of human listeriosis cases are linked with 3 predominant serotypes of 4b, 1/2b, and 1/2a (McLauchlin., 1997). These 3 serotypes are considered as important because of their prevalence in all important niches, especially serotype 4b has been found to be responsible for major food borne outbreaks (Buchrieser *et al.*, 1993), while majority of the foods and food processing environment isolates belongs to serotype 1/2a or 1/2b (Farber and Peterkin, 1991). *L. monocytogenes* is an intracellular pathogen which resides within monocytes and neutrophils (Gray and Killinger, 1966). *L. monocytogenes* shows tumbling motility when cultured at ambient temperature and loses it when infects and resides in host cells. Inside the host *Listeria* makes movement possible by polymerizing actin to form actin tails and propels with it (Salyers and Whitt, 2002).

2.2.4 <u>The Disease</u>

Listeriosis in humans is generally caused by consumption of food contaminated with *L. monocytogenes*. According to United States Department of Agriculture (USDA) Economic Research Services, there are 15 major foodborne illnesses with considerable economic burden due to health and productivity losses including high medical costs every year. Among those 15 major foodborne illnesses, listeriosis is the 2nd costliest disease per case with estimated $2.8 billion economic burdens in a typical year (Hoffman *et al*., 2015). Out of this total economic burden, $2.1 billion is due to deaths and that too only from 1,600 illnesses (Hoffman *et al*., 2015). The infection occurs mostly in immune-compromised individuals. The elderly people, pregnant woman, children below 5 years of age, cancer patients and patients under immunosuppressive therapy are at risk (Goulet *et al*., 2012; Silk *et al*., 2012). Initial symptoms of listeriosis are variable including fever, muscle ache, nausea, and diarrhea. In untreated cases or on the immune status of infected individuals; these symptoms can further develop to septicemia, meningitis, encephalitis and may lead to death (CDC, 2012). There are two different forms of listeriosis with respect to age band of patients: listeriosis in adults and fetomaternal listeriosis (Schuchat *et al*., 1996; Vázquez-Boland *et al*., 2001). The feto-maternal listeriosis is thought to be blood-borne colonization of placenta; this can subsequently result in a listerial invasion of the fetus and may lead to abortion or stillbirth (Farber *et al*., 1991). Listeriosis in adults; which is in severe form is typically associated with infections of central nervous system, manifests meningitis or meningoencephalitis (Brouwer *et al*., 2006). Overall listeriosis has 20-30% case fatality rate, 50% neonatal death rate and 91% hospitalization rate (Farber and

Peterkin, 1991; Sartor *et al*., 2015). Out of 91% hospitalized cases, about 3% cases are moderate. The 80% of adult hospitalizations needs ICU care (Batz *et al*., 2014).

2.2.5 <u>Food as Major Source of Transmission</u>

The listerial infection in humans can be due to direct contact with the contaminated environment, direct contact with infected part of an animal or transfer from infected mother to fetus (Vázquez-Boland *et al*., 2001). The wide categories of foods have been identified as principal routes of transmission. Especially foods which do not require cooking before consumption or foods stored in the refrigerator to extend shelf-life have a higher risk of *L. monocytogenes* growth and transmission (Lianou and Sofos, 2007). These food categories include raw foods, finished food products and RTE foods; such as raw meat, poultry and its products, raw and processed vegetables; raw and/or inadequately pasteurized milk, dairy products, including ripened soft cheese (Amato *et al*., 2017); ice-cream; salads including coleslaw; retail-cook-chill meals; shellfish, raw fish and fish products (Ramaswamy *et al*., 2007; Swaminathan and Gerner-Smidt, 2007; Barbuddhe *et al*., 2012). In US deli meat has found to be a greater vehicle of transmission leading to outbreaks and RTE foods at greater risks (FSIS 2014; Zang *et al*., 2012). Recently, the rate of listeriosis outbreaks observed to be increased in many European countries (Koch and Stark 2006; Antal *et al*., 2007; Garrido *et al*., 2008; Goulet *et al*., 2008; Cairns and Pyrne, 2009; Kasper *et al*., 2009; Smith *et al*., 2009). Outbreak associated with Tome cheese in Switzerland (Bille *et al*., 2006), sandwich associated outbreak in the UK (Dawson *et al*., 2006), soft cheese related outbreak in the Czech Republic and a multinational outbreak in Austria and Germany by Quargel Cheese (Fretz *et al*., 2010) have been reported. In India, 6.66% to 7.08% goat meat (Barbuddhe *et al*., 2000; Rekha *et al*., 2006), 7.4% sheep meat (Barbuddhe *et al*., 2000), 3.07% to 6%

buffalo meat (Bramhabhatt and Anjaria, 1993; Barbuddhe *et al*., 2002) and 8.l% poultry meat samples (Barbuddhe *et al*., 2003) found to be positive for *L. monocytogenes*. In the case of milk and dairy products, 4.82% of raw milk found to be carrying *L. monocytogenes* (D'Costa *et al*., 2012), Incidence of *L. monocytogenes* in wide range of food processing environments/plants was evident to be involved in post-processing contamination (Leong *et al*., 2014). Although organisms are killed by normal cooking regimes; some cheeses made from pasteurized and/or unpasteurized milk, inadequate cooking; contamination after cooking; partially cooked foods; foods served uncooked or with minimal processing (fruits, dairy products), foods processed to be served without further processing (RTE foods) may carry *L. monocytogenes* and sustain the pathogen easily (Chan and Wiedmann, 2008). Additionally abilities of this pathogen like osmotolerance (up to 12.5%) (Farber *et al*., 1991; Liu *et al*., 2005; Shabala *et al*., 2008), diverse pH stress tolerance (4.0 to 9.5) and low temperature tolerance (down to 0°C) (Buchanan *et al*., 2004; Gandhi and Chikindas, 2007) makes *L. monocytogenes* to survive in different foods and food processing environments and increases the chances of foodborne transmission (Pérez-Rodríguez *et al*., 2008). Interestingly, similar hurdles are often being used in food processing and/ or food preservation. This may lead to the development of cross protection from different stresses (Durack *et al*., 2013). Once colonized in food processing plants, *L. monocytogenes* strains persist in those conditions; however, the mechanism for persistence is unclear. Further storage of contaminated food at lower temperatures may support the growth of *L. monocytogenes* and lead to increase a load of the pathogen in foods (Havelaar *et al*., 2010). The incidence in such wide range of foods that to tolerating capacity to deleterious conditions have made *L. monocytogenes* as alarming foodborne pathogen

around the globe. The high mortality associated with this pathogen makes *L. monocytogenes* the deadliest food borne pathogen in comparison with common food borne pathogens such as *Salmonella enteritidis*, *Campylobacter* sp., and *Vibrio* sp. (Painter *et al*., 2013).
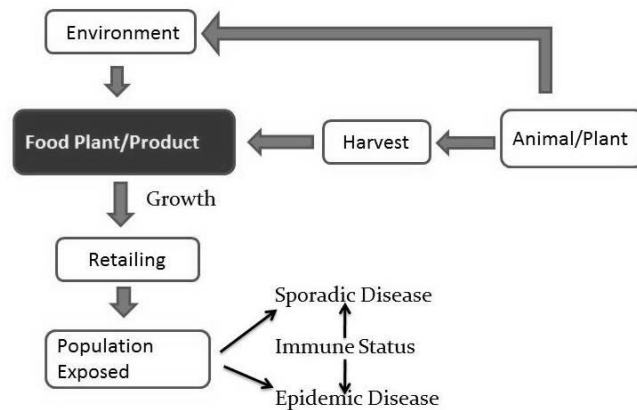
Fig. 2.1: Ecological interrelationships of *L. monocytogenes*, the environment, food, and clinical cases of listeriosis. (Adapted from - Handbook of *Listeria monocytogenes* by Dongyou Liu (2008))

2.2.6 Stressors and listerial stress adaptation

The deleterious effect of external factors on the physiology of bacterial cells leading to the reduction of growth rate or at extreme levels can result in inhibition/killing of bacteria is called as 'environmental stress' (Archer, 1996; McMahon *et al*., 2007). These stresses can be varying conditions in the external environment of the bacteria challenging their optimal growth. Foodborne pathogens experience and need to overcome different stresses during transitions from environment to the gastrointestinal tract of mammalian hosts via foods. Once bacteria survives and grows this resistance helps them to persist in different niches (NicAogáin and O'Byrne, 2016). In a case of foodborne pathogens, these stressors are mostly hurdles used in food processing and/or food preservations. The most important and commonly used hurdles in food preservations include low

temperature, high salt concentrations, extreme pH and food preservatives (Leistner, 2000). The basic concept behind using such stressors as hurdles is putting microorganisms under stressful environmental conditions to cause inhibition of their growth, reducing the numbers or to death. But the limitation is these stressors cannot be used at extreme concentrations/levels that can alter the nature, texture, taste, flavor or appearance of the foods (Lucera *et al*., 2012). So the key phenomenon arises in this food preservation is an adaptation to these stresses by microorganisms. Bacteria can adapt to these stresses by modulating internal mechanisms which help them mainly to maintain homeostasis and in developing stress reactions. Homeostasis is a self-regulating process where bacteria maintain uniformity and integrity inside the cells irrespective of changes in external environmental conditions that surround them. This helps bacteria to perform all intrinsic metabolic processes required for survival and growth without affecting due to changes in external environment. Homeostasis is generally achieved by mainly with ion transport mechanisms across the membrane, compatible solute transporters or adjusting the lipid in the cell membrane, thus altering their permeability (Wang *et al*., 2010; Krulwich *et al*., 2011; Yoon *et al*., 2015; Hoffmann and Bremer, 2017). In stress reactions, some bacteria generate the stress shock proteins and with adaptation, they become more stress tolerant to the severe stresses. Even there are some stress response proteins generated by bacteria help them to tolerate more than one stress. Likewise in *L. monocytogenes* there are classes of proteins generated during the stress response, namely, class I heat shock response regulated by HrcA response and class III heat shock response, which is regulated by the ctsR response (Pleitner *et al*., 2014). The heat shock response specifically activated in cold shock (Liu *et al*., 2002), increase in temperature (Weibezahn *et al*., 2004; Shen *et al*., 2014), high salt

stress (Duche *et al*., 2002; Mataragas *et al*., 2014) or in acid shock (Metselaar *et al*., 2015). Heat shock response encodes for molecular chaperones and proteases that protect cell proteins from aggregation and misfolding (Lee et al., 2003; Abee *et al*., 2016) and helps to reactivate protein aggregates. In *L. monocytogenes* there is another important stress response mechanism, the class II response regulated by sigB protein. This response gets activated by different stresses and help *Listeria* to adapt different stressed conditions (Ferreira *et al*., 2001). In class II stress response, there are genes encoding for decarboxylases for acid resistance (Ferreira *et al*., 2003; Smith *et al*., 2012); osmolyte transporter proteins including OpuC, DtpT, Gbu, and BetL for high salt and low temperature stress and reductases for oxidative stress resistance (Soni *et al*., 2010; Begley and Hill 2015). Class I response includes the genes which encode molecular chaperones and include *dnaK*, *dnaJ*, *grpE*, *groES*, and *groEL*. These chaperone products are in two forms, namely KJE (DnaK, DnaJ, GrpE) and GroESL. GroESL are involved in the refolding of denatured proteins after exposure to the stresses (Hecker *et al*., 1996; Sharma *et al*., 2003). GroESL proteins get induced after exposure to the low-temperature stress and acid stress. DnaK protein induction has been observed after exposure to the salt stress, cold stress and disinfectant stress (Duche *et al*., 2002; van der Veen and Abee., 2010; Soni *et al*., 2011). Recently, down-regulation of DnaK under high salt stress has been reported (Payne *et al*., 2013; Bergholz *et al*., 2012). The class III response consists of molecular chaperones as well as ATP dependent Clp proteases, which includes two ClpP ATPases ClpB, ClpC and ClpE. The Clp ATPases play important role in survival under stressed environment as well as in virulence (Gaillot *et al*., 2000; Begley and Hill 2015). ClpB is a chaperon which reactivates the protein aggregates in collaboration with KJE system. ClpB also found to be induced by low-

temperature stress (Liu *et al*., 2002; Lee *et al*., 2003). The ClpC and ClpE play important roles commonly in virulence and in internalization and cell division, respectively (Nair *et al*., 2000; Van Boeijen *et al*., 2010). These factors help *Listeria* for survival under different stressful environmental conditions.

2.2.7 <u>Salt stress adaptation</u>

Salt is most commonly used additive in foods which act as a preservative, enhances the flavor (Silva *et al*., 2003) and improves the water adsorption (Lawrence *et al*., 2003). Even if common salt does not have antimicrobial property; it reduces the water activity (a_w) at that level, at which metabolism or vital intracellular processes of microorganisms slow down. A high salt concentration generates osmotic effect and affects microbial cellular metabolism. Sodium Chloride (NaCl) is commonly used salt in food processing and preservations, and a total 75% of dietary salt comes from processed foods (Appel and Anderson, 2010). Salt plays different roles in different kinds of foods. In vegetables, salt is mainly used as a preservative (Luck and Pager, 2000), a softening agent (Van Buren, 2006), and also to achieve the dry-salting process (Panagou, 2006). In dairy products, specifically in cheese, salt is added to control the growth of lactic acid bacteria and to prevent contamination of undesirable microorganism; it also adds the flavor in cheese (Rowney *et al*., 2004). There are variety of salting procedures, which have been developed for preservation and taste development of different meat products such as cured ham, Italian Parma ham (Cobe, 2002; Pastorelli *et al*., 2003), Serrano ham (Barat *et al*., 2005, 2006; Luna *et al*., 2006). Salt is used as preservative in variety of fish and fish products, including sea bream (Chouliara *et al*., 2004; Goulas and Kontominas, 2007), chub mackerel (Goulas and Kontominas, 2005), salted cod (Thorarinsdottir *et al*., 2002; Barat *et al*., 2003), and smoked salmon

(Sigurgisladottir *et al*., 2000; Gallart-Jornet *et al*., 2007). Because of different properties of NaCl, like reducing water activity values, antioxidant effect, modifications in water-holding capacity, taste enhancer and most importantly preventing contamination and/ or growth of undesirable microorganisms in the food; NaCl has been empirical part of food processing and/or preservation (Caly *et al*., 2009).

Being widely used as a primary food preservative; development of resistance against the salt used in foods by different microorganisms and importantly by foodborne pathogens is major public health concern (Kim *et al*., 2017). *L. monocytogenes* is one of the important robust, persistent foodborne pathogens prevalent in food supply chain due to the ability to adapt various food and food processing environments (Shabala *et al*., 2008; Durack *et al*., 2013; Ferreira *et al*., 2014). A high salt concentration is one of the important conditions among foods and in food processing. *L. monocytogenes* is known to adapt and survive at osmotic stresses at that extent where other common pathogens cannot survive (Makaritit *et al*., 2015; Poimenidou *et al*., 2016). *L. monocytogenes* is known to have primary and secondary stress response to the osmotic shock. The influx of K^+ and glutamate into the cell is a primary response; while secondary response involves uptake of small molecules known as compatible solutes (Kallipolitis and Ingmer, 2001; Brøndsted *et al*., 2003). These mechanisms help bacterium specifically with maintaining turgor pressure, cell volume and stabilizing protein structure and activity. The compatible solutes generally accumulated in hyperosmotic conditions are glycine, betaine, and carnitine (Sleator *et al*., 2003; Lebre *et al*., 2017). Many bacteria are able to synthesize these compatible solutes, but *L. monocytogenes* lacks this ability and hence these compounds need to be transported inside the cells during hyperosmotic

conditions (Burgess *et al*., 2016). The uptake of such compatible solutes is driven by the transporters namely, Gbu, BetL, and OpuC. These transporters are also known to contribute to low-temperature stress response (Angelidis and Smith, 2003; Wemekamp-Kamphuis *et al*., 2004; Watson *et al*., 2009; Alvarez-Ordonez *et al*., 2015). Interestingly; SigB, a class II response has found to be a regulator of these transporter genes (Cetin *et al*., 2004; Utratna *et al*., 2011). In addition, K^+ import by the kdpE system facilitates the rapid adaptation to the salt stress (Brondsted *et al*., 2003). Schmid *et al*. (2009) reported the up-regulation of *csp* genes in cold shock as well as osmotic shock. This indicates the possible role of Csp proteins adaptation under osmotic up-shift. In absence of compatible solutes, Ctc proteins are reported to be involved in salt stress response. The exact role of Ctc protein in this salt stress response is not clearly known yet (Garden *et al*., 2003). The transcription study by Bergholz *et al*. (2012) revealed the important role of the MrpABCDEFG sodium/proton antiporter in adaptation to salt stress in *L. monocytogenes*. The mutagenesis study by Burall *et al*. (2012) revealed the involvement of *iap* gene in salt adaptation. As trehalose is also known to be involved in osmotic stress adaptation of many bacteria, characterization of the *tre* (*lmo1254*) gene (phosphotrehalose) indicates the role of the trehalose metabolism in stress resistance of *L. monocytogenes* (Ells and Hansen, 2011). Proline is known to be good osmoprotectant and in *L. monocytogenes* increased proline biosynthesis by ProBA observed under osmotic stress (Sleator *et al*., 2001). The Opp and DtpT oligopeptide transport systems are the alternative sources of osmoprotectants, where peptides are provided which can subsequently hydrolyse in amino acids, such as proline (Wouters *et al*., 2005). Two genes namely, *lmo2085* (a putative peptidoglycan bound protein) and *lmo1078* (a putative UDP-glucose phosphorylase) responsible for cell

envelope modifications have been involved in salt stress response (Chassaing and Auvray, 2007; Utratna *et al*., 2011). Recently, transposon mutagenesis study identified effect of osmotic shock on a growth of *L. monocutogenes lstC* gene deletion mutant (Burall *et al*., 2015), however, the exact function of the *lstC* gene is unknown. In another study, Δ*lmo0501* showed alteration in a growth of *L. monocytogenes* under osmotic stress (Michel *et al*., 2011). A guanosine tetra/penta-phosphate [(p)ppGpp] synthetase known to be important under various environmental conditions, the appropriate concentration of (p)ppGpp inside the cell is needed for osmotolerance (Okada *et al*., 2002; Braeken *et al*., 2006). So many researchers have characterized different candidate genes involved in osmotic stress response of *L. monocytogenes* by proteomic (Abram *et al*., 2008; Melo *et al*., 2013a, 2013b; Pittman *et al*., 2014), transcriptional (Bae *et al*., 2012; Bergholz *et al*., 2012; Durack *et al*., 2013; Ribeiro *et al*., 2014) and mutagenesis studies (Burall *et al*., 2012; Gardan *et al*., 2003). Still, further functional characterization of these genes is needed to clarify their exact role in the osmotic stress response of *L. monocytogenes*. Most of such studies are performed with standard strains or few field strains and that to mostly at moderate conditions. Scanty studies are available considering variation in response of large number of strains and least of them are available with attempt of correlation of stress response with serotype and/or diverse origin within large number of strains (Shabala *et al*., 2008; van der Veen *et al*., 2008; Durack *et al*., 2013). The variable stress response analysis of strains from diverse sources needs to be characterized for assessment of the presence of widely disseminated strain type (serotype/origin). The further characterization of dominant strains will give insights for designing of better control measures.

2.2.8 <u>Acid stress adaptation</u>

Acid stress is combined biological effect of low pH and weak acids present in the environment (Liu, 2008). Lowering the pH of the foods for prevention from spoilage as well as pathogenic microorganisms is one of the widely used methods. The pH of the food can generally drop by two methods; the first method is acidification of the food by direct addition of edible acids or acidic compounds to the foods and second method is fermentation (Alvarez-Ordóñez *et al*., 2015). Food borne pathogens need to combat with acidic environmental stress at various stages. Many bacteria have evolved in response to such changing environmental conditions with adaptive strategies to ensure their survival in inhospitable niches. *L. monocytogenes* is well-known acid stress tolerant among the major foodborne pathogens (Cotter and Hill, 2003; Rando and Verstrepen, 2007; Metselaar *et al*., 2013). There are three main mechanisms of acidic stress tolerance followed in foodborne pathogens; actions of enzymes involved in raising of intracellular pH by upgrading the amino acids breakdown and proton pump (proton efflux), acid shock proteins (ASPs) responsible for protecting and repairing proteins and DNA, and modifications in the composition of cell membranes (Alvarez-Ordonez *et al*., 2011; 2012). After exposure of *L. monocytogenes* to the acidic environment, there is the increase in the concentration of straight chain fatty acids and reduction of branched chain fatty acids in the membranes. This compositional change is achieved to comprise cellular proton permeability (Giotis *et al*., 2007). The acceleration of electron transfer through increased oxidation-reduction potential has been reported as one of the possible mechanisms used by bacteria to cope up with acid stress. In *L. monocytogenes* cells the adaptation to the low pH stress is triggered by dehydrogenases (GuaB, PDuQ and Lmo0560), reductases (YegT) and respiratory

enzymes, which includes the active cellular proton flow (Phan-Thanh *et al*., 2006). *L. monocytogenes* maintains the intracellular pH by expelling protons from cytosol via F_0F_1-ATPase proton pump mechanism (Merrell and Camilli, 2002). The glutamate decarboxylase system is well characterized acid stress adaptation mechanism in *L. monocytogenes* and plays a very important role in the survival and adaption to the low pH environments. *L. monocytogenes* fails to survive in the acidic environment when mutants of the *gadA*, *gadB* and *gadC* genes were constructed, which are involved in glutamate decarboxylase metabolism pathways (Merrell and Camilli, 2002; Wemekamp-Kamphuis *et al*., 2004; Cotter *et al*., 2005; Feehily and Karatzas 2013; Feehily *et al*., 2014). There is arginine deiminase (ADI) system in *L. monocytogenes* comprised of three enzymes, namely arginine deiminase (ADI), catabolic ornithine carbamoyl transferase (cOTC) and carbamate kinase (CK) (Ryan *et al*., 2008; Smith *et al*., 2012). These three enzymes are encoded by *arcA*, *arcB* and *arcC*, respectively. This system converts arginine into ornithine, NH_3, CO_2 and ATP. Ornithine transferred out of the cell in exchange of arginine and this process in conducted by the transporter encoded by *arcD* (Fulde *et al*., 2014). The ammonia produces in this reactions takes another proton to form NH_4^+. This results in the increase of cytoplasmic pH and helps bacteria to maintain intracellular pH under extracellular acidic environment. The negative effect on the lifetime in *argR* and *arcA* deletion mutant of *L. monocytogenes* is observed under low pH stress (Ryan *et al*., 2009; Cheng *et al*., 2013; Cheng *et al*., 2017). Involvement of *lmo0038* has also been observed in low pH stress response of *L. monocytogenes* (Chen *et al*., 2009). Madeo *et al*. (2011) demonstrated that thiamine played an important part in the acid resistance mechanisms of *L. monocytogenes*. They showed that thiamine depleted cultures affected significantly under acid stress than that of thiamine sufficient

cultures. Following the transposon based mutagenesis studies, it is hypothesized that cells failed to produce acetoin without thiamine, where acetoin is a proton-consuming compound acquired from pyruvate, which is important for pH homeostasis. It has been also observed that HtrA system and LisRK system play a significant role in the stress response of *L. monocytogenes*. The experimental deletion of *lisRK* gene encoding the system of two component signal transmission showed the increased sensitivity to the acid stress (Merrell and Camilli, 2002; Wonderling *et al*., 2004; Sleator and Hill, 2005). Transcriptomic analysis of *L. monocytogenes* under low pH stress showed changes in the expressions of genes related to protection from oxidative stress, DNA repair, structural modulations in cell wall as well as induction of genes of cofactors and fatty acid biosynthesis, and activation of *δB*, *prfA*, *hrcA* and *ctsR* regulons (Bowman *et al*., 2010). A ribosomal protein S21 encoded by *rpsU* found to be responsible for variation in the acid stress response of different *L. monocytogenes* (Metselaar *et al*., 2015). Recent studies reported the role of NmlR$_{lm}$ regulator in *L. monocytogenes* in survival at highly acidic environments (Supa-amornkul *et al*., 2016). Many researchers have studied the acid stress response in *L. monocytogenes* and it has been observed that microbial population is not isogenic showing resistant variants for stresses. So it is important to understand the variation in stress response of *L. monocytogenes* strains from diverse sources with different subtypes. There is need to understand the phenotypic and genotypic differences in stress response. This will help to emphasize the diversity of stress resistance in *L. monocytogenes* and to identify stable resistant dominance of particular subtype in unsuitable niches (Metselaar *et al*., 2015).

2.2.9 <u>Alkaline Stress adaptation</u>

The alkaline cleaners and sanitizers are used widely in food processing plants and food service industry. The foodborne pathogens may get exposed to alkaline stress at variety of pre and post-processing stages in food processing environments. Strongly alkaline cleaners are being used to clean the surfaces and/or equipments in meat processing industry (Marriott and Gravani 2006). Several different type of sanitizers used in food processing plants have alkaline pH, such as quaternary ammonium salts. Benzalkonium chloride and Cetrimide are among these widespread sanitizers (Sharma *et al*., 2003). Generally Chlorine compounds are used extensively for cleaning in the food industry. Many alkaline sanitizers are used to be applied directly on food and have varying success rate in killing target microorganisms (Fonseca, 2006). A commercial washing aid containing water, oleic acid, glycerol, ethanol, potassium hydroxide, sodium bicarbonate, citric acid, and distilled grape fruit oil found to be reducing *Salmonella* count significantly as compare to water control, when applied on tomatoes (Harris *et al.*, 2001). These different types of sanitizers have been employed to kill *L. monocytogenes* and other bacterial load in food facilities, surfaces coming in contact with raw/finished food products and raw food surface itself (Somers & Wong 2004; Nakamura *et al*., 2013). A proper and recommended use of disinfectants should inhibit the food borne pathogens, but the failure of these procedures has been observed. There are some factors such as inadequate cleaning and faulty disinfection procedures, food debris, biofilm formation, dosage failure or most importantly, acquired resistance characters responsible for a significant reduction of the efficacy of disinfection procedures (Müller *et al*., 2013). The repeated exposure to the sub-optimal concentrations of these disinfectants induces the resistance in the pathogens like *L. monocytogenes* (To

et al., 2002). Giotis *et al*. (2008) reported that the pre-exposure of *L. monocytoges* to pH 9.5 for 1h enhanced the survival to lethal alkaline pH 12.0. Another study by Zang *et al*. (2011) reported that the pre-exposure to pH 9.0 for 30 min. enhanced resistance of *L. monocytogenes* to the sub-lethal concentration of bile salt. Many researchers have reported resistance of *L. monocytogenes* to the alkaline disinfectants mainly used in food industries such as benzalkonium chloride (Romanova *et al*., 2006; Mullapudi *et al*., 2008; Ratani *et al*., 2012; Nakamura *et al*., 2013). Alkaline stress resistant strains have been isolated from many foods and food processing facilities; such as turkey processing plants (Mullapudi *et al*. 2008), dairy products, vegetables (Popowska *et al*. 2006) and cold smoked fish (Soumet *et al*. 2005). *L. monocytogenes* has an important property of extruding toxic ions of ionic disinfectants such as benzalkonium chloride (Moorman *et al*. 2005). This development of resistance to hardened alkaline conditions after exposure to mild alkali stress and contributing failure of removal of *L. monocytogenes* has increased the concerns (Sharma *et al*., 2003; Giotis *et al*., 2008). *L. monocytogenes* has been observed to be developing higher proportion of branched chain fatty acids; which includes more of *anteiso* forms and this is important for adaptation to adverse pH (Giotis et al., 2007). There are four broad categories of alkali stress adaptation, (i) increased metabolic acids production by deamination which helps in pH homeostasis (ii) ATP synthase activity coupled with H^+ entry to ATP generation is increased under alkaline stress (iii) alteration in the cell surface properties (iv) increased expression and activity of the monovalent cation/proton antiporters (Padan *et al*., 2005; Giotis *et al*., 2010). A proteomic study (Giotis *et al.,* 2008) reported the repression of a large number of proteins along with induction of 8 novel proteins under alkaline stress using *L. monocytogenes* 10403S strain. In exoproteome study

of persistent *L. monocytogenes* revealed up-regulation of four proteins suggested to be linked to the stress response: The membrane anchored lipoprotein Lmo2637; AA3-600 quinol oxidase (*qoxA*); formate tetrahydrofolase ligase (*lmo1877*) and the NADPH dehydrogenase (*namA*) (Rychli *et al*., 2016). The transposon based mutagenesis study in *L. monocytogenes* LO28 reported 12 mutants sensitive to alkaline stress. The identification of these mutation targets found to be putative genes (Gardan *et al*., 2003). A microarray analysis reported differential expression of total 390 genes. These genes involved in encoding for multiple metabolic pathways (including those associated with Na^+-H^+ antiporters), ATP-binding cassette transporters of functional compatible solutes, motility and the *sigB* controlled stress resistance network and virulence-associated factors. It is also stimulating *L. monocytogenes* chaperones such as *dnaK* and *groEL* (Giotis *et al*., 2010; Kocaman and Sarimehmetoglu, 2016). Compared to acid, osmotic and temperature stress, alkali stress adaptations in *L. monocytogenes* have not received much attention. However, alkaline stress is still one of the most important stresses need to be studied; as *L. monocytogenes* gets encountered to such stress very frequently, due to extensive use of alkaline agents to clean and/or sanitize food facilities, food contact surfaces and even raw food surfaces also (Shen *et al*., 2016).

2.2.10 <u>Cold stress adaptation</u>

The modern food industry aims to reduce the use of food preservative or preservative treatment to increase the shelf life of food. Refrigeration is very common and widespread method used in food industry for storage, in processing, and for transportation, as it is a common hurdle in microbial growth. Although refrigeration or cold storage are effective measures to inhibit many foodborne pathogens, this method has found to be ineffective in the case of *L. monocytogenes*

(Tasara and Stephan 2006; Melo *et al*., 2015; Cordero *et al*., 2016). *Listeria* faces different forms of cold stress challenges at various stages of food processing and storage and needs to come up with the response of effective molecular strategies for survival, adaptation, and proliferation. Cold stress affects several cellular events such as slowdown of metabolic processes, alteration in membrane fluidity by changes in lipid bilayer composition, which comprises cell membrane associated functions (Tasara and Stephan, 2006; Chan *et al*., 2007; Bajerski *et al*., 2017). After exposure to the low-temperature stress, bacteria pass through two stages, namely acclimation (cell arrest) and adaptation (Barria *et al*., 2013). In a case of *L. monocytogenes* various researchers have evaluated different mechanisms behind acclimation and growth at low temperature (Chan and Wiedmann, 2008; Soni *et al*., 2011; Durack *et al*., 2013; Hingston *et al*., 2017). As exposure to the low temperatures decreases the metabolic rate; in response, bacteria modulates the gene expression pattern towards stabilizing cells and fitness under low-temperature stress. These changes in gene expression also induces the several modifications, including membrane composition (Mastronicolis *et al*., 2006; Yoon *et al*., 2015; Hingston *et al*., 2017), cold shock proteins synthesis (Thieringer *et al*., 1998; Schmid *et al*., 2009; Chaikam and Karlson, 2010) and transporters synthesis facilitating uptake of osmolytes that act as cryoprotectants (Angelidis and Smith, 2003; Pittman *et al*., 2014). The capacities to tolerate low-temperature stresses and existing mechanisms involved in the acclimation and adaptation phases suggests that *L. monocytogenes* have plasticity in genome events and transcriptional regulation (Bresolin *et al*., 2008; Rantsiou *et al*., 2012; Durack *et al*., 2013). The DNA helicases and cold shock proteins play important role in growth of *L. monocytogenes*, especially cold shock proteins play important role in replication, transcription, and translation under low-

temperature stress (Samara and Koutsoumanis *et al*., 2009). Out of these cold shock proteins, CspA and CspD have been confirmed as very necessary proteins for growth at low-temperature stress. The deletion mutants of these two proteins failed to grow under cold stress (Schmid et al., 2009). The *lisR*, *lmo1172* and *lmo1060* genes are also found to be playing important role in survival of *L. monocytogenes* at low temperature (Chan *et al*., 2008). Moreover, *lhkA*, *yycJ* and *yycF* genes observed to be transcriptionally very active under cold stress (Liu *et al*., 2014). Cacace *et al*. (2010) studied the proteome of *L. monocytogenes* strain grown at 4°C and subsequently analyzed with Matrix Assisted Laser Desorption Ionization (MALDI). There was over-expression of total of 57 proteins in comparison with strain grown at 37°C. The proteome showed increased synthesis of proteins linked to energy production, oxidative stress resistance, nutrient uptake, lipid synthesis, and protein synthesis and folding. Ctc is another general stress response protein found to be playing a vital role in low-temperature adaptation (Garden *et al*., 2003). The gene *lmo1078* encoding for UDP glucose pyrophosphorylase proposed to promote cold adaptation. UDP glucose is essential for lipoteichoic acid production hence may have a role in the maintenance of structural integrity under cold stress (Chassaing and Auvray, 2007). Analysis of the metabolomes of *L. monocytogenes* strain 10403S at 8°C and 37°C revealed a total of 56 metabolites to be increased and concentrations of 8 metabolites to be decreased at 8°C as compared to 37°C (Singh *et al*., 2011). The metabolites with increased concentration at 8°C were amino acids, sugars, organic acids, urea cycle intermediates, polyamines, and different compatible solutes. The results of this study indicated that increased solute concentration in cytoplasm may be associated with cold stress adaptation in *L. monocytogenes* (Singh *et al*., 2011). Recently Miladi *et al*., (2017) demonstrated carnitine and betaine to act

as cryoprotectant and essential for rapid induction of the stress response under conditions like cold stress, typically encountered during food preservation. Till date, many studies have demonstrated that involvement of induction of cold stress proteins under cold stress as one of the main response mechanisms in *L. monocytogenes* (Durack *et al*., 2013). However, currently, limited knowledge is available about functional contribution of many proposed factors in alleviation of cold stress constraints at the molecular level.

2.2.11 Diversity and Stress response

The recent advancements in sequencing technologies have made it easier and reliable to get a full picture of whole genome sequences (WGS) (Stasiewicz *et al*., 2015). There are more than 100 complete WGS of *L. monocytogenes* strains available in Database Resources of the National Center for Biotechnology Information including standard strains of each serotype (NCBI database). The sequenced genomes ranges in the size of 2.84-3.24Mb, encode 2908 to 3235 genes with approximately 89% of coding sequences. *L. monocytogenes* genomes are conserved with maintaining high synteny in genetic organization and content (Hain *et al*., 2008; den Bakker *et al*., 2013; Barbuddhe *et al*., 2016). No large genetic shifting or inversions have been observed in the genome and that could be due to a low number of Insertion Sequence (IS) elements (Buchrieser, 2007). However, some genetic variations have been observed within strains and serotypes; this indicates despite lower genetic differences possible phenotypic traits can vary (Nelson et al., 2004; Bécavin *et al*., 2014). Some studies have revealed the presence and/or absence of specific genes restricted to serotypes and type strains (van der Veen *et al*., 2008; Burall *et al*., 2017). Genetic variations among strains and serotypes have showed the correlation with stress response to temperature (Bergholz

et al., 2010), acids, osmotic shock (van der Veen *et al*., 2008), bile salts (Barmpalia-Davis *et al*., 2008) and detergents (Müller *et al*., 2013; Meier *et al*., 2017); also to pathogenesis or to motility (Grundling *et al*., 2005). In most of the previous research, there is a lack of linkage between stress tolerance pattern and serotype and/or origin of strains (Vialette *et al*., 2003; Lianou *et al*., 2006). Fewer studies are available which have attempted such linkage. Most of the studies available with such linkage demonstrations have attempted with a low number of strains; which again limits the diversity of strains under the study (Makariti *et al*., 2015; Metselaar *et al*., 2015).

2.3 Materials and Methods

2.3.1 *Listeria monocytogenes* strains

A total of 104 *Listeria monocytogenes* strains were selected from the Indian *Listeria* Culture Collection (ILCC). The strains included in the study comprised of the strains isolated from different geographical regions of India and from diverse sources such as human as well as animal clinical cases (n=35), food processing and natural environment (n=28) and ready to eat (RTE) and raw foods (n=41) (Table 2.1). All the strains were characterized previously biochemically and for their serogroups (Doumith *et al*., 2004). The *L. monocytogenes* strains selected were belonging to serogroup 4b, 4d, 4e (n= 58), serogroup 1/2a, 1/2c, 3a, 3c (n=34) and serogroup 1/2b, 3b, 4b, 4d, 4e (n=12); considering their importance in foodborne outbreaks (Buchrieser *et al*., 1993). All the strains were maintained at -80°C in brain heart infusion (BHI) broth (Himedia, India) with 15% sterile glycerol (v/v) (Himedia, India).

2.3.2 Inocula preparation

Listeria monocytogenes strains were cultured on PALCAM agar (Himedia, India) at 37°C for 24 h. A single colony for each strain was inoculated in 10 ml of BHI broth and incubated at 37°C for 18 h. The cell densities of overnight grown culture were approximately 10^9 CFU/ml. The grown cultures were further diluted 1:100 with fresh BHI broth and used for inoculation in microplates.

2.3.3 Salt tolerance

Each strain was tested in duplicate for the salt tolerance in 96 well flat bottom microplates (GenAxy, India). BHI broth medium supplemented with additional

sodium chloride (Himedia, India) concentrations of 0.5%, 2.5%, 5%, 7.5%, 10% and 12.5% were prepared. Each well (containing media 190μL) was inoculated with 10μL of each diluted inocula. Plates were covered with sterile lid and then sealed with parafilm.

The duplicate sets were included for each salt concentration in each 96 well flat bottom microplates and a set of three plates was prepared for each experimental set-up. The inoculated plates were incubated at 37°C and growth was followed at O.D. 600nm after 24 h, 48 h, and 72 h (Multiscan Ascent, Thermofisher, USA) and compared with two un-inoculated wells serving as negative controls. The purity of cultures was checked by cultivating on BHI agar at the end of the experiment. In case of *Listeria monocytogenes*, growth/no growth boundary limit is not an absolute cut-off point but it represents a region where the probability of rapid decrease in growth as conditions become more extreme (Pascual *et al*., 2001). Hence in this study stains showing continuous increment of O.D.$_{600nm}$ up to 72h at highest stress of each type were considered as highly stress tolerant.

2.3.4 pH tolerance

BHI broth was prepared with the pH range of 4.0 to 9.5 with the increments of 0.5 pH units. The pH of the medium was adjusted using 1N HCl (Merck, Germany) for acidic pH and 1N NaOH (Merck, Germany) for alkaline pH. Each well (containing media 190μL) was inoculated with 10μL of each diluted inoculants and were incubated at 37°C. The procedures were carried out as explained for salt tolerance experiments.

2.3.5 Low temperature tolerance

The inocula of each *L. monocytogenes* strain were prepared as described earlier. Each strain was tested for its low temperature tolerance by inoculating in wells containing media 190μL for each strain in each 96 well flat bottom microplates in duplicate, and a set of three plates was prepared for each experimental set-up. In relation to refrigeration the temperature ranges mostly from 4°C to 6°C (refrigerators) and 10°C to 12°C (open chiller display units/ transport). This thermal range of 4°C to 12°C is generally in practice domestically and industrially. The plates were incubated at 4°C, 10°C, 18°C, 24°C and 30°C. The further observation procedures were carried as explained for salt tolerance experiments.

Table 2.1: List of *Listeria monocytogenes* strains from Indian *Listeria* Culture Collection (ILCC) used in this study

ILCC ID	PCR serogrouping	Source	Year of Isolation
ILCC001	4b, 4d, 4e	Food	2006
ILCC003	4b, 4d, 4e	Animal	2001
ILCC004	4b, 4d, 4e	Animal	2001
ILCC006	4b, 4d, 4e	Animal	2001
ILCC007	4b, 4d, 4e	Food	2007
ILCC010	4b, 4d, 4e	Food	2007
ILCC012	4b, 4d, 4e	Food	2007
ILCC013	4b, 4d, 4e	Food	2007
ILCC014	4b, 4d, 4e	Food	2007
ILCC015	4b, 4d, 4e	Animal	2001
ILCC016	4b, 4d, 4e	Animal	2006
ILCC017	4b, 4d, 4e	Human	2009
ILCC022	4b, 4d, 4e	Animal	2001
ILCC025	4b, 4d, 4e	Animal	2006
ILCC026	4b, 4d, 4e	Human	2006
ILCC028	4b, 4d, 4e	Human	2006
ILCC029	1/2b, 3b, 4b, 4d, 4e	Human	2006
ILCC032	4b, 4d, 4e	Human	2006
ILCC035	4b, 4d, 4e	Human	2009
ILCC036	4b, 4d, 4e	Human	2005
ILCC037	4b, 4d, 4e	Human	2005
ILCC038	4b, 4d, 4e	Human	2005

ILCC040a	4b, 4d, 4e	Animal	2001
ILCC042	4b, 4d, 4e	Animal	2006
ILCC043	4b, 4d, 4e	Animal	2006
ILCC045	4b, 4d, 4e	Animal	2007
ILCC051a	1/2a, 1/2c, 3a, 3c	Animal	2002
ILCC142	4b, 4d, 4e	Human	2005
ILCC145a	4b, 4d, 4e	Animal	2005
ILCC146	4b, 4d, 4e	Animal	2005
ILCC148	1/2a, 1/2c, 3a, 3c	Animal	2005
ILCC149a	4b, 4d, 4e	Animal	2005
ILCC150a Continued…	4b, 4d, 4e	Animal	2005
	1/2a, 1/2c, 3a, 3c	Food	2004
ILCC158	4b, 4d, 4e	Food	2006
ILCC161	4b, 4d, 4e	Food	2006
ILCC171	4b, 4d, 4e	Animal	2006
ILCC173	4b, 4d, 4e	Animal	2006
ILCC174a	1/2a, 1/2c, 3a, 3c	Animal	2006
ILCC175a	4b, 4d, 4e	Environmental	2002
ILCC176	4b, 4d, 4e	Environmental	2002
ILCC177a	4b, 4d, 4e	Environmental	2002
ILCC179	4b, 4d, 4e	Environmental	2002
ILCC183	4b, 4d, 4e	Environmental	2002
ILCC185	1/2a, 1/2c, 3a, 3c	Food	2008
ILCC187	4b, 4d, 4e	Food	2008
ILCC190	4b, 4d, 4e	Food	2008
ILCC192	1/2a, 1/2c, 3a, 3c	Food	2008
ILCC195	4b, 4d, 4e	Food	2008
ILCC196a	1/2a, 1/2c, 3a, 3c	Food	2005
ILCC264	4b, 4d, 4e	Food	2008
ILCC265	4b, 4d, 4e	Food	2008
ILCC266	4b, 4d, 4e	Food	2008
ILCC267	4b, 4d, 4e	Food	2008
ILCC269	4b, 4d, 4e	Food	2008
ILCC270	4b, 4d, 4e	Food	2008
ILCC272	4b, 4d, 4e	Food	2008
ILCC273	4b, 4d, 4e	Food	2008
ILCC274	4b, 4d, 4e	Food	2008
ILCC276	4b, 4d, 4e	Animal	2001
ILCC277	4b, 4d, 4e	Food	2008
ILCC279	4b, 4d, 4e	Food	2008
ILCC285	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC289	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC293	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC297	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC298	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC301a	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC302a	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC303a	1/2b, 3b, 4b, 4d, 4e	Food	2004

Continued…

ILCC304a	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC305	1/2b, 3b, 4b, 4d, 4e	Food	2004
ILCC312	1/2a, 1/2c, 3a, 3c	Food	2004
ILCC317	1/2a, 1/2c, 3a, 3c	Food	2007
ILCC325	1/2a, 1/2c, 3a, 3c	Food	2007
ILCC373	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC374	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC375	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC376	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC377	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC378	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC479	4b, 4d, 4e	Food	2008
ILCC494	4b, 4d, 4e	Animal	2006
ILCC496	4b, 4d, 4e	Environmental	2002
ILCC521	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC529	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC530	1/2a, 1/2c, 3a, 3c	Environmental	2010
ILCC619	4b, 4d, 4e	Human	2013
ILCC622	1/2b, 3b, 4b, 4d, 4e	Human	2013
ILCC624	4b, 4d, 4e	Human	2013
ILCC629	1/2a, 1/2c, 3a, 3c	Human	2013
ILCC767	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC768	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC769	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC770	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC771	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC772	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC773	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC774	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC775	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC776	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC777	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC778	1/2a, 1/2c, 3a, 3c	Environmental	2013
ILCC779	1/2a, 1/2c, 3a, 3c	Environmental	2013

2.4 Results and Discussion

2.4.1 Tolerance to different salt concentrations

Listeria monocytogenes, a ubiquitous pathogen, has been reported to survive in different harsh conditions. Because of its ability to adapt to adverse environmental conditions, control of *L. monocytogenes* in food processing facilities is difficult task (Gandhi and Chikindas, 2007).

As food is major route of transmission, it is likely to assume the hypothesis that observed epidemiological trends are reflections of the better adaptive response and subsequent infection by particular subtypes of *L. monocytogenes.* To identify such subtypes it is required to study stress response of large number of strains from diverse sources. To design adequate mild processing methods that can help for ample inactivation of pathogens like *L. monocytogenes*, in sights of this bacterium with diversity in relation to stress resistance need to understand. This understanding will help to decide level of exposure to certain stresses or treatment for effective inactivation of *L. monocytogenes*.

Indian *Listeria* Culture Collection (ILCC) has a large collection of strains of *Listeria* that have been isolated from various sources and diverse geographical areas of India. Therefore, the present study was conducted to assess the innate capacity of *L. monocytogenes*, belonging to different serotypes and isolated from various sources, to tolerate food-related stresses. Furthermore, the study attempted to correlate the stress tolerant strains with a source of isolation and serogroups identifying dominant serogroup.

It is well understood that *L. monocytogenes* have the extraordinary fitness to adapt diverse environmental conditions; including higher salinity, extreme pH and

colder temperatures. We analyzed a total of 104 strains isolated from clinical sources (n=35), food processing and natural environment (n=28) and RTE and raw foods (n=41) belonging to three epidemiologically significant serogroups 4b,4d,4e (n=58); serogroup1/2a,1/2c,3a,3c (n=34) and serogroup 1/2b,3b,4b,4d,4e (n=12) (Table 2.1). Strains exhibiting growth at 12.5% NaCl concentration were considered as 'high' stress tolerant (Table 2.2) (Makarti *et al*., 2015). Out of 104 strains studied a total of 12 (11.5%) strains were found to be tolerant up to 12.5% high salt concentration followed by 65 (62.5%) strains tolerant to up to 10% salt concentration and all the strains showed tolerance up to 7.5% salt (Fig. 2.2a). Six (17.14%) strains from clinical cases, 5 (17.85%) from environmental sources and 2 (4.87%) from food were found to be tolerant to the high salt concentration. Salting is the indispensable method used in the manufacturing of many foods such as cheese types; it is also used as additive for flavoring and preservation (Lou and Yousef, 1997). The salt concentrations generally used in such procedures are inadequate for inhibiting the growth of *L. monocytogene*s. In this study, all test strains were assessed without any previous adaptive exposure to the any of these high salt concentrations. The results showed the innate high salt tolerance by *L. monocytogenes* strains. This capability of the pathogen may explain its ubiquitous nature through survival and adaptation to diverse environment from soil to a eukaryotic host with the capacity to tolerate hardy conditions (Freitag, 2009) and also supports the use of *L. monocytogenes* as a model for understanding the switching life as environmental bacterium to pathogen inside the human cell (Xayarath and Freitag, 2012). As percent tolerant strains from clinical and food sources are similar, and the percentage of strains from environmental sources is low, there was no any exact correlation observed for salt stress tolerance and source of isolation of the strains.

Table 2.2: Average turbidity (a measure of stress tolerance capability) of *L. monocytogenes* strains of different serogroups from diverse sources at highest stress of 10% and 12.5% salt (Color indication: Red-high stress tolerant).

