Vidya Prasarak Mandal's
B. N. Bandodkar College of Science (Autonomous)
Thane

NAAC Re-accredited 'A' Grade, College With Potential for Excellence (CPE), Best College Award, ISO 9001: 2015 Certified, Recipient Of RBNQA Commendation Certificate From IMC, Recipient Of Jagar Janivancha Awards Of Govt. Of Maharashtra, Supported Under - Fist O Level (Min. Of Sci. & Tech.) & DBT Star College Scheme, BEQET Special Citation Award

Proceedings of the National Conference

Microbiome: The Story Untold !

7th & 8th January 2022

Organized by

Departments of Biotechnology, Microbiology, Biochemistry, Botany, Human Science & Interdisciplinary Science

In association with

Mycological Society of India (Mumbai Unit)

Edited by

Dr. Moses Kolet, Ms. Sayali Daptardar, Ms. Zahera Momin, Dr. Kalpita Mulye, Dr. Jayashree Pawar

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Jnanadweepa, Chendani Bunder Road, Thane (W) 400 601. Maharashtra

in association with

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Chief Patron's Address…….

'प्रज्वलितो ज्ञानमय: प्रदीप:' is the vision with which Vidya Prasarak Mandal is striding as a premier educational trust in Thane.

VPM's group of institutions are serving the nation tirelessly, creating a strong and able workforce. Each institution on the campus is adapting itself with ever changing requirements of education system and creating better knowledge ecosystem for learners.

B. N. Bandodkar College of Science is committed to provide quality education in science. The fast pace with which the technologies are changing, keeping learner updated with current happenings in the field is mandatory. Anticipating this, annual hosting of National conference has been included in academic calendar as one of the most important events.

Conferences provide a common platform for teachers, researchers, and industry professionals along with learners to come together and exchange ideas, discuss novel concepts and technologies as well. From B. N. Bandodkar College of Science, this year the national conference titled Microbiome: The story untold!' is getting hosted by lead of Department of Biotechnology and Microbiology along with other departments of Biochemistry, Botany, Human Science and interdisciplinary science. In past, the lead Department has organized a national conference on diagnostics (2012) and an international conference on Emerging Technologies for Sustainable Agriculture (2017). In spite of the backdrop of enforced partial lockdown, with the same spirit and passion the Department has come ahead to host one more enriching event in form of this national conference.

Microbiology is a newer branch of biology and the world has and is witnessing dynamic develo0pmets in this field in terms of basic and applied research. I am sure this conclave would bring together young amateur microbiologists, learners of different faculties and stalwarts of this field. We wish to create a rare but the most important connect across all disciplines.

I wish the departments and the College All the best for successful organization of the conference.

Happy learning!

Dr. Vijay Bedekar

Convener's Address…….

I am very happy to present this volume on Microbiome: The story untold. Technology has always fascinated the human race which has always been involved in betterment of available techniques, technology and conventional practices since ancient times. The speed and quantum of this change over a period of time, which was earlier inconspicuous by virtue of being very gradual, slow and steady has now suddenly assumed colossal proportions. The world is changing at a very fast pace; rapid changes are visible all over us and these changes have become more conspicuous during the recent pandemic… and are here to stay. In recent years due to combined advances in research and pedagogy, and the new normal, we have witnessed collapse of isolated disciplines. With the blurring of traditional strong boundaries of disciplines, 'Inter-disciplinary', 'trans-disciplinary' and multi-disciplinary' have become the buzz-words which are commonly encountered while exploring recent research. Realizing this recent development, which is also highlighted in the National Education Policy 2020, institutions of higher learning are shaping young minds in developing these approaches and mindset.

Vidya Prasarak Mandal, Thane, with its visionary approach, has been among the few educational conglomerates to anticipate this changing scenario and has since long, inculcated the culture of hosting of conferences in all the institutions under its umbrella. VPM's B.N. Bandodkar of Science is regularly organizing such conclaves annually and uninterruptedly since the year 2002. Now, with its Autonomous status, in addition to upgrading and modernizing curricula, the institution is committed to develop innovative cross-disciplinary structures of knowledge creation and upgradation, going well beyond conventional boundaries.

Through this venture, 'Microbiome: The story untold', we intend to create a platform for vibrant and dynamic debates over various aspects of technology, research and development as well as create ripples and reflections to uncover hidden truths, far-fetched knowledge and untapped potentials of technologies.

I thank the VPM management and all teams working ceaselessly towards success of this venture. Thanks are due to all stake holders. Students have always been a stronghold and I thank them for their selfless contributions.

Wish you success and fulfillment.

Dr. Moses J. Kolet

Organizing Secretaries' Address……

Microbiome - the catalogue of microbes and their genes, has been the priority research area being extensively explored in last two decades. The term 'Microbiome', was coined by Joshua Lederberg in 2001. It is often used interchangeably with Microbiota – which refers to microbial taxa associated with a particular environment. The field has brought paradigm shift away from studying 'single, specific' microbes to a more holistic microbiome approach. It is evolving rapidly and has generated information that is of great interest, not only for scientific fraternity, but for general public at large. Microbiome research encompasses studies on understanding the behaviour, interactions and functions of microbial communities within a specific environment. Innovative, cost-effective, high-throughput screening technologies and modern computational skills has proved conducive for massive data flood in this area.

Extensive research in this arena has made us aware that humans are known to be colonized with more than trillions of bacteria, with the gut microbiome being equipped with a metabolic potential equivalent to a 'virtual/ exteriorized organ' within the gastrointestinal tract! The microbiota changes according to diet, age, lifestyle, climate and geography, genetic make-up, early microbial exposure and health status of the host. Its role in health, immune status and disease has been the area of intense research. Understanding microbiome of a population, integrated with artificial intelligence, has not only led to newer strategies of diagnosis, management, and treatment of diseases, but has also widened the doors of personalized medicine. India especially holds an unparallel setup for human microbiome studies because of the extensive genetic diversity, dietary habits, ethnicity, cultural connections and changing lifestyle of the population.

Diverse geographical locations and ecosystems also hold a promising potential to undertake research related to plant systems. It has been reported that, an effective plant microbiome can offer benefits to its host, including plant growth promotion, nutrient use efficiency, and control of pests and phytopathogens. This necessitates the immediate need to utilize functional potential of plant-associated microbiome and its innovation into crop production and unlock new opportunities for the design of more efficient microbial consortia for enhancing crop productivity and the restoration of soil health.

Other than fundamental interests to improve Human health and Agriculture, microbiome research holds great promises in various fields like ecology, personalized modern medicine,

forensics to even exobiology. Advances in engineering of environmental microbiomes will replace toxic chemicals in agri-, horti-, and aquaculture in the future and stimulate a more sustainable use of environmental resources, as well as improve food processing. Agricultural products based on the microbiota are one of the fastest growing sectors in agronomy with a Compound Annual Growth Rate (CAGR) of 15–18% and a predicted value of over 10 billion US dollars by 2025.

We are organising the conference on 'Microbiome: The Story Untold!' to uncover this 'hidden treasure', wherein we would host invited talks, research paper presentations and discussions on Human Microbiome, Environmental Microbiome, Plant-Microbe interactions, Probiotics and Functional Foods and advances in computational approaches in the field of microbiome research. The conclave aims to bring together scientists, research scholars, industry professionals, academicians and students to exchange their experiences about various aspects of microbiome research. In view of microbiome research in India being in infancy, such platform would initiate a dialogue amongst all the stakeholders and be instrumental in generating and motivating young students to explore further 'this' field of research!

Dr. Kalpita Mulye and Dr. Jayashree Pawar

Editor's Note....

This publication contains the proceedings of the National Conference on "Microbiome-The story untold!" held at VPM's B. N. Bandodkar College of Science, Thane on January 7-8, 2022.

Over the years, B. N. Bandodkar College of Science has been well-known for its efforts in hosting state and national level scientific gatherings for inculcating research aptitude in students. We believe that this national conference will help in widening the horizons further.

The theme of the conference is Microbiome which as the priority research area since last two decades. The human microbiota consists of the 10-100 trillion symbiotic microbial cells harbored by each person, primarily bacteria in the gut; the human microbiome consists of the genes these cells harbor. Microbiome projects worldwide have been launched with the goal of understanding the roles that these symbionts play and their impacts on human health. Just as the question, "what is it to be human?", has troubled humans from the beginning of recorded history, the question, "what is the human microbiome?" has troubled researchers since the term was coined by Joshua Lederberg in 2001. Hence, one of the aims of the conference is to kindle interest amongst the young minds about Human Microbiome, Environmental Microbiome, Plant-Microbe interactions, Probiotics and Functional Foods and advances in computational approaches in the field of microbiome research.

The conference seeks to provide a platform for an open dialogue amongst the eminent personalities contributing to the field of Microbiome viz. Academicians, Industry professionals, Scientists from Research Institutions, and all the other participants. This scientific gathering will prove to be a great opportunity for getting acquainted with the most recent innovations through invited talks, research paper presentations and poster presentations.

Through this proceeding we have made a humble effort to put together the vast knowledge disseminated through the conference as well as the pre-conference workshops.

Editorial Team

Acknowledgements

We would like to gratefully acknowledge help and support received from all quarters in publishing this volume of 'Microbiome: The story untold'. First and foremost, we are thankful to Dr. Vijay V. Bedekar, Chairman, Vidya Prasarak Mandal, Thane, whose support and guidance have motivated us to constantly introspect ourselves, raise our standards and benchmarks to achieve new heights and deliver with precision to our stake holders.

We are thankful to the esteemed advisory committee, national organizing committee members and speakers, for being part of this journey and contributing towards its success. We thank review committee members for reviewing the research papers and articles. We also express sincere gratitude towards the editorial team for their efforts towards enabling this volume see light of the day. We are thankful to all authors who have contributed to this volume.
We are highly obliged to Perfect Prints for providing hard copies of the Proceedings of the conference in a very short time.

Dr. Moses J. Kolet

Dr. Kalpita Mulye

Dr. Jayashree Pawar

SCHEDULE OF THE CONFERENCE

Day I: 7th January 2022

Time	Contents & Topic
10.00 am – 10.30 am	Inauguration & Release of Proceeding
10.30 am – 11.30 am	**Key Note Address: Dr. Yogesh Shouche** Human Microbiome: Indian Perspective
	Interactive Session
11.30 am – 12.30 pm	**Dr. Ashwin Kotnis** Microbiome of the skin: partners in Health and Disease
	Interactive Session
12.30 pm – 1.30 pm	**Dr. Naveen Arora** Microbe based inoculants and their role in enhancing crop productivity and reclamation of saline soil
	Interactive Session
1.30 pm – 2.30 pm	**Lunch Break**
2.30 pm – 3.30 pm	**Dr. Vineet Sharma & Vishnu Prassodanan PK** Western and non-western gut microbiomes reveal new roles of *Prevotella* in carbohydrate metabolism and mouth-gut axis
	Interactive Session
3.30 pm – 4.30 pm	**Dr. Anil Kumar** Gut microbiota-derived metabolites as potential biomarkers in different diseases
	Interactive Session

Day II: 8th January 2022

Time	Contents & Topic
10.00 am – 10.15 am	Review of Day I
10.30 am – 11.30 am	**Dr. Parikshit Prayag** FMT and its role in the management of Clostridium infection
	Interactive Session
11.30 am – 12.30 pm	Oral Presentations Part I
12.30 pm – 1.30 pm	**Dr. Shilpa Verekar** Mycoflora of Keratin Enriched Soil
	Interactive Session
1.30 pm – 2.00 pm	**Lunch Break**
2.00 pm – 3.00 pm	Oral Presentations Part II
	Interactive Session
3.00 pm – 4.00 pm	**Valedictory: Dr. Hemant Purohit** Microbiome: an unexplored wealth
	Interactive Session
	Vote of Thanks & Pasayadan

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CONTENTS

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ISBN: 978-81-923628-9-2

Section I
Keynote Address & Invited Talks

ISBN: 978-81-923628-9-2

Keynote Address

Human Microbiome: Indian Perspective

Dr. Yogesh Shouche
Emeritus Scientist, NCCS, Pune

The human gut microbiota is "the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our gastrointestinal tract". Dominated by eubacteria, the metabolic activities performed by the gut microbiome is often as complex as an organ and hence it is now being appreciated and studied in much detail. Increasing evidence suggests that the human gut microbiota changes according to diet, age, lifestyle, climate and geography, genetic make-up, early microbial exposure and health status. Studying the Indian population is relevant given the known dietary and geographical variety, unique family structure and ethnic diversity.

In traditional Indian familial system, where three generations can be studied for changes in the gut microflora with age, it has been shown that the gut microbiota changes according to age within individuals of the same family and a shift in the *Firmicutes/Bacteroidetes* ratio with age is observed, which is different than previously reported in European population. With the incoming wave of lifestyle changes observed now in India and given the availability of sugar-rich diet, the population is at high risk of developing obesity and diabetes. In the case of Diabetes, a consolidated dysbiosis of not just eubacterial but also of archaeal and eukaryotic components is seen in the gut microbiota of newly-diagnosed and known-diagnosed diabetic individuals as compared to healthy individuals.

Comparative analysis of gut microbiota of healthy Indian subjects with other populations highlights that the gut microbiomes of Indians is different from that of other Western populations and even cluster separately from Asian populations. The distinctive feature of the healthy Indian gut microbiome is the predominance of genus *Prevotella* and *Megasphaera.*

Taken together, the relevance of studying the Indian microbiome is justified given its unique microbiome features and further studies are necessitated to understand the determinants shaping the Indian microbiome. This will be helpful to develop microbial consortia for prebiotic and probiotic application and devise population specific microbiome therapies.

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Microbiome of the skin: Partners in Health & Disease

Dr. Ashwin Kotnis
Associate Professor, Dept of Biochemistry, AIIMS, Bhopal

Skin is the largest organ in human body providing physical barrier from invasion of foreign microorganisms and protection from the environment. Skin is colonized by numerous commensal microbes that play a crucial role in interaction with immune system, metabolism on the skin thus maintaining the skin homeostasis. The constant interaction of skin surface with external environment and skin type at different sites of the body makes skin microbiome highly diverse and challenging to study. Changes and role of skin microbes has been demonstrated in ageing, different dermatological disorders, wound healing etc. Updates on various types of microbes on the skin, methods such as whole genome metagenomics and culturing the skin microbes to identify the microbes, mechanism of how the microbes participate in immune surveillance and how microbial dysbiosis is responsible in ageing, chronic skin disorders such as vitiligo, psoriasis and its future prospects in treatment will be discussed.

Ongoing collaboration with Dr Dinesh Asati, Dermatology, AIIMS Bhopal and Dr Vineet Sharma, Biological Sciences, IISER Bhopal and funding from MPCST is acknowledged.

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Microbe based inoculants and their role in enhancing crop productivity and reclamation of saline soil

Dr. Naveen Kumar Arora
Department of Environmental Science, School of Earth and Environmental Sciences
Babasaheb Bhimrao Ambedkar University, Lucknow, UP

Land marginalization due to increase in population and fast pace of climate change has retrogressed the progress of Sustainable Development Goals (SDGs) and challenged the food security. Globally researchers have discussed about various methods to tackle climate change and reduce the losses in agriculture through sustainable approaches. However, injudicious use of agro-chemicals has added to salinization of lands at an unprecedented rate and is currently one of the most hassling issues of agro-ecosystems around the globe (after soil erosion). Conventional agricultural practices and development of genetically engineered plant varieties are not sufficient to tackle the problems of salinity and also are time consuming and sometimes unsustainable. Integrated crop management (ICM) using microbial technology, can be way forward in reclaiming saline soils and reduce the chemical abuses in agriculture. Microbes being the most diverse inhabitants on Earth, have tremendous stress adaptive properties and play substantial roles in soil ecology. The rich spectra of beneficial microbes residing in close association with roots *i.e.,* in the rhizosphere, popularly known as 'plant growth promoting rhizobacteria', show various direct and indirect mechanisms to enhance the growth and stress resilience in plants. Mechanisms assigned by microbes such as nutrient chelation, phytohormone production along with stress-responsive actions through exopolysaccharides, antioxidants, osmolytes synthesis enable these potential candidates to establish equilibrium in agro-ecosystem and sustain crop productivity. With impressive progress in the production and commercialization of microbe-based bioinoculants, the technology is gaining attention among the farmers and multi-purpose elite strains are now available in the market. Fluorescent pseudomonads and rhizobia have immense potential as biostimulants even under saline conditions. Through consecutive field applications on various crops, the role of salt tolerant-PGPR was successfully established in large-scale reclamation of saline and marginal lands. Novel bioformulations were developed using microbes and their metabolites to reduce the episodes of environmental stresses in plants and achieve the 'One Health Approach' to sustain the agro-ecosystems.

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Western and non-western gut microbiomes reveal new roles of *Prevotella* in carbohydrate metabolism and mouth-gut axis

Dr. Vineet K. Sharma &Vishnu Prassodanan PK
MetaBioSys Group,
IISER, Bhopal, Madhya Pradesh

Prevotella is a highly diverse genus that exhibits compositional variations in both inter-individual and inter-population comparisons of human gut microbiome. The abundance and diversity of host associated *Prevotella* species have a profound impact on human health. To investigate the composition, diversity, and functional roles of *Prevotella* in human gut, we carried out a population-wide analysis on 586 healthy samples belonging to western and non-western populations, and generated gut microbiome data comprising of 200 samples for the largest Indian cohort. A higher abundance and diversity of *Prevotella copri* species enriched in complex plant polysaccharides metabolizing enzymes, particularly pullulanase containing polysaccharide-utilization-loci (PUL), were found in Indian and non-western populations. A higher diversity of oral inflammations-associated *Prevotella* species and an enrichment of virulence factors and antibiotic resistance genes in the gut microbiome of western populations speculates an existence of a mouth-gut axis. We also included 189 Inflammatory Bowel Disease samples from western populations including US, Netherlands, and Spain to examine the association of *Prevotella* with inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease. This study provided new insights into the role of diversity, composition, and function of *Prevotella* in gut microbiome and their impact on human health. The study also revealed the landscape of *Prevotella* composition in the human gut microbiome and its impact on health in western and non-western populations.

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Gut Microbiota-Derived Metabolites as Potential Biomarkers in Different Diseases

Dr. Anil Kumar[1] & Dr. Pratima Solanki[2]
[1]Gene Regulation Laboratory, NII, New Delhi
[2]Special Centre for Nano Sciences, JNU, New Delhi

Recent reports propose the use of gut microbiota derived metabolites such Trimethylamine N-oxide, para-cresol, Trimethylamine etc. as prognostic and diagnostic biomarkers. Their early detection in body fluids have been presumed to be significant in understanding the pathogenesis and treatment of many diseases. Hence, the development of reliable and rapid technologies for their detection may augment our understanding of pathogenesis and diagnosis of different diseases in which these metabolites have been implicated. Many conventional techniques are available which can be used to detect these metabolites but they are laborious, expensive, time-consuming and require sophisticated equipment with skilled labor. Therefore, there is a need of point of care devices which are affordable, sensitive, specific, user friendly, robust, hassle-free and deliverable. Several studies have been published on molecularly imprinted polymers (MIPs) which can be used as artificial receptors for making electrochemical sensors to detect small molecules, helping in diagnosing the disease with excellent sensitivity and performance. The MIPs possess unique physical and chemical stability in making specific cavities for binding analytes in the polymeric matrix. In comparison to synthetic receptors, as in the case of MIP, biological receptors were also used widely in chemo/biosensors, but they involve complex protocols, high cost, and poor stability. Because of these limitations, the recent trend can be seen in MIP's preference for making artificial recognition receptors in sensor development. MIPs offer rapid, inexpensive, and selective receptors to make electrochemical/optical sensors that seem suitable to promptly detect small metabolites. Our group has experience in developing sensor/biosensors and recently we developed MIP based sensor for the detection of TMAO (Trimethylamine N-oxide), a microbiota-derived metabolite which has been implicated in human health and disease.

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Role of FMT in Infectious diseases - A Clinical Perspective

Dr. Parikshit Prayag, MD, ABIM, ABMS
American Board Certified in Internal Medicine and Infectious Diseases
Fellowship in Transplant Infectious Diseases (Stanford University, USA)
Consultant, Transplant Infectious Diseases, Deenanath Mangeshkar Hospital, Pune

Fecal microbiota transplantation is an emerging therapy for infections such as *Clostridium difficile*. In *Clostridium difficile* therapy, fidaxomicin and vancomycin remain first line therapies.

However, data for FMT is emerging. In recurrent *Clostridium difficile*. FMT can be used as a vital modality. There is data to suggest that chances of bacteremia are reduced, and also the effect of FMT is fairly well preserved one year after therapy.

There is also emerging data to support its use in the first episode, especially for fulminant or severe/refractory *Clostridium difficile*. In this talk, we will explore the mechanism via which FMT works, how the microbiome plays an important role, and how we are performing it in India.

Given the challenges of stool colonization in the Indian population and the lack of availability of stool capsules, FMT can be particularly challenging. We will discuss our own in-house protocol and experience. We will also discuss the role of the microbiome in other infectious conditions.

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Mycoflora of Keratin Enriched Soil

Dr. Shilpa A. Verekar
Parle Agro Pvt. Ltd.

Keratinophilic fungi constitute an important ecological group of microbes, which are able to colonize and degrade structurally very hard and stable animal protein – the keratin. Keratinophilic fungi are distributed in wide range of habitats including road side, cattle farm, poultry farm, river streams, school, parks, forests, pastures, etc. These can be differentiated by several traditional parameters including microscopic and colony morphology, nutritional requirement, growth temperature, pigmentation, hair perforation, and mating reactions etc. The morphology and microscopy-based identification is confirmed by 18S rDNA sequence comparisons or internal transcribed spacer (ITS1 and ITS2) and 5.8S rDNA sequence examination. Several of these fungi have known teleomorphs in order Onygenales of Ascomycetes and majority of them are the representative of families Arthrodermaceae, Gymnoascaceae, Myxotrichaceae, and Onygenaceae.

Keratinophilic fungi are of great importance for three main reasons. Firstly, these fungi play a very important role in ecosystem functioning and degrade a major portion of soil keratin. Secondly these fungi are potential producer of industrially important secondary metabolites. Thirdly, they are very important medically. The need of an extensive survey of this group of fungi from unexplored areas and exploitation of their ability to produce secondary metabolites is expressed. The need of culture collection of this group of fungi will be highlighted.

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Microbiome: an unexplored wealth

Dr. Hemant Purohit
Former Head, Environmental Biotechnology Genomics Division,
National Environmental Engineering Research Institute
NEERI, (CSIR) Nagpur

Dynamically, the microbial communities in different environments are dictated by the availability of organic carbon and sharing amongst the member that creates a well-organized biochemical structure. To capture these microbial community intelligence Metagenomics as an approach provides the collective data or metadata to draw meaningful biological conclusions. This could be understood using various models and exploiting microbial community for desired biochemical performance. Since, every ecological sample represents the major part of its microbial community unexplored, hence to understand microbial community structure and function, there is a lot more innovation required in metagenome analysis. It requires assigning the knowledge to unidentified sequences which includes the information for microbial community structure as well as its functional characteristics.

Another area of microbial exploration research is through mega-culturomics approach. The microbes in different environment survives as a typical community. In these communities the secondary metabolites produced by different members selectively allows a typical enrichment regime. This salient property could be exploited for identifying novel molecules. This approach uses combination of biochemical properties with genetic discriminating features to select novel microbes which are having capacities to produce novel bio-molecules.

Exploration of microbial diversity and its utilization in different domain of human welfare has taken new dimension due to changes in mining approached for microbial wealth. The new genomics tools are still evolving and trying to identify the unexplored functional capacities associated with microbes. The recent developments in microbial genomics will usher the different and innovative approaches in the field of human health, microbial products and management of environmental issues in particular.

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Section II
Logo Competition

Logo competition

To uncover the hidden talent of students and build creative connect a logo competition was organized for the national conference "Microbiome: The Story Untold!".

Students were appealed to make a logo either a handmade or a digital format that would represent various themes of the conference viz., human microbiome, plant microbiome, environmental microbiome, functional foods and microbiome engineering. Total 62 entries were received from 14 different colleges from Mumbai, Navi Mumbai, Pune and Thane. Total number of participants were 40.

With a completely blind assessment, the best Five entries were short listed by the panel of three members of Organizing team of faculties, Ms. Purvi Shah, Ms. Rucha Khadake and Ms. Judith Talker. Ten members of the organizing committee then voted to select the one best.

The logo designed by **Ms. Isha Pisal**, second year Microbiology, B. N. Bandodkar college of science was declared as the winner of the competition. The logo then represented conference on brochure, proceedings and certificates.

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Winning Logo

Thought Behind the Logo

The logo looks quite easy but has a deep meaning in it. The catchy thing about the logo is the shape of simple microscope, which represents the basic instrument used by the microbiologist showing the initial stage and gradual evolution in microbiology. Instead of the circular rim of the microscope i replaced it with the shape of the 'settings' symbol and colored it like the earth. 'Settings' which we can see in our mobile devices, where it is used to alter the functions of the device as per our convenience, so here, in the same way the settings symbol represents the evolution and the changes man has made worldwide for the betterment of human life, through excellent research work and in future we will be doing. Next, inside the microscope, microorganism, are shown, which basically represents the microbiome. Then surrounding the microscope, a human, animal(cow), plant, syringe black structure is shown as a representation of research and application of microbiome in many different fields successfully.

Thus, the logo overall explains about the research, evolution and intense development in various fields of science, overall, in the world.

Ms. Isha Pisal, S Y B Sc Microbiology

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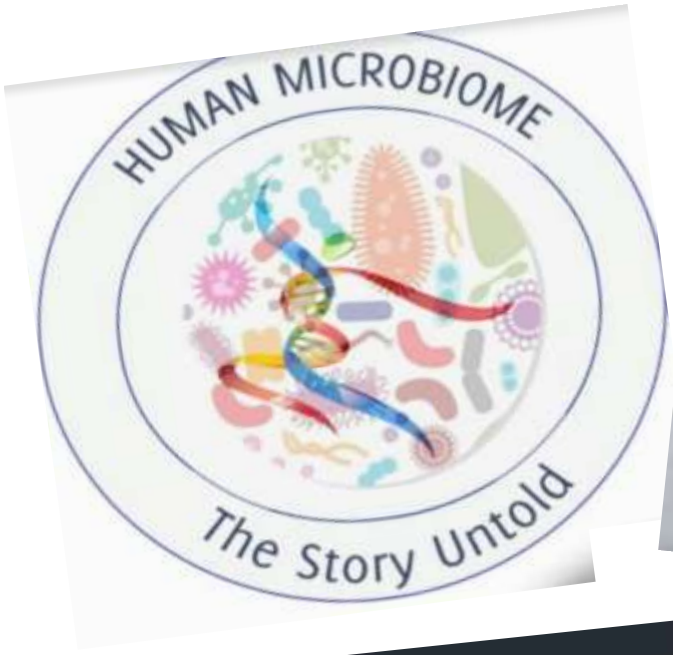
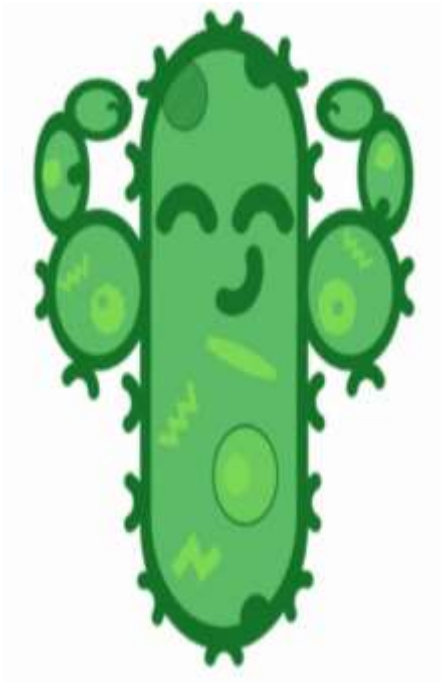
Shortlisted Logos





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Section III
Research Papers

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THE LESSER-KNOWN OPPORTUNISTIC FACET OF FUNGAL GENUS CHAETOMIUM: A BRIEF REVIEW

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ABSTRACT

Fungi are omnipresent organisms in nature. While some cause allergies, others may be pathogens, a huge majority exhibit neutral behavior in their coexistence with humans and some also have valuable positive applications. Fungal diseases usually are difficult to cure, requiring perfect diagnosis and prolonged treatments. The levels of mycosis have appropriately been identified however this important topic, affecting mankind, is apparently and largely ignored in academic curricula. A matter of concern is those saprophytic fungi, not featuring in the usual groups of pathogenic fungi, and considered as 'not dangerous' for human beings. are increasingly found in the most unlikely locations, inside vital organs. The predominantly saprophytic genus *Chaetomium* is one such fungus, species of which have been isolated from unnatural and most unlikely locations, namely, from inside vital human organs and cutaneous infections indicating opportunistic behaviour. Reports of such case studies have increased in last 30 years and have generated concern and interest leading to this review study.

