Philippine **N**etwork of **M**icrobial
Culture **C**ollections, Inc.

in partnership with

Trinity University of Asia

21st Annual Symposium and General Assembly

Culture collections in the new normal and beyond

October 2, 2021 8:30 AM
October 9, 2021 2:00 PM
October 16, 2021 8:30 AM
October 23, 2021 2:00 PM

pilak

PNMCC @ 25

Image used in the cover taken from: https://poweredtemplate.com/07446/0/index.html
The culture shown is *Phyllosticta capitalensis* by C.C.S. Apurillo.

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Philippine Network of Microbial Culture Collections, Inc. (PNMCC)

Headquarters
Philippine National Collection of Microorganisms, BIOTECH
University of the Philippines Los Baños College, Laguna

My warmest greetings to all the participants of the 21st Annual Symposium and General Assembly of the Philippine Network of Microbial Culture Collections!

Since last year, we have experienced the repercussions of the COVID-19 pandemic, forcing us to reinvent the way we do things. This has not only affected aspects of our personal lives, but has also challenged the way we conduct research. However, the central role of culture collections in research and development never changed. This year's annual symposium is anchored on the theme: "Culture collections in the new normal and beyond". In this symposium, we discuss the challenges faced by culture collections during this pandemic to come up with strategies that will ensure that the functions of culture collections are not compromised by the limitations brought about by this pandemic. As we look into the future, we continue to maintain the thrust of culture collections to serve as repositories of strains of microorganisms for instruction, research, taxonomy and technological applications.

This year, we also celebrate the 25th year of PNMCC. The organization was established in 1996 to serve as the inter-agency arm of the Philippine National Collection of microorganisms through the sponsorship of DOST-PCASTRD (currently known as DOST-PCIEERD). Aside from serving as the permanent secretariat of Philippine microbial culture collections, the network was also envisioned to serve as the central contact point for Philippine scientists and institutions for information on culture collection-related matters. Through the years, the PNMCC has conducted various symposia, trainings and workshops to equip its members with knowledge and skills in culture collection. This year, we are preparing for the release of the updated Directory of Microbial Strains of our affiliate culture collections.

As we hold this symposium and celebrate PNMCC's 25th year anniversary, I would like to express my sincerest gratitude to the members of the board of directors of PNMCC for year 2021 for their hard work despite their busy schedules. Let me also express my thanks to our resource persons for sharing their time, knowledge and expertise and to the College of Arts Sciences and Education of the Trinity University of Asia for hosting this symposium. The same gratitude goes to our sponsors, members and participants for joining us in this annual event.

Mabuhay ang PNMCC!

CARLO CHRIS S. APURILLO, RMT, M.Sc.
President

Trinity University of Asia
Cathedral Heights, 275 E. Rodriguez Sr. Avenue
New Manila, Quezon City

OFFICE OF THE PRESIDENT

Congratulations to the Philippine Network of Microbial Culture Collections (PNMCC) as it celebrates its 25th Anniversary.

