



HR EXCELLENCE IN RESEARCH

**13 PhD Fellowship positions available in the
Marie Skłodowska-Curie Actions
Innovative Training Network
"PATHSENSE"
(H2020-MSCA-ETN-721456)**

Ref. No. NUIG 072-17

*Training Network to Understand and Exploit Mechanisms of
Sensory Perception in Bacteria*

Located in Ireland, Germany, Netherlands, Spain, Sweden, Switzerland and UK.

Project background and goal: The PATHSENSE (Pathogen Sensing) ETN investigates the molecular mechanisms of sensory perception in bacterial pathogens. Rapid and sensitive systems to sense and respond to environmental changes are a cornerstone of a bacterium's survival apparatus, and understanding these sensory systems is central to predicting their behaviour. The success of bacterial pathogens is underpinned by their ability to sense their environment in order to protect themselves and then to deploy their virulence mechanisms at the appropriate time. A deep knowledge of how their first line of defence (sensory perception) functions is a vital step in developing strategies to subvert their survival apparatus, and ultimately to preventing human, animal and plant infections.

The overall objective of this project is to focus on understanding a highly sophisticated but poorly understood sensory organelle called the "stressosome". The relationship between molecular structures and biological function is central to understanding any living system; however the research methodologies required to unravel these relationships are often complex and fast-changing. The team participating in the PATHSENSE Network will recruit and train 13 early stage researchers (ESRs) in state-of-the art methodologies, including structural biology, proteomics & protein biochemistry, molecular biology, bacterial genetics, food microbiology, mathematical modelling, cell biology, microscopy and comparative genomics. This inter-sectoral Network comprises 8 leading Universities, 1 public research institution, 4 companies (from spin-off to large multi-national) and 1 governmental agency. A major objective of this Network will be to exploit the fundamental research to develop novel antimicrobial treatments that have applications in the food and public health sectors.

Career Stage

Early Stage Researcher (ESR):

ESR are those who, at the time of recruitment, are in the first four years (full-time equivalent) of their research careers. This time is measured from the date when they obtained the degree which formally entitles them to embark on a doctorate, either in the country in which

the degree was obtained or in the country in which the research training is provided, irrespective of whether or not a doctorate was envisaged. Qualifications required for entry into the PhD program in each partner country can be found on each partner's website or by contacting the partner by email (see links in the project descriptions section).

Benefits and salary

The MSCA-ITN programme offers a highly competitive and attractive salary and working conditions. The successful candidates will receive a salary in accordance with the MSCA regulations for early stage researchers. Exact salary will be confirmed upon offer and will be based on a Living Allowance of €3110/month to be paid in currency of country where based and with a correction factor to be applied per country + mobility allowance of €600/month. Additionally researchers may also qualify for a family allowance of €500/month depending on family situation. Taxation and Social Contribution deductions based on National and Institutional regulations will apply and will be deducted from the gross payment highlighted above. In addition to their individual scientific projects, all fellows will benefit from further continuing education, which includes the opportunity to register for a PhD degree, scientific skills courses, transferable skills courses, active participation in workshops and conferences, and secondments to partner labs.

Applicants need to fully comply with the three eligibility criteria:

- 1) Early-stage researchers (ESR)** are those who are, at the time of recruitment by the host, in the first four years (full-time equivalent) of their research careers. This is measured from the date when they obtained the degree which formally entitles them to embark on a doctorate, either in the country in which the degree was obtained or in the country in which the research training is provided, irrespective of whether or not a doctorate was envisaged. Please note applicants cannot already hold a PhD.
- 2) Conditions of international mobility of researchers:** Researchers are required to undertake trans-national mobility (i.e. move from one country to another) when taking up the appointment. At the time of appointment by the host organisation, researchers must not have resided or carried out their main activity (work, studies, etc.) in the country of their host organisation for more than 12 months in the 3 years immediately prior to their recruitment. Short stays, such as holidays, are not taken into account.
- 3) English language:** Network fellows (ESRs) must demonstrate that their ability to understand and express themselves in both written and spoken English is sufficiently high for them to derive the full benefit from the network training. Non-native English speakers are required to provide evidence of English language competency before the appointment is made. An IELTS score of 6.5, or equivalent, is the minimum requirement.