Introduction: Fungi are omnipresent organisms in nature. Most of them exhibit neutral behavior in their coexistence with human beings while sharing the planet while some have positive applications for wellbeing of mankind. Some fungi are also known to negatively affect human beings by way of causing allergies and mild discomfort. Yet others are known offenders causing invasive infections and mycosis of a more serious nature. Most fungal diseases and disorders are very difficult to cure, requiring perfect diagnosis and prolonged treatments. Besides the trauma, some disorders originating from fungi cause social inferiority complexes, social discomfort and negatively affect social interactions of the affected. Some infections such as those affecting finger and toe nails are also largely ignored unless severe repercussions arise in rare cases. The levels of mycosis have appropriately been identified from superficial to deep mycosis however this important topic, affecting all mankind, is apparently and largely ignored in academic curricula. Fungi causing mycosis are identified and grouped into functional groups such as dermatophytic fungi, keratinophilic fungi or simply disease-causing fungi. Most of such organisms are well documented in case studies.

A matter of concern is those saprophytic fungi, not featuring in the above groups, and considered as 'not dangerous' for human beings are increasingly being found inside vital organs, reports of which are rising in the last 30 years, raising doubts over whether entry and presence of fungi turning opportunists and ending up in form of invasive infections inside the organ can prove fatal to the individual. Such questions largely go unanswered and reasons are attributed to

compromised immunity of the patient along with some other predisposing factor or medical condition of the individual. The predominantly saprophytic genus *Chaetomium* is one such fungus, species of which have been isolated from unnatural and most unlikely locations, namely, from inside vital human organs, leading to questions on safety and life preservation. Reports of such case studies have increased in last 3 decades, sparking need for concern and have generated interest leading to this review study.

The Genus Chaetomium: The genus *Chaetomium* (Family Chaetomiaceae) was named so after its ascomatal hairs resembling plumes of helmet (Ames, 1961) used by soldiers in olden times. *Chaetomium globosum* Kunze was the first species described and this genus now has around 100 species according to current estimates; all of them predominantly saprophytic. The genus is a known member of the highly consistent group of cellulose degrading fungi and has widespread applications in disposal of agricultural residues and municipal solid wastes.

Species of *Chaetomium* are commonly found as saprophytes in soil, plant debris, decaying plant matter, dung, cellulosic substrates and organic wastes, residues and deteriorating cellulosic materials and articles of human origin and usage. Its ascospores are a common occurrence in air samples. *Chaetomium*, apart from its role as an indoor allergen, and few rare human pathogenic attributes of *C. atrobrunneum* Ames, was largely considered 'not dangerous' from human point of view; however in spite of its saprophytic nature, rare instances of pathogenicity, external as well as internal organ infections due to probable opportunistic pathogenic nature of this genus, even resulting in fatalities, have been reported in the last 30 years, mostly attributed to immunocompromised nature of patients and presence of predisposing factors. This study was taken up with this aim to shed light on invasive mycosis, the *Chaetomium* connection and this rare and untold story of genus *Chaetomium*.

Materials and Methods: Case studies on pathogenicity reports of genus *Chaetomium* were reviewed in order to determine the opportunistic pathogenic roles of its various species as probable causal organisms of invasive infections in external parts of the human body as well as internal organs. Selected indicative case studies from PubMed and NCBI literature sources were taken into consideration for the study, and limited to the ascomycetous genus *Chaetomium*.

Results and Discussion: The various instances of species of the saprophytic genus *Chaetomium* behaving as opportunistic pathogens and causing fatal infections to internal human organs are depicted in Table 1. The genus *Chaetomium* is fairly common in the air samples of indoor environments and a potential allergen (Apetrei et al., 2009; Salo et al, 2020). Some of its species are implicated in invasive infections and cutaneous infections suggesting opportunistic nature. Huang *et al*. (2018) indicated *C. globosum, C. atrobrunneum, C. strumarium* and *C. perlucidum* in invasive and superficial infections. Schulze *et al*. (1997) and Lesire *et al*. (1999) suggested that fungal invasion especially that of Chaetomium occurs only in presence of predisposing factors and is a consequence of

some other serious medical condition; which was observed in all cases reviewed during this study. Guppy *et al.* (1998) indicated *Chaetomium atrobrunnnium* also to be responsible for cerebral abscesses, which were till then assumed to be solely caused by Aspergilli. Thomas *et al*. (1999) hinted that the rapid development of infection in the brain is suggestive of the brain tissue providing favourable environment for growth and proliferation of the opportunistic invasive fungus. Barron *et al*. (2003) added *Chaetomium perlucidum* to the list of invasive species of genus *Chaetomium* and documented ability of this organism to spread beyond the central nervous system.

Yu *et al.* (2006) isolated *C. globosum* from necrotic tissues on the face and established its connection with painful erythema. *C. brasiliense* and *C. globosum* were reported from the ear canal and nail infection respectively (Hubka *et al*., 2011). *C. strumarium* (Reddy *et al*., 2017) and *Chaetomium* sp (Jayaraman *et al*., 2011) were reported to be associated with corneal infections. *Chaetomium* sp. was indicated in peritonitis (Issa *et al*., 2013).

Tap *et al*. (2015) reported *C. globosum* to cause cutaneous infection. This first described species of *Chaetomium* was reported from different parts of the world as the causal organism of onychomycosis (Stiller *et al*., 1992; Aspiroz *et al*., 2007; Latha *et al*., 2010; Hubka *et al*., 2011; Kim *et al*., 2013; Shi *et al*., 2016).

C. atrobrunneum was linked with causing pneumonia (Wang *et al*., 2016) and black grain eumycetoma (Madura foot) (Mhmoud *et al*., 2019). Earlier, Yeghen *et al.* (1996) linked *C. globosum* with pneumonia while Capoor *et al.* (2016) associated this species with invasive pulmonary infection stating *Chaetomium* has hidden clinical potential for causing invasive infections. Most of the cases cited were fatal while some reported cure.

Conclusion: It is apparent that emerging opportunistic fungal infections are rising and opportunistic fungi such as species of *Chaetomium* are being detected from most unlikely places such as inside human organs albeit in presence of predisposing factors, risk factors and compounded by immunocompromised status of patients. This is matter of great concern considering the rising pollution, predisposing factors and rising numbers of immunocompromised patients; and the fatal nature of such invasive infections.

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Table 1: Indicative instances of *Chaetomium* causing invasive infections in Human beings

No.	Species	Isolated from	Reported by and Year
1	*C. globosum*	Brain	Anandi et al., 1989
2	*C. strumarium,* *C. atrobrunneum*	Brain	Abbott et al., 1995
3	*C. globosum*	Lungs	Yeghen et al., 1996; Paterson et al., 2005; apoor et al 2016
4	*Chaetomium* sp.	Sinus	Aru et al., 1997
5	*C. homopilatum*	Trachea	Schulze et al, 1997
6	*C. atrobrunneum*	Brain	Guppy et al., 1998; Thomas et al., 1999
7	*C. perlucidum*	Brain, Heart, Lungs, Spleen	Barron et al., 2003
8	*C. globosum*	Lymph nodes	Teixeira et al., 2003
9	*C. globosum*	Necrotic tissue on Face	Yu et al., 2006
10	*C. atrobrunneum* *Chaetomium sp.*	Cerebro spinal fluid Lungs	Al-Aidaroos et al., 2007
11	*C. atrobrunneum*	Retina	Tabbara et al., 2010
12	*C. brasiliense* *C. globosum*	Ear canal Nail infection	Hubka et al., 2011
13	*Chaetomium* sp.	Cornea	Jayaraman et al., 2011
14	*Chaetomium* sp.	Abdomen, peritoneum	Issa et al., 2013
15	*C. globosum*	Cutaneous infection on foot	Tap et al., 2015
16	*C. globosum*	Toe nails	Stiller et al., 1992; Shi et al., 2016
17	*C. atrobrunneum*	Trachea	Wang et al. 2016
18	*C. strumarium*	Cornea	Reddy et al., 2017
19	*C. atrobrunneum*	Eumycetoma	Mhmoud et al., 2019
20	*C. strumarium*	Brain, CSF	Del Castillo et al., 2021
21	*C. globosum*	infection on legs	Cronin et al., 2021

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ISOLATION AND SCREENING OF POTENTIAL CELLULOLYTIC FUNGAL FLORA FROM DETERIORATINGWOODEN PANELS

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ABSTRACT

Introduction: Cellulose is the most over abundant natural substance on earth, found largely in plants. Several cellulosic primarily based objects have found their way into human needs and became articles essentially. Fungi inflicting deterioration of wood and wood objects; additionally referred to as wood-destroying fungi will breakdown cellulose by their cellulolytic enzymes, into simple sugars. Cellulase group of enzymes even have vital applications within the management of municipal solid wastes, agricultural residues and all organic residues and also additionally give organic matter in a readily usable form, for boosting soil fertility and productivity.

Materials and Methods: Isolation of fungal organisms from the wood samples was done by serial dilution method (Pramer and Schmidt, 1966). Cellulolytic activity of the isolates was determined by loss in weight of filter paper method (Fergus, 1969). The isolated fungi were identified using standard literature.

Result and conclusion: In the present work, deteriorating wooden panels were screened for the existence of cellulolytic fungi. Throughout the study the usually encountered fungal genera were *Aspergillus, Chaetomium* and *Penicillium*. Determination of the loss in weight of filter paper was evaluated for determining the cellulolytic activity of fungal organisms, throughout that5 isolates revealed promising cellulolytic activity.

Keywords: biodeterioration, wood samples, cellulolytic activity, cellulose degrading fungi.

INTRODUCTION

Plant biomass is the most overabundant carbon source on Earth and thus plays an important role in ecology and therefore the world carbon cycle. Fungi are extremely adequate and efficient degraders of plant biomass. Fungi play a vital role in the worldwide carbon cycle because of their ability to utilize plant biomass (polysaccharides) as carbon supply. Degradation of cellulose in nature by fungal organisms could be a well-studied phenomenon. Amongst the numerous subdivisions of fungi could be a comparatively little however extremely consistent group of cellulose degrading fungi, particularly the cellulolytic species. Over one hundred cellulolytic fungi are reported and this variety is increasing with advances in research analysis. Besides cellulolytic fungi, the cellulose utilizing population of microorganisms includes aerobic and anaerobic mesophilic bacterium, thermophilic and alkaliphilic bacterium, Actinomycetes and few protozoa. Among the foremost studied wood and cellulose degrader generaare *Chaetomium, Coriolous, Phanerochaete, Poria, Serpula, Aspergillus, Fusarium, Penicillium* and *Trichoderma*

[Viikari and Ragauskas, 2009] whereas *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma*, and *Aspergillus* are amongst the extensively studied producers of enzyme cellulase [Kuhad et al., 2011; Sukumaran et al., 2005].

Cellulose is the main polymeric component part of the plant cell wall. Cellulose is the most overabundant polysaccharide on Earth, and a vital natural resource. Its chemical composition consists of D-glucose residues joined by β-1,4-glycosidic bonds to make a linear polymeric compound chains of over 10,000 glucose residues. Cellulose contains both highly crystalline regions where individual chains are linked to each other and less-ordered amorphous regions. Though with the chemicals simple, the intermolecular bonding pattern might result in a very complex morphology [Hon, 1994]. Ascomycetous and Basidiomycetous fungi are the most potent degraders of this chemical compound as a result; several species grow on dead wood or litter, in environments rich in cellulose. Fungal cellulolytic systems differ from those of bacteria while the differences between individual taxonomic groups are less pronounced [Lynd et al., 2002].Cellulase because of its numerous and large relevance has been utilized in various industrial processes [Ekperigin, 2007; Chakraborty et al., 2000; Vaithanomsat et al., 2009] as well as in agricultural and plant waste management [Mswaka and Magan, 1998; Lu et al, 2004]. With the help of cellulolytic systems, cellulose can be converted to glucose which can be a multi-utility product, during a low-cost and biologically favorable method [Gupta et al., 2012]. The search for economical microorganisms which might manufacture all the 3 styles of cellulases that may facilitate the breakdown of cellulose to glucose is of dominant importance[Maki et al., 2009].

Considering the importance and applications of fungal cellulases, this study was designed to isolate and characterize economical and efficient cellulose degrading fungi from deteriorating wooden panels to put a base for agricultural application of cellulase producing fungi.

MATERIALS AND METHODS

Isolation of fungal organisms

Portions of wooden panels showing few signs of deterioration were collected from Thane and Bhiwandi in a western part of Maharashtra state. The samples once collected were stored in sterilized polythene bags at room temperature for more process. Deteriorated parts of the sample were scraped and serial dilution method [Pramer and Schidmt, 1966] was used for isolation of cellulolytic fungi on selective media CzepekDox Agar (CZA) for isolation of pure cultures of fungal organisms from the samples. Suspensions from the samples were diluted up to 10^5 and 1 ml each of the respective dilutions was plated on nutrient medium (CzapexDox Agar, CzapexDox Agar with cellulose and PDA) in separate petri plates. The plates were incubated 37° C room temperature for expression of fungal growth. Antibiotic like Streptomycin (50 mgl^{-1}) was supplemented to suppress bacterial growth and contamination. The isolated fungi were identified using standard literature and the standard system of fungal classification[Ames, 1969; Ellis, 1971; Pitt,

1979; Tzean et al., 1990].

Loss in weight of filter paper

The experiment was totally based on the method described by Fergus [Fergus, 1969]. Isolates were fully grown on Czapek Dox Broth [Difco Manual, 1969] on Whatman No. 1paper (circular discs, with diameter90mm) as the sole source of carbon, at pH 5.2 before autoclaving. Mean dry weight of the filter paper was recorded. The experiment was conducted in petri plates of 90mm diameter. Every petri plate contained one filter paper disc of known weight and 1 ml of CzapekDox Broth without sucrose. A skinny uniform mat of surgical cotton was placed on every petri plate below the filter paper for retention of wetness. The experiment was conducted with twelve replicates i.e. triplicates were maintained for the observance of the results at 7 day intervals up to 28days. The same set maintained in identical conditions functioned as the controlset. All plates were autoclaved at 15 lbs psi pressure for 20 minutes. The inoculum was prepared in the form of suspension of spores from 15-day old cultures of isolates fully grown on CzapekDox Agar medium by adding 10ml of sterile water. The mixture was shaken well using a vortex mixer to obtain a uniform spore suspension (10^5 spores/ml). 0.5ml of the suspension was added to each plate as inoculum. In case of the control set an equal quantity of sterile distilled water was used instead of suspension. The plates were incubated at room temperature (average mean temperature 28° C) for 7, 14, 21 and 28 days respectively.

At the end of the respective incubation periods the filter paper discs were oven dried at 80° C, allowed to cool down to ambient temperature in a desiccator and then weighed to the closest mg on an electronic weighing balance instrument. The difference in weight of every filter paper disc was calculated by comparing it with the original dry weight and additionally by considering the mean difference in weight shown by the control set. The net loss in weight was attributed to cellulose degradation. The results were recorded in terms of loss in weight further as a sign of deterioration on filter paper discs. The percentage loss in weight caused by every isolate was calculated by using the formula [Ghewande, 1977]

$$\% \text{ loss in weight} = \frac{\text{Difference in weight}}{\text{Initial Weight}} \times 100$$

RESULTS AND DISCUSSION

Table 1: Screening of isolated fungal organisms for their cellulolytic activity in terms of loss in weight of filter paper

No.	Organism	% loss in weight during period of incubation			
		7 Days	14 Days	21 Days	28 Days
1	*Aspergillusniger*	2.6	8.0	11.6	15.5
2	*Aspergillusterreus*	2.1	7.6	10.1	11.8
3	*Penicillium* sp.1	3.3	6.9	8.3	11.4
4	*Aspergillus* sp.1	5.6	9.3	13.7	15.3
5	*Chaetomium* sp. 1	6.3	11.5	14.3	16.9

6	*Penicillium* sp.2	3.1	7.3	11.6	16.4
7	*Penicillium* sp. 3	3.5	4.6	6.9	10.5
8	*Chaetomium* sp.2	2.7	5.5	7.9	11.5
9	*Aspergillus* sp.2	2.5	5.9	8.3	11.5
10	*Penicillium* sp. 4	3.5	13.6	16.3	18.3
Control	Control set	1.66 (+)	1.66 (+)	1.66 (+)	1.66 (+)

Mean initial weight of filter paper 853 mg +gain in weight 10 mg (1.66%) in control set.

A total of 10 isolates were obtained from the samples collected from wooden panels. Three completely different genera of fungi were encountered within the study on deteriorating wooden panels. Table 1 reveals progressive report of percentage of loss in weight of filter paper in the succeeding weeks. Five isolates, namely, *Chaetomium* sp.1, *Aspergillus niger*, *Aspergillus* sp.1, *Penicillium* sp. 2 and *Penicillium* sp.4 revealed superior activity in terms of percent loss in weight of filter paper.

Determination of the loss in weight of filter paper is one amongst tactic method commonly reported in order to evaluate the cellulolytic activity of fungal organisms, whereby the loss in weight is alleged to correspond to the amount of cellulose degraded, that successively correlate with the cellulolytic activity of the individual organism. Results obtained are in agreement with those of similar studies carried out [Lintang et al., 2021].

CONCLUSION

Fungal organisms isolated from deteriorating wood panels were individually tested for their cellulolytic capability in terms of percent loss in weight of filter paper. The efficient organisms can further be utilized for degradation of other forms of organic cellulosic waste which can be converted into compost which will benefit organic farming and help in restoration of soil fertility.

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STUDY OF SELECT ISOLATES FROM VETIVER ROOT MICROBIOTA AS POTENTIAL IMMOBILIZED BIOFERTILIZER

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ABSTRACT

Introduction: Rhizosphere, colonizes PGPR that promote plant growth and development. *Vetiver (Vetiveria zizanoides)* is a fast growing graminaceous plant native to India. It is known for its role in soil erosion control, phytoremediation, production of essential oils and tolerance to extreme climatic variations.
Materials & Methods: Plant growth-promoting rhizobacteria (PGPR) associated with *Vetiveria zizanoides,* were characterized and growth-promoting properties of selected isolates in free and immobilised form on *Vigna radiata* (moong) seeds was investigated. 16SrRNA gene analysis of the isolates were carried out.
Results & Conclusion: The endophytic isolate E1 and rhizospheric isolate R1 were capable of producing Indole Acetic Acid (IAA) and ammonia along with fixing nitrogen and solubilizing phosphate. Molecular identification by 16SrRNA gene analysis speculated isolate R1 and E1 to be 'yet uncultured bacteria'. Efficiency of E1 and R1 as potential biofertilizer on growth of *Vigna radiata* was evaluated in free suspension and immobilised (Calcium alginate and Polyvinyl alcohol) systems maintained in controlled environment (*in vitro* tube setup) and open pot setup. Comparable results were obtained with suspension system in pot and tube setup. Alginate immobilized cultures were able to promote plant growth in pot system when compared with *in vitro* system indicating involvement of environmental factors in determining final effect of isolates on promoting plant growth. However, PVA system were unable to germinate Moong seeds possibly because of reduction in cell viability due to exposure to boric acid during immobilization.

Keywords: PGPR, Vetiver, Immobilization

INTRODUCTION

Meeting the ever-increasing food demand is a serious concern in developing countries. In view of the well-known harms to environment and humans by the chemical fertilizer, use of biofertilizer is an important strategy in safe organic farming.

'Rhizosphere', 'the soil influenced by roots', colonizes PGPR that promote plant growth and development, by nutrient acquisition and assimilation (nitrogen, phosphorus, potassium, other essential minerals), phytohormone modulation, suppression of plant pathogens, combating abiotic stress (Pereira S. *et al*, 2020). Use of PGPR as an active ingredient of biofertilizers is an eco-friendly way to increase soil fertility and crop productivity.

Endophytes, the microorganisms found within plant tissues, are subset of the root microbiome that can be beneficial or non-

beneficial to the host plant. Many endophytic bacteria, however, have been shown to have PGP properties. Though many 'yet-unculturable' endophytes have been known, they have not been isolated in pure culture to facilitate their use in various applications (Gaiero et al., 2013).

The PGPR inoculant formulation can be either in free or immobilised form. Alginate and PVA polymers were selected as entrapping agents for present study. Alginate cross linked with $CaCl_2$ is most commonly used to immobilise microorganisms due to its low cost, non-toxic and biodegradable properties. Immobilisation of the bacteria in alginate beads leads to slow release of microbes for colonization of roots (Bashan Y., 2002). PVA cross linked with boric acid is used to prepare elastic beads with high strength and durability. Addition of calcium alginate helps to eliminate the agglomerization of PVA (Zhan J. *et al.*, 2013).

Vetiver (Vetiveria zizanoides) is a fast growing graminaceous plant native to India. It is known for its role in soil erosion control, phytoremediation, production of essential oils and tolerance to extreme climatic variations (Truong *et al*, 2000). At least few of the unique physiological characteristics of this plant are attributed to the microbiome associated with the plant rhizosphere (Chen et al., 2020). In our previous study (Pawar *et al.,* 2017), we have reported the isolation of endophytic bacterium isolate E1, and rhizospheric bacterium isolate R1 from roots of *Vetiver* using 'plant based dilute cultivation media'. These isolates were also shown to exhibit nitrogen fixation and phosphate solubilization activity. Present study aimed at molecular identification of these cultures, further study of the plant growth promoting activities of these isolates, along with determination of their efficiency as free-living cultures and immobilized biofertilizer.

MATERIALS & METHODS

Production of IAA:

IAA produced by the isolates E1 and R1 was determined using the colorimetric method described by Gordon & Weber (1951).

Ammonia production:

The isolates were inoculated in 1:100 dilute nutrient broth and incubated at 27±2°C for one week. 1ml of culture was added to 0.5ml of Nessler's reagent (Agbodjato *et al.*, 2015). The development of brown to yellow colour indicated ammonia production. Positive control with NH_4Cl and negative control with distilled water were maintained.

Molecular Identification and Phylogenetic Analysis of isolate E1 and R1:

Isolate E1 and R1 culture pellets were outsourced for molecular identification to Chromous Biotech Pvt. Ltd., Bengaluru, Karnataka, India.

For molecular identification, genomic DNA was extracted from the isolates followed by 16S rRNA gene amplification by Polymerase Chain Reaction (PCR) using consensus primers.

Amplification of bacterial 16S rRNA genes: The PCR mix contained the following: DNA, 1 µl; dNTPs 2.5mM each, 4µl; 10X Taq DNA polymerase Assay Buffer, 10 µl; Taq DNA polymerase, 3.0U/µl, 1µl; 16S rRNA gene

primers forward [5'–AGHGTBTGHTCMTGNCTCAS–3'] and reverse [5'–TRCGGYTMCCTTGTWHCGACTH – 3'], 400ng each; PCR grade D/W to make volume to 100 µl. All PCR reagents were of Chromous make. Amplification conditions were as follows: initial denaturation, 95°C, 5 min; 35 cycles (denaturation, 94°C, 30 sec; annealing, 50°C, 30 sec; extension, 72°C, 1.30 min); final extension, 72°C, 7 min. The PCR product of 1.5 kb was separated by agarose gel electrophoresis along with molecular weight marker.

Amplicon sequencing: The 1.5 kb 16S rRNA gene amplification product was sequenced bi-directionally by Sangers method. Sanger's sequencing was carried out in ABI 3500 Genetic Analyzer according to the BigDye Terminator v.3.1 Cycle Sequencing Kit protocol (Applied Biosystems, United States). The reaction mixture contained the following: Ready Reaction Premix, 4 µl; primer, 2 µl; Template,100 ng/ µl, 1µl; Milli Q Water, 3µl. Reaction conditions: 25 cycles (96°C, 5 mins; 96°C, 30 sec; 50°C for 30 sec; 60°C, 1.30 min).

Phylogenetic analysis: The 16S rRNA gene sequences were analysed by nucleotide BLAST and 'Seqmatch' tool of the Ribosomal Database Project. The sequences have been deposited in the ENA (European Nucleotide Archive) sequence repository (accession numbers LT984792.1 and LT984793.1). Phylogenetic analyses were conducted using the MEGA7 software package (Kumar et al. 2016). Sequences were aligned with closest BLASTn matches using the CLUSTALW algorithm. Molecular phylogenetic analyses were inferred by using the Maximum Likelihood (ML) method. Genetic distance between homologous sequences was calculated using the Tamura 3- parameter nucleotide substitution model. Both trees were rooted with the outgroup *Deinococcus radiodurans* strain DSM 20539 (NR 026401.1) and *Clostridium perfringens* strain ATCC 13124 (NR 121607.2).

Immobilization of the cultures: Both the isolates were inoculated in 1: 100 dilute nutrient broth and incubated at 27±2°C for one week. They were, individually and in consortium, were immobilized with the following two immobilizing systems: (i) 4% sodium alginate and chilled 6% calcium chloride (Schoebitz M. *et al.*, 2013) (ii) 8% Polyvinyl alcohol (PVA) + 1% sodium alginate and chilled 5% boric acid + 6% calcium chloride (Zhan J. *et al.*, 2013). The immobilized beads were stored overnight at 4°C and washed with sterile distilled water. Control beads were made with sterile distilled water.

***In vitro* and Pot system:**
Moong seeds were washed under tap water for 5 minutes followed by washing with diluted detergent Tween 20 for 20 minutes. Seeds were washed with distilled water 3 times; surface sterilized with 70% ethanol for 30 seconds, washed with sterile distilled water 3 times. They were transferred to 0.1% mercuric chloride with intermittent shaking for 4 minutes, washed with sterile distilled water 3 times and dried on sterile filter paper. Surface sterilized seeds were then transferred to sterile soil in tube. Following systems were maintained to evaluate the efficacy of immobilized isolate with suspension of the isolate:

(A) Suspension system:

- Control for suspension system (C): Soil fed with only water.
- Sus (E), Sus(R), Sus(E+R): Soil inoculated with free cell suspension of E1, R1, E1+R1 respectively.

(B) Immobilized PVA system:

- PVA (C): Soil with PVA beads without culture
- PVA (E), PVA (R), PVA (E+R): Soil with PVA immobilized E1, R1, E1 + R1 respectively.

(C) Immobilized Calcium alginate system:

- Alg (C): Soil with calcium alginate beads without culture
- Alg (E), Alg(R), Alg (E+R): Soil with calcium alginate immobilized E1, R1, E1+R1 respectively.

Each system maintained in duplicates was supplied with 5ml sterile distilled water on Day 1 to maintain sufficient moisture level in soil. After 7 days of incubation, seedlings were taken out of each of the system and its root length, shoot length was measured. Vigour index for each of the system was calculated.

$$Vigor\ index = \frac{Germination\ percentange * mean\ of\ seedling\ length\ (root + shoot)}{100}$$

For evaluation of efficacy of free suspension and immobilized endophytic and rhizospheric isolate as PGPR on *Vigna radiata* in natural environment, same systems as above were maintained in plastic bags containing non-sterile soil. Three seeds of *Vigna radiata* were sown in each of the plastic bag and watered daily. Root length and shoot length was measured and vigour index was calculated.

Statistical analysis:

The *in vitro* seed germination data obtained was subjected to Kruskal Wallis in SPSS software whereas data of pot system was analysed using ANOVA in SPSS software. The difference among various treatments means were compared using least significance difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION:

In spite of several reports demonstrating the application of microbial products as biofertilizers, the full potential of several beneficial rhizobacteria as biofertilizers remains largely unexplored. Isolating efficient PGP strains that are part of different soil and root microbiomes, in formulations with longer shelf-life, would certainly play major role in this area.

Different PGP activities of isolate R1 and E1 were determined.

