MUTATION OF *katG* IN A CLINICAL ISOLATE OF *Mycobacterium tuberculosis*: EFFECTS ON CATALASE-PEROXIDASE FOR ISONIAZID ACTIVATION

PURKAN[1], IHSANAWATI[2], D. NATALIA[2], Y. M. SYAH[2],
D. S. RETNONINGRUM[3], H. S. KUSUMA[4]

[1]*Biochemistry Research Division, Department of Chemistry, Faculty of Sciences*
and Technology, Airlangga University; Surabaya, Indonesia;
e-mail: purkan@fst.unair.ac.id;
[2]*Biochemistry Research Division, Faculty of Mathematics and Natural Sciences,*
Bandung Institute of Technology, Bandung, Indonesia;
[3]*School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia;*
[4]*Department of Chemical Engineering, Institut Teknologi*
Sepuluh Nopember, Surabaya, Indonesia;
e-mail: heriseptyakusuma@gmail.com

Mutations in katG gene are often associated with isoniazid (INH) resistance in Mycobacterium tuberculosis strain. This research was perfomed to identify the katG mutation in clinical isolate (L8) that is resistant to INH at 1 µg/ml. In addition to characterize the catalase-peroxidase of KatG L8 and perform the ab initio structural study of the protein to get a more complete understanding in drug activation and the resistance mechanism. The katG gene was cloned and expressed in Escherichia coli, then followed by characterization of catalase-peroxidase of KatG. The structure modelling was performed to know a basis of alterations in enzyme activity. A substitution of A713G that correspond to Asn238Ser replacement was found in the L8 katG. The Asn238Ser modification leads to a decline in the activity of catalase-peroxidase and INH oxidation of the L8 KatG protein. The catalytic efficiency (K_{cat}/K_M) of mutant $KatG_{Asn238Ser}$ respectively decreases to 41 and 52% for catalase and peroxidase. The mutant $KatG_{Asn238Ser}$ also shows a decrease of 62% in INH oxidation if compared to a wild type KatG (KatGwt). The mutant Asn238Ser might cause instability in the substrate binding site of KatG, because of removal of a salt bridge connecting the amine group of Asn238 to the carboxyl group of Glu233, which presents in KatGwt. The lost of the salt bridge in the substrate binding site in mutant $KatG_{Asn238Ser}$ created changes unfavorable for enzyme activities, which in turn emerge as INH resistance in the L8 isolate of M. tuberculosis.

K e y   w o r d s: Mycobacterium tuberculosis, INH resistance, katG, catalase-peroxidase.

Tuberculosis (TB) is well known as an infectious disease that is caused by *Mycobacterium tuberculosis*. The disease is currently ranked as the seventh most common causes of death in the world, and still estimated to remain in the top 10 causes of death untill 2030. Recently, TB is reported as the second leading cause of death in adults and recorded as the deadliest of all infectious diseases. Indonesia is classified as a country with the large number of TB cases and occupies the fifth rank of the 22 countries with high potential of TB. In the country, there are about 500,000 new cases of TB annually and 175,000 of them are deaths [1, 2]. Two percent of new cases and twelve percent of the recurring cases of TB found in Indonesia are the multid-rug-resistant (MDR) cases [1]. A better understanding in the antituberculous drug resistance is needed to make easy in the TB therapy.

Isoniazid (isonicotinic acid hydrazide; INH) has commonly been recommended by the World Health Organization (WHO) to treat tuberculosis since 1952 because the drug has a high bactericidal effect and a low price [3]. The bactericidal effect of INH as TB drug depends on catalase-peroxidase of *M. tuberculosis* which is encoded by *katG* gene. The enzyme plays a role to convert isoniazid absorbed by the *M. tuberculosis* to be in active form, an isonicotinoyl acyl radical, to trigger the death of mycobacteria. This might occur because an isonicotinoyl acyl radical is a potential inhibitor for enoyl-acyl

carrier protein reductase (InhA) and β-ketoacyl-acyl carrier protein synthase (KasA), the two key enzymes for the biosynthesis of mycolic acids, a cell wall component of mycobacteria [4, 5].

KatG mediates the sensitivity of *M. tuberculosis* to INH. The *katG* deficiency strain of *M. tuberculosis*, which is resistant to INH, can restore the sensitivity to INH when it is complemented with a functional *katG* [6]. Meanwhile, total deletion of *katG* gene in *M. tuberculosis* raised resistance to INH [6-8]. Nevertheless, the loss of catalase-peroxidase in *M. tuberculosis* has not yet explained completely the mechanism of INH resistance, because the total deletion of *katG* is rarely found in clinical isolates [4, 9].

It has been reported that 50-70% of INH-resistant *M. tuberculosis* strains are associated with the mutation in the *katG* gene [3, 4]. The mutation types are very diverse, with missense mutations being the most common alteration. The mutations in *katG* also reveal unique types in INH resistant strain from different geographical areas. The frequency of mutation types in *katG* is often only associated with drug resistance and rarely linked directly to the change of INH sensitivity or to the effect of the enzymatic activity of KatG. The *katG* mutations that affect catalase-peroxidase activity have been found in either all part of the gene and encode the N-terminal or the C-terminal part of the protein [10, 5]. Many variants of KatG associated with INH resistance exhibits a decrease in catalase-peroxidase activities. The decreasing scale of the activity among the mutants of *katG* does not directly correlate with INH resistance [11, 12]. This is the basis of the argument that the INH resistance in clinical isolates is not linked directly to the ability of variants of KatG in INH activation.

