ATTRACTIVE PhD OPPORTUNITIES WITHIN THE H2020 MARIE-SKLODOWSKA-CURIE EUROPEAN TRAINING NETWORK LIST_MAPS

11 PhD STUDENTS (EARLY-STAGE-RESEARCHERS) POSITIONS

PROJECT TITLE: List_MAPS "Training and research in *Listeria monocytogenes* Adaptation through Proteomic and Transcriptome deep Sequencing Analysis"

Post Duration: 36 months
Closing Date for Applications: 26th June 2015
Contract Type: Fixed Term Whole-Time
Job Type: Research
Number of positions available: 11
Researcher profile: PhD students, Early-stage-researchers

THEMES:

Environment, resources and sustainability, microbiology

OBJECTIVES:

List_MAPS is a Horizon 2020-funded European Training Network. It is dedicated to the training of innovative young researchers in the field of Microbiology and Systems Biology. It focuses on *Listeria monocytogenes*, a ubiquitous pathogen that is in the EU the leading cause of mortality and food recalls due to foodborne pathogens, costing the EU millions of euro per annum in medical care and associated costs in the food sector. List_MAPS is recruiting 11 Early-Stage Researchers (ESR)who will develop scientific expertise through PhD training, mobility of researchers, summer schools, workshops and transfer-of-knowledge in the areas of Transcriptomics, Proteomics, Sequencing and Systems Biology. Working in this dynamic state-of-the-art field will provide for training of ESRs to the highest level.

The network recruits 11 PhDs with the overall objective to understand the ecology of *Listeria monocytogenes* through the combination of high throughput Epigenetics, Deep sequencing of transcripts, Proteomics, Bioinformatics, Mathematics and Microbiology to decipher the transcriptional regulatory circuitry that drives adaptation and virulence of *L. monocytogenes* from farm to fork.

In addition to excellent scientific competences, the network will provide training in the transferable skills required for competitive research to secure funding, optimise management of working teams and exploit research results. ESRs will be trained in these transferable skills. This will secure world-class training for creative, entrepreneurial and innovative ESRs.

DESCRIPTION OF THE INDIVIDUAL RESEARCH PROJECTS:

Sub-project 1: Investigation of the adaptive strategies of *L. monocytogenes* in soil/plants mesocosms

Host Organization	University of Bourgogne (France)
Objectives	Project 1 will focus on adaptation of *L. monocytogenes* EGD-e to soil and plant rhizosphere. Transcriptomes during adaptation to the soil and to plant rhizosphere will be compared. Similarly, transcriptomes will be compared in the presence and absence of microbial community (soil versus g-irradiated soil). We will identify putative regulated proteins (proteomic). Mutagenesis will be implemented on selected targets in order to explore the mechanisms involved in the sensing of the environment and to characterise the phenotype of the deletion mutants in the defined environments.
Expected Results	Global transcriptomic response during adaptation to soil and plant roots. Characterisation of the response to soil microbiote. Determination of TSS. Identification of proteins critical for survival in this habitat. Evaluation of the role of the soil biotic environment on the behaviour of *L. monocytogenes*
Duration (months)	36
Contact	Dr. Pascal PIVETEAU: piveteau@u-bourgogne.fr

Sub-project 2: Stress pre-adaptation and virulence potential of *L. monocytogenes* in the food matrix

Host Organization	University College Cork (Ireland)
Objectives	Project 2 will examine the effects of important food components (including glutamate, arginine and carnitine) upon virulence potential in *L. monocytogenes*. Virulence assys will be performed with the ECadhum transgenic mouse system as a means to study the influence of food constituents upon pathogenesis using the pathogen in suspension (+/- specific food components)and also using special defined chow containing particular food components as additives (from Teklad/Harlan custom diets). The project will combine classical microbiological approaches as well as bioluminescent imaging of *L. monocytogenes* to track the pathogen in this system (IVIS system luminescence imaging). Deep sequencing approaches will be used to determine the effects of food constituents upon gene expression profiles in *L. monocytogenes* with a particular focus upon gene systems known to be induced *in vivo* during growth in the GI tract and necessary for virulence (e.g. SigmaB, BSH, bile, ADI, OpuC, GAD etc).
Expected Results	Global transcriptomic response to food matrix constituents. Determination of TSS. Identification of proteins critical for survival in this habitat.
Duration (months)	36
Contact	Dr. Cormac GAHAN: c.gahan@ucc.ie website: http://www.ucc.ie/en/hr/vacancies/research/full-details-541328-en.html