IAA production:

A wide range of processes in plant development are known to be regulated by exogenous IAA in which a low amount of IAA can stimulate primary root elongation, whereas high IAA levels decrease primary root length, increase root hair formation, and

stimulate the formation of lateral roots, giving plants greater access to soil nutrients. IAA produced by isolate E and R was found to be 4.75 and 3.625 µg/ml respectively after 48 hours of incubation.

Ammonia production:

Both isolate E1 and R1 showed change in colour to yellowish brown with Nessler's reagent indicating their ability to produce ammonia, affirming their nitrogen fixation ability.

Molecular Identification of the cultures:

BLASTn analysis of 16S rRNA gene of isolate R1 showed sequence similarity to 16S rRNA gene sequence of *Acinetobacter pittii* strain L3/3, a PGPR isolated from rice rhizosphere; as well as to that of uncultured bacteria. SeqMatch RDP (Sab score 1.000) tool supported these results, showing similarity to *Acinetobacter* as well as uncultured bacteria. Phylogenetic analysis in MEGA7 software, however, revealed its closest match to bacterium strain BS0171 and bacterium strain WG90917, both 'yet uncultured bacteria'.

BLASTn analysis of 16S rRNA gene of isolate E1 showed sequence similarity to uncultured bacterial 16S rRNA gene clones. RDP analysis confirmed its similarity to 'yet uncultured bacteria' (Sab score 1.000). Phylogenetic analysis in MEGA7 software also showed closest match to *Chryseobacterium geocarposphaerae* strain pgl4, isolated from rhizosphere soil of *Magnolia officinalis*.

Considering all these results, we speculate that isolate R1 and E1 might be 'yet uncultured bacteria' from rhizosphere and root tissue of *Vetiveria zizanioides* respectively. Further genetic analysis of the isolates needs to be performed to confirm the same.

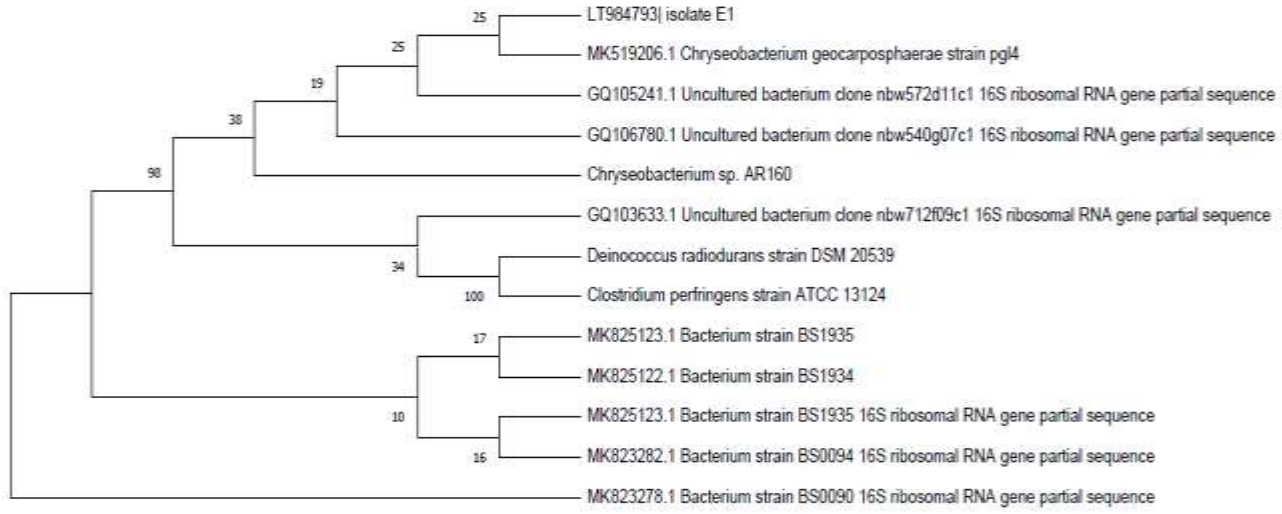

Fig.1 Phylogenetic tree of isolate E1

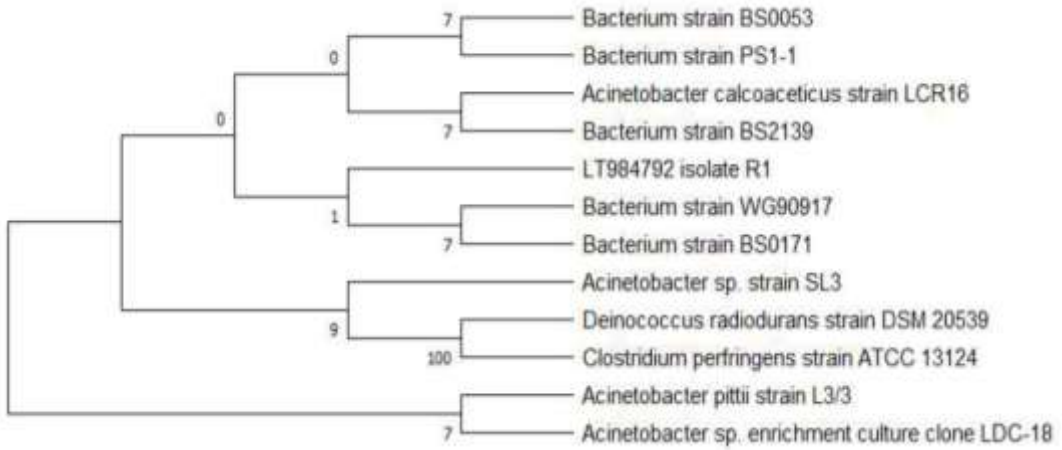

Fig.2 Phylogenetic tree of isolate R1

***In vitro* and Pot system:**

Sus (C) was found to give better vigour index than suspension E, R and E+R using *in vitro* setup (Kruskal Wallis, $p \leq 0.05$). Similarly, Sus (C) promoted plant growth significantly compared to other suspension systems in pot system setup. Hence, results obtained with E1, R1 and consortia of E1 and R1 were less promising in both the suspension systems. It might be because the isolates would be involved in enhancing the plant growth at later developmental stages and not in breaking seed dormancy.

However, unlike *in vitro* setup, Sus (E+R) showed significant effect in inhibiting plant growth compared to Sus (C) and Sus (E) (ANOVA, SPSS, $p \leq 0.05$) in pot system setup. This result might be attributed to the combined inhibitory effect of environmental factors plus consortium of endophytic and rhizospheric isolate on the growth of moong in open pot system. Similar effect in suspension system of *in vitro* setup was not seen probably because of controlled parameters.

Moong seeds were unable to germinate in PVA system in both *in vitro* and pot system setup. During immobilization, bacterial cells might be exposed to boric acid reducing cell viability. Sodium sulphate might be used as an inducer in crosslinking of PVA beads which can reduce the severe impact of boric acid on the cell viability (Takai T. *et al*, 2011).

In the *in vitro* setup, alginate immobilized R and E+R showed no significant difference in promoting growth of moong when compared with the control. Alginate immobilized E did not show any growth in *in vitro* system (Kruskal Wallis, $p \leq 0.05$). This might be because of inability of endophytic isolate to promote plant growth under controlled environmental conditions of *in vitro* setup (Kloepper *et al.,* 1980).

In pot system setup, calcium alginate immobilized E, R and E+R showed significant effect in promoting plant growth when compared with Alg (C) (ANOVA, SPSS, $p \leq 0.05$) indicating that the treatment might be enhanced by environmental conditions (soil characteristics and weather)

of pot system. Vigour indices also were found to be in parallel with the statistical analysis for both the systems.

System	(C)	Sus (E)	Sus (R)	Sus (E+R)	Alg (C)	Alg (E)	Alg (R)	Alg (E+R)	PVA (C)	PVA (E)	PVA (R)	PVA (E+R)
Vigour index (*in vitro* system)	228	137	134.5	224	146.5	0	57.25	75	0	0	0	0
Vigour index (Pot system)	254.3	249.3	230.3	159.7	23.7	183.3	167.7	165.7	0	0	0	0

Fig.3 Vigour indices of *in vitro* and pot system

Symbiotic bacteria are known to be present in the intercellular spaces of the host plant and may form mutualistic interactions with their hosts and penetrate plant cells (Vejan et al., 2016). Since isolate E1 was obtained as an endophyte from the roots of *Vetiveria zizanioides*, it's possible that it is highly specific in forming association and showing PGP activities with this particular plant only, and hence lower PGP activity was observed when *Vigna radiata* was used as the test system. Also, the isolates were obtained from *Vetiver*; a monocot plant whereas tested for efficacy in *Vigna radiata*; a dicot plant.

Different chemicals and signalling molecules in the form of root exudates released by the plant are known to elicit the interaction between plant root and soil microorganisms. Also, single change in plant genotype might alter the rhizosphere microbiome (Berendsen

R., *et al*, 2012). Thus, possible specificity of root exudates to deter or attract microorganisms might be an important factor responsible for the poor vigour indices.

Although PGPR are effective at promoting plant growth and development, few bacterial species may inhibit growth (Vejan et al., 2016). Hence, to peruse the plant growth promoting ability of the isolates, the experiments need to be repeated with *Vetiver* as test system.

CONCLUSION

Present study highlights the varied efficiency of probable yet uncultured bacteria isolated from *Vetiveria zizanoides*, on the growth of *Vigna radiata*. The results indicate that the efficiency of the microbiota as PGPR might depend on soil characteristics and plant species along with the form in which they are applied. Better understanding in context of host specificity, plant–microbial interactions, study based on randomized block design, prolonged observation of effect of isolate on plant growth and detailed characterization of yet uncultured bacteria is required.

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ISOLATION OF NOVEL PGPR FROM VERMIWASH MICROBIOTA

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ABSTRACT:

Introduction: Vermiwash is leachate collected from vermicompost pile. It is a rich source of vital nutrients that promote plant growth. A vast brigade of microbes flourishing in vermiwash contributes greatly to its nourishing potential. Regular farming practices involve use of diluted vermiwash and rarely its neat preparation. It is noted that Plant growth promotion capacities of vermiwash vary with respect to its concentration and plant species to which it is applied.
Materials & methods: Limited literature is available on exploring the possibility of exclusive microbial community thriving at different concentrations of vermiwash that might be contributing in rendering growth promoting attributes. Present study explores different 'Culturing' approaches for isolation of novel Plant Growth Promoting Rhizobacteria (PGPR) and for analyzing exclusive bacterial flora of vermiwash at different concentrations and their individual nourishing potential. Different concentrations of vermiwash viz. 10%, 20%, 50% and neat sample were used and subjected to culturomics approaches namely- media based variation and sample- based variation. Select microbial isolates thriving exclusively at specific vermiwash concentrations were considered for molecular analysis using 16s rRNA gene sequencing studies.
Results and Conclusion: The results revealed, four of the six isolates to be probable novel PGPR. All the isolates could fix atmospheric nitrogen, four isolates could produce auxin IAA and one could also solubilize phosphate. The use of dilute media simulating natural environment was found to be effective approach for cultivation of 'yet uncultivated' bacteria. Vermiwash concentration seemed to be an important factor determining the prevailing microbiome.

***Keywords*:** vermiwash, microbiome, media-based variation, sample-based variation, 16S rRNA gene analysis

INTRODUCTION:

Uncontrolled applications of chemical fertilizers for maximizing yield outputs have worsened soil health during the era of green revolution, so much so that one may term this era as era of "greed evolution". Besides posing severe pollution and health hazards to community, the same has also devastated agro-economy of the country. Thus, emphasizing on increasing use of effective biofertilizers as alternate or complementary measure is a priority. Organic compost, humus, vermicompost and vermiwash are proven bio fertilizers which were and are still reliable ones among most frequently applied biofertilizers.

Contribution of earthworms in conventional farming is incomparable. In natural habitats, earthworms help degrade organic waste material to form Humus and manage nutrient flux through

'vermicomposting'. Besides humus, it generates another valuable liquid manure namely vermiwash. Vermiwash is a liquid that can be collected after the passage of water through an active vermicompost pile. It is a collate of excretory products, mucus secretion of earthworms along with micronutrients from the soil organic molecules. Earthworms indirectly influence the dynamics of soil chemical processes and affect the activity of the soil micro-flora. Vermiwash as well, harbor a vast brigade of microbes that contributes greatly to its nourishing potential. Number of studies reported plant growth promoting effect of vermiwash used at lower concentration. Efficacy of dilute vermiwash when used as foliar spray and liquid manure has also been substantiated. (Mohammad H., 2014; Gopal M., 2010; Mujeera, F., and Malathy, S. 2014.) In present study, we aim to isolate novel Plant Growth Promoting Rhizobacteria from Vermiwash microbiota, hypothesizing that specific concentrations of vermiwash would have unique microbial communities. These novel PGPR isolates can be further explored as potential biofertilizers.

MATERIALS AND METHODS:

Collection of vermiwash:

To active vermicompost pile (earthworm species*: Eisenia fetida*), water was evenly sprinkled and allowed to seep through the vermicompost bed overnight. The dark brown colored, seepage drained from the compost bed was collected in the collector which was then filtered through Whatmann Filter paper No. 1 and stored in sterilized glass bottle. The sample was kept in refrigerator till use.

Preparation of Vermiwash samples

Vermiwash samples were diluted using sterile Distilled water to get 10%, 20%, 50% concentrations and one sample used for undiluted or Neat. The samples were incubated at room temperature overnight before they are used as samples for culturing the microflora.

Media based variation:

To study the influence of concentration of media components and presence of known and unknown growth factors on microbial recovery three media formulations were used.

A) Use of General-purpose medium:

Conventional nutrient agar medium was prepared mixing 1.3g dehydrated Nutrient Broth powder (Himedia laboratories) and 3g Agar Agar powder in 100mL distilled water. The media was sterilized and then used for making the culture plates by pouring 20 ml molten medium in individual plate.

B) Use of 1:100 diluted medium containing 10% vermiwash:

To make dilute medium simulating natural environment 0.013g dehydrated Nutrient Broth powder (Himedia laboratories) was mixed with 90mL Distilled water and 10mL vermiwash. The medium was autoclaved at 121°C for 15 min. before making plates.

C) Use of Gradient plate technique:

Concentration gradient of vermiwash microenvironment was established using basal agar which was overlayed with vermiwash medium.

Basal medium: 0.013 g Nutrient Broth + 100 mL Distilled Water + 3 g Agar agar

Overlayed medium: 0.013 g Nutrient Broth+ 100 mL Vermiwash + 3 g Agar agar

The vermiwash present in top layer would diffuse vertically to form smooth gradient of vermiwash concentration.

Sample Preparation

After overnight incubation at room temperature the samples viz. 10%, 20%, 50% dilute samples and Neat sample (i.e. undiluted vermiwash) were used for streaking general purpose media , 1:100 diluted nutrient media plates and for spreading the gradient media plates. All the plates were incubated at room temperature for three weeks and observed daily for colonies and micro colonies.

Analysis of Plant Growth Promoting Ability:

Nitrogen Fixation: All the isolates were screened for nitrogen fixation capacity by culturing them on Ashby's Mannitol Agar.

Phosphate Solubilization: Pikovaskayas's Agar (Himedia laboratories) was prepared and used for culturing and screening phosphate solubilization capacities. The isolates revealing zone of clearance were considered as phosphate solubilizers.

Indole Acetic Acid production (Plant Growth hormone): IAA assay

IAA produced by the isolates was determined quantitatively using colorimetric method described by Gordon and Weber (1951). The isolates were grown in nutrient broth containing 0.1g/L of L-tryptophan at 27± 2°C for 48 hours. After incubation, culture was centrifuged at 8000 rpm for 10 minutes. 1mL of the supernatant was added to 2mL of Salkowaski's reagent (0.5M $FeCl_2$ and 35% H_2SO_4) and incubated at room temperature for 25 minutes. The pink coloration developed was measured colorimetrically at 530nm.

Gram staining and basic biochemical profiling:

The selected isolates were Gram stained by following the standard protocol. Standard biochemical tests IMViC, catalase and oxidase were performed.

16s rRNA gene analysis:

The isolates were selected on the basis of their exclusive presence and growth promoting features and were maintained on dilute nutrient medium. After incubation period they were subjected to 16s rRNA gene analysis.

Molecular Identification and Phylogenetic Analysis of Cultures:

Cloning based on 16S rRNA gene identification technique was employed for molecular identification of the cultures. The 16s rRNA gene sequences were further analyzed by nucleotide BLAST and SeqMatch tool of Ribosomal Database Project. The sequences have been deposited in the ENA (European Nucleotide Archive) sequence repository. Phylogenetic analyses were conducted using the MEGA7 software package (Kumar et al.,2016)[1]. Sequences were aligned with closest BLASTn matches using the Clustal W algorithm. Molecular phylogenetic analyses were inferred by using the Maximum Likelihood (ML) method. Genetic distance between homologues

sequences was calculated using Tamura 3-parameter nucleotide substitution model.

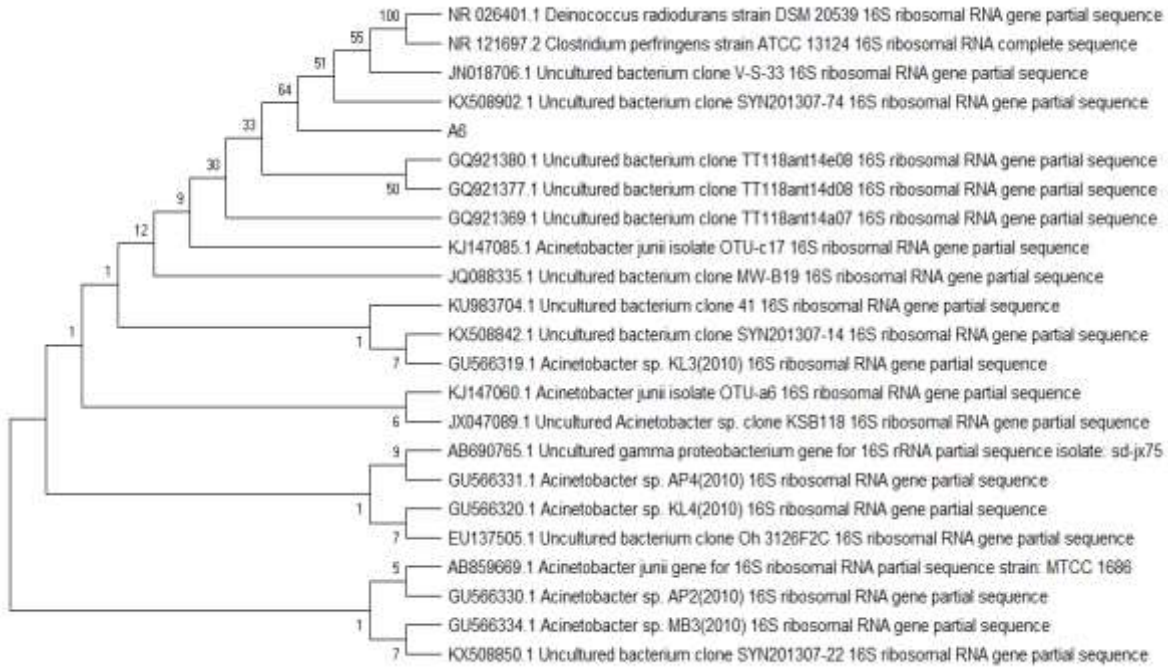
Observation table:

Isolate/ Accession number	Colony Characteristics	Gram Nature	BLASTn	RDP	Phylogenetic Analysis
A6 LR596009	Concentric colony, fluorescent	Gram negative	Uncultured /*Acinetobacter* (100%)*	*Acinetobacter* / uncultured (1.000)#	Uncultured bacterium
A8 LR596011	Pink, circular, fluorescent, centre raised	Gram negative, bacilli, capsule like structure	*Methylobacterium aqaticum* (99%)	Uncultured *Alpha proteobacterium* (1.000)	Uncultured bacterium
B1 LR596010	Agar embedded, slightly raised, irregular	-	Uncultured / *Pseudomonas* (100%)	*Pseudomonas* / Uncultured (1.000)	*Pseudomonas stutzeri*
B4 LR596012	Small colony, raised, irregular	Gram positive, Diplococci in cluster	Uncultured / *Acinetobacter* (99%)	*Acinetobacter* / Uncultured (1.000)	Uncultured bacterium
C3 LR596013	Faded, concentric, slightly irregular, fluorescent, flat	Gram negative, coccobacilli / rods (might be mixed)	Uncultured bacterium / *Rheinheimera sp*. (99%)	Uncultured bacterium / *Rheinheimera sp*. (0.986)	Uncultured bacterium
D2 LR596789	Fluorescent, diffused growth, irregular, Flat (Pseudomonas like)	Gram negative, rods	*Pseudomonas* (99%)	*Pseudomonas* (0.983)	*Pseudomonas sp.*

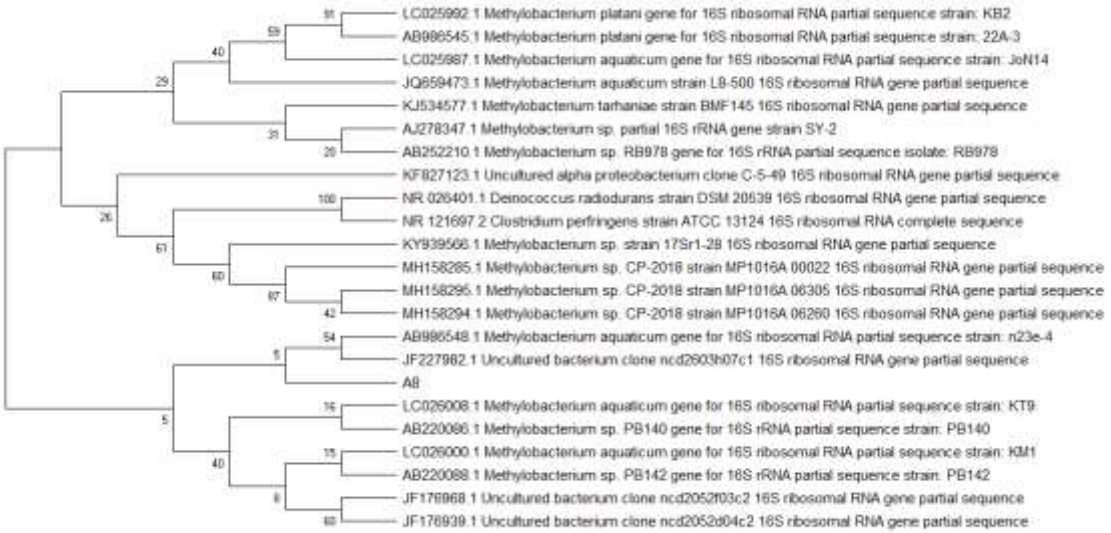
[* - Score, # - S_ab]

Phylogenetic trees:

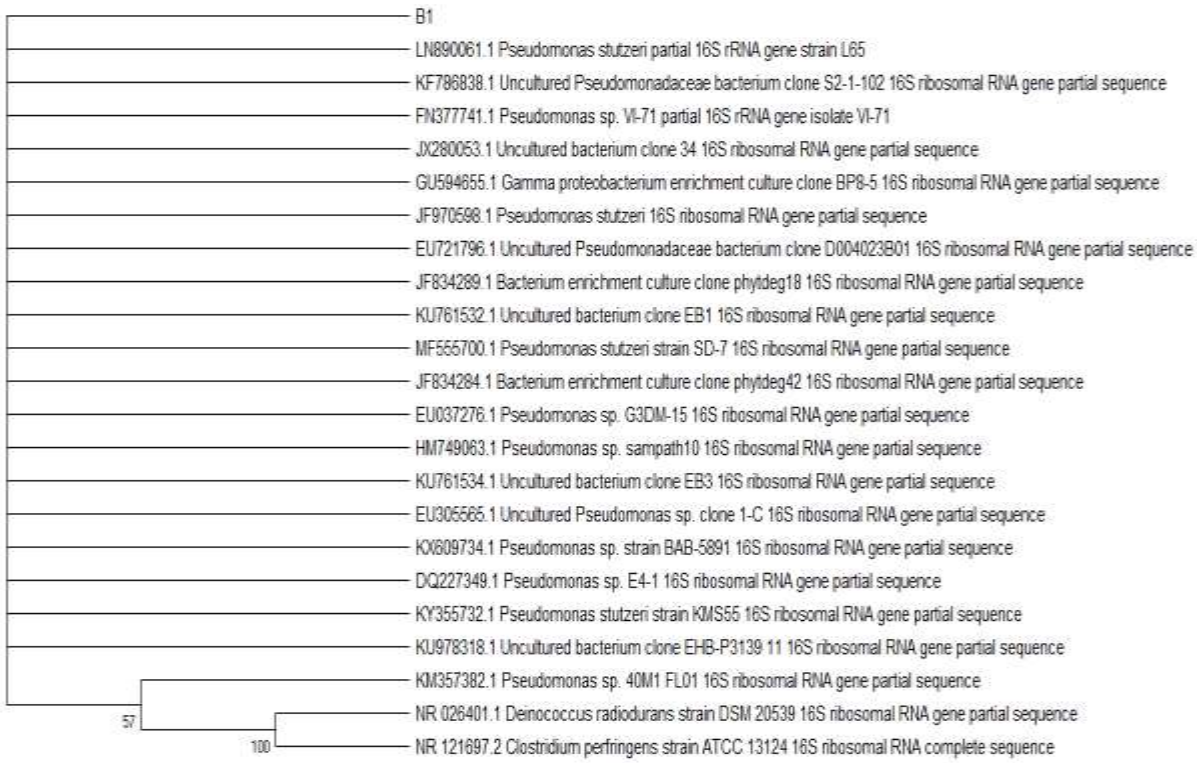
Isolate A6:



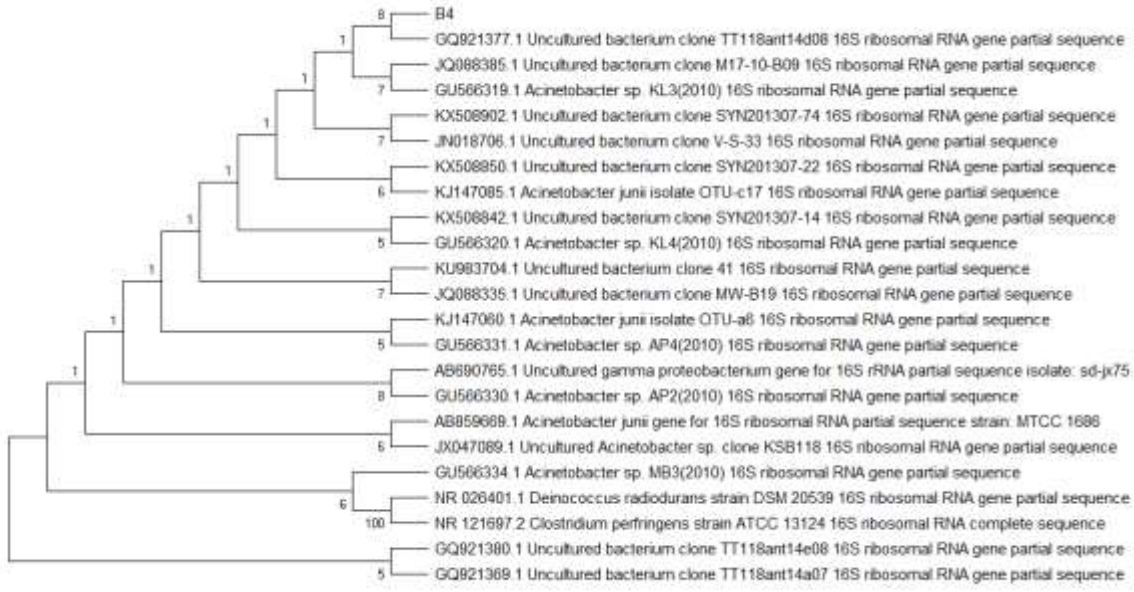
Isolate A8:



