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

Global Journal of Science Frontier Research: C

Biological Sciences

Highlights

- Yield Traits in Cowpea
- Study on Enzyme Activity
- Influence of Colchicine Treatments
- Characterization of Lactobacillus Sakei

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 5 VERSION 1.0

© 2001-2014 by Global Journal of Science Frontier Research , USA

Global Journal of Science Frontier Research: C
Biological Science

Global Journal of Science Frontier Research: C
Biological Science
Volume 14 Issue 5 (Ver. 1.0)

Open Association of Research Society

© Global Journal of Science Frontier Research. 2014.

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 Headquarters
301st Edgewater Place Suite, 100 Edgewater Dr.-Pl,
Wakefield MASSACHUSETTS, Pin: 01880,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, 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: *investors@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)

Integrated Editorial Board
(Computer Science, Engineering, Medical, Management, Natural Science, Social Science)

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 Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Evaluation of Antifungal and Phytochemical Properties of Violet Tree (*Securidaca Longepedunculata* Fres). *1-6*
2. Study on Enzyme Activity in the Production and Optimization of High Temperature Alkaline α-Amylase Enzyme by *Bacillus Lichenoformis* using Low Cost Medium Derived from Agricultural byproducts. *7-13*
3. Influence of Colchicine Treatments on Character Expression and Yield Traits in Cowpea (*Vigna Unguiculata* L. Walp). *15-20*
4. Isolation and Phenotypic Characterization of *Lactobacillus Sakei* and *Pediococcusspp*. Antagonists from Algerian Meat. *21-25*

v. Fellows and Auxiliary Memberships
vi. Process of Submission of Research Paper
vii. Preferred Author Guidelines
viii. Index

Global Journal of Science Frontier Research: C
Biological Science
Volume 14 Issue 5 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Evaluation of Antifungal and Phytochemical Properties of Violet Tree (*Securidaca Longepedunculata* Fres)

By Junaidu, S., Shehu K., Aliero, A. A., Bawa, J. A. & Suleiman, I.
Federal University Dutsin-Ma, Nigeria

Abstract- This study was undertaken to investigate the antifungal activities of aqueous and ethanol extracts of Securideca longepedunculata leaves and root bark against two Aspergillus species (Aspergillus niger and Aspergillus flavus). Agar incorporation method was used for antifungal testing. The results of phytochemical screening demonstrated the presence of flavonoid, saponin, alkaloids, cardiac glycoside and saponins glycosides. Highest growth inhibition (1.16+1.15mm) at 300 mg/ml, (1.67+2.88mm) and higher increase (3.16+0.57mm) at 100 mg/ml were observed. The results showed significant effect ($p<0.05$) of antifungal activities. In the same study, the results however, applied that the phytochemical constituents of S. longepedunculata leaves and root bark extracts can be used as potential antimicrobial agents in the management of microbial diseases caused by pathogenic Aspergillus species which can become an alternative to chemical antibiotics.

Keywords: antifungal, violet tree, phytochemical, aqueous, ethanol extracts, aspergillus.

GJSFR-C Classification : FOR Code: 780105

EVALUATIONOFANTIFUNGALANDPHYTOCHEMICALPROPERTIESOFVIOLETTREESECURIDACALONGEPEDUNCULATAFRES

Strictly as per the compliance and regulations of :

© 2014. Junaidu, S., Shehu K., Aliero, A. A. , Bawa, J. A. & Suleiman, I. 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.

Evaluation of Antifungal and Phytochemical Properties of Violet Tree (*Securidaca Longepedunculata* Fres)

Junaidu, S.[α], Shehu K. [σ], Aliero, A. A.[ρ], Bawa, J. A.[ω] & Suleiman, I.[¥]

Abstract- This study was undertaken to investigate the antifungal activities of aqueous and ethanol extracts of Securideca longepedunculata leaves and root bark against two Aspergillus species (Aspergillus niger and Aspergillus flavus). Agar incorporation method was used for antifungal testing. The results of phytochemical screening demonstrated the presence of flavonoid, saponin, alkaloids, cardiac glycoside and saponins glycosides. Highest growth inhibition (1.16+1.15mm) at 300 mg/ml, (1.67+2.88mm) and higher increase (3.16+0.57mm) at 100 mg/ml were observed. The results showed significant effect ($p<0.05$) of antifungal activities. In the same study, the results however, applied that the phytochemical constituents of S. longepedunculata leaves and root bark extracts can be used as potential antimicrobial agents in the management of microbial diseases caused by pathogenic Aspergillus species which can become an alternative to chemical antibiotics.

Keywords: antifungal, violet tree, phytochemical, aqueous, ethanol extracts, aspergillus,

I. Introduction

Violet tree (*Securidaca longepedunculata*) commonly called Krinkhout in Africa. It is a slender tree with beautiful flowers, belonging to the family polygalaceae. The tree is highly regarded for its medicinal purpose, especially by the vhaVenda people of the Limpopo Province where it occurs (Ndou, 2006). A cooperative approach by ethnobotanists, ethnopharmacologists, physicians and phytochemists is thereby essential to spur the progress of medicinal plants research (Gilani and Rahman, 2005). Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives in Sudan (Nelson-Harrison *et al.,* 2002). Through its long history, the Sudan has witnessed the fusion of many cultures, Pharonic, Islamic and Christianity along with the local indigenous cultures. With this unique history and vast variety of climate and flora, traditional medicine together with use of medicinal plants became an important part of the cultural heritage of the Sudan (Elkalifa *et al*., 1999). The abundance of information on traditional medicinal uses of plants in Africa is in danger of disappearing since the knowledge of how to use medicinal plants is mostly passed down orally and even to date is poorly documented (Gurib-Fakim, 2006), although written information has been produced for some specific regions. Moreover, the most serious threat to local medicinal plant knowledge, however, appears to be cultural change, particularly the influence of modernization and the western world view (Voeks and Leony, 2004) which has contributed to under mining traditional values among the young (Giday *et al*., 2003).

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since Ancient times (Moriita *et al.,* 2011). Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world's population and Over 50% of all modern clinical drugs are of natural product origin (Baker *et al*., 1995: Kumar and Chandrashekar, 2011). The effect of plant extracts on microorganisms have been studied by a very large number of researchers in different parts of the world (Kumar *et al*., 2006; Mathabe *et al*., 2006) and the use of a variety of plant extracts and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, such as, the phenolic compounds which are part of the essential oils, as well as in tannin (Nascimento *et al.,* 2000).

This study was design to investigate and determine the phytochemical properties and antifungal activities of violet tree *Securidaca longepedunculata* on *Aspergillus* species.

II. Materials and Method

a) Description of Study Area

Katsina State, covering an area 23,938 sq. km., is located between latitudes 11Â°08'N and 13Â°22'N and longitudes 6Â°52'E and 9Â°20'E. The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by

Author α ¥: Department of Biology, Isa Kaita College of Education, Dutsin-ma Katsina Nigeria.
Author σ ρ: Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto Nigeria.
Author ω: Department of Biological Sciences, Federal University Dutsin-ma, Katsina Nigeria. e-mail: abdulhadi_jibia@yahoo.com

© 2014 Global Journals Inc. (US)

Zamfara State to the West. A cool dry (harmattan) season from December to February; a hot dry season from March to May; a warm wet season from June to September; a less marked season after rains during the months of October to November, characterized by decreasing rainfall and a gradual lowering of temperature.

b) Collection, Identification and Processing of Plant Material

Fresh roots and leaves of *Securidaca longepedunculata* were collected during the month of May, 2013 at 5:30pm-6:05pm from Kudewa, Kurfi Local Government Area, Katsina State, Nigeria.

The plant was preserved, identified and authenticated at the Herbarium Section, in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Samples deposited at the Herbarium have a Voucher No. D-01SL-7.

The plant materials were properly washed under tap water, rinsed with distilled water, dried under shade and pulverized with a pestle and mortar and kept in a transparent sterile polyethene bag at room temperature for use.

i. *Preparation of Extract*

Two hundred grams (200g), each of dried plant material was extracted by soaking in 1000 ml of ethanol and water (solvent) in 1000 ml of conical flask, and covered with aluminum foil and allowed for 24 hours.

The extracts were filtered and the solvents removed by warming in oven at 40^{0}C for 3 days. The evaporated extract was stored for 48-hours in sterile universal bottles at room temperature, this methods is adopted by Okogun (2000) and Shariff (2001).

c) Qualitative Phytochemical Tests

The plant extracts was screen for the presence of secondary metabolites using standard method (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

i. *Source and Maintenance of Microbial Test Strains*

Stocked isolate of fungal strains *Aspergillus* species (*A. niger, A. flavus*) was obtained from Mycology Laboratory of Botany Unit Usmanu Danfodiyo University Sokoto, Nigeria. The isolate was maintained on Potato Dextrose Agar.

ii. *Sterilization of Glassware*

The glassware were adequately washed with liquid soap and sufficiently rinsed with tap water and distilled water respectively, air dried and sterilized in hot air oven at 160°C for 1hour, while the conical flask was autoclaved

d) Preparation of Media

i. *Preparation of Sarboroud Dextrose Agar (SDA)*

The sarbouraud dextrose agar (SDA) was prepared according to manufacturer's instructions, SDA (65g) was dissolved in 1000 ml distilled water and 0.5g streptomycin solution was added to inhibit bacterial growth. The conical flask was plugged with cotton and capped with aluminum foil, sterilizing using lender autoclave at 121^{0}C for 15 minutes, cooled to 45^{0}C before been poured into sterilized plates and kept at 30^{0}C (Cheesebrough, 1985).

ii. *Preparation of Potato Dextrose Agar (PDA)*

Potato dextrose agar (PDA) was prepared according to manufacturer's instructions, 39g PDA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized and 0.5g of streptomycin was added to inhibit the growth of bacteria. The conical flask containing the media were plugged with cotton wool and capped with aluminum foil, sterilized using lender autoclave at 121^{0}C for 15 minutes, cooled for 45^{0}C and pouring in to sterile plates. The plates were kept at 30^{0}C (Cheesebrough, 1985).

e) Antifungal Testing

Antifungal testing of the aqueous and ethanolic extracts of leaves and root bark were determined by Agar incorporation method as described by Brantner (1994) for antifungal testing.

i. *Activity of S. longepedunculata*

Food poison technique was used to determine the antifungal effects of different concentrations of the extracts. Into 100 ml conical flask, 15 ml of media were added. The flasks were plugged with cotton wool, capped with aluminum foil and allowed to stand for 24 hours. The four flasks of 100mg, 200mg, and 300mg of the extract were added. The fourth flask contained only the media.

Five (5ml) each of varying concentration of the leaves and root bark extracts were incorporated in to each flask containing 15 ml of the media, this was then poured in to pre-sterilized petri-dishes and kept at room temperature of 27^{0}C to 30^{0}C, the growing cultures was punch with sterile inoculating needle and then deposited in the centre of the petri dishes containing varying concentration. The control plate was sterilized and containing 20 ml of the media were place at the centre of treated plates.

The results was measured in millimeters (mm) by measuring the fungal growth from two lines vertical and horizontal, the mean were recorded (Singh and Tripatti, 1999). For each treatment 3 replicate were maintained. Mean of three 3 replicates served as the result of each of the varying concentration.

Results obtained were subjected to statistical analysis using one-way analysis of variance ANOVA, with SPSS 16.0 Version. $p<0.05$ considered as significant followed by Duncan's Multiple Range Test to detect significant differences among the means as well as the interactions between the variable.

© 2014 Global Journals Inc. (US)

III. Results

a) *Antifungal activities of the different solvent extracts of S. longepedunculata*

The growth inhibitions of *Aspergillus* species due to the application of *S. longepedunculata* are presented which revealed that leaves and root bark extracts with different solvents inhibited the growth of all the fungal species Table 1. It was also indicated in the same Table 1, that the phytochemical properties of the extracts appeared more unless where they are not present in the composition.

Table 1 : Phytochemical Composition of S. *longepedunculata* Leaves and Root bark Extracts

Phytochemical	Leaves	Root
Flavonoid	+	+
Tannins	+	-
Saponin	+	+
Glycosides	+	-
Alkaloids	+	+
Cardiac glycosides	-	+
Steroids	+	+
Saponin Glycosides	+	+
Balsams	+	-
Anthraquinones	-	-
Volatile oil	+	+

Keys: -Not present, + Present

Antifungal activities of S. *longepedunculata* was exhibited in root bark extracts on *A. niger* appeared high (at 300 mg/ml 8.83+2.46) and 13.00+00 root bark extract. In *A. niger,* the highest growth inhibition was found in the ethanol leaf extract at 300mg/ml of 3.33 + 0.57mm, as seen in Table 2.

Table 2 : Antifungal activities of S. longepedunculata Leaves and Root bark Extracts on A. niger

Extract	Conc. (mg/ml)	Leaf extracts M ±SD(mm)	Root bark extracts M ±SD(mm)
Aqueous	0	21.3^{fg} + 2.46	21.33^{fg} + 2.46
	100	10.00^{abcd}+1.00	14.00^{d}+3.50
	200	9.17^{abcd}+3.25	13.00^{ef} + 0.50
	300	8.83^{abc}+ 2.46	13.00^{fg}+00
Ethanol	0	21.33^{e} + 2.47	21.33^{de} + 2.46
	100	9.00^{bc}+ 3.12	4.33^{b}+1.52
	200	6.00^{ab} + 2.59	4.33^{b} + 1.75
	300	4.83^{ab}+1.15	3.33^{ab} + 0.57

[a,b,c] Means in a column with different superscripts are significantly different ($p<0.05$) Values are means + standard error of three replications

The antifungal activity of S. *longepedunculata* in both the root bark and leaf extracts increase as a result of increase in the concentrations in mg/ml, Table 1. It could be deduced that, he aqueous and ethanolic extracts of the root bark and leaf significantly different ($P<0.05$) in increase of the antifungal activities in *A. niger* and *A.* f*lavus* respectively.

