Online ISSN: 2249-4626
Print ISSN: 0975-5896

Global Journal

of Science Frontier Research : G

Bio-Tech & Genetics

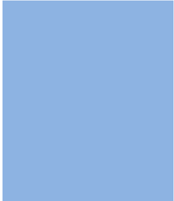
DISCOVERING THOUGHTS AND INVENTING FUTURE

Volume 12 | Issue 4 | Version 1.0

ENG

© 2001-2012 by Global Journal of Science Frontier Research, USA

Global Journal of Science Frontier Research: G
Bio-Tech & Genetics



Global Journal of Science Frontier Research: G

Bio-Tech & Genetics

Volume 12 Issue 4 (Ver. 1.0)

© Global Journal of Science Frontier Research .2012 .

All rights reserved.

This is a special issue published in version 1.0 of "Global Journal of Science Frontier Research." By Global Journals Inc.

All articles are open access articles distributed under "Global Journal of Science Frontier Research"

Reading License, which permits restricted use. Entire contents are copyright by of "Global Journal of Science Frontier Research" unless otherwise noted on specific articles.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without written permission.

The opinions and statements made in this book are those of the authors concerned. Ultraculture has not verified and neither confirms nor denies any of the foregoing and no warranty or fitness is implied.

Engage with the contents herein at your own risk.

The use of this journal, and the terms and conditions for our providing information, is governed by our Disclaimer, Terms and Conditions and Privacy Policy given on our website http://globaljournals.us/terms-and-condition/menu-id-1463/

By referring / using / reading / any type of association / referencing this journal, this signifies and you acknowledge that you have read them and that you accept and will be bound by the terms thereof.

All information, journals, this journal, activities undertaken, materials, services and our website, terms and conditions, privacy policy, and this journal is subject to change anytime without any prior notice.

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: *Open Association of Research Society*
Open Scientific Standards

Publisher's Headquarters office

Global Journals Inc., Headquarters Corporate Office, Cambridge Office Center, II Canal Park, Floor No. 5th, ***Cambridge (Massachusetts)***, Pin: MA 02141
United States
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Open Association of Research Society, Marsh Road, Rainham, Essex, London RM13 8EU
United Kingdom.

Packaging & Continental Dispatching

Global Journals, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investers@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Editorial Board Members (HON.)

John A. Hamilton,"Drew" Jr.,
Ph.D., Professor, Management
Computer Science and Software Engineering
Director, Information Assurance Laboratory
Auburn University

Dr. Henry Hexmoor
IEEE senior member since 2004
Ph.D. Computer Science, University at Buffalo
Department of Computer Science
Southern Illinois University at Carbondale

Dr. Osman Balci, Professor
Department of Computer Science
Virginia Tech, Virginia University
Ph.D.and M.S.Syracuse University, Syracuse, New York
M.S. and B.S. Bogazici University, Istanbul, Turkey

Yogita Bajpai
M.Sc. (Computer Science), FICCT
U.S.A.Email:
yogita@computerresearch.org

Dr. T. David A. Forbes
Associate Professor and Range Nutritionist
Ph.D. Edinburgh University - Animal Nutrition
M.S. Aberdeen University - Animal Nutrition
B.A. University of Dublin- Zoology

Dr. Wenying Feng
Professor, Department of Computing & Information Systems
Department of Mathematics
Trent University, Peterborough,
ON Canada K9J 7B8

Dr. Thomas Wischgoll
Computer Science and Engineering,
Wright State University, Dayton, Ohio
B.S., M.S., Ph.D.
(University of Kaiserslautern)

Dr. Abdurrahman Arslanyilmaz
Computer Science & Information Systems Department
Youngstown State University
Ph.D., Texas A&M University
University of Missouri, Columbia
Gazi University, Turkey

Dr. Xiaohong He
Professor of International Business
University of Quinnipiac
BS, Jilin Institute of Technology; MA, MS, PhD,. (University of Texas-Dallas)

Burcin Becerik-Gerber
University of Southern California
Ph.D. in Civil Engineering
DDes from Harvard University
M.S. from University of California, Berkeley & Istanbul University

Dr. Bart Lambrecht
Director of Research in Accounting and FinanceProfessor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of ReginaPh.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and FinanceLancaster University Management SchoolPh.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical NeuroscienceNorthwestern University
Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic
Radiology
M.D., State University of New York at
Buffalo,School of Medicine and
Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical
Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric
SciencesPurdue University Director
National Center for Typhoon and
Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences,
National Central University, Chung-Li,
TaiwanUniversity Chair Professor
Institute of Environmental Engineering,
National Chiao Tung University, Hsin-
chu, Taiwan.Ph.D., MS The University of
Chicago, Geophysical Sciences
BS National Taiwan University,
Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and
Hypertension Division (PMC)
Penn Medicine, University of
Pennsylvania
Presbyterian Medical Center,
Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of
Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D
Marketing
Lecturer, Department of Marketing,
University of Calabar
Tourism Consultant, Cross River State
Tourism Development Department
Co-ordinator , Sustainable Tourism
Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer
Science
AUST - American University of Science &
Technology
Alfred Naccash Avenue – Ashrafieh

President Editor (HON.)

Dr. George Perry, (Neuroscientist)
Dean and Professor, College of Sciences
Denham Harman Research Award (American Aging Association)
ISI Highly Cited Researcher, Iberoamerican Molecular Biology Organization
AAAS Fellow, Correspondent Member of Spanish Royal Academy of Sciences
University of Texas at San Antonio
Postdoctoral Fellow (Department of Cell Biology)
Baylor College of Medicine
Houston, Texas, United States

Chief Author (HON.)

Dr. R.K. Dixit
M.Sc., Ph.D., FICCT
Chief Author, India
Email: authorind@computerresearch.org

Dean & Editor-in-Chief (HON.)

Vivek Dubey(HON.)
MS (Industrial Engineering),
MS (Mechanical Engineering)
University of Wisconsin, FICCT
Editor-in-Chief, USA
editorusa@computerresearch.org

Sangita Dixit
M.Sc., FICCT
Dean & Chancellor (Asia Pacific)
deanind@computerresearch.org

Suyash Dixit
(B.E., Computer Science Engineering), FICCTT
President, Web Administration and
Development , CEO at IOSRD
COO at GAOR & OSS

Er. Suyog Dixit
(M. Tech), BE (HONS. in CSE), FICCT
SAP Certified Consultant
CEO at IOSRD, GAOR & OSS
Technical Dean, Global Journals Inc. (US)
Website: www.suyogdixit.com
Email:suyog@suyogdixit.com

Pritesh Rajvaidya
(MS) Computer Science Department
California State University
BE (Computer Science), FICCT
Technical Dean, USA
Email: pritesh@computerresearch.org

Luis Galárraga
J!Research Project Leader
Saarbrücken, Germany

Contents of the Volume

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Table of Contents
v. From the Chief Editor's Desk
vi. Research and Review Papers

1. Studying Genetic Diversity of Pistachio Cultivars in Kerman Province Based on Morphological Traits Using Fourier series And Cluster Analysis *1-4*
2. Exhaustive Sliding-Window Scan Strategy for Genome-Wide Association Study via Pca-Based Logistic Model. *5-16*
3. Bioleaching of Copper Concentrate and Pyrite by Using Native Bacterium Acidithiobacillus Ferrooxidans IRL.8F and Evaluating the LPS Role in Bioleaching Process. *17-26*
4. Prevalence of Parasitic Eggs and Parasites Cysts on Computer Mouse and Keyboard in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria. *27-30*
5. Crystal Structure and Kinetic Studies on Met244Ala Variant of KatG from *HALOARCULA MARISMORTUI*. *31-46*

vii. Auxiliary Memberships
viii. Process of Submission of Research Paper
ix. Preferred Author Guidelines
x. Index

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 12 Issue 4 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Studying Genetic Diversity of Pistachio Cultivars in Kerman Province Based on Morphological Traits Using Fourier Series and Cluster Analysis

By Amin Baghizadeh & Reza Haghi
Kerman Graduate University of Technology, IRAN

Abstract - Presentation of results obtained from tests in a diagram is one of the best ways to illustrate laboratory results. Pistachio is one of the most important nuts in the world which has high nutritional and economical values. Iran is the largest pistachio producer in the world and is one of the richest regions regarding pistachio germplasm. Studying genetic diversity is important to protect germplasm storages and also as a prerequisite for breeding. Fourier series can be defined as a function in terms of sin and cosine series. The function can be plotted as a curve in coordinate system. In order to cluster plant genotypes, measured characters of each genotype are replaced with sin and cosine factors of Fourier series so that a function can be determined for it. Plotting all functions in coordinate system and from obtained curves, the genotypes can be clustered. In present study, genetic varieties of some pistachio cultivars in Kerman province have been investigated based on morphological characters. Results obtained from Andrews' plotted curves were similar with those using Fourier series and cluster analysis. Both classified genotypes in 5 groups. It is concluded that using Fourier series and cluster analysis complement each other and it is recommended that in order to choose cultivars, results of both approaches are applied.

Keywords : Andros's curves; Fourier series; Genetic diversity; Pistachio.

GJSFR-G Classification ; FOR Code: 060406

Strictly as per the compliance and regulations of :

© 2012. Amin Baghizadeh & Reza Haghi. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Studying Genetic Diversity of Pistachio Cultivars in Kerman Province Based on Morphological Traits Using Fourier Series and Cluster Analysis

Amin Baghizadeh [α] & Reza Haghi [σ]

Abstract - Presentation of results obtained from tests in a diagram is one of the best ways to illustrate laboratory results. Pistachio is one of the most important nuts in the world which has high nutritional and economical values. Iran is the largest pistachio producer in the world and is one of the richest regions regarding pistachio germplasm. Studying genetic diversity is important to protect germplasm storages and also as a prerequisite for breeding. Fourier series can be defined as a function in terms of sin and cosine series. The function can be plotted as a curve in coordinate system. In order to cluster plant genotypes, measured characters of each genotype are replaced with sin and cosine factors of Fourier series so that a function can be determined for it. Plotting all functions in coordinate system and from obtained curves, the genotypes can be clustered. In present study, genetic varieties of some pistachio cultivars in Kerman province have been investigated based on morphological characters. Results obtained from Andrews' plotted curves were similar with those using Fourier series and cluster analysis. Both classified genotypes in 5 groups. It is concluded that using Fourier series and cluster analysis complement each other and it is recommended that in order to choose cultivars, results of both approaches are applied.

Keywords : Andros's curves; Fourier series; Genetic diversity; Pistachio.

I. Introduction

Pistacia sp.L belongs to the Anacardiaceae family and is a dioecious tree (Zohary, 1952; baghizadeh, 2010). In 1952, Zohary et al. identified 11 varieties of pistacia using morphological traits, among which P.Vara variety has an edible fruit. Pistachio fruit (P.Vara*)* is one of the most important nutsin the world and has high nutritional and economical values (Baninasab, 2008).In breeding, some varieties of pistacia wild type are used as rootstock (Sheibani, 1987; AhmadiAfzadi, 2007).

P.atlanticasub sp.mutica (common name, Baneh) and P.khinjuksub sp.Chatlanqush, Kasor, Golkhunak and khinjuk are grown in Kerman province (Sheibani, 1987).

Chatlanqush and Baneh are accounted as suitable rootstocks for transplanting domestic pistachio trees (Sheibani, 1987). According to FAO statistics, Iran is the largest pistachio producer in the world (F.A.O, 2005). Kerman province with 270000 acres produces 77 percent of pistachio in Iran and is considered as the most important region for growing pistachio in Iran and in the world(statistic center of Iran; Baninasab et al. 2008). Some genetic studies have been done on Pistacia diversity in the world (Hormoza et al. 1997; Parfitt-Dan et al. 1997; Caruso et al. 1998; Kafkas et al. 2001; Kafkas et al. 2002; Golan-Goldhirsh et al. 2004). Some researchers have done isozymic studies in order to investigate and identify genetic varieties among pistachio cultivars (Aalami et al. 1996; Barone et al. 1996). Studies showed that using this method to study genetic diversity of similar cultivars is not suitable (Golan-Goldhirsh et al. 2004). Limited studies have been done on pistachio germplasm in Iran. Tajabadi et al. has done morphological and molecular studies on pistachio varieties in Iran. In 2005, Mirzayi et al. studied genetic diversity of some pistachio cultivars using RAPD molecular marker. Arabnejad et al. (2008) studied genetic diversity of some pistachio cultivars using microsatellite markers of Pistaciakhinjuk Stocks. Norouzi et al. (2009) studied genetic diversity of some pistachio cultivars using ISSR molecular marker. Hajirezaee et al. (2009) studied germplasm diversity of pistachio cultivars using RAPD molecular marker. Tagizad et al. (2010) studied genetic diversity in Iranian pistachio cultivars using ISSR and RAPD markers. In 2010, Baghizadeh et al. Studied genetic diversity of some Iranian Pistachio cultivars using RAPD, ISSR and SSR markers.

Studying genetic diversity is important to protect germplasm storages and as a prerequisite for breeding. Presentation of results obtained from tests in a diagram is one of the best ways to illustrate laboratory results so that the reader reaches the results easily and in the shortest time. Andrews (1972) stated that a p-dimensional vector with amounts of $[x_1, x_2, \dots, x_p]$ can be shown by Fourier series in range of$\pi \leq t \leq -\pi$. Fourier series can be defined as a function in terms of sin and cosine as following:

$$f(t) = \frac{x1}{2} + x2\sin t + x3\ \cos t + x4\ \sin 2t + x5\cos 2t + \dots$$ (Arfken et al. 1985)

Author α : Assistant professor, International Center for Science, High Technology & Environmental Sciences, Kerman, I.R. of IRAN.
E-mail : amin_4156@yahoo.com
Author σ : M.Sc. Student, Department of plant breeding, Kerman Graduate University of Technology,Kerman, I.R. of IRAN.

© 2012 Global Journals Inc. (US)

This function can be plotted as a curve in coordinate system. Obtained diagram results from fitting sin and cosine waves which exist in the function. Limited morphological studies have been done on pistachio cultivars in Iran. Kerman pistachio cultivars have not been studied that is one of the richest regions regarding germplasm in the world. It is necessary to implement this research considering importance of morphological traits in clustering pistachio genotypes and advantages of using Fourier series and Andrews' plotted curves.

II. Materials and Methods

In order to choose plant substances, Kerman province is divided into 5 regions for growing pistachio (Rafsanjan, Zarand, Kerman, Ravar and Sirjan) and each region is subdivided into 5 areas. 5 trees were chosen randomly from each cultivar in every area. It is attempted to select trees with same ages from orchards with same conditions. 29 different genotypes include OhadiRafsanjan, OhadiSirjan, OhadiRavar, Ohadi Kerman, OhadiZarand, Fandoghirafsanjan, AkbariSirjan, Ibrahimababdi, Kaleghochi Kerman, Mohseni, KaleghochiRafsanjan, KaleghochiZarand, BadamiRavar, BadamiSirjan, Italiaei, Baneh, KhanjariRavar, Akbari Kerman, Kasour,R23, N1, BadamiZarand, Shasti, Sirizi, MomtazZarand, gholamrezaii, MomtazSirjan, Amiri and SefidPesteNogh. R23 and N1 cultivars were chosen from collection of Iran pistachio research institute in Rafsanjan. In appropriate time 20 morphological traits were measured including average yield per tree, the 100 grain weight with shell, split pistachio percentage, void pistachio percentage, wet 100 grain weight without shell, day numbers from flowering to harvest, percentage of three leaflet leaves, length of fruit raceme, raceme width, flowering period, tree height, kernel percentage from dry weight of the fruit, leaf length, leaf width, grain numbers per raceme, mixed ounce, dry pistachio length, dry pistachio width, dry pistachio diameter and kernel percentage from dry weight of the fruit. Genotypic Clustering was done by Andrews' plotted curves using Fourier series and cluster analysis. Results obtained from two approaches were compared.

In this research for the first time, Andrew's plotted curves using Fourier series have been used to cluster plant genotypes. In this method a function of Fourier series is defined for each genotype and measured variables for each one are replaced instead of present Xs in this series. So the defined function for each genotype will contain measured characteristics of that genotype in such a way that each trait measured for a special genotype will be the factor of one of sin and cosine terms in defined function for that genotype. Then each function will be plotted as a curve in coordinate system in the range between π and $-\pi$. This curve is the result of fitting sin and cosine terms in functions. Curve coordination depends on sin and cosine factors that are our measured variables. For all studied genotypes a function will be defined and their curves will be plotted for all functions. Similarities between curves and theirpositions in coordinate system indicate their close genetic relations. Genotypes curves being away from each other suggest their genetic difference. Genotypes can be simply classified in different groups from mentioned curves. These curves were plotted by MATLAB software. In order to compare results obtained by Fourier series with those of cluster analysis, genotypes were grouped using SPSS software, average connection approach and based on of Euclidian distance similarity coefficient.

III. Results

Results obtained from clustering by Andrew's plotted curves using Fourier series were compared with results obtained from cluster analysis. Both results were the same and genotypes were placed in 5 groups by both approaches (fig 1 & 2). Shasti cultivar made a group by itself. Baneh and Sefidpestenogh cultivars formed another group so that their genetic similarities were identified in molecular studies of Hajirezaee.et.al (2009). Cultivars of MomtazZarand, MomtazSirjan, Amiri and Kasor were placed in one group. Others were placed in two groups that are shown in fig 1 and 2. All Ohadicultivars were placed in one group and their raphs were next to each other that were observed in molecular studies of Hajirezaee.et.al (2009). Graphs of AkbariSirjan and AkbariKerman cultivars overlapped indicating their close similarities in spite of geographical distance. It was also observed in molecular studies of Hajirezaee.et.al (2009). All cultivars of Badamiand Kaleghochiwere placed in one group. According to molecular studies of Hajirezaee.et.al (2009) and Ahmadi.et.al, Shasti and Sirizi cultivars were placed in one group but they were in different groups in this research and studies of Norouzi.et.al (2009) and they need further molecular researches.

© 2012 Global Journals Inc. (US)

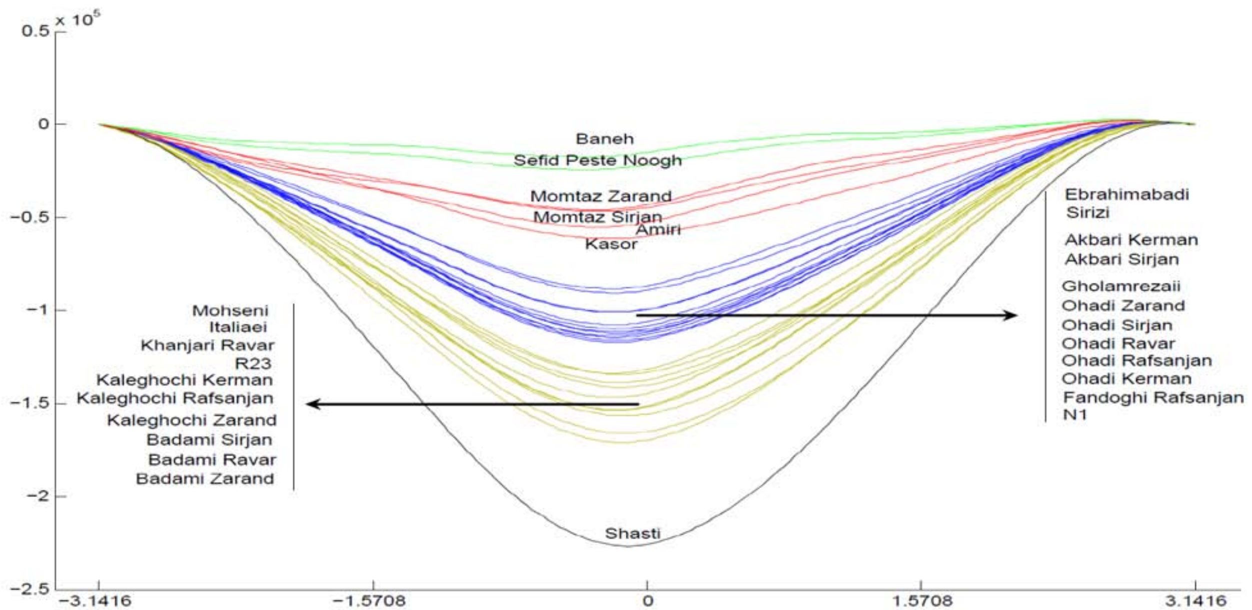


Figure 1 : Andrew's curves plotted by using Fourier series

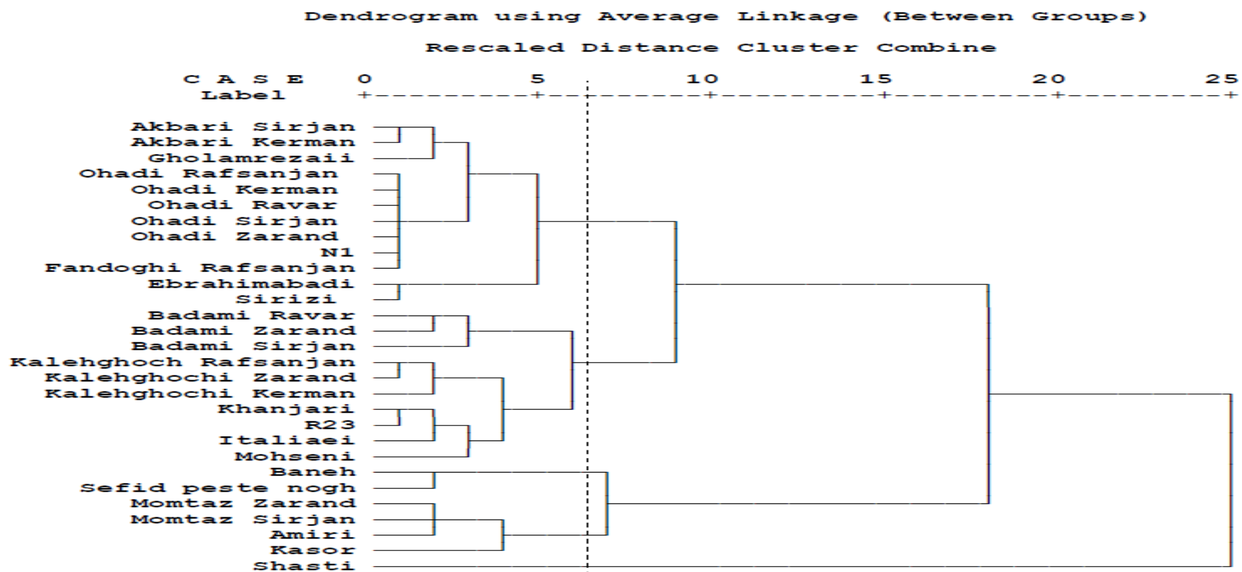


Figure 2 : Dendrogram resulted from cluster analysis for studied cultivars

IV. Discussion and Conclusion

In many aspects, using Fourier series and has more advantages than cluster analysis. In plant breeding, when we want to make a variant population by crossing two cultivars and have the most varieties in segregation generations in order to select them desirably, it is necessary that the parent cultivars have the appropriate genetic difference. From Andrew's curves, genetic difference of cultivars can be observed better than cluster analysis. For example, as it can be seen in the figure 1, Shasti cultivar has the most distance from Baneh cultivar curve that shows phenotype difference of these cultivars, whereas in the graph obtained from cluster analysis, in addition of *Baneh*, *Shasti* cultivar has the same distance with 9 other cultivars. Thus in addition of clustering genotypes, using Fourier series and Andrew's plotted curves cause the breeder to understand clearly the genotypic differences. It is appropriate for choosing suitable cultivars for plant breeding. In general, the curves obtained from plotting Fourier functions present better differences of genotypes and show all measured

© 2012 Global Journals Inc. (US)

characteristics of a genotype in a curve. Another advantage of this approach is that in cluster analysis, it is possible that some genotypes despite significant difference in some characters are placed in unit groups, while all measured characters are affected by curves obtained from Fourier series and identify more differences and similarities. Considering above explanation, it is concluded that using Fourier series and cluster analysis complementeach other and it is recommended that for the purposes of breeding, results obtained from two approaches are used in order to choose the best cultivars.

References Références Referencias

1. AhmadiAfzadi M, Tabatabaei B.E.S, Mohammadi S A, Tajabadipur A. (2007). Comparison of genetic diversity in species and cultivars of pistachio (Pistaciasp L.) based on Amplified Fragment Length Polymorphism (AFLP) markers. Iranian Journal of Biotechtology**5(3)**, 142-157.
2. Aalami A and Nayeb M. (1996). Using isozyme for genetic diversity analysis of Iranian pistachio. M.Sc. Thesis, Faculty of Agriculture, TarbiatModares University, Iran.
3. Anonymous. (2001). Statistical yearbook of statistic center of Iran. Blears MJ.
4. Arfken G. Mathematical methods for physicists. (1985). Third edition. Academic press, Inc.
5. Baghizadeh A, Noroozi SH, JalaliJavaran M. (2010). Study on genetic diversity of some Iranian Pistachio (Pistaciavera L.) cultivars using random amplified polymorphic DNA (RAPD), inter sequence repeat (ISSR) and simple sequence repeat (SSR) markers: A comparative study. African Journal of Biotechnology **9(45)**, 7632-7640, 8 November.
6. Baninasab B and Mobli M. (2008).Morphological attributes of root systems and seedling growth in three species of Pistacia. Silva Lusitana **16(2)**, 175-181.
7. Barone E, Di Marco L, Marra F.P, Sidari M. (1996). Isozymes and canonical discrinant analysis to identify pistachio (Pistaciavera L.) germplasm. HortSci**31(1)**, 134-138.
8. CarusoT, Iannini C, Monastra F, Zakynthinos G, Rouskas D, Barone E, Marra F.P, Sottile F, Batlle I, Vargas F, Romero M, Padulosi S, Greco C.I, Cabina M.R, Martelli G, Ak B.E and Laghezali M. (1998). Genetic and phenotypic diversity in pistachio (Pistaciavera L.) germplasm collected Mediterranean countries. Acta. Hort**470**, 168-178.
9. F.A.O.(2005). Production Year Book. Vol. 58. F. A. O. Rome, Italy.
10. Golan-Goldhirsh A, Barazani O, Wang Z.S, Khadka D K, Saunders J.A, Kostikosky V, and Rowland L J. (2004). Genetic relationships among Mediterranean pistacia species evaluated by RAPD and AFLP markers. Plant Syst**246**, 9-18.
11. Hajirezayi M, Baghizadeh A, Javadi GH, and Sadeghizadeh M. (2009). Genetic diversity assessment of a few numbers of pistachio cultivars in Kerman province based on RAPD markers. Iranian Journal of Biotechtology **22**(3), 462-469.
12. Hormoza J I, Pinney K and Polito V.S. (1998). Genetic diversity of Pistachio (pistaciavera, Anacardiaceae) germplasm based on randomly amplified polymorphic DNA (RAPD) markers. Economic Botany **52(1)**, 78-87.
13. Kafkas S and Perl-Treves R. (2001). Morphological and molecular phylogeny of Pistacia species in Turkey. TheorAppl Genet **102**, 908-915.
14. Kafkas S and Perl-treves R. (2002). Inerspecific relationships in pistacia based on RAPD fingerprinting. HortScience**37**, 168-171.
15. Mirzaei S, Bahar M, Sharifnabi B (2005). A phylogenetic study ofIranian wild pistachio species and some cultivars using RAPDmarkers. ActaHortic**726**, 39-43.
16. Noorozi, sh, Baghizadeh A, JalaliJavaran, M. 2009. The genetic diversity of Iranian pistachio (PistaciaveraL.) cultivars revealed by ISSR markers. Biological Diversity and Conservation, BioDiCon**2(2)**, 50-56.
17. Parfitt Dan E, Badenes Maria, L. (1997). Phylogeny of the genus pistacia as determined from analysis of the chloroplast genome, Proc. Natl. Acad. Sci. USA **94**, 7987-7992.
18. Sheibani A. (1987). Characteristics of Iranian pistachio varieties In Iranian. Pomology Seminar Seed and Plant Improvement Institute, Rafsanjan, Iran.
19. Tajabadipur A. (1997). Identification of some pistachio cultivars. M.Sc. thesis, Faculty of Agriculture, Tehran University, Tehran, Iran.
20. Tagizad A, Ahmadi J, Haddad R and Zarrabi M. (2010). A comparative analysis of ISSR and RAPD markers for studying genetic diversity in Iranian pistachio cultivars. Iranian Journal of Genetics and Plant Breeding **1(1)**, 6-16.
21. Zohary M.A. (1952). Monographical study of the genus Pistacia. Palestin J. Bot **5**, 187-228.

