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Page: 21-29.

Cloning and expression of recombinant MPB70 protein antigen from Mycobacterium bovis BCG for diagnosis of tuberculosis

Y A Soliman [1], M Ghonim, E A Nasr, I Mousse, E A Ebiary, S A Selim

Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt.

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In a search for developing new skin test reagents, MPB70 protein antigen was a candidate antigen for the Diagnosis of bovine tuberculosis. First M. bovis BCG genomic DNA was extracted purified and the mpb70 gene was amplified by PCR. The gene was then ligated to an expression vector, PQE. After transformation of the expression E. coil M15 host strain with the PQE plasmid, the expression was induced using 10 mM of IPTG. Two bands were seen in the SDS-PAGE analysis the 44 and 50 KDa represents the dimmers of the nonglycosylated and glycosylated form of the reMPB70 antigen. The His-tagged reMPB70 antigen was then purified by metal affinity chromatography using Ni-NTA agarose. Protein refolding was done by the use of the co solvent Polyethylene glycol MW 3000. The diagnostic potential of the re-MPB70 was evaluated using sera from experimentally sensitized guinea pigs with different strains of mycobacteria (M. bovis BCG, M. tuberculosis, M. kansasii and M. intracellular) using ELISA test. The results indicated the efficiency of MPB70 but not bovine PPD to discriminate between M. bovis sensitized guinea pigs and those sensitized with other mycobacterial strains at serum dilution of 1150. In a field trials to using reMPB70 antigen for the serodiagnosis of bovine tuberculosis using ELISA test. Fifty serum samples from tuberculin +ve and 6 from tuberculin -ve cattle were used as well as 10 tuberculin +ve buffaloes. All +ve animals were confirmed to be M. bovis infected by P/M analysis, bacteriological examination. ELISA results revealed that reMPB70 could recognize the tuberculin +ve infected animals at serum dilution of 1/50 and that it could diagnose tuberculosis in cattle as well as buffaloes.