ILCC ID	Serogroup	Source	10%				12.5%		
			24h	48h	72h		24h	48h	72h
ILCC001	4b, 4d, 4e	Food	0.008	0.004	0.006		0.007	0.007	0.005
ILCC003	4b, 4d, 4e	Animal	0.009	0.016	0.015		0.006	0.008	0.008
ILCC004	4b, 4d, 4e	Animal	0.207	0.471	0.581		0.006	0.007	0.004
ILCC006	4b, 4d, 4e	Animal	0.005	0.010	0.009		0.005	0.007	0.005
ILCC007	4b, 4d, 4e	Food	0.003	0.006	0.002		0.004	0.008	0.008
ILCC010	4b, 4d, 4e	Food	0.172	0.359	0.514		0.006	0.007	0.004
ILCC012	4b, 4d, 4e	Food	0.010	0.011	0.013		0.009	0.005	0.006
ILCC013	4b, 4d, 4e	Food	0.008	0.007	0.007		0.009	0.007	0.004
ILCC014	4b, 4d, 4e	Food	0.013	0.016	0.015		0.010	0.007	0.005
ILCC015	4b, 4d, 4e	Animal	0.011	0.017	0.014		0.007	0.008	0.004
ILCC016	4b, 4d, 4e	Animal	0.008	0.007	0.010		0.002	0.006	0.003
ILCC017	4b, 4d, 4e	Human	0.256	0.459	0.561		0.005	0.005	0.013
ILCC022	4b, 4d, 4e	Animal	0.376	0.567	0.782		0.096	0.275	0.325
ILCC025	4b, 4d, 4e	Animal	0.193	0.343	0.534		0.003	0.004	0.009
ILCC026	4b, 4d, 4e	Human	0.107	0.255	0.358		0.009	0.005	0.010
ILCC028	4b, 4d, 4e	Human	0.251	0.482	0.594		0.003	0.004	0.007
ILCC029	1/2b, 3b, 4b, 4d, 4e	Human	0.302	0.522	0.787		0.135	0.272	0.364
ILCC032	4b, 4d, 4e	Human	0.163	0.264	0.456		0.003	0.005	0.002
ILCC035	4b, 4d, 4e	Human	0.143	0.338	0.372		0.006	0.007	0.006
ILCC036	4b, 4d, 4e	Human	0.185	0.322	0.496		0.003	0.006	0.008
ILCC037	4b, 4d, 4e	Human	0.219	0.486	0.616		0.002	0.001	0.002
ILCC038	4b, 4d, 4e	Human	0.102	0.345	0.564		0.005	0.005	0.008
ILCC040a	4b, 4d, 4e	Animal	0.225	0.392	0.411		0.009	0.012	0.014
ILCC042	4b, 4d, 4e	Animal	0.157	0.269	0.447		0.007	0.005	0.006
ILCC043	4b, 4d, 4e	Animal	0.236	0.442	0.527		0.001	0.004	0.007
ILCC045	4b, 4d, 4e	Animal	0.240	0.431	0.682		0.010	0.007	0.011
ILCC051a	1/2a, 1/2c, 3a, 3c	Animal	0.264	0.412	0.597		0.007	0.002	0.004
ILCC142	4b, 4d, 4e	Human	0.342	0.589	0.768		0.128	0.272	0.317
ILCC145a	4b, 4d, 4e	Animal	0.349	0.522	0.714		0.117	0.237	0.308
ILCC146	4b, 4d, 4e	Animal	0.301	0.510	0.695		0.007	0.004	0.009
ILCC148	1/2a, 1/2c, 3a, 3c	Animal	0.013	0.006	0.005		0.008	0.004	0.007
ILCC149a	4b, 4d, 4e	Animal	0.180	0.383	0.563		0.006	0.007	0.005
ILCC150a	4b, 4d, 4e	Animal	0.241	0.317	0.528		0.005	0.005	0.006
ILCC152	1/2a, 1/2c, 3a, 3c	Food	0.164	0.343	0.583		0.007	0.001	0.008
ILCC158	4b, 4d, 4e	Food	0.241	0.419	0.602		0.007	0.004	0.009
ILCC161	4b, 4d, 4e	Food	0.269	0.468	0.661		0.008	0.004	0.007
ILCC171	4b, 4d, 4e	Animal	0.175	0.397	0.574		0.004	0.007	0.002
ILCC173	4b, 4d, 4e	Animal	0.285	0.468	0.665		0.011	0.006	0.007
ILCC174a	1/2a, 1/2c, 3a, 3c	Animal	0.343	0.519	0.647		0.118	0.271	0.309
ILCC175a	4b, 4d, 4e	Environmental	0.361	0.569	0.719		0.124	0.295	0.315
ILCC176	4b, 4d, 4e	Environmental	0.116	0.348	0.498		0.057	0.059	0.072
ILCC177a	4b, 4d, 4e	Environmental	0.287	0.409	545		0.051	0.061	0.065
ILCC179	4b, 4d, 4e	Environmental	0.174	0.272	0.426		0.062	0.065	0.055
ILCC183	4b, 4d, 4e	Environmental	0.314	0.506	0.745		0.155	0.315	0.410
ILCC185	1/2a, 1/2c, 3a, 3c	Food	0.285	0.398	0.547		0.053	0.061	0.069

ILCC187	4b, 4d, 4e	Food	0.318	0.576	0.716		0.041	0.065	0.065
ILCC190	4b, 4d, 4e	Food	0.353	0.545	0.717		0.126	0.259	0.322
ILCC192	1/2a, 1/2c, 3a, 3c	Food	0.244	0.463	0.595		0.050	0.061	0.065
ILCC195	4b, 4d, 4e	Food	0.354	0.594	0.748		0.131	0.265	0.405
ILCC196a	1/2a, 1/2c, 3a, 3c	Food	0.013	0.019	0.012		0.007	0.006	0.015
ILCC264	4b, 4d, 4e	Food	0.011	0.016	0.007		0.013	0.018	0.005
ILCC265	4b, 4d, 4e	Food	0.010	0.003	0.001		0.006	0.007	0.009
ILCC266	4b, 4d, 4e	Food	0.33	0.516	0.602		0.007	0.006	0.004
ILCC267	4b, 4d, 4e	Food	0.172	0.259	0.314		0.002	0.001	0.005
ILCC269	4b, 4d, 4e	Food	0.011	0.015	0.004		0.004	0.003	0.004
ILCC270	4b, 4d, 4e	Food	0.268	0.396	0.557		0.009	0.011	0.004
ILCC272	4b, 4d, 4e	Food	0.113	0.356	0.586		0.007	0.002	0.004
ILCC273	4b, 4d, 4e	Food	0.159	0.318	0.432		0.006	0.014	0.011
ILCC274	4b, 4d, 4e	Food	0.254	0.423	0.569		0.017	0.008	0.006
ILCC276	4b, 4d, 4e	Animal	0.016	0.009	0.005		0.001	0.005	0.004
ILCC277	4b, 4d, 4e	Food	0.176	0.307	0.502		0.003	0.007	0.008
ILCC279	4b, 4d, 4e	Food	0.293	0.443	0.534		0.005	0.008	0.007
ILCC285	1/2b, 3b, 4b, 4d, 4e	Food	0.107	0.315	0.458		0.004	0.008	0.005
ILCC289	1/2b, 3b, 4b, 4d, 4e	Food	0.012	0.005	0.004		0.011	0.003	0.006
ILCC293	1/2b, 3b, 4b, 4d, 4e	Food	0.112	0.302	0.477		0.005	0.004	0.008
ILCC297	1/2b, 3b, 4b, 4d, 4e	Food	0.011	0.004	0.006		0.009	0.003	0.002
ILCC298	1/2b, 3b, 4b, 4d, 4e	Food	0.243	0.438	0.572		0.005	0.008	0.007
ILCC301a	1/2b, 3b, 4b, 4d, 4e	Food	0.017	0.011	0.005		0.003	0.004	0.001
ILCC302a	1/2b, 3b, 4b, 4d, 4e	Food	0.279	0.406	0.336		0.007	0.005	0.002
ILCC303a	1/2b, 3b, 4b, 4d, 4e	Food	0.016	0.018	0.014		0.008	0.002	0.008
ILCC304a	1/2b, 3b, 4b, 4d, 4e	Food	0.015	0.012	0.011		0.003	0.005	0.002
ILCC305	1/2b, 3b, 4b, 4d, 4e	Food	0.157	0.269	0.347		0.009	0.006	0.005
ILCC312	1/2a, 1/2c, 3a, 3c	Food	0.136	0.342	0.427		0.006	0.007	0.004
ILCC317	1/2a, 1/2c, 3a, 3c	Food	0.214	0.303	0.542		0.007	0.011	0.009
ILCC325	1/2a, 1/2c, 3a, 3c	Food	0.015	0.014	0.009		0.003	0.005	0.004
ILCC373	1/2a, 1/2c, 3a, 3c	Environmental	0.362	0.529	0.692		0.113	0.223	0.325
ILCC374	1/2a, 1/2c, 3a, 3c	Environmental	0.249	0.322	0.574		0.013	0.012	0.018
ILCC375	1/2a, 1/2c, 3a, 3c	Environmental	0.391	0.562	0.715		0.138	0.264	0.344
ILCC376	1/2a, 1/2c, 3a, 3c	Environmental	0.124	0.213	0.325		0.002	0.005	0.006
ILCC377	1/2a, 1/2c, 3a, 3c	Environmental	0.318	0.543	0.679		0.110	0.218	0.314
ILCC378	1/2a, 1/2c, 3a, 3c	Environmental	0.141	0.307	0.428		0.003	0.009	0.007
ILCC479	4b, 4d, 4e	Food	0.216	0.443	0.583		0.013	0.006	0.007
ILCC494	4b, 4d, 4e	Animal	0.114	0.299	0.402		0.007	0.005	0.004
ILCC496	4b, 4d, 4e	Environmental	0.271	0.418	0.581		0.011	0.006	0.005
ILCC521	1/2a, 1/2c, 3a, 3c	Environmental	0.175	0.387	0.574		0.006	0.003	0.007
ILCC529	1/2a, 1/2c, 3a, 3c	Environmental	0.285	0.468	0.595		0.010	0.007	0.011
ILCC530	1/2a, 1/2c, 3a, 3c	Environmental	0.211	0.438	0.581		0.009	0.012	0.008
ILCC619	4b, 4d, 4e	Human	0.243	0.409	0.647		0.011	0.012	0.010
ILCC622	1/2b, 3b, 4b, 4d, 4e	Human	0.356	0.548	0.788		0.119	0.266	0.352
ILCC624	4b, 4d, 4e	Human	0.287	0.409	0.645		0.009	0.013	0.011
ILCC629	1/2a, 1/2c, 3a, 3c	Human	0.274	0.392	0.572		0.007	0.004	0.007
ILCC767	1/2a, 1/2c, 3a, 3c	Environmental	0.003	0.006	0.005		0.002	0.004	0.005
ILCC768	1/2a, 1/2c, 3a, 3c	Environmental	0.185	0.298	0.347		0.004	0.005	0.007
ILCC769	1/2a, 1/2c, 3a, 3c	Environmental	0.178	0.316	0.412		0.005	0.004	0.002
ILCC770	1/2a, 1/2c, 3a, 3c	Environmental	0.013	0.005	0.007		0.003	0.004	0.002

ILCC771	1/2a, 1/2c, 3a, 3c	Environmental	0.014	0.003	0.005		0.001	0.003	0.001
ILCC772	1/2a, 1/2c, 3a, 3c	Environmental	0.124	0.343	0.542		0.009	0.005	0.006
ILCC773	1/2a, 1/2c, 3a, 3c	Environmental	0.010	0.003	0.005		0.003	0.002	0.004
ILCC774	1/2a, 1/2c, 3a, 3c	Environmental	0.241	0.407	0.528		0.009	0.007	0.009
ILCC775	1/2a, 1/2c, 3a, 3c	Environmental	0.116	0.343	0.383		0.006	0.009	0.010
ILCC776	1/2a, 1/2c, 3a, 3c	Environmental	0.014	0.011	0.012		0.005	0.007	0.008
ILCC777	1/2a, 1/2c, 3a, 3c	Environmental	0.185	0.302	0.396		0.003	0.011	0.018
ILCC778	1/2a, 1/2c, 3a, 3c	Environmental	0.005	0.007	0.007		0.005	0.005	0.008
ILCC779	1/2a, 1/2c, 3a, 3c	Environmental	0.206	0.395	0.464		0.005	0.003	0.004

2.4.2 Tolerance to different pH

Effect of diverse pH range (4.0 to 9.5 with an increment of 0.5 units) was studied on 104 strains of *L. monocytogenes*. The strains showing growth at pH ≤ 4.5 or ≥ 9.0 were considered as 'high' stress tolerant (Makarti *et al*., 2015). A total of 26 isolates were found to be tolerant to the extreme pH (acidic=13 and alkaline=13). Out of 104 strains tested 13 (12.5%) strains showed growth at pH 4.5, while, 76 (73.07%) strains showed tolerance up to pH 5.0 (Table 2.3) and all strains were tolerant up to pH 5.5 (Fig. 2.2b). While, 13 (12.5%) strains showed tolerance at pH 9.5 and 70 (67.3%) strains showed growth up to pH 9.0 (Table 2.4). All the strains showed the tolerance up to pH 8.5 (Fig. 2.2c). The tolerance exhibited by *L. monocytogenes* strains to the diverse pH range supported the earlier observations of incidence and persistence of the pathogen in different food processing facilities (Moorhead and Dyes 2004; Zang *et al*., 2011; Larsen *et al*., 2014) such as milk and/or cheese production facilities (Lomonaco *et al*., 2009; Doijad *et al*., 2015; Stessl *et al*., 2014), meat processing plants (Martin *et al*., 2011; Wang *et al*., 2015), seafood industry (Holch *et al*., 2013; Leong *et al*., 2014). This may partly explain the survival of the pathogen at extreme pH conditions in a host, like gastrointestinal environment (McClure *et al*., 1997). When considered with a source of isolation, seven (17.07%) strains from food showed tolerance to each acidic and alkaline pH.

Surprisingly, only 1 (3.57%) strain from environmental source found to be tolerant to acidic and alkaline pH stress. From clinical sources, 5(14.28%) strains showed high tolerance to acidic pH, while, 4 (11.42%) strains were tolerant to high alkaline pH.

Table 2.3: Average turbidity (a measure of stress tolerance capability) of *L. monocytogenes* strains of different serogroups from diverse sources at highest stress of pH 5.0 and pH 4.5 (Color indication: Red-high stress tolerant).

ILCC ID	Serogroup	Source	pH 5.0				pH 4.5		
			24h	48h	72h		24h	48h	72h
ILCC001	4b, 4d, 4e	Food	0.328	0.598	0.797		0.122	0.291	0.317
ILCC003	4b, 4d, 4e	Animal	0.208	0.429	0.579		0.009	0.003	0.004
ILCC004	4b, 4d, 4e	Animal	0.215	0.465	0.598		0.005	0.011	0.007
ILCC006	4b, 4d, 4e	Animal	0.010	0.009	0.009		0.009	0.002	0.004
ILCC007	4b, 4d, 4e	Food	0.261	0.468	0.643		0.006	0.004	0.001
ILCC010	4b, 4d, 4e	Food	0.141	0.288	0.458		0.007	0.001	0.006
ILCC012	4b, 4d, 4e	Food	0.007	0.009	0.010		0.006	0.005	0.002
ILCC013	4b, 4d, 4e	Food	0.194	0.355	0.466		0.003	0.007	0.008
ILCC014	4b, 4d, 4e	Food	0.215	0.457	0.538		0.013	0.018	0.007
ILCC015	4b, 4d, 4e	Animal	0.272	0.345	0.318		0.004	0.008	0.008
ILCC016	4b, 4d, 4e	Animal	0.362	0.576	0.614		0.007	0.003	0.006
ILCC017	4b, 4d, 4e	Human	0.067	0.056	0.218		0.005	0.004	0.008
ILCC022	4b, 4d, 4e	Animal	0.422	0.668	0.821		0.159	0.313	0.342
ILCC025	4b, 4d, 4e	Animal	0.148	0.272	0.424		0.015	0.018	0.017
ILCC026	4b, 4d, 4e	Human	0.227	0.413	0.598		0.013	0.008	0.012
ILCC028	4b, 4d, 4e	Human	0.139	0.251	0.456		0.007	0.005	0.002
ILCC029	1/2b, 3b, 4b, 4d, 4e	Human	0.266	0.474	0.524		0.008	0.002	0.008
ILCC032	4b, 4d, 4e	Human	0.356	0.511	0.692		0.143	0.285	0.312
ILCC035	4b, 4d, 4e	Human	0.113	0.231	0.431		0.009	0.006	0.005
ILCC036	4b, 4d, 4e	Human	0.214	0.393	0.341		0.006	0.007	0.004
ILCC037	4b, 4d, 4e	Human	0.011	0.011	0.009		0.002	0.008	0.001
ILCC038	4b, 4d, 4e	Human	0.231	0.434	0.351		0.008	0.009	0.009
ILCC040a	4b, 4d, 4e	Animal	0.188	0.321	0.589		0.003	0.003	0.005
ILCC042	4b, 4d, 4e	Animal	0.288	0.398	0.607		0.013	0.012	0.008
ILCC043	4b, 4d, 4e	Animal	0.248	0.488	0.669		0.008	0.014	0.014
ILCC045	4b, 4d, 4e	Animal	0.478	0.691	0.862		0.152	0.295	0.376
ILCC051a	1/2a, 1/2c, 3a, 3c	Animal	0.142	0.298	0.472		0.003	0.008	0.004
ILCC142	4b, 4d, 4e	Human	0.232	0.488	0.658		0.006	0.009	0.007
ILCC145a	4b, 4d, 4e	Animal	0.209	0.379	0.436		0.005	0.003	0.001
ILCC146	4b, 4d, 4e	Animal	0.179	0.312	0.472		0.007	0.011	0.004
ILCC148	1/2a, 1/2c, 3a, 3c	Animal	0.103	0.253	0.499		0.006	0.006	0.004
ILCC149a	4b, 4d, 4e	Animal	0.203	0.354	0.543		0.016	0.001	0.007

ILCC150a	4b, 4d, 4e	Animal	0.306	0.511	0.682		0.091	0.221	0.334
ILCC152	1/2a, 1/2c, 3a, 3c	Food	0.212	0.381	0.484		0.009	0.012	0.008
ILCC158	4b, 4d, 4e	Food	0.003	0.005	0.005		0.008	0.008	0.005
ILCC161	4b, 4d, 4e	Food	0.344	0.547	0.743		0.119	0.236	0.332
ILCC171	4b, 4d, 4e	Animal	0.206	0.403	0.584		0.015	0.013	0.001
ILCC173	4b, 4d, 4e	Animal	0.203	0.383	0.565		0.003	0.004	0.007
ILCC174a	1/2a, 1/2c, 3a, 3c	Animal	0.175	0.316	0.478		0.012	0.009	0.005
ILCC175a	4b, 4d, 4e	Environmental	0.202	0.341	0.551		0.009	0.003	0.001
ILCC176	4b, 4d, 4e	Environmental	0.173	0.332	0.484		0.004	0.014	0.006
ILCC177a	4b, 4d, 4e	Environmental	0.276	0.372	0.485		0.005	0.004	0.007
ILCC179	4b, 4d, 4e	Environmental	0.115	0.251	0.482		0.001	0.003	0.001
ILCC183	4b, 4d, 4e	Environmental	0.193	0.336	0.582		0.003	0.005	0.006
ILCC185	1/2a, 1/2c, 3a, 3c	Food	0.194	0.332	0.578		0.008	0.002	0.001
ILCC187	4b, 4d, 4e	Food	0.365	0.616	0.869		0.161	0.287	0.409
ILCC190	4b, 4d, 4e	Food	0.265	0.378	0.675		0.006	0.009	0.008
ILCC192	1/2a, 1/2c, 3a, 3c	Food	0.143	0.282	0.486		0.004	0.007	0.008
ILCC195	4b, 4d, 4e	Food	0.272	0.445	0.518		0.002	0.001	0.001
ILCC196a	1/2a, 1/2c, 3a, 3c	Food	0.162	0.315	0.474		0.009	0.008	0.009
ILCC264	4b, 4d, 4e	Food	0.267	0.356	0.518		0.009	0.006	0.007
ILCC265	4b, 4d, 4e	Food	0.332	0.548	0.691		0.079	0.199	0.278
ILCC266	4b, 4d, 4e	Food	0.248	0.372	0.424		0.006	0.006	0.001
ILCC267	4b, 4d, 4e	Food	0.327	0.513	0.688		0.107	0.217	0.325
ILCC269	4b, 4d, 4e	Food	0.316	0.507	0.671		0.093	0.210	0.322
ILCC270	4b, 4d, 4e	Food	0.331	0.524	0.690		0.113	0.231	346
ILCC272	4b, 4d, 4e	Food	0.156	0.311	0.492		0.003	0.009	0.002
ILCC273	4b, 4d, 4e	Food	0.213	0.401	0.591		0.004	0.009	0.008
ILCC274	4b, 4d, 4e	Food	0.194	0.323	0.501		0.007	0.003	0.008
ILCC276	4b, 4d, 4e	Animal	0.276	0.407	0.612		0.005	0.007	0.002
ILCC277	4b, 4d, 4e	Food	0.193	0.313	0.434		0.009	0.005	0.012
ILCC279	4b, 4d, 4e	Food	0.207	0.405	0.588		0.005	0.009	0.005
ILCC285	1/2b, 3b, 4b, 4d, 4e	Food	0.101	0.282	0.414		0.003	0.007	0.005
ILCC289	1/2b, 3b, 4b, 4d, 4e	Food	0.002	0.002	0.007		0.007	0.001	0.001
ILCC293	1/2b, 3b, 4b, 4d, 4e	Food	0.163	0.264	0.456		0.008	0.005	0.009
ILCC297	1/2b, 3b, 4b, 4d, 4e	Food	0.143	0.258	0.432		0.003	0.003	0.006
ILCC298	1/2b, 3b, 4b, 4d, 4e	Food	0.185	0.322	0.496		0.009	0.009	0.005
ILCC301a	1/2b, 3b, 4b, 4d, 4e	Food	0.179	0.306	0.516		0.006	0.003	0.006
ILCC302a	1/2b, 3b, 4b, 4d, 4e	Food	0.008	0.007	0.005		0.002	0.003	0.009
ILCC303a	1/2b, 3b, 4b, 4d, 4e	Food	0.005	0.007	0.006		0.008	0.001	0.007
ILCC304a	1/2b, 3b, 4b, 4d, 4e	Food	0.217	0.469	0.547		0.003	0.002	0.002
ILCC305	1/2b, 3b, 4b, 4d, 4e	Food	0.012	0.007	0.008		0.003	0.004	0.001
ILCC312	1/2a, 1/2c, 3a, 3c	Food	0.199	0.343	0.532		0.008	0.002	0.001
ILCC317	1/2a, 1/2c, 3a, 3c	Food	0.164	0.312	0.487		0.002	0.003	0.005
ILCC325	1/2a, 1/2c, 3a, 3c	Food	0.142	0.289	0.482		0.003	0.009	0.007
ILCC373	1/2a, 1/2c, 3a, 3c	Environmental	0.149	0.302	0.474		0.003	0.005	0.004
ILCC374	1/2a, 1/2c, 3a, 3c	Environmental	0.182	0.362	0.575		0.005	0.003	0.005
ILCC375	1/2a, 1/2c, 3a, 3c	Environmental	0.325	0.543	0.702		0.114	0.253	0.344
ILCC376	1/2a, 1/2c, 3a, 3c	Environmental	0.118	0.223	0.425		0.006	0.002	0.004
ILCC377	1/2a, 1/2c, 3a, 3c	Environmental	0.141	0.297	0.484		0.006	0.009	0.002

ILCC378	1/2a, 1/2c, 3a, 3c	Environmental	0.181	0.258	0.458		0.001	0.008	0.004
ILCC479	4b, 4d, 4e	Food	0.218	0.462	0.611		0.011	0.009	0.010
ILCC494	4b, 4d, 4e	Animal	0.266	0.488	0.654		0.008	0.002	0.003
ILCC496	4b, 4d, 4e	Environmental	0.199	0.323	0.545		0.009	0.006	0.006
ILCC521	1/2a, 1/2c, 3a, 3c	Environmental	0.196	0.317	0.493		0.005	0.009	0.007
ILCC529	1/2a, 1/2c, 3a, 3c	Environmental	0.216	0.342	0.566		0.003	0.005	0.002
ILCC530	1/2a, 1/2c, 3a, 3c	Environmental	0.161	0.441	0.431		0.002	0.009	0.001
ILCC619	4b, 4d, 4e	Human	0.274	0.471	0.568		0.009	0.003	0.002
ILCC622	1/2b, 3b, 4b, 4d, 4e	Human	0.247	0.408	0.534		0.004	0.008	0.004
ILCC624	4b, 4d, 4e	Human	0.313	0.505	0.712		0.085	0.252	0.368
ILCC629	1/2a, 1/2c, 3a, 3c	Human	0.196	0.239	0.373		0.001	0.003	0.002
ILCC767	1/2a, 1/2c, 3a, 3c	Environmental	0.142	0.319	0.489		0.009	0.002	0.008
ILCC768	1/2a, 1/2c, 3a, 3c	Environmental	0.005	0.006	0.005		0.008	0.005	0.003
ILCC769	1/2a, 1/2c, 3a, 3c	Environmental	0.205	0.412	0.557		0.001	0.008	0.008
ILCC770	1/2a, 1/2c, 3a, 3c	Environmental	0.011	0.005	0.007		0.006	0.008	0.005
ILCC771	1/2a, 1/2c, 3a, 3c	Environmental	0.004	0.005	0.007		0.004	0.001	0.005
ILCC772	1/2a, 1/2c, 3a, 3c	Environmental	0.258	0.434	0.605		0.005	0.004	0.007
ILCC773	1/2a, 1/2c, 3a, 3c	Environmental	0.207	0.418	0.569		0.001	0.003	0.001
ILCC774	1/2a, 1/2c, 3a, 3c	Environmental	0.143	0.313	0.491		0.009	0.005	0.006
ILCC775	1/2a, 1/2c, 3a, 3c	Environmental	0.179	0.328	0.525		0.008	0.012	0.006
ILCC776	1/2a, 1/2c, 3a, 3c	Environmental	0.243	0.371	0.603		0.001	0.007	0.009
ILCC777	1/2a, 1/2c, 3a, 3c	Environmental	0.013	0.009	0.009		0.006	0.009	0.008
ILCC778	1/2a, 1/2c, 3a, 3c	Environmental	0.006	0.007	0.005		0.004	0.007	0.008
ILCC779	1/2a, 1/2c, 3a, 3c	Environmental	0.002	0.003	0.003		0.002	0.001	0.001

Table 2.4: Average turbidity (a measure of stress tolerance capability) of *L. monocytogenes* strains of different serogroups from diverse sources at highest stress of pH 9.0 and pH 9.5 (Color indication: Red-high stress tolerant).

ILCC ID	Serogroup	Source	pH 9.0				pH 9.5		
			24h	48h	72h		24h	48h	72h
ILCC001	4b, 4d, 4e	Food	0.169	0.203	0.361		0.082	0.129	0.261
ILCC003	4b, 4d, 4e	Animal	0.107	0.197	0.264		0.003	0.005	0.005
ILCC004	4b, 4d, 4e	Animal	0.171	0.256	0.306		0.002	0.004	0.005
ILCC006	4b, 4d, 4e	Animal	0.005	0.003	0.007		0.005	0.005	0.007
ILCC007	4b, 4d, 4e	Food	0.006	0.006	0.007		0.006	0.006	0.001
ILCC010	4b, 4d, 4e	Food	0.164	0.268	0.322		0.007	0.007	0.005
ILCC012	4b, 4d, 4e	Food	0.004	0.005	0.002		0.006	0.003	0.002
ILCC013	4b, 4d, 4e	Food	0.168	0.207	0.291		0.003	0.001	0.006
ILCC014	4b, 4d, 4e	Food	0.202	0.295	0.371		0.003	0.009	0.002
ILCC015	4b, 4d, 4e	Animal	0.156	0.247	0.316		0.004	0.001	0.005
ILCC016	4b, 4d, 4e	Animal	0.162	0.231	0.306		0.007	0.003	0.005
ILCC017	4b, 4d, 4e	Human	0.002	0.007	0.007		0.005	0.007	0.002
ILCC022	4b, 4d, 4e	Animal	0.095	0.181	0.276		0.002	0.0002	0.001
ILCC025	4b, 4d, 4e	Animal	0.082	0.197	0.259		0.005	0.004	0.005
ILCC026	4b, 4d, 4e	Human	0.177	0.281	0.387		0.103	0.167	0.251
ILCC028	4b, 4d, 4e	Human	0.105	0.162	0.204		0.007	0.005	0.004
ILCC029	1/2b, 3b, 4b, 4d, 4e	Human	0.116	0.184	0.237		0.006	0.005	0.009

ILCC032	4b, 4d, 4e	Human	0.106	0.176	0.268		0.003	0.003	0.006
ILCC035	4b, 4d, 4e	Human	0.115	0.172	0.266		0.005	0.003	0.005
ILCC036	4b, 4d, 4e	Human	0.101	0.161	0.209		0.006	0.003	0.006
ILCC037	4b, 4d, 4e	Human	0.009	0.001	0.004		0.002	0.003	0.009
ILCC038	4b, 4d, 4e	Human	0.117	0.168	0.213		0.006	0.005	0.007
ILCC040a	4b, 4d, 4e	Animal	0.088	0.159	0.201		0.003	0.002	0.002
ILCC042	4b, 4d, 4e	Animal	0.102	0.162	0.231		0.003	0.004	0.001
ILCC043	4b, 4d, 4e	Animal	0.083	0.158	0.217		0.005	0.002	0.001
ILCC045	4b, 4d, 4e	Animal	0.193	0.261	0.359		0.102	0.212	0.302
ILCC051a	1/2a, 1/2c, 3a, 3c	Animal	0.083	0.164	0.239		0.003	0.004	0.007
ILCC142	4b, 4d, 4e	Human	0.127	0.218	0.279		0.003	0.003	0.005
ILCC145a	4b, 4d, 4e	Animal	0.106	0.196	0.311		0.083	0.193	0.296
ILCC146	4b, 4d, 4e	Animal	0.076	0.159	0.227		0.007	0.003	0.004
ILCC148	1/2a, 1/2c, 3a, 3c	Animal	0.121	0.202	0.253		0.006	0.002	0.004
ILCC149a	4b, 4d, 4e	Animal	0.109	0.158	0.215		0.006	0.009	0.002
ILCC150a	4b, 4d, 4e	Animal	0.088	0.127	0.203		0.001	0.004	0.004
ILCC152	1/2a, 1/2c, 3a, 3c	Food	0.065	0.112	0.196		0.009	0.006	0.002
ILCC158	4b, 4d, 4e	Food	0.002	0.006	0.007		0.008	0.002	0.003
ILCC161	4b, 4d, 4e	Food	0.114	0.205	0.276		0.009	0.006	0.006
ILCC171	4b, 4d, 4e	Animal	0.073	0.159	0.203		0.005	0.005	0.009
ILCC173	4b, 4d, 4e	Animal	0.071	0.162	0.217		0.003	0.005	0.002
ILCC174a	1/2a, 1/2c, 3a, 3c	Animal	0.103	0.208	0.343		0.102	0.189	0.231
ILCC175a	4b, 4d, 4e	Environmental	0.078	0.155	0.199		0.009	0.003	0.002
ILCC176	4b, 4d, 4e	Environmental	0.073	0.150	0.191		0.004	0.006	0.004
ILCC177a	4b, 4d, 4e	Environmental	0.101	0.158	0.203		0.005	0.002	0.008
ILCC179	4b, 4d, 4e	Environmental	0.071	0.128	0.185		0.001	0.003	0.003
ILCC183	4b, 4d, 4e	Environmental	0.164	0.259	0.388		0.119	0.212	0.308
ILCC185	1/2a, 1/2c, 3a, 3c	Food	0.077	0.124	0.188		0.008	0.005	0.003
ILCC187	4b, 4d, 4e	Food	0.171	0.262	0.414		0.131	0.258	0.328
ILCC190	4b, 4d, 4e	Food	0.102	0.186	0.246		0.006	0.008	0.005
ILCC192	1/2a, 1/2c, 3a, 3c	Food	0.106	0.192	0.257		0.004	0.001	0.005
ILCC195	4b, 4d, 4e	Food	0.169	0.263	0.311		0.002	0.001	0.001
ILCC196a	1/2a, 1/2c, 3a, 3c	Food	0.117	0.167	0.264		0.007	0.008	0.007
ILCC264	4b, 4d, 4e	Food	0.171	0.256	0.316		0.004	0.005	0.001
ILCC265	4b, 4d, 4e	Food	0.128	0.193	0.307		0.007	0.005	0.007
ILCC266	4b, 4d, 4e	Food	0.116	0.186	0.277		0.006	0.006	0.001
ILCC267	4b, 4d, 4e	Food	0.164	0.228	0.342		0.098	0.207	0.311
ILCC269	4b, 4d, 4e	Food	0.154	0.215	0.336		0.086	0.193	0.301
ILCC270	4b, 4d, 4e	Food	0.128	0.217	0.340		0.073	0.171	0.286
ILCC272	4b, 4d, 4e	Food	0.152	0.225	0.310		0.003	0.006	0.002
ILCC273	4b, 4d, 4e	Food	0.110	0.207	0.289		0.004	0.006	0.005
ILCC274	4b, 4d, 4e	Food	0.162	0.257	0.316		0.005	0.003	0.005
ILCC276	4b, 4d, 4e	Animal	0.122	0.079	0.057		0.005	0.005	0.002
ILCC277	4b, 4d, 4e	Food	0.001	0.001	0.006		0.005	0.008	0.002
ILCC279	4b, 4d, 4e	Food	0.102	0.07	0.059		0.003	0.002	0.005
ILCC285	1/2b, 3b, 4b, 4d, 4e	Food	0.107	0.217	0.357		0.083	0.181	0.295
ILCC289	1/2b, 3b, 4b, 4d, 4e	Food	0.005	0.002	0.004		0.007	0.001	0.001
ILCC293	1/2b, 3b, 4b, 4d, 4e	Food	0.086	0.174	0.287		0.005	0.375	0.119

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ILCC297	1/2b, 3b, 4b, 4d, 4e	Food	0.116	0.236	0.371		0.083	0.186	0.276
ILCC298	1/2b, 3b, 4b, 4d, 4e	Food	0.095	0.172	0.256		0.002	0.003	0.001
ILCC301a	1/2b, 3b, 4b, 4d, 4e	Food	0.090	0.161	0.249		0.006	0.003	0.006
ILCC302a	1/2b, 3b, 4b, 4d, 4e	Food	0.009	0.001	0.004		0.002	0.003	0.009
ILCC303a	1/2b, 3b, 4b, 4d, 4e	Food	0.007	0.008	0.003		0.008	0.005	0.007
ILCC304a	1/2b, 3b, 4b, 4d, 4e	Food	0.088	0.159	0.271		0.003	0.002	0.002
ILCC305	1/2b, 3b, 4b, 4d, 4e	Food	0.002	0.002	0.001		0.003	0.004	0.001
ILCC312	1/2a, 1/2c, 3a, 3c	Food	0.083	0.158	0.237		0.008	0.002	0.005
ILCC317	1/2a, 1/2c, 3a, 3c	Food	0.103	0.161	0.249		0.002	0.002	0.003
ILCC325	1/2a, 1/2c, 3a, 3c	Food	0.083	0.144	0.240		0.003	0.002	0.007
ILCC373	1/2a, 1/2c, 3a, 3c	Environmental	0.127	0.238	0.305		0.003	0.003	0.005
ILCC374	1/2a, 1/2c, 3a, 3c	Environmental	0.106	0.258	0.301		0.003	0.004	0.002
ILCC375	1/2a, 1/2c, 3a, 3c	Environmental	0.116	0.239	0.357		0.077	0.183	0.284
ILCC376	1/2a, 1/2c, 3a, 3c	Environmental	0.121	0.262	0.303		0.006	0.002	0.004
ILCC377	1/2a, 1/2c, 3a, 3c	Environmental	0.109	0.198	0.275		0.006	0.006	0.002
ILCC378	1/2a, 1/2c, 3a, 3c	Environmental	0.118	0.197	0.301		0.001	0.003	0.004
ILCC479	4b, 4d, 4e	Food	0.165	0.252	0.316		0.004	0.004	0.001
ILCC494	4b, 4d, 4e	Animal	0.002	0.006	0.007		0.002	0.002	0.003
ILCC496	4b, 4d, 4e	Environmental	0.004	0.001	0.002		0.005	0.005	0.006
ILCC521	1/2a, 1/2c, 3a, 3c	Environmental	0.073	0.149	0.210		0.006	0.005	0.009
ILCC529	1/2a, 1/2c, 3a, 3c	Environmental	0.111	0.262	0.317		0.003	0.005	0.002
ILCC530	1/2a, 1/2c, 3a, 3c	Environmental	0.117	0.198	0.283		0.002	0.005	0.001
ILCC619	4b, 4d, 4e	Human	0.158	0.255	0.319		0.002	0.003	0.002
ILCC622	1/2b, 3b, 4b, 4d, 4e	Human	0.103	0.206	0.291		0.004	0.006	0.004
ILCC624	4b, 4d, 4e	Human	0.151	0.258	0.323		0.005	0.002	0.006
ILCC629	1/2a, 1/2c, 3a, 3c	Human	0.121	0.228	0.315		0.001	0.003	0.003
ILCC767	1/2a, 1/2c, 3a, 3c	Environmental	0.084	0.189	0.288		0.007	0.002	0.005
ILCC768	1/2a, 1/2c, 3a, 3c	Environmental	0.007	0.007	0.004		0.008	0.005	0.003
ILCC769	1/2a, 1/2c, 3a, 3c	Environmental	0.001	0.006	0.006		0.001	0.008	0.008
ILCC770	1/2a, 1/2c, 3a, 3c	Environmental	0.008	0.003	0.007		0.006	0.005	0.005
ILCC771	1/2a, 1/2c, 3a, 3c	Environmental	0.002	0.002	0.001		0.004	0.001	0.005
ILCC772	1/2a, 1/2c, 3a, 3c	Environmental	0.001	0.005	0.003		0.004	0.005	0.003
ILCC773	1/2a, 1/2c, 3a, 3c	Environmental	0.098	0.168	0.275		0.001	0.005	0.006
ILCC774	1/2a, 1/2c, 3a, 3c	Environmental	0.074	0.159	0.238		0.006	0.005	0.005
ILCC775	1/2a, 1/2c, 3a, 3c	Environmental	0.087	0.194	0.258		0.004	0.001	0.005
ILCC776	1/2a, 1/2c, 3a, 3c	Environmental	0.001	0.002	0.004		0.002	0.001	0.001
ILCC777	1/2a, 1/2c, 3a, 3c	Environmental	0.002	0.006	0.006		0.007	0.008	0.007
ILCC778	1/2a, 1/2c, 3a, 3c	Environmental	0.006	0.002	0.007		0.004	0.005	0.003
ILCC779	1/2a, 1/2c, 3a, 3c	Environmental	0.005	0.003	0.007		0.005	0.003	0.002

2.4.3 Tolerance to low temperature

Considering varied temperature ranges used in food processing, storage as well as the distribution of food products, tolerance was studied at different temperatures (4°C, 10°C, 18°C, 24°C, and 30°C). The lowest temperature tested was 4°C selected as representative of domestic as well as retail refrigerators (Kennedy *et al*., 2005). The strains showing growth at 4ºC were selected as highly tolerant strains to low temperature stress. Out of 104 strains tested, a total of 22 (21.15%) strains showed growth at 4°C whereas, 64 (61.53%) showed growth at 10ºC (Table 2.5) (Fig. 2.2d). While all the strains grew well at temperature of 18°C and above.

Storage at low temperature is extensively used method for food preservation at domestic, retail as well as industrial levels. In this study, the strains showed varied tolerance to low temperature. The maximum number of strains found to be highly tolerant to the low temperatures which are widely used for food storage, processing and/or distribution in industries as well as at domestic and retail levels. The temperatures at which *L. monocytogenes* found to be tolerant are unusual temperatures for any pathogenic bacterium. Many RTE foods such as milk, milk products are stored at these temperatures may permit the growth of *L. monocytogenes* to increase a load of pathogen thereby increasing chances of infection (Chan and Wiedmann, 2008). Modern food industries are attempting to minimize the use of food preservatives. Therefore, shelf-life and food safety mainly rely on maintenance of the cold chain. Cold stress tolerance explains that ability to proliferate at low-temperature benefits *L. monocytogenes* to overcome other pathogens in the environment or in food making it major food borne pathogen (Durack *et al*., 2013). Earlier findings revealed frequent linkage of industrially processed and refrigerated foods than raw foods to *L. monocytogenes* outbreaks

(Gianfranceschi *et al.*, 2003). Among the low temperature tolerant strains, 11 (31.42%) strains were from clinical sources followed by 10 (24.39%) from food and 1 (3.57%) were from the environmental source.

Table 2.5: Average turbidity (a measure of stress tolerance capability) of *L. monocytogenes* strains of different serogroups from diverse sources at highest stress of 10°C and 4°C. (Color indication: Red-high stress tolerant).

ILCC ID	Serogroup	Source	10°C				4°C		
			24h	48h	72h		24h	48h	72h
ILCC001	4b, 4d, 4e	Food	0.282	0.391	0.561		0.003	0.004	0.002
ILCC003	4b, 4d, 4e	Animal	0.005	0.007	0.005		0.002	0.004	0.002
ILCC004	4b, 4d, 4e	Animal	0.189	0.258	0.417		0.006	0.004	0.01
ILCC006	4b, 4d, 4e	Animal	0.179	0.249	0.378		0.003	0.004	0.003
ILCC007	4b, 4d, 4e	Food	0.006	0.006	0.001		0.007	0.005	0.005
ILCC010	4b, 4d, 4e	Food	0.207	0.317	0.535		0.002	0.002	0.004
ILCC012	4b, 4d, 4e	Food	0.216	0.343	0.552		0.006	0.002	0.002
ILCC013	4b, 4d, 4e	Food	0.213	0.331	0.526		0.009	0.003	0.003
ILCC014	4b, 4d, 4e	Food	0.179	0.319	0.482		0.002	0.004	0.002
ILCC015	4b, 4d, 4e	Animal	0.194	0.325	0.518		0.005	0.002	0.007
ILCC016	4b, 4d, 4e	Animal	0.207	0.373	0.558		0.001	0.001	0.003
ILCC017	4b, 4d, 4e	Human	0.005	0.007	0.002		0.001	0.003	0.003
ILCC022	4b, 4d, 4e	Animal	0.329	0.489	0.692		0.156	0.226	0.355
ILCC025	4b, 4d, 4e	Animal	0.235	0.429	0.585		0.108	0.219	0.317
ILCC026	4b, 4d, 4e	Human	0.123	0.317	0.465		0.003	0.002	0.002
ILCC028	4b, 4d, 4e	Human	0.327	0.471	0.651		0.119	0.209	0.331
ILCC029	1/2b, 3b, 4b, 4d, 4e	Human	0.318	0.575	0.779		0.159	0.291	0.421
ILCC032	4b, 4d, 4e	Human	0.273	0.443	0.516		0.096	0.176	0.247
ILCC035	4b, 4d, 4e	Human	0.219	0.339	0.505		0.004	0.004	0.005
ILCC036	4b, 4d, 4e	Human	0.186	0.293	0.496		0.002	0.004	0.001
ILCC037	4b, 4d, 4e	Human	0.202	0.393	0.519		0.008	0.007	0.004
ILCC038	4b, 4d, 4e	Human	0.003	0.002	0.006		0.003	0.003	0.005
ILCC040a	4b, 4d, 4e	Animal	0.313	0.492	0.672		0.088	0.157	0.299
ILCC042	4b, 4d, 4e	Animal	0.193	0.314	0.481		0.003	0.007	0.002
ILCC043	4b, 4d, 4e	Animal	0.005	0.002	0.002		0.005	0.006	0.005
ILCC045	4b, 4d, 4e	Animal	0.002	0.002	0.003		0.002	0.006	0.007
ILCC051a	1/2a, 1/2c, 3a, 3c	Animal	0.113	0.239	0.397		0.009	0.006	0.004
ILCC142	4b, 4d, 4e	Human	0.373	0.603	0.808		0.165	0.275	0.418
ILCC145a	4b, 4d, 4e	Animal	0.173	0.293	0.445		0.004	0.003	0.004
ILCC146	4b, 4d, 4e	Animal	0.267	0.403	0.644		0.076	0.188	0.282
ILCC148	1/2a, 1/2c, 3a, 3c	Animal	0.106	0.282	0.364		0.008	0.005	0.009
ILCC149a	4b, 4d, 4e	Animal	0.276	0.469	0.672		0.121	0.236	0.303
ILCC150a	4b, 4d, 4e	Animal	0.261	0.448	0.584		0.009	0.005	0.002
ILCC152	1/2a, 1/2c, 3a, 3c	Food	0.249	0.449	0.619		0.075	0.174	0.257
ILCC158	4b, 4d, 4e	Food	0.218	0.392	0.503		0.006	0.005	0.004
ILCC161	4b, 4d, 4e	Food	0.209	0.386	0.516		0.008	0.061	0.075
ILCC171	4b, 4d, 4e	Animal	0.190	0.289	0.499		0.007	0.005	0.007

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ILCC173	4b, 4d, 4e	Animal	0.213	0.405	0.592		0.002	0.005	0.002
ILCC174a	1/2a, 1/2c, 3a, 3c	Animal	0.270	0.389	0.531		0.106	0.264	0.314
ILCC175a	4b, 4d, 4e	Environmental	0.119	0.213	0.402		0.005	0.005	0.008
ILCC176	4b, 4d, 4e	Environmental	0.114	0.310	0.424		0.006	0.009	0.008
ILCC177a	4b, 4d, 4e	Environmental	0.105	0.262	0.368		0.006	0.002	0.001
ILCC179	4b, 4d, 4e	Environmental	0.081	0.163	0.203		0.002	0.262	0.21
ILCC183	4b, 4d, 4e	Environmental	0.329	0.562	0.718		0.117	0.231	0.315
ILCC185	1/2a, 1/2c, 3a, 3c	Food	0.098	0.165	0.303		0.002	0.002	0.001
ILCC187	4b, 4d, 4e	Food	0.391	0.618	0.818		0.189	0.303	0.442
ILCC190	4b, 4d, 4e	Food	0.286	0.458	0.705		0.115	0.264	0.321
ILCC192	1/2a, 1/2c, 3a, 3c	Food	0.284	0.461	0.665		0.121	0.237	0.390
ILCC195	4b, 4d, 4e	Food	0.294	0.478	0.686		0.110	0.223	0.342
ILCC196a	1/2a, 1/2c, 3a, 3c	Food	0.151	0.291	0.367		0.006	0.003	0.001
ILCC264	4b, 4d, 4e	Food	0.228	0.342	0.484		0.007	0.007	0.004
ILCC265	4b, 4d, 4e	Food	0.221	0.387	0.508		0.001	0.003	0.003
ILCC266	4b, 4d, 4e	Food	0.378	0.532	0.738		0.118	0.253	0.407
ILCC267	4b, 4d, 4e	Food	0.288	0.473	0.575		0.088	0.186	0.277
ILCC269	4b, 4d, 4e	Food	0.219	0.425	0.574		0.004	0.006	0.002
ILCC270	4b, 4d, 4e	Food	0.206	0.416	0.559		0.003	0.005	0.006
ILCC272	4b, 4d, 4e	Food	0.182	0.315	0.456		0.006	0.007	0.007
ILCC273	4b, 4d, 4e	Food	0.315	0.558	0.708		0.102	0.225	0.311
ILCC274	4b, 4d, 4e	Food	0.231	0.458	0.658		0.006	0.007	0.006
ILCC276	4b, 4d, 4e	Animal	0.158	0.302	0.511		0.002	0.003	0.006
ILCC277	4b, 4d, 4e	Food	0.166	0.308	0.534		0.006	0.007	0.006
ILCC279	4b, 4d, 4e	Food	0.209	0.423	0.595		0.002	0.007	0.007
ILCC285	1/2b, 3b, 4b, 4d, 4e	Food	0.276	0.437	0.593		0.091	0.191	0.281
ILCC289	1/2b, 3b, 4b, 4d, 4e	Food	0.196	0.322	0.466		0.002	0.007	0.005
ILCC293	1/2b, 3b, 4b, 4d, 4e	Food	0.161	0.310	0.431		0.007	0.007	0.006
ILCC297	1/2b, 3b, 4b, 4d, 4e	Food	0.224	0.371	0.618		0.105	0.222	0.304
ILCC298	1/2b, 3b, 4b, 4d, 4e	Food	0.227	0.408	0.564		0.003	0.004	0.007
ILCC301a	1/2b, 3b, 4b, 4d, 4e	Food	0.213	0.395	0.442		0.002	0.001	0.002
ILCC302a	1/2b, 3b, 4b, 4d, 4e	Food	0.196	0.239	0.373		0.005	0.002	0.006
ILCC303a	1/2b, 3b, 4b, 4d, 4e	Food	0.142	0.219	0.349		0.005	0.001	0.005
ILCC304a	1/2b, 3b, 4b, 4d, 4e	Food	0.175	0.266	0.373		0.001	0.001	0.004
ILCC305	1/2b, 3b, 4b, 4d, 4e	Food	0.005	0.002	0.005		0.001	0.003	0.003
ILCC312	1/2a, 1/2c, 3a, 3c	Food	0.205	0.306	0.418		0.003	0.005	0.005
ILCC317	1/2a, 1/2c, 3a, 3c	Food	0.172	0.324	0.485		0.004	0.005	0.002
ILCC325	1/2a, 1/2c, 3a, 3c	Food	0.158	0.244	0.455		0.002	0.002	0.001
ILCC373	1/2a, 1/2c, 3a, 3c	Environmental	0.117	0.288	0.419		0.003	0.005	0.007
ILCC374	1/2a, 1/2c, 3a, 3c	Environmental	0.143	0.303	0.441		0.003	0.001	0.001
ILCC375	1/2a, 1/2c, 3a, 3c	Environmental	0.179	0.318	0.425		0.003	0.004	0.003
ILCC376	1/2a, 1/2c, 3a, 3c	Environmental	0.143	0.251	0.403		0.002	0.003	0.003
ILCC377	1/2a, 1/2c, 3a, 3c	Environmental	0.158	0.306	0.463		0.006	0.005	0.001
ILCC378	1/2a, 1/2c, 3a, 3c	Environmental	0.131	0.309	0.489		0.005	0.001	0.002
ILCC479	4b, 4d, 4e	Food	0.211	0.373	0.546		0.001	0.002	0.003
ILCC494	4b, 4d, 4e	Animal	0.197	0.316	0.554		0.001	0.005	0.005
ILCC496	4b, 4d, 4e	Environmental	0.005	0.002	0.001		0.002	0.005	0.003
ILCC521	1/2a, 1/2c, 3a, 3c	Environmental	0.119	0.295	0.365		0.005	0.005	0.001
ILCC529	1/2a, 1/2c, 3a, 3c	Environmental	0.194	0.308	0.477		0.005	0.002	0.006
ILCC530	1/2a, 1/2c, 3a, 3c	Environmental	0.198	0.311	0.502		0.002	0.001	0.002

Continued…

ILCC619	4b, 4d, 4e	Human	0.259	0.405	0.589		0.104	0.255	0.316
ILCC622	1/2b, 3b, 4b, 4d, 4e	Human	0.004	0.003	0.003		0.003	0.005	0.003
ILCC624	4b, 4d, 4e	Human	0.008	0.003	0.004		0.001	0.002	0.004
ILCC629	1/2a, 1/2c, 3a, 3c	Human	0.004	0.001	0.003		0.003	0.005	0.003
ILCC767	1/2a, 1/2c, 3a, 3c	Environmental	0.002	0.003	0.001		0.004	0.005	0.002
ILCC768	1/2a, 1/2c, 3a, 3c	Environmental	0.103	0.248	0.407		0.003	0.006	0.004
ILCC769	1/2a, 1/2c, 3a, 3c	Environmental	0.120	0.265	0.412		0.001	0.005	0.003
ILCC770	1/2a, 1/2c, 3a, 3c	Environmental	0.007	0.006	0.003		0.001	0.002	0.001
ILCC771	1/2a, 1/2c, 3a, 3c	Environmental	0.001	0.002	0.002		0.004	0.005	0.003
ILCC772	1/2a, 1/2c, 3a, 3c	Environmental	0.001	0.005	0.005		0.007	0.006	0.004
ILCC773	1/2a, 1/2c, 3a, 3c	Environmental	0.008	0.006	0.006		0.001	0.003	0.002
ILCC774	1/2a, 1/2c, 3a, 3c	Environmental	0.116	0.208	0.334		0.001	0.003	0.001
ILCC775	1/2a, 1/2c, 3a, 3c	Environmental	0.119	0.223	0.395		0.007	0.007	0.004
ILCC776	1/2a, 1/2c, 3a, 3c	Environmental	0.006	0.003	0.003		0.004	0.006	0.006
ILCC777	1/2a, 1/2c, 3a, 3c	Environmental	0.078	0.195	0.299		0.001	0.003	0.003
ILCC778	1/2a, 1/2c, 3a, 3c	Environmental	0.003	0.006	0.004		0.006	0.005	0.003
ILCC779	1/2a, 1/2c, 3a, 3c	Environmental	0.121	0.258	0.383		0.002	0.001	0.003

A total of 37 (35.57%) strains were found to be tolerant to at least one of stresses tested. Of these 16 strains were tolerant to more than one stress. Among the tolerant strains, 12 (11.5%) strains were tolerant to high salt, 26 (25%) to extreme pH and 22 (21.15%) were tolerant to low temperature (Table 2.6). When compared to their serogroups/serotypes; 46.55% (27/58) serogroup 4b, 4d, 4e strains, 33.33% (4/12) serogroup 1/2b, 3b, 4b, 4d, 4e strains and 17.64% (6/34) serogroup 1/2a, 1/2c, 3a, 3c strains were found to be stress tolerant (Fig. 2.3). While comparing the sources of isolation, 18 (51.52%) strains from clinical, 15 (36.58%) from food and 5 (23.80%) from environmental sources were found to be stress tolerant. Analyzing the percent tolerance with respect to a source of isolation for each stress of high salt, pH and low temperature, there was no exact correlation found among tolerance patterns and sources of isolation as observed earlier (Lianou *et al*., 2006). However, interestingly, serotype 4b strains were observed to be more stress tolerant than that of serotype 1/2b and 1/2a. Earlier studies (van der Veen *et al*., 2008; Makarti *et al*., 2015) also observed a high number of serogroup 4b strains showing tolerance

followed by serotype 1/2b and serotype 1/2a strains. This could be a possible

explanation for the dominance of serotype 4b strains in clinical cases.