© 2012 Global Journals Inc. (US)

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 12 Issue 4 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Exhaustive Sliding-Window Scan Strategy for Genome-Wide Association Study via Pca-Based Logistic Model

By Qingsong Gao , Zhongshang Yuan , Yungang He , Jinghua Zhao Xiaoshuai Zhang, Fangyu Li, Bingbing Zhang & Fuzhong Xue
Shandong University, China

Abstract - In genome-wide association study (GWAS), various sliding-window scan approaches have been proposed recently. How to determine the optimal window size, which is influenced by the underlying linkage disequilibrium (LD) patterns, minor allele frequency (MAF) of the causal SNP, and others, is crucial for these methods. However, it is difficult to clarify the theoretical relationship between the optimal window size and these factors. In this regard, we proposed exhaustive strategy with ergodic window sizes along the genome matter whatever the relationship is. Simulations are conducted to assess statistical powers under different sample sizes, relative risks, MAF, LD patterns and window sizes, followed by a real data analysis to evaluate its performance. The simulation results suggested that it was difficult to determine the optimal window size because it was influenced by many factors such as MAF and LD pattern. Real data analysis indicated that the p-values with different window sizes were quite different. Furthermore, with the development of multiprocessor computational technique, the proposed exhaustive strategy combined with the cluster computer technique computationally efficient and feasible for analyzing GWAS data.So the exhaustive strategy is a powerful tool for GWAS data analysis regardless of the relationship between the window size and LD.

Keywords : Minor allele frequency; Causal SNP; Cluster computer..

GJSFR-G Classification : FOR Code: 060407

Strictly as per the compliance and regulations of :

© 2012 By Qingsong Gao , Zhongshang Yuan , Yungang He , Jinghua Zhao Xiaoshuai Zhang, Fangyu Li, Bingbing Zhang & Fuzhong Xue. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Exhaustive Sliding-Window Scan Strategy for Genome-Wide Association Study via Pca-Based Logistic Model

Qingsong Gao [α], Zhongshang Yuan [σ], Yungang He [ρ], Jinghua Zhao [Ѡ], Xiaoshuai Zhang[¥], Fangyu Li[§], Bingbing Zhang [χ] & Fuzhong Xue [ν]

Abstract - In genome-wide association study (GWAS), various sliding-window scan approaches have been proposed recently. How to determine the optimal window size, which is influenced by the underlying linkage disequilibrium (LD) patterns, minor allele frequency (MAF) of the causal SNP, and others, is crucial for these methods. However, it is difficult to clarify the theoretical relationship between the optimal window size and these factors. In this regard, we proposed exhaustive strategy with ergodic window sizes along the genome matter whatever the relationship is. Simulations are conducted to assess statistical powers under different sample sizes, relative risks, MAF, LD patterns and window sizes, followed by a real data analysis to evaluate its performance. The simulation results suggested that it was difficult to determine the optimal window size because it was influenced by many factors such as MAF and LD pattern. Real data analysis indicated that the p-values with different window sizes were quite different. Furthermore, with the development of multiprocessor computational technique, the proposed exhaustive strategy combined with the cluster computer technique computationally efficient and feasible for analyzing GWAS data.So the exhaustive strategy is a powerful tool for GWAS data analysis regardless of the relationship between the window size and LD.

Keywords : Minor allele frequency; Causal SNP; Cluster computer.

I. Introduction

With rapid improvements in high-throughout genotyping techniques, the cost of genome-wide association study. In recent years, sliding-window methods, in which (GWAS) has been greatly reduced and a boom of large studies of common diseases is underway , which results in an increasing need for new analytical methods to the association mapping study several neighboring single nucleotide polymorphisms (SNPs) together included in a 'window', have been a popular strategy of automated GWAS data analysis (Shaet al., 2009, Manentiet al., 2009, Yanget al., 2009, Liet al., 2007, Tanget al., 2009, Browning, 2006, Lin et al., 2004). In these sliding-window approaches, the candidate region or the whole genome is divided into many contiguous overlapping windows, followed by multi-locus association tests in each window. Sliding-window approach is commonly used with the fixed window size. For example, Manenti et al used a three-SNP sliding window (Manentiet al., 2009), while Yang et al applied multiple moving window sizes: 3, 5, 7, 9 (Yanget al., 2009). One major conc often encountered in these methods, i.e. how to determine the optimal window size. A large window may include too many non-informative markers while a small window may ignore informative markers, both of which will lead to a reduction in testing power (Yanget al., 2006). The potential problem of small windows is that they do not model the untyped, potentially causal, markers so well. The optimal window size is always influenced by the underlying linkage disequilibrium (LD) patterns, which are certainly variable across a large genomic region or the whole genome (Liet al., 2007, Tanget al., 2009). Variable-sized sliding-window approaches with variable window sizes determined by the underlying LD pattern have been proposed in large-scale data analysis (Browning, 2006, Liet al., 2007, Tanget al., 2009). However, how to clarify the potential theoretical relationship between the window size and LD remains unsolved. Furthermore, most of the variable-sized methods have to go through some computationally intensive phasing program to account for uncertain haplotype phases (Sha et al., 2009). Lin et al proposed that an exhaustive search of all possible windows of SNPs at the genome level is not only computationally practical but also statistically sufficient to detect common or rare genetic-risk alleles (Linet al., 2004). With the development as well as the extensive applications of multiprocessor and multithreading computational technique, the 'exhaustive' methods have been more feasible. At present study, based on the above concerns, we proposed an exhaustive strategy

Author α σ ¥ § χ : Department of Epidemiology and Health Statistics, School of Public Health, Shandong University, Jinan 250012, China .
Author ρ : CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.
Author ρ : Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Chinese Academy of Sciences.
Author Ѡ: MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK.
Author ν : postal address: 44 Wenhuaxi Road, Jinan, China.
E-mail : xuefzh@sdu.edu.cn.

© 2012 Global Journals Inc. (US)

with ergodic window sizes along the genome no matter whatever the relationship is. Simulations are conducted to assess statistical powers under different window sizes, followed by a real data analysis to evaluate its performance.

Recently, several sliding-window approaches of GWAS have been developed, including PCA-based methods (Shaet al., 2009, Tanget al., 2009, Wanget al., 2009),P-value combination methods (Sun et al., 2009), haplotype-based methods (Trégouëtet al., 2009), and data mining methods (Jianget al., 2009). In particular, PCA-based methods have been proved to have better performance (Shaet al., 2009, Tanget al., 2009, Wang et al., 2009). We, therefore, proposed to apply PCA-based logistic model to perform the exhaustive methods.

II. Methods

a) Exhaustive sliding-window procedure

Consider a case-control study with total M individuals in a data set and assume each individual has been genotyped at N SNPs. Let $G_i \ \left(g_{i1}, g_{i2,} ..., g_{iN}\right)\left(i \ \ 1, 2, ..., M\right)$ denote the N SNP loci of the ith individual, where g_{ij} denote the genotype of the ith individual at j^{th} SNP. Let y_i denote the trait value of the individual i (1 for cases and 0 for controls).In the exhaustive sliding-window frame, we first set the largest window size L. Then, we scan the candidate region or the whole genome from the first SNP with sliding-window of all possible sizes s, ranging from 2 to L. (Notice that sliding-window approach with window size 1 is the same as single-locus association test.)

b) PCA-based logistic regression procedure

Let w_s^b denote the window with s neighboring SNPs $\{b, b+1, ..., b+s-1\}$ beginning from SNP b. To carry out the PCA in this region, we let Σ_s^b denote the sample variance-covariance matrix of genotypic numerical codes in window w_s^b and λ_j^b denote the jth largest eigenvalue of Σ_s^b. The cumulative contributing proportion of the total variability explained by the first k principal components (PCs)is $C = \left(\lambda_1^b + \lambda_2^b + ... + \lambda_k^b\right) / \left(\lambda_1^b + \lambda_2^b + ... + \lambda_s^b\right)$. The value of k can be chosen such that C exceeds a threshold (80% here). Then we can get our PCA-based logistic model as follows:

$$Logit[\Pr(D \quad 1 | PC_1, PC_2, ..., PC_k)] = \beta_0 + \beta_1 PC_1 + \cdots + \beta_k PC_k$$

where $\Pr\left(D \quad 1 | PC_1, PC_2, \ldots, PC_k\right)$ denotes the probabilities of disease given the first k PCs.

III. Simulation

To assess statistical powers with different window sizes and illustrate thenecessity of exhaustive sliding-windows, we conducted a statistical simulation based on HapMap data under the null hypothesis (H_0) and alternative hypothesis (H_1). The corresponding steps for the simulation are as follows:

Step1 : Download the phased haplotype data of a genome region from the HapMap web site (http://snp.cshl.org): we selected a region near the Protein tyrosine phosphatase, non-receptor type 22 (PTPN22) gene to generate the simulating genotype data of CEU population using HapMap Phase 1& 2 full dataset. This region is located at Chr 1: 114021124.. 114291292, including 96 SNPs. Figure 1shows their pairwise R^2 structure.

Step 2 : Based on the HapMap phased haplotype data, we generated large samples with 100,000 cases and 100,000 controls as CEU populations using the software HAPGEN (Marchini et al., 2007). Toinvestigate the performance of the exhaustive sliding window strategy on different causal SNPs with different minor allele frequencies (MAF), we defined two SNPs as the causal variant respectively: the 45th SNP (rs1746853, MAF=0.433) and the 46th SNP (rs2185827, MAF=0.208). To assess the indirect association with disease via correlated markers, we removed the causal SNP in the simulation. The SNPs in the simulated region were coded according to the additive genetic model.

Step 3 : For the remained SNPs, we set the window size from 2 to 15 for each sample. Single-locus association test was also performed for comparison (set window size as 1). To perform the exhaustive strategy, for each defined window size, all of the windows covering the causal SNP were considered. Correspondingly, single-locus association test was conducted on each of the SNPs involved in these windows. For example, for the causal SNPrs1746853 (45th) with window size 4, windows $\{42, 43, 44, 46\}$, $\{43, 44, 46, 47\}$, $\{44, 46, 47, 48\}$ were tested and the corresponding single-locus test was performed on $\{42, 43, 44, 46, 47, 48\}$ respectively.

For the exhaustive strategy, overlapping sliding windows and correlated neighboring SNPs were tested, which might lead to the issue of multiple testing. In present work, we employed simulations under H0 to construct the null distribution of this strategy, rather than correction methods, to solve the multiple comparison problem. Such simulations have been widely used to

© 2012 Global Journals Inc. (US)

establish significance levels while accounting for multiple tests (Zondervan & Cardon, 2004, Deng et al., 2009). For each set of parameters and a given false-positive error rate ($\alpha = 0.05$), 10000 replications were first generated to construct the null distribution and to determine the critical P value over the simulated region, that is, the smallest P-value of each replication over the simulated region were collected to form the null distribution. Based on the established critical values, we then assessed the power to detect the disease association under different relative risk levels (RR= 1.1, 1.2, 1.3, 1.4 and 1.5 per allele).

To investigate how the optimal window size depended on the underlying LD pattern, the density of genotyped SNPs was used as a surrogate for underlying LD of the region (i.e. the higher LD would be where every SNP is counted as a marker, and lower LD could come from only considering every three SNP). Specifically, we chose the 45th SNP as the causal SNP again, and selected every three SNP of the original data set, i.e. the $3^{rd}, 6^{th}, \ldots, 96^{th}$ SNPs. For this particular subset, we conducted the same simulation procedure as above.

Step 4 : For each window, we sampled the simulation data from the population and performed the PCA-based logistic regression under different sample sizes N ($N/2$ cases and $N/2$ controls, N $1000, 2000, \ldots, 5000$) using the R package *Design*(http://cran.rproject.org/web/packages/Design/index.html).

IV. Application

The proposed method was applied to rheumatoid arthritis (RA) data from GAW16 Problem 1. The data consisted of 2062 Illumina 550k SNP chips from 868 RA patients and 1194 normal controls collected by the North American Rheumatoid Arthritis Consortium (NARAC) (Plengeet al., 2007). At present study, only 1493 females (641 cases and 852 controls) were analyzed to avoid potential bias with the fact that rheumatoid arthritis is two to three times more common in women than in men(Firestein, 2003). We only analyzed chromosome 1 of the data.

Before the sliding-window approach, we excluded data from SNPs that had extensive missingness (missingness$>10\%$), deviations from Hardy-Weinberg equilibrium (<0.00001), and low minor allele frequency ($<0.2\%$) using the software PLINK (Purcellet al., 2007). After this quality control filtering, 38829 SNPs remained. No individuals were excluded for missingness. Then, we applied MACH to impute the missing data (Liet al., 2009).

V. Results

a) Data Simulation

i. Critical values under null distribution

Table 1, Table S1 and Table S2 display the critical values for the three cases (different MAF or different LD patterns) based on the given significant level of $\alpha = 0.05$ over the simulated region. For different sample sizes, the critical values for the same case and the same window size are almost identical. However, for different cases or different window sizes, the critical values are different.

ii. Power

Under the case of defining the 45th SNP (MAF=0.433) as the causal variant including every SNP in the region, Figure 2 shows the powers with different window sizes under different sample sizes at the given relative risk of 1.3, while Figure 3 shows the powers with different window sizes under different relative risks at the given sample size of 1000. As expected, the powers are monotonically increasing functions of sample sizes and the relative risk levels for each window size. With fixed sample size 2000 and the relative risk 1.3, Figure 4 shows the powers of PCA-based logistic model under different window sizes compared with the corresponding results of single-locus test. Generally, the sliding-window approach is more powerful than the corresponding single-locus test except for window size 11, and the optimal window size is 10.

Under the case of defining the 46th SNP (MAF=0.208) as the causal variant including every SNP in the region, Figure 5 shows the powers of PCA-based logistic model under different window sizes compared with the corresponding result of single-locus test. The optimal window size is 3. Nevertheless, the single-locus tests are more powerful for other window sizes.

Under the case of defining the 45th SNP (MAF=0.433) as the causal variant including every three SNP in the region, which creates different LD pattern between SNPs by adjusting the density of SNPs, Figure 6 shows the powers of PCA-based logistic model under different window sizes compared with the corresponding results of single-locus test. In this case, the sliding-window approach is less powerful than the corresponding single-locus test except for window size 4.

These simulation results indicate that the powers of both sliding-window approach and single-locus test are influenced by the minor allele frequency of the causal SNP as well as the LD pattern between SNPs, and it is difficult to decide the optimal window size.

b) Application

Figure 7 shows the exhaustive results to the chromosome 1 of the RA data with window sizes from 1 to 20. The 'win=1' panel denotes the scan results from single-locus association test, while the other panels (from win=2 to win=20') denote the results from PCA-

© 2012 Global Journals Inc. (US)

based logistic model. It is clear that the rs2476601 SNP within PTPN22 gene region was detected at 10^{-7} level (p-value=2.30*10^{-8}) by single-locus association test, which has been identified association with RA (Kllberget al., 2007, Begovichet al., 2004, Carltonet al., 2005). However, when the sliding window size was from 2 SNPs to 9 SNPs, no region showed significant at 10^{-7} level, while the same significant region with the rs2476601 SNP involved was re-detected when the sliding window size was from 10 SNPs to 20 SNPs at 10^{-8} level. On the other hand, the p-values were similar when window sizes ranged from 10 to 20.

VI. Discussion

As the potential theoretical relationship between the optimal window size and LD is difficult to clarify, and the LD varies across the whole genome, the LD-based sliding-window approaches (Browning, 2006,Li et al., 2007, Tang etal., 2009)may not be always the optimal strategy. We, therefore, proposed an exhaustive strategy with ergodic window sizes along the genome no matter whatever the relationship is. Simulation results suggest that, althoughthe powers are monotonically increasing functions of sample sizes and the relative risk levels for each window size, the powers are also influenced by various factors, including MAF of the causal SNP, LD pattern between the SNPs and window sizes. From Figure 4 and Figure 5, the sliding-window approach seems more powerful than the corresponding single-locus test, but the optimal window sizes are various and heavily depend on the data. Further comparison between Figure 4 and Figure 5 shows that sliding-window approach is generally much more powerful than the corresponding single-locus test when the MAF of the causal SNP is higher. However, this may be attributed to different LD patterns which can be affected by the MAF of causal SNP. To investigate whether the powers are influenced by the LD patterns, we design additional simulations by adjusting the density of SNPs under the same MAF of the causal SNP (0.433). Clearly, the optimal window size changes from 10 to 1 (i.e. single-locus test) when the LD is lower. All the simulation results suggest that it is difficult to determine the optimal window size because it is influenced by so many factors. We, therefore, propose the exhaustive sliding-window strategy to detect various associated genome region with the disease.Real data analysis (Figure 7) results indicate that the p-values with different window sizes are also quite different. In particular, single-locus association test (win=1) identifies a significant SNP (rs2476601) at 10^{-7} level (p-value=2.30*10^{-8}), which has been detected as a RA-associated variation by different methods (Kllberget al., 2007, Begovichet al., 2004, Carltonet al., 2005). However, when the sliding window size is from 2 SNPs to 9 SNPs, no region shows significant at 10^{-7} level, while the same significant region with the rs2476601 SNP involved is re-detected when the sliding window size is from 10 SNPs to 20 SNPs at 10^{-8} level. In practice, it is difficult to capture whole information in genome using the approaches with fixed window size (Yanget al., 2009, Manentiet al., 2009) or with variable window sizes (Liet al., 2007, Tang et al., 2009, Browning, 2006) by the specific algorithms. Our exhaustive strategy does not require a prior knowledge of the optimal window size and genetic factors, such as MAF, LD patterns. On the other hand, both fixed and variable sliding-window approach are just special cases of the exhaustive strategy.Thus, our exhaustive sliding-window strategy is reasonable and essential in GWAS.

Recently, cluster computer, usually known as a multiprocessor based on the chip multithreading architecture, has been widely used for scientific applications. It is very fast because several central processing units (CPUs) inside it can each execute a task's instructions independently of the others. In this article, we used this cluster computer system, and run each sliding-window scan task with one CPU simultaneously. When exhaustively scanning the chromosome 1 of the RA data, it took less than half an hour, so it would only take about 10 hours if scanning the whole genome. Therefore, the proposed exhaustive strategy combined with the cluster computer technique is computationally efficient and feasible for GWAS data analysis.

There are several limitations about the proposed method. First, the proposed exhaustive strategy is still less powerful when the effect of the causal SNP is minor (e.g. relative risk is less than 1.2), and only one causal SNP is considered in present work. Second, the frequencies of both causal SNPs selected are higher than 0.05, so it is hard to decide whether the proposed method is powerful for rare variants. Further work to solve such problems will certainly be warranted.

VII. Acknowledgements

This work was supported by the grant from National Natural Science Foundation of China (30871392). We thank NARAC for supplying us with the data.

References Références Referencias

1. Begovich, A., Carlton, V., Honigberg, L., Schrodi, S., Chokkalingam, A., Alexander, H., Ardlie, K., Huang, Q., Smith, A. & Spoerke, J., 2004. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. The American Journal of Human Genetics. 75, 330-337.
2. Browning, S.R., 2006. Multilocus association mapping using variable-length Markov chains. American Journal of Human Genetics. 78, 903-913.
3. Carlton, V., Hu, X., Chokkalingam, A., Schrodi, S., Brandon, R., Alexander, H., Chang, M., Catanese, J., Leong, D. & Ardlie, K. ,2005. PTPN22 genetic

© 2012 Global Journals Inc. (US)

variation: evidence for multiple variants associated with rheumatoid arthritis. The American Journal of Human Genetics. 77, 567-581.
4. Deng, H.W., Guo, Y.F., Li, J., Bonham, A.J. & Wang, Y.P. , 2009. Gains in power for exhaustive analyses of haplotypes using variable-sized sliding window strategy: a comparison of association-mapping strategies. Eur J Hum Genet.17, 785-792.
5. Firestein, G.S. , 2003. Evolving concepts of rheumatoid arthritis. Nature. 423, 356-61.
6. Jiang, R., Tang, W., Wu, X. & Fu, W. , 2009. A random forest approach to the detection of epistatic interactions in case-control studies. BMC bioinformatics. 10, S65.
7. Kllberg, H., Padyukov, L., Plenge, R., R Nnelid, J., Gregersen, P., Van Der Helm-Van Mil, A., Toes, R., Huizinga, T., Klareskog, L. & Alfredsson, L. ,2007. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. The American Journal of Human Genetics. 80, 867-875.
8. Li, Y., Sung, W.K. & Liu, J.J. ,2007. Association mapping via regularized regression analysis of single-nucleotide-polymorphism haplotypes in variable-sized sliding windows. Am J Hum Genet. 80, 705-15.
9. Li, Y., Willer, C., Sanna, S. & Abecasis, G. ,2009. Genotype imputation. Annual review of genomics and human genetics. 10, 387.
10. Lin, S., Chakravarti, A. & Cutler, D. ,2004. Exhaustive allelic transmission disequilibrium tests as a new approach to genome-wide association studies. Nature genetics. 36, 1181-1188.
11. Manenti, G., Galvan, A., Pettinicchio, A., Trincucci, G., Spada, E., Zolin, A., Milani, S., Gonzalez-Neira, A. & Dragani, T.A. ,2009. Mouse genome-wide association mapping needs linkage analysis to avoid false-positive Loci. PLoS Genet. 5, e1000331.
12. Marchini, J., Howie, B., Myers, S., Mcvean, G. & Donnelly, P. ,2007. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet. 39, 906-13.
13. Plenge, R., Seielstad, M., Padyukov, L., Lee, A., Remmers, E., Ding, B., Liew, A., Khalili, H., Chandrasekaran, A. & Davies, L. ,2007. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. New England Journal of Medicine. 357, 1199-1209.
14. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M., Bender, D., Maller, J., Sklar, P., De Bakker, P. & Daly, M. ,2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics. 81, 559-575.
15. Sha, Q., Tang, R. & Zhang, S. ,2009. Detecting susceptibility genes for rheumatoid arthritis based on a novel sliding window approach. BMC Proc. 3 Suppl 7, S14.
16. Sun, Y., Jacobsen, D., Turner, S., Boerwinkle, E. & Kardia, S. ,2009. Fast implementation of a scan statistic for identifying chromosomal patterns of genome wide association studies. Computational statistics & data analysis. 53, 1794-1801.
17. Tang, R., Feng, T., Sha, Q. & Zhang, S. ,2009. A variable-sized sliding-window approach for genetic association studies via principal component analysis. Ann Hum Genet. 73, 631-7.
18. Trégouët, D., König, I., Erdmann, J., Munteanu, A., Braund, P., Hall, A., Gro Hennig, A., Linsel-Nitschke, P., Perret, C. & Desuremain, M. ,2009. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nature genetics. 41, 283-285.
19. Wang, X., Qin, H. & Sha, Q. ,2009. Incorporating multiple-marker information to detect risk loci for rheumatoid arthritis. BMC Proc. 3 Suppl 7, S28.
20. Yang, H.C., Liang, Y.J., Wu, Y.L., Chung, C.M., Chiang, K.M., Ho, H.Y., Ting, C.T., Lin, T.H., Sheu, S.H., Tsai, W.C., Chen, J.H., Leu, H.B., Yin, W.H., Chiu, T.Y., Chen, C.I., Fann, C.S., Wu, J.Y., Lin, T.N., Lin, S.J., Chen, Y.T., Chen, J.W. & Pan, W.H. ,2009. Genome-wide association study of young-onset hypertension in the Han Chinese population of Taiwan. PLoS One. 4, e5459.
21. Yang, H.C., Lin, C.Y. & Fann, C.S. ,2006. A sliding-window weighted linkage disequilibrium test. Genet Epidemiol. 30, 531-45.
22. Zondervan, K.T. & Cardon, L.R. ,2004. The complex interplay among factors that influence allelic association (vol 5, pg 89, 2004). Nat Rev Genet. 5, 238-238.

© 2012 Global Journals Inc. (US)

Table 1 : Empirical critical values (α=0.05) of the exhaustive sliding-window approach for MAF=0.433 including every SNP under different window sizes and their corresponding single locus test.

Window size	Sample size				
	1000	2000	3000	4000	5000
win=2	0.053(0.042)	0.049(0.040)	0.051(0.041)	0.048(0.037)	0.047(0.038)
win=3	0.044(0.018)	0.039(0.015)	0.040(0.016)	0.038(0.016)	0.038(0.015)
win=4	0.039(0.016)	0.036(0.014)	0.039(0.014)	0.038(0.014)	0.037(0.014)
win=5	0.034(0.013)	0.032(0.011)	0.032(0.011)	0.032(0.011)	0.028(0.010)
win=6	0.032(0.013)	0.030(0.011)	0.030(0.011)	0.031(0.011)	0.027(0.011)
win=7	0.031(0.012)	0.028(0.011)	0.028(0.011)	0.028(0.011)	0.024(0.009)
win=8	0.029(0.012)	0.026(0.010)	0.027(0.010)	0.027(0.010)	0.026(0.009)
win=9	0.027 (0.012)	0.025(0.010)	0.024(0.010)	0.024(0.010)	0.025(0.010)
win=10	0.026(0.012)	0.023(0.010)	0.022(0.010)	0.023(0.010)	0.022(0.009)
win=11	0.033(0.011)	0.029(0.009)	0.028(0.009)	0.028(0.009)	0.028(0.010)
win=12	0.033(0.009)	0.029(0.008)	0.029(0.008)	0.029(0.008)	0.028 (0.007)
win=13	0.032(0.009)	0.028(0.008)	0.027(0.007)	0.027(0.007)	0.027(0.007)
win=14	0.031(0.008)	0.027 (0.007)	0.026(0.007)	0.026 (0.007)	0.027(0.007)
win=15	0.033(0.008)	0.028(0.007)	0.029(0.007)	0.028(0.007)	0.028(0.007)

Note: the number in ( ) denotes the results of the corresponding single locus test

Table 2 : Empirical critical values (α=0.05) of the exhaustive sliding-window approach for MAF=0.208 including every SNP under different window sizes and their corresponding single locus test.

