

Cloning Open Reading Frame (ORF) of Rv2430c *Mycobacterium tuberculosis* Indonesian Isolate in *Escherichia coli* JM 109

Rosana Agus^{1*}, Muhammad Nasrum Massi², Francisca Srioetami Tanoerahardjo³

¹Department Of Biology, Faculty of Life Sciences, Hasanuddin University, Indonesia

²Department of Microbiology, Faculty of Medicine, Hasanuddin University, Indonesia

³Development Ministry of Health of Republic of Indonesia, Central Basic Biomedical,
Health Technology National Institute of Health and Research, Indonesia

*Corresponding author: rosana_agus@yahoo.com

Abstract Various strategies have been implemented to prevent tuberculosis. Vaccination with Bacille Calmette Guerin (BCG) vaccine is still used around the world. Generally, most people in Indonesia have gained BCG vaccine as an infant, but the effectiveness of these vaccines do not survive to adulthood. Therefore, the necessary replacement BCG vaccine more effective to eliminate tuberculosis. *Mycobacterium tuberculosis* is an intracellular pathogen and it was inside the macrophage, which is considered to be the most important component of the immune system. *M. tuberculosis* has two sets of genes are highly polymorphic referred to as PE and PPE families. These unique families of proteins account for about 10% of the mycobacterial genome and have attracted great interest from a variety of different studies around the world. One member of the PPE as a vaccine candidate is Rv 2430c. It is known that the sera of all patients infected with TB showed strong antibody responses against Rv 2430c compared to healthy individuals. The existence of these antibodies indicates that this protein is found in vivo during infection and is a native immunogenic molecule. The purpose of this study was to clone the Open Reading Frame (ORF) Rv 2430c *M. tuberculosis* Indonesian isolates to host cells *Escherichia coli* JM 109. The method used is by isolating -chromosomal DNA from clinical isolates from Indonesia, amplifying the ORF Rv 2430c with PCR, ligating into cloning vectors pGEM-T and transform to *E.coli* JM 109. Characterization of clones do with migration analysis, restriction analysis and PCR. The results obtained are recombinant clones that carry DNA insertion was Rv 2430c. **Keywords:** *Mycobacterium tuberculosis*, Rv 2430c, ORF, PPE, JM 109.

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1. Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is responsible for 9 million new cases of active TB and 1–1.5 million deaths annually [1]. It is estimated that nearly 2 billion people suffer from latent TB around the world, which potentially become an active TB. The only vaccine currently used is BCG, which has been developed since almost a century ago. BCG is given in newborns routinely in TB endemic countries. However, the effectiveness of BCG was reduced in adolescents, and the vaccine does not consistently prevent the development of active pulmonary TB in adults (efficacy estimated between 0 and 80% with the lowest efficacy rates often found in countries with the highest burden of TB) [2,3,4]. Thus, there is an urgent need for a new TB vaccine to either boost immunity primed by BCG or replace BCG [5,6].

The variation in sequence and length of C-terminal region among members of the unique PE (Pro-Glu) and PPE (Pro-Pro-Glu) protein families of *Mycobacterium tuberculosis* is a likely source of antigenic variation. Based on in-silico analysis, we selected a hypothetical open reading frame (ORF) encoding a protein belonging to the PPE family and having epitopes with predictably higher antigenic indexes. Reverse transcriptase PCR from in vitro-cultured *M. tuberculosis* H37Rv generated an mRNA product corresponding to this gene, which is indicating the expression of this ORF (Rv2430c) at the mRNA level. Our results reveal that this PPE ORF induces a strong B-cell response compared to that generated by *M. tuberculosis* Hsp10 or PPD, pointing to the immunodominant nature of the protein [7].

Cloning of ORF Rv 2430 performed on pGEM-T cloning vector and host *Escherichia. coli* JM 109. It is expected that this gene can be used as candidate antigens for TB vaccine search.

2. Materials and Methods

2.1. Bacterial Strains and Plasmids

The cloning vectors pGEM-T Easy (Promega) was used. The strains *M. tuberculosis* was obtained from clinical isolate from Indonesia. *E. coli* JM 109 was obtained from Center For Biomedical and Basic Technology of Health.

