COLLEGE DOCTORAL PAYS DE LA LOIRE ED BIOLOGIE SANTE

Year 2024 /2025

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin:** CDE
Thesis title: **Characterization of interkingdom interactions between *E. coli* and *Candida*, implications for intestinal colonization by multidrug resistant organisms, and exploration of the potential of the microbiota as a resource for small molecule discovery.**		3 keywords: Bacteria, fungi, interkingdom interactions, antibiotics, antifungals, small molecule discovery.
Unit / team: **UR1155 – IICiMed – Cibles et médicaments des infections et de l'immunité**		
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Socio-economic and scientific context (approximately 10 lines):

The Word Health Organization (WHO) prioritized a list of multi-resistant bacteria that pose the greatest threat to human health and recently publish critical and high-risk fungal pathogens to guide research, development and public health actions (WHO, 2017, 2022). Among these lists are bacteria of the *Enterobacterales* family and yeasts of the *Candida* genus that are key priorities based on the urgency and need for new antibiotics and antifungals. Infections due to these pathogens have common features as they occur in immunocompromised patients and represent redoubtable healthcare-associated complications, associated with prolonged therapy and hospital stays, and high mortality. In most cases, single *Enterobacterales*, single *Candida* or co-infections are preceded by the intestinal opportunistic expansion of the pathogen from polymicrobial communities before its translocation into the bloodstream.

Significant advances in the metagenomic characterization of the human microbiome have allowed the identification of the bacterial and fungal microbiota composition (Aggarwal N et al, 2022). Microbial homeostasis is important for normal physiological functions and changes are associated with many human diseases. It is important to note that members of the microbiota interact and influence each other's growth and pathogenicity. In addition, the use of broad-spectrum antibiotics and antifungals, also modify the interaction networks. Recent clinical observations show that bacterial factors influence fungal proliferation (Delavy M et al., 2022; MacAlpine G et al., 2022). However, the understanding of the mechanisms involved between *Enterobacterales* and *Candida* interkingdom interactions are very limited. Nevertheless, the study of the mechanisms underlying antagonistic and synergistic interkingdom interactions would provide a better understanding of human health and disease.

Working hypothesis and aims (approximately 8 lines):

E. coli and *Candida* species are commensal and opportunistic pathogens often co-isolated from intestinal microbiota. Despite the billions of years of co-existence, this pair of microorganisms is an example on how little is known about cross-kingdom interactions. Our working hypothesis is that the complex interactions between these two types of microorganisms are variable depending on the genetic and phenotypic characteristics within the genus, species and even at the strain level. Thus, we propose to characterize *in vitro,* interactions between *E. coli* and *Candida*, by examining the mechanisms by which bacteria govern fungal growth and virulence, as well as how fungi regulate bacterial growth and pathogenesis. Another important feature is that the use of a large number of antibiotics and systemic antifungals, can alter interkingdom interactions in intestinal microbiota. We also propose to study the impact of these molecules on bacteria-fungi crosstalk and persistence. Based on the interaction profiles, we propose to study *in vivo* the impact of these interkingdom interactions on host immunomodulation.
The work expected from this thesis will allow progress towards the understanding of chemical and physical interactions, and their role in exacerbating or impeding intestinal colonization by multidrug-resistant organisms. Manipulating these polymicrobial interactions should allow us to explore the potential of the human microbiota as a resource for small molecule discovery.

Main milestones of the thesis (approximately 12 lines):

This thesis subject is a central project of the thematic axis 2 of the UR1155 - IICiMed, which was evaluated as very relevant and innovative by the HCERES committee for the current contract. This project is an opportunity to combine the laboratory's expertise in antibiotic resistance and medical mycology, in order to consolidate this innovative and interdisciplinary research axis. It also fits into the framework of the EUniWell University as we have established a European research consortium entitled "Interkingdom interactions in microbiomes linked to human health", which will allow the PhD student to collaborate, notably with colleagues from Birmingham (UK) and Semmelweis University (Hungary).

We will generate different *in vitro* bacterial-yeast co-culture systems from a biocollection of commensal and pathogenic *Candida* species in interaction with *E. coli* strains with different degrees of virulence, and susceptibility to antibiotics. Preliminary results have allowed us to identify some *Candida* isolates capable of inhibiting the proliferation of multi-resistant *E. coli*. Multiparametric analyses (survival, proliferation curves, virulence, resistance) will allow the identification of the different types of agonistic, synergistic and neutral interactions.

The characterization of the cellular and soluble fractions of the cocultures will be carried out by non-targeted and targeted analytical approaches in collaboration with the technological platforms of the SFR Bonamy. We will study the composition of these fractions by advanced mass spectrometry (lipidomics and proteomics), flow cytometry and sequencing analyses to determine potential candidate molecules exacerbating or blocking infection.

Finally, we will develop a panel of *in vivo* models of intestinal co-colonization by MDR *E. coli* and *Candida* in mice dysbiosed by antibacterial or antifungal agent. Through combinatorial experimental approaches using *in vitro* cocultures with human immune cells and murine models with antimicrobial-induced digestive dysbiosis, we will study how interkingdom interactions between microbiota, host immune system, antibacterial

and antifungal agents, and prebiotic candidates (currently tested in the lab), can modulate the persistent digestive colonization by multi-drug resistant bacteria and fungi.

Scientific and technical skills required by the candidate (2 lines):
The candidate must have skills in microbiology, cell biology, cell culture, biochemistry, molecular biology, imaging and flow cytometry.

3 publications from the team related to the topic (last 5 years):

Grégoire M, Berteau F, Bellouard R, Lebastard Q, Aubert P, Gonzales J, Javaudin F, Bessard A, Bemer P, Batard É, Lepelletier D, Neunlist M, Montassier E, Dailly É. A murine model to study the gut bacteria parameters during complex antibiotics like cefotaxime and ceftriaxone treatment. Comput Struct Biotechnol J. 2021 Mar 4;19:1423-1430. doi: 10.1016/j.csbj.2021.02.019.

Ishnaiwer M, Bezabih Y, Javaudin F, Sassi M, Bemer P, Batard E, Dion M. In vitro and in vivo activity of new strains of Bacillus subtilis against ESBL-producing Escherichia coli: an experimental study. J Appl Microbiol. 2022 Mar;132(3):2270-2279. doi: 10.1111/jam.15329.

Alvarez-Rueda N, Rouges C, Touahri A, Misme-Aucouturier B, Albassier M, Pape PL. In vitro immune responses of human PBMCs against Candida albicans reveals fungal and leucocyte phenotypes associated with fungal persistence. Sci Rep. 2020 Apr 10;10(1):6211. doi: 10.1038/s41598-020-63344-6.

National and international collaborations:

Pr. Frédéric DALLE, PU-PH, Université de Bourgogne Franche-Comté à Dijon. Pr. Christine IMBERT, PU, Université de Poitiers. Pr. Boualem SENDID, PU-PH, Université de Lille.

EUniWell Consortium : Prof. Dóra Szabó - Head of the Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary.

Dr Jan-Ulrich Kreft, Associate Professor in Computational Biology, and Timothy Foster, PhD Student, University of Birmingham, UK.