Table 2.6. *L. monocytogenes* strains and their growth observed at maximum stress conditions. (Red highlights indicate strains exhibiting tolerance).

Clinical

ILCC ID	Serotype	Salt Stress Tolerance (%)	Cold stress tolerance (°C)	Acidic pH tolerance	Basic pH tolerance
51a	1/2a	10	10	5	9
148	1/2a	7.5	10	5	9
174a	1/2a	12.5	4	5	9.5
629	1/2a	10	18	5	9
29	1/2b	12.5	4	5	9
40	4b	10	4	5	9
622	1/2b	12.5	18	5	9
3	4b	7.5	18	5	9
4	4b	10	10	5	9
6	4b	7.5	10	5	9
15	4b	7.5	10	5	9
16	4b	10	10	5	9
17	4b	10	18	5	8.5
22	4b	12.5	4	4.5	9
25	4b	10	4	5	9
26	4b	10	10	5	9.5
28	4b	10	4	5	9
32	4b	10	4	4.5	9
35	4b	10	10	5	9
36	4b	10	10	5	9
37	4b	10	10	5	9
38	4b	10	18	5	9
42	4b	10	10	5	9
43	4b	10	18	5	9
45	4b	10	18	4.5	9.5
142	4b	12.5	4	5	9
145a	4b	12.5	10	5	9.5
146	4b	10	4	5	9
149a	4b	10	4	5	9
150a	4b	10	10	4.5	9
171	4b	10	10	5	9
173	4b	10	10	5	9
494	4b	10	10	5	8.5
619	4b	10	4	5	9
624	4b	10	18	4.5	9

Food

ILCC ID	Serotype	Salt Stress Tolerance (%)	Cold stress tolerance (°C)	Acidic pH tolerance	Basic pH tolerance
185	1/2a	10	10	5	9
158	1/2a	10	10	5	9
196a	1/2a	7.5	10	5	9
152	1/2a	10	4	5	9
289	1/2b	7.5	10	5	9
302a	1/2b	10	10	5	9
298	1/2b	10	10	5	9
285	1/2b	10	4	5	9.5
293	1/2b	10	10	5	9
297	1/2b	7.5	4	5	9.5
301a	1/2b	7.5	10	5	9
269	4b	7.5	10	4.5	9.5
274	4b	10	10	5	9
264	4b	7.5	10	5	9
161	4b	10	10	4.5	9
190	4b	12.5	4	5	9
195	4b	12.5	4	5	9
267	4b	10	4	4.5	9.5
270	4b	10	10	4.5	9.5
272	4b	10	10	5	9
273	4b	10	4	5	9
276	4b	7.5	10	5	9
277	4b	10	10	5	9
279	4b	10	10	5	9
1	4b	7.5	10	4	9.5
7	4b	7.5	18	5	9
10	4b	10	10	5	9
12	4b	7.5	10	5	9
13	4b	7.5	10	5	9
187	4b	10	4	4.5	9.5
192	1/2a	10	4	5	9
265	4b	7.5	10	4	9
266	4b	10	4	5	9
14	4b	7.5	10	5	9
325	1/2a	7.5	10	5	9

Food

ILCC ID	Serotype	Salt Stress Tolerance (%)	Cold stress tolerance (°C)	Acidic pH tolerance	Basic pH tolerance
312	1/2a	10	10	5	9
317	1/2a	10	10	5	9
303a	1/2b	7.5	10	5	9
304a	1/2b	7.5	10	5	9
305	1/2b	10	18	5	9
479	4b	10	10	5	9

Environment

ILCC ID	Serotype	Salt Stress Tolerance (%)	Cold stress tolerance (°C)	Acidic pH tolerance	Basic pH tolerance
378	1/2a	10	10	5	9
529	1/2a	10	10	5	9
769	1/2a	10	10	5	8.5
774	1/2a	10	10	5	9
779	1/2a	10	10	5	9
777	1/2a	10	10	5	9
374	1/2a	10	10	5	9
376	1/2a	10	10	5	9
377	1/2a	12.5	10	5	9
521	1/2a	10	10	5	9
530	1/2a	10	10	5	9
767	1/2a	7.5	18	5	9
768	1/2a	10	10	5.5	9
770	1/2a	7.5	18	5.5	9
771	1/2a	7.5	18	5	9
772	1/2a	10	18	5	8.5
773	1/2a	7.5	18	5	9
775	1/2a	10	10	5	9
776	1/2a	7.5	18	5	8.5
778	1/2a	7.5	18	5.5	9
175a	4b	10	10	5	9
176	4b	10	10	5	9
177a	4b	10	10	5	9
179	4b	10	10	5	9
183	4b	12.5	4	5	9.5
373	1/2a	12.5	10	5	9
375	1/2a	12.5	10	4.5	9.5
496	4b	10	18	5	8.5

Noteworthy, the majority of studies available concerning *L. monocytogenes* tolerant behavior to food-related stresses usually dealt with as a result of short-term exposure to the stress conditions, that is, shock or adaptation for a few hours, before the experiments are conducted (Lou and Yousef, 1997; Phan-Thanh and Montagne, 1998; van Schaik *et al*., 1999; Wemekamp-Kamphuis *et al*., 2004; Wałecka-Zacharska *et al*., 2013). However, this approach does not possibly reflect the real conditions in food-related stresses, as the contaminating bacteria might present in the foods and/or on the food surfaces for several hours or even days' prior to consumption or decontamination, respectively. Thus present study was conducted for understanding inherently present tolerance of *L. monocytogenes* towards different food related stresses. The innate resistance by *L. monocytogenes* strains to the stresses commonly employed in food preservation and/or food processing has been reported. The data showed that strains varied remarkably with respect to stress tolerance abilities under different stresses. There was no correlation observed between stress tolerance pattern and origin of the strains for all stresses. The investigation underlined significant stress tolerance by strains of serogroup 4b, 4d, 4e (serotype 4b). This improved our understanding that how specific strains or subtypes of *L. monocytogenes* become resident to selected niches by better adaptive response. Hence the food and/or food processing environment related stresses may result in selection of particular subtypes possessing the appropriate adaptive physiological attributes, promoting efficient adaptation, survival and proliferation during stressed environmental conditions.
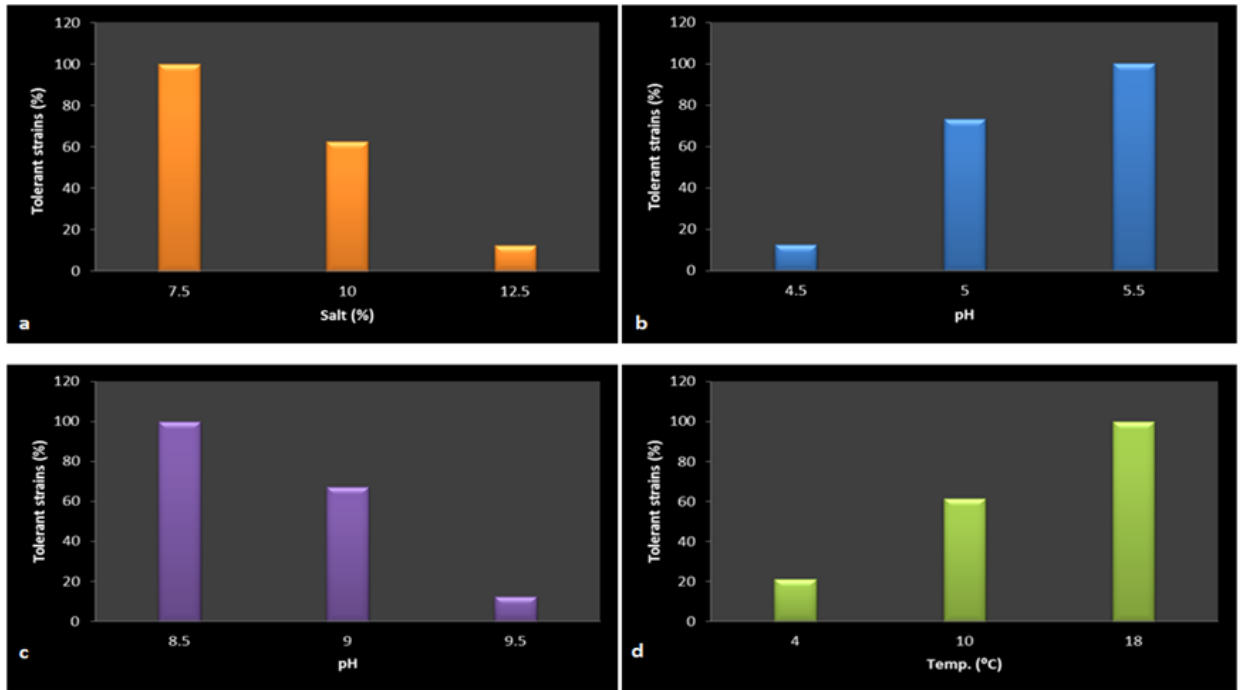
Fig.2.2 Distribution of strains of *Listeria moncytogenes* with reference to their tolerance to various stresses (a) The percentage of tolerant strains to different salt concentrations. (b) The percentage of tolerant strains to different acidic pH. (c) The percentage of tolerant strains to different alkaline pH. (d) The percentage of tolerant strains at different low temperatures.
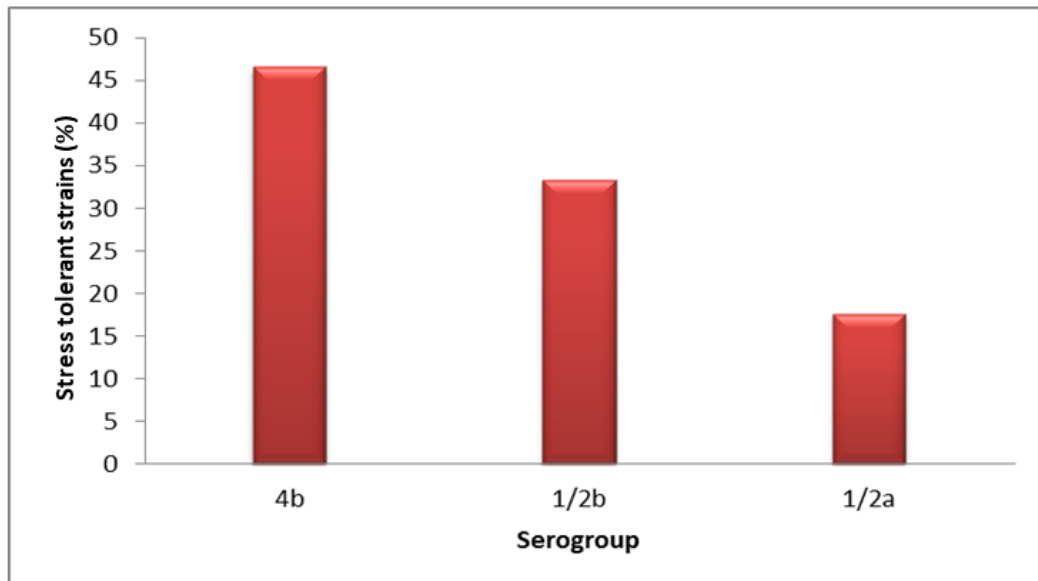

Fig. 2.3: The overall percentage of stress tolerant serogroups.

Chapter 3

Determination of morphological and physico-chemical parameters of the stress tolerant strains

3.1 Introduction-

In various foods and food preparation processes, microorganisms constantly experience the kinds of environmental stresses. Depending upon the form and degree of stresses microorganism devise mechanisms that assist them in overcoming stresses. The stress induces alterations in phenotypic and physiological characters of bacteria. These alterations are generally two types; one is the direct effect of stress resulting in alterations in morphology and a functional properties of cells; the other type is an adaptive attempt by the cells to induce alteration in cell's properties towards combating with the stress (Silhavy *et al*., 2010; Rowlett *et al*., 2017). The degree and type of alteration in the physiological properties of cells depend upon severity and type of stress. This change in the physiology can be the altered morphology with respect to size and shape bacterial cells (Giotis *et al*., 2007; Visvalingam *et al*., 2012; Jones *et al*., 2013). Membranes are the first line of defense against adverse physical environmental conditions; hence stability and permeability of cellular membranes are one of the important factors playing a crucial role in the adaptation of bacteria to various environmental stresses (Miladi *et al*., 2013). The structural integrity and functional stability of membranes are associated with lipids and fatty acids composition of the membranes under that stress. In response to the stresses like osmotic, acidic, alkaline or thermal there is an alteration in the cell membrane, particularly in the lipids and fatty acids (Murga *et al*., 2000; Guerzoni *et al*., 2001; Krawczyk-Balska and Lipiak 2013; Zhang *et al*., 2015). These modifications in the membrane lipids are achieved through a change in length of fatty acids, alteration in the degree of fatty acid unsaturation and change in the type of branching at the methyl end of the fatty acids. The change in the protein expression, especially in cell-membrane surface associated proteins is another

potential mechanism used by bacterium for modified physiological status towards adapting altered environmental conditions. Many stress associated proteins are required for proper cellular functioning under stressed environmental conditions, but they are also involved in sensing the change in external environment, transduction of signals accordingly and maintenance of cell physiology to cope up with changed environmental conditions (Wlliams *et al*., 2005; Soni *et al*., 2011; Lei *et al*., 2015).

There are many attempts made towards understanding the factors involved in physiology and stress response in *L. monocytogenes*; which included induced alterations in cell morphology, cell membrane, cell-surface associated proteins under various stresses (Bereksi et al., 2002; Jydegaard-Axelsen *et al*., 2005; Giotis *et al*., 2007; Zhang *et al*., 2015). Studies so far highlighted the contribution of many potential but improperly understood common factors involved in the physico-chemical response of *L. monocytogenes* under various stresses.

3.2 Review of Literature

The life of microorganisms goes through fierce competition, nutritional hardship and often life-threatening exposure to the changing external environment. Adaptive response of the bacterium to such deleterious changes in external environmental conditions defines the foundation of microbial life in a natural milieu (Diard and Hardt, 2017). Stressful environment leads to many alterations at morphological, physiological, cellular and biochemical levels in bacteria (Rowlett *et al*., 2017). Those species which adapt to the changing environmental conditions survive, while other species vanish completely. When grown under optimal conditions, the dimensions such as cell diameter, length, shape are strictly controlled in each bacterial species. There is genetic machinery to control and maintain these

parameters in bacterial species and hence shape, size, and biochemical property became the 'classical' descriptions for a particular type of species (Zaritsky, 1975; Young, 2006). Despite having such controlled mechanisms bacteria also possesses the genes which encode for the protein for purposeful modulations in the cell dimensions under certain conditions (Harry *et al*., 2006). There are different types of morphological alterations have been observed acquired by many microbes to cope up with changing environment. Chakraborty *et al*. (2008) observed the change in the length, width and radius of *Acidocella* strain after exposure to the heavy metals. Bron *et al*. (2004) reported the shrunken shape with the empty appearance of *Lactobacillus plantarum* WCFS1 when grown on media supplemented with 0.1% bile. After starvation of *Enterococcus feacalis*, cells developed a rippled cell surface with irregular shapes (Hartke *et al*., 1998). Exposure to the salt stress leading to elongations of cells was observed in *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* (Gandhi and Shah, 2016). Survival and adaptation under chlorine containing disinfectants stress with morphological changes of cells from short rod to coccoid were observed in *L. monocytogenes* strains (Gao and Liu, 2014). Elongations of the cells with increased adhesion property were observed in *Pseudomonas aeruginosa* as an effect of nalidixic acid (Al Bahry *et al*., 2012). Varying morphotypes: rod, filamentous and spherical forms were observed in *Aeromonas hydrophila* under salt and cold stress (Pianetti et al., 2009). *Salmonella* strains exposed to acidic pH followed with exposure to cetylpyridinium chloride and nisin found to be lost their normal rod shape (Thongbai *et al*., 2006). After exposure to the cold stress and carbon starvation, *Vibrio parahaemolyticus* cells shape changed from rod-like to coccoid with waning-shape (Chen *et al*., 2015). Scanning electron microscopic (SEM) studies revealed changes in mean cell length, radius,

and volume of *L. monocytogenes* subjected to sublethal alkaline stress (Giotis *et al.*, 2007). Notably, a number of laboratories have converged similar observation of bacterial morphology under different stresses and that is filamentous form providing a survival advantage. There are many conditions that induce bacterial filamentation including environmental stress due to physicochemical changes in surroundings or in host defense (Justice *et al.*, 2006; Liu *et al.*, 2014). Filament formation in foodborne pathogens such as *Escherichia coli, Listeria monocytogenes, Salmonella enterica* and *Bacillus cereus* has been observed in conditions involving sublethal stress (Jydegaard-Axelsen *et al.*, 2005; Kieboom *et al.*, 2006; den Besten *et al.*, 2009, Jones *et al.*, 2013). This formation of a filament is reported to be reversible change and believed to be the cause of problems in cell division as well as changes in gene and protein expression (Bereksi *et al.*, 2002; Kieboom *et al.*, 2006, Jones *et al.*, 2013). A filament grows as a single colony when plated on solid media misleading assessment of an actual number of bacteria in food samples. It has been opined that when filamentous cells are exposed to less stressful conditions, there is a rapid division of filaments into a number of individual cells (Giotis *et al.*, 2007) which may pose major implications for the food industry. This is a major public health concern in the case of the foodborne pathogen since the potential viable number of the pathogen can be underestimated due to filamentous form leading to food safety risk for the consumer (de Sarrau *et al.*, 2013). The mechanism behind the filament formation under stresses has been studied in Gram negative bacteria, however, studies are largely lacking in Gram positive bacteria (Jones *et al.*, 2013).

The bacterial cell envelope is one of the crucial structures that surround and protect the cytoplasm. The cell envelope of bacteria falls into two major categories, namely, Gram positive and Gram Negative. A Gram-negative bacterium has thin

peptidoglycan cell wall, with an outer membrane containing lipopolysaccharide. Gram-positive bacteria lack an outer membrane but peptidoglycan layer is thicker than that of found in the Gram-negatives (Silhavy *et al*., 2010; Hong and Hong, 2016). Bacteria have evolved with sophisticated envelope structure which not only protects them from unpredictable changes in surroundings but allows selective passage of nutrients from the outside, waste products from the inside and communication interface mediating information exchange from cell to surroundings (Uppu and Haldar, 2016). An important mechanism acquired by many bacteria to maintain this sophisticated cell envelope with structural and functional integrity is the maintenance of lipid fluidity in cell membranes (Yoon *et al*., 2015). Likewise, under low-temperature stress, there is reduced membrane fluidity. Microbes respond to this transition of temperatures by altering fatty acid compositions (Bajerski *et al*., 2017). The lipids in the bacterial cell membrane are in fluid, crystalline state and this physical state needs to maintain for proper enzyme activity and transfer of solutes across the cell membrane (Gennis, 2013). Bacteria encounters the extracellular stress by adapting membrane fluidity via a change in the fatty acid chain lengths, change in the degree of fatty acid unsaturation, and changing type of branching at the methyl end of the fatty acids (Brown, 2016). In *Streptococcus mutans* strains exposed to acidic stress increased levels of monounsaturated fatty acids and longer chain fatty acids were observed than grown at neutral pH (Quivey *et al*., 2000). Under salt stress bacterium *Klebsiella* sp. SBP-8 reported synthesizing long chain alkanes and fatty alcohols (Singh and Jha, 2017). In *L. monocytogenes* there is an increase in unsaturated fatty acids were observed under cold stress (Hingston *et al*., 2017). Unsaturated fatty acids are known to help enhance the fluidity of the membrane. The temperature downshift also leads to shortening of fatty acids, this shortening of fatty

acids opined to help in maintaining the degree of membrane fluidity for growth at low temperatures (Beales, 2004). The importance of branched chain fatty acids has been reported for the survival of *L. monocytogenes* under pH stress (Giotis *et al*., 2007) and under trans-cinnam aldehyde exposure (Rogiers *et al*., 2017). The alterations in the cell membrane of *L. monocytogenes* were observed over exposure to bile salts (Merritt *et al*., 2010). Recently, there was an increase in the expression of cell-wall-associated proteins following bile-salt exposure in the *L. monocytogenes* 1/2a, it suggested the involvement of proteins with fatty acids in alterations to cell-wall composition over exposure to the stresses (Zhou *et al*., 2012). Proteomics plays an important and decisive role in connecting genome and transcriptome to potential biological functions (Armengaud *et al*., 2014). Protein analysis under different stresses have been done for better understanding of stress response in *L. monocytogenes* to cold, heat, acidic, salt, alkali, detergent and redox stresses (Duche *et al*., 2002; Giotis *et al*., 2008; Agoston *et al*., 2009; Cacace *et al*., 2010; Bowman *et al*., 2012; Ignatova *et al*., 2013). There could be adaptation via reconstruction of metabolic pathways of pathogen forming de-novo end products such as lipids, proteins, and high-energy intermediates. Proteins involved in cell wall maintenance and amino acid metabolism, like osmolyte transporters, osmolyte synthesis enzymes, and lipid biosynthesis proteins, found to be over-expressed under cold temperatures stress and salt stress (Pittman *et al*. 2014). A set of proteins has been reported recently, which could potentially support survival and adaptation of *L. monocytogenes* in abiotic niches in food processing environments. This data has also helped towards better understanding and supported elucidation of the mechanism of the phenomenon of persistence of *L. monocytogenes* (Rychli *et al*., 2016). The protein analysis of biofilm form in *L. monocytogenes* revealed that there were

proteins found to be more highly expressed involved in stress response and envelope. Apparently cell envelope proteins contribute in surface attachments and stress tolerance mechanisms as well (Hefford *et al.*, 2005). Comparative proteomic analysis of *L. monocytogenes* showed a similar pattern of stress proteins (chaperones) with some differences in both cellular states when exposed to enterocin AS-48 in planktonic and sessile states (Gómez *et al.*, 2013). This observation remarks the possible involvement of some common proteins in the response to different stresses. Even though proteomic strategies have been applied in understanding mechanisms behind stress response of *L. monocytogenes*, least information is available about the involvement of common proteomic factors in tolerance response of *L. monocytogenes* in different stresses. It is important to understand commonly involved a proteomic response to diverse stresses.

Although structural changes in cell envelope of *L. monocytogenes* under different kinds of stresses have been studied; the majority of studies concerning *L. monocytogenes* physiological behavior as a result of exposure to food-related stresses usually at short-periods exposure or adaptive response to the stress conditions, such as shock or adaptation for a couple of hours, before the experiments, are conducted (NicAogáin and O'Byrne, 2016). It does not give the idea about innate response and innate changes in physiological factors in dominant stress tolerant subtype among strains of diverse origin; when characterized for their tolerance to different stresses of high salt, extreme pH and cold stress. This information can be applied to the mechanistic studies for understanding the molecular factors such as mechanisms involved in bacterial resistance to various foods related stressors.

3.3 Materials and Methods

3.3.1 Determination of morphological changes under stress

The strains exhibiting highest stress tolerance (high salt, low temperature, low pH and high pH) were selected to determine the morphological changes under particular stressed environmental conditions. The *L. monocytogenes* ILCC183 showing tolerance to 12.5% salt concentrations was selected for morphological analysis after exposure to high salt concentrations. The *L. monocytogenes* ILCC187 with tolerance capacity to low temperature of 4°C, acidic pH 4.5 and alkaline pH 9.5 was selected for morphological analysis under respective stresses.

3.3.2 Light Microscopy

To determine the morphological changes under high salt stress, 16-18 h grown culture of *L. monocytogenes* strain ILCC 183 in BHI broth (2 ml) and in BHI broth supplemented with 12.5% NaCl (high salt stress) were centrifuged, washed twice with PBS to remove media particles, re-suspended in 2 ml of PBS and a loopful of culture was taken on glass slide. The culture was stained with Gram's Method and observed under compound microscope. Similarly, strain ILCC187 was grown at respective stresses of low temperature (4°C), acidic pH (4.5), and alkaline pH (9.5) and at optimum conditions; which further Gram stained and analyzed.

3.3.3 Scanning electron microscopy (SEM)

Strains exhibiting high tolerance for each of stress (high salt concentration 12.5%, low temperature (4°C) and extreme pH of 4.5 and pH 9.5) were analyzed for morphological changes using scanning electron microscopy (SEM). Bacterial strains were incubated in BHI broth (2 ml) under respective stress conditions. After 16-18h,

of growth cells were harvested, washed twice with PBS, re-suspended in 2 ml of PBS and a loopful of culture was taken on cover slip and allowed to air dry. After air drying, the smear was fixed with 2.5% of glutaraldehyde for overnight and then dehydrated via successive passages of 10 minutes through 30%, 50%, 75%, 85%, 90%, 95% and 100% of ethanol. This preparation was then allowed to air dry and then sputter-coated with gold. The gold coated smears were examined by scanning electron microscope (EVO 18, Carl Zeiss, Germany).

3.3.4 Determination of genetic basis of filament formation

The *minC* gene in *L. monocytogenes* was detected by PCR in all high salt (12.5%) stress tolerant strains (n=13). The primers for amplification of the *minC* gene were designed using Primer 3 (ver. 4.0) software. A 50µl reaction mixture was prepared consisting of 55 ng of bacterial genomic DNA, 15pmol of each primer (Table 3.1), 25µl of 2X PCR master-mix (Sigma, USA). The reaction was performed in an thermal cycler (Eppendorf, Germany) with initial denaturation at 95°C, 5.0 min; denaturation 95°C, 45 s; annealing 51°C, 30 s; and extension 72°C, 1 min. (35 cycles); final extension at 72°C, 10 min. PCR products were stored at 4°C till further analysis.

3.3.5 Electrophoresis

The 7µl of PCR products (+2 µl Loading dye) were loaded on 1% agarose gel (added with ethidium bromide) and run at 80V in 0.5x TBE for 30 min. After electrophoresis gel was visualized and photographed in G:Box gel documentation system (Syngene).

3.3.6 Relative gene expression of the *minC* gene under high salt stress

The actual effect of salt stress on the *minC* gene expression was determined by qPCR. The strains that formed filaments under salt stress were selected for the study. After growing the strains at mid-exponential phase under salt stress, RNA was isolated by using TRIzol® reagent (Invitrogen, USA) following the manufacturer's protocol. The extracted RNA was further processed for removal of DNA with Turbo DNA free kit™ (Invitrogen, USA) according to manufacturer's protocol. RNA yield was determined using the NanoDrop® ND-1000 instrument (Thermo Scientific, USA) and RNA quality was checked by resolving it on 1.5% agarose gel. The cDNA synthesis was performed using SuperScript III First-Strand Synthesis SuperMix (Invitrogen, USA) for Quantitative-Real Time PCR (qRT-PCR). Total 200ng RNA was converted into cDNA in 20 μL reaction mixture according to manufacturer's protocol. The contamination of residual of DNA was checked in each RNA sample by a control reaction which includeded a cDNA synthesis without reverse tran-scriptase enzyme (no RT control). The primers used in this reaction (Table 3.1) were designed by using Primer 3 software version 4.0 and optimized to achieve specific target gene amplification (product with a single melting peak). qRT-PCR mixture was prepared by using 10μL of 2x SYBR green master mix (Sigma, USA), 0.5μL (10 nM) of each forward and reverse primers 1μL of template cDNA and 8μL of nuclease free water making volume to 20μL. As negative controls, water (no template) and the no RT control were applied. The reaction was performed in Light Cycler 96 (Roche, Switzerland). The reaction conditions were pre-incubation at 95°C for 10 min, then 40 cycles (95°C for 10s; 56°C for 20s and 72°C for 20s) followed by melting curve (65-97°C with increment rate of 2.2°C/s and a continuous fluorescent measurement) and cooling. The expression of *minC* gene was

normalized against expression of reference *23s rRNA* gene (Romanova *et al*., 2006). The transcript levels of the *minC* gene were determined in strain exposed to high salt stress as well as optimal (no salt stress control) conditions. The fold induction of the *minC* transcript in response of high salt stress was calculated relative to optimal conditions transcript level.

Table 3.1: List of Primers used in this study

Gene	Primer Sequence	Amplicon Size	Reference
Primers used for amplification of *minC* gene			
minC	**F** 5'AGAACTAACTCAATTGCTTGCAG 3' **R** 5'CAAATCTGTTTCAGTGACCTCTTT 3'	475 bp	This study
Primers used in relative expression analysis of *minC* gene			
23s rRNA	**F** 5'GTGTCAGGTGGGCAGTTTG 3' **R** 5'CATTCTGAGGGAACCTTTGG-3'	76 bp	Romanova *et al*., 2006
minC	**F** 5' GGAGGACAAATACGTTCAAATG 3' **R** 5'GGATAAAGAAATTTCCCTGCTACAA 3'	110 bp	This study

3.3.7 FAME analysis

A single strain *L. monocytogenes* ILCC187 exhibiting the stress tolerance to acidic pH (4.5), alkaline pH (9.5), low temperature (4°C) and salt (10%) was selected for the comparative analysis of fatty acids under respective stresses and at optimum conditions. Fatty acid profiles were analyzed as described by Ichihara and Fukubayashi (2010) using Fatty Acid Methyl Esterase (FAME) analysis. In brief, the overnight grown cultures were diluted 1:50 in 50 ml fresh BHI broth with respective stresses and in optimal conditions, which were further grown until reached to mid-

exponential phase. Then cells were pelleted by centrifugation and washed twice with PBS. Approximately 40-50 mg of cell pellet was taken in screw-capped glass tube and hydrolyzed with 1 ml of 1 M KOH in 70% ethanol at 90°C for 1 h. Then reaction mixture was acidified with 0.2 ml of 6 M HCl, and added with 1 ml of water. Then 1 ml of hexane was added to the mixture for release FFAs. Hexane was allowed to evaporate and then FFAs were methylated with 1 ml of 10% Boron trifluoride (BF_3) in methanol at 37°C for 20 min. Finally 1ml of water was added to the solution, and then FAMEs were extracted with 1 ml of hexane. FAMEs for each stress and control were extracted separately and analyzed on Agilent 6850 Gas Chromatography system.

3.3.8 Protein profiling

To study the protein based response of *L. monocytogenes*, protein profiles of cell wall and cytoplasmic proteins under the stresses were analyzed separately. *L. monocytogenes* ILCC187 exhibiting the stress tolerance to acidic pH (4.5), alkaline pH (9.5), low temperature (4°C) and salt (10%) was selected for the comparative analysis of protein profiles under stress and control conditions by SDS-PAGE.

3.3.9 Protein Extraction

The isolation of cell wall and cytosolic protein fractions was performed as described by Mraheil *et al*. (2017) with little modifications. The overnight grown cultures were diluted 1:50 in 50 ml fresh BHI broth with respective stresses (BHI broth with 12.5% salt, BHI broth of pH 4.5, BHI broth of pH 9.5 and control BHI broth) using 100 ml flasks and incubated at 37°C with shaking at 180 rpm until mid-exponential phase (A_{600} ~1.0). For low temperature stress, inoculated BHI broth was incubated at 4°C with shaking at 180 rpm until it grew to mid-exponential phase

(A_{600} ~1.0). Subsequently the cells were harvested by centrifugation at 6200g for 20 min at 4°C. For extraction of cell-wall proteins the cell pellet was re-suspended in 1.8 ml of 1% SDS and incubated at 37°C with shaking (300 rpm) for 45 min. This preparation was then centrifuged at 14,000g for 20 min at room temperature, and the supernatant was transferred into a fresh tube. Cell wall proteins were precipitated by addition of 200µl of TCA to make final concentration to 10% and incubated at 4°C overnight. Finally, the proteins were pelleted by centrifugation at 14,000g for 20 min at 4°C and resuspended in 1 M Tris-HCl (pH = 8.8). For extraction of cytosolic proteins, 50 ml culture was pelleted by centrifugation at 6000g for 20 min at 4°C. Then cell pellet was suspended of 5 ml of 1X SMMP (Appendix-7) buffer and added with 100µl of lysozyme solution (10mg/ml). This preparation was then incubated at 37°C for 12-16h. After incubation solution was centrifuged at 6000rpm for 20 min at 4°C and protoblast washed with 1X SMMP (Appendix-7) buffer. Finally cells were lysed by adding 200µl of cold ultrapure water and centrifuged at 15000 rpm for 30 min at 4°C. The supernatant (protein) was collected in fresh tube and same step was repeated and supernatant (protein) collected again. All protein preparations were stored at -20°C till further analysis.

3.3.10 Estimation of Proteins

Extracted proteins were estimated by Bradford's method (Kruger, 1994). Briefly, the standards were prepared (1 ml each) containing 0, 10, 20, 30, 40 and 50 µg ml^{-1} bovine serum albumin (BSA). Sample proteins were diluted at a range of dilutions (1, 1:10, 1:100, 1:1000). Then 100µl of each sample and known standards were taken in separate tubes in duplicates, distilled water was used as blank. This solution is then added with 1 mL of protein reagent (Appendix-8) to each tube and mixed thoroughly. The samples were incubated at room temperature for 5 min. and

then absorbance was measured at 595nm. Standard curve was plotted with readings of known standards and protein concentrations of samples were estimated.

3.3.11 SDS-PAGE

Profiles of cell wall and cytoplasmic proteins isolated from strains grown under stress and control were separated and analyzed by SDS-PAGE (Sambrook *et al.*, 1989). Samples were diluted 1:1 with sample buffer containing 2% (w/v) SDS and 4% (v/v) 2-mercaptoethanol (Appendix-17). A total of 30 µg of each sample and Precision Protein Standard (Bio-Rad) was loaded on polyacrylamide gel comprising of 4% stacking gel with 12% resolving gel (Appendix-18) (SureCast Gel Handcast System, Thermo Fischer) in 1X Tris-Glycine electrophoresis buffer (Appendix-16). Electrophoresis was carried out in Mini Gel Tank (Thermo Fischer) at conditions 60V for 3 h. Finally bands were visualized by staining with 1% Coomassie Brilliant Blue R 250 in 40% methanol with 10% acetic acid and 50% Milli-Q water (Appendix-19) for 30 min. with shaking 50 rpm. Then gel was de-stained with de-staining solution (40% methanol with 10% acetic acid and 50% Milli-Q water) until distinct blue bands were appeared with clear background.

3.4 Results and Discussion

3.4.1 Determination of morphological changes under stress

Long filament formations were observed under salt stress (Fig. 3.1a and 3.1b), while, no structural changes could be observed for isolates grown under pH and temperature stresses. Therefore, effect on morphology under salt stress was further studied for longer time (till 72 h). With the increase in time, there was an increase in the length of filament (Fig. 3.1c). Compared to control, cells were present singularly (Fig. 3.1a) and few elongated cells were observed. Therefore, it appeared that under stress conditions there could be inhibition of cell division. To verify this phenomenon, the bacteria grown under salt stress (BHI with 12.5% of NaCl) were transferred to normal BHI broth and incubated for 2 h and observed for cell morphology. Interestingly, all the cells were observed to occur singularly (Fig.3.1d) as observed in control. These observations were further confirmed by Scanning Electron Microscopy (SEM).
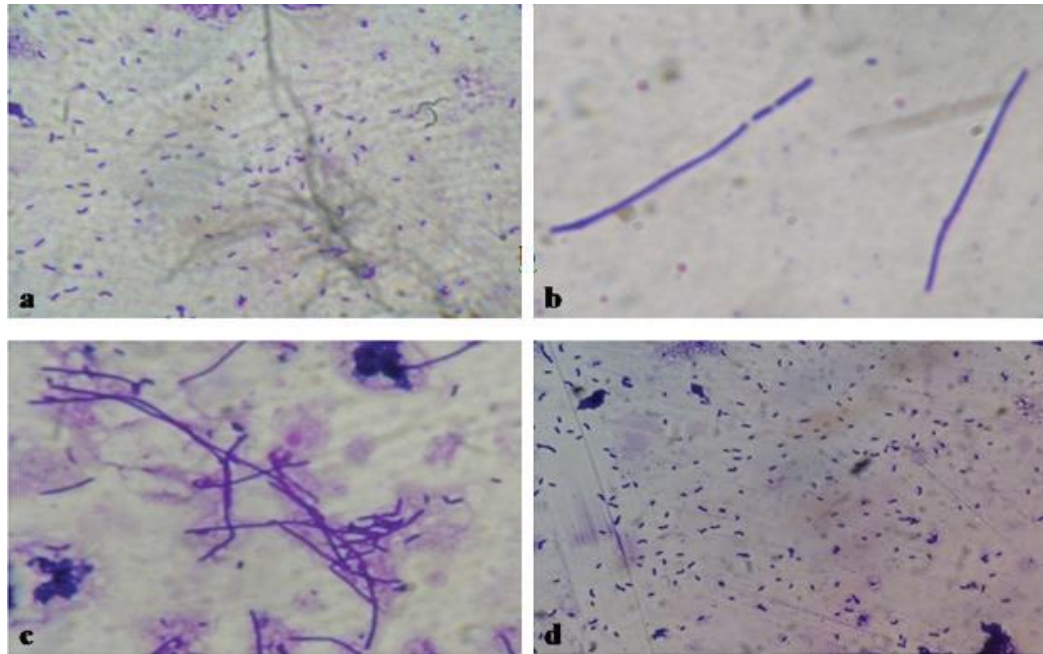
Fig. 3.1: Changes in morphology of *Listeria monocytogenes* after osmotic shifts.
a) Cell morphology at control (optimum) conditions b) Cell morphology at 12.5%
salt stress. c) Cells increases in length with longer incubation under stress (72h) (d)
Cells regains original size and shape after removal of stress.

3.4.2 Scanning electron microscopy (SEM)

Scanning electron microscopic studies showed 3-10 times elongation of cells under stress which got reverted to normal size after removal of stress. The length of the cell found to be increased (8.45 μm) (Fig. 3.2A) as compared to control (1.53μm) (Fig. 3.2B) under high salt stress. Under pH and low temperature stresses, there were no significant changes observed in morphology. The length of filament under high salt stress was observed to be increased (13.84μm, 14.16μm, 16.03μm) (Fig. 3.2C and 3.2D) with the longer time of incubation up to 48 h to 72 h indicating a positive correlation (R^2=0.9145) with filament formation with stress duration. The filament formation ability have been observed previously in *L. monocytogenes* under different stresses by different researchers including high osmotic stress, low

temperature stress, acidic stress and alkaline stress (Bereksi *et al.*, 2002; Giotis *et al.*, 2007; Vail *et al.*, 2012). It has been observed that as stress increased filamentation also increased (Giotis *et al.* 2007). These morphological changes under stress might infer that filament formation under stressed environmental conditions could be the one of the mechanisms of stress tolerance in *L. monocytogenes.* Changes in morphology of *L. monocytogenes* were observed after osmotic shifts. There was increase in length of the filament as with longer incubation (72 h). The bacteria regained normal size after removal of stress. This type of phenomenon was also observed earlier (Pratt *et al.*, 2012).
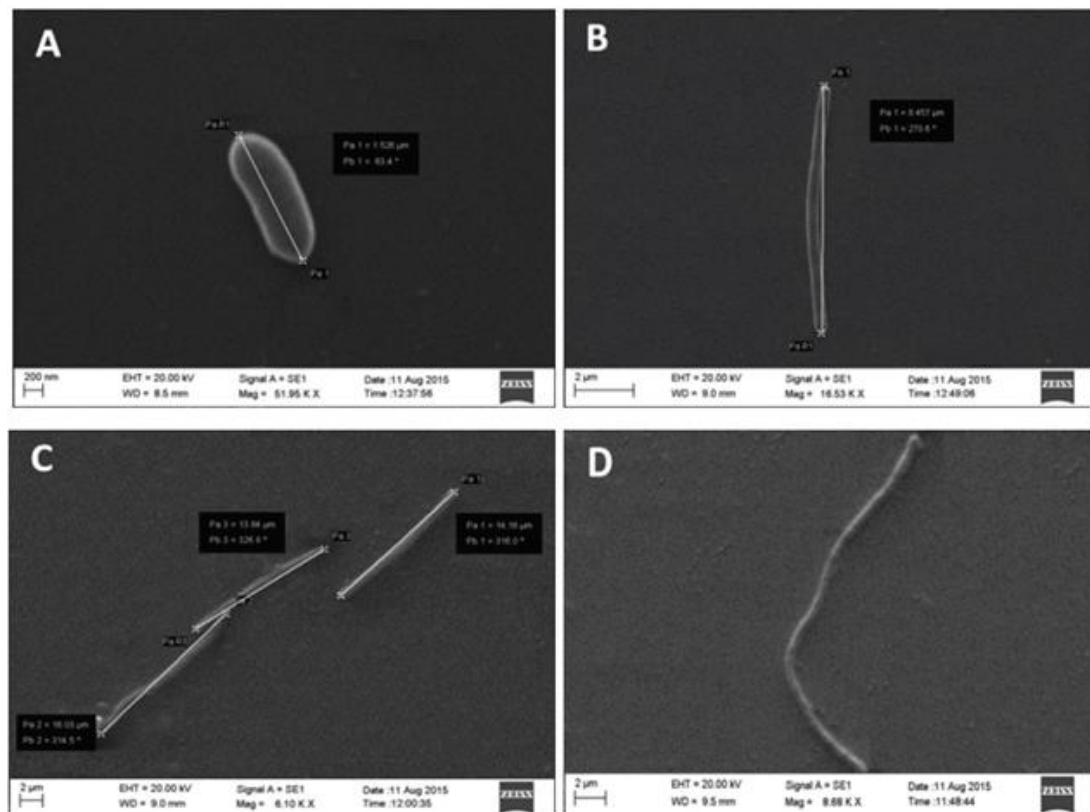

Fig. 3.2: Scanning Electron Microscopy of *Listeria monocytogenes* ILCC187 (A) Cell under optimum conditions (B) Cell with increased length under high salt stress after 24 h incubation (C) Cell under high salt stress after 48 h incubation (D) Elongated cell after 72 h incubation under salt stress.

When it comes to the food processing and/or food preservation by using high salt, bacteria which forms the filaments under such stress could be the problem. Since after exposure to the less stressed conditions filamentous cells immediately divides in individual cells, ultimately there is an increase in the number of bacteria in the food (Bereksi *et al.*, 2002; Jones *et al.*, 2002). The formation of filamentous cells leads to forming a single colony on solid media. This may result in a false estimation of bacteria in particular food product by cultural methods and false readings in predictive models for growth kinetics of bacteria based on CFU methods (Giotis *et al.*, 2007), significantly increasing the risk to consumers. Though filament formation under stress has been observed in *L. monocytogenes*; however, studies are largely lacking about further mechanisms (Jones *et al.*, 2013). *L. monocytogenes* is normally exposed to various stresses during food processing and disinfection procedures which could influence its response and ability to persist in these environments, and thus contribute to defining conditions for better control in food processing plants (Magalhaes *et al.*, 2016).

3.4.3 Determination of genetic basis of filament formation under high salt stress

Considering the inhibition of cell division, study was further focused at genomic level. There are 10 proteins involved in bacterial cell division - FtsA, FtsB, FtsI, FtsK, FtsL, FtsN, FtsQ, FtsW, FtsZ, and ZipA (de Boer., 2010). The key stages are formation of FtsZ septal ring, positioning of the ring, maturation of the ring and then cell division. This septal ring positioning is needed to be on right place and on right time which is controlled by two different systems namely, Min system and nucleoid occlusion. The Min system plays a role in prevention of cell division at cell poles in which MinC and MinD form a cell inhibitor which is topologically regulated by DivIVA. The MinC inhibits FtsZ ring formation at poles resulting in

septation at middle position of cell and blockage of other possible sites of cell divison (Rothfield *et al*., 2005). It has been reported that there is an over-expression of *minC* in *E. coli* during its filamentous morphology (de Boer *et al*., 1989; de Boer *et al*., 1988). Studies in *Bacillus subtilis* had shown that effect of MinC on FtsZ was pH dependant (Scheffers, 2008).

We hypothesized that in salt stress the *minC* gene gets over expressed and inhibits the formation of FtsZ ring which ultimately inhibits the septa formation in *L. monocytogenes.* To test this hypothesis, the *minC* gene was first detected by PCR and then its expression was analysed by qPCR under stress in comparison with control. All the 13 salt stress tolerant strains studied showed the presence of 475 bp band of the *minC* gene (Fig 3.3). The induction of the *minC* gene transcription level under high salt stress was examined by qPCR. All tested 13 strains showed the 6 to 11 fold expression of the *minC* gene under high salt stress (Fig. 3.4), suggesting the possible involvement of the *minC* gene in altered morphology with filamentous structure in *L. monocytogenes* under high salt stress. The over expression of *minC* gene supported the hypothesis that the MinC protein over-expression could be the factor behind filamentous morphology of *L. monocytogenes* under high salt stress and forming a filament could be the one of the mechanisms by bacteria to tolerate high salt stress.
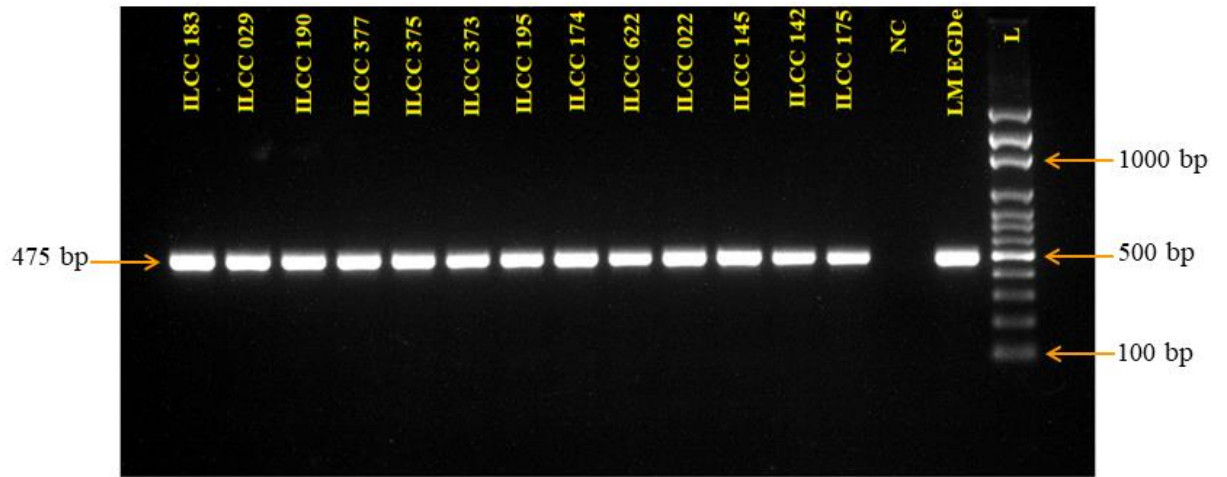
Fig. 3.3: PCR amplified product targeting the *minC* gene (475bp) from filamentous cells former strains, run on 1% agarose gel. ILCC183, ILCC029, ILCC190, ILCC377, ILCC375, ILCC373, ILCC195, ILCC174, ILCC622, ILCC022, ILCC145, ILCC142, ILCC175 – the *L. monocytogenes* strains tested. NC- Negative Control. LM EGDe - Positive Control and L - 100bp Ladder.
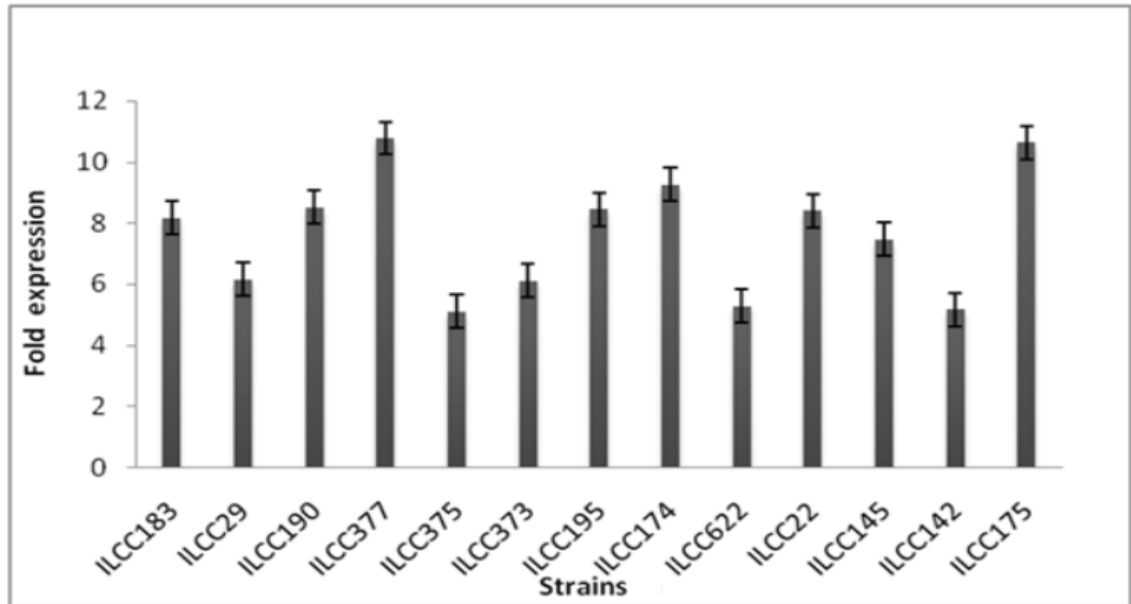

Fig. 3.4: Transcriptional induction of the *minC* gene expression in response to high salt stress.

