

## Generation and phenotypic characterization of *SIN3* mutants of *Cryptococcus neoformans*

Parente, L.S.<sup>1</sup>; Coelho, CM<sup>1</sup>; Poças-Fonseca, M. J<sup>1\*</sup>.  
<sup>1</sup>Department of Genetics and Morphology, University of Brasília  
\* mpossas@unb.br

### Abstract

*Cryptococcus neoformans* is a haploid pathogenic yeast that can reproduce asexually or by sexual cycle. Scientific literature has shown the increase in annual cases of cryptococcosis, a disease with high lethality, mostly on immunodeficient patients, even with use of common antifungal drugs. Despite that, there has been a rise in cases presented in immunocompetent individuals. This disease is acquired by the inhalation of spores found in nature. Our research group already demonstrated that inhibitors of histone deacetylase (HDAC), proteins that participate in the remodeling of chromatin, are important for regulation of virulence attributes. *sin3* is a subunit of a HDAC complex called RPD3s. It has been described in *Saccharomyces cerevisiae* but not in *C. neoformans*. Obtaining mutants in *C. neoformans* can be challenging since this pathogen presents low rates of homologous recombination, the pathway usually used for genetic mutation. This work uses a variant of the CRISPR-Cas9 system to obtain mutants of the gene *SIN3* in *C. neoformans* and subsequently studies variation on key virulence phenotypes. Plasmid constructs of the CRISPR-Cas9 suicide technique were transformed via electroporation on H99, a clinical isolate of *C. neoformans*, and then selected via PCR for the insertion of a DNA fragment, 2 colonies were positive for the insertion. These colonies had reduced growth in optimal conditions (30°C with shaking), in 37 and 39 °C, temperatures tested for thermotolerance related to infection on the human host, and in different types of cell stress. The thickness of the polysaccharide capsule, essential for evasion of phagocytosis in host immune cells, was half of the measured in the reference strain. *SIN3* was also found to be essential for completion of the mating cycle. These findings indicate that *SIN3* causes an impact on important virulence phenotypes, agreeing with the previous discovery that HDACs attenuate virulence phenotypes, and will be useful for understanding the biology of the fungus and designing alternative therapeutic strategies.

**Keywords:** Epigenetics; CRISPR-Cas 9; Virulence.

### References:

ARRAS, S. D. M. et al. Targeted Genome Editing via CRISPR in the Pathogen *Cryptococcus neoformans*. **PLOS ONE**, v. 11, n. 10, p. e0164322, 6 out. 2016. Available in:

<https://doi.org/10.1371/journal.pone.0164322>.

BRANDÃO, F.A.S et al. Histone Deacetylases Inhibitors Effects on *Cryptococcus neoformans* Major Virulence Phenotypes. **Virulence**, v. 6, n 6, p. 618-360, 07 jul 2015. Available in: <https://doi.org/10.1080/21505594.2015.1038014>.

BRANDÃO, F. et al. HDAC genes play distinct and redundant roles in *Cryptococcus neoformans* virulence. **Scientific Reports**, v. 8, n. 1, 26 mar. 2018. Available in: <https://doi.org/10.1038/s41598-018-21965-y>.

CORRÊA PINHEIRO, M. et al. Cryptococcosis in the Amazon: A current overview and future perspectives. **Acta Tropica**, v. 197, p. 105023, 2019. Available in: <https://doi.org/10.1016/j.actatropica.2019.05.014>.

FU C., SUN S., BILLMYRE R. B., ROACH K. C., HEITMAN J. Unisexual versus bisexual mating in *Cryptococcus neoformans*: Consequences and biological impacts. **Fungal Genetic Biology**. 78: 65-75, 2014. Available in: <https://doi.org/10.1016%2Fj.fgb.2014.08.008>.

GIL, Y.; GIL, Y. D.; MARKOU, T. The Emergence of Cryptococemia in COVID-19 Infection: A Case Report. **Cureus**, 20 nov. 2021. Available in: <https://doi.org/10.7759%2Fcureus.19761>.