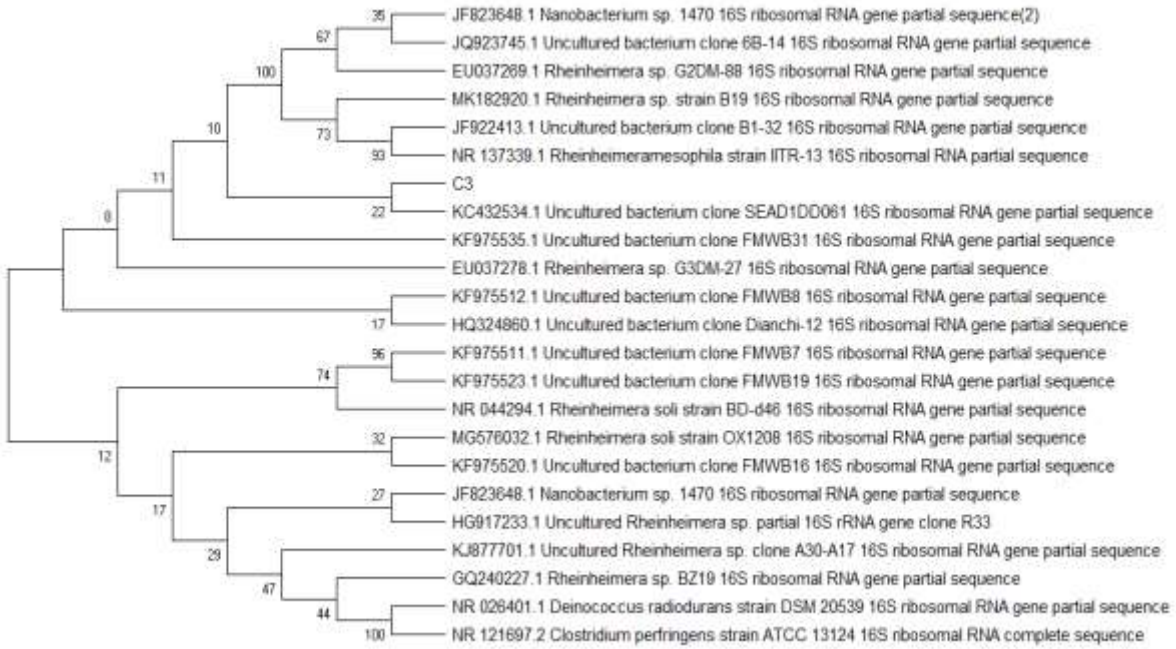
Isolate B1:



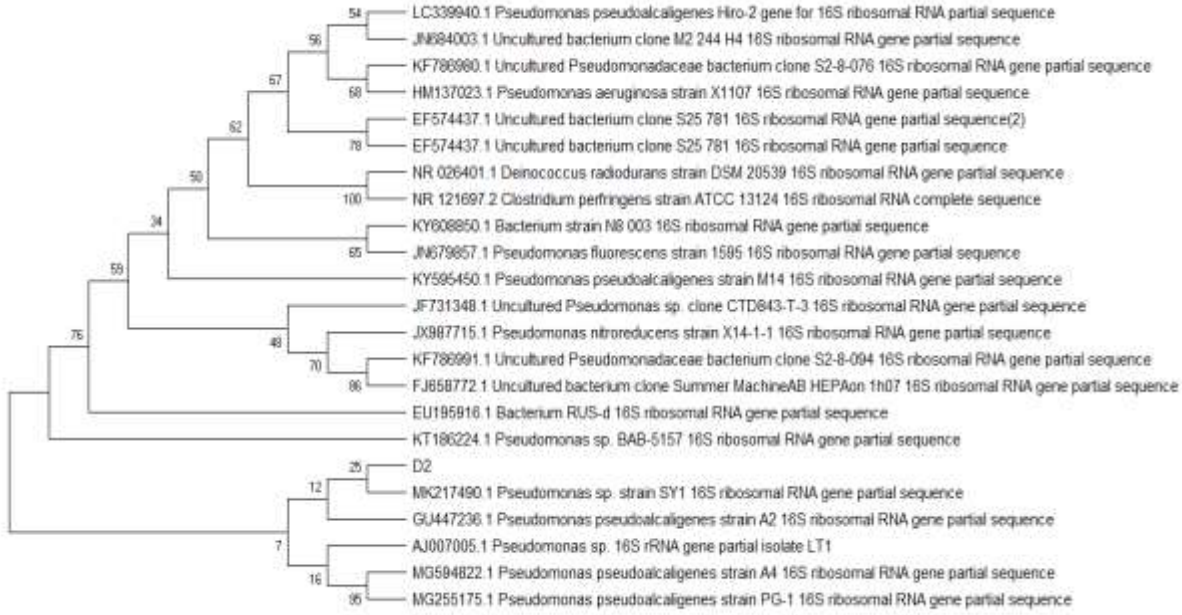
Isolate B4:



Isolate C3:



Isolate D2:



DISCUSSION:

Vermiwash is one of the most popular organic fertilizers that are being used effectively in the organic farming practices. Studies have reported different modes of preparation of vermiwash and its varied application as well. Numerous studies have reported Its efficacy as a soil additive and foliar spray for various crops and even for ornamental flowers. (Chattopadhyay A. (2015), Murali G. et al (2010)

In present study, we attempted to compare, the microbial recovery on general purpose media and that on dilute medium simulating natural environment was drastically different. General purpose medium revealed many of *Pseudomonas sp.* dominating. In contrast, the dilute medium showed enormous microbial diversity in form of vast number of micro-colonies that were observed to be slow growers.

It is observed that classical microbiological strategy involves study of microbes in isolation however in reality, the microbes tend to work closely and not in isolation. This is identified as one of the major hurdles, why few species resist to get recovered during their culturing *in vitro*.

All four dilutions viz. 10 %, 20 %, 50 % and neat or 100% samples when streaked on general purpose medium and dilute medium showed microbial diversity which was noted in 10%, 20% and 50% whereas, the neat samples showed dominant presence of *Pseudomonas spp.*

Reason of this observation is probably the chance that these microbial communities perform Cell-Cell Communication, and the environment has a low nutrient concentration where oligophiles can flourish.

Also, we used gradient plate technique where we created the gradient of concentration of vermiwash on which an attempt was made to check if specific types of organisms get cultured at specific concentration of vermiwash. When vermiwash samples were spread on such gradient medium preferential growth trends were noted, through microcolonies appearing at lower concentrations of vermiwash and prominent presence of *Pseudomonas sp.*

Gradient plate technique is conventionally being used to study antibiotic resistance. Apart from this, the technique has been used to study the effect of variable environmental factors on the growth of particular organism [Panagou E. *et al* (2005)] ; to screen a potential strain for biomineralisation of contaminants[Gajendiran A. *et al* (2017)] [Bhalerao T. *et al* (2007)]. We have used Gradient Plate technique to isolate novel PGPR from Vermiwash. As per our literature study, prior to us only Webster N. *et al* (2000) has used this technique to isolate previously uncultured bacteria associated with a marine sponge. Thus, our approach of isolating novel bacteria using Gradient plate technique is somewhat a less explored application of this technique.

Depending on exclusive presence and striking morphological features 6 isolates were selected for further analysis.

Using nucleotide BLAST and SeqMatch tool of Ribosomal Database Project, 16s rRNA gene sequences were analysed and compared with their microbial and cultural morphologies for final phylogenetic analysis.

Isolate A6, with BLASTn (similarity score of 100%) and SeqMatch RDP (Sab score 1.000) showed to have similarity with Acinetobacter and Uncultured bacterium. Phylogenetic analysis revealed it to be an Uncultured bacterium. Isolate A8 was also found to be uncultured bacterium based on RDP analysis (Sab score 1.000). Both these isolates were obtained from 10% vermiwash sample.

From 20 % diluted vermiwash sample, two exclusive isolates obtained were B1 and B4. Isolate B1 was identified as *Pseudomonas stutzeri.* Isolate B4 was having a close similarity with uncultured Bacterium and *Acinetobacter* BLASTn (similarity score of 99%) and SeqMatch RDP (Sab score 1.000), however when Gram stained, the bacterium was found to be Gram positive diplococci unlike *Acinetobacter*, hence found to be an uncultured bacterium.

From 50% Vermiwash sample C3 isolate was obtained. BLASTn (similarity score of 99%) and SeqMatch RDP (Sab score 0.986) indicated it to be Uncultured bacterium or *Rheinmeinhera spp*. Obtaining Phylogenetic tree it was found to have close similarity with uncultured bacterium and not with *Rheinmeinhera spp.*

Isolate D2 from neat sample was found to be of the genus *Pseudomonas* based on BLASTn (similarity score of 99%) and SeqMatch RDP (Sab score 0.983)

All these isolates, when screened for their ability to fix nitrogen, could grow on Ashby's Mannitol Agar plates indicating nonsymbiotic Nitrogen fixation capacity of all six isolates.

Phosphate solubilization capacity was checked by culturing these isolates on Pikovaskaya medium. Out of six isolates only C3 isolate, identified as uncultured bacterium, showed phosphate solubilization ability.

Indole-3-acetic acid is an auxin that has been implicated in plant growth promotion by stimulating root growth and increasing root branching. IAA production is one of the very important properties that contribute positively for plant growth promotion. Out of six isolates that were screened for IAA production isolates C3, A6, A8, and B1 showed IAA production.

CONCLUSION:

Vermiwash concentration seemed to be an important factor determining the prevailing microbiota. All the isolates could fix atmospheric nitrogen and 4 isolates could produce auxin IAA which is an important plant growth hormone. One isolate could also solubilize phosphate that is required for root growth and flowering. These qualities indicate that the isolates have promising future as plant growth promoters. Four out of the six isolates, were found to be yet uncultured PGPR. Hence, use of dilute media simulating natural environment is an effective approach for cultivation of 'yet unexplored' bacteria. Gradient medium can be further explored to check the influence of concentrations of active environmental constituent in simulated environment and for isolating novel bacteria. The potential of the prevailing microbiota from particular concentration of vermiwash and their specific role as PGPR supporting specific plants needs to be explored further.

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INHIBITORY EFFECT OF BIOGENICALLY SYNTESIZED GOLD NANOPARTICLES OF METHANOLIC EXTRACT OF STEM BARK OF ACACIA LEUCOPHOLEA (ROXB.) WILLD. ON VARIOUS VIRULENCE FACTORS OF *CANDIDA ALBICANS*

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ABSTRACT

Introduction: - Planktonic growth, adhesion, cell surface hydrophobic and mainly biofilm formation are the important virulence factors caused by *Candida albicans* is serious clinical challenge for immune compromised population. Present investigation reports that the gold nanoparticles have promising effect on various virulence factors of *Candida albicans.*

Materials and Methods:-The biosynthesis of gold nanoparticles (GNPs) through the reduction of HAuCl4 using methanolic extract of stem bark of *Acacia leucophloea* (Roxb.) Willd. (Latitude 19°06'08" N; Longitude 77°17'16"E) of family Mimosaceae. The gold nanoparticles were characterized by Nano spectrophotometer for identification of gold specific peak, X-Ray Diffraction (XRD) for the size and nature of nanoparticles and Transmission Electron Microscopy (TEM) and HORIBA SZ-100 Particle Size Analyzer and Calorimetric assay (XTT metabolite assay) ; biofilm formation was analysed using inverted light microscope and Scanning of Electron Microscope and the cell surface hydrophobicity assay was carried out to check the cell surface hydrophobicity for biosynthesised gold nanoparticles. It was checked for various anti-virulence factors on two strains of *Candida albicans.* One strain is standard culture obtained from Chandigarh (ATCC90028) and other strain is a clinical isolate No.3 of *Candida albicans* of Government Medical College (GMC-3).

Result and Conclusion:-The particles size analysis of biosynthesized gold nanoparticles by various methods showed that Transmission Electron Microscopy (TEM) reveals that the size of gold nanoparticles was 46 nm and by particle size analysed using HORIBA SZ-100 Particle Size Analyzer it is 71.8 nm, while 13.66 nm as per , X-Ray Diffraction (XRD) analysis.

Anti-virulence effect of biosynthesized gold nanoparticles showed inhibitory effect on standard culture obtained from Chandigarh (ATCC90028). It showed planktonic growth (MIC 50 µg/ml), adhesion (MIC 0.391 µg/ml), developing biofilm formation (MIC 25 µg/ml after 24 hours and 150 µg/ml after 48 hours), and cell surface hydrophobicity (50 µg/ml) of *C. albicans* was observed while strain obtained from Government Medical College (GMC-3) showed planktonic growth (insensitive), adhesion (insensitive), developing biofilm formation (MIC 12.5 µg/ml after 24 hours and insensitive after 48 hours), and cell surface hydrophobicity of *C. albicans* was insensitive. The biosynthesized of gold nanoparticles (GNPs) of methanolic extract of stem bark of *Acacia leucophloea* did not show cytotoxicity effect on RBCs even at 200 µg/ml.

Key words: Hydrophobicity, Biofilm, Adhesion, *C. albicans*, Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), Gold Nanoparticles (GNP).

INTRODUCTION

Candida albicans is forth common cause of bloodstream infection in India, a major cause of morbidity and mortality in hospitalized patients (Chander et al., 2013). *C. albicans* is a member of flora of human microbiome, under the immune compromised individual they causes the candidiasis. *C. albicans* cause the infection from the superficial to life threatening, the ability of *C. albicans* to infect the host by using the various virulence factor like yeast to hyphal form, adhesion, investigation, biofilms formation and secretion hydrolytic enzyme (Mayer et al., 2013). There are 15 *Candida* species that cause human disease, but 90 % of invasive disease is caused by the 5 common pathogens like *C. albicans, C. krusei, C. glabrata, C. tropicalis,* and *C. parapsilosis*, (Pappas et al., 2016).

Biofilm-associated infections are responsible for the high rate of mortality and morbidity (Pfaller and Diekema, 2007) and major cause of nosocomial infections (Privett et al. 2010) and they can be formed on various prosthetic devices which includes, urinary catheters, intrauterine devices, artificial heart valves, central venous catheters, joint prostheses, pacemakers, dentures and soft contact lenses (Pierce *et al*., 2015).

Extracts of various plants have been found to be suitable for biosynthesis of gold nanoparticles (Sujitha and Kannan, 2013; Noruzi *et al*., 2011; Philip *et al*., 2011; Basavegowda *et al*., 2015; Velmurugan *et al*., 2014; Aromal and Philip, 2012; Firdhouse *et al*., 2014). Das *et al.,* (2015) reported biological synthesis of GNPs using bark extract of *Abroma augusta L* and suggested that, polyphenols present in the bark extract acts as reducing and stabilising agents in the synthesis of GNPs. Similarly the use of bark extract of *Pterocarpus santalinus* has also been suggested for synthesis of GNPs by Keshavamurthyi *et al.,* (2017). *Acacia leucophloea (Roxb.) Willd.* stem bark extract have aldehyde, ketone, aromatic, azo, and nitro compounds which act as reducing and stabilizing the agent for the synthesis of silver nanoparticles (Murugan *et al*., 2014). That why we have selected the this bark for the biosynthesis of gold nanoparticles and it is the first report for it as per our knowledge. GNPs inhibit growth of human pathogenic bacteria like *St*aphylococcus and *Bacillus* (Velmurugan *et al*., 2014). Present investigation was undertaken to assess antifungal activity of Gold nanoparticles

MATERIAL AND METHODS

Bark of the tree *Acacia leucophloea* was collected from S. R. T. Marathwada University campus, Nanded (M.S.) The prepared herbarium of the same was submitted for authentication to Prof. Dr. R. M. Mulani (SRTM Univeristy, Nanded.), who confirmed the identity of the same. *A leucophloea* is small tree present along the rode side or on hill slopes in the forest with white or ash-coloured bark. The leaves 6-8 cm long with -15 pairs of subsessile pinnae. The corolla dull white. Bark has strong fiber and yield sprit after distillation (Naik, 1998). The collected *A. leucophloea* stem barks were shade-dried for 10 days after the removal of adhered soil and it is washed with sterilized distilled water and were finally dried at 50°C in a hot-air oven for 6 hours. The dried stem barks were chopped into small pieces and powdered using a pulverizer, and the active compounds were extracted by a reflux method in a Soxhlet apparatus with methanol. The extraction was repeated until a colorless solvent appeared in the siphon tube. At this point, the extract was vacuum-dried and concentrated using a hot-air drier, the semi-solid extract was stored at 4^0C in deep freezer.

Biosynthesis of gold nanoparticles was standardize in which on 1 % of a methanolic extract of the bark of *A. leucophloea* was prepared with into milli-Q deionized water then vertexes to complete dissolve the powder and filtered with a 0.22 um syringe filter. From this, 16 ml were added into 184 ml of 3 mM of HAuCl4 solution and kept at 20^{o}C for 30 min. The colour change was monitored using the Nanophotometer in the range of 400 nm to 800 nm gold specific peak was observed 549 nm at pH. 4.

The gold colloidal solution was centrifuged at 12500 rpm for 20 min then the pallets were suspended into milli-Q deionized water and again centrifuged two times and washed with ethanol. The pallets were dried at 60^{0}C for half an hour it was added in DMSO for study the anti-*Candida.*

Characterization of gold nanoparticles

Gold nanoparticles were monitored using a Nano spectrophotometer in the range of 400 to 800 nm. The powder XRD pattern was recorded using the Powder X-ray Diffractometer (PXRD), Bruker D8, High-Resolution X-ray Diffractometer in NCL, Pune, Maharashtra state (Sujitha & Kannan 2013). Methanolic bark extracts of *A. leucophloea* & GNPs were analyzed by FTIR (SHIMADZU 8400S model in University College Of Technology, Osmania University, Hyderabad, India.). (Ankamwar *et al*., 2017). Gold colloid solutions were taken in a cuvet and particle size and zeta potential were measured (HORIBA model no. SZ-100) and scanning electron microscopy (HITACHI model no. S-3700N, University College of Technology, Osmania University, Hyderabad, India.). FEI Make Quanta 200-3D Dual Beam with EDAX make EDS system were used for EDX for determination the content of GNPs at NCL Pune. The shape and size of particles were measured by using TEM (Model: Hitachi, H-7500) at RUSKA Lab, Hyderabad (John *et al.,* 1998).

Culture conditions of *C. albicans*

Authentic culture of *C. albicans*, ATCC 90028 was obtained from the Institute of Microbial Technology, Chandigarh, India. Clinical isolate (GMC-3) were obtained from Government Medical College, Nanded, Maharashtra state, India. Both the cultures were maintained on Yeast-Peptone-Dextrose (YPD, 1:2:2 %) agar slant at 4^{0}C (Raut *et al.,* 2013).

The effect of gold nanoparticles biosynthesized from methanolic extract of bark of *Acacia leucophloea* on Planktonic Growth, Adhesion Assay, Biofilm formation and its evaluation on *candida albicans* was done.by using method suggested by is based on the Chandra *et al*., 2001 and Mukherjee *et al*., 2003 and it was modified by Raut *et al*., 2013.

Cell surface hydrophobicity (CSH)

The method suggested by Rosenberg in 1984 was modified by Hazen and Hazen, 1987 and further modification was suggested by Raut *et al*., 2010 for analysis of *candida albicans*

Hemolytic assay of blood

Hemolysis of was done by using the detail method suggested by Helmerhorst *et al,*1999 and subsequent modification of Wei & Bobek, 2004 and Rajput *et al***.,** 2013.

Sample preparations of *C. albicans* developing biofilm for Scanning Electron Microscopy.

The method suggested by Bozzola & Russell in 1998 was as modified by Chauhan *et al.*,2011 further modification suggested by Kathwate & Karuppayil 2013

Statistical analysis

Statistical analysis values are the mean with standard deviations obtained from three dissimilar observations. This result compared using Student's t - test. A value of $P < 0.05$ was considered statistically significant (He *et.al.,* 2007; Bansode *et al*., 2016).

Results and discussion

Nanomaterials are well known for their antimicrobial efficacy. The antibacterial activities of gold nanoparticles are widely reported. GNPs have been shown to inhibit growth of various bacteria including the human pathogenic bacteria like *Staphylococcus* and *Bacillus* (Velmurugan *et al*., 2014), and pathogenic fungi like *C. albicans* (Yu *et al*., 2016). We have successfully synthesized gold nanoparticles using methanolic extract of the bark of the tree *A. leucopholea.* This biological way of synthesis does not involve toxic chemicals and this process is eco-friendly in nature. The bioprocess variables such as pH 4 (Fig. 3 A), incubation time 30 min(Fig. 3A), Molarity 3 Mm Fig.2 and temperature 20^0C (Fig. 3B) favored the biogenic synthesis of gold nanoparticles. The bioprocess variables affecting synthesis of gold nanoparticles are standardized. We have achieved conditions, which carry out the reaction fast and completes in 30 min. Hence, 30 min of incubation time is ideal for biogenic synthesis of gold nanoparticles.

Scanning electron micrographs of gold nanoparticles showed nanoparticles ranging from 51 – 77 nm in size (Fig. 4A) were as TEM showed that spherical having size 46 nm (Fig. 4 B and C) and XRD indicated crystalline structure having size 13.66 nm (Fig.5 A) this result is indicate that capping and stabilizing agent may increase the size GNPs (Bahram *et al.,* 2014). Das *et al* (2015) reported the biological synthesis of GNPs using bark extracts of *Abroma augusta L* and suggested that, polyphenols present in the bark extract acts as reducing and stabilising agents in the synthesis of GNPs. The use of bark extract of *Pterocarpus santalinus* is reported for the synthesis of GNPs (Keshavamurthyi *et al.,* 2017). However, ours is the first report on biosynthesis of gold nanoparticles where methanol extract of *A. leucopholea* bark is used. FTIR analysis indicates the presence of biological molecules on the nanoparticles suggesting capping of the gold nanoparticles may occur by phytochemicals present in *A. leucopholea* extracts. Wankhade & Mulani, 2015 analysis the bark extract of *Acacia leucophloea* Roxbs contain carbohydrate, saponin, tannins, flavonoids, steroids, alkaloids, glycosides, terpenoids and protein. Zeta potential analysis of gold nanoparticles negative potential indicate that dispersion in medium and stability of nanoparticles and more positive or negative value indicate the stability of gold nanoparticles (Ivanov *et al.,* 2009).

Relatively very few studies are there on the inhibitory properties of GNPs against the growth *of C. albicans*. Most of the studies are restricted to the colony growth of *C. albicans* where disc diffusion assay is used. The mode of action of GNPs is hypothesized and reported **(**Yu *et al*., 2016**).** Studies on gold nanoparticles against *C. albicans* are very few. In this work, we have tested the efficacy of GNPs on the growth and different virulence factors of *C. albicans*. Biogenically synthesized GNPs showed pronounced inhibitory activity against planktonic growth of *C. albicans* and MIC was achieved at 50 µg/ml (Fig.7). Mature biofilm of *C. albicans* were insensitive (Fig.10) to gold nanoparticles while developing biofilms of *C. albicans* ATCC 90028 was inhibited at 200 µg/ml of concentration (Fig. 9 A). However, biofilms of a clinical isolate of *C.*

albicans remained insensitive to treatment with gold nanoparticles. These differences in sensitivities may be due to strainal variation. Gold nanoparticles considerably inhibited cell surface hydrophobicity (Fig.12 A) and adhesion in *C .albicans* ATCC 90028 (Fig. 11 A). Decreased cell surface hydrophobicity on treatment with GNPs may contribute to inhibiting adhesion. A recent study reported the effect of gold nanoparticles on the biofilms of *C. albicans*. They have reported an MIC value of 63.38 ppm against biofilms of *C. albicans* (Yu *et al*., 2016). Ours is the first systematic study done on the efficacy of gold nanoparticles on the planktonic growth and different virulence factors such as developing biofilm formation, adhesion and hydrophobicity.

GNPs considerably inhibited adhesion and developing biofilm. These are considered as important virulence factors in *C. albicans* (Yu et al.,2016). Decreasing hydrophobicity on treatment with GNPs may contribute to inhibition of pathogenicity of *C. albicans*. This is the first report on cell surface hydrophobicity. As hemolytic GNPs are not advisable to treat humans, we have studied hemolytic properties of gold nanoparticles. We found that, the inhibitory concentrations of gold nanoparticles did not exhibit hemolytic properties against human red blood cells (Fig.12 B). These results indicate the non-toxic nature of GNP (Goodman *et al*., 2004).

Yu et al. (2016) suggested that inhibitory activities of GNPs against biofilm and invasion are not due to these factors. They hypothesized that inhibitory activity of GNPs on *C. albicans* may be due to binding of GNPS to extracellular hydrolytic enzymes such as secreted aspartyl proteinase (Sap), phospholipases, and lipases that may lead to inactivation of these enzymes. However, Yu et al. (2016) has not clearly explored the mechanism of GNPs against *C. albicans*. Hence, not much information is available on the mode of action of GNPs against *C. albicans* and its virulence factor.

Conclusions this study explored the efficacy of bark extract of *A. leucopholea* for the biogenic synthesis of gold nanoparticles which is eco-friendly and a less harmful way of synthesis of nanoparticles. Also efficacies of GNPs against growth and virulence factors of *C. albicans* are explored. It revealed GNPs possess inhibitory activity against growth and virulence factors such as developing biofilm, hydrophobicity, and adhesion. These findings may contribute to application of GNPs in fighting candidiasis. However, studies in animal models and toxicity studies need to be done.

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EXTRACTION OF EXOPOLYSACCHARIDE AND SCREENING OF ANTIOXIDANT ACTIVITY OF MARINE BACTERIA

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ABSTRACT

Introduction: Marine microorganisms are a rich source of natural products with potential applications in drug discovery, environmental remediation, and the development of new resources for industrial processes. The present study aimed to isolate bacterial strains from marine fish and screen their potential for various applications.

Materials and Methods: The primary aim of the project is to isolate bacteria from marine water fish that is able to produce Exopolysaccharide and screening for Antioxidant activity of marine bacteria.

1. Isolation and characterization of marine bacteria from the marine samples.
2. Extraction of exopolysaccharide from marine isolate and it's quantification using phenol-sulphuric acid method followed by spectroscopy.
3. .Screening of antioxidant activity of isolated marine bacteria by DPPH method.

Result and conclusion: EPS was estimated as carbohydrate by phenol-sulphuric acid method. Further the isolates were screened for antioxidant production using DPPH assay. All isolated organism from different organs of marine fish showed different scavenging activity. Marine bacteria constitute a diverse group, which has received attention only in recent years, as potential natural antioxidant producers in terms of their ability to act as efficient radical scavengers and it is believed to be mainly due to their redox properties.

Keywords: Marine bacteria, exopolysaccharide (EPS) extraction, screening of antioxidants.

INTRODUCTION

Marine biotechnology is the study of using marine organisms to create or change goods, improve plants or animals, or produce microorganisms for specialized applications. Humans have been able to elaborate numerous biological procedures relevant to microorganisms, whether aquatic or terrestrial, with the use of various molecular and biotechnological approaches. The maritime environment has shown to be a significant source of biological and chemical variety. The seas, which occupy around 71 percent of the earth's surface or 361 million square kilometers (Whitman et al., 1998), include nearly 300 000 recognized species, although it is anticipated that this number represents just a small proportion of the total number of species still to be discovered and described (Malakoff., 1997). This becomes more challenging when more than 99 percent of bacteria are present (Amann et al.,1995).

Marine microorganisms account for the majority of undescribed marine species (Pomponi., 1999). This aids in the identification of novel chemicals that have the potential to be used as medications, nutritional supplements, cosmetics, agrichemicals, metabolites/compounds, molecular probes, enzymes, and fine chemicals. Marine bio-

products from these classes have a market worth in the billions of dollars (Carte., 1996). Revenues from cosmeceutical, nutraceutical, medical, and pharmaceutical businesses are expected to reach $3.78 billion by 2012 (Bird., 2008).

Marine microorganisms have acquired unique metabolic and physiological capacities that not only permit survival in a wide range of severe settings, but also provide the possibility for the creation of metabolites not seen in terrestrial microorganisms (David H. et al., 2013). Marine creatures provide an unlimited supply of beneficial chemical substances for the production of new pharmaceuticals; among these species are marine bacteria that dwell in the seas and are important organisms utilised in biotechnology for the discovery of novel compounds from marine origin.

Exopolysaccharide (EPS) polymers have a high molecular weight and are made up of sugar residues that vary greatly in structure and function. Microbial EPS are vital in cellular relationships, nutrition, and micro and macroenvironments. They are utilised in the food, textile, detergent, and beverage industries, pharmaceutical, biotechnology, agricultural, paper, paint, and petroleum sectors, medication delivery and cancer therapy, and culture media formulation. EPS generation by LAB varies greatly in terms of amount, chemical content, molecular size, charge, presence of side chains, and molecule stiffness (Uchechukwu et al., 2012). When exposed to UV light, ionising radiation, or chemicals, reactive oxygen species are produced (ROS). During normal physiological circumstances, cells also create reactive oxygen species.