In *A. flavus* highest growth inhibition was observed in the aqueous root extract at (300mg/ml of 3.00 +0.50mm), least growth inhibition (13.83+2.51mm) was observed in aqueous leaves extract at 100mg/ml, to the more higher concentrations (at 300mg/ml) of leaf (6.33+1.44) and root bark (3.16+0.57) as seen in Table 3.

© 2014 Global Journals Inc. (US)

Table 3 : Antifungal activities of S. *longepedunculata* Leaves and Root bark Extracts on *A. flavus*

Extract	Conc. (mg/ml)	Leaf extract M ±SD(mm)	Root extract M ±SD(mm)
Aqueous	0	24.66^{g}+ 2.02	24.67^{f} + 2.02
	100	13.83^{cde}+2.51	8.50^{bc}+ 3.50
	200	8.17^{ab}+4.80	7.00^{ab}+ 4.92
	300	4.67^{a}+2.75	3.00^{a}+0.50
Ethanol	0	6.67^{ab} + 4.31	6.16^{b}+2.56
	100	6.67^{ab} + 4.31	6.16^{b}+2.56
	200	6.33^{ab} + 5.39	4.50^{b} + 0.00
	300	6.33^{ab} + 1.44	3.16^{ab} + 0.57

[a,b,c] Means in a column with different superscripts are significantly different ($p<0.05$)

Values are means + standard error of three replications

IV. Discussion

The present study revealed the phytochemical and antifungal screening of *Securidaca longepedunculata* samples, which co-opt the rich sources of bioactive compounds in potential use of diseases management. It was reported in this study, that the presence of various secondary metabolites like tannins, saponins, alkaloids, flavonoids and others in qualitative analysis extracted from *S. longepedunculata* might be responsible for great medicinal importance. These findings are in conformity with those reported by (Donald *et al.*, 2011; Auwal *et al.*, 2012) on phytochemical composition and acute toxicity of root bark extracts of *S. longepedunculata*. The present of bioactive compounds is an indication that *S. longepedunculata* has medicinal potential; this is due to the fact that each of the compounds identified has one or more therapeutic usage. Absent of anthraquinone worth nothing medically as earlier observed by (Ajiboye *et al.*, 2010).

Results of the antifungal activities of ethanol and aqueous extracts of *S. longepedunculata* root bark and leaves ware tested against the organisms *A. niger and A. flavus* at three different concentrations, the extracts indicate significant effects ($P<0.05$) inhibitory activities of aqueous and ethanol extracts. This might be due to the fact that the extracts can exhibit remarkable activity. Antifungal activities of the ethanol extracts appeared to be more effective then aqueous extracts, since ethanol could extracts a wide variety of active component as compared to aqueous. Flavonoid together with the other secondary metabolites identified in the presence study have been severally reported to show curative activity against diverse pathogens, used traditionally analgesic antimicrobial, anti tumor headache, venereal diseases, constipation and coughs. This report is in line with findings of Abubakar *et al.* (2011) who investigated the growth inhibition and broad spectrum activity (14 to 27 mm) of *Vernonia* spp., from the crude ethanol extracts and chloroform fractions against some clinical bacterial strains and found the activity of chloroform fraction to be higher on *Corynbacterium ulcerans* and *Klebsiella pneumoniae* (27 mm), while the chloroform fractions of *V. oocephala* and *V. ambigua* were more active on *Proteus mirabilis* (27 mm) and *Salmonella typhi* (22 mm), respectively. They added that the minimum inhibitory concentration (MIC) values ranged from 1.25-2.5 mg/mL for all the organisms tested.

However, the phytochemical screening and antifungal activities of the concentrations at 100, 200 and 300mg/ml of the extracts used in this research revealed the presence of active compounds like tannins, saponins, alkaloids, flavonoid, steroids/terpenes, tannins and glycosides. The antifungal activity exhibited against the organisms *A. niger* and *A. flavus* and the susceptibility of these organisms may be a pointer to their potentials as a component or drug against the organisms tested in this study.

V. Conclusion

The results of this study confirm the potential use of Violet tree, *Securidaca longepedunculata* as antifungal agents against infections caused by *Aspergillus niger* and *Aspergillus flavus*. The presence of these importance substances suggests that *S. longepedunculata* may possess myriads of therapeutic tendencies and ability to manage numerous malaises caused by *Aspergillus* species. The overall result concludes that the extracts used in this research are of potent antifungal activity. Thus, should be explored further for pharmaceutical uses as this is important in combating the recent observed emergence of drug resistance organisms.

VI. Acknowledgement

The Authors appreciated the effort of Mr. Auwal Umar (Biological Sciences, Botany Unit, Usmanu Danfodiyo University Sokoto Nigeria) in carrying out the field work of this research.

© 2014 Global Journals Inc. (US)

References Références Referencias

1. Ajiboye TO, Salau AK, Yakubu MT, Oladiji AT, Akanji MA, Okogun JI (2010) Aqueous Extract of *Securidaca longepedunculata Root induce Redox* Imbalance in Male Rats Liver and Kidney. *Journal of Human and Experimental Toxicology* 29 (8): 67-688.
2. Abubakar BA, Mikhail SA, Hamisu I, Adebayo OO (2011) Phytochemical Screening and Antibacterial Activities of *Vernonia ambigua*, *Vernonia blumeoides* and *Vernonia oocephala* (Asteraceae). *Acta Poloniae Pharmaceutica Drug Research*, 68 (1): 67-73.
3. Auwal SM, Atiku MK, Wudil AM, Sule MS (2012) Phytochemical composition and Acute Toxicity Evaluation of Aqueus Root Bark Extract of *Securidaca longepedunculata. Bayero Journal of Pure and Applied Sciences,* 5 (2): 67-72.
4. Baker J, Borris R, Carte B (1995) Natural product drug discovery and development. New Perceptive on International Collaboration, *Journal of National Production*, 58: 1325-1328.
5. Brantner A, Pfeiffer K, Brantner H (1994) Application of Diffusion methods required by Phamacopoeiasis for testing Antibacterial activity of Natural compounds. *Phamazie*, 49 (7): 512-516.
6. Cheesebrough M (1985) Medical Laboratory Manual for Tropical Countries, 17: 203-305.
7. Donald Z, Blackson LK, Thokozani-Gudeta WS, Zewge T, Dominic SB, Gondwez VS, Philip CS (2011) Propagation of the African medicinal and pesticidal plant, *Securidaca longepedunculata; African Journal of Biotechnology* 10 (32): 5988-5992.
8. Duncan RC, Knapp RG, Miller MC (1977) Test of hypothesis in population Means. In: Introductory Biostatistics for the Health Sciences. John Wiley and Sons Inc. NY: 71- 96.
9. Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical Constituents of Some Nigerian Medicinal Plants. *African Journal of Biotechnology*, 4(7): 685-688.
10. El Khalifa MY (1999) Home remedies in Khartoum State. Unpublished Research Data, APRI Reports.
11. Evans CW (1996) Trease and Evans Pharmacognosy, 14th edition. W.B. Saunders Company Ltd., London. pp 268-270.
12. Giday M, Asfaw Z, Elmqvist T, Woldu Z (2003) An Ethnobotanical Study of Medicinal Plants used by the Zay People in Ethiopia. *Journal of-Ethnopharmacology.*, 85: 43-52.
13. Gilani AH, Rahman AU (2005). Trends in ethnopharmacology. *Journal of Ethnopharmacology*, 100: 43-49.
14. Gurib-Fakim A (2006) Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. *Mol. Aspi. Medicine*, 27: 1-93.
15. Harborne JB (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd., London. pp 279.
16. Hassan LG, Kamba AS (2010) Phytochemical Screening and Antimicrobial activities of *Euphorbia balsamifera* Leaves, Stem and Root against Some Pathogenic Microorganisms. *African Journal of Pharmaceutical Sciences and Pharmacy* 1:85-95.
17. Kamba AS, Hassan LG (2010) Anti-bacterial screening and brine shrimp (*Artemia salina*). Toxicity of *Securidaca longepedunculata* (Polygalaceae) Root bark, *African Journal of Pharmaceutical Science pharmacology*, 1(1): 85-95.
18. Kumar P, Chauhan S, Padh H, Rajani M (2006) Search for Antibacterial and Antifungal agents from Selected Indian Medicinal plants. *Journal of Ethnopharmacology*, 107: 182-188.
19. Kumar T, Chandrashekar K (2011) *Bauhinia purpurea* Linn. A Review of its Ethnobotany, Phytochemical and Pharmacological Profile. *Research Journal of Medicinal plants* 5(4) 420-431.
20. Mariita R, Ogal C, Oguge N, Okemo P (2011) Methanol Extract of Three medicinal plants from Samburu in Northern Kenya show significant Antimycobacterial, Antibacterial and Antifungal Properties. *Research Journal of Medicinal Plants*, 5(1) 54-64.
21. Mathabe M, Nikolova R, Laly N, Nyazema N (2006) Antibacterial activities of Medicinal Plants used for the treatment of diarrhoea in Limpopo Province, South *Africa*, *Journal of Ethnopharmacology*, 107: 286-293.
22. Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000) Antibacterial Activity of Plant Extracts and Phytochemical on Antibacterial-resistant Bacteria. *Brazilian Journal of Microbiology*, 31(4): 247-256.
23. Ndou PA (2006) Walter Silsilu: National Botanical Guarden. Retrived from www.plantzafrica.com/-plantqrs/securidlong.htm.
24. Nelson-Harrison ST, King SR, Limbach C, Jackson C, Galiwango A, Kato SK, Kanyerezi BR (2002) Ethnobotanical Research into the 21st century. In: Iwu MM, Wootton JC (Eds.), Ethnomed. Drug Discov. Elsevier, Amsterdam.
25. Okogun JI (2000) Methods of Medicinal Plant Extract Preparation. National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja, Nigeria. 20:145-48.
26. Ojewole JAO (2008) "Analgesic, Anti-inflammatory and Hypoglycaemic Effects of *Securidaca longepedunculata* (Fresen.) [Polygalaceae] Root Aqueous Extract." *Inflammopharmacology* 16(4): 174-181.
27. Ojowole JAO, Ilesanmi ORS, Olayiwola G (2000) Pharmacology of African Medicinal plants: Neuromuscular and cardiovascular properties of

© 2014 Global Journals Inc. (US)

Securidaca longepedunculata, *Nigerian Journal of Natural Product and Medicine*. 4 (Abstract)

28. Ojowole JAO, Olayiwola G, Ilesanmi ORS (2001) Pharmacological properties of *Securidaca longepedunculata*: Neuromuscular and cardiovascular properties of *Securidaca*. *Nigerian Journal of Natural Product and Medicine* 2001: 5 (Abstract)
29. Shariff ZU (2001) Modern Herbal therapy for Common ailments. Nature Pharmacy Series, 1 Ibadan, Nigeria/ United Kingdom: in Association with Safari Books (Export) Limited Spectrum Books Limited; pp. 79–84.
30. Trease GE, Evans WC (1989) Pharmacognosy. 13th Edition, Bailliere Tindal Ltd, London. pp 176-180.
31. Voeks RA, Leony A (2004) Forgetting the Forest: Assessing Medicinal plant erosion in Eastern Brazil. Economic Botany 58: 294-306.
32. WHO (1999) World Health Organization: Consultation Meeting on Traditional Medicine and Modern Medicine: Harmonizing the Two Approaches. Geneva, WHO, TM/ICP/TM/001/RB/98-RS/99/GE/32(CHN).

© 2014 Global Journals Inc. (US)

Global Journal of Science Frontier Research: C
Biological Science
Volume 14 Issue 5 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Study on Enzyme Activity in the Production and Optimization of High Temperature Alkaline α-Amylase Enzyme by Bacillus Lichenoformis using Low Cost Medium Derived from Agricultural byproducts

By M. Sathiyamoorthy & Dr. S. Theneshkumar
Himalayan University, India

Abstract- Production of **α**-amylase enzyme by Bacillus Lichenoformis using stirred tank fermentor (BIOSTAT – E) was carried out. The strain was obtained from National Chemical Laboratory, Pune, India. Corn starch is used as the substrate. The enzyme production was studied by changing the various parameters like temperature, pH, rpm and substrate concentration. The enzyme activity shows maximum at a temperature of 350C – 370C, pH 8 and 300 rpm. The maximum enzyme production was achieved, for 1% concentration of cornstarch at 35.60C and pH 9 using the fermentation medium contains yeast extract and peptone and the enzyme activity was found to be 55.93 DUN/ml.

Keywords: ***α**-amylase, bacillus lichenoformis, low cost medium, agricultural by products, fermentation, alkaline enzyme.*

GJSFR-C Classification : ***FOR Code: 860399***

STUDYONENZYMEACTIVITYINTHEPRODUCTIONANDOPTIMIZATIONOFHIGHTEMPERATUREALKALINEAMYLASEENZYMEBYBACILLUSLICHENOFORMISUSINGLOW COSTMEDIUMDERIVEDFROMAGRICULTURALBYPRODUCTS

Strictly as per the compliance and regulations of :

© 2014 M. Sathiyamoorthy & Dr. S. Theneshkumar. 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.