2.2. Culture Condition

The *E. coli* JM 109 were incubate with stirring over night in LB medium in the presence of ampicillin (1µg/ml) at 37°C. *E.coli* JM109 competent cells prepared in advance by the CaCl₂ method [8] modified.

2.3. PCR

The following primer was design by [7]

Rev 5'-AAGCTTCTAAGTGTCTGTACGCGATGA-3'
Fwd 5'-GGATCCATGCATTTTGAAGCGTAC-3'

The PCR reactions were performed with 1 µl of template in a total volume of 25 µl. Generally, the PCR profiles consisted of a hot start at 94°C, an initial denaturation at 94°C for 1 minute, a denaturation at 94°C

for 30 second, an annealing at 55°C for 1 minute and an extension step at 72°C for 40 second. After 25 cycles an extension was carried out at 72°C for 7 minutes. PCR products were examined by agarose gel electrophoresis and staining with ethidium bromide.

2.4. Transformation

Isolation of DNA and transformation of the *E. coli* JM 109 cells were performed as described in the guidebook [8], with the following modifications. Transformed cells were spread on the appropriate indicator plates containing ampicillin. Colonies were scored for phenotype on Luria Bertani agar plates after 24 hours at 37°C.

2.5. Creation of Plasmids of the Recombinant Rv 2430c in *E. coli* JM 109

Fragment of the ORF Rv 2430c were obtained by PCR using two primers pair and DNA of the *M. tuberculosis* Indonesian strain as a template. The pGEM-T vector and PCR product were cut with *Bam*HI and *Hind*III, mixed, and treated with T4 DNA ligase. The resulting recombinant plasmid pGEM-T-Rv2430c was transformed in *E. coli* JM 109.

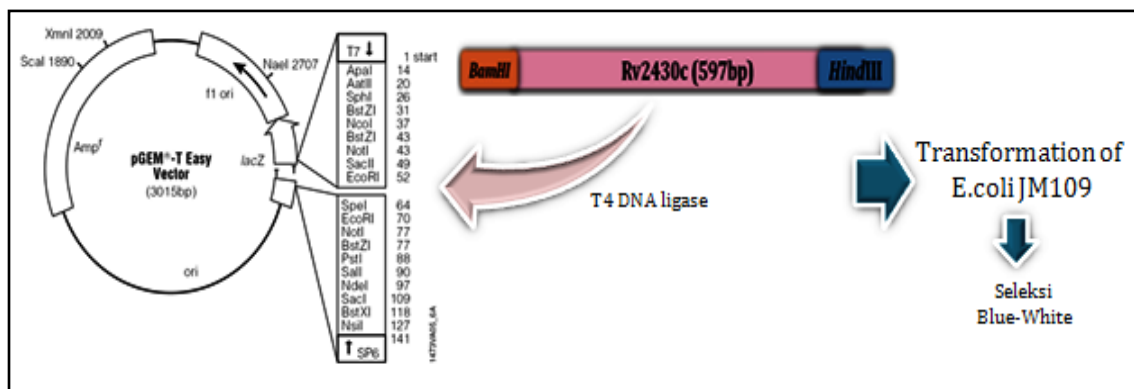


Figure 1. Ligation scheme of ORF Rv2430c into cloning vector pGEM T-Easy

3. Results and Discussion

3.1. PCR Product and Purification

The results of PCR amplification of ORF Rv 2430 observed by electrophoresis agarosa. A band was revealed which corresponded to the gene with the apparent of 597 bp. It is appropriate that obtained Choudhary *et al*, 2003 that 597bp band was observed upon staining with ethidium bromide, indicating that the ORF was expressed at the mRNA level in the liquid cultures of *M. tuberculosis*

3.2. Characterization of Recombinant Plasmid of pGEM-T-Rv 2430c

Isolation of plasmid were performed according to the procedure instructions (BioRad). Characterization was done by migration analysis, restriction analysis, and PCR analysis. Migration analysis was done by comparing pGEM-T (3000bp) and pGEM-T-Rv 2430c (3597bp). A

band shows difference length while pGEM-T-Rv2430c had slower migration than pGEM-T (Figure 3).