The mutant KatG Ser315Thr, that is commonly found in clinical isolates and associated with INH resistance, decreases the activities of catalase-peroxidase and INH oxidation [13, 14]. The amino acid Ser315 in KatG is closely put in the active environment. So that, the genetic modifications in this part are easily understood as the producer of importantly affected enzymatic function and thus isoniazid resistance. As many as 50% variants of *katG* associated with INH resistance are not modified in Ser315Thr. Biochemical analysis of variants of *katG* other than Ser315Thr is necessary to examine the relation between INH resistance with the changes in the function and structure of the mutants.

Some clinical isolates of INH-resistant *M. tuberculosis* from Indonesian TB patients are not mutated at codon 315 of *katG*. Among these isolates, an isolate, namely L8 isolate, shows resistance to INH at 1 µg/ml. This paper shows the correlation of *katG* mutation in the isolate with the biochemical properties of catalase-peroxidase, especially for isoniazid oxidation. This paper also examines the ab initio structural study of the mutant KatG L8 with the aim of gaining a more complete understanding of drug activation and the resistance mechanism.

Materials and Methods

Bacterial strains and plasmids. A clinical isolate of *M. tuberculosis* was obtained from sputum of a TB patient in Bandung, Indonesia. *Escherichia coli* TOP10 (Invitrogen, Carlsbad, CA) was used as hosts for cloning of *katG* of INH resistant and sensitive *M. tuberculosis*. The *E. coli* BL21 (*DE3*) (Promega, Madison, USA) was employed as an expression host of KatG. The plasmid pGEM®-T (Invitrogen) and pCold II-DNA (Kinki University) were used as a cloning and expression vector, respectively.

Preparation of chromosomal DNA. Chromosomal DNAs of *M. tuberculosis* of wild type and clinical isolates were prepared by an alkaline lysis method in 5 mM Tris-Cl buffer (pH 8.5) containing 0.5% (b/v) Tween-20 and 0.2 mg/ml proteinase K at 50 °C for 60 min. The mixture then was heated at 95 °C for 5 min. Cellular debris was collected at 12,000 g for 10 min and the supernatant containing chromosomal DNA of *M. tuberculosis* was used for PCR [15].

Amplification of *katG* gene. The full length of the *katG* gene was amplified by the polymerase chain reaction (PCR) technique using FG and RG primers (Table 1). PCR was performed in a total reaction volume of 50 µl containing 50 ng cromosomal DNA; 1xPCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl); 0.1 mM of each primer, 200 µM dNTPs; 1.5 mM MgCl$_2$; and 0.25 unit of *Taq* DNA polymerase (Amersham). The amplification process was done by a GeneAmp® PCR System 2700 (PerkinElmer), and set at the following steps: pre-denaturation at 94 °C for 4 min, 25 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, and an extension at 72 °C for 3 min. The process was terminated by post-elongation at 72 °C for 7 min. All PCR products were analyzed using agarose gel electrophoresis and purified by GFX purification kit (Amersham).

T a b l e   1. The oligonucleotide primers for sequencing of katG gene

Name of Primer	Nucleotide sequence of primers (5′→3′)
SP6 promoter	catacgatttaggtgacactatag
T7 promoter	taatacgactcactataggg
FG	gttattgaattcgatgcccgagcaacacccac
RG	ttcatagcggccgcgcgcacgtcgaacctgtc
KF	gcagatggggctgatctacg
FDPRK	cgacgagttcgccaaggc

Cloning of the *katG* gene. The PCR products corresponding to the *katG* gene was inserted into pGEM®-T vector, then the recombinant vector was used to transform *E. col*i TOP10 using the CaCl₂ method. [16] Transformants were screened in Luria Bertani (LB) solid medium containing ampicillin 100 µg/ml, 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside (X-gal) and isopropyl β-D-thiogalactoside (IPTG). The target transformants which carried recombinant pGEM-T-*katG* plasmid were selected by a restriction analysis.

DNA sequencing. The nucleotide of the *katG* gene in the recombinant plasmid was sequenced by an automatic nucleotide sequencer (ABI PRISM, Macrogen, Seoul, Korea). The recombinant pGEM®-T, namely pGEM-T-*katG* was used for DNA template. All oligonucleotides primers used for the sequencing were presented in Table 1.

Alignment analysis. The *katG* genes of wild type and clinical isolate of *M. tuberculosis* were analyzed *in silico* by aligning the nucleotide sequences of the genes and their deduced amino acid sequences, using the SeqManTMII and MegAlignTM DNASTAR program (Lasergene). The nucleotides of the genes were also compared with *katG* nucleotides in Genbank (accession number X68081).

Subcloning of the *katG* gene. The *katG* gene fragment in pGEM-T-*katG* was taken by digestion of plasmid recombinant with NdeII and XbaI. The digestion product was purified and inserted into plasmid pCold II DNA, which previously had been digested with the same restriction enzymes. The ligation product was transformed to *E. coli* BL21 (*DE3*) and the transformed bacteria were then grown on a selective LB agar plate.