Study on Enzyme Activity in the Production and Optimization of High Temperature Alkaline α-Amylase Enzyme by *Bacillus Lichenoformis* using Low Cost Medium Derived from Agricultural byproducts

M. Sathiyamoorthy [α] & Dr. S. Theneshkumar [σ]

Abstract- Production of α-amylase enzyme by Bacillus Lichenoformis using stirred tank fermentor (BIOSTAT – E) was carried out. The strain was obtained from National Chemical Laboratory, Pune, India. Corn starch is used as the substrate. The enzyme production was studied by changing the various parameters like temperature, pH, rpm and substrate concentration. The enzyme activity shows maximum at a temperature of 35^{0}C – 37^{0}C, pH 8 and 300 rpm. The maximum enzyme production was achieved, for 1% concentration of cornstarch at 35.6^{0}C and pH 9 using the fermentation medium contains yeast extract and peptone and the enzyme activity was found to be 55.93 DUN/ml. Since the cost of yeast extract and peptone is very high, so the further work was done using some low cost carbon and nitrogen sources like defatted cotton seed, defatted soya flour and mustard seed which are extracted from agricultural byproducts. The enzyme activity for using the low cost medium was found to be nearly triple such as 121.49 DUN/ml. The enzyme production reaches the steady phase at 24 hours. So it is highly recommended that using the low cost medium for the α-amylase enzyme gives better biomass cell concentration and enzyme activity as well.

Keywords: α-amylase, bacillus lichenoformis, low cost medium, agricultural by products, fermentation, alkaline enzyme.

I. Introduction

Enzymes are proteins which catalyze variety of reactions in the biological system. When enzymes were first intensively studied in the last two centuries this chemical nature was obscure and even the reactions catalyzed were frequently ill defined. It was natural and therefore, that individual enzymes were given names by their discoverers. Most enzymes are studied and need to be named before any significant information about their structures exists. Whenever the 'same' enzyme from different organism is studied, it is found that Proteins different in detailed structure (and some times in gross structure) can have essentially the same catalytic properties. In the recommendations of the "International Union of Biochemistry Nomenclature Committee (1984), therefore, an enzyme name does not specify a structure but instead defines the Principal reaction catalyzed.

Enzymes are classified in to six classes. Enzymes in the first three classes all catalyze transfer reactions, with stoichiometry A+B → P+Q, but differ in other respects. Oxidoreductases catalyze reaction in which one or more electronics (usually two) are transferred from a donor (reducing agent) to an acceptor (Oxidizing agent). In many oxidoreductases the oxidized substrate can be regarded as a hydrogen donor, and for these enzymes the term dehydrogenase is preferred. Hydrolases catalyze hydrolytic reaction, i.e. reactions in which water is the acceptor of the transferred group. The transferases thus comprise all enzymes catalyzing transfer reaction that are not oxide reductases or hydrolases. Lyases catalyze elimination reaction, where the bond is broken without oxidoreduction or hydrolysis and in most cases have stoichiometry. A → P+Q.

The six classes are further sub divided in to subclasses, to specify the type of reaction more fully and to indicate the reactants. All the enzymes have a property of either intra cellular or extra cellular in nature. But most of them are extra cellular in nature.

a) Intracellular Enzymes

Enzymes occur in all living cells, where they catalyze and regulate reactions of Biochemical pathways essential to the existence of the living system. In general substrates for these enzymes are small molecular weight molecules, e.g. Sugars, amino acids, carboxylic acids, which are able to permeate the membrane. Their catalytic properties are regulates by conformational changes in their three dimensional structure accomplished by allosteric cofactor molecules.

Author α: Research Scholar, Department of Chemical Engineering, Himalayan University, Arunachal Pradesh, India.
e-mail: sathyachemical@gmail.com
Author σ: Department of Chemical Engineering, SRM University, Kattankulathur, Chennai, Tamilnadu, India.

© 2014 Global Journals Inc. (US)

b) Extracellular enzymes

Extra cellular enzymes were originally defined as enzymes which are external to the cell wall and in contact with surrounding medium. At present we consider transport the membrane as the primary secretion event. Thus for the purpose of this review the term & erection is used to refer to the transmembrane passage of protein and the term extra cellular to those proteins that have undergone this process. The biological function of this kind of enzymes may be seen in the hydrolysis of macro molecules which are too large to be transported in to the cell.

c) Animal tissue Enzyme

Enzymes used in Industry are isolated from animal and plant tissues, as well as from Micro organisms. One of these three sources may be favored for a given enzyme. For example, some proteolytic enzymes isolated from animals may be advantageous in special fields of application. The enzyme chymosin, also known as rennet, is an acid protease used in the milk-clotting step of cheese production. A mixture of chymosin and its zymogen prochymosin, which may be converted chymosin by low pH treatment, are currently obtained from the abo-masum of an unweaned calf. Animal glands, e.g. the pancreas, are sources for hydrolyzing enzymes used as a digestive acids. The pancreas is a very rich sources of enzymes. It contains about 23% of trypsinogen and 10 -14% of chymotrypsinogen. So called pancreatin, a digestive aid, contains several enzymes such as amylase, lipase and protease.

d) Plant tissue enzymes

Plant protease isolated from pineapple (bromelain) and the papaya plant (papain) have been used for meat tenderizing and chill proofing beer. Useful amylolytic enzymes occur in plant tissues such as barely, wheat, rye, Potatoes, sweet potatoes, beans, soy beans, α - amylase, β - amylase, which starts at the non-reducing ends of the outer chains of the starch and proceeds by gradual removal of maltose units and de branching enzyme which hydrolyzes the α -1 - 6 linkages of starch, were detected in these plants.

e) Microbial enzymes

Microorganisms have become increasingly important as producers of industrial enzymes and in fact most enzymes used in industry today are of Microbial origin. Attempts are now being made to replace enzymes which traditionally have been isolated from animal tissue and plant tissues with enzymes from Microorganisms. Examples for partial replacement of plant and animal enzymes in dudes. Amylases and endo - β - glucanases of malted Barley and wheat by enzymes from Bacillus and Aspergillus in the beer, distillery, baking and textile industries. Plant and animal proteases by Aspergillus and Thermoactinomyces protease for meat tenderization and for chill proofing beer.

f) Uses of α – amylase

The enzyme α–amylase is used as a biocatalyst in many small scale and large scale industries some of the uses are.

- The Bacterial α–amylase used in starch hydrolysis industries, Brewing industries, Detergents industries and textile industries.
- The fungal α–amylase used in starch industries and baking industries.
- The α–amylase from Malt used as a digestive aid and supplement to bread.
- The α–amylase from Aspergillus Orygaze is used to produce starch liquefying syrups.
- The α–amylase from Bacillus Subtillis used in Desizing textile industries, Alcohol fermentation industries and glucose producing industries.
- The α–amylase produced from Aspergillus Niger is highly acid resistant is used as a digestive acid at pH-5.
- The α–amylase from Bacillus lichenoformis is used in all starch industries and detergent industries and to produce starch sizing pastes for use in paper coatings.

II. Objective of the Study

Enzymes are Proteins which catalyze variety of reaction in the Biological systems. There are many methods used to produce the enzymes among that the biological methods are widely used. In this type of biological method of production, solid state fermentation is applied for the production. In all the types of fermentation processes, the cultures has been prepared using yeast extract and peptone etc. These are added to the culture in terms of nutrients as a carbon and nitrogen sources for the microorganism. The cost of these chemicals are much expensive. So the alternative method has been proposed for the preparation of culture medium using some low cost agricultural byproducts such as defatted cotton seed, defatted soybean, mustard seed etc. The fermentation has to carryout using these type of low cost medium to check the productivity and enzyme activity.

III. Experimental Setup

a) Biostat E fermentor

The fermentation was carried out in a B. BRAUN CO, Biostat E fermentor. It is a compact and comprehensive fermentation system on a laboratory scale, which can be used in microbiological and biotechnological research and development. Biostat E fermentors are designed for use in discontinuous fermentation (Batch operations) as well as in continuous

© 2014 Global Journals Inc. (US)

process. The measurement and control system used in compatible with computers. The Biostat E is protected against unauthorized use with a main key. All modules of the measurement and control section are separately switched on. Therefore they can be installed or removed independently from the control in spite of the central mains switch. Additional modules can be inserted without interruption or disturbance of operations.

The lower front panel of the basic device is provided with installation ports for at least 4 dosing pumps of the four, three are peristaltic pumps for the supply of acid, alkali and antifoam agent, the fourth is prepared to install precision dosing pumps.

The arrangements of the various technical appliances in the basic devices are:

- Thermostat system which containing heating and cooling water circuit for tempering as well as for sterilization.
- Gas supply system including exhaust equipment.
- Motor and drive system for the stirrer shaft drive.

The recorder, of 6 channels dot printer records the following measurement values in the basic devices.

- Temperature
- Speed
- pH Value & Antifoam consumption

The culture vessel is mounted on the console laterally fixed at the fermentor where there are the corresponding borings for the feet of the culture. Simultaneously the connection to the stirrer drive is guaranteed. For starting operating the device the filling state of the fermentor thermostat is to be checked. The set point temperature is adjusted at the corresponding digital switch of the module. A good mixing of the culture vessel is a prerequisite. For that a stirrer system is provided which is driven by a controlled DC motor. The stirrer speech can be directly adjusted by the digital switch of the speed controlled module. The adjustable speed range is 50 – 1500 minutes^{-1}.

The pH – value in the culture medium can be electro chemically determined via a combined – glass electrode. The pH set point desired can be adjusted with the digital switch of the pH controller.

b) Dimensions of the fermentor

Total volume of the fermentor	:	6 lit.
Working volume	:	5 lit.
Max working temperature	:	138° C
Max working pressure	:	124° C
Diameter of the fermentor	:	17.5 cm
Height of the fermentor	:	40 cm
Agitator type	:	6 Blade, Paddle type Agitator

IV. Materials and Methods

a) Microbial strain

Bacillus Lichenoformis, NCIM 2051 Received from National Chemical Laboratory, Pune, India.

b) Chemicals

Beef extract
Peptone
NaCl
$MgSO_4$
KH_2PO_4
$CaCl_2$
Yeast extract
Agar
Corn Starch
Defatted Cotton Seed
Defatted Soya flour
Mustard Seed

c) Medium

i. *Universal medium for bacteria*

Beef extract	:	1.0 %
Sodium Chloride	:	0.5 %
Peptone	:	1.0 %
pH	:	7.0 - 7.2

Sterilize the medium, and adjust the pH at 7.2. Add 2% Agar for making slants.

ii. *Corn starch medium: (Basal Medium)*

Corn starch	:	1 %
Yeast extract	:	0.2 %
Peptone	:	0.5 %
$MgSO_4$	:	0.05 %
KH_2PO_4	:	0.05 %
NaCl	:	0.15 %
$CaCl_2$	:	0.015 %

iii. *Low cost medium*

Corn starch	:	1 %
$MgSO_4$	:	0.05 %
KH_2PO_4	:	0.05 %
NaCl	:	0.15 %
$CaCl_2$	:	0.015 %
Soya bean	:	0.5 %
Mustard Seed	:	2 %
Cotton seed	:	3 %

© 2014 Global Journals Inc. (US)

d) Procedure

Shake flask cultures were operated at constant temperature of 37^{0}C and fixed rpm with 100 ml of medium in a 500 ml Erlenmeyer flask and inoculated with the culture. Fermentation studies were carried out in above described B. Braun Biostat E fermentor with the cultural conditions of 37^{0}C, pH 7, and 300 rpm. Since it is an aerobic fermentation, the aerobic rate was maintained at 1 vvm. For every six hours the sample were collected from the sampling point provided in the top of the culture vessel, and analyzed.

i. *Stock Culture*

Bacillus Lichenoformis NCIM 2051 was maintained in an Agar slant at 4^{0}C.

ii. *Sub Culture Maintenance*

Subculture was prepared using a universal Bacteria medium and it was maintained in an incubator at 37°C.

iii. *Pre inoculum*

Take 100 ml of the Universal medium inoculate this with a stock agar culture in a 500 ml Erlenmeyer flask and kept in a shaker at 300 rpm and 37^{0}C. It is also called as seeding of culture.

iv. *Enzyme activity*

One unit of enzyme activity (DUN) is defined as the quantity of enzyme that causes 1 % reduction of blue color intensity of starch-iodine solution in 1 min. The optical density was first measured at 660 nm using an UV spectrophotometer.

V. Results and Discussion

a) Enzyme Activity determination

Different techniques have been used to measure enzyme activities. There is no general method equally applicable to all enzymes. The enzyme activity may be depends on the time, enzyme concentration, substrate concentration.

Extra cellular amylase activity was determined by measuring the decrease in iodine color reaction showing dextrinization of starch. The reaction contained 1 ml of enzyme (cell free supernatant) and 10ml of 1% starch solution incubated at 40^{0}C for 10 min. The reaction was stopped by adding 10ml of 0.1N HCl. 1 ml of this acidified solution was added to 10ml 0.1N HCl. From this 1ml was added to iodine solution (0.05% iodine in 0.5% KI). The optical density of the blue colored solution was determined of 660 nm one unit of enzyme activity (DUN) is defined as the quantity of enzyme that causes 1% reduction of blue color intensity of starch iodine solution at 40^{0}C in 1 min.

