The following conclusions may be drawn from the present study on truncated hemoglobin.

- A hypothetical ORF "yjbl" in *Bacillus subtilis* genome was identified as truncated hemoglobin using bioinformatic tools.

- Structure based sequence alignment of trBHb with various microbial trHbs suggests that the presence of strongly conserved residues among proteins in the trHb family is mainly of three types: glycine rich motifs, hydrophobic residues, heme binding residues.

- The truncated hemoglobin yjbl gene was cloned in *E. coli* cloning pKS' vector and sub-cloned in expression vector pGS100 under transcriptional control of heat inducible bacteriophage λ P_R and P_L promoters in tandem, expressed in the cytoplasm of *E. coli* DH5a in HCDM and purified to homogeneity level by using anion exchange and gel filtration chromatography.

- Purified trBHb was monomeric protein as concluded from the repeated gel filtration chromatography, molecular weight was determined by MALDI-ToF and found 14.912 kDa.

- Under heat and salt stress conditions, expression of yjbl gene in *B. subtilis* was not detected in our experimental conditions checked by ELISA and western blot.
The attribute of trBHb as a heme protein confirmed by recording CD and optical spectra.

Reduced iron (Fe^{2+}) of heme proteins generally binds extrinsic gaseous ligands such as CO, O_2, and NO. When the oxidized heme iron (Fe^{3+}) of the trBHb protein is reduced to Fe^{2+} by adding Sodium dithionite in the presence of CO, the spectrum changes with a characteristic red shift of the Soret band to 424 nm. These results strongly suggest that trBHb is indeed a heme protein.

The in vitro stability of trBHb, expressed as ΔG(ΔH_2O) (i.e., the free energy difference between the folded and the unfolded state) is ~5.45 kcal mol^{-1}. This value for the stability of trBHb compares closely with the stability of myoglobin.

Truncated BHb exhibits low peroxidase like activity. Activity is enhanced in the presence of urea and guanidine hydrochloride, more so in the presence of the latter.
References