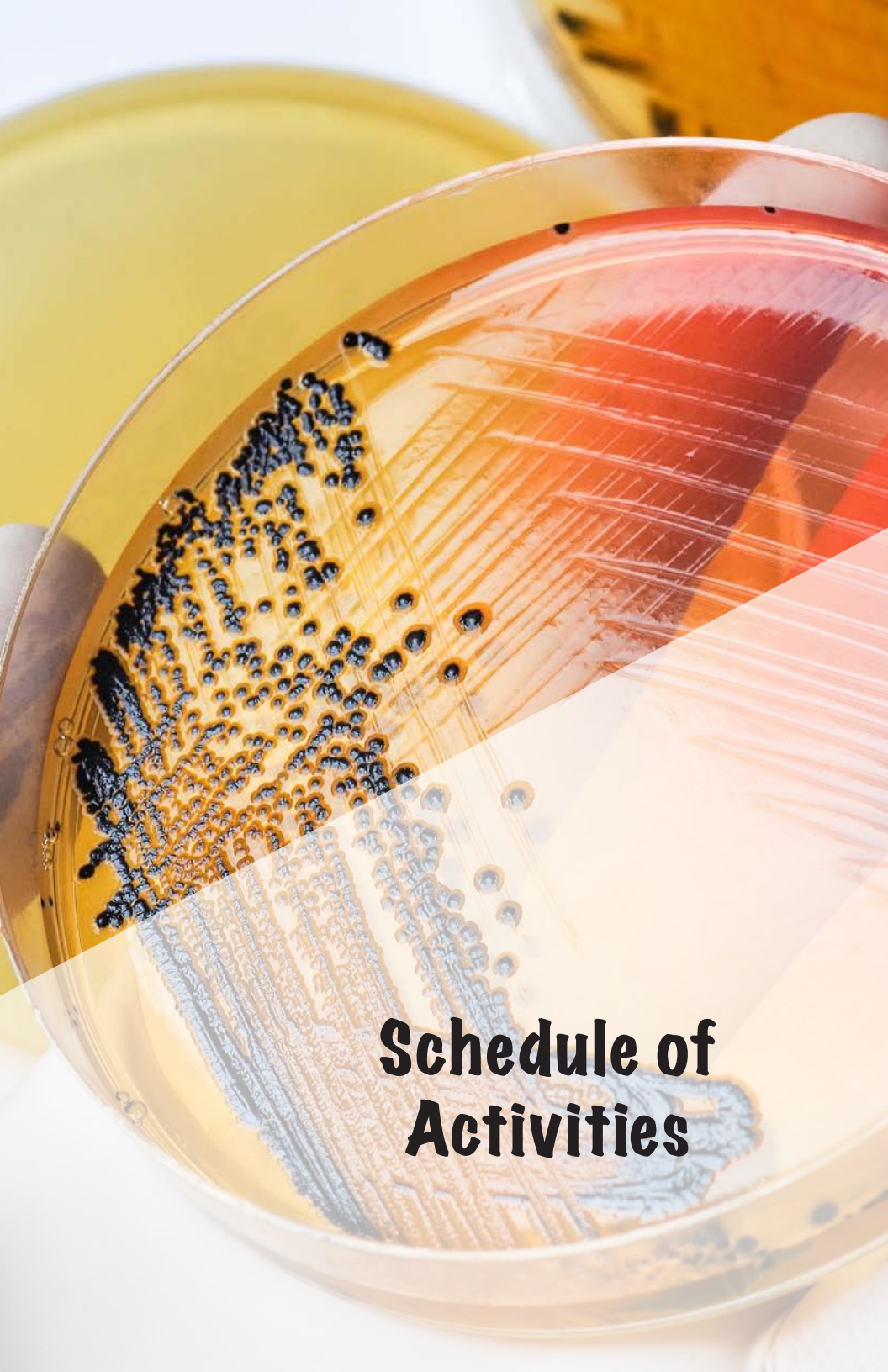
The Trinity University of Asia community is honored to be a constant partner to the PNMCC and serving as host institution to this year's 21st Annual Symposium and General Assembly with the theme "Culture Collections in the New Normal and Beyond".

Microorganisms are important to society and have their impact on the life of everyone. Our knowledge of microorganisms is enhanced by its study and is made possible by having microbial cultures. The validity of any microbiology-related project is primarily dependent on reliable sources of microbial collections. Thus, the presence of the PNMCC in safeguarding and guiding microbial culture collections in the Philippines is invaluable.

We are looking forward to the sharing of knowledge and expertise by our esteemed speakers in this year's symposium. We are excited for the many research papers submitted by microbiologists and microbiology students around the country. As well as anticipating the many collaborations to result from interactions among the participants to this year's celebration.

God Bless and Stay Safe Always.

WILFRED U. TIU, Ph.D.
President



Schedule of Activities

PROGRAM OF ACTIVITIES

Day 1: October 2, 2021
Overview of Type Collections

8:00 AM — Zoom Login

8:30 AM — Opening Ceremony
- Invocation
  - **Fr. Echanes Cadiogan**
  - *University Chaplain*
  - *Trinity University of Asia*
- National Anthem
- Welcome Message
  - **Dr. Howell T. Ho**
  - *Dean, College of Arts, Sciences and Education*
  - *Trinity University of Asia*
- Opening Remarks
  - **Carlo Chris S. Apurillo, RMT, M.Sc.**
  - *President, PNMCC*

8:50 AM — **Keynote Speech: The PNCM: 25 Years of Microbial Banking and Providing the Country's Needs for Microbial Resources**
Dr. Rosario G. Monsalud
Philippine National Collection of Microorganisms
University of the Philippines Los Baños

9:40 AM — **Lecture: Conserving Fungal Diversity: Integration of a herbarium and living collection**
Dr. Connie Fe C. Gibas
UT Health San Antonio, Texas

10:10 AM — Open Forum

10:30 AM — **Lecture: Microbial Research and Culture Collections, a leap towards strengthening Scientific Research in PUP in the New Normal**
Dr. Lourdes C. Alvarez
Polytechnic University of the Philippines

