

J Pure Appl Microbiol

Volume 13, Issue 3, September 2019

ISSN: 0973-7510

E-ISSN: 2581-690X

Journal of Pure and Applied Microbiology

An International Peer Reviewed Open Access Research Journal



www.microbiologyjournal.org

Journal of Pure and Applied Microbiology

Country India -  SIR Ranking of India

Subject Area and Category Biochemistry, Genetics and Molecular Biology
Biotechnology

Immunology and Microbiology
Applied Microbiology and Biotechnology
Microbiology

Publisher Oriental Scientific Pub. Co.

Publication type Journals

ISSN 09737510

Coverage 2007-ongoing

Scope Journal of Pure and Applied Microbiology (JPAM) is a peer-reviewed, open access, quarterly published international journal, dedicated to serve the scientific community and the wider scientifically interested general public by providing a platform for worldwide researchers, intellectuals and microbiologists for publication of high quality reviews, research articles and clinical studies pertaining to all aspects of microbiology and its allied disciplines.

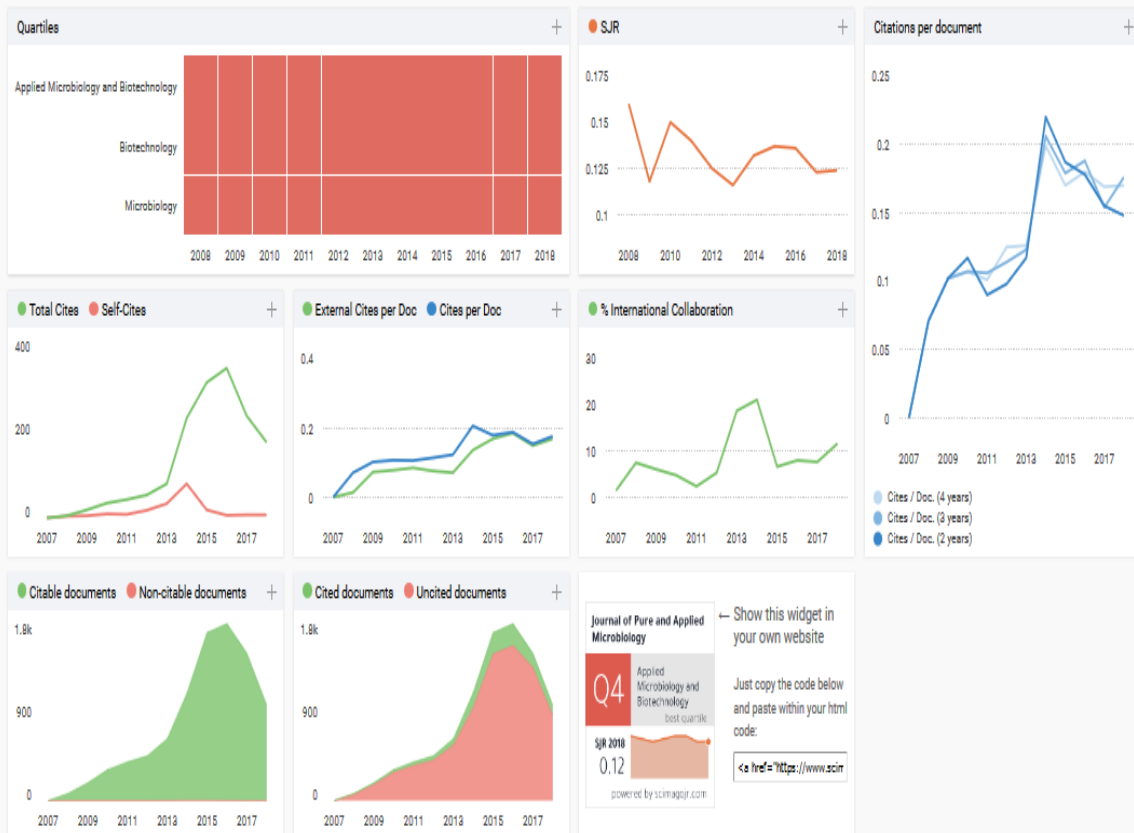
12

H Index

This website uses cookies to ensure you get the best experience on our website

Got it!

 Join the conversation about this journal



This website uses cookies to ensure you get the best experience on our website

Got it!

Editorial Board

Editor-in-Chief

Dr. Iqbal Ahmad

Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India

Email: iahmad.am@amu.ac.in

<https://orcid.org/0000-0001-8447-4497>



Iqbal Ahmad is currently Professor of the Department of Agricultural Microbiology at Aligarh Muslim University in Aligarh, India. He received his Ph.D. degree from Aligarh Muslim University in Agriculture Microbiology, during his Ph.D. research work he worked at the Division of Microbiology, CSIR-Central Drug Research Institute, Lucknow, India with pioneer veterinary bacteriologist, Late Dr. JNS Yadava, FNA, FNASC. He first worked as a Research Scientist at The Himalya Drug Co., New Delhi in 1994 and then joined as a Lecturer at the Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh in 1995 and then promoted to Professor in 2010. He was the Section Incharge of Microbiology during 1999-2000 and has also chaired the Department during 2011-2013 and in 2017. He has done pioneering research on plasmid mediated drug resistance and virulence factors in enteric bacteria and strategies to combat drug resistance and virulence of pathogenic microbes through screening and

evaluation of Indian medicinal plant derived herbal products/extracts and phytochemicals especially targeting Quorum sensing, biofilm and virulence of pathogenic microorganisms. His research work on agriculturally important microorganisms on diazotrophs, biofilm forming PGPR and Impact of wastewater on soil health has been well documented. His recent interest on Interdisciplinary microbiological works and drug-macromolecule interactions are gaining importance in academic world.

Deputy Editor-in-Chief

Dr. Kuldeep Dhama

Division of Pathology, ICAR – Indian Veterinary Research Institute, Bareilly, India

Email: kdhama@rediffmail.com

<https://orcid.org/0000-0001-7469-4752>



Dr. Faisal Masood Khan

Associate Professor

Department of Pathology and Laboratory Medicine, Faculty of Cumming School of Medicine, Foothills Campus, Alberta Canada

fkhan@ucalgary.ca

Kuldeep Dhama is currently working as Principal Scientist in ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, India. With 22 years of research and teaching experience in the areas of microbiology, immunology and virology, he has developed several diagnostics, vaccines, immunomodulatory modules and hypothesis to counter infectious diseases of animals, poultry and public health concerns. He also has been awarded NAAS Associateship (National Academy of Agricultural Science, India). To his credit he has handled 20 research projects and guided 17 M.V.Sc. and P.D. scholars.



Dr. Ruchi Tiwari

Assistant Professor

Department of Veterinary Microbiology, College of Veterinary Sc. & A.H. DUVASU, Mathura, U.P.

