Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral : ***Mechanophysiology of Bacterial Microcolonies***

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

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Adresse professionnelle :

Unité de Recherche :

Intitulé : **LJP - *Laboratoire Jean Perrin***

Code : **UMR8237**

Equipe de Recherche *(au sein de l'unité)* **:**

Intitulé : **MécanoMicroBiologie**

Thématique de recherche : **Biophysique, Mécanobiologie des bactéries.**

Responsable d'équipe :

NOM : **BIAIS** Prénom : **Nicolas**

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED PIF**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :

1 en cinquième et dernière année et 1 en deuxième année, 100%

CO-DIRECTION *(obligatoire)*

Co-Directeur de Thèse (titulaire d'une HDR) :

NOM : **HALIYO** Prénom : **Sinan**

Titre : **MCF HC Sorbonne Université** **HDR** ☒

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Unité de Recherche :

Intitulé : **ISIR - *Institut des Systèmes Intelligents et de Robotique***

Code: **UMR 7222**

Equipe de Recherche *(au sein de l'unité)* **:**

Intitulé : **Interactions Multi-Échelles**

Thématique de recherche : **micro-robotique, robotique interactive, instrumentation scientifique**

Responsable d'équipe :

NOM : **HALIYO** Prénom : **Sinan**

Ecole Doctorale de rattachement : **EDSMAER (ED391)**

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :

1e année :1 étudiant 50 % – 2e année : 1 étudiant 50 % - 3e année : 2 étudiants 100 % et 50 %

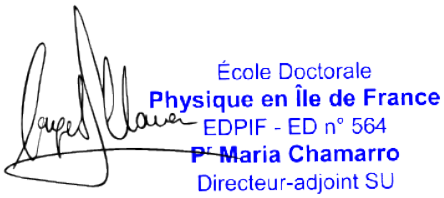
Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Résumé (2 000 caractères maximum) :

The role of physical cues in shaping the development of multicellular eukaryotic organisms is now firmly established. Even though it is now also appreciated that bacteria mostly live within dense multicellular communities called biofilms, the understanding of the role of physical cues within these communities is still in its infancy. This doctoral project will aim at studying the role of physical forces in the early biofilm formation of species of the *Neisseria genus*. Focusing on a pair of species, one pathogen and one commensal, the PhD candidate will combine molecular biology, genetics, biophysics and microscopy to tackle the role of physical cues in the physiology of these members of the human microbiota in order to both unravel the fundamental role of mechanical cues in bacterial physiology and understand how to use these cues to control the spread of these bacteria.

Joindre en annexe un descriptif du PRD avec références au format pdf
(« NOM_2_IPV_2021 » / 3 pages maximum, taille police 11)

AVIS et VALIDATION de l'ECOLE DOCTORALE :



Avis favorable

à envoyer simultanément par e-mail à l'ED de rattachement et au programme : interfaces_pour_le_vivant@listes.upmc.fr avant le lundi 15 février minuit.

Doctoral Project

Title: Mechanophysiology of Bacterial Microcolonies

Abstract : The role of physical cues in shaping the development of multicellular eukaryotic organisms is now firmly established[1]. Even though it is now also appreciated that bacteria mostly live within dense multicellular communities called biofilms[2,3], the understanding of the role of physical cues within these communities is still in its infancy[4]. This doctoral project will aim at studying the role of physical forces in the early biofilm formation of species of the *Neisseria genus*. Focusing on a pair of species, one pathogen and one commensal, the PhD candidate will combine molecular biology, genetics, biophysics and microscopy to tackle the role of physical cues in the physiology of these members of the human microbiota in order to both unravel the fundamental role of mechanical cues in bacterial physiology and understand how to use these cues to control the spread of these bacteria.

Context and objective:

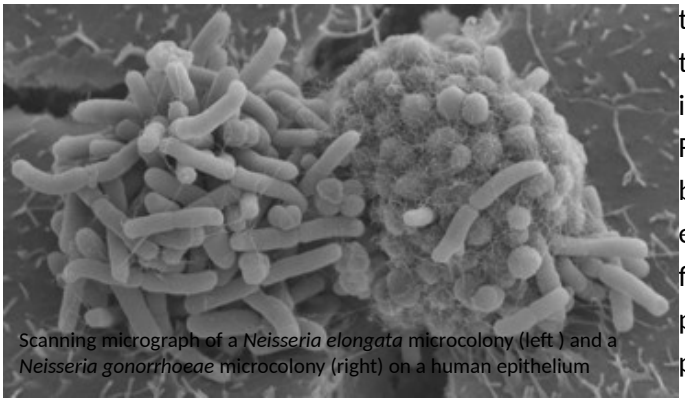
Most of what is known about bacterial physiology has been figured out for bacteria living a planktonic lifestyle, free-floating in relative low density in liquids. We now understand that the majority of bacteria live a sessile lifestyle attached to surfaces where they form organized physically connected communities called biofilms[5]. One of the main differences in these two lifestyles is the existence of physical forces between bacteria and between bacteria and surfaces in the case of the biofilms. In line with the realization that the development of multicellular eukaryotic organisms is not solely due to the chemical signals within and in between cells but might involve at least a feedback of physical signals[6], it is time to understand the development of the biofilm superorganism as integrating both chemical and physical signals.