KUO, Min-Hao; ALLIS, C. David. Roles of histone acetyltransferases and deacetylases in gene regulation. **Bioessays**, v. 20, n. 8, p. 615-626, 7 dez. 1998. Wiley. Acesso em: 23 jul. 2024. Available in: [https://doi.org/10.1002/\(sici\)1521-1878\(199808\)20:8%3C615::aid-bies4%3E3.0.co;2-h](https://doi.org/10.1002/(sici)1521-1878(199808)20:8%3C615::aid-bies4%3E3.0.co;2-h).

I. YU. LEBOVKA et al. Sin3 histone deacetylase controls level of spontaneous and UV-induced mutagenesis in yeast *Saccharomyces cerevisiae*. **Russian Journal of Genetics**, v. 50, n. 1, p. 1–6, 1 jan. 2014. Available in: <https://doi.org/10.1134/S1022795413110124>.

LI, W. et al. Structure of histone deacetylase complex Rpd3S bound to nucleosome. **Nature Structural & Molecular Biology**, v. 30, n. 12, p. 1893–1901, 1 dez. 2023. Available in: <https://doi.org/10.1038/s41594-023-01121-5>.

MARKERT, J. W.; VOS, S. M.; FARNUNG, L. Structure of the complete *Saccharomyces cerevisiae* Rpd3S-nucleosome complex. **Nature communications**, v. 14, n. 1, 8 dez. 2023. Acesso em: 23 jul. 2024. Available in: <https://doi.org/10.1038/s41467-023-43968-8>.

MESQUITA DA COSTA, M. et al. Cryptococcosis, A Risk for Immunocompromised and Immuno competent Individuals. **The Open Epidemiology Journal**, v. 6, p. 9–17, 2013. Available in: <http://dx.doi.org/10.2174/1874297101306010009>.

RAJASINGHAM, R. et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. **The Lancet Infectious Diseases**, v. 17, n. 8, p. 873–881, ago. 2017. Available in: [https://doi.org/10.1016/s1473-3099\(17\)30243-8](https://doi.org/10.1016/s1473-3099(17)30243-8).

RANJAN, K. et al. The role of *Cryptococcus neoformans* histone deacetylase genes in the response to antifungal drugs, epigenetic modulators and to photodynamic therapy mediated by an aluminium phthalocyanine chloride nanoemulsion in vitro. **Journal of Photochemistry and Photobiology. B, Biology**, v. 216, p. 112131, 1 mar. 2021. Available in: <https://doi.org/10.1016/j.jphotobiol.2021.112131>.

SCOTT, Kenneth L.; PLON, Sharon E.. Loss of Sin3/Rpd3 Histone Deacetylase Restores the DNA Damage Response in Checkpoint-Deficient Strains of *Saccharomyces cerevisiae*. **Molecular And Cellular Biology**, v. 23, n. 13, p. 4522-4531, jul. 2003. Available in: <https://doi.org/10.1128%2FMCB.23.13.4522-4531.2003>.

STEENBERGEN, J. N.; CASADEVALL, A. The origin and maintenance of virulence for the human pathogenic fungus *Cryptococcus neoformans*. **Microbes and Infection**, v. 5, p. 667-675, 2003. Available in: [https://doi.org/10.1016/s1286-4579\(03\)00092-3](https://doi.org/10.1016/s1286-4579(03)00092-3).

TUGUME, L. et al. Cryptococcal meningitis. **Nature Reviews Disease Primers**, v. 9, n. 1, p. 1–18, 9 nov. 2023. Disponível em: <https://doi.org/10.1038/s41572-023-00472-z>. Acesso: 23 de julho de 2024.

WANG, Y. et al. A “suicide” CRISPR-Cas9 system to promote gene deletion and restoration by electroporation in *Cryptococcus neoformans*. **Scientific Reports**, 2016. Disponível em: <https://doi.org/10.1038/srep31145>. Acesso: 23 de julho de 2024.

WIEDENHEFT, B.; STERNBERG, S. H.; DOUDNA, J. A. RNA-guided genetic silencing systems in bacteria and archaea. **Nature**, v. 482, n. 7385, p. 331–338, 16 fev. 2012. Disponível em: <https://doi.org/10.1038/nature10886>. Acesso: 23 de julho de 2024.