Isolation and cloning of Rv3204 of *Mycobacterium tuberculosis* to *Escherichia coli* BL21 as vaccines tuberculosis: A preliminary Study

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Abstract. Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). TB prevention is done through vaccination using the Bacille Calmette-Guerin (BCG) vaccine. There are weaknesses in this vaccine, so research is still needed in the search for specific antigens. One potential is the Rv3204 gene that encodes the methylated DNA-protein cysteine methyltransferase protein 11 kDa (MTSP11). This protein is immunogenic because it can produce interferon-γ (IFN-γ). The purpose of this study was to isolate and clone the Rv3204 *Mycobacterium tuberculosis* into *Escherichia coli* BL21 as vaccine tuberculosis. The research stage was amplification of Rv3204, ligation to pQE-30 Xa expression vector and transformation to *Escherichia coli* BL21 host cells on LB medium. Characterization was carried out by PCR analysis to recombinant plasmid isolation. The results showed that Rv3204 encoding MTSP11 was successfully cloned to *E. coli* BL21 with white colony formation and characterization showed that the band was 306 bp as the Rv3204 gene.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by an intracellular bacillus *Mycobacterium tuberculosis* (*Mtb*). This disease can be pulmonary TB also extra pulmonary TB and spread by people who have sick, example by coughing caused bacteria into the air [1]. Approximately, 1.7 billion people in the world are infected with *Mtb*. Among of them, approximately 10% or 170 million can be a spreader in their lifetime [2]. There are many cases of TB, approximately 4.1% cases globally are rifampicin-resistant, 82% cases Multidrug Resistant (MDR-TB) and 6.2% of them also Extensively Drug Resistant (XDR-TB) [3].

Globally, there are 30 high TB burden countries who are giving 87% cases in the world and 8 countries of them are giving 66% cases. They are India (27%), China (9%), Indonesia (8%), Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). This data indicated that Indonesia in third as burden country [1]. Moreover in the last year Indonesia in Second after India [4]. TB become an epidemic disease and have a high cost for therapy. Because of that, the effort for prevention is needed.

TB is prevented by Bacille Calmette-Guerin (BCG). Using this vaccine can not be controlling this disease because BCG have weaknesses. BCG can cause TB disease in immunosuppressed individuals after vaccinated. Infant with unknown HIV infection status or born to women with HIV infection
should not receive BCG. But, countries with a high burden of TB should be given a single dose of BCG as soon as possible after birth as prevention of TB [5] The new innovation for development of vaccines candidate for TB is needed.

One of them is potential of Methylated DNA-protein cysctein Methyltransferase 11 kDa (MTSP11) which coding by gene Rv3204. Its proteins were known that strongly induced Interferon Gamma (IFN-γ) and interleukin (IL)-12p40 in peripheral mononuclear cells from healthy tuberculin reactors [6]. In TB cases, IFN-γ is needed to activated macrophage who phagocyte Mtb [7]. Protein MTSP 11 as antigen from Mycobacterium tuberculosis have a potential to development of vaccines candidate. Isolation, ligation, transformation and characterization of gene Rv3204 in Escherichia coli BL21 is needed to do to get a recombinant plasmid pQE-30 Xa – Rv3204. This result can be used to production of MTSP11 protein as vaccines candidate for tuberculosis.

2. Materials and Methods

2.1. Bacterial strains and plasmid

Genes Rv3204 were isolated from Mycobacterium tuberculosis clinical isolate from Makassar. Plasmid as cloning vector was using pGEM-T (Promega) and plasmid as expression vector was using pQE-30 Xa (Qiagen). Bacterial as hosts for cloning vector were using Escherichia coli JM109 and hosts for expression vector were Escherichia coli BL21.

2.2. Culture condition

The Escherichia coli JM109 and Escherichia coli BL21 were incubate with stirring over night in Luria Bertani (LB) medium in the presence of ampicillin (1 μg/ml) at 37°C. The competent cells were prepared with CaCl₂ method [8].

