

Mukhlisin Jefri Ainul Ma'ruf, 2014, **Isolasi dan Sequencing Gen *pncA* penyandi PZase serta Pemodelan Struktur 3 Dimensi PZase dari Isolat Klinis Lokal *M. tuberculosis* Resisten Pirazinamid.** Skripsi ini dibawah bimbingan Dr. Purkan M.Si dan Prof. Dr. Afaf Baktir M.S, Departemen Kimia, Fakultas Sains dan Teknologi, Universitas Airlangga, Surabaya.

ABSTRAK

Telah dilakukan isolasi gen *pncA* penyandi protein pirazinamidase dari isolat lokal *M. tuberculosis* resisten pirazinamid. Protein pirazinamidase merupakan enzim yang bertanggung jawab terhadap perubahan pirazinamid menjadi asam pirazinoat sehingga dapat bertindak aktif sebagai agen obat anti tuberkulosis. Resistensi terhadap obat jenis pirazinamid memerlukan penjelasan terkait mutasi pada gen *pncA* serta perubahan struktur pirazinamidase. Tahap penelitian ini terdiri dari isolasi DNA genom *M. tuberculosis* H37RV yang merupakan isolat alami sensitif pirazinamid dan DNA genom R2 yang merupakan isolat resisten pirazinamid, amplifikasi gen *pncA* dengan PCR, kloning gen *pncA* ke dalam *E. coli* dengan vektor pGemT, isolasi DNA plasmid rekombinan, sekuensing, serta pemodelan struktur 3 dimensi pirazinamidase. Gen *pncA* berhasil dikloning dan disekuensing urutan nukleotidanya menurut metode *dideoxy Sanger*. Hasil analisis sekuensing menunjukkan adanya 3 titik mutasi yang menyebabkan resistensi isolat R2 pada urutan nukleotida gen *pncA* yaitu T40C, G419A dan A535G. Perubahan basa ini menyebabkan perubahan susunan asam amino pada protein pirazinamidase berturut-turut Ser14Arg, Arg149His, Ser179Gly. Hal ini menyebabkan perubahan struktur 3 dimensi pirazinamidase dari isolat lokal *M. tuberculosis* resisten pirazinamid.

Kata kunci: *M. tuberculosis* resistant pirazinamid, gen *pncA*, pirazinamid, pirazinamidase.

Mukhlisin Jefri Ainul Ma'ruf, 2014, **Isolation and Sequencing *pncA* gene Encoding PZase and Modelling 3 Dimensional Structure of PZase from Local Isolate *M. tuberculosis* Resistant Pyrazinamide**. Skripsi were supervised of Dr. Purkan M.Si and Prof. Dr. Afaf Baktir M.S, Departement of Chemistry, Fakulty of Science and Technology, University of Airlangga, Surabaya.

ABSTRACT

It had been cloned of *pncA* gene encoding pyrazinamidase from local isolate of pyrazinamide resistant *M. tuberculosis*. Pyrazinamidase was an enzyme that responsible to changed of pyrazinamide becomes pyrazinoic acid, so it could be active as agent of anti tuberculosis. Pyrazinamide resistant *M. tuberculosis* need an explanation about mutation of *pncA* gene and also changed of pyrazinamidase structure. This research was from isolation DNA genome of *M. tuberculosis* H37RV that was wild type isolate of sensitive pyrazinamide and R2 DNA genome that was resistant of pyrazinamide, amplification *pncA* gene with PCR, cloning *pncA* gene in *E. coli* with PgemT vector, isolation of DNA plasmid recombinant, sequencing and also modelling 3 dimensional structure of pyrazinamide. *pncA* gene was success isolated an sequenced level of its nucleotide based on dideoxy sanger method. Sequence analyze showed three spot of mutation in *pncA* gene from R2 that cause resistance of pyrazinamide, that are T40C, G419A and A535G. Change of nucleotide base give an effect of amino acid structure in Sis14Arg, Arg149His, Ser179Gly and it also changed three dimensional structure of pyrazinamidase from local isolate.

Keyword: **pyrazinamide resistant *M. tuberculosis*, *pncA* gene, pyrazinamide, pyrazinamidase**