3.4.4 FAME analysis

There is large diversity in structures of phopsholipids in the bacterial world, still most membrane phospholipids contains two fatty acid chains. The viscosity of the membrane is determined by such phospholipidacyl chains, which in turn influences many crucial membrane associated functions, including the passive permeability, active solute transport and protein–protein interactions. The alterations in fatty acid compositions towards maintaining the biophysical properties of membranes are called as homoviscous adaptation (Zhang and Rock, 2008). The studies showed that amount and type of fatty acids influences the stress tolerance properties of *L. monocytogenes.* Considering the role of membrane lipids in protecting an organism from damaging effect of salt, acidic/alkaline pH and cold stress on fatty acids profile, we made attempt to determine whether there is in difference in fatty acids profile of *L. monocytogenes* strain after exposure to the stress in comparison with control by GC-MS analysis. The samples were compared with reference standards and cell surface fatty acids ranging from 12:0 to 18:2 were recognized and their relative amounts were estimated. A significant increase in the amounts of $C_{16:0}$ (Table 3.2) was observed in *L. monocytogenes* strain grown under acid pH and alkaline pH stress as compared to that of control. There was also incorporation of $C_{14:0}$ fatty acids under acidic stress and $C_{13:0}$ fatty acids under alkaline stress. The structures of these fatty acids determine the biophysical properties of the membrane bilayer. The $C_{16:0}$, $C_{14:0}$ and $C_{13:0}$ are straight chain saturated fatty acids and being linear they pack together efficiently to produce a bilayer that has a high phase transition and low permeability property. As observed earlier by Mastronicolis *et al*. (2010) with such alterations in fatty acid composition *L. monocytogenes* decreases the membrane fluidity and under acid stress. Since

under acidic/alkaline pH stress, it is necessary for bacterial survival to maintain cytoplasmic pH irrespective of ionic conditions outside the cell. Hence bacteria like *L. monocytogenes* counter with such stresses by controlling the ionic diffusion across the cell membrane by alteration in fatty acids composition. Earlier in *L. monocytogenes,* alteration in membrane fluidity after exposure to the low temperature stress has been reported (Beales, 2004; Najjar *et al*., 2007). Under low temperature stress significant increase in the amount of $C_{16:0}$ were observed, this again underlined the decrease in membrane fluidity. This might be one of the important mechanisms of stress adaptation in *L. monocytogenes*. In cold stress exposed strain there is also increase in the amount of unsaturated fatty acids ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$) (Table 3.2). Unsaturated fatty acids have one carbon–carbon double bond. The presence of double bond restricts the mobility of acyl chains at that position (Rustan and Drevon, 2005). This modification of unsaturated fatty acids helps in order to stabilize membrane fluidity which is known as 'homeoviscous adaptation' (To *et al*., 2011); and plays crucial role in major bacterial adaptive response to low temperature stress. Interestingly under high salt stress, the amount of $C_{16:0}$ observed to decline in comparison with control (Table 3.2). This could be the attempt by bacteria to enhance the membrane fluidity than that of at optimum conditions, as under osmotic stress bacteria needs to accumulate different solutes across the membrane to maintain the osmosis.

Table 3.2: Fatty Acids Profile of *L. monocytogenes* ILCC187 under control and different stresses conditions by FAME analysis.

Fatty Acids	Control				Acid Stress				Alkaline stress				Cold stress				Salt stress		
	Run time	Area	Results (%)		Run time	Area	Results (%)		Run time	Area	Results (%)		Run time	Area	Results (%)		Run time	Area	Results (%)
$C_{12:0}$	19.14	13428	0.0013		19.65	15647	0.0011		19.71	18968	0.0009		19.91	22514	0.0007		19.91	22514	0.0007
$C_{13:0}$	0	0	0		0	0	0		21.79	213514	0.0087		0	0	0		0	0	0
$C_{14:0}$	0	0	0		29.18	13428	0.0013		0	0	0		0	0	0		0	0	0
$C_{16:0}$	34.28	11478562	1.1181		34.57	71478562	6.9627		34.91	121478562	4.7333		34.83	61367451	5.3635		33.64	32154188	0.9369
$C_{18:0}$	38.81	225145	0.0219		39.36	10212665	0.3512		39.64	10794163	0.4206		38.82	12514528	0.4114		38.21	10526984	0.3067
$C_{18:1}$	38.23	32147852	3.1315		39.21	5147852	0.5015		39.41	7158635	0.8732		38.31	121514528	3.9943		37.58	102141526	2.9761
$C_{18:2}$	38.11	58794163	5.7271		38.74	18794163	1.8307		38.96	12516227	0.9266		38.23	210794163	6.9291		37.44	148596312	4.3296

3.4.5 Protein Profiling

Protein is a vital component of a living organisms required for normal functioning of many physiological metabolic processes within the cells. When a bacterium is exposed to the stressed environmental conditions, proteins are one of the very important factors involved in maintaining physiology of cells under those stresses (Krawczyk-Balska and Lipiak 2013). Proteins maintain the physiology and also help to counter such stressed environmental conditions and help bacteria in survival, adaptation and to acquire the resistance (Soni *et al*., 2011). To study the protein based response of *L. monocytogenes*, protein profiles of cell wall proteins and cytoplasmic proteins for both control and stressed strains were analyzed by SDS-PAGE. After comparing the protein profiles of *L. monocytogenes* grown under controlled and under stressed conditions, over-expression of proteins in cell wall of stressed cells as compared to control conditions was observed. Even though there were some cell wall proteins observed differentially expressed specific to the stresses, some same size cell wall proteins were also observed to be expressed in different stresses. A protein of size ~25 kDa observed to be overexpressed in cell wall of high salt stressed strain (Fig. 3.5a) and cell wall acid stressed strain as well (Fig. 3.5b). In case of cytoplasmic proteins over expression of protein sized ~45 kDa under high salt (Fig. 3.5a), acidic pH (Fig. 3.5b) and alkaline pH stress (Fig.3.5c) were observed. This indicated possible involvement of common factors in regulation of stress response to high salt and acidic pH. Under low temperature stress (Fig.3.5d), there was lower expression of proteins observed in cell wall indicating uncommon modifications in cell envelope structures under cold stress than that of other stresses.

Although this study did not fully detect the major proteins involved in stress response, it provided valuable partial picture of both differences and similarities of induced and/or expressed proteome of *L. monocytogenes* strain under four different stresses. This protein profiling was attempted as a step towards robust determination of physiological factors used by *L. monocytogenes* in adaptation strategies to high salt, extreme pH and low temperature stresses.
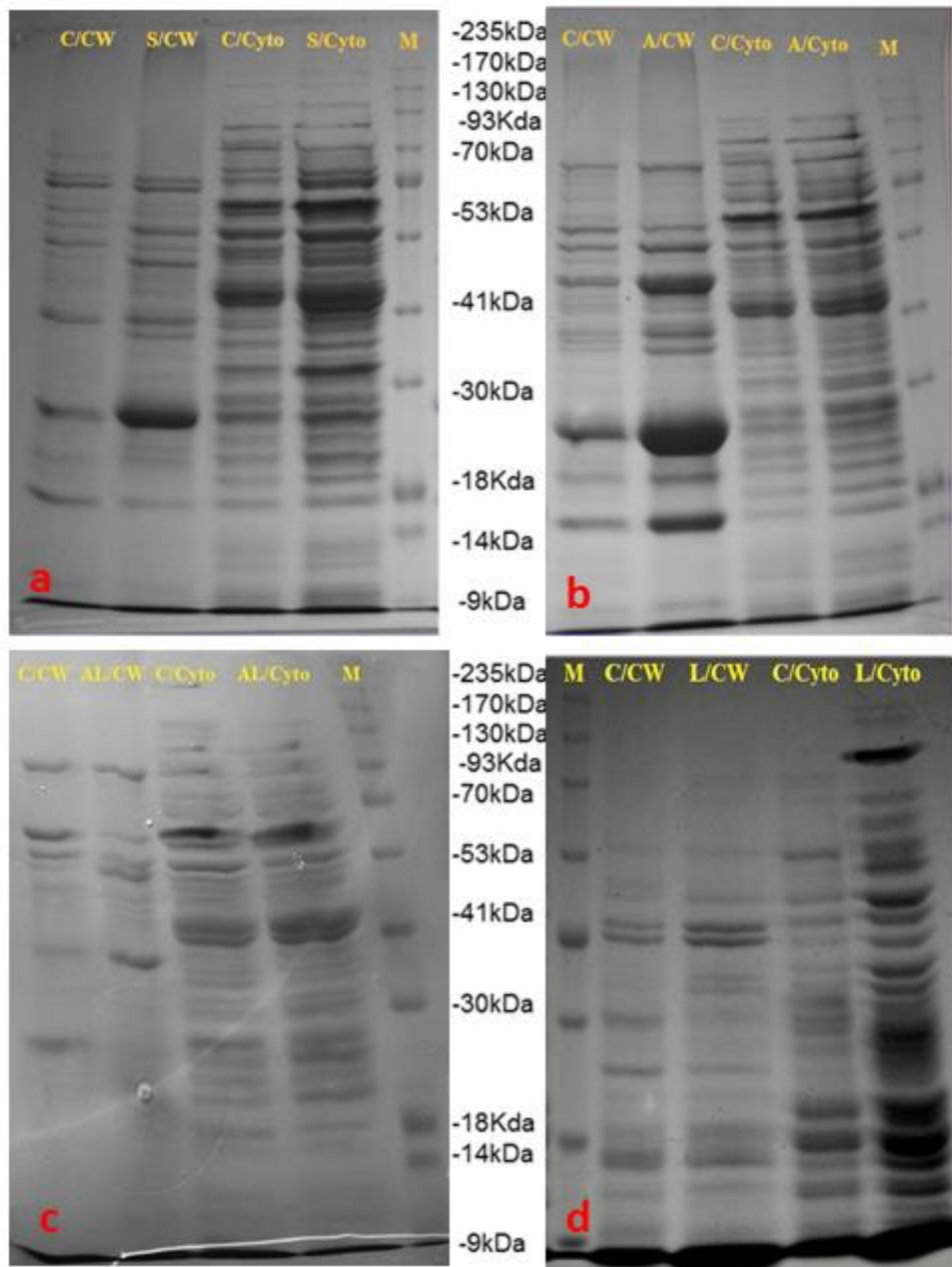
Fig 3.5: SDS-PAGE analysis of cell wall and Cytoplasmic Proteins under (a)high salt stress (C/CW – Control Cell wall proteins; S/CW-salt stress cell wall; C/Cyto-Control Cytoplasm proteins; S/Cyto- salt tress cytoplasm) (b) acid stress (C/CW – Control Cell wall proteins; A/CW-acid stress cell wall; C/Cyto- Control Cytoplasm proteins; A/Cyto- acid tress cytoplasm) (c) alkaline stress (C/CW – Control Cell wall proteins; AL/CW- alkaline stress cell wall; C/Cyto- Control Cytoplasm proteins; AL/Cyto- alkaline tress cytoplasm) (d) low temperature stress (C/CW – Control Cell wall proteins; L/CW-Low temperature stress cell wall; C/Cyto- Control Cytoplasm proteins; L/Cyto- Low temperature stress cytoplasm). M- Protein Marker

Chapter 4

Determination of genetic basis for stress tolerance

4.1 Introduction

Sophisticated sensing mechanisms and signal transduction systems have been evolved by bacteria that senses dramatic shift in the environmental conditions/ stresses. This sense activates intracellular signaling pathways, which can produce accurate dynamic outcomes in response to changing environmental conditions and controls almost any aspect of cell physiology. The major component of this response is modulation of gene expression along with alterations in metabolism, protein homeostasis, cytoskeletal organization and modification of enzymatic activities (De Nadal *et al*., 2011; Reverchon *et al*., 2015). The knowledge of coordination of interactions between the regulatory factors such as proteins and/or sRNA molecules with the structural entities that is genes and/or groups of genes is required for the better understanding of bacterial genetic regulation. The recent advances in experimental technology in association with bioinformatics tools have implemented high-throughput approaches to study the genetic regulation systems in bacteria (Conway *et al*., 2014). As observed earlier, these adaptive responses controlled by gene expressions has reversibility; which enables the cells to change transcriptional mode with exposure to stresses and revert to original state after removal of the stresses. This helps bacteria to cope up with ups and downs in the external environmental conditions. In *L. monocytogenes,* several studies have been attempted to understand genetic basis of stress response. Several researchers have attempted to identify uniformity and variation in genes specific to the stresses. The candidate genes involved in response of different stresses, such as cold stress (Chan *et al*., 2007; Liu *et al*., 2014); high salt stress (Begley *et al*., 2002; Gardan *et al*., 2003; Utratna *et al*., 2011; Bergholz *et al*., 2012; Burall *et al*., 2015); acid stress response (Ryan *et al*., 2009; Cheng *et al*., 2013; Cheng *et al*., 2017) and alkaline stress (Giotis

et al., 2010; Kocaman and Sarimehmetoglu, 2016; Rychli *et al.*, 2016) have been identified. Some studies have attempted to correlate variations in stress response with genetic makeup in *L. monocytogenes* (van der Veen *et al.*, 2008; Bergholz *et al.*, 2010). However, it is also not well understood that whether subtype dominating different niches with exceptional stress tolerance capabilities have genetic relatedness. Studies are largely lacking with attempt of identification of common genetic mechanisms under different stresses. So, it is important to expand our knowledge of *Listeria* to be more inclusive with respect to genetic factors involved in survival, adaptation and physiological response of *L. monocytogenes* under different stresses. A study with the combined physico-chemical and genetic approach gives new insights in physiology of stress response of *L. monocytogenes* to the food related stresses.

4.2 Review of Literature

L. monocytogenes contains single circular genome of average size 2.84-3.24Mb with 2908 to 3235 coding genes (approx. 89%) and low G+C content of 36-42% (Glaser *et al.*, 2001; Nelson *et al.*, 2004; Hain *et al.*, 2006). There are several fully sequenced genomes available on different databases; multiple sequences of single standard strains are also available on same and/or different database such as *L. monocytogenes* EGDe. Generally *L. monocytogenes* strains are very similar with respect to genetic content and organization; however, strains or serotype specific genes have been identified. *L. monocytogenes* F2365 (4b) was identified with 51 strain specific genes (Nelson *et al.*, 2004). Similarly, 97 genes were found to be restricted to the *L. monocytogenes* F6854 (1/2a), 69 genes to the *L. monocytogenes* H7858 (4b) and 61 genes found to be restricted to the *L. monocytogenes* EGDe (1/2a) strains. Similarly 83 genes were restricted to the serotype 1/2a and 51 genes

were restricted to the serotype 4b. *L. monocytogenes* EGDe has 2,944,528 base pairs (bp) sized genome with an average 39% of G+C content and 2853 protein-coding genes. *L. monocytogenes* F2365 contains genome of 2,905,310 bp in length with G+C content of 38% and total of 2847 predicted coding regions in the genome (Glaser *et al*., 2001). The majority of these genomic differences are opined due to phage insertion, transposable elements, scattered unique genes and islands coding for proteins of unknown functions (Nelson *et al.*, 2004). The genomics has opened new avenues in which the search for stress response factors can be pursued using comparative genomic approaches of different subtypes of stress tolerant *L. monocytogenes*. Although there are total 12 serotypes of *L. monocytogenes,* only 3 serotypes (1/2a, 1/2b, 4b) are considered as important because of their prevalence in all important niches, especially serotype 4b (serogroup 4b,4d,4e) has found to be responsible for major food borne outbreaks (Buchrieser *et al*., 1993), while, majority of the food isolates belong to serotype 1/2a (serogroup 1/2a,1/2c,3a,3c) or serotype 1/2b (serogroup 1/2b,3b,4b,4d,4e) (Farber and Peterkin 1991). Thus, many researchers have suggested that there may be a link between serotype and stress response (van der Veen *et al*., 2008; Makaritti *et al*., 2015). Variation in virulence (Wiedmann *et al*., 1997) and in growth capabilities under adverse conditions (De Jesus and Whiting, 2003; Lianou *et al*., 2006; van der Veen *et al*., 2008; Metselaar et al., 2015) have been observed among different subtypes of *L. monocytogenes*, which may, in part, explain the predominance of some subtypes in human disease. In Shiga Toxin-producing *Escherichia coli* O157, variation in stress tolerance patterns among different genotypes have been observed (Lee *et al*., 2012). Hingston *et al* (2017) reported that the stress tolerance of *L. monocytogenes* is associated with serotype, clonal complex and full length *inlA* profiles within 55% of the isolates. Metselaar *et*

al, (2015) reported that different adverse conditions drive for selection of different variants. The variation in salt stress tolerance capability of *L. moncytogenes* with difference in genotype has been reported (Bergholz *et al*., 2010). These phenotypic differences among *L. monoocytogenes* strains could be due to genetic differences of individual *L. monocytogenes* strains and/or subtypes. The molecular typing method such as PFGE analysis of different isolates is used critically in epidemiological studies (Swaminathan *et al*., 2001; Olsen *et al*., 2005). Such techniques can be also used in characterization of *L. monocytogenes* strains of diverse origins with respect to determine the clonality and genetic diversity of the stress tolerant *L. monocytogenes* strains and their adaptation to specific niches (Vogel *et al*., 2001; Autio *et al*., 2002). The genetic analysis can be further extended towards finding involvement common factors in different stress tolerance. The gene expression and quantification studies are required to identify genetic elements associated with stress response (Hain *et al*., 2007). This gene expression includes a flow of steps from DNA to RNA to proteins and it is provided with potential control points for self-regulation and function. This system helps cells to respond changes in environmental conditions and maintain and/or modulate their cell type expressions (Rieger *et al*., 2012). The process of transfer of genetic information from the DNA into a RNA molecule is designated as transcription and performed by an enzyme called RNA polymerase. The regulation at transcriptional level assumes paramount control in gene expression. It is only regulation point in the process of gene expression where no synthesis of unnecessary intermediates is ensured. In translational regulation, there is less control and less stability in all over the process (Khor and Hibberd, 2011). Even after protein synthesis, there is protein degradation, mis-folding of proteins or short life of proteins. This makes transcriptional regulation point best for

the gene expression studies (van Vliet, 2010). Gene expression studies under stress in *L. monocytogenes* have been performed using different technologies such as qRT-PCR, Microarray or RNAseq (Durack *et al*., 2013; Casey *et al*., 2014; Mataragas *et al*., 2015).

Gene expression using RNA sequencing data/transcriptome is exceptionally powerful tool in modern biology. RNAseq allows unequivocal mapping of the sequences in a single region of the genome (Voelkerding *et al*., 2009; Fang *et al*., 2013). It provides extent of coverage with a large range detection of transcripts, as it is able to detect from one to numerous copies of RNA per cell. The transcriptome also provides information on operon structures and mapping transcriptional start and stop sites (Wang *et al*., 2010). Transcriptomics data also can be used to elucidate the regulatory roles of noncoding RNAs (Storz *et al*., 2011; Morris and Mattick, 2014). The transcrptomic analysis of *L. monocytogenes* EGDe at different growth phases revealed that 7.6% of the genome was regulated of σ^B (105 genes upregulated and 111 genes downregulated) (Hain *et al*., 2008). Microarray based transcriptomic analyses of listerial stress response under salt and cold stress revealed the role of cell wall synthesis proteins, transcriptional regulators, and multiple biochemical pathways involved in protecting *L. monocytogenes* (Durack *et al*., 2013). Sengupta and Chattopadhyay (2013) observed up-regulation in cell synthesis genes and decrease in overall metabolic turnover and suppression of genes associated with motility when subjected to cold stress. The transcriptional response of persistent and non-persistent strains to a sublethal dose of the QAC benzethonium chloride was analyzed (Casey *et al*., 2014; Fox *et al*., 2011). About 600 genes were reported to be up-regulated upon exposure to benzethonium chloride, which included the genes involved in cell wall reinforcement, sugar metabolism, pH regulation, transcription,

and biosynthesis of cofactors (Fox *et al*., 2011). A transcriptome of *L. monocytogenes* persisted strain 6179 was compared in presence and absence of benzalkonium chloride and genes involved in peptidoglycan biosynthesis, carbohydrate uptake, chemotaxis, and motility were observed to be overexpressed (Casey *et al*., 2014). Tessema *et al*. (2012) reported the up-regulation of several genes encoding virulence factors, transport proteins, and transcriptional regulators (including σ^L) in presence of organic acids. Transcriptomic analysis by microarray in *L. monocytogenes* under alkali stress showed rapid change in expressions with up-regulation of genes encoding for multiple metabolic pathways, ATP-binding cassette transporters, motility as well as the σ^B controlled stress resistance network (Giotis *et al*., 2010). Mraheil *et al*. (2011) analyzed the intracellular sRNA transcriptome of *L. monocytogenes* during growth in macrophages and reported 150 putative regulatory RNAs; 71 of those have not been previously described and 29 regulatory RNAs, including small non-coding antisense RNAs, are specifically expressed intracellular. Transcriptome analysis targeted to particular genes or systems under different stresses have been performed by some researchers (Oliver *et al*., 2009; Nielsen *et al*., 2012; Supa-amornkul *et al*., 2016). Although various genetic factors have been studied for their role in stress response using different methodologies, numbers of observations are inconsistent and hence our understanding of *L. monocytogenes* stress response is still fragmented. None of the studies are available for comparative transcriptional analysis under food related stresses of epidemiologically important subtype of *L. monocytogenes*. Therefore encyclopedic picture of transcripts of *L. monocytogenes* under different food related stresses is required towards understanding global response as well as common factors in the response to these various stresses.

4.3 Materials and Methods

4.3.1 Pulsed Field Gel Electrophoresis analysis (PFGE)

A total of 37 strains which exhibited tolerance to one of the stress were further investigated for their genomic pattern by Pulsed Field Gel Electrophoresis (PFGE). The PFGE was performed according to the Pulsenet standardized protocol (Graves and Swaminathan, 2001). In brief, cultures were grown in BHI broth overnight and cells were pelleted by centrifugation. Standardized cell suspension and genomic DNA was prepared by mixing 240μL of standardized cell suspension with 20μL of 10mg/ml lysozyme solution (Sigma, St. Louis, Mo), followed by incubation at 56°C for 20 min. An equal volume of molten cooled (55°C) 1.2% PFGE grade agarose, 1% SDS and 20μL of 20mg/ml Proteinase-K were mixed with standardized cell suspension. This mixture was immediately dispensed in volume of 200μL in plug molds and allowed to cool for 5 min. The plugs were transferred to 4ml of cell lysis buffer (50 mM Tris and 50 mM EDTA, pH 8.0, 1% Sodium lauryl sarcosine and 0.15 mg/ml Proteinase-K) and incubated at 55°C for 2h in water bath shaker at 200 rpm. After incubation, lysis buffer was removed and plugs were washed with preheated (50-55°C) sterile ultrapure water. This washing with sterile ultrapure water was done twice in water bath shaker at 55°C at 200 rpm for 10 min. Then plugs were washed with preheated Tris EDTA (TE) buffer (50-55°C) as described above for 15 min four times. Once washings were completed, plugs were transferred to 1.5 ml tubes in TE buffer and held at 4°C until processed for restriction digestion. The plugs were digested either with 25U of *Asc*I (New England BioLabs, Beverly, MA, USA) at 37°C for 3h or 25U of *Apa*I (New England BioLabs, Beverly, MA, USA) at 25°C for 5h. After digestion the plugs were loaded on 1% PFGE grade agarose gel in 0.5X Tris Borate EDTA (TBE) buffer and electrophoresed on CHEF-DR® III apparatus

(Bio-Rad Laboratories, Hercules, USA). The gel also loaded with Lambda ladder (New England Biolabs, Beverly, MA). The generated DNA fragments were separated using following electrophoresis conditions: voltage, 6V; initial switch time, 4.0s; final switch time 40s; runtime 19h and temperature at 14°C. After electrophoresis gel was stained for 30 min in 400 ml of 0.5x TBE containing 25ml (10 mg/ml) of ethidium bromide and destained by two washes of 20 min each using 400 ml of deionized water and visualized under gel documentation system (Bio-Rad, USA). Genomic fingerprints were then analyzed by Phoretix Software (Total labs, UK).

4.3.2 Transcriptome analysis under different stresses

4.3.2.1 Bacterial strain and growth conditions

Considering the epidemiological significance, dominance of subtype in stress tolerance study and PFGE data; *L. monocytogenes* ILCC187 belonging to the predominant serotype 4b and with stress tolerance capability to all tested stress was selected for transcriptomic studies. A single isolated colony of test culture was inoculated in BHI broth and incubated at 37°C till reached to early stationary phase (18h). This was used as inoculum and 1ml was inoculated in 49 ml of BHI broth with pre-adjusted pH 4.5 (designated as 'AP') as well as at optimal BHI conditions which was used as control (designated as 'CT'). In the same way 1ml culture was inoculated in 49 ml of BHI broth with salt concentration of 10% (designated as 'HS'), BHI broth with pre-adjusted pH 9.0 (designated as 'AL'). All the preparations were incubated on shaker with 180 rpm at 37°C until reached to mid-exponential phase (A_{600nm}~ 0.9). For low temperature stress, 1ml culture was inoculated in 49 ml of BHI broth and incubated on shaker with 180rpm at 4°C until reached to mid-

exponential phase (A_{600nm}~ 0.9) (designated as 'LT'). All the cells were separated by centrifugation at 6000g for 10 min. at 4°C. Cell pellet was immediately suspended in RNA*later* (Sigma, USA) and stored at -20°C till further step.

4.3.2.2 Qualitative and Quantitative analysis of total RNA

Total RNA was isolated from all samples using Trizol® (Invitrogen, USA) as per the manufacturer's protocol. The quality of the isolated RNA was checked on 1% agarose gel for the presence of 16S and 23S bands. Further, total RNA was quantified using Qubit fluorometer (Invitrogen, USA).

4.3.2.3 Illumina NextSeq 2 x 150 PE library preparations

The RNAseq paired end (PE) sequencing libraries were prepared from the isolated Total RNA using illumina TruSeq stranded mRNA sample preparation kit. Briefly, mRNA was enriched from the total RNA using MICROBExpress™ kit as per manufacturer's protocol (Ambion, USA), followed by enzymatic fragmentation and 1st strand cDNA conversion. The 1st strand cDNA was then synthesized to second strand using second strand mix and Act-D mix to facilitate RNA dependent synthesis. The ds cDNA samples were then purified using Ampure XP beads followed by A-tailing, adapter ligation and then enriched by limited number of PCR cycles.

4.3.2.4 Quantity and quality check (QC) of library on Tape-station

The PCR amplified libraries for all samples were analyzed in Tape Station 4200 (Agilent Technologies) using High Sensitivity (HS) D1000 Screen Tape assay kit as per manufacturer's instructions.

4.3.2.5 Cluster Generation and Sequencing

After obtaining the Qubit concentration for the libraries and the mean peak size from Agilent Tape Station profile, the Paired End (PE) libraries were prepared from RNA samples using TruSeq stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA). The means of the library fragment size distribution is 306 bp to 401 bp. The library was sequenced on NextSeq 500 using 2 x 150 bp chemistry. The PE illumina library was loaded onto NextSeq 500 for cluster generation and sequencing. Paired End sequencing allowed the template fragments to be sequenced in both the forward and reverse directions on NextSeq 500. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment. The contigs were assembled using bwa-0.7.12 mem with default parameters. The putative coding sequences (CDS) were identified with Prodigal tool which is a microbial gene finding program. Prodigal is a highly accurate gene finder.

4.3.3 Bioinformatics Analysis

4.3.3.1 Functional Annotation of Predicted CDSs

The predicted CDSs data were analyzed by Rock Hopper Software (McClure *et al.*, 2013) by blasting the sequence data against reference strain *Listeria monocytogenes* F2365.

4.3.3.2 Differential Gene Expression

The DESeq package was used to identify significantly and differentially expressed genes between control and treated samples and genes were defined as significantly differentially expressed based on a p-value ≤0.05. The comparative analysis of the differentially expressed genes and their pathways was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://pathways.embl.de/iPath2.cgi#). KEGG database (Fig. 4.2a and Fig. 4.2b) is knowledge base for systematic analysis of gene functions, linking genomic information with higher order functional information. The experimental gene sequence data is used to produce knowledge in computable forms; namely, in the forms of molecular networks called KEGG pathway maps, BRITE functional hierarchies and KEGG modules and are developed as networks of KO (KEGG Orthology) nodes, representing high-level functions of the cell and the organism.

4.4 Results and Discussion

4.4.1 Pulsed Field Gel Electrophoresis analysis (PFGE)

Analysis of whole genome patterns of 37 tolerant strains with both the enzymes (*Asc*I and *Apa*I) revealed 15 pulsotypes (Fig.4.1). Two strains could not be typed with the *Asc*I enzyme. The Simpson's Diversity index was low (0.6873), indicating low diversity of strains that were capable of tolerating the stress. The observed 15 pulsotypes were labelled serially and alphabetically from 'A' to 'O'. The strains with pulsotype 'M' were observed to be dominant clustering 15 strains belonging to serotype 4b. Apparently, the possibility of single ubiquitous stress tolerating 4b clone cannot be denied. Also, in the case of serotype 1/2a and 1/2b strains very low genomic variation was noted. Although PFGE profiles showed correlation with the serotypes, there were no associations found with the stress tolerance capacities. Interestingly, the stress tolerance pattern of the similar pulsotype strains was different. For example, the strains with pulsotype 'M' were found to tolerate variable pH, salt, and low temperature. Similarly, in the case of serotype 1/2a strains and 1/2b strains were not consistent with their tolerance pattern. Considering the clonal or narrow genetic profile of the strains exhibiting tolerance to different stresses, it can be inferred that these tolerances must have been controlled by some common factor. Those common factors could be the presence some genes playing a role in survival and adaptation during exposure to the stressful environment. *In-silico* bioinformatics analysis of *L. monocytogenes* whole genomes have suggested several such gene-clusters present at distinct regions of the genome that altogether play significant roles in stress tolerance. (Kazmierczak *et al*., 2003; Hain *et al*., 2008).

The studies of distinct pattern of genotypes among stress tolerant *L. monocytogenes* strains might help to identify similarities and/or diversity based on genetic traits in *L .monocytogenes* strains of different origin and subtypes. Further studies with transcriptional analysis will help to confirm this hypothesis. *L. monocytogenes* is normally exposed to various stresses during food processing and disinfection procedures which could influence its response and ability to persist in these environments and thus contributes to defining conditions for better control in food processing plants (Magalhaes *et al*., 2016).

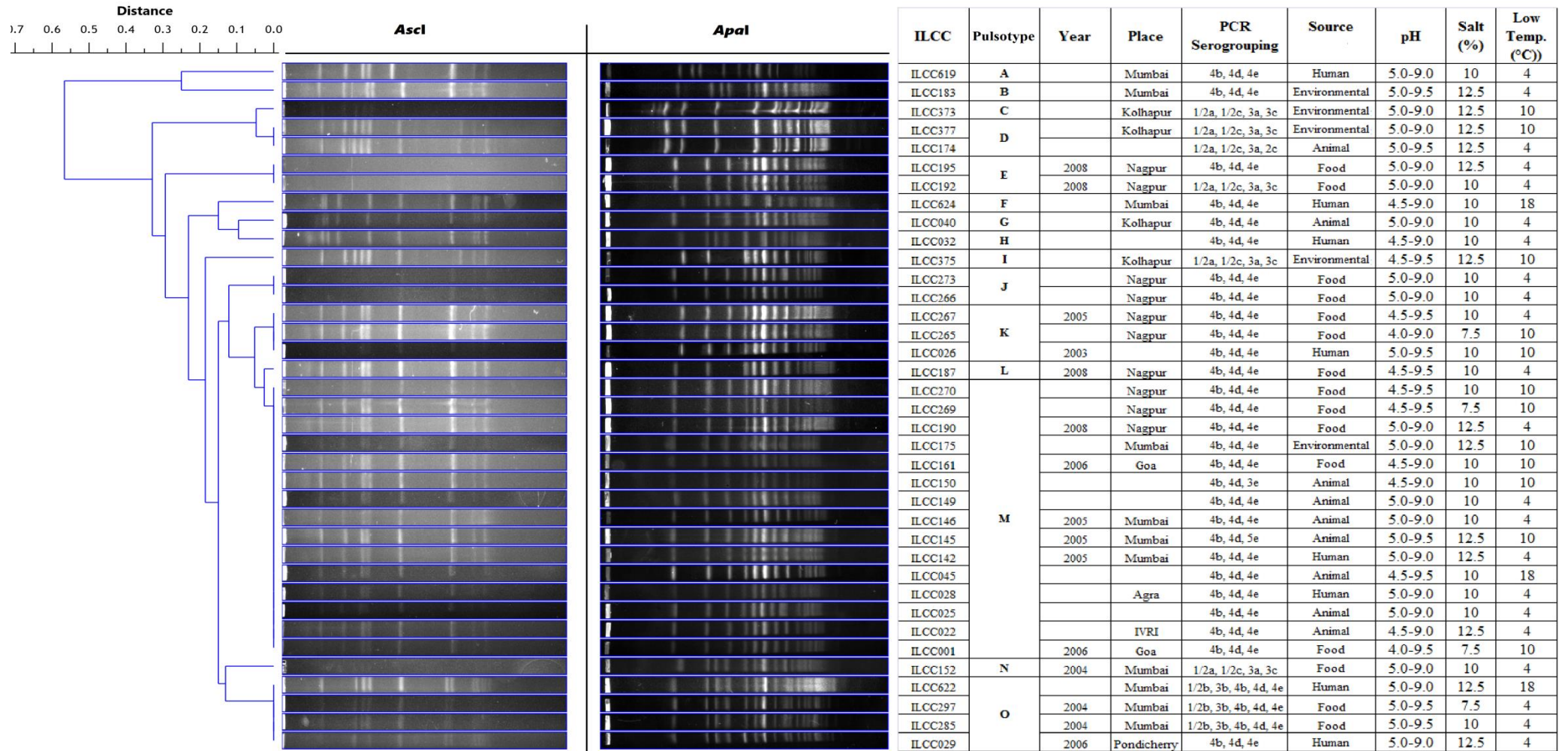| ILCC	Pulsotype	Year	Place	PCR Serogrouping	Source	pH	Salt (%)	Low Temp. (°C))
ILCC619	A		Mumbai	4b, 4d, 4e	Human	5.0-9.0	10	4
ILCC183	B		Mumbai	4b, 4d, 4e	Environmental	5.0-9.5	12.5	4
ILCC373	C		Kolhapur	1/2a, 1/2c, 3a, 3c	Environmental	5.0-9.0	12.5	10
ILCC377	D		Kolhapur	1/2a, 1/2c, 3a, 3c	Environmental	5.0-9.0	12.5	10
ILCC174				1/2a, 1/2c, 3a, 2c	Animal	5.0-9.5	12.5	4
ILCC195	E	2008	Nagpur	4b, 4d, 4e	Food	5.0-9.0	12.5	4
ILCC192		2008	Nagpur	1/2a, 1/2c, 3a, 3c	Food	5.0-9.0	10	4
ILCC624	F		Mumbai	4b, 4d, 4e	Human	4.5-9.0	10	18
ILCC040	G		Kolhapur	4b, 4d, 4e	Animal	5.0-9.0	10	4
ILCC032	H			4b, 4d, 4e	Human	4.5-9.0	10	4
ILCC375	I		Kolhapur	1/2a, 1/2c, 3a, 3c	Environmental	4.5-9.5	12.5	10
ILCC273	J		Nagpur	4b, 4d, 4e	Food	5.0-9.0	10	4
ILCC266			Nagpur	4b, 4d, 4e	Food	5.0-9.0	10	4
ILCC267		2005	Nagpur	4b, 4d, 4e	Food	4.5-9.5	10	4
ILCC265	K		Nagpur	4b, 4d, 4e	Food	4.0-9.0	7.5	10
ILCC026		2003		4b, 4d, 4e	Human	5.0-9.5	10	10
ILCC187	L	2008	Nagpur	4b, 4d, 4e	Food	4.5-9.5	10	4
ILCC270			Nagpur	4b, 4d, 4e	Food	4.5-9.5	10	10
ILCC269			Nagpur	4b, 4d, 4e	Food	4.5-9.5	7.5	10
ILCC190		2008	Nagpur	4b, 4d, 4e	Food	5.0-9.0	12.5	4
ILCC175			Mumbai	4b, 4d, 4e	Environmental	5.0-9.0	12.5	10
ILCC161		2006	Goa	4b, 4d, 4e	Food	4.5-9.0	10	10
ILCC150				4b, 4d, 3e	Animal	4.5-9.0	10	10
ILCC149				4b, 4d, 4e	Animal	5.0-9.0	10	4
ILCC146	M	2005	Mumbai	4b, 4d, 4e	Animal	5.0-9.0	10	4
ILCC145		2005	Mumbai	4b, 4d, 5e	Animal	5.0-9.5	12.5	10
ILCC142		2005	Mumbai	4b, 4d, 4e	Human	5.0-9.0	12.5	4
ILCC045				4b, 4d, 4e	Animal	4.5-9.5	10	18
ILCC028			Agra	4b, 4d, 4e	Human	5.0-9.0	10	4
ILCC025				4b, 4d, 4e	Animal	5.0-9.0	10	4
ILCC022			IVRI	4b, 4d, 4e	Animal	4.5-9.0	12.5	4
ILCC001		2006	Goa	4b, 4d, 4e	Food	4.0-9.5	7.5	10
ILCC152	N	2004	Mumbai	1/2a, 1/2c, 3a, 3c	Food	5.0-9.0	10	4
ILCC622			Mumbai	1/2b, 3b, 4b, 4d, 4e	Human	5.0-9.0	12.5	18
ILCC297	O	2004	Mumbai	1/2b, 3b, 4b, 4d, 4e	Food	5.0-9.5	7.5	4
ILCC285		2004	Mumbai	1/2b, 3b, 4b, 4d, 4e	Food	5.0-9.5	10	4
ILCC029		2006	Pondicherry	4b, 4d, 4e	Human	5.0-9.0	12.5	4

Fig. 4.1: Dendrogram (UPGMA) showing PFGE patterns of 37 stress tolerant *Listeria monocytogenes* strains restricted by *AscI* and *ApaI* enzymes with details of the source of isolation, serotype and stress tolerance patterns

4.4.2 Transcriptomic analysis

To understand the genomic context of phenotypic observations in various studied stresses, we used RNA-Seq approach to comprehensively examine the expression of genes in presence and in absence of different stresses.

4.4.2.1 Transcriptional response under acidic/low pH stress

In presence of low pH stress, ~55% of the genome of *L. monocytogenes* ILCC187 was mobilized. In brief, 668 (20.75%) and 1109 (34.45%) genes were found to be up- and down- regulated while, 1442 (44.79%) genes remained unaffected. Nevertheless, 12% and 7% genes were differentially up- and down-regulated (Table: 4.1).

Among the significantly differential regulation we observed that particularly, genes that are involved in metabolic pathways, such as pentose phosphate and glutathione metabolism were up regulated (Fig4.2a and 4.2b). The pentose phosphate pathway is a process of glucose turnover that produces NADPH as reducing equivalents and pentoses as essential parts of nucleotides. Glutathione known to act as the most important intracellular redox buffer, that plays a crucial role in osmoadaptation of bacteria which in turn maintains the pH homeostasis of cells (Schafer and Buettner, 2001, Smirnova and Oktyabrsky, 2005). Differential up regulation of 6 phosphogluconate dehydrogenase (LMOf2365_1395) was indicative of both the pathway (Table 4.1). Then *glnA* gene showed significant up regulation coding for glutamine synthetase, type I; which is involved in different pathways of GABA biosynthesis and metabolism as well as in two component system. GABA-gamma-Aminobutyrate metabolism is known to be integral part of acid stress stress and response to multiple other stresses in many bacteria (Feehily and Karatzas,

2012). Two components system, which is well characterized signal transduction system, enable bacteria to sense, respond, and adapt to changes in their environment including acid stress (Cotter *et al*., 2003; Sleator and Hill, 2005; Gao and Stock, 2009; Pöntinen *et al*., 2017). Similarly, differential up-regulation of *cobC* (LMOf2365_1156) was indicative of active involvement of cobalamin biosynthetic pathway. Although the exact reason for up-regulation of cobalamin biosynthesis is unknown, it is well documented in many bacteria, including *L. monocytogenes* (Buchrieser *et al*., 2003) that biosynthesis of cobalamine or intermediates of cobalamine biosynthesis pathways acts as cofactors for various different biological processes, including source of carbon and energy, and likewise in the synthesis of acetyl-CoA (Roth *et al*., 1996). The acetyl-CoA synthesis pathway may also serve further to fatty acid biosynthesis pathway; this suggests the contribution of genes to the maintenance of membrane under severe environmental stress (Casey *et al*., 2014). The observation of acetyl-CoA is supported by another observation of up-regulation of gene LMOf2365_0293 (Table 4.1) coding for acetyltransferase, the Gcn5-related N-acetyltransferases (GNAT) family. The acetyltransferase, GNAT family known to play critical role in peptidoglycan synthesis and structural modifications towards escaping from deleterious conditions (Aubry *et al*., 2011; Burall *et al*., 2015; Favrot *et al*., 2016). Beside metabolic pathways, differential up-regulation of genes encoding for phosphotransferase system (PTS), ATP transport systems and DNA repair systems were indicative of altered carbohydrate-metabolism of cells at stressed conditions.

The ability of *L. monocytogenes* to colonize and grow in diverse ecological niches is correlated with the presence of 331 (11.6%) genes encoding for different transport proteins. Interestingly, out of 331 genes, 88 (26%) transporter genes

specifically code for carbohydrate transporters, mediated by phosphoenolpyruvate-dependent phosphotransferase systems (PTSs) (Glaser *et al*., 2001). The components of the PTS-system LMOf2365_0024, LMOf2365_0025, LMOf2365_1743, LMOf2365_1744, and LMOf2365_2020 (Table 4.1) were significantly up-regulated. These proteins are specifically involved in the uptake of carbohydrates which are further involved in energy producing metabolic pathways (Barabote and Saier, 2005; Stoll and Goebel, 2010; Casey *et al*., 2014). The gene LMOf2365_0416 encoding ABC transporter ATP binding protein; also the genes coding for proteins involved in the membrane-associated zinc metalloprotease (LMOf2365_1335), oligopeptide ABC transporter permease (LMOf2365_2228) and efflux pump (LMOf2365_2548) (Table 4.1) that are involved in the exporting H^+ ions to maintain the cytoplasmic homeostasis observed to be significantly up-regulated. The involvement of ABC transporters in acid stress response of *Acetobacter aceti* and *L. monocytogenes* has been reported earlier (Nakano *et al*., 2006; Stasiewicz *et al.*, 2011). The gene LMOf2365_1188 (Table 4.1) encoding ethanolamine utilization protein showed significant up-regulation. This might helping *L. monocytogene* to survive through host gastrointestinal tract as observed in *Clostridium difficile* (Sebaihia *et al*., 2006). Interestingly, the gene *accC* was up-regulated by 8 folds, encoding subunit of acetyl Co-A carboxylase enzyme; this enzyme is known to be the major rate-limiting enzyme in fatty acid biosynthesis (Brownsey and Denton, 1987; Polyak *et al*., 2012). As observed earlier by Mastronicolis *et al.* (2010), such alterations in fatty acid composition help *L. monocytogenes* to decrease the membrane fluidity under acid stress. These observations of differential regulation in the genes related to fatty acids also support the earlier observations of FAME analysis in this study. Single stranded-DNA-binding protein, *ssb2* has up-regulated significantly (Table 4.1);

which is opined to be protecting and repairing of chromosomal DNA in cellular stresses (Weber *et al.*, 2007). As observed earlier by Durack *et al*. (2013), cell division proteins significant accumulation under low temperature and osmotic stress, similarly there was up-regulation in *ftsA* (4.6 fold) and *divIVA* (59.5 folds) was observed under acidic stress. Besides this, genes encoding proteins for ribosomal subunits (*rplM, rplU*), DNA repair (*recJ*), cell wall surface anchor family protein (*LMOf2365_2052*), uridylate kinase (*pyrH*), segregation and condensation protein A (*scpA*) and many hypothetical proteins showed significant up-regulation under low pH stress test strain. Interestingly, there was up-regulation in antisense: LMOf2365_2548 (Table 4.1)*; which is antisense RNA of cation efflux family protein. There was up-regulation in antisense: LMOf2365_2495, again antisense RNA for LysM family protein which is motif of peptidoglycan known for influencing peptidoglycan structural stability (Krawczyk-Balska *et al*., 2014). As antisense RNAs known for transcription termination, codegradation, control of translation, and transcriptional interference (Georg and Hess, 2011); these observations suggested their significant accumulation in cells was towards reducing cation efflux for maintaining pH homeostasis and to inhibit influence on cell wall structure, respectively under this low pH stress.

A significant down-regulation in the genes LMOf2365_0148 and LMOf2365_2139 encoding serine/threonine phosphatase was observed (Table 4.1). Serine/threonine phosphatase coupled with serine/threonine kinase is involved in peptidoglycan modulation in Gram positive bacteria (Débarbouillé *et al*., 2009; Dworkin, 2015). Deletion mutant study of serine/threonine kinase showed increased resistance to Triton X-100 in *Staphylococcus aureus* (Débarbouillé *et al*., 2009). Hence, this down-regulation could be helping bacteria for inhibiting modulations in

cell wall which further helping towards low pH stress tolerance. The small nucleotide cyclic di-3′,5′-adenosine monophosphate (c-di-AMP) is essential molecule in ubiquitous signaling, with essential roles in bacterial physiology, growth, host–pathogen interactions and virulence. HD domain protein acts in cooperative hydrolysis of c-di-AMP towards reducing elevated c-di-AMP levels (Huynh *et al*., 2015). Interestingly down-regulation of gene LMOf2365_0504 (Table 4.1) encoding for HD domain proteins underlined that increased concentration of c-di-AMP might be helping for growth in such stressed conditions. Glyoxalase are the enzymes that catalyses the conversion of toxic metabolites to corresponding nontoxic acids, leading to acidification of cytoplasm (Suttisansanee *et al*., 2011). Since the pH of cytoplasm is already lowered with accumulation of H^+ ions across the membrane towards achieving pH homeostasis; the excessive acidification of cytoplasm could be avoided by down-regulation of gene LMOf2365_1726 (Table 4.1). Van Boeijen *et al.* (2010) and Liu *et al*. (2014) observed down-regulation of genes associated with flagellar assembly in *L. monocytogenes* under high hydrostatic pressure (HHP) and to a sublethal dose of Carnocyclin A, respectively. Similarly here, under low pH stress *fliC* gene was observed to be down-regulated (Table 4.1). Although exact mechanism of inhibition of flagella synthesis is unknown; it might be the channeling energy to other cellular processes that aid the survival of the organism in low pH stress, as large amount of energy is required in flagellar assembly synthesis. Surprisingly, we observed considerable down-regulation of gene *thiM* (Table 4.1) involved in thiamine metabolism. Under low pH stress tryptophan operon was affected very significantly with down-regulation of genes of tryptophan operon namely *trpA*, *trpB* (Table 4.1). Besides this, genes encoding for transcriptional regulators such as AraC family transcriptional regulator

(LMOf2365_1198) and TetR family transcriptional regulator (LMOf2365_2437) showed significant down-regulation. Some uncharacterized proteins of and ABC-2 type transporter system permease protein (LMOf2365_2247) were down-regulated (Table 4.1). Besides this, genes encoding threonine aldolase (LMOf2365_0330), Zn-dependant alcohol dehydrogenase (LMOf2365_2827), SsrA binding protein (*smpB*) and M20/M25/M40 family peptidase (LMOf2365_2810) showed significant down-shift (Table 4.1) under this stress. An interesting observation made and it was, the 15/67 tRNA did not express. Some of these tRNAs have been shown to associated with genomic variation forming genomic hot-spot of *L. monocytogenes* (Kuenne *et al*., 2013). Moreover, some antisense RNAs were also observed to be regulated negatively indicating suppression of metabolic pathways.

Thus, at low pH stress, ABC transporter, ATP dependat PTS systems, carbohydrate utilization and energy producing pathways, structural component synthesis and/or modulating pathways and DNA repair systems were up-regulated. While amino acids and flagella component synthesis pathways, some regulatory proteins and tRNAs were down-regulated to tackle the stress (Fig. 4.2a and 4.2b). Nevertheless, involvement of several accessory pathways and systems needs to be further investigated to determine their actual role in the low pH stress.

Table 4.1: Fold change (up-regulation/down-regulation) of differentially regulated genes under acidic/low pH stress in *L. monocytogenes* ILCC187.