India

ruchi.vet@gmail.com

Associate Editors



Dr. Hafiz M. N. Iqbal

Professor

Tecnologico de Monterrey
School of Engineering and Sciences
Campus Monterrey.C.P. 64849
Monterrey, Nuevo León
Mexico
hafiz.iqbal@tec.mx



Dr. Munir Aktas

Professor

Faculty of Veterinary Medicine,
Department of Parasitology,
Firat University, Elazig
Turkey
maktas@firat.edu.tr



Dr. Yashpal Singh Malik
Principal Scientist
ICAR-Indian Veterinary Research Institute,
Izatnagar – 243 122
Bareilly, Uttar Pradesh
India
malikyps@ivri.res.in



Dr. T. Muthukumar
Assistant Professor
Root and Soil Biology Laboratory
Department of Botany
Bharathiar University, Coimbatore
India
tmkum@yahoo.com



Dr. Vodnar Dan-Cristian
Associate Professor
Department of Food Science
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca
Romania
dan.vodnar@usamvcluj.ro



Dr. Ahmet Adiguzel
Professor
Faculty of Science
Department of Molecular Biology
and Genetics, Ataturk University
25240-Erzurum
Turkey
adiguzel@atauni.edu.tr



Dr. Maryam Dadar
Assistant Professor
Agricultural Research, Razi Vaccine and Serum Research Institute
(RVSRI), Karaj
Iran
dadar.m77@gmail.com

Editorial Board Members



Dr. Nikhat Manzoor
Associate Professor
Department of Biosciences
Jamia Millia Islamia, New Delhi
India
nmanzoor@jmi.ac.in



Dr. Abd El-Latif Hesham
Professor
Faculty of Agriculture, Department of Genetics, Microbial Genetics &
Environmental Meta-Genome Biotechnology, Assiut University, Assiut
Egypt
hesham_egypt5@aun.edu.eg



Dr. Pramod W. Ramteke
Professor and Head
Department of Biological Sciences
Sam Higginbottom University of Agriculture
Technology & Sciences (SHUATS), Allahabad
India
pramod.ramteke@shiats.edu.in



Dr. Pongsak Rattanachaikunsopon

Professor
Department of Biological Science
Faculty of Science
Ubon Ratchathani University, Ubon Ratchathani
Thailand
rattanachaikunsopon@yahoo.com



Dr. Mona Ibrahim Shaaban

Associate Professor
Department of Microbiology and Immunology
Faculty of Pharmacy, Mansoura University
Egypt
mona_ibrahem@mans.edu.eg



Dr. Vasudeo Zambare

Scientist
Biorefining Research Institute
Lakehead University, Thunder Bay, Ontario
Canada
vpzambar@lakeheadu.ca



Dr. Amir Sasan Mozaffari Nejad

Research Scholar
Department of Virology
Hamadan University of Medical Sciences, Hamadan
Iran
asmozafarnejad@yahoo.in



Dr. Debdulal Banerjee

Associate Professor
Department of Botany and Forestry
Vidyasagar University, Midnapore, West Bengal
India
db@mail.vidyasagar.ac.in



Dr. Pawan K Dadheech

Professor
Department of Microbiology
School of Life Sciences
Central University of Rajasthan, Rajasthan
India
pdadheech@curaj.ac.in



Dr. Nour Shafik Emam El-Gendy

Professor
Department of Petroleum and
Environmental Biotechnology
Egyptian Petroleum Research Institute, Cairo
Egypt
nourepri@yahoo.com



Dr. Hussein Hasan Abulreesh

Associate Professor
Environmental and Public Health Microbiology, Department of Biology
Umm Al-Qura University, Makkah
Saudi Arabia
hhabulreesh@uqu.edu.sa



Dr. Pranav Kumar Prabhakar
Assistant Professor
Faculty of Applied Medical Sciences
Lovely Professional University
Phagwara, Punjab
India
pranav.16113@lpu.co.in



Dr. Pushpanathan Muthuirulan
Research Associate
Department of Human Evolutionary Biology
Harvard University Cambridge, Massachusetts
USA
muthuirulanp@fas.harvard.edu



Dr. Koshy Philip
Associate Professor
Faculty of Science, Institute of Biological Sciences, University of
Malaya, Kuala Lumpur
Malaysia
kphil@um.edu.my



Dr. Prashant Khare
Scientist D & Ramalingaswami Fellow
Department of Microbiology
All India Institute of Medical Sciences, Bhopal
India
prashantkhare.microbiology@aiimsbhopal.edu.in



Dr. A.K. Srivastava
Principal Scientist
Soil Science
ICAR- Central Citrus Research Institute, Nagpur
India
aksrivastava2007@gmail.com



Dr. Guohua (Karen) Yin
Research Associate
Department of Plant Biology and Pathology
Rutgers University, New Jersey
USA
gy78@scarletmail.rutgers.edu



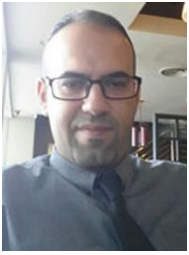
Dr. Kunal
Post Doctorate Research Associate
Department of Soil Science
Punjab Agricultural university, Ludhiana
India
kunal_pau@yahoo.co.in



Dr. Hesam Kamyab
Post Doctoral Fellow
UTM RAZAK School of Engineering and Advanced Technology
University Teknologi Malaysia (UTM)
Malaysia
khesam2@live.utm.my



Dr. Parvez Akhtar
Research Scientist
Aurora Research Institute
Milwaukee, WI 53233
USA
Parvez.Akhtar@aurora.org



Dr. Belal J. Muhiuddin
Post Doctoral Fellow
Faculty of Food Science and Technology
Universiti Putra Malaysia, UPM
Malaysia
belal@upm.edu.my



Dr. Mohd M. Khan
Associate Faculty
School of Medicine
University of Maryland, Baltimore
USA
mohsin.khan@umaryland.edu



Dr. Godfred A. Menezes
Associate Professor and Clinical Microbiologist
Department of Medical Microbiology and Immunology
RAK College of Medical Sciences
RAKMHSU, Ras Al Khaimah
UAE
godfred@rakmhsu.ac.ae

Editor
Dr. M.N. Khan
editor@microbiologyjournal.org

CONTENTS

- 637-648** **Barbara Blyskal, Anna Lenart-Borod and Piotr Borod**
Approaches to Taxonomic Studies of Actinomycetes Isolated from Historic and Contemporary Materials
- 649-660** **Hai-Ming Tang, Xiao-Ping Xiao, Wen-Guang Tang, Chao Li, Ke Wang, Kai-Kai Cheng and Geng Sun**
Dynamic Change of Soil Enzyme Activities and Soil Microbe During Rice Main Growth Stages in Different Long-term Fertilizer Regimes
- 661-668** **Daniel Parrott, Kevin M. Ringelman and Michael S. Chaussee**
Anti-Microbial Effects of Conductive Copper Nanoparticle Film
- 669-675** **Erman Munir, Nunuk Priyani, Dwi Suryanto and Zulfatun Naimah**
Using Biomass of Basidiomyceteous Fungi in Decolorization of Wastewater of Textile Industry
- 677-683** **Sara El-Hossain Aly Reda, Mona A. Abd El-Messiah, Lamiaa A. Madkour and Abd El- Meguid Kassem**
Serology versus Real Time PCR in the Diagnosis of Human Brucellosis
- 685-694** **Moustafa Y. El-Naggar, Wegdan Ramadan and Ramy A. El-Hamamsy**
The Application of Mediated Biosynthesized Green Silver Nanoparticles by *Streptomyces griseorubens* in Water Treatment
- 695-701** **Purkan Purkan, Sri Puji Astuti Wahyuningsih, Wiwin Retnowati, Diah Amelia and Alfain Noerdin Alimny**
Structure - Activity Relationship of Mutant KatG from INH resistant *Mycobacterium tuberculosis*
- 703-709** **Il-Doo Kim, Sanjeev Kumar Dhungana, Jeong-Ho Kim and Dong-Hyun Shin**
Persimmon Fruit Affects Bacterial Growth, Hardness, Vitamin C and Chlorophyll Content of Soybean Sprouts during Storage
- 711-716** **Maryam Hosseini, Fateme Babaha, Mushtaq Talib Shawi Al-Rubaye, Javad Fakhari and Mastafa Heilo Jabber Al-Musawi**
Urease-Producing Halophilic Bacteria Isolated from Bahr Al-Milh Salt Lake, Karbala, Iraq
- 717-723** **Dwi Suryanto, Rani Artha Munthe, Isnaini Nurwahyuni and Erman Munir**
An Assay On Potential of Local *Trichoderma* Spp. to Control White Root Rot Disease Caused by *Rigidoporus microporus* in Rubber Plant Stump
- 725-732** **A.M. Zaki, A.H. Zaki, A.A. Farghali and Elham F. Abdel-Rahim**
Sodium titanate - Bacillus as a new Nanopesticides for Cotton Leaf-Worm
- 733-741** **M. E. Uddin, T Ahmad, MM Ajam, M Moniruzzaman, D Mandol, SK Ray, A Sufian, MA Rahman, E Hossain and R Ahammed**
Thermotolerant Extracellular Proteases Produced by *Bacillus subtilis* Isolated from Local Soil that Representing Industrial Applications