Sub-project 3: Regulation of the virulon of *L. monocytogenes* by carbohydrates

Host Organization	University Of Copenhagen (Denmark)
Objectives	Preliminary data suggest that host sugar-molecules have chitin-resembling moieties and that these are substrates for both bacterial and human chitinases. Considering that the *L. monocytogenes* chitinases are important in both environmental and host adaptation, we wish to identify the host chitin-type molecules and determine the role of these molecules as well as of the listerial chitinases for attachment, invasion and long-term survival in host cells. To assess the environmental role of the *L. monocytogenes* chitinases the project will address the regulation of chitinase expression and investigate the conditions at which they are expressed. The role of chitinases in adaptability of *L. monocytogenes* will be determined by investigation of the respective mutants in growth and survival in the various environments and the ability of the mutants to survive and initiate growth after abrupt changes in environments. Regarding the critical role of carbohydrates in regulation, this project will use combinations of proteomic and transcriptomic approaches to dissect the intricate regulatory mechanisms controlling chitinase expression. This will be studied using transposon mutagenesis and site-directed mutagenesis.
Expected Results	Global transcriptomic response to chitin. Determination of TSS. Identification of proteins critical for regulation of chitinase expression. Evaluation of the role of chitinases during infection. Contribution of chitinases to soil and plant adaptation.
Duration (months)	36
Contact	Dr. Hanne INGMER: hi@life.ku.dk

Sub-project 4: Role of σB regulon of *L. monocytogenes* in environmental stress resistance

Host Organization	National University of Galway (Ireland)
Objectives	This project will use a combination of proteomic and transcriptomic approaches to elucidate the light stimulon of *L. monocytogenes*. This analysis will be performed in both wild-type and *sigB* mutant backgrounds in order to define the contribution of σB to the light response. The role of novel light-inducible components will be determined using genetic approaches (deletion and overexpression studies). The behaviour of these mutants will be performed during secondments. The role of novel light-inducible components will be determined using genetic approaches (deletion and overexpression studies). A 3-month secondment to UB will be used to investigate the possibility that light can influence biofilm formation and soil survival. At NUIG a series of innovative experiments will be conducted to investigate the effectiveness of blue light in controlling the growth and survival of *L. monocytogenes* on food processing surfaces and on food surfaces (vegetables, cheese and fish). Together these approaches will give new insights into the molecular response of *L. monocytogenes* to light, and establish whether visible light might be used as a means of controlling this organism in the food chain.
Expected Results	Global transcriptomic response to light stimulus. Together these approaches will give new insights into the molecular response of *L. monocytogenes* to light, which may have important implications for food producers.
Duration (months)	36
Contact	Dr. Conor O'BYRNE: conor.obyrne@nuigalway.ie

Sub-project 5: Role of protein secretion in adaptation of *L. monocytogenes*

Host Organization	INRA (France)
Objectives	This project will connect the role of secreted protein, particularly the cell surface-associated proteins named surfaceome (or surfome) and the extracellular proteins named exoproteome during biofilm formation and adaptation to controlled environments (eg low air relative humidity). A combination of classical and innovative proteomic approaches by using different protein extraction methods (precipitation, cell fractionation, cell enzymatic shaving), in-gel and off-gel protein separation and analysis (2-DE and Maldi-Tof MS; label-free quantitative LC-MS/MS) and Maldi imaging MS of proteins, peptides and lipids in biofilms will be implemented to explore the molecular response of *L. monocytogenes*. The analyses will be performed both with a reference sequenced strain and a strain qualified as persistent due to its identification during several years in a foodstuff plant (but never found on output food products). Deletion mutants will be constructed to understand the regulation and role of some proteins of interest.
Expected Results	Quantitative proteome allowing linkage between mRNA levels and protein levels in order to strengthen interpretation of transcriptome data. Decipher molecular response in the interface cell/environment.
Duration (months)	36
Contact	Dr. Michel HEBRAUD: hebraud@clermont.inra.fr

Sub-project 6: Biodiversity and transmission of *L. monocytogenes* in the food chain

