Group III Hybrid Histidine Kinases :

New therapeutic target for *Scedosporium apiospermum* ?

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Context

Scedosporium apiospermum is the second filamentous fungus colonizing chronically the respiratory tract of patients with cystic fibrosis[1]. This fungus constitutes a real threat for patients because of its thermotolerance, its ability to disseminate in the systemic circulation, and its low susceptibility to antifungals. It is thus necessary to identify new potential therapeutic targets in this fungus. Here, we focused on hybrid histidine kinases (HHKs) since this family of proteins progressively emerge as prominent environmental sensors in the fungal kingdom and as ideal targets for future therapeutics. The group III HHK is of main interest since they were demonstrated to play important roles in host-pathogen interaction and virulence in some yeast and mold models[2].

HHK III roles

The group III HHK is one of the most documented among fungi. Its involvement in different physiological process had been studied such as adaptation to the osmotic stress, morphogenesis or virulence (**Fig. 1**)[3].

+++ Involvement largely demonstrated
\+ Involvement demonstrated in few species

Physiological process	HHK III
Osmosensing	+++
Oxidant adaptation	+
Morphogenesis	+++
Conidiation	+++
Cell wall integrity	+++
Melanin	+
Virulence	+++

Figure 1. Fungal HHK III and physiological process[3].

HHK III disruption

We were first interested in generating a HHK III mutant strain (**Fig. 2A**). A disruption cassette containing the *hph*^{R} gene (which confers resistance to hygromycin B in transformed cells) bordered by 5' and 3' homologous sequences of the *HHK III* gene was inserted by homologous recombination in the genome of the fungus following an adapted transformation procedure of protoplasts. The correct insertion of the disruption cassette at the corresponding locus and thus the disruption of the *HHK III* gene was finally validated by Southern blot analysis (**Fig. 2B**).

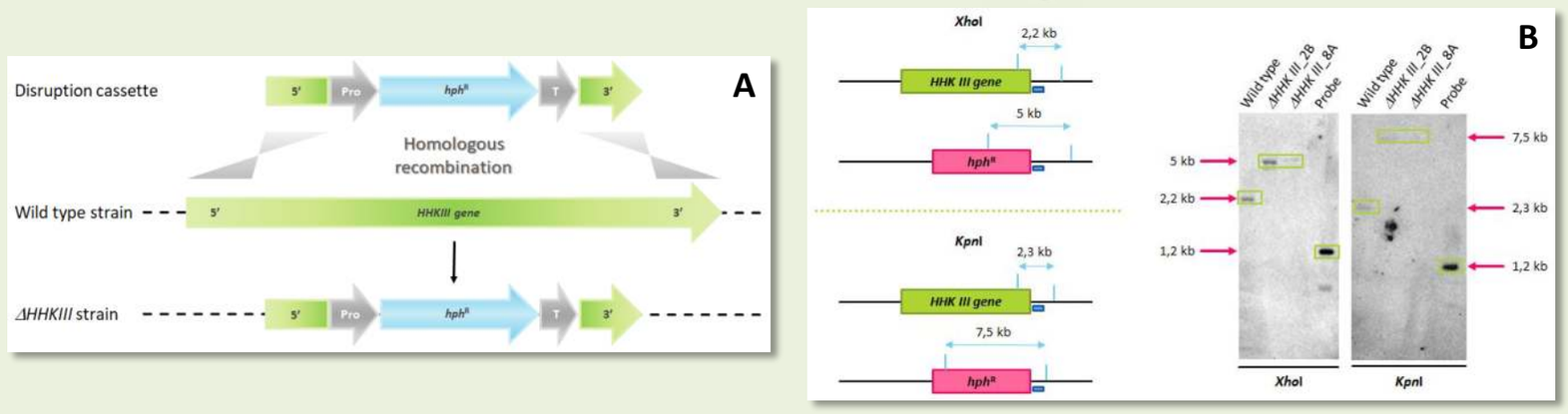

Figure 2. Generation of a HHK III mutant strain in *S. apiospermum*. **A.** Integration of a disruption cassette in the genome of *S. apiospermum* by homologous recombination. **B.** Southern blot analysis of the *HHK III* gene in transformants.