11:00 AM — Open Forum

11:15 AM Product Demo: ESCO

11:30 AM Synthesis and Closing Remarks
Dianne L. Dizon, RMT, M.Sc.
Corresponding Secretary, PNMCC

Moderator:
Dr. Marian P. De Leon
Treasurer, PNMCC

Master of Ceremony:
Prof. Caleb Donne Coniate
Trinity University of Asia

1:00 PM Viewing of Posters and Exhibits

Day 2: October 9, 2021
Network and Linkages

10:00 AM Viewing of Posters and Exhibits

1:30 PM Zoom Login

2:00 PM Opening Ceremony
Invocation
Fr. Echanes Cadiogan
University Chaplain
Trinity University of Asia
National Anthem
Welcome Message
Dr. Wilfred U. Tiu
President
Trinity University of Asia
Opening Remarks
Carlo Chris S. Apurillo, RMT, M.Sc.
President, PNMCC

2:20 PM **Lecture: Transition of culture collections into Biological Resource Centres: The catalyst role of the WFCC**
Dr. Ipek Kurtboke
President, World Federation of Culture Collections (WFCC)

3:05 PM Open Forum

3:25 PM **Lecture: PNMCC, 25 years of Culture Collections**
The adaptation of culture collections to the scientific and socio-economic evolution
Dr. Philippe Desmeth
Ex-Officio member, World Federation of Culture Collections

3:55 PM Open Forum

4:15 PM **Lecture: How Can WDCM Help PNMCC**
Dr. Juncai Ma
Director, World Data Center of Microorganisms

4:45 PM Open Forum

4:55 PM Synthesis and Closing Remarks
Eldrin DLR. Arguelles, M.Sc., SMicro, DPAM
Auditor, PNMCC

Moderator:
Dr. Gina R. Dedeles
Past President, PNMCC

Master of Ceremony:
Prof. Dino Cantal, Jr.
Trinity University of Asia

Day 3: October 23, 2021
Viruses

8:00 AM Zoom Login

8:30 AM Opening Ceremony
Invocation
Fr. Echanes Cadiogan
University Chaplain
Trinity University of Asia
National Anthem
Welcome Message
Dr. Mark Francisco
Dean, College of Medical Technology
Trinity University of Asia

Opening Remarks
Carlo Chris S. Apurillo, RMT, M.Sc.
President, PNMCC

8:50 AM **Lecture: Current Advances in African Swine Fever Research**
Dr. Homer Pantua
Department of Infectious Diseases, Genentech

9:20 AM Open Forum

9:40 AM **Lecture: Bacteriophage Isolation and Preservation Techniques**
Prof. Jayme R. Encabo, M.Sc.
University of the Philippines Los Baños

10:10 AM Open Forum

10:25 AM Business Meeting
Oath Taking of New Members
Election of Officers

11:10 AM Poster Pitches and Q & A

11:40 AM Product Demo

11:50 AM Synthesis and Closing Remarks
Dr. Esperanza C. Cabrera
Member, Board of Directors, PNMCC

Moderator:
Dr. Marilen P. Balolong, DrPH, RMicro, DPAM
Member, Board of Directors, PNMCC

Master of Ceremony:
Dr. Ma. Cecilia Ycong
Trinity University of Asia

1:00 PM Viewing of Posters and Exhibits

Day 4: October 23, 2021
Actinomycetes and Microbial References

10:00 AM	Viewing of Posters and Exhibits
1:30 PM	Zoom Login
2:00 PM	Opening Ceremony Invocation **Fr. Echanes Cadiogan** *University Chaplain* *Trinity University of Asia* National Anthem Welcome Message **Dr. John Michael Lorena** *Dean, St. Luke's College of Nursing* Opening Remarks **Carlo Chris S. Apurillo, RMT, M.Sc.** *President, PNMCC*
2:20 PM	**Lecture: Actinomycetes** **Dr. Doralyn S. Dalisay** *University of San Agustin*
2:50 PM	Open Forum
3: 10 PM	**Lecture: Maximizing the Use of Microbial Reference Cultures in a Testing Laboratory** **Marlon S. Aguinaldo, RMT** *Industrial Technology Development Institute*
3:25 PM	Open Forum
3:40 PM	Product Demo
3:50 PM	Closing Program Awarding of Winners Oath-Taking of Elected Officers Synthesis and Closing Remarks **Dr. Ursela G. Bigol** *Vice-President, PNMCC*

