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# Variations in Stress Tolerance Abilities of Diverse *Listeria monocytogenes* Isolates

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## ABSTRACT

*Listeria monocytogenes* is an important foodborne pathogen with the ability to survive and grow in different foods and food processing environments. The variability in innate stress tolerance abilities of *L. monocytogenes* strains (n=104) isolated from clinical (n=35), environment (n=28) and food (n=41) sources was investigated against salt (2.5% to 12.5%), pH (pH 4.0 to 9.5) and low temperature (down to $4^0$C). The stress tolerance abilities were correlated with the source of isolation, serogroups and identifying the prevalent stress tolerant genotype. A total of 37 (35.57%) strains could tolerate different stresses of which 19 (18.26%) strains showed multi-stress tolerance capability. No correlation was observed among tolerance pattern and sources of isolation, while, 46.55% strains of *L. monocytogenes* serogroup 4b, 4d, 4e were tolerant to different stresses. The subtyping of stress tolerant strains employing pulsed-field gel electrophoresis revealed 15 pulsotypes. Multiple stress tolerant strains belonging to serogroup 4b, 4d, 4e (n= 21) revealed to be clonal with unique pulsotypes. However, no correlation was observed for particular stress and pulsotypes. The data showed that strains varied remarkably with respect to stress tolerance abilities under different stresses without any correlation between stress tolerance pattern and origin of the strains for all studied stresses. This study is a significant step towards dissecting the variability of stress response in *L. monocytogenes* and understanding the dominance and prevalence of particular serogroup among different niches.

**Keywords**

*Listeria monocytogenes*, Serogroups, Stress tolerance, pH, Salt, Low temperature

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## Introduction

*Listeria monocytogenes*, a Gram-positive, ubiquitous bacterium is a well known and important foodborne pathogen (Hoffmann *et al.*, 2015). The extraordinary capabilities of the pathogen to survive in the gastrointestinal tract of animals and humans and its intracellular multiplication eventually can develop into a disease makes this bacterium a major concern (Olier *et al.*, 2003; Cossart, 2012). Although the pathogen can infect healthy individuals, listeriosis is more common in immune-compromised individuals, pregnant women, neonates, elderly people, children, cancer patients and patients on immunosuppressive therapy (Silk *et al*., 2012; Feng *et al*., 2013). Listeriosis has 20-30% case fatality rate, 50% neonatal death rate and 91% hospitalization rate (Sartor *et al*., 2015). Being ubiquitous, *L. monocytogenes* easily enters in the food chain, contaminates foods and food processing environments. It has unique capabilities such as tolerance to high salt concentrations (as high as 10-14%), low temperature (down to $0^0$C) and diverse pH range (pH 4.5 to 9.5) (Buchanan *et al*., 2004; Gandhi and Chikindas, 2007) which make *L. monocytogenes* a versatile and pervasive in nature and also help to survive even in sub-optimal environmental conditions (Shabala *et al*., 2008). Ironically, these abilities allow the pathogen to grow selectively in harsh conditions in food processing industries. Contaminated foods that are stored in a refrigerator ($4^0$C-$7^0$C) enrich growth of *L. monocytogenes* making it difficult to control (Angelidis *et al*., 2002; Makariti *et al*., 2015).

Earlier studies reported large variations in stress tolerance of *L. monocytogenes* under different conditions of high salt, acidic and/or alkaline pH and low temperature (De Jesús and Whiting, 2006; Valero *et al*., 2014).

Limited studies have been done demonstrating the relation between stress tolerance and serotype or origin of isolation of *L. monocytogenes*. Numerous investigations are based on the physiological basis of stress tolerance, but most of these studies are available with a limited number of strains (Lianou *et al*., 2003; Liu *et al*., 2005, Vermeulen *et al*., 2007). This approach limits investigation for the comprehensive scenario for determination of variation in stress phenotypes under different stresses.

In order to control the spread of the pathogen, the stress tolerance mechanisms of *L. monocytogenes* have been a focus of research worldwide. Several universal stress mechanisms such as efflux pump also have been identified in *L. monocytogenes*, which help cells get adapted easily to low level stresses inducing tolerance capabilities (Romanova, 2006).