Name	Synonym	Product	Fold Change
-	LMOf2365_1226	peptidase	4.02
rplM	LMOf2365_2570	50S ribosomal protein L13	4.15
gnd	LMOf2365_1395	6-phosphogluconate dehydrogenase	4.17
ftsA	LMOf2365_2065	cell division protein FtsA	4.67
-	LMOf2365_1418	phosphodiesterase	5.20
-	LMOf2365_2087	hypothetical protein	5.71
pyrH	LMOf2365_1330	uridylate kinase	6.10
-	LMOf2365_2548	cation efflux family protein	6.15
glnA	LMOf2365_1317	glutamine synthetase, type I	6.41
-	LMOf2365_2228	oligopeptide ABC transporter permease	6.65
recJ	LMOf2365_1544	single-stranded-DNA-specific exonuclease RecJ	6.75
accC	LMOf2365_1374	acetyl-CoA carboxylase biotin carboxylase subunit	8
greA	LMOf2365_1515	transcription elongation factor GreA	8.27
rplU	LMOf2365_1561	50S ribosomal protein L21	8.59
-	LMOf2365_0697	cof family hydrolase	9
rpsS	LMOf2365_2601	30S ribosomal protein S19	9.13
-	predicted RNA	antisense: LMOf2365_2495	9.68
-	LMOf2365_1095	glycosyl transferase family protein	10.33
-	LMOf2365_0621	hypothetical protein	15.18
-	LMOf2365_1532	carbon-sulfur lyase	17
-	predicted RNA	antisense: LMOf2365_2548	17.51
-	LMOf2365_0024	PTS system fructose-specific transporter subunit IIA	31
-	LMOf2365_0112	hypothetical protein	31
-	LMOf2365_0933	hypothetical protein	31
scpA	LMOf2365_1981	segregation and condensation protein A	33
-	LMOf2365_1188	ethanolamine utilization protein	38
-	LMOf2365_2061	hypothetical protein	39
-	LMOf2365_0025	PTS system mannose/fructose/sorbose family transporter subunit IIB	40
-	LMOf2365_1638	phosphotransferase enzyme family protein	40
-	LMOf2365_0416	ABC transporter ATP-binding protein	41
-	LMOf2365_0514	hypothetical protein	41
cobC	LMOf2365_1156	alpha-ribazole-5'-phosphate phosphatase	41
rbfA	LMOf2365_1344	ribosome-binding factor A	41
-	LMOf2365_1744	PTS system beta-glucoside-specific transporter subunit IIB	41
-	LMOf2365_0609	phospholipase/carboxylesterase	43
-	LMOf2365_2020	PTS system mannose/fructose/sorbose family transporter subunit IIA	46
-	LMOf2365_1664	hypothetical protein	48
-	LMOf2365_1879	carboxypeptidase	50
-	LMOf2365_2052	cell wall surface anchor family protein	51
-	LMOf2365_1508	hypothetical protein	52
-	LMOf2365_1743	PTS system beta-glucoside-specific transporter subunit IIA	52
-	LMOf2365_1335	membrane-associated zinc metalloprotease	55
-	LMOf2365_0608	hypothetical protein	56
divIVA	LMOf2365_2045	cell division protein DivIVA	59.5
-	LMOf2365_1300	hypothetical protein	60

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-	LMOf2365_1077	hypothetical protein	63
-	LMOf2365_0657	hypothetical protein	66
-	LMOf2365_0293	acetyltransferase	71
ssb-2	LMOf2365_2496	single-strand binding protein	72
-	LMOf2365_1581	hypothetical protein	87
-	LMOf2365_1475	hypothetical protein	133
-	LMOf2365_1198	AraC family transcriptional regulator	-450.14
-	LMOf2365_0740	hypothetical protein	-124
-	predicted RNA	antisense: fruB	-121.14
-	LMOf2365_1726	glyoxalase	-113.75
thiM	LMOf2365_0334	hydroxyethylthiazole kinase	-111
-	LMOf2365_0220	hypothetical protein	-97
-	LMOf2365_2437	TetR family transcriptional regulator	-90
-	LMOf2365_tRNA-Leu-2	Leu tRNA	-88
trpA	LMOf2365_1649	tryptophan synthase subunit alpha	-86
-	predicted RNA	antisense: LMOf2365_2307	-78.16
-	LMOf2365_tRNA-Ser-4	Ser tRNA	-78
-	LMOf2365_2791	hypothetical protein	-74
-	LMOf2365_tRNA-Glu-2	Glu tRNA	-72
-	predicted RNA	antisense: LMOf2365_2305 fruB	-65.43
-	LMOf2365_2827	zinc-dependent alcohol dehydrogenase	-60
-	LMOf2365_0330	threonine aldolase	-56
-	LMOf2365_tRNA-Leu-5	Leu tRNA	-56
-	predicted RNA	antisense: LMOf2365_0726 cheY cheA	-54.98
-	LMOf2365_2689	hypothetical protein	-52
trpB	LMOf2365_1650	tryptophan synthase subunit beta	-51
-	LMOf2365_2510	UDP-N-acetylglucosamine 2-epimerase	-51
-	LMOf2365_2840	sugar transporter	-50
-	LMOf2365_2810	M20/M25/M40 family peptidase	-47
-	LMOf2365_tRNA-Leu-6	Leu tRNA	-47
-	LMOf2365_tRNA-Lys-1	Lys tRNA	-47
-	LMOf2365_0046	amino acid permease	-46
-	LMOf2365_2139	Ser/Thr protein phosphatase	-45.34
-	predicted RNA	-	-44.61
-	LMOf2365_2539	hypothetical protein	-44
-	LMOf2365_tRNA-Arg-1	Arg tRNA	-44
-	LMOf2365_tRNA-Asp-3	Asp tRNA	-44
-	LMOf2365_tRNA-Glu-1	Glu tRNA	-44
-	LMOf2365_tRNA-Lys-4	Lys tRNA	-44
-	LMOf2365_tRNA-Met-4	Met tRNA	-44
-	LMOf2365_tRNA-Pro-1	Pro tRNA	-44
-	LMOf2365_tRNA-Ser-3	Ser tRNA	-44
-	LMOf2365_tRNA-Tyr-2	Tyr tRNA	-44
-	LMOf2365_0313	hypothetical protein	-42
-	LMOf2365_0412	hypothetical protein	-42
-	LMOf2365_2028	hypothetical protein	-39
-	LMOf2365_tRNA-Ile-1	Ile tRNA	-38
-	LMOf2365_1427	PadR family transcriptional regulator	-37.35
-	LMOf2365_0064	hypothetical protein	-36
-	LMOf2365_2427	hypothetical protein	-36

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-	LMOf2365_0504	HD domain-containing protein	-30.08
-	LMOf2365_1139	hypothetical protein	-28.39
-	LMOf2365_0148	Ser/Thr protein phosphatase	-26.97
-	predicted RNA	antisense: LMOf2365_2313	-24.62
-	LMOf2365_2247	ABC transporter permease	-23.51
-	LMOf2365_0726	flagellin	-19.91
smpB	LMOf2365_2421	SsrA-binding protein	-19.44
-	LMOf2365_1122	hypothetical protein	-17.43
-	LMOf2365_0688	hypothetical protein	-16.98
-	LMOf2365_1267	hypothetical protein	-15.79
-	predicted RNA	antisense: LMOf2365_tmRNA1	-1.06
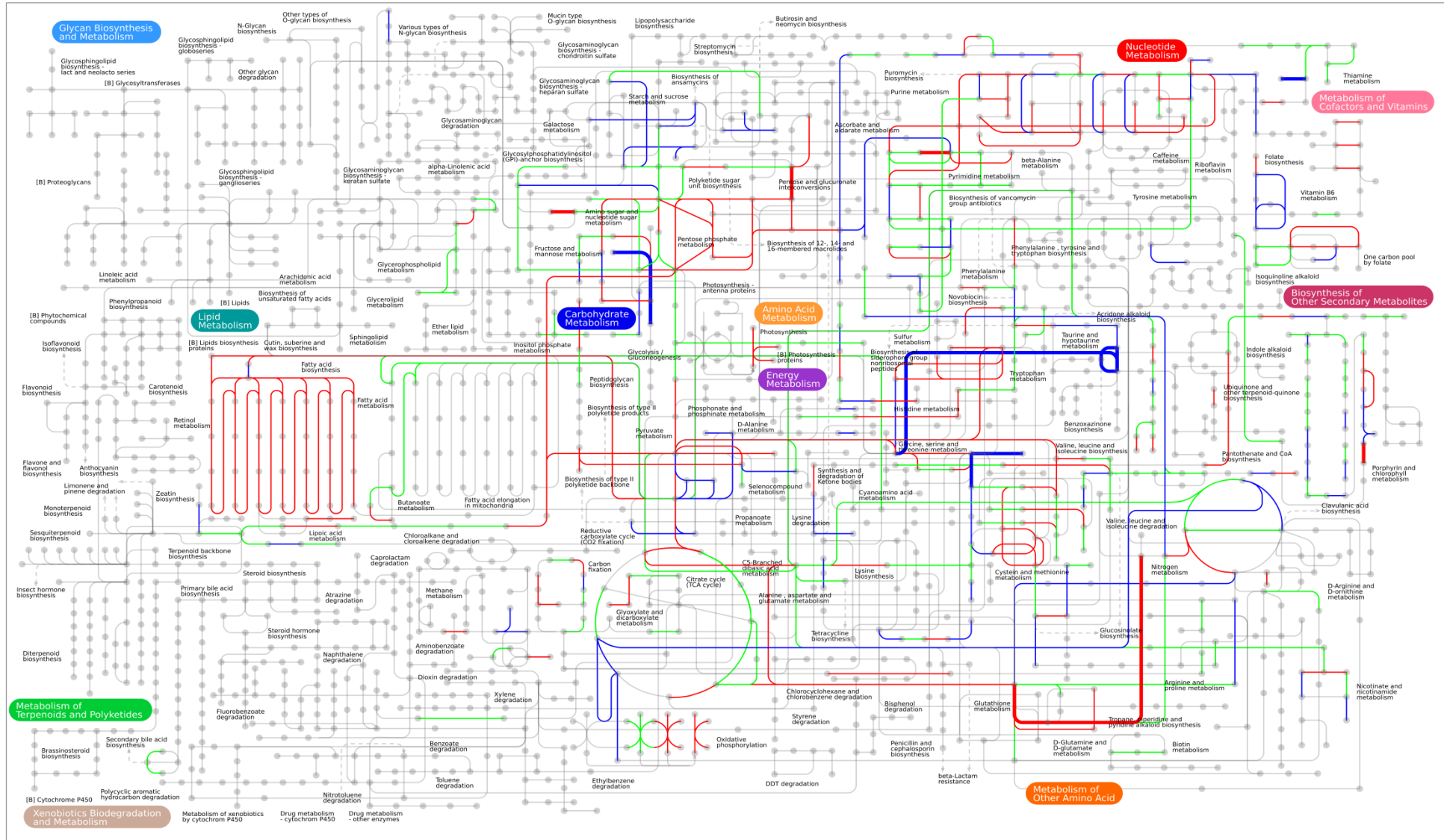
Fig. 4.2a : Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under low pH stress. (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)

Fig. 4.2b: Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under low pH stress (Zoomed view). (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)
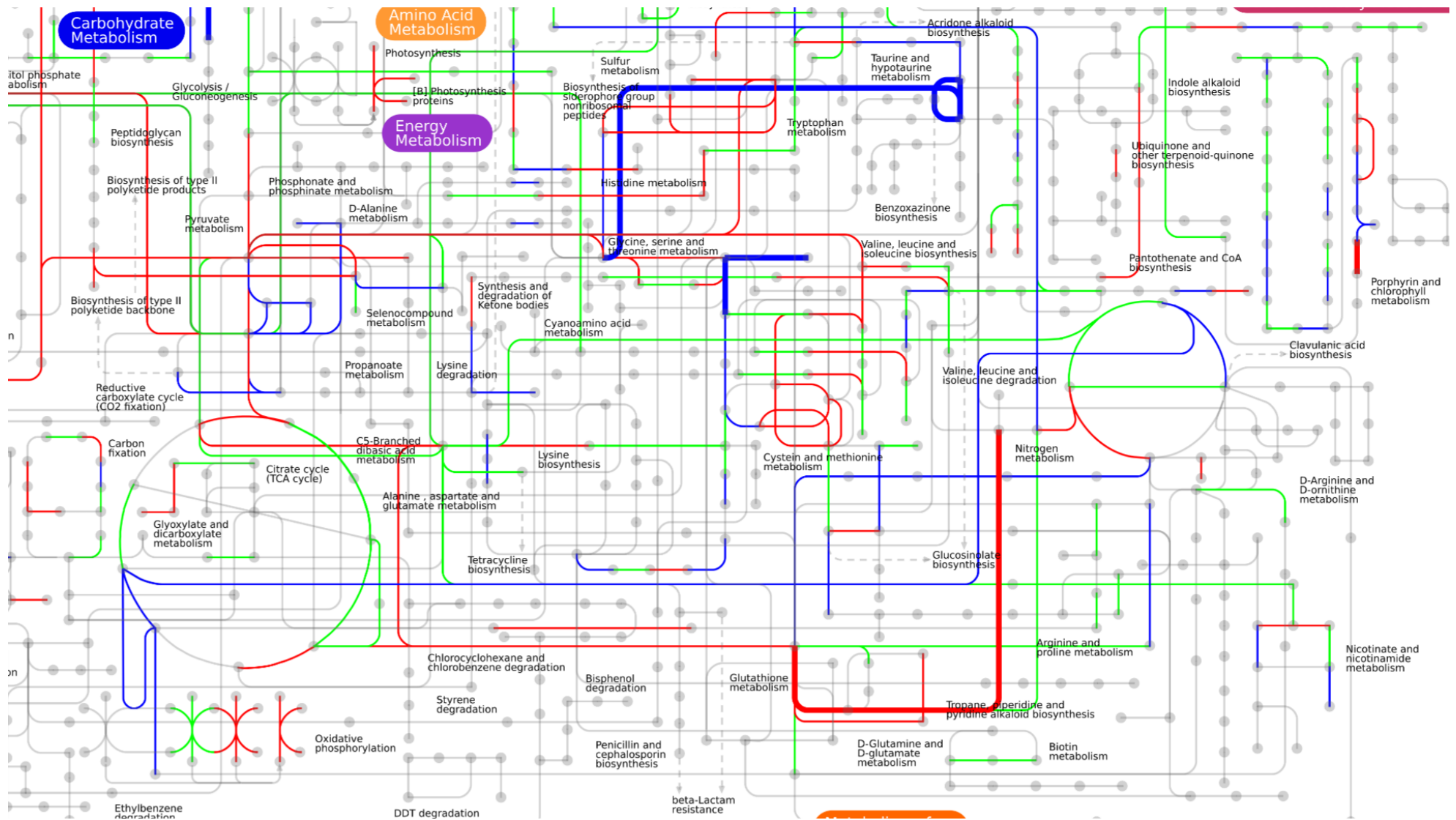
4.4.2.2 Transcriptional response under alkaline/high pH stress

The alkaline pH stress resistance of *L. monocytogenes* is of matter of concern in the food industries, particularly when mild alkali treatments are used for cleaning of surfaces and/or equipment coming in contact with foods. It also accounts for the listerial persistence in such environments. The ability of *Listeria* to resist high pH stress also makes this organism capable of escaping pH-related human defense mechanisms, e.g. alkaline conditions due to presence of pancreatic secretions and, rise and fall of the vacuolar pH in the phagolysosomes (Gray *et al.,* 2006).

In this study, under alkaline stress, the genome of the strain *L. monocytogenes* was deployed to higher extent with thousands of genes to be up and down regulated. Among the differentially regulated genes with glaring degree there were 60 genes to be differentially over-expressed and 23 genes were differentially down-regulated.

As per earlier observations, there are four broad categories of alkali stress adaptation, (i) increased metabolic acids production which helps in pH homeostasis (ii) ATP synthase activity coupled with H^+ entry to ATP generation is increased under alkaline stress (iii) alteration in the cell surface properties (iv) increased expression and activity of the transporters (Padan *et al*., 2005; Giotis *et al*., 2010). As primary strategy of bacteria towards maintaining pH homeostasis is increased production of acids; here highly differential up-regulation of glutamate synthase (*gltB*) could have been alike strategy of *L. monocytogenes* to resist alkaline stress. Similar phenomenon has been observed in other bacteria under same alkaline stressed conditions (Wilks *et al*., 2009). In accordance, we observed the induction of genes related to carbohydrate and energy metabolism which could be helping *L. monocytogenes* with increased ATP generation to cope-up with alkaline stress (Fig.

4.3a and 4.3b). The genes related to glycolysis (*bglA*) and energy metabolism (LMOf2365_2417, LMOf2365_1896, LMOf2365_1901, LMOf2365_0270) were observed to be highly up-regulated (Table 4.2). There was a significant up-regulation in the expression of genes (*talA, talB*) encoding enzymes involved in pentose phosphate pathway (Table 4.2). The alteration in cell surface properties is another well-known mechanism in *L. monocytogenes* under alkaline stress. Interestingly, in this study differential up-regulation was observed in the expression of genes involved in pathways related to cell wall, such as fatty acids biosynthesis, peptidoglycan biosynthesis (LMOf2365_1093) and techoic acid synthesis (LMOf2365_1097). The genes encoding for lipoprotein (LMOf2365_0495) as well as wall associated protein (LMOf2365_0450) showed differential over-expression under alkaline stress. Surprisingly, large number of cell wall surface anchor family proteins (LMOf2365_0349, LMOf2365_0350, LMOf2365_0656, LMOf2365_0693, LMOf2365_0694, LMOf2365_0768, LMOf2365_0805, LMOf2365_0869, LMOf2365_1254, LMOf2365_1974, LMOf2365_2210, LMOf2365_2211, LMOf2365_2212, LMOf2365_2370, LMOf2365_2638) were expressed with differential up-regulation (Table 4.2). The observations differential regulation of genes related to fatty acids supports earlier data of FAME analysis in this study. Under alkali stress there was induced differential regulation of many ORFs coding for ABC transporters. These ABC transporters are known to play very important role in diverse physiological processes of cells (Geourjon *et al*., 2001), which includes the uptake of oligopeptides and other solutes during exposure and growth under alkaline pH stress (Padan *et al*., 2005). Earlier reports in many bacteria have shown the import and break down of peptides into free amino acids as significant sources of protons (Padan *et al*., 2005). This phenomenon suggests a possible basis of the

increased uptake of peptides under alkaline stressed conditions. This process not only acts as source of amino acids, it might be helping with structural adaptation by incorporating stress resistant polymers in cell wall of bacteria (Wood *et al*., 2001; Takami *et al*., 2002). In this study, there was very significant up-regulation observed in ABC transporter ATP-binding proteins (LMOf2365_0279, LMOf2365_0280) and ABC transporter permease (LMOf2365_1233, LMOf2365_1525) (Table 4.2). An up-regulation of gene encoding thiamine-pyrophosphate-requiring enzyme (LMOf2365_0398) was observed, which is opined to be involved in microbial metabolism in diverse environments. Also up-regulation of S1 RNA-binding protein domain (LMOf2365_0917) was observed (Table 4.2), possibly helping in modulation of translation via binding mRNA under stress as previously reported for other RNA binding proteins (Michaux *et al*., 2012). The two component system is well known to playing important role in sensing stress and regulation of response accordingly under different stresses of high salt, low pH and low temperature (Sleator and Hill, 2005; Tessema *et al*., 2012; Pöntinen *et al*., 2015). It was interesting to note the significant over-expression in the genes of sensor (*resE*, LMOf2365_2035) and response regulator (LMOf2365_2034) of two component systems in here under alkaline stress. There was notable up-regulation in PRD/PTS system IIA 2 domain-containing protein under alkaline stress. The role of PTS in alkaline stress response is incompletely understood in case of *Listeria* and more work is required to clarify the relation of differential regulation of PTS under alkali stress.

Besides this, there was induced differential regulation were observed in the genes of DNA repair system such as ATP-dependent nuclease subunit A and B (*addA*, *addB*), DNA segregation ATPase FtsK/SpoIIIE, S-DNA-T family

(LMOf2365_0072) and SbcC family exonuclease (LMOf2365_1666). Also dolichyl-phosphate-mannose-protein mannosyltransferase (LMOf2365_0954), type II restriction enzyme *Sau3*AI (LMOf2365_0325), amidase (LMOf2365_0866), and many hypothetical proteins showed high expression under alkaline stress in *L. monocytogenes.*

It was surprising to note less number of genes to be differentially down-regulated under alkaline stress in comparison with transcriptome of *L. monocytogenes* under other stresses.

The pre-protein translocase subunits (*secY* and *yajC*) were observed to be down-regulated very significantly (Table 4.2). These proteins are very well known to play role as membrane transporter for export of peptides (du Plessis *et al.*, 2011; Durack *et al.*, 2015). Since import and break down of peptides into free amino acids is significant sources of protons (Padan *et al.*, 2005), these are thought to be important for better adaptation under alkaline stress; hence the transfer of peptides out of the cell need to be controlled. This could have resulted in repression of the genes *secY* and *yajC* to avoid peptide transfer under alkaline stress. It was interesting to note significant repression in bifunctional acetaldehyde-CoA/alcohol dehydrogenase (*adhE*); which is known to be involved in various pathways including fatty acid degradation. As cell wall need to be intact under various stresses, the inhibition of this enzyme could be helping bacteria under alkaline stress. Similar observation of down-regulation of bifunctional acetaldehyde-CoA/alcohol dehydrogenase have been reported in *L. monocytogenes* in response to carnocyclin A exposure (Liu *et al.*, 2014); where carnocyclin A is bacteriocin believed to act by disruption of the integrity of the membrane of target cells (van Belkum *et al.*, 2011). There was down-regulation of GatB/Yqey domain-containing protein

(LMOf2365_1487) observed under alkaline stress. The exact function of this protein is not yet known and need to be studied further. Interestingly, proteins such as peroxide resistance protein Dpr (LMOf2365_0963) and redox-sensing transcriptional repressor Rex (LMOf2365_2104) repressed under alkaline stress; which are known to be involved in tolerance of various stresses (Tsou *et al*., 2008; Bitoun *et al*., 2011). The exact relation of down-regulation of these proteins under alkaline pH stress in *L. monocytogenes* needs to be explored. As previously reported in *L. monocytogenes* under alkaline stress; repression of different ribosomal proteins (*rpsE, rpsF, rpsM, rpsC*) was observed (Giotis *et al*., 2008) (Table 4.2). Although there is up-regulation of various ABC transporter genes in *L. monocytogenes* under alkaline stress; surprisingly, ABC transporter substrate-binding proteins (LMOf2365_1877, LMOf2365_2229) were down-regulated. Same phenomenon has been reported previously for same genes and even under similar stress in *L. monocytogenes* (Giotis *et al*., 2008). It was also interesting to observe the repression of heat shock protein related; co-chaperonin GroES (*groES*) and TetR family transcriptional regulator (LMOf2365_2120) genes under alkaline stress (Table 4.2). Still there is no knowledge of its relation with alkaline stress tolerance in *L. monocytogenes*, which need to be elucidated.

Besides, down-regulation of many hypothetical proteins was observed under alkaline stress in this study. Unlike low pH stress the repression of some tRNAs were observed under high pH stress (Table 4.2); which could have been shown to associated with genomic variation forming genomic hot-spot of *L. monocytogenes* (Kuenne *et al*., 2013).

Thus there was up-regulation of the genes related to energy production systems (Fig. 4.2b), cell wall component modifications/biosynthesis systems, transporter systems, RNA binding proteins, stress sensor and response regulator systems. Also there was up-regulation of many uncharacterized proteins observed under this stress. However, there was repression of pre-protein translocase subunits, fatty acid degrading proteins, few ABC transporters and many other proteins; which are needed to study further for their role in alkaline stress. Though, role of many other genes related to different pathways and systems needs to be explored further towards advancement of knowledge for their actual role in the alkaline pH stress tolerance.

Table 4.2: Fold change (up-regulation/down-regulation) of differentially regulated genes under alkaline/high pH stress in *L. monocytogenes* ILCC187

Name	Synonym	Product	Fold Change
-	LMOf2365_2417	glycosyl hydrolase	3.48
-	LMOf2365_2211	cell wall surface anchor family protein	3.8
-	LMOf2365_0072	diarrheal toxin/FtsK/SpoIIIE family protein	4.35
-	LMOf2365_0345	hypothetical protein	4.75
-	LMOf2365_0350	cell wall surface anchor family protein	4.84
gltB	LMOf2365_1758	glutamate synthase	5
-	LMOf2365_1233	ABC transporter permease	12.25
-	LMOf2365_0656	cell wall surface anchor family protein	12.43
-	LMOf2365_0103	hypothetical protein	14
-	LMOf2365_1690	hypothetical protein	17
addB	LMOf2365_2301	ATP-dependent nuclease subunit B	22.5
-	LMOf2365_0805	cell wall surface anchor family protein	22.6
-	LMOf2365_0859	cell wall surface anchor family protein	23.5
-	LMOf2365_2212	cell wall surface anchor family protein	29.33
-	LMOf2365_0398	thiamine-pyrophosphate-requiring enzyme	73
-	LMOf2365_0917	S1 RNA-binding domain-containing protein	78
-	LMOf2365_2055	hypothetical protein	78
-	LMOf2365_0495	lipoprotein	81
-	LMOf2365_1825	hypothetical protein	81
-	LMOf2365_2056	hypothetical protein	81
-	LMOf2365_0693	cell wall surface anchor family protein	82
-	LMOf2365_0450	wall-associated protein	83
-	LMOf2365_1093	N-acetylmuramoyl-L-alanine amidase	84
ppdK	LMOf2365_1896	pyruvate phosphate dikinase	84
-	LMOf2365_0768	cell wall surface anchor family protein	85
-	LMOf2365_0349	cell wall surface anchor family protein	86
-	LMOf2365_0494	hypothetical protein	86
-	LMOf2365_1901	phosphoglucomutase/phosphomannomutase	86
-	LMOf2365_1525	ABC transporter permease	87
-	LMOf2365_2034	DNA-binding response regulator	87
-	LMOf2365_0694	cell wall surface anchor family protein	88
-	LMOf2365_1974	cell wall surface anchor family protein	89
-	LMOf2365_0422	PRD/PTS system IIA 2 domain-containing protein	90
malL-2	LMOf2365_0270	oligo-1,6-glucosidase	91
-	LMOf2365_0280	ABC transporter ATP-binding protein/permease	92
-	LMOf2365_0833	hypothetical protein	92
-	LMOf2365_0954	dolichyl-phosphate-mannose-protein mannosyltransferase	92
-	LMOf2365_1097	glycosyl transferase family protein	92
glyS	LMOf2365_1477	glycyl-tRNA synthetase subunit beta	92
resE	LMOf2365_1977	sensor histidine kinase ResE	92
-	LMOf2365_2035	sensor histidine kinase	92

Continued…

| -	LMOf2365_0493	hypothetical protein	93
-	LMOf2365_2210	cell wall surface anchor family protein	93
addA	LMOf2365_2300	ATP-dependent nuclease subunit A	94
-	LMOf2365_2370	cell wall surface anchor family protein	94
bglA	LMOf2365_2761	6-phospho-beta-glucosidase	94
-	LMOf2365_0687	hypothetical protein	96
-	LMOf2365_0279	ABC transporter ATP-binding protein	97
-	LMOf2365_0325	type II restriction enzyme Sau3AI	97
-	LMOf2365_0866	amidase	97
-	LMOf2365_0096	hypothetical protein	98
-	LMOf2365_1666	SbcC family exonuclease	98
-	LMOf2365_1096	hypothetical protein	100
-	LMOf2365_2057	hypothetical protein	100
-	LMOf2365_0605	hypothetical protein	101
-	LMOf2365_2766	bacteriocin immunity protein	101
-	LMOf2365_0118	hypothetical protein	106
-	LMOf2365_0374	internalin	106
-	LMOf2365_2638	cell wall surface anchor family protein	107
-	LMOf2365_2740	hypothetical protein	110
-	LMOf2365_1254	cell wall surface anchor family protein	119
-	LMOf2365_2120	TetR family transcriptional regulator	-0.0569
groES	LMOf2365_2100	co-chaperonin GroES	-0.0363
-	LMOf2365_1877	manganese ABC transporter ATP-binding protein	-0.2718
rpsC	LMOf2365_2599	30S ribosomal protein S3	-0.0612
rpsM	LMOf2365_2581	30S ribosomal protein S13	-0.0370
rpsF	LMOf2365_0053	30S ribosomal protein S6	-0.0432
rpsE	LMOf2365_2588	30S ribosomal protein S5	-0.0503
-	LMOf2365_1711	hypothetical protein	-0.0563
-	LMOf2365_2104	redox-sensing transcriptional repressor Rex	-0.0967
-	LMOf2365_0963	peroxide resistance protein Dpr	-0.0150
-	LMOf2365_1487	GatB/Yqey domain-containing protein	-0.0574
adhE	LMOf2365_1656	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	-0.0152
yajC	LMOf2365_1548	preprotein translocase subunit YajC	-0.0447
-	LMOf2365_1622	hypothetical protein	-0.0723
-	LMOf2365_1324	hypothetical protein	-0.0607
-	LMOf2365_1266	hypothetical protein	-0.0022
-	LMOf2365_1916.5	hypothetical protein	-0.0038
-	LMOf2365_1180	hypothetical protein	-0.0139
-	LMOf2365_0963	peroxide resistance protein Dpr	-0.0150
-	LMOf2365_1179	hypothetical protein	-0.0168
-	LMOf2365_2229	oligopeptide ABC transporter substrate-binding protein	-0.0355
secY	LMOf2365_2585	preprotein translocase subunit SecY	-0.0608
rpsC	LMOf2365_2599	30S ribosomal protein S3	-0.0612
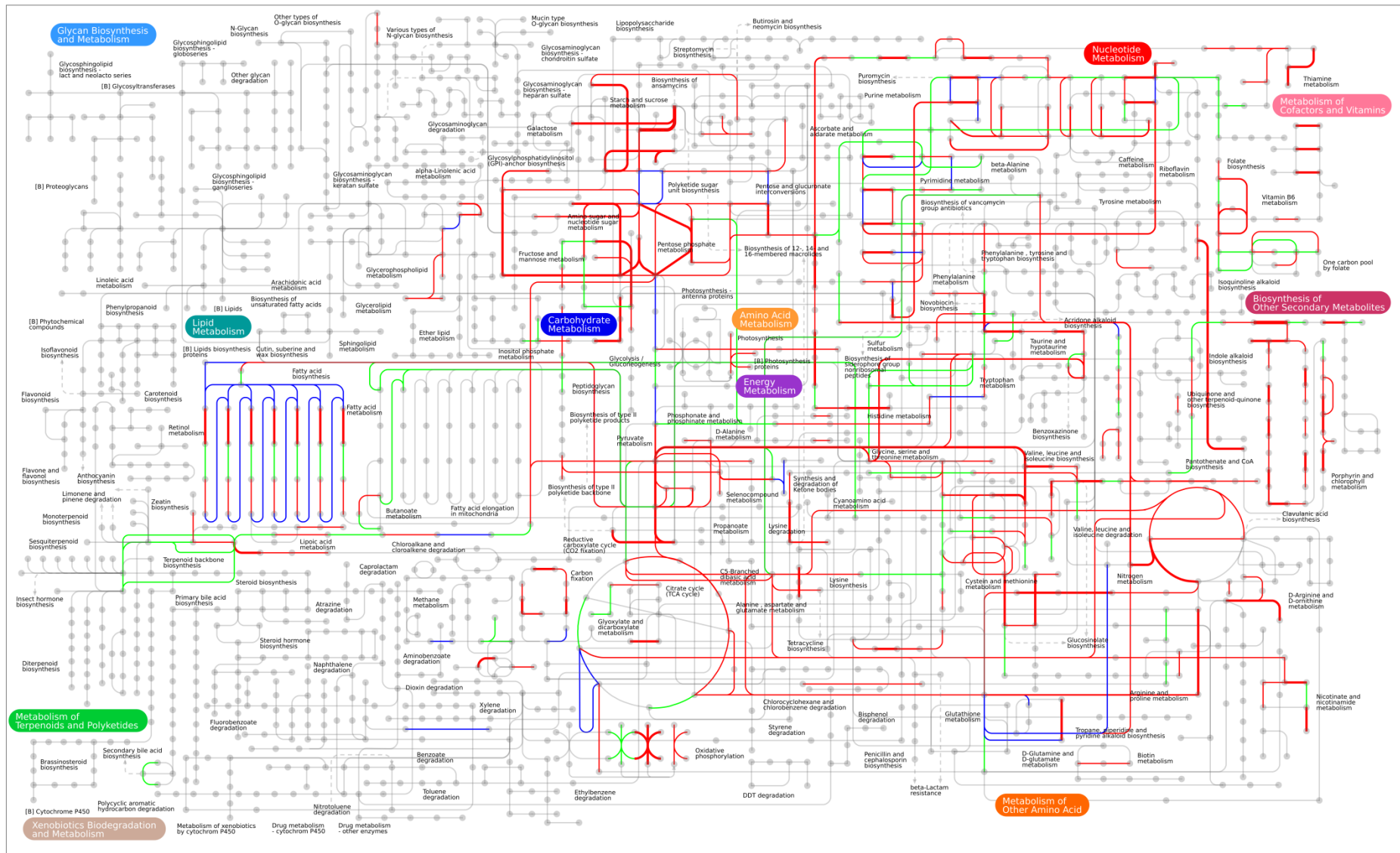
Fig. 4.3a: Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under high pH stress. (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)
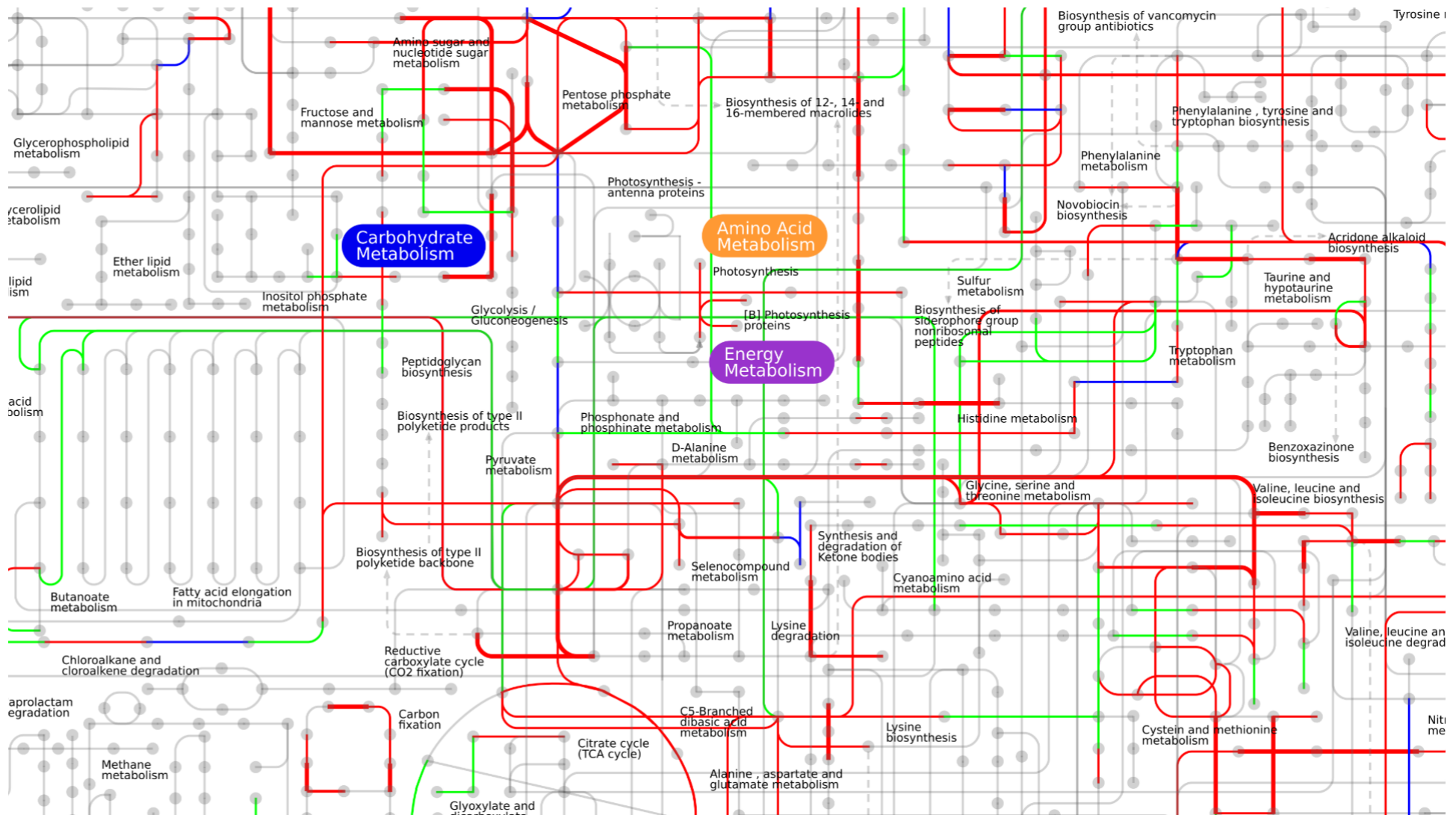
Fig. 4.3b: Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under high pH stress (Zoomed view). (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)

4.4.2.3 Transcriptional response under high salt stress

L. monocytogenes ILCC187 grown under high salt stress transcribed with up-regulation of 1667 (51.78%) genes and down-regulation of 612 (19.01%) genes, keeping 940 (29.20%) genes at normal. Under this stress, 70.79% of genome was deployed with differential up-regulation of 59 genes; while, 51 genes were differentially down-regulated (Table 4.3).

The ABC transporters form the largest group of paralogous genes in bacterial and archaeal genomes (Tatusov *et al*., 1996). It constitutes a large superfamily of multi-subunit permeases that actively involved in transportation of diverse molecules across the biological membranes (Braibant *et al*., 2000). There are reports of solute transport based involvement of ABC transporters in osmotic stress tolerance in different microorganisms (Perroud and Le Rudulier, 1985; Fraser *et al*., 2000; Heide *et al*., 2001; Du *et al*., 2011). In our study, significant up-regulations were observed in genes namely, LMOf2365_0416, LMOf2365_0780, LMOf2365_0877, *cbiQ* (LMOf2365_1215) and LMOf2365_2319 (Table 4.3). Energy pool management appeared to be critically important for osmo-adaptation of *L. monocytogenes* cells (Duche *et al*., 2002). This conclusion is supported by up-regulation of genes such as *atpC* (LMOf2365_2501), LMOf2365_2558, LMOf2365_2321 and different genes of PTS system family proteins (Fig. 4.4a and 4.4b) (Table 4.3). Also a significant overexpression was observed in genes coding for PTS system transporter subunit IIB (LMOf2365_1109) and PTS system transporter subunit IIA (LMOf2365_2785) involved in carbohydrate metabolism pathways (Table 4.3). Interestingly, a factor associated with virulence of *L. monocytogenes* that is invasion associated secreted endopeptidase encoded by the *iap* gene significantly activated by 8 folds in cells grown under hyperosmotic stress. Cold shock family proteins are nucleic acid binding

proteins that presumably have important roles in regulation of various microbial physiological processes (Ermolenko *et al*., 2002). A gene LMOf2365_1908 encoding for cold-shock domain-family protein was observed to be up-regulated (Table 4.3) under high salt stress. It was interesting to note the up-regulation in the *minC* gene under high salt stress; which defended the earlier data of SEM and qPCR analysis of *minC* gene (Chapter 3) and also supported hypothesis of possible involvement of MinC protein in filamentous morphology of *L. monocytogenes* under salt stress. In bacterial pathogens, cell wall surface anchor family proteins are typically involved in promoting bacterial attachment to the host tissues or preventing from phagocytosis and plays important role in epithelial invasion and survival through macrophage (Reddy *et al*., 2016). Here in strain challenged with osmotic stress showed over-expression of gene LMOf2365_0543 coding for cell wall surface anchor family protein. The carboxypeptidase family proteins are involved in variety of processes including the modifications of peptidoglycan in bacteria to maintain elasticity in the cell wall under the stressed environmental conditions (Bergholz *et al*., 2009). Likewise in our study, LMOf2365_1879 gene encoding carboxypeptidase inducted with up-regulation (Table 4.3) in response of high salt stress. The gene LMOf2365_1532 coding for enzyme involved in thiamine metabolism pathway up-regulated significantly; thiamine uptake and biosynthesis of thiamine precursors are reported to be required for pathogen's intracellular replication (Schauer *et al*., 2009). Controlled DNA replication and coordination of chromosome duplication with cell cycle is a crucial step in survival of microorganisms under stressed environmental conditions (Durack *et al*., 2013). A deletion mutant study of *lstC* coding for GNAT acetyltransferase showed led to a reduction in growth of *L. monocytogenes* in 7% NaCl (Burall *et al*., 2015). Similarly in our study, the genes encoding for acetyltransferases (LMOf2365_0152 and

LMOf2365_0293) observed to be up-regulated significantly. The genes (*purN, scpA, rnhB, ssb2,* LMOf2365_1418) encoding for proteins involved in nucleotide biosynthesis pathways, chromosome condensation and segregation, RNA degradation, DNA replication as well as in DNA repair were inducted with very significant up-regulation (Table 4.3). Interestingly, genes encoding for large and small ribosomal subunits (LMOf2365_2600, LMOf2365_2601) also observed to be over-expressed. Apart from this genes encoding phospholipase/carboxylesterase (LMOf2365_0609), MobB operon encoding molybdenum cofactor biosynthesis (LMOf2365_1064), membrane-associated zinc metalloprotease (LMOf2365_1335), cell division protein (*divIVA*), general stress proteins (LMOf2365_2340), NADPH dependant FMN reductase (LMOf2365_2321) and many hypothetical proteins showed significant activation in cells under the hyperosmotic stress (Table 4.3).

Similarly to acidic stress LMOf2365_0504 encoding for HD domain-containing protein observed to be repressed under high salt stress. HD domain protein already known for its role in cooperative hydrolysis of cyclic di-3′,5′-adenosine monophosphate (c-di-AMP) towards reducing its elevated levels (Huynh *et al*., 2015). However, in *L. monocytogenes* c-di-AMP coordinates bacterial growth, cell wall stability, and responses to stress and plays a crucial role in the establishment of bacterial infection and virulence (Witte *et al*., 2013; Zeevi *et al*., 2013). In case of glyoxalase protein suppression was observed under high salt stress; although reason behind downshift of glyoxalase under salt stress was not clearly known; but unlike in low pH stress glyoxalase (LMOf2365_1726) significantly down-regulated under osmotic stress (Table 4.3). Being under stress, more energy is required by *L. monocytogenes* for survival and adaptation (Duche *et al*., 2002). AraC family transcriptional regulator (LMOf2365_1198) also observed to be drastically down-

regulated under high salt stress; which could be part of energy-pool management. AraC family proteins are transcriptional regulators and widespread among bacteria known for regulating genes having diverse functions; including responsible for negative auto-regulation of arabinose operon (Martin and Rosner, 2001; Madar *et al*., 2011). As L-arabinose metabolism is involved in different energy related pathways including pentose phosphate pathway. Surprisingly, PRD/PTS system IIA2 domain-containing protein (LMOf2365_0318*)* which is part of phosphoenolpyruvate-dependent sugar phosphotransferase system also observed to down-regulated under high salt stress (Table 4.3). Enzyme IIA is known to play a central role in the mechanism of carbon catabolic repression; which allows microorganisms to adapt quickly to the rapidly metabolisable carbon and energy source first (Stülke *et al*., 1998; Deutscher *et al*., 2008). The catabolic repression could have been activated to cope up with increased energy requirements resulting down-regulation of PRD/PTS system IIA 2 domain-containing protein. A gene LMOf2365_0757 encoding for fibronectin-binding protein was observed with significantly repressed. The fibronectin-binding protein from *L. monocytogenes* does not share any similarity with previously known bacterial fibronectin-binding proteins and is recognized for its ability to bind human fibronectin; thus may facilitate the entry of bacteria into mammalian cells (Gilot and André, 1999). Any relationship between reduced fibronectin binding protein and high salt stress in *L. monocytogenes* remains to be elucidated. Interestingly, gene encoding for SNF2 family protein involved in DNA remodelling, also observed to be down-regulated. The activities of this enzyme need to study in depth to find out relation of its down-regulation under stress. The cation efflux family proteins are responsible for efflux of positively charged ions from the cell cytoplasm. Increased efflux activity may remove excessive ions required as

cofactors in high energy consumption situation. These situations may have altered cation efflux family protein expression with significant down-regulation of gene LMOf2365_2264 (Table 4.3). Another gene LMOf2365_2162 coding LacI family transcriptional regulator, the repressor protein of sugar metabolism pathways was down-regulated under high salt stress (Table 4.3). This repression of repressor proteins could be due to energy demanding situation under stress. Besides this, the down-regulation of genes (LMOf2365_1761, LMOf2365_0382, LMOf2365_1412) encoding some uncharacterized proteins and many hypothetical proteins were observed under high salt stress. Surprisingly, some genes related to structural component (LMOf2365_1730), metabolic pathways (LMOf2365_2822, LMOf2365_2143) (Table 4.3) were observed to be down-regulated under high salt stress. Similar to low pH, in the presence of high salt concentration 10/67 tRNA and antisense RNA for regulation of fructose utilization and chemotaxis were observed to be suppressed.

Thus, at high salt stress, ABC transporters, ATP dependent PTS systems, carbohydrate utilization and energy producing pathways, structural component synthesis and/or modulating pathways and DNA repair systems were up-regulated. While, carbon catabolic repression, regulator proteins and tRNAs were down-regulated while tackling the stress by *L. monocytogenes*. Nevertheless, involvement of several accessory pathways and systems needs to be further investigated to determine their actual role in high salt stress response.

Table 4.3: Fold change (up-regulation/down-regulation) of differentially regulated genes under high salt stress in *L. monocytogenes* ILCC187.

Name	Synonym	Product	fold change
-	predicted RNA	antisense: rrlE	2.22
minC	LMOf2365_1564	septum formation inhibitor	7.33
-	LMOf2365_1418	phosphodiesterase	7.74
iap	LMOf2365_0611	invasion associated secreted endopeptidase	8.22
-	predicted RNA	antisense: LMOf2365_2495	10.02
rplX	LMOf2365_2594	50S ribosomal protein L24	10.78
-	LMOf2365_2319	amino acid ABC transporter substrate-binding protein	10.98
atpC	LMOf2365_2501	F0F1 ATP synthase subunit epsilon	11.63
-	LMOf2365_2558	formate dehydrogenase subunit alpha	12.11
rplV	LMOf2365_2600	50S ribosomal protein L22	13.80
-	LMOf2365_1908	cold-shock domain-contain protein	14.24
-	LMOf2365_2403	hypothetical protein	19.53
-	LMOf2365_2192	hypothetical protein	24
-	LMOf2365_2340	general stress protein 13	24.17
-	LMOf2365_2190	hypothetical protein	24.67
-	LMOf2365_1916.5	hypothetical protein	25.30
rrfB	LMOf2365_5SB	5S ribosomal RNA	25.31
-	LMOf2365_0543	cell wall surface anchor family protein	26
-	LMOf2365_1532	carbon-sulfur lyase	26
rpsS	LMOf2365_2601	30S ribosomal protein S19	26.2
-	LMOf2365_0416	ABC transporter ATP-binding protein	28
-	LMOf2365_1879	carboxypeptidase	28
-	LMOf2365_0785	hypothetical protein	30
-	LMOf2365_1461	AzlC family protein	30
scpA	LMOf2365_1981	segregation and condensation protein A	31
rrfD	LMOf2365_5SD	5S ribosomal RNA	31.27
-	LMOf2365_0650	hypothetical protein	32
-	LMOf2365_0780	ABC transporter ATP-binding protein	32
cbiQ	LMOf2365_1215	cobalt transport protein CbiQ	34
rrfF	LMOf2365_5SF	5S ribosomal RNA	35.35
rrfE	LMOf2365_5SE	5S ribosomal RNA	40.88
-	LMOf2365_0877	sugar ABC transporter permease	42
lmaC	LMOf2365_0134	antigen C	43
-	LMOf2365_1638	phosphotransferase enzyme family protein	44
purN	LMOf2365_1791	phosphoribosylglycinamide formyltransferase	47
tyrA	LMOf2365_1953	prephenate dehydrogenase	47
-	LMOf2365_2785	PTS system transporter subunit IIA	47
rnhB	LMOf2365_1291	ribonuclease HII	49
-	LMOf2365_0620	hypothetical protein	51
ssb-2	LMOf2365_2496	single-strand binding protein	53
rrfC	LMOf2365_5SC	5S ribosomal RNA	55.78
rrfA	LMOf2365_5SA	5S ribosomal RNA	57.90
-	LMOf2365_1109	PTS system transporter subunit IIB	62
-	LMOf2365_1335	membrane-associated zinc metalloprotease	65
-	LMOf2365_0369	hypothetical protein	66

Continued…

-	LMOf2365_0293	acetyltransferase	70
divIVA	LMOf2365_2045	cell division protein DivIVA	74.5
-	LMOf2365_0152	acetyltransferase	77
mobB	LMOf2365_1064	molybdopterin-guanine dinucleotide biosynthesis protein MobB	79
-	LMOf2365_0609	phospholipase/carboxylesterase	80
nrdR	LMOf2365_1584	NrdR family transcriptional regulator	82
-	LMOf2365_2163	hypothetical protein	82
-	LMOf2365_1885	hypothetical protein	112
-	LMOf2365_0711	hypothetical protein	121
-	LMOf2365_0933	hypothetical protein	128
-	LMOf2365_1508	hypothetical protein	155
-	LMOf2365_0608	hypothetical protein	159
moaD	LMOf2365_1066	molybdenum cofactor biosynthesis protein D	177
-	LMOf2365_2321	FMN reductase, NADPH-dependent	279
-	LMOf2365_1198	AraC family transcriptional regulator	-3151
-	LMOf2365_1122	hypothetical protein	-1429
-	LMOf2365_0688	hypothetical protein	-747
-	LMOf2365_2182	hypothetical protein	-701
-	LMOf2365_0090	hypothetical protein	-546
-	LMOf2365_0217	hypothetical protein	-486
-	LMOf2365_2822	beta-phosphoglucomutase	-407
-	LMOf2365_2379	hypothetical protein	-388
-	LMOf2365_2813	hypothetical protein	-378
-	LMOf2365_2413	hypothetical protein	-300
-	LMOf2365_1716	hypothetical protein	-284.75
-	LMOf2365_0382	S51 family peptidase	-242.5
-	LMOf2365_0483	hypothetical protein	-219.21
-	LMOf2365_1761	alcohol dehydrogenase, iron-dependent	-162
-	LMOf2365_1219	hypothetical protein	-150.65
-	LMOf2365_0607	hypothetical protein	-139
-	LMOf2365_0667	hypothetical protein	-130
-	LMOf2365_1726	glyoxalase	-130
-	LMOf2365_1941	diguanylate cyclase	-130
-	predicted RNA	antisense: LMOf2365_2307	-128.84
-	predicted RNA	antisense: LMOf2365_0678	-128.27
-	LMOf2365_0031	hypothetical protein	-119
-	LMOf2365_0887	hypothetical protein	-116.35
-	predicted RNA	antisense: LMOf2365_2331	-114.98
-	predicted RNA	antisense: LMOf2365_2305 fruB	-110.64
-	LMOf2365_2162	LacI family transcriptional regulator	-99.30
-	LMOf2365_1328	SNF2 family protein	-99
-	predicted RNA	antisense: *fruB*	-94.00
-	LMOf2365_0318	PRD/PTS system IIA 2 domain-containing protein	-91.4
-	LMOf2365_1412	M16 family peptidase	-83
-	LMOf2365_2264	cation efflux family protein	-82
-	LMOf2365_0757	fibronectin-binding protein	-76.81
-	predicted RNA	antisense: LMOf2365_0726 *cheY cheA*	-68.51
-	LMOf2365_1947	hypothetical protein	-67.21
-	predicted RNA	-	-53.02

Continued…

-	LMOf2365_tRNA-Lys-1	Lys tRNA	-47
-	LMOf2365_tRNA-Arg-1	Arg tRNA	-44
-	LMOf2365_tRNA-Asp-3	Asp tRNA	-44
-	LMOf2365_tRNA-Met-4	Met tRNA	-44
-	LMOf2365_tRNA-Pro-1	Pro tRNA	-44
-	LMOf2365_tRNA-Thr-2	Thr tRNA	-44
-	LMOf2365_tRNA-Tyr-2	Tyr tRNA	-44
-	LMOf2365_tRNA-Val-2	Val tRNA	-44
-	LMOf2365_0504	HD domain-containing protein	-42.61
-	LMOf2365_0687	hypothetical protein	-38
-	LMOf2365_tRNA-Ile-1	Ile tRNA	-38
-	LMOf2365_1730	ribonuclease BN	-35.82
gltD	LMOf2365_1757	glutamate synthase	-35.73
manA	LMOf2365_2143	mannose-6-phosphate isomerase	-35.49
-	LMOf2365_1636	metallo-beta-lactamase	-34.04
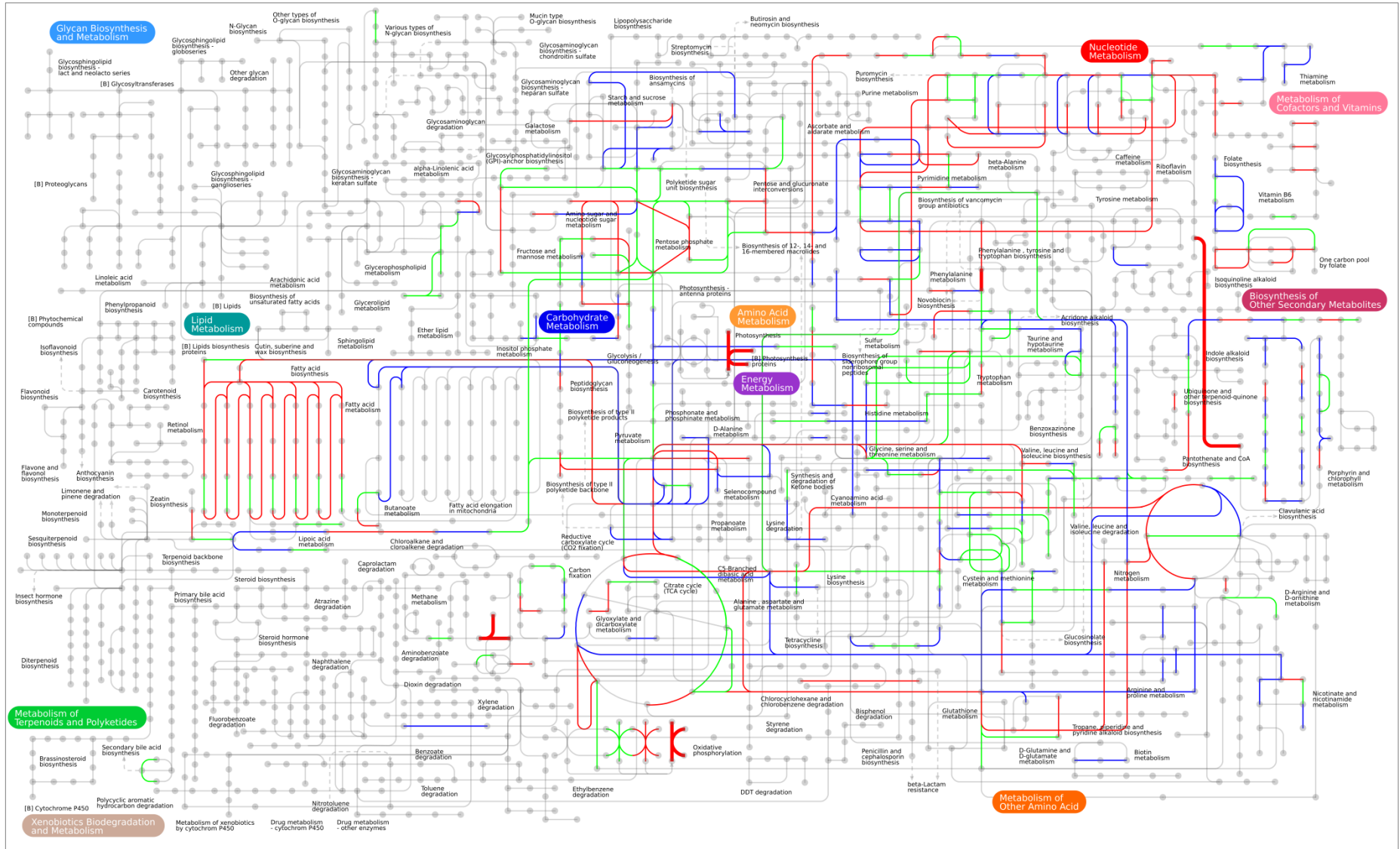
Fig. 4.4a: Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under high salt stress. (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)

Fig. 4.4b: Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under high salt stress (Zoomed view). (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)
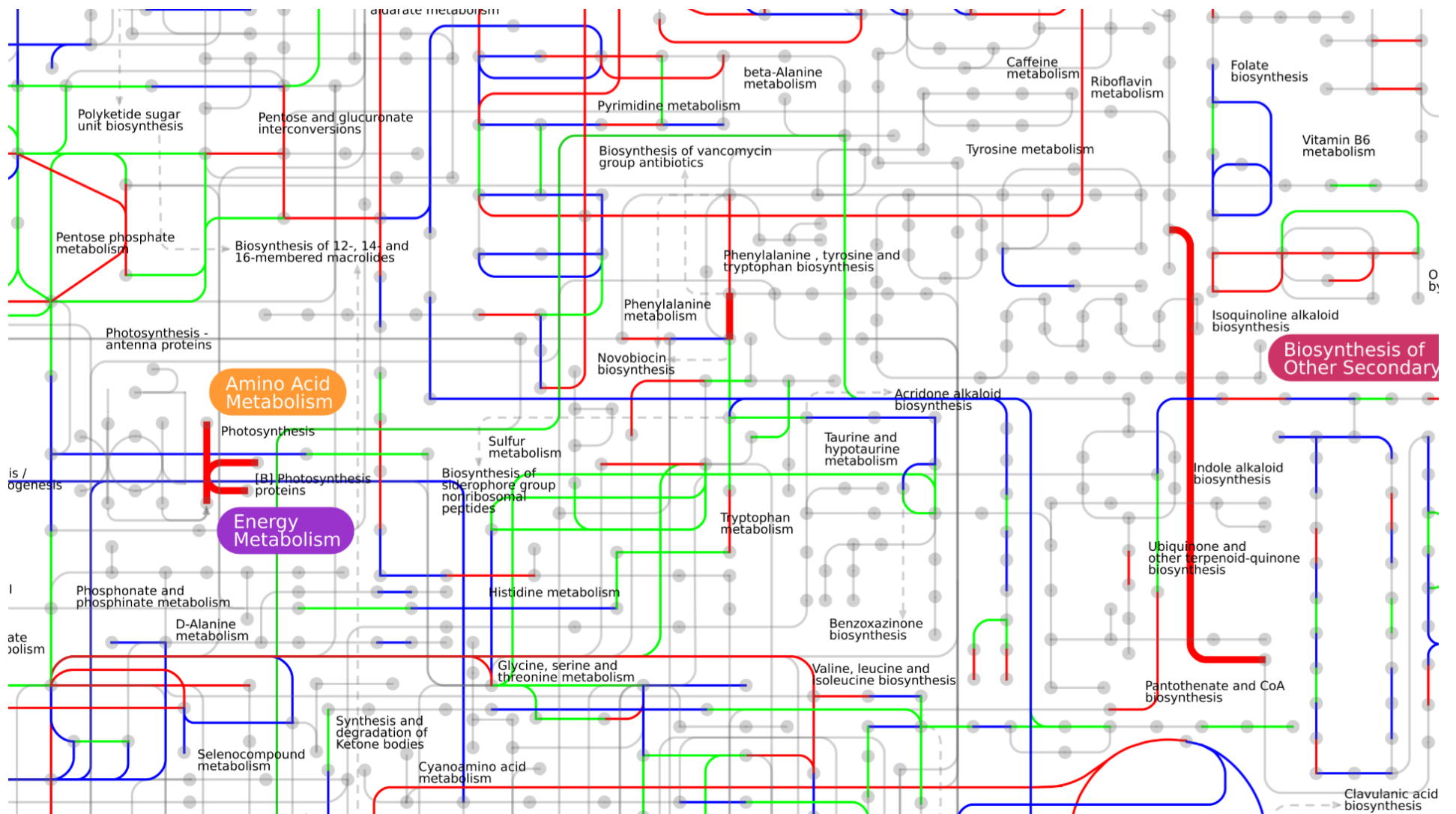
4.2.2.4 Transcriptome analysis under low temperature stress

There were many different metabolic and regulatory pathways affected under cold stress in *L. monocytogenes*. Total 60% of genome was mobilized under low temperature stress with up-regulation of 37% genes, down-regulation of 23% genes and 39% genes were unaffected.

