(An Annual Peer-Reviewed Research Journal of Life-Sciences)

JOURNAL OF BIOSCIENTIA

DEPARTMENT OF LIFE SCIENCES
CHAITANYA (DEEMED TO BE UNIVERSITY)
KISHANPURA, HANAMKONDA, WARANGAL 506 001
TELANGANA STATE INDIA

Vol.I January 2022

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DEPARTMENT OF MICROBIOLOGY
CHAITANYA (DEEMED TO BE UNIVERSITY)
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Executive Summary

JOB

Journal of Bioscientia is an annual peer-reviewed journal which provides a good platform to publish original research articles, case studies, comments, reviews, and books in a nonprofit, innovative and open access way. The journal implements the scientific mode of collection, curation, and projection of the ideas, discoveries, and inventions in an eloquent manner to understand the phenomena of the scientific hypothesis and justification. It becomes an integrated showcase to publish all scientific evidences, research pursuits at one place to disseminate the original research. Its data accumulation process ensures the rigidity and smoothness of the quality of the research.

The scrutiny of the articles in the journal maintains the standards of peer review process. The perfect editorial team and uninterrupted flow of the peer review at various levels in scheduled time prevent the unwanted, irrelevant, redundant contents of the research. The professional editorial board with collective effort makes the journal prestigious.

Background

Viswa Bharati Education Society is a premier group of educational institutions in the area of Higher Education and Technical Education in the Southern India. With the modest beginning, the Education Society has reached new heights. It is committed and dedicated to its vision and mission and constantly evolves itself to the future needs and imparts an education that makes the world a better place to live in. The pillar of its strength is innovative teaching and learning experiences offered by experienced faculty backed with high-quality resources.

Rationale

JB aims to provide a rigidity of publishing the original, peer reviewed contents of the expanding domains of the science. It is primarily aimed to nurture the scientific attitude among the young investigators. It reflects the quality research publications in a way to synthesize quality, knowledge, and sustainable utilization. The editorial committee gives importance to the aims and maintains the academic as well as the research integrity.

Aims and Scope

JB publishes the scientific research investigations, reviews, original research, comments, and case studies. The major themes of the journal are the variety subjects that reflect the fundamentals of the basic phenomena of life sciences. It allows the investigators to explore the existing knowledge in a sustainable way for a better tomorrow.

Areas

1. Agricultural sciences
2. Ayush// Natural products/ Biosimilars
3. Biochemistry/Nanotechnology
4. Bioengineering / Biopolymers
5. Bioinformatics
6. Biophysics/instrumentation engineering
7. Botany/Zoology/ Microbiology
8. Biotechnology/ Microbial biotechnology
9. Environmental sciences/ Earth sciences
10. Forensic sciences
11. Horticulture
12. Medical sciences
13. Pharmaceutical sciences
14. Veterinary sciences

Companies active in this area:

1. Reich India Pharma limited
2. Fortune biotech
3. Ramkey environ engineers
4. Sri biotech

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13. Pharmaceutical sciences
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Dr. Ch. V. PURUSHOTHAM REDDY
Founder & Chancellor
Chaitanya (Deemed to be University)
Kishanpura, Hanamkonda, Warangal

MESSAGE

Over the past three decades Chaitanya Institutions have made steady and phenomenal progress in imparting quality education with several awards and accolades. Our vision is to take Chaitanya (Deemed University) (CDU) to greaterheights with good digital governance and sound academic standards. Eventually, we want to make CDU a Center of Academic Excellence with creativity, productivity and accountability for knowledge society.

All our laboratories have state-of-the-art equipment and facilities. Modernizing and removing obsolete equipment are done from time to time. They meet the requirements of updated practicals designed for present-day demands. Students are trained in recent techniques keeping in view the job requirements in the industriesand other sectors of today's world.

Our University is Research and Innovative driven one. The Management facilitates both the teachers and the students to present their research papers in seminars and symposia and also publication in national and international journals. The faculty members have already been granted three patents and published fourteen patents papers with eight inventions and innovations besides publishing 305 research papers during 2020-2021. Now there are 71 staff members with Ph.D. degrees. They haveattended 2329 Seminars, Webinars and Virtual Conferences.

Patents of the Staff

We are glad to state that our teachers have the following 10 Patents Granted and 43 Patent Publications, making a total of 53 as under:

Patents Granted

1. Prof. M. Ravinder of Chemistry on "Metal Complexes with Anti-Cancer Activity" (USA Patent No. US20100209537A1, 2010)
2. Dr Sreedhar Gundekari of Chemistry on "Process for the preparation of Gamma-valerolactone by catalytic hydrogenation of levulinic acid using Ru-based catalysts" (USA Patent No: US 10221149 B2, March 5, 2019)
3. Dr Sreedhar Gundekari of Chemistry on "An improved fast and selective process for the preparation of □-valerolactone by catalytic hydrogenation of levulinic acid using Ru-based catalysts" (Indian Patent No: IN 337459, June 5, 2020)
4. Dr Sreedhar Gundekari of Chemistry on "An eco-friendly process for hydrogenation of organic molecules using hydrous ruthenium oxide catalyst"(Indian Patent No. 338850, June 26, 2020)
5. Dr Sreedhar Gundekari of Chemistry on "Preparation of value added products by catalytic organic transformations of biomass derived levulinic acid with aromatics" (Indian Patent No: IN 350735, November 6, 2020)
6. Dr Ratansingh Atkar of EEE on "Artificial Intelligence Based Cooling System for Managing the Energy Efficiency" (Australian Patent No. 2021100960, May12, 2021) valid for eight years.
7. Dr Sreedhar Gundekari of Chemistry on "Eco-friendly process for hydrogenation or/and hydrodeoxygenation of organic compound using hydrous ruthenium oxide catalyst" (USA Patent No: US 10954185 B2, March 23, 2021)
8. Dr Banoth Mohan, Dr Mukuloth Srinivasnaik, B. Rajkumar, G. Sagar, A. Harish, U. Raghupathi, S. Raju of Mechanical Engineering, Dr K. Vinay Kumar(Electrical) on "Sliding Particles Solar Receiver" (Australian Patent No: 2021101791, May 26,2021). Valid for 8 years.

Patent Publications

1. Dr Ch. Sathvika of Bio-technology on "Isolation and Characterization of Anticancer Compound from Sesuvium Portulacastrum (L.)L., (Indian Patent No. 201941007336, February 25, 2019).
2. Prof A. Raju of Physics & Electronics on "Experimental Analysis of Natural Composites Materials"

3. Dr Gundekari Sridhar of Chemistry on "A Process for the Preparation of Gama □-valeorlactone by Catalytic Hydrogenation of Levulinic Acid Using Ru-based Catalysts" (USA Patent No. US10221149B2, March 5, 2019)

4. Dr Gundekari Sridhar of Chemistry on "An Echo-Friendly Process for Hydrogenation or/and Hydrodeoxygenation of Organic Compounds Using Hydrous Ruthenium Oxide" (USA Patent No. 0084918A1, March 21, 2019)

5. Dr Gundekari Sridhar of Chemistry on "Preparation of Value-Added Products by Catalytic Organic Transformations of Biomass Derived Levulinic Acid with Aromatics" (Indian Patent No. 201611023585, July 11, 2016)

6. Prof Jagadeesh Kumar Ega of Chemistry on "Design and Implementation of Smart Air Quality Detection System Based on IOT" (Indian Patent No. 202121018506A, May 7, 2021)

7. Prof Jagadeesh Kumar Ega of Chemistry on "Deep Learning Based Detection of Abnormality Scenarios for Computer Vision-Based Har Systems" (Indian Patent No. 202141024137A, June 26, 2021)

8. Prof. Jagadeesh Kumar Ega Professor of Chemistry, under Collaborative Interdisciplinary Research entitled "Machine Learning and IOT Based Smart Wearable System for Autonomous Management of Diabetes Mellitus" (Indian Patent No: 202141030571A, July 16, 2021)

9. Mr B. Rajkumar of Mechanical Dept on "Solar Power Based Robotic Autonomous Vehicle Using Raspberry PI with Live Streaming Using IOT Control" (Indian Patent No: 202041028499, July 4, 2020)

10. Dr T. Anveshand, Dr G. Santosh Reddy of ECE has an Indian Patent on "A Novel Automatic Sleep Staging Features Analysis Using Machine Learning" (Indian Patent No. 202141026196A, June 25, 2021)

11. Prof V. Mallikarjun of Pharmacy on "Synthesis of N-Substituted Benzylidene- 1H-Pyrazolo (3, 4-D) Pyrimidin-4-Amine" (Indian Patent No: 201841032284B, September 7, 2018)

12. Mr Kanjarla Narasimha of Pharmacy on "Ayurvedic Herbal Formulation for Infertility" (Indian Patent No. 202141025016A, June 11, 2021).

13. Mrs M. Aravinda of Commerce and Business Management on "Virus Killer Liquid Herbal Disinfectant (Liquid Powder) for Pandemic Condition" (Indian Patent No. 202041028094, July 31, 2020)

14. Dr. Rajender Boini of EEE on Scalable Multisensor Networking Protocol to Interface Upcoming Internet of Everything (IoE) Devices. (Indian Patent ID: 202141035033 App.No: 202141035033A, Issue No: 33/2021, August 13, 2021)

15. Prof G. Shankar and Ms B. Shoba Rani on A System and Method for Data Encryption between IOT Devices and a Network Gateway (Indian Patent No. 202141030003, July 16, 2021)

16. Prof G. Shankar and Dr Seetharam Khetavath on A System and Method for Analysing Biological Effects of Molecules Using Monte Carlo Method (Indian Patent No. 202141031063, July 16, 2021)

17. Dr Sreedhar Gundekari (with Kannan Srinivasan) on "A process for the preparation of Gamma-valerolactone by catalytic hydrogenation of levulinic acid using Ru-based catalysts" (European Patent No: EP 3204366 A1, August 16, 2017)

18. Dr Sreedhar Gundekari (with Kannan Srinivasan) on "A process for the preparation of Gamma-valerolactone by catalytic hydrogenation of levulinic acid using Ru-based catalysts" (World Patent No: WO 056030 Al, April 14, 2016)

19. Dr Sreedhar Gundekari (with Kannan Srinivasan) on "An eco-friendly process for hydrogenation or/and hydrodeoxygenation of organic compound using hydrous ruthenium oxide catalyst" (World Patent No: WO 060922 Al, April 13, 2017)

20. Ms M. Swapna of ECE on “A System and Method of Image-based Assay Using AI and Machine Learning” (Indian Patent No: 202141035988, August 13, 2021)

All our 291 research scholars of the PhD program are enthusiastic to pursue their research seriously from the date of joining their research program due to our research facilities, fortnightly review meetings. All the 71 supervisors are uploading screenshots of their meetings with details in the CDU Research Group. The research scholars are submitting their monthly progress reports and finally a yearly consolidated report to the Dean, Research and Development. Within ten months of joining the PhD program, our 141 research scholars of the first batch have published 54 Research Papers in refereed journals. The faculty have attended 2329 Seminars, Webinars and Virtual Conferences. We have so far conducted 18 national and international webinars, 45 UKTI sessions and 60 standard quizzes covering all branches.

Best Paper Publication Award

Our University instituted the Best Paper Publication Award (BPPA) to encourage high impact factor papers with original ideas, and the Best Book Publication Award (BPPA) to promote serious and sustained academic work. Four CDU faculty members (Dr Ch Sathvika, Dr T Narasimha Swamy, Prof. B Hari Prasad and Dr G Vamshikrishna) were chosen to receive Chaithanyam-2020 Best Publication Award (with a cash prize and a certificate) last year on 72nd Republic Day. This year Prof M. Sunder Ram (Maths), Dr S. Narasimha (Patent in Chemistry), Dr N. Satish (Chemistry), Dr G. Kumara Swamy (Patent Granted in Pharmacy) and Prof V. Mallikarjun (Pharmacy for Publication). We hope these awards will create healthy competition promoting serious and sustained academic work on the campus.

Faculty Development Program

The Department of Pharmacy organized a five-day National Virtual Faculty Development Program on a current topic titled *"COVID-19 & GLOBAL HEALTH"*. More than 400 academicians, teachers, researchers, and pharmacy students took part in the FDP during July 16-21, 2021.

Workshop

Prof G. Damodar conducted a Workshop in English to the faculty of Pharmacy and Engineering for three days to improve their communication skills in English.

International Conference

We conducted a mega international conference on Disciplinary, Interdisciplinary, and Multidisciplinary Research-2020-2021. (IVC-DIMR 20-21) during October, 14 & 15, 2021 with the thrust areas of Ecology, Culture, Language, Education, Communication, Technology, Innovation, Collaboration (ECLECTIC), fully supported by some Scopus-Index Journals in collaboration with Research India Foundation (RIF), a Government of India approved research foundation in India.

Video Lessons on YouTube

We have so far uploaded about a hundred video lessons by the faculty of CDU on all branches of learning on our YouTube Channel.

Ten MoUs

We have MoUs with

— Four Reputed State Universities of the USA;

— In India, TCS for the Introduction of Academic Interphase Programs, B.Sc. with Cognitive Systems and B.Com. with Business Process Management;

— IBM to offer its popular courses for B.Tech (CSE and ECE), MBA, MCA, BBA and BCA with internationally recognized certifications for global placements. CDU is one among nine universities selected in India.

— Indian Institute of Chemical Technology (IICT) Hyderabad, and Council of Scientific Industrial Research (CSIR) Lab, Government of India to offer nanotechnology courses;

— Hindustan BEC Tech Pvt Ltd, Punjab, Dept of Scientific and Industrial Research (DSIR) recognised R&D Laboratory;

— Board of Apprenticeship Training (SR), Chennai;

— Center of Sustainable Agriculture (CSA), Hyderabad, Tesla Diagnostics, Hyderabad, VCARE Bio Labs, Sri Laxmi Nursing Home and Ajara Health and Medicare Pvt. Ltd.

Research Plans

We have plans to start Atal Chaitanya Incubation and Innovation Centre involving Engineering, Pharmacy, and other departments on thrust areas of farming, app development, manufacturing, etc. We are planning for BIRAC under Bio-Nest in collaboration with University of Hyderabad involving the departments of Bio-technology, Micro-biology, Bio-chemistry, Chemistry, Maths, Computer Science, Engineering Pharmacy, on thrust areas of agriculture, poultry, animal husbandry, food processing and value addition, bio-medical engineering, etc. We were shortlisted for DBT Skill Vigyan Program monitored by TSCOST. We have submitted DBT Builder Program for financial assistance involving all the departments of science. We have also submitted proposals for CRG, Startup Grants and Matrix under SERB-DST. Departments of Engineering, Pharmacy, Computer Science, Commerce and Business Management have submitted Research Project

Scheme (RPS) under AICTE Program. We have successfully conducted second PhD Eligibility Test and admissions are in process.

Our Inventions and Innovations

Our Mechanical Engineering faculty, Dr Srinivas Naik, Mr Harish and Mr Santhosh, have converted a petrol engine car into a battery operated eco-friendly electric car without gears. A motor of 48 volts, 700 watts and 500 rpm has been replaced with an engine of Maruti 800 car. The weight of the engine was reduced by removing the gearboxes. The motor is connected to the drive shaft by a chain. The vehicle runs at a speed of 60 kmph. The car is economical and it costs just 20 paise per km. The conversion of the car costs two lakh rupees. Coming to other innovations, a unique Chaitanya App was designed by Prof. G. Shankar Lingam and Mr. K. Praveen in 2020 for attendance, results, timetables and general information. Our mechanical branch staff has designed a solar bike and agri-cultivators. A cost-effective electronic bike has been designed by Mr G. Sagar. A digital electronic clock was designed by Dr Santhosh Reddy of ECE. The wing of ECE has also designed a Digital Display Board and devised Easy Vehicle Lifting Mechanism. The process of making transparent mementoes with a special material is in the pipeline.

Chaitanya At-Home-Library

Students have access to massive open online courses in MP3 format based on MHRD model MOOCS, Commonwealth Education Services, cec.nic.in for all lessons. As students do not have access to the physical library due to Pandemic, a Digital Library called *"Chaitanya At-Home-Library"* was launched. It is a new initiative with all prescribed e-books made available with the efforts of the Faculty on our University Website and on Chaitanya App. We have procured URKUND Anti-Plagiarism Check Software from UGC.

Way Forward

We are committed and dedicated to our vision and mission and constantly evolve ourselves to the future needs and impart education that makes the world a better place to live in. Our syllabus is skill-based and industry focussed with contemporary curriculum, choice-based credit system (CBCS) and continuous assessment and grading pattern (CAGP). We always remember our core vision of empowering our future generations to be morally, ethically and intellectually strong with LOCF and following some provisions of National Education Policy 2020.

Social outreach programs, eco-friendly environment, diversified student community, education scholarships for deserving and meritorious students, internal quality assurance, enriching projects and internships, corporate linkages, global alumni network, learning management system, highly accomplished faculty members and levitating research culture are some of our salient features.

I am glad to know that the Department of Microbiology under the scholarly guidance of Prof B. S. Anuradha, Prof. S. Jeevan Chandra, Editor, is bringing out this maiden issue of Journal of Bioscientia. I compliment him and the members of the Advisory Board for their guidance, members of the Editorial Board for their review, and the contributors for their contributions.

Editorial

Life Sciences is a multi-disciplinary subject, requires a systematic approach to query the survivability of life and dynamics of struggle. It do not allow swindles of interrogation, it requires a concrete evidence to envisage the underlying forces. Hitherto, our knowledge is very limited to comprehend the natural life and its fullest possibility. In a pinch of soil, a million bacteria, fungi, and viruses are there that articulates the enormous volumes of species richness. Our technology and knowledge can provides the chance to realize only 1% of the diversity itself, but 99 percent of the diversity still unexplored. To harness the gap, it strongly necessitated the invention of various methods to understand the microbial interactions. Since last 40 years, there is high priority in invention of most effective, reliable, fast, accurate methodology, and tools. Conventional methods are replaced with molecular technologies, which could deal with the signature molecules of life i.e DNA, RNA proteins and metabolites. However, capacity-building programmers equipped with conceptualization, understanding, training, handing, and analysis and reporting are the main. A scientific approach made to conduct, segregate, polish, and project the recent advances in the life sciences field in the form of a scientific journal.

The first paper “Current trends in designing of microbial enzymes; protein engineering, directed evolution and metagenomics approaches” by by S Jeevan Chandra and his scholars is a persistent version of the main concepts of molecular evolution. This paper ventures many advances in computational designing of enzymes. In view of the author, present knowledge about enzymes is not sufficient to realize the molecular architecture and evolution of peptides into the most splendid enzymes. “The miniature, subcellular biocatalysts, chemically proteins, but they behave strategically and meticulously”. Further, we must be update the knowledge on how to utilize the recent advances of directed evolution and metagenomics in understanding, discovery and synthesis of the novel artificial peptides, enzymes with added advantage, that can cater the present and future needs of the society. At the same time, academia and industrial sector at various levels should implement the capacity building and training to scientific community of life sciences is obligatory to achieve the sustainable goals of enzyme research for better tomorrow.

Vaddem Kiran Kumar from Central Pollution Control Board, Regional Directorate, Kolkata, conveys the uncultivable diversity conquers in the Ganga and its tributaries ecosystem. Metagenome analysis of various bacteria found in river Ganga and its tributaries - an integrative study. This review provides insights into how river Ganges is polluted. Ganges is the one of the holy and longest river in the world. Ganga is originates at Uttarakhand and travels to West Bengal before voiding into Bay of Bengal. The mighty Gangetic basin a great fertile basins and provides livelihood for millions of people, who dwell in on its outer banks. However, due to waste water contamination, there is high incidence of metal resistant and antibiotic resistant bacteria through water chain would eventually cause human infections which are untreatable. The present review aims at summarizing the various studies for identifying various contaminating bacteria with metagenomic methods. This would help in identifying the pollution hotspots and help in controlling the pollution of the river.

R. Kishore’s paper “A rare case of klinefelter's syndrome with ISO X chromosome’ this article projects the classical as well as the modern FISH-Flurecent Insitu Hydridization with Cytovision. It explanations the

rarity of Klinefelter syndrome disease and the phenotype of subjects associated with isochromosome Xq. Klinefelter syndrome is important to provide meaningful genetic counseling. However, due to only further evaluation with a larger group of such patients would be able to determine more clearly the prevalence and clinical features and appropriate genetic counseling required.

Shikha Kapil and his scholars from University Institute of Biotechnology, Chandigarh University "Isolation of Bio-surfactant Exhibiting Bacteria from the Northern Regions of India and Their Phylogenetic Relationships" this paper reveals the effective methodologies to elucidate the Phylogenetic Relationships of novel Bio-surfactant producing bacteria.

Lavaynya and Pogu Srinivas in their article "Chemical analysis of renal stones in Telangana population" provides a greater insights into the renal crystals and its chemical composition. The article also throws light on gender wise discrepancies among the population.

Anuradha's "Isolation of Keratonophillic fungi and related dermatophytes from the soil" explains the associaton of Keratonophillic fungi prevailing in the soil, they also explained the methdology to isolate and investigate. .

Raja Abhilash's article explains various development and validation of new rp-hplc method for the quantitative estimation of secnidazole in tablet dosage form, A novel, rapid, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative estimation of Secnidazole in bulk and tablet dosage form. The developed HPLC. method and validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

Shilpaveni and her colleagues enlighten the concept of drought resistance, they hythesized the "Role of rhizobacteria in induced drought resistance in crop plants". Root associated microbes help to stabilize the phytobiont drought. At the same time plants regulate the composition of signature bacterial community. These microbes provide the carbon, nitrogen compounds, and other metabolites required to harness the drought.

-- Editor

CONTENTS

Contents lists available at www. chaitanya.edu.in/job.

JOURNAL OF BIOSCIENTIA

Journal homepage: www. chaitanya.edu.in/job.

Current trends in designing of microbial enzymes; protein engineering, directed evolution and metagenomics approaches

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ARTICLE INFO

Key words
Microbial enzymology, bioinformatics tools, protein engineering, directed evolution and metagenomics

ABSTRACT

Enzymes are the proteins in bimolecular features that can catalyze various reactions as like chemical catalysts perform. Global demand and supply chain expected to cross 13.79 billion USD, by 2026. Plant and animal-based enzymes do not prefer to explore due to biodiversity loss. Hence, an alternative bio resource, like microbial enzymes gains the reputation for their sustainable availability. Several advances in molecular approaches, bioinformatics, computational tools, protein engineering, directed evolution, and metagenomics have become mandatory to screen and design various novel extremozymes. In the current review, an attempt was made to present the recent advances in microbial enzymology and future prospects in a comprehensive way.

Introduction

The word **'Enzyme'** that signifies the genesis of an idea of Wilhelm Kühne who coined the term. He just privileged unique observations made by senior researchers. The true literal meaning is "inside the yeast" i.e En = leaven, zyma = yeast, that nothing but, the yeast responsible for leavening of bread. Later, the vernacular name with suffix "ase" was popularized to denote the enzyme. The term enzyme was synonymous to ascertain the pragmatic relationship of the molecular phenomenon and biocatalytic efficiency. Enzymes are biochemically protein biomolecules which execute a variety of biochemical reactions in various systems of biology. Hence, they regarded as biocatalysts, which can perform biochemical reactions in a specific and selective approach, with a fine tuned velocity with high turnover number of product delivery under defined conditions. [1, 2, 3, 4, 5].

Hitherto 5000 different reaction types of enzymes have been discovered and some of them are engineered [6, 7]. All the civilizations have been utilized these enzymes knowingly or unknowingly ranging from household to sophisticated industrial applications for welfare of the human being [8]. There is an increased demand for the enzymes by all sectors and by the year 2026 expected to cross 13.79 billion USD [9]. Hence, there is an urgent need to create high skilled human resource personnels and rationalized good laboratory practices for sustainable development and welfare of the enzyme sector.

Biodiversity of enzymes:

Enzymes are in biological nature they found in all most all domains of life including viruses to humans [10]. They span from a single poly peptide to multiple protein domains, simple to complex enzymes [11].

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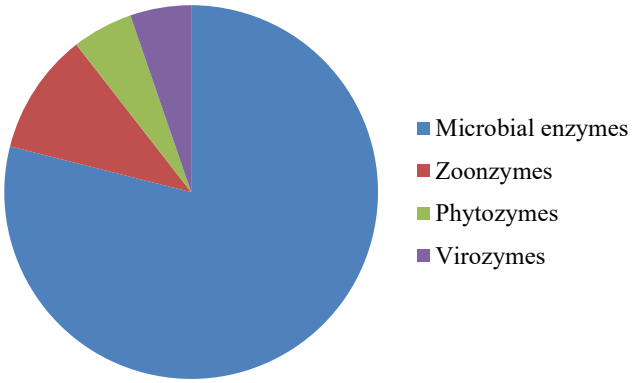

Figure: 1. Utilization share percentage of enzymes by industrial sector

Microbial enzymes in utilization, more than 80% in usage by microbial enzymes followed by animal zoozymes, phytozymes and least by virozymes. The enzymes present in viruses are termed as virozymes, archaeozymes from archea, bacteriozymes from bacteria, mycozymes from fungi, phycozymes from algae, phytozymes from plants and zoozymes from animals and extremozymes from extremophiles of all domains of life.