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. The objective of this study was to assess the innate capacity of *L. monocytogenes*, belonging to different serogroups and isolated from various sources to tolerate food-related stresses. Furthermore, the study attempted to the study attempted to correlate the stress tolerant strains with a source of isolation and serogroups identifying dominant serogroup with the particular genotype. In this study, 104 *L. monocytogenes* strains from ILCC of different origins representing the epidemiologically important serotypes were studied for their stress tolerance capacities using several food-related stresses.

## Materials and Methods

### *Listeria monocytogenes* strains

A total of 104 *Listeria monocytogenes* strains were selected from the Indian *Listeria* Culture Collection (ILCC). The collection 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 1). All the strains were characterized previously biochemically and for their serogroups (Doumith *et al*., 2004). The *L. monocytogenes* strains were belonging to serogroups of *L.*

*monocytogenes* as 4b, 4d, 4e (n= 58), 1/2a, 1/2c, 3a, 3c (n=34) and 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$^{0}$ C in brain heart infusion (BHI) broth (Himedia, India) with 15% sterile glycerol (v/v) (Himedia, India).

## Inocula preparation

*Listeria monocytogenes* strains were cultured on PALCAM agar (Himedia, India) at 37$^{0}$ C for 24 h. Single colony for each strain was inoculated in 10 ml of BHI broth and incubated at 37$^{0}$ 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.

## 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 190 µL) was inoculated with 10 µL 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 $OD_{600nm}$ 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.

## 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$^{0}$ C.The procedures were carried out as explained for salt tolerance experiments.

## Low temperature tolerance

The inoculants 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. The plates were incubated at 4ºC, 10ºC, 18ºC, 24ºC and 30ºC. The further observation procedures were carried as explained for salt tolerance experiments.

## Pulsed Field Gel Electrophoresis (PFGE)

A total of 37 strains which exhibited tolerance at least one of the stress factors studied were further investigated for their genomic patterns using pulse field gel electrophoresis (PFGE). The PFGE was performed according to the Pulse Net standardized protocol (Graves and Swaminathan, 2001). In brief, bacterial cell suspension was embedded in 1.2% PFGE grade agarose (Bio-Rad, USA). The plugs were digested either with 25U of *Asc*I (New England BioLabs, Beverly, MA, USA) at 37$^{o}$C for 3h or 25U of *Apa*I (New England BioLabs, Beverly, MA, USA) at 25$^{o}$C for 5h. After digestion the plugs were loaded on 1% PFGE grade agarose gel in 0.5X TBE buffer and electrophoresed on CHEF-DRIII Mapper 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 $14^0$ C. After electrophoresis gel was stained for 30 min in 400 ml of 0.5x TBE containing 25 ml (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 analyzed by Phoretix Software (Total labs, UK).

## Results and Discussion

### 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). 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 ready to eat (RTE) and raw foods (n=41) belonging to three epidemiologically significant serogroups 4b,4d,4e (n=58); 1/2a,1/2c,3a,3c (n=34) and 1/2b,3b,4b,4d,4e (n=12) (Table S1). Strains exhibiting growth at 12.5% NaCl concentration were considered as 'high' stress tolerant (Makarti *et al*., 2014). Out of 104 strains studied a total of 13 (12.5%) strains were found to be tolerant up to 12.5% high salt concentration followed by 65 (62.5%) strains tolerant to up to10% salt concentration and all the strains showed tolerance up to 7.5% salt (Fig. 1a). Total 6 (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.

### pH tolerance

Effect of diverse pH range (4.0 to 9.5 with an increment of 0.5 units) was studied on 104 isolates of *L. monocytogenes*. The strains showing growth at pH $\leq$ 4.5 or $\geq$ 9 were considered as 'high' stress tolerant (Makarti *et al*., 2014). A total of 25 isolates were found to be tolerant to the extreme pH (acidic=13 and alkaline=12). 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 and all strains were tolerant up to pH 5.5 (Fig. 1b). While 12 (11.53%) strains showed tolerance at pH 9.5 and 70 (67.3%) strains showed growth up to pH 9.0. All the (Fig.1c) strains showed the tolerance up to pH 8.5. 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*., 2014; 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, total 7 (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.

## Tolerance to low temperature

Considering varied temperature ranges used in processing, storage as well as the distribution of food products (4ºC, 10ºC, 18ºC, 24ºC, and 30ºC), tolerance was studied at different temperatures. The lowest temperature tested was 4ºC 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. Out of 104 strains tested a total of 22 (21.15%) strains showed growth at 4ºC and, whereas, 64 (61.53%) showed growth at 10ºC (Fig. 1d). While all the strains grew well at 18ºC 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 a pathogenic bacterium. Many ready-to-eat 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*., 2002). Among the low temperature tolerant strains, 10 (28.57%) strains were from clinical sources followed by 10 (24.39%) from food and 2 (7.14%) from the environment.