CONTENTS

- 743-752** **Khaled E. El-Gayar, Mohamed A. AlAbboud and Ashraf M. M. Essa**
Characterization of Thermophilic Bacteria Isolated from two Hot Springs
in Jazan, Saudi Arabia
- 753-758** **Babak Asghari, Hamid Reza Sadeghi and Mohammad Jalilian**
Intestinal Colonization of Vancomycin-Resistant *Enterococci* Isolates
among Patients in an Iranian Hospital
- 759-772** **Sahar AbdEl-Mogheith, Ahmed Osama El-Gendy, Serageldeen Sultan
and Khalid A. El-Nesr**
Exploring the Antimicrobial and Hepatoprotective Effects of Kefir;
A Probiotic Fermented Milk
- 773-778** **Maryam Hosseini, Javad Fakhari, Mushtaq T. Sh. Al-Rubaye
and Ehsan Ansari Dezfouli**
Screening of Halophilic Bacteria Able to Degrade Crude Oil Contamination
from Alborz Oil Field, Qom, Iran
- 779-792** **Masoumeh Navidinia, Mehdi Goudarzi, Samira Molaei Rameshe, Zahra Farajollahi,
Pedram Ebadi Asl, Saeed Zaka khosravi and Mohammad Reza Mounesi**
Molecular Characterization of Resistance Genes in MDR-ESKAPE Pathogens
- 793-801** **Sandra L. Villarreal Morales, Nagamani Balagurusamy,
Raúl Rodríguez Herrera, Alejandro Zugasti Cruz, Mayela Govea Salas
and Jesús Morlett Chávez**
Anaerobic Biodegradation of Polyaromatic Hydrocarbons by a Sulfate
Reducing Bacteria C1Fd Strain
- 803-809** **Pornthep Niamphithak, Siripavee Chareonwattanasak and
Sompong Doolgindachbaporn**
Effect of Dietary Supplement of Probiotic (*Lactobacillus plantarum*) on
Growth Performance, Feed Utilization and Survival Rate in Bocourti Catfish
(*Pangasius bocourti* Sauvage, 1880)
- 811-819** **Elham Zarifi, Gilda Eslami, Azad Khaledi, Mahmood Vakili,
Hossein Vazini and Hengameh Zandi**
Prevalence of ESBLs in *Acinetobacter baumannii* Isolated from
Intensive Care Unit (ICU) of Ghaem Hospital, Mashhad, Iran
- 821-827** **Magdah Ganash**
Genotoxicity and Molecular Response of Biotechnological agent
Trichoderma harzianum as a Result of Silver Nanoparticles Application
- 829-836** **Gaganpreet Kaur, Sanjeev Kumar Soni and Rupinder Tewari**
Optimization of Phospholipase A1 (PLA1) Production from a Soil Isolate
Bacillus subtilis sub sp. in *aquosorum* RG1 via Solid State Fermentation
- 837-845** **Rizki A. Nasution, Agustina M. Tangapo, Intan Taufik and Pingkan Aditiawati**
Comparison of Plant Growth Promoting Rhizobacteria (PGPR) Diversity and Dynamics
During Growth of Cilembu Sweet Potato (*Ipomoea batatas* L var. Rancing) in Cilembu and
Jatinangor Site, Indonesia

CONTENTS

- 847-869 S.M. Wakil, B.C. Adebayo-Tayo, O.A. Odeniyi, K.O. Salawu, S.A. Eyiolawi and A.A. Onilude**
Production, Characterization and Purification of Laccase by Yeasts Isolated from Ligninolytic Soil
- 871-878 Tina Delsouz Bahri, Mehdi Goudarzi, Seyed Mohammad Ghafoori, Nasrin Ebrahimi, Arezoo Asadi and Hossein Goudarzi**
Comparing Culture and Multiplex PCR Methods to Examine Fastidious Bacteria in Otitis Externa and Media
- 879-884 Vishnuvardhan Reddy Sultanpuram and Thirumala Mothe**
Genome Sequences of *Salisediminibacterium haloalkalitolerans* 10 nlg, *Bacillus lonarensis* 25 nlg, *Bacillus caseinilyticus* SP, *Pelagihabdus alkalitolerans* S5, *Salibacterium halotolerans* S7 and *Salipaludibacillus aurantiacus* S9 six novel, Recently Described Compatible Solute Producing Bacteria
- 885-889 Swagata Roy and Debadatta Dhar (Chanda)**
Isolation, Characterization and Antibiotic Sensitivity Pattern of Different Bacteria in Pus Sample
- 891-901 Sathyaprabha Govindaraj and Senthil Renganathan**
Molecular Characterization and Phytochemical Studies of *Pleurotus* sp. to Unveil Species Diversity and Pharmacological Activities
- 903-906 Vinay Hajare, H. Anandkumar and R.S. Rajeshwari**
Rate of Isolation of *Helicobacter pylori* from Different Clinical Samples in Patients Suffering from Gastritis Attending Tertiary Care Hospital
- 907-912 Roya Rouhi, Mohammad Reza Koushki and Seyed-Ahmad Shahidi**
Microbiological and Physicochemical Properties of Raw Milk Produced from Milking to Delivery to Milk Plant
- 913-919 B.S. Nalini, L. Krishna Naik, Nausheen Saba, Priyanka Prasad and G. Ashiwini**
Studies on the Effect of Microbial Inoculants on Growth and Yield of *Capsicum*, *Capsicum annum L.*
- 921-932 Vikas Kaushik, Bhupender Singh and Joginder Singh**
Bioinformatics Techniques used in Hepatitis C Virus Research
- 933-939 M.D. Shrimali, N.M. Shah, B.S. Chandel, H.C Chauhan, S.S. Patel, K.B. Patel, B.K Patel, A.G. Bhagat, S.I. Patel, A.I. Dadawala, J.D. Shah, Manish Rajgor, R.P. Pandya, A.C. Patel, M.A. Patel, J.K. Kala and M.G. Patel**
Isolation, Identification and Molecular Characterization of *Brucella abortus* from Bovines
- 941-948 Rina Mohanty, Manorama Swain, Sanjib Kumar Kar, Shivaram Prasad Singh and Niranjana Rout**
An Audit of Endoscopic RUT and Treatment for *Helicobacter pylori* in Clinical Practice
- 949-951 Manoj P. Dohat, R. A. Patel, V.Y.Patel and H. K. Patel**
Effect of Irrigation and Nitrogen on Growth and Yield of Linseed (*Linum usitatissimum L.*)