PhD Projects

ESR1: Stressosome expression, localisation and diffusion in response to osmotic stress in *Listeria monocytogenes*

Objectives: The biochemical processes that characterize all living cells, such as energy provision, gene expression, and cell division, take place in a confined and highly crowded space. The reaction conditions in the cytoplasm are very different from those in the test tube. The high concentration of macromolecules impacts individual proteins and provides a unique environment for catalysis and the co-evolution of cell components. We will study the formation of protein complexes and the structure of the cytoplasm, using state-of-the-art super-resolution fluorescence microscopy (<http://www.membraneenzymology.com/our-equipment/>). We will track the activity, localization and mobility of individual protein complexes (e.g. the stressosome) in cells perturbed by osmotic stress. In parallel, we will use recently developed biosensors for crowding, ionic strength, pH and ATP to probe the physicochemistry of the cytoplasm.

Expected Results: A quantitative description of the physical biology of the cell, and a comparison of the reaction environment of pathogenic and non-pathogenic bacteria. A model

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of the structure of the bacterial cytoplasm, which clearly is not a bag of randomly organized enzymes.

Host: University of Groningen – RUG (Groningen, Netherlands)

Supervisor: Prof Bert Poolman (For information on this lab and more detail of the project please see www.membraneenzymology.com or contact b.poolman@rug.nl)

ESR2: Structural analysis of *Listeria monocytogenes* and *Vibrio vulnificus* stressosomes

Objectives: To: (1) establish the architecture of the core *L. monocytogenes* stressosome (comprising RsbR, S, T) using cryo-electron microscopy; (2) characterise the influence of RsbR paralogues and sensing defective mutants of RsbR on the overall structure of the stressosome; (3) compare the structures of the *V. vulnificus* and *L. monocytogenes* stressosomes.

Expected Results: A model describing the stoichiometry and structure of the *L. monocytogenes* stressosome, incorporating both core stressosome subunits and paralogues of RsbR. An understanding of how sensing defective stressosome mutants influence the overall structure. A comparative model of stressosomes from distantly related food-borne pathogenic bacteria.

Host: Universität Regensburg – UR (Regensburg, Germany)

Supervisor: Prof Christine Ziegler (For information on this lab and more detail of the project please see <http://www.biologie.uni-regensburg.de/Biophysik2/Ziegler/> or contact christine.ziegler@ur.de)

ESR3: Genetic characterisation of the *Listeria monocytogenes* stressosome and role in virulence and food survival

Objectives: In *L. monocytogenes* the stressosome contributes to σ^B activation in response to different environmental stimuli (e.g. acidic pH, visible light, osmotic stress) but the molecular mechanisms of stress sensing are largely not understood. In this project the sensory components of the stressosome, thought to involve RsbR and its paralogues, will be dissected genetically. Mutants lacking these components will be phenotypically characterised to determine the role of each in stress sensing. The possibility that the stressosome may interact with other regulatory elements will be investigated by screening a transposon library for mutants that fail to induce σ^B in response to stress. The contribution of stress sensing to virulence and survival in the food chain will be investigated using established food and animal model systems.

Expected Results: This project will define the sensory elements of the stressosome in *L. monocytogenes* and establish what role these play in the saprophytic and host-associated phase of this pathogen's life cycle. The project will also give new insights into the mechanisms underpinning stress sensing, which in the long term should help devise new control measures for this important pathogen.

Host: National University of Ireland Galway – NUIG (Galway, Ireland)

Supervisor: Dr Conor O'Byrne (For information on this lab and more detail of the project please see <http://www.nuigalway.ie/microbiology/cpoblab/> or contact conor.obyrne@nuigalway.ie).

ESR4: Role of the *Listeria monocytogenes* stressosome during intracellular pathogenesis

Objectives: To: (1) determine the role played by stressosome components in the invasion and intracellular life cycle of *L. monocytogenes*; (2) measure the influence of the stress sensing via the stressosome on the *L. monocytogenes* proteome in the vacuole and

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cytoplasm of the host cell; (3) determine the influence of the stressosome on the cell envelope structure of *L. monocytogenes* (protein and glycolipid).

