Comunicazione Orale

SURVIVAL DYNAMICS OF LISTERIA MONOCYTOGENES IN LONG-TERM CULTURES

Dip. BCA, UniPD, Legnaro, Padova, Italy.

The foodborne pathogen Listeria monocytogenes (Lm) poses a significant threat to the food industry, due to both its high mortality rate and its persistence. Its adaptive strategies range from genomic modifications (e.g., mutations, epigenetic changes, differential gene expression), to the entry of its cells into dormancy states (e.g., VBNC- viable but nonculturable- and persister cells), and to the arrangement of cells into communities, such as in a biofilm. The aim of this study was to investigate the survival capabilities after long-term incubation of Lm at its optimal temperature and at different initial concentrations, by looking for the presence of cells at their dormancy states and by studying the ability of cultures to produce biofilm.

The cultures at three different initial concentrations (9-7-5 log10 cells/mL) were incubated in minimal medium at 30°C for up to 60 days. After selected time points, survival of cells was checked by quantitative PCR combined with propidium monoazide (PMAxx) dye for the detection of total viable population, and it was combined with traditional plating methods with or without the presence of gentamicin at the MIC for the detection of persisters and culturable cells, respectively. In the cultures with lower initial concentration, both planktonic and biofilm populations were tested for cell composition (VBNC, persister, culturable cells). In addition, resuscitation assays on cultures presenting only VBNC cells were carried out by using media supplemented with amino acids, pyruvic acid and other nutrients to test whether these cells were able to regain culturability.

Results revealed that cultures with lower initial concentrations became unculturable in bacteriological media faster compared to cultures at higher initial concentrations, and that all cultures developed a high portion of cells in the VBNC state, while no persister cells were detected throughout the experiment. The number of culturable cells declined quicker for the planktonic population, while the biofilm population maintained culturability for two additional weeks.

This study demonstrated that the initial density of the culture influences the fraction of active cells of the long-term cultures and that cells in the biofilm maintain culturability longer compared to planktonic cells confirming the resilience of cells while present in protected communities.