Project title: **Structural Elucidation of Siderophores from Bacteria of the genus *Achromobacter*.**

Research teams and supervisors:

- Institut des Biomolécules Max Mousseron : équipe des **Sciences Analytiques des Biomolécules (F12) : Dr Adrien Chouchou**

- HydroSciences Montpellier (UMR5151 HSM) : équipe **Pathogènes Hydriques-Santé-Environnements (PHySE) : Pr Hélène Marchandin**

Context and issues:

Iron is an essential nutrient for the homeostasis of living organisms, from microorganisms like bacteria to more complex organisms like humans, participating in various processes such as DNA, RNA, and protein synthesis, cellular respiration, cell proliferation, and gene expression regulation[1]. Iron's ability to gain or lose electrons (transitioning from ferrous Fe^{2+} to ferric Fe^{3+} forms or vice versa) plays a fundamental role in *in vivo* redox reactions. To mitigate iron's intrinsic toxicity, it is primarily found in a bound form within different organisms. Chelation can occur via macromolecules such as ferritin, which serves as a storage form, or through smaller compounds (under 2,000 Daltons) that constitute a pool of free transit iron[2]. Bacteria have developed various strategies to efficiently acquire iron based on its availability, nature, and oxidation state in the environment[3]. For example, when iron is limited, bacteria can synthesize and secrete siderophores. These are low molecular weight compounds (200 to 2,000 Daltons) that chelate ferric iron (Fe^{3+}) from the environment with very high affinity[4].

Currently, a great diversity of **bacterial siderophores** has been identified, with compounds exhibiting different chemical structures specific to certain bacterial genera[5]. Siderophores are classified into three main families with diverse chelating functions: phenolate-catecholates, hydroxamates, and hydroxycarboxylic acids[6]. Due to iron's importance for bacterial growth, siderophores are considered virulence factors for many human pathogenic bacterial species and represent an innovative target in the fight against these microorganisms[7]. Consequently, a first molecule combining a siderophore and an antibiotic, cefiderocol, is now available for treating certain infections caused by multidrug-resistant bacteria[8,9]. Other therapeutic strategies could also involve reducing siderophore production, inhibiting their secretion or the transport of siderophore-iron complexes from the extracellular medium to the bacterial cytoplasm, or altering mechanisms regulating iron homeostasis. Therefore, understanding the structural diversity of siderophores produced by pathogenic bacterial species is crucial.

In the proposed project, bacteria of the **genus *Achromobacter*** will be studied. These environmental bacteria are also opportunistic human pathogens, particularly in cystic fibrosis patients and immunocompromised patients where invasive infections have been reported[8]. Moreover, these bacteria are naturally resistant to many antibiotics, such as aminoglycosides and certain cephalosporins[10]. Recently, a population study (163 *Achromobacter* strains from various species) conducted by the PHySE team showed that: i) siderophore production is common in the genus *Achromobacter*, ii) this production is particularly observed in clinical strains from cystic fibrosis patients, and iii) the species predominantly identified in human infections, *Achromobacter xylosoxidans*, presents specific characteristics (highest prevalence of siderophore-producing strains and strains producing the highest amounts of siderophores)[11].

To date, the siderophore molecules produced by bacteria of the genus *Achromobacter* remain unknown.

Project objectives:

- **Identification and characterization**: i) elucidate the structure of siderophores produced by bacteria of the genus *Achromobacter* ii) characterize the diversity of siderophore production according to the origin of the strains (environmental, cystic fibrosis patient-derived, or non-cystic fibrosis patient-derived) and species.
- **Understanding mechanisms**: understand the mechanisms of siderophore production and regulation in *Achromobacter* to identify potential therapeutic targets.

