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Ligation, Transformation and Characterization of Rv 1926c *Mycobacterium tuberculosis* to *Escherichia coli* JM 109 For Latent Tuberculosis Immunodiagnostic

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Abstract

Tuberculosis caused by *Mycobacterium tuberculosis* is the biggest infectious disease causing human death in the world. The main challenge in controlling tuberculosis is to quickly and accurately diagnose tuberculosis infection. Several kits have been produced to diagnose tuberculosis, but have different sensitivity and specificity. This shows that the kit is not yet ideal for diagnosing tuberculosis, so the search for candidates for specific antigens still needs to be done. One potential antigen is the Rv 1926c encoding MPT 63 protein. This protein is known to induce Th1 cells and produce IFN λ from PBMC cells of patients infected with tuberculosis. The purpose of this study was to clone the Rv 1926c from Mycobacterium tuberculosis as a tuberculosis immunodiagnostic kit. The method used is isolating Rv 1926c with PCR, ligation to pGEM-T vector and transformation to *E.coli* host cell JM 109. Clone characterization was carried out by PCR and migration analysis. The results obtained are the recombinant clones obtained have successfully inserted with the Rv 1926 c

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Introduction

Tuberculosis (TB) is the ninth leading cause of death worldwide. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people and an additional 374.000 deaths among HIV-positive people. In 2016 an estimated 10.4 million people fell ill with TB: 90% were adults, 65% were male, 10% were people living with HIV (74% in Africa) and 56% were in five countries: India, Indonesia, China, the Philippines and Pakistan (WHO, 2017).

Generally most of the people in Indonesia have received the BCG vaccine when they were toddlers. But the effectiveness of this vaccine does not last until adulthood, so it is suspected that everyone can be infected by *M. tuberculosis* and is latent. Latent TB infection

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has the potential to become active TB and people with active TB can be a source of new infections

Latent tuberculosis infection (LTBI) is the presence of tuberculosis in the body without symptoms or radiographic evidence or bacteriological examination. It is estimated that up to 13 million people in the United States are latent TB, and 5-10% of infected people will suffer from TB, which is equivalent to 650,000 to 1,300,000 (CDC, 2013).

The guidelines recommend that either Tuberculin Skin Test (TST) or Interferon gamma release assays (IGRA) can be use to test for latent TB in in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100 000. Consistent with existing WHO recommendations, the guidelines reiterated that IGRA should not replace tuberculin skin test in low-income and other middle-income countries (WHO, 2018).

Tuberculin Skin Test (TST), also referred to as the Mantoux or Purified Protein Derivative (PPD) test, detects TB exposure through the skin. Advantages of TST are simple and easy to administer and can detect TB infection. Disadvantages of TST are false negatives: the failure of a bump to develop can be the result of a compromised immune system, false positives: the bacterial particles in the BCG vaccine can stimulate the production of TB antibodies, causing a false positive, sensitivity varies widely across populations and is inconclusive for children, HIV patients with low CD4 counts (TAG, 2018).

Some weaknesses of TST include not being able to distinguish between active and latent TB patients. This test will be positive in people who are BCG vaccinated and who are in contact with other mycobacteria (Pai *et al*., 2016). Therefore the specificity of PPD is questionable, especially in endemic areas such as Indonesia.

Due to the limitations of the TST test, the study was directed to find the specific antigen to be used as an immunodiagnostic. Especially the availability of TB diagnostic reagents that can identify new and latent infected individuals with high risk that can develop into active tuberculosis. One potential antigen is the Rv1926c encoding MPT 63 protein. This protein is known to induce Th1 cells and produce IFN λ from PBMC cells of patients infected with TB.The purpose of this study was to clone the Rv 1926c from *M. tuberculosis* to *E.coli* JM 109 as immunodiagnostic latent tuberculosis

Materials and Methods

Bacterial Strains and Plasmids

The cloning vectors pGEM-T Easy and bacterial strain JM 109 (Promega) was used. The strains *M. tuberculosis* was obtained from clinical isolate from Indonesia.

Culture Condition

Bacterial strain JM 109 is a useful host for transformation of pGEM-T vector. Bacterial were incubate with stirring over night in Luria- Bertani medium in the presence of ampicillin (1µg/ml) at 37°C. The clinical isolate of *M. tuberculosis* is cultured in the Lowenstein-Jensen medium.

Chromosome DNA extraction

M.tuberculosis chromosome DNA extracted using Qiagen DNeasy kit. DNA extraction were measured using a spectrophotometer at absorbance of 260 nm.

Amplification

Amplification of Rv 1926c was carried out by PCR using a specific primer. The primer sequence used in this study according (5) as follow Forward: 5'-CAGCAGGATCCCGCCTATCCCATCACCGGA-3' and Reverse : 5'-GCCCAAGCTTCGGCTCCCAAATCAGCAG-3' .The PCR conditions for amplification were pre denaturation 94^0C 10 minutes, denaturation 94^0C 1 minute, annealing 56^0C 1 minute and elongation 72^0C 1 minute in 30 cycles.

Creation of Plasmid of the Recombinant Rv 1926c in *E. coli* JM 109

Fragment of the Rv 1926c 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-Rv1926 was transformed in *E. coli* JM 109.

Transformation

Isolation of DNA and transformation of the *E. coli* JM 109 cells were performed as described in the guidebook [6], 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.

Results and Discussion

PCR Product and Purification

The results of PCR amplification of Rv 1926 observed by electrophoresis agarosa. A band was revealed which corresponded to the gene with the apparent of 412 bp.

