THE EFFECT OF VARIABLE NUMBER OF TANDEM REPEAT (VNTR) OF MYCOBACTERIUM TUBERCULOSIS IN GENE EXPRESSION

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M.Sc. (BIOTECHNOLOGY)

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ABSTRACT

The genome of Mycobacterium tuberculosis contains various kinds of repetitive DNA sequences including the variable number of tandem repeats (VNTR). A previous study on VNTR identification in the M. tuberculosis genome revealed that most of the VNTR loci were located in the intergenic regions. The functions of VNTR loci are still not well characterized. Nine loci of VNTR located outside coding regions were found to contain ribosomal binding sites (RBS), implying they may influence the downstream gene expression. In this study, two recombinant plasmids, pSP0960C and pSP0960, containing VNTR0960C and inverted VNTR0960C DNA fragments, respectively, from chromosomal DNA of M. tuberculosis H37Ra were derived from pFPV2 vector by replacing the heat shock protein 60 promoter (Phsp60) in order to investigate the effect of VNTR0960C containing RBS in its sequence on gfp expression using M. smegmatis as a host. A promoterless GFP vector, pSP1 was also derived from the plasmid pFPV2 and used as a negative control for GFP measurement. The gfp expression was determined and measured by fluorescence microscopy, microplatefluorometry, and flow cytometry. The levels of GFP expression of plasmid pSP0960C and pSP0960 were compared with plasmid pFPV2, pSP1, and a wild type strain. It was found that only pFPV2 and pSP0960C strains were fluorescent and the level of gfp expression of pSP0960C was 3 times lower than the pFPV2 strain. These results demonstrated that the VNTR0960C DNA fragment can function as a promoter. It has been reported by others that the VNTR0960C DNA fragment was polymorphic, having 7 alleles (0, 1, 2, 3, 4, 5, and 7 complete copies). The effects of polymorphism of VNTR0960C DNA fragment on gfp expression were also studied in M. smegmatis. The polymorphic alleles of VNTR0960C DNA fragments were cloned from the chromosomal DNA of clinical isolates from Amnat Charoen province and inserted into plasmid pFPV2 by replacing Phsp60, resulting in the plasmids pSP09C0, pSP09C1, pSP09C3, pSP09C4, pSP09C5, and pSP09C7. Comparison of the levels of GFP expression among these recombinants, including plasmid pSP0960C and wild type strains demonstrated that all recombinants were fluorescent and pSP09C4 gave the highest signal of gfp expression whereas pSP09C1 was the lowest. The other recombinant strains permit the similar level of GFP. Therefore, the level of gfp expression does not associate with the copy number of the VNTR0960C DNA fragment. It demonstrated that copy number does not influence promoter activity of the VNTR0960C DNA fragment.

KEY WORDS : MYCOBACTERIUM TUBERCULOSIS / VNTR / GENE EXPRESSION / GFP / POLYMORPHISM

97 P. ISBN 974-04-6118-2