ABSTRAK

Analisis Karakteristik Molekular dan Imunogenisitas Protein Rekombinan dan Epitop Imunodominan P*olymorphic GC-Rich Repetitive Sequences* (*PE-PGRS*) 24 dan 35 *M. tuberculosis* Isolat Lokal

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Mycobacterium tuberculosis merupakan bakteri intraselular patogen yang berdiam di dalam makrofag yang merupakan komponen paling penting dalam sistem imun. *M. tuberculosis* menghasilkan 2 kelompok protein yang sangat polimorfik, yaitu kelompok *Proline-Glutamic acid (PE)* dan *proline-proline-glutamic acid (PPE)*. Protein kelompok ini merupakan 10% dari total protein *M tuberculosis*. Protein ini sangat terkait dengan virulensi dan menjadi target pengembangan diagnosis dan terapi.

Penelitian ini bertujuan untuk menganalisis karakteristik molekular dan imunogenisitas protein PE, khususnya *PE-PGRS* 24 dan 35 dari *M. tuberculosis* isolat lokal.

Tahap awal penelitian adalah genotyping *M. tuberculosis* isolat lokal, sehingga dapat dideferensiasi *Strain Beijing* dan bukan. Analisis genomik dilakukan terhadap variasi molekular gen *PE-PGRS* 24 dan 35 berdasarkan hasil sekuensing. Beberapa analisis *in silico* dilakukan untuk mengetahui lokalisasi protein dan identifikasi epitop imunogenik. Rekombinan protein diproduksi menggunakan teknik *Gateway*. Dilakukan uji potensi imunogenik dalam bentuk respon antibodi secara *ELISA* terhadap *epitop* peptida dan protein rekombinan *PE-PGRS* 24 dan 35 pada penderita TB dan non TB.

Analisis molekular dilakukan terhadap 10 sampel, yang terdiri dari 5 sampel *M. tuberculosis Strain Beijing* dan 5 non *Beijing* serta 10 sekuen pembanding. Analisis terhadap *PE-PGRS* 24 memperlihatkan adanya 0.83% variasi dibandingkan dengan isolat referen sedangkan pada *PE-PGRS* 35 ditemukan 1.13% variasi *Rv1983*. Analisis lokalisasi memperlihatkan bahwa PE-PGRS 24 adalah protein sekresi sedangkan *PE-PGRS* 35 merupakan protein membran. Respon antibodi terlihat berbeda pada *epitop* peptida 024*A, 035*A dan 035*C antara penderita TB maupun non TB. Kondisi yang sama juga ditemukan antara *epitop* peptida dengan protein rekombinan.

Berdasarkan hasil penelitian ini dapat disimpulkan terdapat variasi molekuler gen *PE-PGRS* 24 dan 35, *PE-PGRS* 24 merupakan protein ekstrasel sedangkan *PE-PGRS* 35 adalah bagian dari protein membrane. Kedua protein dan epitop linearnya bersifat imunogenik dan mampu membedakan TB dengan non TB serta berpotensi dikembangkan sebagai model diagnostik.

Key words : *Tuberkulosis*, *PGRS* protein, Molekular, Imunogenik, *epitop*

ABSTRACT

Analysis of Molecular Characteristics and Immunogenicity of Recombinant and Epitope Immunodominant of PE-PGRS 24 and 35 proteins M. tuberculosis local isolates

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Mycobacterium tuberculosis is an intracellular pathogen and it resides inside the macrophage, which is considered to be the most important component of the immune system. *M. tuberculosis* possesses two highly polymorphic sets of genes called the *Proline-Glutamic acid (PE)* dan *proline-proline-glutamic acid (PPE)* families. These unique families of proteins account for about 10% of the *Mycobacterial* genome. PE and PPE proteins are closely related to bacterial virulence and and become the target of the development of diagnostics and vaccines.

This study aims to assess the characteristics genomic and proteomic of PE family proteins, especially *PE- PGRS* 24 and 35.

At the first stage this research, we have done genotyping analysis from *M. tuberculosis*, so we can differentiate *Beijing* and non *Beijing* strain. Molecular analyzes have performed on molecular variation *PE- PGRS* 24 and 35 genes based on the sequencing results. Some *in silico* analysis have conducted to determine protein sublocalization and identification of immunogenic epitopes. Recombinant proteins are produced using *Gateway* techniques. Immunogenic potency have conducted based on antibody responses against recombinant protein *epitope* peptide and *PE- PGRS* 24 and 35 in patients with TB and non-TB using *ELISA* method.

Molecular analysis have performed on 10 samples, consist of 5 *Beijing strains* and 5 *non-Beijing* and 10 comparison sequences. Molecular variation on *PE- PGRS* 24 was 0.83% that was compared with the referent isolates, while on *PE- PGRS* 35 was 1.13%. Localization analysis have showed that PE-PGRS 24 is a secretion protein while *PE- PGRS* 35 is a membrane protein. Antibody responses on peptide epitope 024*A, 035*A and 035*C were different between TB with non-TB. The same condition was also found between the peptide epitope with recombinant proteins.

Based on these results we can conclude there are molecular variations in *PE- PGRS* 24 and 35. *PE- PGRS* 24 is , extracellular protein while *PE-PGRS* 35 is part of a membrane protein. Both proteins recombinan and immunogenic linear *epitopes* were immunogenic and able to distinguish TB and non TB an also potent as diagnostic candidate.

Key words : *Tuberculosis*, *PGRS* proteins, Molecular, immunogenic, *epitope*