Moderator:
Joel C. Cornista, M.Sc., SMicro
Business Manager, PNMCC

Master of Ceremony:
Prof. Rommel Agbayani
Trinity University of Asia

Abstracts of Symposium Lectures

Day 1:
October 2, 2021

The PNCM: 25 Years of Microbial Banking and Providing the Country's Needs for Microbial Resources

Rosario G. Monsalud*, Armi R. Creencia, Noel H. Tan-Gana, Eldrin DLR. Arguelles, Nik Shawn S. Tabao and Rose May Ann D. Capanzana
National Institute of Molecular Biology & Biotechnology (BIOTECH)
University of the Philippines Los Banos (UPLB), College 4031, Laguna

The Philippine National Collection of Microorganisms (PNCM) is the National Repository of Microbial Resources of the Philippines. It started as a Microbial Culture Collection of BIOTECH, UPLB. It was given the national repository status by the Department of Science and Technology in 1996. The PNCM accepts culture deposits and distributes them according to prescribed protocols. It preserves microbial cultures for long-term use by at least two methods for backup. These are presented along with quality controls and other management procedures being practiced. The current research activities, challenges and future directions are likewise highlighted.

The PNCM has also evolved into a microbiological testing laboratory and got the ISO 17025 accreditation in 2014. Currently, it has accreditation for 22 test methods including identification of microbial cultures. Moreover, it provides training on culture preservation techniques, identification of bacteria and fungi, and various microbiological test methods.

Dr. Rosario G. Monsalud is the Head of the Philippine National Collection of Microorganisms (PNCM). She is a Scientist I and Affiliate Professor at the Microbiology Division and Agricultural Systems Institute of the University of the Philippines Los Baños. She is a recipient of the National Research Council of the Philippines 2013 Achievement Award for Biological Sciences and the UP Alumni Association 2015 Distinguished Alumni Award in Science and Technology. She has served as the President of PNMCC for the years 2005, 2006, 2009 and 2012.

Day 1:
October 2, 2021

Conserving fungal diversity: Integration of a herbarium and living collection

Connie Fe Cañete-Gibas, Ph.D.
UT Health San Antonio, Texas

The kingdom fungi is a vast and species rich kingdom and the most recent estimate of the number of species is about 10-12 million. However, only about 2% are known. Fungi play important roles in our daily lives. Many benefits in food, medicine and agriculture have been recognized to be contributed by fungi as well as the ability to recycle nutrients in all habitats. Many diversity studies and other areas of research i.e., clinical, agricultural, food, environmental, and industrial, often result in the huge numbers of isolates obtained that can be used for screening for a variety of other uses. To prevent the loss of these isolates, microfungal biorepositories are a good system in place. Biorepositories aim to preserve these isolates to ensure genetic stability and long-term viability. They also serve as a core and shared resource available to multiple internal and external researchers of an institution and support teaching and research by providing living cultures, mycology expertise and hands-on laboratory training. Biorepository management involves careful curation of holdings to ensure authenticity and purity of cultures. Curation involves extensive collection of strain provenance, validation of strain species and preservation.

Dr. Connie Fe C. Gibas is a Clinical Research Project Manager and Mycologist at the Fungus Testing Laboratory, Depts. of Pathology & Laboratory Medicine, Long School of Medicine of the UT Health San Antonio Texas. Her field of specialization is on fungal systematics. She has discovered 18 new fungus species and established a new order and family of fungi. She has received numerous awards such as the Excellence in Teaching Award in 2002 and commendations for Excellence in Teaching from 2001 to 2004 from the Dept. of Biological Sciences, University of Alberta.

Day 1:
October 2, 2021

Microbial Research and Culture Collections, a leap towards strengthening Scientific Research in PUP in the New Normal

Lourdes V. Alvarez, PhD, RM
Polytechnic University of the Philippines

Culture collections serve an important role for both teaching and experimentation. In the Polytechnic University of the Philippines (PUP), culture collections serve as basis for the morphological identification, molecular, and phylogenetic analysis of microbial collections used by both students and faculty in their research. It is also intended to be used in future studies that involve solving taxonomic problems and utilization of isolates for medical and biotechnology research, among others. Microbial research is gaining interest in PUP as many of its BS Biology students and faculty have developed keen interest and fascination with research in microbiology and mycology. These interests have inspired us to conduct more microbial research in PUP and build a culture collection of our own.

