First Virtual Workshop on the **Developments in the Italian PhD Research on Food Science Technology and Biotechnology**

Palermo, 14-15 September 2021

Genomic, transcriptomic and proteomic study on hypovirulent and hypervirulent strains of *Listeria monocytogenes* subjected to several modulatory agents of virulence

Federica D'Onofrio (fdonofrio@unite.it)
University of Teramo, Via R. Balzarini 1, 64100 Teramo, Italy
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy.
Tutor: Prof.ssa Maria Schirone, Co-tutor: Dott.ssa Mirella Luciani.

The following PhD thesis research project is aimed at studying hypovirulent and hypervirulent strains of *Listeria monocytogenes* isolated from different sources: the strains, subjected to several modulatory agents of virulence, will be characterised by sequencing of the entire genome, transcriptomic and proteomic analysis.

State-of-the-Art

Listeria monocytogenes is the ubiquitous microorganism responsible of listeriosis, a severe foodborne disease mostly transmitted through contaminated foods. Such pathogen can attack the nervous system causing encephalitis, meningitis, acute sepsis and eventually death (Desai *et al*., 2019). The risk of contracting listeriosis involve mainly immunocompromised people, elderly, children and pregnant women (Brouwer *et al*., 2006; Becattini *et al*., 2017) .

In addition to the individual sensitivity of the subject, the risk of illness can vary according to the food level contamination, the strain and its relative capacity to infect the host cells (EFSA, 2018).

Furthermore, the virulence of microorganism is supported by a complex intracellular life cycle, modulating by multiple virulence factors which regulate motility, adhesion, invasion and multiplication of the pathogen in the human cells.

These factors are regulated by the expression of genes coding for specific proteins that are involved in the infection process of host cells by *L. monocytogenes*.

The biochemical and molecular pathways are object of study that need to be investigated in order to manage, prevent and reduce the risk of listeriosis outbreaks.

Several environmental stress conditions and chemical substances, are already reported in literature, as possible positive or negative modulator, of *L. monocytogenes* virulence genes expression (Good *et al*., 2016).

Nowadays analytical methods, such as whole genome sequencing (WGS), allow to characterize the bacterial strains genome and identify the presence of virulence and antibiotic – resistance genes; proteomics techniques (mono and two-dimensional electrophoresis, 1D and 2DE, combined with mass spectrometry analysis), permit to analyze the proteome of different strains of *L. monocytogenes* and identify the proteins coded by the genome and, consequently, the factors of virulence expressed in the phenotype of the different strains (Chen *et al*., 2020; Lanciotti *et al*., 2019).

PhD Thesis Objectives and Milestones

The PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in **Table 1**:

A1) Bibliographic research. The study will be carried out analyzing data already available in the literature on virulence factors and methods of characterization of *L. monocytogenes*; the study will be mainly focused on the evaluation of genomic, transcriptomic and proteomic techniques currently available for the characterization of pathogen.

A2) Strain selection and characterization. *Listeria monocytogenes* strains, from nosocomial and food origin, will be performed on the base of specific phenotypic and genotypic characteristics; in particular, a first characterization of the selected strains will be performed by serotyping and PFGE (Pulsed-Field Gel Electrophoresis) techniques (Park *et al*., 2016).

A3) Genomic, transcriptomic and proteomic analysis. The genome sequencing will be carried out by WGS analysis; the results will be analyzed through specific bioinformatics programs, in order to highlight the presence of virulence genes (Fagerlund *et al*., 2020). The transcriptomic analysis will be carried out using the NGS (Next-Generation Sequencing) method: the data will be analyzed by specific bioinformatics programs, focusing on the different genes expressed by the strains and the mechanisms related to non-coding RNA, in order to define specific mechanisms of gene regulation (Shuyan *et al*., 2018). The proteomic analysis will be performed by means of 1D and 2DE electrophoresis mass spectrometry in order to identify the differences in protein expression of selected strains (Lanciotti *et al*., 2019).

A4) Data analysis.

A5) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project.

Activity		Months																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1	**Bibliographic research**																								
A2	**Strain selection and characterization**																								
	1) Strains collection																								
	2) Serotyping and PFGE																								
A3	**Genomic, transcriptomic, proteomic analysis**																								
	1) WGS																								
	2) NGS																								
	3) 1D-2DE - Mass spectrometry																								
A4	**Data analysis**																								
A5	**Writing and editing**																								

References

Desai A.N., Anyoha A., Madoff L.C., Lassmann B. (2019). Changing epidemiology of *Listeria monocytogenes* outbreaks, sporadic cases, and recalls globally: a review of ProMED reports from 1996 to 2018. Int. J. Infect. Dis. 84, 48–53.

Brouwer M.C., Van de Beek D., Heckenberg S.G.B., Spanjaard L., de Gans J. (2006). Community- acquired *Listeria monocytogenes* meningitis in adults. Clin. Infect. Dis., 43(10), 1233–1238.

Becattini S., Littmann E., Carter R.A., Kim S.G., Morjaria S.M., Ling L., Gyaltshen Y., Fontana E., Taur Y., Leiner I.M., Pamer E.G. (2017). Commensal microbes provide first line defense against *Listeria monocytogenes* infection. J. Exp. Med., 214(7), 1973-1989.

EFSA (2018). *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA J. 16(1), 5134.

Good J.A.D., Andersson C., Hansen S., Wall J., Krishnan K.S., Begum A., et al. (2016). Attenuating *Listeria monocytogenes* virulence by targeting the regulatory protein PrfA. Cell Chem. Biol., 23(3), 404-414.

Chen Y., Chen Y., Pouillot R., Dennis S., Xian Z., Luchansky, J.B., et al. (2020). Genetic diversity and profiles of genes associated with virulence and stress resistance among isolates from the 2010-2013 interagency *Listeria monocytogenes* market basket survey. PLoS One, 15(4), e0231393.

Lanciotti R., Braschi G., Patrignani F., Gobbetti M., De Angelis M. (2019). How *Listeria monocytogenes* shapes its proteome in response to natural antimicrobial compounds. Front. Microbiol., 10, 437.

Park S., Jung H., Lee M., Choi H., Kim J., Jung J., et al. (2016). Detection of *Listeria monocytogenes* in foods and characterization by PFGE. Adv. Microbiol., 6(4), 343-349.

Fagerlund A., Langsrud S., Møretrø T. (2020). In-depth longitudinal study of *Listeria monocytogenes* ST9 isolates from the meat processing industry: resolving diversity and transmission patterns using whole-genome sequencing. Appl. Environ. Microbiol., 86(14), e00579- 20.

Wu S., Yu P.L., Wheeler D., Flint S. (2018). Transcriptomic study on persistence and survival of *Listeria monocytogenes* following lethal treatment with nisin. J. Glob. Antimicrob. Res. 15, 25-31.