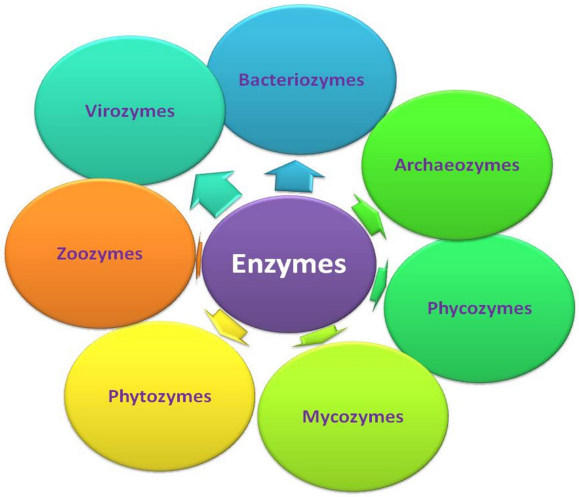

Figure 2: Biodiversity of enzymes

The current demand and supply of enzymes is not saturated and it needs sustainable resources for constant providing, where the plant and animal origin is unsuitable. Because of the consumption of huge quantities of economy and human resources input, land utilization and natural resources. An ever increased demand for microbial enzymes was witnessed. Hence, microbial enzymes grasped the importance over the other [12].

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Table 1: Enzymes and their industrial applications

ENZYMES	INDUSTRIAL APPLICATIONS
Glucoamylase, Protease, Xylanase, α-amylases	Household care, textiles, food and beverages, oil and fats, baking
Amylase, Phytase, Protease, Xylanase, α-galactosidase β-mannanase	Food and beverages, Pharma, Animal feed, Textiles, Detergent, Biofuel, etc.
Amylase, Amyloglucosidase, Catalases,Pectinase, Protease, Xylanase	Paper, Grain processing, Beverages, Textiles, Baking, Animal feed, Pharmaceuticals, etc.
Amidase, Lipase, Pectinase, Penicillin	Textile, Pharma, Food
Lysozyme, Papain,	Beverages, Feed, Detergent, etc.
Amylase, Cellulase, Lipase, Nattokinase, Phytase, Protease,	Textile, Food, Nutraceuticals, Animal feed, etc.
Amylases, Phytase, Catalase, Lipase, Glucoamylase, Pectinase, Proteases, Cellulases and Xylanase.	Textile, leather, feed, etc.
Amylase, Catalase, Cellulase, Laccase, Mannanase, Pectinases, Protease.	Textile, pharma, Food and Beverages, feed, detergent, chemical, et
Alkaline Pectinase, Amylase, Amylases, Catalase, Cellulase, Cellulases, Laccase, lipase, Protease, Proteases, Xylanase, Xylanase and β-glucanase.	Textiles, Food and Beverages, feed, etc.

Table 2: Enzyme market rankings based on CAGR from 2019 to 2025	
Continental ranking	1. North America 2. Europe 3. Asia Pacific, 4. Central & South America 5. Middle East & Africa
Country ranking	1. U.S. 2. Canada 3. Mexico 4. U.K. 5. Germany 6. France 7. Spain 8. Italy 9. China 10. Japan 11. India 12. South Korea 13. Indonesia 14. Australia 15. Brazil 16. Argentina 17. Saudi Arabia 18. Turkey

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Table-3: Microorganisms and their enzymes employed in various industrial applications

Name of the Microorganisms	Enzyme	Application purpose
Bacillus subtilis, A. oryzae	Neutral proteinase	Cheese ripening, debittering
Aspergillus niger, A. oryzae	Lipase	Cheese ripening, flavoring
Escherichia coli, Kluyveromyces sp.	Lactase (b- galactosidase)	Lactose reduction in whey and milk products
Lactobacillus sp.	Aminopeptidase	cheese ripening
Aspergillus niger	catalase	Cheese processing
Streptomyces sp.	Transglutaminase	Cross linking of Protein
Bacillus stearothermophilus	Maltogenic α-Amylase	breads shelf life enchancement
Aspergillus niger	Xylanase	conditioning
Aspergillus niger	Lipase	stability and conditioning of Dough
Aspergillus niger, Penicillium chrysogenum	Glucose oxidase	Strengthening of Dough
Streptoverticillium sp., streptomyces sp.	Transglutaminase	dough strength and Lamination
Aspergillus niger	Glucose oxidase	Oxygen removal from beer
Aspergillus niger, Trichoderma atroviride	Cellulase	Fruit juice clarification and liquefaction
Bacillus, Aspergillus	a-Amylase	Starch hydrolysis
Bacillus, Streptomyces, Rhizopus	b-Amylase	Starch hydrolysis
Bacillus subtilis, Aspergillus spp.	b-Glucanase	Inhibition of mist formation
Aspergillus niger	protease	Inhibition of mist formation
Bacillus sp., Klebsiella sp.	Pullulanase	Saccharification of Starch
Aspergillus niger	Naringinase	Debittering
Aspergillus niger, A. oryzae	limoninase	Debittering
Lactobacillus brevis, L. plantarum	Aminopeptidases	Protein degradation during mashing
Aspergillus sp., Bacillus sp.	Xylanase	Starch digestibility
Aspergillus niger	b-glucanase	Digestive tonic
Bacillus subtilis	Protease	Biofilm removing and drainage clearing
Bacillus licheniformis	Amylase	Deinking and drainage clearing
Trichoderma reesei, Thermomyces lanuginosus, Aureobasidium pullulans	Xylanase	Paper Bleach boosting Paper Bleach boosting
Bacillus subtilis	Laccase	Non-chlorine bleaching, delignification
Bacillus sp., Aspergillus niger	Cellulase	Deinking, drainage clearing
Trametes hirsuta	Laccase	bisphenol A Polymerization
Aspergillus niger, Penicillium chrysogenum	Glucose oxidase	anilines Polymerization
Streptomyces mobaraensis	Transglutaminase	Crosslinking of protein
Trichoderma reesei	Tyrosinase	lignin and chitosan Polymerization
Aspergillus oryzae, A. flavus,	Lipase	Oil stain elimination
Aspergillus oryzae, Bacillus subtilis	Protease	Protein stain removal
Aspergillus niger, Bacillus sp.	Cellulase	Color removal
Fusarium solani f. pisi	Cutinase	Oil, Triglyceride removal
Bacillus sp.	Mannanase	Mannan ans soil spot removal
Aspergillus niger	Phytase	Hydrolyze phytic acid to release phosphorous, soil spot removal
Aspergillus sp., Bacillus sp.	Amylase	Flour adjustment, bread softness
Aspergillus oryzae, Penicillium funiculosum	Pectinase	Removal of pectin
Aspergillus sp.	Acid proteinase	Milk coagulation and curdling
Aspergillus sp., Bacillus subtilis	Amylase	Carbohydrate stain removal
Candida Antarctica	Lipase	Polycondensation, ring-opening polymerization of
Candida Antarctica	Lipase	Pitch control

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Reasons to consider Microbes as Sustainable Resources of Enzymes:
Microbes are available in nature hence the microbial systems ranging from viruses, bacteria, archaea fungi, algae and protozoa are contain a range of enzyme encoding genes [13]. Hence, enzymes play a significant role in species evolution [14]. Microbial systems can be cultivated in a natural and artificial laboratory scale ranging from open fermentation to sophisticated industrial fermentation [15, 16]. Hence, the microbial resources of enzymes are economically feasible with sustenance of availability throughout the year [17, 10].

Location of the enzyme:
Enzymes are ubiquitous but they available in two locations in biological systems either extracellular location or intracellular location [18].

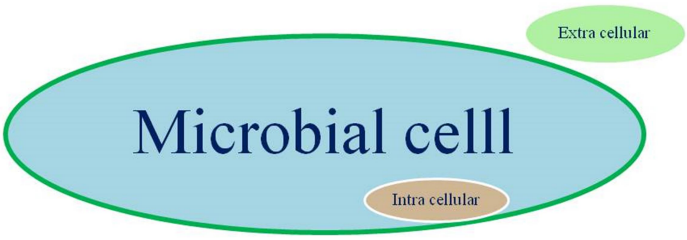

Figure 3: Enzymes locations in microbial cells.

Most of the enzymes said to be constitutional or inducible in manner, expressible and repressible status, secretable and non secretable status in a given biological circumstance [19]. Even these enzymes are found to be inactive or active form later they might be activated into zymogenic form [20]. However, all the enzymes happened to be proenzyme or active enzyme conformation with greater degree of biological specificity and selectivity [21].

Screening of enzymes:
Screening for microbial enzymes requires an adequate knowledge regarding the occurrence and easy techniques available for laboratory demonstration [22]. Sometimes, it necessitates an innovative principles and hypothesis. Most of the screening tests are encompass the utilization of specific substrates to isolate the microorganisms that could produce the enzyme. Some enzymes require a range of sophisticated tests like immunofluorescence, flow cytometry and computer aided biosensor [23,24].

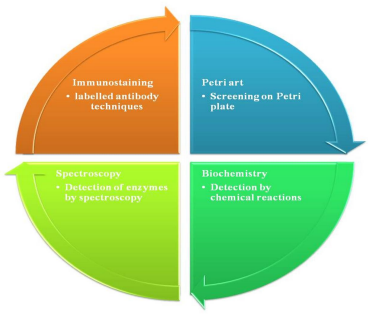

Figure 4: Enzyme detection approach consists of conventional Petri art (zone of clearance) , enzyme detection by chemical species, spectroscopy and fluorescence dye labeled immunoglobulin based techniques for detection disable enzyme

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In general, the substrate degradation followed by detection of product chemical species in a cheapest way of biophysical approach. Assay of extracellular microbial enzymes made easy by incorporating a specific substrate and chromogenics to the zone of clearance around

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the microbial colonies [25]. Several enzyme detection tests relies on biochemical changes that they execute. The key component of the enzyme reaction can easily detectable by the spectroscopy methods [26].

Purification of enzymes:

Purification of enzymes is a state of art, the purification strategies often requires a meticulous plan [27, 28]. These methodologies are ranging from simple filtration to sophisticated chromatographic techniques [29]. These purification techniques broadly divided into physical, chemical and molecular techniques. Purification of enzymes into their native form confers the catalytic efficiency and increases the specific activity several folds as compared to the crude [30].

Characterization:

Characterization of enzymes is a broad area of interest to elucidate the efficiency and kinetics [31]. The purified fraction was checked for its molecular weight and allowed to assess the reaction kinetic parameters like Vmax, Km K cat, and turn over number [32]. At the same time the effect of individual concentrations of substrate, enzyme, activator, inhibitor and influence of pH, temperatures were determined to know the competence of the enzyme [32].

Reaction kinetics:

Kinetic activities of enzyme explain the abilities of enzyme to fit into the specific industrial or applied aspects can be easily inquired by conduction of simple reactions in test tubes followed by ne variable at a time [33]. Then, the data was feed into computer software to determine the accurate kinetic constants [34,35].

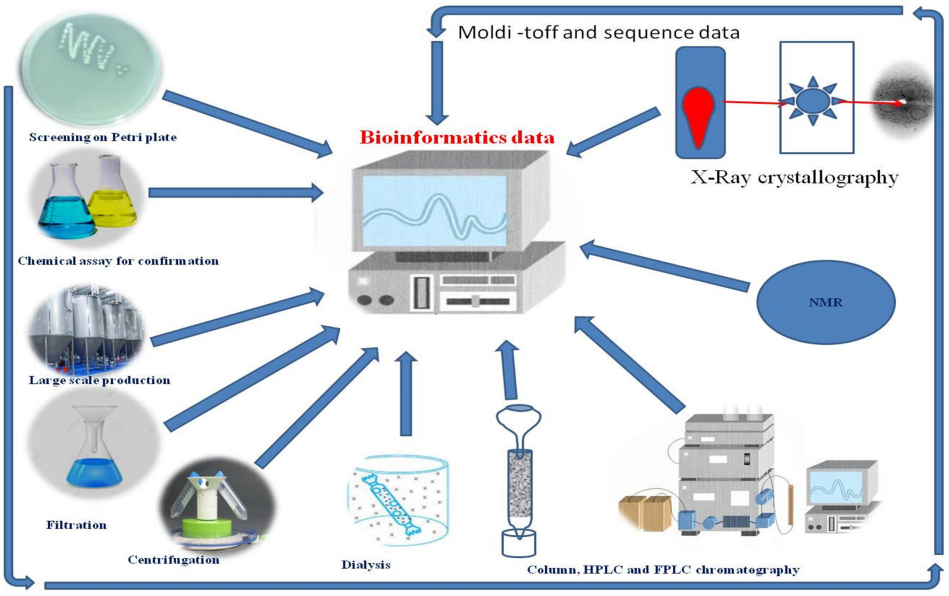

Figure 5: An integrated information deposition approach consists of various leves of biophysical techniques and computer based information for Enzyme data bases in bioinformatics.

Modification of the enzyme active site

Enzyme modifications specific sites other than active site often required to nullify the autolysis and extension of the stability and enhanced enzyme activity also the central theme [36]. Nevertheless, all types of chemical modifications are impedes the enzyme activity or active site at least partially [37].

Discovery of extremozymes:

In the past enzyme research relied on the conventional techniques ranging from petriart,

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mutagenesis to protein sequencing [38]. Recently, the discovery of computational and bioinfrmatics tools like Abinitio, Protein threading and Hidden Markowney Model, have been popularized for discovery of enzymes with enhanced extreme tolerance [39, 40, 41]. Site directed mutagenesis through crisper cas9 is also an eminent approach to create the desirable character and novel extremozymes [42]. Protein engineering and protein domain editing and enzyme morphing are the recent techniques used to create the desirable enzyme for desirable character [43, 44].

Directed mutagenesis

Computer based generation of the random protein sequences from genetic code is highly beneficial to ascertain the happening of novel enzymes with possible desirable characters [45]. But it requires a range of sophisticated biophysical techniques and computer based model simulations [46].

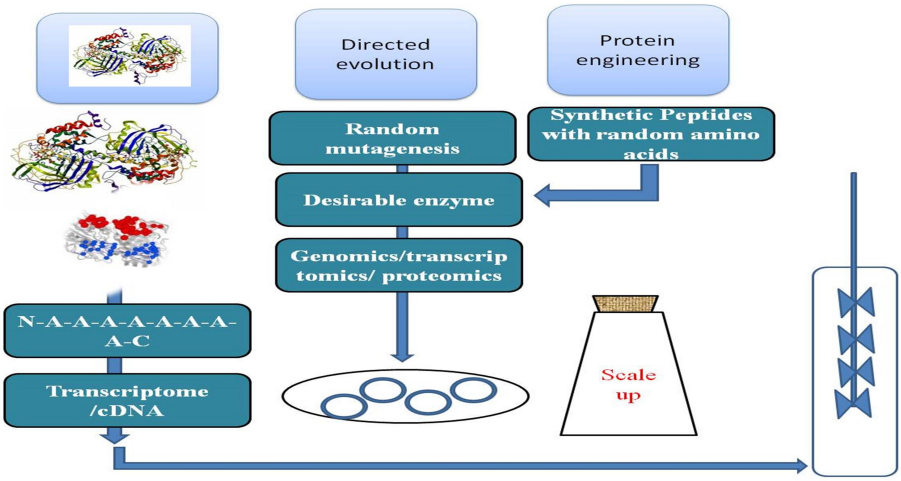

Figure 6: An integrated Directed mutagenesis approach consists of sophisticated biophysical techniques and computer based protein engineering model simulations for disable character.

Metagenomics:

Recently, the metagenomic approaches became an essential strategy to discover unusual enzymes which would not possible to isolate by conventional techniques [47]. Metagenomics has revolutionized the entire life science branches to discover unusual enzymes from insect intestine to rumen of the animals and from various environmental samples [48, 49, 50, 51].

Metagenomics also render the investigators to expression of enzyme genes in heterologus host from uncultivable diversity [52, 53, 54]. Hence, the occurrence of unculturable diversity present in biosphere and exobiological samples made easy by the metagenomics approach and often it is envisaged as shortcut road map and indispensible tool to discover novel enzymes [55].

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Table 4. Extremozymes targeted by metagenomic approaches

Organism Name	Enzyme	Specific activity U/mg of protein	V_{max} at		Kinetic Stability ($T_{1/2}$)
			Temp	pH	
***Acidothermus cellulolyticus* 11B**	Endoxylanase	350	90 ºC	6.0	90 ºC/90 min
Archaeal enrichment	Endoglucanase	4	109 ºC	6.8	100 ºC/4.5 hours
Bacillus thuringiensis* subsp. *kurstaki	Chitinase	4.7	110 ºC	9.0	Not detected
Coprothermobacter proteolyticus	Protease	4	85 ºC	9.5	Not detected
Dictyoglomus thermophilum	Endoglucanase	5	60-85 ºC	5	70 ºC/336 hours
***Geobacillus toebii* E134**	α-Glucosidase	5	70 ºC	6.8	Not detected
Halorubrum xinjiangense	α-Amylase	487	70 ºC	8.5	80ºC/60 min
Hydrothermal spring metagenome	β-Glucosidase	3195	90 ºC	6.5	90 ºC,>90 min
Metagenomic enrichment culture	Lipase	12	70 ºC	8.0	90 ºC, >4 hours
***Salimicrobium halophilum* LY20**	β-Amylase	573	70 ºC	10.0	70 ºC, >24 hours
Sulfolobus tokodaii	Chitinase	0.08	70 ºC	6.0	Not detected
Thermoanaerobacter thermohydrosulfuricus	Lipase	12	75 ºC	8.0	90 ºC, >50 min
***Thermococcus kodakarensis* KOD1**	Pullulanase/amylase	118	100 ºC	5.6-6.0	Not detected
Thermococcus sp	α-Amylase	1000	80 ºC	7.0	70 ºC >3 hours
Thermotoga neapolitana	Pullulanase	25	80 ºC	5.0–7.0	80 ºC/ 88 min
Thermotoga petrophila	Endoxylanase	2600	95 ºC	6.0	96 ºC/55 min
Thermotoga thermarum	Endoxylanase	146	95 ºC	7.0	90 ºC/60 min
***Thermotoga thermarum* DSM 5069T**	β-Glucosidase	142	90 ºC	4.8	90 ºC/2 hours

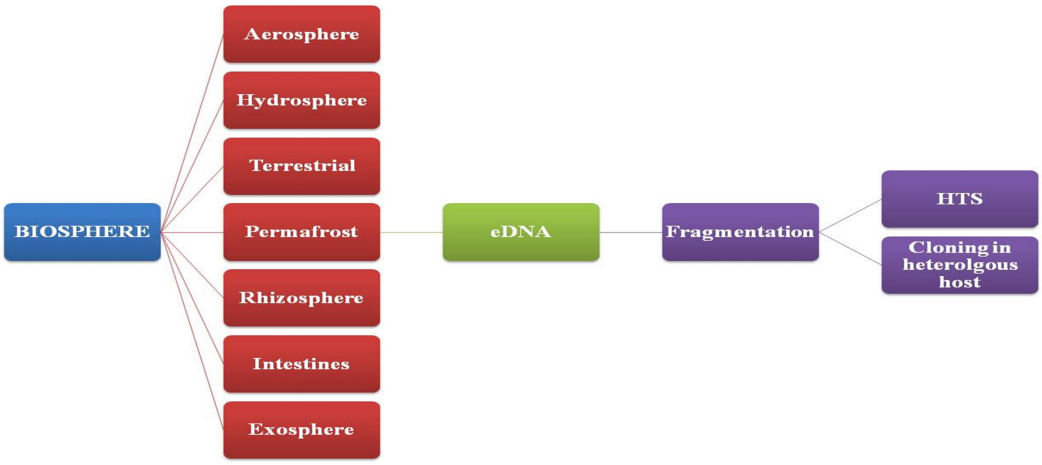

Figure 7: Biodiversity of eDNA for isolation, detection and expression in heterologus host in Metagenomic approaches

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Table 5: Essential tools and web links available for successful metagenomic approaches

Metagenomic tools	*Recommended Annotation pipelines*	*Web links available*
CAMERA	FragGeneScan, MetaGene, COG, Pfam, TIGRfam, GO, KEGG	http://camera.calit2.net/
CoMet	Pfam, GO	http://comet.gobics.de/
EBI Metagenomics	RDP, Greengenes database, InterPro protein signature database	https://www.ebi.ac.uk/ metagenomics/
IMG/M	COG, KOG, KEGG, KO, Pfam, TIGRfam, TIGR, MetaCyc, GO	http:// img.jgi.doe.gov/m
MetaABC	Database of reference genomes (NCBI)	http://metaabc.ii s.sinica.edu.tw/
METAGENassist	BacMap, GOLD, NCBI Taxonomy, PubMed	http://www.metagenassist.com/METAGENassist/
metaMicrobesOnline	TIGRfam, COG, Pfam	http://meta.microbesonline.org/
METAREP	GO, NCBI Taxonomy	http://jcvi.org/metarep/
METAVIR	Pfam, RefSeq virus database	http://metavir-meb. univ-bpclermont.fr/
MG-RAST	KO, NOG, eggNOG, M5RNA, KEGG, PATRIC, RefSeq SEED subsystem, COG, SwissProt, GenBank,TrEMBL, SEED,	https://metagenomics.anl.gov/
MyTaxa	Database of reference genes and genomes (NCBI)	http://enveomics.ce.gatech.edu/mytaxa/
VIROME	SEED, ACLAME, COG, GO, KEGG, MGOL, UniRef 100	http://virome.dbi. udel.edu/

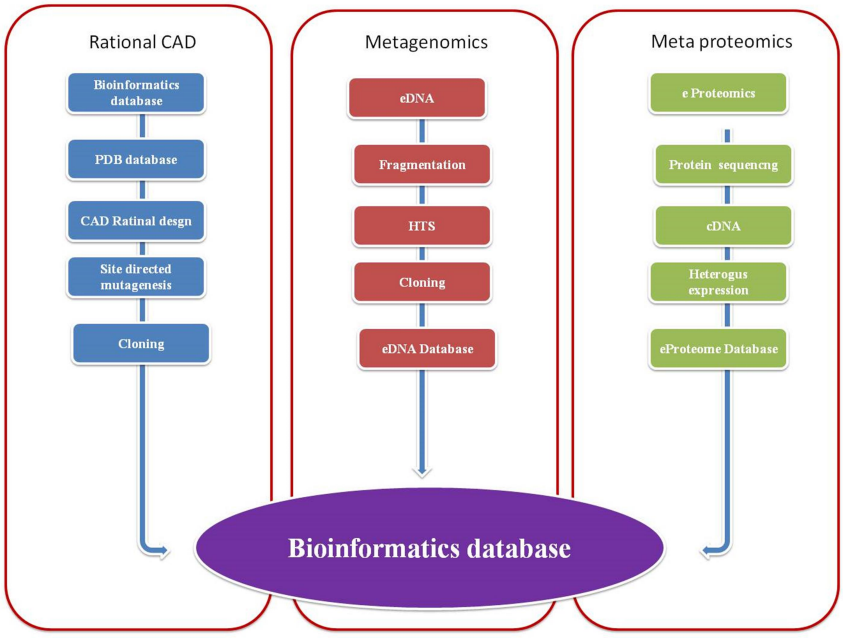

Figure 8: Integrated approaches for designing a novel extremozymes

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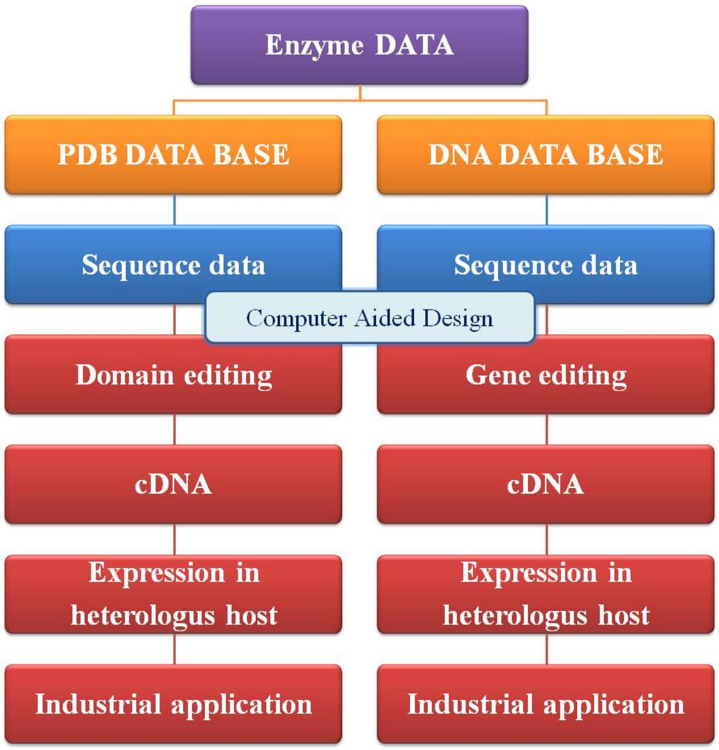