CONTENTS

- 953-961** **Kishan Lal, Pappu Singh, S.K. Biswas, Supriya Yadav, Virendra Kumar and Narender Kumar**
Suitable Integrated Approach for Management of Fusarium Wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.)
- 963-968** **Charlie C. Luchen, Percy M. Chimwamurombe and Larry Hale**
Isolation and Characterization of Fungi Associated with Disease Symptoms on *Ziziphus mucronata* leaves and *Phaseolus vulgaris* pods in Windhoek, Namibia
- 969-973** **Lakshyasri Baishya, Madhab Kalita, Ajanta Sharma, Bornali Sarma Dutta and Debeeka Hazarika**
White Grain Eumycetoma due to *Aspergillus flavus* in Infancy: A Rare Case Report from Assam
- 975-979** **Nadim Chishty, Garima Verma and Chandra Veer**
Isolation and Characterization of *Pseudomonas stutzeri* as Lead Tolerant Bacteria from Water Bodies of Udaipur, India using 16S rDNA Sequencing Technique
- 981-986** **L. Sharma, U. Biswas, C. Guha, A. Chatterjee, P.S. Jana and R. Pandey**
Seroconversion of a Thermostable live Attenuated Lentogenic Strain Newcastle Disease Vaccine (Local isolate) in Chicken
- 987-991** **R.K. Tiwari, Gaurav Mahajan, Amit Jha, S.K. Singh and S.K. Tripathi**
Growth Efficiency, Productivity and Economics of Direct Seeded Rice as Influenced by Nitrogen Level and Weed Management
- 993-1007** **Sarvjeet Kukreja, Kajal Thakur, Neha Salaria and Umesh Goutam**
Changing Trends in Microalgal Energy Production-Review of Conventional and Emerging Approaches
- 1009-1025** **Fazilath Uzma and Srinivas Chowdappa**
Antimicrobial and Antioxidant Potential of Endophytic Fungi Isolated from Ethnomedicinal Plants of Western Ghats, Karnataka
- 1027-1032** **K.Hemalatha, A.V. Ramana, Neelam Bisen and Meena Rani**
Effect of Weed Management Practices on Yield and Economics of Semidry Rice
- 1033-1038** **E.S. Challaraj Emmanuel, Apoorva Udayashankar and Jyoti Mishra**
A Study on Bacteriocin Producing Lactic Acid Bacteria with Antibacterial and Antioxidant Properties Isolated from Plant Wastes
- 1039-1043** **Satish Patil, Praveen C. Shetty, Raghavendra D. Kulkarni, Ajantha G.S., Anuradha Kalabhavi, Deepa Patil, Manjunath Hosamani, Pavithra Jain, A.K. Chakraborti and Shubhada C.**
Antibiogram and Serotyping of *Vibrio cholerae* O1 Isolates from a Tertiary Care Centre in South India
- 1045-1052** **T.K. Radha, D.L.N. Rao and K.R. Sreeramulu**
Actinobacteria of Arid and Semi-arid Soils: Antagonism to Fungal Pathogens and Plant Growth Promoting Potential

CONTENTS

- 1053-1059 Chandra Kant Singh, Ichini Sudhir, Ramesh Chand, Vineeta Singh and Mamta Sharma**
Variability in *Phytophthora drechsleri* f. sp. cajani and Effect of Temperature
- 1061-1066 Ajay Kumar P. and VinodKumar C.S**
Detection of Carbapenem Resistance Encoding Genes Among Gram Negative Bacteria from Urinary Tract Infection in Patients with Type 2 Diabetes Mellitus
- 1067-1077 Jeetendra Kumar Gupta and Pradeep Mishra**
Antimicrobial Screening of Some Newly Synthesized Triazoles
- 1079-1090 Sangeeta Pandey**
Prospects of Metagenomic Cellulases for Converting Lignocellulosic Biomass into Bio-ethanol
- 1091-1097 G.J. Christian, S. Elansekaran, M. Ramamurthy, V. Srinivasan, P. Shanmugapriya and M. Nijavizhi**
Anti-Microbial Screening and Elemental Analysis of the Siddha Medicinal oil 'Oon Poochu Thailam' - A Debriding Agent used in Sloughing Ulcers and Fistulae
- 1099-1103 Krishna D. Kurubetta, R.K. Mesta, M.H. Tatagar and M. Abdul Kareem**
Response of Chilli (*Capsicum annum L.*) for Graded Levels of Fertilizers and Jeevamruta Application
- 1105-1112 Mothe Thirumala and Sultanpuram Vishnuvardhan Reddy**
Isolation of a Cellulolytic Bacterium from the Lonar Soda Lake and Genomic Analysis of it
- 1113-1116 Praveen Solanki, Maitreyie Narayan, Shiv Singh Meena and R.K. Srivastava**
Floating Raft Wastewater Treatment System: A Review
- 1117-1122 K.D. Mevada, S.J. Chaudhary, K.C. Ombase and M.M. Chaudhary**
Effect of Time of Sowing, Row Spacing and Variety on Summer Cluster Bean (*Cymopsis tetragonoloba* (L.)Taub.) Under Middle Gujarat Conditions
- 1123-1128 Praveen C. Shetty and K. Gopalkrishna Bhat**
Comparative Evaluation of Microscopy and Culture Methods in the Diagnosis of Pulmonary Tuberculosis in HIV Infected Patients
- 1129-1134 Deepak Parashar, Divya Bhatia and Deepak Kumar Malik**
Optimization of Keratinase Production by *Bacillus oleronius* Isolated from Poultry Farm Soil
- 1135-1139 Davinder, Anis Mirza, Anjil Kumar, Rupinder Singh, Sudhir Pratap and Bhupinder Singh**
Impact of Zinc and Boron on Growth, Yield and Quality of Kinnow (*Citrus deliciosa* X *Citrus nobilis*) in Sub-tropical Conditions of Punjab
- 1141-1147 Parul Sharma, Pankaj Prakash Verma and Mohinder Kaur**
Comparative Effect of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas putida* on the Growth of Replanted Apple

CONTENTS

- 1149-1154 Kanwaljit Singh, Madhu Sharma and Shailesh Kumar Singh**
Effect of Plant Growth Regulators on Fruit Yield and Quality of Guava
(*Psidium guajava*) cv. Allahabad Safeda
- 1155-1159 Kumar Lambani and Shamarao Jahagirdar**
Morphological, Cultural, Physiological and Biochemical Characteristics of Bacterial
Pustule of Soybean *Xanthomonas axonopodis* pv. *glycines*
- 1161-1168 P. Suneeta, K. Eraivan Arutkani Aiyathan and S. Nakkeeran**
Evaluation of *Trichoderma* spp. and Fungicides in the Management of Collar
Rot of Gerbera Incited by *Sclerotium rolfsii*
- 1169-1173 Anjil Kumar, Anis Mirza, Davinder, Bhupinder Singh, Sudhir Pratap
and Rupinder Singh**
Effect of Pruning, Micronutrients and Plant Growth Regulators on
Kinnow Mandarin Fruits
- 1175-1181 K.C. Ombase, K.D. Mevada, R.B. Kadu, P.G. Gamar and H.L. Ghadage**
Influence of Planting Patterns and Intercropping on Growth and Yield of
suru sugarcane (*Saccharum officinarum* L.)
- 1183-1188 Rameetha Rajan, Dharmalingam Jothinathan Mukesh Kumar,
Palani Perumal and Abhay Kumar**
Effect of Bio-Availability of Magnetized Water on Different Biological Systems
- 1189-1198 Md. Hasanul Karim, Sayad Md. Didarul Alam and Tanzima Yeasmin**
Molecular identification of TEM and SHV Genes in Extended Spectrum
Beta-lactamase Producing *Escherichia coli* and *Klebsiellae pneumoniae* Isolates
in a Tertiary Care Hospital, Bangladesh
- 1199-1213 Prasad Rasane, Rekha Kailey and Shailesh Kumar Singh**
Fermented Indigenous Indian Dairy Products: Standards, Nutrition,
Technological Significance and Opportunities for its Processing

Structure - Activity Relationship of Mutant KatG from INH resistant *Mycobacterium tuberculosis*

Purkan Purkan^{1*}, Sri Puji Astuti Wahyuningsih³, Wiwin Retnowati²,
Diah Amelia¹ and Alfain Noerdin Alimny¹

¹Biochemistry Research Division, Department of Chemistry, Faculty of Sciences and Technology, Airlangga University; Jl. Mulyorejo, Surabaya, 60115, Indonesia.