Among the significantly differentially expressed genes, the gene encoding for bifunctional glutamate-cysteine ligase/glutathione synthetase (LMOf2365_2760) was one of differentially up-regulated genes; this enzyme known to be involved biosynthesis pathway of glutathione (Table 4.4). Glutathione known to act as the most important intracellular redox buffer that plays a crucial role in cold stress response (Zang *et al*., 2016). The gene *kdpA* (LMOf2365_2662) was significantly over-expressed; encoding K$^+$ transporting ATPase A chain, A subunit. This enzyme plays the crucial role of signal transduction in two component systems; a well characterized system for sensing change in external environment and helping bacteria to accommodate with the change. Interestingly, genes *leuB* (LMOf2365_2011) and *ispG* (LMOf2365_1460) encoding for enzymes involved in diverse metabolites synthesis; known to be involved in many cellular processes were inducted under cold stress (Table 4.4). Thiamine metabolism was observed to be increased under cold stress via gene LMOf2365_1532 encoding for carbon-sulphur lyase. Thiamine metabolism has been investigated for its role in acid stress tolerance; its exact mechanism in cold stress yet to elucidated. A gene LMOf2365_1013 coding for enzyme TerC family membrane protein was observed to be up-regulated (Table 4.4). A membrane protein, TerC family is known for involvement in bacterial responses to different stresses (Ponnusamy *et al*., 2011; Anantharaman *et al*., 2012). Being membrane protein it might also involve in maintenance of membrane under

stressful environments. The genes encoding for enzymes involved cobalt transport *cbiQ* (LMOf2365_1215) and *cbiO* (LMOf2365_2573) were up-regulated significantly (Table 4.4). Although, the exact mechanism of cobalt transfer in low temperature stress tolerance is unknown; still nickel and cobalt are known to be essential cofactors for a number of prokaryotic enzymes involved in a variety of metabolic processes (Mulrooney *et al*., 2004). Increased expression of genes involved in amino acid synthesis, uptake and transfer (LMOf2365_2619, LMOf2365_1953, LMOf2365_1461, LMOf2365_1864, and LMOf2365_1703) observed in strain challenged with cold stress (Table 4.3) (Fig 4.5a and 4.5b). This expression can be correlated with increased protein synthesis to compensate lowered activity of essential cell enzymes under low-temperature conditions. Towards compensating the demand of energy under stress unlike observed in acidic and high salt stresses; in this study, genes involved in porphyrin and chlorophyll metabolism (LMOf2365_1178), carbohydrate metabolism (LMOf2365_0365), phosphotransferase system (PTS) uptake of cellobiose (Lmof2365-2774) and fructose and mannose (LMOf2365_0114) showed increased expressions (Table 4.4) (Fig. 4.5a and 4.5b). Additionally, the genes of ABC transporter system (LMOf2365_780; LMOf2365_0877) were up-regulated significantly under cold stress. As stated previously these transporters are involved in transportation of diverse molecules across the biological membranes (Braibant *et al*., 2000) and also known to be playing crucial role in bacterial low temperature stress response (Angelidis *et al*., 2003; Wemekamp-Kamphuis *et al*., 2004). Apart from this genes encoding for nitrogen regulatory protein P-II (LMOf2365_1536), exonuclease SbcD (LMOf2365_1667), peptidase (LMOf2365_0485) and many hypothetical proteins were significantly over-expressed under cold stress in *L. monocytogenes.*

Generally inhibition of flagella synthesis is opined as energy retrenchment mechanism under different stresses (Durack *et al*., 2013; Cordero *et al*., 2016). In accordance with and as observed earlier in low pH stress, the flagellin (LMOf2365_0717) gene was observed to be significantly repressed under cold stress. It was interesting to note that genes LMOf2365_0407 and LMOf2365_2490 (Table 4.4) were annotated in KEGG database as acetyltransferases of GNAT family proteins; were observed to be supressed at 4°C. OsmC/Ohr family proteins are induced by ethanol and osmotic stresses or by organic peroxide and are involved in organic hydroperoxide detoxification (Atichartpongkul *et al*., 2001). The gene LMOf2365_0924 encoding for OsmC/Ohr family protein was down-regulated under low temperature stress. The reason behind its suppression under cold stress is yet obscured and needs to be explicated. As cell wall is first structural part of bacterial cells to be challenged with fluctuations in external environmental conditions, the genes related to structural components of cell wall also observed to be repressed *in L. monocytogenes* grown at 4°C. The N-acetylmuramoyl-L-alanine amidase (LMOf2365_2236), cell wall teichoic acid glycosylation protein GtcA (LMOf2365_2522) and lipoprotein (LMOf2365_2610) encoding genes were down-regulated significantly (Table 4.4). These are involved in peptidoglycan synthesis, the decoration of cell wall teichoic acid with galactose and glucose and cell wall associated lipoproteins. Apart from this genes encoding fumarate reductase flavoprotein subunit (LMOf2365_0376) and tRNA delta(2)-isopentenylpyrophosphate transferase (LMOf2365_1311) involved in synthesis of secondary metabolites; also the transcriptional regulators such as ArsR family transcriptional regulator (LMOf2365_0119) and transcritptional regulator (LMOf2365_2433) were down-regulated significantly (Table 4.4). The down-

regulation of these genes also could be the reduced turnover rate towards conservation of carbohydrates for alternative uses. Besides this, the genes of lipid kinase (LMOf2365_2529), exodeoxyribonuclease VII small subunit (LMOf2365_1379), thymidylate synthase (LMOf2365_1904), quorum sensing (LMOf2365_2303) and many hypothetical proteins (Table 4.4) were observed to be suppressed under Low temperature stress.

Overall under cold stress, there were many systems related to conservation of energy observed to differentially regulate. Also there were transporters systems of variety of molecules, gene related to protein synthesis machinery and many metabolic processes observed to be significantly activated with any other genes. While the expression of genes related to flagellar system, secondary metabolite synthesis, structural components and GNAT family proteins were greatly affected under to low temperature stress.

Table 4.4: Fold change (up-regulation/down-regulation) of differentially regulated genes under low temperature stress in *L. monocytogenes* ILCC187.

Name	Synonym	Product	Fold Change
-	predicted RNA	antisense: rrlB rrfB rrsB ctsR	1.91
-	predicted RNA	antisense: rrlA LMOf2365_tRNA-Leu-1 LMOf2365_tRNA-Val-1 rrsA	1.92
-	predicted RNA	antisense: rrlC	2.11
-	predicted RNA	antisense: rrlD	2.11
-	predicted RNA	antisense: rrlF	2.11
-	predicted RNA	antisense: rrlE	2.11
-	predicted RNA	-	4.52
-	LMOf2365_2760	bifunctional glutamate--cysteine ligase/glutathione synthetase	4.9
kdpA	LMOf2365_2662	potassium-transporting ATPase subunit A	4.96
-	LMOf2365_1139	hypothetical protein	5.69
-	LMOf2365_2558	formate dehydrogenase subunit alpha	6.11
leuB	LMOf2365_2011	3-isopropylmalate dehydrogenase	6.75
ispG	LMOf2365_1460	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	10.9
-	LMOf2365_2774	PTS system beta-glucoside-specific transporter subunit IIC	11.75
-	LMOf2365_0485	peptidase	12.2
-	LMOf2365_1095	glycosyl transferase family protein	17.5
-	LMOf2365_2567	hypothetical protein	26.61
-	LMOf2365_1532	carbon-sulfur lyase	28
-	LMOf2365_1667	exonuclease SbcD	30.5
-	LMOf2365_0265	hypothetical protein	36
-	LMOf2365_0780	ABC transporter ATP-binding protein	47
-	LMOf2365_0114	PTS system mannose/fructose/sorbose family transporter subunit IIC	52
-	LMOf2365_1703	cystathionine beta-lyase	52
carA	LMOf2365_1864	carbamoyl phosphate synthase small subunit	55
-	LMOf2365_1013	TerC family membrane protein	56
-	LMOf2365_1335	membrane-associated zinc metalloprotease	60
-	LMOf2365_0877	sugar ABC transporter permease	61
-	LMOf2365_1461	AzlC family protein	61
cbiQ	LMOf2365_1215	cobalt transport protein CbiQ	63
tyrA	LMOf2365_1953	prephenate dehydrogenase	70
-	LMOf2365_0778	hypothetical protein	73
cbiO	LMOf2365_2573	cobalt transporter ATP-binding subunit	76
-	LMOf2365_0609	phospholipase/carboxylesterase	78
-	LMOf2365_2619	creatinine amidohydrolase	78
-	LMOf2365_0717	flagellar biosynthesis regulator FlhF	80
-	LMOf2365_1178	propanediol utilization protein PduX	88
-	LMOf2365_0369	hypothetical protein	125
rpiB-1	LMOf2365_0365	ribose 5-phosphate isomerase B	130
-	LMOf2365_1536	nitrogen regulatory protein P-II	181
-	LMOf2365_2368	hypothetical protein	-324
-	LMOf2365_0995	hypothetical protein	-269
rplN	LMOf2365_2595	50S ribosomal protein L14	-116
xseB	LMOf2365_1379	exodeoxyribonuclease VII small subunit	-115
-	LMOf2365_0835	hypothetical protein	-112
-	LMOf2365_2847	hypothetical protein	-100

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bsaA	LMOf2365_1004	glutathione peroxidase	-82
-	LMOf2365_2490	acetyltransferase	-82
-	LMOf2365_0739	hypothetical protein	-71
-	LMOf2365_0575	hypothetical protein	-67
deoC	LMOf2365_2018	deoxyribose-phosphate aldolase	-53
thyA	LMOf2365_1904	thymidylate synthase	-45
miaA	LMOf2365_1311	tRNA delta(2)-isopentenylpyrophosphate transferase	-38
-	LMOf2365_2236	N-acetylmuramoyl-L-alanine amidase	-38
-	LMOf2365_2379	hypothetical protein	-35.27
-	predicted RNA	antisense: LMOf2365_2333	-34.18
-	LMOf2365_0726	flagellin	-19.91
-	LMOf2365_0119	ArsR family transcriptional regulator	-17.28
-	LMOf2365_0817	hypothetical protein	-17.04
-	LMOf2365_0376	fumarate reductase flavoprotein subunit	-15.57
-	LMOf2365_2610	lipoprotein	-14.03
-	LMOf2365_1598	hypothetical protein	-12.125
gtcA	LMOf2365_2522	cell wall teichoic acid glycosylation protein GtcA	-10.03
-	LMOf2365_2529	lipid kinase	-10
-	LMOf2365_2361	hypothetical protein	-9.85
-	LMOf2365_1996	hypothetical protein	-9
-	LMOf2365_0222	50S ribosomal protein L25/general stress protein Ctc	-8.20
-	LMOf2365_0924	OsmC/Ohr family protein	-6.78
-	LMOf2365_2433	transcriptional regulator	-6.70
-	LMOf2365_0407	acetyltransferase	-6.04
-	LMOf2365_2303	transcriptional regulator	-5.93
-	LMOf2365_tRNA-Arg-5	Arg tRNA	-5.45
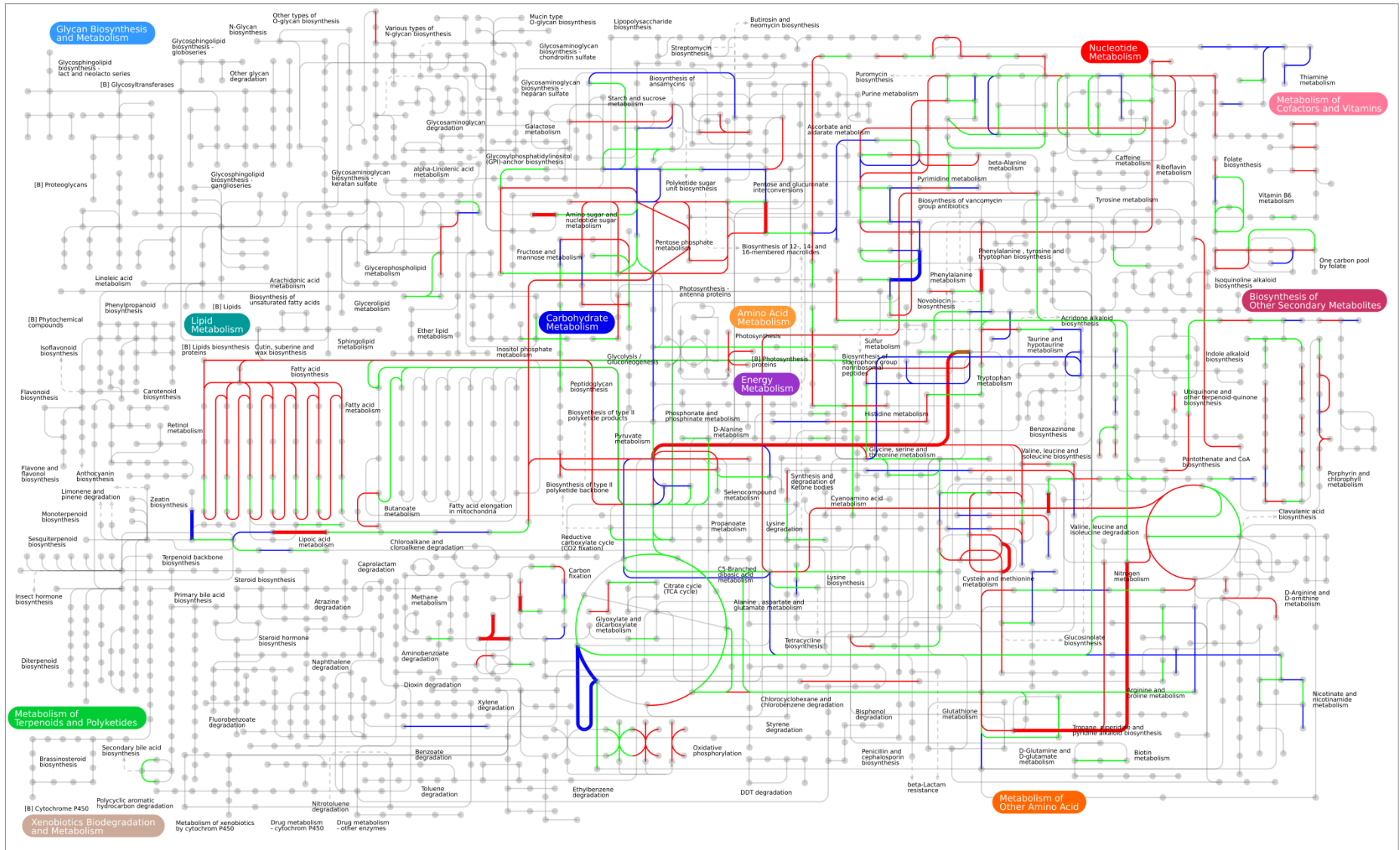
Fig. 4.5a : Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under low temperature stress. (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)
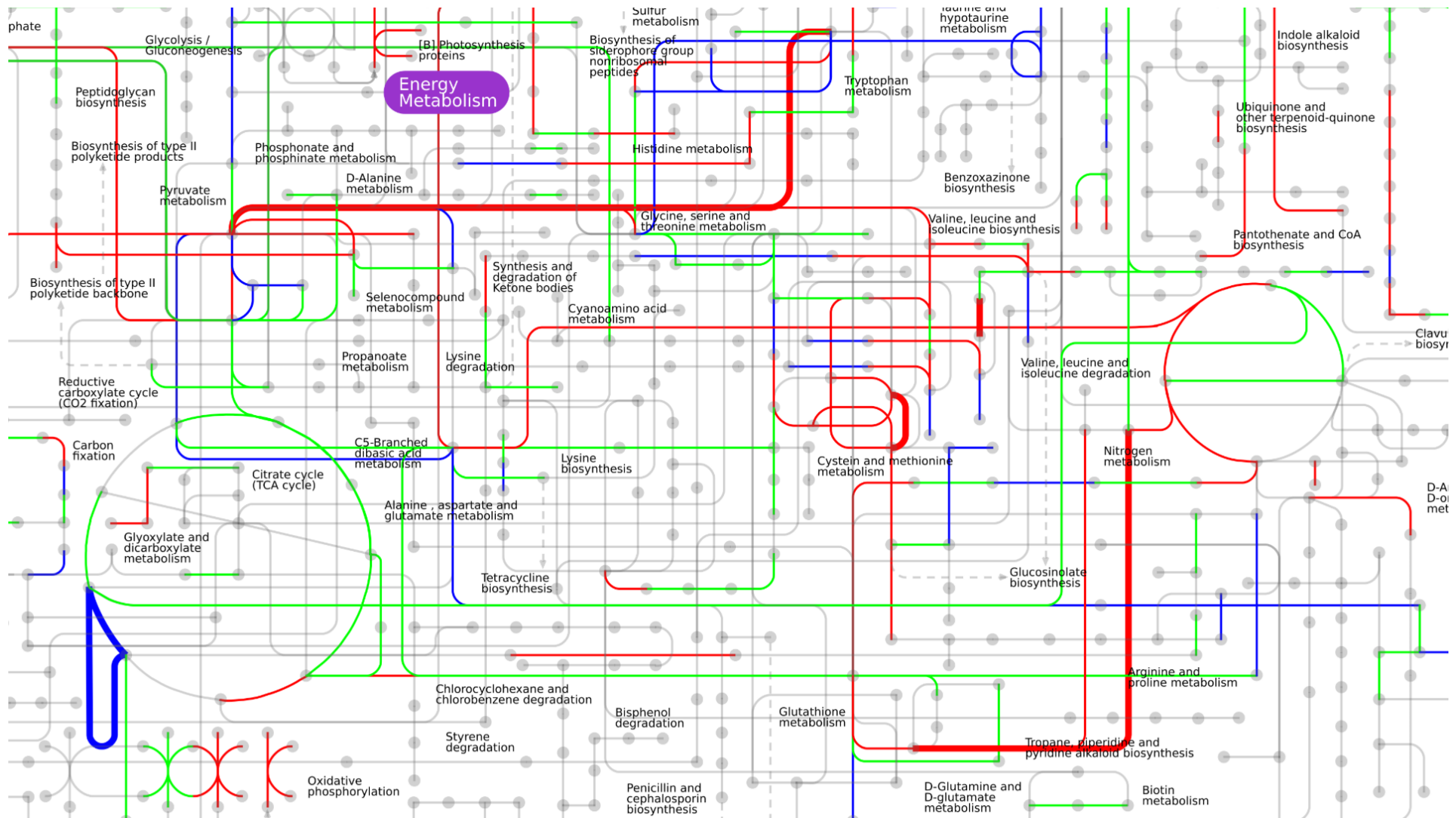
Fig. 4.5b: Kyoto Encyclopedia of Genes and Genomes (KEGG) database based analysis of Transcriptome under low temperature stress (Zoomed view). (Color indication: Red-Up-regulated, Blue – Down-regulated, Green – Unaffected) (http://pathways.embl.de/iPath2.cgi#)

Each stress was observed to be tackled by switching metabolic systems on or off. The differentially regulated genes under all stresses showed common involvement of various systems under two or more stresses. Genes related to ABC transporter systems, associated PTS systems, energy producing pathways, structural component synthesis pathways and/or modulating pathways and DNA repair systems were profoundly found to be differentially up-regulated under all the stresses. Genes for acetyl-transferase, membrane associated zinc metalloprotease showed differential up-regulation under low pH and high salt stress. While genes related to cell wall surface anchor family proteins were reported to be commonly induced under low pH stress, high pH stress and high salt stress also. Genes for AzlC family protein and cobalt transport protein CbiQ showed significant up-regulation under high salt and low temperature stresses. Furthermore, among commonly suppressed genes, flagellin gene was found to be commonly down-regulated under low pH and low temperature stress. While genes for AraC family transcriptional regulator and glyoxalase showed significant down-regulation under low pH and high salt stresses. The gene encoding TetR family transcriptional regulator was observed to be commonly repressed under low pH and high pH stress. Overall survival and adaptation to various stress conditions in *L. monocytogenes* strain were found to have many parallel approaches under low pH, high pH, high salt and low temperature stresses.

The observations made in whole transcriptomic analysis of *L. monocytogenes* ILCC187, supported the earlier observations made in PFGE analysis that these tolerances must have been controlled by some common factors. Further analysis with two-dimensional tandem mass spectrometry-based proteomics assessment of *L. monocytogenes* in response to these various stresses will help for advanced

elucidation of results presented in this work. These future aspects will give better insights about reliable correlation of transcriptomic data coupled with protein abundance changes. It may give excelled knowledge of common factors involved in survival and adaption of *L. monocytogenes* to high salt, adverse pH and cold stresses.

Chapter 5
Summary and Conclusions

5.1 Summary and Conclusions

Listeria monocytogenes is an important foodborne pathogen and nagging public health hazard. The disease, listeriosis, caused by this pathogen is rare but serious and life-threatening with 20-30% case fatality rate, 50% neonatal death rate and 91% hospitalization rate in human cases. The disease mainly occurs in the immuno-compromised individuals including pregnant women, neonates, elderly persons, cancer patients, and patients undergoing immunosuppressive therapy.

In this study, we attempted and demonstrated different approaches to elucidate the function of stress resistance diversity in *L. monocytogenes* strains of diverse origin and of epidemiologically important serotypes. The analysis of the collection of 104 strains of *L. monocytogens* revealed extraordinary innate resistance capability to the stresses commonly employed in food preservations. The data showed that the strains varied remarkably with respect to stress tolerance abilities under different stresses. The exact correlation of stress tolerance pattern with the origin of the strains could not be drawn for all the stresses. The investigation underlined significant stress tolerance capability of certain serotype with respect to intensity and number of stresses; indicating that certain serotype such as *L. monocytogenes* 4b might be epidemiologically dominant due to capacity to adapt these stressful environments. It supported the proposition that observed epidemiological trends might be the reflection of superior adaptive response with successive infection by particular subtype of *L. monocytogenes*. Morphological analysis of the strains under stress inferred that filament formation under stressed environmental conditions could be one of the mechanisms for stress tolerance of *L. monocytogenes*. Gene expression studies supported the hypothesis that filament formation could be the result of over-expression of the *minC* gene under stressed environmental conditions.

The FAME analysis and cell wall proteomic analysis illustrated various cell surface modifications and/or alterations needed under the variety of stresses; culminating potential but lesser known contribution of cell membrane associated molecular factors in adaption of *L. monocytogenes* to the variety of stressful environmental conditions. Although protein profiling could not fully detect major proteins; it provided a valuable picture to develop a partial map of possible common proteins to be involved in physico-chemical response of bacteria under different stresses. The PFGE analysis showed clonality among the strains with different stress tolerance abilities suggesting the possible involvement of common factors in control of tolerance abilities. Whole genome transcriptome analysis suggested that a broadly similar genetic regulatory mechanism could be operating in response to high salt, extreme pH and low temperature stresses. The data depicted possible direct role of some genetic elements in stress survival. The transcriptomic data also supported the observations made in the genetic basis of the morphological analysis, fatty acids profiling, partial proteome and PFGE analysis as well. These transcriptomic results have established yet another convergence within various pathways of *L. monocytogenes* under different stresses. This also manifested the complex nature and intelligibility of these regulatory units. Hereafter, even though *L. monocytogenes* strains varied in intensity of stress tolerance, there might be common genetic factors involved in regulation of physiology of stress response with the innate capability of tolerance under different stresses. This makes *L. monocytogenes* a versatile and pervasive pathogen, capable to equally adapt from soil to eukaryotic host. This study is a significant step towards understanding the innate stress response of *L. monocytogenes* and adaptive dominance of particular subtypes to the extreme environmental conditions encountered during food preservation or in the

human/animal host. The study also aided in scrutinizing the molecular events with better insights into function and connection of specific genetic elements to the stress response mechanisms of *L. monocytogenes*. The results increased our comprehension of the induction and function of the complex stress response networks in *L. monocytogenes*.

Future Perspective

Future Perspectives

Listeria monocytogenes is an emerging foodborne pathogen that shows its existence in a variety of food products. *L. monocytogenes* enters in food processing environments from several routes and survives through resisting types of stresses. Considering the change in life style, a demand for RTE foods and increased incidences of listeriosis; it is important to devise the strategies to control *L. monocytogenes* in foods and food processing premises. The present study has enhanced our knowledge and understanding that the physiological changes appeared in *L. monocytogenes* in response to particular environmental stresses are the consequences of modulation at transcription and/or protein expression levels. Further study with detailed proteomic analysis coupled with identification and characterization of stress inducted proteins promises to lead to the discovery of other intriguing factors. This advancement of knowledge will help to devise the strategies to control the stress tolerant *L. monocytogenes* in foods and food processing premises.

L. monocytogenes is one of the most versatile pathogen and able to adapt to different type of environments including the human body. *Listeria* uses various virulence mechanisms that enable them to conquer different conditions inside host during the course of infection. Many of deleterious effects such as high salt, extreme pH are tolerated by the pathogen in the foods and inside the hosts also. *L. monocytogenes* employ many strategies to adhere, invade and multiply within their hosts. It is important to understand response by the host after infection of such stress adapted *L. monocytogenes.* Considering similarity of stresses experienced by *L. monocytogenes* in foods as well in hosts; it is important to understand the virulence

of *Listeria* after exposure to such stresses. Furthermore studies of stress tolerant strains with employing animal model will give better insights about modulation of virulence of *L. monocytogenes* after stress adaptation. Additionally, the study of cytokine profiling employing over-expressed proteins from stress tolerant strains will offer a wide range of data, which can be useful for understanding immunomodulation.

Bibliography

- Abee, T., den Besten, H.M.W., Koomen, J.S., Metselaar, K.I. and Zwietering, M.H., 2016. A systems-based approach to determine the impact of *Listeria monocytogenes* stress resistant variants on food safety. 25th International ICFMH Conference. Food Micro 2016. July 19-22, 2017. Dublin, Ireland. Abstract no. 14. http://library.wur.nl/WebQuery/wurpubs/fulltext/403875.

- Abram, F., Starr, E., Karatzas, K.A.G., Matlawska-Wasowska, K., Boyd, A., Wiedmann, M., Boor, K.J., Connally, D. and O'Byrne, C.P., 2008. Identification of components of the sigma B regulon in *Listeria monocytogenes* that contribute to acid and salt tolerance. *Applied and Environmental Microbiology*, *74*(22), pp.6848-6858.

- Agoston, R., Soni, K., Jesudhasan, P.R., Russell, W.K., Mohácsi-Farkas, C. and Pillai, S.D., 2009. Differential expression of proteins in *Listeria monocytogenes* under thermotolerance-inducing, heat shock, and prolonged heat shock conditions. *Foodborne Pathogens and Disease*, *6*(9), pp.1133-1140.

- Al Bahry, S., Sivakumar, N. and Al-Khambashi, M., 2012. Effect of nalidixic acid on the morphology and protein expression of *Pseudomonas aeruginosa*. *Asian Pacific Journal of Tropical Medicine*, *5*(4), pp.265-269.

- Altekruse, S.F., Cohen, M.L. and Swerdlow, D.L., 1997. Emerging foodborne diseases. *Emerging Infectious Diseases*, *3*(3), p.285-293.

- Álvarez-Ordóñez, A., Begley, M., Prieto, M., Messens, W., López, M., Bernardo, A. and Hill, C., 2011. *Salmonella* spp. survival strategies within the host gastrointestinal tract. *Microbiology*, *157*(12), pp.3268-3281.

- Alvarez-Ordonez, A., Broussolle, V., Colin, P., Nguyen-The, C. and Prieto, M., 2015. The adaptive response of bacterial food-borne pathogens in the

environment, host and food: Implications for food safety. *International Journal of Food Microbiology*, *213*(3), pp.99-109.

- Álvarez-Ordóñez, A., Prieto, M., Bernardo, A., Hill, C. and López, M., 2012. The acid tolerance response of *Salmonella* spp.: an adaptive strategy to survive in stressful environments prevailing in foods and the host. *Food Research International*, *45*(2), pp.482-492.

- Amato, E., Filipello, V., Gori, M., Lomonaco, S., Losio, M.N., Parisi, A., Huedo, P., Knabel, S.J. and Pontello, M., 2017. Identification of a major *Listeria monocytogenes* outbreak clone linked to soft cheese in Northern Italy–2009-2011. *BMC Infectious Diseases*, *17*(1), p.342-350.

- Ananchaipattana, C., Hosotani, Y., Kawasaki, S., Pongsawat, S., Md. Latiful, B., Isobe, S. and Inatsu, Y., 2012. Prevalence of foodborne pathogens in retailed foods in Thailand. *Foodborne Pathogens and Disease*, *9*(9), pp.835-840.

- Anantharaman, V., Iyer, L.M. and Aravind, L., 2012. Ter-dependent stress response systems: novel pathways related to metal sensing, production of a nucleoside-like metabolite, and DNA-processing. *Molecular BioSystems*, *8*(12), pp.3142-3165.

- Angelidis, A.S. and Smith, G.M., 2003. Role of the glycine betaine and carnitine transporters in adaptation of *Listeria monocytogenes* to chill stress in defined medium. *Applied and Environmental Microbiology*, *69*(12), pp.7492-7498.

- Angelidis, A.S. and Smith, G.M., 2003. Three transporters mediate uptake of glycine betaine and carnitine by *Listeria monocytogenes* in response to

hyperosmotic stress. *Applied and Environmental Microbiology*, *69*(2), pp.1013-1022.

- Antal, E.A., Høgåsen, H.R., Sandvik, L. and Mæhlen, J., 2007. Listeriosis in Norway 1977–2003. *Scandinavian Journal of Infectious Diseases*, *39*(5), pp.398-404.

- Appel, L.J. and Anderson, C.A., 2010. Compelling evidence for public health action to reduce salt intake. *The new England Journal of Medicine*, *362*(7), pp.650-652.

- Archer, D.L., 1996. Preservation microbiology and safety: evidence that stress enhances virulence and triggers adaptive mutations. *Trends in Food Science & Technology*, *7*(3), pp.91-95.

- Armengaud, J., Trapp, J., Pible, O., Geffard, O., Chaumot, A. and Hartmann, E.M., 2014. Non-model organisms, a species endangered by proteogenomics. *Journal of Proteomics*, *105*, pp.5-18.

- Asselt, E.D., der Fels-Klerx, H.J., Marvin, H.J.P., Bokhorst-van de Veen, H. and Groot, M.N., 2017. Overview of Food Safety Hazards in the European Dairy Supply Chain. *Comprehensive Reviews in Food Science and Food Safety*, *16*(1), pp.59-75.

- Atichartpongkul, S., Loprasert, S., Vattanaviboon, P., Whangsuk, W., Helmann, J.D. and Mongkolsuk, S., 2001. Bacterial Ohr and OsmC paralogues define two protein families with distinct functions and patterns of expression. *Microbiology*, 147(7), pp.1775-1782.

- Aubry, C., Goulard, C., Nahori, M.A., Cayet, N., Decalf, J., Sachse, M., Boneca, I.G., Cossart, P. and Dussurget, O., 2011. OatA, a peptidoglycan O-

acetyltransferase involved in *Listeria monocytogenes* immune escape, is critical for virulence. *Journal of Infectious Diseases*, *204*(5), pp.731-740.

- Autio, T., Lundén, J., Fredriksson-Ahomaa, M., Björkroth, J., Sjöberg, A.M. and Korkeala, H., 2002. Similar *Listeria monocytogenes* pulsotypes detected in several foods originating from different sources. *International journal of food microbiology*, *77*(1-2), pp.83-90.

- Bae, D., Liu, C., Zhang, T., Jones, M., Peterson, S.N. and Wang, C., 2012. Global gene expression of *Listeria monocytogenes* to salt stress. *Journal of Food Protection*, *75*(5), pp.906-912.

- Bajerski, F., Wagner, D. and Mangelsdorf, K., 2017. Cell Membrane Fatty Acid Composition of *Chryseobacterium frigidisoli* PB4T, Isolated from Antarctic Glacier Fore field Soils, in Response to Changing Temperature and pH Conditions. *Frontiers in Microbiology*, *8*.

- Barabote, R.D. and Saier, M.H., 2005. Comparative genomic analyses of the bacterial phosphotransferase system. *Microbiology and Molecular Biology Reviews*, *69*(4), pp.608-634.

- Barat, J.M., Rodríguez-Barona, S., Andrés, A. & Fito, P. 2003. Cod salting manufacturing analysis. *Food Research International*, 36, 447–453.

- Barbour, A.H., Rampling, A. and Hormaeche, C.E., 2001. Variation in the infectivity of *Listeria monocytogenes* isolates following intragastric inoculation of mice. *Infection and Immunity*, *69*(7), pp.4657-4660.

- Barbuddhe, S.B., Chaudhari, S.P. and Malik, S.V.S., 2002. The occurrence of pathogenic *Listeria monocytogenes* and antibodies against listeriolysin-O in buffaloes. *Zoonoses and Public Health*, *49*(4), pp.181-184.

- Barbuddhe, S.B., Malik, S.V.S., Bhilegaonkar, K.N., Kumar, P. and Gupta, L.K., 2000. Isolation of *Listeria monocytogenes* and anti-listeriolysin O detection in sheep and goats. *Small Ruminant Research*, *38*(2), pp.151-155.

- Barbuddhe, S.B., Malik, S.V.S., Kumar, J.A., Kalorey, D.R. and Chakraborty, T., 2012. Epidemiology and risk management of listeriosis in India. *International Journal of Food Microbiology*, *154*(3), pp.113-118.

- Barbuddhe, S.B., Swain, B.K., Chakurkar, E.B. and Sundaram, R.N.S., 2003. Microbial quality of poultry meat with special reference to *Listeria monocytogenes*. *Indian Journal of Poultry Science*, *38*(3), pp.305-307.

- Barbuddhe, S.B., Doijad, S.P., Goesmann, A., Hilker, R., Poharkar, K.V., Rawool, D.B., Kurkure, N.V., Kalorey, D.R., Malik, S.S., Shakuntala, I, Chaudhari, S., Waskar, V., D'Costa, D., Kolhe, R., Arora, R., Roy, A., Raorane, A., Kale, S**.**, Pathak, A., 2016. Presence of a widely disseminated *Listeria monocytogenes* serotype 4b clone in India. *Emerging Microbes & Infections*, *5*(6), p.e55.

- Barmpalia-Davis, I.M., Geornaras, I., Kendall, P.A. and Sofos, J.N., 2008. Differences in survival among 13 *Listeria monocytogenes* strains in a dynamic model of the stomach and small intestine. *Applied and Environmental Microbiology*, *74*(17), pp.5563-5567.

- Barria, C., Malecki, M. and Arraiano, C.M., 2013. Bacterial adaptation to cold. *Microbiology*, *159*(12), pp.2437-2443.

- Batz, M., Hoffmann, S. and Morris Jr, J.G., 2014. Disease-outcome trees, EQ-5D scores, and estimated annual losses of quality-adjusted life years (QALYs) for 14 foodborne pathogens in the United States. *Foodborne Pathogens and Disease*, *11*(5), pp.395-402.

- Beales, N., 2004. Adaptation of microorganisms to cold temperatures, weak acidpreservatives, low pH, and osmotic stress: a review. *Competitive Reviews in Food Science and Food Safety 3* (1), pp.1–20.

- Bécavin, C., Bouchier, C., Lechat, P., Archambaud, C., Creno, S., Gouin, E., Wu, Z., Kühbacher, A., Brisse, S., Pucciarelli, M.G. and García-del Portillo, F., 2014. Comparison of widely used *Listeria monocytogenes* strains EGD, 10403S, and EGD-e highlights genomic differences underlying variations in pathogenicity. *MBio*, *5*(2), pp.e00969-14.

- Begley, M. and Hill, C., 2015. Stress adaptation in foodborne pathogens. *Annual Review of Food Science and Technology*, *6*, pp.191-210.

- Begley, M., Gahan, C.G. and Hill, C., 2002. Bile stress response in *Listeria monocytogenes* LO28: adaptation, cross-protection, and identification of genetic loci involved in bile resistance. *Applied and Environmental Microbiology*, *68*(12), pp.6005-6012.

- Bereksi, N., Gavini, F., Benezech, T. and Faille, C., 2002. Growth, morphology and surface properties of *Listeria monocytogenes* Scott A and LO28 under saline and acid environments. *Journal of Applied Microbiology*, *92*(3), pp.556-565.

- Bergholz, P.W., Bakermans, C. and Tiedje, J.M., 2009. Psychrobacter arcticus 273-4 uses resource efficiency and molecular motion adaptations for subzero temperature growth. *Journal of Bacteriology*, *191*(7), pp.2340-2352.

- Bergholz, T.M., Bowen, B., Wiedmann, M. and Boor, K.J., 2014. *Listeria monocytogenes* shows temperature-dependent and-independent responses to salt stress, including responses that induce cross-protection against other stresses. *Applied and Environmental Microbiology*, *78*(8), pp.2602-2612.

- Bergholz, T.M., den Bakker, H.C., Fortes, E.D., Boor, K.J. and Wiedmann, M., 2010. Salt stress phenotypes in *Listeria monocytogenes* vary by genetic lineage and temperature. *Foodborne Pathogens and Disease*, *7*(12), pp.1537-1549.

- Bhunia, A.K., 2008. *Bacillus cereus* and *Bacillus anthracis*. In Bunia A.K. (Ed), Foodborne Microbial Pathogens: Mechanisms and Pathogenesis, pp.135-148. Chapter VII: Springer New York.

- Bille, J., Blanc, D.S., Schmid, H., Boubaker, K., Baumgartner, A., Siegrist, H.H., Tritten, M.L., Lienhard, R., Berner, D., Anderau, R. and Treboux, M., 2006. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. *Euro surveillance: bulletin Europeen Sur Les Maladies Transmissibles= European Communicable Disease Bulletin*, *11*(6), pp.91-93.

- Bitoun, J.P., Nguyen, A.H., Fan, Y., Burne, R.A. and Wen, Z.T., 2011. Transcriptional repressor Rex is involved in regulation of oxidative stress response and biofilm formation by Streptococcus mutans. *FEMS Microbiology Letters*, *320*(2), pp.110-117.

- Bowman, J.P., Chang, K.J.L., Pinfold, T. and Ross, T., 2010. Transcriptomic and phenotypic responses of *Listeria monocytogenes* strains possessing different

growth efficiencies under acidic conditions. *Applied and environmental microbiology*, *76*(14), pp.4836-4850.

- Bowman, J.P., Hages, E., Nilsson, R.E., Kocharunchitt, C. and Ross, T., 2012. Investigation of the *Listeria monocytogenes* Scott A acid tolerance response and associated physiological and phenotypic features via whole proteome analysis. *Journal of Proteome Research*, *11*(4), pp.2409-2426.

- Braeken, K., Moris, M., Daniels, R., Vanderleyden, J. and Michiels, J., 2006. New horizons for (p) ppGpp in bacterial and plant physiology. *Trends in Microbiology*, *14*(1), pp.45-54.

- Brahambhatt, M.N. and Anjaria, J.M., 1993. Analysis of market meats for possible contamination with *Listeria*. *Indian Journal of Animal Sciences*, *63,*pp. 522-530.

- Braibant, M., Gilot, P. and Content, J., 2000. The ATP binding cassette (ABC) transport systems of *Mycobacterium tuberculosis*. *FEMS Microbiology Reviews*, *24*(4), pp.449-467.

- Bresolin, G., Trček, J., Scherer, S. and Fuchs, T.M., 2008. Presence of a functional flagellar cluster Flag-2 and low-temperature expression of flagellar genes in *Yersinia enterocolitica* W22703. *Microbiology*, *154*(1), pp.196-206.

- Bron, P.A., Marco, M., Hoffer, S.M., Van Mullekom, E., De Vos, W.M. and Kleerebezem, M., 2004. Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters in vitro and in situ in the gastrointestinal tract. *Journal of Bacteriology*, *186*(23), pp.7829-7835.

- Brøndsted, L., Kallipolitis, B.H., Ingmer, H. and Knöchel, S., 2003. kdpE and a putative RsbQ homologue contribute to growth of *Listeria monocytogenes* at high osmolarity and low temperature. *FEMS Microbiology Letters*, *219*(2), pp.233-239.

- Brouwer, M.C., Beek, D.V.D., Heckenberg, S.G., Spanjaard, L. and Gans, J.D., 2006. Community-acquired *Listeria monocytogenes* meningitis in adults. *Clinical Infectious Diseases*, *43*(10), pp.1233-1238.

- Brown, J., 2016. Cell wall morphogenesis in *Bacillus subtilis*. (Unpublished doctoral thesis). Newcastle University, UK.

- Brownsey, R. W., and Denton, R. 1987. Acetyl-coenzyme A carboxylase. *Enzymes 18*(3), pp123–146.

- Buchanan, R., Lindqvist, R., Ross, T., Smith, M., Todd, E., Whiting, R., 2004. Risk assessment of Listeria monocytogenes in ready-to-eat foods. Microbiological Risk Assessment Series, 4. Food and Agriculture Organization of the United Nations, Rome (Italy).

- Buchrieser, C., 2007. Biodiversity of the species *Listeria monocytogenes* and the genus *Listeria*. *Microbes and Infection*, *9*(10), pp.1147-1155.

- Buchrieser, C., Brosch, R., Catimel, B. and Rocourt, J., 1993. Pulsed-field gel electrophoresis applied for comparing *Listeria monocytogenes* strains involved in outbreaks. *Canadian Journal of Microbiology*, *39*(4), pp.395-401.

- Buchrieser, C., Rusniok, C., Kunst, F., Cossart, P. and Glaser, P., 2003. Comparison of the genome sequences of *Listeria monocytogenes* and *Listeria*

innocua: clues for evolution and pathogenicity. *Pathogens and Disease*, *35*(3), pp.207-213.

- Burall, L.S., Grim, C.J. and Datta, A.R., 2017. A clade of *Listeria monocytogenes* serotype 4b variant strains linked to recent listeriosis outbreaks associated with produce from a defined geographic region in the US. *PloS one*, *12*(5), p.e0176912.

- Burall, L.S., Simpson, A.C., Chou, L., Laksanalamai, P. and Datta, A.R., 2012. A novel gene, *lstC*, of *Listeria monocytogenes* is implicated in high salt tolerance. *Food Microbiology*, *48*, pp.72-82.

- Burgess, C.M., Gianotti, A., Gruzdev, N., Holah, J., Knøchel, S., Lehner, A., Margas, E., Esser, S.S., Sela, S. and Tresse, O., 2016. The response of foodborne pathogens to osmotic and desiccation stresses in the food chain. *International Journal of Food Microbiology*, 221, pp.37-53.

- Cacace, G., Mazzeo, M.F., Sorrentino, A., Spada, V., Malorni, A. and Siciliano, R.A., 2010. Proteomics for the elucidation of cold adaptation mechanisms in *Listeria monocytogenes*. *Journal of Proteomics*, *73*(10), pp.2021-2030.

- Cairns, B.J. and Payne, R.J., 2009. Sudden increases in listeriosis rates in England and Wales, 2001 and 2003. *Emerging Infectious Diseases*, *15*(3), p.465.

- Caly, D., Takilt, D., Lebret, V. and Tresse, O., 2009. Sodium chloride affects *Listeria monocytogenes* adhesion to polystyrene and stainless steel by regulating flagella expression. *Letters in Applied Microbiology*, *49*(6), pp.751-756.

- Carpentier, B. and Cerf, O., 2011. Persistence of Listeria monocytogenes in food industry equipment and premises. *International journal of food microbiology*, *145*(1), pp.1-8.

- Casey, A., Fox, E.M., Schmitz-Esser, S., Coffey, A., McAuliffe, O. and Jordan, K., 2014. Transcriptome analysis of *Listeria monocytogenes* exposed to biocide stress reveals a multi-system response involving cell wall synthesis, sugar uptake, and motility. *Frontiers in Microbiology*, *5*.

- Centers for Disease Control and Prevention (CDC). 2012. National Listeria Surveillance Annual Summary, 2010. Atlanta, Georgia: US Department of Health and Human Services, CDC.

- Cetin, M.S., Zhang, C., Hutkins, R.W. and Benson, A.K., 2004. Regulation of transcription of compatible solute transporters by the general stress sigma factor, σB, in *Listeria monocytogenes*. *Journal of Bacteriology*, *186*(3), pp.794-802.

- Chaikam, V. and Karlson, D.T., 2010. Comparison of structure, function and regulation of plant cold shock domain proteins to bacterial and animal cold shock domain proteins. *BMB Reports*, *43*(1), pp.1-8.

- Chakravarty, R., Manna, S., Ghosh, A.K. and Banerjee, P.C., 2007. Morphological changes in an *Acidocella* strain in response to heavy metal stress. *Research Journal of Microbiology*, *2*(10), pp.742-748.

- Chan, M. 2014. Food safety must accompany food and nutrition security. *Lancet*. *384* (9958), pp.1910–1911.

- Chan, Y.C. and Wiedmann, M., 2008. Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. *Critical Reviews in Food Science and Nutrition*, *49*(3), pp.237-253.

- Chan, Y.C., Hu, Y., Chaturongakul, S., Files, K.D., Bowen, B.M., Boor, K.J. and Wiedmann, M., 2008. Contributions of two-component regulatory systems,

alternative σ factors, and negative regulators to *Listeria monocytogenes* cold adaptation and cold growth. *Journal of Food Protection*, *71*(2), pp.420-425.

- Chan, Y.C., Raengpradub, S., Boor, K.J. and Wiedmann, M., 2007. Microarray-based characterization of the *Listeria monocytogenes* cold regulon in log-and stationary-phase cells. *Applied and Environmental Microbiology*, *73*(20), pp.6484-6498.

