Study on gene expression of Na^+-ATPase encoding gene *enaA* during stress response in *Aspergillus fumigatus*

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INTRODUCTION

Major factors that makes the opportunistic mold *Aspergillus fumigatus* effective in establishing diseases in human include sophisticated adaptation mechanism to host environment and immune evasion strategies. During infection in human the fungus is exposed to toxic concentration of calcium ions and slightly alkali pH. While several researches have been focused on adaptation to calcium, limited data is available for how *A. fumigatus* responses to alkaline pH. In *Aspergillus nidulans* the sodium ATPase encoded by the gene *enaA* is involved in response to toxic sodium concentration and alkaline pH [1-2]. In this study, expression of *A. fumigatus* orthologous gene (AFUA_6G03690), termed here as *enaA* was assessed in response to cationic (in the presence of Na^+, Ca^{2+}, Mn^{2+}), osmotic (in the presence of sorbitol) and alkaline stress by quantitative real-time PCR.

METHODS

Culture condition:

Aspergillus fumigatus strain AF293 (Fungal Genetics Stock Center) was grown in GMM (glucose minimal media) broth overnight at 37° C and 230 rpm. Fungal cells were collected by centrifugation at 4,500 rpm for 15 minutes. Afterward, 10 ml of GMM broth containing either 0.8 M NaCl, 1 M Sorbitol, 200 mM $CaCl_2$, 10 mM $MnCl_2$, 1 M KCl or GMM that was adjusted to pH 9, was added to the cells. Cell suspensions were shaken at 37°C for 20 minutes, centrifuged at 4,500 rpm for 15 minutes and washed once with PBS buffer. The cells were harvested and lyophilized overnight prior to RNA extraction.

RNA extraction & Realtime PCR:

Total RNA was extracted using conventional Plant RNA Extraction Kit (Smart Science). To assess expression of *enaA* gene, real-time PCR was carried out using LightCycler® 480 Instrument (Roche Life Science) with *benA* (beta-tubulin) serving as reference gene. The experiment was done in duplicates and was repeated once with an independent cell culture. Relative quantification was analyzed by $2^{-\Delta\Delta Ct}$ method using LightCycler® 480 software version 1.5.

RESULTS

Real-time PCR results revealed that under normal growth condition expression of *enaA* gene was suppressed. However, when exposed to NaCl, $CaCl_2$ and $MnCl_2$, expression of *enaA* gene was increased to approximately 12 fold, 3 fold and 6 fold, respectively, while *enaA* showed expression level similar to that of *benA* under osmotic and alkali stress (Fig. 1, 2, Tab. 1, 2). For KCl exposure, the applied amount of 1 M probably severely compromised fungal growth, so that expression of both *benA* and *enaA* was not detectable within 35 amplification cycles (Fig. 2 and Table 2).

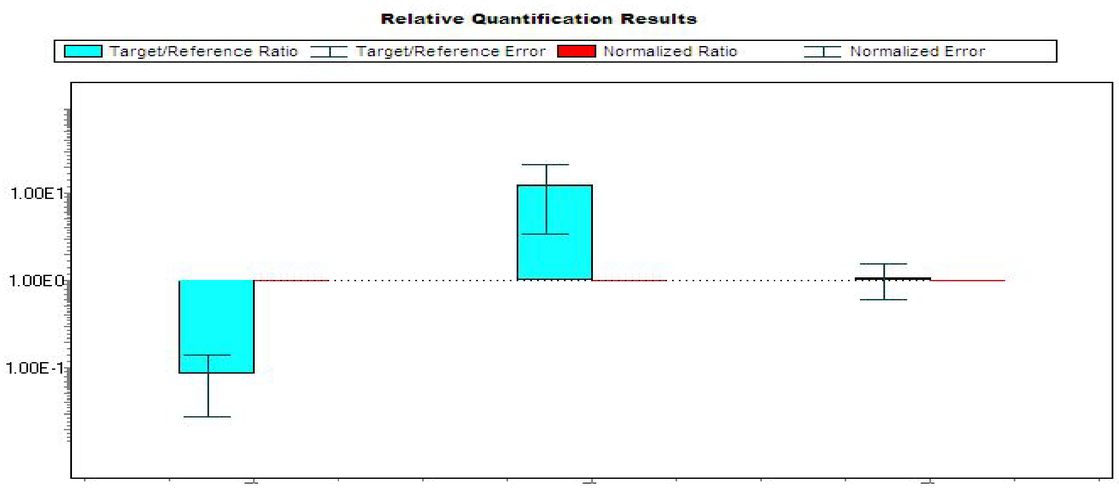

Fig. 1 Expression of *enaA* gene under sodium and osmotic stress. The results revealed that when exposed to NaCl, expression of *enaA* was increased to approximately to 12 fold, while was expressed at equivalent level to that of *benA* under osmotic stress.

Sample Name	Target Name		Tgt Cp	Ref. Cp	Ratios	
	Targets	References	Mean	Mean	Tgt/Ref.	Norm
WT GMM only	enaA	tubulin	22.08	18.53	8.54E-2	
WT+NaCl	enaA	tubulin	20.37	23.98	12.21	
WT+Sorbitol	enaA	tubulin	17.09	17.20	1.084	

Tab. 1 Basic relative quantification.