²Genetic Research Division, Department of Biology, Faculty of Sciences and Technology, Airlangga University; Jl. Mulyorejo, Surabaya, 60115, Indonesia.

³Microbiology Laboratory, Faculty of Medicine, Airlangga University; Jl. Prof Moestopo, Surabaya, 60115, Indonesia.

<http://dx.doi.org/10.22207/JPAM.11.2.07>

(Received: 20 January 2017; accepted: 15 April 2017)

Mutation in *katG* gene of *Mycobacterium tuberculosis* encoding catalase-peroxidase that damage its enzyme activities is well associated with isoniazid (INH) resistance. The *katG* gene from INH resistant strain of *M. tuberculosis* clinical isolate L19 has been observed in previous study, carrying mutations of G₂₃₄A and C₆₂₅T, and changed the arginine residue at position 209 with cysteine in its KatG protein. However the activities of the mutant protein has been not known yet. Expression of the *katG* gene L19 that was done in *Escherichia coli* BL21(DE3) using pCold II-DNA generated KatG protein with 80 kDa in SDS PAGE electroforegram. The mutant protein of KatG L19 decreased 43% of catalase activity and 11% of peroxidase activity against to KatG wild type (H37RV). The model structure of the mutant KatG protein had deviation structure toward KatG wt as 0,13 for number of *Root Mean Square Deviations* (RMSD). The mutant KatG (Arg209Cys) lost two hydrogen interactions and a van der Waals bond which present in KatG wild type. In the KatG wt protein, the both hydrogen bonds was formed between the Arg209 residu to Glu201, while the van der Waals bond occured by linking of Arg209 residu to Glu217. Disruption in the some chemical interactions might trigger the decline in catalase-peroxidase activities of mutant KatG L19 and further it bring out the INH resistance in the clinical isolate L19.

Keywords: *katG*, catalase-peroxidase, isoniazid resistance, *M. tuberculosis*.

Mycobacterium tuberculosis, the causing agent of tuberculosis (TB) disease, recently many strains have been found resistant to the TB drugs. This events caused the TB cases more difficult to resolve. Multi-drug resistant tuberculosis (MDR-TB) is TB resistant to at least two potent anti-TB drugs, such as isoniazid and rifampicin together, or with resistance to first-line anti-TB

drugs, namely, pyrazinamide, ethambutol and streptomycin.¹ Indonesia posed the third ranks for TB cases in the world after India and China, having the total of TB burden are around 395,000 per one thousand populations in 2015 and 126,000 of them are deaths. Of TB cases, as many as 32,000 are cases of MDR-TB^{1,2}. A better understanding in the antituberculous drug resistance is needed to make easy in the TB therapy.

The mechanism of drug resistance in *Mycobacterium tuberculosis* can occur in several ways, (1) a decrease in the effectiveness of the drug due to over production of its target protein,

* To whom all correspondence should be addressed.
Tel.: +62-31-5922427; Fax: +62-31-5922427;
E-mail: purkan@fst.unair.ac.id

(2) drug efflux that cause the drug could not reach to its target, and (3) alteration of in the drug target protein due to mutations of their genes. Most research showed that the main cause of *M. tuberculosis* resistance to antibiotics were due to mutations of the genes targeted by anti-tuberculosis drugs, such as gene *rpoB* for rifampin, *katG* gene for isoniazid, *pncA* gene for pyrazinamide, and *embB* gene ethambutol^{3,4}.

Isoniazid (hidrazid acid 4-piridinkarboksilat, INH) is a mainstay chemical agent used for the treatment of TB, since first introduced in 1951 to the present. This is caused by it having a high bactericidal effect and relatively cheap. Isoniazid is a prodrug that is converted to an active intermediates (Isonicotinoyl acyl radical) by the catalase-peroxidase enzyme encoded by the *katG* gene of *M. tuberculosis*. Isonicotinoyl acyl radical which formed, then reacts with NADH to form INH-NADH complex in the enzyme active site of enoyl ACP reductase (*InhA*) and 2-ketoacyl ACP synthase (*KasA*), then impede the activity of two enzymes in the biosynthesis of mycolic acid, the main component in the cell wall of mycobacteria^{5,6,7}. Disruption in the biosynthesis of mycolic acid can cause the mycobakteri cells death. The analysis of of catalase-peroxidase activities is required to determine the involvement of the enzyme in the emerging of isoniazid.resistance in *M. tuberculosis*. Disruption of catalase-peroxidase function in isoniazid activating can cause INH resistance to *M. tuberculosis*^{7,8}.

Purkan (2011) have identified a *katG* gene of INH resistant *M. tuberculosis* clinical isolates (L19). The clinical isolate which had resistant to INH at a level of 0.2 mg / mL carried double mutations of G234A and C625T in *katG* gene. The *katG* mutations changed the amino acid of arginine at position 209 to Cysteine in *KatG* protein. Although *katG* mutation in the clinical isolate L19 has been connected with INH resistance, but there is no explanation for the catalytic activity of the *KatG* protein. It is important to clarify, because mutational event can be linked with the emergence of other traith in the cells that are not associated with INH resistance phenotype. The *katG* gene of *M. tuberculosis* clinical isolate L19 has been cloned in *Escherichia coli* using pCold II-DNA vector. To explain in detail the causes of INH resistance in clinical isolates of L19, since it was determined the

catalase-peroxidase activity of the mutant protein of *KatG* (Arg209Cys), then followed by modelling of its structure.

MATERIALS AND METHODS

Samples

The *Escherichia coli* BL21 (DE3) carrying of pCold II-*katGwt* and pCold II-*katG* L19 recombinant respectively was obtained from the laboratory of biochemistry, Faculty of Sciences and Technology, Airlangga University. The isolates were resulted from the previous research.