KatG gene expression. A single colony of *E. coli* BL21 (DE3) containing recombinant plasmid (pCold II-*katG*) was cultured in LB liquid medium containing ampicillin 100 µg/ml, then followed by shaking at 37 °C to obtain an optical density (OD) at λ 600 nm approximately of 0.4-0.5. The culture was then immediately cooled at 15 °C for 30 minutes without shaking. To induce the expression of recombinant protein, the culture was added by 0.1 mM IPTG, and followed by shaking at 15 °C for 24 h. The culture was then centrifugatedat 5,000 g for 10 min to harvest the cells. The resulted cells were washed and re-suspended in 50 mM potassium phosphate buffer (pH 7.0), and then disrupted by sonication. The cellular debris was removed by centrifugation at 12,000 g for 15 min. The KatG protein that remained in the supernatant was then purified.

Protein purification. The recombinant protein of KatG was purified by affinity chromatography using HisTrapTM-HP column (GE Healthcare, Freiburg, Germany) containing Ni-sepharose matrix. The purification steps were run according to the manufacturer's protocol. The recombinant protein was eluted gradually by 50 mM K-phosphate buffer, pH 7.0, containing imidazole of 50-200 mM. The purified protein was analyzed by a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Catalase-peroxidase activity and INH oxidation assays. The recombinant KatG was assayed regarding its activity on catalase-peroxidase and INH oxidation. Catalase activity was determined by the Patti and Bonet-Maury method, based on the formed color from the reaction of titanium with H_2O_2 [17]. The reaction was carried out in 10 mM K-phosphate buffer pH 7.0 with a total volume of 1 ml containing 12.5 mM H_2O_2 substrate and KatG protein. The enzymatic reaction was stopped by the addition of 2.5 ml of titanium reagent, in turn the formed yellow color was observed at λ 410 nm [17]. One unit of catalase activity was defined as the amount of enzyme decomposing 1 mmol of H_2O_2 per min.

Peroxidase activity was determined by reacting of 100 µM o-dianisidine in 100 ml of 50 mM potassium buffer (pH 4.5) containing 25 mM *tert*-butyl hydroperoxide (t-BHP) with 12.5 mM H_2O_2. The absorbance of the reaction product, i.e. o-dianisidin quinone diimine was observed at λ 460 nm ($\varepsilon_{460} = 11.86$ mM⁻¹·cm⁻¹) [13]. One unit of peroxidase activity was defined as the amount of enzyme that catalyzed the formation of one µmol product per min at 30 °C.

The activity of *katG* on INH oxidation was carried out by the Shoeb method [18]. A mixture

of 0.65 g KatG protein in 10 mM phosphate buffer pH 7.0 was reacted with 13.1 mM phenol and 6.25 mM H_2O_2 in a total volume of 3 ml at 37 °C for 7 min, then followed by addition of 2.4 mM INH. The reaction was continued at 37 °C for 1 h [18]. The absorbance of the reaction product, i.e. benzo-quinone was monitored at λ 444 nm (ε_{444} = 0.393 $M^{-1}\cdot cm^{-1}$). One unit of the activity was defined as the amount of enzyme that catalyzes the formation of benzo-quinone product per min at 37 °C.

Structure alignment. Three-dimensional structure of the mutant KatG protein was generated by SWISS-MODEL, an automated protein homology modeling server, using the known crystal structure of a wild type KatG protein structure (PDB code 1SJ2) as a template. The minimization of the structural model was conducted with Amber 10 [19]. The modeled structure was visualized using PyMOL 1.3 (www.pymol.org). Root mean square deviation of the model was compared to the 1SJ2 structure by Super Pose version 10.

Results and Discussion

Cloning of *katG* gene from INH-resistant *M. tuberculosis* strain. The open reading frame (ORF) of *katG* gene from L8 Isolate of INH-resistant *M. tuberculosis* strain was amplified by PCR using FG and RG primers. The 2.2 kb fragment of PCR-product then was inserted in pGEM-T vector (3.0 kb) to construct pGEMT-*katG* recombinant. The recombinant plasmid exhibited two fragments (3.0 and 2.2 kb) then it was digested by both *Eco*RI and *Not*I restrction enzymes (Fig. 1). The 3.0 kb fragment corresponded to pGemT vector, and the 2.2 kb fragment had the size of the *katG* gene. The 2.2 kb fragment of L8 isolate carried a mutation, the guanine instead of adenine at position 713 (Fig. 3).

The *katG* gene was then subcloned to pColdII-DNA as expression vector. Insertion of *katG* fragment (2.2 kb) in vector pCold II-DNA (4.3 kb) yielded a pCold II-*katG* recombinant (6.5 kb). Single digestion of the recombinant with *Nde*I and *Xba*I restriction enzymes yielded a DNA fragment (6.5 kb), respectively, while the digestion with both the enzymes yielded two fragments consisting of 2.2 and 4.3 kb (Fig. 2). The nucleotides sequences of the *katG* gene from the L8 *M. tuberculosis* isolate showed a substitution of adenine to guanine at position 713 compared to the *katG* from INH susceptible *M. tuberculosis*, the H37Rv strain (Fig. 3). The mutation altered the amino acid of KatG protein, the serine instead of asparagine at posistion 238 through *in silico* translation (Fig. 3).