MATERIALS AND METHODS

A. Sample:

Mugil cephalus Linnaeus (Common name: Grey Mullet Fish)

B. Sample collection:

Marine Grey Mullet Fish was collected from Teen Batti Road and Fish Market Road from Bhiwandi, Dist; Thane 421302, Maharashtra in a sterile glass bottles and carried to the laboratory in icebox and processed within one hour of collection.

C. Sample processing:

The fish were dissected in aseptic condition; swabs were taken from various organs like scale, gills and intestine.

D. Enrichment and isolation of organism:

Swabs of different organs like scale, gills and intestine collected from marine fish were enriched. Microflora was grown on sterile Zobell Marine Agar plates by swabbing. Further, pure culture of the various colonies thus obtained, were also maintained on the same medium.

E. Characterization of isolates:

Standard bacterial analysis was used for characterization of bacterial isolates from fish.

F. Extraction of Exopolysaccharide (EPS):

A baseline medium broth was made to extract exopolysaccharide from marine microorganisms. A loopful of culture was inoculated into each flask containing basal media and incubated for three days on a rotator shaker with continual shaking. Three days after incubation, the medium was centrifuged for 20 minutes at 5000 rpm. By adding an equivalent volume of cold ethanol to the supernatant and keeping it at 40° C for 24 hours, the

exopolysaccharide was precipitated from the supernatant. To avoid a high concentration of precipitate, the mixture was stirred with ethanol and kept overnight at 4° C before being centrifuged at 7000 rpm for 20 minutes. The protein content of the EPS was isolated by precipitating it on ice for two hours with 25% (wv) trichloroacetic (Al- Nahas et al., 2011; Edward et al., 2011; Orsod et al., 2012).

G. Estimation of Exopolysaccharide:

The modified phenol-sulphuric acid technique was used to calculate exopolysaccharide as total carbs (Dubois et al., 1956). 1 mL of 5 percent (w/v) phenol was added to 1 mL of sample, followed by 2 mL of concentrated sulphuric acid. While adding sulphuric acid to the sample tubes, they were maintained on ice. After 20 minutes of incubation at room temperature, the absorbance at 490 nm was measured. In the concentration range of 100-1000 g, glucose was employed as the standard.

H. Antioxidant assay:

The isolated cells were injected in nutritional media (with the appropriate concentration of NaCl) and incubated on a shaker at room temperature for 24 hours. Centrifugation was used to separate the bacterial cell mass, and cell-free supernatants were lyophilized and kept at 4°C. 100 g of these lyophilized crude extracts were reconstituted in sterile distilled water, filtered through a Millipore filter, and used to screen for antioxidant production using the DPPH radical scavenging test. 2 ml of extract was combined with 2 ml of newly made DPPH solution (0.03 mM in methanol) and incubated for 30 minutes at room temperature in the dark. At 517 nm, the absorbance was measured. Ascorbic acid was utilised as a control.

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RESULT AND DISCUSSION

1. **Number of bacteria isolated from marine fish:**

   One pure well isolated bacterial culture from each organ of fish *Mugil cephalus* L were used for further studies in this project.

2. **Colony Characteristic:**

   After isolating the pure culture from each part of the fish under study, one pure culture with prominent colony characteristics were taken for further studies in the project and the observed characteristic are depicted in table no.1 in which scale isolate was Gram positive cocci while gills and intestine showed Gram negative rod and cocci respectively which is in accordance with earlier studies done elsewhere.

Sr. No	Fish Organ	Isolate No.	Size (mm)	Shape	Margin	Elevation	Colour	Opacity	Gram nature
1	Scales	Isolate S1	5	Irregular	Entire	Concave	Cream	Opaque	+ve cocci
2	Gills	Isolate G1	3	Circular	Irregular	Flat	Off White	Opaque	-ve rod
3	Intestine	Isolate I1	4	Circular	Undulated	Raised	Cream	Opaque	-ve cocci

Table 1. Colony characteristics of Bacterial isolates obtained from *Mugil cephalus* Linnaeus were studied

3. Estimation of standard exopolysaccharide

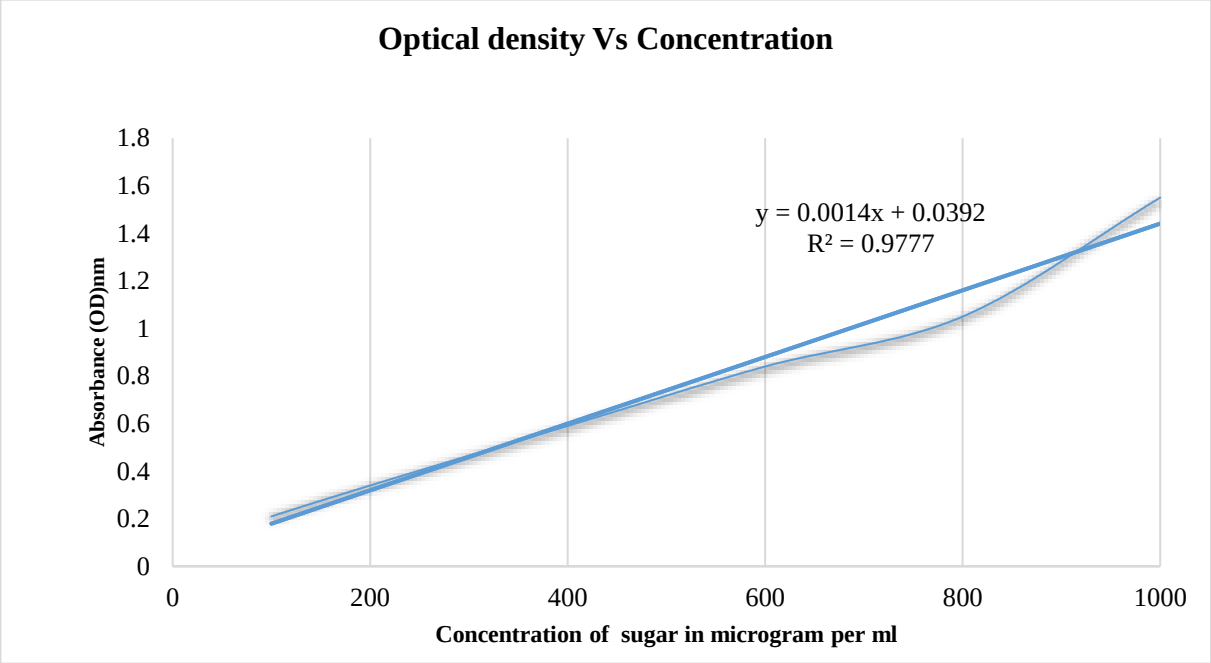
Conc. µg /ml	Stock (ml)	Diluent (ml)	Total vol. (ml)	5% (W/V) Phenol (ml)		Sulphuric Acid (ml)	OD (490)
1000	2	-	2	1		2	1.55
800	1.8	0.2	2	1		2	1.05
600	1.6	0.4	2	1		2	0.84
400	1.4	0.6	2	1	ICE BATH	2	0.59
200	1.2	0.8	2	1		2	0.34
100	1	1	2	1		2	0.21
Isolate S1	2	-	2	1		2	0.72
Isolate G1	2	-	2	1		2	0.65
Isolate I1	2	-	2	1		2	0.98

Table 2. Estimation of standard exopolysaccharide

4. Extraction and Estimation of EPS by phenol sulphuric acid method

Extraction of EPS from bacterial isolate under study the estimation of it was done using Phenol sulphuric acid method with Glucose as standard at 490 nm on spectrophotometer. After plotting standard curve using observed optical density of known concentration against absorbance the unknown EPS of isolate of fish scale, gills and intestine was found to be 564.2, 514.28 and 750 microgram per ml which was appreciable amount for further analysis and characterization. This disparity in the EPS producing ability of these isolate may be dependent on the strains of bacteria in question, as well as the growth environment. Similar studies by other investigators also showed similar results. Results obtained by

(Lebeer et al., 2007) indicated that glucose was the initial raw material for producing EPSs, it was the most important part of the biofilm matrix, and the efficiency of producing EPS production and biofilm construction changed with changes in the quantity of glucose.



Tube Number	Concentration in microgram per ml	Optical density
1	100	0.21
2	200	0.34
3	400	0.59
4	600	0.84
5	800	1.05
6	1000	1.55
7	564.2	0.72
8	514.28	0.65
9	750	0.98

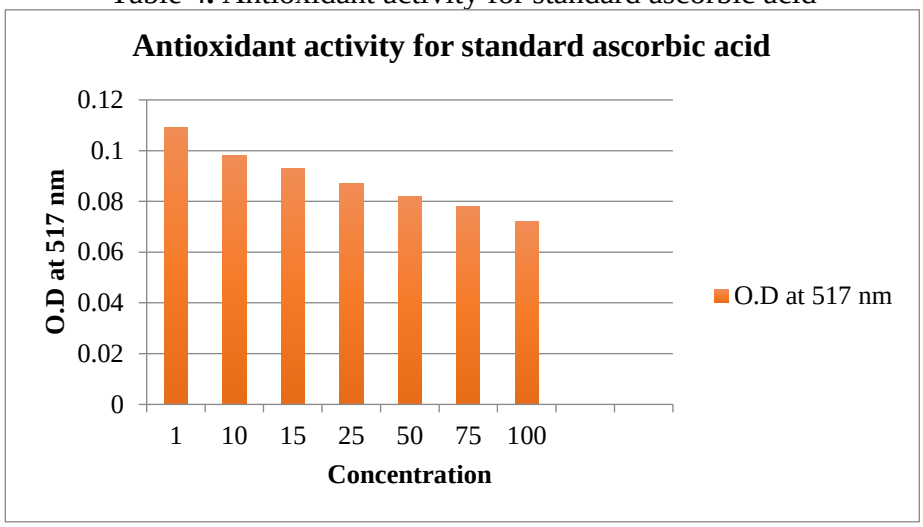
Table 3. Extraction and Estimation of EPS by phenol sulphuric acid method

5. Antioxidant Assay:

Sr. No	Concentration (µg/ml)	OD 517 nm
01	1	0.109
02	10	0.098
03	15	0.093

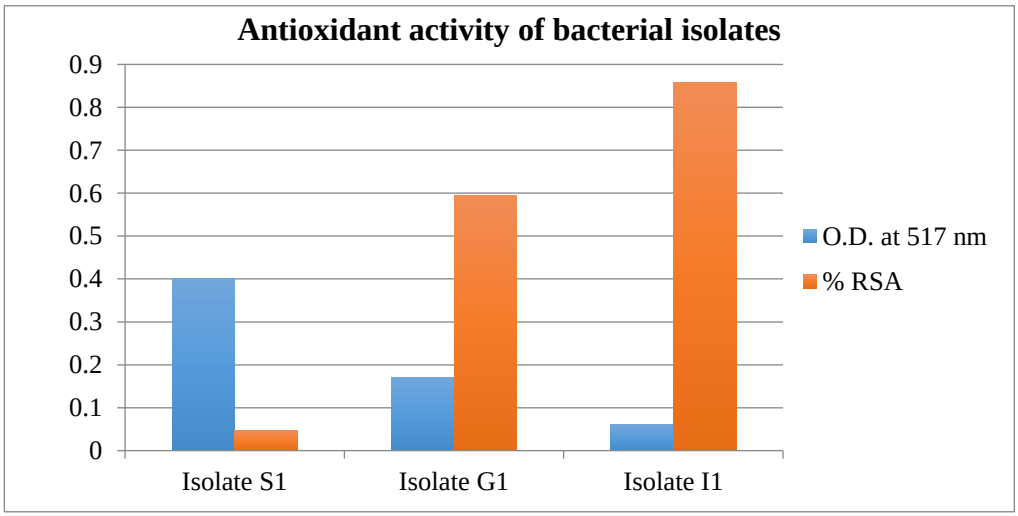
04	25	0.087
05	50	0.082
06	75	0.078
07	100	0.072

Table 4**.** Antioxidant activity for standard ascorbic acid



Name of the fish sample	**Bacterial isolate**	O.D. at 517 nm	% RSA
Mugil cephalus Linnaeus	Isolate S1	0.40	4.7%
	Isolate G1	0.17	59.5%
	Isolate I1	0.06	85.7%

Table 5. Antioxidant activity of bacterial isolates and % RSA



Analysis of antioxidant property of bacterial isolate under investigation using standard DPPH assay, percent radical scavenging activity of extracted EPS was found to be 4.7%, 59,5% and 85.7% of scale ,gills and intestine isolates respectively. The study by (Zhang et al., 2016) also showed that the EPS exhibited strong scavenging activities for 1, 1-diphenyl-2-picrylhydrazyl and hydroxyl radicals. Two EPS fractions (EPS-1 and EPS-3) exhibited higher antioxidant activities than EPS-2.

The research focused on marine microbes and their qualities. The fish market was used to bring in marine fish. Various marine bacterial isolates were isolated from Mugil cephalus Linnaeus organs using Zobell Marine agar. The materials were selectively plated and grown on Zobell Marine agar. Gram staining of these isolates revealed the Gram's nature, which aided in the isolate's characterisation. Furthermore, EPS was extracted from well-characterized isolates based on the sticky material formed principally in the Zobell Marine agar during the isolation and screening of EPS generating bacteria strains. The phenol-sulphuric acid technique was used to calculate EPS as carbohydrate. On a colorimeter, absorbance was measured at 490nm. These isolates were then tested for antioxidant production utilizing DPPH method and ascorbic acid as standard.

CONCLUSION

The maritime environment has shown to be a promising source of antioxidant chemicals, with some isolated organisms from the marine environment exhibiting strong antioxidant characteristics, despite the fact that secondary metabolites of marine organisms are exploding globally. The antioxidant activity of isolated marine organisms was investigated in this study. However, further research is needed to determine the structure of these antioxidant molecules as well as their prospective use in the pharmaceutical business. Microbial EPS is a non-toxic, biodegradable, and renewable material found in nature. Under all ideal circumstances, isolated organisms from marine fish produced the most EPS.

FUTURE PROSPECTS

Over the last few decades, the ocean has been discovered as a reliable supply of human needs. The marine environment is rich in biodiversity and has immense scientific application potential. Secondary metabolites originating from the sea have emerged as a prospective source for medication design and development. Capsular polysaccharide and exopolysaccharide are the two types of microbial polysaccharides (EPS). EPSs of microbial origin are abundant in nature, have distinct features, and may be isolated from bacteria in fresh water, seawater, harsh circumstances, and so on. Exopolysaccharides are made up of sugar moeties that are repeated and connected to a carrier lipid, chemical and inorganic substances, metal ions, and DNA. Bacterial EPSs have enormous potential, and the physiochemical properties of EPS determine its potential. Over the last few decades, the ocean has been discovered as a reliable supply of human needs. The marine environment is rich in biodiversity and has immense scientific application potential. Secondary metabolites originating from the sea have emerged as a prospective source for

medication design and development. Capsular polysaccharide and exopolysaccharide are the two types of microbial polysaccharides (EPS). EPSs of microbial origin are abundant in nature, have distinct features, and may be isolated from bacteria in fresh water, seawater, harsh circumstances, and so on. Exopolysaccharides are made up of sugar moeties that are repeated and connected to a carrier lipid, chemical and inorganic substances, metal ions, and DNA. Bacterial EPSs have enormous potential, and the physiochemical properties of EPS determine its potential.

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DETERMINATION OF ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF *Myristica fragrans* (NUT MEG)

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ABSTRACTS:

Introduction: It is a regular occurrence for harmful bacteria to develop resistance to antibiotics that are currently accessible. As a result, plant-based bioactive compounds might be a viable option for developing novel formulations. Myristica fragrans seed has been utilised in everyday life for health care from ancient times. The study's objectives were to investigate the antioxidant and antibacterial properties of the seed's essential oil.

Methodology: The Antimicrobial Assay was done using Well Diffusion Method with Acetone as a solvent system and Antioxidant Activity was studied using the standard DPPH Assay and presence of phytochemicals were evaluated by phytochemical analysis by standard methods (Vogel et.al.).

Results: Phytochemical analysis showed the presence of all phytochemicals such as Alkaloids, Flavonoids, Phenols, Terpenes, & Carbohydrates. The nutmeg Acetone Extract showed high antimicrobial activity against *E. coli*, *Bacillus* & *Aspergillus Niger* where as 100% of conc was effective against *S. Typhi*. *Myristica Fragrans* showed higher ability to scavenge free radicals at all concentrations and the values were found to be near to Std. ascorbic acid.

Conclusion: It can be concluded that nutmeg can be incorporated for medical advantages, other bioactive properties and existed studied properties needs to evaluated for further advancements on a large industrial scale basis.

Keywords: Myristica fragrans, Antimicrobial, Antioxidant activity, phytochemical analysis

INTRODUCTION:

The facts that using synthetic antibiotics or antioxidants such as butylated hydroxytoluene (BHA) or butylated hydroxyl anisole (BHA) can cause catastrophic adverse reactions such as liver or kidney damage, or could be carcinogenic have raises questions over their utility and resulted in limitations in their implements in the treatment. (Senevirathne M, 2006). Apart from these psychophysiological deuteriations their constant subsequent

usage can also impart resistance in the bacterial population against that intervention which is the real deal. Hence the search for new alternatives that can outweigh benefits over risks is from Nature that nurtures elements that are of humongous applicabilities and utilities, gifted from nature plants have become a reliable resource for exploring options for plant derived herbal formulations in the therapeutics, which is one of the most trustworthy less harmful, economical, and abundant resource because they can very well protect against biological oxidative damages. Spice can be considered as one of the most resourceful bioactive antimicrobial compounds as they have their fair share in the treatment of key disorders of the body. (Jyothiprabha, 2016). Among spices *Myristica-Fragrans* is exotic not just because of its bioactive properties such as antimicrobial or antioxidant activities yet it's of much greater economical values.

PLANT MORPHOLOGY:

Nutmeg and mace are produced by Myristica fragrans, which belongs to the magnoliales family. Nutmeg is the tree's seed, which is dark brown, oval-shaped, 2-3 cm long, and weighs 5 to 10 grammes. Nutmeg seeds have ruminating endosperm and are the oldest flowering plant seeds. Mace is the seed's dried lacy reddish aril. The entire potential of a nutmeg tree takes about 20 years to reach, however the first harvest can happen as early as 7-9 years after planting. (Deep Gupta (2011))

BIOACTIVE PROPERTIES:

Although dried formulations of nutmeg and mace are used in medicine and spices, the essential oils and oleoresin have far more potential and intrigue. Because of their anti-inflammatory, narcotic, carminative, astringent, and aphrodisiac properties, both spices are used as pharmacological interventions in India for the treatment of stomach aches, nausea, malaria, flatulence, dysentery, and rheumatisms. (B Krishnamoorthy, 2001) Alcoholic extracts and essential oils of nutmeg have been shown to have antioxidant and antimicrobial properties, and are effective against a wide range of gramme positive and negative bacteria, including E. coli, Aeromonas hydrophila, Salmonella choleraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes, Listeria innocua, and others: Acinebacter calco , *Citrobacter fruendii, Enterobacter aerogenes, Erwinia carotovora, Flavobacterium suaveolens, Klebsiella pneumonia, Micrococcus luteus, Moraxella sp., Proteus vulgaris, Serratia marcescens and Yersinia enterocolitica.*(B Krishnamoorthy, 2001, Lima, 2012, Dorman, 2004). Many nutmeg compounds have shown greater antioxidant activity, and compounds with a catechol structure, such as caffic acid, can quickly lose hydrogen or electrons to acceptors, such as reactive oxygen species or lipid peroxyl groups, and hence are thought to be effective antioxidants. (Deep Gupta, (2011)). Eugenol and -caryophyllene have hydrogen atoms in the benzylic & / or allylic sites, which makes the extraction of

atomic hydrogen by peroxy radicals (formed under oxidative stress) from these functional groups easier, and due to eugenol's catalytic properties towards enzymes such as catalase superoxide, glucose-6-phosphate dehydrogenase, glutamine transferase, and glutathione peroxidase they are thought to be good coordinator of overall nutmeg antioxidant activity. (Kumaravelu, 1996).

Several alcoholic extracts and essential oils of nutmeg have demonstrated excellent antibacterial and antioxidant properties. Takikawa et al (2002) revealed antibacterial properties of nutmeg chloroform extract against enterohemorrhagic E. coli O157, which was found to be extremely sensitive to b-pinene(Takikawa, A., K. Abe, M. Yamamoto, S. Ishimaru, M. Yasui & Yokoigawa, 2002). "A chloroform extract of nutmeg seeds was identified by Narasimhan and Dhaka (2006) to have strong antibacterial activity against both gram-positive and gram-negative bacteria. (Indian Pharmacopoeia, 1996) (2006, B. Narasimhan)". The main antibacterial components identified from nutmeg seeds were trimyristin and myristic acid(Pharmacopoeia of India, 1996). Cho et al. (2007) used a methanolic extract to isolate three lignans (erythron-austrobailignan-6, meso-dihydroguaiaretic acid, and nectandrin-β) as antifungal compounds that inhibit rice blast and white leaf rust growth.(Cho, J. Y., Choi, G. J., Son, S. W., Jang, K. S., Lim, H. K., Lee, S. O., 2007) According to Dorman & Deans, a-pinene, b-pinene, p-cymene, carvacrol, and b-caryophyllene are some additional key antibacterial moieties, Membrane disruption by lipophilic chemicals is a mode of action for these agents on microbes(Dorman, 2004).

As previously stated, the seed's antioxidant properties are due to chemical substances such as caffic acid, eugenol, and -caryophyllene, which can be measured using various chemical assays such as total phenolic attention estimation, capacity to scavenge stable free revolutionaries DPPH (-diphhenyl-1-picrylhydrazyl), ferric reduction antioxidant power assay (FRAP), and so on. Numerous experimenters have discovered the antioxidant eventuality of nutmeg by analysing the factors using the below- mentioned tests. Jukic etal. (2006) revealed that the aglycone part of nutmeg essential canvas, which is insulated enzymatically from glycosidically connected volatiles, had more antioxidant exertion than free volatiles. The friction was produced by different quantities of eugenol and isoeugenol. After heating, Tomaino etal. (2005) examined the antioxidant effectiveness and chemical composition of nutmeg seed essential canvas. They plant that hotting increased free revolutionary scavenger exertion, which might be related to the hydrocarbons in the canvas volatilizing at advanced temperatures, performing in the attention of phenolic factors in the residual canvas. (Deep Gupta, 2011).

Indeed though there are numerous studies put head regarding essential parcels of nutmeg, still the evaluation of nutmeg

bioactive property with acetone as a solvent system haven't caught enough eyes of the science community hence there aren't numerous reports stating with acetone as a rooting principle hence this study is conducted to find the implicit use of acetone for nutmeg antimicrobial and antioxidant analysis as a solvent system and to estimate its efficacy as a good detergent grounded on total inhibition and RSA exertion given by the nutmeg-acetone.

MATERIALS AND METHODOLOGY:

Preparation of acetone extract of nutmeg:

20gms of nutmeg sample were taken and crushed with the help of mortar and pestle and 100 ml of acetone was added to the crushed sample and kept at room temp for 3 days. After incubation the admixture was filtered first with muslin cloth and also by Whatman No. 1 sludge paper and incubated at 500C for evaporation. The final Extract attained after evaporation was of 100 attention, dilutions (75, 50,) were made by adding needed attention of nutmeg in mg to 100 ml of acetone.

Phytochemical Analysis:

Test for Alkaloids:

400 mg of excerpt was dissolved in 20 ml of HCL, and also filtered. 1 ml of Mayer's reagent was added to the 1 ml of filtrate by the side of the tube. Presence of alkaloids can be detected by appearance of white delicate precipitate

Test for carbohydrates:

400 mg of crude excerpt was dissolved in 20 ml of acetone & also filtered. 1 ml of solvent excerpt was mixed with 1 ml of Benedict's reagent. A red precipitate indicates presence of sugars.

Test for phenolic and tannin compounds:

100 mg of excerpt was dissolved in 10 ml of Acetone and filtrate was attained 2 ml of filtrate and0.5 ml of lead acetate was mixed together. Presence of unheroic precipitate indicates presence of phenolic and tannin composites

Test for flavonoids:

100 mg of excerpt was dissolved in 10 ml of acetone and 2 ml of attained filtrate was suspend with 3 ml of 2 NaOH. Unheroic color which becomes tintless on addition of adulterated H2SO4 indicates presence of flavonoids.

ANTIBACTERIAL SCREENING:

Nutrient Agar Plates were used for antibacterial analysis0.1 ml of culture suspense (*E. coli, S. typhi, Bacillus subtilis*) used for inoculation and plates were kept at room temp for prolixity, 4 wells were created with cork and borer and0.1 ml of each conc (100, 75, 50, 25) were added to each well the plates were kept in fridge for 15 twinkles for prediffusion and also incubated at 37^0C for 24 hrs. After incubation zone of inhibition were measured.

ANTIFUNGAL ACTIVITY:

Potato dextrose agar plates were used for antifungal analysis with0.1 ml of inoculation size of *Aspergillus niger* fungi, plates were kept at room temp for prolixity, 4 wells were created with cork and borer and0.1 ml of each conc (100, 75, 50, 25) were added to each well the plates were kept in fridge for 15 twinkles for pre-diffusion and also incubated at 37^0C for 24 hrs. After incubation zone of inhibition were measured.

