Phenotypic analysis of *Aspergillus fumigatus* mutant lacking the P-Type Na^+-ATPase encoding gene *enaA*

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Abstract

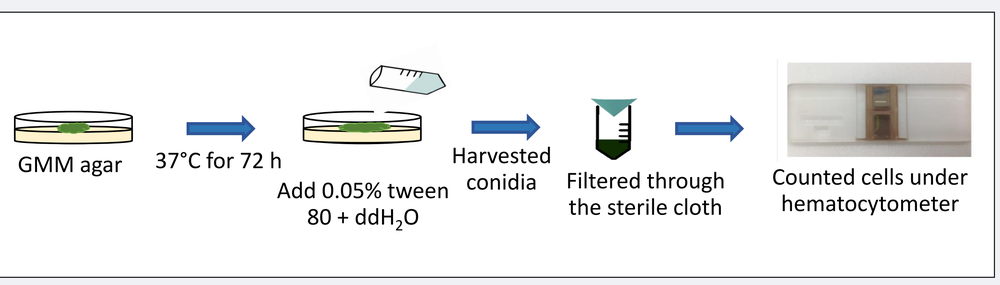
Aspergillus fumigatus is a hyaline septate mold, commonly found in the in-door environment and is important cause of opportunistic infection in immuno-compromised individuals. During infection in the host, the fungus is exposed to alkaline pH and toxic cations. Thus, an efficient detoxification system is crucial for survival. In most fungi, sodium ATPases play important role in pumping the exceed Na^+ out of the cells and are also important for growth under alkaline condition. In the pathogenic yeast, *Cryptococcus neoformans* the gene *ENA1*, encoding the P-type Na^+-ATPase has been implicated in response to alkaline stress (1). However, data about how pathogenic molds are coping with alkaline environment are still limited. In a recent study, a homologue of *ENA1* in *A. fumigatus* was investigated and the results showed that *ENA1* homologue in *A. fumigatus* was rapidly upregulated when exposed to alkaline condition (2). Nevertheless, the role of *A. fumigatus ENA1* homologue in adaptation to cationic stress has not been studied. The aim of this research is therefore to explore the involvement of *ENA1* homologue in *A. fumigatus*, termed in this study as *enaA*, in response to cationic stress, acidic and alkaline pH.

To observed the phenotype of *A. fumigatus* mutant strain lacking the *enaA* gene, 10^6 conidia/ml of both mutant and wild-type strain were inoculated on GMM medium under different kind of stress conditions, including cationic stress (NaCl,$MnCl_2$), alkaline, acidic pH and osmotic stress. The colony diameters were measured after 72 hours.

The results showed statistically significant reduction of hyphal growth of Δ*enaA* strain under high concentration of Na^+, Mn^{2+} and alkaline pH, when compared to the wild type strain, suggesting that *Aspergillus fumigatus enaA* gene may play important role in response to certain cationic stress and alkaline environment.

Methods

Radial hyphal growth analysis



10 µl of 10^6 conidia/ml from wild type and mutant strain

Inoculated on GMM agar containing difference ingredients, as indicated below

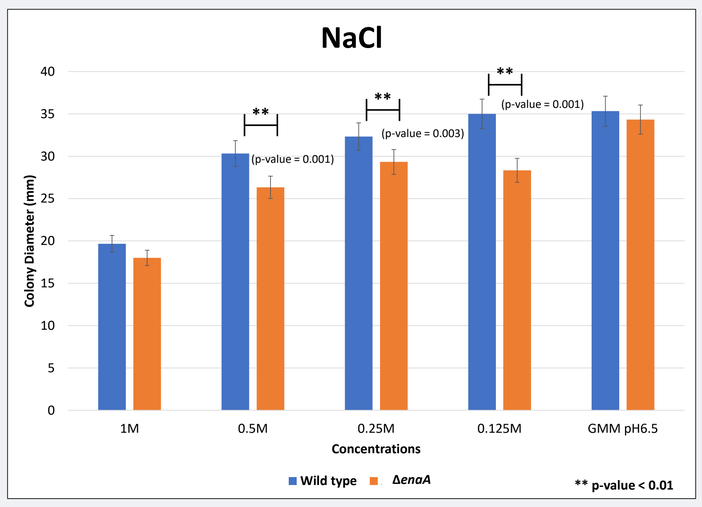
No.	Conditions
1.	GMM agar pH 6.5 , 37°C
2.	GMM agar pH 6.5 + 0.125 M, 0.25 M, 0.5 M and 1 M of NaCl
3.	GMM agar pH 6.5 + 0.005 M, 0.01 M, 0.02 M and 0.04 M of $MnCl_2$
4.	GMM agar pH 4
5.	GMM agar pH 9