The KatG expression. To express the *katG*, the *E. coli* BL21 (*DE3*) that bring pCold-*katG* recombinant was grown in LB medium containing amphicilin, then followed the induction of the culture with IPTG. After the cell of *E. coli* was collected by centrifugation, the cell pellets were lysed to release its extract protein. Analysis of the extract protein in SDS PAGE showed a high intensity of protein band with a molecular mass of 80 kDa that belonged to KatG protein (Fig. 4).

Catalase-peroxidase activities of mutant KatG$_{Asn238Ser}$. The kinetic properties of catalase-peroxidase of mutant KatG$_{Asn238Ser}$ and wild type had been determined, i.e. K_M, K_{cat} and K_{cat}/K_M. The mutant KatG$_{Asn238Ser}$ exhibited the K_{cat}/K_M value for catalase and peroxidase lower than KatGwt. The K_{cat}/K_M value mean as the catalytic efficiency of enzyme. The high K_{cat}/K_M suggested that an enzyme could bind its substrate effectively and decompose its substrate into a product well, and vice versa. KatGwt has K_{cat}/K_M value as 8.62×10^4 $M^{-1}\cdot S^{-1}$ for catalase and 1.99×10^5 $M^{-1}\cdot S^{-1}$ for peroxidase. Meanwhile KatG$_{Asn238Ser}$ mutants have a low K_{cat}/K_M value, i.e. 5.12×10^4 $M^{-1}\cdot S^{-1}$ for catalase and 0.96×10^5 $M^{-1}\cdot S^{-1}$ for peroxidase (Table 2). In addition to the kinetic properties, the mutant KatG$_{Asn238Ser}$ took also a declining activity to oxidize INH. The mutant KatG$_{Asn238Ser}$ activity in oxidation of isoniazid remained 42.5% from that of KatGwt (Fig. 5).

Structural Model of mutant KatG$_{Asn238Ser}$. Structure modeling of KatG$_{Asn238Ser}$ was done to know the effect of amino acid substitution in the mutant protein. Superposition of Cα framework of the mutant model with the KatGwt structure exhibited root mean square deviations (RMSD) of 0.073. This result showed that The mutant KatG$_{Asn238Ser}$ shared similiar structure with KatGwt of *M. tuberculosis* H37Rv. Moreover, the amino acid substitution of Asn238Ser found in the mutant KatG L8 eliminate the salt bridge interaction around the substrate binding site, which connects between the amine group in side chain of Asn238 residue with the carboxyl group of Glu233 in KatGwt (Fig. 6).

KatG is the only enzyme in *M. tuberculosis* that could generate isoniazid susceptibility. Therefore it plays a central role in the development of at least one type of isoniazid resistance [20]. Mutations in *katG* are often associated with INH resistance. A substitution of adenine to guanine at position 713 in
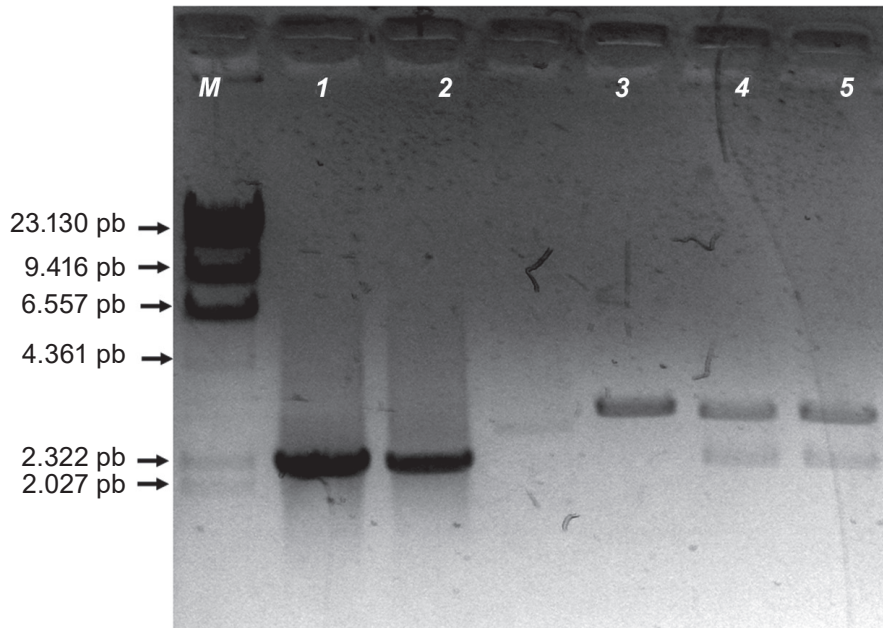
Fig. 1. The restriction map of pGemT-katG recombinat. Marker DNA λ/HindIII (M); the 2.2 kb fragment of PCRproduct from L8 and H37Rv isolate (1 and 2); The purifykatG (3); the pGemT-katG pGemT-katG (L8 and H37Rv) which digested by EcoRI and NotI (4 and 5). Each digestion resulted two fragmens at 3.0 kb and 2.2 kb. The 3.0 fragment correspond to pGemT vector, then the 2.2 kb fragment correspond to the katG gene

katG gene that correspond to Asn238Ser replacement was found in the L8 clinical isolate which had a resistance level to INH at 1 μg/ml. Minimal inhibitor concentration (MIC) of INH for growing *M. tuberculosis* has been reported as 0.02 to 0.06 μg/ml [3]. The INH resistance is classified as a low level for MIC value < 1 μg/ml, and a high level for MIC value ≥ 1 μg/ml [21]. Many papers showed that the level of INH resistance of clinical isolates of *M. tuberculosis* are not directly correlated with the number of mutations in *katG*. There are many clinical isolates resistant to INH at low level, having multiple mutations in *katG*, and vice versa, single mutation is connected with high resistance to INH [5, 21, 22].