Window size	Sample size				
	1000	2000	3000	4000	5000
win=2	0.052(0.025)	0.049(0.025)	0.056(0.025)	0.057(0.025)	0.045(0.023)
win=3	0.030(0.014)	0.032(0.014)	0.025(0.012)	0.022(0.012)	0.027(0.015)
win=4	0.036(0.012)	0.035(0.011)	0.035(0.010)	0.032(0.009)	0.034(0.011)
win=5	0.035(0.012)	0.032(0.011)	0.031(0.010)	0.031(0.009)	0.031(0.011)
win=6	0.028(0.012)	0.025(0.011)	0.026(0.010)	0.023(0.009)	0.027(0.010)
win=7	0.030(0.011)	0.027(0.010)	0.027(0.009)	0.025(0.008)	0.026(0.009)
win=8	0.029(0.011)	0.026(0.009)	0.026(0.009)	0.023(0.007)	0.025(0.009)
win=9	0.027 (0.011)	0.025(0.009)	0.025(0.009)	0.021(0.007)	0.022(0.009)
win=10	0.028(0.011)	0.026(0.009)	0.026(0.009)	0.023(0.007)	0.026(0.008)
win=11	0.032(0.009)	0.030(0.008)	0.028(0.007)	0.025(0.006)	0.026(0.006)
win=12	0.032(0.008)	0.029(0.007)	0.028(0.006)	0.025(0.005)	0.024 (0.005)
win=13	0.031(0.007)	0.029(0.006)	0.027(0.006)	0.025(0.005)	0.025(0.005)
win=14	0.027(0.007)	0.026 (0.006)	0.024(0.006)	0.021 (0.005)	0.022(0.005)
win=15	0.031(0.007)	0.029(0.006)	0.026(0.006)	0.022(0.005)	0.022(0.005)

Note: the number in ( ) denotes the results of the corresponding single-locus test.

© 2012 Global Journals Inc. (US)

Table 3 : Empirical critical values (α=0.05) of the exhaustive sliding-window approach for MAF=0.433 including every three SNP under different window sizes and their corresponding single locus test.

Window size	1000	2000	3000	4000	5000
win=2	0.041(0.041)	0.035(0.035)	0.027(0.027)	0.023(0.023)	0.021(0.021)
win=3	0.032(0.020)	0.026(0.015)	0.020(0.012)	0.018(0.010)	0.014(0.008)
win=4	0.020(0.009)	0.015(0.006)	0.009(0.004)	0.007(0.003)	0.006(0.002)
win=5	0.024(0.008)	0.017(0.006)	0.011(0.004)	0.009(0.002)	0.007(0.002)
win=6	0.021(0.007)	0.015(0.005)	0.010(0.003)	0.007(0.002)	0.006(0.002)
win=7	0.021(0.007)	0.015(0.005)	0.009(0.003)	0.007(0.002)	0.005(0.002)
win=8	0.019(0.006)	0.013(0.004)	0.008(0.002)	0.006(0.002)	0.004(0.002)
win=9	0.022 (0.005)	0.015(0.003)	0.009(0.002)	0.007(0.002)	0.005(0.001)
win=10	0.019(0.005)	0.013(0.003)	0.008(0.002)	0.006(0.001)	0.005(0.001)
win=11	0.019(0.004)	0.012(0.003)	0.008(0.002)	0.006(0.001)	0.005(0.001)
win=12	0.018(0.004)	0.012(0.003)	0.008(0.002)	0.006(0.001)	0.005 (0.001)
win=13	0.017(0.004)	0.012(0.003)	0.008(0.002)	0.005(0.001)	0.004(0.001)
win=14	0.017(0.004)	0.012 (0.002)	0.008(0.001)	0.006 (0.001)	0.004(0.001)
win=15	0.017(0.003)	0.012(0.002)	0.008(0.001)	0.006(0.001)	0.004(0.001)

Note: the number in ( ) denotes the results of the corresponding single-locus test.

Figure Legends

.

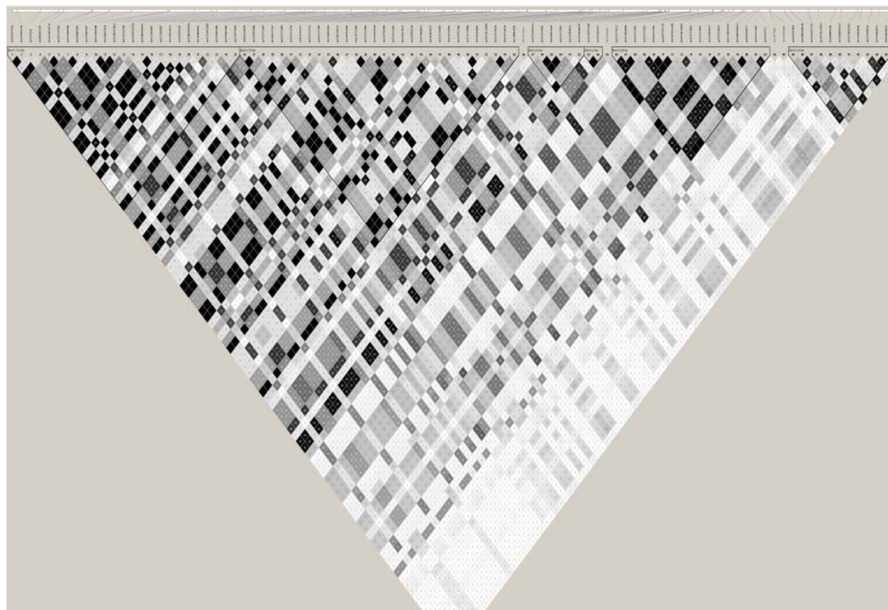


Figure 1 : Pairwise R^2 among the SNPs in the selected region.

© 2012 Global Journals Inc. (US)

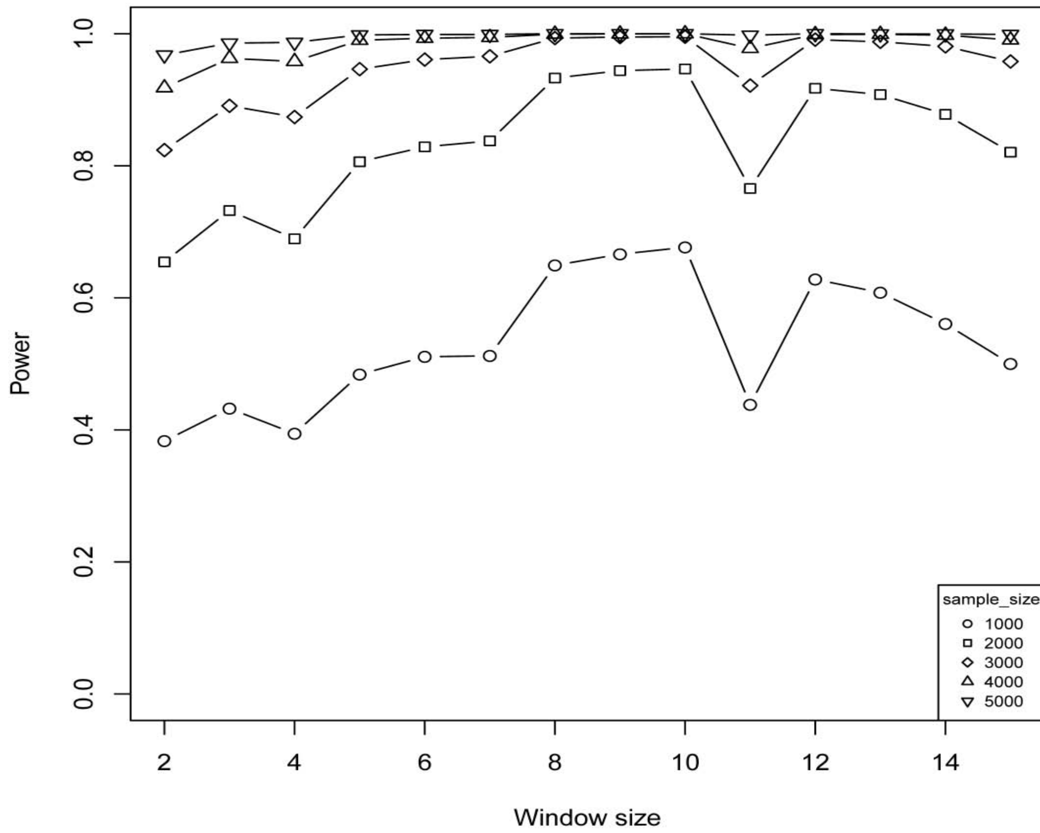


Figure 2 : The powers of the exhaustive sliding-window approach under different window sizes (2-15) and different sample sizes. The horizontal axis denotes the window sizes and the vertical axis denotes the powers of PCA-based logistic regression model with different window sizes.

© 2012 Global Journals Inc. (US)

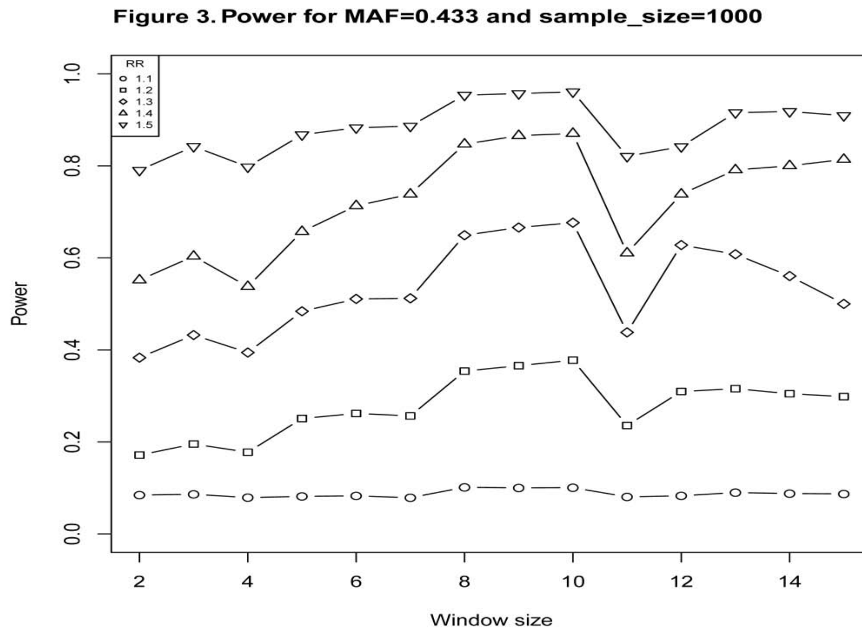


Figure 3 : The powers of the exhaustive sliding-window approach under different window sizes (2-15) and different relative risks. See Figure 2 for the figure legends.

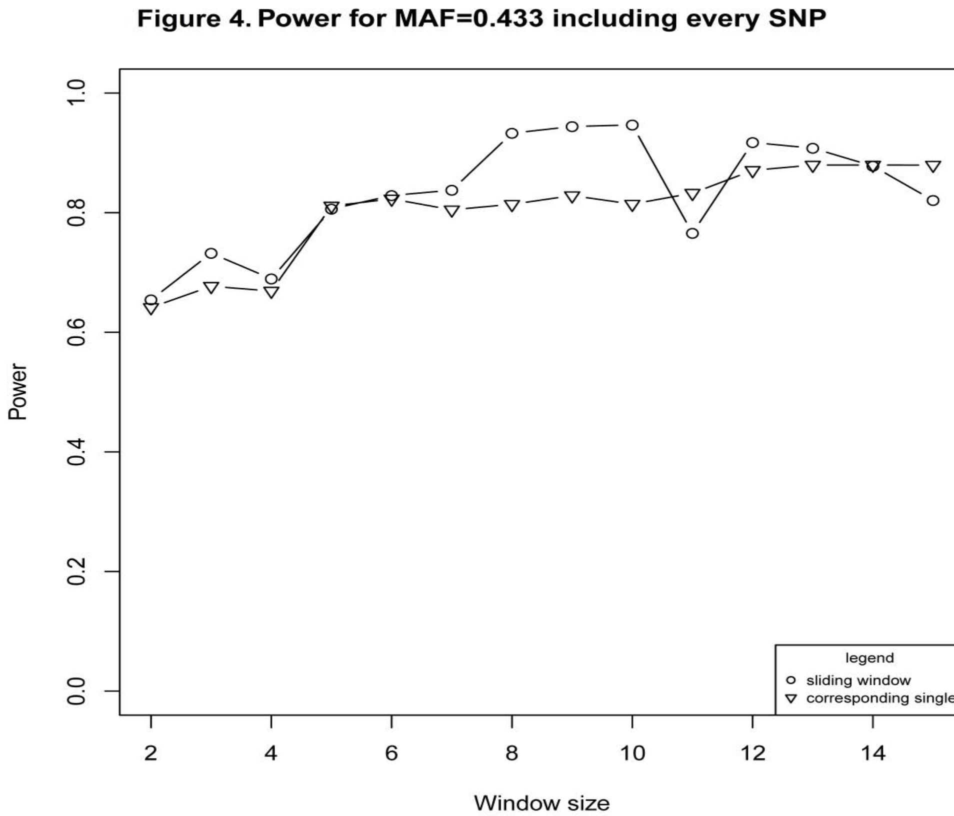


Figure 4 : The powers of the exhaustive sliding-window approach for MAF=0.433 including every SNP under different window sizes and their corresponding single locus test. See Figure 2 for the figure legends.

© 2012 Global Journals Inc. (US)

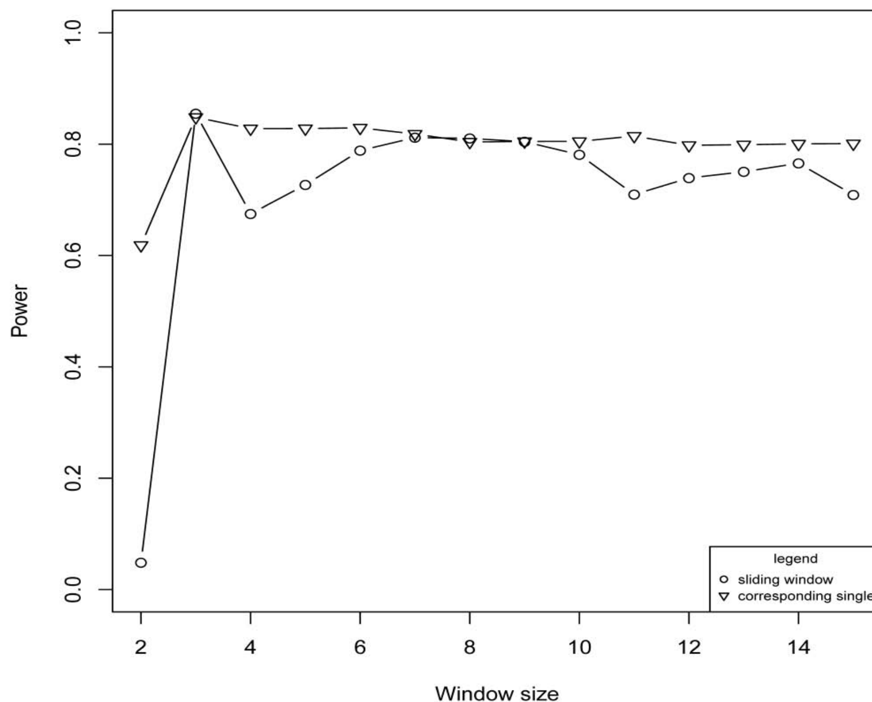


Figure 5 : The powers of the exhaustive sliding-window approach for MAF=0.208 including every SNP under different window sizes and their corresponding single locus test. See Figure 2 for the figure legends.

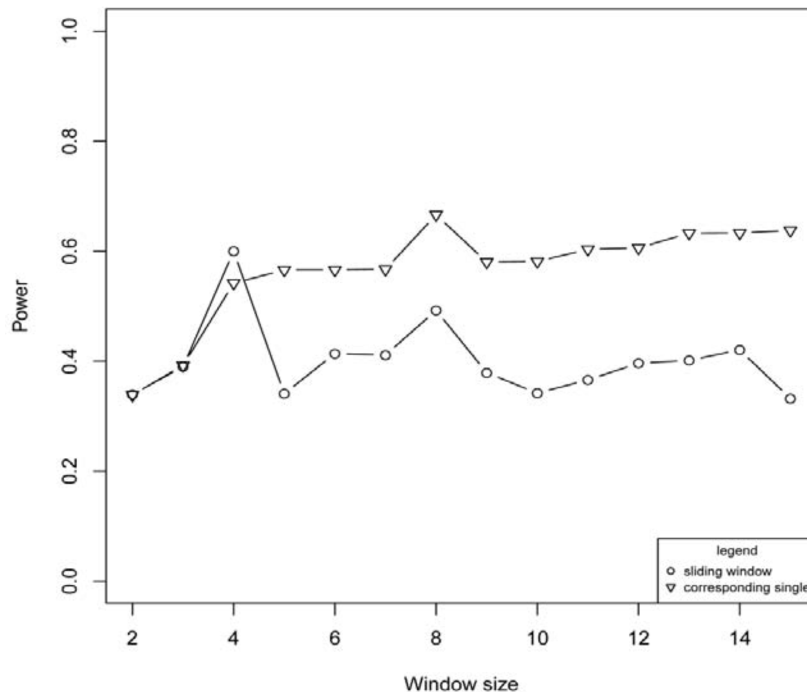


Figure 6 : The powers of the exhaustive sliding-window approach for MAF=0.433 including every three SNP under different window sizes and their corresponding single locus test. See Figure 2 for the figure legends.

© 2012 Global Journals Inc. (US)

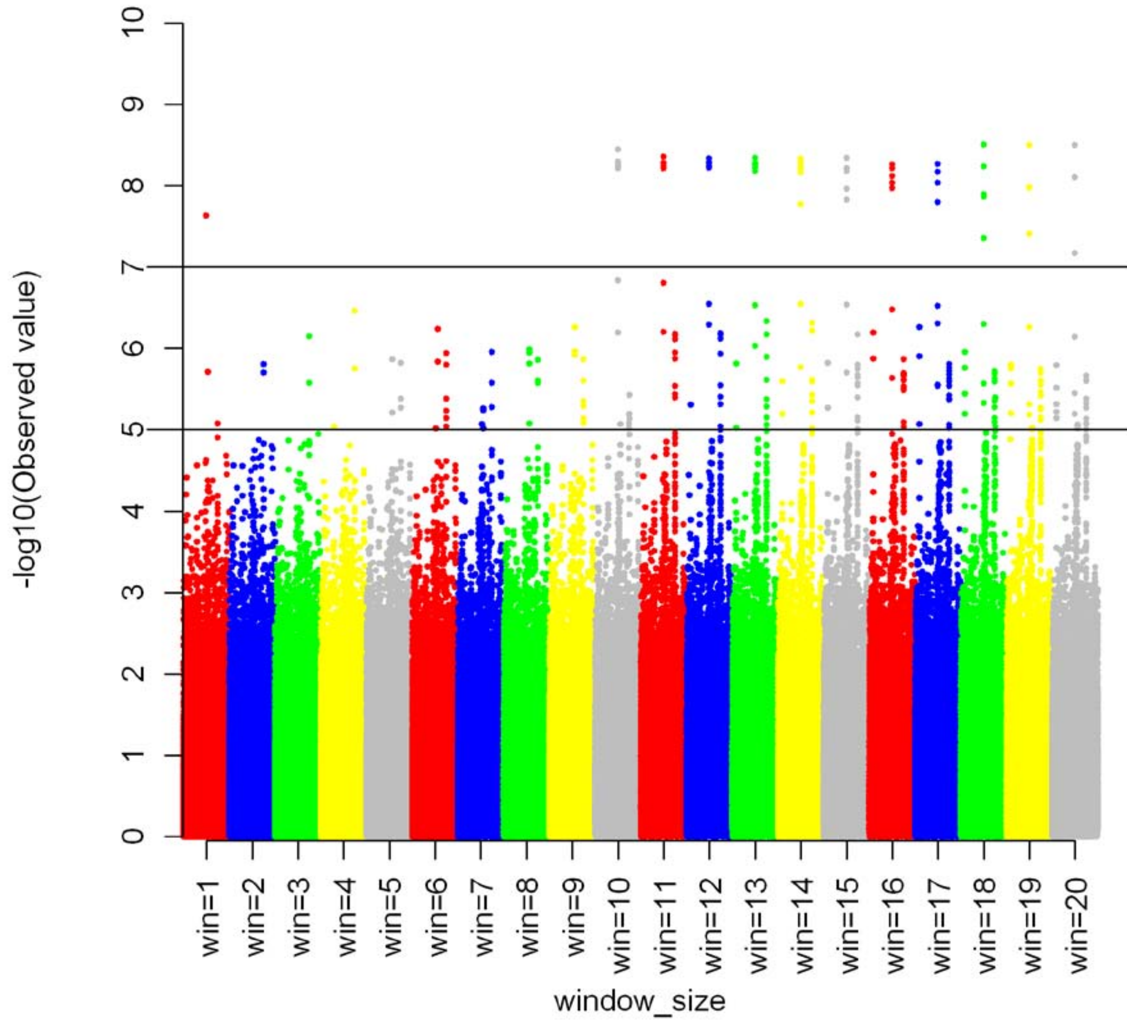


Figure 7 : The results for chromosome 1 of the RA data using single-locus association test (the 'win=1' panel) and exhaustive sliding-windows (from panel 'win=2' to panel 'win=20'.)

© 2012 Global Journals Inc. (US)

This page is intentionally left blank

© 2012 Global Journals Inc. (US)

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 12 Issue 4 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

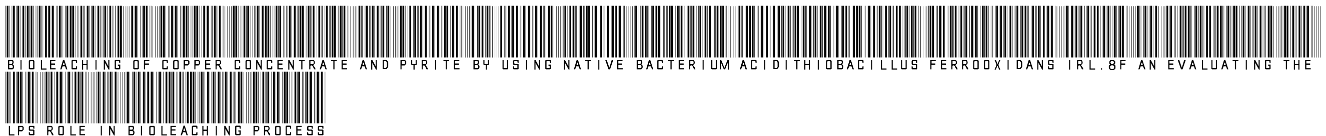
Bioleaching of Copper Concentrate and Pyrite by Using Native Bacterium Acidithiobacillus Ferrooxidans IRL.8F and Evaluating the LPS Role in Bioleaching Process

By Dr. Ali Mohammad Latifi , Ahmadi . M & Olad .G
Baqiyatallah Medical Sciences University

Abstract - This study was performed to evaluate the ability of native bacterium to extract copper and iron from their ores. This bacterium was isolated from iron mineral springs in Iran's Larzan region and was named Acidithiobacillus ferrooxidans IRL.8F based on morphological and physiological characteristics and 16S rRNA molecular analyses. The results from bioleaching of copper concentrate showed that the amount of extracted copper and iron was 71.4% and 29.3%, respectively. Furthermore, in comparison with control samples, these amounts increased by 93.5% and 92%, respectively. In the control samples minor amount of metals were extracted due to spontaneous leaching. To assess the importance of bacterial lipopolysaccharydes(LPS) role, LPS of bacterium was removed. When ethylenediaminetetraacetic acid (EDTA) in concentrations of 5 and 10% was used during the bioleaching process of pyrite, process efficiency decreased to 61 and 70%, respectively. The cells lacking LPS were led to 59.4 % decrease in the amount of bacterial leaching, in contrast to whole cells. Therefore, it can be concluded that: 1. EDTA causes a drastic reduction in the efficiency of leaching process, 2. Bacterial LPS have a key role in attachment to particles of ore and 3.This bacterium is capable of leaching metals through the direct mechanism.

Keywords : Bioleaching, LPS, Ore, Copper, Pyrite.

GJSFR-G Classification : FOR Code: 060501



Strictly as per the compliance and regulations of :

© 2012 By Dr. Ali Mohammad Latifi , Ahmadi . M & Olad .G. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Bioleaching of Copper Concentrate and Pyrite by Using Native Bacterium Acidithiobacillus Ferrooxidans IRL.8F and Evaluating the LPS Role in Bioleaching Process

Dr. Ali Mohammad Latifi [α] , Ahmadi . M [σ] & Olad .G [ρ]

Abstract - This study was performed to evaluate the ability of native bacterium to extract copper and iron from their ores. This bacterium was isolated from iron mineral springs in Iran's Larzan region and was named *Acidithiobacillus ferrooxidans IRL.8F* based on morphological and physiological characteristics and 16S rRNA molecular analyses. The results from bioleaching of copper concentrate showed that the amount of extracted copper and iron was 71.4% and 29.3%, respectively. Furthermore, in comparison with control samples, these amounts increased by 93.5% and 92%, respectively. In the control samples minor amount of metals were extracted due to spontaneous leaching. To assess the importance of bacterial lipopolysaccharydes(LPS) role, LPS of bacterium was removed. When ethylenediaminetetraacetic acid (EDTA) in concentrations of 5 and 10% was used during the bioleaching process of pyrite, process efficiency decreased to 61 and 70%, respectively. The cells lacking LPS were led to 59.4 % decrease in the amount of bacterial leaching, in contrast to whole cells. Therefore, it can be concluded that: 1. EDTA causes a drastic reduction in the efficiency of leaching process, 2. Bacterial LPS have a key role in attachment to particles of ore and 3.This bacterium is capable of leaching metals through the direct mechanism.

Keywords : Bioleaching, LPS, Ore, Copper, Pyrite.

I. Introduction

Bioleaching is a general term used to refer to the conversion of insoluble to soluble metals (usually in sulfated form) through biological oxidation by using microorganisms (Rawlings., 2002; Makita et al., 2004).

Bacteria of the genus Thiobacillus, like Thiobacillus ferrooxidans retrieve the energy from ores via enzymatic oxidation. Biological oxidation of sulfide ores and the electron transport occur in three forms, including direct (or enzymatic or contact), indirect (mediated by compounds such as Fe^{3+} ions) and cooperative (which includes both direct and indirect) mechanisms. In the indirect mechanism, Fe^{3+} iron plays major role, while in the direct mechanism, the bacterium should have access to the ore, bind it and then the reaction will occur at the ore-water interface (Donati and Sand, 2007; Wolfgang and Edgardo, 2007). In this mechanism, the microbial attachment to the ore surface is necessary for the bioleaching process. As the micro-organism approaches.

The minerall, the cell surface changes and this accompanies with expression of extracellular polymeric substances (EPS) which lead to the attachment (Scobar et al., 1997; Clausen, 2003). EPS forms chemical bonds with the surface and mediates or promotes respiration and nutritional chemical reactions (Scobar et al., 1997). These bonds are made stronger with the attachment of microorganism to the ore and the reactions are followed by oxidation of reduced mineral compounds or reduced Fe^{2+} or sulfate ions. Attaching to the ores may be mediated by forming EPS on the surface of solid particles such as lipopolysaccharide (LPS), phospholipids or other macromolecules like the polypeptides in the outer membrane of the bacterium. These compounds are released by the organism when it is in contact with the ores (Donati and Sand., 2007; Scobar et al., 1997). The Mechanism of the electron transport from pyrite to molecular oxygen has been identified in detail. The primary stages occur in the EPS, in which electrons are extracted by means of the Fe^{3+} ion in complex with glucoronic acid (Rangin and Basu., 2004). Attachment to hydrophobic substrates such as sulfur is mediated by van der Waals forces, while for binding to charged substrates like pyrite, cations or molecules which act as Lewis acids accept the un-charged electron pair of the pyrite sulfur, followed by formation of a complex between different iron species and the exopolysaccharide and finally the attachment of the bacterium to the substrate (Gehrke et al,. 1998)

II. Materials and Methods

a) Media

Types and compositions of the media used for culturing, isolating and screening included: (1)SF or T.F.

