Involvement of central catabolic pathways for aromatic compound degradation in the pathogenesis and polluted environment adaptation of *Scedosporium apiospermum*

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Abstract: Fungi of the *Scedosporium* genus are saprophytes, opportunistic pathogens in humans. This globally ubiquitous fungus is predominantly found in human-impacted environments contaminated with aromatic pollutants (wastewater effluents from sewage treatment plants, urban playgrounds as well as on roadsides, and petrol stations). Several studies have revealed their ability to degrade polyaromatic molecules derived from environmental pollutants. Our previous work (Poirier et al, 2021) demonstrates that species of the genus *Scedosporium* can grow in the presence of lignin. In the environment, the catabolic steps of polyaromatic molecules converge on a limited number of simple aromatic molecules (catechol, protocatechuate, hydroxyquinol and gentisate), which are handled by central intermediate pathways, also known as fission pathways. Bioinformatics analysis enabled us to characterize the gene clusters degrading these central molecules in *S. apiospermum*. Experimental results demonstrate the functionality of the gentisate pathway cluster in the presence of this molecule. The dioxygenases that catalyze benzene ring opening, a key step in the catabolic mechanism, are prime targets for the design of deletion strains. Deletion strains for the gene encoding dioxygenase were generated for each pathway by using CRISPR-Cas9 technology (Ravenel et al, 2025). These deletions have a different impact on the growth of these strains on media in the presence of the corresponding core molecules. Under certain conditions, these results suggest the implementation of compensatory mechanisms that remain to be defined. Moreover, this work establishes for the first time a link between aromatic compound catabolism and pathogenesis of a human opportunistic pathogen *S. apiospermum*.

Objective of the project.

To follow the study of phenotypes associated with dioxygenase deletion we would like to study different aspects of fungal growth:

- Comparison of biofilm formation of deletion strains with wild type strains
- Culture of deleted strains in presence of different chemical compounds
- Interaction with lung epithelial cells
- qRT-PCR experiments

The results obtained will help us to understand the function of dioxygenases and their associated pathways in pathogenesis and adaptation to polluted environments of *S. apiospermum*.

Another aspect of the project il also to go on with the development of tools for functional genetics in *Scedosporium* and to obtain complemented strain for dioxygenase mutants. In fact, if the CRISPR Cas 9 technology has been adapted with success to obtain deleted strains, to date only one complemented strain has been constructed in the laboratory for an adenylate cyclase deleted strain and this technic need to be optimized. So, the student will work also to adapt CRISPR Cas9 technology to create complemented strain and multiple deletion strains.

References

Poirier, W., Ravenel, K., Bouchara, J.-P. et Giraud, S. (2021). Lower Funneling Pathways in *Scedosporium* Species. *Frontiers in Microbiology*, *12*, 630753.

Ravenel K, Poirier W, Razafimandimby B, Bouchara J.P., Gastebois A. and Giraud S. Optimization of the Genome Editing CRISPR-Cas9 Technology in *Scedosporium apiospermum*. *Mycopathologia* , 190, 94.