Direct relationship between mutation in *katG* with INH resistance phenotype is still insufficient to understand the emergence of INH resistance in clinical isolates, because the mutation events might connect with other biological traits. As confirmation that the substitution Asn238Ser connected with INH resistance in clinical isolate of L8, it was determined the activities of catalase-peroxidase and INH oxidation by the mutant.

The mutant KatG$_{Asn238Ser}$ of the L8 isolate exhibited lower catalase and peroxidase activity than that of KatGwt. The mutant has a binding affinity
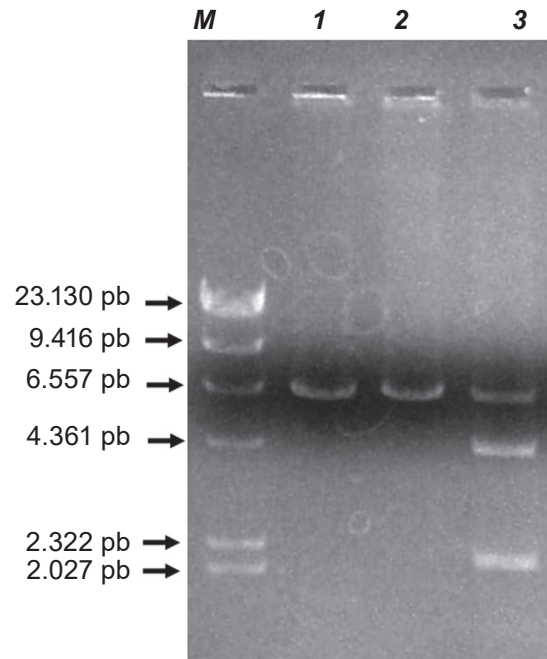

Fig. 2. The restriction map of pCold II-katG recombinant. Marker DNA λ/HindIII (M), pCold II-DNA/XbaI (1), pCold II-katG/NdeI (2), pCold II-katG/NdeI + XbaI (3). Dygestion of the recombinant with both the enzymes yielded two fragments consisting of 2.2 and 4.3 kb
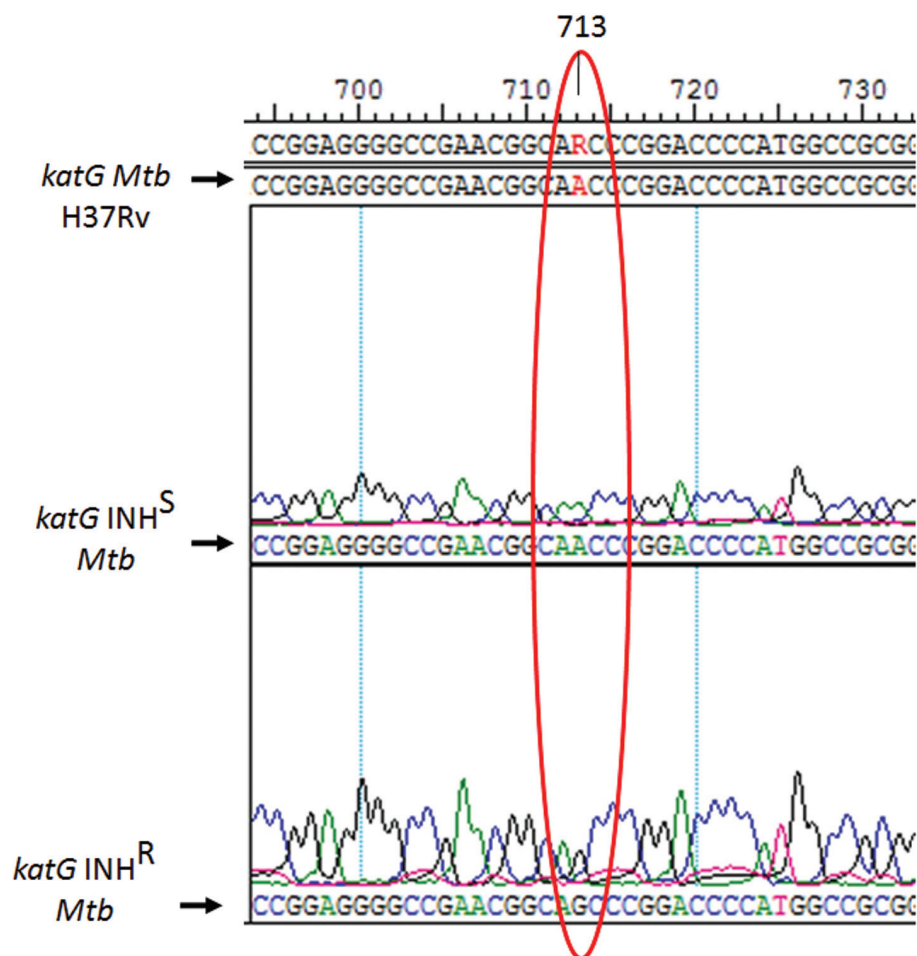
Fig. 3. Nucleotides alignment of the katG of INH resistant M. tuberculosis strain (L8) againts the katG from INH sensitive strain (H37Rv) and GenBank. Comparing with H37Rv and genbank katG, The L8 katG exhibited a varian nucleotide at position 713, guanine instead of adenine, then substituted Asn with Ser at position 238. Mutation is marked with a red oval