For amylase activity determination requires the standard chart for starch iodine solution. Take six test tubes in that add 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of 1% starch solution respectively and add 1, 0.8, 0.6, 0.4, 0.2 and 0 ml of water reply. Add 10ml of iodine solution (0.05% I_2 and 0.5% KI) in all the six text tubes. The inference is the light blue color formation. The optical density of the blue colored solution was measured at 660 nm in the UV spectrophotometer. The standard graph was drawn by plotting starch concentration Vs absorbance. From the standard graph the enzyme activity was calculated.

b) Production of enzyme

The growth pattern of Bacillus Lichenoformis NCIM 2051 and α–amylase production was observed for three days in basal medium with 1% cornstarch as a carbon source. The formation of α–amylase started from 4 hours. The maximum enzyme production was achieved at 24 hours. The pH of the broth increased from 7 at the beginning to 8.9 at the end of fermentation. The maximum yield was achieved at 35^{0}C.

c) Effect of corn starch concentration

The effect of corn starch concentration was further studied. The α–amylase production was studied, by changing the Corn starch concentration at 0.5%, 1% and 2.5%. It was found that with an increase of starch concentration in the medium beyond 1%, enzyme production did not increase. At higher starch concentration, enzyme production was comparatively lower and the time required to reach the maximum enzyme level was longer.

d) Effect of pH

The bacterium was found to grow at pH 3-11, with growth resulting in an increase of the patient's media's pH. Enzyme production started at 5.0 and ceased at pH 10.0. Maximum enzyme production occurred at pH 6-9. Very little enzyme production in the medium at initial pH of 3 - 4. At higher pH values (10-11), growth was quite high, but the amount of enzyme production was very low.

e) Effect of temperature

The strain was found to grow and produce enzyme at temperatures from 25 to 50^{0}C. Maximum enzyme production was observed at 35^{0}C. Growth and enzyme production both started decreasing drastically above 40^{0}C.

f) α–amylase production in low cost medium

The α–amylase production was further studied by using the low cost medium which containing the carbon and nitrogen sources like corn flour, mustard seeds. Since the cost of yeast extract and peptone in the Basal medium is very high, we can replace the yeast extract and peptone with the above mentioned things. The low cost medium produced 2 times more enzyme than the high cost synthetic medium (yeast extract and peptone). The medium containing 0.5% defatted, 2% mustard seed in the place of yeast extract and peptone, was found to yield high enzyme activity of α–amylase. The experiments were conducted for 6 different batches

© 2014 Global Journals Inc. (US)

with various concentrations, which are given in the below table and graph.

Table 1 : Enzyme production for 1% corn starch concentration

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	36.9	104.7	300	0.003
3	7.1	36.8	101.8	300	2.01
6	7.2	37.0	100.2	300	3.37
12	5.8	37.1	22.6	300	4.51
18	7.3	35.7	89.3	300	29.31
24	8.2	35.6	86.8	300	55.84
48	9.0	35.6	96.4	300	55.93
72	8.9	36.2	98.7	300	56.11

Table 2 : Enzyme production for 2.5% corn starch concentration

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	6.3	37.0	100.8	300	0.0007
3	5.8	36.4	100.1	300	1.37
6	6.1	35.9	92.7	300	2.56
12	6.7	35.7	95.6	300	8.48
18	6.9	35.4	97.9	300	33.49
24	7.1	35.3	98.3	300	40.83
48	7.9	35.1	88.5	300	40.71
72	8.5	34.8	84.3	300	40.74

Table 3 : Enzyme production for 0.5% corn starch concentration

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	6.1	37.0	120.3	300	0.0007
3	6.1	36.7	110.8	300	1.53
6	6.9	36.6	102.6	300	4.92
12	7.5	35.9	100.9	300	12.19
18	7.8	35.8	98.7	300	39.43
24	7.9	35.6	98.5	300	43.38
48	8.3	35.7	98.1	300	42.31
72	8.8	35.4	83.6	300	42.34

Table 4 : Enzyme production using Basal medium + 0.5% defatted soya flour

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	37.0	120.3	300	0.98
3	7.1	38.3	110.4	300	11.91
6	7.3	37.1	93.6	300	27.56
12	8.1	36.3	83.9	300	48.29
18	8.7	36.1	70.8	300	61.49
24	8.9	35.9	64.7	300	81.24
48	8.7	36.3	53.9	300	81.31
72	8.9	37.8	48.7	300	80.9

Table 5 : Enzyme production using Basal medium + 3% defatted cotton seed

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	37.0	120.1	300	1.90
3	7.3	38.1	117.3	300	18.53
6	7.9	37.5	93.5	300	46.93
12	8.5	36.3	83.8	300	64.71
18	9.1	35.3	77.9	300	84.18
24	9.7	35.5	64.2	300	91.5
48	9.9	36.1	56.9	300	92.3
72	10.3	38.5	28.5	300	91.9

Table 6 : Enzyme production using Basal medium + 2% mustard seed

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	37.0	120.1	300	2.41
3	7.3	38.1	117.3	300	12.49
6	7.9	37.5	93.5	300	32.33
12	8.5	36.3	83.8	300	65.91
18	9.1	35.3	77.9	300	95.41
24	9.7	35.5	64.2	300	121.49
48	9.9	36.1	56.9	300	120.83
72	10.3	38.5	28.5	300	121.10

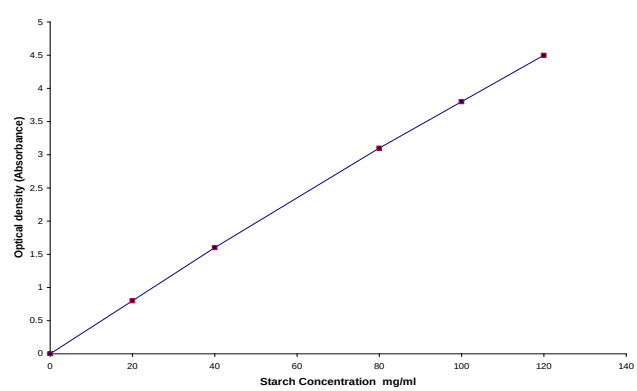

Figure 1 : Standardization graph of starch iodine solution for α–amylase activity

© 2014 Global Journals Inc. (US)

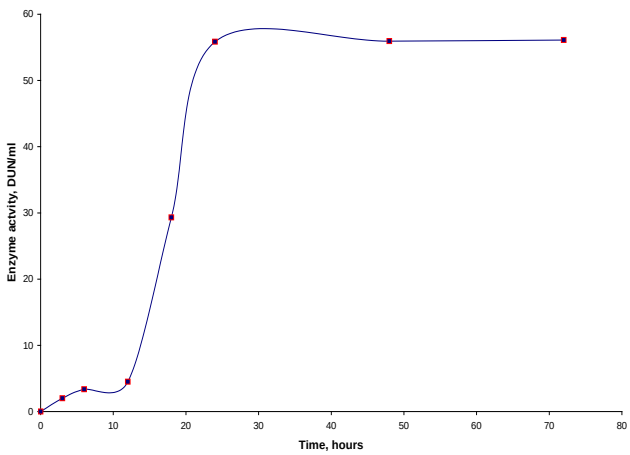

Figure 2 : Enzyme activity for 1% corn starch concentartion

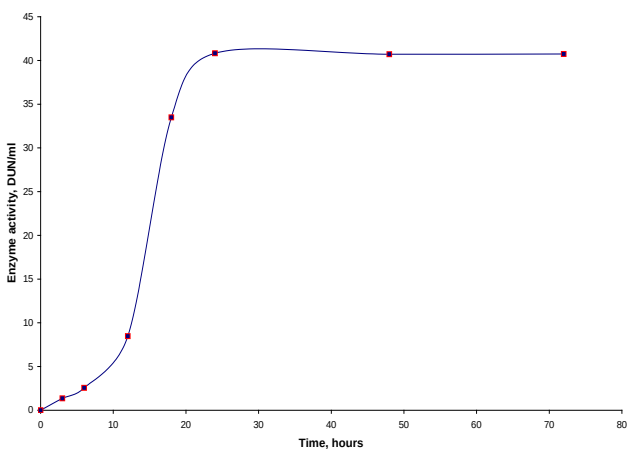

Figure 3 : Enzyme activity for 2.5% corn starch concentartion

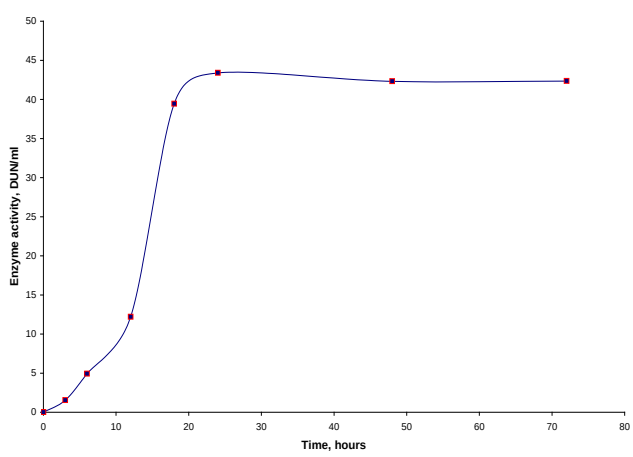

Figure 4 : Enzyme activity for 0.5% corn starch concentartion

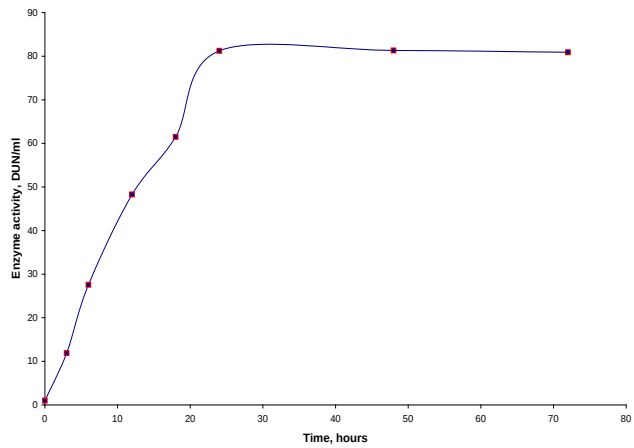

Figure 5 : Enzyme activity for Basal medium with 0.5% defatted Soya flour

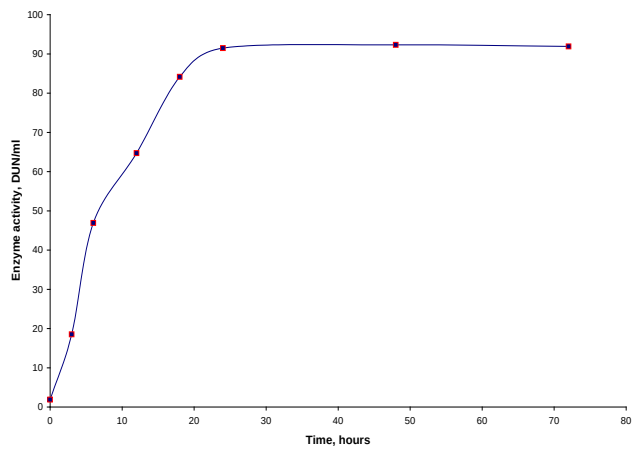

Figure 6 : Enzyme activity for Basal medium with 3% defatted Cotton seed

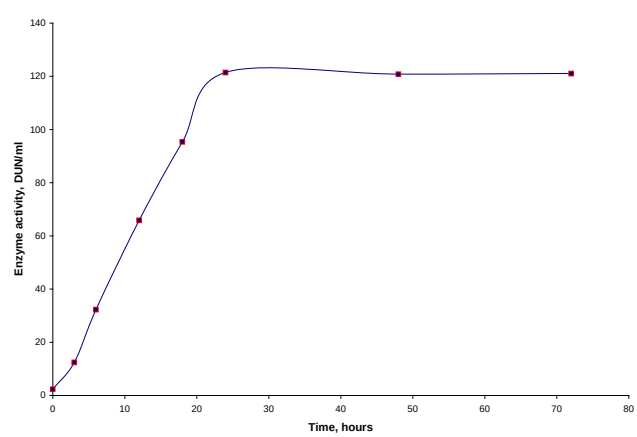

Figure 7 : Enzyme activity for Basal medium with 2% Mustard seed

© 2014 Global Journals Inc. (US)

VI. Conclusion

The Bacterial strain, *Bacillus lichenoformis* NCIM 2051 was obtained from National Chemical Laboratory, Pune, which produced high temperature alkaline α–amylase enzyme. The optimum cultural conditions are found to be 35°C, pH 7 and 300 rpm. The α–amylase produced from this Bacterial strain, *Bacillus lichenoformis* was quite active even at 100°C, however it showed optimum activity at 90°C, and also it exhibited optimum activity in the broad pH range 5.5 – 10, thus α-amylase *of Bacillus lichenoformis* seems to have a very broad pH range. A low cost synthetic medium producing large quantities of α–amylase has been developed from *bacillus lichenoformis* was used for α–amylase production. The α–amylase of this strain showed excellent stability at high temperatures and over a wide pH range. The enzyme activity were determined and optimized. The low cost medium which contains, Defatted soya flour, Defatted cottonseed, and Mustard seed, produces around three times more enzyme than the high cost synthetic medium using yeast extract and peptone in the B. Braun Biostat E fermentor. So it is further suggested to change the cheapest different nitrogen sources components in this low cost medium like corn steep liquor etc.