Figure 9: Flow chart for designing a novel extremozymes in Computer Aided Designing CAD

Table 6: Genes discovered through metagenomic approaches with high biotechnological potential

Functional gene target	eDNA source	Size	Screening method	Optimized hits found	Biotechnological relevance
Extreme tolerance genes					
Acid resistance genes	Plankton and rhizosphere from Tinto River	-2.3 Gb	Function based	15	Genes involved in acid resistance
Arsenic resistance genes	Head water from Tinto River	151Mb	Function based	18	Genes involved in arsenic
Nickel resistance genes	Rhizosphere andTinto River	2.15 Gb	Function based	13	Genes related to nickel resistance
Salt resistance genes	Brine and rhizosphere and saltern	2.15 Gb	function-based	11	Genes conferring salt resistance
Regulatory sequences					
Constitutive promoters	Soil from Atlantic Forest	-500 Mb	Function based	33	Use as "biobricks
Dioxygenase-degradincluster	Forest soil	260-815 bp	Sequencing based	11	Pathways/systems/operons (PAA) herbicides avoiding groundwater contamination
Naphthalene-degrading system	Naphthalene contaminated groundwater	~283Mb	Sequencing based	3	Pollutant-degrading enzyme
NRPS biosynthetic pathway	Tunicate consortium	-280 Mb	Sequencing based	1	ET-743 biosynthetic pathway;anticancer molecule
Bioactive molecules					
Pigmentation producing and antibacterial activity	Soil	Not found	Function based	45	Potential new molecules to be used as antibiotics
Turbomycin A and B	Soil	1Gb	Function based	3	Antibiotic activity
Antimicrobial small molecules		~720Mb	Function based	4	Antibiotic activity

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Conclusion

Rationale conventional enzyme isolation, characterization sequencing and expression and processing and purification is a complex activity which requires huge economical input and a time consuming process. Hence, biobusiness industries relies on extraordinary enzyme molecules that suites the current demand and future prospects are always in high demand

Present knowledge about enzyme technology must be updated to utilize the directed evolution and metagenomics in large scale to discover the novel enzymes that can cater the needs. At the same time capacity building of academia to industrial sector at various levels is necessitated for sustainable enzyme resource

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Metagenome analysis of various bacteria found in river Ganga and its tributaries - an integrative study

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Keywords

Ganga, antibiotic resistant bacteria, metal resistant bacteria, anthropogenic sources, Yamuna

ABSTRACT

Ganga is the one of the longest river in the world. It originates in Uttarakhand and travels from Uttarakhand to West Bengal before emptying into Bay of Bengal. The Gangetic basin is one of the fertile basins and millions of people reside on its outer banks. However due to the enhanced population and release of untreated wastewater, effluents from industry and pharmaceutical companies and agricultural waste the river has significant levels of pollution. Due to the release of sewage there is enhanced levels of pathogenic bacteria such as *Bacillus anthraces, Pseudomonas aeruginosa* and *Escherichia coli* in these waters. Apart from the pathogenic bacteria there is high incidence of metal resistant and antibiotic resistant bacteria seen in both surface water and in sediments of Ganga. The New Delhi beta lactam metallo resistance (NDM) gene containing bacteria are a new class of superbug which are extremely dangerous and have been observed in tributaries of Ganga in Delhi region. The presence of such antibiotic resistant bacteria and their transmission through water chain would eventually result in human infections which are untreatable. Metagenomic analysis is a fast and reliable method for large scale identification of various bacterium present in given sample. The present review aims at summarizing the various studies done utilising Ganga for identifying resident bacteria and various contaminating bacteria with special emphasis on metagenomic methods. This would help in identifying the pollution hotspots and help in controlling the pollution of the river.

Introduction

River Ganga is one of the major rivers operating in the Indian sub-continent. It is one of the oldest rivers with great cultural, economic, spiritual and social significance. It originates in the Himalayas and flows a distance of 2525km before it drains into Bay of Bengal. Ganga river basin is one of the largest in the world with around 400 million people depending on this river for multiple purposes. The river originates in the Gangotri glacier near Gomukh of Uttarkashi which is situated in the Uttarakhand state. The Ganga basin though flows mostly through Indian sub-continent tributaries of it are also associated with Bangladesh, Nepal and China (Kumar *et al.,* 2017). Due to the vast area covered by this river there is a wide variety of flora and fauna associated with the river at any given point of time. The Ganga river basin accounts for 26% of total land mass, 40% of Indian population and 30% of total water resource of the country (Location, National mission for clean Ganga). In the state of Uttarakhand Bhagirathi river originates from Gangotri glacier. The river unites with Alaknanda to form Ganga river which flows through Uttarkashi. The river flows uninterrupted on the plains of Uttarakhand, Uttar Pradesh and Bihar as Ganga. While approaching West Bengal the river

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bifurcates into two. One of its tributaries Padma enters Bangladesh whereas the other tributary enters as Hooghly river in West Bengal. The Hooghly river empties into Bay of Bengal. In its course of flow the river forms multiple estuaries and huge delta region which comprises of a highly fragile ecosystem which supports a large life line (Bonnaile *et al.,* 2019, Lodrick *et al.,* 2011). Water from Ganga river and its tributaries feed a large number of plants and animals identified in biodiversity hotspot regions. It is estimated that 10,000 plants species, 300 types of mammals and 1000 types of birds are fed by Ganga and its tributaries (Conservation international, 2008). The river has a large number of tributaries which are important source of feed. The Yamuna river is the major right bank tributary of river Ganga. It flows through the National capital regions and in Uttar Pradesh before meeting other rivers in Allahabad. The left bank tributaries of Ganga include Ram Ganga, Gomati, Ghagra, Gandak and Koshi river (Alam *et al.,* 2020). Ganga basin has average population density of 520 persons per square kilometre. In highly fertile basin regions of Ganga the density is as high as 900 per square kilometre. A large number of activities such as agriculture, fisheries, navigation, transport and religious activities are associated with the river. (Demography of Ganga basin, 2011).

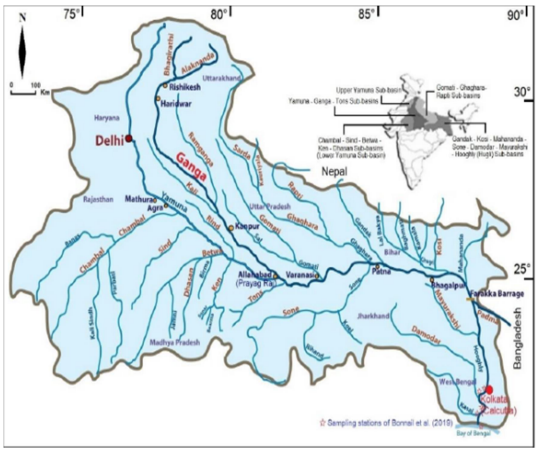

Fig 1: Major tributaries of River Ganga depicted withing Map of India (Adapted from Alam *et al.,* 2020)

The major concern with Ganga is its pollution arising from both human activities and by various industries. As it flows through major cities and towns there are large number of industries that release toxins and effluents in to water. Anthropogenic causes such as sewage release and throwing of dead bodies into river results in highly contaminated water bodies. Associated with such activities the release of pharmaceutical waste from industries and hospitals also has become major concern of late. Owing to the cultural and social importance of river the Government of India has set "Mission clean Ganga" in 2009. The objective is clean the river by 2020 and prevent industrial and sewage effluent mixing with Ganges river (National mission for clean Ganga). The present review is aimed at understanding the nature of bacteria that are observed in polluted waters of Ganga and its tributaries. This would help in identifying the nature and extent of pollution and thus would help in suggesting appropriate methods to control it.

Approach to review

In the present review an in depth understanding of the various contributing factors associated with pollution of the Ganga river are presented. The various aspects associated with identification of the contributing factors for pollution are described. A special emphasis is given on the advanced technologies for detection which includes the metagenomic methods for identification of pollutants depending on the nature of micro organisms that flourish in such polluted waters

The mass utilisation of water for various purposes has significant impact on ecology of the river. Ganges flows through a large number of cities and hence the pollution is amplified due to discharge at multiple points across several cities. One such example is amplified by pollution of the river in Varanasi. In Varanasi alone around 200 million litres of human sewage are discharge into the river. This increases the coliform count of river by a huge magnitude. Coliform counts of upto 10^8/ml are observed in Ganga waters in and around Varanasi. The most common pathogenic

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bacteria encountered include *Vibrio cholerae*, *Salmonella typhi, Leptospira sps*., *Clostridium botulinum* and *Wolbachia sps*. Release of high numbers of such bacteria into water makes the water unfit for bathing and for human consumption. (Abraham, 2011)

The extent of pollution of Ganga in West Bengal was studied by identifying the coliform content in the Hooghly estuary. Samples collected at eight different locations in and around Sundarbans region which included some of the major places such as Haldia, Diamond Harbour and Namkhana. The total coliform count ranged from 24-393MPN/100ml with Haldia showing the highest coliform count. The major coliforms observed included *E.coli* and *Enterococci*(Mitra, 2019).

The water quality of Ganga in Garhwal region was assessed in distinct seasons such as summer, monsoon and winter to understand the anthropogenic contribution in contaminating the water. The two major tributaries of Ganga the Bhagirathi and Alaknanda were studied for this purpose. The total coliform content and the various physio chemical properties were assessed. The study found that total viable count of coliform bacteria was high in different regions analysed. Badrinath showed highest coliform content for Alaknanda whereas the Gangotri showed highest content for Bhagirathi river. Coincidentally the higher contaminated sites were the region where there was more annular pilgrimage associated with river. (Sati *et al.,* 2011). A similar such study was done by Ahmmad *et al.,* to associate the increase in pollution with enhanced human activity. The water samples were collected from seven distinct regions of the Upper Ganges constituting of the Rishikesh- Haridwar region. The water and sediment were analysed for coliform content and for total viable coliforms in the water samples. Across the various months of analysis only in June highest coliform content was seen which was associated with pilgrimage and in the rest of seasons the content were low and water had good quality. (Ahammad *et al.,* 2014).

In order to understand the extent of contamination of Ganges water a distinctive study was performed across the various months of year in order to understand the effect of anthropogenic sources on pollution of river. Faecal count, coliform count and physical parameters were assessed in West Bengal across different seasons. The study observed that the coliform count and faecal count exceeded the standard values thus making water unfit for consumption. The month of December showed a high count. (Sengupta *et al.,* 2014)

The release of coliforms possesses a distinctive threat for infection of humans who consume such water. Another distinctive threat that arises is from the anti-biotic resistant bacteria that are encountered. The emergence of antibiotic resistant bacteria has become one of the most common features associated with hospital infections. Among the different antibiotics widely used β lactams represent a broad group to which resistance is developed. Many of the resistant bacteria host β lactamases which have the capacity to neutralised a wide variety of antibiotics. The unlimited use of antibiotics and their release into environment has resulted in development of these resistant forms which pose a significant threat to mankind. Water samples of Ganges tested in Varanasi and Aurihar showed bacteria which host the resistant genes. The soil sediment also showed for antibiotic resistant bacteria. The predominant bacterium isolated were *Pseudomonas* and *Klebsiella pneumoniae*. (Anand *et al.,* 2006)

Similar such study was carried out in Varanasi for identification of metal resistant and antibiotic resistant genes in polluted water of Ganga. Genomic sequencing and phylogenetic alignment of polluted water showed bacteria belonging to α, β and γ *Proteobacter* respectively. *Pseudomonas, Serratia, Enterobacteria* and *Proteus* were seen selectively in Dashashwamedh Ghat and Assi Ghat. The metal tolerant bacteria observed belong to *Comamonas sps.* These bacteria show exceptional tolerance to wide range of metals and are able to grow even at concentrations of 400mg/ml. (Niveshika *et al.,* 2016).

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Findings of the review

Understanding the extent of contamination of river system by antibiotic resistant and metal resistant bacteria by analysing samples at individual level is cumbersome and unduly long. With the advancement of the technologies high throughput methods have gained significant importance. Whole genome shot gun sequencing methods are rapid and help in identification and phylogenetic labelling of the bacteria in given sample. Such metagenomic methods are of immense importance in characterising the organisms that are harboured in the river water ecosystems. Such studies have been done in order to understand the nature of pollutants in Ganges river. (Mukesh Kumar Awasthi *et al.,* 2020)

In one such novel research Samson *et al.,* studied the changes of microbial community at confluence of Ganges with Yamuna. The identification was carried out using MinIon sequencing method. Native Ganges before confluence and immediate upon confluence showed similar profile. *Proteobacter* was the most prominent followed by *Firmicutes* and *Bacteroides*. Upon confluence the *Proteobacter* was still abundant however *Bacteroides* and *Firmicutes* showed lower levels. However, post confluence and further travel of the river the community showed similar profile before confluence with Yamuna. The study utilised sediment samples from 21 distinct sites for analysis. Thus, this was a landmark study which helped in understanding the community dynamics before and after confluence of the rivers. (Samson *et al.,* 2019)

Yamuna is the major tributary of Ganga which flows through Delhi city. It is one of the most polluted rivers in the world. In order to explore the nature of microbes that are inhabiting such polluted waters a metagenome analysis was performed during pre-monsoon and post monsoon seasons. The study observed methyl chemotaxis accepting protein (MCP) as major component of the microbiome. Sulphur and nitrogen assimilation pathways were seen as predominant components of the microbial system. The study shows a large number of antibiotic and metal resistant gene expressing bacteria in the polluted waters. Bacterial genes expressing resistance to fluoroquinolones, rifampicin, macrolides and β-lactams were observed. A wide spectrum of β-lactamases including bla_{NDM-1}, bla_{NDM-8}, DMB-1, bla_{imp-1}, bla_{imp-2} and imiH were discovered in the genomes of bacteria. The discovery of New Delhi metallo beta lactamase (NDM-1 and NDM-8) in the waters has significant impact. Most of these bacteria have found to have tolerance to very high levels of metals. The bacteria have been shown to express genes of mer operon (Mercury tolerance) and arsenic operon genes. (Mittal *et al.,* 2019).

In order to understand the microbial community and the various polluting bacteria in Ganges sediments were collected from different major cities which include Bijnor, Narora, Kannauj, Kanpur, Allahabad, Allahabad Sangam, Mirzapur and Varanasi. Library preparation and Ilumina sequencing were performed to identify the major bacteria inhabiting the sediments. The metagenomic analyses observed that *Proteobacter* and *Actinobacter* as major bacterial species of sediment community. Approximately 84% of the Illumina reads showed these bacteria. A small fraction of the reads showed for the presence of antibiotic resistant genes. The most abundant genes were found to be for multi drug resistance which includes resistance for macrolide-lincosamide-streptogamin (MLS), bacitracin, fosidomycin, vancomycin, tetracycline, quinoline, aminoglycosides and β lactams. Genes for multidrug resistant transporters and related efflux proteins have also been observed in sediments. (Kumar *et al.,* 2020)

Pesticide contamination of waters is the major problem associated with Ganges river. The presence of Xenobiotic degrading bacteria could be a powerful indicator of the extent of the contamination. These bacteria could also help in cleaning up of the contaminating pesticides in a given system. In one such study Raju and Bidlan performed a metagenomic analysis of Yamuna and Godavari to identify the novel bacteria which can degrade organochlorine pesticides such as DDT and Lindane. The study identified two predominant bacteria *Brevundimonas dimunita* and

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Strenotrophomanas acidaminiphila as the major group of bacteria responsible for degrading organochlorine group of pesticides. (Raju and Bidlan.,2017)

In order to understand the spread of antibiotic resistant genes(ARG) and metal resistant genes (MRG) in Ganges sediments of the river were collected from five major Ghats of Ganga in Varanasi. High throughput genomic analysis was performed of DNA obtained. The study observed high concentration of ARG in sediments than in water. ARG concentration were high in Harishchandra ghat whereas MRG concentrations were high in Bhadaini ghat. The major bacterial genes were found to be resistant for aminocoumarins, mupirocin, polymyxin and tetracycline. The metal resistant bacteria exhibited resistance to wide range of metals including copper, zinc, iron, nickel, cadmium, arsenic, tellurium, mercury and tungsten. There was a significant correlation between ARG and MRG bacteria with some of the bacteria possessing both the properties. (Reddy and Dubey, 2019)

Metagenome analysis of river Ganga was performed at different regions in Allahabad. Surface water samples were used for DNA isolation and metagenomic study. Surface water study showed four distinct types of bacteria namely *Bacillus sps, Bacillus anthracis*, *Pseudomonas aeruginosa* and *Eschericia coli*. Of the four only *Bacillus sps* is endogenous rest all are pathogenic. (Zaed & Singh, 2017)

Zhang et al., in a pioneering study performed metagenomic analysis of Ganges across various cities to identify the anthropogenic sources contributing to its pollution. The study clearly showed that of the total transcripts identified for bacteria 11-32% belonged to human input and sewage effluent discharge into Ganges. The presence of high number of microbiome of human gut in water was clear indication of the sewage discharge. There was significant amount of antibiotic resistant bacteria that were seen which were primarily from human inputs. As like many other metagenome studies *Proteobacter* and *Actinobacter* were found to be the most abundant species seen in water. However, a small but significant portion of the genome was contributed by *Pseudomonas aeruginosa*, *Acinetobacter sps* and *Chthoinobacter* sps. Human gut microbes such as *Prevotella*, *Escherichia*, *Oxalaobacter* and *Bacteroides* were found in these waters. Genes conferring resistance to common antibiotics such aminoglycosides, coumarins, flour quinones and beta lactams have been found in the genome of identified bacteria. (Zhang *et al.,* 2019)

Discussion

Contamination with various anthropogenic sources possess as distinct threat to i novel ecosystem of Ganga and thus preservation of the system is important. Understanding the microbiome of polluted water thus plays an important role. Several of the research work has been done to identify the presence of antibiotic resistant genes and further spread of such bacteria in waters of Ganga (Chakraborty *et al,*, 2018, Biswas *et al.,* 2015). The direct transmission of human waste to river bodies possess a significant challenge. In case of Ganga annual pilgrimages and mass bath in the river results in extensive pollution with antibiotic resistant bacteria along with the introduction of human gut microbiome. (Fouz *et al.,* 2020) Along with the discharge of untreated waste the discharge of metal waste from industries has results in development of metal resistant bacteria. In fact, the waters of Ganga at many places have shown to have both antibiotic and metal resistant bacteria. The development of such tolerant bacteria is a grave indicator of extent of pollution. (Paul, 2017, Ram & Kumar, 2020). Apart from Ganga studies done in its various tributaries such as Gomati, Yamuna and others have shown good load of the resistant bacteria implicating the seriousness of the problem (Singh and Singh, 2014, Anand *et al.,* 2016, Azam *et al.,* 2018 & Biswas *et al.,* 2019). Apart from the bacteria the changes seen associated with fungal community also have shed light on the extent of modification of resident microbiome by the pollutants (Samson *et al.,* 2019 & 2020). The various metagenomic studies that have been highlighted above only emphasize the fact that there is extensive human intervention in the Ganges water and this has effect on the resident

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flora and fauna. Since the waste is used for drinking and bathing in different seasons high loads of pathogenic and metal resistant bacteria could pose a significant threat to the people who are using such water.

Future perspective

Ganga action plan has been formulated for cleaning the river and to make it free of industrial and sewage waste. However, the plan is still at its infancy. Metagenomic analysis is a powerful method for understanding and helping in pointing out the sources of contamination. Based on precise locations utilising metagenomic studies one could then develop strategies for controlling the pollution.

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A RARE CASE OF KLINEFELTER'S SYNDROME WITH ISO X CHROMOSOME

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KEY WORDS: Chromosome analysis, Klinefelter Variant, Genetic counseling

ABSTRACT: Klinefelter's syndrome (KS) is the most common chromosome aneuploidy in males, characterized by at least one supernumerary X chromosome. Klinefelter's syndrome (KS) with an isochromosome Xq is a rare condition. In all cases reported so far, patients showed the classic phenotype. Early diagnosis and intervention with proper treatment can improve the quality of life and will prevent serious consequences in Klinefelter's Syndrome.

BACKGROUND: Klinefelter's variant with an additional iso-chromosome Xq is a rare event and only a few cases are reported. Majority of these patients show the main features of KS, one of the remarkable difference between these men and 47,XXY KS patients, is only height where the former show a normal height when compared to standard klinefelter's syndrome patients. Due to less number of cases reported so far , with a 47,X,i(Xq), Y karyotype, the spectrum of clinical manifestations has not been fully elucidated.

LH levels, hypogonadism, normal or increased height, , osteoporosis, obesity, dyslipidemia, impaired glucose tolerance,cognitive ariant with an additional isochromosome Xq is between 0.3–0.9% in Klineflter's patients.(4). First case of a 47,X,i(Xq),Y male was reported in 1969 (5). In literature, so far 21 cases of isochromosome Xq have been reported . (6). We are reporting a rare variant of Klinefelter syndrome with a 47,X,i(Xq),Y karyotype with infertility.

Introduction:

Klinefelter syndrome (KS) is one of the most common numerical chromosomal abnormality among men, with an incidence of 1 out of 660 newborn males(1). Klinefelter syndrome (KS) was first described by Harry F. Klinefelter in 1942 (2). In 1959, this was found to be the result of an additional X chromosome (3). The important clinical features are infertility mainly charecterised by azoospermia, increased FSH and

impairment, and absence of mental retardation. The incidence of the Klinefelter v

Materials And Methods:

Chromosome analysis was performed as per standard procedure –GTG banding with 550 band resolution and was analyzed for chromosome identification.

Case Report:

The 40-year-old patient, who had a married life of 20 years was referred from an infertility clinic with a history of azoospermia for karyotyping. The patient generally looked healthy with a normal male appearance. His height was 165 cm, body weight 59.5 kg, and body mass index 22 kg/m^2. The semen

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analysis showed semen volume of 1.6ml and no sperm count, and he was diagnosed with complete azoospermia. Endocrinological investigation revealed elevated FSH and LH levels with FSH 71.2 mIU/mL (normal range, 1.4-18.1 mIU/mL), LH 23.21 mIU/mL (normal range, 1.5-9.3 mIU/mL), prolactin 25.84 mIU/mL (normal range, 2.64-13.13 standard protocol [7] . 25 G-banded metaphases were analyzed using Cytovision software, and designated as per ISCN (2013) at 550 bands per haploid genome. The investigation revealed 47,X,i(Xq),Y karyotype on 25 metaphase chromosomes with no mosaicism, and all isochromosomes were apparently monocenteric.

Discussion: Klinefelter syndrome, first described in 1942 as an endocrine disorder, is the most common sex chromosomal aneuploidy disease and occurs in one of every 500-600 newborn males. The typical karyotype of Klinefelter syndrome is 47,XXY, but chromosome mosaics with 46,XY/47,XXY and complements with multiple X chromosomes like 48,XXXY are known [3-5]. KS is the most frequent genetic cause of male infertility, and is found in 11% of azoospermic men and 4% of infertile men [7]. In most of the cases KS is not diagnosed before puberty and even in adulthood it was estimated that only a fourth of affected males are diagnosed, mostly due to their infertility [1]. In most of the published cases, infertility, elevated plasma LH and FSH levels, low or normal testosterone levels, sometimes gynecomastia, normal to short body height, and average intelligence are reported. Our patient's features are similar to those described in literature. The 47,X,i(Xq),Y describe here is a very rare variant form of Klinefelter syndrome. Isochromosome Xq is a structural rearrangement frequently observed in Turner syndrome, but it is apparently rare in males . It is suggested that the most probable origin of an Xq isochromosome is misdivision of the centromere or sister chromatid exchange of one X chromosome. Due to the limited number of cases, the prevalence of this Klinefelter syndrome is still unclear. So far, 21 patients with i(Xq) have been reported in literature, and reports on Klinefelter syndrome with an isochromosome Xq have been discussed briefly in the literature; therefore, mIU/mL), and testosterone 2.08 mIU/mL (normal range, 2.80-8.00 mIU/mL). Cytogenetic analysis was carried out on phytohemagglutinin stimulated peripheral blood lymphocytes, cultured in Roswell Park Memorial Institute (RPMI) 1640 medium, using modified

genetic counseling is difficult for such cases [6].In general, all 47,X,i(Xq),Y patients have been reported to exhibit the main clinical features of Klinefelter syndrome, including reduced androgenization, small testes, azoospermia, gynecomastia, and elevated FSH and LH levels[8]. In Klinefelter syndrome, when testosterone serum concentrations in patients are low, lifelong substitution therapy is indicated and should be started as early as possible to avoid symptoms and sequelae of androgen deficiency. For fertility issues, a testicular sperm extraction procedure may be considered for any viable sperm before androgen replacement therapy. It is well-documented that 47, XXY males show a mean height of about 6.5 cm more than their male relatives [9]. The only clinical difference between the 47, XXY and 47,X,i(Xq), Y Klinefelter syndrome patients is normal-to-short stature in the latter yet its pathophysiological mechanism remains unclear [10]. Most of the literature indicated that the normal height is due to the presence of only one Xp carrying the growth gene *SHOX* (short stature homeobox-containing gene) and other putative Xp-specific growth genes[11] . The height of the patient in our case is compatible with the previous reports. The difference between 47,XXY males and the 47,X,i(Xq),Y is difficult to validate, since only few data from male relatives are documented in the latter group . However, normal stature is confirmed when the mean height of our patient is compared with age-matched normal controls from the same ethnic population (mean 165.38 cm)[12] .The delineation of the phenotype of subjects with isochromosome Xq Klinefelter syndrome is important to provide meaningful genetic counseling. However, due to the rarity of this disease, only further evaluation with a larger group of such patients would be able to determine more clearly the clinical features and appropriate genetic counseling for these patients.