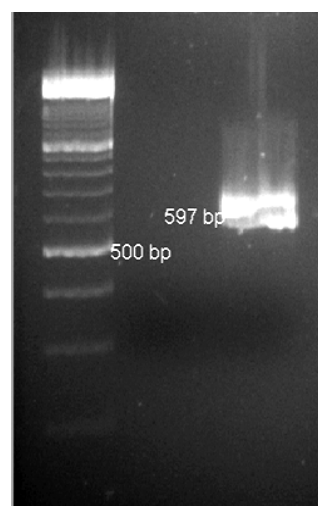


Figure 2. Purification of PCR product: 1) Marker 100 bp, 2) ORF Rv 2430c



Figure 3. Migration analysis: 1) pGEM-T: 3000 bp, 2) pGEM-T-Rv2830: 3597bp

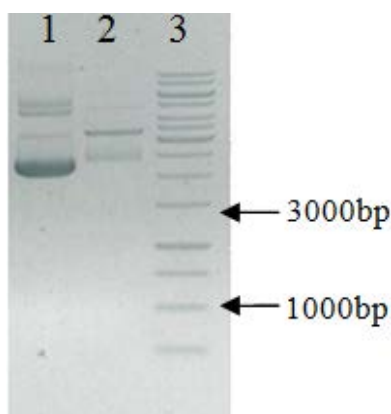


Figure 4. Restriction analysis: 1) pGEM-T-Rv2430c (3597bp) uncut; 2) pGEM-T-Rv2430c (3597bp) cut; 3) Marker 1 kb

This suggests that the plasmid without insert DNA (pGEM-T) will move faster than the recombinant plasmid (pGEM-T-Rv 2430). This means that the DNA inserts ORF Rv 2430 has been successfully ligation into the vector pGEM-T.

Restriction analysis was done using 2 restriction enzymes *Bam*HI and *Hind*III in pGEM-T-Rv2430c. By electrophoresis, a linear band showed a different shape with uncut recombinant plasmid which has supercoiled and relaxed band (Figure 4).

Uncut plasmids can be in two forms: relaxed and **supercoiled** (or superhelical). The lane with the digested plasmid has a single band, while the lane with the undigested sample has three bands. If the lower band had moved most rapidly because it was a smaller fragment than the others, then we would expect that it would be much dimmer than the others because, in general, less fluorescent dye is intercalated by smaller fragments. However, in this case the lower band is much brighter than the other bands, either because the supercoiled shape holds dye better or because a higher fraction of the molecules in that lane were in the supercoiled form than in other forms [9].

PCR analysis was done by using exactly the same cycle as it mentioned before for amplified the Rv 2430c gene. Electrophoresis showed that plasmid recombinant pGEM-T-Rv2430c contain the Rv 2430c gene as DNA insert was 597 bp (Figure 5).

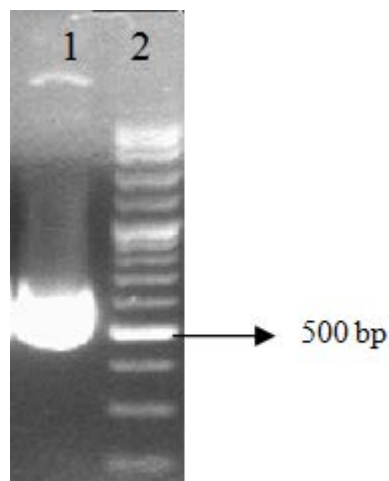


Figure 5. PCR analysis: 1) Rv 2430c :597bp 2) Marker 100 bp

4. Conclusion

Cloning ORF of Rv 2430c *M. tuberculosis* Indonesia isolates to *Escherichia coli* JM 109 has been successfully and characterized DNA insert by PCR, migration analysis and restriction analysis.

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