ANTIOXIDANT ACTIVITY; DPPH ASSAY:

1 ml of0.1 mM DPPH was added in 3 ml of test sample having attention (25, 50, 75, 100). kept in darkness for 30 twinkles. Thirty twinkles latterly, the absorbance was measured at 517nm

RESULTS AND DISCUSSION:

Phytochemical analysis

Table 1 Phytochemical analysis results

Phytochemical	**Nutmeg**
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Phenolics and tannins	+

Keys

+ ⟶ Presence of compounds

- ⟶ Absence of compound

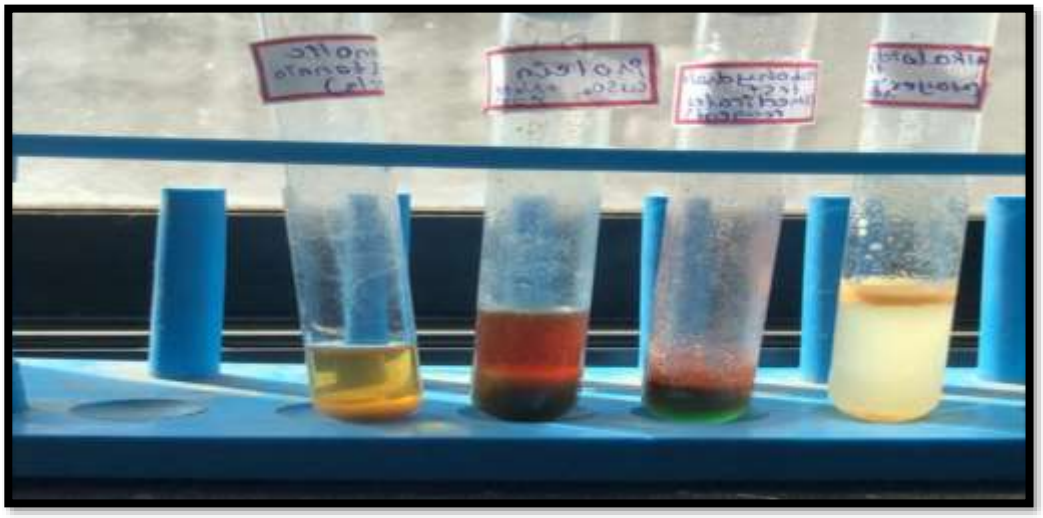

Figure 1 Phytochemical Analysis

Antimicrobial susceptibility test (AST):

Sr.no	Tested bacteria and fungi	Concentration of acetone extract of nutmeg %	Mean inhibition zone of diameter(mm)	Sensitivity
1.	*E. coli*	100% 75% 50% 25%	–	+,+
2.	*S. typhi*	100% 75% 50% 25%	14 9 6 0	+
3.	*Aspergillus niger*	100% 75% 50% 25%	19 17 14 11	+
4.	*Bacillus subtilis*	100% 75% 50% 25%	22 20 17 15	+

Table 2 The in vitro activity of acetone extract of nutmeg against *E. coli, S. typhi, Aspergillus niger, Bacillus subtilis.*

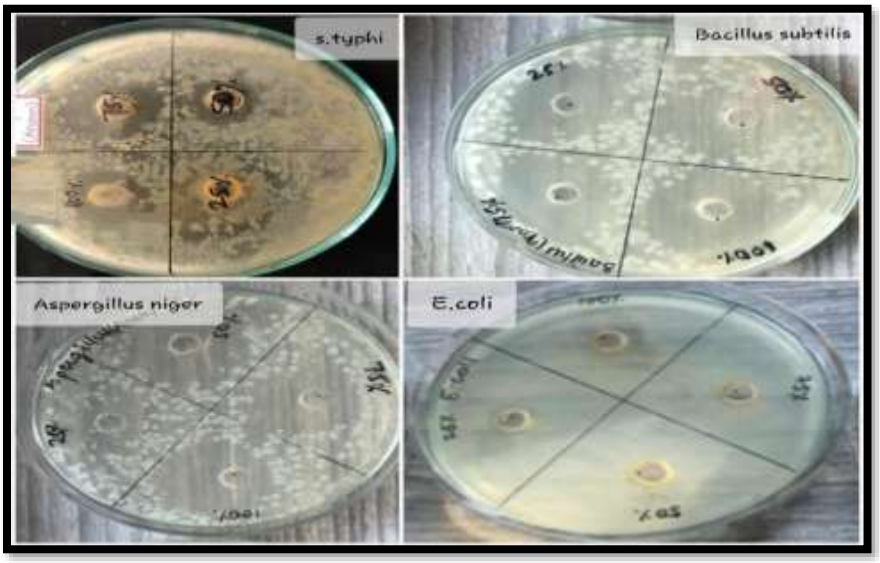

Figure 2 Antimicrobial Assay

Antioxidant activity by DPPH assay:

OD of control=0.151 Wavelength=517 nm

Concentration (%)	OD of test	% Radical scavenging
25%	0.106	29.801
50%	0.093	38.410
75%	0.091	39.735
100%	0.085	43.708

Table 2 Calculation of antioxidant activity for standard ascorbic acid

Concentration (%)	OD of test	% Radical scavenging
25%	0.145	3.953
50%	0.130	13.907
75%	0.115	23.841
100%	0.096	36.423

Table 3 Calculation of antioxidant activity for nutmeg

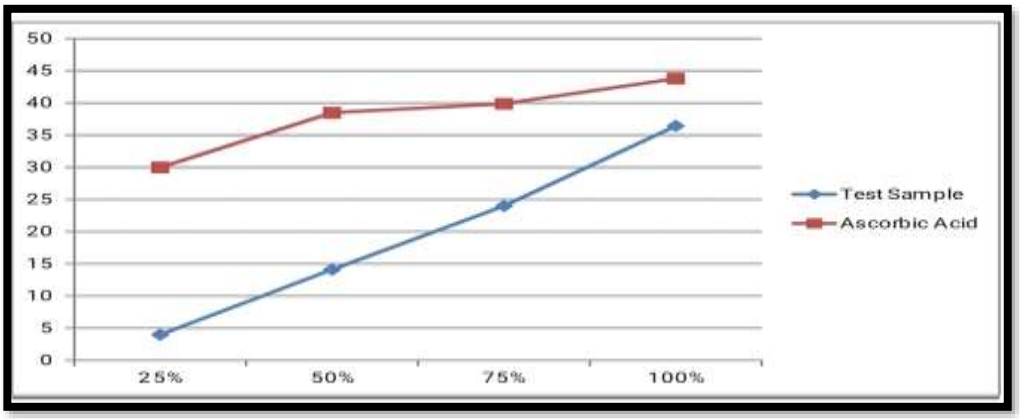

Figure 3 line showing the antioxidant (DPPH radical scavenging activity)

DISCUSSION:

The phytochemical characteristics of nutmeg were tested and are epitomized in the table1. The results revealed the presence of medially active composites in the nutmeg. From the table 1, it could be seen that alkaloids, carbohydrates, phenolics and tannins is present in the nutmeg. while nutmeg shows the absence of protein performing the trial the result of antimicrobial exertion of acetone excerpt of nutmeg against *E. coli* revealed that this bacterium is largely sensitive to all the attention and showed no growth on the plate. The result of nutmeg excerpt (acetone excerpt) on *Salmonella typhi* show respectively the bacteria was sensitive to all the concentration of acetone extract of nutmeg respectively the bacterium was sensitive to all or any the concentration of solvent extract of nutmeg. Acetone extract of nutmeg showed high activity against *Aspergillus niger*, the diameter of inhibition in 100% was 19 mm in seventy fifth it had been 17mm but, in five hundredth and twenty fifth it had been fourteen and 11mm. solvent extract showed a most impact on hay bacillus, its diameter of inhibition in 100% and seventy fifth was twenty two and 20mm and in five hundredth and twenty fifth there was slight decrease in inhibition to seventeen to 15mm.As dynamic criteria, nutmeg oil includes monoterpenes like -pinene, camphene, -pinene, sabinene, myrcene, a-phellandrene, a-terpinene, limonene, 1, 8-cineole, g-terpinene, linalool, terpinen-4-ol, safrole, alkyl eugenol Its ability to inactivate microorganism bonds, catalysts, and cell wall proteins identifies their antimicrobial action approach (Pharmacopoeia of Asian country, 1996). gram-negative enteric bacteria' cell divider auxiliary nature may well be answerable for the ascertained blockage. The capability of antioxidants to present gas is assumed to be the rationale for his or her action on DPPH. The DPPH check is employed to see the inhibitor content of nutmeg extract. The DPPH radical scavenging activity of nutmeg were compared with a regular antioxidant answer. At concentrations of 25%, 50%, 75%, 100%, the scavenging activity of nutmeg were

found to be capable commonplace antioxidant.

CONCLUSION:

Acetone extraction of nutmeg seed yielded the highest-grade extract. Carbohydrates, alkaloids, proteins, phenolics, and tannins are among the bioactive substances found in nutmeg seed extract. Antimicrobial activity of nutmeg seed extract against *Bacillus Subtilis, E. coli, Aspergillus Niger*, and *Salmonella Typhi*. It also has anti-oxidant properties. It is possible to conclude that nutmeg can be utilized to treat a variety of diseases. It may be concluded that nutmeg can be used for medicinal purposes; nevertheless, other bioactive qualities and previously documented features must be investigated for future developments on a big industrial scale.

FUTURE ASPECT OF NUTMEG AND MACE:

Myristica fragrans is an annual spice from the Myristicaceae family. It's grown all over the world and is utilized in culinary flavoring, essential oil uses, and traditional medicine. Terpenes and phenylpropenes are mostly found in nutmeg. Due to varying growth circumstances, the chemical makeup of these elements fluctuates. Nutmeg is an important component in a variety of commercial uses, including food and cosmetics. Because of its antioxidant and antibacterial characteristics, its medicinal products are extremely essential. Nutmeg byproducts have a growing number of uses and applications. Nutmeg is used in the creation of remedies for diarrhea, flatulence, stomachache, nausea, vomiting, rheumatism, sciatica, malaria, and leprosy in its early stages. There is a need for more study into optimal production, best preservation, and oil extraction methods. (Namra Naeem, Rafia Rehman, Ayesha Mushtaq1, 2016)

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Section IV

Pre-Conference Workshops


Vidya Prasarak Mandal's
B N Bandodkar College of Science
(Autonomous), Thane
Departments of Biotechnology, Microbiology, Biochemistry, Botany & Interdisciplinary Science Present
25th September 2021
3 – 5 pm
On Microsoft Teams
Join Here
1st Pre-Conference Workshop
Of National Conference on
Microbiome: The Story Untold !
Topic:
The Curtain Raiser
Dr. Kalpita Mulye
Head, Dept of Microbiology
Topic:
16S rRNA gene analysis: Important Tool in Microbiome Research
Dr. Jayashree Pawar
Head, Dept of Biotechnology
E-certificates will be given to all participants who attend the workshop completely and fill the feedback form.
Join 15 minutes before to avoid any technical issues.
Themes of the conference:
Human Microbiome
Plant Microbiome
Environmental Microbiome
Probiotics & Functional Foods
Advances in Computational Research
Upcoming Events
2nd Pre-Conference Workshop
27th November, 2021
National Conference
7th & 8th January 2022
Contact : mbtsu2022@gmail.com
VPM's B.N. Bandodkar College of Science (Autonomous), Thane
Departments of Biotechnology, Microbiology, Biochemistry, Botany, Human Science and Interdisciplinary Science in collaboration with Mycological Society of India (Mumbai Chapter) present
National Conference on,
2nd Pre-conference Workshop
'Understanding The Tools of Trade'
MICROBIOME : THE STORY UNTOLD!
10th- 11th December 2021
Join Here
10th December 2021
Ms. Purvi Shah
"Unravel the DNA sequence"
Timing : 3.00 pm-5.00 pm
11th December 2021
Ms. Judith Talker
Timing : 3.00 pm-5.00 pm
11th December 2021
Dr. Ashwini Tilak
Timing: 11.00 am-1.00 pm
"Essential Bioinformatics"
For any queries contact:
mbtsu2022@gmail.com
E- certificates will be provided to all participants attending the workshop after filling the feedback form. Join 15 minutes prior to avoid any technical glitches

First Pre-Conference Workshop

The first pre-conference workshop for National Conference on 'Microbiome: The Story Untold!' was conducted on 25th September, via an online platform of Microsoft teams between 3.00-5.00 pm. Total 81 students and 12 faculty members of B. N. Bandodkar College of Science participated in the workshop. Ms. Zahera Momin, Assistant Professor, Department of Biotechnology and Microbiology, compered the event. The event began by reciting Sarasvati Vandana to worship Goddess Sarasvati.

Honorable Principal Dr. Moses Kolet, while addressing the audience, emphasized on the concept of microbiome in various fields and its significance in upcoming research work. Ms. Shruti Gudekar, student of TY Biotechnology introduced first speaker of the workshop, Dr. Kalpita Mulye, Head of Department of Microbiology. She enlightened the students on the basic concepts of Microbiome. She raised the curiosity of students by initiating the dialogue on evolution and devolution. She even shed light on the significance of the microbiome in diverse fields.

The second speaker for the workshop was Dr. Jayashree Pawar, Head of Department of Biotechnology, VPM's B.N. Bandodkar College of Science, who delivered a talk on '16S rRNA gene analysis: Important tool in microbiome research'. Ms. Shruti Gudekar briefly introduced Dr. Jayashree Pawar. Dr. Jayashree Pawar initiated her talk by introducing the concept of 'The Great Plate Count Anomaly'. She made the audience cognizant about 16SrRNA gene, its significance in microbial typing, process of 16SrRNA gene-based microbiome analysis. Pros & Cons of 16SrRNA gene analysis and concept of alpha & beta diversity were also weighed upon.

The audience were amazed with the world of Microbiome. The session has definitely trained the participants so that they are all set to explore the vast ocean of knowledge about Microbiome to be built gradually in the upcoming second preconference workshop and national conference. Talk was followed by a brainstorming question-answer session. Queries based on intricacies in 16SrRNA gene analysis procedure, criteria of donor selection in FMT, significance of FMT in treatment of Autism, plant related microbiome study and many more

were asked.

Speaker of First Preconference Workshop

The Curtain Raiser: Introduction to Microbiome

Dr. Kalpita Mulye
In-Charge, Department of Microbiology,
VPM's B N Bandodkar College of Science, Thane

Microbiome research has emerged from environmental microbiome research (microbial ecology) and provides an interdisciplinary platform. With Human Microbiome Project, it was soon realised that the human microbiota consists of the 10-100 trillion symbiotic microbial cells harboured by each person, primarily bacteria in the gut; the human microbiome consists of the genes these cells harbour. It has been also referred as 'the last organ'.

Whipps et al. in 1988 first coined the term microbiome. With redefining of concept of Microbiome by Joushua Lederberg 2001, the interest about this field got accelerated. Defining the human microbiome has been a challenging task. 'Microbiota' (the microbial taxa associated with humans) and 'microbiome' (the catalog of these microbes and their genes) are often used interchangeably. Now all know that Microbiome is microbiota and theatre of activity resulted through the communications in the individual members of it.

Many different living systems viz. plants, animals, humans and environmental samples have been studied with respect to their microbiome, to find some major leads in areas like personalized medicines, unconventional therapies, plant-microbe interaction and interdependence, pollution management etc.

Significant progress has been made in the field with advent of technology. Newer Technologies like Fluorescence in situ hybridization, Real time PCR, Full cycle rRNA approach, NGS, Third generation sequencing are getting utilized for the best possible extent to generate valuable data.

Each technique has unique contribution to field. DNA based approaches tell us about who is there and what can they do? RNA based approaches make us know how do they respond and using which pathway? Protein based approaches can help us know the kind of proteins that are getting produced. Metabolite based approaches determine chemical outcomes of the activity.

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Speaker of First Preconference Workshop

16S rRNA gene Analysis Important Tool in Microbiome Research

Dr. Jayashree Pawar
In-Charge, Department of Biotechnology,
VPM's B N Bandodkar College of Science, Thane

Conventional methods of studying microorganisms are limited to those that can be actively grown in laboratory culture; organisms that cannot be cultivated in the laboratory are not represented by this approach. Molecular methods, in contrast, don't necessitate cultivation of the microorganisms, are rapid and accurate. Shotgun metagenomics and multi-omics approaches are important molecular methods that answer the question: 'Who is there??' in the given environment.

Marker is a DNA sequence that identifies the genome that contains it, without the need to sequence the entire genome! Use of a taxonomic marker gene like the 16S ribosomal RNA (rRNA) gene is another widely used molecular approach in microbiome analysis. rRNA gene-based analysis remains a central method in microbiology, used not only to explore microbial diversity, but also as a day-to-day method for bacterial identification and taxonomy.

16S ribosomal RNA is part of the 30S ribosomal small subunit (SSU), present in all bacteria. 16S rRNA gene is 1542 bp long, easy to sequence due to small size, at the same time large enough for bioinformatics-based analysis. The 16S rRNA gene consists of eight highly conserved regions and nine variable regions across the bacterial domain. Primers can be constructed for amplification of the gene by polymerase chain reaction; while characteristic oligonucleotide signature sequences present in variable regions are used for molecular identification of the bacterium. The function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolutionary clock!). 16S rRNA gene sequencing has been largely responsible for revealing the status of our lack of knowledge of microbial world and is considered to be the 'Gold standard' in microbial typing. It provides information about both, species richness as well as their relative abundance. Many reference databases are available for 16S rRNA gene sequence analysis.

Steps in 16S rRNA gene-based microbiome analysis involve sample selection, isolation of gDNA from the samples, PCR using universal 16S rRNA gene primers (Amplicon size/ read length important), amplicon sequencing, computational analyses (BLASTn, RDP, GreenGenes, Silva etc.), phylogenetic identification and binning 16S rRNA Sequences into OTUs. High throughput sequencing (HTS) technologies capable of sequencing multiple DNA molecules in parallel have enabled hundreds of millions of DNA molecules to be sequenced at a time with reduced sequencing cost specialized software and algorithms used to convert raw sequencing data into biologically meaningful information and required representation format.

Though, 16S rRNA gene analysis is a cost-effective, high throughput technique with well-developed statistical tools and curated databases, varying number of copies of 16S rRNA gene in bacteria can skew estimates of community composition. Also, the technique is not quantitative.

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Second Pre-conference Workshop

"Understanding the tools of trade"

The second pre-conference workshop "Understanding the tools of trade" for National Conference on 'Microbiome - The Story Untold!' was organized on 10th and 11th December 2021.

The pre-conference workshop was organized with an aim to enable students to prepare for the conference and equip them with basic understanding of techniques. The Second Pre conference workshop had 3 sessions –

- Session I – Unravel the DNA sequence by Ms Purvi Shah.
  10th December 2021, 3.00-5.00pm
- Session II- Essential Bioinformatics by Ms Ashwini Tilak.
  11th December 2021, 11.00-1.00pm
- Session III- Essential Bioinformatics by Ms Judith Talker.
  11th December 2021, 3.00-5.00pm

Ms. Meetali Chinnkar, Assistant Professor, Department of Biochemistry, compered the event. The event began with Sarasvati Vandana. After the welcome address, Dr. Moses Kolet, Principal, VPM's B.N. Bandodkar College of Science, Thane gave his introductory remarks. Sir welcomed the participants and gave best wishes to the organizing committee for smooth conduct of the event. He also highlighted the importance of these preparatory sessions and motivated students to participate in large number. Dr. Jayashree Pawar, Organizing Secretary briefly explained the conceptualization of the National Conference. She also elaborated on various themes and gave an overview of all the activities that were organized till date under the umbrella of Microbiome- the Story Untold.

The first session of workshop 'Unravel the DNA sequence' was taken by Ms Purvi Shah, Assistant Professor Department of Biotechnology and Microbiology. Mr Omkar Barve, third year biotechnology student gave a brief introduction of Ms Purvi Shah. The session threw light upon central dogma of molecular biology and importance of sequencing 16s rRNA gene in Microbes. Ms. Purvi Shah elaborated on the use 16S rRNA gene sequencing is as a tool to identify bacteria at the species level and assist with differentiating between closely related bacterial species. An overview of a timeline on the study of DNA was discussed. She also elaborated on the various DNA sequencing methods that are available and continued her session with detailed explanation of the Sanger Sequencing method. Ms. Purvi Shah mentioned that Next-Generation Sequencing (NGS) of 16S rRNA gene is now one of the most widely used application to investigate the

microbiota. She explained the techniques of Illumina sequencing technology, sequencing by synthesis (SBS), which are widely adopted next-generation sequencing (NGS) technology worldwide. This session concluded with interaction with the participants. 112 Participants attended the session.

The second session of workshop "Essential bioinformatics "was taken by Dr. Ashwini Tilak, Assistant Professor Department of Biotechnology and Microbiology on 11th December 2021 from 11.00 am to 1.00 pm. The session began with formal introduction of the speaker by Ms Komal Rai, TY Biotechnology student. Dr. Ashwini Tilak explained the broad concept of bioinformatics and need for amalgamation of wet lab techniques and virtual tools of data assessment. She mentioned various tools that are widely used today for data analysis. She emphasized on the need to analyze data with more precision and fastness. She also demonstrated the use of Basic Local Alignment Search Tool (BLAST) in nucleotide and protein sequencing. Dr. Ashwini Tilak stepwise guided the participants to perform sequence alignment and analyze the results. This session concluded with interaction with the participants. 90 Participants attended the session.

The third session of workshop "Essential bioinformatics "was taken by Ms Judith Talker, Assistant Professor Department of Biotechnology and Microbiology on 11th December 2021 from 3.00 pm to 5.00 pm. The session began with formal introduction of the speaker by Ms Sayali Malvankar, TY Biotechnology student. Ms Judith Talker focused on Phylogenetic Analysis in the talk. She introduced the participants with concept of phylogenetic as the study of the evolutionary development of a species or a group of organisms or a particular characteristic of an organism. Taking recent example of Covid Outbreak, she uncovered an otherwise difficult topic with very easy and relatable example. She also demonstrated the use of Molecular Evolutionary Genetics Analysis (MEGA) computer software for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees. This session concluded with interaction with the participants. 98 Participants attended the session. The two-day pre conference workshop concluded by vote of thanks and concluding remarks from Dr. Kalpita Mulye, Organizing Secretary.

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Speaker of Second Preconference Workshop

Unravel the DNA Sequence

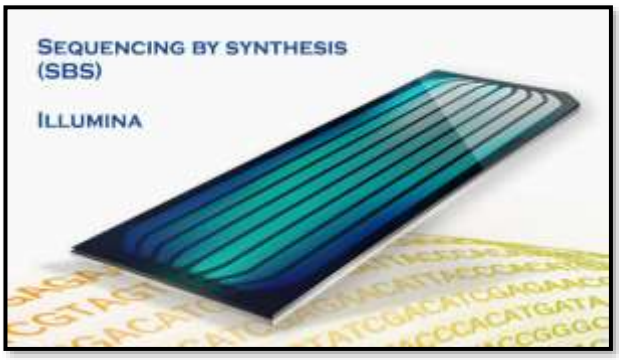
Ms. Purvi Shah
Assistant Professor,
VPM's B. N. Bandodkar College of Science (Autonomous), Thane

In 1977, Frederick Sanger developed a procedure for DNA sequencing also known as Chain termination sequencing or Dideoxy sequencing. The classical Sanger sequencing method requires ssDNA template, dNTP, DNA polymerase and primer. Components are added in four tubes, each containing a different radioactively labelled ddNTP. After reaction, content of tubes is loaded in PAGE. PAGE is used for separation of DNA fragments differing in a single nucleotide. Sequence of template DNA can be decoded by reading the gel. Sanger sequencing was upgraded to automated DNA sequencing in which all four ddNTPs labelled with different fluorescent tags were added in a single tube. After the reaction, the sample would be run in capillary electrophoresis and DNA sequence would be read. This forms the era of 'First generation sequencing.'

Later, Illumina majorly with HiSeq and MiSeq sequencing platforms dominates second generation sequencing or next generation sequencing (NGS) market by more than 70%. This platform is based on the principle of sequencing by synthesis (SBS) using fluorescently labelled reversible terminators. Library preparation, cluster generation, sequencing and data analysis forms four major steps of SBS. Apart from SBS, the remaining NGS platform works on pyrosequencing, proton detection, ligation, etc. The choice of NGS platform for sequencing depends on number of criteria like read length per run, error rate, number of reads per run, cost etc.

After NGS, third generation sequencing platforms have emerged to simplify the preparatory procedures and sequencing methods along with cost reduction. These platforms are capable of sequencing even single molecule of DNA in real-time.

One of the important applications of sequencing is identification of microbe. This has been significant in the field of microbiome research especially which deals with unculturable bacteria.



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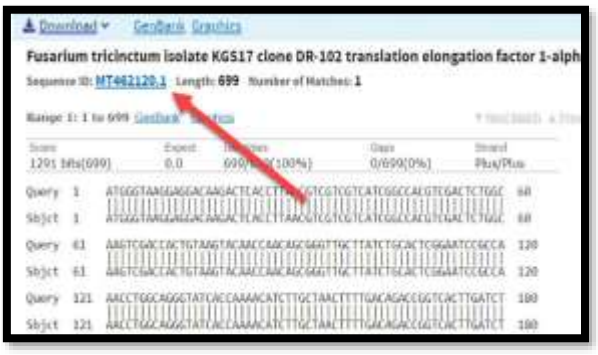
Speaker of Second Preconference Workshop

BLAST (Basic Local Alignment Sequence Tool): A Tool for Sequence Alignment

Dr. Ashwini Tilak
Assistant Professor
VPM's B. N. Bandodkar College of Science (Autonomous), Thane

Sequencing is an inevitable part of any Microbiome study, and so is the analysis of these sequences. This can only be achieved with the aid of computers and Bioinformatics software tools. The two approaches viz. shotgun metagenomic sequencing and 16s rRNA sequencing, both generate raw data that has to be analyzed for making a meaningful conclusion out of them. The raw data has to be compared with plethora of sequences with known identity that are already present in the global databases. We can also identify genes or their function, another feat that can be achieved by aligning them with homologous sequences whose functionality is already known

Alignment i.e. identification of residue–residue correspondences can be carried out with the help of algorithms supplemented with suitable biological information. NCBI BLAST is a one such useful tool to accomplish both the goals mentioned above, as it helps to find out local regions of similarity in biological sequences. It takes a heuristic approach for aligning the sequences based on identity/similarity scores. This approach is faster as compared to the dynamic approach for alignment wherein an entire sequence is aligned with every sequence in the database- a mammoth task which might take several days to years depending on the length of the sequence. Instead, BLAST algorithm breaks down the query in the form of short words of a designated length (e.g. default word size is 11 for Nucleotides BLAST) and searches the matches (or similarities) for these words in the target entries in the database. If there is a good match (depending on T value calculated using an identity or substitution matrix), it extends these words while giving appropriate score for each match or mismatch as the query is being aligned with database sequences. If a particular extension score drops below a drop off value, BLAST does not consider that alignment to be viable and discontinues its extension. BLAST calculates the statistical significance of matches that are above the said threshold value, and displays an output in the form of alignments. The more identical the sequences are, the more possibility they have to be closely linked. To make sure that the alignments are 'true' and not 'by chance', BLAST gives user an option of selecting the sequences according to E value (expect value), a parameter that depends on the drop off value and the size of sequence database used.



A novel sequence would not show exact match with any sequence in the database. Such sequences, however, should be thoroughly scrutinized for all the parameters offered by BLAST. The sequences that are novel can be deposited in the sequence databases (EMBL/GenBank/DDBJ).

BLAST gives many options for query-target combinations such as BLASTn (nucleotide-nucleotide), BLASTp (protein-protein), and translated BLAST that can translate the nucleotide sequence to protein and align.

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Speaker of Second Preconference Workshop

Essential Bioinformatics- Introduction to phylogenetics by MEGA

Ms. Judith Talker
Assistant Professor
VPM's B. N. Bandodkar College of Science (Autonomous), Thane

Genome sequencing provides a pool of data from a wide variety of organisms. To accommodate these large volumes of data, databases have evolved to store, annotate and retrieve the information. However, to analyse this vast amount of data, there is a need to use computer tools. The Molecular Evolutionary Genetics Analysis (MEGA) software is a desktop application designed for comparative analysis and understanding the evolutionary relationship between species. It is a tool for sequence alignment and studying the phylogenetic relationships between sequences. It provides many convenient tools to explore the primary data with a user-friendly interface. The two steps in MEGA included Multiple sequence alignment and construction of phylogenetic tree. Multiple sequence alignment is a sequence alignment method used to align more than three sequences which can be used to derive the relatedness between them and construction of phylogenetic tree by using the information obtained from the alignment. MEGA makes available many methods of evolutionary analysis to students and scientific community. MUSCLE and CLUSTAL W are two tools that can be used for sequence alignment. MEGA allows the user to choose between different methods and algorithms for building of the tree. Phylogenetic tree can be constructed using either the distance-based methods or Character- based methods. Maximum likelihood, Distance methods, Bayesian method and Maximum parsimony method based on a set of assumptions can be used to construct evolutionary tree using this software. It can be used to make a rooted or an unrooted tree. A guide file can also be added to make a rooted tree and find the distance between the all sequences from its root. Thus, the software allows the user to get a true tree using the different method. It gives results in the form of a phylogenetic tree. Phylogenetic tree depicts the line of evolutionary descents of different species. It gives the results in the form of p-values. P-value is a hypothetical value on a scale of 1 to 100 to denote the evolutionary relatedness between sequences. The branch lengths can be used to know the evolutionary distance between the species of interest. MEGA is thus a software that can be used for conducting user-friendly statistical analysis of evolution.

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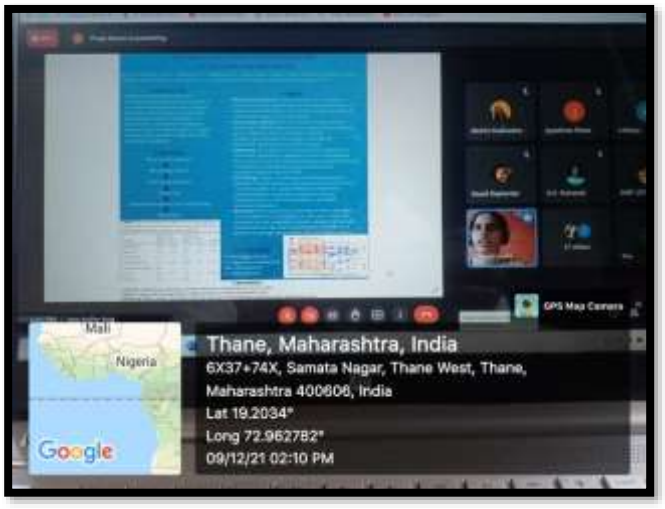
ISBN: 978-81-923628-9-2

Section V
Poster Competition

Poster competition

Online Poster competition was organised for national conference on Microbiome: The story untold on 9th of December 2021 on google meet platform. A guidance session for poster making was held for the undergraduate students on 23rd November, 2021 by faculty members Dr. Ashwini Tilak and Ms. Purvi Shah. Total of 21 registrations were received under three categories namely; Category 1- undergraduate students, Category 2- Masters students and Category 3- Ph.D. / Faculty/ Industrial personnel. Registration for poster competition was received from nine different institutions including organizing college. A scrutiny committee of faculty members from organizing team Ms. Sayali Daptardar, Ms. Zahera Momin and Mrs. Rucha Khadke selected 16 relevant posters for final presentation.