Culturing of *Escherichia coli*

The recombinant of *E. coli* was cultured in liquid and solid Luria Bertani (LB) media containing 100 mg/mL of ampicillin. The liquid LB media had compositions of 0,5% (w/v) yeast extract, 1% (w/v) tryptone and 1% (w/v) NaCl. The solid media had the same compositions with the liquid media, but it contained 2% (w/v) agar.^[9]

Expression of *katG* gene and protein isolation

The *E. coli* BL21 (DE3) carrying of (pCold II-*katG*) recombinant plasmid was grown in 50 mL liquid LB media containing ampicillin 100 mg / mL, then incubated with shaking at 150 rpm in the temperature of 37 °C. The obtained cultures was immediatly treated with cool shock at a temperature of 15°C for 30 minutes without shaking. The culture cells then was added with 0.1 mM IPTG and re-incubated with shaking at 150 rpm in the temperature of 15°C for 24 hours. The culture cells was separated by centrifugation at 5.000g in the temperature 4°C for 10 minutes. After the cell pellet was washed with lysis buffer (50 mM Tris-Cl pH 7,4; 200 mM NaCl), they was centrifugated at 5.000g in the temperature 4°C for 10 minutes. The obtained cell pellet was resuspended in 7-10 mL of 0.02 M phosphate buffer pH 7, then lysed with the sonicator for 10 minutes on the frequency scale 4. The sonication process was carried out in cold conditions (incubated in ice). The obtained cell suspension then was centrifuged at 10.000g in the temperature of 4°C for 20 minutes. The supernatant containing of *KatG* protein was stored at -20°C^{10,11}

Protein Purification

The purification of *KatG* recombinant protein was performed by affinity chromatography using HP HisTrap column containing Ni-sepharose

matrix. The protein sample was firstly adjusted in 0.02M phosphate buffer pH 7.4 containing 25-50 mM NaCl and 10 mM imidazole. The matrix in the HiTrap HP column was balanced with binding buffer (50 mM NaH₂PO₄ pH 7.4, 25 mM NaCl, 10 mM imidazole). The crude extract protein which has undergone initial preparation was filled in the chromatographic column slowly. All the liquid coming out of the column was collected. The column was washed with binding buffer as much as 5 times of the column volume. In the final step,

the target protein was eluted with elution buffer (50 mM NaH₂PO₄ pH 7.4, 25-50 mM NaCl) containing 50-200 mM imidazole. Each fraction was accommodated per 1 mL, then the presence of katG protein in the fraction was detected by polyacrylamide gel electrophoresis^{10,11}

SDS-PAGE Electrophoresis

The Extract protein was analyzed by SDS PAGE using 12% (w/v) acrylamide for separating gel and 4% (w/v) acrilamide for *stacking* gel. The running process was done in 120 mV for 1,5 hours.

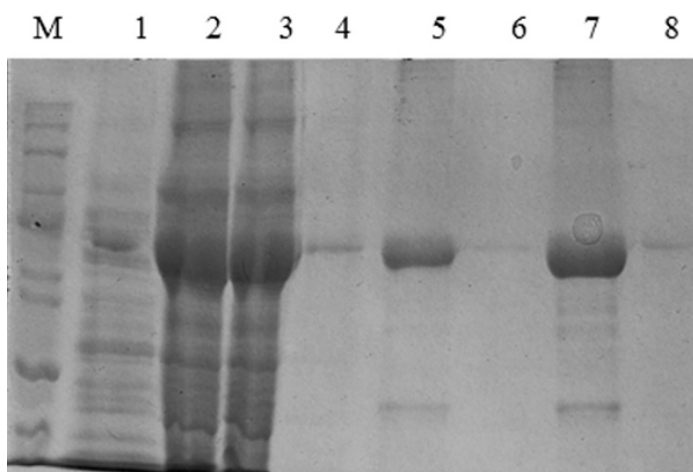


Fig. 1. The SDS PAGE Electroforegram of the KatG protein. (M), Marker protein marker; 1, crude protein obtained from *E.coli* BL21 (DE3); 2&3, extract protein from *E.coli* BL21 (DE3)[pCold-*katG*wt] and [pCold-*katGL19*]; 4&5, the fraction of KatG wt protein eluted with imidazole 100 and 150mM; 6-8, the fraction of KatG L19 protein eluted with imidazole 100 dan 150mM

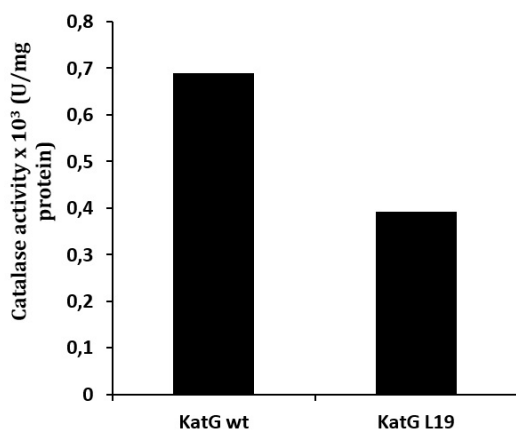


Fig. 2. The catalase activity of KatG. The mutant KatG L19 had catalase activity 43% lower than the KatG wt

This method was adopted from Sambrook et al, 1989⁹

Assay of Enzyme Activities

Catalase activity is determined by the method of Patti and Bonnet-Maury (1953)¹² based on the color formed from the reaction of titanium with H₂O₂ reagents in the reaction mixture containing substrate of 12.5 mM H₂O₂ and enzyme extract, The test is performed in 10 mM potassium phosphate buffer pH 7 with a total volume of 1 mL containing 12.5 mM substrate H₂O₂. The amount of remaining substrate in the mixture was determined by the addition of 2.5 ml of titanium reagent, then the absorbance of the yellow colour formed was determined at λ 410 nm. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the decomposition

of 1 mol of hydrogen peroxide per minute at the experimental conditions¹¹

Peroxidase enzyme activity is determined by reaction of 100 μ M O-dianisidine in the presence

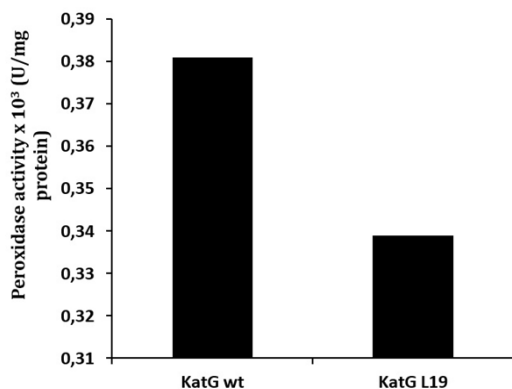


Fig. 3. The peroxidase activity of KatG. The mutant KatG L19 had catalase activity 11% lower than the KatG wt

of 25 mM tert-butyl hydroperoxide (t-BHP) in 50 mM potassium buffer (pH 4.5) and 12.5mM H₂O₂ substrate¹³. The product of O-dianisidine quinonediimine formed was determined at \gg 460 nm ($\mu_{460} = 11.3\text{mM}^{-1} \text{cm}^{-1}$). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 mol product per min on the experimental conditions^{10,11}.

Modeling of the Three Dimensional Structure of the mutant KatG Protein

Construction of the mutant katG protein structure model was performed by a server automatically modelers for protein structure

Table 1. The model structure RMSD of mutant KatG (Arg209Cys) toward KatG wild type

| KatG | Totalmuatan | RMSD (C \pm) |
|---------------------|-------------|-----------------|
| KatG wild type (wt) | -21 | 0 |
| KatG (R209C) (L19) | -22 | 0.13 |

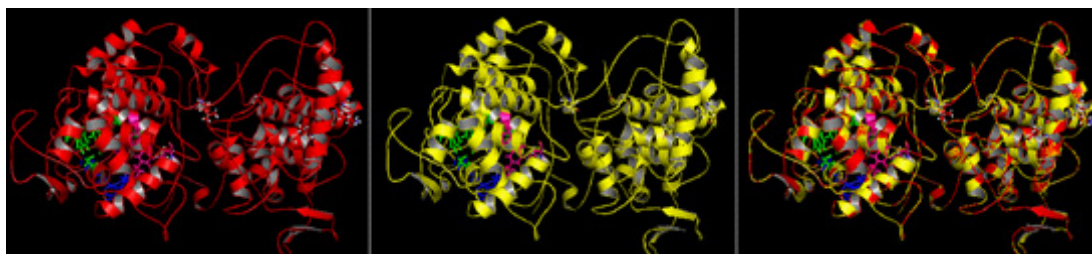


Fig. 4. The alignment product of structure model of mutant KatG L19 toward KatG wt. The KatG *wild type* structure (A); the model structure of mutant KatG L19 (B); the superposition result of the KatG L19 toward KatG wt (C)

