Functional characterization of truncated Haemoglobin(HbO) of Mycobacterium smegmatis

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Introduction

The ability of *Mycobacterium tuberculosis* to utilize different defence mechanisms in response to the varied environmental challenges during the course of its intracellular infection, latency, and reactivation cycle makes it one of the most successful human pathogen. India has the highest burden of TB in the world, with an estimated 2 million cases annually. So, there is an urgent need to develop a drug that specifically targets the intracellular bacterium and therefore, research into the survival and pathogenesis of the bacterium is of utmost importance. *MSMEG_HbO* (trHbO) is having 110-130 amino acid residues in each chain and it is conserved in pathogenic and non-pathogenic mycobacteria. HbO plays vital roles in adaptation of Mtb under hypoxia and environmental stress, suggesting that it may be crucial for the pathogenicity of Mtb. An important role for trHbO in oxygen transfer is based on the observation that expressing trHbO significantly enhanced the cell growth, oxygen uptake rate, and ATP level in *E. coli* and *Mycobacterium smegmatis* cells (Pathania *et al.*, 2002). Basal level of the HbO is present during all phases of growth, which suggests that it is crucial for the cellular metabolism. Present study is focused on establishing the essentiality of *MSMEG HbO* in survival, physiology and metabolism of *Mycobacterium smegmatis.*

Materials and methods

Cloning

MSMEG_HbO was amplified from *Mycobacterium smegmatis* mc^2155. The gene was cloned in p19Kpro vector and cloning was confirmed by colony PCR and restriction digestion.

Overexpression of *MSMEG_HbO* under stress (oxidative) in M. smegmatis.

MSMEG_HbO facilitated the intracellular survival of these bacteria.

Mycobacterial surface properties were studied.

Results

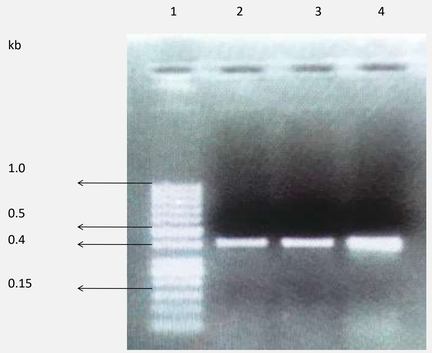

Figure1: Amplification of *MSEMG_HbO* on 1.2% agarose gel. Lane 1-50bp DNA ladder, Lane 2,3,4 – Amplified *HbO* gene product

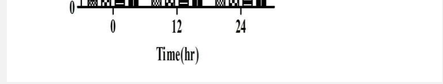

Figure 2: Survival of *MSEMG_HbO* under oxidative stress. *Msmeg-p19Kpro* and *Msmeg- p19Kpro- HbO* were grown in the presence of M7H9 media and M7H9 media supplemented with 5 mM H_2O_2 and grown for 12 h and 24 h with shaking at 37 °C at 180 rpm.

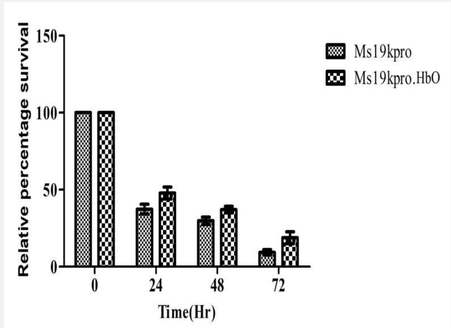

Figure 3: Relative percentage survival of *Msmeg*-p19Kpro and *HbO* in THP-1 macrophages after 0, 24, 48 and 72 h of infection.

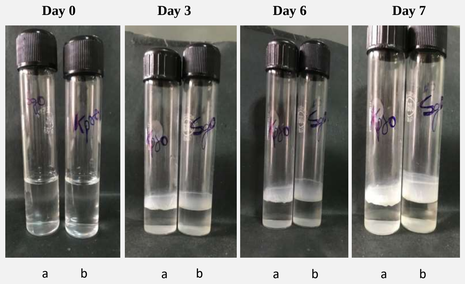

Figure 4: Pellicle formation; (a-*MSMEG p19Kpro*), (b-*MSMEG p19Kpro.HbO*)

Conclusions

- Expression pattern of *MSMEG_HbO* under stress conditions pointed towards its importance in intracellular survival of mycobacteria.
- Pellicle formation also pointed towards its role in pathogenesis. Therefore, findings of this study may provide new insight into functional properties of *MSMEG_HbO*, that may let us establish the importance of HbO protein as an attractive novel system to target mycobacteria for the development of anti-mycobacterial agents.

Acknowledgements

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References
Pathania, R., Navani, N.K., Rajamohan, G. and Dikshit, K.L., 2002. Mycobacterium tuberculosis Hemoglobin HbO Associates with Membranes and Stimulates Cellular Respiration of Recombinant Escherichia coli* 210. *Journal of Biological Chemistry*, *277*(18), pp.15293-15302.