- Chassaing, D. and Auvray, F., 2007. The *lmo1078* gene encoding a putative UDP-glucose pyrophosphorylase is involved in growth of *Listeria monocytogenes* at low temperature. *FEMS Microbiology Letters*, *275*(1), pp.31-37.

- Chen, J., Jiang, L., Chen, Q., Zhao, H., Luo, X., Chen, X. and Fang, W., 2009. lmo0038 is involved in acid and heat stress responses and specific for *Listeria monocytogenes* lineages I and II, and *Listeria ivanovii*. *Foodborne Pathogens and Disease*, *6*(3), pp.365-376.

- Chen, S.Y., Jane, W.N., Chen, Y.S. and Wong, H.C., 2009. Morphological changes of *Vibrio parahaemolyticus* under cold and starvation stresses. *International Journal of Food Microbiology*, *129*(2), pp.157-165.

- Chen, Y., Ross, W.H., Gray, M.J., Wiedmann, M., Whiting, R.C. and Scott, V.N., 2006. Attributing risk to *Listeria monocytogenes* subgroups: dose response in relation to genetic lineages. *Journal of Food Protection*, *69*(2), pp.335-344.

- Cheng, C., Chen, J., Shan, Y., Fang, C., Liu, Y., Xia, Y., Song, H. and Fang, W., 2013. *Listeria monocytogenes* ArcA contributes to acid tolerance. *Journal of medical microbiology*, *62*(6), pp.813-821.

- Cheng, C., Dong, Z., Han, X., Sun, J., Wang, H., Jiang, L., Yang, Y., Ma, T., Chen, Z., Yu, J. and Fang, W., 2017. *Listeria monocytogenes* 10403S arginine repressor ArgR finely tunes arginine metabolism regulation under acidic conditions. *Frontiers in Microbiology*, *8*.

- Chouliara, I., Savvaidis, I.N., Panagiotakis, N. & Kontominas, M.G. 2004. Preservation of salted, vacuum-packaged, refrigerated sea bream *(Sparus aurata)* fillets by irradiation: microbiological, chemical and sensory attributes. *Food Microbiology*, 21, 351–359.

- Cobe, P. 2002. Dry-cured ham. *Restaurant Business*, 101, pp. 67–71.

- Conway, T., Creecy, J.P., Maddox, S.M., Grissom, J.E., Conkle, T.L., Shadid, T.M., Teramoto, J., San Miguel, P., Shimada, T., Ishihama, A. and Mori, H., 2014. Unprecedented high-resolution view of bacterial operon architecture revealed by RNA sequencing. *MBio*, *5*(4), pp.e01442-14.

- Cordero, N., Maza, F., Navea-Perez, H., Aravena, A., Marquez-Fontt, B., Navarrete, P., Figueroa, G., González, M., Latorre, M. and Reyes-Jara, A., 2016. Different transcriptional responses from slow and fast growth rate strains of *Listeria monocytogenes* adapted to low temperature. *Frontiers in Microbiology*, *7*.

- Cotter, P.D. and Hill, C., 2003. Surviving the acid test: responses of Gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, *67*(3), pp.429-453.

- Cotter, P.D., Ryan, S., Gahan, C.G. and Hill, C., 2005. Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated

with an ability to grow at low pH. *Applied and Environmental Microbiology*, *71*(6), pp.2832-2839.

- Cotter, P.D., Ryan, S., Gahan, C.G. and Hill, C., 2005. Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. *Applied and Environmental Microbiology*, *71*(6), pp.2832-2839.

- Czuprynski, C.J., 2005. *Listeria monocytogenes*: silage, sandwiches and science. *Animal health research reviews*, *6*(2), pp.211-217.

- D'Costa, D., Bhosle, S., N., Dhuri, R., B., Kalekar, S., Rodrigues, J., Doijad, S. P. and Barbuddhe, S. B. 2012. Occurrence and characterization of *Listeria* species isolated from the milk production chain. *Milchwissenschaft-Milk Science International*, *67*(1), pp.43–46.

- Dahl, J.U., Gray, M.J. and Jakob, U., 2015. Protein quality control under oxidative stress conditions. *Journal of Molecular Biology*, *427*(7), pp.1549-1563.

- Dawson, S.J., Evans, M.R., Willby, D., Bardwell, J., Chamberlain, N. and Lewis, D.A., 2006. *Listeria* outbreak associated with sandwich consumption from a hospital retail shop, United Kingdom. *European Surveillance*, *11*(6), pp.89-91.

- de Boer, P.A., 2010. Advances in understanding *E. coli* cell fission. *Current Opinion in Microbiology*, *13*(6), pp.730-737.

- de Boer, P.A., Crossley, R.E. and Rothfield, L.I., 1988. Isolation and properties of *minB*, a complex genetic locus involved in correct placement of the division site in *Escherichia coli*. *Journal of Bacteriology*, *170*(5), pp.2106-2112.

- de Boer, P.A., Crossley, R.E. and Rothfield, L.I., 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli. Cell*, *56*(4), pp.641-649.

- De Jesús, A.J. and Whiting, R.C., 2003. Thermal inactivation, growth, and survival studies of *Listeria monocytogenes* strains belonging to three distinct genotypic lineages. *Journal of Food Protection*, *66*(9), pp.1611-1617.

- De Nadal, E., Ammerer, G. and Posas, F., 2011. Controlling gene expression in response to stress. *Nature reviews. Genetics*, *12*(12), p.833.

- de Noordhout, C.M., Devleesschauwer, B., Angulo, F.J., Verbeke, G., Haagsma, J., Kirk, M., Havelaar, A. and Speybroeck, N., 2014. The global burden of listeriosis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *14*(11), pp.1073-1082.

- Débarbouillé, M., Dramsi, S., Dussurget, O., Nahori, M.A., Vaganay, E., Jouvion, G., Cozzone, A., Msadek, T. and Duclos, B., 2009. Characterization of a serine/threonine kinase involved in virulence of *Staphylococcus aureus*. *Journal of Bacteriology*, *191*(13), pp.4070-4081.

- den Bakker, H.C., Manuel, C.S., Fortes, E.D., Wiedmann, M. and Nightingale, K.K., 2013. Genome sequencing identifies Listeria fleischmannii subsp. coloradonensis subsp. nov., isolated from a ranch. *International journal of systematic and evolutionary microbiology*, *63*(9), pp.3257-3268.

- den Bakker, H.C., Warchocki, S., Wright, E.M., Allred, A.F., Ahlstrom, C., Manuel, C.S., Stasiewicz, M.J., Burrell, A., Roof, S., Strawn, L.K. and Fortes, E., 2014. *Listeria floridensis* sp. nov., *Listeria aquatica* sp. nov., *Listeria cornellensis* sp. nov., *Listeria riparia* sp. nov. and *Listeria grandensis* sp. nov.,

from agricultural and natural environments. *International Journal of Systematic and Evolutionary Microbiology*, *64*(6), pp.1882-1889.

- Den Besten, H.M., Mols, M., Moezelaar, R., Zwietering, M.H. and Abee, T., 2009. Phenotypic and transcriptomic analyses of mildly and severely salt-stressed *Bacillus cereus* ATCC 14579 cells. *Applied and Environmental Microbiology*, *75*(12), pp.4111-4119.

- Derra, F.A., Karlsmose, S., Monga, D.P., Mache, A., Svendsen, C.A., Félix, B., Granier, S.A., Geyid, A., Taye, G. and Hendriksen, R.S., 2013. Occurrence of *Listeria* spp. in retail meat and dairy products in the area of Addis Ababa, Ethiopia. *Foodborne Pathogens and Disease*, *10*(6), pp.577-579.

- Deutscher, J., 2008. The mechanisms of carbon catabolite repression in bacteria. *Current opinion in microbiology*, *11*(2), pp.87-93.

- DeWaal, S., Tian, X., and Plunkett, D. 2012. Outbreak alert! Analyzing foodborne outbreaks 1999-2008. Center for Science in the Public interest, Washington DC, United States, Available at www.cspinet.org. (January 19, 2017)

- Diard, M. and Hardt, W.D., 2017. Evolution of bacterial virulence. *FEMS Microbiology Reviews*, p.fux023.

- Doijad, S., Barbuddhe, S. B., Garg, S., Kalekar, S., Rodrigues, J., D'Costa, D., Bhosle, S., & Chakraborty, T. 2011. Incidence and genetic variability of *Listeria* species from three milk processing plants. *Food Control*, 22(12), pp.1-6.

- Doijad, S.P., Barbuddhe, S.B., Garg, S., Poharkar, K.V., Kalorey, D.R., Kurkure, N.V., Rawool, D.B. and Chakraborty, T., 2015. Biofilm-forming abilities of Listeria monocytogenes serotypes isolated from different sources. *PLoS One*, *10*(9), p.e0137046.

- Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of clinical microbiology*, *42*(8), pp.3819-3822.

- du Plessis, D.J.F., Nouwen, N., Driessen, A.J.M. 2011. The Sec translocase. *Biochim Biophysica Acta, 1808*(3), 851–865.

- Du, Y., Shi, W.W., He, Y.X., Yang, Y.H., Zhou, C.Z. and Chen, Y., 2011. Structures of the substrate-binding protein provide insights into the multiple compatible solute binding specificities of the *Bacillus subtilis* ABC transporter OpuC. *Biochemical Journal*, *436*(2), pp.283-289.

- Duché, O., Trémoulet, F., Glaser, P. and Labadie, J., 2002. Salt stress proteins induced in *Listeria monocytogenes*. *Applied and Environmental microbiology*, *68*(4), pp.1491-1498.

- Durack, J., Burke, T.P. and Portnoy, D.A., 2015. A prl mutation in SecY suppresses secretion and virulence defects of *Listeria monocytogenes* secA2 mutants. *Journal of Bacteriology*, *197*(5), pp.932-942.

- Durack, J., Ross, T. and Bowman, J.P., 2013. Characterisation of the transcriptomes of genetically diverse *Listeria monocytogenes* exposed to hyperosmotic and low temperature conditions reveal global stress-adaptation mechanisms. *PLoS One*, *8*(9), p.e73603.

- Dworkin, J., 2015. Ser/Thr phosphorylation as a regulatory mechanism in bacteria. *Current Opinion in Microbiology*, *24*, pp.47-52.

- Dyda, F., Klein, D.C. and Hickman, A.B., 2000. GCN5-related N-acetyltransferases: a structural overview. *Annual Review of Biophysics and Biomolecular Structure*, *29*(1), pp.81-103.

- Ells, T.C. and Hansen, L.T., 2011. Increased thermal and osmotic stress resistance in *Listeria monocytogenes* 568 grown in the presence of trehalose due to inactivation of the phosphotrehalase-encoding gene treA. *Applied and Environmental Microbiology*, *77*(19), pp.6841-6851.

- Ermolenko, D.N. and Makhatadze, G.I., 2002. Bacterial cold-shock proteins. *Cellular and Molecular Life Sciences*, *59*(11), pp.1902-1913.

- Esteban, J.I., Oporto, B., Aduriz, G., Juste, R.A. and Hurtado, A., 2009. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *BMC Veterinary Research*, *5*(1), p.2.

- Fang, G., Passalacqua, K.D., Hocking, J., Llopis, P.M., Gerstein, M., Bergman, N.H. and Jacobs-Wagner, C., 2013. Transcriptomic and phylogenetic analysis of a bacterial cell cycle reveals strong associations between gene co-expression and evolution. *BMC Genomics*, *14*(1), p.450.

- Farber, J.M. and Peterkin, P.I., 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews*, *55*(3), pp.476-511.

- Farber, J.M., Coates, F. and Daley, E., 1992. Minimum water activity requirements for the growth of *Listeria monocytogenes*. *Letters in Applied Microbiology*, *15*(3), pp.103-105.

- Farber, J.M., Peterkin, P.I., Carter, A.O., Varughese, P.V., Ashton, F.E. and Ewan, E.P., 1991. Neonatal listeriosis due to cross-infection confirmed by isoenzyme typing and DNA fingerprinting. *Journal of Infectious Diseases*, *163*(4), pp.927-928.

- Favrot, L., Blanchard, J.S. and Vergnolle, O., 2016. Bacterial GCN5-related N-acetyltransferases: From resistance to regulation. *Biochemistry*, *55*(7), pp.989-1002.

- FDA. 2012. Risk Assessment /Safety Assessment - *Listeria monocytogenes* Risk Assessment: II. Hazard Identification. Center for Food Safety and Applied Nutrition.

  http://www.fda.gov/food/scienceresearch/researchareas/riskassessmentsafetyassessment/ucm183981.htm (April 20, 2014).

- FDA-BAM. 2013. Laboratory Methods - Microbiological Methods & Bacteriological Analytical Manual (BAM). Center for Food Safety and Applied Nutrition.

  http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm

- Feehily, C. and Karatzas, K.A.G., 2012. Role of glutamate metabolism in bacterial responses towards acid and other stresses. *Journal of Applied Microbiology*, *114*(1), pp.11-24.

- Feehily, C., Finnerty, A., Casey, P.G., Hill, C., Gahan, C.G., O'Byrne, C.P. and Karatzas, K.A.G., 2014. Divergent evolution of the activity and regulation of the glutamate decarboxylase systems in *Listeria monocytogenes* EGD-e and 10403S: roles in virulence and acid tolerance. *PloS One*, *9*(11), p.e112649.

- Ferreira, A., O'Byrne, C.P. and Boor, K.J., 2001. Role of ςB in Heat, Ethanol, Acid, and Oxidative Stress Resistance and during Carbon Starvation in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *67*(10), pp.4454-4457.

- Ferreira, A., Sue, D., O'byrne, C.P. and Boor, K.J., 2003. Role of *Listeria monocytogenes* σB in survival of lethal acidic conditions and in the acquired acid tolerance response. *Applied and Environmental Microbiology*, *69*(5), pp.2692-2698.

- Ferreira, V., Wiedmann, M., Teixeira, P. and Stasiewicz, M.J., 2014. Listeria monocytogenes persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection*, *77*(1), pp.150-170

- Fleming, D.W., Cochi, S.L., MacDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V. and Reingold, A.L., 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New England Journal of Medicine*, *312*(7), pp.404-407.

- Fonseca, J. M. 2006. Postharvest handling and processing: Sources of microorganisms and impact of sanitizing procedures. In Matthews, K. R. (Ed.). Microbiology of Fresh Produce. Chapter IV, pp. 85-120. American Society of Microbiology.

- Food Safety and Inspection Service/U.S. Department of Agriculture (FSIS/USDA). 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eatfoods.http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM19733 0.pdf.

- Fox, E., Hunt, K., O'Brien, M. and Jordan, K., 2011. *Listeria monocytogenes* in Irish Farmhouse cheese processing

environments. *International Journal of Food Microbiology*, *145*, pp.S39-S45.

- Fox, E.M., Leonard, N. and Jordan, K., 2011. Physiological and transcriptional characterisation of persistent and non-persistent *Listeria monocytogenes* isolates. *Applied and Environmental Microbiology*, pp.AEM-05529.

- Fraser, K.R., Harvie, D., Coote, P.J. and O'Byrne, C.P., 2000. Identification and Characterization of an ATP Binding Cassette l-Carnitine Transporter in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *66*(11), pp.4696-4704.

- Freitag, N.E., Port, G.C. and Miner, M.D., 2009. *Listeria monocytogenes*—from saprophyte to intracellular pathogen. *Nature Reviews Microbiology*, *7*(9), pp.623-628.

- Fretz, R., Sagel, U., Ruppitsch, W., Pietzka, A.T., Stöger, A., Huhulescu, S., Heuberger, S., Pichler, J., Much, P., Pfaff, G. and Stark, K., 2010. Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009.

- FSIS Compliance Guideline (2014): Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products. Available at https://www.fsis.usda.gov/wps/wcm/connect/d3373299-50e6-47d6-a577 e74a1e549fde/Controlling-Lm-RTE-Guideline.pdf?MOD=AJPERES.

- Fulde, M., Willenborg, J., Huber, C., Hitzmann, A., Willms, D., Seitz, M., Eisenreich, W., Valentin-Weigand, P. and Goethe, R., 2014. The arginine-ornithine antiporter ArcD contributes to biological fitness of *Streptococcus suis*. *Frontiers in Cellular and Infection Microbiology*, *4*.

- Gaillot, O., Pellegrini, E., Bregenholt, S., Nair, S. and Berche, P., 2000. The ClpP serine protease is essential for the intracellular parasitism and virulence of *Listeria monocytogenes*. *Molecular Microbiology*, *35*(6), pp.1286-1294.

- Gallart-Jornet, L., Barat, J.M., Rustad, T., Erikson, U., Escriche, I. & Fito, P. 2007. Influence of brine concentration on Atlantic salmon fillet salting. *Journal of Food Engineering*, 73, pp. 9–19

- Galsworthy, S.B., Girdler, S. and Koval, S.F., 1990. Chemotaxis in *Listeria monocytogenes*. *Acta microbiologica Hungarica*, *37*(1), pp.81-85.

- Gandhi, A. and Shah, N.P., 2016. Effect of salt stress on morphology and membrane composition of *Lactobacillus acidophilus, Lactobacillus casei*, and *Bifidobacterium bifidum*, and their adhesion to human intestinal epithelial-like Caco-2 cells. *Journal of Dairy Science*, *99*(4), pp.2594-2605.

- Gandhi, M. and Chikindas, M.L., 2007. *Listeria*: a foodborne pathogen that knows how to survive. *International Journal of Food Microbiology*, *113*(1), pp.1-15.

- Gao, H. and Liu, C., 2014. Biochemical and morphological alteration of *Listeria monocytogenes* under environmental stress caused by chloramine-T and sodium hypochlorite. *Food Control*, *46*, pp.455-461.

- Gao, R. and Stock, A.M., 2009. Biological insights from structures of two-component proteins. *Annual Review of Microbiology*, *63*, pp.133-154.

- Gardan, R., Cossart, P. and Labadie, J., 2003. Identification of *Listeria monocytogenes* genes involved in salt and alkaline-pH tolerance. *Applied and Environmental Microbiology*, *69*(6), pp.3137-3143.

- Gardan, R., Duché, O., Leroy-Sétrin, S. and Labadie, J., 2003. Role of ctc from *Listeria monocytogenes* in osmotolerance. *Applied and Environmental Microbiology*, *69*(1), pp.154-161.

- Garrido, V., Torroba, L., Garcia-Jalon, I. and Vitas, A.I., 2008. Surveillance of listeriosis in Navarre, Spain, 1995-2005--epidemiological patterns and characterisation of clinical and food isolates. *European Surveillance, 13(49).* pp. *19058.*

- Garrido,V., Vitas, A.I. and García-Jalón, I. 2010. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. A. Méndez-Vilas (Ed.). Chapter III, pp. 62-93. Formatex Research Center press.

- Gawade, L., Barbuddhe, S.B. and Bhosle, S., 2010. Isolation and confirmation of *Listeria* species from seafood off Goa region by polymerase chain reaction. *Indian Journal of Microbiology*, *50*(4), pp.385-389.

- Gennis, R.B. 2013. , Introduction: The structure and composition of biomembranes. Gennis, R.B (ed), In Biomembranes: molecular structure and function. Chapter I, pp. 1-35. Springer Science & Business Media.

- Georg, J. and Hess, W.R., 2011. cis-antisense RNA, another level of gene regulation in bacteria. *Microbiology and Molecular Biology Reviews*, *75*(2), pp.286-300.

- Geourjon, C., Orelle, C., Steinfels, E., Blanchet, C., Deléage, G., Di Pietro, A. and Jault, J.M., 2001. A common mechanism for ATP hydrolysis in ABC transporter and helicase superfamilies. *Trends in Biochemical Sciences*, *26*(9), pp.539-544.

- Gianfranceschi, M., Gattuso, A., Tartaro, S. and Aureli, P., 2002. Incidence of *Listeria monocytogenes* in food and environmental samples in Italy between 1990 and 1999: serotype distribution in food, environmental and clinical samples. *European Journal of Epidemiology*, *18*(10), pp.1001-1006.

- Gill, D.A., 1933. Circling Disease: A Meningo-Encephalitis of Sheep in New Zealand. Notes on a New Species of Pathogenic Organism. *Veterinary Journal*, *89*, pp.258-270.

- Gilot, P. and André, P., 1999. *Listeria monocytogenes* possesses adhesins for fibronectin. *Infection and Immunity*, *67*(12), pp.6698-6701.

- Gilot, P., Jossin, Y. and Content, J., 2000. Cloning, sequencing and characterisation of a *Listeria monocytogenes* gene encoding a fibronectin-binding protein. *Journal of Medical Microbiology*, *49*(10), pp.887-896.

- Giotis, E.S., Blair, I.S. and McDowell, D.A., 2007. Morphological changes in *Listeria monocytogenes* subjected to sublethal alkaline stress. *International Journal of Food Microbiology*, *120*(3), pp.250-258.

- Giotis, E.S., Julotok, M., Wilkinson, B.J., Blair, I.S. and McDowell, D.A., 2008. Role of sigma B factor in the alkaline tolerance response of *Listeria monocytogenes* 10403S and cross-protection against subsequent ethanol and osmotic stress. *Journal of food protection*, *71*(7), pp.1481-1485.

- Giotis, E.S., McDowell, D.A., Blair, I.S. and Wilkinson, B.J., 2007. Role of branched-chain fatty acids in pH stress tolerance in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *73*(3), pp.997-1001.

- Giotis, E.S., Muthaiyan, A., Blair, I.S., Wilkinson, B.J. and McDowell, D.A., 2008. Genomic and proteomic analysis of the Alkali-Tolerance Response (AlTR) in *Listeria monocytogenes* 10403S. *BMC Microbiology*, *8*(1), p.102.

- Giotis, E.S., Muthaiyan, A., Natesan, S., Wilkinson, B.J., Blair, I.S. and McDowell, D.A., 2010. Transcriptome analysis of alkali shock and alkali adaptation in *Listeria monocytogenes* 10403S. *Foodborne Pathogens and Disease*, *7*(10), pp.1147-1157.

- Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., Brandt, P., Chakraborty, T. and Charbit, A., 2001. Comparative genomics of *Listeria* species. *Science*, *294*(5543), pp.849-852.

- Gómez, D., Azón, E., Marco, N., Carramiñana, J.J., Rota, C., Ariño, A. and Yangüela, J., 2014. Antimicrobial resistance of *Listeria monocytogenes* and Listeria innocua from meat products and meat-processing environment. *Food Microbiology*, *42*, pp.61-65.

- Gómez, N.C., Abriouel, H., Ennahar, S. and Gálvez, A., 2013. Comparative proteomic analysis of *Listeria monocytogenes* exposed to enterocin AS-48 in planktonic and sessile states. *International Journal of Food Microbiology*, *167*(2), pp.202-207.

- Goulas, A.E. & Kontominas, M.G. 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *Food Chemistry*, 93, pp. 511–520.

- Goulas, A.E. & Kontominas, M.G. 2007. Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): biochemical and sensory attributes. *Food Chemistry*, 100, pp.287–296.

- Goulet, V., Hebert, M., Hedberg, C., Laurent, E., Vaillant, V., De Valk, H. and Desenclos, J.C., 2012. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clinical Infectious Diseases*, *54*(5), pp.652-660.

- Goulet, V., Hedberg, C., Le Monnier, A. and De Valk, H., 2008. Increasing incidence of listeriosis in France and other European countries. *Emerging Infectious Diseases*, *14*(5), p.734.

- Graves, L.M. and Swaminathan, B., 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *International Journal of Food Microbiology*, *65*(1), pp.55-62.

- Gray, M.J., Freitag, N.E. and Boor, K.J., 2006. How the bacterial pathogen *Listeria monocytogenes* mediates the switch from environmental Dr. Jekyll to pathogenic Mr. Hyde. *Infection and Immunity*, *74*(5), pp.2505-2512.

- Gray, M.L. and Killinger, A.H., 1966. *Listeria monocytogenes* and listeric infections. *Bacteriological reviews*, *30*(2), p.309.

- Gründling, A., Burrack, L.S., Bouwer, H.A. and Higgins, D.E., 2005. *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(33), pp.12318-12323.

- Guerzoni, M.E., Lanciotti, R. and Cocconcelli, P.S., 2001. Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. *Microbiology*, *147*(8), pp.2255-2264.

- Guillet, C., Join-Lambert, O., Le Monnier, A., Leclercq, A., Mechaï, F., Mamzer-Bruneel, M.F., Bielecka, M.K., Scortti, M., Disson, O., Berche, P. and Vazquez-

Boland, J., 2010. Human listeriosis caused by *Listeria ivanovii*. *Emerging Infectious Diseases*, *16*(1), p.136.

- Hain, T., Hossain, H., Chatterjee, S.S., Machata, S., Volk, U., Wagner, S., Brors, B., Haas, S., Kuenne, C.T., Billion, A. and Otten, S., 2008. Temporal transcriptomic analysis of the *Listeria monocytogenes* EGD-e σ B regulon. *BMC Microbiology*, *8*(1), p.20.

- Hain, T., Steinweg, C. and Chakraborty, T., 2006. Comparative and functional genomics of Listeria spp. *Journal of biotechnology*, *126*(1), pp.37-51.

- Harris, L.J., Beuchat, L.R., Kajs, T.M., Ward, T.E. and Taylor, C.H., 2001. Efficacy and reproducibility of a produce wash in killing *Salmonella* on the surface of tomatoes assessed with a proposed standard method for produce sanitizers. *Journal of Food Protection*, *64*(10), pp.1477-1482.

- Harry, E., Monahan, L. and Thompson, L., 2006. Bacterial cell division: the mechanism and its precison. *International Review of Cytology*, *253*, pp.27-94.

- Havelaar, A.H., Van Rosse, F., Bucura, C., Toetenel, M.A., Haagsma, J.A., Kurowicka, D., Heesterbeek, J.H.A., Speybroeck, N., Langelaar, M.F., van der Giessen, J.W. and Cooke, R.M., 2010. Prioritizing emerging zoonoses in the Netherlands. *PloS One*, *5*(11), p.e13965.

- Hecker, M., Schumann, W. and Völker, U., 1996. Heat-shock and general stress response in *Bacillus subtilis*. *Molecular Microbiology*, *19*(3), pp.417-428.

- Hefford, M.A., D'aoust, S., Cyr, T.D., Austin, J.W., Sanders, G., Kheradpir, E. and Kalmokoff, M.L., 2005. Proteomic and microscopic analysis of biofilms formed by *Listeria monocytogenes* 568. *Canadian Journal of Microbiology*, *51*(3), pp.197-208.

- Heide, T.V.D., 2002. On the osmotic signal and osmosensing mechanism of an ABC transport system. *System*, *93*, pp.12786-12790.

- Heide, T.V.D., Stuart, M.C. and Poolman, B., 2001. On the osmotic signal and osmosensing mechanism of an ABC transport system for glycine betaine. *The EMBO Journal*, *20*(24), pp.7022-7032.

- Hill, C., Cotter, P.D., Sleator, R.D. and Gahan, C.G., 2002. Bacterial stress response in *Listeria monocytogenes*: jumping the hurdles imposed by minimal processing. *International Dairy Journal*, *12*(2), pp.273-283.

- Hingston, P., Chen, J., Allen, K., Hansen, L.T. and Wang, S., 2017. Strand specific RNA-sequencing and membrane lipid profiling reveals growth phase-dependent cold stress response mechanisms in *Listeria monocytogenes*. *PloS One*, *12*(6), p.e0180123.

- Hingston, P., Chen, J., Dhillon, B.K., Laing, C., Bertelli, C., Gannon, V., Tasara, T., Allen, K., Brinkman, F.S., Hansen, L.T. and Wang, S., 2017. Genotypes Associated with *Listeria monocytogenes* Isolates Displaying Impaired or Enhanced Tolerances to Cold, Salt, Acid, or Desiccation Stress. *Frontiers in Microbiology*, *8*.

- Hoffmann, S., Maculloch, B. and Batz, M., 2015. Economic burden of major foodborne illnesses acquired in the United States. *Current Politics and Economics of the United States, Canada and Mexico*, *17*(4), p.543.Available at: http://www.ers.usda.gov/media/1837786/eib140_summary.pdf.

- Hoffmann, T. and Bremer, E., 2017. Guardians in a stressful world: the Opu family of compatible solute transporters from Bacillus subtilis. *Biological Chemistry*, *398*(2), pp.193-214.

- Holch, A., Webb, K., Lukjancenko, O., Ussery, D., Rosenthal, B.M. and Gram, L., 2013. Genome sequencing identifies two nearly unchanged strains of persistent Listeria monocytogenes isolated at two different fish processing plants sampled 6 years apart. *Applied and environmental microbiology*, *79*(9), pp.2944-2951.

- Hong, H.J. and Hong, 2016. Microarray Analysis to Monitor Bacterial Cell Wall Homeostasis *Bacterial Cell Wall Homeostasis*. Hong H.J. (Ed) Chapter III, pp. 31-46. Springer New York.

- Huillet, E., Velge, P., Vallaeys, T. and Pardon, P., 2005. LadR, a new PadR-related transcriptional regulator from *Listeria monocytogenes*, negatively regulates the expression of the multidrug efflux pump MdrL. *FEMS Microbiology Letters*, *254*(1), pp.87-94.

- Hurst, A., 1984. Revival of vegetative bacteria after sublethal heating. *The Revival of Injured Microbes*.

- Huynh, T.N., Luo, S., Pensinger, D., Sauer, J.D., Tong, L. and Woodward, J.J., 2015. An HD-domain phosphodiesterase mediates cooperative hydrolysis of c-di-AMP to affect bacterial growth and virulence. *Proceedings of the National Academy of Sciences*, *112*(7), pp.E747-E756.

- Ichihara, K.I. and Fukubayashi, Y., 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research*, *51*(3), pp.635-640.

- Ignatova, M., Guével, B., Com, E., Haddad, N., Rossero, A., Bogard, P., Prévost, H. and Guillou, S., 2013. Two-dimensional fluorescence difference gel electrophoresis analysis of *Listeria monocytogenes* submitted to a redox shock. *Journal of Proteomics*, *79*, pp.13-27.

- Ivanek, R., Gröhn, Y.T., Tauer, L.W. and Wiedmann, M., 2005. The cost and benefit of *Listeria monocytogenes* food safety measures. *Critical Reviews in food Science and Nutrition*, *44*(7-8), pp.513-523.

- Jay, J.M., 2000. High-temperature food preservation and characteristics of thermophilic microorganisms. In *Modern Food Microbiology* . Jay J. M. (Ed), Chapter XV, pp. 347-369. Springer New York.

- Jones, T.H., Vail, K.M. and McMullen, L.M., 2013. Filament formation by foodborne bacteria under sublethal stress. *International Journal of Food Microbiology*, *165*(2), pp.97-110.

- Justice, S.S., Hunstad, D.A., Seed, P.C. and Hultgren, S.J., 2006. Filamentation by *Escherichia coli* subverts innate defences during urinary tract infection. *Proceedings of the National Academy of Sciences*, *103*(52), pp.19884-19889.

- Jydegaard-Axelsen, A.M., Aaes-Jørgensen, A., Koch, A.G., Jensen, J.S. and Knøchel, S., 2005. Changes in growth, rRNA content, and cell morphology of *Listeria monocytogenes* induced by CO_2 up-and downshift. *International Journal of Food Microbiology*, *98*(2), pp.145-155.

- Kallipolitis, B.H. and Ingmer, H., 2001. *Listeria monocytogenes* response regulators important for stress tolerance and pathogenesis. *FEMS Microbiology Letters*, *204*(1), pp.111-115.

- Karunasagar, I. and Karunasagar, I., 2000. *Listeria* in tropical fish and fishery products. *International Journal of Food Microbiology*, *62*(3), pp.177-181.

- Kasper, S., Huhulescu, S., Auer, B., Heller, I., Karner, F., Würzner, R., Wagner, M. and Allerberger, F., 2009. Epidemiology of listeriosis in Austria. *Wiener Klinische Wochenschrift*, *121*(3), pp.113-119.

- Kazmierczak, M.J., Mithoe, S.C., Boor, K.J. and Wiedmann, M., 2003. Listeria monocytogenes σB regulates stress response and virulence functions. *Journal of Bacteriology*, *185*(19), pp.5722-5734.

- Kempf, B. and Bremer, E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Archives of Microbiology*, *170*(5), pp.319-330.

- Kennedy, J., Jackson, V., Blair, I.S., McDowell, D.A., Cowan, C. and Bolton, D.J., 2005. Food safety knowledge of consumers and the microbiological and temperature status of their refrigerators. *Journal of food protection*, *68*(7), pp.1421-1430.

- Khor, C.C. and Hibberd, M.L., 2011. Revealing the molecular signatures of host-pathogen interactions. *Genome biology*, *12*(10), p.229-237.

- Kieboom, J., Kusumaningrum, H.D., Tempelaars, M.H., Hazeleger, W.C., Abee, T. and Beumer, R.R., 2006. Survival, elongation, and elevated tolerance of *Salmonella enterica* serovar Enteritidis at reduced water activity. *Journal of Food Protection*, *69*(11), pp.2681-2686.

- Kim, N.H., Cho, T.J. and Rhee, M.S., 2017. Sodium Chloride Does Not Ensure Microbiological Safety of Foods: Cases and Solutions. *Advances in Applied Microbiology*.

- Kocaman, N. and Sarimehmetoglu, B., 2016. Stress responses of *Listeria monocytogenes*. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *63*(4), pp.421-427.

- Koch, J. and Stark, K., 2006. Significant increase of listeriosis in Germany--epidemiological patterns 2001-2005. *Euro surveillance: bulletin Europeen sur les maladies transmissibles= European communicable disease bulletin*, *11*(6), pp.85-88.

- Kramarenko, T., Roasto, M., Meremäe, K., Kuningas, M., Põltsama, P. and Elias, T., 2013. *Listeria monocytogenes* prevalence and serotype diversity in various foods. *Food Control*, *30*(1), pp.24-29.

- Krawczyk-Balska, A. and Lipiak, M., 2013. Critical role of a ferritin-like protein in the control of *Listeria monocytogenes* cell envelope structure and stability under β-lactam pressure. *PloS One*, *8*(10), p.e77808.

- Krawczyk-Balska, A., Korsak, D. and Popowska, M., 2014. The surface protein Lmo1941 with LysM domain influences cell wall structure and susceptibility of *Listeria monocytogenes* to cephalosporins. *FEMS Microbiology letters*, *357*(2), pp.175-183.

- Krishna, U., Desai, M.W. & G., D. V. (1966). Listeriosis: a clinico-bacteriological study. *Journal of obstetrics and Gynecology of India*, 16(1), pp.304–310.

- Kruger, N.J., 1994. The Bradford method for protein quantitation. *Basic Protein and Peptide Protocols*, *32*, pp.9-15.

- Krulwich, T.A., Sachs, G. and Padan, E., 2011. Molecular aspects of bacterial pH sensing and homeostasis. *Nature Reviews Microbiology*, *9*(5), pp.330-343.

- Kuenne, C., Billion, A., Mraheil, M.A., Strittmatter, A., Daniel, R., Goesmann, A., Barbuddhe, S., Hain, T. and Chakraborty, T., 2013. Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome. *BMC Genomics*, *14*(1), p.47.

- Lamont, R.F., Sobel, J., Mazaki-Tovi, S., Kusanovic, J.P., Vaisbuch, E., Kim, S.K., Uldbjerg, N. and Romero, R., 2011. Listeriosis in human pregnancy: a systematic review. *Journal of Perinatal Medicine*, *39*(3), pp.227-236..

- Lawrence, T.E., Dikeman, M.E., Hunt, M.C., Kastner, C.L. and Johnson, D.E., 2003. Effects of calcium salts on beef longissimus quality. *Meat Science*, *64*(3), pp.299-308.

- Lawrence, T.E., Dikeman, M.E., Stephens, J.W., Obuz, E. & Davis, J.R. 2003. In situ investigation of the calcium-induced proteolytic and salting-in mechanisms causing tenderization in calcium-enhanced muscle. *Meat Science*, 66, pp.69–75.

- Lebre, P.H., De Maayer, P. and Cowan, D.A. (2017). Xerotolerant bacteria: surviving through a dry spell. *Nature Reviews Microbiology*, *15*(5), pp.285-296.

- Lee, K.I., French, N.P., Jones, G., Hara-Kudo, Y., Iyoda, S., Kobayashi, H., Sugita-Konishi, Y., Tsubone, H. and Kumagai, S., 2012. Variation in stress resistance patterns among *stx* genotypes and genetic lineages of shiga toxin-producing *Escherichia coli* O157. *Applied and Environmental Microbiology*, *78*(9), pp.3361-3368.

- Lee, S., Sowa, M.E., Watanabe, Y.H., Sigler, P.B., Chiu, W., Yoshida, M. and Tsai, F.T., 2003. The structure of ClpB: a molecular chaperone that rescues proteins from an aggregated state. *Cell*, *115*(2), pp.229-240.

- Leistner, L., 2000. Basic aspects of food preservation by hurdle technology. *International journal of food microbiology*, *55*(1-3), pp.181-186.

- Leong, D., Alvarez-Ordóñez, A. and Jordan, K., 2014. Monitoring occurrence and persistence of Listeria monocytogenes in foods and food processing environments in the Republic of Ireland. *Frontiers in microbiology*, *5*, p.436.

- Lianou, A. and Sofos, J.N., 2007. A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. *Journal of Food Protection*, *70*(9), pp.2172-2198.

- Lianou, A., Stopforth, J.D., Yoon, Y., Wiedmann, M. and Sofos, J.N., 2006. Growth and stress resistance variation in culture broth among *Listeria monocytogenes* strains of various serotypes and origins. *Journal of Food Protection*, *69*(11), pp.2640-2647.

- Liu, D. 2008. Epidemiology. In Handbook of *Listeria monocytogenes*. Liu, D. (Ed), pp. 27-60. CRC press.

- Liu, D., Lawrence, M.L., Ainsworth, A.J. and Austin, F.W., 2005. Comparative assessment of acid, alkali and salt tolerance in Listeria monocytogenes virulent and avirulent strains. *FEMS Microbiology Letters*, *243*(2), pp.373-378.

- Liu, S., Graham, J.E., Bigelow, L., Morse, P.D. and Wilkinson, B.J., 2002. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. *Applied and Environmental Microbiology*, *68*(4), pp.1697-1705.

- Liu, X., Basu, U., Miller, P. and McMullen, L.M., 2014. Stress response and adaptation of *Listeria monocytogenes* 08-5923 exposed to a sublethal dose of carnocyclin A. *Applied and Environmental Microbiology*, *80*(13), pp.3835-3841.

- Liu, X., Miller, P., Basu, U. and McMullen, L.M., 2014. Sodium chloride-induced filamentation and alternative gene expression of fts, murZ, and gnd in

Listeria monocytogenes 08-5923 on vacuum-packaged ham. *FEMS Microbiology Letters*, *360*(2), pp.152-156.

- Lomonaco, S., Decastelli, L., Nucera, D., Gallina, S., Bianchi, D.M. and Civera, T., 2009. Listeria monocytogenes in Gorgonzola: subtypes, diversity and persistence over time. *International journal of food microbiology*, *128*(3), pp.516-520.

- Lou, Y. and Yousef, A.E., 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Applied and Environmental Microbiology*, *63*(4), pp.1252-1255.

- Lucera, A., Costa, C., Conte, A. and Del Nobile, M.A., 2012. Food applications of natural antimicrobial compounds. *Frontiers in Microbiology*, *3*.

- Luna, G., Aparicio, R. & García-González, D.L. 2006. A tentative characterization of white dry-cured hams from Teruel (Spain) by SPME-GC. *Food Chemistry*, 97, pp. 621–630.

- Luo, G., Huang, L., Su, Y., Qin, Y., Xu, X., Zhao, L. and Yan, Q., 2016. flrA, flrB and flrC regulate adhesion by controlling the expression of critical virulence genes in *Vibrio alginolyticus*. *Emerging Microbes & Infections*, *5*(8), p.e85.

- Madar, D., Dekel, E., Bren, A. and Alon, U., 2011. Negative auto-regulation increases the input dynamic-range of the arabinose system of *Escherichia coli*. *BMC Systems Biology, 5*(1), p.111.

- Madeo, M., O'Riordan, N., Fuchs, T.M., Utratna, M., Karatzas, K.A.G. and O'Byrne, C.P., 2011. Thiamine plays a critical role in the acid tolerance of *Listeria monocytogenes*. *FEMS Microbiology Letters*, *326*(2), pp.137-143.

- Magalhães, R., Ferreira, V., Brandão, T.R., Palencia, R.C., Almeida, G. and Teixeira, P., 2016. Persistent and non-persistent strains of *Listeria monocytogenes*: A focus on growth kinetics under different temperature, salt, and pH conditions and their sensitivity to sanitizers. *Food microbiology*, *57*, pp.103-108.

- Mahajan, M.R., 1936. Some observations on circling disease of sheep in Hyderabad state. *Indian Journal of Veterinary Science and Animal Husbandry*, *5*, pp.350-354.

- Makariti, I.P., Printezi, A., Kapetanakou, A.E., Zeaki, N. and Skandamis, P.N., 2015. Investigating boundaries of survival, growth and expression of genes associated with stress and virulence of *Listeria monocytogenes* in response to acid and osmotic stress. *Food microbiology*, *45*, pp.231-244.

- Marriott, N. and Gravani, R.B., 2006. Cleaning Compounds. In *Principles of food sanitation*. Marriott, N. and Gravani, R.B (Ed), Chapter VI, pp. 141-164. Springer Science & Business Media.

- Martin, R.G. and Rosner, J.L., 2001. The AraC transcriptional activators. *Current Opinion in Microbiology*, *4*(2), pp.132-137.

- Martins, E.A. and Germano, P.M.L., 2011. *Listeria monocytogenes* in ready-to-eat, sliced, cooked ham and salami products, marketed in the city of São Paulo, Brazil: Occurrence, quantification, and serotyping. *Food Control*, *22*(2), pp.297-302.

- Mastronicolis, S.K., Berberi, A., Diakogiannis, I., Petrova, E., Kiaki, I., Baltzi, T. and Xenikakis, P., 2010. Alteration of the phospho-or neutral lipid content and

fatty acid composition in *Listeria monocytogenes* due to acid adaptation mechanisms for hydrochloric, acetic and lactic acids at pH 5.5 or benzoic acid at neutral pH. *Antonie Van Leeuwenhoek*, *98*(3), pp.307-316.

- Mastronicolis, S.K., Boura, A., Karaliota, A., Magiatis, P., Arvanitis, N., Litos, C., Tsakirakis, A., Paraskevas, P., Moustaka, H. and Heropoulos, G., 2006. Effect of cold temperature on the composition of different lipid classes of the foodborne pathogen Listeria monocytogenes: focus on neutral lipids. *Food microbiology*, *23*(2), pp.184-194.

- Mataragas, M., Greppi, A., Rantsiou, K. and Cocolin, L., 2014. Gene transcription patterns of pH-and salt-stressed *Listeria monocytogenes* cells in simulated gastric and pancreatic conditions. *Journal of Food Protection*, *77*(2), pp.254-261.

- Mataragas, M., Rovetto, F., Bellio, A., Alessandria, V., Rantsiou, K., Decastelli, L. and Cocolin, L., 2015. Differential gene expression profiling of *Listeria monocytogenes* in Cacciatore and Felino salami to reveal potential stress resistance biomarkers. *Food Microbiology*, *46*, pp.408-417.

- McClure, P.J., Beaumont, A.L., Sutherland, J.P. and Roberts, T.A., 1997. Predictive modelling of growth of *Listeria monocytogenes* The effects on growth of NaCl, pH, storage temperature and NaNO2. *International Journal of Food Microbiology*, *34*(3), pp.221-232.

- McClure, R., Balasubramanian, D., Sun, Y., Bobrovskyy, M., Sumby, P., Genco, C.A., Vanderpool, C.K. and Tjaden, B., 2013. Computational analysis of bacterial RNA-Seq data. *Nucleic Acids Research*, *41*(14), pp.140-152.

- McLauchlin, J., 1997. The pathogenicity of *Listeria monocytogenes*: a public health perspective. *Reviews in Medical Microbiology*, *8*(1), pp.1-14.

- McMahon, M.A.S., Xu, J., Moore, J.E., Blair, I.S. and McDowell, D.A., 2007. Environmental stress and antibiotic resistance in food-related pathogens. *Applied and Environmental Microbiology*, *73*(1), pp.211-217.

- Meier, A.B., Guldimann, C., Markkula, A., Pöntinen, A., Korkeala, H. and Tasara, T., 2017. Comparative Phenotypic and Genotypic Analysis of Swiss and Finnish *Listeria monocytogenes* Isolates with Respect to Benzalkonium Chloride Resistance. *Frontiers in Microbiology*, *8*.

- Melo, J., Andrew, P.W. and Faleiro, M.L., 2013. Different assembly of acid and salt tolerance response in two dairy *Listeria monocytogenes* wild strains. *Archives of Microbiology*, *195*(5), pp.339-348.

- Melo, J., Andrew, P.W. and Faleiro, M.L., 2015. *Listeria monocytogenes* in cheese and the dairy environment remains a food safety challenge: the role of stress responses. *Food Research International*, *67*, pp.75-90.

- Melo, J., Schrama, D., Andrew, P.W. and Faleiro, M.L., 2013. Proteomic analysis shows that individual *Listeria monocytogenes* strains use different strategies in response to gastric stress. *Foodborne pathogens and disease*, *10*(2), pp.107-119.

- Merrell, D.S. and Camilli, A., 2002. Acid tolerance of gastrointestinal pathogens. *Current Opinion in Microbiology*, *5*(1), pp.51-55.

- Merritt, M. E., Lawrence, A. M. & Donaldson, J. R. 2010. Comparative study of the effect of bile on the *Listeria monocytogenes* virulent strain EGD-e and avirulent strain HCC23. *Archives of Clinical Microbiology* 1, pp.4–9.

- Metselaar, K.I., den Besten, H.M., Abee, T., Moezelaar, R. and Zwietering, M.H., 2013. Isolation and quantification of highly acid resistant variants of *Listeria monocytogenes*. *International Journal of Food Microbiology*, *166*(3), pp.508-514.

- Metselaar, K.I., den Besten, H.M., Boekhorst, J., van Hijum, S.A., Zwietering, M.H. and Abee, T., 2015. Diversity of acid stress resistant variants of *Listeria monocytogenes* and the potential role of ribosomal protein S21 encoded by rpsU. *Frontiers in Microbiology*, *6*.

- MHFW. Food safety and standards authority of India 2011. India.

- Michaux, C., Martini, C., Shioya, K., Lecheheb, S.A., Budin-Verneuil, A., Cosette, P., Sanguinetti, M., Hartke, A., Verneuil, N. and Giard, J.C., 2012. CspR, a cold shock RNA-binding protein involved in the long-term survival and the virulence of *Enterococcus faecalis*. *Journal of Bacteriology*, *194*(24), pp.6900-6908.

- Michel, E., Stephan, R. and Tasara, T., 2011. The lmo0501 gene coding for a putative transcription activator protein in *Listeria monocytogenes* promotes growth under cold, osmotic and acid stress conditions. *Food Microbiology*, *28*(7), pp.1261-1265.

- Miladi, H., Bakhrouf, A. and Ammar, E., 2013. Cellular lipid fatty acid profiles of reference and food isolates *Listeria monocytogenes* as a response to refrigeration and freezing stress. *Journal of Food Biochemistry*, *37*(2), pp.136-143.

- Miladi, H., Elabed, H., Slama, R.B., Rhim, A. and Bakhrouf, A., 2017. Molecular analysis of the role of osmolyte transporters opuCA and betL in *Listeria monocytogenes* after cold and freezing stress. *Archives of Microbiology*, *199*(2), pp.259-265.

- Moorhead, S.M. and Dyes, G.A. 2004. Influence of the sigB gene on the cold stress survival and subsequent recovery of two *Listeria monocytogenes* serotypes. *International Journal Food Microbiology*., 91, pp.63–72.

- Moorman, M., Nettleton, W., Ryser, E., Linz, J. and Pestka, J., 2005. Altered sensitivity to a quaternary ammonium sanitizer in stressed *Listeria innocua. Journal of Food Protection*, *68*(8), pp.1659-1663.

- Morris, K.V. and Mattick, J.S., 2014. The rise of regulatory RNA. *Nature Reviews. Genetics*, *15*(6), p.423.

- Mraheil, M.A., Billion, A., Mohamed, W., Mukherjee, K., Kuenne, C., Pischimarov, J., Krawitz, C., Retey, J., Hartsch, T., Chakraborty, T. and Hain, T., 2011. The intracellular sRNA transcriptome of *Listeria monocytogenes* during growth in macrophages. *Nucleic Acids Research*, *39*(10), pp.4235-4248.

- Mraheil, M.A., Frantz, R., Teubner, L., Wendt, H., Linne, U., Wingerath, J., Wirth, T. and Chakraborty, T., 2017. Requirement of the RNA-binding protein SmpB during intracellular growth of *Listeria monocytogenes*. *International Journal of Medical Microbiology*, *307*(3), pp.166-173

- Mullapudi, S., Siletzky, R.M. and Kathariou, S., 2008. Heavy-metal and benzalkonium chloride resistance of *Listeria monocytogenes* isolates from the environment of turkey-processing plants. *Applied and environmental microbiology*, *74*(5), pp.1464-1468.

- Müller, A., Rychli, K., Muhterem-Uyar, M., Zaiser, A., Stessl, B., Guinane, C.M., Cotter, P.D., Wagner, M. and Schmitz-Esser, S., 2013. Tn6188-a novel transposon in *Listeria monocytogenes* responsible for tolerance to benzalkonium chloride. *PLoS One*, *8*(10), p.e76835.

- Mulrooney, S.B. and Hausinger, R.P., 2004. Nickel uptake and utilization by microorganisms. *FEMS Microbiology Reviews*, *27*(2-3), pp.239-261.

- Murga, M.L., Cabrera, G.M., De Valdez, G., Disalvo, A. and Seldes, A.M., 2000. Influence of growth temperature on cryotolerance and lipid composition of *Lactobacillus acidophilus*. *Journal of Applied Microbiology*, *88*(2), pp.342-348.

- Murray, E.G.D., Webb, R.A. and Swann, M.B.R., 1926. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. spp.). *The Journal of Pathology*, *29*(4), pp.407-439.

- Nair, S., Milohanic, E. and Berche, P., 2000. ClpC ATPase is required for cell adhesion and invasion of *Listeria monocytogenes*. *Infection and Immunity*, *68*(12), pp.7061-7068.

- Najjar, M. B., Chikindas, M., and Montville, T. J. 2007. Changes in *Listeria monocytogenes* membrane fluidity in response to temperature stress. *Applied and Environmental Microbiology, 73* (20), pp. 6429-6435.

- Nakamura, H., Takakura, K.I., Sone, Y., Itano, Y. and Nishikawa, Y., 2013. Biofilm formation and resistance to benzalkonium chloride in *Listeria monocytogenes* isolated from a fish processing plant. *Journal of Food Protection*, *76*(7), pp.1179-1186.

- Nakano, S., Fukaya, M. and Horinouchi, S., 2006. Putative ABC transporter responsible for acetic acid resistance in *Acetobacter aceti*. *Applied and Environmental Microbiology*, *72*(1), pp.497-505.