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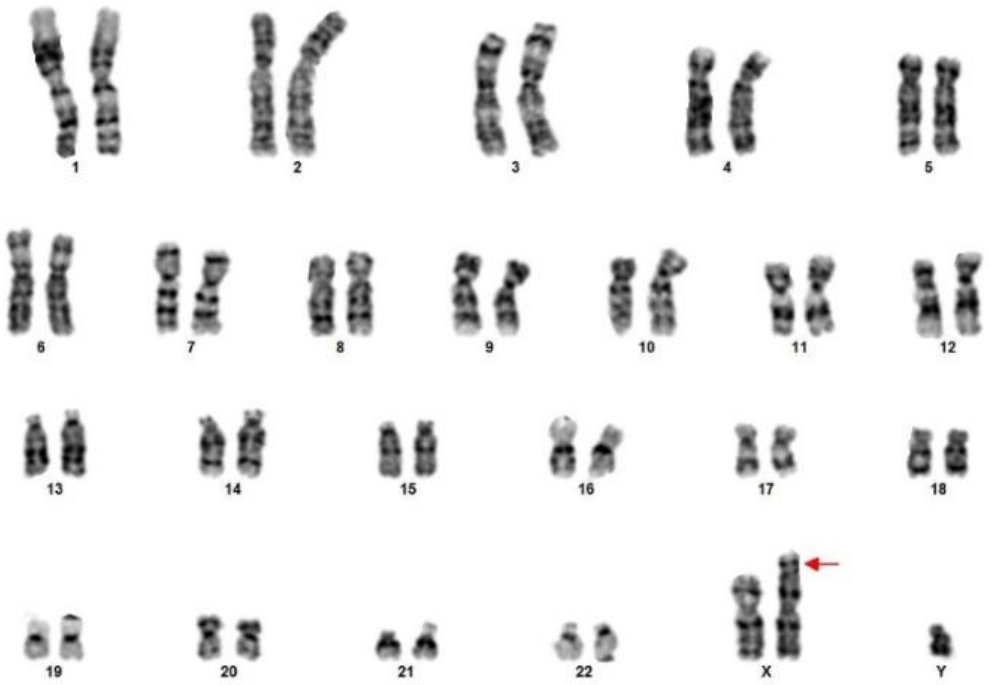

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Isolation of Bio-surfactant Exhibiting Bacteria from the Northern Regions of India and Their Phylogenetic Relationships

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ABSTRACT
Two strains of bacterial isolate, *Bacillus cereus* CH1, and *Bacillus cereus* G1 were isolated from the northern part of India, namely Chandigarh and Delhi respectively. Both of them showed potential biosurfactant activity. There was a considerable displacement of oils globules by the *Bacillus cereus* CH1 and *Bacillus cereus* G1 within 45 seconds when 10 µl of bacterial suspensions were spread on the water sample containing cooking oil. To identify their emulsification activity, the bacterial suspension of both isolates were added by taking 20 µl sample of each in test tubes containing water and 1 ml of cooking oil separately. The froth formed was found to be stable for 1 h. The phylogenetic analysis showed both strains 99% identical with *Bacillus cereus* strain CCM but interestingly there were 94% identical similarities between *Bacillus cereus* CH1 and *Bacillus cereus* G1. In addition, both isolates shared a different clade and have evolved recently.

INTRODUCTION

The use of renewable resources for the replacement of chemical resources has attracted people worldwide. One of them is the use of bio-surfactant in place of chemical surfactant and has been observed as promising alternative in parallel to their eco-friendly nature as they do not pollute the environment (Nitschke and Costa, 2007). Instead of their importance in food and pharmaceutical industries, they are also expected to have potential in various environmental applications such as emulsification, foaming, wetting, detergency, dispersion, and degradation of hydrophobic compounds (Banat, Makkar and Cameotra, 2000; Luna et al., 2013).

Apart from their potential in replacing the synthetic surfactants, their Functional and structural diversity has attracted scientists to understand the various bio-emulsifiers, exopolysaccharides, and biosurfactants characterized from various resources (de Sousa and Bhosle, 2012). Under which the few investigations have led into the elucidation of high surface activity of bio-surfactants over synthetic surfactants. In parallel to their physiochemical properties, they are also observed with efficient surface activity, less toxicity, efficient biodegradability, high specificity, and a tendency to retain activity under enormous conditions. With these characteristic features, such emulsifiers and biosurfactants have been broadly applied in various environment conservation practices such as remediation of water, soil, oil spill, etc. along with commercial applications (Lima et al., 2011; Sivapathasekaran et al., 2010; Xu et al., 2011).

Biosurfactants effectively reduce surface & interfacial tension among different phases such as liquid-solid, liquid-liquid, and liquid-air. Looking into the ideality of effective surfactants, they possess low critical micelle concentration as well as a tendency to generate stable emulsions. How bio-surfactant lowers the surface and interfacial tensions are considerably taken care of by estimating its adsorption onto the various phases leading to enhanced interaction and mixing of dissimilar phases. Therefore, into the accountability of the adsorption capability of biosurfactant, it is calculated by the lowest concentration of the biosurfactant necessitate to lower down the surface tension between water and micelle, also known as CMC. With these

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properties, they can be utilized in various industrial processing by acting as foaming, wetting, and solubilizing agents. Moreover, their application in environmental bioremediation is seen by enhancing the dispersion and microbial access by providing bioavailability of hydrophobic pollutants. Even more, they are capable of moving hydrophobic molecules that are present on the solid substrate. Biosurfactants are amphiphilic compounds having hydrophobic and hydrophilic moieties produced on living cell surfaces or secreted extracellularly, thus reducing surface and interfacial tension by accumulating between two fluid phases. Biosurfactants are capable to increase cell surface hydrophobicity on the producing living cell. With increased hydrophobicity, various insoluble substrates like hydrocarbons (hydrophobic) get easily diffused inside living cells (Perfumo et al., 2010; Satpute et al., 2008). Due to the toxicity of these insoluble compounds, these biomolecules are used for different bioremediation technologies (Mulligan and Gibbs, 2004). Biosurfactant classification depends upon its chemical structure, molecular weight, microbial origin, and physiochemical activity. They are further characterized into low molecular weight biosurfactants such as glycolipids, lipopeptides, phospholipids and high molecular weight bioemulsifiers such as amphipathiclipopolysaccharides, polysaccharides, protein and lipoproteins (Calvo et al., 2009; Rosenberg and Ron, 1999).

In other term, they are also classified as extracellular polymeric molecules or exopolysaccharides. These EPS are high molecular weight polymers consisting of monosaccharides. With very little concentration of emulsifiers have one advantage of emulsifying two different immiscible liquids like hydrocarbon and hydrophobic pollutants, good in solubilizing inadequately soluble substrates but with reduced effectiveness at surface tension reduction. Hence as the name suggests they only have emulsifying activity but unfortunately not well at the surface activity. Hence, at oil-polluted sites emulsifiers are good at degrading and preventing mixing of the insoluble substrate with oil by increasing their kinetic stability with the help of the highly reactive group present in them. The process of preventing merging is also called stabilization of the emulsion. Not only in degrading pollutants but emulsifiers are also useful in cosmetic, food, pharmaceutical, and petroleum industries (Calvo et al., 2009; Monteiro et al., 2010). Willumsen and Karlson (1997) also described that molecules that have activity on the surface are further distinguished into two surfactants and emulsifiers, where surfactants lower surface tension, their emulsifiers form & stabilize emulsions. But some biomolecules possess both & contribute their unique functions in broad industrial uses (Willumsen and Karlson, 1996). Some of the microorganisms that are producing a class of bio-surfactants and bio-emulsifiers along with their physicochemical property are enlisted in table-1.

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Table-1 In the given table there is a list of various microorganisms that are producing a class of bio-surfactants and bio-emulsifiers along with their physicochemical property.

Biosurfactant	Class of biosurfactant	Microbial strain	Physiochemical properties	Application	References
Low molecular weig Glycolipids	Rhamnolipids	*Pseudomonas aerugino DS10-129*	1 or 2 rhamnose sugars linked 3-hydroxydecanoic acid. ST- 28 mN/m EI24- 53–73%	Emulsification, degradation, and dispersi of different classes of hydrocarbons, vegetable oils, metals from the soil	(Herman, Artiola and Miller, 1995; Itoh al., 1971; Maier and Soberon-Chavez, 2000; Rahim et al., 2001; Rahman et al., 2010; Sifour, Al-Jilawi and Aziz, 2007; Whang et al., 2008)
	Sophorolipids	*Torulopsisbombicola*, *Torulopsispetrophilum*, *Torulopsisapicola* *Candida bombicola* *Candida tropicalis*	Disaccharidesophoroses (2-O-β D-glucopyranosyl D-glucopyranose) are linked to fa acids. ST-32.1-34.2mN/m	Recovery of soil and hydrocarbons from dregs and muds, heavy metal removal fro sediments	(Baviere, Degouy, and Lecourtier, 1994; Develter and Lauryssen, 2010; Joshi-Navare, Khanvilkar and Prabhune, 2013; Pesce, 2002; Whang et al., 2008)
	Trehaloselipid	*Rhodococcus, wratislaviensis BN38, Norcardiafarcinica BN*	Non-reducing disaccharides wi 2 glucose units linked with α α 1,1-glycosidic linkage. ST-24.4 mN/m EI- 23-70%	Increase bioavailability of hydrocarbons	(Christova et al., 2014; Franzetti et al., 2010; Tuleva et al., 2008)
Lipopeptides	Surfactin	Bacillus subtilis K1 *Bacillus siamensis*	Heptacyclicdepipeptides consis of 2 acidic amino acids, 4 hydrophobic amino acids, and $C_{13\text{-}17\beta}$-hydroxy fatty acids. ST- 22–27.9 mN/m	Increase effectiveness of phytoextraction biodegradation of hydrocarbon, chlorinat pesticides, heavy metals from the soil, water, and sediments	(Arima, Kakinuma and Tamura, 1968; Arrebola, Jacobs and Korsten, 2010; Awashti et al., 1999; Jenneman et al., 19 Ongena and Jacques, 2008; Pathak and Keharia, 2014; Varadavenkatesan and Murty, 2013)
	Iturin	*Bacillus subtilisK1* *Bacillus amylofaciens*	Cycloheptapeptide with 7 amir acids and $C_{13\text{-}16\beta}$-hydroxy fatty acids. ST-30–37 mN/m EI- 32–66%	Antifungal and biopesticides	(Lukondeh, Ashbolt and Rogers, 2003; Monteiro et al., 2010; Mulligan and Gibl 2004; Ongena and Jacques, 2008)
	Fengycin	*Bacillus subtilis*	Cyclic deca-peptides with $C_{14\text{-}}$ hydroxy fatty acid and 10 amir acids.	Strong immunomodulating activities and fungi toxic agent against filamentous fun	(Arrebola, Jacobs and Korsten, 2010; Pathak and Keharia, 2014)
	Lichenysin	*Bacillus licheniformis*		Increase in oil recovery	(Gerson and Zajic, 1978)
Fatty acids, phospholipids, and neutral lipids	Corynomycolic acid	*Corynebacteriumlepus*	β-hydroxy-α-branched fatty ac	Enhancement of bitumen recovery	(Cooper, Zajic and Gracey, 1979)
	Spiculisporic acid	*Penicilliumspiculisporu*		Removal of heavy metal and metal ions, dispersion of hydrophilic pigments, preparation of emulsion-type organogels, superfine microcapsules vesicles, and liposomes	(Nitschke and Costa, 2007; Ongena and Jacques, 2008; Pathak and Keharia, 2014 Rahim et al., 2001; Rahman et al., 2010; Ron and Rosenberg, 2001)
	Phosphatidylethanolam e	*Acinetobacter* sp., *Rhodococcuserythropo*	*Acinetobacter* sp. Strain HO1-N phosphatidylethanolamine IT <1 mN/m CMC-30mg/liter	Enhance bacterial tolerance to heavy met	(Appanna, Finn and Pierre, 1995; Käppe and Finnerty, 1979; Kretschner, Bock an Wagnee, 1982)
Polymeric biosurfactant /High molecular weight-emulsifiers	RAG-1 emulsan	*Acinetobacter*sp. *ATCC31012* (RAG-1)	Lipopolysaccharides. Lipid moiety of $C_{10\text{-}18}$ unsaturated fat acids and polysaccharide moie of D-galactosamine, D-galactosaminuronic acid, di-amino-6-deoxy-D-glucose	Increase binding to heavy metals and bioavailability of less soluble substrates	(Choi, Choi and Lee, 1996; Ron and Rosenberg, 2001; Zosim, Gutnick and Rosenberg, 1982)
	BD4 Emulsan	*Acinetobactercalcoacet us BD4 13*	Protein polysaccharides compl in which polysaccharide is a repeating heptasaccharides of l rhamnose, D-glucuronic acid, l mannose	A stable oil-in-water emulsion	(Kaplan and Rosenberg, 1982; Kaplan, Zosim and Rosenberg, 1987)
	Alasan	*Acientobacterradioresi ns KA53*	Polysaccharides and proteins containing alanine amino acid residues	Emulsify and solubilize mixture	(Navon-Venezia et al., 1995; Toren et al. 2001; Walzer, Rosenberg and Ron, 2006
	Mannoproteins	*Saccharomyces cerevis Kluyveromycesmarxian*	A complex of polysaccharides and proteins	Stabilizes emulsification process with hydrophobic	(Cameron, Cooper and Neufeld, 1988; Casanova et al., 1992; Lukondeh, Ashbo and Rogers, 2003)
	Bioemulsifiers containi uronic acid	*Halomonaseurihalina* *Klebsiella*sp.	A complex of polysaccharides-proteins-uronic acids	Emulsify and detoxify hydrocarbons	(Jain et al., 2013; Martínez-Checa et al., 2002)
	Biodispersan	*Acinetobacter calcoaceticus*A2		Dispersion of limestone in water	(Rosenberg et al., 1988)

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MATERIAL AND METHODS

COLLECTION OF SOIL SAMPLE

The soil samples were collected from two different regions, motor market Chandigarh and motor market Delhi, India. The soil sample was collected from 10 cm below the surface of the soil. The whole procedure was done with precautions and samples were collected in sterile bags using sterile equipment.

9ml distilled water. Serial dilution was done 8 times with the dilution factor of 10^{-1}. The soil suspension was stored at 37°C for 24 hrs. After 24 hrs of incubation of 100ul of soil, the suspension was spread on a freshly prepared nutrient agar plate and incubated at 37°C for 24 hrs.

PURIFICATION OF BACTERIA STREAK METHOD

After 24 hrs of incubation, the distinct bacterial isolates were streaked on freshly prepared nutrient agar plates and incubated at 37°C for 24 hrs. the procedure was repeated 3 times to get the purified single distinct colonies. The purified colonies were further characterized for Gram's staining.

QUALITATIVE TEST FOR BIOSURFACTANT ACTIVITY

BATH test: 40ml of distilled water was poured into Petri plates, to which 10ul of cooking oil was added and to this 10ul of bacterial isolates was added and left to stand for a few seconds to observe the displacement of oil globules.

Emulsification activity test: A qualitative test was performed for selected isolates to identify their emulsification activities. For that purpose, 20ul of cell culture of selected isolates was added into the conical flask containing 10ml of distilled water and 1ml of cooking oil. The mixture was vortex for 5 minutes and allowed to stand for 1hr. The basis of emulsification activity was on the formation of froth over the surface of the water and its stability for 1 h.

IDENTIFICATION OF BACTERIA BY 16S RDNA SEQUENCING

ISOLATION OF BACTERIA BY SPREAD METHOD

3gm of soil sample was taken and dissolved in 10ml autoclaved distilled water. After 5 minutes of vortexing sample was allowed to stand for 5 minutes then after 1ml of suspension was taken and further added in

Selected isolates were pursued 16s rDNA sequencing which was done by the contractual party, Applied Biosciences, Bangalore, India. Data were interpreted using Sequence Scanner Software, 2v2.0.

Multiple sequence alignment

Multiple sequence alignments (MSA) were constructed using the program Clustal X version 2.0.11 (Thompson et al., 1997). Nucleotide sequence alignment was performed with the following parameters: Gap Opening penalty=10.0, Gap Extension penalty=0.05nucleotide, and IUB DNA weight matrices. The multiple sequence alignment was scrutinized by the eye and edited using GeneDoc 2.7.000 software (Nicholas, 1997).

PHYLOGENETIC ANALYSIS

To find sequence homology of isolated bacterial sequence with other related nearby sequences, various computational-based software Clustal X 2.0.11, GeneDoc, Mega 7, and BLAST were used. The measure of similarity score between sequences provides us with the function and biologically significant relationships between query and subject sequences. The DNA sequences showing 95-98% similarity score were used in phylogenetic analysis and obtained from NCBI using BLAST (Altschul et al., 1990). Then DNA sequences aligned using Clustal 2.0.11 and GeneDoc with default setting followed by a visual inspection. The evolutionary history among progeny was theorized by using the Maximum Likelihood method built on the Tamura-Nei model (Tamura and Nei, 1993). The tree with maximum log-likelihood (-4275.00) is shown. The initial tree for the heuristic results was obtained automatically by using Neighbor-Join and BioNJ algorithms to a matrix of pairwise

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distances. The maximum Composite Likelihood method was used to calculate average evolutionary divergence overall sequence pairs (Tamura, Nei, and Kumar, 2004) and then select the topology with superior log likelihood value. The number of base substitutions per site from averaging over all sequence pairs was shown. The analysis contains 22 nucleotide sequences. The tree is drawn according to branch length which was estimated by applying the pathway method (Nei & Kumar, 2000). The measure of evolution is in numerical values that denotes changes over time. Codon positions involved were 1st+2nd+3rd+Noncoding. Gaps and missing data were eliminated from all 1282 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, Stecher, and Tamura, 2016, p.7).

RESULTS AND DISCUSSION

ISOLATION, PURIFICATION, AND CHARACTERIZATION OF BACTERIAL ISOLATES

Total 7 different pure colonies were isolated which were classified based on Gram staining. 4 different isolates were purified from Delhi regions *viz*. CH1, CH2, CH3, and CH4. From Chandigarh 3 different isolates were purified and named G1, G2, and G3. All of those isolates given in table 2 were screened for the biosurfactant activities.

From the Bath test it was observed that when cultures of CH1, CH3, G1, and G3 isolates were poured over the Petri plates containing water and oil, they significantly displaced the oil globules within 45 seconds. Whereas in the culture of *E. coli*, CH2, CH4, and G2 no such event was observed. Amongst CH1, CH3, G1, and G3 isolates, CH1 and G1 were found to be potential isolates exhibiting biosurfactant activities (Fig. 1 and 2).

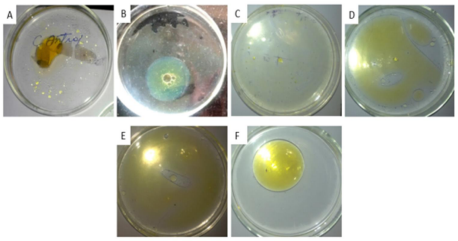

Figure. 1 Determination of biosurfactant activities using Bath test, A: Control (water+ oil without isolate), B. water+ oil + Culture of *E.coli*, C. water+ oil Culture of CH1, D. water+ oil+ Culture of CH2, E. water+ oil+ Culture of CH3, F. water+ oil+ Culture of CH4.

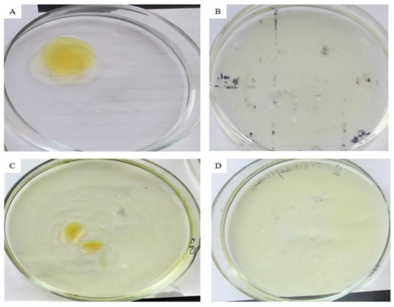

Figure. 2 Determination of biosurfactant activities using Bath test, A: Control (water+ oil without isolate), B. water+ oil + Culture of G1, C. water+ oil Culture of G2, D. water+ oil+ Culture of G3

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EMULSIFICATION ACTIVITY OF SELECTED ISOLATES

After determining the biosurfactant activities, two isolates CH1 and G1 isolates were pursued the emulsification activity. During experimentation, we used SDS as a positive control and compared it with the bacterial culture. After experimenting, it was observed that however SDS had a significant emulsion layer but comparatively both isolates also form a significant emulsion layer and were stable for 1 h (Fig.3).

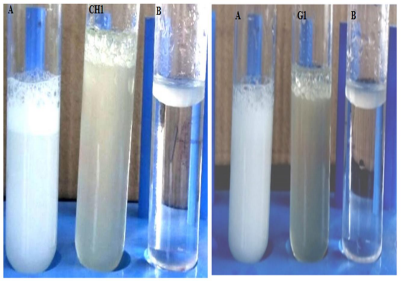

Figure 3. Emulsification activity of CH1 and G1 isolates wherein A SDS was used for emulsification activity, CH1 and G1 are test samples and B is *E. coli.*

PHYLOGENETIC ANALYSIS

The occurrence of a homologous sequence of 20 bacterial species was investigated using the BLAST similarity analysis tool. The topmost hits were evaluated in terms of their statistical measure. The DNA sequence of *Bacillus cereus* strain G1(gene ID 1465395717) and *Bacillus cereus* strain CH1(GI: 1465395718) used for phylogenetic analysis were submitted at NCBI databank under the accession numberMH810304.1 and MH810305.1 respectively. Data were obtained from NCBI to study the phylogenetic relationship of *Bacillus cereus* strain G1and *Bacillus cereus* strain CH1with related homologous sequences. The maximum likelihood method was used to measure the phylogenetic relationship that grouped various descendants into separate clade A and Clade B in Figure-4. Clade A and clade B includes various descendants that have shared a common ancestral lineage. *Bacillus cereus strain* CCM 2010, *Bacillus cereus* strain JCM 2152, *Bacillus thuringiensis* strain IAM 12077, *Bacillus cereus* strain ATCC 14579, *Bacillus* *Bacillus cereus* strain ATCC 14579, *Bacillus cereus* strain IAM 12605, *Bacillus wiedmannii* strain FSL W8-169, *Bacillus toyonensis* strain BCT-7112, *Bacillus cereus* strain NBRC 15305, *weihenstephanensis* strain DSM 11821, *Bacillus licheniformis* strain DSM 13, *Bacillus licheniformis* strain ATCC 14580, *Bacillus sonorensis* strain NBRC 101234, *Bacillus licheniformis* strain NBRC 12200, *Bacillus subtilis* subsp. *inaquosorum* strain BGSC 3A28, *Bacillus subtilis* strain DSM 10, *Bacillus aerius* strain 24K, *Bacillus licheniformis* strain BCRC 11702, *Bacillus halotolerans* strain DSM 8802, *Bacillus subtilis* strain IAM 12118 are grouped in to clade A. *Bacillus cereus* strain G1 and *Bacillus cereus* strain CH1 grouped in to clade B. The estimated average evolutionary divergence over all sequence pairs is 0.163. Evolutionary distance from ancestral lineage till speciation event of clade A is 0.35 and of clade B is 0.37 (Fig. 4).

