INTRODUCTION

Listeriosis, a Zoonotic bacterial disease, due to it high fatality rate, has emerged as a major food borne disease during the past two decades. The causative agent of Listeriosis, *Listeria monocytogenes* is one among the six species of *Listeria* Genus (Doyle, 2003). Since 1981 there have been more than 20 dreadful reported outbreaks of food borne Listeriosis in the United States and Europe. Due to the high risk and public health concern it poses, the international community and establishments such as the WHO, FAO, Codex Alimentarius etc., invoked a qualitative risk assessment process for RTE processed food, which resulted in many countries to adopt a ‘Zero tolerance’ policy on *Listeria monocytogenes* in RTE processed foods (Todd., 1999). Based on this policy, the detection of any *L. monocytogenes* in RTE food makes the food adulterated (Gandhi & Chickindas, 2007) and unfit for human consumption. However, to achieve and observe Zero tolerance has been an uphill task due to the ubiquitous nature of *L. monocytogenes*, cross contamination through different sources and especially because *L. monocytogenes* form biofilms. Formation of biofilm is to the advantage of the microbes forming it, as within the biofilms, the microbial cells grow resistant to antimicrobial agents. Thus biofilm formation by pathogens has been considered a major risk. Biofilms of Listeria are of particular concern, since they are more resistant to disinfectants and sanitizing agents compared to planktonic cells. (Mah & O’Toole, 2001; Lewis, 2001). Despite all possible approaches for control of *L. monocytogenes*, the fact remains that it is difficult to eliminate *L. monocytogenes* from the food processing plants, because it has the propensity to adhere to food contact surfaces and to form biofilms, which makes implementing effective sanitation procedures difficult and their elimination, a challenge (Kornacki and Gurtler, 1999).

Therefore, for these obvious reasons, understanding biofilm formation at the gene expression level was pivotal. Data when generated, on the transcriptome of *L. monocytogenes* in biofilms with observable variations from that of the transcriptome of the bacterium in its planktonic state, will open wider vista and understanding on the specific metabolic pathways and concurrent gene expressions up regulated during and, for biofilm formation which will be
fundamental for many studies. Thus the primary objective of the research work carried out was, to study and profile the gene expression by sessile cells (biofilms) of *L.monocytogenes*.

The expression profile of *L.monocytogenes* in biofilms, inevitably had to be compared with the expression profile of the bacterium in its planktonic state, therefore the gene expression by *L.monocytogenes* as planktonic cells and its comparison with that of the cells (sessile) in biofilms, in order to extract the variation in the genes expressed (by up regulation or down regulation) as sessile cells was essential. Hence, the second objective of the research work was to compare the gene expression with sessile cells to planktonic cells of *L.monocytogenes*.

Further, unlike the lab grown cultures, microbes almost always exist as a mixed population comprising of microbes of other genus and species, in their natural environment. There have been studies on the gene expression of lab grown cultures of pathogens. However, in their naturally occurring state pathogens also share resources with other co-inhabitants and seldom exist in a pure culture state. Therefore, it could be perceived that the gene expression of the pathogens in their natural niche environment would be different from the expression of lab grown pure culture. In addition, Kathariou.S (2007) in summing up the future research perspective, has emphasised on the need for understanding the expression of special genome components by *L.monocytogenes* in a mixed community. The behaviour of most organisms living in close proximity will be governed by competition. (Alexander, R.D., 1974). Under such conditions reproduction of bacteria can be suppressed merely because of shared and limited resources (Nadel et al, 2009). *B.subtilis*, having been reported to be exhibiting potential antagonistic behavior towards other pathogenic species of *Salmonella* and *Clostridium* (Roberto and Martin 2003), was yet to be screened individually against *L.monocytogenes* both in the planktonic state and as biofilms. Screening for possible antagonism by *B.subtilis*, a potent antibiotic producer (Gabriella and Simon 2002; Stein 2005) towards *L.monocytogenes* in simulated laboratory conditions may not only yield direct application but may also yield unfathomable data on the stress response of
L. monocytogenes. Thus, the necessity to study the gene expression of L. monocytogenes in a mixed population, the third objective of this research work.

Bacterial response to surrounding microorganisms are specifically tailored says, Christian Van Ooij (March 2011) referring to current research reports on bacterial transcriptional and antagonistic responses (Garbeva et al, 2011). Also, Kathariou.S (2007), in summing up the future research perspective on Listeria, had prophesied that characterization of microbial communities in association with Listeria would be the focus of research, with hopefully interesting revelations. In this study the emphasis was to under the special genome components expressed under stress by Listeria. Upon initiation of the study in the direction, towards deciphering the molecular expression of L. monocytogenes as a member of a community, it was realized that the need for understanding species level interaction as organismal behavior and response was primarily essential and fundamental. Therefore in order to proceed to expression studies of genes in a mixed population it was required that interaction between L. monocytogenes and B. subtilis, be understood primarily. Experiments for interaction studies between the two microbes which fell within the scope of the work, were also carried out and the results of which have been discussed as a part of the research work.
EXPERIMENTAL DESIGN & METHODOLOGY

a) Interaction studies
   a.1. Interaction between *L. monocytogenes* and *B. subtilis* as broth cultures
       (Planktonic state)

   a.2 Interaction studies between *L. monocytogenes* and *B. subtilis* in
       biofilms.

b) Gene expression studies

   B.1. Gene expression of *L. monocytogenes* as planktonic cells (Broth
        culture)
   b.2. Gene expression of *L. monocytogenes* as sessile cells (in biofilms)

The strain of *L. monocytogenes* used for the gene expression study was strain
NRRL B 33419. The strain in the *L. monocytogenes* gene database of the Broad’s
Institute is with the reference J 0161. The strain is of the 1/2a serotype.

