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Secretome analysis of *Rhizopus delemar* identified a novel immunomodulatory peptide with enhanced phagocytic and iron homoeostasis activity

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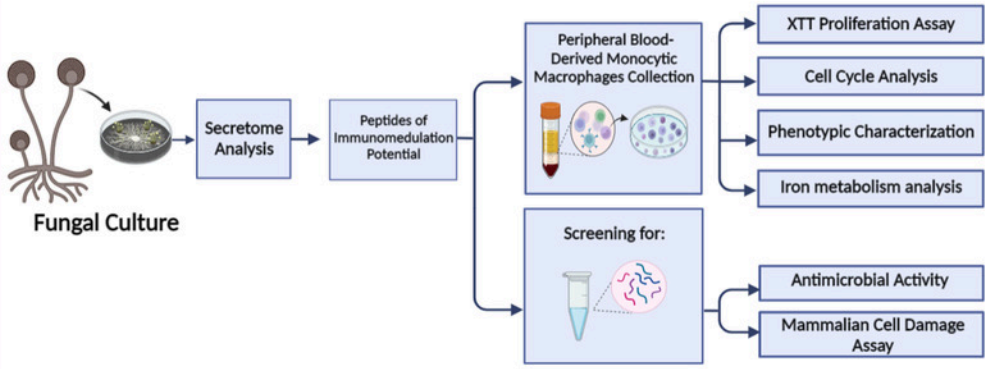
Introduction

Rhizopus species are responsible for ~70% of all mucormycosis cases, a disease with high mortality rates in immunocompromised patients and among significant risk factors causing nosocomial infections [1,2]. Analysis of secreted peptides that are released into the extracellular space by a pathogen during the infection provides a unique avenue to study pathogen-specific metabolic signatures [3]. This study provides for the first time an insight on the secreted peptides with immunomodulatory activity by *R. delemar* when incubated in a dissemination-like condition.

Objectives

- This study aimed to screen and evaluate secreted peptides from the fungus *R. delemar* with immunomodulatory activity and significantly involved in pathogenesis.

Methodology



Results

1. *R. delemar* Secretes Variable Peptides at Initiation of Infection and Hematogenous Dissemination.

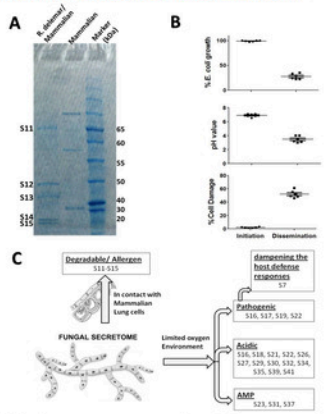


Figure 1: (A) Culture supernatants collected from mammalian cell cultures and co-cultures of *R. arrhizus var. delemar* and lung epithelial cells.(B) Effect of culture supernatants extracts pH, anti-bacterial activity against E. coli, and on mammalian cell survival. (C) *R. arrhizus var. delemar* secretome analysis in response to growth at two different environmental conditions

2. Peptide S27 Induced an M2-Like Polarization by Binding to IL-4/IL-13 Receptor.

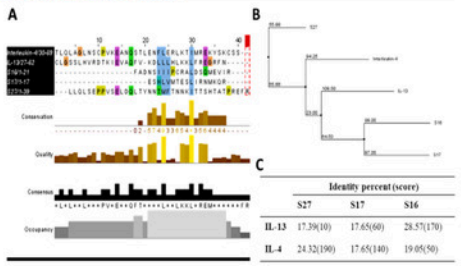


Figure 2: (A) Presence of a shared domain between S27 and IL-4. (B) S27 was more closely related to IL-4, and S16 and S17 were grouped with a closer relation to IL-13. (C) S27 has 24.32 and 17.39% identity with IL-4 and IL-13, respectively.

3. Peptide S27 Induced Activation of STAT-6 Protein.

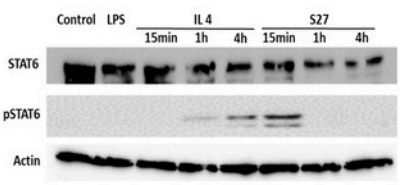


Figure 3: S27 showed early STAT-6 phosphorylation in the S27-treated macrophages at 15min. IL-4-treated initiate STAT-6 phosphorylation at 1 h.

4. PBMM response to S16, S17, and S27 peptides.

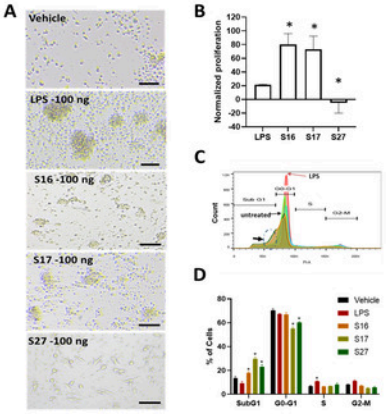


Figure 4: (A and B) peptides induced dose-dependent morphological changes and significant proliferation in PBMMs. (C and D) shift in cells population from G1 phase to sub G0 phase indicating terminal differentiation.

5. *R. arrhizus var. delemar* Peptide S27 Induces Non-classical M2-Like Polarization in Macrophages.

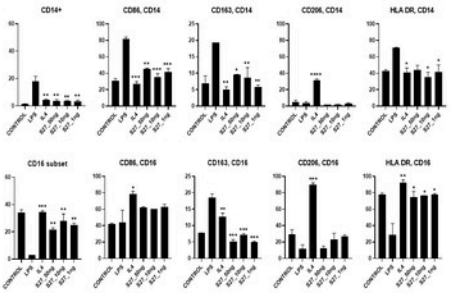


Figure 5: S27-treated PBMMs show high expression of CD16. As CD86, and HLA-DR were reduced. Expression of CD163 and CD206 was downregulated, indicative of specific immunomodulatory effects.

6. S27 Modulates Cellular Iron Metabolism in PBMMs.

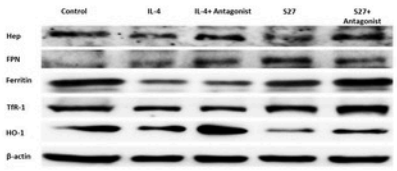
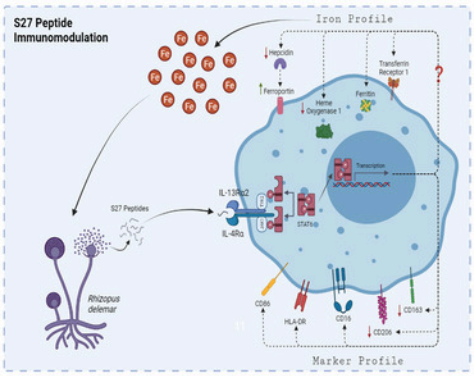


Figure 6: IL-4- and S27-treatment showed significant downregulation of HEP, and significantly upregulated. S27 showed no significant change in Ferr and TfR-1. Also resulted in reduction in heme oxygenase (HO-1) expression.

Conclusion



- *R. delemar* secretes peptides with immunomodulatory activities that support fungal pathogenesis.
- S27 peptide induces macrophage polarization toward an M2 phenotype by interacting with IL-4/IL-13R complex.
- S27 peptide induces switching of iron metabolism to increase the cellular iron availability that support further persistence and dissemination.
- Targeting the IL-4/IL-13R/STAT-6 axis is a potential therapeutic approach to enhance PBMM-mediated fungal phagocytosis.

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References

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