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Shortening of Amino Acids from C-terminal of PZase as Basis of Pyrazinamide Resistance in P14 Isolate of *Mycobacterium tuberculosis* Strain

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Abstract

Pyrazinamide (PZA) is one of the mainstays WHO-recommended drugs for therapy of tuberculosis (TB). The emergence of PZA resistance in clinical isolates of *M. tuberculosis* is often associated with *pncA* gene mutations encoding PZase. A local clinical isolate of *Mycobacterium tuberculosis* strain showed phenotipe resistant to PZA at concentration of 10 μg/mL. The ORF of *pncA* gene of the isolate showed deletion of guanine base at position 81, then followed by shortening of 70 amino acids from C-terminal of PZAse which has 186 amino acid residues. The mutant of PZase took frame shift of amino acids after the residue at position 27. The *pncA* gene mutation at the level of genotype, that produced a physical-chemical alteration of the active site or the metal-binding site of PZase, in this case perturbing or lossing its activity was proposed as trigering the PZA resistance in P14 clinical isolate of *M. tuberculosis* strain.

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Keywords: PZA resistance; *pncA* gene; *Mycobacterium tuberculosis*

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Nomenclature

TB	Tuberculosis
PZA	Pyrazinamide
MDR	Multidrug-resistant
PZase	Pyrazinamidase

1. Introduction

Tuberculosis (TB) is a bacterial infectious disease, caused by *Mycobacterium tuberculosis*. Currently, TB has been the seventh most common cause of death in the world. Indonesia has been clasified as a "high burden country" for tuberculosis, cited in the highest fifth rank of the 22 countries related to high burden. There are about 500,000 new cases of TB annually and 175,000 of them are deaths in Indonesia[1,11]. Of the TB cases in Indonesia, about 2 percent of new cases and 12 percent of the recurring cases are the multidrug-resistant (MDR) strain[1,11].

Pyrazinamide (PZA) is a potential drug that is commonly used to threat active tuberculosis (TB), along with other drugs during the initial phase of therapy (generally the first two months of treatment). The PZA has a spesifically antibacterial merit only to resist against *Mycobacterium tuberculosis* and *M. africanum*[2,8.]

PZA is adsorpted by *M. tuberculosis* by passive diffusion, then converted to be active form by pyrazinamidase (PZase) enzyme that is encoded by *pncA* gene, to resist against dormant or semidormant microorganisms although the role of PZA against intracellular organisms remains uncertain[4,8,10]. In one study, pyrazinoic acid remained outside of *M. tuberculosis* cells at a neutral or alkaline pH but accumulated within cells at an acidic pH[5,6]. In the same study, *M. smegmatis* which is resistant to PZA, and was found converting PZA to pyrazinoic acid via an active efflux mechanism, did not accumulate the metabolite at an acidic pH. Other mycobacterial strains exhibited to be resistant to PZA, due to lack of PZase activity or absence of transport mechanisms to take up the drug[3,6].

PZA resistance in *M. tuberculosis* is commonly associated with mutations in the *pncA* gene encoding pyrazinamidase (PZase)[7,13]. Mutation of *pncA* in mycobacterial isolates is unique from each different geographical region[7,10,11]. Determination of *pncA* mutation is needed to make genetic marker. The paper reported a novel mutation in *pncA* gene of a PZA-resistant *M. tuberculosis* from local isolate (P14) which linked to its protein model as a basis of the emergence of PZA resistance in the isolate.

2. Methods

2.1. Bacteria and plasmid

An isolate of PZA-resistant *M. tuberculosis* (P14) and a PZA-sensitive isolate named as H37Rv strain were obtained from Health Research Center, Bandung, Indonesia.

2.2. Mycobacterium tuberculosis growth condition

M. tuberculosis was grown in a solid medium, named as Lowenstein-Jensen (LJ) at 37ºC for 2 weeks until single colony was found. The LJ medium is composed of egg suspension 60% (v/v) ; malachite green 1% (w/v); glycerol 0.8% (v/v); KH_2PO_4 0.2% (w/v); $MgSO_4.7H_20$ 0.02% (w/v); and citrate magnesium 0.04% (w/v)[10,11].

2.3. Chromosomal DNA isolation

The chromosomal DNA of *M. tuberculosis* was isolated by alkali lyses method using wizard genomic DNA purification kit (Promega). The pellet cells were suspended in 480 µl 50 mM EDTA, then added with 120 µl lysozyme and incubated at 37ºC for 1 hour. The mixture was centrifuged for 2 minutes at 13.000g to separate the pellet cells from their supernatant. The cell pellet was added by *Nuclei lysis Solution* and *Protein Precipitation solution* solvent, incubated for 5 minutes at 80°C, then cooled in room temperature. After it was centrifuged for 3 minutes, it was followed by isopropanol additon to obtain DNA precipitate. DNA precipitate formed was then washed by ethanol 70% (v/v), after it was centrifuged for 2 minutes. Etanol was thrown; DNA pellet was dissolved in *Rehydration Solution* DNA solvent to use DNA amplification[10].