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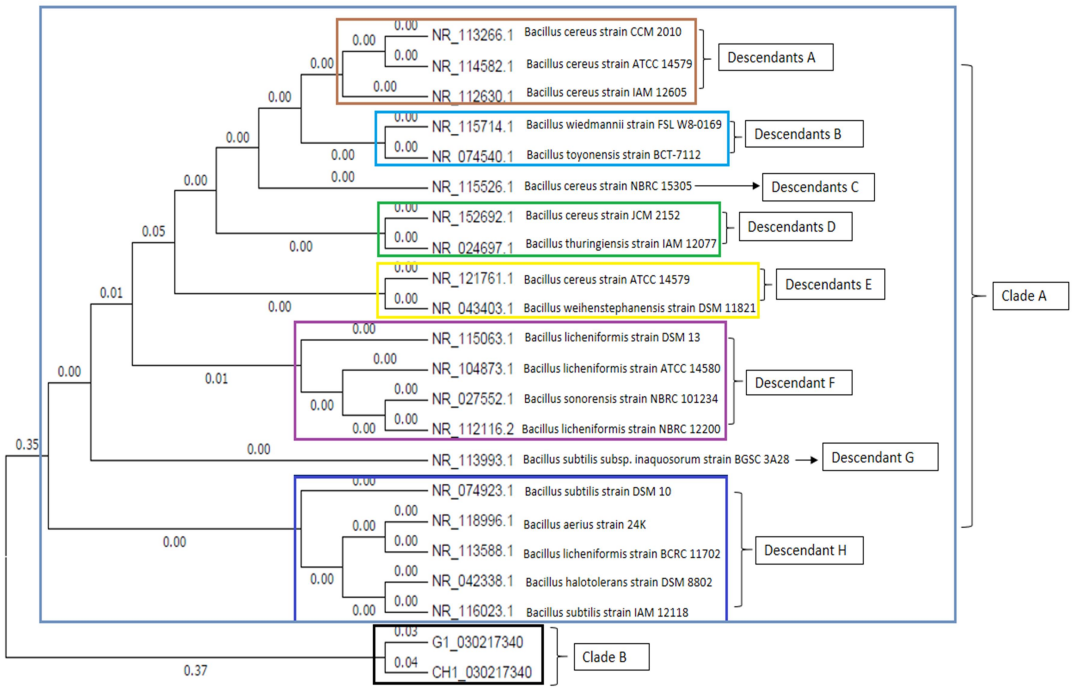

Figure-4 The DNA sequence of *Bacillus cereus* strain G1(gene ID 1465395717) and *Bacillus cereus* strain CH1 (GI: 1465395718) used for phylogenetic analysis.

The maximum likelihood method was used to measure the phylogenetic relationship that grouped various descendants into separate clade A and Clade B in Fig.4.

Clade A and clade B includes various descendants that have shared a common ancestral lineage. *Bacillus cereus* strain CCM 2010, *Bacillus cereus* strain CCM 2010, *Bacillus cereus* strain ATCC 14579, *Bacillus cereus* strain IAM 12605, *Bacillus wiedmannii* strain FSL W8-0169, *Bacillus toyonensis* strain BCT-7112, *Bacillus cereus* strain NBRC 15305, *Bacillus cereus* strain JCM 2152, *Bacillus thuringiensis* strain IAM 12077, *Bacillus cereus* strain ATCC 14579, *Bacillus weihenstephanensis* strain DSM 11821, *Bacillus licheniformis* strain DSM 13, *Bacillus licheniformis* strain ATCC 14580, *Bacillus sonorensis*strain NBRC 101234, *Bacillus licheniformis* strain NBRC 12200, *Bacillus subtilis* subsp. *inaquosorum* strain BGSC 3A28, *Bacillus subtilis* strain DSM 10, *Bacillus aerius* strain 24K, *Bacillus licheniformis*strain BCRC 11702, *Bacillus halotolerans* strain DSM 8802, *Bacillus subtilis* strain IAM 12118 is grouped into clade A. *Bacillus cereus* strain G1 and *Bacillus cereus* strain CH1 grouped into clade B. The estimated average evolutionary divergence over all sequence pairs is 0.163. Evolutionary distance from ancestral lineage till speciation event of clade A is 0.35 and of clade B is 0.37 (Fig. 4).

MULTIPLE SEQUENCE ALIGNMENT

Complete Multiple sequence alignments were constructed using the program Clustal X version 2.0.11 and inspected by eye and edited using GeneDoc 2.7.000. The aligned sequence has color-coding that represents the presence of a conserved region in all the sequences (Fig. 5).

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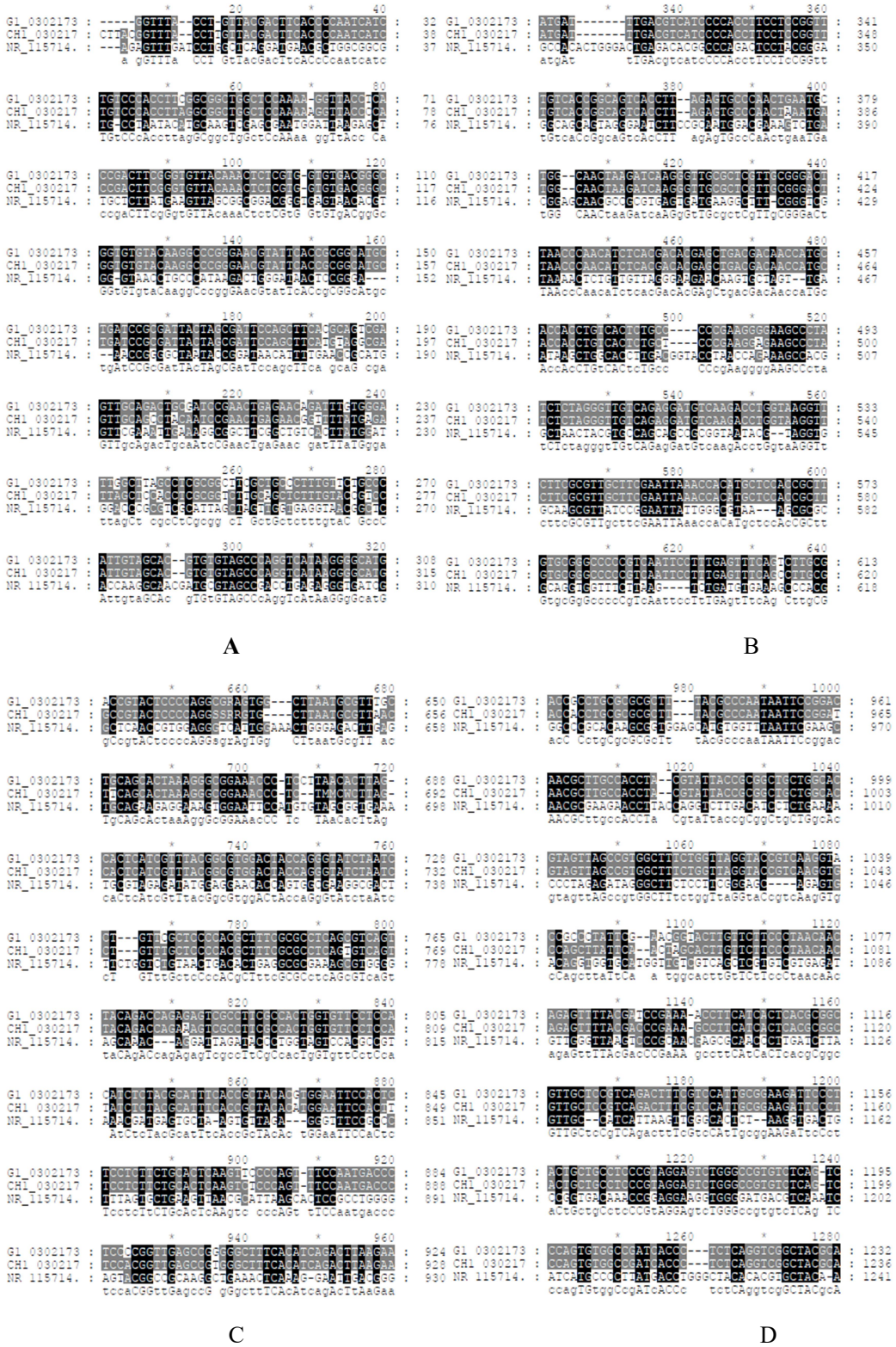

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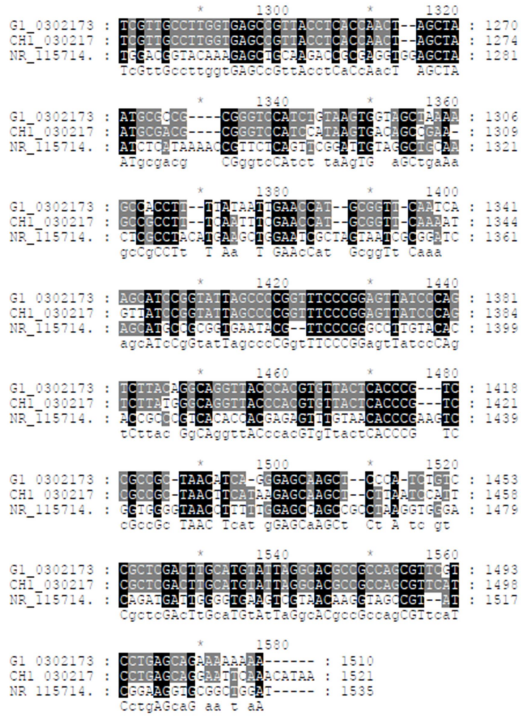

E

Conserved & quantify mode colors		Conserved percent (%)
Primary color	G	100
Secondary colors	G	80
Tertiary colors	G	60
Base color	G	-

Ⓐ

Similarity score in %	CH1	G1	Bacillus cereus strain CCM
CH1	100	94	99
G1	94	100	94
Bacillus cereus strain CCM	99	94	100

Ⓑ

F

Figure-5 Complete multiple sequence alignment of three prokaryotic sequences. Two sequences of isolated bacterial strain CH1 and G1 whereas the third one Bacillus cereus strain CCM is taken from BLAST hits showing highest similarity score with both the isolated bacterial sequences. Legends of **Fig. 5** i.e. **A**, **B**, **C**, **D,** and **E** are shown in the form of images having aligned sequences of nucleotides. Conserved nucleotide sequences are colored according to their distribution in all sequences explained in legend **F** of **Fig. 5**. **'-'** showing the presence of gaps in a specific sequence. Legends **F(A)** alternative color scheme for nucleic acid and **F(B)** BLAST similarity score between each of the sequences. **F(A)** Primary color is given to sequences that are conserved throughout all sequences i.e. 100% conserved sequence. The secondary color is given to those sequences having 80% conserved region. Tertiary color i.e. 60% conserved region and base color to the sequences having very little or no conserved region accordingly.

F(B) correlation coefficient of each sequence with another sequence. Strain CH1 is showing 94% and 99% similarity with G1 and Bacillus cereus strain CCM respectively. Whereas, G1 is showing 94% similarity with each of the strains CH1 and Bacillus cereus strain CCM. The dissimilarity is due to the presence of a 3.8% average gap in all sequences. Gaps are present according to nucleotide position that denotes the deletion of nucleotide from a specific location of a specific sequence.

DISCUSSION

Bio-surfactant activity evaluation possesses a significant role in replacing chemical technology with green technology and is a high priority. In our observation we have found two same species from two different regions, which are classified as *B. cereus* CH1 and *B. cereus* G1, interestingly a similarity score was found 94% between them. In literature, reports on *B. cereus* exhibiting

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biosurfactant activity are already published but there have different origins. Tuleva et al., 2005, have identified a new strain of *Bacillus cereus* 28BN exhibiting biosurfactant activity and naphthalene degradation (Tuleva et al., 2008). In another example, Sriram et al., 2011 have identified strain *Bacillus cereus* NK1 exhibiting biofilm inhibition and anti-microbial action of lipopeptide biosurfactant (Sriram et al., 2011). Cooper et al., 1987 successfully quantified the biosurfactant activity of strain *Bacillus* sp. strain IAF 343. 1987 showing significant biosurfactant activity (Cooper and Goldenberg, 1987). Phylogenetic analysis of all the reported bacterial species having biosurfactant property in the present invention also has proven significant divergence among each other. That is due to the presence of gaps in a nucleotide sequence. Gaps represent the deletion and insertion of gene i.e. point mutation. Now, these variances come in existence to bacterial survival with enhanced existing capability. Each bacterial species has a set of the conserved gene, hence representing its importance and family belongings. *Bacillus* species are important for degrading major class of biosurfactant i.e. lipoproteins. In this study, isolates were showing biosurfactant and emulsification activity as well as they had identical NCI database. For example, Bacillus cereus strain CCM 2010, ATCC 14579, and IAM 12605 had 94% sequence similarity with isolates CH1 and G1. This means they are not identical but are different with a 6% dissimilarity score. Complete sequence alignment was performed against standard sequence length had 1583 base pair. The gap score was around 3.8% to that of the overall alignment score. Phylogenetic analysis also has proven that these isolated sequences have shared a different clade and also have evolved recently.

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Analysis of Chemical profile of renal stones in selected surgical subjects Telangana population

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ABSTRACT

Renal Stones may occur in the kidney, ureter and urinary bladder The present study was carried out to investigate the chemical analysis of kidney stones to provide assistance for appropriate preventive treatment of the patient and to prevent a recurrence of stone formation in Telangana population. A total of 98 kidney stone samples were recovered from patients who were admitted to Krishna Institute of Medical Sciences, Secunderabad, who were subjected to surgical operation 25 females and 73 males; age range 5 to 85 years during 2016 -2018. Gender wise comparison revealed that majority of the stones (78.0%) analyzed were recovered from males 71.54% and females 24.25% The study reported that renal stones were more common in the age group of 31 to 60 years with male predominance than females (3:1). The chemical analysis of renal stones showed that all the assessed stones were of mixed heterogeneous type with calcium oxalate most common (51%) and followed by urate (36.73%) calcium phosphate (8.16%)and combination of calcium, urate with phosphate & carbonates was (2%) each.

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Introduction

Urolithiasis is a common problem worldwide[1,2,3]with a prevalence of 7% in the adults and with ≥30% recurrence rate within 10years[4].The incidence of kidney stones is globally increasing with an estimated prevalence ranging up to 15%[5]. During lifetime, approximately 7% of women and 13% of men will develop a kidney stone [6]

It is common among all age groups, sex and races[7, 8] but occurs more frequently in men than in women within the age of 20–49 years[9].

Kidney stone disease is a crystal concretion formed usually within the kidney. The mechanism of stone formation is a complex process which results from several physicochemical events including super saturation, nucleation, growth, aggregation and retention of urinary stone constituents within tubular cells.

Kidney stones have been associated with an increased risk of chronic kidney diseases[10],

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end-stage renal failure [11, 12], cardiovascular diseases[13, 14], diabetes and hypertension[15]. It has been suggested that kidney stone may be a systemic disorder linked to the metabolic syndrome. Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis[16].

Initially, stone formation will not cause any symptom. Later it presents with renal colic (intense cramping pain), flank pain (pain in the back side), hematuria (bloody urine), obstructive uropathy (urinary tract disease), urinary tract infections, blockage of urine flow and hydronephrosis (dilation of the kidney). These conditions may be associated with nausea and vomiting [17].

Stone composition has a great role in selecting the modality of treatment to be offered. India is the second most populous country in the world for renal stones. The incidence of renal stones in various parts of the country logically varies due to the differences in climate, dietary habits, quality of ground water etc.

We in this study utilized chemical analysis to assess the composition of stones, to find out the most common type of stone. Hence, providing the clinician the information with which he may help the patient in avoiding stone formation.

Materials & methods

Sources of data collection: The data was collected in the KIMS Hospitals from May 2016 -May 2018. It is an observational study.

Categories of patients: A total of 98 patients with renal stones were identified. Clinical data including age, gender, symptoms, signs and composition of stones were evaluated.

The stones obtained from urology department to clinical Biochemistry laboratory were washed with distilled water to remove the debris, dried completely and weighed. The stones were cut and crushed; the powdered form was qualitatively analysed for composition by manual end point colorimetric method adopting standard methods [18] using chemicals of Analytical reagent grade.

The results were analyzed using MS-excel and were presented in tabular form.

Results

Clinical study of renal stone

The overall male to female ratio was 2.95 (Table 1). The age distribution ranged from 5 to 85 years with a mean of 42.42 ± 15.10 years (Table 2). The Table 3 shows the percentage of various stone components in the whole series of 98 stones. Calcium oxalate,(51%), Mixed components of calcium oxalate and Urate were second most common form (36.73 %) and the third most common form was calcium phosphate (8.16%). Mixed form of Calcium oxalate, urate, carbonate components & mixed form with phosphate accounted for 2% & 2% of all stones.

Table 1: Distribution of patients according to gender.

S.No	Gender	Number of patients	Percentage (%)
1	Males	73	71.54
2	Females	25	24.25

Table 2: Distribution of patients according to age.

S No	Group (age)	Number of patients	Percentage (%)
1	1-30	20	19.4
2	31-60	65	63.70
3	61-90	13	12.6

Table 3: A Composite table with distribution of renal stone patients according to age and gender.

S No	Group (age)	Number of patients	Number of males	Number of females	Percentage (%)
1	1-30	20	14	6	19.4
2	31-60	65	47	18	63.70
3	61-90	13	10	3	12.6

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Table 4: Distribution of stones based on composition.

S No	Stone composition	Number of patients	Percentage (%)
1	Calcium oxalate	50	51.00
2	Calcium oxalate and urate	36	36.73
3	Calcium phosphate	8	8.16
4	Calcium oxalate, urate, carbonate	2	2.0
5	Calcium oxalate, urate, carbonate, phosphate	2	2.0

Table 5: Composition of renal stones and their valid percentages

Sex	Frequency	Organic constituents		Inorganic constituents		
		Oxalate	Uric acid	Calcium	Phosphate	Carbonate
Males	72	68	31	71	6	1
Females	26	23	11	26	5	3
Total (%)	98	91(93%)	42 (43%)	97 (99%	11 (11%)	4 (4%)

Table 5 shows the percentage values of major constituents for the chemical analysis. As clearly shown, the major detected components were calcium, oxalate and uric acid. Magnesium, cysteines are not found in all samples. Uric acid also found in males with higher frequency than in females. In contrast, the carbonate stones were more likely to occur in females than in males while the sex of patients showed a difference as far as the composition of kidney stones is concerned for calcium and oxalate.

Discussion

The stone composition can also be an important factor in deciding the modality of the treatment offered to the patients[19].

The stone compositions vary in different populations and are dependent on various factors like geographic location, environmental conditions, dietary patterns, socioeconomic status, water quality etc. The stone composition in our country is different from the rest of the world

In our present study, renal calculi were more common in men when compared to women. As in Table 1, out of 98 patients 71.54 % were male and 24.25% were female. This result is comparable to the studies made by Rajput *et al.*, 2002 who found the male to female ratio is 3:1. Similarly Khan *et al.,* 2004; Nazir*et al*., 2007 also reported the same incidents.

The higher incidence of calculi in men than females may be due to the fact that androgen increases, while estrogens decrease urinary oxalate excretion and kidney calcium oxalate deposition.

As in Table 2, In our present study, prevalence of renal stone was highest for age group of 31-60 years with 62%, 1-30 years of age group of 19.40 % and stone formation in the age group above 61-90 years is (12.61 %). This result was comparable to the study conducted by Rajput *et al* 2002. Similarly Arian *et al*., 1997 reported men in the age of 29 years were more prone for renal stone formation, while Ahmed *et al.,* 1999 reported a maximum incidence of renal stone in the age group of 30-50years.

Composition of renal stones

In our study it was found that (table 3) calcium oxalate crystals (49%) are more common than other crystals which are in positive correlation with Pak et al.,2003[20] who reported that the most common form of kidney stones were

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calcium oxalate (74.8%). Studies in the Durban area, with its high incidence of kidney and bladder stones, indicate that Calcium Oxalate is the overwhelmingly dominant (80%) followed by urate[21]. It is in positive correlation with our study with calcium oxalate being more common. This positive correlation may be due to dietary habits.

Our study is also in positive correlation with Akram et al., (2012) in which most common form is calcium oxalate with 52.6% [22].

Limitations

Study is done in narrow range of population that is in hospital setting. A multicentre study is required to find out in southern India.

Conclusion

Our results emphasize a high percentage of calcium oxalate stones and it was found that the

major risk factors that contribute to the stone formation and its re occurrence are age,

Gender, diet they follow. This study provides assistance for appropriate preventive treatment of the patient and to prevent a recurrence of stone formation.

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ISOLATION OF KERATONOPHILLIC FUNGI AND RELATED DERMATOPHYTES FROM THE SOIL

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isolation, Fungi, Tinea, keratin

ABSTRACT: Dermatophycoses are a group of closely related filamentous fungi that infect only superficial keratised tissue like skin, hair and nails. They are known as keratonophillic fungi, Tinea or ring worm fungi. These fungi occur in many natural and manmade habitats and utilize chiefly products of keratin decomposition. In our investigation various soil samples collected from different habitats were screened for the presence of keratophillic fungi. Soil samples were collected from the vicinity of animal houses, poultry farms, hospitals, barber shops, etc.From the soil samples examined 60% were found positive for the presence of keratophillic fungi and related dermatophytes. Among soil samples collected from a variety of barber shops 100% samples were recorded positive. Soil Samples of other habitats like poultry farm, hospitals, animal house were also found to be positive for keratonophillic fungi.

INTRODUCTION:

Fungi had been recognized as causative agent of human disease earlier than bacteria. Fungi causing favus (*Trichophyton schonleinii*) and thrush (Candida *albicans*) had been described as early as in1839. In spite of the earlier beginning the study of pathogenic fungi has received only scant attention in comparison with the study of other pathogens. This is probably due to relatively benign nature of common mycotic diseases. Fungal infection, however are common and some of them are serious and fatal. With the control of bacterial infections in developed countries fungal infections have assumed greater importance, Keratonophillic fungi along with dermatophytes are responsible for various cutaneous mycoses. Dermatophytes require keratin for growth. These fungi can cause different types of tinea in humans. The majority of the fungi producing diseases in human beings and animals exist freely in nature as soil saprophytes [Kumari et al 2005]. The soils represent the main reservoir of fungi. Some soil fungi are potential pathogen to both human and animals. Soils that are rich in keratinous materials are the most conductive for the growth and occurrence of keratonophillic fungi. The potentially pathogenic keratonophillic fungi and allied geophilic-dermatophytic species are widespread worldwide. The forest, farmyard, park soils, as well as sediments of the rivers and oceans contained humus and organic material are the best candidate for growth of keratinolytic and saprophytic fungi (Mohamed S Ali *et.al* 2000).Most fungi are soil saprophytes and human infections are mainly opportunistic. Modern advances I treatment such as antibiotics, steroids and immunosuppressive agents have led to an increase in opportunistic fungal infections.

Dermatophytes are spread by direct contact from infected people (Anthropophilic organisms), animals (Zoophilic organisms), and soil (geophilic organisms), and indirectly from fomites [Hainer 2005]. Several studies have been shown that soils

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are important sources of dermatophytes and keratonophillic fungi [Hedayati et al 2004]. The presence of dermatophytes in soil can be a reservoir for infection in human beings. In the recent years, many workers have reported the distribution of keratonophillic fungi and related dermatophytes in soils [Ramesh et al 1998; Papini et al 1998].To date, little epidemiological data on fungal flora of soil in this area has become available. The present paper reports the prevalence of dermatophytes and related keratonophillic fungi in the various locations in Warangal T.S India .

Material & Methods

Collection of soil samples

In the present investigation, various soil samples collected from different habitats were screened for the presence of keratonophillic fungi and related dermatophytic fungi. Soil samples were collected from the vicinity of animal houses, poultry farms,hospitals,barber shops, chicken centres. The soil samples were collected from the superficial layers with the help of a spatula in sterile polythene bags. All samples were carried to the laboratory and processed immediately for the isolation of keratophillic and related dermatophytes using baiting technique [Vanbreuseghem 1952; Rahul Sharma and Rajak 2003].

Isolation of keratonophillic fungi can also be done by the other techniques such as the dilution plate method or pour plate method although the hair baiting method is better as the keratinolytic ability is automatically checked if the fungus grows on the de-fatted natural keratin substrate. Once the fungus grows on the keratin substrate in the hair baited plate it can then be transferred onto agar media as these fungi generally can grow on various artificial media

Preparation of keratinic subsrates (Keratin baits)

The following substrates were used as keratin baits. Human hair, Nails, hen feathers, buffalo horn pieces. The samples were collected, washed with distilled water to remove the dust particles and then air dried. The were then cut into pieces and were soaked in diethyl ether for 24 hours. They were then sterilized by dry autoclaving at 15 lbs pressure for 15 minutes and are used as keratin baits.