RESULTS

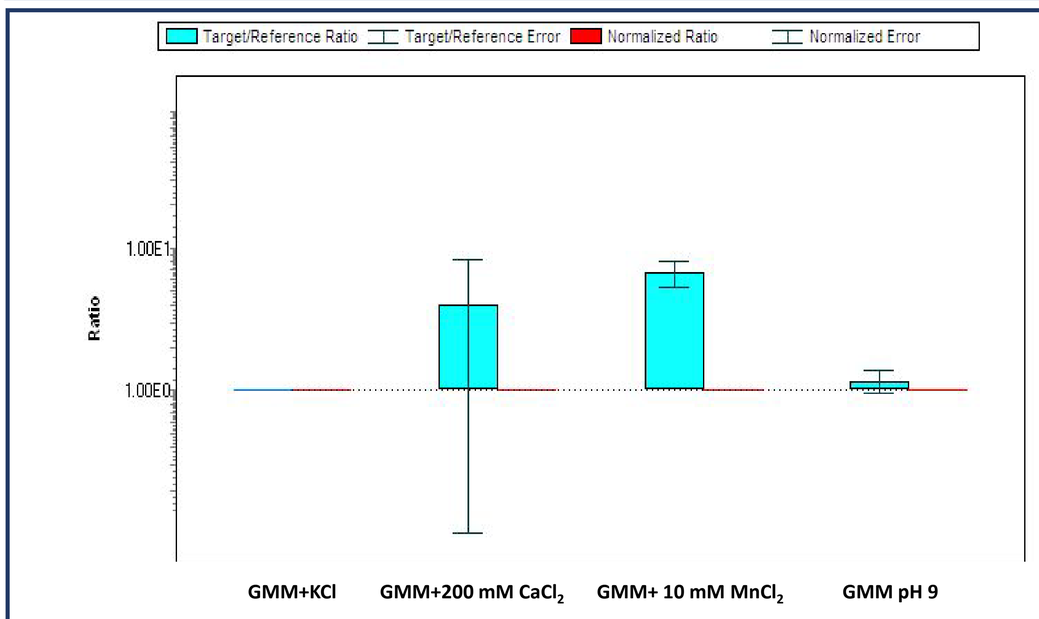

Fig. 2 Expression of *enaA* gene under cationic and alkali stress. Exposure to $CaCl_2$ and $MnCl_2$ resulted in increased expression of *enaA* to 3.9 and 6.6 fold, respectively, while under alkali pH, *enaA* showed expression level similar to that of *benA.*

Sample Name	Target Name		Ratios		
	Targets	References	Tgt/Ref.	Error	Norm
WT(2)+KCl	enaA	tub	Invalid	Invalid	
WT(2)+Ca	enaA	tub	3.925	4.322	
WT(2)+Mn	enaA	tub	6.647	1.460	
WT(2) pH9	enaA	tub	1.157	0.2180	

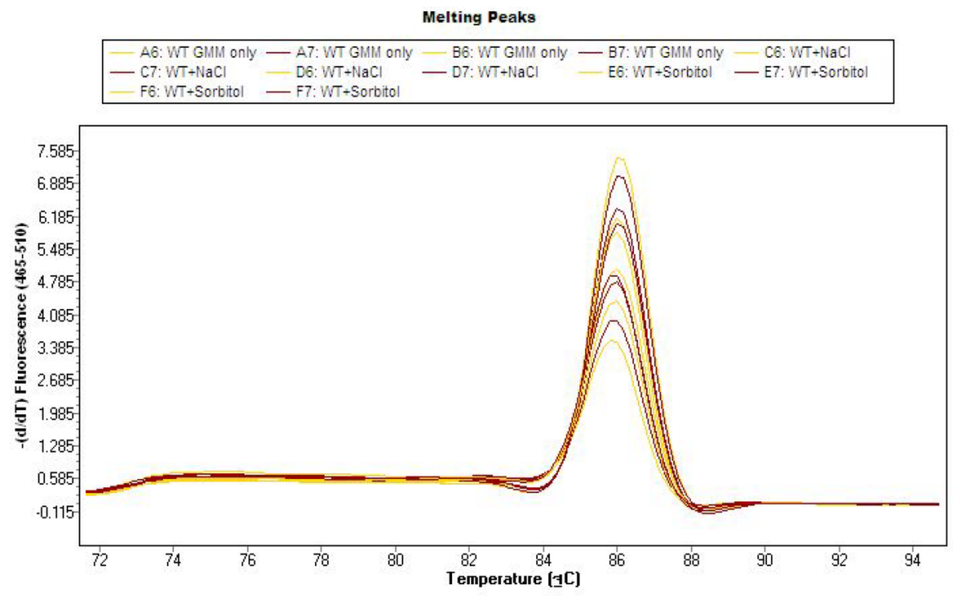

Figure 3. Representative melting curve analysis

DISCUSSION & CONCLUSION

Sodium ions are essential for diverse cellular processes. Nevertheless, high Na^+/K^+ ratio is toxic to living cells. In fungi, Na^+ and K^+ are transported by ENA-ATPases. Fungal ENA-ATPases mediate not only the efflux of Na^+ and K^+ uptake, but also mediate the transport of other alkali ions out of the cells. The ability of ENA-ATPases to pump diverse alkali ions out of the cells, enable fungi to grow under a wider range of natural environments including decaying organic matters enriched with alkali cations. This study aims to assess possible role of *Aspergillus fumigatus* gene encoding Na^+-ATPase, termed here as *enaA* in adaptation to cationic, osmotic and alkali stress by investigation *enaA* gene expression using real-time PCR. The results revealed increased expression of *enaA,* when exposed to Na^+, Ca^{2+} and Mn^{2+}, suggesting possible common or linked pathway involved in detoxification of metal cations.

REFERENCES

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