to the substrate is lower than the KatGwt, because it has a value of K_M for each catalase and peroxidase, 1.4 times higher than KatGwt (Table 2). Substitution Asn238Ser reduced 40% of substrate binding affinity for catalase-peroxidase of the mutant. Comparing with KatGwt, the mutant KatG$_{Asn238Ser}$ also displayed a decrease of K_{cat} value by 17% for catalase and 32% for peroxidase. This means that the mutant lost 17 and 32% ability of catalase and peroxidase respectitively in the converting of substrate into product. The catalytic efficiency that symbolized by K_{cat}/K_M for the mutant KatG$_{Asn238Ser}$ decreased by 41% for catalase and 52% for peroxidase activity (Table 2). The mutant KatG$_{Asn238Ser}$ decreased in both the binding affinity and the converting activity of substrate into product. Several papers reported that the catalase-peroxidase activity among variants of katG were not correlated with the resistance level of INH [11, 12]. This can be triggered due to the measurement of enzyme activity *in vitro,* but it is connected directly with INH resistance phenotype which is actually derived from the *in vivo* process.

The INH oxidation test of KatG$_{Asn238Ser}$ showed that the mutant lost 62% of the activity compared with that of KatGwt (Table 2). The Asn238Ser modification in KatG of INH resistant isolate (L8) is connected with the decline in the activity of catalase-peroxidase and INH oxidation of the protein. Decreasing of catalytic efficiency (K_{cat}/K_M) of mutan KatG$_{Asn238Ser}$ as 41% for catalase and 52% for peroxidase accompanied the reduction of 62% in INH oxidation activity of the mutant. The decline of INH activation in many variants of KatG, i.e. Ala110Val, Asp735Ala, Ala139Pro, Ser315Asn, Leu619Pro, and
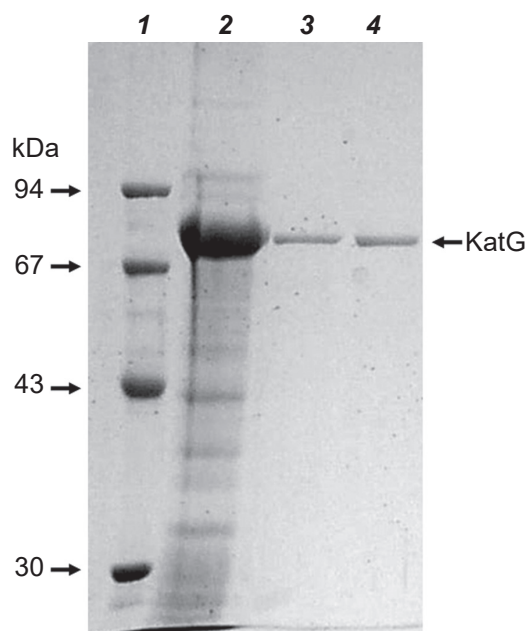
Fig. 4. Electrophoregram of KatG protein in SDS PAGE. Protein marker (1), crude extract of KatG (2), purify product of L8 and H37Rv KatG (3 and 4). The KatG protein was eluted by 100 mM imidazole buffer

Fig. 5. The INH oxidation activity of wild type and mutant KatG

Leu634Phe have exhibited a highly correlated with the change level of catalase-peroxidase activity for the mutants [23].

The catalytic function of KatG is carried out effectively by amino acid residues in the active site, i.e. Arg-104, Trp-107 and His-108 residues in the distal pocket, and His270, Trp321 and Asp381 residues in the proximal pocket. INH binds to KatG via interacting with amino acids in the distal pocket. Other residues such as Val230, Asn231, Pro232 and Ser315 have been reported to be involved in interacting with INH [24-26]. The stability in the active site environ-
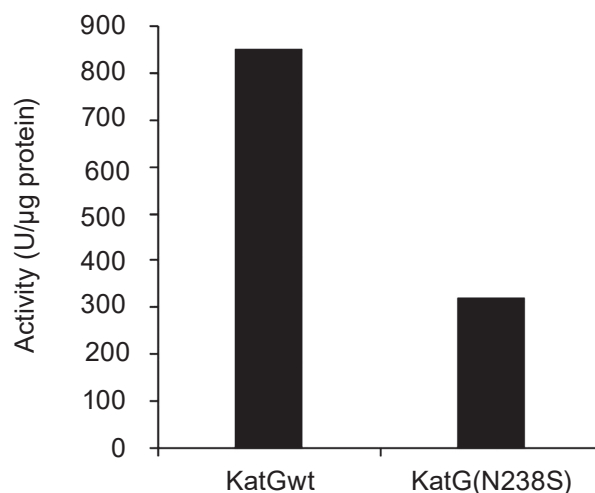
ment of KatG is required to support the INH activation by the protein.