References Références Referencias

1. A.P. Gandhi and L. Kjaergaard, "Effect of CO_2 on the formation of α -amylase by *Bacillus subtillis* growing in continuous and batch cultures, Biotechnology & Bioengineering, Vol.17, pp. 1109-1118 (1975).
2. Fogarty .W.M, Griffon and A.M. Joyce, "Enzymes of *Bacillus* species process" Biochemistry, Vol.9, pp. 11-24 (1974).
3. H.J. Rehm and G. Read, Biotechnology, Volume 7a, Enzyme technology, VCH publishers (1987).
4. J. Jayaraman, "Laboratory manual in Biochemistry", (1981).
5. James E. Bailey and David F.Ollis, "Biochemical engineering fundamentals", McGraw.Hill international editions, second edition, (1986).
6. Martha H.M.Oseley and Leonard Keay, "Purification and characterization of the α -amylase of *Bacillus subtillis* NRRL B3411, Biotechnology & Bioengineering, Vol.12, pp. 251-271 (1970).
7. Peter F. Stanbury and Allen Whitkar, "Principles of Fermentation technology", Pergamon press, (1984).
8. Pratima Bajpai and Promod Bajpai, "High temperature alkaline α-amylase From *Bacillus lichenoformis*", Biotechnology & Bioengineering, ol.33 pp. 72-78 (1989).
9. Pratima Bajpai and Umender Sharma, "Production of α -amylase in a low cost medium by *Bacillus lichenoformis",* Journal of Fermentation & Bioengineering, Vol.67, No 6, pp.422-423 (1989).
10. Seung-Hyeon moon and Satish J .Parulekar, "A parametric study of x-amylase production in Batch, Fed batch and continuous suspension culture of *Bacillus firmus*", Biotechnology & Bioengineering, Vol.41, pp. 43-54 (1993).
11. Yong Hee Lower et al, "Production of alkaline protease by *Bacillus lichenoformis* in an aqueous two phase system, Journal of fermentation and Bioengineering, Vol.69, pp. 89-92 (1990).

© 2014 Global Journals Inc. (US)

This page is intentionally left blank

© 2014 Global Journals Inc. (US)

Global Journal of Science Frontier Research: C
Biological Science
Volume 14 Issue 5 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Influence of Colchicine Treatments on Character Expression and Yield Traits in Cowpea (*Vigna Unguiculata* L. Walp)

By Abiola T. Ajayi, Akinlolu O. Ohunakin, Oluwatoyin S. Osekita & Opeyemi C. Oki.

Adekunle Ajasin University, PMB, 001, Akungba-Akoko, Nigeria

Abstract- Mutagenesis has been exploited to enhance genetic variability in cowpea (Vigna unquiculata L. Walp.); an important legume in the tropical and subtropical regions of the world. Mutation is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Effects of 0.1% aqueous solution of colchicine for different periods of time, viz; - 0, 2, 4 and 6 hours were tested on the quantitative and yield characters of a cowpea variety popularly known as 'Oloyin' in M1 generation. Lethal dose value (LD40) of 59% was observed at 2 hours treatment. Treatment was significant (P = 0.05) for seedling emergence percentage (67 – 13%), plant height (21.52 – 15.63 cm), number of leaves (11.08 – 4.98), number of nodes on main stem (6.26 - 4.5), survival percentage (63.50 – 12.50%) and number of days to first flowering (55.52 – 47.30) while treatment was not significant for all other characters studied.

Keywords: mutagenesis, genetic variability, induced variation.

GJSFR-C Classification : FOR Code: 780105

INFLUENCEOFCOLCHICINETREATMENTSONCHARACTEREXPRESSIONANDYIELDTRAITSINCOWPEAVIGNAUNGUICULATALWALP

Strictly as per the compliance and regulations of :

© 2014. Abiola T. Ajayi, Akinlolu O. Ohunakin, Oluwatoyin S. Osekita. 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.

Influence of Colchicine Treatments on Character Expression and Yield Traits in Cowpea (*Vigna Unguiculata* L. Walp)

Abiola T. Ajayi [α], Akinlolu O. Ohunakin [σ], Oluwatoyin S. Osekita [ρ] & Opeyemi C. Oki. [ω]

Abstract- Mutagenesis has been exploited to enhance genetic variability in cowpea (Vigna unquiculata L. Walp.); an important legume in the tropical and subtropical regions of the world. Mutation is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Effects of 0.1% aqueous solution of colchicine for different periods of time, viz; - 0, 2, 4 and 6 hours were tested on the quantitative and yield characters of a cowpea variety popularly known as 'Oloyin' in M1 generation. Lethal dose value (LD40) of 59% was observed at 2 hours treatment. Treatment was significant (P = 0.05) for seedling emergence percentage (67 – 13%), plant height (21.52 – 15.63 cm), number of leaves (11.08 – 4.98), number of nodes on main stem (6.26 - 4.5), survival percentage (63.50 – 12.50%) and number of days to first flowering (55.52 – 47.30) while treatment was not significant for all other characters studied. The results revealed that colchicine can be used to induce variations in cowpea which may be of agronomic importance in the production of this crop.

Keywords: mutagenesis, genetic variability, induced variation.

I. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of six major cultivated crop species of the family *Leguminosae* distributed throughout the tropics (Padulosi and Ng, 1997; Pasquet, 2001). It is the second most important grain legume crop after groundnut (Blade *et al*., 1997). Cowpea has been reported as an important food crop throughout Sub –Saharan Africa (SSA) (Kitch *et al*., 1998) and one of the major sources of plant protein in the developing countries including Nigeria (Adekola and Oluleye, 2007). Its grain and leaves have high quality protein and vitamins which serves as an excellent food supplement in developing countries (Kitch *et al*., 1998). Millions of relatively poor people in low income countries in the tropics rely on it for their livelihood and as protein supplement (Ajayi, 2014). Hence, it is a key staple food crop for ever-increasing population both in the rural and urban areas. Cowpea has a great ability to fix atmospheric nitrogen in the soil, thereby improving soil nutrients (Adetiloye *et al*., 2013). Ajayi and Adesoye (2013) reported that cowpea production has been consistently hindered by low grain yields and quality, and lack of improved cultivars. Dhanavel *et al* (2012) reported induced mutation as a valuable supplement to conventional breeding in crop improvement programs, but has been least applied in grain legumes like cowpea. Induced mutations have been used successfully to improve yield and yield components of many crops like *Oryza sativa, Hordeum vulgare, Triticum durum, Vicia faba, Cicer arietinum, Cajanus cajan,* in the world (Khan and Wani, 2006). Improvement of legumes such as cowpea through induced mutation could make it possible to identify new genes and thus broaden the spectrum of heritable changes and expand cowpea germplasms.

To enhance the limited genetic variability in cowpea, mutagenesis has been exploited and efforts made at identifying the proper mutagens in cowpea breeding which can produce mutants for future breeding programs (Achaya *et al*., 2007). Acceleration of frequency of mutation in cowpea has been accomplished by exposure of seeds to mutagenic agents like ionizing radiation and / or chemical mutagens (Natarajan, 2005). Colchicine treatment was reported as one of the best tools of inducing and enhancing genetic variability in some food crops within a very short period of time (Gnanamurthy *et al*., 2013). Hence, this study focused on the influence of colchicine treatments on characters expression and yield of cowpea.

II. Materials and Methods

This research was conducted at the Research Laboratory of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Modified techniques of Khan and Wani (2006); Kumar and Verma (2011); Ajayi (2014) were employed for this study.

A cowpea variety popularly known as "Oloyin" was obtained from a local farmer in Akungba-Akoko, Ondo State, Nigeria. A total of 800 healthy seeds of uniform size were used for this study. Two hundred seeds per treatment were soaked in distilled water for 12 hours, after which the water was drained, and seeds spread and air dried on a filter paper before being treated with colchicine.

Author α σ ρ ω: Department of Plant Science and Biotechnology, Adekunle- Ajasin University, PMB 001, Akungba-Akoko, Nigeria.
e-mail: akin_biyi2001@yahoo.com

© 2014 Global Journals Inc. (US)

Two hundred seeds per treatment were soaked in 0.1% (w/v) aqueous colchicine solution for 2, 4 and 6 hours at room temperature while the control was left untreated. Seeds were later transferred into a sterile cloth, tied and rinsed in running water for 20 min to terminate the residual effect of colchicine. The treated seeds were later air dried for 15 hours before sowing.

Treated seeds along with the control were sown in the field to generate M1 generation in a randomized complete block design with five replications, during the rainy season of 2013 at the Research Field of Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. Two hundred seeds from each treatment and control were planted in the field adopting an intra-row spacing of 50cm and inter- row 30cm.

a) Data collection

Data on 15 quantitative traits were collected from 10 randomly selected plants per replicate for control and from all surviving plants per replicate for the treated plants. The quantitative traits included;- seedling emergence percentage (7- 20 days after sowing); plant height (cm) taken at 4weeks and 8 weeks after sowing; number of leaves; terminal leaflet length (cm); terminal leaflet width (cm); number of main branches; number of nodes on main stem taken at 7 weeks after sowing; survival percentage; days to first flowering; number of peduncles per plant; peduncle length (cm); number of pods per plant; pod length (cm); number of seeds per pod and 100-seed weight (g) were taken at maturity.

Data were analyzed using Statistical Package for Social Science (SPSS) version 20 (SPSS, Inc., Chicago IL). Analysis of variance (ANOVA) was performed, followed by least significant difference (LSD; P = 0.05% level of significance) computation for mean separation.

III. Results and Discussions

Chemical mutagenesis has been a beneficial technique in the improvement of yield characters in crop breeding. Variability of quantitative traits influencing yield have been greater in mutagenic progenies than in control. Ability of mutagens to enter the cell of the living organisms to interact with DNA produces the general toxic effects associated with their mutagenic properties (Mensah *et al.*, 2007). It has been widely proved that chemical mutagens induce physiological damages (injury), gene mutations and chromosomal aberration in M1 individuals which can be detected and measured from seed germination or emergence of seedlings, survival reduction (lethality), plant height reduction (injury) and fertility reduction or sterility (reduction in pod and seed formation) (Kumar *et al.*, 2009) which might not be restricted to M1 generation (Mak *et al.*, 1986). Analysis of variance revealed that treatment effect was significant ($P \leq 0.05$) for emergence percentage, plant height, number of leaves, number of nodes on main stem, survival percentage, and number of days to flowering in the M1 generation whereas treatment effect was not significant for all other traits (Table 1). Means for emergence percentage from 7th day after sowing to 20th day after sowing established that colchicine treatment reduced emergence of seedlings with direct correlation with the duration of exposure. At 20days after sowing, emergence of seedlings ranged from 67% (control) to 25% (6 hours). Colchicine significantly decreased the emergence of seedlings compared with the control. About 40% reduction of emergence was found at 2 hours of exposure; therefore, LD_{40} value (59%) was fixed at 2 hours duration of treatment (Table 2). Reduction in emergence of seedlings and survival as a result of colchicine treatments agrees with the findings of many workers on cowpea (Kumar *et al.*, 2009; Girija and Dhanavel, 2009; Gnanamurthy *et al.*, 2013) and in many other crops like black gram (Deepalaskshmi, 2000; ThangaHamavathy, 2002). The damage to the biological materials according to these findings might be considered as an indication of the mutagenic effects.

Survival percentage at maturity ranged from 63.50% (control) to 12.5% (6 hours). Survival was significantly reduced with increased duration of colchicine treatment (Table 4). The linear relationship of treatment duration on survival has been observed by many workers. In most cases, the mortality may be due to poor seedling vigor resulting from inability to overcome the toxic effect of colchicine (Zlesak *et al.*, 2005).

Plant height was significantly reduced by all treatments generally compared with the control, with the highest duration of time producing the shortest heights, 7.46cm (4 weeks) and 15.63cm (8 weeks); whereas the control had the highest heights of 15.57cm at week 4 and 21.52cm at week 8 (Table 3). The reduction of growth observed with increase in duration of treatment is very common in M1 generation of many mutated crops, which is actually as a result of reduction in the rate of cell division among the treated plants and also linked to chromosomal abnormality, reduction of auxin levels, inhibition of auxin synthesis and failure of assimilation mechanisms (Riley, 1954).

Number of leaves was more in the control than other treatments: the number of leaves being 11.08 (control), 8.86 (2 hours), 4.98 (4 hours) and 6.79 (6 hours). There was a significant difference in treatment effects for number of leaves. The treated plants were found to possess longer leaflets and wider leaflets compared to control as also confirmed by Ajayi *et al.* (2010). This may actually be a useful trait for breeding for its potential to increase the net photosynthetic area which may lead to increase in photosynthetic assimilates going into grains as a positive effect of seed yield (Priya, 2006). It has also been proved that

© 2014 Global Journals Inc. (US)

successful colchicine treatment has been found to result in plants that possess characters such as thicker-wider leaves with bigger and fewer stomata number (Uhlik, 1981).

Number of nodes on main stem showed a significant difference among the treatments, with the 2 hours having the highest number of nodes (6.26), followed by the control (6.22), and while the lowest number of leaves was for the 4hour treatment (4.50). Control flowered earlier (47.30 days) than all other treatments. Increase in duration of exposure delayed flowering. The mean number of days to flowering among the treated plants was 47.50 (2 hours), 54.97 (4 hours) and 55.12 (6 hours). The difference between the control and other treatments was significant. This delay in flowering with direct correlation with increased exposure period is not novel as it has been observed by many workers especially in soybean (Maheshwari *et al.*, 2003; Pavadai and Dhanavel, 2005), in mungbean (Khan and Wan, 2005).

The decrease in peduncle length, number of pods per plant, pod length and number of seeds per pod all contradict the results of Odeigah (1998) on M1 generation of cowpea but consistent with Kumar *et al.* (2009), who reported that reduction in pod number may be as a result of inhibiting action of enzymes, changes in enzymatic activities and toxicity of the mutagen on these traits, while reduced seed yield can be attributed to high seed sterility and reduced pod number, also as consequences of physiological and biochemical disturbances in the development of plants (Prabhakar, 1985) resulting from mutagenic treatment (Ajayi *et al.*, 2014). Seed weight was however enhanced by 2hour duration of exposure but was further reduced by treatment at higher exposure of time. Some of the characters studied decreased linearly as the duration of treatment increased why most of them showed irregular pattern of behaviour with increase in duration of treatment.