Expected Results: A comprehensive understanding of the role of stress sensing via the stressosome on the intracellular behaviour of *L. monocytogenes*. A full description of the role of the stressosome in regulating the proteome and cell surface properties during the intracellular stage of infections.

Host: Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas - CNB-CSIC (Madrid-Spain)

Supervisor: Prof Francisco Garcia del Portillo (For information on this lab and more detail of the project please see <http://www.cnb.csic.es/index.php/es/investigacion/departamentos-de-investigacion/biotecnologia-microbiana/laboratorio-de-patogenos-bacterianos-intracelulares> or contact fjportillo@cnb.csic.es)

ESR5: The design principles of the stressosome signalling module at the single-cell level

Objectives: Gene expression is noisy; single cells often display dynamic and heterogeneous gene expression, even in an isogenic population under uniform environmental conditions. This variability can be obscured by traditional 'bulk' approaches that average away dynamics over 1000's of cells. To tackle this in this project we will combine single-cell time-lapse microscopy, synthetic biology approaches, and mathematical modelling to understand the design principles of the stressosome signalling module. Broadly, the project goals will involve: (1) Perform single-cell time lapse microscopy on *B. subtilis* cells carrying a fluorescent reporter of σ_B activity and measure σ_B activity in response to acid pH, osmotic stress and ethanol; (2) measure the effects of stressosome mutants with impaired sensing ability on the activation of σ_B at the single cell level; (3) produce a mathematical model describing the sensory behaviour of *B. subtilis* and test this model by re-wiring the stressosome circuit based on model predictions.

Expected Results: A systems level understanding of how stressosome-mediated stress sensing and signal transduction influences the σ_B activation in *B. subtilis* in response to stress. A mathematical model that predicts the sensory behaviour of bacteria that has the potential to be validated in a food-related setting.

Host: University of Cambridge - UC (Cambridge, UK).

Supervisor: Dr James Locke (For information on this lab and more detail of the project please see <http://www.slcu.cam.ac.uk/directory/locke-james> or contact james.locke@slcu.cam.ac.uk).

ESR6: Structure and role of the *Vibrio vulnificus* stressosome

Objectives: Due to its oxygen-sensing activity, the *V. vulnificus* stressosome is an excellent model system to explore the structure-function relationship of this macromolecular signalling hub. In this project, and in a joint effort with ESR7, a series of mutants within the stressosome genes and downstream encoded signalling proteins will be constructed to a) establish the stressosome-dependent signalling pathway and b), using a gel-free proteomic approach, to identify proteins expressed in a stressosome dependent manner in *V. vulnificus*. Based on these results, reporter systems for stressosome activity in *V. vulnificus* will be generated, which will allow the investigation of stressosome signalling and phosphorylation in a variety of in vitro and in vivo contexts. In addition, proteins that constitute the stressosome signalling pathway will be overexpressed and purified for structural analysis in close collaboration with ESR2. Results from structural analysis will guide genetic work in order to evaluate predicted mechanisms of stressosome activation.

Expected Results: A structural model of the *V. vulnificus* stressosome including a predicted sensory mechanism. A detailed understanding of the role of the stressosome in modulating the *V. vulnificus* proteome in response to stress. Elucidation of the downstream signalling events triggered by the stressosome in *V. vulnificus*.

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Host: Universität Greifswald - UG (Greifswald, Germany)

Supervisor: Dr Jan Pané-Farré (For information on this lab and more detail of the project please see <https://mikrobiologie.uni-greifswald.de/staff/pane-farre/> or contact janpf@uni-greifswald.de)

ESR7: Genetic characterisation of the *Vibrio vulnificus* stressosome and role in virulence

Objectives: *V. vulnificus* is a bacterial pathogen that possesses the core stressosome components, but the downstream targets of the stress sensing module are unknown. ESR7 will determine the role of the stressosome in controlling the key virulence characteristics of this bacterium. In collaboration with ESR6, ESR7 will first construct a series of defined stressosome mutants in *V. vulnificus* and then characterise these mutants in terms of stress resistance, biofilm formation, surface properties and virulence. Virulence functions that will be assessed include cell adhesion, cell cytotoxicity, resistance to serum and induction of host inflammatory response.