Isolation of keratonophillic fungi by baiting technique

Approximately 50g of each soil sample was placed into a sterile Petri dish and baited with sterilized small pieces of hair. Each Petri dish was moistened with 5–10mlsterile distilled

water and incubated at room temperature for up to five weeks before being discarded. The growth was observed under the microscope . The isolates were then transferred to sabouraaud's dextrose agar (SDA) medium of the following composition (Peptone 10g, Dextrose 40g,Agar 20g, Distilled water 1000ml)

Results and Discussion

The results obtained are given in the Table 1 and the organisms isolated are given in Table 2.

All the soil samples examined were positive for keratonophillic fungi. Among all the soil samples, the soil obtained from the barber shops exhibited maximum keratonophillic fungi followed by the soil collected from poultry farms and animal houses. The soil obtained near the hospitals only 2 samples exhibited the presence of keratonophillic fungi.

Table 1: Distribution of keratophillic fungi in the soilsamples collected in Warangal

S.No	Source ofsoil sample	Number of soil samples examined	Numberof samples found positive
1	Poultryfarm	05	03
2	Animalhouses	05	03
3	Barber shops	05	05
4	Near hospitals	05	02
5	Chickencentres	05	02

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Keratonophillic fungi are important ecologically and recently have attracted the attention throughout the world .They play a significant role in the natural degradation of keratinized residues (Sharma R, Rajak RC 2003, Fillipello MV, Fusconi A, Rigo S 1994, Fillipello MV 2000.), have many properties in common with dermatophytes and some can probably cause human and animal infections (Connole M 1990,Ali- shatayeh MS *et al*,1989, Filipello MV,*et al*1996, Spiewak R, Szostak W 2000, Spiewak R 1998, Restrepo A *et al* ,1976, Cano J,*et al* 1991).

Keratonophillic fungi are presented in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance. Reports on the presence of these fungi in different soil habitats from different countries, e. g., Egypt, Australia, Palestine, Spain, India, Kuwait, Ukraine and Malaysia, have indicated that this group of fungi are distributed worldwide(Mohamed S Ali et al 2000, Anbu P,*et al* 2004).

Table 2: Distribution of various fungal isolated from different soils in Warangal

S.No	Fungi isolated	Poultry farm	Animal house	Barber shops	Hospitals	Chicken centres	Distribution (%)
1	*Aspergillus niger*	+	+	+	-	+	7.25
2	*Aspergillus flavus*	+	+	+	+	-	7.50
3	*Aspergillus fumigates*	-	-	+	-	-	6.50
4	*Mucor sps*	+	+	+	-	+	8.65
5	*Fusarium sps*	-	-	+	+	-	4.1
6	*Penicillium sps*	+	+	-	+	-	5.25
7	*Microsporum gypseum*	+	+	+	+	+	20.0
8	*Trichophyton rubrum*	+	-	+	-	+	6.5
9	*Epidermophyton floccosum*	-	-	+	-	-	4.5
10	*Microsporum sps*	+	+	+	+	+	12.5

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Keratonophillic fungi like to grow and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof beak of the birds etc. They utilize keratin as carbon source (Cooke 1990). Keratin is highly insoluble protein having fibrous helical structure and numerous disulfide linkages which make it resistant to many proteases but is easily digested by keratinase enzyme [Grant and Long 1981]. The keratinous materials in or on soil are attacked by these keratonophillic microbes, therefore biodegradation takes place. Keratinases also provide the virulence to certain fungi such as dermatophytes to cause dermatophytoses or ringworm in human and animals [Dexter 1983; Fry L and Cornell 1985]. The data revealed that out of 25 samples, maximum number (05/25 ;100%) of keratonophillic fungi was isolated from soils of barber shops; followed by the soil samples from poultries farms (03/25; 75%) and from the soil samples from animal houses (03/25; 75%) . Isolation rates of keratonophillic fungi from soils from hospitals(02/25; 50%) and from soil isolated from chicken centres (02/25; 50%) were almost similar. The least number of keratonophillic fungi was isolated from hospital areas and chicken centres Out of the total isolates most of the isolates contained *Aspergillus niger* as the most common species (7.25%). The soil samples of poultry houses, animal houses, barber shops and hospitals contained *Aspergillus flavus* (7.50%).The soil isolated from barber shops contained *Aspergillus fumigates* which was not present in other isolates.(6.5%).*Mucor* sps was also identified in all the isolates expect the soil isolated near the hospitals.(8.65%).*Fusarium* sps was isolated only from soils isolated from barber shops and hospitals(4.1%).*Pencillium* sps was identified from the isolates of poultry farms,animalhouses and hospitals but not found in the isolates of soil from barber shops and chicken centres.

The keratonophillic fungi *Microsporum* sps and *Microsporum gypseum* was isolated from all the soil samples (20%, 12.5% respectively).*Trichophyton rubrum* was isolated from the soil samples of Poultry farms and barber shops only.(6.5%).*Epidermophyton flocossum* was isolated from barber shops (4.5%).

Figure 1: A and B Colonies showing *Epidermophyton flocossum,C and D* Colonies showing *Microsporum sps and E* Colony showing *Trichophyton rubrum*

Shukia *et al* reported that geophilic *M. gypseum* was only isolated from soil in all the cases. [Shukia 2003] believed that increasing temperature and decreasing humidity lead to providing a less favourable condition for the growth of *M. gypseum* in the soil. *M. gypseum* is a common geophilic dermatophyte widely distributed in soil globally. It causes ringworms of scalp and glabrous skin in human and animal [Mohamed Ali *et al* 2000]. Irshad 2007 reported that that *Aspergillus niger* is the most prevalent keratonophillic fungus and also dominant species that isolated from 51 soil samples of five different regions like fertile lands, animal herds, slaughter houses, poultries and barbers' shops. Isolation rate of keratonophillic fungi including *A. niger* was higher in soil samples collected from the farm lands and poultries.

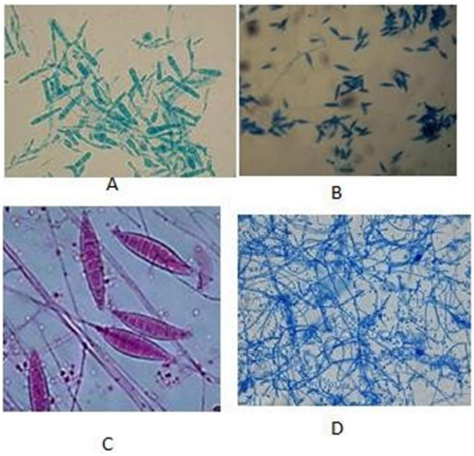

Figure 2: Observations under microscope of A.*Epidermophyton floccosum B. Microsporum gypseum*

C. Microsporum sps .and D. Trichophyton rubrum

Aspergillus flavus was the second dominant species in soils of Gorgan (19.5%) and Gonbade Kavus (19%) areas(Moallaei *et al* 2006). Velasco Benito *et al* 1979 reported the presence of prevalence of *Trichophyton verrucosum* and *Epidermophyton floccosum* was strikingly high in relation to other fungi. Youssef YA *et al* 1992 reported the isolation of keratonophillic fungi by "ToKaVa" hair baiting technique. 22 species belonging to 6 genera were isolated viz.: *Chrysosporium tropicum,C. indicum,*

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C. keratinophilum, C. queenslandicum, C. merdarium, C. anamorph of Arthroderma curreyi, C. pannicola, C. lobatum, C. anamorph of Renispora flavissima, C. pseudomerdarium, Microascus mangini, Malbranchea gypsea, *reesii, Coccidioides immitis, Microsporum gypseum, Mr. distortum Mr. audouinii, Mr. fulvum, Trichophyton mentagrophytes, T. terrestre,T. verrucosum and Epidermophyton floccosum.*

Conclusion

Hence in the present investigations keratonophillic fungi have been isolated in different types of soil in Warangal and they have been identified.

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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE QUANTITATIVE ESTIMATION OF SECNIDAZOLE IN TABLET DOSAGE FORM

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KEY WORDS: *Secnidazole, RP-HPLC, new method development, validation*

ABSTRACT A novel very rapid, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative estimation of Secnidazole in bulk and tablet dosage form. The developed HPLC has several advantages over reported HPLC methods with respect to speed, solvent consumption, resolution and cost of analysis.It was resolved by using a mobile phase of Acetonitrile and phosphate buffer in the ratio of 70:30 v/v at a flow rate of 1.0 mL/min. using UV - Visible detector at the wavelength of 365 nm for quantification. Efficient separation was achieved for secnidazole on Cosmosil C_{18} (100 × 2.1 mm, 5 µm). The retention time of secnidazole was 3.11 min. The calibration graphs were linear and the method showed excellent recovery for tablet dosage form. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness

1. INTRODUCTION

Secnidazole chemically is1-(2-methyl-5-nitro-1H-imidazol-1-yl)propane-2-ol [1] (figure.1). Its empirical formula is $C_7H_{11}N_3O_3$with a molecular weight 185.180. Secnidazole is a nitro imidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria. It is aanti infective drug[2].

Structure of drug

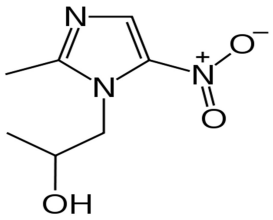

Figure No 1: Structure of secnidazole

The survey of literature suggests few methods for the determinations of secnidazole in bulk and tabletsof which two UV methods available for the determination of secnidazole [3,4]. It was to be noted that secnidazole was determined by two HPLC methods [5,6]. It was also found two UPLC methods for the estimation of secnidazole[7,8]. The reported UVmethods were time consuming and accurate, the UPLC methods were expensive and the HPLC methods were not possessing well resolution and requires expensive solvents.

So, therefore, it was felt necessary to develop a new rapid, economical and sensitive method for the determination of Secnidazole by HPLC method. Hence a reproducible RP-HPLC method was developed for the quantitative determination of Secnidazole tablets by using Cosmosil C_{18} column (100 × 2.1 mm, 5 µm).HPLC column. The proposed method was

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validated as per the guidelines suggested by ICH[9].

Material and methods

2.1 Reagents

Secnidazole Working Standard was procured from Aurabindho laboratories, Hyderabad, India. Commercially available Secnidazole purchased from local pharmacy. Methanol HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

2.2 Chromatographic conditions

Chromatography separation was performed on LC Solution HPLC with UV detector. The output signal was monitored and processed using Chrom- work station HPLC V4.0 software. The chromatographic column usedCosmosil C_{18} (100 × 2.1 mm, 5 µm).The mobile phase of Acetonitrile and phosphate buffer in the ratio of 70:30 v/v at a flow rate of 1.0 mL/min. The detection was monitored at the Wavelength of 365 nm. The injection volume was 20.0 µL and the chromatographic runtime of 8 min was used.

2.3 Preparation of solutions

2.3.1 Preparation of Sodium Phosphate buffer: Weighed 7.0 grams of Potassium di hydrogen phosphate into a 1000mLbeaker, dissolve and diluted to 1000mL with milli pore water. Adjusted the pH to 4.0 with ortho phosphoric acid.

2.3.2 Preparation of mobile phase: Mixed a mixture of above buffer 300mL (30%) and 700 mL of acetonitrile (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4 Preparation of the Secnidazole Standard & Sample Solution:

2.4.1 Standard Solution Preparation:

Accurately transferred 10mg of Secnidazole working standard into a 10 mL volumetric flask and take 1 mL of diluent added 9ml of mobile phase then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent(Stock solution). Further pipetted 0.5 mL of the above stock solution into a 10mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.4.2 Sample Solution Preparation:

Accurately transferred the sample equivalent to 10 mg of Secnidazole into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Further pipetted 5 mL of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipetted 3 mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

2.5 Method validation

2.5.1 Precision:

The precision of the method was evaluated by carrying out five independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated (% RSD should not be more than 2%).

2.5.2 Intermediate Precision/Ruggedness:

2.5.2.1 Intra-day precision: The precision of the assay method was evaluated by carrying out five independent assays of Secnidazole (50,100, 150% i.e. 5.0, 7.5, 10. µg/ml.) test samples against qualified reference standard. The percentage of RSD of five assay values was calculated.

2.5.2.2 Intermediate precision (inter-day): Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the five samples of Secnidazole against qualified reference standard. The percentage of RSD of five assay values was calculated. The %RSD for the area of five replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

2.5.3 Accuracy:

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Recovery of the assay method for Secnidazole was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was injected thrice (n=3) into HPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

2.5.4 Linearity:

Test solutions were prepared from stock solution at 5 concentration levels (10,20,30, 40,50, and 50µg/ml). The peak area vs. concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

2.5.5 Limit of Detection (LOD) Limit of Quantification (LOQ):

LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

2.5.6 Robustness:

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

3. RESULTS

3.1 Method development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Cosmosil C_{18} (100 × 2.1 mm, 5 µm).column used for the elution, but the peak eluted before 3.11 minutes with a tailing factor of 2. Cosmosil C_{18} (100 × 2.1 mm, 5µm) column ended with inconsistent retention time and peak fronting. Buffers like sodium dihydrogen orthophosphate, dipotassiumhydrogenorthophosphate, and disodium hydrogen orthophosphate did not yield desired results. Use of ion pair reagents also did not yield the expected peak.

At 70:30 (solvent : buffer) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 70:30 (solvent :buffer) in an isocratic mobile phase flow rate. The typical chromatogram obtained for Secnidazole from final HPLC conditions are depicted in Figure2.

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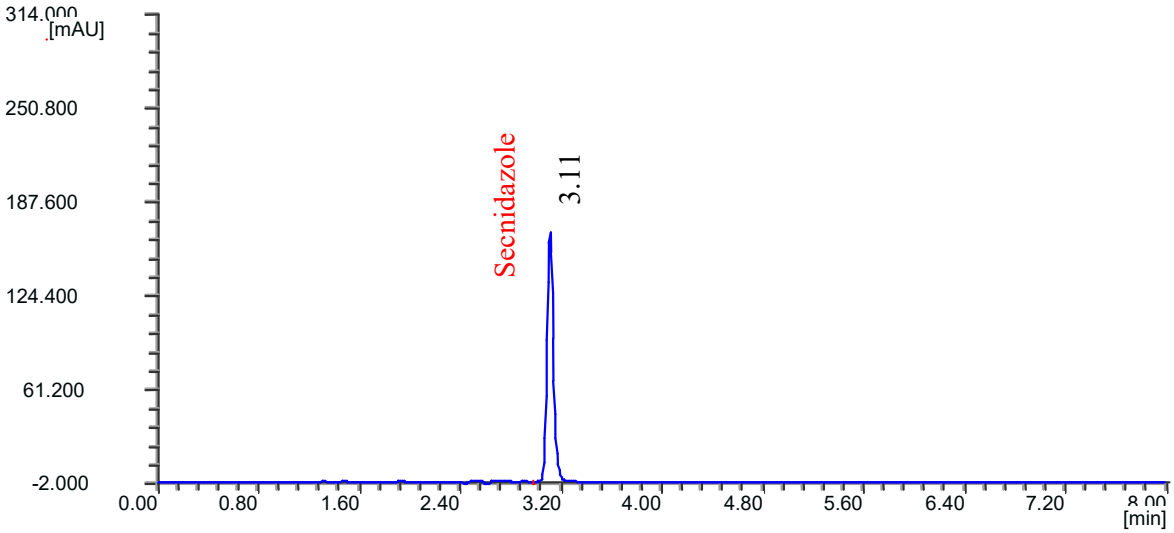

Figure No : 2 typical chromatogram of Secnidazole by proposed method

3.2 Method validation

Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ , robustness and sensitivity as follows.

3.2.1 System suitability

The system suitability results for the proposed HPLC method are Tailing factor Obtained from the standard injection is 1.4 Theoretical Plates Obtained from the standard injection is 13684.41. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

3.2.2 Precision

The % R.S.D. of Secnidazole assay during the method precision was found to be 1.63%, indicating good precision of the method. The results are summarized in table 1.

Table 1- Results of precision

Injection	Area
Injection-1	70271.4
Injection-2	70823.8
Injection-3	71652.9
Injection-4	73434.7
Injection-5	73212.1
Injection-6	72503.7

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Average	71983.1
Standard Deviation	1174.133
%RSD	1.63%

3.2.4 Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for Secnidazole were 0.01 and 0.05µg/ml, respectively. Since the LOQ and LOD values of Secnidazole are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

3.2.5 Accuracy

Percentage recovery of Secnidazole samples ranged from 100.0% to 101.2% and the mean recovery is 100.5%, showing the good accuracy of the method. The result is shown in Table 2.

Table No: 2 Results of Accuracy

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	23634.6	5.0	5.0	99.94%	99.83%
100%	48221.2	7.5	7.5	99.94%	
150%	75883.7	10.0	10.2	99.83%	

3.2.6 Linearity

The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 10 - 50 µg/ml for three times, and the correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

3.2.7 Robustness

In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 20 -60 µg/ml, (n=3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% and change in the Mobile phase ±10%. The results are summarized in table 3.

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Table- 3- Results of Robustness

Chromatographic changes	*USP Plate Count*	*USP Tailing*
Flow rate(ml/min)		
0.8	12017.73	1.36
1.0*	7145.78	1.29
1.2	11495.57	1.42
Change in organic composition in the mobile phase		
10% less	8441.17	1.33
70:30*	11127.27	1.38
10% more	97901.14	1.27
UV wavelength(nm)		
363	11568.29	1.36
365*	11977.79	1.68
367	11275.60	1.40

* optimized parameters

3.2.8 Application of the developed method to commercial Secnidazole tablets

When the developed method was used to analyze a commercial brand of Secnidazoletablet formulation, the mean recovery of five replicates was 99.69 % with % R.S.D. of 0.52. The % recovery value indicates non-interference from the excipients present in the dosage form.

DISCUSSIONS

Method development and optimization

The main aim of the developed method was to achieve separation and quantification of Secnidazole using an isocratic mobile phase with HPLC system. Developing a HPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Secnidazole was adequate at 365 nm. The initial trial was conducted using HPLC and chromatographic separation was obtained on a Cosmosil C_{18} (100 × 2.1 mm, 5 µm). Secnidazole is an acid labile compound and to avoid any degradation, a mobile phase with basic pH was selected. The mobile phase was optimized in the ratio of Acetonitrile and phosphate buffer in the ratio 70:30% v/v at a flow rate of 1 ml/min. While developing the HPLC method, basic chromatographic conditions such as the column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the HPLC column, its stability at the lower pH was taken into consideration to preserve the long life of the column. Most commercial C_{18} columns are not stable at high pH on the longer run, thus shortening their life span. Cosmosil C_{18} (100 × 2.1 mm, 5 µm).column was found to be more suitable and stable at this pH. The peak was sharp and acceptable. The flow rate also is scaled down from 2.0 to 1.0 mL/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for Secnidazole, which eluted at around 3.11 min giving a total run time of 8 min.

4. CONCLUSION

The new, isocratic RP-HPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 3.11 min allows the analysis of a large number of samples in a short period of time

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and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of Secnidazole in tablet formulations.

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Pivotal Role of rhizobacteria in induced drought resistance in crop plants.

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ABSTRACT

Soils are generally a moist environment rich in highly reduced carbon, which wires wide-ranging soil microbial populations. Generally, microbes have affinity to companion with plants to survive and alleviate the stress. Plants normalize the structure and activity of their linked microbial communities carefully. These microbes manage to compensate a wide range of facilities and benefits to the plant, in return, the plant provides the microbial communities with soluble carbon, nitrogen compounds, and other metabolites. Rhizobacteria plays a vital role in induced drought resistance in crop plants. In the preset review deal with the microbial induced drought resistance.

Introduction

The underlying concept of organic farming is to nutritional enrichment of crops and food security in the 21st century. Hence, it requires the sustainable agriculture strategies. The signal exchange between the plant roots and PGPR relationships would greatly descripts the modulatory mechanism of plant abiotic stress responses via induced systemic resistance. The improved PGPR inoculants with survive more under stress conditions is need some.

Soil microorganisms directly or indirectly render by conserving soil profile and plant health. Various groups of biofertilizers are specialized in ability of plant health promotion. Varieties of biofertilizers are specialized in various biofertilizers activates includes:

(1) Biofertilizers with Nitrogen-fixing ability

These biofertilizers consist of a) free-living microbes viz., *Azotobacter, Bejerinkia, Clostridium,Klebsiella, Nostoc* and symbiotic microbes viz., *Anabaena, Azospirillum, Frankia, Rhizobium*. These transform atmospheric dinitrogen into bioavailable organic nitrogen;

(2) Biofertilizers with phosphate solubilizing ability

In soil phosphate is present in biologically non available insoluble form of rock phosphate. Soil flora such as *Bacillus subtilis, Bacillus circulans*, *Phosphaticum and Pseudomonas putida* are capable f solubilize phosphate these microbes used as phosphate biofertilizers for crops. Fungi like *Penicillium* spp. and *Aspergillus* spp. are also phosphate solubilizing in nature.

(3) Biofertilizers with phosphate mobilizing ability

These biofertilizers include numerous classes of mycorrhizal fungi, for instance ectomycorrhiza (Amanita spp., Boletus spp., Laccaria spp., closely associated with plant roots. Endomycorrhiza incudes arbuscular mycorrhiza

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(*Acaulospora* spp., *Glomus* spp., *Sclerocystis* spp., etc.)

(4) **Biofertilizers with potassium solubilizing ability**

Generally, potassium present in mineral silicates form and is not easily acquisition to the plant, hence soil microorganisms solubilize these silicates and releasing the potassium from the metals and mobilize it to make available to plant's acquisition

(5) **Biofertilizers with Sulphur oxidizing ability**

Microbes like *Thiobacillus* spp. and used as sulphur oxidizers as these microbes oxidize sulphur into sulphates, which are nicely utilized by the plants;

(6) **Biofertilizers with Plant growth-promoting ability**

Plant growth-promoting biofertilizer are composed of plant growth-promoting rhizobacteria, which play a vital role in plant growth and devel- opment. They promote the synthesis and concentration of plant growth hormones and enhance plant growth by secrete auxins, gibberellins, ethyl- ene, indole acetic acid; *Pseudomonas* promote root growth and influence the decomposition of organic matter for better nutrient availability

Development of drought tolerant crop varieties through conventional breeding is a long and anxious process. Whereas, the PGPR induced drought stresses in plants opens a new avenue in the dry land agriculture. The captivating current clues available in PGPR induced drought stress technology will be abridged the gap for future research in terms of identification of potential PGPMs and designing better, field evaluation and supply systems.

Table-1: **Contribution of drought tolerance by PGPR in plants,** they induce physical and chemical changes in plants that results in improved tolerance to abiotic stresses (Yang et al, 2009).

Plant species	PGPR	Mode of Action
Arabidopsis	*Phyllobacterium brassicacearum*	ABA enhanced to decrease the leaf transpiration capacity
Lavandula dentate	*B. thuringiensis*	IAA improves K-content, proline and decreases the glutathione reductase and ascorbate peroxidase
Maize	*Azospirillum lipoferum*	Gibberellins increases ABA levels to alleviate drought stress
Platycladus orientalis	*Bacillus subtilis*	Cytokinin directed ABA enhancement levels in shoot, increases the stomata conductance
Soybean	*P. putida*	Gibberellins improves the plant growth
Tomato	*A. brasilense*	No as a signaling to IAA then enhance the lateral root and root hair growth
Wheat	*Azospirillum sp.*	IAA enhanced root growth, lateral roots and increased uptake of nutrients and water under drought stress condition
Wheat	*Rhizobium leguminosarum* , *Mesorhizobium ciceri* and *Rhizobium phaseoli*	The consortia improved the IAA production then improves the growth, biomass and drought tolerance index

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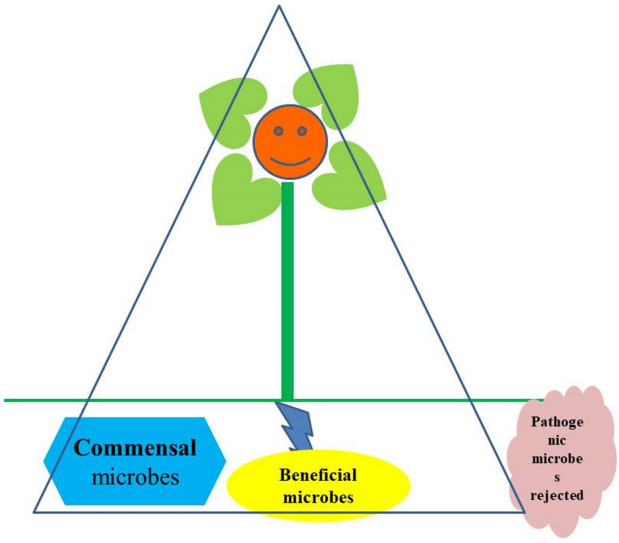

Figure-1: Protection offer by indigenous PGPR

The rhizosphere microbiome

The rhizosphere microbiome of given crops have a great importance to agriculture, due to presence of rich variety of root exudates and plant cell remains, that attracts a diverse and unique form of microflora to colonize.