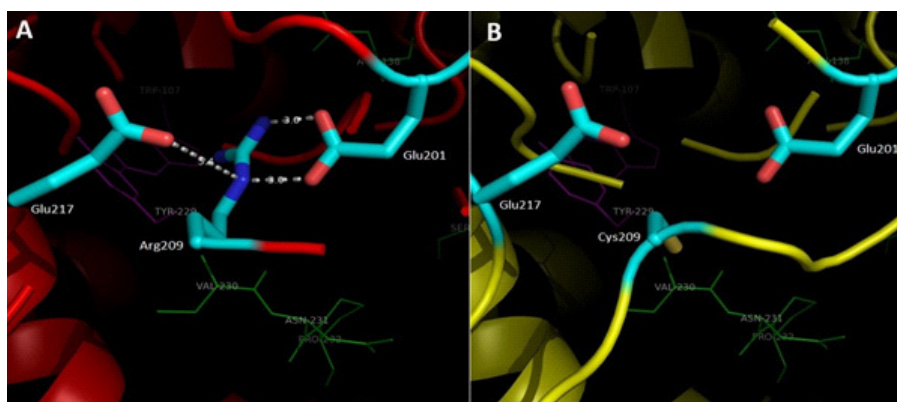


Fig. 5. Illustration of the effect for Arg209Cys substitution in mutant KatG L19. Superposition result of mutant KatG L19 structure (yellow) toward KatG wt (red). The green residues like Val230, Asn231 and Pro232 constituted amino acid residues for binding sites, while the magenta residues like Trp107 and Tyr229 represented as active sites in KatG. The residu Arg209 in KatG wt make two hidrogen interactions with Glu201 and van der Waal interaction with Glu217 (A). Replacement of Arg209Cys in KatG L19 eliminated the interactions (B)

(<http://www.expasy.org/Swiss-Model>). The structure templates used in this modeling is three-dimensional structure of KatG wild type protein (1sj2b). The model structure of the mutant katG were then minimized by Amber program 10, and aligned with the structure of the katG wild type using PyMOL 1.3 program. Deviations structure of the mutant KatG toward KatG wild type was analyzed by SuperPose program version 10 to determine the amount *Root Mean Square Deviations* (RMSD). Interaction between amino acid residues both mutant and wild type katG was visualized with the 1.3 PyMOL program^{10,11}.

RESULTS AND DISCUSSION

Expression and Purification of mutant KatG

Catalase-peroxidase is one of the enzymes in the *M. tuberculosis* that is running the activation of isoniazid^{14,15}. Mutations in katG gene encoding the catalase-peroxidase enzyme is often associated with the emergence of resistance to INH in *M. tuberculosis*. To associate the katG mutations in clinical isolates of *M. tuberculosis* L19 with the catalytic activity of KatG protein produced, it is necessary to express the gene and test its activity of catalase-peroxidase.

The expression of *katG* from INH sensitive *M. tuberculosis* (H37Rv) and from INH resistant clinical isolate (L19) were performed in the host cell of *Escherichia coli* BL21 (DE3) using pCold II-DNA vector, the work could generate the KatG protein 80 kDa in SDS PAGE electropherogram (Fig 1, lane 2 and 3). The 80 kDa protein band was not resulted by the control *E. coli* without pCold II-DNA plasmid (Fig 1, lane 1). Therefore, the expression system of pCold-II-DNA vector is controlled by the promoter *cspA*, a gene derived from cold shock and *lac* promoter of *E. coli*,¹⁶ then to express the *katG* gene, it was done the addition of IPTG inducer and cold shock treatment to the culture of recombinant *E. coli* cells. The KatG protein extract from expression result was further purified by methods Immobilized Metal Affinity Chromatography (IMAC), using HP HisTrap column containing Ni-sepharosa matrix. This purification technique could produce the pure katG protein, as indicated by the presence of a single band of protein 80 kDa in SDS PAGE electropherogram for KatG of *M.*

tuberculosis H37Rv (Fig 1, lane 4-5) and for KatG L19 (Fig 1, lane 6-8). The KatG protein band that observed in SDS PAGE electropherogram was very significantly dominant, and almost invisible presence of the other non-target protein. The purity level of katG protein sulted from the purification step was over 90%.

Catalase-peroxidase activities of KatG protein from clinical isolate L19

Determination of catalase-peroxidase activities for KatG protein was done by using substrate of H_2O_2 for catalase and o-dianisidin for peroxidase activity. The mutant of KatG L19 exhibited diminishing in catalase-peroxidase activities comparing with KatG wild type (H37RV). The mutant KatG L19 had specific activity $0,393 \times 10^3$ U/mg for catalase, and $0,69 \times 10^3$ U/mg for peroxidase activity. The catalase activity of mutant KatG L19 reduced for 43% toward KatG wt (Fig 2). Meanwhile, the mutant KatG L19 had peroxidase activity as $0,339 \times 10^3$ U/mg, but the KatG wt exhibited $0,381 \times 10^3$ U/mg. The mutant KatG L19 had peroxidase activity 11% lower than KatG wt (Fig 3). The change of Arg209Cys amino acids in the KatG L19 might trigger the decreasing in the catalase-peroxidase activities for the mutant KatG L19.

A decrease in catalase-peroxidase activities in some variant katG of *M. tuberculosis* strains resistance to INH was also reported by other researchers. The mutant KatG (S315T) which was found in clinical isolates of *M. tuberculosis* isoniazid resistance, decreased 50% catalase-peroxidase activity of KatG wt^{17,18}. Meanwhile, the mutant KatG (Asn238Ser) decreased the catalytic efficiency as 41% for catalase and 52% for peroxidase respectively¹¹. Wei (2003) reported that the change of catalase-peroxidase activity of the variants katG directly correlated with activity activates INH¹⁷. To know the basis of the change in catalase-peroxidase activity in KatG L19, it was created the structure modeling of the mutant protein.

The Structure Model of Mutant KatG L19 Protein

The model structure of mutant katG L19 (Arg209Cys) was constructed using three-dimensional protein structure template katG wt (1sj2b) of *M. tuberculosis* (H37Rv). The structure of the mutant katG L19 resulted from modeling

method indicated similar folds with KatG wt structure (1sj2b) (Fig 4, A-B). Superposition of two structures KatG showed that all residues between mutant and wild type katG impinge upon each other well (Fig 4C). Superposition of C α skeleton between mutant KatG L19 to KatG wt indicated root mean square deviations (RMSD) of 0:13 (Table 1), which meant that there was a change in the structure of mutant katG L19 compared with the structure of katG wt. The other effect from the mutations of Arg209Cys in the KatG L9 was the change on the total charge in protein molecule, i.e. -21 in the KatG wt and -22 in the KatG L19 (Table 1).

The interpretation of the model structure for KatG L19 show Arg209Cys amino acid modifications lead to changes in interactions at multiple amino acid residues around the substrate binding and catalytic side of KatG (Fig 5, A). In the structure of KatG protein, the amino acid residues Arg209 located in around of substrate binding side that composed by amino acid residues Asn137, Val230, Asn231, Pro232 and Ser315. Three of the five amino acid residues for substrate binding, namely Val230, Asn231, and Pro232 is located very close to Arg209 (Fig 5, A). The Arg209 residue has an important role to support the stability of the environment in which the substrate is bound and catalyzed. In the structure of KatG wt, amino acid residues Arg209 appears to form two hydrogen bonds with residues Glu201 and a van der Waal interaction with Glu217 (Fig 5, A). Replacement of Arg209 Cys in the mutant KatG L19 eliminated these types of interaction which present in KatG wt (Fig 5, B). The loss of two hydrogen interactions and a van der Waals interaction might cause disruption in the function of catalase-peroxidase KatG L19, in turn it decreased the enzym activity. Reduction in the catalase-peroxidase activities might further trigger the emergence of INH resistance in clinical isolates L19.