Expected Results: A defined set of mutants that allow the role of the *V. vulnificus* stressosome to be elucidated. A detailed understanding of the contribution of the stressosome to stress tolerance, biofilm and virulence in *V. vulnificus*.

Host: National University of Ireland Galway – NUIG (Galway, Ireland)

Supervisor: Dr Aoife Boyd (For information on this lab and more detail of the project please see <http://www.nuigalway.ie/science/school-of-natural-sciences/disciplines/microbiology/stafflist/aoifeboyd/> or contact Aoife.Boyd@nuigalway.ie).

ESR8: Identification of sensory elements of the *Listeria monocytogenes* stressosome and elucidation of role in virulence

Objectives: RsbR and its paralogues are believed to constitute the sensory part of the stressosome in *Listeria monocytogenes*. To identify interacting partners of RsbR (and its paralogues) a bacterial two-hybrid system approach together with a genetic screen will be undertaken. Mutants lacking these novel partners will be phenotypically characterised to determine their role in stress sensing/tolerance and in virulence, using both human cell lines and a chicken embryo model. Also, a genetic approach will be undertaken to elucidate the mechanistic basis for the overlap between PrfA-controlled virulence gene expression and the σ^B -regulated stress response in *L. monocytogenes*.

Expected Results: This project will identify novel interaction partners to the *L. monocytogenes* stressosome and give a further understanding of these interactions in the physiology and virulence of this pathogen. The project will also give a comprehensive model explaining the overlap between virulence and the general stress response in *L. monocytogenes*.

Host: Umeå Universitet - UmU (Umeå, Sweden)

Supervisor: Dr Jörgen Johansson (For information on this lab and more detail of the project please see <http://www.molbiol.umu.se/english/research/researchers/jorgen-johansson/> or contact jorgen.johansson@umu.se)

ESR9: Identification and characterisation of novel antimicrobial plant extracts using stress-sensing biosensors

Objectives: To: (1) exploit stress-sensing biosensors to screen plant extracts and an existing compound library for antimicrobial activity against *L. monocytogenes* and *B. subtilis*; (2) extract, purify and chemically characterise novel plant compounds that have antimicrobial activity; (3) characterise the effects of the newly isolated antimicrobial compounds on cell physiology and virulence; (4) examine the effectiveness of the novel antimicrobials in food trials.

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Expected Results: Development of a new stress-sensing biosensor screen for antimicrobial compounds. Isolation of novel antimicrobial plant extracts and characterisation of their effects on bacterial cells. Validation of the new antimicrobial compounds in a food trial.

Host: Natac Biotech – Natac (Madrid, Spain)

Supervisor: Dr José Carlos Quintela (For information on this lab and more detail of the project please see <http://www.natac.es/?lang=en> or contact jcquintela@natac.es)

ESR10: Comparative and functional genomics of the general stress response in genus *Bacillus*

Objectives: *Bacillus* species form resistant spores that are ubiquitously present in the environment. Spores often survive heating processes and can lead to spoilage or food safety issues in foods upon germination and outgrowth. To prevent outgrowth, multiple hurdles can be applied in foods, but some *Bacillus* species are better adapted to overcome such stress factors than others. NIZO has an extensive collection of species that have led to spoilage in foods. The objective of this project is to: (1) perform an extensive comparative genomic study of the σ^B operon and regulon in the genus *Bacillus* using a large collection of newly available genomes sequences from wild isolates; (2) use σ^B reporters to correlate genotypic differences with altered σ^B activity and stress tolerance phenotypes; (3) perform proteomic comparisons on wild isolates with altered stress responses.

Expected Results: A model describing the evolution of the general stress response within the *Bacillus* genus. An understanding of how genetic changes in the σ^B regulon influence the sensory properties and phenotype of the organism, ultimately leading to improved control and prevention of outgrowth in foods.