A total of 37 (35.57%) strains were found to be tolerant to at least one of stress tested. Of these 16 strains were tolerant to more than one stress. Among the tolerant strains, 13(12.5%) strains were tolerant to high salt, 25 (24.03%) to extreme pH and 22 (21.15%) were tolerant to low temperature. When compared to their serotypes, 46.55% (27/58) serogroup 4b strains, 33.33% (4/12) serogroup 1/2b strains and 17.64% (6/34) serogroup 1/2a strains were found to be stress tolerant (Fig. 2). 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*., 2003). However, interestingly, serogroup 4b strains were observed to be more stress tolerant than that of serogroup 1/2b and 1/2a. Earlier studies (van der Veen *et al*., 2008; Makarti *et al*., 2014) also observed a high number of serotype 4b strains showing tolerance followed by serptype 1/2b and 1/2a strains. This could be a possible explanation for the dominance of serotype 4b in clinical cases.

## PFGE

Analysis of whole genome patterns of 37 tolerant strains with both the enzymes (*Asc*I and *Apa*I) revealed 15 pulsotypes (Fig. 3). Two strains could not be typed with the *Asc*I enzyme. The Simpson's Diversity index was low (0.6873), indicating very few of strains were capable of tolerating the stress. The observed 15 pulsotypes were labeled serially and alphabetically from 'A' to 'O'. The strains with pulsotype 'M' were observed to be dominant clustering 15 strains belonging to serogroup 4b. Apparently, the possibility of single ubiquitous stress tolerating 4b clone cannot be denied. Also, in the case of serogroup 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 serogroup 1/2a strains and 1/2b strains were not consistent with their tolerance pattern.

**Table.1** List of *Listeria monocytogenes* isolates 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 | 4b, 4d, 4e | Animal | 2005 |
| ILCC152 | 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 |
| 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 |

**Fig.1** (a) The percentage of salt stress tolerant strains to the different salt concentrations. (b) The percentage of low pH stress tolerant strains to respective acidic pH. (c) The percentage of high pH stress tolerant strains to respective alkaline pH. (d) The percentage of cold stress tolerant strains at different low temperatures