IV. Conclusion

From the results obtained, seed treatment has proven to be a viable method for inducing variability in cowpea through mutagenesis. The level of dissimilarity among the plants as a result of treatment was high and this indicates a possible improvement through this approach. Although colchicine reduced most of the morphological characters at M1, this is always expected as it is a usual phenomenon in M1 generation.

Table 1 : Mean square values of all traits for colchicine treatment

CHARACTER	DF	REPLICATION	TREATMENT	ERROR
EP (20 DAS)	3	452.66*	3753.75*	47.24
PH(8 WAS)	3	50.04*	36.04*	5.99
NL	3	2.45	34.64*	3.86
NN	3	1.34*	5.66*	0.78
TLL (cm)	3	1.21	1.78	2.31
TLW (cm)	3	0.84	0.56	0.82
NMB	3	0.63	0.83	1.19
SUP	3	358.91*	3176.98*	32.45
PPP	3	39.36	94.48	53.51
PEL (cm)	3	55.85*	50.19	14.42
NDF	3	45.07	97.51*	19.94
NPP	3	5.62	2.58	8.22
PL (cm)	3	2.88	1.48	1.35
SP	3	0.86	3.52	3.67
SW (g)	3	0.04	0.81	0.89

*: Significant

EP: Emergence percentage; PH: Plant height; NL: Number of leaves per plant; NN: Number of nodes on main stem; TLL: Terminal leaflet length; TLW: Terminal leaflet width; NMB: Number of main branches; SUP: Survival percentage; PPP: Peduncle per plant; PEL: Peduncle length; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; SP: Seeds per pod; SW: Seed weight; DAS: Days after sowing; WAS: Weeks after sowing

© 2014 Global Journals Inc. (US)

Table 2 : Emergence percentage of colchicine treated cowpea and control from 7 DAS to 20 DAS (Mean ±Standarderror)

Treatment	7DAS	8DAS	9DAS	13DAS	18DAS	20DAS
Control	55.00±7.70[a]	62.00±5.55[a]	63.50±5.45[a]	65.00±5.53[a]	67.00±5.26[a]	67.00±5.26[a]
2Hrs	28.50±4.07[b]	38.50±7.85[b]	44.00±7.05[b]	55.00±8.60[b]	55.50±8.11[b]	59.00±7.81[b]
4Hrs	11.00±2.44[c]	11.50±2.44[c]	12.00±3.00[c]	15.00±4.10[c]	16.80±4.58[c]	18.50±5.35[c]
6Hrs	11.50±2.31[c]	12.00±2.12[c]	12.50±2.50[c]	14.00±2.80[c]	13.00±2.42[c]	13.00±2.42[c]
LSD	12.27	13.48	11.46	11.76	9.66	9.47
CV (%)	33	32	25	23	18	18

Mean values followed by same letters within a column are not significantly different

DAS: Days after sowing; LSD: Least significant difference; CV: Coefficient of variation

Table 3 : Mean and Standard error values of plant height of colchicine treated and untreated cowpea

Week	Control	2Hrs	4Hrs	6Hrs	LSD	CV (%)
4WAS	15.57±0.6[a]	10.93±0.7[b]	6.69±0.50[c]	7.46±0.78[c]	1.93	13.9
8WAS	21.52±1.8[a]	17.53±1.4[b]	16.05±2.03[b]	15.63±2.04[b]	3.37	13.84

Mean values followed by same letters within the same row are not significantly different

LSD: Least significant difference; CV: Coefficient of variation; WAS: Weeks after sowing

© 2014 Global Journals Inc. (US)

Table 4 : Quantitative traits (Mean ± Standard error) of control and colchicine treated cowpea

Treatment	NL	NN	TLL	TLW	NMB	SUP	PPP	PEL	NDF	NPP	PL	NSP	SW
Control	11.08±1.29a	6.22±0.43b	11.73±0.07a	7.57±0.16a	7.30±0.41a	63.50±4.23a	27.56±3.29a	24.90±1.59a	47.30±1.43c	9.00±0.89a	14.65±0.54a	10.78±0.75a	12.92±0.51a
2hrs	8.06±0.42b	6.26±0.26a	15.04±0.70a	8.38±0.56a	7.84±0.42a	52.00±6.49b	28.52±4.27a	25.32±0.86a	47.50±0.83c	8.76±1.09a	14.62±0.36a	9.37±0.68a	12.50±0.32a
4hrs	4.98±0.35c	4.26±0.22c	14.36±0.81a	7.84±0.41a	6.40±0.60a	17.50±5.06	18.89±2.69a	24.85±2.36a	54.97±1.53b	8.58±1.65a	13.84±0.68a	11.06±0.73a	13.30±0.81a
6hrs	6.79±0.90c	4.50±0.36c	14.91±0.55a	7.96±0.36a	6.94±0.34a	12.50±2.37	24.25±1.91a	18.70±3.32a	55.52±3.99a	7.38±1.16a	13.58±0.71a	11.19±0.90a	12.44±0.38a
LSD	2.7	0.84	NS	NS	NS	7.85	NS	NS	6.15	NS	NS	NS	NS
CV (%)	24.78	11.52	10.43	11.41	15.34	15.66	29.49	16.19	8.72	34	8.21	18.06	7.37

Mean values followed by same letters within the column are not significantly different

NL: Number of leaves per plant; NN: Number of nodes on main stem; TLL: Terminal leaflet length; TLW: Terminal leaflet width; NMB: Number of main branches; SUP: branches; SUP: Survival percentage; PPP: Peduncle per plant; PEL: Peduncle length; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; SP: Seeds per pod; SW: Seed weight; LSD: Least significant difference; CV: Coefficient of variation.

© 2014 Global Journals Inc. (US)

References Références Referencias

1. Acharya, S.N., Thomas, J.E and Basu, S.K 2007. Improvement in medicinal and nutritional properties of fenugreek (*Trigonella foenum graecum* L). in: S.N. Acharya, J.E. Thomas (eds) Advances in medicinal plant research, Research Signpost, Trivandrum, Kerala, India.
2. Adekola, O.F and Oluleye, F. 2007: Induction of genetic variation in cowpea (*Vigna unguiculata* L.Walp) by Gamma Irradiation. Asian journal of plant sciences 6 (5): 869-873.
3. Adetiloye, I.S., Ariyo, O.J., Alake, C.O., Oduwaye, O.O and Osewa, S.O. 2013. Genetic diversity of some selected Nigeria cowpea using simple sequence repeats (SSR) marker, *Afr. J. Agric. Res*. 8 (7): 586 – 590.
4. Ajayi, A.T. 2014: Variations in germination, survival and yield characters of a colchicine-induced m1 generation of cowpea [*Vigna unguiculata* (L.) Walp]. Applied science research journal. 2014 vol 2 (1) 1 – 9.
5. Ajayi, A.T and Adesoye, A.I. 2013. Cluster analysis technique for assessing variability in cowpea *(Vigna unguiculata* L. Walp).
6. Blade S.F, Shetty S.V.R, Terao T, Singh B.B. 1997. Recent developments in cowpea cropping systems research In: Singh BB, Mohan Raj DR, Dashiell K.E, and Jackai L.E.N. (eds). Advances in Cowpea.
7. Deepalakshmi, A.J. 2000. Creation of variability in black gram (*Vigna mungo* L. Hepper) through induced mutagenesis. MSc. (Ag.) Thesis, Tamil Nadu Agric. Univ. Coimbatore, India.
8. Dhanavel, D., Gnanamurthy, S and Girija, M. 2012. Effect of gamma rays on induced chromosomal variation in cowpea (*Vigna unguiculata* L. Walp.). *Int. J. Curr.* Sci. 245 – 250.
9. Girija, M and Dhanavel, D . 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatment in cowpea (*Vigna unguiculata* L. Walp.). Glo. *J. Mol. Sci.* 4 (2): 68 – 75.
10. Gnanamurthy, S., Dhanavel, D and Girija, M. 2013. Effect of gamma irradiation on the morphological characters of cowpea (*Vigna unguiculata* L. Walp.). *Int. J. Cur. Tr. Res.* 2 (1): 38 – 43. IITA-JIRCAS, Ibadan, pp 1–12.
11. Khan, S and Wani, M.R. 2005. Comparison on the effect of chemical Mutagens on mungbean. *Adv. Plant Science* 18 (11): 533-535.
12. Khan, S and Wani, M.R 2006. Estimate of genetic variability in mutated populations and scope of selection for yield attributes in *Vigna radiata* (L.) Wilczek. *Egyptian Journal of Biology,* 8 pp. 1 – 6.
13. Kitch, L.W., Boukar, O., Endondo, C. & Murdock, L.L., *Expl Agric.*, 1998, 34: 475–486.
14. Kumar, G and Verma, S. 2011. Induction of quantitative variability through EMS treatment in *Vigna unguiculata Rom. J. Biol* – Plant Biol. 56 (2): 91 – 97.
15. Kumar, V.A., Kumari, R.U., Amutha, R., Kumar, T.S., Hepziba, S.J and Kumar, C.R.A. 2009. Effect of chemical mutagen on expression of characters in arid legume pulse – cowpea (*Vigna unguiculata* L. Walp.). *Research Journal of Agriculture and Biological Sciences*, 5 (6): 1115 – 1120.
16. Maheshwari, J.J., Patil, S., Dhole, V.J and Rathod, D.R. 2003. Radiation induced variability for quantitative characters in soybean . *J. soils* and *crops* 13(12): 314-316.
17. Mak, C., Teoh, S.B., and Ratnam, A .1986. The influence of Gamma rays on the injury and chromosomal aberrations of long bean (*Vigna sesquipedalis Fruw.*). Journal of Petranika, ((1): 109-117.
18. Megloire, N. 2005. The genetic, morphological and physiological evaluation of African cowpea genotypes. University of Free State, South Africa.
19. Mensah, J.K., Obadoni, B.O., Akomeah, P.A., Ikhajiagbe, B and Ajibolu, J. 2006. The effects of sodium Azide and Colchicine treatments on morphological and yield traits of sesame. African Journal of Biotechnology 6(5), pp534-538.
20. Natarajan, A.T. 2005. Chemical mutagenesis: from plants to human. *Curr. Sci.* 89(2):312-316.
21. Padulosi S, Ng N.Q. 1997. Origin, taxonomy and morphology of *Vigna unguiculata* [L.] Walp). In: Singh B.B, Mohan Raji D.R, Dashiell K.E, Jackai L.E.N. eds. Advances in Cowpea Research 1997.
22. Pasquet R. *Vigna savi.* In: Mackinder B, Pasquet R, Polhill R, Verdcourt B eds. Flora zambesiaca, volume part *Phaseoleae.* 2001; Royal Botanic Gardens, Kew, pp 121–156.
23. Pavadai, P and Dhanavel, D. 2005. Effect of gamma rays on yield and its components in Soybean (Glycine max L.) Merrill. Var.Co. *Crop Research* 30 (3): 459-461.
24. Prabhakar, L.V .1985. Studies on induced mutagenesis in *Sesamum indicum* L. MSc. (Ag). Thesis, Tamil Nadu Agricultural University, Coimbatore, India.
25. Priya R.T. 2006. Induced macromutation in mungbean (*Vigna radiata* (L) Wilczek). *International Journal of Botany* 2(3): 219-228.
26. Riley, E.F .1954. The effect of X-rays upon growth of Avena seedlings. *Rad. Res.* 1: 227 – 228
27. ThangaHemavathy, A .2002. Creation of variation in black gram (*Vigna mungo* L. Hepper). M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ. Coimbatore.
28. Uno, G., Storey, R., and Moore, R. 2001. *Principles of Botany.* Mc Graw Hill New York 1-550pp.
29. Zlesak, D.C., Thill, C.A and Anderson, N.O. 2005. Trifluralin-medxiated polyploidization of *Rosa chinensis* minima (Sims) Voss seedlings. Euphytica 141: 281 – 290.

© 2014 Global Journals Inc. (US)

Global Journal of Science Frontier Research: C
Biological Science
Volume 14 Issue 5 Version 1.0 Year 2014
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Isolation and Phenotypic Characterization of Lactobacillus Sakei and Pediococcusspp. Antagonists from Algerian Meat

By M. Naimi & M. B. Khaled

Djillali Liabes University of Sidi Bel Abbes, Algeria

Abstract- The aim of the present work was to isolate antagonist cultures in order to use them in biopreservation. LAB were isolated from Algerian meat and characterized at the genus level based on phenotypic characteristics. That following these spot agar test was achieved to assess their potential antagonistic towards pathogens: Bacillus cereus, Bacillus subtilis ATCC6633, Escherichia coli ATCC8739, Salmonella Typhimurium ATCC14028, Staphylococcus aureus ATCC6538, and Pseudomonas aeruginosa. Biochemical tests had ended this study to characterize the potent isolates at the spice level. As a results, thirty LAB had been differentiated to: 53% belong to Lactobacillus or Lactobacillus-like; 23% to Pediococcus; 20% to Lactococcus or Vagococcus; and 4% to Streptococcus. The antagonist test had observed activity of five isolates against only St. aureus with inhibition zone ranging from 0.58 to 5.16 mm.

Keywords: exploratory test, lab, crude bacteriocins, spoilage, pathogens.

GJSFR-C Classification : FOR Code: 069999

ISOLATIONANDPHENOTYPICCHARACTERIZATIONOFLACTOBACILLUSSAKEIANDPEDIOCOCCUSSPPANTAGONISTSFROMALGERIANMEAT

Strictly as per the compliance and regulations of :

© 2014. M. Naimi & M. B. Khaled. 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.

