Exploring Enct Efflux Pump Functionality and their Role in Lipid Signaling

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Introduction

Cryptococcus neoformans, a fungal pathogen mainly affecting immunocompromised individuals, has sparked interest in lipid signaling research due to its role in pathogenesis. Eicosanoids, derived from fatty acids, are crucial in virulence and immune modulation; with *C. neoformans* lacking human enzyme homologs for eicosanoids biosynthesis, we want to identify the enzymes involved in the biosynthesis of cryptococcal eicosanoids and test their potential as antifungal targets. This project is focused on the EncT gene, encoding an efflux pump, which we observed to be upregulated in response to lipid precursors. Using CRISPR technology, we produced an EncT knockout (KO) strain and the corresponding reconstituted strain, aiming to discern shifts in virulence factors like melanin production, capsule formation, and urea production, among others, comparing the knockout, wild-type, and reconstituted strains and, subsequently, employing a mouse model of pulmonary cryptococcosis to delve deeper into virulence dynamics. Our initial results show early production of melanin EncT KO compared to the WT strain and no changes in the capsule formation or growth at 37° C.

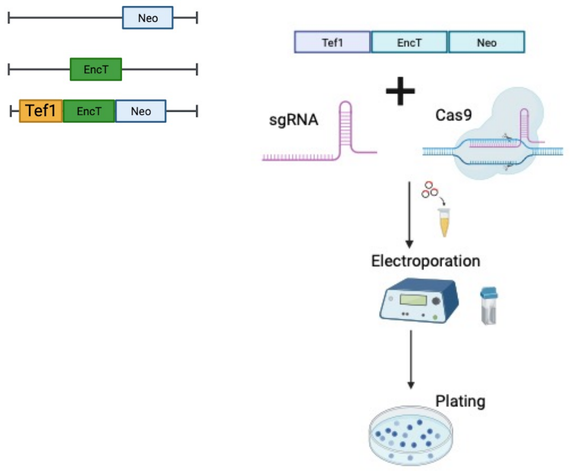
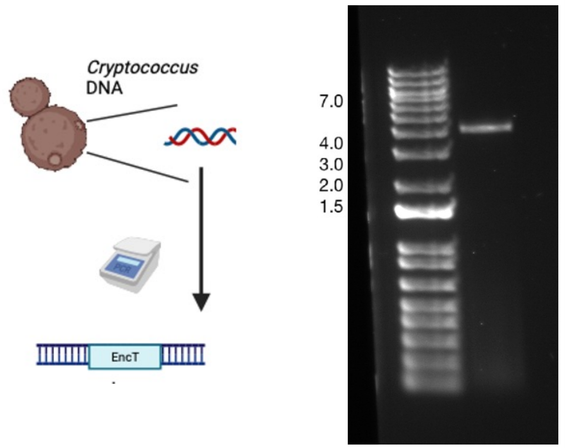


Figure 2. Amplification of the EncT gene



This gene encodes an efflux protein.

Conclusion and Future Directions

To assess the role of EncT, a knockout strain has been created.

Preliminary results show that the EncT KO strain can grow a host conditions (37° C) and is able to produce melanin and capsule, major virulence factors in *C. neoformans*.

In order to assess the role of EncT in the virulence of *C. neoformans*, we need to create a reconstituted gene.

We have made a construct by binding the Tef1 promoter, EncT gene, and the neomycin gene, the selection marker, our next step is to insert this construct into the EncT KO to reestablish the functionality of the gene in the previous KO created.

By putting this construct inside a plasmid with a *C. neoformans* promoter (TEF1) we will be able to obtain enough DNA to proceed to the transformation by electroporation using the Cas9, sgRNA.

We will isolate DNA from the colonies obtained in the transformation to corroborate if the EncT was inserted.

Once we corroborate the insertion of the gene, different phenotypic tests (production of melanin, capsule, among others) will be conducted in both the KO and the reconstituted strain.

Experimental Methods

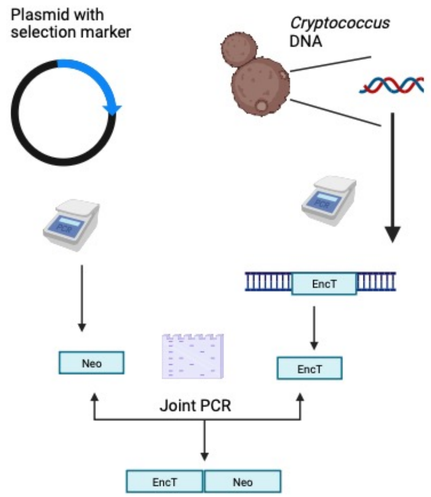
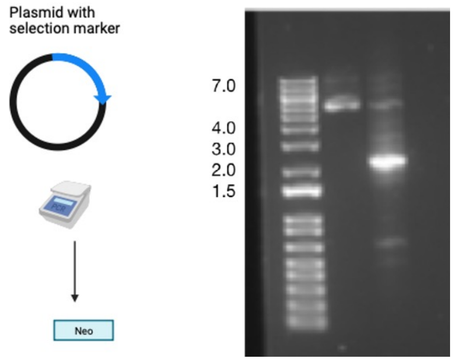
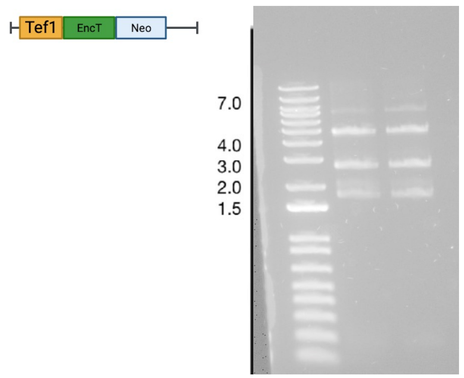


Figure 1. Amplification of the selection marker (neomycin)



The section marker helps us to identify the colonies that might be transformed because the cells that don't have the neo gene wont be able to grow when this antibiotic is present in the media.

Figure 3. Ligation of the construct to be added to the EnCT KO strain.



The three pieces together Tef1 promoter, EnCT gene and the selection marker neomycin (Neo). Amplified from the plasmid. Total size 6.2 Kb

References & Acknowledgements

Fan Y, Lin X. Multiple Applications of a Transient CRISPR-Cas9 Coupled with Electroporation (TRACE) System in the *Cryptococcus neoformans* Species Complex. Genetics. 2018 Apr;208(4):1357-1372. doi: 10.1534/genetics.117.300656. Epub 2018 Feb 14. PMID 29444806; PMCID: PMC5887135.

Lin J, Fan Y, Lin X. Transformation of *Cryptococcus neoformans* by electroporation using a transient CRISPR-Cas9 expression (TRACE) system. Fungal Genet Biol. 2020 May; 138: 103364 doi: 10.1016/j.fgb.2020.103364. Epub 2020 Mar 3. PMID: 32142753; PMCID: PMC7153975

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