Thw KatG failure in running the catalytic activity was also demonstrated in the mutant katG (Ser315Thr).^[13,19,20,21] Modification of the amino acid serine at position 315 into threonine emerge an impact on changing the conformation of the inlet substrate, thus the mutant KatG (Ser315Thr) could not bind well its substrate^{13,21} Modification of Ser315Thr shifted the substrate channel from 6 Å in the KatG wt to 4.7 Å in the mutant

KatG (Ser315Thr)²⁰ As a result of this structural change, the mutant KatG (Ser315Thr) had dropped catalase-peroxidase activity around 50%¹⁹ In future, the crystal structure of the mutant KatG L19 is importantly developed to detect the real changes in its protein structure.

ACKNOWLEDGMENTS

The research was supported by DIPA DITLITABMAS Project fiscal year 2015 in accordance with the Decree of the Rector of Airlangga University, Number: 519 / UN3 /2015. We would also thank to Go Bambang Sugiarto, Ph.D, California University, Davis for critically reading the manuscript

REFERENCES

1. Anonymous. Tuberculosis. <https://www.expat.or.id/medical/tuberculosis>. Retrieved 2016-11-25
2. Anonymous, Indonesia Tuberculosis Profile. www.who.int/tb/data. Retrieved 2016-11-25
3. Shi, R., Koji, O., Hiroyuki, Y., Taiga, T., Isamu, S. Temperature-Mediated Heteroduplex Analysis for The Detection of Drug-Resistant Gene Mutations in Clinical Isolates of *Mycobacterium tuberculosis* by Denaturing HPLC, SURVEYOR Nuclease, *Microbes and Infection.*, 2006; **8**:128–135
4. Parka, T.Y.K., Sonya, S., Sungweon R., Sang N.C., Won-Jung K., Kwond, O.J., Young S.S., Woo, J.L., dan Gill, H.B. Comparison of Drug Resistance Genotypes between Beijing and Non-Beijing Family Strains of *Mycobacterium tuberculosis* in Korea., *Journal of Microbiological Methods.*, 2005; **63**:165–172
5. Yu S., Chouchane, M., Magliozzo, R.S. Characterization of the W321F mutant of *Mycobacterium tuberculosis* catalase-peroxidase KatG, *Protein Science.*, 2002; **11**: 58–64
6. Atalay, F.M.D., Nejat, A., Dilek, E.T., Derya, A., P1nar, E., Yurdanur, E.A.N. Catalase-Peroxidase Gene (KatG) Deletion in Isoniazid Resistant Strains of *Mycobacterium Tuberculosis.*, *T Klin J Med Sci.*, 2004; **24**: 243-246.
7. Cade, C.H., Adrienne C., Dlouhy, Katalin, F., Medzihradzky, Saida, P.S.C., Ghiladi, R.A. Isoniazid-resistance conferring mutations in *Mycobacterium tuberculosis* KatG: Catalase, peroxidase, and INH-NADH adduct formation activities, *Protein Science.*, 2005; **19**: 458—474.
8. Ando, H., Yuji, K., Toshinori, S., Emiko, T., Seiya, K., Toru, M., dan Teruo, K. Identification of *katG*

- Mutations Associated with High-Level Isoniazid Resistance in *Mycobacterium tuberculosis*, *Antimicrobial Agents And Chemotherapy*, 2010; **54**(5): 1793–1799.
9. Sambrook, J. F., Maniatis, T. *Molecular Cloning Laboratory Manual*, 2nd edn. USA: Cold Spring Harbour Laboratory Press, 1989; pp 87-111
 10. Purkan., Ihsanawati., Syah YM., Retnoningrum D., Noer A., Shigeoka S., Natalia D. Novel mutations in *katG* gene of a clinical isolate of isoniazidresistant *Mycobacterium tuberculosis*. *Biologia*, 2012; **67**(1): 41-47.
 11. Purkan, Ihsanawati, Natalia D, Syah YM, Retnoningrum DS, Kusuma HS. Mutation of *katG* in a clinical isolate of *Mycobacterium tuberculosis*: effects on catalase-peroxidase for isoniazid activation. *Ukr Biochem J.*, 2016; **88** (N5): 71-81.
 12. Patti, F., Bonet-Maury, P. Methode Colorimetrique pour le Dosage de la Catalase. *Bull. Soc. Chim. Biol.*, 1953; **35**: 1177-80
 13. Wengenack, N.L., Brian, D.L., Preston, J.H., James, R.U., Gudrun, S.L.R., Leslie, H., Glenn, D.R., Franklin, R.C., Patrick, J.B., Kenton, R.R., John, J.B., Frank, R. Purification and Characterization of *Mycobacterium tuberculosis* KatG, KatG(S315T), and *Mycobacterium bovis* KatG(R463L), *Protein Expression and Purification*, 2004; **24**: 232-243
 14. Bertrand, T., Eady, A.J.N., Jones, N.J., Jesmin, Nagy, M.J., Gregoire, J.N., Rave, L.E., Brown, A.K. Crystal Structure of *Mycobacterium tuberculosis* Catalase-Peroxidase., *J. Biol. Chem.*, 2004; **279**(37): 38991-38999.
 15. Wilming, M., dan Johnsson, K. Inter- and Intramolecular Domain Interactions of The Catalase-Peroxidase KatG from *M. tuberculosis*, *FEBS Letters*, 2001; **509**: 272-276
 16. Anonymous. Cold Shock Expression System pCold™ DNA; TAKARA BIO Inc. <http://www.takara-bio.com>. Retrieved: 2015-2-12
 17. Wei, J., Benfang, L., James M.M., Shiao-Chun T.C. Isoniazid Activation Defects in Recombinant *Mycobacterium tuberculosis* Catalase-Peroxidase (KatG) Mutants Evident in InhA Inhibitor Production *Antimicrobial Agents And Chemotherapy*, 2003; **47**(2): 670–675
 18. Devito, J.A., Morris, S. Exploring the Structure and Function of the Mycobacterial KatG Protein Using *trans*-Dominant Mutants., *Antimicrobial Agents And Chemotherapy*, 2003; **47**(1): 188–195
 19. Kapetanaki, S.M., Salem, C., Shengwei Y., Richard S.M., Johannes, P.M.S. Resonance Raman spectroscopy of Compound II and its decay in *Mycobacterium tuberculosis* catalase-peroxidase KatG and its isoniazid resistant mutant S315T, *Journal of Inorganic Biochemistry*, 2005; **99**: 1401–1406
 20. Zhao, X., Yu, H., Yu, S., Wang, F., Sacchettini, J.C., Magliozzo, R.S. Hydrogen peroxide-mediated isoniazid activation catalyzed by *Mycobacterium tuberculosis* catalase-peroxidase (KatG) and its S315T mutant, *Biochemistry Journal*, 2006; **45**: 4131–4140.
 21. Kapetanaki, S.M., Xiangbo, Z., Shengwei Y., Richard, S. M., Johannes, P.M.S. Modification of The Active Site of *Mycobacterium tuberculosis* KatG After Disruption of the Met–Tyr–Trp Cross-Linked Adduct, *Journal of Inorganic Biochemistry*, 2007; **101**: 422–433.