Isolation and Phenotypic Characterization of *Lactobacillus Sakei* and *Pediococcusspp*. Antagonists from Algerian Meat

M. Naimi [α] & M. B. Khaled [σ]

Abstract- The aim of the present work was to isolate antagonist cultures in order to use them in biopreservation. LAB were isolated from Algerian meat and characterized at the genus level based on phenotypic characteristics. That following these spot agar test was achieved to assess their potential antagonistic towards pathogens: Bacillus cereus, Bacillus subtilis ATCC6633, Escherichia coli ATCC8739, Salmonella Typhimurium ATCC14028, Staphylococcus aureus ATCC6538, and Pseudomonas aeruginosa. Biochemical tests had ended this study to characterize the potent isolates at the spice level. As a results, thirty LAB had been differentiated to: 53% belong to Lactobacillus or Lactobacillus-like; 23% to Pediococcus; 20% to Lactococcus or Vagococcus; and 4% to Streptococcus. The antagonist test had observed activity of five isolates against only St. aureus with inhibition zone ranging from 0.58 to 5.16 mm. The five potent isolates vary mainly by the fermentation of: raffinose, sorbitol, dulcitol, l'esculine and D-mannitol, thus one had been identified as Lactobacillus sakei and four as Pediococcusspp.. This work showed our isolates as potential inhibitors to the growth of pathogens, suggesting the possibility to improve the hygienic quality of meat.

Keywords: exploratory test, lab, crude bacteriocins, spoilage, pathogens.

I. Introduction

Meat is rich in nutrients, so provided a desired environment for growth for different groups of micro-organisms (Guiraud et al. 1980; Stiles 1994; Bibek et al. 2008). Lactic acid bacteria are part of the initial microbiota, typically mesophilic which can grow easily at 5-45°C, under aerobic, anaerobic or microaerobic terms. These bacteria form a group of diverse genera with *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* that form the core of the group. However, from a practical food-technology point of view, the following genera are considered the principal: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella*. Lactic acid bacteria; may be characterized as Gram-positive sphere or rod shaped, non-spore-forming, oxidase and catalase negative, do notreduce nitrates to nitrite and sulfate to sulphide, able to produce lactic acid either by Homofermenter or Heterofermenter way; and are associated not only with meat but also with beverages, vegetables and dairy as well normal micobiota of mouth, intestinal and vaginal microbiota of mammals (Carr et al. 2002; Axelsson 2004; Doyle et al. 2006). Since do not pose any health risk to human there are designated as GRAS « Generally Recognized As Safe » organisms (Klaenhammer et al. 2005; Castellano et al. 2008; Dortu et al. 2009; Jeevaratnam et al. 2005). At this time, lactic acid bacteria are exploited as one of three cultures: probiotic, protective or starter (Carr et al. 2002; Castellano et al. 2008; Lücke 2000; Holzapfel 1995; Gálvez et al.2007). Also using theirs antimicrobial end products such bacteriocins as dual anti- and probiotics is well-known. Lactic acid bacteria have been isolated and characterized from meat and meat products (Schillinger, and Lücke 1987; Morishita, and Shiromizu 1986; Samelis et al. 1994; Najjari et al. 2008; Bromberg et al. 2004; Jones et al. 2008; De-Martinis, and Freitas 2003; Al-Allaf et al. 2009; Chaiyana 2007; Castellano et al. 2004). As no universal selective medium exists for the cultivation of all genera, elective media appear for more than one genus and selective media assigned for well-defined genera wile changing pH, addition of inhibitory agents, or used with other temperature-time terms (Reuter 1985), as well as some color indicators (Najjari et al. 2008; Dallbelo et al.). Moreover, the choice of the medium is related to the biotope, and for example, for meat and meat products MRS medium are often used. Therefore, this medium has been recommended for the isolation of "LLPW" group (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weissella*) other secondary genera as *Lactoccucs* and *Streptococcus* can grow over (Reuter 1985; Schillinger and Holzapfel 2003; Carr et al. 2002). Self-evidently, characterization is largely based on morphology, mode of glucose fermentation, lactic acid produce, ability to grow at different temperatures, at high salt concentrations, and acid or alkaline tolerance. These characteristics are a basic and still very important to identify lactic acid bacteria (Axelsson 2004; Doyle et al. 2006). The objective of this study was to isolate and characterize, through phenotypic characteristics, *Lactobacillus* and *Pediococcus* from Algerian meat, in

Author α σ: Department of life and natural sciences, Djillali Liabes University, B.P N° 89 Sidi Bel Abbes, Algeria.
e-mail: mostecoc@yahoo.fr.

© 2014 Global Journals Inc. (US)

order to initiate an isolates collection and to probable use as bioprotective agents for meat products.

II. Material and Methods

a) Sample Meat Collection

Six samples, each one in three units, every unit about one hundred gram measuring five centimeters cub of fresh lamb meat, liver, and small intestine were cut out according to destructive technique using sterile instruments (scalpel, clamp) under aseptic condition (Larpent 1997), from retail stores and butchers in Saida region, (Algeria). Samples were introduced in label sterile bags, immediately transferred in isotherm box at 4°C to the laboratory, being analyzed on arrival.

b) LABMeatIsolation

Samples have been prepared for analysis according to ISO 6887-2, for each sample 25 g of meat cutting into small cubes was aseptically transferred to a sterile stomacher bag homogenized with 225 ml of saline-peptone water (NaCl 8.5 g/l; bactopeptone 1g/l) for 1 min using stomacher (LAB BLINDER © 400) to obtain a 1:10 dilution. Serial dilutions 10^{-1} -10^{-6} were then made directly or after enrichment for 1 day at room temperature, and 100 µl aliquots were spread onto duplicate plates of MRS-BG agar (bromocresol green: 0.0025% (w/v)). Plates were incubated microaerobically at 30°C for 2 days (Najjari et al. 2008; Dallbelo et al.) Bacterial count was performed according to ISO 4833. Colonies were selected from plates on the basis of theirs colors and size. Such colonies were sub cultured differentially on MRS-BG agar and pure isolates were maintained on MRS-BG as slants agar at temperature of 4°C for short-term use. Stock cultures were maintained frozen at -18°C on 20% glycerol (De valdez 2001). Each isolate was propagated twice on MRS broth before use. Overnight culture was employed in the tests. All isolates were initially subjected to macroscopic exams and orientation tests; Gram stain (Chaskes 2009), catalase (Hart and Shears 1997) and endospore (Guiraud et al. 1980).

c) Characterization and Differentiation of LAB Meat Isolates tothe Genus Level

A preliminary identification in order to differentiate isolates at the genus level (Axelsson 2004; Doyle et al. 2006) was carried out using the following tests: CO_2 from glucose, growth at different temperatures, salt tolerance at 6.5-10 % (w/v) and pH tolerance at pH 3.9, 4.4, 9.6. Incubations were made at: 30°C for 3 days, 7-10°C for 7 to 10 days, 15-45°C for 3 to 5 days, 30°C for 2 to 3 days, in the same order (Schillinger and Lücke 1987). Lactic acid production was determined according to NF V.04.206.

d) Antagonism Test

To select antagonists among lactic acid bacteria isolates an antagonism test was achieved based on the agar spot test, according to (Schillinger and Lücke 1989) originally described by Fleming et al. (1975), on TSA-YE medium (Tryptic Soy Agar supplemented with 0.6% Yeast Extract) towards the following pathogens: *B. cereus, B. subtilis ATCC 6633, E. coli ATCC 8739, S. typhimurium ATCC 14028, St. aureus ATCC 6538,* and *P. aeruginosa.* Incubation was carried out at 30°C for 2 days under anaerobic conditions means to reduce lactic acid and hydrogen peroxide effect. Isolates were selected on the basis of positive results showed the presence of clear zone around spots.

e) Characterization of the Selected LAB Meat Isolates to Species Level

A secondly identification at the species level was carried out by both assimilation and production tests: Arginine (Schillinger and Lücke 1987), Nitrate, Urea, and Hydrogen sulfide (Guiraud et al. 1980; Larpent and Gourgaud 1990; Forouhandeh et al. 2010). Incubations were made at: 30°C for 2 days, 30°C for 3 days, and 30°C for 2 weeks, in the same order.Carbohydrate fermentation profile was determined on MRS-BCP (bromocresol purple: 0.017% (w/v)). Sterile solutions of the sugars at 10 % (w/v) were added at final sugar concentration of 2 % (w/v). All strains were tested for fermentation of the following sugars: L-Arabinose, D (+) Glucose, Starch, D (+) Maltose, D (+) Galactose, Saccharose, D Mannitol, L Rhamnose, D (+) Lactose, Esculine, Arabinose, D Fructose, Raffinose, D Xylose, Sorbitol, D Cellubiose, Ducitol. 100 µl aliquots of sterile liquid paraffin were added to ensure anaerobic conditions. Incubations were made at 37°C for 2 days.

III. Results

Thirty-three isolates were pricked from dilutions 10^{-4} and 10^{-5}. In the case without enrichment only eight isolates were pricked from dilutions 10^{-1}. So a total of forty-one bacteria were isolated from different parts of fresh lamb meat, including liver and small intestine. Loads of 2.10^2, 2.10^6 and 3.10^6 UFC / g for directly, after enrichment, liver and small intestine, were taken in. Colonies macroscopic exams show five colors (green, light green with green center, white, white with green center and grey), two forms (punctiform and circular), opaque with smooth surface, of sizes from 1 to 3 mm. The colonies were picked from plates with 100 to 150 total colonies. Thirty isolates were non-spore-forming, catalase negative and Gram-positive (bacilli/coccobacillior cocci, some of them form tetrad). These lactic acid bacteria isolates produce various ratios of lactic acid of 0.74 to 1.26% and were farther characterised at the genus level, most (66.66%) seems to be true psychrotrophic growing at 7°C, as: fifteen mesophilichomofermentative bacilli (*atypical Streptobacteria*), eight among them were able to grow at 10-15°C but not at

© 2014 Global Journals Inc. (US)

45°C, failed to stand in the presence of 10-6.5% NaCl, as well to different pH except 9.6, thus, they appears belong to the genera: *Lactobacillus* and or *Lactobacillu-slike.* While the others were able to grow at 10-15°C but not at 45°C, also in the presence of 10% but not at 6.5% of NaCl, unable to bear different pH except 9.6 other than one isolate, they appears belong to the genus *Lactobacillus*; one thermophilichomofermentative bacilli (*Thermobacteria*) the only able to grow at 45°C but not at 15°C, stand in the presence of 6.5% NaCl and to pH 9.6, such description be like the genus *Lactobacillus*; seven mésophilichomofermentativecocci (*Streptococcus*), six of whom were able to grow at 10 15 °C but not at 45°C, do not with 10-6.5% NaCl and on different pH except 9.6, that to say *Lactococcus* or *Vagococcus*,only one isolate are unable to grow at 10°C appears to be *Streptococcus;* and seven mésophilicho-mofermentativecocci (*Tetracoccus*) able to grow at 10°C except for two, grow all at 15°C but not at 45°C, can't do it at 10-6.5% NaCl, as well to different pH except 9.6, characteristics of genus *Pediococcus.* The antagonist test point out five isolates potency bacteriocinogenic, were antagonistic to Gram-positive target strains: *B. cereus, B. subtilis ATCC 6633* and *St.aureus ATCC 6538* with inhibition diameters ranging from 0.5 to 5.16 mm. On the basis of biochemical tests carried towards their characterization at the spice level; one isolate assumed *Lactobacillus* or *Lactobacilluslike* are arginine positive, urea negative, are neither nitrite nor H_2S producer, ferment weakly esculine, mannitol, D-sorbitol, are negative reaction for L-rhamnose, L-arabinose, raffinose and dulcitol, take these specific characters with *Lactobacillussakei*. Another are arginine and urea negative, are neither nitrite nor H_2S producer, ferment all sugars except for L-rhamnose, L-arabinose and sorbitol, weakly reaction for dulcitol; the three other isolates are Nitrate, H2S, arginine and urea negative, ferment all sugars except for L-rhamnose, L-arabinose, raffinose, sorbitol and dulcitol. These four isolates were *Pediococcus. spp*.