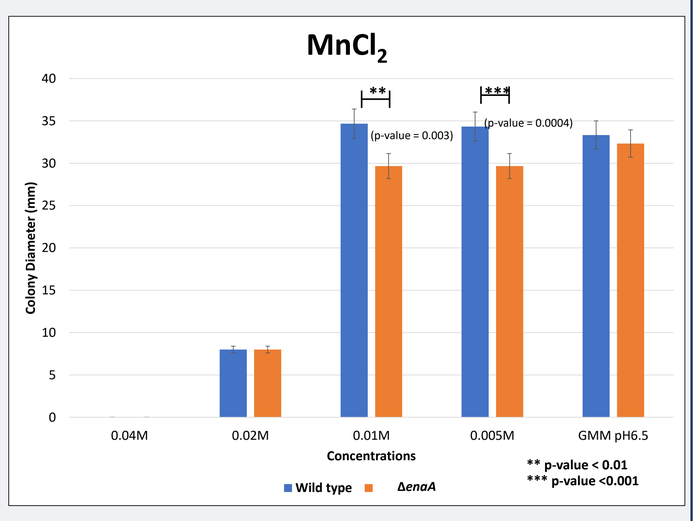
Incubated at 37°C for 72 hours

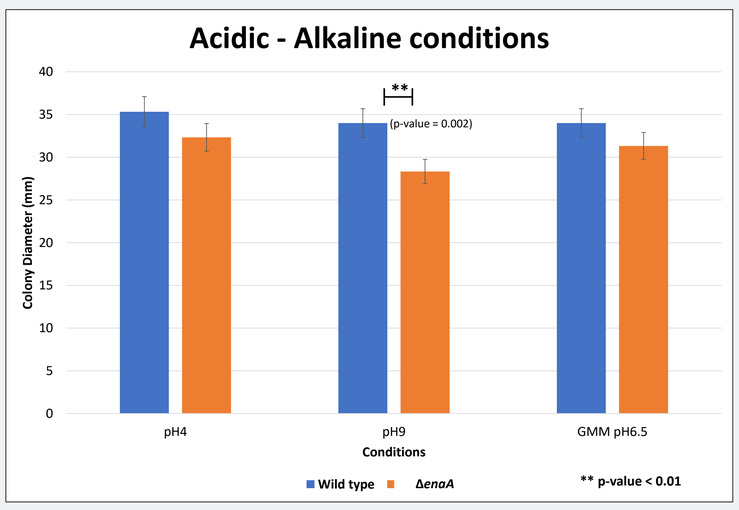
Measured the colony diameter

Results

The results showed statistically significant reduction of hyphal growth of Δ*enaA* strain at 0.5 M, 0.25 M and 0.125 M of NaCl, at 5 mM and 10 mM of $MnCl_2$ and at alkaline pH, when compared to the wild type strain.







Discussion & Conclusion

Aspergillus fumigatus mutant strain, lacking the P-type Na^+-ATPase encoding gene *enaA* revealed significantly increased susceptibility to sodium ion at three different concentrations (0.125 M, 0.25 M and 0.5 M), suggesting that the sodium pump EnaA most likely plays major role in detoxification of Na^+. At 1 M NaCl, however, both wild-type and Δ*enaA* strains showed similar susceptibility, indicating that at this concentration the ability of EnaA to pump excess Na^+-ion out of the cells may be exceeded. Surprisingly, Δ*enaA* strain was also significantly more susceptible to Mn^{2+} and alkaline pH, suggesting that tolerance to sodium, manganese and alkaline pH might be controlled by the same pathway, which needs to be further investigated in future works.

References

1. Idnurm A, Walton FJ, Floyd A, Reedy JL, Heitman J. Identification of *ENA1* as a virulence gene of the human pathogenic fungus *Cryptococcus neoformans* through signature-tagged insertional mutagenesis. Eukaryot Cell. 2009;8(3):315-26.

2.Loss O, Bertuzzi M, Yan Y, Fedorova N, McCann BL, Armstrong-James D, et al. Mutual independence of alkaline- and calcium-mediated signalling in *Aspergillus fumigatus* refutes the existence of a conserved druggable signalling nexus. Mol Microbiol. 2017;106(6):861-75.