Type IV pili (Tfp) are ubiquitous prokaryotic polymers that have the unusual capacity to be highly dynamical[7]. With a diameter of 6 to 10 nm, Tfp can extend hundreds of microns away from the cellular body. When Tfp retract, they can exert mechanical forces on their surroundings whether an abiotic surface, host cells or other bacterial cells[8,9]. Tfp are the only outside organelles present in most bacteria from the genus *Neisseria*, hence are the main way to mediate physical interactions between cells. In the case of WT cells, the stochastic cycles of elongation and retractions of Tfp are what enables and structures the early biofilm formation. Within hours, WT bacteria form little balls of cells comprised of a few tens to a few thousands bacteria, called microcolonies[10]. The bacterial microcolonies are, in respect to biofilms, not dissimilar to the embryo in respect to a developed metazoan.

In the case of the causative agent gonorrhea, *Neisseria gonorrhoeae*, we have demonstrated that the dynamics of Tfp and the forces they can generate can induce a heterogeneous motility within microcolonies that leads to spatially differentiated gene expression[11]. We have also demonstrated that dynamics of Tfp and the forces Tfp can generate affect globally the physiology of *Neisseria gonorrhoeae* cells whether in their survival in stationary phase or their sensitivity to antibiotics. The close cousin commensal bacterium, *Neisseria elongata*, shows a similar dependence on Tfp for the early formation of biofilm and its general physiology[12].

Mechanical interactions generated by Tfp appears to be the main driver in the formation of microcolonies of these two evolutionarily closely related bacterial species, as well as in their physiologies. **The main objective of this PhD project is to investigate the relationship between the mechanical parameters and the biological functions within bacterial microcolonies.**

In both species, intercellular forces can be modified by either changing the adhesion forces between Tfp or their ability to retract. The adhesion forces between pili can be modified most efficiently by changing the sequence or post-translational modifications on the monomer constituting the pilus, the major pilin pilE. The retraction forces can be modified most efficiently by removing or mutating the molecular motor pilT. Cells deprived of Tfp stay planktonic, do not interact with each other and don't form microcolonies. We have already characterized biophysically quite a few of these mutants and the **first axis of this PhD project** will be dedicated to fully characterize them in terms of modification of the spatio-temporal expression of genes and the impact of these mutants on the bacteria physiology. For each mutant, we will be able to correlate the biophysical intercellular forces with the pattern of gene expression across microcolonies as exhibited by fluorescent gene reporters and the impact on physiology as exhibited by survival during stationary phase.



Scanning micrograph of a *Neisseria elongata* microcolony (left) and a *Neisseria gonorrhoeae* microcolony (right) on a human epithelium

In the case of *Neisseria gonorrhoae*, knocking out the molecular motor pilT abrogates Tfp retraction[13,14]. The pili are still extended but they don't retract. The ΔpilT mutant cells still adhere to each other and can form microcolonies but they don't exert retraction forces on each other. ΔpilT cells survive less in stationary phase. It is thus possible to find conditions where WT cells are still thriving while ΔpilT cells are all dead. This is the perfect situation to utilize the power of genetics. We can mutagenize ΔpilT cells and look for compensatory mutations that would enable the cells to survive. As the molecular motor pilT is responsible for retraction, mutations should either restore retraction or point out to the mechanisms linked to mechanosensation in these cells. A saturated screen for compensatory mutations and their characterization will be the **second axis of this PhD project**.

In order to fully understand the role of mechanical stimuli on the *Neisseria* microcolonies, it doesn't suffice to genetically modify the internal forces that are at play in their self-assembly. It will be important to apply external mechanical stimuli and follow their impact on both gene expression and cell physiology. To this end, we will use a microrobotic system to apply very localized forces on single microcolonies. This device is based on a robotic evolution of optical trapping techniques, which offers contactless nanonewton range 3D force generation and sensing with micrometer resolution. We will also design and implement an robotized cell stretcher device compatible with microscopy and long term incubator growth to apply controlled cyclical strain to a surface of 6 well plate well covered with microcolonies[15]. This will be the **third axis of this PhD project**.

T he three axes of this PhD project (characterization of Tfp mutants, genetic screen and application of external forces) are largely independent and can be started in parallel. These three axes will all converge in a

complementary fashion towards the main objective of the project. The relative advances along the different axes will be monitored in close partnership with the Thesis Advisory Committee, and will guide, in an organic manner, the advancement of the doctoral student and the emphasis to be given to them as the thesis progresses.

Role of each supervisor / skills provided: Nicolas Biais is a leading expert on the characterization of Tfp biophysical properties. He has also studied Neisseria species for over 15 years and designed genetic tools to easily genetically modify these organisms. He will be the leading supervisor on the first two axes. Sinan Haliyo is a leading expert on microrobotics and micro-manipulation of biological samples. He was at the helm of the conception and realization of many scientific instruments around force-sensing and control at microscales. He will be the leading supervisor on the third axis. Constant communication between the two coordinators will be facilitated by the physical closeness of the two labs. The student will spend time in both labs.

Profile of the desired student: The ideal student will have a background in biology, physics or engineering with previous experience in microbiology, microscopy and experimental biophysics. But more importantly we are interested in students that are ready to embrace the challenge of this truly interdisciplinary project.

References:

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