The poster presentation was divided into two sessions; morning and evening, with 8 participants judged by two judges; one internal and one external judge, in each session. The morning session commenced at 10 am followed by an evening session at 2 pm. A blindfolded marking system was implemented, where identity of the participants was not revealed to the judges. Ms. Mohini Khushwaha hosted the event. The event started with a welcome address by principal Dr. Moses Kolet. Dr. Jayashree Pawar, organizing secretary then threw light on the various activities conducted under the National conference Microbiome- The story Untold to be held on 7th and 8th Jan, 2021. Ms. Mohini Khushwaha introduced the judges Dr. Abhishek Mule, Field Application specialist, Eppendorf,

India and Dr. Urmila Kumavat, Assistant professor Botany, B. N. Bandodkar College of Science. She then reminded the participants about the rules of the competition. Each student was given 7 minutes time to present his/ her poster followed by a few questions asked by the judges. A massive audience of 125 viewers were present at the event. At the end of all the 8 presentations for the morning session, judges, Dr. Abhishek mule and Dr. Urmila Kumavat appreciated the effort of the committee in organising such an event and appreciated the students for their participation. Dr. Urmila Kumavat also mentioned the marking system to all the participants. The session was concluded by a formal vote of thanks by Dr. Kalpita Mulye, organizing secretary.

The afternoon session began sharp at 2 pm by welcoming everyone and introducing the judges Mr. Amit Jethwa, Assistant Professor, Biotechnology, K. J. Somaiya college of science and commerce and Dr. Urmila Kumavat, Assistant professor, B. N. Bandodkar college of science. Rules of the poster competition were recalled for all the participants followed by poster presentation of the all the 8 participants. After completion of the all the poster presentations, judges Mr Amit Jethwa and Dr. Urmila Kumavat appreciated the efforts of the students in presenting on very diverse topics and urged the students to participate in such competition irrespective of the results. Formal vote of thanks was proposed by organizing secretary Dr. Kalpita Mulye.

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Dr. Preeti Vinayak Phate

National Conference On Microbiome: The Story Untold !

Abstract: The present work describes spore to spore agar culture of *Stemonitis axifera*, a myxomycetous species from order Stemonitales. The species was successfully cultured on 1.5 water agar and apparently the first report from Maharashtra India.

Introduction:

Myxomycetes are the eukaryotic microorganism comprising of about 1000 species (Lado 2005-2018) distributed worldwide. Current classification place them in Super class Amoebozoa (Adl et al 2005). From known species only 10% species were cultured spore to spore so far and of those most belong to order Physarales.

Material and Method:

- The specimen was collected from Tala village from living tree bark and was studied morphologically and on the basis of relevant literature the species was identified as *Stemonitis axifera*.
- For composition of agar medium and techniques the paper by Haskin and Wrigley de Basanta 2008 was referred.
- The fruiting bodies were gently picked from the substratum and crushed by tapping gently them on surface of water agar germination plates in order to release spores.
- The plates were then incubated at 22-25°C at relative humidity 95%.
- The plates were regularly observed for germination and plasmodial formation.

Results:

- Spore Germination – 4th day after sowing spores on 1.5 WA plates.
- Plasmodium formation: After 12 days, Aphanoplasmodium type.
- Fruiting body formation: After 15 days color change from white-pink-dark brown.
- Spore to spore life cycle thus completed in about 34 days on 1.5 water agar.

Figure 1: *Stemonitis axifera* on tree bark

Figure 2. Agar culture results on 1.5 W A plates. a. Myxamoebae and swarmers, b. Aphanoplasmodium, c & d. Color change in fruiting bodies.

Conclusion: Most of the studies related to myxomycetes in India are taxonomy based while the literature on their laboratory culture and nutrition is negligible. Moreover myxomycetes were found to be source of about 100 novel secondary metabolites showing biological activities, hence it is the need of the present to culture these organisms and understand their biology.

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Netkar Vaishali Mahendra

PLANT MICROBIOME PARADIGM

ABSTRACT: Plant-associated microbes interact with their host in essential functional contexts. They can stimulate germination and growth, help plants fend off disease, promote stress resistance, and influence plant fitness. The metabolism and morphology of plants and their microbiota are intensively connected with each other, and the interplay of both maintains the functioning and fitness of the holobiont. Understanding identity, ecology, and physiology of such keystone microbial species and their interactions within the microbial network could be crucial for both sustaining plant health and soil fertility and could provide a potential target for novel agricultural management strategies.

KEYWORDS: Microbiota, Holobiont, Plant-Microbe Interaction, Phyllosphere

OBJECTIVE

- Microbiota are important for plant growth, health and stress resilience.
- Inoculation with key microbiota members can improve plant traits.
- Tailored selection and delivery of microbial strains or consortia is required.
- Microbiome improvement may be achieved by appropriate agro-management practices.
- Plant breeding for improved interaction with microbiota will be of benefit.

TECHNIQUES

- Numerous culture-independent
- Microscopy
- Molecular techniques
- Sequencing technologies
- Shotgun metagenome

ADVANTAGES

- Production of antibiotics
- Lytic enzymes production
- Pathogen-inhibiting volatile compounds production
- Siderophores production
- Modulate plant hormones
- Develop disease-suppressive soils
- Disease antagonists
- Protects plant from biotic stress
- Protect plant from abiotic stress
- Plant productivity

INTRODUCTION:

A wide range of other microbes, including arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, root endophytes, and a range of disease-suppressive bacteria do impact plant growth or nutrient acquisition positively. The plant genotype has a big impact on microbiome composition indicates that breeding and genotype selection offers opportunities to select for specific (beneficial) microbiomes (finding by Agler et al.). Many microbes simply inhabit the plant microbiome as colonizers of the phyllosphere, or the rhizosphere using plant exudates for their growth. Such symptomless colonizers usually have no major impact on plant growth or fitness. microbes interact and influence plant growth and the fact that many plant species cannot survive in absence of microbes. Having more information on plant microbiota in regard to biotic and abiotic stresses, plant genotype, and environmental conditions, it might be feasible to find better suitable candidates or approaches for inoculation in a given environment.

PHYLLOSPHERE OR CARPOSPHERE

- *Pseudomonas*
- *Sphingomonas*
- *Frigoribacterium*
- *Curtobacterium*
- *Bacillus*
- *Enterobacter*
- *Acinetobacter*
- *Erwinia*
- *Cirrobacter*

FUTURE PROSPECTS

- Identify link microbiome composition to function
- Investigation of microbial interaction and influence plant growth
- Several unexpected functions and metabolic interaction

AFFECTING FACTORS

- Soil Ph
- Salinity
- Soil Type
- Soil Structure
- Soil Moisture
- Soil Organic Matter
- Exudates
- Climate
- Pathogen Presence
- Human Practices

PLANT PATHOGENS

- *Pseudomonas syringae*
- *Erwinia amylovora*
- *Xanthomonas sp.*
- *Ralstonia solanacearum*
- *Xylella fastidiosa*

ROOT MICROBIOTA

- *Acidobacteria*
- *Verrucomicrobia*
- *Bacteroidetes*
- *Proteobacteria*
- *Planctomycetes*
- *Actinobacteria*
- *Chloroflexi*
- *Firmicutes*
- *Gemmatimonadetes*

CONCLUSION:

The metabolism and morphology of plants and their microbiota are innately connected with each other. The plant performance depends on the presence of particular microbial species, and that alternative stable states with high or low plant performance can be identified, depending on microbial community composition. The time is ripe to move on to the next challenge. Due to the interesting functional potential of plant microbiota as well as due to current challenges in crop production there is an urgent need to bring microbial innovations into practice.

ENDOPHYTES

- *Ralstonia*
- *Burkholderia*
- *Pseudomonas*
- *Staphylococcus*
- *Mesorhizobium*
- *Propionibacterium*
- *Dyella*
- *Bacillus*

CORE MICROBIOME

- *Bradyrhizobium*
- *Sphingobium*
- *Microvirga*
- *Deltaproteobacteria*
- *Alphaproteobacteria*
- *Actinobacteria*
- *Zarrasonandia*

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Winner

Mridula Leela Gangadharan Nair

REPRODUCTIVE TRACT MICROBIOME AND ITS ROLE IN FEMALE INFERTILITY

Introduction

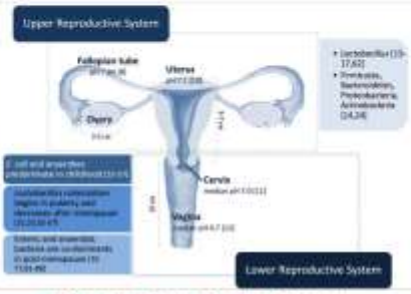
- The human body harbors microorganisms that inhabit surfaces and cavities exposed or connected to the external environment.
- Human vaginal microbiota seem to play a key role in preventing a number of urogenital diseases.
- Many different species of *Lactobacillus* are present in the vaginal tract, with a few that predominate.
- Other represented genera being *Prevotella*, *Bifidobacterium*, *Gardnerella*, *Atopobium*, *Megasphaera*, *Sneathia*, and *Anaerococcus*.
- *Lactobacillus* comprises 90 to 95% of the total bacterial count in the reproductive tract
- Female reproductive microbiota has also been suggested to affect infertility.

Upper reproductive system

- Metagenomic studies have shown that the female upper reproductive system is not sterile.
- The upper reproductive system hosts 10,000 times less bacteria than the vagina.
- *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* being the most abundant phyla
- They are able to modulate the functions of both endometrial cells and the local immune system, to prevent uterine infections
- A continuum along the reproductive system with the ascension of microbes from the vagina, also have been suggested.

Lower reproductive system

- Vaginal microbiome accounts for about 1 billion bacteria/gram of vaginal fluid
- The *Lactobacillus* predominance seems to be age-dependent and strictly related to the reproductive age
- Estrogen level reduction causes an increase of vaginal pH allowing the colonization of enteric bacteria and anaerobes.
- Bacterial vaginosis is the most common form of vaginal dysbiosis.
- Dysbiosis has been related to several diseases like endometritis, pelvic inflammatory disease, and gynecological cancers



Female Reproductive System Microbiome and Infertility

- An increasing number of studies is highlighting a correlation between infertility and the microbiota
- Infertile women host a different microbiota, both in the lower and/or in the upper reproductive system.
- *Ureaplasma* and *Gardnerella* were more abundant in the vagina and in the cervix of the infertile women.

Female Reproductive System Microbiome and IVF Outcomes

- A high number of oocytes and embryo quality are positively associated with good IVF outcome
- Vaginal dysbiosis may negatively impact IVF pregnancy rates.
- The culture of the tips of the catheter used for embryo transfer has revealed that, while the presence of *Lactobacilli* is associated to a better reproductive outcome.
- The efficacy of antibiotic administration in the treatment of chronic endometritis also had a positive impact on the implantation and pregnancy rate and improved IVF success.

Conclusions

- To correctly evaluate the reproductive status of a couple, microbiota status of male partner is mandatory.
- Need to assess the semino-vaginal microbiome in infertility and in IVF outcomes.
- Microbiome thus, offers a unique opportunity to develop specific treatments.

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Winner

Pranali Amol Mohite

THE HIDDEN SUPERHEROES - HUMAN MICROBIOME

ABSTRACT

The human microbiome is the collection of microorganisms living in association the huamn body. The symbiotic relationship with the microbial flora inhabiting our plays an immmerse role in maintaining our vitality. The microbiota protect us from pathogens, hardwires our immunity and enages in the production of essential micronutrient components. The unique diversity of the human microbiota accounts for the specific metabolic activities and functions of these micro-organisms within each body site.

KEYWORDS - Human Microbiome, Micro-organisms, Symbiotic, Pathogen.

Functions

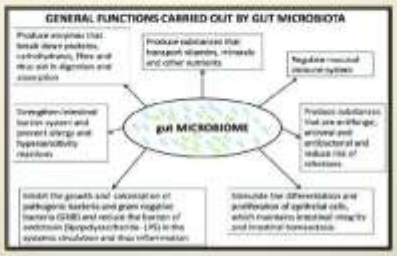
- Influences mental health
- Promotes skin health
- Helps digest food
- Boost immune system
- Protects against toxic
- Controlling metabolism and nutrient storage
- Decreasing inflammation
- Producing antimicrobials
- Maintaining tissue integrity

INTRODUCTION

The human microbiota is defined as a set of organisms inhabiting and interacting with the human body. The various interactions may be commensalistic, mutualistic, or pathogenic. An alteration in the intestinal microbial community play a major role in human health and disease pathogenesis. It is therefore to understand the microbial composition and activities of the human microbiome as they contribute to health and diseases

GUT MICROBIOME

- Gut microbiome is made up of trillions of bacteria, fungi and other microbes. For ex. *E.coli, H.pylori, L.acidophilus, S.aureus, C.albicans* etc.
- Most of the microbes in your intestines are found in a "pocket" of your large intestine called the cecum, and they are referred to as the gut microbiome.
- The gut microbiome plays a very important role in your health by helping control digestion and benefiting your immune system and many other aspects of health.



CONCLUSION

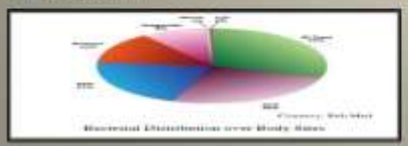
- The study of the human microbiome is important, and it gives an in-depth understanding of the interplay between humans and its indigenous microbiota.
- This gives valuable insight into further research studies in optimizing these organisms to combating life-threatening diseases.
- In addition, further studies should emphasize on the effects of the human microbiome on mental health and also the impacts of mycobiome and the virome community on indigenous microbiota as they may contribute to dysbiosis.
- International human microbiome studies using metagenomics have highlighted the functional role of the microbiota in the body, and it promises new clinical applications

OBJECTIVES

- To make you all generally conversant in the language of microbiomes.
- To provide examples how microbial communities affect health and cure diseases.
- The role of the microbiomes in our organs.
- To give practical examples of how medical interventions interact with the microbiome and change outcomes.

HUMAN MICROBIOME PROJECT

- The Human Microbiome Project was a United States National Institutes of Health research initiative to improve understanding of the microbial flora involved in human health and disease.
- To understand the microbial components of the human genetic and metabolic landscape and how they contribute to normal physiology and predisposition to disease.
- To understand the range of human genetic and physiological diversity, the microbiome and the factors that influence the distribution and evolution of the constituent microorganisms must be characterized.



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ISBN: 978-81-923628-9-2

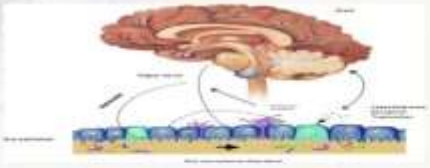
Winner

Komalkumari Ashok Rai

"ALTERATIONS IN ME,CAN 'DEPRESS' YOU SAYS GUT MICROBIOME!!"

INTRODUCTION:

- Depression affects more than 300 million people of all ages globally , and is one of the leading causes of psychiatric disability.
- linkage between gut microbiota pattern and depression through the brain-gut microbiome axis.
- α-diversity and β-diversity of the microbiota existed in people with depression compared to healthy controls
- Changes in gut microbiota patterns result in immune activation through the bidirectional interactions between the gut and the brain, potentially yielding the generation of various types of psychiatric symptoms.



- Aim of this systematic review was to identify the alterations of the gut microbiota patterns in people with depression compared to healthy controls.

MATERIAL & METHODS :

Data Analysis :-

Gut microbiome was grouped based on α-diversity (the richness and evenness of the microbial community), β-diversity (the compositional dissimilarity among the microbiome community), the abundance and proportion of bacteria at phylum, family, and genus levels.

METHODOLOGY USED:

Mini-International Neuropsychiatric Interview (MINI), Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), Diagnostic and Statistical Manual of Mental Disorders Text Revision (DSM-IV-TR), and International Classification of Diseases (ICD-10).

Depression severity was assessed with;

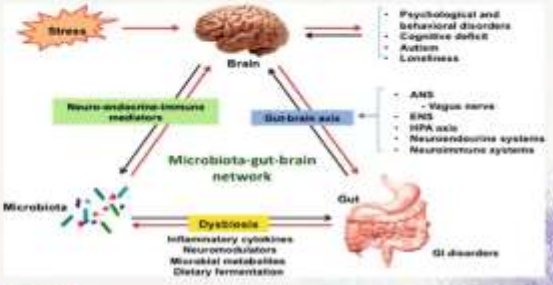
- Hamilton Depression Rating Scale (HDRS), Montgomery–Åsberg Depression Rating Scale (MADRS)
- microbiome analysis involved sequencing of hypervariable regions V1–V5 of the 16S rRNA genes.

TECHNIQUES USED FOR DNA SEQUENCING:

PCR, reverse transcription–quantitative PCR (RT-qPCR), pyrosequencing, Illumina sequencing, and Illumina sequencing MiSeq platform .

RESULT & DISCUSSION :

- No consensus in the α-diversity and β-diversity was evident.
- Additionally, different quantities of bacterial abundance were present at the family and genus levels.
- inconsistencies in in the abundance of *Firmicutes*, a lower abundance of *Bacteroidetes* and a higher abundance of *Actinobacteria* phyla among people with depression
- High abundance of *Oscillibacter, Parabacteroide, Klebsiella, Paraprevotella, Veillonella, Desulfovibrio, Parasutterella, and Paraprevotella* as a gram-negative bacteria in people with depression may explain the contribution of microbiota in development/maintenance of depression.
- Gut microbiota are able to influence the body neurotransmitters levels by stimulate the CNS and the gut *via* the production of metabolites.
- use of diverse anti-depressant medications was observed to impose a variety of alterations in the bacterial community, which made it difficult to predict the bacterial community pattern in medicated depressed patients.



LIMITATIONS :

Variations in findings may be attributed to the difference in methodology and population of the included studies.

CONCLUSION :

- The area of research on the role of microbiota in the brain-gut axis in development or maintenance of depression is still limited.
- There are conflicting reports on microbial diversity as well as the abundance of bacteria at phyla, family, and genus taxonomic levels in people with depression.
- inconsistency suggests that there may be confounding factors within these complicated relationships.
- Nonetheless, further studies are strongly suggested..

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Sayli Shyamsundar Malvankar

BIDIRECTIONAL CONNECTION BETWEEN 'GUT MICROBIOME AND BRAIN'

INTRODUCTION:

- Role of microbiome in sleep and mental disease, focusing on the interaction of the microbiome with circadian rhythm and sleep problem

GUT BRAIN AXIS:

- Within Microbiome- Gut- Brain (MGB) axis, the gut microbiota affect brain function through three pathways that are bidirectional in nature, as shown in figure 1 :

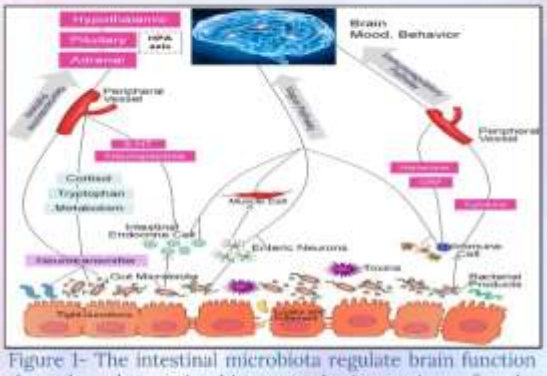

Figure 1- The intestinal microbiota regulate brain function through the microbiome-gut-brain axis of the immunoregulatory, neuroendocrine, and vagus pathways.

EFFECT ON CIRCADIAN RHYTHM:

- Disruption of host circadian rhythm alters Gut microbiome equilibrium
- Circadian clock genes are closely related to the development insomnia

STRESS AND SLEEP DISORDER:

- Microbial dysbiosis- disrupt circadian gene expression- high level of cortical hormone
- Corticosterone- bridge connecting intestinal microbiome to stress and anxiety
- Stress leads to dysbiosis- causing insomnia and depression

CONCLUSION:

- At present, research on the effects of the intestinal microbiota on the pathogenesis of some psychiatric disorders, is incomplete
- Thus further research based on various aspects is necessary for understanding mechanism underlying this relationship

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Mitali Milind Mone

Saliva Microbiota Differs Between Children With Low And High Sedentary Screen Times

Elina Engberg, Sajan C. Raju, Rejane A.O. Figueiredo, Elisabete Weiderpass ,Trine B. Rounge, Heli Viljakainen

Human microbiome journal 20(2021)

INTRODUCTION

The diversity and composition of both the gut and saliva microbiota seem highly important in human health and disease. The saliva microbiota has a similar richness in species to elsewhere in the gastrointestinal tract, and the bacterial taxa are similar to those in stomach fluids and the placenta. Moreover, the gut microbiome is influenced by the oral microbiota given the continuity of the gastrointestinal tract. Physical activity may increase microbial diversity through several mechanisms, including promoting an antiinflammatory state.

Characteristics	All n=376(100%)	Children with low screen times n=193 (51%)	Children with high screen time n= 183 (49%)	p^a
Age (in years), mean (SD)	11.7 (0.4)	11.7 (0.4)	11.8 (0.4)	0.012
Gender, n (%)				
Girl	184 (48.9)	109 (56.5)	75 (41.0)	0.003
Boy	192 (51.1)	84 (43.5)	108 (59.0)	
Language spoken at home, n (%)				
Finnish	319 (84.8)	164 (85.0)	155 (84.7)	0.060
Swedish	30 (8.0)	24 (12.4)	13 (8.2)	
Other	18 (4.8)	5 (2.6)	13 (7.1)	
BMI Categories n (%)				
Underweight	52(13.8)	30 (15.5)	22 (12.0)	0.297
Normal weight	274(72.9)	143 (74.1)	31 (71.0)	
Overweight	41(10.9)	17 (8.8)	24 (13.1)	
Obese	9(2.4)	3 (1.6)	6 (3.3)	
Eating habit groups , n (%)				
Unhealthy	67(17.5)	13 (6.7)	35 (19.1)	<0.001
Vegetable avoider	154(41.0)	63 (32.6)	91 (49.7)	
Healthy	175(46.5)	118 (61.1)	57 (31.1)	
Leisure time physical activity, n (%)				
Low (<4h/week)	88(23.4)	38 (19.7)	50 (27.3)	0.178
Medium (5-9h/week)	196(52.1)	103 (53.4)	93 (50.8)	
High (>9h/week)	92(24.5)	52 (26.9)	40 (21.9)	

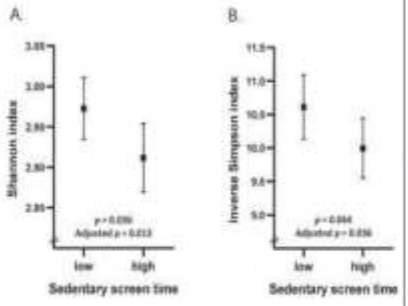
If physical activity provokes beneficial changes in the microbiota, sedentary behavior may, in turn, promote harmful alterations. Sedentary behaviors refer to activities in a reclining, seated or lying position requiring very low energy expenditure and body movement. some health-promoting bacterial species was higher among active women compared to inactive women , a sedentary lifestyle may induce changes in the gut microbiota. Examining the possible relationship of both physical activity and inactivity with the microbiota may lead to further understanding how physical movement diminishes the risk of disease and improves health. We previously showed that high sedentary screen times among children associated with being overweight and central adiposity, and predicted a higher body mass index (BMI) among adolescents . Here, we aim to identify potential differences in saliva microbiota richness, composition and functional capacity between children with high and low sedentary screen times. This study provides novel evidence on the possible factors associated with children's microbiota, and may reveal mechanisms regarding how sedentary behavior relates to health.

METHODS AND MATERIAL

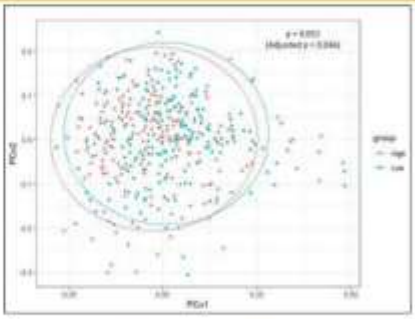
- **Study design and participants**
- **Measures**
  - Sedentary screen timing
  - Demographics
  - Body mass index (BMI)
  - Eating habits
  - Physical activity
  - Saliva samples and microbiota analysis
  - Statistical analysis
  - Covariates

RESULT

- Participants characteristics
- Sedentary screen time and the alpha diversity of saliva microbiome



- Sedentary screen time and functional capacity
- Sedentary screen time and the beta diversity of the saliva microbiota
- Sedentary screen time and saliva bacteria abundance



DISCUSSION AND RESULT

our study carries several strengths, including the large number of participants compared to studies examining the relationship between sedentary time and the gut microbiota. Furthermore, we could include many possible confounding factors in our analyses, including BMI, physical activity and eating habits. However, any residual confounding may persist even after adjustments because of possible measurement errors in the self-reported confounders. This study provides novel evidence on the possible relationship between children's sedentary screen times and the microbiota, suggesting that one mechanism behind the adverse health consequences of sedentary time may associate with the microbiota. This study is the first to suggest that high amounts of sedentary behaviors, i.e. behaviors requiring very low energy expenditure and body movement, may be distinctly related to less diversity, different composition and distinct functional capacity in the children's saliva microbiota.

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- (Engberg et al., 2021) (Beistrøm, 2020)
- (Miele et al., 2015) (Kodukula et al., 2017)
- (Acharya et al., 2017) (Segata et al., 2012)

Arunima Patil

PRESERVING THE MATERNAL-INFANT MICROBIOME

ABSTRACT

Every individual harbors diverse microbe populations The human microbiome is established in a newborn prior to birth as it moves through the intrauterine environment and picks microbiota from the mother. Studies have found that the method of labor and the birth environment have the potential to influence the initial colonization of microbes in the neonate. As compared to newborns delivered vaginally, those delivered by the cesarean section may have future health implications and may be predisposed to chronic health conditions.Thus there is need to promote practices that can preserve the maternal-infant microbiome

FACTORS AFFECTING THE INFANT MICROBIOME

- Labor methods and birth environment
- Antibiotic exposure and post-operative
- Quality and quantity of skin to skin contact
- Delay or absence of breastfeeding
- Vaginal examination frequency

C-SECTION AND THE USE OF ANTIBIOTICS

There are 50% higher chances of admitting a C-section newborn to the ICU or developing pulmonary disorders.In C section, there is no immediate skin-to-skin contact with the mother instead, the newborn is handled by the nurses and comes in contact with the labor room surfaces.The breast feeding is also delayed ,both resulting in an altered seeding. Antibiotics are administered to minimize post-operative infection, while it is necessary it may causes dysbiosis of the microbiome.Antibiotics can decrease the count of *Bifidobacterium* in the breast-milk and increase the abundance of *Firmicutes* associated with increased fat and weight gain in the infant.The use of Antibiotics on a large scale can also result in MRSA and *Clostridium difficile* infection to the mother and infant.

BACKGROUND

The placenta, fetal membranes, amniotic fluid, and the umbilical cord blood were considered sterile until microbes were isolated from these regions. Varied bacterial populations such as *E.coli*, *Prevotella tannerae*, and *Bacteroidetes*, etc present in lower numbers indicate a separate placental microbiota.

In the US 68% births take place vaginally.A distinct microbiome was observed in newborns delivered by the C-section as compared to those born vaginally. The vaginal microbiome keeps the vaginal environment in homeostasis and prevents the growth of bacteria that may induce pre-term labor and chorioamnionitis. The maternal microbiome profile is dynamic throughout the period of gestation.During a period of 18 to 40 weeks the species diversity and abundance lower.Although an overall dominance of protective bacteria such as *Lactobacillus* species,*Clostridiales*,*Bacteriodales* and *Actinomycetes* orders is observed.