Author α σ ρ : Baqiyatallah Medical Sciences University
E-mail : amlatify@yahoo.com

© 2012 Global Journals Inc. (US)

medium containing: Solution A: K_2HPO_4 (0.5g/l), $(NH_4)_2SO_4$ (0. 5g/l), $MgSO_4$ (0.5g/l), H_2SO_4 0.5M (5 ml/l) and D.W (1000ml) ; Solution B: $FeSO_4 . 7H_2O$ (167g/ l), H_2SO_4 . 0.5M (50 ml) and D.W (1000ml).One unit volume of solution B is mixed with four unit volumes of solution A and the pH is adjusted on 2-2.5 by using H_2SO_4 . 0.5M. (2)TSB medium containing: KH_2PO_4 (3g/l), $(NH_4)_2SO_4 . 7H_2O$ (0.4 g/l), $MgSO_4 . 7H_2O$ (0.5 g/l), $CaCl_2 . 2H_2O$ (0.25 g/l), $FeSO_4 . 7H_2O$ (0.01g/l), $Na_2S_2O_3 . 5H_2O$ (5 g/l), Agar powder (16g/l) and D.W (1000 ml).(3)TTB medium which contains (g/l) : ($(NH_4)_2SO_4$ 0.3; K_2HPO_4 , 0.5; $MgSO_4.7H_2O$, 0.5; and 0.5M H_2SO_4 .After autoclaving, sterilizing and cooling the medium, 5% sulfur powder separately sterilized in an aluminum foil, was added(Chen and Lin, 2000; Sasaki et al., 2009). During the preparation of these media, the iron sulfate was sterilized with a (0.22μ) filter and added to the solution. The cells were collected from 10-day media centrifuged in 50 ml falcon tubes at 15000 rpm for 20 min. (Elzeky an

III. The Bacterium

The bacterium used in this study was isolated from mineral springs in Larzan, Qazvin province, Iran. With this purpose, the mixed samples of water and precipitations deposited at the bottom of the spring were collected, transferred to the laboratory and incubated into 250 ml Erlenmeyer flasks containing 50 ml of broth TF and TT media (respectively containing elemental iron and sulfur as the sole sources of electron and energy). The samples were placed in shaker incubator at 30°C and in 200 rpm for 7 days, and then recultured in fresh media. The oxidation power of Fe and S elements were evaluated. During the cell culture period, essential parameters including the daily measure of pH, titration of produced acid, solution rate of elemental sulfur in medium, the amount of oxidized Fe, macroscopic and microscopic study of samples and counting and calculating the cell concentration were also considered or evaluated.

IV. Oxidation of Fe^{2+} to Fe^{3+}

The oxidation of Fe^{2+} by the bacterium was investigated in a 250 ml Erlenmeyer flask containing 50 ml SFB medium. With this purpose, 5 ml of 14-day bacterium culture (comprising $\sim 9\times10^8$ cells) was inoculated into the medium and incubated at 30°C at 200 rpm. The control was without bacterium inoculation. The initial pH was adjusted to 2.5 using 0.5M sulfuric acid. As Fe^{2+} is oxidized to Fe^{3+} iron, the medium turns from lime green to yellow, brown and brick red. Orth-ophenanthroline method and atomic adsorption spectroscopy analysis systems were used to analyze the iron. The total iron content (Fe^{2+} + Fe^{3+}), the converted Fe^{2+} iron to Fe^{3+} and the Fe^{3+} content of the medium were measured.

V. Sulfur Oxidation and Sulfuric Acid Production

The medium in this study was TTB. The inoculation and growth conditions were similar to those of iron oxidation. The initial pH was adjusted to 4.5. Sulfur oxidation, pH reduction and sulfuric acid production were measured. The control was prepared in a similar way without bacterium inoculation. The sulfuric acid content of the medium was measured after the drastic decrease of pH by titration using 0.1 M NaOH.

VI. Bacteria Identification By 16s Rrna

To identify the bacteria, the sequencing of 16S rRNA gene fragments was applied. Considering that these bacteria have a slow growth rate and are extremophilic species, the alkaline lysis and lysosyme methods were used in hybrid to extract their genomes. At first, the bacterial genomes were purified. With this purpose, 500 ml of 7-day culture of bacteria was prepared in TTB medium. The sample was centrifuged at 15000 rpm and the bacterial biomass was obtained. One hundred microliter of the SET cold buffer was added to the bacteria and 100μl of lysosyme was added to the above mixture and it was vortexed thoroughly. The mixture was incubated at 37°C for 30 min, and then, 200μl of lysis buffer (NaOH (5M), SDS (10%), H_2O) was added to the mixture and placed in ice for 10 min. In the next stage, as much phenol as the volume of the solution in the tube was added. The mixture was blended thoroughly and centrifuged at 10000 rpm and 4°C for 3 min. Furthermore, the supernatant was transferred to another tube and as much as its volume, phenol-chloroform (1:1) was added. The mixture was centrifuged again in a similar way as mentioned above. The supernatant was transferred to another tube, chloroform was added as much as its volume and the sample was again centrifuged as above. The supernatant was removed and isopropanol stored at -20°C was added as much as 0.6 of volume of the supernatant. This solution was stored at -20°C for 1 h. In the next stage, the sample was centrifuged at 14000 rpm in 4°C for 15 min. Isopropanol was immediately removed and 1 ml of 70% alcohol was added and then the sample was centrifuged at 14000 rpm at 4°C for 10 min. The sample was drought at room temperature and 20 to 30μl of TE buffer or distilled water and 3 to 5μl of RNase A was added. The tube containing the sample was stored at 37°C for 1 h and the sample was then stored at 4°C(Ohba and Owa., 2005). After electrophoresis, the PCR was performed. The primers required to identify the bacteria were universal primers with the following sequences (Hong et al.,2006 ; Yeats et al 1998): Forward: FORB: 5́ AGAGTTTGATCCTGGCTCAG3́

© 2012 Global Journals Inc. (US)

reverse : REVB: 5́ GGTTACCTTGTTACGACT3́. Using the purified genome as the template and the *Taq* polymerase, the 16S rRNA gene fragment was amplified as the defined program and the final product was investigated on agarose 1% gel. After confirming the quality of the PCR product, the samples were sequenced using Genetic analyzer 31030 - Accessories Applied Biosystem.

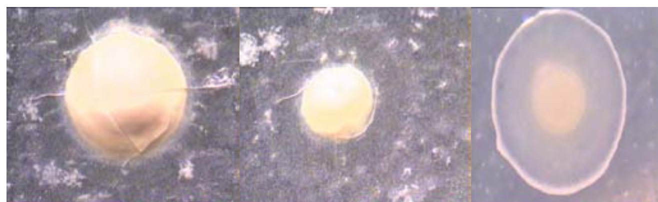


Figure 1 : A. ferrooxidans IRL.8F micro-colony and production of exopolysaccharide (white corona) on TSA medium (magnified ×100 – by A.M. Latifi). From right to left: fresh colony, semi- dried colony, dried colony.

The ore

The pyrite and copper ore were used in this study. The ore was powdered using the crusher or mortar-and-pestle, and the samples were prepared using special sieves with appropriate gridding. The elemental and compositional analysis of the ore powder

VII. Bioleaching Media and Conditions

To measure the metal produced from ore, 10 g of ore powder in flasks containing 250ml medium without any of energy resources (iron, sulfur, etc) was used. The base medium for all samples was 100ml water. The gridding of the particles was 200 and their

VIII. Study of The Effect of Edta on Bioleaching Process

To study the effect of EDTA on bioleaching process, four samples were prepared as followed: Sample 1: leaching medium without bacterium inoculation (as control), Sample 2: leaching medium inoculated with bacterium without EDTA, Sample 3: leaching medium inoculated with bacterium and EDTA (5%) and Sample 4: leaching medium inoculated with bacterium and EDTA (10%) test samples, the 0.2M EDTA solution was used.

All samples were placed in a shaker-incubator at 28°C and 200 rpm. Bacterium compatibility with new media was investigated by measuring pH of media during the bioleaching process. The initial pH at the start time of the process was also recorded. During its growth period, the bacterium reduces the pH and produces sulfuric acid through oxidation of the sulfur ore.

IX. LPS Removal In *Acidithiobacillus Ferrooxidans IRL.8F*

To remove the bacterial LPS, 0.2M EDTA and Tris-HCl at pH4.5 were used. (Ramadas et al., 1991; Scobar et al.,1997). Bacterial biomass was collected from 50 ml of bacterial suspension comprising a 10-day culture. The biomass was converted to a homogenous suspension in the EDTA and Tris-HCl solution and incubated at 37°C for 1 h. The tube containing the sample was then centrifuged at 11000 rpm and the supernatant containing EDTA, Tris-HCl and lipopolysaccharide (LPS) was removed. Bacterial cells lacking LPS were extracted from the solution containing EDTA and LPS by centrifugation at 11000 rpm for 10 min, and were inoculated with the bioleaching medium previously prepared .

X. Assessing The Activity of Lps-Lacking Bacteria In Oxidation of Fe^{2+}

Two samples containing TF medium with Fe^{2+} as the source of energy, were inoculated as follows: (1) The control in which the normal *A. ferrooxidans* IRL.8F was inoculated into the medium without any treatment, and (2) The test sample with LPS-lacking *A. ferrooxidans* IRL.8F inoculated into the medium.

To investigate the restoring of LPS production ability of the bacteria, they were collected from 10-day culture of the second sample and inoculated into the fresh TF medium.

XI. Assessing The Activity of Lps-Lacking Bacteria In Bioleaching Of The Iron From Pyrite Soil

To assess such an activity, the pyrite ore with mesh of 200 and mesh size of 0.074mm was used and 10g/l of the ore was added to each of Erlenmeyer flasks. The base medium of all samples was water. pH of all samples was adjusted on 4.5. Prepared samples included: leaching medium inoculated with normal bacteria (having LPS), leaching medium inoculated with LPS-lacking bacteria and leaching medium without any bacterium.

The samples were placed in a shaker-incubator at 200 rpm, and 25°C for 14 days, and after precipitation the supernatant was used to analyze the amount of

XII. Results

During screening stage, we could isolate a bacterial strain with remarkable enzymatic ability to oxidize the iron and sulfur as its sole energy and electron source. It is noteworthy that the mineral spring

© 2012 Global Journals Inc. (US)

from where the bacterium was isolated have a brick-red solution and fawn deposits. This results from the natural activity of the bacterium in oxidation of Fe^{2+} iron in the nearby soils to Fe^{3+}, leading to color change and generation of jarosite (iron hydroxide) deposits. The isolated bacterium produces small colonies similar to fried egg in TSA agar medium which are hardly visible with naked eyes. Applying an innovative method using optical microscope and simultaneous lighting from up and down in this study, we could produce high-quality pictures of bacterial colonies (Fig. 1).

Results obtained from morphological, physiologic and molecular identification based on 16S rRNA revealed that this bacterium is mostly similar to A. ferrooxidans strain. Therefore, the bacterial strain was named A. ferrooxidans IRL.8F. It is chemautotroph and uses CO_2 in the air as its carbon source. Fig 2 shows what was obtained from extraction of bacterial genome.

XIII . Fe Oxidation

The medium was observed to turn from lime green to brick red (confirming the conversion of Fe^{2+} to Fe^{3+}) (Fig. 3). After 18 h, the medium turns to yellow as a result of bacterial activity and the brick red color observed within 48 to 72 h represents complete oxidation of Fe^{2+} to Fe^{3+}. Results of cell counting showed that bacterial cell concentration has begun to increase when the color changes started and it increased from 9×10^8 cells per ml in the first 24-h period to 18×10^8 in the second day. Within this period, pH of the medium decreased from 2.5 to 2. Deposits in fawn color were observed on the wall of flask, which increased in amount daily. . They can trap the leached metals in the solution in their lattices and thereby disturb the bioleaching process. Meanwhile, this problem can be overcome by retaining the low pH.

This experiment demonstrates the bacterial capability to leaching the minerals containing iron compounds.

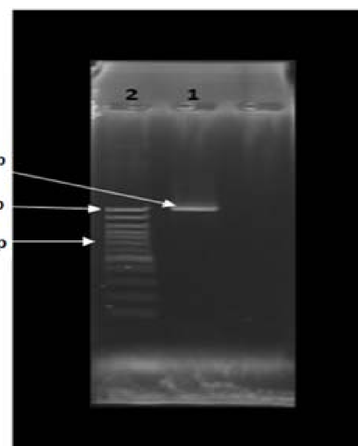


Figure 2 : Electrophoresis of the PCR product of IRL.F8 bacterium on 1% agarose gel. 1. PCR product, 2. DNA Ladder.

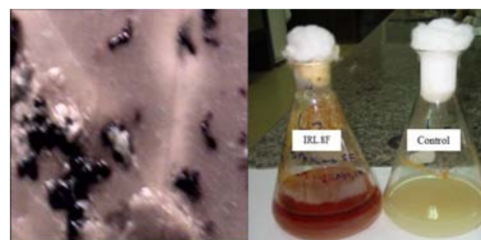


Figure 3 : Oxidation of Fe^{2+} to Fe^{3+} in SF broth medium and iron oxide particles formed on the colonies surface in the solid TSA medium (magnified ×100 – by A.M. Latifi).

XIV . Oxidation of Sulfur and Production of Sulfuric Acid

Results obtained from this experiment (Figure 4) revealed the high capability of the bacterium to produce acid, reduce the pH and make strong acidic conditions in TTB medium, such that in the third day the pH reached 1.5, in tenth day it decreased below 1 and in the 18th day it was 0.75. As the pH decreases, the number of bacterial cells progressively increased, such that it doubled (to 6.13×10^8) with pH decrease from 4 to 1.6 and it triples with pH decrease from 1.6 to 0.9. For the fact that the most populated cell colony is observed in 14th day, we used the 14-day suspension to produce the bacterial seed. The maximum acid production rate in 25th day is 20 g/l. The pH changes and the sulfuric acid production are shown in the plot(Figure 4) .

© 2012 Global Journals Inc. (US)

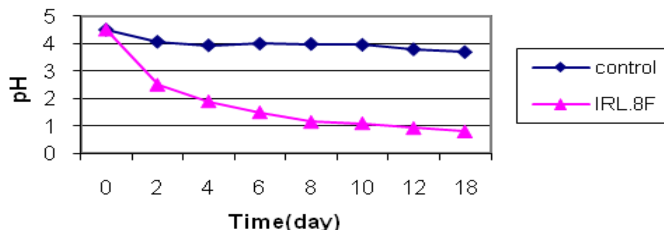


Figure 4 : Decrease of pH by the bacterium in TT broth medium.

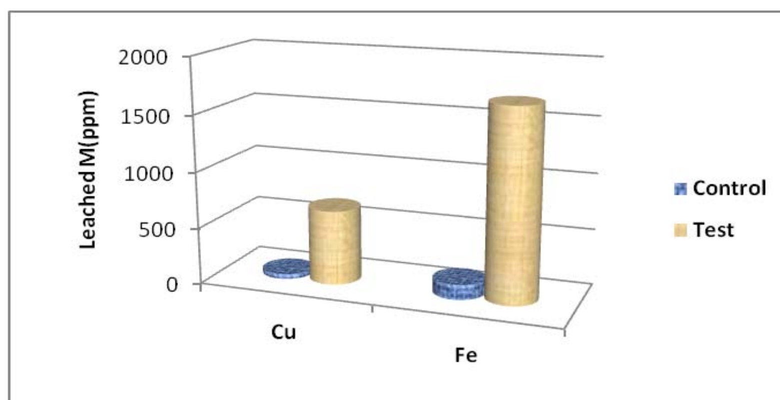


Figure 5 : Bioleaching of Cu and Fe from Copper concentrate.

XV. Bioleaching of Iron And Copper From Its Concentrate

X-ray fluorescence (XRF) and X-ray diffraction (XRD) analyses of pyrite ore showed that it contains 23.91% iron, 23.11% copper, 0.052% manganese, 0.001% nickel, 5.55% gold, 0.014% molybdenum, 22.79% sulfur.

Bioleaching of this ore for copper and iron elements showed that this mine is a highly appropriate medium for growth and activity of this bacterium. The bacterial cells consume and oxidize the sulfur element available in the mineral soil to sulfuric acid and drastically decrease the pH to as low as 1.98; thereby they provide.

Appropriate conditions for extraction of insoluble metals in the mineral soil. Analysis of the leaching solution showed 1690 and 663ppm rates of iron and copper extraction, which equal 29.3 and 71.4%, respectively. The values showed 92 and 93.5% increase in comparison with control samples, respectively (Fig.5).

The small amounts of extracted metals in control samples have resulted from spontaneous leaching. Note that in comparison with control samples (without inoculated bacteria), bioleaching medium of copper concentrate came in green and with development process the intensity of color was increased (Fig.6) .

Figure 6 : Color change due to bacterial activity and extraction of Cu in medium.

Bioleaching of pyrite ore

XRF and XRD analyses of pyrite ore showed that it contains ti iron and 25% sulfur. The mineralogical analysis showed its composition as $CaCO_3$ (Calcite), FeS_2 (Pyrite), ZnS (Sphalerite) and $CaMg(CO_3)_2$ (dolomite). Bioleaching of this type of ore demonstrated the decrease of pH as a consequence of sulfur consumption, resulting in the efficient metal extraction. The amount of metal extracted from the test sample showed a 60 to 70% increase in comparison with control sample (without bacterium).

© 2012 Global Journals Inc. (US)

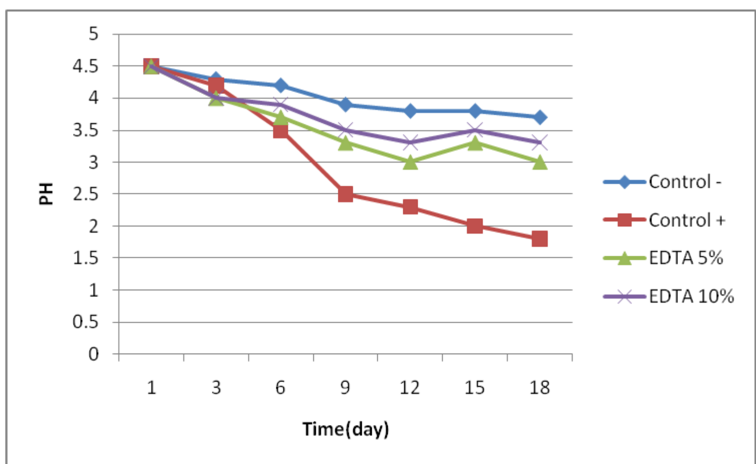


Figure 7 : pH changes in the investigation of EDTA effect on bacterial activity of *A. ferrooxidans IRL.8F* and rate of Fe^{3+} extraction from pyrite ore.

XVI. Study of EDTA Effect on LPS in the Outer Membrane of *A. Ferrooxidans* IRL.8F and on Rate of Fe^{3+} Extraction from the Pyrite Ore

To investigate the effect of EDTA on bioleaching through damaging the bacterial cell membrane, 5 and 10% concentrations of EDTA were simultaneously added to the leaching medium (the base medium of water). Decrease of pH in the sample without EDTA was observed to follow a slower slope and it directly depends on rate of bioleaching process (Fig.7). The results also confirm the severe reductive effect of EDTA on efficiency of the bioleaching process, such that the bioleaching rate in 5 and 10% concentrations of EDTA decreased by 61and 70%, respectively in comparison with the sample without EDTA (Fig.8).

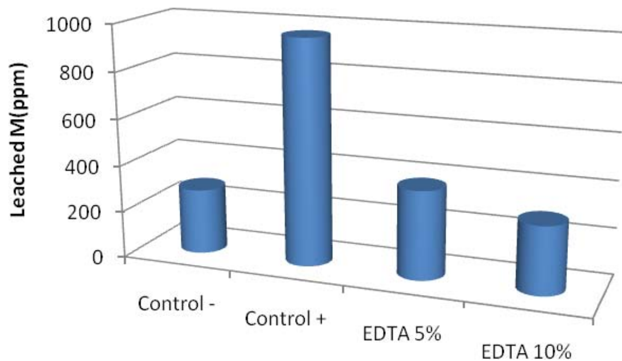


Figure 8 : EDTA effect on extraction of iron from pyrite ore
Control - = leaching medium without bacterium and EDTA
Control + = leaching medium + bacterium without EDTA.

© 2012 Global Journals Inc. (US)

XVII. Study of The Activity of Lps-Lacking Bacteria And Oxidation of Fe^{2+} Iron In Tf Medium

For this purpose, the bacterial LPS was first removed and the LPS-lacking bacteria were inoculated into the TFB medium. No color change was observed after 10 days, which represents the inability of the bacteria in the oxidation of Fe^{2+} to Fe^{3+}, whilst in the control sample with normal bacterium, the color began to change in the 5 day and it remarkably turned from green to red after the 10th day (Fig.9, right). To ensure that the EDTA + Tris-HCl treatment has not killed the bacteria and the cells just have lost their LPS, in the second stage, the LPS-lacking bacteria used in this experiment were transferred to a fresh TF medium. After 10 days, the bacterium turned the medium from lime green to red, indicating that the bacteria have restored the ability of LPS synthesis and have oxidized Fe^{2+} to Fe^{3+} (Fig.9, left).

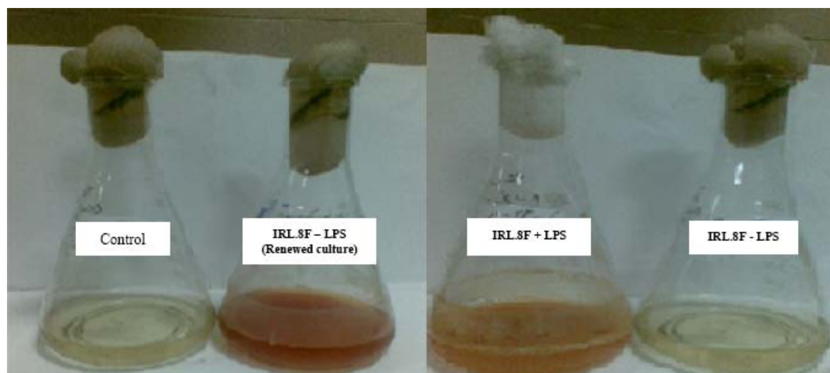


Figure 9 : Two stages of bacterial cell culture in TF medium. Right: the initial culture of LPS-lacking bacteria in TF medium, Left: re-culturing the LPS-lacking bacteria in the fresh TF medium.

XVIII. Study of The Capability of Lps-Lacking Bacteria in Leaching of Fe^{3+} From Pyrite or

The samples were analyzed after 14 day from beginning of the process and the amount of leached iron was obtained as shown in Figure 10. As it can be observed from the figure, LPS-lacking bacteria have remarkably lost their ability to leaching the iron.

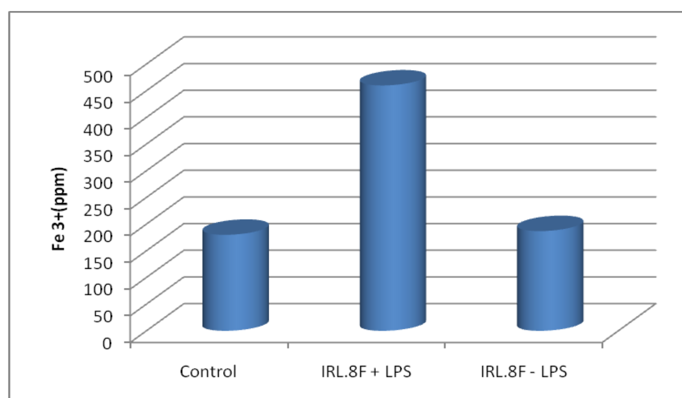


Figure 10 : Concentration of extracted Fe^{3+} from pyrite ore, in presence of LPS-lacking *A. ferrooxidans IRL.8F.*

© 2012 Global Journals Inc. (US)

XIX. Discussion

In the present study with the aim of evaluating the ability of native bacterium to extract copper and iron from their ores, an acidophilic strain was isolated from an iron mineral spring in Larzan, Iran. The isolated strain shows a remarkable enzymatic activity in Fe and S oxidation and is highly capable with bioleaching the copper and pyrite.

The results from bioleaching of copper concentrate showed that the amount of extracted copper and iron was 71.4 and 29.3%, respectively. Furthermore, in comparison with control samples, without bacteria, these amounts increased by 93.5 and 92%, respectively. In the control samples, however, a minor amount of metals were extracted due to spontaneous leaching.

One of the factors influencing the quality and quantity of the bioleaching process is the bacterial ability to attach to the mineral surface. The microbial contact with the ore surface stimulates the expression and production of extracellular polymers which entrap the bacterium at the side of the ore and attach it to the mineral surface (Dispirito et al., 1983; Bagdigian and Meyerson, 1986).

In addition, EPS can form chemical bonds with the mineral surface and mediate or promote respiration and nutritional reactions (Scobar., 1997; Ehrlich and Brierley., 1990). The molecules constituting the EPS can be made of LPS, phospholipids or other macromolecules such as poly-peptides. These compounds are released by the organism when it is attached to the mineral (Scobar, 1997).

There are varioustechniques to isolation of LPS from bacteria, Such as phenol-water method or by the phenol-chloroform-petroleum ether extraction methods, but these methods usually cause cellular damag or death. (Ramadas et al., 1991).The purpose of this study was to isolate and remove bacterium LPS without causing bacterial cell damage or death. Studies show that EDTA treatment negatively affects the adherence of the cell to mineral by the loss of part of LPS, without cell lysis. (Arredondo et al., 1994; Scobar et al 1997).

Scobar et al (1997) investigated the effect of EDTA on iron extraction from the pyrite ore. They believed that this substance removes the LPS from bacterial outer membrane and this leads to a remarkable decrease in the attachment of bacterium to its substrate. In the investigation of chalcopyrite and pyrite ores, they observed 85 and 77% decrease in attachment, respectively for bacterial cells treated with EDTA (Scobar, 1997). Such substances as EDTA absorb bivalent cations attached to phosphate groups in LPS and transform it from natural form to aggregated form, which obstruct the subsequent reactions (Rangin and Basu, 2004). Results of the present study revealed that samples with EDTA treatments show a remarkable decrease in bioleaching rate of the metal of interest. In direct mechanism where contact and attachment of the bacterium to the mineral surface is mediated by releasing exopolymers (Vandevivere and Kirchman., 1993) , EDTA removes part of this exopolymer and thereby, to a great extent decreases the efficiency of iron extraction from pyrite ore (Arredondo et al., 1994; Scobar et al 1997).

