Title of thesis: Molecular Characterization of *Bacillus anthracis*

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By

Rajnee

Department of Biosciences, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi, INDIA

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Supervisor:

Prof. Tasneem Fatma,

Department of Biosciences,

Jamia Millia Islamia, New Delhi – INDIA

Co-supervisor:

Dr. Syed Tazeen Pasha,

Division of Biotechnology and Biochemistry,

National Institute of Communicable Diseases,

Ministry of Health, Government of India,

22 Shamnath Marg, Delhi - INDIA

ABSTRACT

The complete genome of *Bacillus anthracis Ames* (about 5.23 mega bases) was released on May 7, 2002. The present thesis attempts at functional genomics of *B.anthracis* to study a selected hypothetical ORF from its genome using various approaches to validate the *in-silico* predicted functions and characterize the protein encoded by the ORF. Possible primary and secondary functions of encoded protein have been proposed.

The gram-positive, rod shaped, and spore forming *Bacillus anthracis* is the etiologic agent of anthrax, an acute, often fatal infection in both animals and humans. Virulent strains of *B. anthracis* are encapsulated and produce toxins. They harbor two virulence plasmids pXO1 and pXO2.

The present work started with the identification of Indian clinical isolate of *Bacillus anthracis* and then selecting a hypothetical ORF from the published genome sequence, cloned in *E. coli* plasmid vector and heterogeneously expressed protein in *E. coli* host cells was purified to homogeneity for characterization.

Identification of Indian clinical isolate of *B. anthracis* was confirmed using PCR amplification and analysis of 16S rDNA which was later followed with those of S-layer, PA and Capsule genes as per the WHO guidelines. One of the many ORFs in genome database of *B. anthracis*, a 399 bp ORF located at locus BA1209 [deposited under Accession nos. AE016879 and AAP25170] was predicted to be a protozoan/cyanobacterial globin family protein and showed homology to Class II truncated hemoglobins was selected. The ORF was named BA-Glb, the *Bacillus anthracis* globin, in this study.

The ORF “BA-Glb” has been analyzed using various softwares to know about its primary and secondary structure, the presence conserved protein domain. The phylogenetic tree of BA-Glb was constructed with current database available for the truncated hemoglobins. 3D homology model of BA-Glb was also constructed and analyzed for it’s proximal, distal and heme cavity structures. The purified recombinant protein obtained upon cloning and expression in *E. coli* was used for biochemical and biophysical characterization.

The optical analysis on UV/VIS spectrophotometer and Circular Dichroism established the characteristics structural and ligand binding properties of BA-Glb which are typical to hemoglobins. The weak peroxidase activity determined in BA-Glb also

supports the evidences collected from optical methods for its identification as hemoglobin protein. BA-Glb was confirmed as an active and monomeric hemoglobin protein of ~15 kDa.

The presence of weak peroxidase activity of BA-Glb hints at one of the important possible functions of this truncated hemoglobin in the pathogen *B. anthracis* for its survival within the host cells and scavenging of peroxides. The non-linear changes in peroxidase activity upon subjection to chemical denaturants were compared with the unfolding properties of BA-Glb. The BA-Glb followed typical two steps unfolding pattern when measured using optical methods like UV/VIS, CD and Florescence spectrometry. However, the fraction denature calculated from individual methods did not superimpose, indicating the presence of certain intermediates which escaped detection in these optical methods. The weak peroxidase activity which showed alteration upon unfolding with chemical denaturants, were studied in little detail to correlate peroxidase activity in different denaturants with its unfolding pattern observed using classic optical methods. That concludes the hemoglobin protein BA-Glb unfolds in more than two steps with urea and guanidine hydrochloride, and it is thermally stable at high temperature.

A possible physiological role of BA-Glb was determined by its in-vivo expression in attenuated *B. anthracis* vaccine culture. The expression of protein was estimated qualitatively and quantitatively by western blotting and ELISA of cell culture extract using highly specific polyclonal antibody raised against recombinant BA-Glb. The expression of BA-Glb was compared with the growth pattern in different condition of growth. The expression of BA-Glb was through out the growth period of 10 days in both aerobic and oxygen stressed conditions. The change in growth pattern in oxygen-stressed condition was correlated with the BA-Glb expression. It suggested the primary role of the BA-Glb in storage and supply of oxygen in *B. anthracis*.

The present findings have attempted to understand the very presence and need of hemoglobin like molecule in *B. anthracis*. The information obtained through such initiatives and successive advanced investigations might in future yield to a probable drug target to restrain the hemoglobin of organism specifically within the host in disease management.