HHK III phenotypes

We then focused on the phenotypical comparison between the wild type and the *ΔHHKIII* mutant strains. According to **Fig. 1**, we would like first to appreciate the involvement of the *HHK III* gene in osmosensing (**Fig. 3A**), oxidant adaptation (**Fig. 3B**), morphogenesis (**Fig. 3C**) and cell wall integrity (**Fig. 3D**). Our preliminary results show a marked susceptibility of the mutant strain compared to wild type (**Fig. 3A**) when cultured under high osmolarity conditions. A marked difference is noted in the morphogenesis of both strains (**Fig. 3C**). Long and straight filaments are observed in the wild type strain while shorter and tortuous filaments harboring chlamydospores are observed in the mutant strain. No significant differences were noted between the mutant and the wild type strains in the presence of oxidant compounds (**Fig. 3B**) or cell wall inhibitors (**Fig. 3D**).

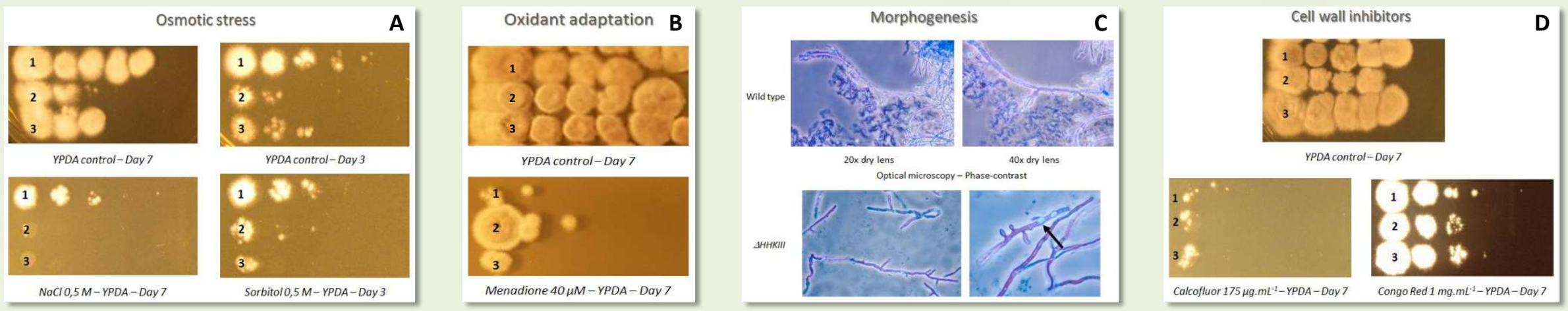

Figure 3. Preliminary results obtained after phenotype assays comparing the wild type strain and the *ΔHHKIII* mutants of *S. apiospermum*. *YPDA*: Yeast Peptone Dextrose Agar. *1*: Wild type strain. *2-3*: *ΔHHKIII* mutant strains. **A.** Involvement of the *HHK III* gene in osmosensing. **B.** Involvement of the *HHK III* gene in oxidant adaptation. **C.** Involvement of the *HHK III* gene in morphogenesis: the arrow indicates chlamydospores, a phenomenon which is never described in *S. apiospermum* wild type strain. **D.** Involvement of the *HHK III* gene in cell wall integrity.

Perspectives

Encouraging outcomes were obtained with the preliminary phenotype assays on the *ΔHHKIII* strain. Indeed, *HHK III* gene seems to play a role in the adaptation to osmotic stress and in the morphogenesis of *S. apiospermum*. Additional phenotypical analysis will be undertaken and in any case, we need to confirm these preliminary results with the reintegrant strain (that remains to be generated) in order to ensure that the observed phenotypes in the mutant strain exclusively derive from the loss of the *HHK III* gene.

References

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3. Hérivaux A, *et al*. Major sensing proteins in pathogenic fungi : the hybrid histidine kinase family. PLoS Pathog. 2016 Jul 28;12(7):e1005683.