Since the bioleaching drastically decreased with LPS removal, in the present study, it can be concluded that the most amount of metal has been extracted through direct mechanism. The bacterium secretes such substances as LPS when approaching the mineral surface in order to be able to attach to the mineral surface; however, when LPS is removed the attachment cannot occur and the leaching by the bacterium will decrease (Pogliani and Donati., 1999; Arredondo et al., 1994; Scobar et al 1997). In the next experiments, to ensure that the decrease in bioleaching has resulted from LPS removal by EDTA, the bacterial LPS was removed by use of EDTA treatment and LPS-lacking bacteria were transferred to the leaching medium. Cultures of these bacteria in leaching media containing pyrite soil also significantly showed the decrease in extration of Fe^{3+}. These bacterial cells were also cultured in iron-containing TF medium. As it was expected, the treated bacteria with EDTA could not oxidize the iron, whereas the iron oxidation was observed in the Erlenmeyer flask containing non-treated bacteria.

Furthermore, EDTA in the leaching medium may act as a chelator absorbing the iron cations and decrease the oxidation of iron from Fe^{2+} to Fe^{3+}; therefore, the bacterial LPS may be of no role in decrease of leaching. LPS removal and inoculation of LPS-lacking bacteria into the leaching medium led to a 60.1% decrease in extracted metal. The less decrease in metal in comparison with when EDTA was used can be attributed to three possible reasons: 1) Some bacterial cells have restored their ability to produce LPS, 2) EDTA has acted as an iron chelator, or 3) EDTA has decreased the enzymatic oxidation of the iron.

In bacterial bioleaching, Thiobacillus Thiooxidans is used together with T. ferrooxidans, for the following reasons: It can release metallic elements by oxidation of reduced and semi-reduced sulfur compounds of the minerals and can promote the leaching of metals by producing the sulfuric acid as an oxidant. In addition, it provides the optimal acidic conditions for growth and activity of T. ferrooxidans. In bioleaching processes, that bacterial strain is of the greater importance which produces more amount of acid (Qiu et al., 2005).

In conclusion, results obtained from the present study indicate that: 1) EDTA drastically

© 2012 Global Journals Inc. (US)

decreases the efficiency of the bioleaching process, 2) LPS in the isolated bacterial strain in this study has a key role in bacterial attachment to mineral particles, and 3) the bioleaching process in this case promotes through the direct mechanism.

In Fe oxidation in TFB , pH of the medium decreased from 2.5 to 2, Probably because of consumption of sulfur compounds in the medium. Deposits in fawn color were observed on the wall of flask, which increased in amount daily; probably the iron hydroxide(jarosite) appeared in pH<2 (Qiu et al., 2005).

References Références Referencias

1. Arredondo R, Garcia A, Jerez C (1994). Partial Removal of Lipopolysaccharide from *Thiobacillus ferrooxidans* Affects Its Adhesion to Solids. Applied And Environmental Microbiology. 60: 2846-2851.
2. Bagdigian RM, Meyerson AS (1986). The adsorption of *Thiobacillus ferrooxidans* on coal surfaces. Biotechnol Bioeng, 28: 467-479.
3. Chen S, Lin JG, (2000). Influence of solid content on bioleaching of heavy metals from contaminated sediment by Thiobacillus sp. Chemistry technology biotechnology. 75: 649-659.
4. Clausen C (2003). Reusing Remediated CCA-Treated Wood, Special Seminar sponsored by American Wood-Preservers' Association Utility Solid Waste Activities Group.
5. DiSpirito AA, Dugan PR, Tuovinen OH (1983). Sorption of *Thiobacillus ferrooxidans* to particulate material. Biotechnol Bioeng. 25: 1163-1168.
6. Donati ER, Sand W (2007). Microbial Processing of Metal Sulfides . Springer book.
7. Ehrlich EC, Brierley CL (1990). Microbial Mineral Recovery. McGraw-Hill, New York.
8. Elzeky M, Attia YA, (1989). Bioleaching of gold pyrite tailing with adapted bacteria. Hydrometallurgy. 151-159.
9. Gehrke T, Telegdi J, Thierry D, and Sand W (1998). Important of extracellular polymeric substances from *Thiobacilus ferrooxidans* for bioleaching. Appl. Environ. Microbiol. 2743-2747.
10. Hong P., Yang Y., Li X., Qiu G., Liu X., Huang J. and Hu Y. Structure analysis of 16s rDNA sequences from strains of *Acidithiobacillus ferrooxidans*. Journal of biochemistry and molecular biology 39: 178-182, 2006.
11. Makita M, Esperón M, Pereyra B, Lopez A, Orrantia E (2004). Reduction of arsenic content in a complex galena concentrate by *Acidithiobacillus ferrooxidans*. BMC Biotechnology. 1-23.
12. Ohba H, Owa N (2005).Isolation and identification of sulfur-oxidizing bacteria from the buried layer containing reduced sulfur compounds of a paddy field on sado island in niigata prefecture. Nigata Unit. 5: 55-61,.
13. Pogliani C, Donati E (1999). The role of exopolymers in the bioleaching of a non-ferrous metal sulphide. Journal of Industrial Microbiology & Biotechnology. 22:88-92.
14. Qiu M, Xiong S, Zhang W, Wang G (2005). A comparison of bioleaching of chalcopyrite using pure culture or a mixed culture. Mineral Engeneering. 18: 987-990.
15. Ramadas U , Carlson RW, Busch M, Mayer H, (1991). Distribution and Phylogenetic Significance of 27-Hydroxy-Octacosanoic Acid in Lipopolysaccharides from Bacteria Belonging to the Alpha-2 Subgroup of *Proteobacteria*. International Journal Of Systematic Bacteriology. 41: 213-217.
16. Rangin M. and Basu A (2004). Lipopolysaccharide Identification with Functionalized Polydiacetylene Liposome Sensors. American Chemical Society. 126: 5038-5039.
17. Rawlings DE (2002). Heavy metal mining using microbes. Annual Review Microbiology. 56: 65-91.
18. Sasaki K, Nakamuta Y, Hirajima T, Tuovinen OH (2009). Raman characterization of secondary minerals formed during chalcopyrite leaching With *Acidithiobacillus ferrooxidans*. Hydrometallurgy. 95:153-158.
19. Scobar B, Huerta G and Rubio J (1997). Short communication :influence of lipopolysaccharides on the attachment of *Thiobacillus ferrooxidans* to minerals. W.J of Microbiol & Biotechnol. 13: 593-594.
20. Vandevivere P, Kirchman DL (1993). Attachment stimulates exopolysaccharide synthesis by a bacterium. Appl Environ Microbiol. 59: 3280-3286..
21. Wolfgang S, Edgardo RD (2007). Microbial Processing of Metal Sulfides. University of La Plata, Argentina. 169-191.
22. Yeats C, Gillings M, Davison A, Altavilla N, Veal DA (1998). Methods for microbial DNA extraction from soil for PCR amplification. Biological Procedures Online. 1: 40-45.

© 2012 Global Journals Inc. (US)

This page is intentionally left blank

© 2012 Global Journals Inc. (US)

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 12 Issue 4 Version 1.0 Year 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

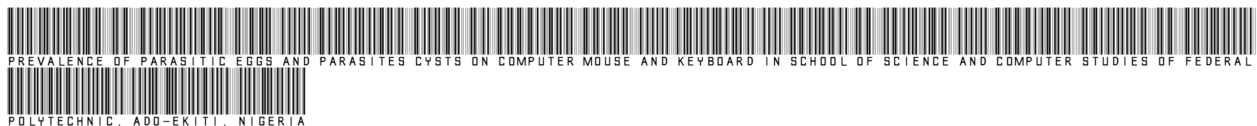
Prevalence of Parasitic Eggs and Parasites Cysts on Computer Mouse and Keyboard in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria

By Ajenifuja, Oluwafemi A & Ajibade, V.A
Federal Polytechnic, Ado-Ekiti

Abstract - The prevalence of parasitic eggs and parasite cysts on computer mouse and keyboard in School of Science and Computer Studies, Federal Polytechnic, Ado-Ekiti, Nigeria was investigated. The total number of samples examined was one hundred and eighty (180) of which twenty nine (29) were positive. The result showed that the samples collected during the first, second, and third weeks had 13, 10, and 6 numbers of cysts and ova respectively. The highest incidence was observed during the first week. Some bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aureginosa* and *Enterococcus faecalis* were also isolated from the sample with the highest incidence found in *Staphylococcus aureus*. It was concluded that the mouse and keyboards could be a source of disease transmission and should be disinfected appropriately and often.

Keywords : Parasitic eggs, Parasite cysts, Staphylococcus aureus, Computer keyboard and mouse.

GJSFR-D Classification : FOR Code: 060501



Strictly as per the compliance and regulations of:

© 2012. By Ajenifuja, Oluwafemi A & Ajibade, V.A .This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction inany medium, provided the original work is properly cited.

Prevalence of Parasitic Eggs and Parasites Cysts on Computer Mouse and Keyboard in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria

Ajenifuja, Oluwafemi A[α] & Ajibade, V.A[σ]

Abstract – The prevalence of parasitic eggs and parasite cysts on computer mouse and keyboard in School of Science and Computer Studies, Federal Polytechnic, Ado-Ekiti, Nigeria was investigated. The total number of samples examined was one hundred and eighty (180) of which twenty nine (29) were positive. The result showed that the samples collected during the first, second, and third weeks had 13, 10, and 6 numbers of cysts and ova respectively. The highest incidence was observed during the first week. Some bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aureginosa* and *Enterococcus faecalis* were also isolated from the sample with the highest incidence found in *Staphylococcus aureus*. It was concluded that the mouse and keyboards could be a source of disease transmission and should be disinfected appropriately and often.

Keyword : Parasitic eggs, Parasite cysts, Staphylococcus aureus, Computer keyboard and mouse.

I. Introduction

Parasite is an organism that lives in or on a second organism, called a host, usually causing it some harms. It is generally smaller than the host and of different species (Yusuf, 1990). Parasites are dependent on the host for some or all of their nourishment (Martins *et al*, 1980). Parasite can also be seen as an organism that has a deleterious symbiotics relationship with another organism or host species. A flea or tick is a parasite, bacteria can be parasitic, mistletoe is a parasite (Tanko *et al*, 1999). Parasite sometimes cause the eventual death of the host although not always and this can lead to the parasites demise if it cannot leave or find a new host (Kramer, 2006). Parasites are just about everywhere in our environment, so it's easy to become infected (World Health Organization).

In the 1993, world development report intestinal helminthes rank first as the main cause of disease burden in children aged 5 – 4 years and also rank highly as the disease that can be efficiently control by cost effective intervention (Lawande, 1983). Multiple infectious with several different parasites e.g. hookworms, roundworms and amoebae are common, and their harmful effects are often aggravated by co-existence malnutrition or micronutrient deficiencies (Akogun, 1989).

In America, parasitic infections are not as widespread but these infections are on the rise for various reasons. For example people bring parasites with them when they migrate to the U.S and soldiers often return to the U.S bringing parasites with them from overseas (Kucik *et al*, 2006). Parasitic infections are common in rural or developing areas of Africa, Asia, and Latin America and less common in developed areas. A person who visits such an area can unknowingly acquire a parasitic infection when the person returns home. In developed areas, parasite infections may also affect immigrants and people with a weakened immune system (such as those who have AIDS or who take drugs that suppress the immune system).

The infections may occur in places with poor sanitation and unhygienic practices. Parasites increase their fitness by exploiting host for resources necessary for the parasites survival i.e. food, water, heat, habitat, soil and dispersal. Parasites reduce host fitness in many ways, ranging from general or specialization pathology such as parasitic castration, impairment of secondary sex characteristic, to the modification of host behaviour (Rufala, 2006).

The Nigeria environment has been described as poor, based on personnal, community and environmental hygiene (Akogun *et al*, 1989). This poor state of hygiene is accounted for by the presence of immature stages of parasite (egg and cysts) in the soil (Ali, 1993), in the air (Lawande, 1983) on toilet door handles, on water closet handles (Nock and Geneve, 2003), on becks and legs of domestic chicken (Abuja, 1997) and on the sole of shoes (Tanko, 1999) demonstrating the indiscriminate nature of faecal disposal system. As these show the dynamic transmission network that exist in the Nigeria

Author α σ : Microbiology Unit, Department of Science Technology, Federal Polytechnic, P.M.B. 5351, Ado-Ekiti, Ekiti State Nigeria. E-mail : joseyajenifuja@yahoo.com

© 2012 Global Journals Inc. (US)

environment, through which parasites infect human and animal hosts; because once they are introduce into the soil, parasites eggs and cysts can be transported on contact with any subject. This accounted for the high prevalence and incidence of parasitic infection in both humans and animals (Hopkins, 1992).

The internet is progressively becoming an effective means of communication in Nigeria, thus there is an upsurge of people visiting the internet cafes, some reason to browse. During the use of the computer, the keyboard and mouse are used for input of commands with the fingers and palms of the hands, thus acting as points of contact between the internet and its users. The internet café is proposed, as a suitable model to test the role it plays in the transmission of parasite cysts and eggs in Federal Polytechnic, Ado-Ekiti, Nigeria.

II. Materials And Methods

a) *Collection of Samples*

A total number of 180 samples were collected from keyboard and mouse in School of Science and Computer Studies of Federal Polytechnic, Ado-Ekiti, Nigeria, over a period of three weeks. Sixty (60) samples were collected in each of the three weeks, 10 samples in the morning and 10 samples in the afternoon, which made up of 20 samples for each week.

b) *Preparation of Culture Medium*

2.8g of Nutrient Agar was dissolved in 100ml of distilled water and heat to melt. The conical flask was plugged with cotton wool and it was wrapped with foil paper and autoclave for 15mins. It was allowed to cool to between 45°C – 50°C after autoclaving it was poured into sterile Petri dishes and allowed to solidify. It was also poured into McCartney bottles which was half filled and the bottles were placed slantingly on the bench tops to allow the agar to set inform of slopes. The plates were labeled with Date and Name of the organisms to be inoculated. The swab samples collected from computer accessories were inoculated into the various grow media by streaking each nutrient agar plate and the plate were incubated at 30°C for 2 days. The plates were observed after incubation.

c) *Preparation of Unstained Wet Mount*

A sterile swab stick moistened with normal saline solution was moved over the keyboard and the buttoms of computer mouse. Special attention was given to the swabbing of the most commonly used keys for examples 'Enter', 'Spacebar', 'Delete', 'Shift key', etc. These swabs were taken to laboratory in sterile test tube containing 10ml of normal saline and each samples were labeled Day 1, 2, 3, etc. Each sample was further centrifuged at 2000rpm for 3 minutes. The supernatant was discarded and the sediment re-suspended. Little quality was taken with a Pasteur pipette and placed on a clean microscope glass slide. A drop of lugol's iodine solution was added and a clean cover slip was placed on the surface and examined under florescent microscope x400 magnification.

d) *The Gram Staining*

The bacterial smear was taken from the prepared Nutrient agar plates into the slide. The slide was placed on the staining rack and a drop of distilled water was added and mixed with the bacterial smear. The smear was flooded with crystal violet stain and leaf for 60secs. The smear was flooded again with Gram iodine and leaf for 60secs, after which the iodine was washed off with distilled water. Acetone-alcohol was added until no more colouration is seen to come up; it was washed immediately with distilled water and left for 10 – 15secs. The slides were flooded with carbol fuchsin and left for 1 minute, it was then washed off. It was gently dried between sheets of clean blotting paper and allowed to air-dry. It was examined under the florescent microscope x100 oil immersion.

Organisms isolated are;

- Staphylococcus aureus
- Streptococcus pryogenes
- Pseudomonas aeruginosa
- Enterococcus feacalis

e) *Catalase test*

A loopful of the isolate was placed on a clean sterile slide and a drop of hydrogen peroxides was added. The effervescence of gas is shown by bubbling.

f) *Oxidase test*

An oxidase strip (i.e. a strip that has been impregnated in the reagent) was smeared with the test organism and left for 10 seconds. Purple colouration is a sign of oxidase.

III. Results And Discussion

Table 1 : Prevalence of parasite eggs and cysts on keyboard, and mouse for three days per week in the first week

Days	Number of samples	Positive No.
1	20	6
2	20	3
3	20	4
Total	60	13

Table 2 : Parasite eggs and cysts on keyboard, and mouse for three days per week in the second week.

Days	Number of samples	Positive No.
1	20	4
2	20	3
3	20	3
Total	60	10

© 2012 Global Journals Inc. (US)

Table 3 : Parasite eggs and cysts on keyboard, and mouse for three days per week in the third week.

Days	Number of samples	Positive No.
1	20	3
2	20	3
3	20	0
Total	60	6

Table 4 : Bacterial encountered during the study.

Bacterial isolated	No (%)
Staphylococcus aureus	8 (57)
Streptococcus pryogenes	2 (14)
Pseudomonas aeruginosa	3 (21)
Enterococcus feacalis	1 (7)

Table 5 : Characteristic of the test bacteria.

Test bacteria	Shape	Size (μm)	Motility	Gram reaction	Appearance	Temperature (ºC)	Characteristic
S. aureus	Cocci	0.7-1.0	+ve	+ve	White, yellow	37	Anaerobic
Str. pryogenes	Cocci	0.6-1.0	–ve	+ve	Greenish	37	Anaerobic
P. aeruginosa	Rod shape	0.6-1.0	+ve	–ve	Pink-red	37	Aerobic
Ent. feacalis	cocci	1 – 2	–ve	+ve	Yellow pigment	37	Aerobic

IV. Discussion

Overall samples examined were one hundred and eighty (180) and twenty nine (29) of the samples were positive. The results show that first week samples had (13) highest occurrence number of positive samples having eggs and cysts, then followed by second week samples has (10), followed by third week samples which had (6) with the lowest prevalence. Bacteria encountered during the study are; Staphylococcus aureus (08) with the highest occurrence number, then followed by Pseudomonas aeruginosa (3), followed by Streptococcus pryogenes (2) and Enterococcus feacalis (1) with the lowest occurrence number.

Computer technology for the management of individual has become an essential part in all aspect of modern medicine (Fukatat *et al*, 2008). Consequently, the computer keyboard and mouse in the Departments of School of Science and Computer Studies' laboratory in Federal Polytechnic may act as a reservoir for microorganisms. And contribute to the transfer of pathogens from one individual to the other unknowingly. (Hartman *et al*, 2004).

Most of the keyboards examined in the study were contaminated with non pathogenic microorganisms such resident skin flora or environmental bacteria. Long survival time of potentially pathogenic microorganism, particularly on desks, contribute to the hypothesis of computers acting as reservoir of pathogenic (Kassem, 2007). Hence, the process of correct hand disinfection is still the main stay of any preventive measure for the reduction of infections. Hand disinfection policy should not be reserved to student or internet users (Nock and Geneve, 2002). Beside to improve hand hygiene compliance, improvement of cleaning service could admonished as an infective infection control measure (Nock and Brown, 1994). Disinfectant including chlorine, alcohol, phenol and quarternary ammonium are all effective against Staph. aureus and Enterococcus spp. Species on keyboards of computers and even sterile water is effective to remove more than 95% bacteria (Rutala, 2006). Although keyboard can be safety and successfully disinfected, the need to clean computer interface surface as routine practice is generally accepted, no specific cleaning and disinfection frequency and procedure for computer accessories has been defined. Daily cleaning and hygiene regularly for using computer is of great significance and could help in the reduction of parasite eggs/cysts and pathogenic bacteria and also reduce keyboard contamination (Williams, 2006).

Computer should be disinfected daily and well visibly soiled, Health care workers should not touch computer keyboard and mouse with contaminated hands. Preventive measure should be adopted particularly when the number of people visiting the operating room daily are considered.

The isolation cysts from samples collected on keyboard is an indication that it could be source of transmission of pathogens Krammer *et al*, 2006). These findings correlate with that of (Hartman *et al*, 2004) where it was observed that keyboard houses a lot of parasites.

Staphylococcus aureus which are antibiotic-resistant are found to be predominance bacteria found on keyboard and mouse because they are normal floral of humans found on nasal passage, skin and mucurs membrane, pathogen of humans, causes a wider range of superlative infections, as well as food poisoning and toxic shock syndrome.

The isolation of some bacteria from the keyboard and mouse is an indication that they could be a source of the transmission of diseases. The predominance of Staphylococcus aureus explains the

© 2012 Global Journals Inc. (US)

long standing believe that the skin houses Staphylococcus aureus. The isolation of Streptococcus pyogens which is found in nasal passages is an indication that the bacteria could have been dispersed through droplets from the mouth.

V. Conclusion

This study showed that a fairly large number (i.e. 95%) of the computer keyboard and mouse devices which are in use in various areas of the school is contaminated and the discovery of Staphylococci on computer keyboards draw much needed attention to good sanitary habit after utilizing the keyboard and mouse. Additionally, touch of the mouth or the nose while operating the keyboard could have contributed to the contamination because humans can transport staphylococci from the nasal passage.

References Références Referencias

1. Aetlas. Illexas.edu (1996):What is computer virus, Retrieved 2010-08-27.
2. Adleman L.M (1988): An abstract theory of computer viruses advances in Cryptology. crypto LNCS; 403, 354-374.
3. Akogun O. B (1989): Some social aspects of helminthiasis among the people of Gumaru district, Bauchi State. Nigeria journal of tropical medicine and hygiene; 92 (3): 193-196.
4. Fukata T (2008): Anaethetists role in computer keyboard contamination in an operating room. J. Hosp. infect; 3 (5): 10-1016.
5. John Von Neuman, (1949): Theory of self reproducing automata part 1 Transcripts of lectures given at the (University of Ilinius Press) Editor A.W. Burks University of Illinius U.S.A·
6. Jussi Parika (2001): Digital contagious A media Archeology of computer viruses Digital formation series. Jour. of Com. Sci. & Envir. 8 (10) 2 – 19.
7. Hartman B. (2004): Computer keyboard and mouse as a Reservoirs of pathogen in an intensive care units. J clin; 18, 7 – 12.
8. Kassem Issmat (2007): Public computer surfaces are reservoirs for methicillin-resistant staphylococci. The ISME journal 1, 265 – 268.
9. Kramer (2006): License Biomed Central LTD, How long do nosocomical pathogens persist on inaminate surfaces? A systematic review. BMC infectious Diseases pg 6: 130.
10. Kucik C, Corry J, Martin G. L, and Sortor B. (2006): Communication in intestinal parasites. America Family Physician; 69 (5): 2004 – 2020.
11. Lawande R. V (1983): Recovery of soil amoeba from the air during the harmattan in Zaria, Nigeria. Annals of Tropical medicine and parasitology; 77 (1): 45 – 49.
12. Nock I. H and Geneve A. I (2002): Public health significance of parasite cysts and egg on water closet handles. The Nigeria Journal of Parasitology; 17: 1 – 94.
13. Rutala Williams (2006): Bacterial contamination of keyboards: Efficiecny and functional impact of disinfectants. Infection control and Epidemiology; 27 (4): 231 – 249.
14. Schultz, Maureen (2003): Bacteria contamination of computer keyboards in a Teaching Hospital. Infection Control and Hospital Epidemiology; 27 (4): 420 – 432.
15. Tanko D. (1998): Isolation of parasite ova and cysts from sole of shoes. Department of Biological Science, Ahmadu Bello University, Zaria.
16. Tiller, Joerge C (2001): Designing surface that kill bacteria on contact. PNAS; 98 (11): 5981 – 5985.
17. Yusuf M and Hussein A. M. Z (1990): Sanitation in rural communities in Bangladesh. Bulletin of World Health Organization; 68 (5): 619– 624.

© 2012 Global Journals Inc. (US)

Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 12 Issue 4 Version 1.0 Year 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Crystal Structure and Kinetic Studies on Met244Ala Variant of KatG from *HALOARCULA MARISMORTUI*

By Takao SATO, Wataru Higuchi, Katsuhiko Yoshimatsu & Taketomo Fujiwara

Tokyo modify Institute of Technology, Japan

Abstract - KatG from *HALOARCULA MARISMORTUI* (*Hm*), used concomitantly with initiator (H_2O_2), exhibits high *catalase* and *peroxidase* activities with substrate (*O*DA). The distal side M244–Y218–W95 covalent adduct and M244 centered octahedral coordination complexes in the active site are essential for the *catalase* activity. Mass spectroscopic analysis of the M244A shows cleavage of the covalent adduct between Y214–W95 and M244 without its sulfer atom. Crystal structure of M244A variant in *Hm*KatG has the geometrically dimeric subunits that disrupted or not a π–interaction which is linked between heme edge (C*1C*) to the adduct end W95 (Nε*1*). The isoenzyme pattern of *peroxidase* was determined by fitting the kinetic data to non-linear (mixed) Michaelis-Menten equation and then governed by the hetero-dimeric characters. Respective *peroxidase* catalytic efficiency for two subunits was 2.5 and 4.8 -fold increased with higher binding affinity for *O*DA. It was enhanced by rotating the dihedral angle χ2 of D125.

Keywords : Structural and Functional heterodimers/Kinetics on isoenzyme pattern of peroxidase activity / X-ray Crystallography / mass spectrometry.

GJSFR-G Classification : FOR Code: 060107

Strictly as per the compliance and regulations of:

© 2012. By Takao SATO, Wataru Higuchi, Katsuhiko Yoshimatsu & Taketomo Fujiwara .This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction inany medium, provided the original work is properly cited.

Crystal Structure and Kinetic Studies on Met244Ala Variant of KatG from *HALOARCULA MARISMORTUI*

Takao SATO [α], Wataru Higuchi [σ], Katsuhiko Yoshimatsu [ρ] & Taketomo Fujiwara [ω]

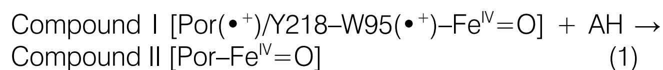
Abstract - KatG from *HALOARCULA MARISMORTUI* (*Hm*), used concomitantly with initiator (H_2O_2), exhibits high *catalase* and *peroxidase* activities with substrate (*O*DA). The distal side M244–Y218–W95 covalent adduct and M244 centered octahedral coordination complexes in the active site are essential for the *catalase* activity. Mass spectroscopic analysis of the M244A shows cleavage of the covalent adduct between Y214–W95 and M244 without its sulfer atom. Crystal structure of M244A variant in *Hm*KatG has the geometrically dimeric subunits that disrupted or not a π–interaction which is linked between heme edge (C*1C*) to the adduct end W95 (Nε*1*). The isoenzyme pattern of *peroxidase* was determined by fitting the kinetic data to non-linear (mixed) Michaelis-Menten equation and then governed by the hetero-dimeric characters. Respective *peroxidase* catalytic efficiency for two subunits was 2.5 and 4.8 -fold increased with higher binding affinity for *O*DA. It was enhanced by rotating the dihedral angle $\chi 2$ of D125.

Keywords : Structural and Functional heterodimers /Kinetics on isoenzyme pattern of peroxidase activity / X-ray Crystallography / mass spectrometry.