The failure role of KatG in the INH activating has been shown in detail by the mutant KatG$_{Ser315Thr}$ [12, 13, 27]. The Ser315Thr substitution in KatG impacts on the shifting of substrate binding channel from 6.0 to 4.7 Å [27]. Consequently the mutant failed to bind the INH, and subsequently decreased 160 times in the INH activation compared with KatGwt [14].

By using a structure model, the basis of a decrease in the catalytic efficiency for catalase-peroxidase and INH oxidation activity in the mutant KatG$_{Asn238Ser}$ was described. In the KatGwt structure, the amino acid 238 is put closely to the substrate binding site, i.e. Asn137, Val230, Asn231, Pro232 and Ser315 residues. It is found that Asn231 residue makes hydrogen bond with Glu233 and Van Der Walls interaction with Asn236. These interactions

T a b l e   2. Kinetic parameters of catalase-peroxidase of KatGwt and KatG$_{Asn238Ser}$

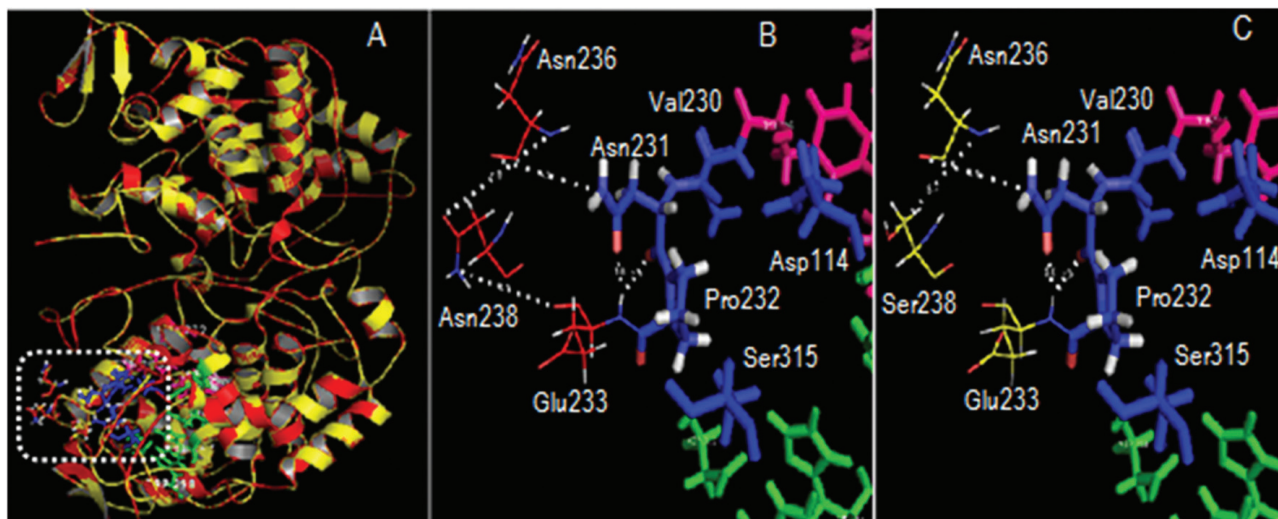| Activity	Isolate	Kinetic parameters		
		K_{cat} (S^{-1})	K_M (mM)	K_{cat}/K_M (M^{-1}·S^{-1})
Catalase				
Wild type KatG	H37Rv	272.3	3.16	8.62×10^4
KatG (Asn238Ser)	L8	227.5	4.44	5.12×10^4
Peroxidase				
Wild type KatG	H37Rv	49.0	0.25	1.99×10^5
KatG (Asn238Ser)	L8	33.3	0.35	0.96×10^5

Fig. 6. Illustration of Asn238Ser substitution effect in KatG L8. Superposition of mutant KatG structure (L8) (yellow) to the KatGwt (red) (A). The blue rod-shaped amino acids represented the residues for subtrate binding; green rod-shaped amino acids represented catalytic residues, and magenta are the residue for cross links. In the KatGwt, the Asn238 stabilized the active site environment through linkage to the Glu233 and Asn236 (B). In the mutant KatG L8, the Ser238 residue could not make interaction with Glu233

are stabilized by the presence of salt bridge which connects between the amine group in side chain of Asn238 residue with the carboxyl group of Glu233 in KatGwt (Fig. 6). This salt bridge was lost due to modification of Asn238Ser in the mutant KatG L8. The lost of the salt bridge created unfavorable for enzyme activities, then in turn it emerged the INH resistance in the L8 isolate of *M. tuberculosis*. The model analysis of KatG$_{Asn238Ser}$ should be further confirmed by a real crystal structure of the mutant.

Conflicts of interest. All authors declare that there is no conflict of interest.