Host: NIZO food research – NIZO (Ede, Netherlands)

Supervisor: Dr Marjon Wells-Bennik (For information on this lab and more detail of the project please see <https://www.nizo.com/> <https://www.nizo.com/food-safety-spoilage/> or contact marjon.wells-bennik@nizo.com)

ESR11: Use stress biosensors to develop a predictive model based on pathogen behaviour during food processing

Objectives: Quantitation of growth and survival of pathogens and spoilage organisms in food process environments is challenging. ESR11 will begin by developing a σ^B -based stress reporter system, and then using this system to correlate biosensor measurements with observed growth and survival behaviour. The student will then develop a predictive mathematical model based on these data, which will be validated in novel food systems to help develop new preservation regimes.

Expected Results: A predictive model that uses stress response measurements as an indicator of behaviour in food processing environments, and development of innovative preservation regimes that have applications in real food products.

Host: Nestlé Research Konolfingen - Nestlé (Konolfingen, Switzerland)

Supervisor: Dr Nicholas Johnson (For information on this lab and more detail of the project please see <http://www.nestle.com/jobs> or contact NicholasBrian.Johnson@rdko.nestle.com)

ESR12: Genetic study of the molecular mechanisms of sensory perception in *B. subtilis*

Objectives: The stressosome contributes to σ^B activation in response to different environmental stimuli but the molecular mechanism of stress sensing are largely not understood. The goal of this project is to (1) generate σ^B reporter fusions that allow the quantification of stressosome-dependent stress sensing in *B. subtilis*; (2) use random transposon mutagenesis and targeted mutagenesis of RsbR and its paralogues to identify mutants with defective stress sensing, revealing either locked-on or signal blind variants; and

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(3) in collaboration with Dr. James Locke to develop single-cell and population based mathematical models that account for the stress sensing behaviour of *B. subtilis*.

Expected Results: The development of stress-sensing biosensors for *B. subtilis*. Identification of novel factors influencing stressosome-mediated sensing in *B. subtilis* and a genetic definition of residues important for sensing in RsbR and its paralogues. A model describing the molecular mechanisms of stress sensing in *Bacillus*.

Host: University of Dundee - UD (Dundee, UK)

Supervisor: Prof Nicola Stanley-Wall (For information on this lab and more detail of the project please see <http://www.lifesci.dundee.ac.uk/people/nicola-stanley-wall> contact N.R.Stanleywall@dundee.ac.uk)

ESR13: Structure-function relationships within the sensory domains of the *Listeria monocytogenes* stressosome

Objectives: To: (1) determine the x-ray crystal structure of the amino terminal domains of *L. monocytogenes* RsbRA and its paralogues (Lmo0161, Lmo1642, Lmo1842); (2) to solve the structure of mutant variants with altered sensory functions; (3) perform domain-swap experiments combined with proteomics to determine the capacity for functional interspecies exchange of the sensory subunit.

Expected Results: Structural models of the sensory domains of the *L. monocytogenes* stressosome. A testable mechanistic model explaining the sensory capacity of the stressosome (using data from mutant analysis). An understanding of the structural constraints within the system and between species based on the domain swap studies.

Host: Newcastle University - NU (Newcastle, UK)

Supervisor: Dr Richard Lewis (For information on this lab and more detail of the project please see <http://sbl.ncl.ac.uk/> or contact rick.lewis@newcastle.ac.uk)

Application procedure:

All applications **must be made on the PATHSENSE APPLICATION FORM** which is included in advertisement and available to download from <http://www.nuigalway.ie/about-us/jobs/researchjobs/>

The form should be completed and e-mailed to **PATHSENSE@nuigalway.ie** by 5.00 pm (GMT) on 12th May 2017.

Recruitment process information: Eligible applications will be forwarded to the relevant partners in charge of each project and each partner will shortlist their applicants. Shortlisted candidates will be invited for interview with the PATHSENSE team at a central location in June. Applicants will be informed of the outcome by end of June 2017. Successful applicants will need to prove that they are eligible (three aspects: respect ESR definition, mobility criteria, and English language proficiency). The formal enrolment of the successful applicant as a PhD student will be taken by the Host Institution. The selected ESRs are expected to start 1st September 2017.

Closing date for receipt of applications is 5pm (GMT) on Friday 12th May 2017.

All positions are recruited
Transparent, Merit
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