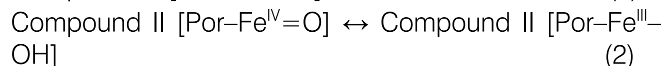
I. Introduction

KatG is a bifurcation enzymes that catalysis *catalase* and *peroxidase*, despite differing from manmalian liver *catalase*. It, indeed, belong to a member of the class I of the plant *peroxidase* superfamily (Welinder, 1992) including the heme-containing active sites which consists of *peroxidase*-conserved amino acids at almost identical positions as in class I *peroxidase*. In *peroxidase*, compound I is reduced in two sequential one-electron transfers, usually from donor (AH) (eq. 4) and involve an intermediate called compound II (eqs. 1 and 3). Two resonance structures for compound II could coexist (eq. 2). By first one electron-transfer (ET), the donor (AH, *O*DA; *o*-dianisidine) at nitrogen atom of quinoneimine groups was excited to the *O*DA cation radical (*O*DA($\cdot^+$)) and then by second one ET oxidation from its intermediate can be completed to the product (A, *O*DA$_{red}$; *o*-dianisidine quinoneimine) (eq. 4).

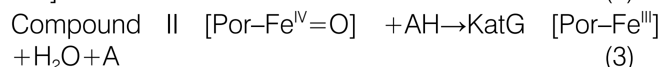
Author α : Depertment of Life Science, Graduate school of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259–B-10 Nagatsuta, Midori-ku, Yokohama, 226-8501, Japan.
E-mail : tsatoh@bio.titech.ac.jp
Author σ ρ ω: Department of Biology and Geosciences, Faculty of science, Shizuoka University, 836 Ohya, Shizuoka, 422-8529, Japan.



Compound I [Por($\bullet^+$)/Y218–W95($\bullet^+$)–Fe^{IV}=O] + AH → Compound II [Por–Fe^{IV}=O] (1)



Compound II [Por–Fe^{IV}=O] ↔ Compound II [Por–Fe^{III}–OH] (2)



Compound II [Por–Fe^{IV}=O] +AH→KatG [Por–Fe^{III}] +H_2O+A (3)

AH [*O*DA / *O*DA($\bullet^+$)] →A [*O*DA$_{red}$] (4)

Haloarcula *marisomortui (Hm)* naturally lives in salt lake and uses sunlight as an energy source. *Hm*KatG shares 55% identity and 69% similarity in its sequence with KatG from *Mycobctrium tuberculosis* (*Mt*) as homologous protein. *Mt*KatG is interesting in its involvement of the activation of *antituberculous* pro-drug isonicotinic acid hydrazide (isoniazide, INH) (Bertrand *et al.*, 2004). INH is activated as its *peroxidase* substrate by *Mt*KatG (Zhang *et al.*, 1992; Johnsson *et al.*, 1995). Resulting radical *via* oxidation prevents growth of the pathogenic microorganism by inhibiting the synthesis of mycolic acid component of the *mycobacterial* cell wall (Heym *et al.*, 1993). Structural and functional information is available for the crystallographic, kinetics and site-directed mutagenesis studies on KatGs (Donald *et al.*, 2003; Jakopitsch *et al.*, 2004; Singh *et al.*, 2004). These structures in combination with the biochemical characterization of variants lead to identify a few of KatG-specific residues (all numbering is for *Hm*KatG), including the cross-linkage covalent adduct among W95, Y218 and M244, unique to KatGs, to coordinate G99 and Y101, D125 and E194, which is known to be mobile in KatG and which is conserved across all KatGs. It is required for [M244A] equivalent variant from *Mt*KatG to be susceptible to INH and known for *peroxidase* reaction. Hence, the M244A mutation in KatGs from *Synechococcus* PCC7942 (*Sy*) and *Bulkhorderia pseudomallei* (*Bp*) expected to be one of the commonest causes of increasing sensitivity to *O*DA and, activating significant *peroxidase* while may be remaining slightly *catalase* activities. Therefore, *Hm*KatG [M244A] variant is also expressed with an attempt at rational catalytic redesign, to elucidate *peroxidase* reaction mechanism, because this variant is expected to exhibit the equal to or higher *peroxidase* efficiency than that of Wild-type (WT) KatG. Substitution for M244A induced the significant change in the active site that

Early View

© 2012 Global Journals Inc. (US)

would trigger a loss of *catalase* activity and a high enhancement of *peroxidase* activity.

In this paper, *Hm*KatG [M244A] variant losts *catalase* but, indeed, reveals the higher *peroxidase* property with isoenzyme pattern that each of subunits exhibits differences between the two kinetic parameters for *O*DA (Ten-I, *et al.*, 2007). This mutation also affected the structure of the access channel and therefore the enzymatic parameters for the *peroxidase* activity. The crystal structure of *Hm*KatG [M244A] variant is reported here. Remarkably, each structure of subunits was not entirely identical. Atypical correlation between the reaction rate of turnover and the substrate affinity was properly and accurately described in terms of its heterodimeric character that can performed separately from two identical subunits (designated A and B) of heterogeneous structural and functional dimer of *Hm*KatG.

II. Resource And Techniques

a) Experimental Procedures

i. Protein Expression, Purification, Crytallization and Kinetics of the [M244A] Variant of HmKatG –

The plasmid pHKH6 katG gene was used as the source of *catalase-peroxidase* from *Haroarcula marismortui* (ATCC43049) with a C-terminal poly-His tag. From producing the M244A substitution in KatG to yield pHKM244AH6, [M244A] variant of *Hm*KatG was prepared, purified and crystallized, as described previously (Ten-I, *et al.*, 2007). Protein concentration was determined by a modified Lowry method (Dulley & Grieve, 1975) using bovine serum albumin as the standard. SDS-PAGE was carried out using the method of Schägger and von Jagow (1987). Spectroscopic measurements in the UV-visible regions were performed using a spectrophotometer model MPS2000 (Shimadzu Co., Kyoto, Japan) with a 1 cm light path cuvette. The level of heme *b* was calculated on the basis of the pyridine ferrohemochrome spectrum using a millimolar extinction coefficient of 34.4 $mM^{-1}cm^{-1}$ at 557 nm (Falk, 1964). *Catalase* activity of the purified recombinants was measured spectrophtometrically. A broad-range buffer, which was composed of 33 mM each of Na-citrate, Na-phosphate and Tris base, was used for pH adjustment of the reaction mixture at 6. The activity was determined using the reaction mixture containing 2.0 M NaCl, 10 mM H_2O_2. The reaction was started by an addition of the enzyme to the reaction mixture, decay of the absorbance at 240 nm was measured. Millimolar extinction coefficient of H_2O_2 was 0.0436 $mM^{-1}cm^{-1}$ at 240 nm (Wei *et al.*, 2003). *Peroxidase* activity was measured as the reduction rate of *o*-dianisidine (*O*DA) in the presence of *tert-butyl*peroxide (*t-Bu*OOH) by monitoring increase of absorbance at 460 nm. Millimolar extinction coefficient of *O*DA was 11.3 $mM^{-1}cm^{-1}$ at 460 nm (Worthington, 1988). The *peroxidase* activity was determined using the reaction mixture containing 2.0 M NaCl, 20 μM *O*DA, 100 mM *t-Bu*OOH at pH8. Estimation of kinetic parameters, velocity constant (k_{cat}) and affinity constant (K_m) for *O*DA, were performed by a fit of the 22 data at each concentration to the mixed Michaelis-Menten equation (eq.5) using nonlinear regression analysis program (Sigmaplot 2000 and systat 7.0, www.systat.com, Systet Software Inc.)

$$[V]=0.5(k_{cat}^{A}\times[S]/(K_m^{A}+[S])+k_{cat}^{B}\times[S]/(K_m^{B}+[S])) \quad (5)$$

In this equation, 0.5 is coefficient constant per number heme *b* in KatG, [V] and [S] are the maximal velocity and H_2O_2 or *O*DA concentrations. The *peroxidase* isoenzyme patterns were independent of each subunit. Each of catalytic centers has significantly different kinetic parameters between subunit A (k_{cat}^{A} and K_m^{A}) and B (k_{cat}^{B} and K_m^{B}), respectively.

ii. Digestion, Fractionation, and Sequence and Mass-Analysis of the Polypeptide –

Endopeptidase-digestion of the two KatG recombinants was performed as follows : the purified sample (0.2 mg protein) was denatured and precipitated by treating with 5 % (w/v) trichloroacetate. The pellet that obtained centrifugatically was dissolved in 100 mM Tris-HCl buffer (pH 9.0) containing 2 M urea to become 0.2 ml in volume. Enzymatic digestion of the polypeptide was performed by treating with *lysyl-endopeptidase* (20 units, Wako Pure Chemical Industries Inc., Osaka, Japan) for 12 hr at 37 °C. Digested peptides thus obtained were fractionated by reverse-phase HPLC in 0.1% tetrafluoroacetate (TFA) with a linear gradient from 0 to 60 % (v/v) acetonitrile over 1 h at 1.0 ml/min with using cosmosil 5C18 packed column (4.6 x 250 mm, Tosoh Co.) equipped with the HPLC system (Shimadzu). N-terminal amino acid sequences of the fractionated peptides were determined by a protein sequencer model PPSQ-21A (Shimadzu). Molecular weight of the fragment was determined by ion spray ionization mass spectrometry using a single quadrupole mass spectrometer (API-150EX, Perkin-Elmer Sciex Instruments, Foster City, CA).

iii. Structure Determination of [M244A] Variant of HmKatG –

The crystal structure of [M244A] variant was solved by native model (PDB code 1ITK) for MOLREP (Vagin & Teplyakov, 1997). Rigid-body refinement in REFMAC5 was performed before any refinement or model building. Several rounds of positional and isotropic B-factor refinement using REFMAC5 (Vagin & Teplyakov, 1997; Murshudov *et al.,* 1997), solvent molecules were added to well defined peaks with ARP/warp (Perrakis *et al.*, 1999) and manual modification were performed for the molecular model, using Xfit of XtalView (McRee, 1999). The quality of the models was analyzed using PROCHECK (Laskowski, 1993). Molecular-graphics figures were produced using PyMOL (DeLano, 2002).

Early View

© 2012 Global Journals Inc. (US)

iv. *Structure Based Substrate Docking and ET Pathway Analyses –*

The substrate docking and semi-empirical molecular orbital calculations were carried out with using a MOPAC2002 program (Stewart, 2002) / AM1 wavefunction (Dewar *et al.,* 1985) in BioMedCAChe ver6.1.12.34 (Fujitsu, Tokyo).

Substrate affinity was quantified by binding energy calculated from the docking study. The binding energy for a given ligand (ΔE_{ligand}) can be expressed in (eq.6) as the difference in the energy between complex and components (Fukuzawa *et al.*, 2003).

$$\Delta E_{ligand} = E_{complex} - (E_{enzyme} + E_{ligand}) \qquad (6)$$

, where are the heat of formation energy of each of three systems, i.e., E_{ligand} of H_2O_2, *O*DA (or *O*DA_{red}) and, E_{enzyme}, of the variant, and $E_{complex}$ of the variant complexes with H_2O_2, *O*DA (or *O*DA_{red}). The binding energy can be estimated to subtract the sum of heat of formation energies of each system from that of pair (of the dipartite) system, exhibiting the value of attractive interaction which is negative for MOPAC-specific calculation and can be discussed by the magnitude of its absolute value.

In addition, the ET pathway from the HOMO (the highest occupied molecular orbital; electrophilic reactivity) to the LUMO (the lowest unoccupied molecular orbital; electron affinity) were searched for the crystal structure–based analysis on the frontier electron theory (Fukui *et al.,* 1954; Fukui *et al.,* 1957) that substrate can approach to active site within 3.4Å (of van der Waals contact), when there is the energy gap within 6eV (Pearson, 1986) and the bonding orbital between HOMO of substrate with electrophilic superdelocalizability (Sr) and LOMO of reactive residue atom with nucleophilic Sr. Starting structure contained for 364 atoms of [M244A], after hydrogen addition to its crystal structure. Geometries were determined by Mechanics optimization using Augmented MM3. All the sets of molecular orbitals (HOMO to LUMO) are generated on the docking model of ligand-protein complexes which involved in the covalent adduct and heme *in vacuo*, to which ligate H_2O_2 as an initiator, *O*DA as a *peroxidatic* substrate and *O*DA_{red} as *peroxidase* product were bound.

v. *Fragment Analysis -*

Fragment approaches were investigated for non-covalent interactions (π-complexes) between fragment 1 (W95 or Y218-W95 adduct) and fragment 2 (heme), using the term “DFT-D3” employing BJ-damping as “DFT-D3 (BJ)” level of theory (Grimme, *et al.*, 2010; 2011). In order to estimate the hole (or electron) mobility calculation between W95 and heme, the electronic coupling term, V (eV), is defined in (eq.7) as follows:

$$V = \{J_{RP} - S_{RP} * (H_{RR} + H_{PP}) * 0.5\} / (1 - S_{RP}^2) \qquad (7)$$

, where are charge transfer intergral (hole) HOMO fragment 1-HOMO fragment 2 for J_{RP}, overlap integral (hole) HOMO fragment 1-HOMO fragment 2 for S_{RP}, site energy (hole) HOMO fragment 1 for H_{RR} and HOMO fragment 2 for H_{PP} . The term of charge transfer can be discussed by the magnitude of its square value (V^2). These calculations for charge transfer integral between WT and [M244A] were performed using the ADF2012.01 program package (Scientific Computing & Modelling)(Baerends *et al.*,2007).

III. Results

a) *[M244A] variant exhibits only peroxidase activity with isoenzyme pattern–*

Steady state kinetic analyses of the activities of the two recombinants were performed by using a nonlinear regression analysis. In the two subunits of WT enzyme, velocity constant (k_{cat}) of *catalase* activity revealed maximum values ($4.48 \times 10^2 \pm 54.8$ sec^{-1}; $7.75 \times 10^3 \pm 2.31 \times 10^3$ sec^{-1}) at pH 6.0. Affinity constants (K_m) for H_2O_2 were also determined as 0.130 ± 0.054 mM; 37.9 ± 15.4mM. The *catalase* activity was completely lost by substitution of M244A; no remaining activity can be detected in this measurement system. Kinetics for *peroxidase* activity was also affected drastically by this mutation. *Peroxidase* activity of the WT enzyme showed its maximum at around pH 6.0. On the other hand, *peroxidase* activity of the [M244A] variant also revealed maximum at pH 8.0. *Peroxidase* catalytic efficiency (k_{cat}/K_m for *O*DA) for two subunits of WT enzyme was calculated as 0.650×10^6 M^{-1} s^{-1} and 0.0196×10^6 M^{-1} s^{-1}, respectively, with the coefficient of determination $R^2 = 0.999$ using 8 data points as shown in Table 1. Interestingly, in the *peroxidase* activity of [M244A] variant, relationship between the substrate concentration [S] and the rate of turnover [v] could not be interpreted by a simple Michaelis-Menten's equation. However, according to the mixed Michaelis-Menten equation (eq.5), which was derived from the model, kinetic parameters for subunit A (k_{cat}^A and K_m^A) and for subunit B (k_{cat}^B and K_m^B) were estimated by nonlinear regression analysis program. As shown in Fig. 1, the [v] is the rate of turnover and [S] are the concentration of *O*DA. [S]– [v] correlation of the *peroxidase* activity in the [M244A] variant at pH 8.0 was reproduced by using the estimated kinetic parameters for subunit A ($k_{cat}^A = 1.6 \pm 0.27$ sec^{-1} and $K_m^A = 0.97 \pm 0.78$ μM) and for center B ($k_{cat}^B = 4.73 \pm 0.20$ sec^{-1} and $K_m^B = 50.8 \pm 7.8$ μM) which is in good agreement with $R^2 = 0.998$. Atypical [S]–[v] curve which was explainable according to the two catalytic center model was also observed. The results indicated the enzymatic feature of the two catalytic centers in *K*atG, one (subunit A) showed low-activity and high-affinity for substrate, while the other (subunit B) is highly active but showed low-affinity.

Early View

© 2012 Global Journals Inc. (US)

As shown in Table 1, the substrate affinity constant of subunit B in [M244A] variant was about 7 fold higher affinity of that in the WT enzyme, whereas that of subunit A was comparable with that in the WT enzyme subunit A (k_{cat}^{A} = 0.528 sec^{-1} and K_m^{A} = 0.814 μM) and for subunit B (k_{cat}^{B} = 6.92 sec^{-1} and K_m^{B} = 352 μM). Each subunit A and B for the *peroxidase* activity is of 0.84 and 6.9-fold lower K_m value for [M244A] which is reflected in the 3.0 and 0.7-fold higher k_{cat} value as compared with those of WT. Therefore, the catalytic efficiency (k_{cat} /K_m) for *O*DA was 2.5 and 4.7-fold increase in *peroxidase* activity, respectively. It was suggested that this site mutation of [M244A] variant is fast rate of turnover for *O*DA compared with WT, by the cleavage adduct between M244-Y218-W95.

b) Identification of the Covalent-adduct and Partial Cleavage Lysyl endopeptidase-digestion and Mass-spectrometry with Reverse-phase HPLC –

To confirm with or without only a covalent link between side W95 (C*η*) and Y218 (C*ε2*) in two subunits, *Lysyl endopeptidase* digestion studies were performed for each of KatG recombinants, WT and [M244A] variant. Following proteolytic digestion, the peptide fragments were separated using reverse-phase HPLC. Fractions were collected, concentrated and submitted for Mass-spectrometry (MS) analysis. Both digests exhibit peptide elution patterns that are very similar, with notable exceptions as indicated in panel A of Fig. 2: the presence of a large peptide cluster in the retention time (r.t.) region of ~45 min in WT KatG is absent in M244A. This difference was highly suggestive of the presence of a M244–Y218–W95 covalent adduct peptide fragment in WT, predicted (from *Lysyl endopeptidase* cleavage sites) to incorporate S66–K131, A184–K235, and N 236–K249, which would be unable to form in [M224A] due to the Met→Ala mutation. Second, several additional peaks, which were not further characterized, were also observed in [M244A] but were absent in WT KatG, and may represent the above uncross-linked peptides. As the HPLC chromatograms were monitored at λ=220nm (peptide backbone), covalent adduct assignments were performed with using the characteristic spectral features and r.t. for either the M244–Y218–W95 or Y218–W95 covalent adducts. The presence of each covalent adducts were confirmed by mass spectrometry for both the M244–Y218–W95 ([S66–K131], [A184–K235], and [N236–K249]) and Y218–W95 ([S66–K131], and [A184–K235]). The M244–Y218–W95 covalent adduct that located on the distal side of the heme, is a structural characteristic common to all the KatGs. *Lyzyl endopeptidase*-digestion and fractionation by HPLC of the two recombinants, both WT KatG and [M244A] variant were performed. The polypeptide in the fraction prepared from the WT exhibited five peaks that include ions at mass/charge (m/z) for the +10 (m/z = 1474.0), +9 (1637.8), +8 (1840.1), +7 (2103.2) and +6 (2453.0) charged states by electrospray mass spectroscopic analysis (Schnölzer *et al.* 1992). Molecular weight of WT was determined as 14681.4 Da by Mass spectroscopy (MS) (upper panel B in Fig. 2). In this structure, demethylation (C*ε*) in the side-chain of M244 was expected; that is because the electrophilic attack of proton to the S*δ* of methionine should dominate in the acidic denaturation of the enzyme by TFA. The value of mass 14681.7 Da calculated for the M244–Y218–W95 covalent adduct that combines three polypeptides ([S66–K131], [A184–K235], and [N236–K249]) is in good agreement with the experimentally determined mass of 14681.4 Da, or a mass 0.3 Da lower than the calculated value. It was indicative of the expected covalent-modification among W95, Y218, and M244 side-chains (left in panel C of Fig. 2). In case of M244A (lower panel B in Fig. 3), there are six peaks for the +10 (m/z = 1305.2), +9 (1450.0), +8 (1629.2), +7 (1863.5), +6 (2173.9) and +5 (2608.7) charged states, corresponding to that of the covalent-adduct composed of two polypeptides ([S66–K131] and [A184–K235]). We attribute this mass to a specific cleavage occurring at position M244A (i.e. loss of residues [N236-K249]) of the M244–Y218–W95 covalent adduct, whose calculated value (12958.8 Da) is also consistent with an experiment based on a mass of 12960.0 Da, or a mass 2 Da more than that calculated for the Tyr−Trp adduct. This profile indicates the presence of the Y218–W95 covalent adduct in [M244A] predicted from *endopeptidase* digest and the combination of Y218–W95 dipeptides are most likely (right in panel C of Fig. 2).

c) N-terminal sequence analysis –

The preparation for this analysis obtained from the two enzymes was shown in Fig. 3. In the sample from the WT enzyme, three amino acid residues appeared in each cycles with almost equimolar ratio, demonstrating that the sample would contain three polypeptides ([S66–K131], [A184–K235], and [N236–K249]), as predicted from *lysyl - endopeptidase* cleavage site. In case of the [M244A] variant, two residues appeared in each cycles, suggesting the presence of two polypeptides ([S66–K131] and [A184–K235]). The result also evidenced the presence of [M244–Y218–W95] covalent adduct in the WT enzyme, and the presence of [Y218–W95] adduct in the [M244A] variant.

In the M244I variant from *Mt*KatG (Ghiladi *et al.*, 2005b; Ghiladi *et al.*, 2005c) and *Sy*KatG (Jakopitsch *et al.*, 2004), analysis of MS data has demonstrated the presence of the covalent adduct between Y218 and W95, corresponding to the result of *Hm*M244A variant. The formation of the dipeptide [Y218–W95] covalent bond has been proposed to occur upon the simultaneous on electron oxidation of both the phenol of Y218 and indole rings of W95, respectively, by KatG Compound I formation. Thus it is suggested that the

Early View

© 2012 Global Journals Inc. (US)

absence of a coordinate centered sulfur atom in position 244 is most likely the reason as to why the *Hm*KatG [M244A] variant did not exhibit a tripeptide [M244–Y218–W95] covalent adduct, in spite of the presence of a redox active side-chain (indole group) of N*ε1* atom in position 95 adjacent to heme. As only one of the INH - resistance conferring *Mt*KatG variants has been found to conclusively cause a complete lack of *catalase* activity ([R409L], Ghiladi *et al.*, 2004), the result is in good agreement with study previously reported which noted any correction between drug susceptibility and the absence of the tri-peptide [M244–Y218–W95] covalent adduct.

d) Heterologously-Structured Dimer Subunits in [M244A] Variant –

The crystal structure of *Hm*KatG [M244A] variant was determined by using Molecular replacement method with WT (PDB code 1ITK) as probe molecule. Structural refinement statics are shown in Table 2. The overall structure is similar to that of the WT. The average r.m.s. deviation between each subunit are 0.67 Å for the backbone Cα atoms, respectively. For [M244A] variant, the electron density maps defined backbone and side-chain atoms of 1380 amino acid residues, two iron atoms, two heme groups and 306 water molecules in two subunits. Residues 1–29, 295–301 and 727–731 of both subunits are not included in the final model because they are invisible or suspense in the electron density map. The model has crystallographic agreement R and R_{free} factors of 28.3% and 32.5% for 71879 reflections in the resolution limit of 2.33Å. On the other hand, an asymmetric unit of [M244A] variant crystal contains two subunits A and B related by non-crystallographic two-fold symmetry. The comparison of the dimer structures in [M244A] reveals remarkable few changes, which are the relative displacement of W95, H96, D125, E194 and E222 for 1.5 Å, 0.82 Å, 0.81 Å, 0.87 Å, 0.814Å and 0.824Å significant for overall 0.49 Å r.m.s. displacement in backbone atoms.

e) Covalent-adduct, Heme Distal Side of the Active Center and Substrate Access Channel –

The electron density maps corresponding to the active centers in subunit A and B of [M244A] variant are clearly evident to be in the different state as shown in Fig. 4. In subunit A, there is no continuous electron density and a link between distal side tryptophane and tyrosine could not be found but distance between Y218 C*ε1* and W95 C*η* is 2.42Å, suggesting the presence of covalent adduct between the Y218 and W95. By contrast, in subunit B, the distance between Y218 C*ε1* and W95 C*η* is 1.76Å, strongly demonstrating the covalent-linkage between Y218 and W95. The lower electron density was caused by the disorder effect of mobile Y218 on the flexible LL1 loop which formed the substrate access channel into the cavity as shown in Fig.6. Such flexibility may be observed in subunit A. Moreover, A244 C*β* moves away from Y218 C*ε2* at 0.95Å and 0.824Å, respectively. By substitution of Met244 to Ala, the covalent adduct between M244 and Y218 was disrupted. This clearly rules out the hypothesis that M244 takes part in the integrity and/or formation of the covalent bond between Y218 and W95. Structural information obtained from X-ray crystallography on the [M244A] variants can confirm these results from MS. In this work, we have demonstrated that M244 variants affect the linkage between W95 and Y218.

f) Mobile D125

Side-chain of D125, which located at the bottom of the channel, showed remarkable structural change in the [M244A] variant. In subunit A, the side-chain D125 is hydrogen (H-) bond interaction with the backbone amino nitrogen of I217, resulted perpendicular rotation of χ2 of D125 side-chain to face the imidazole of H96 (Fig. 5). D125 in subunit B was still fixed as well as the original architecture observed in the WT enzyme by H-bonding with backone of I217 in the LL1 loop. D125 has been known to be important in the H_2O_2 oxidation to date (Jakopitsch *et al.*, 2003a; Singh *et al.*, 2004). However, there is a dramatic reversal of the side chain dihedral angle χ2 of D125 without backbone distortion with respect to that of the WT structure. In subunit A, the larger dihedral angle χ2 of D125 than that in subunit B, which the side-chain of D125 is reoriented to bind *peroxidase* substrate as *O*DA or water molecule as deriving from H_2O_2, respectively. Hence, the mobile D125 residue will also be suggestive of utilizing as both initiator H_2O_2 and substrate recognition, making it effective in binding substrate, though disruption of π-complexes with heme and W95 known to act on as molecular switch from the *catalase* to the *peroxidase* (Carpena *et al.*, 2005). Also the backbone amino N of I217, a proton donor, forms an H-bond to the oxygen O*δ1* of the side-chain carbonyl group of D125 with displacement from at a distance of 2.69Å toward 2.80 Å, rotating by 63.8° with respect to the O*δ1*–C*γ*–O*δ1* (I217) angle. Thus it can be concluded that no H-atom is seen in the C=O*δ1* group (D125). When O*δ2* in the side chain of the D125 can be an ionized carboxyl group at optimum pH6 near pKa value of 4.0, it is implying that O*δ2* is the –OH position and that an ionized carboxyl group of D125 cannot be proton accepter but may be a powerful proton donor for *peroxidase* substrate. Because of possible function of D125 for binding the *peroxidase* substrate, one of two catalytic centers with extremely high affinity (K_m^A = 0.974 μM) for *O*DA in M244A variant would be attributed to rotate the side-chain dihedral angle χ2 by 61.3° of the mobile D125 in the subunit A.

g) Access Channel –

While the WT exhibits *catalase* activity that can detoxify oxidative radicals, stabilizing LL1 loop by covalent adduct linkage between Y218 and M244 on

Early View

© 2012 Global Journals Inc. (US)

helix E, the [M244A] variant lost *catalase* activity due to the cleavage of the linkage and then the mobile upstream residues of LL1 (accompanied by the mobile D125). When the displacement of E194 and E222 was endured by the flexible response of the downstream portion of LL1 loop, it allows the mouth of the channel to open and to facilitate adequate uptake of substrate into the heme cavity. Side-chain of E194 on LL1 loop locates at the entrance of the channel and was also affected by this mutation.

h) π-complexes between W95 and Heme –

The Structural architecture of the distal residues shows significant differences between the subunit A and B. As shown in Fig.6, the distance between indole nitrogen atom of W95 (N*ε1*) and carbon atom (C*1C*) in heme pyrrole ring C is 3.87Å and 3.25Å, respectively, on the vicinity of *γ*- meso heme edge in subunits A and B. It is suggested that the space of heme pocket was extended in subunit A and indole ring of W95 could not form π-complexes with the porphyrin.The π interaction between the distal W95 and heme can stack and may form the ET complex, since resulting from π interaction distance that is slightly shorter 3.25Å in subunit B than the 3.3 Å distance observed in WT enzyme. According to Marcus theory, the electronic coupling term, V, depends on the distance between the electron donor (heme) and electron acceptor (W95). The electronic coupling term of WT is of high square value of 0.06836 eV^2 and 0.79315 eV^2 for subunit A and B, respectively. M244A exhibits 0.00241 eV^2 and 0.04082 eV^2 during *peroxdase cycle* , which would not almost transfer electron from Heme to W95. It is strongly sensitive to the electronic coupling term that the rate of ET in protein controls the *catalase* function.