The plant rhizosphere microbiome shows a pivotal role in nutrient acquisition, absorption, soil texture improvement, exudation and modulation of variety of extracellular biomolecules such as various signal compounds, hormones, antibiotics likes secondary metabolites, etc all are prime to an enhancement of plant growth and development. These rhizosphere microbiome secretes constitutional as well as induced valuable bio stimulants and modulating plant stress responses.

Table-2: PGPR microbiomes Salient Features

1. **Mycorrhiza and Rhizobia**
   These microbiomes help in natural weathering of minerals, degradation of organic matter and recalcitrants. In return, the microbiome is nurtured with soluble nutrients present in rhizodeposits and root exudates.

2. **Mechanisms**
   competition for space and nutrients, production of lytic enzymes, nutrient consumption and antibiotic compounds to reject pathogen

3. **To boost the plant defense.**
   PGPR microbiomes have the natural ablity to boost the plant defense system is primed for induced systemic resistance.

4. **Symbiosis**
   Expression of analogous molecular patterns leads to differentiate associate from antagonist

5. **Pathogensis and Immunity**
   Both associate and pathogens are also known to cntrole plant response to promote their establishment via synthesis of effector molecules-Effector triggered Immunity

6. **Density of rhizosphere microbiomes**
   The microbial density is typically higher than in bulk soil (10^8 to 10^9 bacteria per gram

7. **Host and Geo Specificity**
   The rhizo microbiomes of the plants grown in the gegical sites of same soil differ in plant as wellas microbiomes

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Rhizobacteria Directing the Plant to Produce Signal Compounds

Plant-growth promoting rhizobacteria directs the plant to produce signal compounds that can effectively manage to overcome the unfavorable condition and stimulate growth. Additionally, these schemes can advance crop tolerance against drought, heat, and salinity. Which are more frequent because climate change continues to harness the development of crops. To overcome the problem the multifunctional PGPR-based formulations will save agriculture to minimize the use of synthetic fertilizers and agrochemicals.

PGPR determines resistance to water stress in plants other than the original (Marasaco et al, 2018). Resistance to a wide set of abiotic conditions and were able to perform different plant growth-promoting activities and root colonization of halophilic/halotolerant bacteria inhabiting salty and arid ecosystems have the potential to alleviate salinity and drought conditions (Mapelli et al., 2013).

A variety of rhizobacterial strains reported from degraded environments (Pires et al., 2017), these bacterial strains possess plant growth-promoting traits like siderophore and indole acetic acid (IAA) production, and 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity, and metal pollution resistance (Moreira et al., 2016).

Cis-element	Plant specie	Protein
Drought Responsive Element (DRE)	*Arabidopsis thaliana*	DREBs
5'-TACCGACAT-3'	*Oriza sativa*	OsDREBs
	Triticum aestivum	TaDREB
	Atriplex hortensis	AhDREB1
C-repeat Responsive Element (CRT) 5'-TGGCCGAC-3'	*Arabidopsis thaliana*	CBFs
	Brassica napus	BnCBFs
Drought Responsive Element 1 (DRE1) 5'-ACCGAG -3'	*Zea mays*	Unidentified maize protein
Drought Responsive Element 2 (DRE2) 5'-ACCGAC-3'	*Zea mays*	DBF1 and DBF2

Various Cis -acting elements in drought regulation

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Consequence of Drought Stress

Drought known to upsets plant leaves turgor pressure and water potential it directly restricts the normal physiological and morphological qualities in plant system (Rahdari and Hoseini, 2012). Additionally, it affects the accessibility and transportation of nutrients to the roots. Drought stress consequently declines the water-soluble like Ca, Mg, Si and inorganic nitrate, sulfates, etc (Selvakumar et al., 2012). It also recognized that, drought prompts the accumulation of free radicals superoxide radicals, hydrogen peroxide and hydroxyl radicals and Reactive Oxygen Species (ROS) that results in over burden oxidative stress that badly affects antioxidant defenses.

Mechanisms of Drought Tolerance

Drought Tolerance defined as the capacity of plants to adapt, endure with a consistent functional accomplishment through the temporal, fine modulation of several genes and numerous metabolic pathways to reduce the consequential damage subjected by dryness or adverse environmental conditions (Mitra, 2001). Drought is known to bring drastic effects in plant physiology, where decreased relative water content of the leaf in plants of both cultivars under well-watered conditions and membrane stability.

Microbes dwell at plant roots have very complex ecological groups that provoke plant growth through its metabolic activities, interactions. The ecological selection pressure, that changes the structure and composition of plant- root associated communities at endosphere, rhizosphere and the root surrounding soils towards abiotic stress resistance and drought tolerance to promote health of plants (Schmid et al, 2014).

To better comprehend the PGPR mediated drought stress tolerance in plants a systemic study of strategies is necessitated. Strategies includes, modification of root morphology, action of phytohormones, volatile compounds, accumulation of osmolytes, EPS production,, ACC deaminase activity, antioxidant defense and co-inoculants contribution finally leads to Induced Systemic Tolerance.

Conclusion

Microbes have tendency to associate with plants to stabilize and plants known to regulated by their associated bacteria. Hence, analysis of these microbes shall provide a wide range of interactions and unveil the benefits to the plant. The range of carbon, nitrogen compounds, and other metabolites shuttle will discloses the controlled regulation of druoght. Such findings supports excusive nature of soil microbial communities. The future research needs to be in developing efficient microbial formulation for boosting plant performance under drought stress that substantially reduces the use of chemical fertilizers and pesticides. The research should focus on to isolate indigenous PGPR from the stress-affected soils that could be used as bioinoculants for crops grown in stressed ecosystems.

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Albendazole and its biotransformed metabolites as inhibitors of Glutathione peroxidise (GPx1): A potential target for cancer treatment: An *in silico* study

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ABSTRACT Albendazole, an antihelminthic drug and its biotransformed metabolites were investigated *in silico* for glutathione peroxidise (GPx1), a crucial antioxidant enzyme inhibition potentials. Albendaole sulfoxide (M1) the main metabolite of albendazole and albendazole sulfone (M2) exhibited strong interaction with GPx1 followed by metabolites M3 and M4 of albendazole when compared to the standard reference drug misonidazole. The parent drug albendazole showed moderate interaction with GPx1 compared to all the metabolites in the study. In the present investigation, among all the four metabolites of albendazole, M1 and M2 showed strong binding affinity with GPx1 enzyme which is over expressed and protects the tumour cells. Hence, from the present study, it can be concluded that albendazole can be used as antihelminthic drug and also against tumour arising from helminthic infections as metabolites of albendazole has strong GPx1 inhibition potentials. However, further investigations are needed in vitro and in vivo for further confirmation.

Introduction

Bacteria, viruses and parasitic infections cause more than 20% of cancers (Brindley et al., 2015). Helminthic infections which are the most common in developing countries also induce cancer. Infections with *Opisthorchis viverrini*, *Clonorchis sinensis* and *Schistosome haematobium* are classified as group 1 biological carcinogens which are a definitive causes of cancer (Brindley et al., 2015). Carcinogenesis associated with helminth infections is a complex process involving several different mechanisms, but chronic inflammation is a key feature caused by the presence of parasites or deposition of parasite products in tissues (Vennervaldi and Polman, 2009). In response to parasitic infections, inflammatory cells such as macrophages and eosinophils generate free radicals and nitrogen species which oxidise and damage DNA, initiate DNA mutations and lead to genetic instabilities and malignant transformation (Mayer and Fried, 2007). There is also evidence of high rate of parasitic infections in cancer patients, especially those undergoing chemotherapy and these infections can be a severe threat to immunocompromised patients (Zabolinejad et al., 2013). Bile duct cancer and bladder cancer can be directly attributed to parasites (Cancer Research UK (2019).

Reactive oxygen species (ROS) are generated by all cells during normal oxidative respiration. Cancer cells contain an inherently greater amount of ROS than normal cells because of an enhanced metabolism and mitochondrial dysfunction (Tafani et al., 2016) . Increased ROS levels have been linked to cancer initiation, malignant transformation and resistance to chemotherapy. These ROS levels are countered by the increased activity of

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antioxidant enzymes in cancer cells to avoid the detrimental effects of oxidative stress (Lee et a., 2017). Among the antioxidant enzymes, Glutathione peroxidise (GPx) family is one of them whose main biological function is to protect the organism from oxidative damage and its biochemical function is to reduce hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Baken et al.,2003). Eight isoenzymes of GPx are known, five of which (GPx1–GPx4 and GPx6) make use of an active site selenocysteine while for the other GPx s an active site cysteine is present (Flohe et al.,2013). It has also been observed that increased GPx activity in cancer cells can be involved in the resistance mechanism against anti-cancer drug treatment (Cornwell et al., 2020). Recently, GPx4 has been brought into contention as an antiferroptotic enzyme in the survival of therapy-resistant cancer cells across diverse mesenchymal cell origins (Cornwell et al., 2020). Among the GPx family enzymes GPx1 is a crucial antioxidant enzyme involved in preventing the harmful accumulation of intracellular hydrogen peroxide (Lubos et al., 2011). It has been demonstrated that many cancer cells require oxidant scavenging and the upregulation of antioxidant enzyme expression for tumor progression and metastasis (Chang et al., 2020). Thus, the development of GPx inhibitors could offer a promising avenue to novel anticancer drugs.

Albendazole (ABZ) is a benzimidazole carbamate with a broad anti-parasitic spectrum which was first approved for treatment of helminth infections in sheep in 1977, and subsequently approved for human use in 1983 (De Silva *et al*., 1997). In general, most ascariasis, trichuriasis, enterobiasis and hookworm infections can be successfully treated with single dose of ABZ, and strongyloidiasis with multiple doses of ABZ. ABZ is also used in the treatment of capillariasis, gnathostomiasis, and trichostrongyliasis, the cestode infections hydatidosis, taeniasis, and neurocysticercosis, and the tissue nematodes cutaneous larval migrans, toxicariasis, trichinisis and filariasis (in combination with other antihelmintics). ABZ has also been used successfully against mixed infections.

In our previous study, (Prasad et al., 2011) we reported biotransformation of antihelminthic drug albendazole using Thermophilic fungi *Rhizomucor pusillus* NRRL 28626 into four metabolites. In the present work we report glutathione peroxidase (GPx-1), a crucial antioxidant enzyme inhibition potentials of all the four metabolites viz.M1, M2, M3 and M4 of antihelminthic drug albendazole.

Materials and methods

Preparation of target Protein:

Molecular docking of albendazole and its four metabolites produced by thermophilic fungus *Rhizomucor pusillus* (Prasad et al., 2011) was performed with glutathione peroxidise receptor (GPx1) using Auto dock vina software, an interactive molecular graphics programme to understand the protein-ligand interactions (available from http://viba.scripps.edu/). The crystal structures of (GPx1) (PDB I D: 1GP1) with resolution of 2.0°A was obtained from PDB data base (http://www.rcsb.org./pdb). The bound ligands, hetero atoms, water molecules were removed, and polar hydrogen atoms were then added, Kollman charges and salvation parameters were assigned by default using Auto dock software

Preparation of Ligands:

The metabolites of albendazole viz.M1, M2, M3 and M4 and the standard reference drugs Misonidazole which were used as ligands in the present investigation were drawn using Chem Draw software and converted to 3D PDB format from mol format by Accelrys Discovery Studio 2.3. 2.3.

Validation of Software:

The software Autodock was validated by downloading the X-ray crystal structure of the receptors glutathione peroxidise (PDB I D: 1GP1) from protein data bank and redocking the co-crystallized ligand reproducing the original interactions of the reference protein-ligand complexes comparing the root-mean square distance of the experimentally determined pose with the docked pose.

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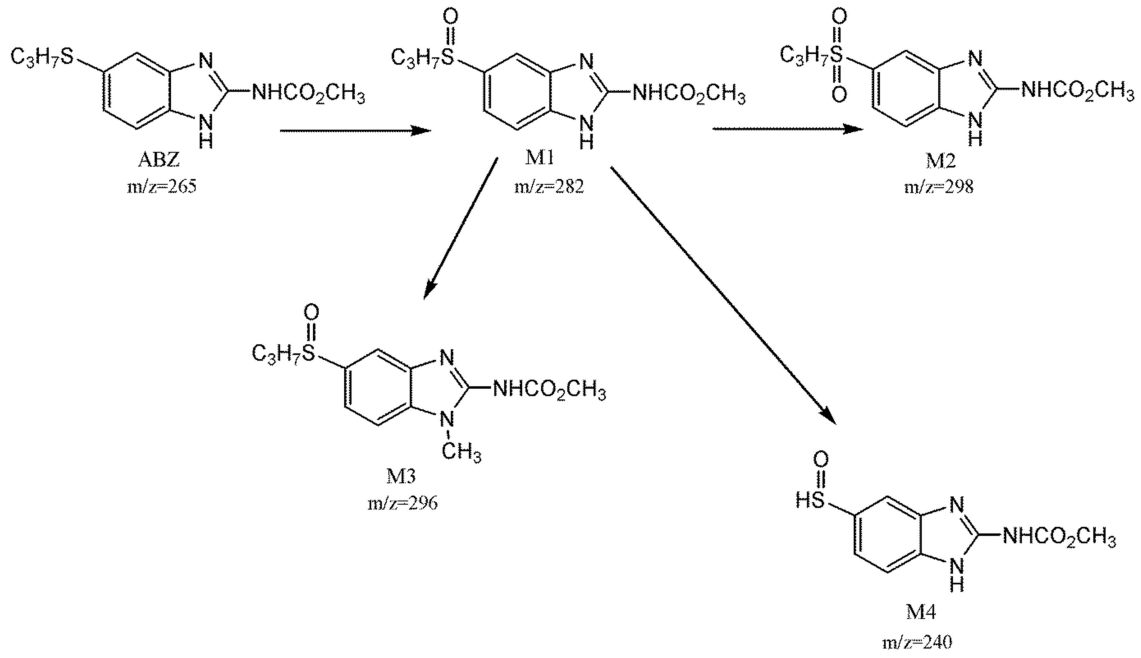
In silico studies:

Virtual screening for interaction of ligands albendazole and its four metabolites with receptor GPx1 (PDB I D: 1GP1) was performed by molecular docking. After preparing the ligands as well as receptor, both were converted into the pdbqt format using the automated docking tool Auto Dock which was later used for docking. A grid box was prepared to cover the pocket with the main residues of protein binding site by maintaining the grid size of X = 40, Y = 40, and Z = 40. Coordinates used for docking the ligands with 1GP1 were x=34.057; y=62.084; z=26.267. An advanced molecular docking program Auto Dock vina, version 1.1.2 available from http://vina.scripps.edu/download.html was used for docking against the receptor to estimate the binding affinities (kcal mol-1). The ligands were evaluated *in silico* against glutathions peroxidase (PDB ID: 1GP1) in triplicates and the average of the best conformation was chosen with the lowest docked energy, based on complete docking search (ten runs). The interaction of glutathions peroxidase with the ligands, hydrogen bonds, bond lengths and Root Mean Square Difference (RMSD) was analyzed using PyMOL software (http://pymol.sourceforge.net/).

Results and discussion

In our previous study, we reported four metabolites (Fig.1) of anti-helmintic drug albendazole viz. M1, M2, M3 and M4 (Prasad et al., 2011).



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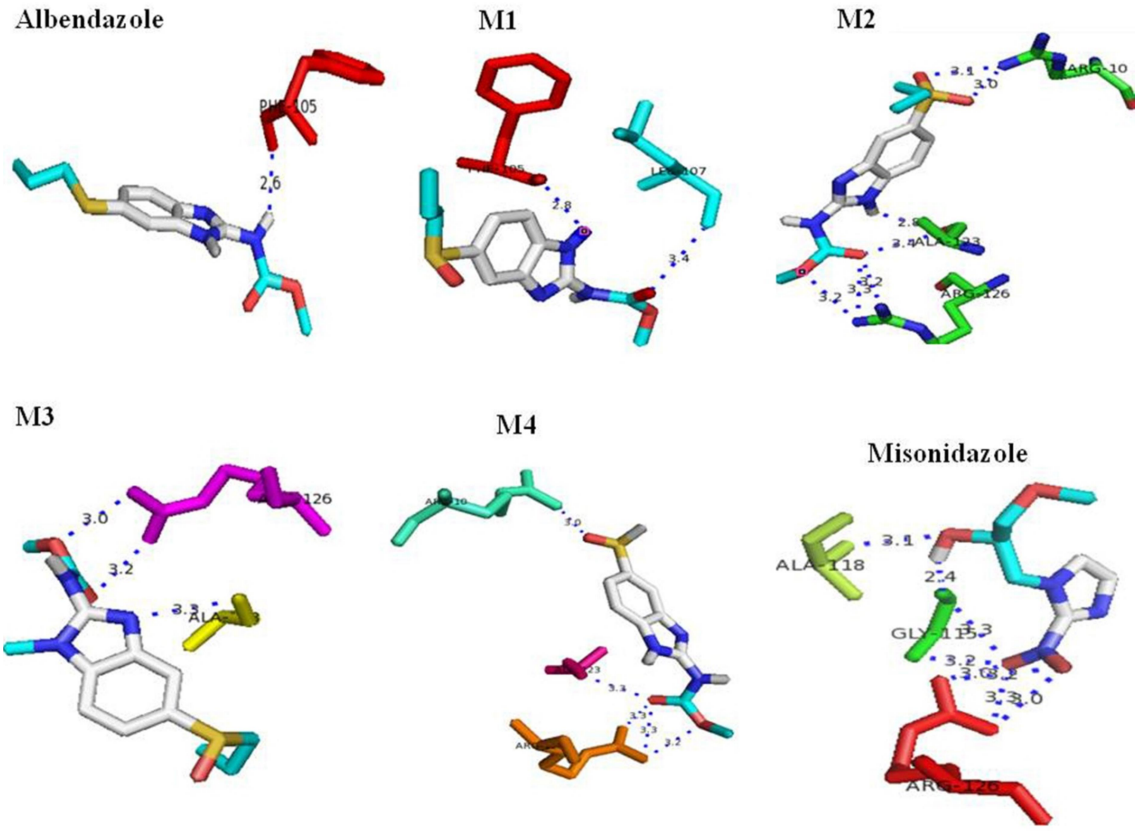

Figure Legends

Fig.1 Proposed metabolic pathway of albendazole in culture broth of *Rhizomucor pusillus* NRRL 28626 (Prasad et al., 2011)

Fig.2 Protein-Ligand complex of GPX1 receptor with different ligands

In the present investigation a preliminary study was performed to find GPx1 inhibition potentials of Albendazole and four of its metabolites to find a dual activity of Albendazole to treat both helminth infections and tumor associated with helminthic infections.

Insilico studies of Albendazole metabolites with receptor GPx1:

Molecular docking of test compounds into the active site of GPx1 was found to be successful based on the formation of complexes of GPx1 with the ligands M1, M2, M3 and M4. The hydrogen bond interactions, binding energy, bond length, RMSD, active site residues and orientation of the docked compounds within the active site were visualized. All the test compounds screened showed the best RMSD value of 0.000, indicating statistically significant interaction. The negative and low value of ΔG indicated a strong favourable bonding between GPx1 and the ligands in their most favourable conformations.

The binding energies of metabolites M1, M2, M3 and M4 recorded were -5.3, -5.1, -4.9, -4.9 respectively, indicating a relatively higher interaction of test compounds with GPx1. The details of binding energies, the number of hydrogen bonds formed and the catalytic site residues involved in the protein-ligand complex of GPx1 with different metabolites of Albendazole were depicted in table 1 and fig.2

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Name of the Ligand	Affinity kcal/mol	Number of hydrogen bonds	Distance (A^0)	amino acids of residues interacting	Interacting Ligand atoms	
Albendazole	-4.4	1	2.6	Phe-105/O	O-HN	
M1	-5.3	2	2.8	Phe-105/O		O-184
			3.4	Leu-107/O		O-238
M2	-5.1	6	3.2	Arg-126NH2		O-238
			3.3	Arg-126/CZ	O-8	
M3	-4.9	3	3.3	Ala-123/O		O-62
			3.0	Arg-126/NH1	O-309	
			3.2	Arg-NH2		O-309
M4	-4.9	4	3.3	Arg-126/NH2		238-O
			3.3	Arg-126/NH1		238-O
			3.2	Arg-126/NH1		309-O
			3.3	Ala-123/O		238-O
Misonidazole	-4.6	6	3.1	Ala-118/N		392-O
			2.4	Gly-115/N	210-OH	
			3.3	Gly-115/N		529-O
			3.2	Gly-115/N	529-O	
			3.0	Arg-126/NH1		529-O
			3.3	Arg-126/NH1		529-O
			3.2	Arg-126/NH2		529-O
			3.0	Arg-126/NH1	529-O	

Table.1 Interacting amino acids, H-bonds, distance and binding scores of Glutathione peroxidise GPx1 (PDB ID 1GP1), with albendazole and its four metabolites viz.M1,M2, M3 and M4.

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In the present *in silico* study, among the metabolites of albendazole, strong binding of with GPx1 was exhibited by metabolite M1 followed by M2 whereas, least interaction was shown by metabolites M3 and M4 while the parent drug albendazole less binding compared to metabolites M3 AND M4. All the metabolites exhibited high binding energies compared to the reference drug misonidazole.

Conclusion

Tumour cells have high levels of ROS than normal cells causing oxidative damage of proteins, DNA and lipid thus acting as toxic agents leading to cell death. But cancer cells survive by over expressing antioxidant enzymes like GPx, thus promoting cell proliferation and differentiation. In the present study, an antihelminthic drug and its four metabolites showed strong inhibition potentials of a crucial antioxidant enzyme glutathione peroxidise (GPx1).Hence, it can be concluded that both helminth infections and tumour associated with helminth infections can be treated with single drug albendazole. However, further *in vitro* and *in vivo* investigations are needed.

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A FRAME WORK FOR THE ISOLATION AND CHARACTERIZATION OF POTENT PLANT GROWTH PROMOTERS

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ABSTRACT:

The present work was conducted to isolate and characterize the plant growth promoting (PGP) bacteria on red gram seeds. The PGP characteristics were evaluated for their beneficial effects on the early growth of red gram. These studies indicated that these Plant growth-promoting bacteria (PGPR) strains provided a significant increase in shoot and root length, The study indicates the potential of these PGPR for inoculums production or biofertilizer for enhancing growth and nutrient content of RED GRAM under greenhouse experimental conditions. The plant growth promoting bacteria (PGPR) are the bacteria that may be utilized to increase plant growth and decrease the plant diseases. The PGPR shows the significantly higher levels of germination seedling fastly, growth in root and shoot tissue. The seed germination studies have done by the paper towel method. The Biofilm quantification and biochemical studies are also performed. The use of plant growth promoting bacteria (PGPR) increasingly meaningful for the development of more environmentally friendly agricultural practices. The objectives of this study were to isolate and characterize PGPRs. The use of our frame work could assist the selection of more competent strains to be tested in green house experiment.

Introduction

The rhizosphere is a nutrient-rich habitat and harbors a huge variety of bacteria and fungi that each can have neutral, beneficial or deleterious effects on the plant [1]. Some of these organisms can improve plant growth by different mechanisms [2, 3]. Fluorescent *Pseudomonas* and *Trichoderma* species are important groups of plant growth-promoting microorganism reported to protect plants against pathogens by evolving various mechanisms such as antagonism, competition and Induced systemic resistance (ISR) [4]. Rhizosphere colonization by certain PGPR and plant growth-promoting fungi (PGPF) can elicit ISR [5]. Induced systemic resistance (ISR) triggered by plant growth-promoting fungi (PGPFs) and Plant growth promoting rhizobacteria (PGPR) confers a broad-spectrum resistance that is effective against different types of pathogens [3]. There are numerous reports of plant growth and yield stimulation by beneficial soil microorganisms [6]. Plant growth promoting rhizobacteria (PGPR) is one among the most effective and best studied soil microorganisms which can promote plant performance. The PGPR can be classified as extracellular bacteria (existing in the rhizosphere, on the root surface or in the spaces between cells) and intracellular bacteria (mainly N2 fixing bacteria) [7]. The PGPR have been demonstrated to increase growth and productivity of many commercial crops including rice [8], Wheat [9], cucumber [10], maize [11], cotton [12], black pepper [13] and banana [14].