Host Organization	Wageningen University (Netherlands)
Objectives	This project will focus on the performance of *L. monocytogenes* food and outbreak isolates (belonging to different lineages) in single and mixed species biofilms using selected secondary species including lactic acid bacteria isolated from food processing environments. A next-generation sequencing-based method that targets a variable intergenic region will be employed to assess biofilm ecology in single and mixed species conditions to assess performance of different *L.monocytogenes* strains when administered in mixtures. Relevant transcriptome and full genome sequences will be obtained and used in gene trait matching with selected biofilm and food process stress resistance-associated phenotypes. Screening will be performed for establishment of stable stress resistant persisters isolated after exposure to disinfection treatments and during maturation of biofilms. The performance of these strains including growth characteristics at low temperatures under aerobic and anaerobic conditions, and stress resistance will be analysed in single and mixed strain competition in relevant conditions encountered during transition from saprophytic to virulent lifestyles.
Expected Results	Transcriptomic response to the biotic environment, assessment of intraspecies diversity.
Duration (months)	36
Contact	Dr. Tjakko ABEE: tjakko.abee@wur.nl

Sub-project 7: Role of non-coding sRNAs in the transmission of *L. monocytogenes* between environments

Host Organization	University of Southern Denmark (Denmark)
Objectives	This project will focus on the characterization of selected non-coding sRNAs in *L. monocytogenes* specifically induced in response to signals encountered by the bacterium in the external environment and/or within the infected host. The role(s) of the sRNAs will be addressed by searching for their cellular targets and mechanism of action. These studies involve i) identification of genes affected by the sRNAs, using transcriptomics and proteomics approaches. ii) Studies of the regulatory mechanism by which the sRNAs affect the translation and/or mRNA stability of their target genes, using experimental and computational approaches. iii) Studies of the role of sRNAs and selected target genes in virulence, using various infection models. Proteome analyses will be performed in NUIG
Expected Results	Characterisation of the regulatory role of selected ncRNAs, identification of regulated genes, assessment of their role in adaptation to selected habitats and virulence.
Duration (months)	36
Contact	Pr. Birgitte KALLOPOLITIS: bhk@bmb.sdu.dk (**Application online on the SDU website** : http://sdu.dk/en/servicenavigation/right/ledige_stillinger/jobs/Soegjob)

Sub-project 8: Investigation of interconnections between AgrA and σB regulons (Joint supervision PhD)

Host Organizations	University of Bourgogne (France) and National University of Galway (Ireland)
Objectives	The objective of this project under the joint supervision of UB and NUIG is to investigate the crosstalk between cell communication (mediated by AgrA) and stress response (mainly mediated by σB). The role of AgrA and σB have been studied for several years by UB and NUIG respectively. Recent evidence suggests that these regulators may interact in a way not previously expected. This project will investigate the crosstalk between these two regulators. Reporter strains will be constructed that allow the activities of the two regulators to be monitored by recording cellular fluorescence, either in the presence or absence of the other regulator. Using a combination of flow cytometry and fluorescence microscopy, the activities of these important regulators will be measured in both planktonic cells and in biofilm. Mutants of a selection of target genes will be constructed and their phenotypes will be assessed under specific environmental conditions such as survival in soil and rhizosphere.
Expected Results	Understanding of the cellular integration of biotic stimuli and harsh conditions. Hierarchy of the cell response under specific environmental conditions.
Duration (months)	36
Contact	Pr. Pascal PIVETEAU: piveteau@u-bourgogne.fr Dr. Conor O'BYRNE: conor.obyrne@nuigalway.ie