**Fig. 1**

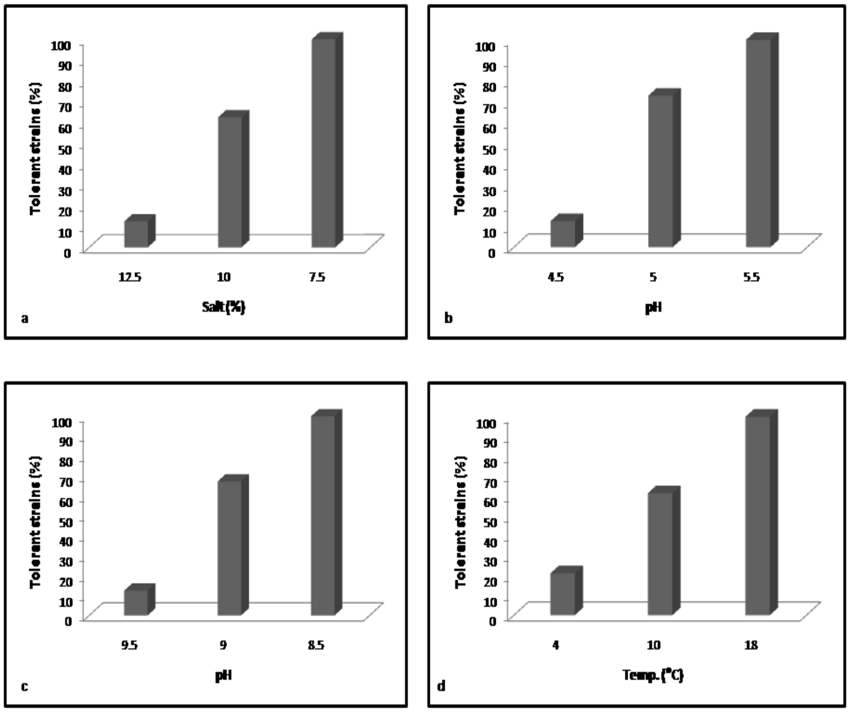


**Fig.2** Stress tolerance pattern of the strains with respect to serotypes

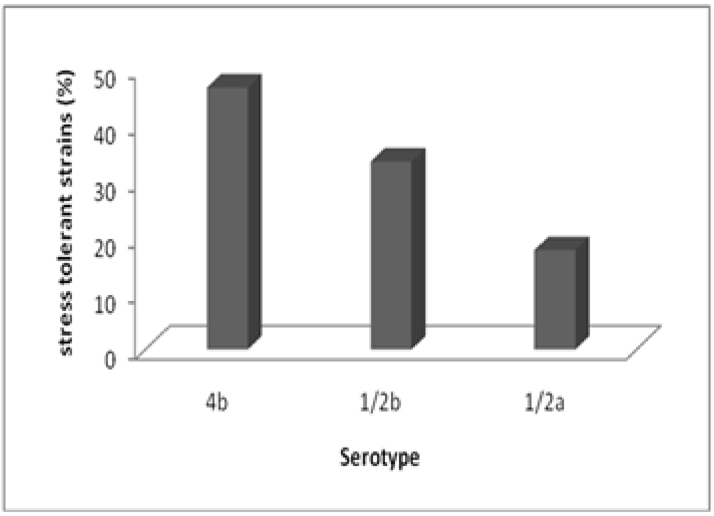
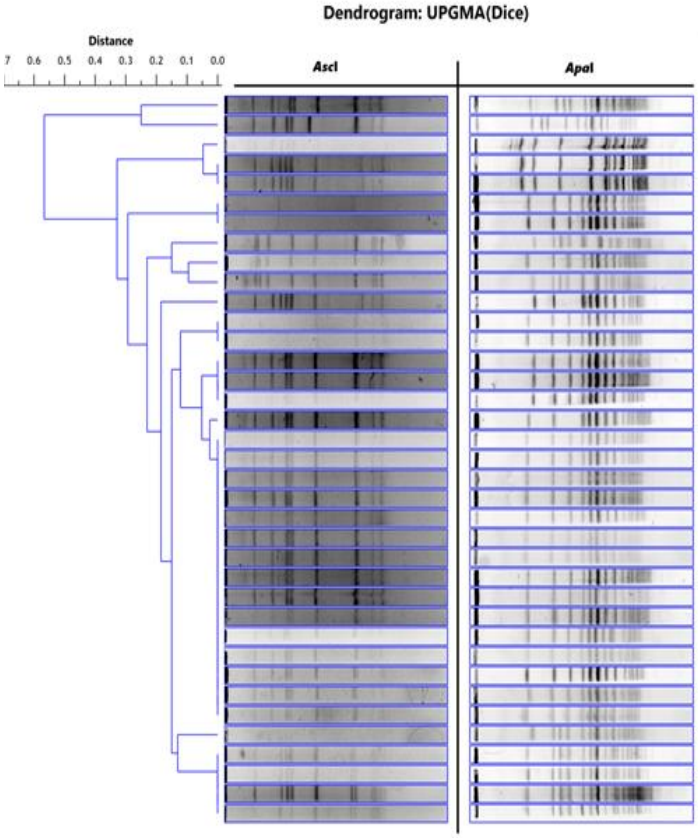


**Fig.3** Dendrogram (UPGMA) showing PFGE patterns of 37 stress tolerant *Listeria monocytogenes* strains restricted by AscI and ApaI enzymes with dtails of the source of isolation, serotype and stress tolerance patterns



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

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. All these gene-clusters, however, appear to be controlled by a single factor known as sigB (Kazmierczak *et al.*, 2003; Hain *et al.*, 2008). Further studies are necessary 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).

It is reported that the innate resistance by *L. monocytogenes* strains to the stresses commonly employed in food preservation and/or food processing. 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 serogroup 4b, 4d, 4e strains. This could be a possible explanation for the dominance of serotype 4b, 4d, 4e strains among clinical cases. This improved our understanding that how specific strains or subtypes of *L. monocytogenes* become resident to selected niches. PFGE analysis showed clonal or less genetic diversity among the stress tolerant strains. This study is a significant step towards dissecting the variability of stress response in *L. monocytogenes* and understanding the dominance and prevalence of particular serogroup among different niches.

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