- National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; (1988) – (Cited- 2017). Available from: https://www.ncbi.nlm.nih.gov/

- NCDC, 2016. The mandate of the Institute. National Centre for Disease Control.

- Nelson, K. E., D. E. Fouts, E. F. Mongodin, J. Ravel, R. T. DeBoy, J. F. Kolonay, D. A. Rasko, S. V. Angiuoli, S. R. Gill, I. T. Paulsen, J. Peterson, O. White, W. C. Nelson, W. Nierman, M. J. Beanan, L. M. Brinkac, S. C. Daugherty, R. J. Dodson, A. S. Durkin, R. Madupu, D. H. Haft, J. Selengut, S. Van Aken, H. Khouri, N. Fedorova, H. Forberger, B. Tran, S. Kathariou, L. D. Wonderling, G. A. Uhlich, D. O. Bayles, J. B. Luchansky, and C. M. Fraser. 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic acid Research, 32*(8), pp.2386-2395.

- Netzler, N.E. and White, P.A. 2017. Introduction. In Foodborne Viral Pathogens. Netzler, N.E. and White, P.A. (Ed). Chaper I, pp. 1-4. CRC Press.

- Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F. and van der Giessen, J., 2010. Food-borne diseases—the challenges of 20years ago still persist while new

ones continue to emerge. *International Journal of Food Microbiology*, *139*, pp.S3-S15.

- NicAogáin, K. and O'Byrne, C.P., 2016. The Role of Stress and Stress Adaptations in Determining the Fate of the Bacterial Pathogen *Listeria monocytogenes* in the Food Chain. *Frontiers in Microbiology*, *7*.

- Nielsen, P.K., Andersen, A.Z., Mols, M., van der Veen, S., Abee, T. and Kallipolitis, B.H., 2012. Genome-wide transcriptional profiling of the cell envelope stress response and the role of LisRK and CesRK in *Listeria monocytogenes. Microbiology*, *158*(4), pp.963-974.

- Nyfeldt, A., 1929. Etiologie de la mononucleose infectieuse. *Comptes Rendus des Seances de la Societe de Biologie*, 101, 590-591.

- Okada, Y., Makino, S.I., Tobe, T., Okada, N. and Yamazaki, S., 2002. Cloning of rel from *Listeria monocytogenes* as an osmotolerance involvement gene. *Applied and Environmental Microbiology*, *68*(4), pp.1541-1547.

- Oliver, H.F., Orsi, R.H., Ponnala, L., Keich, U., Wang, W., Sun, Q., Cartinhour, S.W., Filiatrault, M.J., Wiedmann, M. and Boor, K.J., 2009. Deep RNA sequencing of *L. monocytogenes* reveals overlapping and extensive stationary phase and sigma B-dependent transcriptomes, including multiple highly transcribed noncoding RNAs. *BMC Genomics*, *10*(1), p.641.

- Olsen, S.J., Patrick, M., Hunter, S.B., Reddy, V., Kornstein, L., MacKenzie, W.R., Lane, K., Bidol, S., Stoltman, G.A., Frye, D.M. and Lee, I., 2005. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clinical Infectious Diseases*, *40*(7), pp.962-967.

- Padan, E., Bibi, E., Ito, M. and Krulwich, T.A., 2005. Alkaline pH homeostasis in bacteria: new insights. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1717*(2), pp.67-88.

- Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J. and Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, *19*(3), p.407-423.

- Panagou, E.Z., 2006. Greek dry-salted olives: Monitoring the dry-salting process and subsequent physico-chemical and microbiological profile during storage under different packing conditions at 4 and 20 C. *LWT-Food Science and Technology*, *39*(4), pp.323-330.

- Parihar, V.S., Barbuddhe, S.B., Danielsson-Tham, M.L. and Tham, W., 2008. Isolation and characterization of *Listeria* species from tropical seafoods. *Food Control*, *19*(6), pp.566-569.

- Pascual, C., Robinson, T.P., Ocio, M.J., Aboaba, O.O. and Mackey, B.M., 2001. The effect of inoculum size and sublethal injury on the ability of Listeria monocytogenes to initiate growth under suboptimal conditions. *Letters in Applied Microbiology*, *33*(5), pp.357-361.

- Pastorino, A.J., Hansen, C.L. & McMahon, D.J. 2003. Effect of salt on structure–function relationships of cheese. *Journal of Dairy Science*, 86, pp.60–69.

- Payne, A., Schmidt, T.B., Nanduri, B., Pendarvis, K., Pittman, J.R., Thornton, J.A., Grissett, J. and Donaldson, J.R., 2013. Proteomic analysis of the response of *Listeria monocytogenes* to bile salts under anaerobic conditions. *Journal of Medical Microbiology*, *62*(1), pp.25-35.

- Pérez-Rodríguez, F., Valero, A., Carrasco, E., García, R.M. and Zurera, G., 2008. Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Science & Technology*, *19*(3), pp.131-144.

- Perroud, B.E.R.T.R.A.N.D. and Le Rudulier, D.A.N.I.E.L., 1985. Glycine betaine transport in *Escherichia coli*: osmotic modulation. *Journal of Bacteriology*, *161*(1), pp.393-401.

- Phan-Thanh, L. and Jansch, L., 2006. Elucidation of mechanisms of acid stress in *Listeria monocytogenes* by proteomic analysis. *Methods of biochemical analysis*, *49*, p.75.

- Phan-Thanh, L. and Montagne, A., 1998. Physiological and biochemical aspects of the acid survival of *Listeria monocytogenes*. *The Journal of General and Applied microbiology*, *44*(3), pp.183-191.

- Pianetti, A., Battistelli, M., Citterio, B., Parlani, C., Falcieri, E. and Bruscolini, F., 2009. Morphological changes of *Aeromonas hydrophila* in response to osmotic stress. *Micron*, *40*(4), pp.426-433.

- Pirie, J.H., 1940. *Listeria*: Change of Name for a Genus of Bacteria. *Nature*, *145*.

- Pittman, J.R., Buntyn, J.O., Posadas, G., Nanduri, B., Pendarvis, K. and Donaldson, J.R., 2014. Proteomic analysis of cross protection provided between cold and osmotic stress in *Listeria monocytogenes*. *Journal of Proteome Research*, *13*(4), pp.1896-1904.

- Pleitner, A.M., Trinetta, V., Morgan, M.T., Linton, R.L. and Oliver, H.F., 2014. Transcriptional and phenotypic responses of *Listeria monocytogenes* to chlorine dioxide. *Applied and Environmental Microbiology*, *80*(9), pp.2951-2963.

- Poimenidou, S.V., Chatzithoma, D.N., Nychas, G.J. and Skandamis, P.N., 2016. Adaptive response of *Listeria monocytogenes* to heat, salinity and low pH, after habituation on cherry tomatoes and lettuce leaves. *PloS one*, *11*(10), p.e0165746.

- Polyak, S.W., Abell, A.D., Wilce, M.C.J., Zhang, L. and Booker, G.W., 2012. Structure, function and selective inhibition of bacterial acetyl-coa carboxylase. *Applied Microbiology and Biotechnology*, *93*(3), pp.983-992.

- Ponnusamy, D., Hartson, S.D. and Clinkenbeard, K.D., 2011. Intracellular *Yersinia pestis* expresses general stress response and tellurite resistance proteins in mouse macrophages. *Veterinary Microbiology*, *150*(1), pp.146-151.

- Pöntinen, A., Lindström, M., Skurnik, M. and Korkeala, H., 2017. Screening of the two-component-system histidine kinases of *Listeria monocytogenes* EGD-e. LiaS is needed for growth under heat, acid, alkali, osmotic, ethanol and oxidative stresses. *Food Microbiology*, *65*, pp.36-43.

- Pöntinen, A., Markkula, A., Lindström, M. and Korkeala, H., 2015. Two-component-system histidine kinases involved in growth of *Listeria monocytogenes* EGD-e at low temperatures. *Applied and Environmental Microbiology*, 81(12), pp.3994-4004.

- Popowska, M.A.G.D.A.L.E.N.A., Olszak, M.A.R.T.A. and Markiewicz, Z.D.Z.I.S.Ł.A.W., 2006. Susceptibility of *Listeria monocytogenes* strains isolated from dairy products and frozen vegetables to antibiotics inhibiting murein synthesis and to disinfectants. *Polish Journal of Microbiology*, *55*(4), pp.279-288.

- Pratt, Z.L., Chen, B., Czuprynski, C.J., Wong, A.C. and Kaspar, C.W., 2012. Characterization of osmotically induced filaments of *Salmonella enterica*. *Applied and environmental microbiology*, *78*(18), pp.6704-6713.

- Príncipe, A., Jofré, E., Alvarez, F. and Mori, G., 2009. Role of a serine-type D-alanyl-D-alanine carboxypeptidase on the survival of *Ochrobactrum* sp. 11a under ionic and hyperosmotic stress. *FEMS Microbiology Letters*, *295*(2), pp.261-273.

- Quigley, L., O'sullivan, O., Stanton, C., Beresford, T.P., Ross, R.P., Fitzgerald, G.F. and Cotter, P.D., 2013. The complex microbiota of raw milk. *FEMS Microbiology Reviews*, *37*(5), pp.664-698.

- QuiveyJr, R.G., Faustoferri, R., Monahan, K. and Marquis, R., 2000. Shifts in membrane fatty acid profiles associated with acid adaptation of *Streptococcus mutans*. *FEMS Microbiology Letters*, *189*(1), pp.89-92.

- Ramaswamy, V., Cresence, V.M., Rejitha, J.S., Lekshmi, M.U., Dharsana, K.S., Prasad, S.P. and Vijila, H.M., 2007. *Listeria*-review of epidemiology and pathogenesis. *Journal of Microbiology Immunology and Infection*, *40*(1), p.4.

- Rando, O.J. and Verstrepen, K.J., 2007. Timescales of genetic and epigenetic inheritance. *Cell*, *128*(4), pp.655-668.

- Rantsiou, K., Greppi, A., Garosi, M., Acquadro, A., Mataragas, M. and Cocolin, L., 2012. Strain dependent expression of stress response and virulence genes of *Listeria monocytogenes* in meat juices as determined by microarray. *International Journal of Food Microbiology*, *152*(3), pp.116-122.

- Raorane, A., Doijad, S., Katkar, S., Pathak, A., Poharkar, K., Dubal, Z. and Barbuddhe, S., 2014. Prevalence of *Listeria* spp. in animals and associated environment. *Advances in Animal and Veterinary Sciences*, *2*(2), pp.81-85.

- Ratani, S.S., Siletzky, R.M., Dutta, V., Yilidrim, S., Osborne, J.A., Lin, W., Hitchins, A.D., Ward, T.J. and Kathariou, S., 2012. Heavy metal and disinfectant

resistance of *Listeria monocytogenes* from foods and food processing plants. *Applied and Environmental Microbiology*, pp.AEM-01553.

- Rekha, V.B., Malik, S.V.S., Chaudhari, S.P. and Barbuddhe, S.B., 2006. Listeriolysin O-based diagnosis of *Listeria monocytogenes* infection in experimentally and naturally infected goats. *Small Ruminant Research*, *66*(1), pp.70-75.

- Reverchon, S., Sobetzko, P., Nasser, W. and Muskhelishvili, G., 2015. Rethinking the bacterial genetic regulation. *Biochemistry and Analytical Biochemistry*, *4*(3), p.1-9.

- Ribeiro, V.B., Mujahid, S., Orsi, R.H., Bergholz, T.M., Wiedmann, M., Boor, K.J. and Destro, M.T., 2014. Contributions of σ B and PrfA to *Listeria monocytogenes* salt stress under food relevant conditions. *International Journal of Food Microbiology*, *177*, pp.98-108.

- Rieger, R., Michaelis, A. and Green, M.M., 2012. Glossary of genetics: classical and molecular. Rieger, R., Michaelis, A. and Green, M.M. Chaper III, pp, 56-137. Springer Science & Business Media.

- Robach, M. 2012. Food-borne pathogen control programs. National Academies Press (US). Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK114487/

- Rodionov, D.A., Hebbeln, P., Gelfand, M.S. and Eitinger, T., 2006. Comparative and functional genomic analysis of prokaryotic nickel and cobalt uptake transporters: evidence for a novel group of ATP-binding cassette transporters. *Journal of Bacteriology*, *188*(1), pp.317-327.

- Rodrigues, J., Kalekar, S., Doijad, S., Poharkar, K., D'Costaand, D. and Barbuddhe, S.B., 2015. Prevalence and characterisation of *Listeria* spp. from seafood. *Indian Journal of Fisheries*, *62*(1), pp. 14-17.

- Rodrigues, J., Kalekar, S., Doijad, S., Poharkar, K., D'Costaand, D. and Barbuddhe, S.B., 2015. Prevalence and characterisation of Listeria spp. from seafood. *Indian Journal of Fisheries*, *62*(1). pp. 15-19.

- Rogiers, G., Kebede, B.T., Van Loey, A. and Michiels, C.W., 2017. Membrane fatty acid composition as a determinant of *Listeria monocytogenes* sensitivity to trans-cinnamaldehyde. *Research in Microbiology*, *168* (6), pp.536-546.

- Romanova, N.A., Wolffs, P.F.G., Brovko, L.Y. and Griffiths, M.W., 2006. Role of efflux pumps in adaptation and resistance of *Listeria monocytogenes* to benzalkonium chloride. *Applied and Environmental Microbiology*, *72*(5), pp.3498-3503.

- Roth, J.R., Lawrence, J.G. and Bobik, T.A., 1996. Cobalamin (coenzyme B12): synthesis and biological significance. *Annual Reviews in Microbiology*, *50*(1), pp.137-181.

- Rothfield, L., Taghbalout, A. and Yu-Ling, S., 2005. Spatial control of bacterial division-site placement. *Nature Reviews. Microbiology*, *3*(12), p.959.

- Rowlett, V.W., Mallampalli, V.K., Karlstaedt, A., Dowhan, W., Taegtmeyer, H., Margolin, W. and Vitrac, H., 2017. Impact of membrane phospholipid alterations in *Escherichia coli* on cellular function and bacterial stress adaptation. *Journal of Bacteriology*, *199*(13), pp.e00849-16.

- Rowney, M.K., Roupas, P., Hickey, M.W. and Everett, D.W., 2004. Salt-induced structural changes in 1-day old Mozzarella cheese and the impact upon free oil formation. *International Dairy Journal*, *14*(9), pp.809-816.

- Rustan, A.C. and Drevon, C.A., 2005. Fatty acids: structures and properties. *eLS*.

- Ryan, S., Begley, M., Gahan, C.G. and Hill, C., 2009. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. *Environmental Microbiology*, *11*(2), pp.432-445.

- Ryan, S., Hill, C. and Gahan, C.G., 2008. Acid stress responses in *Listeria monocytogenes*. *Advances in Applied Microbiology*, *65*, pp.67-91.

- Rychli, K., Grunert, T., Ciolacu, L., Zaiser, A., Razzazi-Fazeli, E., Schmitz-Esser, S., Ehling-Schulz, M. and Wagner, M., 2016. Exoproteome analysis reveals higher abundance of proteins linked to alkaline stress in persistent *Listeria monocytogenes* strains. *International Journal of Food Microbiology*, *218*, pp.17-26.

- Ryser, E.T. and Marth, E.H. 1999. The Genus *Listeria* and *Listeria monocytogenes*: Phylogenetic position, Taxonomy and Identification. In *Listeria, listeriosis, and food safety*. Ryser, E.T. and Marth, E.H. (Ed). Chapter I, pp. 1-51. CRC Press.

- Sagot, B., Gaysinski, M., Mehiri, M., Guigonis, J.M., Le Rudulier, D. and Alloing, G., 2010. Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine amide is mediated by a new pathway conserved among bacteria. *Proceedings of the National Academy of Sciences*, *107*(28), pp.12652-12657.

- Salyers, A.A. and Whitt, D.D., 2002. Bacterial pathogenesis: A molecular approach Salyers, A.A. and Whitt, D.D (Ed). Chapter II, pp. 53-100. 2nd edn. Washington, DC: ASM Press.

- Samara, A. and Koutsoumanis, K.P., 2009. Effect of treating lettuce surfaces with acidulants on the behaviour of *Listeria monocytogenes* during storage at 5 and 20 C and subsequent exposure to simulated gastric fluid. *International Journal of Food Microbiology*, *129*(1), pp.1-7.

- Sambrook, J., Maniatis, T., Fritsch, E. F., 1989. Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbor Laboratory Press, New York, USA.

- Sartor, C., Grégoire, E., Albanèse, J., Fournier P.E., 2015. Invasive *Listeria monocytogenes* infection after liver transplantation: a lifethreatening condition, *Lancet,* 6736, pp.61831-61836.

- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, *17*(1), p.7.

- Schafer, F.Q. and Buettner, G.R., 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biology and Medicine*, *30*(11), pp.1191-1212.

- Schauer, K., Stolz, J., Scherer, S. and Fuchs, T.M., 2009. Both thiamine uptake and biosynthesis of thiamine precursors are required for intracellular replication of *Listeria monocytogenes*. *Journal of Bacteriology*, *191*(7), pp.2218-2227.

- Scheffers, D.J., 2008. The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA. *FEBS letters*, *582*(17), pp.2601-2608.

- Schlech III, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V., 1983. Epidemic listeriosis—evidence for transmission by food. *New England Journal of Medicine*, *308*(4), pp.203-206.

- Schmid, B., Klumpp, J., Raimann, E., Loessner, M.J., Stephan, R. and Tasara, T., 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. *Applied and Environmental Microbiology*, *75*(6), pp.1621-1627.

- Schmid, B., Klumpp, J., Raimann, E., Loessner, M.J., Stephan, R. and Tasara, T., 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. *Applied and Environmental Microbiology*, *75*(6), pp.1621-1627.

- Schuchat, A., Swaminathan, B. and Broome, C.V., 1996. Epidemiology of human listeriosis. *Clinical Microbiology Reviews*, *4*(2), pp.169-183.

- Sebaihia, M., Wren, B.W., Mullany, P., Fairweather, N.F., Minton, N., Stabler, R., Thomson, N.R., Roberts, A.P., Wang, H., Holden, M.T. and Wright, A., 2006. The multidrug-resistant human pathogen *Clostridium difficile* has a highly mobile, mosaic genome. *Nature Genetics*, *38*(7), p.779.

- Sengupta, D. and Chattopadhyay, M.K., 2013. Metabolism in bacteria at low temperature: A recent report. *Journal of Biosciences*, *38*(2), pp.409-412.

- Shabala, L., Lee, S.H., Cannesson, P. and Ross, T., 2008. Acid and NaCl limits to growth of *Listeria monocytogenes* and influence of sequence of inimical acid and

NaCl levels on inactivation kinetics. *Journal of Food Protection*, *71*(6), pp.1169-1177.

- Sharma, M., Taormina, P.J. and Beuchat, L.R., 2003. Habituation of foodborne pathogens exposed to extreme pH conditions: genetic basis and implications in foods and food processing environments. *Food Science and Technology Research*, *9*(2), pp.115-127.

- Shen, Q., Jangam, P.M., Soni, K.A., Nannapaneni, R., Schilling, W. and Silva, J.L., 2014. Low, medium, and high heat tolerant strains of *Listeria monocytogenes* and increased heat stress resistance after exposure to sublethal heat. *Journal of Food Protection*, *77*(8), pp.1298-1307.

- Shen, Q., Pandare, P., Soni, K.A., Nannapaneni, R., Mahmoud, B.S. and Sharma, C.S., 2016. Influence of temperature on alkali stress adaptation in *Listeria monocytogenes*. *Food Control*, *62*(3), pp.74-80.

- Silhavy, T.J., Kahne, D. and Walker, S., 2010. The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, *2*(5), p.a000414.

- Silk, B.J., Date, K.A., Jackson, K.A., Pouillot, R., Holt, K.G., Graves, L.M., Ong, K.L., Hurd, S., Meyer, R., Marcus, R. and Shiferaw, B., 2012. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004–2009: further targeted prevention needed for higher-risk groups. *Clinical Infectious Diseases*, *54*(suppl_5), pp.S396-S404.

- Silva, I.M., Almeida, R.C.D.C., Alves, M.A.O. and Almeida, P.F.D., 2003. Occurrence of *Listeria* spp. in critical control points and the environment of Minas Frescal cheese processing. *International Journal of Food Microbiology*, *81*(3), pp.241-248.

- Singh, A.K., Ulanov, A.V., Li, Z., Jayaswal, R.K. and Wilkinson, B.J., 2011. Metabolomes of the psychrotolerant bacterium *Listeria monocytogenes* 10403S grown at 37 C and 8 C. *International Journal of Food Microbiology*, *148*(2), pp.107-114.

- Singh, R.P. and Jha, P.N., 2017. Analysis of fatty acid composition of PGPR *Klebsiella* sp. SBP-8 and its role in ameliorating salt stress in wheat. *Symbiosis*, pp.1-10.

- Singleton, P. 1999. Bacteria in Biology, Biotechnology and Medicine. Singleton, P. (Ed.). Chapter XIV,   pp. 444–454 (5th ed.) .Wiley. ISBN 0-471-98880-4.

- Sleator, R.D. and Hill, C., 2002. Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. *FEMS Microbiology Reviews*, *26*(1), pp.49-71.

- Sleator, R.D. and Hill, C., 2005. A novel role for the LisRK two-component regulatory system in listerial osmotolerance. *Clinical Microbiology and Infection*, *11*(8), pp.599-601.

- Sleator, R.D., Gahan, C.G. and Hill, C., 2001. A postgenomic appraisal of osmotolerance in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *69*(1), pp.1-9.

- Sleator, R.D., Wemekamp-Kamphuis, H.H., Gahan, C.G., Abee, T. and Hill, C., 2005. A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in *Listeria monocytogenes. Molecular Microbiology*, *55*(4), pp.1183-1195.

- Smirnova, G.V. and Oktyabrsky, O.N., 2005. Glutathione in bacteria. *Biochemistry (Moscow)*, *70*(11), pp.1199-1211.

- Smith, B., Kemp, M., Ethelberg, S., Schiellerup, P., Bruun, B.G., Gerner-Smidt, P. and Christensen, J.J., 2009. *Listeria monocytogenes*: maternal-foetal infections in Denmark 1994–2005. *Scandinavian Journal of Infectious Diseases*, *41*(1), pp.21-25.

- Smith, J.L., Liu, Y. and Paoli, G.C., 2012. How does *Listeria monocytogenes* combat acid conditions?. *Canadian Journal of Microbiology*, *59*(3), pp.141-152.

- Somers, E.B. and LEE WONG, A.C., 2004. Efficacy of two cleaning and sanitizing combinations on *Listeria monocytogenes* biofilms formed at low temperature on a variety of materials in the presence of ready-to-eat meat residue. *Journal of Food Protection*, *67*(10), pp.2218-2229.

- Soni, K.A., Nannapaneni, R. and Hagens, S., 2010. Reduction of *Listeria monocytogenes* on the surface of fresh channel catfish fillets by bacteriophage Listex P100. *Foodborne pathogens and disease*, *7*(4), pp.427-434.

- Soni, K.A., Nannapaneni, R. and Tasara, T., 2011. An overview of stress response proteomes in *Listeria monocytogenes*. *Agriculture, Food and Analytical Bacteriology*, *1*, pp.66-85.

- Soni, K.A., Nannapaneni, R. and Tasara, T., 2011. The contribution of transcriptomic and proteomic analysis in elucidating stress adaptation responses of *Listeria monocytogenes*. *Foodborne Pathogens and Disease*, *8*(8), pp.843-852.

- Soumet, C., Ragimbeau, C. and Maris, P., 2005. Screening of benzalkonium chloride resistance in *Listeria monocytogenes* strains isolated during cold smoked fish production. *Letters in Applied Microbiology*, *41*(3), pp.291-296.

- Stasiewicz, M.J., Oliver, H.F., Wiedmann, M. and den Bakker, H.C., 2015. Whole-genome sequencing allows for improved identification of persistent *Listeria monocytogenes* in food-associated environments. *Applied and Environmental Microbiology*, *81*(17), pp.6024-6037.

- Stasiewicz, M.J., Wiedmann, M. and Bergholz, T.M., 2011. The transcriptional response of *Listeria monocytogenes* during adaptation to growth on lactate and diacetate includes synergistic changes that increase fermentative acetoin production. *Applied and Environmental Microbiology*, *77*(15), pp.5294-5306.

- Stessl, B., Fricker, M., *et al.* 2014. Collaborative survey on the colonization of different types of cheese-processing facilities with *Listeria monocytogenes*. *Foodborne Pathogen and Diseases.,* 11, pp.8–14.

- Stoll, R. and Goebel, W., 2010. The major PEP-phosphotransferase systems (PTSs) for glucose, mannose and cellobiose of *Listeria monocytogenes*, and their significance for extra-and intracellular growth. *Microbiology*, *156*(4), pp.1069-1083.

- Storz, G., Vogel, J. and Wassarman, K.M., 2011. Regulation by small RNAs in bacteria: expanding frontiers. *Molecular Cell*, *43*(6), pp.880-891.

- Stülke, J., Arnaud, M., Rapoport, G. and Martin-Verstraete, I., 1998. PRD—a protein domain involved in PTS-dependent induction and carbon catabolite repression of catabolic operons in bacteria. *Molecular Microbiology*, *28*(5), pp.865-874.

- Supa-amornkul, S., Chantratita, W., Srichunrusami, C., Janchompoo, P. and Chaturongakul, S., 2016. *Listeria monocytogenes* MerR-Like Regulator NmlRlm: Its Transcriptome and Role in Stress Response. *Foodborne Pathogens and Disease*, *13*(7), pp.369-378.

- Suttisansanee, U., Lau, K., Lagishetty, S., Rao, K.N., Swaminathan, S., Sauder, J.M., Burley, S.K. and Honek, J.F., 2011. Structural Variation in Bacterial Glyoxalase I Enzymes Investigation of the Metalloenzyme Glyoxalase I from *Clostridium acetobutylicum*. *Journal of Biological Chemistry*, *286*(44), pp.38367-38374.

- Swaminathan, B. and Gerner-Smidt, P., 2007. The epidemiology of human listeriosis. *Microbes and Infection*, *9*(10), pp.1236-1243.

- Swaminathan, B., Barrett, T.J., Hunter, S.B., Tauxe, R.V. and Force, C.P.T., 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging infectious diseases*, *7*(3), p.382.

- Takami, H., Takaki, Y. and Uchiyama, I., 2002. Genome sequence of *Oceanobacillus iheyensis* isolated from the Iheya Ridge and its unexpected adaptive capabilities to extreme environments. *Nucleic Acids Research*, *30*(18), pp.3927-3935.

- Tasara, T. and Stephan, R., 2006. Cold stress tolerance of *Listeria monocytogenes*: a review of molecular adaptive mechanisms and food safety implications. *Journal of Food Protection*, *69*(6), pp.1473-1484.

- Tatusov, R.L., Mushegian, A.R., Bork, P., Brown, N.P., Hayes, W.S., Borodovsky, M., Rudd, K.E. and Koonin, E.V., 1996. Metabolism and evolution of Haemophilus influenzae deduced from a whole-genome comparison with Escherichia coli. *Current biology*, *6*(3), pp.279-291.

- Tessema, G.T., Møretrø, T., Snipen, L., Heir, E., Holck, A., Naterstad, K. and Axelsson, L., 2012. Microarray-based transcriptome of *Listeria monocytogenes*

adapted to sublethal concentrations of acetic acid, lactic acid, and hydrochloric acid. *Canadian Journal of Microbiology*, *58*(9), pp.1112-1123.

- Thieringer, H.A., Jones, P.G. and Inouye, M., 1998. Cold shock and adaptation. *Bioessays*, *20*(1), pp.49-57.

- Thongbai, B., Gasaluck, P. and Waites, W.M., 2006. Morphological changes of temperature-and pH-stressed *Salmonella* following exposure to cetylpyridinium chloride and nisin. *LWT-Food Science and Technology*, *39*(10), pp.1180-1188.

- Thorarinsdottir, K.A., Arason, S., Geirsdottir, M., Bogason, S.G. & Kristbergsson, K. 2002. Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry. *Food Chemistry*, **77**, pp.377–385.

- To, M.S., Favrin, S., Romanova, N. and Griffiths, M.W., 2002. Postadaptational resistance to benzalkonium chloride and subsequent physicochemical modifications of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *68*(11), pp.5258-5264.

- To, T.M.H., Grandvalet, C. and Tourdot-Maréchal, R., 2011. Cyclopropanation of membrane unsaturated fatty acids is not essential to the acid stress response of *Lactococcuslactis* subsp. *cremoris*. *Applied and Environmental Microbiology*, *77*(10), pp.3327-3334.

- Tsou, C.C., Chiang-Ni, C., Lin, Y.S., Chuang, W.J., Lin, M.T., Liu, C.C. and Wu, J.J., 2008. An iron-binding protein, Dpr, decreases hydrogen peroxide stress and protects *Streptococcus pyogenes* against multiple stresses. *Infection and Immunity*, *76*(9), pp.4038-4045.

- Uppu, D.S. and Haldar, J., 2016. Lipopolysaccharide neutralization by cationic-amphiphilic polymers through pseudoaggregate formation. *Biomacromolecules*, *17*(3), pp.862-873.

- Utratna, M., Shaw, I., Starr, E. and O'Byrne, C.P., 2011. Rapid, transient, and proportional activation of σB in response to osmotic stress in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *77*(21), pp.7841-7845.

- Vail, K.M., McMullen, L.M. and Jones, T.H., 2012. Growth and filamentation of cold-adapted, log-phase *Listeria monocytogenes* exposed to salt, acid, or alkali stress at 3 C. *Journal of food protection*, *75*(12), pp.2142-2150.

- Valero, A., Hernandez, M., De Cesare, A., Manfreda, G., González-García, P. and Rodríguez-Lázaro, D., 2014. Survival kinetics of *Listeria monocytogenes* on raw sheep milk cured cheese under different storage temperatures. *International Journal of Food Microbiology*, *184*, pp.39-44.

- Van Belkum, M.J., Martin-Visscher, L.A. and Vederas, J.C., 2011. Structure and genetics of circular bacteriocins. *Trends in Microbiology*, *19*(8), pp.411-418.

- Van Boeijen, I.K., Chavaroche, A.A., Valderrama, W.B., Moezelaar, R., Zwietering, M.H. and Abee, T., 2010. Population diversity of Listeria monocytogenes LO28: phenotypic and genotypic characterization of variants resistant to high hydrostatic pressure. *Applied and Environmental Microbiology*, *76*(7), pp.2225-2233.

- Van Buren, J.P. 2006. Snap bean texture softening and pectin solubilization caused by the presence of salt during cooking. *Journal of Food Science*, 51, pp.131–134.

- Van der Veen, S. and Abee, T., 2010. HrcA and DnaK are important for static and continuous-flow biofilm formation and disinfectant resistance in *Listeria monocytogenes*. *Microbiology*, *156*(12), pp.3782-3790.

- Van der Veen, S., Moezelaar, R., Abee, T. and Wells-Bennik, M.H., 2008. The growth limits of a large number of *Listeria monocytogenes* strains at combinations of stresses show serotype-and niche-specific traits. *Journal of Applied Microbiology*, *105*(5), pp.1246-1258.

- Van Der Veen, S., Moezelaar, R., Abee, T. and Wells-Bennik, M.H., 2008. The growth limits of a large number of *Listeria monocytogenes* strains at combinations of stresses show serotype-and niche-specific traits. *Journal of Applied Microbiology*, *105*(5), pp.1246-1258.

- Van Schaik, W.I.L.L.E.M., Gahan, C.G. and Hill, C., 1999. Acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the lantibiotics nisin and lacticin 3147. *Journal of Food Protection*, *62*(5), pp.536-540.

- Van Vliet, A.H., 2010. Next generation sequencing of microbial transcriptomes: challenges and opportunities. *FEMS Microbiology Letters*, *302*(1), pp.1-7.

- Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J. and Kreft, J., 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews*, *14*(3), pp.584-640.

- Vermeulen, A., Gysemans, K.P., Bernaerts, K., Geeraerd, A.H., Van Impe, J.F., Debevere, J. and Devlieghere, F., 2007. Influence of pH, water activity and acetic acid concentration *on Listeria monocytogenes* at 7 C: data collection for the

development of a growth/no growth model. *International Journal of Food Microbiology*, *114*(3), pp.332-341.

- Vetting, M.W., de Carvalho, L.P.S., Yu, M., Hegde, S.S., Magnet, S., Roderick, S.L. and Blanchard, J.S., 2005. Structure and functions of the GNAT superfamily of acetyltransferases. *Archives of Biochemistry and Biophysics*, *433*(1), pp.212-226.

- Vialette, M., Pinon, A., Chasseignaux, E. and Lange, M., 2003. Growths kinetics comparison of clinical and seafood *Listeria monocytogenes* isolates in acid and osmotic environment. *International Journal of Food Microbiology*, *82*(2), pp.121-131.

- Vishwanathan, G.R. and Ayyar, V.V., 1950. Circling disease of sheep in the Madras State-etiology established. *Indian Veterinary Journal*, *26*, pp.395-402.

- Visvalingam, J. and Holley, R.A., 2012. Temperature-dependent effect of sublethal levels of cinnamaldehyde on viability and morphology of *Escherichia coli*. *Journal of Applied Microbiology*, *113*(3), pp.591-600.

- Voelkerding, K.V., Dames, S.A. and Durtschi, J.D., 2009. Next-generation sequencing: from basic research to diagnostics. *Clinical Chemistry*, *55*(4), pp.641-658.

- Vogel, B.F., Huss, H.H., Ojeniyi, B., Ahrens, P. and Gram, L., 2001. Elucidation of *Listeria monocytogenes* Contamination Routes in Cold-Smoked Salmon Processing Plants Detected by DNA-Based Typing Methods. *Applied and environmental microbiology*, *67*(6), pp.2586-2595.

- Wałecka-Zacharska, E., Kosek-Paszkowska, K., Bania, J., Karpíšková, R. and Stefaniak, T., 2013. Salt stress-induced invasiveness of major *Listeria monocytogenes* serotypes. *Letters in Applied Microbiology*, *56*(3), pp.216-221.

- Wang, C., Chang, T., Yang, H. and Cui, M., 2015. Antibacterial mechanism of lactic acid on physiological and morphological properties of *Salmonella Enteritidis, Escherichia coli* and *Listeria monocytogenes*. *Food Control*, *47*, pp.231-236.

- Wang, Y., Kendall, J., Cavet, J.S. and Giedroc, D.P., 2010. Elucidation of the functional metal binding profile of a CdII/PbII sensor CmtRSc from *Streptomyces coelicolor*. *Biochemistry*, *49*(31), pp.6617-6626.

- Watson, D., Sleator, R.D., Casey, P.G., Hill, C. and Gahan, C.G., 2009. Specific osmolyte transporters mediate bile tolerance in *Listeria monocytogenes*. *Infection and Immunity*, *77*(11), pp.4895-4904.

- Weber, M., Hellmann, I., Stadler, M.B., Ramos, L., Pääbo, S., Rebhan, M. and Schübeler, D., 2007. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nature genetics*, *39*(4), p.457.

- Weibezahn, J., Tessarz, P., Schlieker, C., Zahn, R., Maglica, Z., Lee, S., Zentgraf, H., Weber-Ban, E.U., Dougan, D.A., Tsai, F.T. and Mogk, A., 2004. Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. *Cell*, *119*(5), pp.653-665.

- Weller, D., Andrus, A., Wiedmann, M. and den Bakker, H.C., 2015. *Listeria booriae* sp. nov. and *Listeria newyorkensis* sp. nov., from food processing environments in the USA. *International Journal of Systematic and Evolutionary Microbiology*, *65*(1), pp.286-292.

- Wemekamp-Kamphuis, H.H., Sleator, R.D., Wouters, J.A., Hill, C. and Abee, T., 2004. Molecular and physiological analysis of the role of osmolyte transporters BetL, Gbu, and OpuC in growth of *Listeria monocytogenes* at low temperatures. *Applied and Environmental Microbiology*, *70*(5), pp.2912-2918.

- Wemekamp-Kamphuis, H.H., Wouters, J.A., de Leeuw, P.P., Hain, T., Chakraborty, T. and Abee, T., 2004. Identification of sigma factor σB-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology*, *70*(6), pp.3457-3466.

- Wesche, A.M., Gurtler, J.B., Marks, B.P. and Ryser, E.T., 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *Journal of Food Protection*, *72*(5), pp.1121-1138.

- Wiedmann, M., Bruce, J.L., Keating, C., Johnson, A.E., McDonough, P.L. and Batt, C.A., 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. *Infection and Immunity*, *65*(7), pp.2707-2716.

- Wilks, J.C., Kitko, R.D., Cleeton, S.H., Lee, G.E., Ugwu, C.S., Jones, B.D., BonDurant, S.S. and Slonczewski, J.L., 2009. Acid and base stress and transcriptomic responses in *Bacillus subtilis. Applied and Environmental Microbiology, 75*(4), pp.981-990.

- Williams, G., Khan, A.A. and Schweiger, F., 2005. *Listeria meningitis* complicating infliximab treatment for Crohn's disease. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *16*(5), pp.289-292.

- Witte, C.E., Whiteley, A.T., Burke, T.P., Sauer, J.D., Portnoy, D.A. and Woodward, J.J., 2013. Cyclic di-AMP is critical for *Listeria monocytogenes* growth, cell wall homeostasis, and establishment of infection. *MBio*, *4*(3), pp.e00282-13.

- Wonderling, L.D., Wilkinson, B.J. and Bayles, D.O., 2004. The htrA (degP) gene of *Listeria monocytogenes* 10403S is essential for optimal growth under stress conditions. *Applied and Environmental Microbiology*, *70*(4), pp.1935-1943.

- Wood, J.M., Bremer, E., Csonka, L.N., Kraemer, R., Poolman, B., van der Heide, T. and Smith, L.T., 2001. Osmosensing and osmoregulatory compatible solute accumulation by bacteria. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *130*(3), pp.437-460.

- World Health Organization, 2009. WHO Regional Office for Europe guidance for influenza surveillance in humans. Available at: http://www.euro.who.int/en/media-centre/sections/press-releases/2015/complex-food-chain-increases-food-safety-risks.

- Wouters, J.A., Hain, T., Darji, A., Hüfner, E., Wemekamp-Kamphuis, H., Chakraborty, T. and Abee, T., 2005. Identification and characterization of Di-and tripeptide transporter DtpT of *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology*, *71*(10), pp.5771-5778.

- Xayarath, B. and Freitag, N.E., 2012. Optimizing the balance between host and environmental survival skills: lessons learned from *Listeria monocytogenes*. *Future microbiology*, *7*(7), pp.839-852.

- Yoon, Y., Lee, H., Lee, S., Kim, S. and Choi, K.H., 2015. Membrane fluidity-related adaptive response mechanisms of foodborne bacterial pathogens under environmental stresses. *Food Research International*, *72*, pp.25-36.

- Young, K.D., 2006. The selective value of bacterial shape. *Microbiology and Molecular Biology Reviews*, *70*(3), pp.660-703.

- Yousef, A.E. and Courtney, P.D., 2003. Basics of stress adaptation and implications in new-generation foods. *Microbial Stress Adaptation and Food Safety*, *1*, pp.1-30.

- Zang, Q., Feng, Y., Deng, L., Feng, F., Wang, L., Zhou, Q. and Luo, Q., 2011. SigB plays a major role in *Listeria monocytogenes* tolerance to bile stress. *International journal of food microbiology*, *145*(1), pp.238-243.

- Zaritsky, A., 1975. On dimensional determination of rod-shaped bacteria. *Journal of Theoretical Biology*, *54*(2), pp.243-248.

- Zeevi, M.K., Shafir, N.S., Shaham, S., Friedman, S., Sigal, N., Paz, R.N., Boneca, I.G. and Herskovits, A.A., 2013. Listeria monocytogenes multidrug resistance transporters and cyclic di-AMP, which contribute to type I interferon induction, play a role in cell wall stress. *Journal of Bacteriology*, *195*(23), pp.5250-5261.

- Zhang, L., Moosekian, S.R., Todd, E.C. and Ryser, E.T., 2012. Growth of *Listeria monocytogenes* in different retail delicatessen meats during simulated home storage. *Journal of Food Protection*, *75*(5), pp.896-905.

- Zhang, X.F., Shen, W. and Gurunathan, S., 2016. Biologically synthesized gold nanoparticles ameliorate cold and heat stress-Induced oxidative stress in *Escherichia coli*. *Molecules, 21*(6), p.731.

- Zhang, Y.M. and Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. *Nature reviews. Microbiology*, *6*(3), pp.222-233.

- Zhou, Q., Zhang, Q., Qu, H., Wang, L., Feng, F. and Luo, Q., 2012. Comparative proteomic analysis of *Listeria monocytogenes* tolerance to bile stress. *Annals of Microbiology*, *63*(1), pp.117-128.

Appendix

Media

1. BHI Broth

Components	Quantity for l lt.
Brain Heart, Infusion from (Solids)	8.0 gm
Peptic Digest of Animal Tissue	5.0 gm
Pancreatic Digest of Casein	16.0 gm
Sodium Chloride	5.0 gm
Glucose	2.0 gm
Disodium Hydrogen Phosphate	2.5 gm

pH 7.4 ± 0.2

For stress response studies, Salt content in the medium were adjusted to desired concentration by additional supplement of Sodium Chloride. The pH of the medium was adjusted by addition of 1N HCl for acidic pH stress studies and by addition of 1N NaOH for alkaline pH stress studies.

2. BHI Agar

Components	Quantity for l lt.
Brain Heart, Infusion from (Solids)	8.0 gm
Peptic Digest of Animal Tissue	5.0 gm
Pancreatic Digest of Casein	16.0 gm
Sodium Chloride	5.0 gm
Glucose	2.0 gm
Disodium Hydrogen Phosphate	2.5 gm
Agar	13.5 gm

pH 7.4 ± 0.2

3. *Listeria* Identification Broth Base (PALCAM)

Component	Quantity for l lt.
Peptic digest of animal tissue	23.0 gm
Yeast extract	5.0 gm
Lithium chloride	10.0 gm
Esculin	0.8 gm
Ammonium ferric citrate	0.5 gm
D-Mannitol	5.0 gm
Soya lecithin	1.0 gm
Polysorbate 80	2.0 gm
Phenol red	0.08 gm

Final pH 7.4±0.2

23.69 grams of medium powder were suspended in 500 ml of distilled water. Then it was heated to dissolve the medium completely. It was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes; Cooled to 45-50°C and sterile reconstituted contents of 1 vial of *Listeria* Selective Supplement (PALCAM) were added aseptically. This preparation was mixed well and poured in sterile Petri dishes. (As per manufacturer's instructions).

Buffers and Reagents

1. TRIS stock (1M)

Component	Quantity
TRIS	121.14 gm
D/W	1000 ml

Adjust the pH to 8.0

2. EDTA Stock

Component	Quantity
EDTA	372.24 gm
D/W	1000 ml

Adjust the pH to 8.0

3. TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0):

Component	Quantity
Tris (1M, pH 8.0)	10 ml
EDTA (1M, pH 8.0)	1 ml
D/W	89 ml

Adjust the pH to 8.0

4. 1M KOH in 70% Ethanol

Component	Quantity
KOH	5.610gm
Ethanol	70 ml
D/W	30 ml

5. 4X BPB buffer

Component	Quantity
Nutrient Broth	8.0 gm
Peptone	74.0 gm
Yeast Extract	6.2 gm
Ultrapure water	500ml

Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

6. 2X SMM Buffer

Component	Quantity
Sucrose	64.8 gm
Tris	0.49 gm
$MgCl_2$	0.41gm
Maleic acid	0.93gm
Ultrapure Water	200 ml
pH 6.8	

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

7. 1X SMMP Buffer

Component	Quantity
4X BPB	20 ml
2X SMM	27 ml

  (Keep Sterile)

8. Protein Reagent

Component	Quantity
Coomassie Brillient Blue R-250	10.0 mg
95% Ethanol	5 ml
85% Phosphoric acid	10 ml
De-ionized water (make final volume to)	100 ml

9. Acrylamide-*bis*-acrylamide solution (monomer solution)

Component	Quantity
Acrylamide	29.0 gm
N,N' methylene *bis* acrylamide	1.0 gm
De-ionized water (make final volume to)	100 ml

The solution was stored in amber colour bottle at room temperature.

10. Resolving gel buffer (Tris 1.5 M, pH 8.8)

Component	Quantity
Tris	18.171 gm
De-ionized water (make final volume to)	100 ml
Adjust to pH 8.8 using 6N HCl	

11. Stacking gel buffer (Tris 0.5 M, pH 6.8)

Component	Quantity
Tris	6.57 gm
De-ionized water (make final volume to)	100 ml
Adjust to pH 6.8 using 6N HCl	

12. Ammonium persulphate (APS) (10% w/v)

Component	Quantity
Ammonium persulphate	0.1gm
Deionized water	1ml

13. Sodium dodecyl sulphate (SDS) (10% w/v)

Component	Quantity
Sodium dodecyl sulphate	10.0 gm
Deionized water	100 ml

14. Hydrochloric acid (6N)

Component	Quantity
Concentrated HCl	51 ml
Deionized water (make up to)	100 ml

15. Bromophenol blue (1% w/v)

Component	Quantity
Bromophenol blue	0.1 gm
Deionized water (make up to)	100 ml

16. Tris-glycine electrophoresis buffer 5X (pH 8.3)

Component	Quantity
Tris base (25 mM)	3.02 gm
Glycin (250 mM)	18.8 gm
SDS (10% w/v)	10 ml
De-ionized water (make final volume to)	200 ml

17. Sample buffer 2X (10 ml)

Component	Quantity
Tris-HCl (1 M, pH 6.8)	1 ml
Glycerol	2 ml
Bromophenol blue (1% w/v)	2 ml
SDS (10% w/v)	4 ml
β-mercaptoethanol (200 mM)	284 μl
De-ionized water	716 μl

18. Preparation of resolving and stacking gel

Solution	Resolving gel 12% (10 ml)	Stacking gel 4% (4 ml)
Monomer	4.0 ml	0.67 ml
Tris (1.5 M, pH 8.8)	2.5 ml	-
Tris (0.5 M, pH 6.8)	-	1 ml
SDS (10% w/v)	0.1ml	0.04 ml
APS (10% w/v)	0.1 ml	0.04 ml
De-ionized water	3.3 ml	2.7 ml
TEMED	0.004ml	0.004 ml

19. Staining solution

Component	Quantity
Coomassie Brillient Blue R-250	1.0 gm
Methanol	40 ml
Acetic acid	10 ml
De-ionized water	40 ml

20. Destaining Solution

Component	Quantity
Methanol	40 ml
Acetic acid	10 ml
De-ionized water	40 ml

21. 1% PFGE agarose in TE Buffer:

Component	Quantity
PFGE grade agarose	1.0 g
TE buffer (pH 8.0)	10 ml

22. Preparation of phosphate-buffered saline (0.01 M; pH 7.2)

Component	Quantity
Na$_2$HPO4 (anhydrous)	1.09 gm
NaH$_2$PO4 (anhydrous)	0.32 gm
NaCl	9.0 gm
D/W	1000 ml

Mixed to dissolve and adjusted to pH 7.2

23. Cell lysis buffer (50mM Tris:50mM EDTA, pH 8.0 + 1% Sarcosyl)

Component	Quantity
Tris (1M, pH 8.0)	5 ml
EDTA (1M, pH 8.0)	5 ml
Sarcosyl	1.0 gm
D/W	90 ml

24. Cell Lysis/Proteinase K Buffer

Component	Quantity
Cell lysis buffer	5 ml
Proteinase K (20 mg/ml)	25 μl

25. SSP solution (1.2% PFGE grade agarose:1% Sodium Dodecyl Sulfate: 0.2 mg/ml proteinase K)**:**

Component	Quantity
PFGE grade agarose	0.12 gm
SDS	0.1 gm
Proteinase K (20 mg/ml)	100 μl
D/W	9.9 ml

26. 10X Tris-Borate-EDTA (TBE) buffer

Component	Quantity
Tris	108.0 gm
Boric Acid	55.0 gm
EDTA	9.375 gm
D/W	1000 ml

Adjusted to pH 8.0

27. Phosphate buffered saline

Component	Quantity
Sodium Chloride	8.0 gm
Disodium hydrogen phosphate	1.14 gm
Potassium chloride	0.2 gm
Potassium di hydrogen phosphate	0.2 gm
D/W	1000 ml

Publications

Publications

- **Kale, S.B.**, Doijad, S.P., Poharkar, K.V., Garg, S., Pathak, A.D., Raorane, A.V., Rawool, D.B., Kurkure, N.V. and Barbuddhe, S.B., 2017. Elucidation of the role of the *minC* gene in filament formation by *Listeria monocytogenes* under stress conditions. *International Journal of Current Research and Review*, *9*(9), p.9-13.

- **Kale, S.B.**, Kurkure, N.V., Doijad, S.P., Poharkar, K.V., Garg, S., Rawool, D.B. and Barbuddhe, S.B., 2017. Variations in Stress Tolerance Abilities of Diverse *Listeria monocytogenes* Isolates. *International Journal of Current Microbiology and Applied Sciences, 6*(5), pp.2246-2258.

- Rawool, D.B., Doijad, S.P., Poharkar, K.V., Negi, M., **Kale, S.B.**, Malik, S.V., Kurkure, N.V., Chakraborty, T., Barbuddhe, S.B., 2016. A multiplex PCR for detection of *Listeria monocytogenes* and its lineages. *Journal of Microbiological Methods.130*, pp.144-147.

- Barbuddhe, S.B., Doijad, S.P., Goesmann, A., Hilker, R., Poharkar, K.V., Rawool, D.B., Kurkure, N.V., Kalorey, D.R., Malik, S.S., Shakuntala, I., Chaudhari, S., Waskar, V., D'Costa, D., Kolhe, R., Arora, R., Roy, A., Raorane, A., **Kale, S.**, Pathak, A., Negi, M., Kaur, S., Waghmare, R., Warke, S., Shoukat, S., Harish B., Poojary, A., Madhavaprasad. C., Nagappa, K., Das, S., Zende, R., Garg, S., Bhosle, S., Radriguez, S., Paturkar, A., Fritzenwanker, M., Ghosh, H., Hain, T., Chakraborty, T., 2016. Presence of a widely disseminated *Listeria monocytogenes* serotype 4b clone in India. *Emerging Microbes & Infections*, *5*(6), p.e55.

Conference Participations

Poster Presentations

- **Kale, S.B**., Doijad, S.P., Raorane, A. V., Pathak, A. D., Poharkar, K.V., Kurkure, N.V., Garg, S. and Barbuddhe, S.B. Physiological stress tolerance of *Listeria monocytogenes* and its Mechanisms. Presented at 56th annual conference of Association of Microbiologist of India (AMI) and International Symposium on Emerging Discoveries in Microbiology. December 2015. Held at JNU, New Delhi.

  Awarded with **"Best Poster Award"**

- **Kale, S**., Doijad, S., Garg, S., Kurkure N., Kalorey D., Poharkar, K. and Barbuddhe S. presented at "Problems of Listeriosis"-XIX (ISOPOL XIX), Institute of Pasteur, Paris, France, June 14-17, 2016.