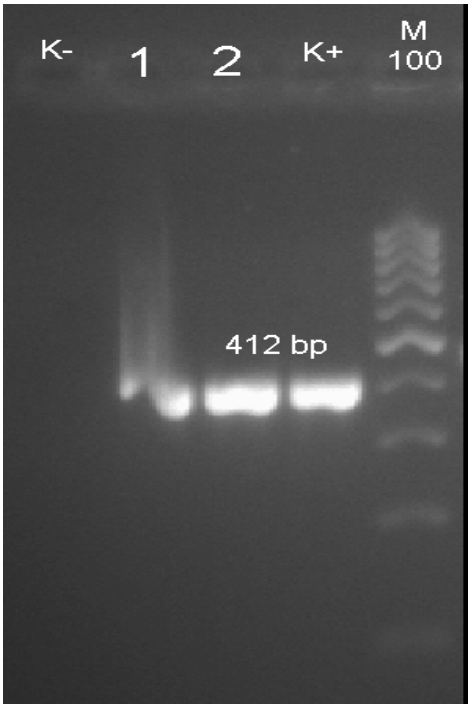

Figure 1. PCR product of Rv 1926c, (K-) Negative control , (1,2) Rv 1926c , (K+) Positive control , (M 100) Marker 100 bp

Ligation and transformation of the Rv 1926c to the pGEM-T vector

The results of the transformation in Figure 1 showed that there were colonies and white colonies in petridish containing LB medium, X-gal, IPTG and ampicillin. White colonies indicate that the insertion DNA has been successfully inserted into the vector, while the blue colony means that the insertion DNA has not been successfully transferred to the vector. White *E. coli* colonies (transformant cells) showed Rv 1926c coding DNA successfully ligated in the MCS (multi cloning site) area found in the lacZ pGEM-T gene. The insertion of this DNA fragment will inhibit the lacZ gene to encode the subunit of β-galactosidase, so that the enzyme cannot degrade the available galactose substrate. The bacterial colonies are blue, meaning they do not have insertion DNA fragments so they can degrade the available galactose substrate (Medical Biochemistry, 2017).

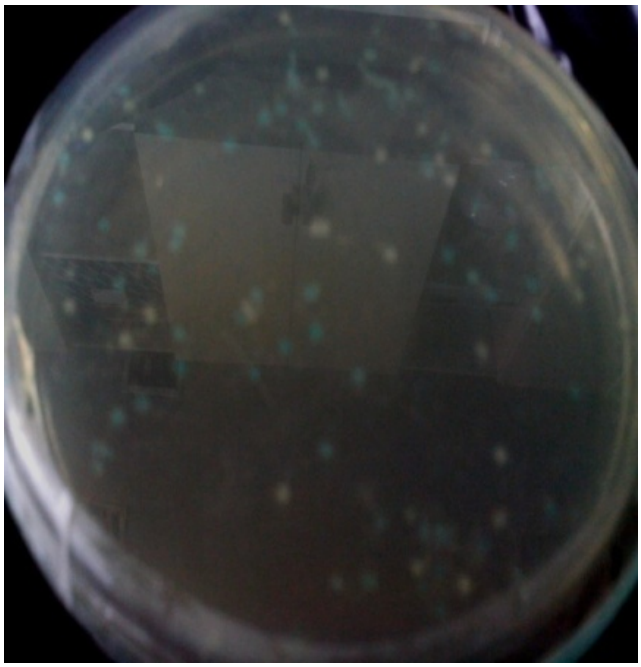

Figure 2. Recombinant clone pGEM-T-Rv 1926c

Characterization of Recombinant Plasmid of pGEM-T-Rv 1926c

Isolation of plasmid were performed according to the procedure instructions (BioRad). Characterization was done by PCR analysis and migration analysis. Migration analysis was done by comparing pGEM-T (3000 bp) and pGEM-T-Rv 1926c (3412 bp). A band shows difference length while pGEM-T-Rv1926c had slower migration than pGEM-T (Figure 3).

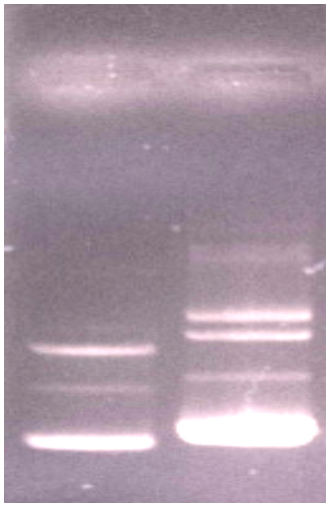

Figure 3. Migration analysis: (1) pGEM-T: 3000 bp (2) pGEM-T-Rv1926: 3412bp

This suggests that the plasmid without insert DNA (pGEM-T) will move faster than the recombinant plasmid (pGEM-T-Rv 1926c). This means that the DNA inserts Rv 1926c has been successfully ligation into the vector pGEM-T. PCR analysis was done by using exactly the same cycle as it mentioned before for amplified the Rv 1926c gene. Electrophoresis showed that plasmid recombinant contain the Rv 1926c gene as DNA insert was 412 bp (Figure 4)

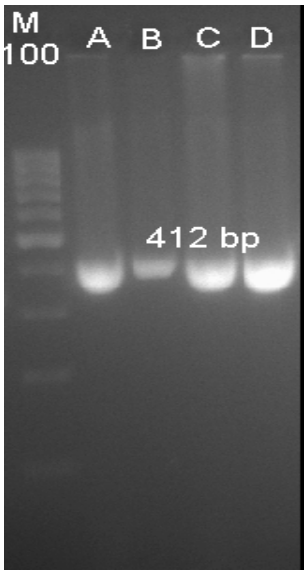

Figure 4. PCR analysis: (A,B,C,D) Rv 1926c : 412bp. (M) Marker 100 bp

Conclusions

Gene of Rv 1926c *M. tuberculosis* Indonesian isolates have been successfully ligated to pGEM-T vectors and transformed into *E. coli* JM 109

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