МУТАЦІЯ *katG* У КЛІНІЧНОМУ ІЗОЛЯТІ *Mycobacterium tuberculosis*: ВПЛИВ НА КАТАЛАЗУ-ПЕРОКСИДАЗУ, ЩО АКТИВУЄ ІЗОНІАЗИД

Purkan[1], Ihsanawati[2], D. Natalia[2], Y. M. Syah[2], D. S. Retnoningrum[3], H. S. Kusuma[4]

[1]Biochemistry Research Division, Department of Chemistry, Faculty of Sciences and Technology, Airlangga University; Surabaya, Indonesia; e-mail: purkan@fst.unair.ac.id;
[2]Biochemistry Research Division, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Bandung, Indonesia;
[3]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia;
[4]Department of Chemical Engineering, Institute Teknologi Sepuluh Nopember, Surabaya, Indonesia; e-mail: heriseptyakusuma@gmail.com

Мутації в гені *katG* часто пов'язані із резистентністю штаму *Mycobacterium tuberculosis* до ізоніазиду. Дослідження проведено з метою виявлення мутації *katG* в клінічному ізоляті (L8) з *M. tuberculosis*, стійкими до ізоніазиду за концентрації 1 мкг/мл. У роботі охарактеризовано каталазу-пероксидазу KatG L8 і проведено вивчення структури протеїну з метою глибше зрозуміти процес активації препарату і механізм резистентності до нього *M. tuberculosis*. Ген *katG* був клонований і експресований в *Escherichia coli*, вивчено властивості каталази-пероксидази KatG. Проведено моделювання структури протеїну для з'ясування причин зміни ензиматичної активності. Встановлено заміщення каталази-пероксидази A713G, що відповідає заміні Asn238Ser в L8 katG. Модифікація Asn238Ser у протеїні L8 KatG призводила до зниження активності каталази-пероксидази і окислення ізоніазиду. Каталітична ефективність (K_{cat}/K_M) мутантного KatG$_{Asn238Ser}$ зменшувалась для каталази і пероксидази до 41 і 52% відповідно. Мутант KatG$_{Asn238Ser}$ також знижував окислення ізоніазиду на 62% порівняно з диким типом KatG (KatGwt). Показано, що мутація Asn238Ser може призвести до нестабільності в сполучній ділянці KatG через вилучення електростатичного зв'язку, що з'єднує аміногрупу Asn238 із карбоксильною групою Glu233, яка представлена в KatGwt. Втрата електростатичного зв'язку в місці зв'язування субстрату в мутанта KatG$_{Asn238Ser}$ знижує активність ензимів, що, в свою чергу, зумовлює резистентність *M. tuberculosis* до ізоніазиду в ізоляті L8.

К л ю ч о в і   с л о в а: *Mycobacterium tuberculosis*, резистентність до ізоніазиду, *katG*, каталаза-пероксидаза.

МУТАЦИЯ *katG* В КЛИНИЧЕСКОМ ИЗОЛЯТЕ *Mycobacterium tuberculosis*: ВЛИЯНИЕ НА КАТАЛАЗУ-ПЕРОКСИДАЗУ, АКТИВИРУЮЩУЮ ИЗОНИАЗИД

Purkan[1], Ihsanawati[2], D. Natalia[2], Y. M. Syah[2], D. S. Retnoningrum[3], H. S. Kusuma[4]

[1]Biochemistry Research Division, Department of Chemistry, Faculty of Sciences and Technology, Airlangga University; Surabaya, Indonesia; e-mail: purkan@fst.unair.ac.id;
[2]Biochemistry Research Division, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Bandung, Indonesia;
[3]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia;
[4]Department of Chemical Engineering, Institute Teknologi Sepuluh Nopember, Surabaya, Indonesia; e-mail: heriseptyakusuma@gmail.com

Мутации в гене *katG* часто связаны с резистентностью штамма *Mycobacterium tuberculosis* к изониазиду. Это исследование проведено для определения мутации *katG* в клиническом изоляте (L8) с *M. tuberculosis*, устойчивыми к изониазиду при концентрации 1 мкг/мл. В работе охарактеризована каталаза-пероксидаза KatG L8 и выполнены первоначальные структурные исследования протеина с целью более полного понимания процесса активации препарата и механизма резистентности к нему *M. tuberculosis*. Ген *katG* был клонирован и экспрессирован в *Escherichia coli*, затем изучены свойства каталазы-пероксидазы KatG. Проведено моделирование структуры протеина для выяснения причин изменения энзиматической активности. Установлено замещение каталазы-пероксидазы A713G, что соответствует замене Asn238Ser в L8 katG. Модификация Asn238Ser в протеине L8 KatG приводила к снижению активности каталазы-пероксидазы и окисления изониазида. Каталитическая эффективность (K_{cat}/K_M) мутантного KatG$_{Asn238Ser}$ уменьшалась для каталазы

и пероксидазы до 41 и 52% соответственно. Мутант KatG$_{Asn238Ser}$ также снижал окисление изониазида на 62%, по сравнению с диким типом KatG (KatGwt). Показано, что мутация Asn238Ser может привести к нестабильности в связывающем участке KatG из-за удаления электростатической связи, соединяющей аминогруппу Asn238 с карбоксильной группой Glu233, которая представлена в KatGwt. Потеря электростатического взаимодействия в месте связывания субстрата в мутанте KatG$_{Asn238Ser}$ снижает активность энзимов, что, в свою очередь, обусловливает резистентность к изониазиду *M. tuberculosis* в изоляте L8.

К л ю ч е в ы е   с л о в а: *Mycobacterium tuberculosis*, резистентность к изониазиду, *katG*, каталаза-пероксидаза.

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