IV. Discussion

Isolation been began with an enrichment so as to increase the initial biomass and to give a better chance to detect lactic acid bacteria. Whereon, total counts are 2.10^2 on fresh lamb meat, at attempt without enrichment, those counts may reflect the exact population of the products at the time of sampling. While, after enrichment counts are ranged from 2.10^6 to 3.10^6 UFC/g on fresh meat, liver and small intestine, respectively. Similar densities from fresh sheep-meat around 10^6 CFU/g were found by Najjari et al. (2008). Liver and small intestine showed the highest bacterial population, these are in agreement with results obtained by Olaoye and Onilude (2009). Five different types of colony were observed on plates, where upon colonies were picked on the basis of theirs colors and size that have the same colony morphology noted by Najjari et al. (2008) and Dallbelo et al. (). Such colonies are: green; light green with green center; white; white with green center and grey. As result, forty-one bacteria were isolated; among them thirty isolates were non-spore-forming, catalase negative and Gram-positive. These results are consistent with the group of genera of lactic acid bacteria (Carr et al. 2002; Schillinger and Holzapfel 2003; Axelsson 2004; Doyle et al. 2006). These isolates displayed various forms: bacilli, coccobacillior cocci, some of them with tetrad formation. As to lactic acid, isomer L/D, and CO_2 production from glucose, such parameters were useful for the characterization (Hayward1957; Axelsson 2004). Thereby, all our isolates converted glucose quantitatively to lactic acid suggesting that belonging to the homofermetative following genera *atypical Streptobacteria*, *Thermo-bacteria*, *Streptococcus* and *Tetracoccus* (Stiles et al. 1997; Axelsson 2004; Doyle et al. 2006), So with a wide prevalence of homofermentative. Similar to that observed by Niemand and Holzapfel (1984) having isolated 67 strains, only two were heterofermentative. In addition, homofermentative lactic acid bacteria are potency to be used for biopreservation of meat (Vermeiren et al. 2004). Titratable acidity shows deferent capacity to produce lactic acid from 0.74 to 1.26%, this may have an effect antagonist, on typical spoilage microbiota mainly Gram-negative bacilli, while decreasing pH (Niemand and Holzapfel 1984). Moreover, (Stiles 1994) noted suitable for use lactobacilli with are aciduric, producing low pH in meats. In fact Inhibitory activity of lactic acid lies in the reduction of pH, and in the action of undissociated acid molecules. Further, (L+) lactic acid is inhibitorier than (D-), since the (D-) isomer is not hydrolyzed by human lactate dehydrogenase and may cause health problems, only strains producing mainly (L+) lactic acid should be selected (Ammor and Mayo 2007). All our isolates except one were able to grow at 15°C most among them were psychrotrophic growing at 7°C. An advantage, since the psychrophilic character is noted as a key in the selection of protective cultures (Vermeiren et al. 2004). Mesophilichomofermentative were *Lactobacillus*, *Lacto-bacilluslike* genus *Carnobacterium*called "atypical meat lactics". This genus resemble lactobacilli but they do not grow on acetate media (Stiles et al. 1997, are unable to grow at 0°C and are arginine positive (Carr et al. 2002). Our *Lactobacillus* and *Lactobacilluslike* were: bacilli/co-ccobacilli; able to grow at pH 9.6 but do not at pH 3.9. Are therefore in agreement with description of *atypicalStreptobacteria*found associated with red meat (Samelis et al. 1994; Carr et al.2002). Growing at pH 9.6, this also was found in previous study (Chaiyana 2007). Such *atypicalStreptobacteria* havebeen found inhibit the growth of *St. aureus*and others undesirable bacteria with bacteriocins (Carr et al. 2002). Bacteriocins can be

© 2014 Global Journals Inc. (US)

used with or without released cultures as food-grad (Hugas 1998; Lücke 2000; Vermeiren et al. 2004; Savadogo et al. 2006; Castellano et al. 2008; Carr et al. 2002). Mésophilichomofermentativecocci were: *Lactococcus* or *Vagococcus*, except one unable to grow at 10°C was *Streptococcus*; those with tetrad formation were *Pediococcus* (Schillinger and Lücke 1987). Antagonism test carried out *in vitro* to asses' isolates potential inhibitor has ended to select five isolatespotency bacteriocinogenic against Gram-positive: *B. cereus, B. subtilis ATCC 6633* and *St.aureus ATCC 6538*, with inhibition diameters ranging from 0.5 to 5.16 mm.. This result isn't wonder; as known the inactivity of bacteriocins against the Gram-negative due to the protective barrier provided by the lipopolysaccharides (Abee et al. 1995; De-Martinis and Freitas 2003; Bromberg et al. 2004) and bacteriocinsare only active against Gram-positive (Dortu et al. 2009). Biochemical characteristics allowed to identify one isolate as *L. sakei*(Schillinger and Lücke 1987;Korkeala and Mäkelä 1989; Carr et al. 2002) this isolate shows properties of the subgroup S6/b include this spice described by Morishita and Shiromizu (1986), therewith weak reaction; fermetingD-xylose, mannitol, sorbitol, esculine; and little growing in 7.5% NaCl. The same identity description of group 4 represented only by *L. sakei*reported by Korkeala and Mäkelä (1989) therewith weak reaction; fermeting D-xylose, sorbitol and little growing in 8% NaCl. Also his pattern agrees with *L. sakei*identified by Samelis et al. (1994) there with fermenting D-xylose and growing in 8 % NaCl. Furthermore, this isolate is saccharose positive, another clean character to *L. sakei* (Carr et al. 2002). So it is of great importance to note that various researchers have used different criteria to describe *a typical Streptobacteria* which sometimes makes it difficult to make a point comparison and to take meaning conclusions (Carr et al. 2002). Others four isolates, were distinctive for tetrad formation, identified at genus as *Pediococcus,* three isolates have common fermenting all sugars except L-rhamnose, L-arabinose, raffinose, dulcitol, while the fourth has ability to ferment all sugars except L-rhamnose, L-arabinose, sorbitol, dulcitol. Due too far differences patterns from those described in the literature, themselves ever changing, sometimes even contradictory, this was mainly attributable to high variability observed in the same species of the genus. It seems at that time not possible to make clear statements about *Pediococcus* sp.

V. Acknowledgment

We are; obligedto the Algerian Centre of Quality Control and Packaging, Saida, Algeria; thankful to messes Hadj Ahmed Belaouni and Mohammed EL Amine Bendaha, for invaluable helpful.

References Références Referencias

1. Abee T, Krockel L, Hill C. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*. 1995; 28: 169-185.
2. Al-Allaf MAH, Al-Rawi AMM, Al-Mola AT. Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria. *Iraqi Journal of Veterinary Sciences.*2009; 23: 115-117.
3. Aly S, Ouattara cat, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria. *African Journal of Biotechnology*. 2006; 5 : 678-683.
4. Ammor MS, Mayo B. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production. *Meat Science.* 2007; 76: 138-146.
5. Axelsson L. Lactic acid bacteria, Classification and physiology. In: Salminen S, Von-Wright A, Ouwehand A. Lactic acid bacteria, third edition. Marcel Dekker: New York; 2004.20-86.
6. Bibek R, Arun B. Fundamental food Microbiology. fourth edition. CRC Press: 2008. 492p.
7. Bromberg R, Moreno I, Zaganini CL, Delboni RR, De Oliveira J. Isolation of bacteriocin-producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. *Brazilian Journal of Microbiology.* 2004; 35:137-144.
8. Carr FJ, Chill D, Nino M. The lactic acid bacteria: A literature survey, *Critical Reviews in Microbiology*. 2002; 28: 281-370.
9. Castellano P, Belfiore C, Fadda S, Vignolo G. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science.*2008;79: 483-499.
10. Castellano PH, Holzapfel WH, Vignolo GM. The control of *Listeria innocua* and *Lactobacillus sakei* in broth and meat slurry with the bacteriocinogenic strain *Lactobacillus casei CRL705.Food Microbiology.*2004; 21: 291-298.
11. Chaiyana J, Boonrang S, Sinsuwongwat S. Isolation and screening of bactériocin producing bacteria from fermented meat products. *Biotechnology for gross national hapipiness.*2007; 108: 245-258.
12. Chaskes S. Stains for Light Microscopy. In: Goldman E, Green LH. Pratical Handbook of Microbiology. Second Edition, CRC Press: 2009; 37-51.
13. De-Martinis ECP, Freitas FZ. Screening of lactic acid bacteria from Brazilian meats for bacteriocin formation.*Food Control.*2003; 14: 197-200.
14. De-valdez GF. Maintenance of lactic acid bacteria. In: Spencer JFT, Spencer ALR. Food Microbiology-protocols, HumanaPress: 2001; 163-172.
15. Dortu C, Thonart P. Les bactériocines des bactéries lactiques: caractéristiques et intérêts pour la

© 2014 Global Journals Inc. (US)

bioconservation des produits alimentaires. *Biotechnol.Agron. Soc. Environ.* 2009; 13: 143-154.
16. Doyle MP and Meng J. Bacteria in food and beverage production. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. The Prokaryotes.Third edition. Springer, 2006; 795-809.
17. Dal-Bello F. Ecological studies of the Lactobacillus biota in the human digestive tract and adaptation of intestinal lactobacilli to the sourdough ecosystem. Thèse de doctorat des sciences, université de Hohenheim. Université de Hohenheim, 2005; 98p.
18. Fleming HP, Etchells JL, Costilow RN. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. *Applied Microbiology.*1975; 30: 1040-1042.
19. Forouhandeh H, Vahed SZ, Hejazi MS, Nahaie MR, Akbari DM. Isolation and phenotypic characterization of lactobacillus species from various dairy products. *current research in bacteriology.* 2010; 3: 84-88.
20. Gálvez A, Abriouel H, López RL, Ben Omar N. Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology.* 2007; 120: 51-70.
21. Guiraud J, Galzy P. L'analyse Microbiologique dans les industries alimentaires. L'usine, 1980 ; 235p.
22. Hart T, Shears P. 1997. Atlas de poche de Microbiologie. Flammarion, Paris. 1997; 314p.
23. Hayward AC. Detection of gas production from glucose by heterofermentative lactic acid bacteria. *J. gen. Microbiol.* 1957; 16: 9-15.
24. Holzapfel WH, Geisen R, Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology.*1995; 24: 343-36.
25. Hugas M. Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. *Meat Science.*1998; 49: 139-150.
26. Jeevaratnam K, Jamuna M, Bawa AS. Biological preservation of foods–bacteriocins of lactic acid bacteria.*Indian journal of biotechnology.*2005; 4: 446-454.
27. Jones RJ, Hussein HM, Zagorec M, Brightwell G, Tagg JR . Isolation of lactic acid bacteria with inhibitory activity against pathogens and spoilage organisms associated with fresh meat.*Food Microbiology.*2008; 25:228-234.
28. Klaenhammer TR , Barrangou TR , Buck R, Azcarate-Peril BL, Altermann MA, Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS Microbiol Rev.* 2005; 29.
29. Korkeala H, Mäkelä P. Characterization of lactic acid bacteria isolated from vacuum-packed cooked ring sausages, *International Journal of Food Microbiology.* 1989; 9 : 33-43.
30. Larpent JP, Microbiologie des viandes. In: Larpent. Microbiologie alimentaire, Technique de laboratoire. Lavoisier. Paris, 1997 ; 860-870.
31. Larpent JP, Larpent- Gourgaud M. Mémento technique de Microbiologie. Lavoisier:paris,1990; 417p.
32. LückeFK .Utilization of microbes to process and preserve meat, *Meat Science.*2000; 56: 105-115.
33. Morishita Y, Shiromizu K. Characterization of lactobacilli isolated from meats and meat products. *International Journal of Food Microbiology.*1986; 3:19_29.
34. Najjari A, Ouzari H, Boudabous A, Zagorec M. Method for reliable isolation of *Lactobacillus sakei* strains originati from Tunisian seafood and meat products. *International Journal of Food Microbiology.*2008; 121: 342-351.
35. Niemand JG, Holzapfel WH. Characteristics of lactobacilli isolated from radurised meat. *International Journal of Food Microbiology.* 1984; 1: 99-110.
36. Olaoye OA, onilude AA. A study on isolation of presumptive technologically important microorganisms from Nigerian beef. *American-Eurasian Journal of Sustainable Agriculture.* 2009; 3: 75-83.
37. Reuter G, Elective and selective media for lactic acid bacteria International, *Journal of Food Microbiology.* 1985; 2: 55-68.
38. Samelis J, Maurogenakis F, Metaxopoulos J. Characterisation of lactic acid bacteria isolated from naturally fermented Greek dry salami. *International Journal of Food Microbiology.*1994; 23:179-196.
39. Schillinger U, Holzapfel WH. Culture media for lactic acid bacteria Chapter 8. In: Corry JEL et al. Handbook of culture media for food Microbiology. 2003; 127-140.
40. Schillinger U, Lücke FK. Antibacterial Activity of *Lactobacillus sakei* isolated from meat. *applied and environmental Microbiology.* 1989; 55:1901_1906.
41. Schillinger U, Lücke FK. Identification of lactobacilli from meat and meat products.*Food Microbiology.4,* 1987, 4: 199-208.
42. Stiles ME, Wilhelm H, Holzapfel B. Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology.* 1997; 36: 1-29.
43. Stiles ME. Potential for biological control of agents of foodborne disease. *Food Research International.*1994; 27:245_250.
44. Vermeiren L, Devlieghere F, Debevere J. Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology.* 2004; 96: 149-164.

© 2014 Global Journals Inc. (US)

Global Journals Inc. (US) Guidelines Handbook 2014

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (FARSS)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards "FARSS" title to individuals. The 'FARSS' title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

- The "FARSS" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSS or William Walldroff, M.S., FARSS.

FARSS accrediting is an honor. It authenticates your research activities. After recognition as FARSB, you can add 'FARSS' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSS designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

Once FARSB title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSS can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSS, you will be given a renowned, secure and free professional email address with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

The FARSS will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSS member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSS, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSS members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSS member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

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

The FARSS is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSS member can decide its price and we can help in making the right decision.

The FARSS member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (MARSS)

The ' MARSS ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The “MARSS” is a dignified ornament which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., MARSS or William Walldroff, M.S., MARSS.

MARSS accrediting is an honor. It authenticates your research activities. After becoming MARSS, you can add 'MARSS' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSS designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSS, you will be given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSS member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSS, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

Auxiliary Memberships

Institutional Fellow of Global Journals Incorporation (USA)-OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone. "

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.

Preferred Author Guidelines

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

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 .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish 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.

The Administration Rules

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

Agalactiae · 16
Aspergillus · 3, 4, 5, 7, 10, 26, 27

C

Chymotrypsinogen. · 26

D

Dulcitol · 46, 50, 52

H

Haemostatic · 15

K

Koraiensis · 19, 22

L

Leuconostoc · 46, 47
Lichenoformis · 24, 27, 34, 35
Limpopo · 3, 12
Longepedunculata · 2, 3

P

Pharonic · 3
Phenotypic · 2, 46
Polygalaceae · 3

R

Raffinose · 46, 50, 52

S

Saponins · 3, 9, 10
Securidaca · 2, 3, 4, 5, 9, 10, 11, 12, 13
Shiromizu · 47, 52, 54
Streptococcus · 16
Streptomycin · 6

save our planet

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

© Global Journals