2.3. PCR

Rv3204 gene was obtained by PCR using a specific primer of 30 cycles. The following are primer used [6]

Forward 5P-CGC GGG ATC CAT GGC GCC GGT GAC CGA CGA-3P
Reverse 5P-CTA AGA ATT CTC AGC CCG GCG GAA ACT CAT-3P

All the PCRs were performed with 5 μL of template in a total volume 25 μL. Each cycle consisted of an initial denaturation 5 min at 94°C, denaturation 1 min at 95°C, annealing 1 min at 58°C, and extension 1 min at 72°C with a final extension step of 7 min at 72°C.

2.4. Construction of recombinant plasmid of pQE-30 Xa – Rv3204

Fragment of Rv3204 from DNA of Mycobacterium tuberculosis Makassar were obtained by PCR. The pGEM-T vector and PCR product were treated with T4 DNA ligase, then transformed in E. coli JM109 for cloning. The pQE-30 Xa vector were cut with BamHI and HindIII and ligate with fragment of Rv3204 to result recombinant plasmid pQE-30 Xa – Rv3204. This plasmid recombinant was transformed in E.coli BL21 for expression.

Figure 1. Construction of recombinant plasmid pQE-30 Xa – Rv3204
2.5. Ligation
Ligation of Rv3204 in the pQE30-Xa expression vector, which had previously been cut with the restriction enzyme *Bam*HI and *Hind*III. Ligation was carried out at 4°C over night.

2.6. Transformation
Transformation on host cells *E. coli* BL 21 with the heat shock method and spreading them on the Luria Bertani medium containing ampicillin, IPTG and X-gal 24 hours at 37°C [8]

2.7. Characterization of recombinant plasmid of pQE-30 Xa – Rv3204
Before the characterization of recombinant plasmids, plasmid isolation was first performed on white colonies. Characterization by PCR was carried out on the plasmids obtained from isolation.

3. Result and Discussion
3.1. PCR product
Isolation of gene Rv3204 from *Mycobacterium tuberculosis* clinical isolate in Makassar. The result of PCR amplification of Rv3204 on *Mycobacterium tuberculosis* H37Rv as positive control obtained band 306 bp. It appropriates with TB Database that 306 bp as size of gene Rv3204 [9].

3.2. Ligation
Ligation of Rv3204 to the pQE 30-Xa expression vector are obtained in Figure 3. The result of ligation showed four bands. First showed DNA circular nicked, it moved slowest on agarose gel. Second showed linear plasmid, it migrated between DNA circular nicked and supercoil DNA. Third showed supercoil DNA as desired species in prep of recombinant plasmid. Fourth showed single strand circular, it migrated ahead of all the other band in agarose [10].

![Figure 2. PCR Product of Rv3204](image2.png)

**Figure 2.** PCR Product of Rv3204
K- = negative control, K+ = positive control, 1-2 = samples, M = marker

![Figure 3. Ligation Rv3204 to expression vector pQE-30 Xa](image3.png)

**Figure 3.** Ligation Rv3204 to expression vector pQE-30 Xa

3.3. Transformation
The result of transformation was shown on solid LB medium with ampicillin, X-Gal and IPTG. White colonies indicate that the insert (Rv3204) has been successfully inserted into the pQE-30 Xa. Ampicillin caused LB as selective medium. Only colonies containing plasmid could grow on the medium. The aim of X-gal as chromogenic substrate caused LB as differential medium. Hydrolysis X-gal by enzyme caused the blue colour. Insert annoyed activity of LacZ operon caused X-gal could not be hydrolysed and showed white colonies. Moreover, white colonies were *E. coli* containing recombinant plasmid pQE-30 Xa – Rv3204.
3.4. Characterization of recombinant plasmid of pQE-30 Xa – Rv3204

Recombinant plasmid characterization was carried out by PCR analysis. In figure 5, the PCR results of the recombinant plasmid isolation showed a band measuring 306 bp. This indicates that the Rv3204 gene was successfully isolated and cloned to E. coli BL21.

4. Conclusion

Isolation and cloning of Rv3204 Mycobacterium tuberculosis to Escherichia coli BL21 was successfully carried out and white colonies were obtained. The results of the clone characterization showed insertion DNA was 306 bp.

Acknowledgment

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