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 responds 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⁺, Ca²⁺, Mn²⁺), 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 CaCl₂, 10 mM MnCl₂, 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 gene (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 2ΔΔCt 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, CaCl₂, and MnCl₂ expression of enaA gene was increased to approximately 12 fold, 3 fold and 6 fold, respectively, while under alkali pH, enaA showed expression level similar to that of benA.

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⁺, Ca²⁺ and Mn²⁺, suggesting possible common or linked pathway involved in detoxification of metal cations.

REFERENCES