## ABSTRAK

Rahmawati, Hemas Abidah. 2013. *Purifikasi Protein Rekombinan Ag38 kDa Mycobacterium tuberculosis dengan Teknik Denaturasi dan Renaturasi Menggunakan Urea*. Tugas Akhir, Fakultas Kedokteran Universitas Brawijaya. Pembimbing: (1) Dr. rer. nat. Tri Yudani M. R., M. App. Sc. (2) dr. Iin Noor Chozin., Sp. P.

Tingginya angka morbiditas dan mortalitas akibat tuberkulosis mendorong berbagai penelitian mengenai *Mycobacterium tuberculosis* untuk dijadikan agen serodiagnostik dan kandidat vaksin. Salah satunya adalah menemukan faktor virulensi yang paling poten menyebabkan dan meningkatkan keparahan tuberculosis, yaitu antigen 38 (Ag38) kDa. Antigen ini bersifat imunodominan sehingga akan merangsang sel-sel imun untuk membuat pertahanan terhadap bakteri jenis ini. Di Laboratorium Biomedik Fakultas Kedokteran Universitas Brawijaya telah dilakukan cloning dan ekspresi gen *pab* pengkode Ag38 kDa *Mycobacterium tuberculosis* yang diisolasi dari sputum pasien tuberculosis di Malang. Namun demikian, pada saat gennya diekspresikan, protein Ag38 yang disintesis berbentuk *inclusion bodies*. Dengan demikian, tidak bisa dipurifikasi secara konvensional. Salah satu cara purifikasi protein rekombinan dari *inclusion bodies* adalah dengan teknik denaturasi dan renaturasi menggunakan urea. Konsentrasi agen denaturasi yang digunakan berbeda-beda. Diawali dengan 8 M memberi hasil visualisasi dengan metode SDS PAGE kurang memuaskan karena terjadi penyumbatan (*clogging*) protein pada proses *binding* dan pada tahap *washing* terjadi *overload* protein sehingga protein ikut tercuci. Berikutnya, menggunakan konsentrasi 4 M. Hasil visualisasi memperlihatkan masih adanya *clogging* pada proses *binding*, namun tidak setebal eksperimen sebelumnya. Pada tahap *washing*, protein masih ikut terbilas. Kedua percobaan tersebut masih menunjukkan adanya pita protein selain pita protein Ag38. Namun, pada konsentrasi yang lebih rendah (2 M), hasilnya cukup memuaskan. Tidak didapatkan *clogging* dan *overload* pada proses *binding* dan *washing*. Serta, pita protein selain Ag38 lebih bersih. Hasil ketiganya dikonfirmasi menggunakan metode *western blot* bahwa protein yang dipurifikasi memang benar protein rekombinan Ag38 kDa.

Kata Kunci: Tuberculosis, *Mycobacterium tuberculosis*, Ag38 kDa, Denaturasi, Renaturasi, Urea

## ABSTRACT

Rahmawati, Hemas Abidah. 2013. *Ag38 kDa Recombinant Protein Purification of Mycobacterium tuberculosis by Denaturation dan Renaturation Technique Using Urea*. Final Assignment, Faculty of Medicine, Brawijaya University. Supervisors : (1) Dr. rer. nat. Tri Yudani M. R., M. App. Sc. (2) dr. Iin Noor Chozin., Sp. P.

The height number of morbidity and mortality because of tuberculosis encourages many researches about *Mycobacterium tuberculosis* to be serodiagnostic agent and vaccine candidate. One of them is finding the most potential virulence factor which causes and increase tuberculosis severity, such as 38 kDa antigen (Ag38). This antigen is immunodominant so that stimulates immune cells to make defence to this bacteria. Biomedical Laboratory of Medical Faculty of University of Brawijaya has cloned and expressed *pab* gene which is coding Ag38 kDa *Mycobacterium tuberculosis* isolated from tuberculosis patient's sputum in Malang. Meanwhile, when the gene was expressed, Ag38 protein is synthesized as inclusion bodies. So, it can not be purified conventionally. One of possible recombinant protein purification of inclusion bodies is denaturation and renaturation using urea. We used different concentration of denaturing agent. Started with 8 M, the visualization by using SDS PAGE method gave unsatisfied result because there was protein clogging at binding process and protein overload at washing process, the protein was washed. The next experiment is using concentration 4 M. The result still gave clogging and overload but not as much as before. At washing process, the protein was washed too. Both of those experiments still visualized that the other protein bands exist. But at lower concentration (2 M), the result was satisfying. There was no clogging and overload at binding and washing process. The other protein bands did not exist. Three of them were confirmed by using western blot method that the purified protein was truly Ag38 kDa.


Keywords: Tuberculosis, *Mycobacterium tuberculosis*, Ag38 kDa, Denaturation, Renaturation, Urea