CONCLUSION-PRESERVING THE MATERNAL-INFANT MICROBIOME

- Nursing staff should be trained about the physiology of childbirth and should provide emotional support
- Hospital environments should support vaginal birth and avoid interventions when not required.
- Fetal heart rate surveillance monitors must be used
- Family centered or immediate skin-skin contact C-section birth should be promoted
- Antibiotics must be administered carefully
- Excessive cervical examinations must be avoided and the microbiome must not be disturbed

DATE-28th November 2021

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Pranoti Pranam Joshi

The Influence Of A Short-Term Gluten-Free Diet On The Human Gut Microbiome

Marc Jan Bonder1† , Ettje F. Tigchelaar1,2† , Xianghang Cai3† , Gosia Trynka4 , Maria C. Cenit1 , Barbara Hrdlickova1 , Huanzi Zhong3 , Tommi Vatanen5,6, Dirk Gevers5 , Cisca Wijmenga1,2, Yang Wang3† and Alexandra Zhernakova1,2*†

❖ INTRODUCTION:

A gluten-free diet (GFD) is the most commonly adopted special diet worldwide. It is an effective treatment for coeliac disease and is also often followed by individuals to alleviate gastrointestinal complaints. It is known there is an important link between diet and the gut microbiome, but it is largely unknown how a switch to a GFD affects the human gut microbiome.

❖ METHODS:

Blood sample collection

↓

Microbiome analysis

↓

Fecal sample collection

↓

Sequencing

↓

Operational Taxonomic Unit (OTU) picking

↓

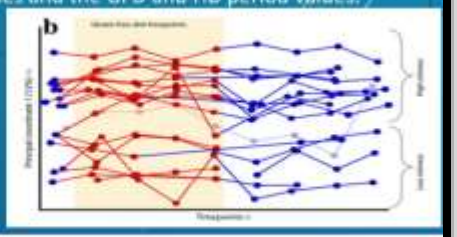
Biomarkers

Table 1 Mean and standard deviation (SD) of energy, protein, carbohydrates, and fat intake during the gluten-free diet (GFD) and habitual diet (HD). g = grams, en% = energy %

	GFD (n = 12)		HD (n = 12)		
Nutrient	Mean	SD	Mean	SD	*p* value
Energy (kcal)	1709.5	344.0	1811.5	433.9	0.243
Protein (g)	73.1	18.4	78.1	18.2	0.401
Protein (en%)	17.1		17.2		
Carbohydrates (g)	211.1	50.3	199.9	63.2	0.275
Carbohydrates (en%)	49.4		44.1		
Fat (g)	63.7	18.1	72.5	24.3	0.109
Fat (en%)	33.6		36.0		

❖ RESULT:

- **Blood sample collection:** There was observed no significant associations with the diet change. All the selected biomarkers are markers of noninflammation or metabolic changes and remained in the normal range in all our participants.
- **Fecal sample collection** : for the microbiota analysis and we observed 114 different taxonomic units. Alpha diversity observed species, (Shannon, Chao1, and Simpson indexes). Therefore, we concluded that a change in diet did not influence the bacterial diversity within a sample.
- **Sequencing** : On a taxonomic level we identified eight bacteria that change significantly in abundance on GFD: *Veillonellaceae*, *Ruminococcus bromii*, and *Roseburia faecis* decreased on GFD, and *Victivallaceae*, *Clostridiaceae*, and genus *Slackia*, and *Coriobacteriaceae* increased on GFD.
- **OTU Picking**: In total, 161 pathways and 100 modules were predicted, all of the pathways and modules were found in at least 1 % of the samples. We applied PICRUSt and HUMAnN for pathway annotation, tryptophan metabolism, butyrate metabolism (Fig. 4a), fatty acid metabolism, and seleno-compound metabolism.
- **Biomarkers :**We measured four biomarkers in feces: calprotectin, human-β-defensin-2, chromogranin A, and a set of short-chain fatty acids (acetate, propionate, butyrate, valerate, and caproate). The Wilcoxon test was used to test biomarker level differences between the average values and the GFD and HD period values.



❖CONCLUSION:

A GFD changes the gut microbiome composition and alters the activity of microbial pathways.

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Sakshi Chavan

Study Of The Human Microbiota In Health And Disease

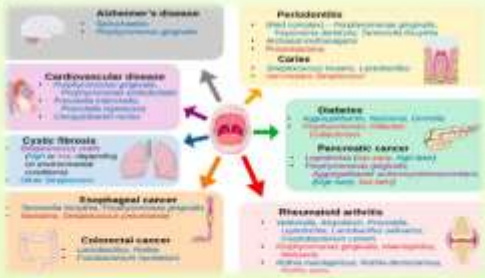
INTRODUCTION

More than 100 trillion symbiotic microorganisms live on and within human begin and play an important and crucial role in human health and disease. The human Microbiota provides a physical barrier, protecting its host against foreign pathogens through competitive exclusion and the production of antimicrobial substances.

Studies have revealed the central role of microbial symbiosis in the development of many diseases such as infection, liver diseases, gastrointestinal(GI) malignancy, metabolic disorders, respiratory diseases, mental or psychological diseases and autoimmune diseases. Immune modulation functions of the Microbiota in the germ free (GF) animals are primarily involved in promoting the maturation of immune cells and the normal development of immune functions.

➢Antibiotics disturb intestinal mucosa homeostasis, thus decreasing resistance against toxin producing clostridium Difficile and promoting the progression of Clostridium Difficile infection (CDI).

➢Alterations in the intestinal Microbiota play an important role in inducing and promoting liver damage progression as well as in direct injury in resulting from different casual agents through mechanisms such as the activation of Kuppfer cells by bacterial endotoxins.



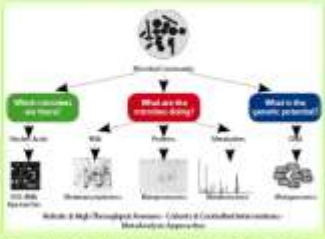
➢The new techniques for Microbiota function prediction, new Microbiota interaction models, and novel analytical and stimulation approaches the future advances to help to clarify the interactions between the Microbiota and human development and the potential roes of those Microbiota involved in the mechanism of various diseases, and metabolic diseases.

OBJECTIVE AND DISCUSSION

➢To study human Microbiota in health and disease.

➢The human Microbiota affects host physiology to a great extent.

➢Symbiotic bacteria metabolise indigestible compounds, supply essential nutrients, defend against colonization by opportunistic pathogens, and contribute to the formation of intestinal architecture.



➢The short chain fatty acids (SCFAs) are quickly absorbed in the colon and serve many diverse roles in regulating gut mobility, inflammation, glucose homeostasis and energy harvesting.

RESULTS

Microbiotic functions and function differ according to different locations, ages, sexes, races and diets of the host.

Short chain fatty acids such as acetic acid, propionic acid and butyric acid serves as energy resources to the host intestinal epithelium.

Not all Microbiota lead to health benefits, some also induce inflammation under certain conditions.

Infection is one of the most common diseases caused by dysbiosis of the Microbiota.

The human microbiome is considered as an important origin of resources for genetic diversity, a modifier of disease, an essential component of immunity, and a functional entity that influences metabolism and modulates drug interactions.

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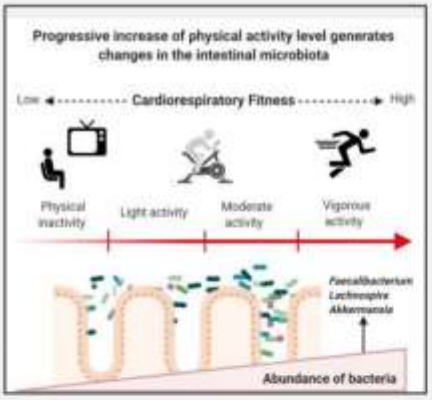
Winner

Omkar Barve

Effect of Physical activity on Intestinal Microbiota Composition

Introduction

- *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia* represent the predominant microorganisms that inhabit the human digestive system
- Microbiota composition depends on extrinsic factor like physical activity
- Active lifestyle improves metabolic and inflammatory parameters in chronic diseases
- Animal models have allowed researchers to explore the effects of physical activity on the microbiome composition of active versus non-active adult human
- Measurements of Physical activity amounts (PAA) and Transcriptional and metabolomic data extracted from feces used to classify results from active, inactive subjects



Exercise induces changes in gut microbiota through enhance CRF in previously inactive individuals

Gut microbiota composition in active individual

- Active people have unique capacity to obtain energy because of increase in gut bacteria
- Active people show Overrepresentation in the methylmalonyl-CoA pathway
- Animal model after inoculation of bacteria and endurance training show reduction in proinflammatory cytokine levels and better performance

Intestinal microbiota improves metabolite synthesis

- Exercise in inactive individual increases beneficial bacteria
- Increase in short chain fatty acids (SCFAs) which is beneficial to gut health
- Inactive people after a half marathon have increased metabolites and increased degradation of lactic acid along with presence of beneficial bacteria

Discussion

- Increased abundance of Butyrate production bacteria in active people utilizes dietary starch as well as resistant starch produced by other bacteria
- Increase of beneficial bacteria in Active individual improves immune response , metabolic activity and play role in immunomodulation, antioxidant and anti-inflammatory
- Certain group of bacteria in sportspeople exhibit a protective function in colon by inhibiting activity of carcinogenic compound
- Depletion of certain bacteria results in onset of bowel disease, Crohn's disease and sarcopenia in inactive individuals
- Some species in athletic/active individual can stimulate pathways between the gut and brain which may reduce mental fatigue, normalization of anxiety like behavior

Limitation

- Need to explore different sequencing method and multi omics analysis to understand adaptation of gut microbiota to exercise

Conclusion

- Exercise may promotes the abundance of bacteria in intestine that could provide benefits to human host
- Exercise could contribute to communication pathways between microbiota members which can influence function of other members of gut ecosystem
- Controlled clinical trials including different exercise, age groups and details about extrinsic factor can provide good amount information for further research
- Role of other microorganisms like viruses, archaea, fungi may help to comprehend the role of gut microbiome on exercise adaptation

Reference- Aya, V., Flórez, A., Perez, L., & Ramirez, J. D. (2021). Association between physical activity and changes in intestinal microbiota composition: A systematic review. PLOS ONE, 16(2), e0247039. https://doi.org/10.1371/journal.pone.0247039

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Isha Jayant Shendye

The World Of Plant Microbiome

INTRODUCTION :

- Plant microbiota/Plant microbiome : Microbial component of the plant holobiont.
- Has important functions supporting plant growth and health.
- Comprises of all the microbial genomes in the rhizosphere, phyllosphere and endosphere.

BELOW GROUND PLANT MICROBIOTA :

- Root microbiota : Derive from the soil environment.
- Contain highly diverse microorganisms.
- Rhizosphere : Hotspot of microbial activity.
- Plant roots are also colonized internally (root endosphere) by a diverse range of bacterial endophytes.

ABOVE GROUND PLANT MICROBIOTA :

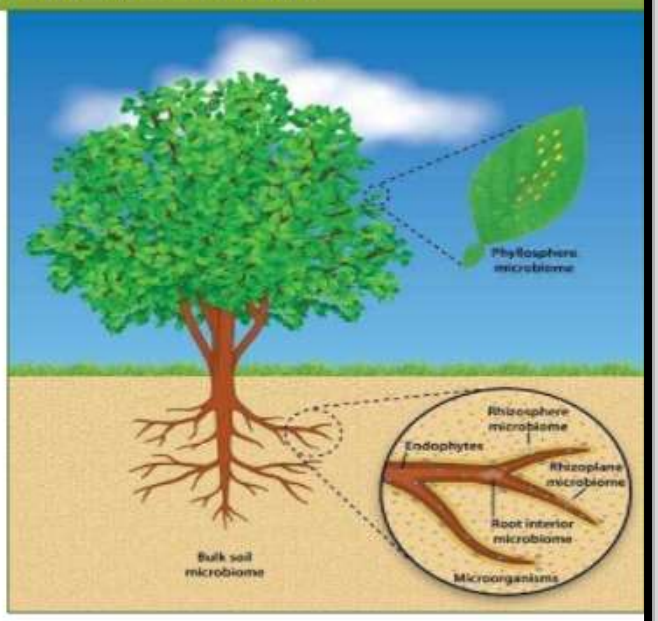
- Above ground plant tissues such as vegetative and floral parts provide unique environments for endophyte and epiphyte diversities.
- Mainly originate from soil, seed and air and adapt for life on or inside the plant tissue.

FACTORS AFFECTING PLANT MICROBIOTA :

- Affected by both biotic and abiotic factors.
- Below ground plant microbiota are mostly affected by soil pH, salinity, soil type, soil structure, soil moisture and soil organic matter.
- Influenced by external environmental factors like climate, pathogen presence and human practices.
- Host related factors like plant age and developmental stage, health and fitness also influence the plant bacterial community.

CORE AND SATELLITE MICROBIOMES :

- Core plant microbiome : Microorganisms that are tightly associated with a certain plant species or genotype, independent of soil and environmental conditions.
- Satellite taxa : Microbial taxa that occur in low abundance in a reduced number of sites.



FUNCTIONS OF PLANT MICROBIOTA :

- Plant growth promoting bacteria (PGPB) promote growth by direct or indirect mechanisms.
- Some PGPB produce phytohormones like auxin which affect plant growth.
- Some bacteria can cause disease causing symptoms through production of phytotoxic compounds. Example : *Pseudomonas syringae* is a well known plant pathogen.
- Some bacteria protect the plant from pathogens through modulating plant hormone levels and inducing plant systemic resistance.
- The continuous use of agricultural soils can build pathogen pressure and also develop disease suppressive soils containing microorganisms mediating disease suppression. Genera like *Streptomyces*, *Bacillus*, *Enterobacter*, *Panoea* have been reported for their role in pathogenic suppression.

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Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. Journal of Advanced Research, 19, 29-37. https://doi.org/10.1016/j.jare.2019.03.004

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Winner

Gowda Kavita Devaraj Shobha

GUT MICROBIOTA AND BILE ACID METABOLISM

Induction of Farnesoid X receptor signaling in germ-free mice colonized with a human microbiota

ABSTRACT: 1) The gut microbiota influences the development and progression of metabolic disease partly by metabolism of bile salts {BA'S} and the modified signaling through the farnesoid X receptor (FXR).2) The aim is to determine how the human gut microbiota metabolizes murine BA'S and affects FXR signaling in colonized mice . 3) It shows that human microbiota can change BA composition and induce FXR signaling in colonizes mice but the level of secondary BA'S produced are lower than in mice colonizes with mouse microbiota.

INTRODUCTION: 1)The gut microbiota influences the development and progression of metabolic disease such as obesity ,diabetes ,and atherosclerosis. 2)The influence of the gut microbiota on metabolic disease may in part be mediated by modifications of the bile acids. 3)FXR Farnesoid is a powerful regulator of lipids and glucose metabolism and is activates by the primary BA's chenodeoxycholic acid (CDCA) and the cholic acid (CA).4)The gut microbiota deconjugate and subsequently metabolizes primary BA's to secondary BA's in the gut and thereby modify to extent of FXR activation. 5)The founding's aimed to determine whether a human gut microbiota can deconjugate and metabolize primary murine BS's and thus increase FXR activation in the recipient mice.

MATERIALS AND METHODS : 1) GF female Swiss Webster mice were maintained in flexible plastic gnotobiotic Isolators under a strict 12 h light cycle and fed an Autoclaved chow diet ad libitum. 2)Colonization was performed by diluting human fecal or mouse cecal samples (0.5 g) in 5 ml reduced PBS; 0.2 ml of this suspension was introduced by gavage into each GF mouse 3). The human fecal sample from the first donor was obtained from a healthy 38-year-old female volunteer, and the sample from the second donor was obtained from a healthy 40-year-old male volunteer.4) The samples were obtained shortly before colonization and immedi-ately (within 5 min) diluted and introduced into the GF mice by gavage within 2 h after dilution.

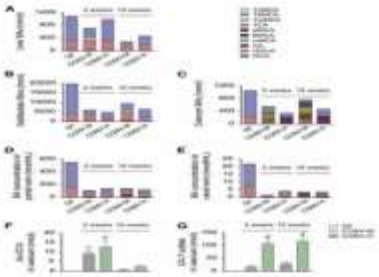
SATISTICAL ANALYSIS : Data are presented as mean ± SEM. Resource equation method was used to determine the adequate sample size

RESULTS :1) Cecal microbiota differs between mice colonized with mouse or human microbiota.2) It was analyzed cecal microbiota after short-term (2 weeks) or long-term (15 weeks) colonization of GF mice with cecal content from a mouse donor (recipient mice termed CONV-M) or feces from a human donor (recipient mice Termed CONV-H) varies in BA'a concentration .

3) In summary, the microbiota composition differs between mice colonized with mouse and human microbiota, and the major differences between the groups are preserved over long-term colonization.

BA composition differs between mice colonized with mouse or human microbiota

. Representation in graphical way as follows. Fig.(1)
Changes in BAs composition in different compartments after colonization. Whole organ amounts of BAs in liver (A), gallbladder (B), and cecum (C). Concentrations of BAs in portal vein (D) and caval vein (E). For statistics on specific BA levels,see supplemental Tables 1–5. Whole organ amounts of iso-DCA (F) and CA-7 sulfate (G) in cecum. Mean values ± SEM are plotted; n = 4–9 samples/group;
a $P < 0.05$, b $P < 0.01$, c $P < 0.001$, d $P < 0.0001$.



the relative amount of unconjugated BAs was higher in the CONV-M mice after 15 weeks' colonization compared with the humanized mice.

DISCUSSION : 1) colonization of mice with a human microbiota reduces total BA levels to a similar extent as colonization with a mouse microbiota, but BA composition differs and fewer secondary BAs are present in the humanized mice. 2) Results indicate that some species-specific divergences in microbiota composition cannot be transferred from one mammalian host to another, likely due to the environment in the recipient gut, and hence the microbiota of the donor sample is more preserved when transferred to a recipient of the same species. 3) In conclusion, we show that a human microbiota can establish a functional community in the mouse gut and that reduces total BA levels to the same extent as a mouse microbiota.

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Aashvin Kumar Das

Introduction

This is a comprehensive analysis of idli fermentation employing modern molecular tools which provided valuable information about the bacterial diversity enabling its fermentation.

The study has demonstrated the relationship between the bacterial population and its functional role in the process.

The nature of idli fermentation was found to be more complex than other food fermentations due to the succession of the bacterial population.

Further studies using metatranscriptomics and metabolomics may enhance the understanding of this complex fermentation process.

Moreover, the presence of microorganisms with beneficial properties plausibly makes idli a suitable functional food.

Conclusion:-

Weissella, key player in the fermentation of idli batter

Genus Weissella emerged as the most important organism. The [1-14C]glucose assay showed maximum carbon dioxide generation from the Weissella isolates. the addition of only Weissella isolates to the batter obtained from surface-sterilized grains led to significant fermentation

The Microbiolgy of Idlies

Diversity and Succession of Microbiota during Fermentation of the Traditional Indian Food Idli

Madhvi H. Mandhania, Dhiraj Paul, Mangesh V. Suryavanshi, Lokesh Sharma, Somak Chowdhury, Sonal S. Diwanay, Sham S. Diwanay, Yogesh S. Shouche, and Milind S. Patole

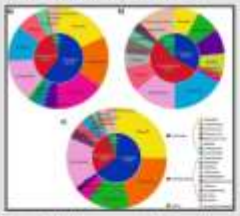
Microbial composition of idli batter by culture-independent methods

The similarity of the microbiota in 10 idli samples obtained from different vendors. Each sample had 13 to 17 major PCR bands. The DGGE profiles of 10 samples of idli obtained from different vendors were similar. Members of each group demonstrated approximately 90% similarity with each other.

The high-intensity PCR bands from both DGGE gels were selected for identification by DNA sequencing. Of the 117 bands analyzed , DNA sequences of 76 bands revealed lactic acid bacteria (LAB). The remaining bands belonged to order Bacillales and Enterobacteriales. At the genus level, most of the bands were identified as Weissella upon sequencing. Amplicon sequencing of the V3 region of 16S rRNA gene using the MiSeq platform was performed.

A total of 4.4 million reads were obtained. A total of 19 bacterial phyla were present at various times of the fermentation process. The first five abundant phyla constitute up to 99.4% of the entire bacterial diversity. Firmicutes were the most dominant, increased from an average 35% in the first 6 hours to 87% in the remaining duration followed by Proteobacteria (22%) and Actinobacteria (4%) which were abundant in the first 6 hours and then decreased thereafter.

Absolute quantification of important bacterial genera (as indicated) using quantitative real-time PCR. The radar plots elucidate the abundance distribution of predominant organisms (expressed as log10) essential during the fermentation of idli batter.



Donut plots representing the bacterial population in fully fermented idli batter samples from (a) Bangalore, (b) Pune, and (c) laboratory fermented batter by the culture-dependent method. The inner and outer circles show the percentage distribution at the phylum and genus levels, respectively

Microbial succession during idli fermentation.

The succession pattern was first studied by a DGGE profile obtained for 9 time points of the fermentation spanning from 0 to 24 hours. The 16S rRNA amplicon sequencing also highlighted the presence of succession in the bacterial diversity. Phylum Firmicutes increased, whereas phyla Proteobacteria and Actinobacteria decreased after the first 6 hours of the process, suggestive of microbial succession.

Family Leuconostaceae showed a gradient which gradually starts increasing from 0th to 12th hour and then decreases thereafter monas, Bacillus, Halomonas, Lactobacillus, Propionibacterium, Pseudomonas, and Shewanella were found to be abundant during the first 6 hours which then declined and were taken over by Weissella, as its abundance increased drastically from 9% to 71% from the 6th to 9th hour of fermentation.

The abundance of Weissella gradually increased from time 0 to the 6th hour, exponentially increased in the 9th and 12th hour, and finally declined thereafter, highlighting the essential role of this heterofermentative organism in acid production and leavening of the idli batter during the major duration of the fermentation process. Genera Enterococcus and Streptococcus succeeded Weissella after the 12 hours of fermentation

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Shivani Mrityunjay Singh

Plastic habitats: Algal biofilms on photic and aphotic plastics

Imogen L. Smith , Thomas Stanton , Antonia Law

1. INTRODUCTION

Plastics are a heterogeneous group of polymers predominantly manufactured from fossil fuels that can be chemically tailored to provide a variety of useful functions. Durable, lightweight, and cheap, plastic materials are integral to much of modern-day life and have been increasingly so since the onset of their mass production in the midtwentieth century. However, at the end of their life plastic products that are not disposed of responsibly may enter aquatic environments. The negative impacts of this plastic debris in the environment are well documented, including ingestion and entanglement , and vary with product type, form and polymer. The majority of plastic pollution research is concerned with the marine environment , but freshwater catchments represent major pathways for the transport of pollution and are a primary source of marine plastic debris. Some biota utilise the plastic waste they encounter in the natural environment, where it represents a novel environmental substrate. Interactions between biota and plastic debris are not limited to animals. Biofilms – assemblages of bacteria, algae, and fungi – also colonise plastic material that enters aquatic environments. Biofilm formation on plastic material can degrade plastic surfaces and can overcome the buoyancy of plastic polymers, causing plastic to sink to aphotic environments .

3.RESULT & DISCUSSION

- **Photic and aphotic photosynthetic pigment concentrations**

Generally, PPCs showed a progressive increase from week 1 to week 6 in the photic zone. In the aphotic zone this not observed, with concentrations peaking in week 2 (Fig. 2). A large rainfall event is recorded prior to the collection of the week 4 samples, followed by an increase in chloride, sulphate and nitrate in week 5, which does not influence PPCs in either zone (Fig. 2). Overall, total PPCs were higher in the photic zone

Fig3 shows the Principle Components Analysis of the pigment compositions throughout this study. Plastic samples located close to each other in the PCA diagram reflect similar pigment assemblages.

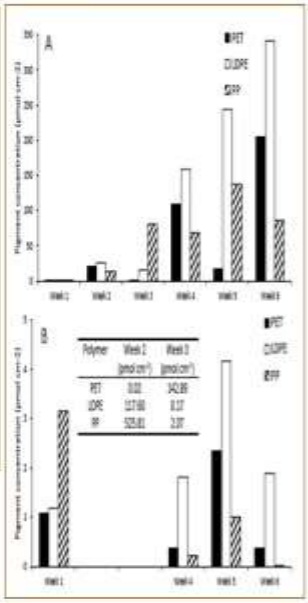
- **Diatom communities**

There was no significant difference in the diatom assemblages that developed on the different plastic types over the course of the study (ANOSIM R value = -0.144; p = 0.9838). However, diatom assemblages demonstrated statistically significant differences between the aphotic and the photic zones (ANOSIM R value = 0.4657; p = 0.0015), and also throughout the six weeks of the study in both the photic (ANOSIM R value = 0.7366; p = 0.0066) and the aphotic zone (ANOSIM R value = 0.9506; p = 0.0034) (Fig. 4)

- **SEM imaging**

Changes to surface structure were apparent across all polymer types in both the photic and aphotic samples by the end of the six-week study (Fig. 5).

Fig. 5 also shows the clear presence of fissures in the polymer surface of PET and PP samples. The extent of changes to the surface structure on the aphotic samples was less apparent that on the photic samples.



4.CONCLUSION

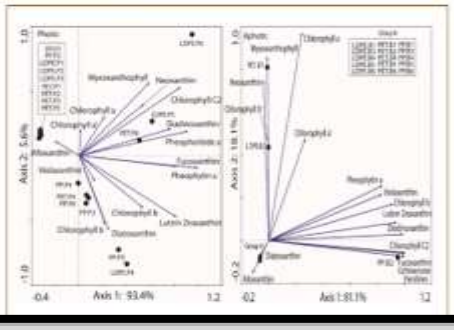
Plastic waste is a diverse and pervasive anthropogenic pollutant in aquatic environments that provides a surface for the development of algal biofilms. Here we present some of the first work to systematically characterise the plastic-biofilm relationship in freshwater environments. Plastic pollution represents an abundant and diverse substrate in aquatic environments that will be colonised by biofilms.

2.METHODS:

- Study Site
- Sample Designing
- Photosynthetic Pigment Preparation And Analysis
- Di Atom Preparation And Analysis
- Scanning Electron Microscopy (SEM) Of Samples
- Statistical Analysis
  - PPCs
  - Di Atom Assemblages

5.REFERENCE

(Andrady,2011)
(Artham et al., 2009)
(Pinto et al., 2017)
(Kettner et al., 2017)



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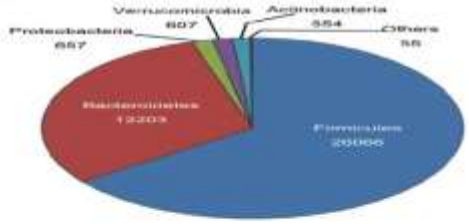
Shrunoti Vishvas Joshi

Human Microbiome : A community on Human host - Life sustainability , Health(*Prakriti*)and disease(*Vikriti*)

Introduction - **Is Human body single or a community on Host? Yes a community with a ratio10(M);1(H).**Main factors that decide Human and intestinal Microbiome are **(a)** Host genetics and metabolism (Heritage) **(b)** Lifestyle (**environment**) **(c)** Diet and Nutritional habits . Microbiome influences human health and disease condition. So environment and its exposure is extremely important as well as Symbiotic environment to help digestion , immunity, protection for Life. **Human Microbiome – General distribution.**



Soil and Human gut contain approximately same number of active microorganisms , However due to modern lifestyle both are getting deteriorated . We tracked a close relationship in between soil and human microbiome and observed a **close link evolved during evolution and still evolving.** In fact **they are a basis for Health (Prakriti) and disease (Vikriti).**

Objective – To Understand the Microbiome diversity in Soil and Human gut- its effect on the health and disease

Materials and Methods- Microbiome measurement was done as under-

Sr No	Habitat	Number of cells per mL
1	Soil	1010
2	Sewage	10^9
3	Marine water	10^6
4	Large intestine	1011
5	Lower small intestine	10^8
6	Upper small intestine	10^4
7	Human mouth Saliva	10^8

Pre-Industrial and post Industrial revolution Microbiome in Human and Soil.

Process –1- Number of microbial cells in Soil , Intestine sewage and mouth were measured . Pre-Industrial show microbial richness , however post industrialization the concentration has reduced , showing less immunity and susceptibility to diseases. **2-** Deliveries through Cesareans and non- Cesareans show difference in concentration and effect on disease conditions.

Summary –Soil contributes to human gut microbiome There are functional similarities in Soil rhizosphere and human intestine microbiome , which has adverse effect on human health .

References – 1- *Does Soil Contribute to the Human Gut Microbiome, Katharina M. Keiblinger Microorganisms , 2019 , 7 , 287.* **Acknowledgement**- and B N Bandodkar College of Science ,Thane .

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