IV. Discussion

a) Functional Prediction guided by Docking Study with H_2O_2 ,ODA (or ODA_{red}) molecule based on the structure of [M244A] –

Though K_m values (affinity) for H_2O_2 to [M244A] cannot be detected from kinetic study, the structure-based docking calculation is useful in distinguishing subunit A from B, in [M244A] variant that has binding H_2O_2 affinity. It cannot estimate only H_2O_2 affinity but can also predict the proposal space among three target residues as W95, H96 and D125. The docking energies defined as the negative value of attractive binding energy, which each subunit A and B is of (-30.3kcal/mol; -23.9kcal/mol) for W95, (-29.9 kcal/mol; -18.2 kcal/mol) for H96 and (-32.1 kcal/mol; -30.3kcal/mol) for D125 as shown in Table 3.

A calculated binding energy in the [M244A] variant (-76.5 kcal/mol for subunit A; -30.9 kcal/mol for B) also reveals to have significantly high affinity for *O*DA and a possible site has been proposed in a cavity on the distal side of the heme. Consequently, the porphyrin carbon atom (C*HB*) of **δ**-meso heme edge , which is in the position to make a 90-degree turn to the right from *γ*- meso edge of the heme plane (in Fig.6), may serve as a docking site for substrate as *O*DA or ODA_{red} (*O*DA cation radical; *O*DA (▪⁺). However, the specific enhancements in *peroxidise* can be influenced by the *O*DA affinity difference between each subunit. The 2-fold higher energy for subunit A than that of subunit B has been predicted from docking with *peroxidise* substrate as *O*DA. The binding site of ODA_{red} had been estimated with -46.6 kcal/mol from docking calculations against D125 for subunit A but repulsion energy for subunit B in Table 3. Thus the active site of subunit A exhibits the higher affinity for the imino (>C=NH) group of ODA_{red} and deprotonate the amino ($-NH_2$) group of *O*DA more efficiently than that of the subunit B. The difference of catalytic efficiency in M244A here is of the 18 fold higher subunit A than that of B. Arising from the H–bonding interaction with either *O*DA or ODA_{red}, the promising *peroxidase* in [M244A] variant may result from lost *catalase* activity by a change in the localized electronic state between C*1C* and C*HB* in the heme edge.

b) Reasonableness of peroxidatic expression evaluated by [M244A] Structure- based Frontier Orbital Calculations

While the difference between WT KatG and [M244A] variant structures are very subtle changes and the structural integrity is highly maintained, the HOMO/LUMO orbital calculation appears to functioning of the enzyme. When the *O*DA binds to **δ**-edge of the heme estimated as of LUMO and then W95 cleavage from the **γ**-heme edge, KatG appears to convert from *catalase* into *peroxidase* function. As shown in Table 4, indeed, it is supported that *catalase* activity lost when ET cannot complete between C*1C* carbon Heme and N*ε1* nitrogen atom W95. Though the C*1C* carbon atoms [for subunit A of -0.96eV and B of -1.27eV] of the heme is mix of LUMO orbital with nucleophilic Sr of (0.523, 0.771) and temporary HOMO with electrophilic Sr of (0.510, 0.437), the C*1C* can either less than 3.3 Å or always link the π-π interaction to W95, if W95 N*ε1* (▪⁺) cation radical show usually HOMO with electrophilic Sr of (0.462, 0.518) and transient LUMO orbital with nucleophilic Sr of (0.487, 0.355) on the covalent adduct of Y218–W95 [-7.57eV, respectively, and -7.62eV], since both energy gaps exceed the capacity of ET over 6 eV, having no *catalase* activity for [M244A]. Having electron-withdrawing (proton donor) group, C*HB* carbon atoms of heme are of LUMO in subunit A [-1.87eV] and B [-1.39eV] and also exhibit most active due to nucleophilic Sr of (1.113, 0.924). The most likely site of binding *peroxidase* substrate would be the C*HB* atom in the **δ**-meso heme edge. The *peroxidase* substrate acts as the electron donor in the *peroxidase* reaction. It is possible to recognize as the *peroxidase* substrate with the presence of the *O*DA. However, without the *O*DA in

Early View

© 2012 Global Journals Inc. (US)

especially subunit B of M244A, Compound I and W95 were elaborated by ET to W95 (•+) cation radical from Por(•+) via π-complex between them. When electron donation to the W95(•+) cation radical from(•)C*1C* atom on Por (•+), compound II reverts to compound I. The working hypothesis of the present study therefore includes the assumption that expression of *catalase* function may be converted by ET pathway for *O*DA into *peroxidase* which is inherent in KatG. In subunit B, the orbital between C*1C* Heme and Nε*1* W95 become the binding orbital (in green and yellow) and promotes the bonding of π-system of between indole rings of Trp and pyrole ring of heme, which may be reclaiming compound I. In subunit A, there is no π-bonding interaction between Nε*1* W95 and C*1C* Heme. There would not be ET at all. The two electrons normally occupy in each orbital (in red and blue) are produced by the excitation of photoreaction using sunlight as energy source and therefore Heme edge become of LUMO and may have reduction of compound II when *O*DA(•+) radical cation bound to C*HB*.

Kinetic parameters in the *Hm*KatG [M244A] variant are determined by fitting the kinetic data to non-linear (mixed) Michaelis-Menten equation and show that isoenzyme pattern of active two catalytic center motifs typical of *peroxidases.* For crystallographic analysis of *Hm*KatG [M244A] variant indicated that KatG is a functional heterodimer in governing KatG dimeric subunits structure. Despite of missing *peroxidase* substrate, no *catalase* reactivity against the second H_2O_2 exhibits at all so that the electron cannot transfer from M244 to the covalent adducts W95 via Y218. In spite of a π-π* electron interaction of the heme with the covalent adduct W95, the ability to transfer electron between an electrophile of tyrosinate of Y218 and the nucleophile of sulfur cation was lost by the deletion mutation at the position 244. Therefore KatG is considered the *catalase* function to use a methionine nucleophile intramolecularly- and octahedrally-coordinated complex with the carbonyl O atoms of Y101 and G99. [M244A] was of not identical electron pathway in two subunits. The phenalic group of Y218 could move its side chain closer to the indole group of W95 in subunit B than that of subunit A. Subunit A disrupted a possible π-π* interaction between W95 and heme. Including the differences in active site geometry, it would be sufficiently stronger to facilitate the oxoferryl (Fe (IV) =O) reduction in the *peroxidase* reaction. And back donation of electron from heme edge to W95 would suffice for compound I revitalization.

*O*DA binding affinity for subunit A was enhanced by χ2 of 61.3° in the carboxyl side chain of D125. On the contrary, in subunit B, the consequent ET from heme to W95 could explain the enhancement of *peroxidase* and iteratively-generated compound I intermediate. The isoenzyme pattern of *peroxidase* was discussed in terms of its hetero-dimeric character of *peroxidatic* subunit A for reduction of compound II and subunit B for reclaiming compound I. The value of catalytic efficiency (k_{cat}/K_m) for the *peroxidatic* reaction catalyzed by the *Hm*KatG [M244A] variant falls within the expected range for an efficient enzyme (Albery & Knowles, 1976).

The M244–Y218–W95 covalent adduct confirms to be essential for the *catalase* activity. It was also constructed to explore the effect of successive triple base substitutes for Met244 to Ala and to cleavage the covalent bond amongst the tri-peptide. The [M244A] variant that coupled with the structure based-evolution within laboratory time scale is not biochemically associated with INH susceptibility. *Catalase* activity of KatG prevents INH oxidation to the active form. Despite of INH resistance-conferring variants, this “unnatural” protein engineering for *Hm*KatG, can confirm the inherent *catalase* functional capability for M244 of capping the C-terminal ends of E-helix in KatG. Perhaps the most intriguing feature of the *Mt*KatG is its ability to mediate INH susceptibility. In the closing discussion, lastly the kinetic characterization of KatG enzyme in this bacterial has been detected isoenzyme pattern of *peroxidases*. For a better understanding of the complex interrelations between *catalase* and *peroxidase* and the oxidation of phenols, *peroxidases* are highly polymorphic enzymes, and the functionality of each isoenzyme depends on its (acidic) nature and its persistent growth phase of the *Mt* clinical strains. In order to facilitate the *peroxidase* activity and to understand the metabolic functions that are needed for the persistence of *Mt*, the structure based compounds can be useful in the design of HIV/anti-tuberculosis drugs that could eradicate persisters effectively.

V. Conclusion

KatG exhibits *catalase* and *peroxidase* Sulfer–centered M244 coordinated complexes with carbonyl oxygens of G99 or Y101, the covalent adduct, and π–conjugated complexes interacted with heme facilitate *catalase* reaction. It is crucial for understanding INH-sensitivity process how KatG functional groups participate in *peroxidase* catalysis.

VI. Abbreviations

INH, isoniazide, isonicotinic acid hydrazide; H_2O_2, hydrogen peroide; *t-Bu*OOH, *tert*-butylperoxide; *O*DA, *o*-dianisidine, 4-(4-amino-3-methoxyphenyl)-2-methoxyaniline; *O*DA (•+), *O*DA cation radical; *O*DA$_{red}$, *o*-dianisidine, quinoneimine,4-(4-imino-3-methoxycyclo-hexa-2,5-dien-1-ylidene)-2 methoxycyclo- hexa-2,5-dien-1-imine; HOMO, the highest occupied molecular orbital (electrophilic reactivity); LUMO, the lowest unoccupied molecular orbital (electron affinity); Sr, superdelocaliza-bility.

Early View

© 2012 Global Journals Inc. (US)

VII. Data Deposition:

The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID codes 3VLM for M244A)

References Références Referencias

1. Albery, E.J., and Knowles,J.R. (1976) Evolution of enzyme function and the development of catalytic efficiency. Biochemistry 15, 5631-5640.
2. Baerends, E. J., Berger, J. A., Bérces, A., Bickelhaupt, F. M., Bo, C., de Boeij, P. L., Boerrigter, P. M., Cavallo, L., Chong, D. P., Deng, L., Dickson, R. M., Ellis, D. E., van Faassen, M., Fan, L., Fischer, T. H., Fonseca Guerra, C., van Gisbergen, S. J. A., Götz, A. W., Groeneveld, J. A., Gritsenko, O. V., Grüning, M., Harris, F. E., van den Hoek, P., Jacob, C. R., Jacobsen, H., Jensen, L., Kadantsev, E. S., van Kessel, G., Klooster, R., Kootstra, F., Krykunov, M. V., van Lenthe, E., Louwen, J. N., McCormack, D. A., Michalak, A., Neugebauer, J., Nicu, V. P., Osinga, V. P., Patchkovskii, S., Philipsen, P. H. T., Post, D., Pye, C. C., Ravenek, W., Rodriguez, J. I., Romaniello, P., Ros, P.,Schipper, P. R. T., Schreckenbach, G., Snijders, J. G., Sola`, M., Swart, M., Swerhone, D., te Velde, G., Vernooijs, P., Versluis, L., Visscher, L., Visser, O., Wang, F., Wesolowski, T. A., van Wezenbeek, E. M., Wiesenekker, G., Wolff, S. K., Woo, T. K., Yakovlev, A. L., and Ziegler, T. (2007) *ADF 2007.01, SCM*, Theoretical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands
3. Bertrand, T., Eady,N.A.J., Jones,J.N., Jesmin,J.M., Nagy,J.M., Jamart-Gregoire,B., Raven,E.L., Brown,K.A. (2004) Crystal structure of *Mycobacterium tuberculosis catalase-peroxidase*. The Journal of Biological Chemistry 279, 38991-38999.
4. Carpena,X., Wiseman,B., Deermagarn,T., Singh,R., Switala,J., Ivancich,A., Fita,I., and Loewen,P.C. (2005) A molecular switch and electronic circuit modulate catalase activity in *catalase-peroxidases* EMBO Report 6, 1156-1162.
5. Dewar M.J.S., Zoebisch E.G., Healy E.F., and Stewart, J.J.P. (1985) Development and use of quantum mechanical molecular models. 76. AM1: a new general purpose quantum mechanical molecular model. Journal of the American Chemical Society 107, 3902-3909.
6. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, Palo Alto, CA.
7. Donald,L.D., Krokhin,O.V., Duckworth,H.W., Wiseman,B., Deemagarn, T., Singh,R., Switala,J., Carpena,J.X., Fita,I., Loewen,P.C. (2003)Characterization of the *Catalase-Peroxidase* KatG from *Burkholderia pseudomallei* by Mass Spectrometry. The Journal of Biological Chemistry 278, 35687-35692
8. Dulley,J.R., and Grieve,P.A. (1975) A simple technique for eliminating interference by detergents in the Lowry method of protein determination. Analytical Biochemistry 64, 136-141.
9. Falk,J.E. (1964) In: Porphyrins and Metalloproteins: Their general, physical, and coordination chemistry and laboratry methods. Biochim Biophys Acta Library Vol.2, New York, Elsevier Co, pp.181-188.
10. Fukui,K., Yonezawa,T., Nagata,C. (1954) Theory of Substitution in Conjugated Molecules. Bull.Chem.Soc.Japan, 27, 423- 427.
11. Fukui,K., Yonezawa,T., Nagata,C. (1957) MO Theoretical Approach to the Mechanism of Charge Transfer in the Process of Aromatic Substitutions. Journal of Chemical Physics., 27, 1247-1259.
12. Fukuzawa, K., Kitaura, K., Nakata, K., Kaminuma, T., and Nakano, T. (2003) Fragment molecular orbital study of the binding energy of ligands to the estrogen receptor. Pure and Applied chemistry, 75, 2405-2410.
13. Ghiladi,R.A., Cabelli,P.R., and Ortiz de Montellano,P.R. (2004) Superoxide reactivity of KatG: Insights into isoniazid resistance pathways in TB. Journal of the American Chemical Society 126, 4772-4773.
14. Ghiladi,R. A., Knudsen, G. M., Medzihradszky, K.F., and Ortiz de Montellano, P. R. (2005b) The Met-Tyr-Trp Cross-link in *Mycobacterium tuberculosis. Catalase-peroxidase* (KatG). The Journal of Biological Chemistry 280, 22651–22663.
15. Ghiladi,R. A., Medzihradszky, K. F., and Ortiz de Montellano, P. R. (2005c) Role of the Met-Tyr-Trp Cross-Link in *Mycobacterium tuberculosisCatalase-Peroxidase* (KatG) As Revealed by KatG(M255I). Biochemistry 44, 15093-15105.
16. Grimme,S.,Anthony,J.,Ehrlich,S.,and Krieg,H. (2010) A consistent and accurate *ab initio* parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *The Journal of Chemical Physics.,***132**, 154104 -154119.
17. Grimme,S.,Ehrlich,S.,and Goerigk,L. (2011) Effect of the Damping Function in Dispersion Corrected Density Function Theory.*Journal of Computational Chemistry.,***32**, 1456-1465
18. Heym,B., Zhang,Y., Poulet,S., Young,D., and Cole,S. (1993) Characterization of the KatG gene encoding *catalase-peroxidase* required for the isoniazid susceptibility of *Mycobacteriumtuberculosis.* Journal of Bacterilogy, 175, 4255-4259.
19. Jakopitsch,C.,Auer,M.,Regelsberger,G.,Jantschko, W., Furtmüller,P.G., Ruker,F., and Obinger,J.

Early View

© 2012 Global Journals Inc. (US)

(2003a) Distal site Aspartate is Essential in the *Catalase* Activity of *catalase-peroxidases*. Biochemistry 42, 5292-5300.
20. Jakopitsch,C.,Ivancich,A., Schmuckenschlager,A., Wanasinghe,A., Furtmüller,P.G., Ruker,F., and Obinger,C. (2004) Influence of the unusual covalent adduct on the kinetics and formation of radical intermediates in Synechocystis catalase peroxidase : A stopped-flow and EPR chracterization of the Met275, Tyr249, and Arg439 variants. Journal of Biological Chemistry 279, 46082-46095.
21. Johnsson K ,King DS, Schultz PG (1995) Studies on the mechanism of action of isoniazid and ethionamide in the chemotheraphy of tuberculosis. Journal of American Chemical Society 117 , 5009-5010.
22. Laskowski, R. A., MacArthur, M. A., Moss, D. S., and Thornton, J. M. (1993) PROCHECK: a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography 26, 283-291.
23. McRee,D. (1999) XtalView/Xfit-A versatile program for manipulating atomic coordinates and electron density. Journal of Structural Biology 125, 156-165.
24. Murshudov,G.N., Vagin,A.A., and Dodson,E.J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallographica Section D 53, 240-255.
25. Pearson RG. (1986) Absolute electronegativity and hardness correlated with molecular orbital theory. Proceedings of the National Academy of Sciences of the United States of America. 83, 8440-8441.
26. Perrakis, A., Morris, R., and Lamzin, V. S. (1999) Automated protein model building combined with iterative structure refinement. Nature Structural Biology 6, 458-463.
27. Schnölzer, M., Jones, A., Alewood, P. F., and Kent, S.B.H. (1992) Ion-spray tandem mass spectrometry in peptide synthesis: Structural characterization of minor by-products in the synthesis of ACP(65–74) *Analytical Biochemistry* **204**, 335–343
28. Schägger,H., and von Jagow,G. (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analytical Biochemistry 166, 368-379.
29. Singh,R., Wiseman,B., Deemagarn,T. Donald,L.J., Duckworth,H.W., Carpena,X., Fita,I., and Loewen,P.C. (2004) *Catalase-peroxidase* (KatG) exhibit NADH *oxidase* Activity. Journal of Biological Chemistry 279, 43098-43106
30. Stewart JJP, MOPAC 2002 release 2.1 Fujitsu Limited,Tokyo, JAPAN(2002).
31. Ten-i,T., Kumasaka,T., Higuchi,W., Tanaka,S., Yoshimatsu,K., Fujiwara,T. and Sato,T. (2007) Expression, purification, crystallization and preliminary X-ray analysis of Met244Ala variant of *catalase-peroxidase* (KatG) from the haloarchaeon *Haloarcula marismortui.* Acta Crystallographica Section F 63, 940-943.
32. Vagin,A.A., and Teplyakov,A. (1997) MOLREP: an automated program for molecular replacement. Journal of Applied Crystallography 30, 1022-1025.
33. Wei,C.J., Lei,B., Musser,J.M., and Tu,S.C. (2003) Isoniazid activation defects in recombinant *Mycobactrium tuberculosis Catalase-Peroxidase* (KatG) mutants evident in InhA inhibitor production. Antimicrobial Agents and Chemotherapy, 47, 670-675.
34. Welinder,K.G. (1992) Superfamily of plant, fungal and bacterial *peroxidases.* Current Opinion in Structural Biology 2, 388-393.
35. Worthington CC, Worthington (1988). In: EnzymeManual: Enzymes and Related Biochemicals. Freehold, New Jersey, pp.254-60.
36. Zhang,Y., Heym,B., Allen,B., Young,D., and Cole,S. (1992) The *catalase-peroxidase* gene and isoniazid resistance of *Mycobacterium tuberculosis.* Nature 358, 591-593.

Early View

Table 1 : Kinetic Constants for *peroxidase* activity associated WT *Hm*KatG and M244A variant.

All the kinetic constants was measured at Optimum pH. The averages and standard deviations were obtained from 8 individual data.

	WT		M244A	
Subunit	A	B	A	B
pH	6		8	
k_{cat} (s^{-1})	$0.528 \pm 5.53 \times 10^{-2}$	6.92 ± 0.641	1.60 ± 0.27	4.73 ± 0.20
K_m(μM)	0.814 ± 0.686	$3.52 \times 10^{2} \pm 58.6$	0.974 ± 0.782	50.76 ± 7.76
k_{cat} / K_m($\times 10^6 M^{-1} s^{-1}$)	0.650	0.0196	1.645	0.092
R^2	0.999		0.998	

© 2012 Global Journals Inc. (US)

Table 2 : Structural refinement statistics

	M244A
Refinement	25.0-2.33
Number of reflections	71879
†Rwork(%)	28.3
‡Rfree(%)	32.5
Number of residues	1325
Number of water molecules	151
R.m.s.d.bond length (Å)	0.019
R.m.s.d.angle(°)	1.796
Average B-factor ($Å^2$)	
Protein atoms	39.79
Water molecules	32.5

‡R_{free} was calculated using a set of reflections where 10% of the total reflections had been randomly omitted the refinement and used to calculate R_{work}.

Table 3 : Binding Energies for [M244A] Variant Associated with Initiator H_2O_2 and Substrates *O*DAs.

It is suggested as binding affinity that the value of ΔE_{ligand} is negative to be predicted by docking calculation with the initiator H_2O_2 and the *Peroxidise* substrate *O*DA and *O*DA$_{red}$, according to eq.6.

M244A variant						
Subunit	A	B	A	B	A	B
	initiator		Substrate for Compound I		substrate for Compound II	
Ligand	H_2O_2		*O*DA		*O*DA$_{red}$	
Target residue						
W95 (kcal/mol)	-30.3	-23.9				
H96 (kcal/mol)	-29.9	-18.2				
D125 (kcal/mol)	-32.1	-30.3	-46. 6	repulsive	-76.5	-30.9
E194 (kcal/mol)	-22.5	-23.7	-70.0	-71.4	-67.9	-66.1
E222 (kcal/mol)	-21.2	-20.1	repulsive	-32.8	-21.2	-44.8
S305 (kcal/mol)	repulsive	-17.7	repulsive	-8.4	repulsive	-9.5
Heme (kcal/mol)	-26.6	-23.9	repulsive	repulsive	-69.3	-26.9

Table 4 : Each Subunit of MO Energy in the π-complexes with Y218-W95 covalent adduct of *Hm*KatG [M244A] Variant.

The frontier molecular orbital of (a) HOMO and (b) LUMO Energies for active site model, [M244A] variant associated with *peroxidase* reaction.

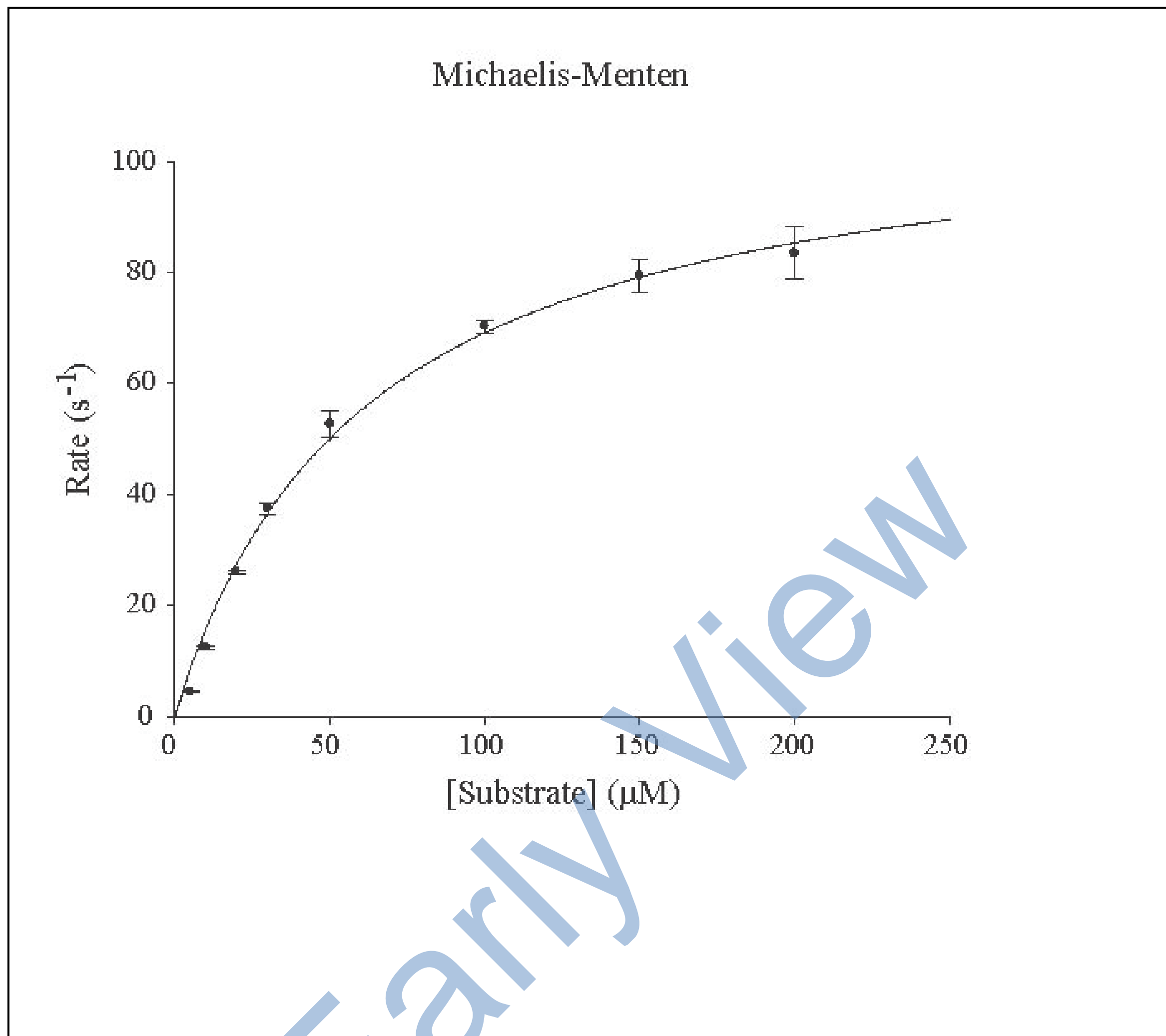
		M244A		*O*DA	*O*DA	*O*DA$_{red}$	*O*DA$_{red}$
	Subunit	A	B	A	B	A	B
reaction	*catalase*						
HOMO(cation radical) (eV)	Y218-W95 Nε1($\cdot^{+}$)	-7.57	-7.62				
LUMO(eV)	Heme C*1C*	-0.96	-1.27				
The energy gap (eV)		6.61	6.35				
Distance (Å)		3.9	3.3				
Phase &		matching	matching				
orbital		bonding	bonding				
ET		impossible	possible				
	peroxidase						
HOMO(cation radical) (eV)				-7.522	-7.400	-7.468	
LUMO(nucleophilic) (eV)	Heme *CHB*	-1.87	-1.39	-1.759	-1.288	-1.639	
The energy gap (eV)				5.76	6.11	5.83	
Distance (Å)				3.16	3.06	3.40	
orbital				bonding	bonding	bonding	
ET				possible	possible	possible	

Early View

© 2012 Global Journals Inc. (US)

Figure Legends

Figure 1 : Mixture Michaelis-Menten Plotting of *Peroxidase* activity of *Hm*katG [M244A] variant.