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Role of PGPR

The Plant growth promoting rhizobacteria, compost and chemical fertilizers significantly affect the growth and yield of different crops. A novel approach could be that composted material may be converted into a value added product such as an effective biofertilizer by blending with PGPR which are free living soil bacteria that can either directly or indirectly facilitate rooting [15] and growth of plants. There are several mechanisms by which PGPR effect plant growth such as ability to produce various compounds (phytohormones, organic acids, siderophores), fix atmospheric nitrogen, solubilize phosphate and produce antibiotics that suppress deleterious rhizobacteria, and production of biologically active substances or plant growth regulators (PGRs) is one of the major mechanisms through which PGPR influence the plant growth and development [16]. Plant growth promoting rhizobacteria, having multiple activities directed toward plant growth promotion vis-a-vis exhibiting bioremediating potentials by detoxifying pollutants like, heavy metals and pesticides and controlling a range of phytopathogens as biopesticides, have shown spectacular results in different crop plants has been observed following PGPR applications (Table 1). The productive efficiency of a specific PGPR may be further enhanced with the optimization and acclimatization according to the prevailing soil conditions [17].

Physiochemical Characterization of PGPR

PGPR are free-living bacteria and some of them invade the tissues of living plants and cause unapparent and symptomatic infections [18], when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens [5]. Rhizobacteria that exert beneficial effects on plant growth and development are referred to as PGPR. In last few decades a wide group of bacterial species including of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have reported to enhance plant growth [18]. The PGPR can effect plant growth by producing and releasing secondary metabolites and facilitate the availability and uptake of certain nutrient from the root environment [19]. Unfortunately, the interaction between associative PGPR and plants can be unstable. The good results obtained from *in vitro* assays cannot be always dependably reproduced under field conditions [20] .The rhizobacteria population was slightly affected by Nickel-Cadmium exposure. However, majority of the isolated strains for both the rhizosphere possessed one or more PGP traits. Isolates tolerant to elevated levels of heavy metals and test bacterium P35 are outstanding for PGP potential. Selection of microorganisms both metal tolerant and efficient in producing PGP compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils [21]. The presence of such growth promoting rhizoflora accountable for the beneficial effects on crop growth and yield. The significance of the study could be stated as the potential of these IAA producing isolates and optimization study for IAA production will flourish the growth and ultimately IAA production in the field and prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields.

PGPR	Plant	Conditions	Results of addition of bacteria to plants
Pseudomonas sp. PS1	Greengram (*Vigna radiate* (L.) wilczek	Pots	Significantly increased plant dry weight, nodule nubers, total chlorophyll content, leghaemoglobin, root N, shoot N, root P, shoot P, seed yield and seed protein
Bradyrhizobium MRM6	Greengram (*Vigna radiate* (L.) wilczek) Soybean, Wheat	Pots	When herbicide tolerant *Rhizobium* strain MRP1 was used with herbicide, it increased the growth parameters at all tested concentrations of herbicides (quizalafop-p-ethyl and clodinafop)
Pseudomonas sp. *Paenibacillus polymyxa*	Pepper *Zea mays* L. (maize)	Fields Gnotobiotic conditions	Significantly increased soil enzyme activities, total productivity, and nutrient uptake. Significantly increased the biomass of plants and elicited induced systemic resistance against bacterial spot pathogen *Xanthomonas axonopodis* pv. Vesicatoria untreated plants.
Enterobacter sakazaki 8MR5		Pots	Inoculation increased growth parameters

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PGPR as a Biofertilizer

Bacterial siderophores from C138 are most effective in supplying Fe to iron-starved tomato plants when delivered to the roots, independent of the bacterial presence. Furthermore, results are similar or even better than with full Hoagland solution, representing a promising candidate to develop an organic Fe chelator. The short period is needed for fermentation appears as an asset for economic feasibility. In summary, strain C138 tested in this experiment can serve as effective organic biofertilizer .The plant-PGPR cooperation plays a major role by enhancing growth and health of widely diverse plants. That plant PGPR independently produced IAA has also been revealed. It is obviously a step forward in our understanding of plant-PGPR cooperation but it does not fully clarify the bacterial functions and plant hormonal networks involved in components of hormonal pathways .Phytohormone-producing *Bacillus* sp., WhIr-15 and *B. subtilis* WhIr-12, isolated in the present study, have potential at field level to improve wheat productivity and may be helpful in the formulation of an effective biofertilizer for wheat .Expected to replace the chemical fertilizers, pesticides and artificial growth regulators which have numerous side effects to sustainable agriculture. Multifaceted bacterium of *Bacillus amyloliquefaciens* was improved growth, yield and nutrition of soybean through the contributions of the bacteria mediated induced mechanisms/ processes in the rhizosphere= of the soybean and also as a broad-spectrum bioinoculant for soybean cultivation in India .

Material and methods

SAMPLING

Different rhizosphere soil samples from different crop fields like Paddy, Cotton, was collected from, company nearby areas.

Serial Dilution:

1. Taken 1 gram of soil sample in a test tube and added 10ml of distilled water
2. Then serial dilution is done in this process 8 test tubes are taken with the first test tube containing 10ml of water and 1gm of sample.
3. 1ml of sample from the 1st test tube is transfer to next test tube and added 9ml diss. Water using micro pipette respectively till the 7th one.
4. The sixth and seventh test tubes are bacterial cultures and they are spread on agar slants then after 24hours of inoculation antifungal activity is observed in the media.
5. The solution of the first test tube containing 1gm of sample is mixed using vortex shaker the sample containing the test tube 10^{-6} and 10^{-7} are taken and the others are discarded all the medium are shifted from autoclave to laminar air flow then uv is switched on for sterilization for 10mins before that is done the laminar air flow is wiped with cotton dipped with ethyl alcohol the sterilized petri plates must be free from bubbles.

Preparation of nutrient agar:

Agar medium is prepared by mixing nutrient medium with commercially available agar powder if nutrient medium is not available then it can be individually prepared by water, peptone, beef extract and agar. 1gm of agar is weighted and it is mixed with nutrient medium mixed with 100ml of water and poured into petri plates then it is kept for autoclave at 121°C at 15lbs for 15 min.

Sub-Culturing:

The colonies are picked up from the 10^{-6} and 10-7 plates and are streaked upon agar slants. Which when inoculated produces active cultures More active cultures are also prepare by inoculating the bacteria in nutrient broth without agar.

Plant growth promoting characteristics

Indole acetic acid production

Luria bertani (LB) (Hi-Media) agar medium was amended with 5mM L- tryptophan. Actively growing (24 h old) bacterial cultures were inoculated into above medium and after incubation for 24-48 h, the inoculated points were overlaid with 10mm-diameter nitrocellulose membrane (NCM) disk. After incubation, the NCM were overlaid on a Whatman No.1 filter paper saturated with Salkowski reagent (Gordon and Weber 1951).

Phosphate solubilization

Pikovskaya's medium containing tri-calcium phosphate was prepared and poured it into sterilized Petri plates. Actively growing

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bacterial culture is spotted on these plates and incubated it at 28º C for 4-7 days. Plates were observed for clearing or solubilization zone around the colonies (Pikovskaya, 1948).

Ammonia production

Ten ml of peptone water was inoculated with 100µl of actively growing bacterial culture and incubated for 48 h. After incubation, 1ml broth culture was taken in a separate test tube and 1ml of Nesslers reagent was added and observed for color change. (Deep orange was considered as excellent ammonia production, orange as good and yellow as fair production of ammonia) (Ahmad et al., 2008).

Phytase activity

Medium containing phytic acid is prepared and poured into sterilized Petri plates. Actively growing bacterial culture was spotted onto these plates and incubated at 37 º C for 2-4 days. Plates were observed for zone of hydrolysis around the colonies (Graf et al., 1987).

Biofilm quantification

LB media was inoculated with 24 h bacterial culture (1%), vortexed well and incubated for 15 days at 28º C. After incubation, quantitative analysis of biofilm production was performed by removing the broth from tubes and the tubes were washed with saline (0.85% NaCl). After washing, tubes were air dried and the cells that were attached to tubes were stained with 1% solution of crystal violet and incubated for 45 minutes. After incubation, crystal violet solution was removed and tubes were rinsed with water. At this point, biofilm was visible as purple rings formed on the sides of each tube. Biomass of attached cells (biofilm) was quantified by solubilization of dye with 95% ethanol and absorbance at 600nm was read using spectrophotometer (Shimadzu, Japan) (Stepanovic et al., 2000).

Biochemical tests:

Carbohydrates fermentation

Principle

Fermentation degradation of various carbohydrates such as glucose (a monosaccharide), sucrose (disaccharide), cellulose (polysaccharide) by microbes, under anaerobic condition is carried out in a fermentation tube. A fermentation tube is a culture tube that contains a Durham tube (i.e. a small tube placed in an inverted position in the culture tube) for the detection of gas production, as an end product of metabolism. The fermentation broth contains ingredients of nutrient broth, a specific carbohydrate (glucose, lactose, maltose, sucrose, or mannitol) and a P^H indicator (phenol red), which is red at a neutral P^H (7) and turns yellow at or below a P^H of 6.8 due to the production of a an organic acid.

Materials

Culture, Sterile fermentation tubes along with Durham's tubes of:, Glucose broth , Sucrose broth , Lactose broth , Inoculating loop

Methodology

Preparation of fermentation medium whose constituents are as follows:

Tryptone /peptone	1.0 g
Carbohydrate*	0.5 g
Sodium chloride	1.5 g
Phenol red	0.0018 g
Distilled water	100.0 ml

(* A specific carbohydrate--- such as glucose, sucrose and lactose is added)

1) Broth was taken into fermentation tubes and Durham's tubes were inserted into the fermentation tube such a way that the broth enters into the Durham's tube also. Autoclaved at 12l^{o}C for 15 minutes.
2) After autoclaving it was cooled at room temperature.
3) The culture was inoculated and all the 6 inoculated and 3 un inoculated tubes at 35^{o}C for 24-48 hours were incubated

Seed germination by paper towel method

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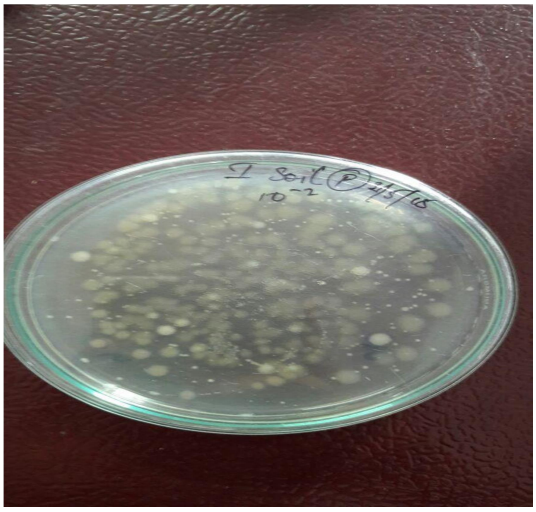
Red grams seeds were treated with different isolates isolate 5: (T1), isolate 5 supernatant: (T2) and control: (T3) were used separately for plant growth using paper towel technique. The percentage of germination by the influence of treatment with isolates was assessed by the procedure recommended by International Seed Testing Association (ISTA) (Yuan, 1992). Seeds of maize were washed thoroughly with sterile distilled water and surface sterilized with 0. 1% (w/v) $HgCl_2$ for 10 min and used in experiments. The treated seeds were incubated for 10 days in a paper towel and assessed for vigor index and compared with control (T3).

Green house experiments

Seeds of redgram were washed thoroughly with sterile distilled water and surface sterilized with 0.1% (w/v) $HgCl_2$ for 10min and used in experiments. Pots (15×10 cm) were filled with optimum weight of sterilized loamy clay soil. Red grams seeds were treated with isolate isolate 5: (T1), isolate 5 supernatant: (T2), and control: (T3) were used for plant growth in greenhouse conditions.

Results Serial dilution



Colony morphology

Source	Isolate no	Shape	Size	Margin	Elevation	Color
soil	1	irregular	small	entire	flat	white
soil	2	irregular	large	c	flat	transparent
soil	3	irregular	large	serrate	flat	white
soil	4	circular	medium	entire	flat	white
soil	5	irregular	medium	entire	raised	transparent
soil	6	circular	medium	entire	raised	white
soil	7	irregular	large	entire	flat	red
soil	8	irregular	large	serrate	flat	white
soil	9	circular	medium	entire	raised	transparent
soil	10	irregular	medium	entire	raised	white

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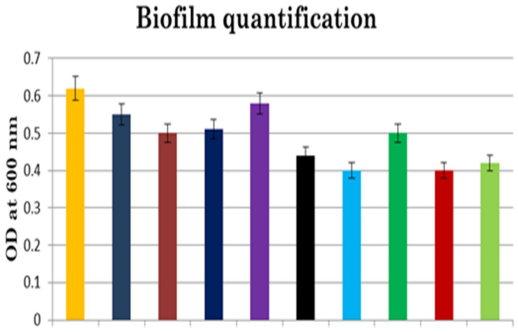
Isolation of bacteria:

Potential strain observed different colony morphology was streaked on nutrient agar slants to obtain pure culture and they were taken for further study.



Biofilm production by biosurfactant producing bacteria.

Efficacy of Isolate 1 on growth of Redgram (greenhouse study)

Greenhouse experiment was performed to evaluate growth of red gram using different treatments (T1= isolate1, T2=isolate 1 supernatant, T3= control). Data revealed that there was increase in shoot and root length when seeds were treated with Isolate 1 and isolate 2when compared to control. Similarly, plant biomass increased with isolate 1 when compared to control.

T1 T2 T3

Efficacy of Isolate on growth of Redgram under greenhouse condition

Treatment	Root length(cm)	Shoot length(cm)	Bio mass weight (gm)
T1	2.5±(0.3)^{a}	6.4±(0.3)^{a}	0.45±(0.2)^{a}
T2	1.8±(0.2)^{b}	4.5±(0.3)^{b}	0.32±(0.3)^{b}
T3	0.5±(0.4)^{c}	1.5±(0.4)^{c}	0.14±(0.3)^{c}

Values superscripted by a-g are ranking highest to lowest of significant, same alphabet are insignificant according to Fischer's least significance difference test ($p<0.05$). Values in the brackets are standard error; values in column are mean two independent experiments of 4 replications

Conclusion :

The Plant growth promoting rhizobacteria, compost and chemical fertilizers significantly affect the growth and yield of different crops. We have shown spectacular results in different crop plants has been observed following PGPR applications .The significance of the study could be stated as the potential of these IAA producing isolates and optimization study for IAA production will flourish the growth and ultimately IAA production in the field and prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields. It is obviously a step forward in our understanding of plant-PGPR cooperation but it does not fully clarify the bacterial functions and plant hormonal networks involved in components of hormonal pathways. The plant growth promoting characteristics are shows best production for Indole acetic acid, phosphate solubilization, phytase activity and Biofilm quantification and also performed Biochemical test for production

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of carbohydrates by fermentation. The percentage of germination by the influence of treatment with isolates was assessed by the procedure recommended by International Seed Testing Association. Red grams seeds were treated with isolate: (T1), isolate 5 supernatant: (T2), and control: (T3) were used for plant growth in greenhouse conditions. Ultimately, the data obtained in this study brings new insights in to the PGPR properties governing the successful beneficial interactions.

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Isolation of microorganisms from various agricultural soils of Warangal district.

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Isolation, Microorganisms , Agriculture soils

ABSTRACT

As agriculture is main occupation in India which is not only affected by natural calamities such as draughts and floods but also by disease causing microorganisms. Plants and soil harbor millions of microorganisms, which collectively known as the microbiome.Out of many some of the microbial communities will offer a wide range of securities to the host plant includes pathogen avoidance, pests and insects repelling and growth promotion. Therefore, in present study we investigated on microbiome of agricultural soils. Soil samples were collected from different fields like Paddy, Cotton, Maize, Red gram and Mirchi. Isolation of the microorganisms was done by using Pour plate method, Spread plate method, Membrane filter technique and Direct inoculation technique (Paul Nijema et.al). A number of bacterial, fungal and actinomycetes isolates were obtained from soil samples. The bacterial isolates were characterized by Gram staining and by several biochemical tests such as Catalase test, MR test, VP test etc. The bacterial isolates were identified as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Shigella sp., Proteus mirabilis, Bacillus anthracis , Bacillus subtilis* and *Staphylococcus aureus.* The fungal isolates such as *Aspergillus niger, Aspergillus flavus,Fusarium oxysporum, Mucor sp., Trichoderma sp*. and *Rhizopus sp.* were cultured on Potato dextrose and sabouraud's dextrose agar media and identified by microscopic method. The actinomycetes isolates were identified as *Streptomycetes luridiscabiei and Streptomyces scabies by* colony morphology , Gram staining and Acid fast staining.

INTRODUCTION

Agriculture is the main occupation of India . India's economy and its pride also depends on agriculture . It is estimated that Global agriculture has to double food production by 2050 in order to feed the growing population of world(Vadakattu V.S.R. Gupta 2012). Soil microbes are the most abundant of all the biota in soil and responsible for driving nutrient and organic matter cycling, soil fertility, soil restoration, factory health and ecosystem primary product. Salutary microorganisms include those that produce symbiotic associations with factory roots. In this environment the study was accepted to insulate different microorganisms from soil. Microorganism are constantly present in soil, ordure and decaying factory napkins which are suitable to degrade wastes that are identified with the substrate organic matter (Alexander,.1977). Agriculture soil is a dynamic medium in which a large number of pathogenic and nonpathogenic bacterial and fungal foliage live in close association. Soil microflora directly participates in nutrient cycles.Microorganisms produce some useful composites that are salutary to soil health, factory growth and play an important part in nutritive chains that are important part of the natural balance in the life in our earth (Paul and Clerk, 1966;. Kummerer, 2004). Along with bacteria and fungi actinomycetes are also important soil microorganisms. Actinomycetes are the most abundant organisms that form thread-suchlike fibers in the soil and are responsible for characteristically" earthy" smell of lately turned healthy soil. Actinomycetes aren't only useful for husbandry but also used in other sectors i.e., medicinal, drugetc., In attempts to develop marketable biocontrol and factory growth

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promoting products using rhizobacteria, it's important to fete the specific challenges they present. To begin with, the commerce between PGPR species and their factory symbionts appears to be specific, indeed within a crop or cultivar (KIoepper 1996). Therefore webbing and insulation of promising strains of actinomycetes with implicit antibiotics is a thrust area of hunt since numerous times. As there's a geographic variation in Indian soil type and their contents, hence it is relatively likely that the distribution of antibiotic producing actinomycetes is also variable.Thus, disquisition of agrarian ecosystems for bacteria, fungi and actinomycetes is necessary to understand agrarian microbiome and their relation to plant growth and yield.

2.0 MATERIAL AND METHODS

2.1 Soil sample collection

All Soil samples were collected from 5-15 cms below the surface of the soil. Soil samples were collected from various agricultural fields of Warangal District like paddy, cotton, maize etc., The collected soil samples were then packed in zip lock bag and stored.

2.2 Determination of physiochemical properties of soil

Freshly collected soil samples were analyzed for physiochemical properties. The moisture content of the sample was removed by placing in hot air oven at 105°C to maintain constant weight. The physiochemical properties i.e., humidity, pH, temperature and aw were determined according to methods suggested by Pramer and Schmidt, (1964).

2.3 Microbial Isolation and Enumeration

In order to avoid the Gram negative bacteria soil samples were heated for 1 week prior isolation.Isolation and enumeration of bacteria was carried out by method described by Oskay et al.,(2004). The soil suspension were 71 ipette and lawn onto Starch Casein Agar (SCA) at Ph 7. All the plates were incubated at 30 Oc for 1 – 2 weeks. Emerging actinomycetes were picked and streaked onto fresh SCA plates and incubated at 30Oc for 1 week. Similarly Bacteria and Fungi were isolated by Potato Dextrose Agar (PDA) and Nutrient Agar Media (NAM). 0.1 ml of serially diluted soil suspension was 71 pette onto plates with PDA and NAM media, spread with a glass spreader and incubated at 28°C for fungal and 37°C for bacterial observation (Nakuleshwar *et al*.,2013). The characteristic colonies were selected and maintained for further screening.

2.4 Identification and characterization of Fungi

The fungal isolates were linked by morphological examination and its characteristics .Morphological characteristics were examined under microscope (Onion *et al*., 1981).

2.5 Identification and characterization of Bacteria

Gram staining was performed to check the morphology of the cells and spore chain morphology was linked by spore staining fashion. The isolates were confirmed based on biochemical profile. (Collins and Lyne, 1989).

3.0 Results and Discussion

This study revealed that soil samples were analysed with respect to different types of bacteria, fungi and actinomycetes. The bacteria found in all fifteen soil samples were biochemically characterized (Table 2) as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Shigella sp., Proteus mirabilis, Bacillus anthracis, Bacillus subtilis* and *Staphylococcus aureus.* In the present study, the isolated fungi were identified on the basis of cultural, microscopic and morphological characteristics (----).The fungal isolates were identified (Table 1) as *Aspergillus niger, Aspergillus flavus,Fusarium oxysporum, Mucor sp., Trichoderma sp*. and *Rhizopus sp* Earlier works reported that potato dextrose agar was most favorable for maximum growth of fungi (Maheshwari, 2000). The isolated fungi were cultured in PDA, SDA and Czapeck Dox agar media. Based on the results PDA and SDA media were most suitable for good growth of *A. niger, A. flavus* and *Trichoderma* sp., .Tricoderma sps., Aspergillus niger, Aspergillus flavus were grown in the Czapek Dox agar media effectively at 7 days of incubation period. The fungal strains have not shown effective growth at 3 and 5 days of incubation on any PDA Media, SDA Media and Czapek

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Dox agar medias.The actinomycetes isolates identified were *Streptomycetes luridiscabiei and Streptomyces scabies.* The physiochemical properties of soil also play an important role in the growth of microorganism. Similarly, Karthik et al. (2011) also reported the isolation, identification of microorganisms such as *Bacillus* species from agricultural waste dump soil. Diversifed microflora was observed .The isolation of various fungal, bacterial species showed that the agricultural soil is quite rich in microbial flora. In agriculture process, soil microorganisms such as bacteria, fungi and actinomycetes may play important roles in soil fertility and in the form of loss and gain in the production of grains, fruits, vegetables. Further, we need to study how these microbes are influencing plant growth and yield. In conclusion the goal of this research was to focus on sustainable agriculture i.e., to identify agriculture friendly microorganisms.

Table 1: Effect of pH and incubation period on the growth of isolated fungi on PDA, SDA and Czapek Dox agar media.

pH of the medium	Isolated fungi	PDA Media Incubation period (days)			Incubation period (days) SDA Media			Czapek Dox agar Media Incubation period (days)		
		3	5	7	3	5	7	3	5	7
	Aspergillus niger	-	+2	+4	+1	+2	+4	+1	+2	+4
6	*Trichoderma sps*	+2	+3	+4	+2	+3	+4	+2	+3	+4
	Rhizopus sps	-	+2	+3	-	+2	+3	-	+2	+3
	Aspergillus flavus	-	+2	+4	+1	+2	+4	-	+2	+4
7	*Trichoderma sps.*	+2	+3	+4	+1	+2	+4	-	+2	+4
	Fusarium oxysporum	-	+2	+4	-	+2	+4	-	-	-
	Aspergillus niger	+1	+2	+4	+1	+2	+4	-	-	+4
8	*Trichoderma sps*	+2	+3	+4	+1	+2	+4	-	-	+4
	Rhizopus sps	-	+1	+2	-	+1	+2	-	-	+3

- no growth +1 poor growth +2 moderate growth +3 good growth +4 massive growth

Table 2 : Biochemical characterization of bacterial isolates.

Identified bacteria	Gram strain	Motility	Indole	VP	MR	Oxidase	Nitrate reductase	Citrate	Catalase	Urease
Klebsiella pneumonia	Gram negative	Non motile	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Enterobacter aerogenes	Gram negative	Motile	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
E..coli	Gram negative	Motile	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
B. anthracis	Gram postive	Non motile	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve
B. subtilis	Gram postive	Motile	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve
Staphylococcus aureus	Gram postive	Non motile	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve

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Table 3 Growth of Fungi on different medias

S.No	Sample	T CFU(colony forming unit)		
		PDA	CZAPDEK DOX AGAR	SDA
1	Cotton	3	4	2
2	Paddy	4	3	5
3	Red gram	5	4	4
4	Ground nut	6	5	3
5	Maize	8	2	3
6	Mirchi	2	3	2

Table 4 Growth of Bacteria on different medias

S. No	Sample	SCA	NAM
1	Cotton	5	8
2	Paddy	3	4
3	Red gram	2	5
4	Maize	2	6

4.0 CONCLUSION

Soil harbours a variety of microbes in which some bacterial species are very fragile and may be destroyed by slight changes in the soil environment. Others are extremely tough, able to withstand severe heat, cold or drying. Some bacteria are dependent on specific plant species. Soil food web supports other soil organisms and the functions of a healthy soil. The isolated strains from agricultural soil were preserved and sent for molecular study to identify . Isolation and screening of microorganisms from such areas in optimum conditions may contribute to the discovery of novel antibiotics.

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