The strain of *L. monocytogenes* was cultured under the desired condition
both as broth cultures and as biofilms. The cultures were grown under optimal
growth conditions in different sets as was required for the study. Incubation
time frames for each of the culture sets were 4, 12 and 24 hrs.

One set of the aforesaid samples were enumerated for *L. monocytogenes*
after each of the time intervals and the results obtained were represented as
Log$_{10}$ values. After the stipulated incubation time (4,12 and 24 hrs) the cultures
were drawn and the cells pelleted. From the pelleted cells (total) RNA was
extracted using *column-based method* (AB systems-Ambion). The RNA
extracted was subject to quality check using *Nano drop technique* (Thermo
Scientific) and *Bioanalyser* (Agilent) and further processed (*PAP tailing*
{Epicentre Biotechnologies}, *cDNA synthesis, cRNA synthesis and labelling*
{Agilent}) for hybridisation and *One Colour Microarray Based Gene
expression analysis*. Post hybridisation, the samples were subject to
*Microarray Scanning* (Agilent). After the microarray scan, the images were
subject to feature extraction using bioinformatics tool. The extracted features as
data were analysed using the software, *Gene Spring* (Agilent).
RESULTS & CONCLUSION

The results section describes the following outcomes.

Interaction studies
a. The growth response of *L.monocytogenes* to the presence of *B.subtilis* in broth.
   *Observation*: Growth of *L.monocytogenes* was suppressed by *B.subtilis*.
b. The growth response of *L.monocytogenes* to the presence of *B.subtilis* in biofilms.
   *Observation*: Growth of *L.monocytogenes* was not affected by *B.subtilis*.

Gene expression studies
a. Expression pattern of *L.monocytogenes* in 4, 12 & 24hrs of co-culture broths in the presence of *B.subtilis*.
b. Expression pattern of *L.monocytogenes* in 4, 12 & 24hrs of in biofilms.
c. Expression pattern of *L.monocytogenes* in 24hrs of co-culture biofilms in the presence of *B.subtilis*.
d. Comparison of gene expression by *L.monocytogenes* in 24hrs of pure culture biofilm to co-culture biofilm.
e. Comparison of gene expression by *L.monocytogenes* in 24hrs of Co-culture broth to co-culture biofilm.

*B.subtilis*, having been reported to be exhibiting potential antagonistic behavior towards other pathogenic species of *Salmonella* and *Clostridium* (Roberto and Martin 2003), was yet to be screened individually against *L.monocytogenes* both in the planktonic state and as biofilms. Our findings distinctly indicate that *B.subtilis* indeed expressed antagonistic behavior against *L.monocytogenes* also, not only in planktonic state but also in a biofilm. Yet, interestingly as has been the earlier reports on *L.monocytogenes*, the bacterium showed pertinacity to life and emerged to survive after initial inhibition for 48 hours within a biofilm. *L.monocytogenes* in our study, when co-cultured to form biofilm along with *B.subtilis* was not detectable after 24 hrs of Incubation and even after 48hrs incubation, however the organism could be detected after 96 hrs of incubation. The phenomenon behind the inhibition of *L.monocytogenes* could also be due to antibiosis apart from competitive exclusion by *B.subtilis*. Our results clearly
signify that *B. subtilis* will prove to be an effective control agent for *L. monocytogenes* atleast in its planktonic state. Further if antibiosis by *B. subtilis* is screened, a potent antimicrobial agent (as antibiotic) could be obtained for control of *L. monocytogenes* in any state.

The gene expression profile of *L. monocytogenes* at different time intervals, states and stages of growth is showing differential gene expression levels. Identifying the groups of genes that are specifically up-regulated at particular conditions is definitely directing us to understand better, the specific functions that are hyperactivated at that particular state. Biologically, these data obtained can now give us information on the specialised genome components that are expressed at each of the specified conditions, states and stages of growth. Since the data gives details on the expression of the whole transcriptome of the organism under states and stages of growth, the data was deposited at the National Centre for Biotechnonology Information (NCBI). The same has been approved and is available globally through the Gene Expression Omnibus (GEO) of the NCBI. The accession number provided for the submission is GSE27936 ([www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo)). The data from this study will be a source for further research work on identifying markers/enzymes for specific functions up-regulated or down-regulated.

**REFERENCES**