Sub-project 9: Transcription regulatory network construction

Host Organizations	INRA (France)
Objectives	A significant part of regulation at the transcriptional level is achieved by modulation of transcription initiation rate. In bacteria, transcription initiation relies on recognition of particular sequence motif by a Sigma-factor approximately 10 bp upstream of the transcription start site (TSS). Tiling array and next generation sequencing transcriptomics provide direct information on the repertoire of TSSs and transcription units and thereby offer new perspectives to address the problem of motif identification. The first step of the project will be to characterise the transcriptional activity of the bacteria (detection of trancripts, delineation of operon structure, identifications of TSS and transcription terminators) by using existing tools. As a second step, new methods will be developed for prediction of TF binding sites by combining expression profiles and precise information on the location of the TSSs. Two approaches based on position weight matrix (PWM) models would be investigated. The classical mixture model will be extended by relaxing the hypothesis that motifs corresponding to different TF binding sites occur independently between TSS regions. In the new model, we will explicitly account for the increased probability of occurrence of a same motif in two promoters when their profiles of activity across conditions are similar. In parallel, Hawkes' processes could be used to screen a list of candidate motifs. Hawkes' processes allow the analysis of the patterns of motif co-occurrence at preferential distances and will also serve here to account for expression patterns.
Expected Results	Construction of a mathematical model of the regulatory network of *L. monocytogenes*. First of all, existing data will be integrated in a first model of regulatory network. In a second step, experimental data will beprocessed to generate the final model. Elucidation of the effect of environmental conditions on virulon expression.
Duration (months)	36
Contact	Dr. Vincent FROMION: Vincent.fromion@jouy.inra.fr Pierre Nicolas pierre.nicolas@jouy.inra.fr Website : http://maiage.jouy.inra.fr/?q=fr%2Fnode%2F394

Sub-project 10: Development of bioinformatic tools for the analysis of MACE data

Host Organizations	GenXPRO (Germany)
Objectives	The ESR will be in charge of standardising pipelines that will be used for RNA-seq and MACE analyses by all the participants. He will be involved in performing next generation sequencing to characterise environmental adaptation. A single pipeline to analyse listerial transcriptomic and proteomic data will be developed and implemented by each partner for the sake of uniformity of all the data produced within List_MAPS. The ESR will be involved in the interpretation of transcriptomic and proteomic data for which pathway analyses and good data visualization will be required. A cytoscape app will be developed as visualization tool.
Expected Results	MACE analysis pipeline. Database. Transcriptome comparisons in selected habitats. Data visualization tool.
Duration (months)	24
Contact	Dr. Bjorn ROTTER: rotter@genxpro.de

Sub-project 11: Development of innovative tools for rapid phenotypic characterisation of intraspecific diversity of *Listeria monocytogenes* (Joint supervision PhD)

Host Organizations	BioFilm Control (France) and GenXPRO (Germany)
Objectives	1. The ESR will develop an assay to test biofilm phenotype in a large array of food processing-related environmental conditions (salt, acides, disinfectants, preservatives) in BFC facilities. He will be in charge of the development and validation of an *in silico* virulence assay. This assay will target specific mRNAs in order to estimate the virulence potential of strains of *L. monocytogenes*. Transcript targets will be selected and tested by qPCR in GXP premises. In the process of validation, virulence results of several strains collected in a humanised mouse model will be compared with the *in silico* analysis. Once these innovative tools will be validated, intraspecific phenotypic diversity (biofilm and virulence) will be assessed on a collection of environmental and clinical isolates of *L. monocytogenes*. Genotypic diversity will be assessed under the supervision of GPX.
Expected Results	Adaptation of the BioFilm Ring test R to test food processing environmental conditions. Development of an innovative *in silico* virulence assay surrogate to animal models. Diversity results will inform stakeholders on the level of health hazard according to the strain. This in turn will help secure food safety all along the shelf life of foodstuff.
Duration (months)	36
Contact	Dr. Thierry BERNARDI: thbe@biofilmcontrol.com Dr. Bjorn ROTTER: rotter@genxpro.de

ELIGIBLE CRITERIA of Marie Sklokowska Curie actions:

- Researchers may be of **any nationality**
- Candidates shall at the time of recruitment by the host organization, **be in the first four years** (full-time equivalent research experience) **of their research careers.** Full-time equivalent research experience is measured from the date when a researcher obtained the degree which would formally entitle him or her to embark on a doctorate, either in the country in which the degree was obtained or in the country in which the researcher is recruited or seconded, irrespective of whether or not a doctorate is or was ever envisaged.
- Candidates must **not have a doctoral degree**
- Mobility rules: candidates shall **not have resided or carried out their main activity** (work, studies) **in the country of their host organization for more than 12 months in the 3 years** immediately **prior their recruitment**.

REQUIREMENTS:

- Academic ability
- Scientific skills and competences
- Labwork experience

- Proficiency in foreign languages
- Level of independence
- **Other experiences**

APPLICATION PROCEDURE:

Application deadline: 26th June 2015. Application should be sent by email to the specific contact named above or the website indicated. Attachments must include:

- Cover letter
- Curriculum vitae
- One reference letter
- Transcript of results of the last 3 years of University studies (including modules and courses)