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Co expression of *hbha* and *mtb32C* genes from *Mycobacterium tuberculosis* H37Rv in prokaryotic system

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Background and Objective: HBHA (heparin-binding hemagglutinin) is a surface adhesin that mediate in binding to host cells by its own unique, carboxyl-terminal region. This methylated region has specific motif that rich of lysine-, alanine-, and proline amino acids. More recently, has been shown that HBHA has potential activity in stimulating immune responses and is a promising new candidate for diagnostic and protective antigen against tuberculosis. Interferon-gamma release assay test (IGRAs) is a new method to identifying latent tuberculosis and in compared to old method, tuberculin skin test (TST), has several advantages. Therefore in this study recombinant heparin-binding haemagglutinin antigen as new antigen in IGRAs test was produced.

Materials and Methods: In present work *hbha* and *mtb32C* genes were isolated from *Mycobacterium tuberculosis* H37Rv genome by PCR. PCR products and pet21+ vector were digested with specific restriction enzymes and then submitted to ligation procedure. *E. coli* BL21-CodonPlus (DE3) competent cells were transformed with recombinant Mtb32C-HBHA –pet21+ vector. Expression of recombinant protein (Mtb32C-HBHA) was confirmed with SDS-PAGE and Western blot methods.

Results: Detection of about 500 bp gene in Colony-PCR method and sequencing of recombinant pet-Mtb32C-HBHA vector all confirmed the accuracy of cloning procedure. Also presence of a 36 KDa protein band was confirmed with Western blotting method(Fig.1,2,3).

Conclusion:

In this study, expression of Mtb32C-HBHA protein in prokaryotic system successfully was done and production of new recombinant protein confirmed by western blot technique and anti His tag antibodies. Other studies are needed to evaluate efficacy of recombinant Mtb32C-HBHA protein in diagnosis of latent tuberculosis**.**

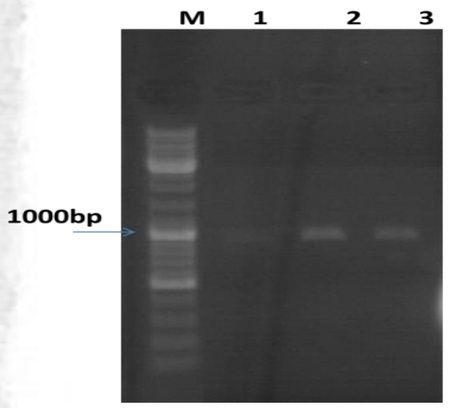

Fig.1: Colony-PCR results that show amplification of about 1000bp of *mtb32C-HBHA* fragment of recombinant *mtb32C-hbha* – pet21+ using *mtb32C-HBHA* specific primers (lane 2, 3); lane M: 100bp DNA size marker

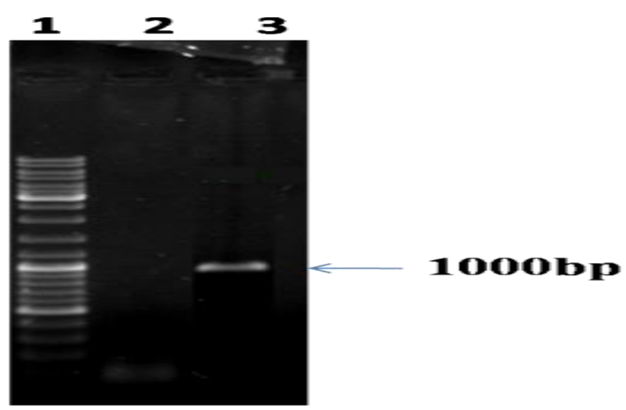

Figure 2: Detection of *mtb32C-hbha* mRNA in transformed and non-transformed. *E. coli* BL21 (DE3) CodonPlus cells by RT-PCR analysis. By using specific primers designated for *mtb32C-hbha* genes showed negative results in non-transformed cells (lane2) and a band with a size of 1000bp in transformed cells with recombinant vector (lane 3). Lane 1: 1kb DNA size marker

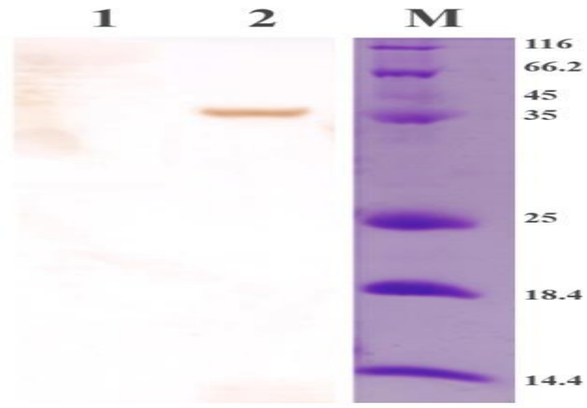

Fig 3: Western blot analysis that confirm presence of recombinant protein (36kDa protein band) in transformed bacteria (lane 1) but not in non transformed bacteria (lane 2); M: protein size marker

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