Methodology:

1. ***Achromobacter* strains:** selection of strains from different species, including *A. xylosoxidans*, and from various origins (environmental strains, cystic fibrosis patient-derived or non-cystic fibrosis patient-derived) from the *Achromobacter* collection published by Sorlin *et al.*[11].
2. **Qualitative and quantitative analysis of siderophores by Mass Spectrometry:** use of high-resolution Mass Spectrometry (Orbitrap ID-X, Thermo Fisher Scientific) for structural elucidation of compounds present in the culture supernatant (based on siderophore spectral signature analysis) and comparison with known compounds in the literature (using Compound Discover software, Thermo Fisher Scientific). The development of a method for quantifying the main compounds by internal calibration will also be developed.

Innovation and impact:

This project constitutes the first study aiming to identify and characterize siderophores from bacteria of the genus *Achromobacter*. The results of this research could contribute to significant advances in the development of **new therapeutic strategies** against infections caused by bacteria of the *Achromobacter* genus, particularly during cystic fibrosis.

Bibliographic references:

(1) Galaris, D.; Pantopoulos, K. Oxidative Stress and Iron Homeostasis: Mechanistic and Health Aspects. Crit Rev Clin Lab Sci 2008, 45 (1), 1–23. https://doi.org/10.1080/10408360701713104.

(2) Vaulont, S.; Schalk, I. Rôles des sidérophores bactériens et de mammifères dans les interactions hôtes-pathogènes. Med Sci (Paris) 2015, 31 (8–9), 756–763. https://doi.org/10.1051/medsci/20153108014.

(3) Schalk, I. J. Innovation and Originality in the Strategies Developed by Bacteria to Get Access to Iron. Chembiochem 2013, 14 (3), 293–294. https://doi.org/10.1002/cbic.201200738.

(4) Hider, R. C.; Kong, X. Chemistry and Biology of Siderophores. Nat. Prod. Rep. 2010, 27 (5), 637–657. https://doi.org/10.1039/B906679A.

(5) Prabhakar, P. K. Bacterial Siderophores and Their Potential Applications: A Review. Curr Mol Pharmacol 2020, 13 (4), 295–305. https://doi.org/10.2174/1874467213666200518094445.

(6) Kramer, J.; Özkaya, Ö.; Kümmerli, R. Bacterial Siderophores in Community and Host Interactions. Nat Rev Microbiol 2020, 18 (3), 152–163. https://doi.org/10.1038/s41579-019-0284-4.

(7) Holden, V. I.; Bachman, M. A. Diverging Roles of Bacterial Siderophores during Infection. Metallomics 2015, 7 (6), 986–995. https://doi.org/10.1039/c4mt00333k.

(8) Isler, B.; Kidd, T. J.; Stewart, A. G.; Harris, P.; Paterson, D. L. *Achromobacter* Infections and Treatment Options. Antimicrob Agents Chemother 2020, 64 (11), e01025-20. https://doi.org/10.1128/AAC.01025-20.

(9) Jean-Pierre V, Sorlin P, Pantel A, Chiron R, Lavigne JP, Jeannot K, Marchandin H; Collaborative study group on antimicrobial resistance of *Achromobacter* spp. Cefiderocol Susceptibility of *Achromobacter* spp.: Study of an Accurately Identified Collection of 230 Strains. Ann Clin Microbiol Antimicrob. 2024, 23(1):54. doi: 10.1186/s12941-024-00709-z.

(10) Menetrey Q, Sorlin P, Jumas-Bilak E, Chiron R, Dupont C, Marchandin H. *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia*: Emerging Pathogens Well-Armed for Life in the Cystic Fibrosis Patients' Lung. Genes (Basel). 2021, 12(5):610. doi: 10.3390/genes12050610.

(11) Sorlin P, Brivet E, Jean-Pierre V, Aujoulat F, Besse A, Dupont C, Chiron R, Jumas-Bilak E, Menetrey Q, Marchandin H. Prevalence and Variability of Siderophore Production in the *Achromobacter* genus. Microbiol Spectr. **2024**, *12*(3):e0295323. doi: 10.1128/spectrum.02953-23.