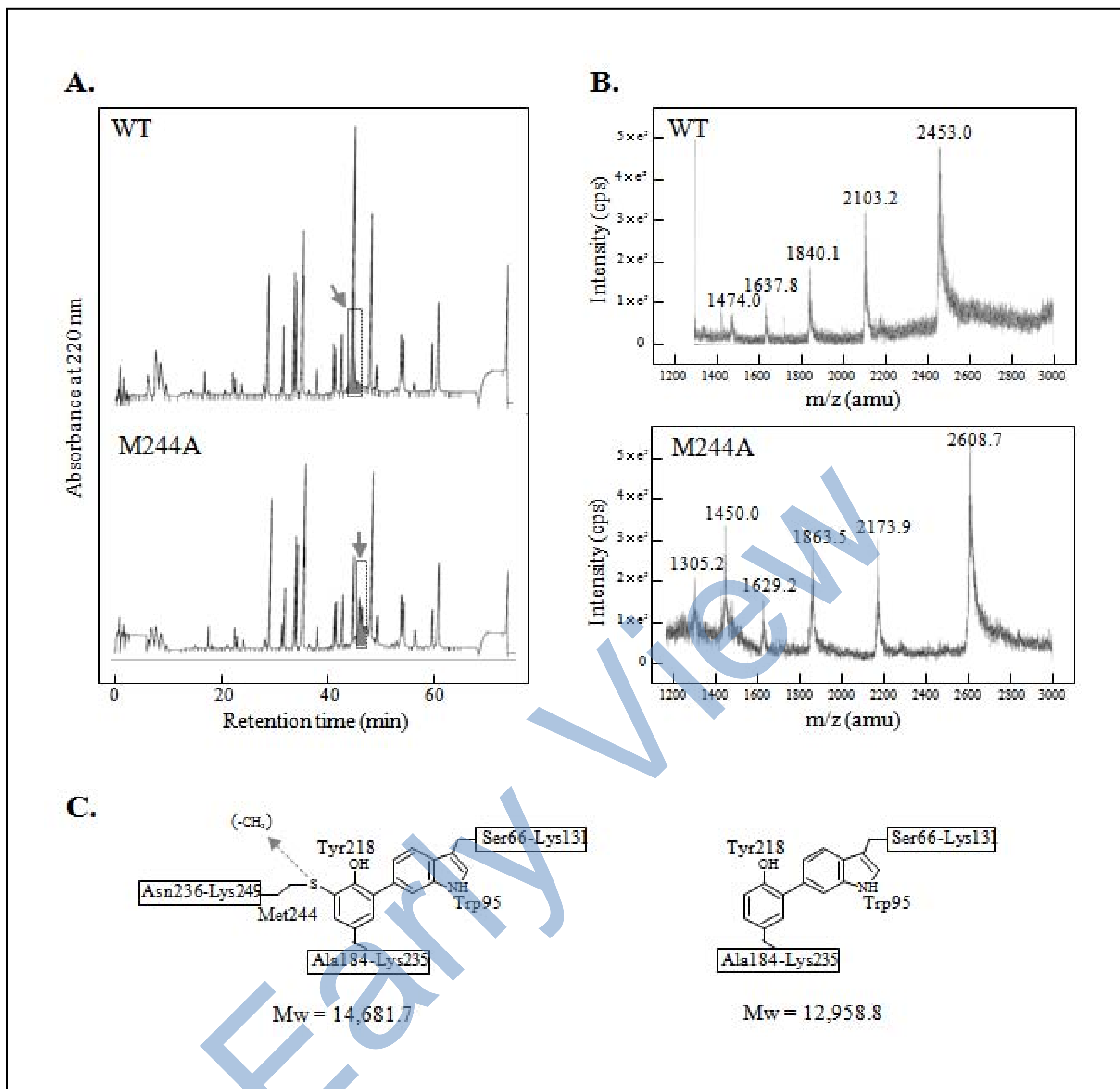


Early View

The reaction velocity [Rate, v] was plotted against the initial *O*DA concentration [Substrate, S]. Data were fitted to the Mixture Michaelis-Menten equation, yielding the kinetic parameters for the two catalytic center models (eq.5).

© 2012 Global Journals Inc. (US)

Figure 2 : Fractionation and Mass spectroscopic Analysis of Polypeptides Obtained by *Lysyl-endopeptidase* Digestion of WT and [M244A] variant of *Hm*KatG.

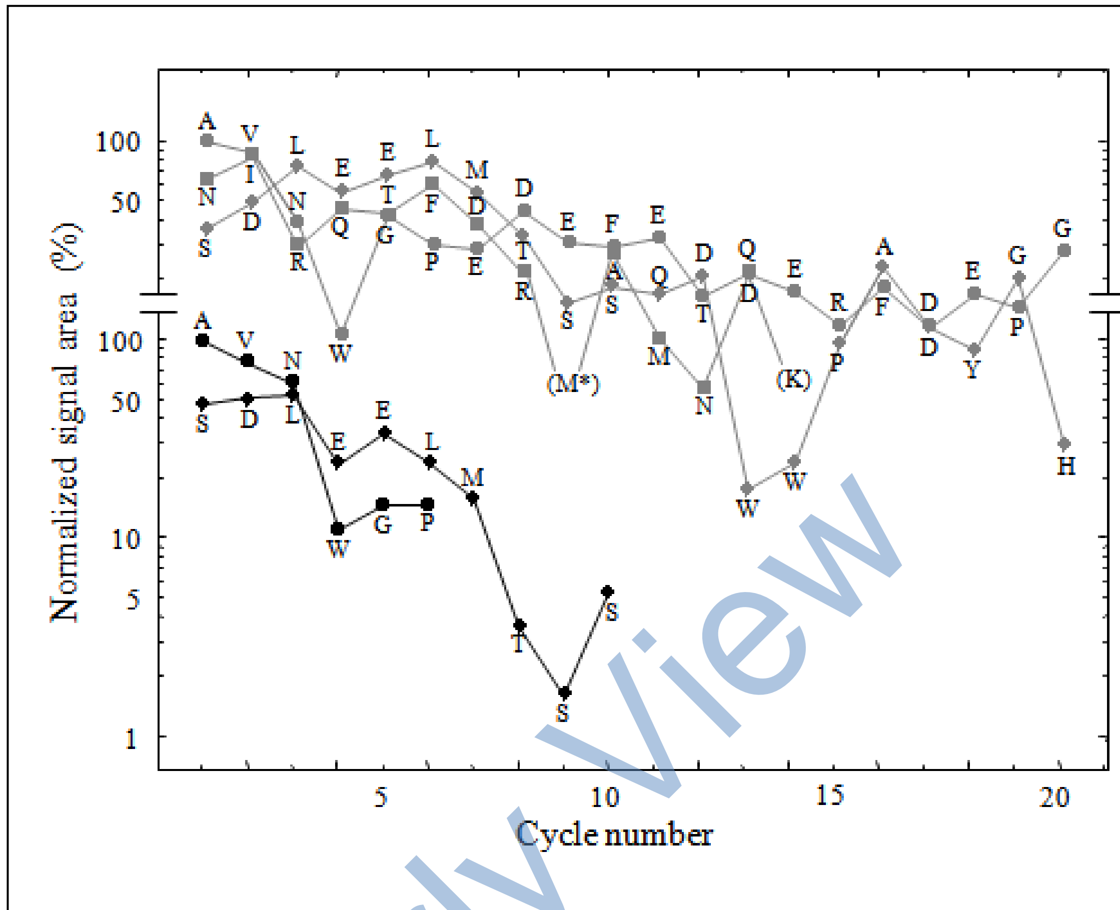


(A)HPLC chromatograms (0–75 min region) of the *lysyl-endopeptidase* digests for WT (top) and[M244A] (bottom) monitored at λ=220 nm. The region (r.t. ~45 min), corresponding to the covalent adducts, is highlighted (Fig.3, boxed area). Their fractions were corrected with the broad signals appeared at the different positions. (B) Mass spectrum (in the m/z range of 1200–3000 Da) of the covalent adducts. The polypeptide prepared for both WT (upper panel) and [M244A](lower) and was shown. (C)Fragment assignment from M244–Y218–W95 covalent adduct (left) and Y218–W95 (right).The cleavage that produced the base peak and the ion at m/z 1305.2 Da is shown in C (right).

Early View

© 2012 Global Journals Inc. (US)

Figure 3 : N-terminal Amino Acid Sequence of the *Endopeptidase*-digested Fragment Containing the Covalent-adduct.

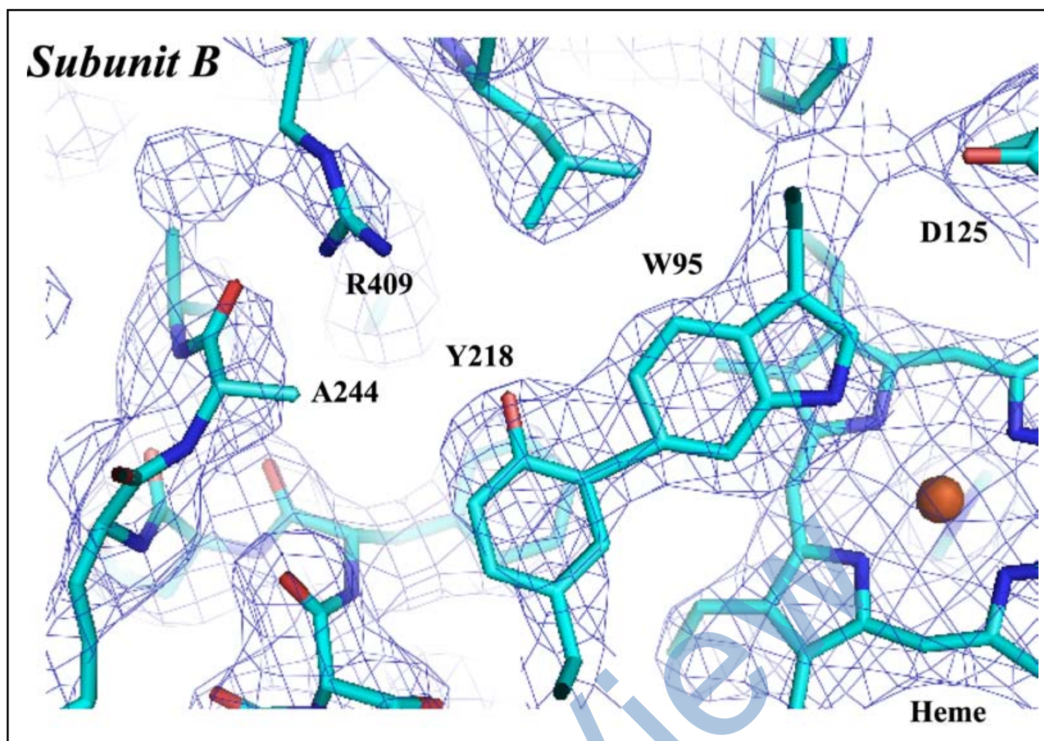


N-terminal amino acid sequence of the fragments purified from the WT (gray line) and [M244A]-variant (black line) of *Hm*KatG were analyzed. Signal areas of each residue were normalized by that of Alanine appeared at the first cycle (corresponding to A184). The signal that corresponded to M244 (M* in the figure) was not observed.

Early View

© 2012 Global Journals Inc. (US)

Figure 4 : The 2|Fo|-|Fc| electron density map around the covalent adduct in [M244A].

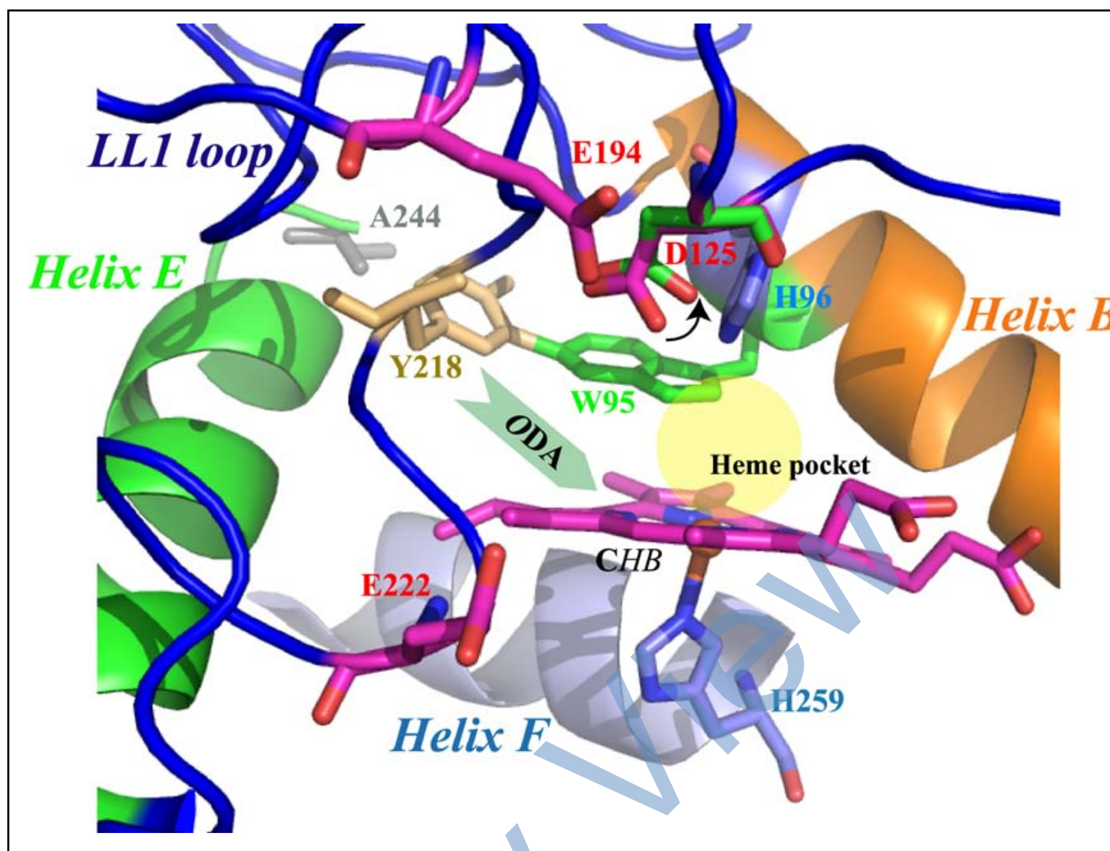


The electron density map in the vicinity of A244, Y218, W95 and heme is shown in subunit B and is contoured at 1σ (pale blue). The atom color is cyan for carbon, dark blue for nitrogen, red for oxygen and orange for heme iron. The figure was constructed using Pymol (DeLano, 2002).

Early View

© 2012 Global Journals Inc. (US)

Figure 5 : Mobile D125 relevant to Active Site Involving LL1 loop and Helices B, E and F in the Subunit B of [M244A] variant.

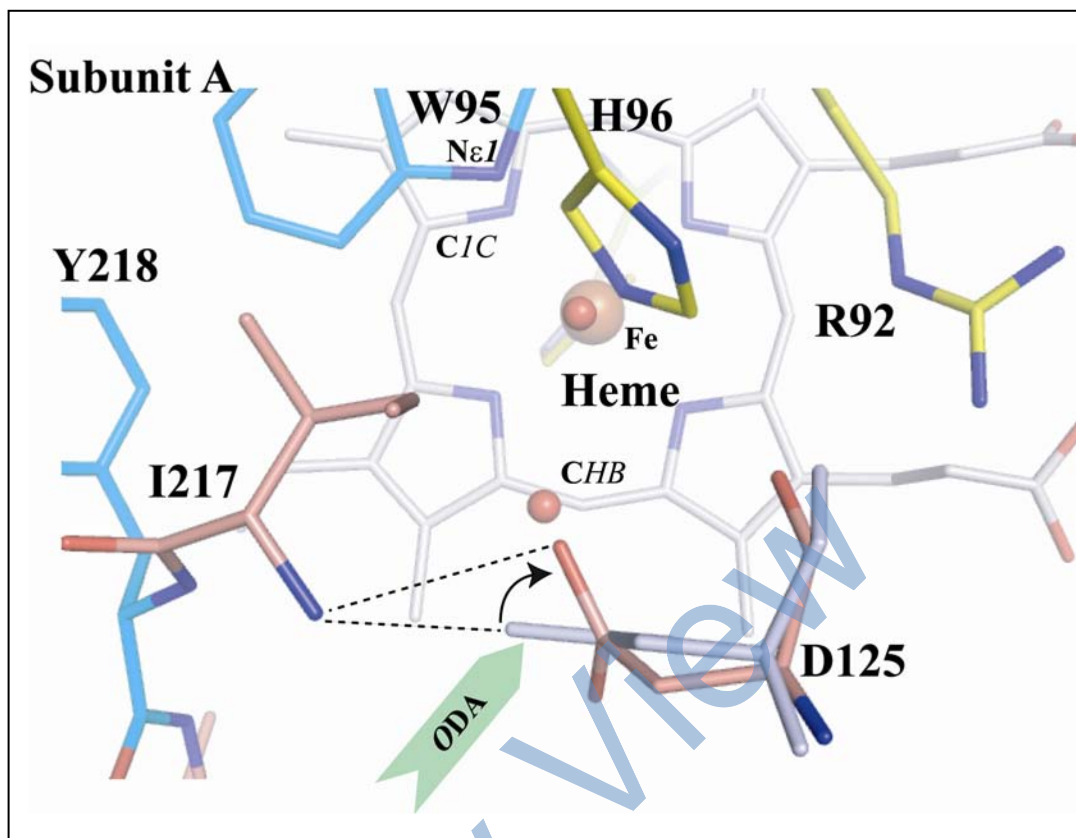


The distal H96 (on helix B; orange) and proximal H259 (on helix F; pale blue) are shown in blue. The W95 (on helix B), Y218 (on LL1 loop; cyan) and A 244 (on helix E; green) residues in green, orange, and gray. The porphyrin and its iron atom of Subunit B are represented in magenta sticks and orange sphere. The latent access channel residues of D125, E194 and E222 locate on LL1 loop, showing in red. The mobile D125 superposed that of Subunit A in WT.

Early View

© 2012 Global Journals Inc. (US)

Figure 6 : π-complex cleavage between W95 and Heme in subunit A of [M244A] to form *O*DA binding site.



The cleavage of π-complex to W95 (Nε*1*) and γ- *meso* heme edge (C*1C*) convert from *catalase* into *peroxidase* function due to arise from the H–bonding interaction with *O*DA near the porphyrin carbon atom (C*HB*) of δ-meso heme edge, accompanied by mobility of D215. The distal R92 and H96 are presented in yellow, the Y218 and W95 covalent adduct in cyan, and D125 and I217 in pink, showing in subunit A. The nitrogen, oxygen and heme iron atoms are colored for dark blue, red, and orange. D125 in subunit B is colored blue-white and water molecule is shown as red spheres. The figure is view from the distal side of Fig 5.

Early View

© 2012 Global Journals Inc. (US)

Global Journals Inc. (US) Guidelines Handbook 2012

www.GlobalJournals.org

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (FARSS)

- 'FARSS' title will be awarded to the person after approval of Editor-in-Chief and Editorial Board. The title 'FARSS" can be added to name in the following manner. eg. Dr. John E. Hall, Ph.D., FARSS or William Walldroff Ph. D., M.S., FARSS
- Being FARSS is a respectful honor. It authenticates your research activities. After becoming FARSS, you can use 'FARSS' title as you use your degree in suffix of your name. This will definitely will enhance and add up your name. You can use it on your Career Counseling Materials/CV/Resume/Visiting Card/Name Plate etc.
- 60% Discount will be provided to FARSS members for publishing research papers in Global Journals Inc., if our Editorial Board and Peer Reviewers accept the paper. For the life time, if you are author/co-author of any paper bill sent to you will automatically be discounted one by 60%
- FARSS will be given a renowned, secure, free professional email address with 100 GB of space eg.johnhall@globaljournals.org. You will be facilitated with Webmail, SpamAssassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
- FARSS member is eligible to become paid peer reviewer at Global Journals Inc. to earn up to 15% of realized author charges taken from author of respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account or to your PayPal account.
- Eg. If we had taken 420 USD from author, we can send 63 USD to your account.
- FARSS member can apply for free approval, grading and certification of some of their Educational and Institutional Degrees from Global Journals Inc. (US) and Open Association of Research,Society U.S.A.
- After you are FARSS. You can send us scanned copy of all of your documents. We will verify, grade and certify them within a month. It will be based on your academic records, quality of research papers published by you, and 50 more criteria. This is beneficial for your job interviews as recruiting organization need not just rely on you for authenticity and your unknown qualities, you would have authentic ranks of all of your documents. Our scale is unique worldwide.
- FARSS member can proceed to get benefits of free research podcasting in Global Research Radio with their research documents, slides and online movies.
- After your publication anywhere in the world, you can upload you research paper with your recorded voice or you can use our professional RJs to record your paper their voice. We can also stream your conference videos and display your slides online.
- FARSS will be eligible for free application of Standardization of their Researches by Open Scientific Standards. Standardization is next step and level after publishing in a journal. A team of research and professional will work with you to take your research to its next level, which is worldwide open standardization.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

- FARSS is eligible to earn from their researches: While publishing his paper with Global Journals Inc. (US), FARSS can decide whether he/she would like to publish his/her research in closed manner. When readers will buy that individual research paper for reading, 80% of its earning by Global Journals Inc. (US) will be transferred to FARSS member's bank account after certain threshold balance. There is no time limit for collection. FARSS member can decide its price and we can help in decision.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (MARSS)

- 'MARSS' title will be awarded to the person after approval of Editor-in-Chief and Editorial Board. The title 'MARSS" can be added to name in the following manner. eg. Dr. John E. Hall, Ph.D., MARSS or William Walldroff Ph. D., M.S., MARSS
- Being MARSS is a respectful honor. It authenticates your research activities. After becoming MARSS, you can use 'MARSS' title as you use your degree in suffix of your name. This will definitely will enhance and add up your name. You can use it on your Career Counseling Materials/CV/Resume/Visiting Card/Name Plate etc.
- 40% Discount will be provided to MARSS members for publishing research papers in Global Journals Inc., if our Editorial Board and Peer Reviewers accept the paper. For the life time, if you are author/co-author of any paper bill sent to you will automatically be discounted one by 60%
- MARSS will be given a renowned, secure, free professional email address with 30 GB of space eg.johnhall@globaljournals.org. You will be facilitated with Webmail, SpamAssassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
- MARSS member is eligible to become paid peer reviewer at Global Journals Inc. to earn up to 10% of realized author charges taken from author of respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account or to your PayPal account.
- MARSS member can apply for free approval, grading and certification of some of their Educational and Institutional Degrees from Global Journals Inc. (US) and Open Association of Research,Society U.S.A.
- MARSS is eligible to earn from their researches: While publishing his paper with Global Journals Inc. (US), MARSS can decide whether he/she would like to publish his/her research in closed manner. When readers will buy that individual research paper for reading, 40% of its earning by Global Journals Inc. (US) will be transferred to MARSS member's bank account after certain threshold balance. There is no time limit for collection. MARSS member can decide its price and we can help in decision.

© Copyright by Global Journals Inc.(US)| Guidelines Handbook

ANNUAL MEMBER

- Annual Member will be authorized to receive e-Journal GJSFR for one year (subscription for one year).
- The member will be allotted free 1 GB Web-space along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted free 500 MB email space.

PAPER PUBLICATION

- The members can publish paper once. The paper will be sent to two-peer reviewer. The paper will be published after the acceptance of peer reviewers and Editorial Board.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

© Copyright by Global Journals Inc.(US)| Guidelines Handbook

MANUSCRIPT STYLE INSTRUCTION **(Must be strictly followed)**

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, “Abstract” word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript’s Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

© Copyright by Global Journals Inc.(US)| Guidelines Handbook

the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. **Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic

principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

ADMINISTRATION RULES LISTED BEFORE SUBMITTING YOUR RESEARCH PAPER TO GLOBAL JOURNALS INC. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.
- Do not give permission to anyone else to "PROOFREAD" your manuscript.
- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

CRITERION FOR GRADING A RESEARCH PAPER (COMPILATION)
BY GLOBAL JOURNALS INC. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

Index

A

Acetone · 51
Adduct · 56, 57, 60, 62, 63, 64, 65, 67, 68, 69, 71, 74, 76, 78, 79,

C

Catalase · 56, 57, 58, 61, 63, 65, 66, 67, 68, 69, 70, 71, 72, 74, I
Chandrasekaran, · 18
Complementeach · 8
Cumulative · 12

D

Davison · 46
Deermagarn · 70

E

Erlenmeyer · 31, 34, 44
Evaluating · 1, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47
Exhaustive · 10, 11, 12, 13, 15, 16, 17, 18, 20, 21, 22, 23, 25, 27
Extraction · 35, 37, 38, 39, 43, 44, 46

F

Ferrooxidans · 29, 33, 34, 35, 39, 41, 44, 45, 46

G

Geometries · 60
Gridding · 33

H

Haloarcula · 72
Haplotype · 11, 12, 19

J

Jakopitsch · 56, 63, 65, 71

K

Kinetic · 1, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 75, 76, 78, 79, 80, I
Kirchman · 44, 46

L

Leaching · 29, 33, 34, 35, 37, 39, 41, 43, 44, 46
Linkage · 10, 18, 19, 57, 64, 65
Logistic · 1, 10, 12, 14, 16, 18, 20, 21, 22, 23, 25, 27, 28

M

MARISMORTUI · 1, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 75, 76, 78, 79, 80, I
Monotonically · 14, 16

O

OhadiZarand · 4
Optimum · 65

P

Pistaciakhinjuk · 2
Polypeptides · 29, 63

R

Recombinants · 58, 59, 61, 62
Residues · 64

S

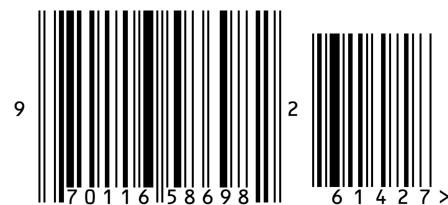
Screening · 30, 34
Spectroscopy · 31, 62
Spontaneous · 29, 37, 43
Streaking · 50
Substrate · 30, 43, 56, 58, 60, 61, 65, 66, 67, 68, 72
Supernatant · 32, 34, 50

T

Tabatabaei · 8
Trigger · 57

Global Journal of Science Frontier Research

Visit us on the Web at www.GlobalJournals.org | www.JournalofScience.org
or email us at helpdesk@globaljournals.org



ISSN 9755896

© 2012 by Global Journals