In this talk, few of the research undertakings conducted and currently being done in PUP including the online investigations using several bioinformatic tools, will be presented. Worthwhile outcomes of the completed studies will be shared to uncover the advantageous benefits that can be gained by the community and society as a whole from these research. Hence, it is essential to take note that research in PUP is on-going even in the midst of this COVID-19 pandemic.

Dr. Lourdes V. Alvarez is a full professor and faculty researcher at the Polytechnic University of the Philippines. She completed he Ph.D. in Biological Sciences (*cum laude*) from the University of Santo Tomas. She has served as project leader of different studies focusing on fungal pathogens. She has received various awards such as the 2019 DOST International Publication Award and the Distinguished Paper Award during the 2018 International Forum-Agriculture, Biology and Life Science in 2018.

Day 2:
October 9, 2021

Transition of culture collections into Biological Resource Centres: The catalyst role of the WFCC

Ipek Kurtböke
WFCC President

Culture collections have evolved from their role as mostly providers of microbiological material for the society at large. While culture collections were essentially seen and run as centres of conservation and distribution of microbiological material in the past, BRCs are now conceived as the source of all essential material for research and development in life sciences. To fulfil their role in basic infrastructure for knowledge-based bio-economy, BRCs must implement quality management systems, overcome legal and administrative obstacles, follow diverse international, supra-national and national laws and regulations and take extra appropriate precautions due to security concerns

Individual BRCs face constraints in terms of personnel and appropriate strategies to meet the challenges, therefore they might opt to join integrated networks at national, regional, or international level. The World Federation for Culture Collections (WFCC) thus accordingly can play a major international role in all matters related to culture collections such as the operation and management of culture collections as well as addressing issues in a wider context. WFCC hence serves as a bridge in this global transition to the BRCs to ensure that collections are in line with the pace of change and do not disappear altogether with their valuable documented microbiological assets

WFCC also emphasizes the importance of (i) standardization and best practice guidelines, (ii) networking, capacity building and education, (iii) postal, quarantine and safety regulations (iv) IP, patent, and commercialization, (v) access, policies, and legal frameworks and (vi) sustainability of endangered collections. Moreover, WFCC in the era of molecular advancements includes genome level characterization of the microorganisms as well as defining criteria to ensure type strain integrity as well as its preservation in a genetically stable form via the WDCM. WFCC also interacts with different global organizations to promote the importance of culture collections with emphasis placed on the contributions and impact culture collections make on science, health, education, and society. This presentation will communicate WFCC's catalyst role in above listed matters.

Dr. İpek Kurtböke is currently the President of the World Federation for Culture Collections (WFCC) and recently re-elected to serve a second term (2021-2024). She has been associated with the WFCC for over 25 years. She is also a Senior Lecturer in Environmnental Microbiology at the University of the Sunshine Coast in Australia. Her most significant contribution to the field has been the development of a novel isolation technique that selectively cultures rare actinomycetes with industrial importance at the University of Liverpool, UK during her PhD studies.

Day 2:
October 9, 2021

PNMCC, 25 years of culture collections

The adaptation of culture collections to the scientific and socio-economic evolution

Dr. Philippe Desmeth
Past-President WFCC
Belgian Coordinated Collections of Micro-organisms
Belgian Science Policy Office, Simon Bolivar Boulevard, 30 1000 Brussels, Belgium
Email: philippe.desmeth@belspo.be

Since 1996, date of the establishment of the Philippine Network of Microbial Culture Collections, scientific knowledge and techniques, the legal framework, the bioinformatics capacity, and the Internet have greatly evolved.

To fulfil their role of basic infrastructure for biosciences in Knowledge Base Bioeconomy, Culture Collections (CC) have evolved from mere centres of conservation and distribution of microbiological material to “Biological Resources Centres (BRC)” conceived as sources of all essentials for Research and Development in life sciences and nowadays as "Biobanks", to support socio-economic activities in bioeconomy.

Those who couldn't negotiate the bend have disappeared, and with them valuable documented microbiological assets. The others still face difficult times: Culture Collections are confronted primarily by scientific and technical challenges, but also, they cannot get away from new legal parameters, and socio-economic constraints. They must constantly adapt, and increasingly integrate new paradigms to propose adequate solutions to new demands.

Some of these developments, especially scientific and technical, have enabled culture collections to reach scientific excellence. A normative framework was created at the initiative of international organizations, starting with the OECD and the ISO, allowing collections to optimize their way of working, to meet the needs of their scientific partners by offering services. and biological material of consistent quality.

Standard ISO 20387:2018 (Biotechnology-Biobanking-General requirements for biobanking) incorporates financial sustainability of biobanks because entrepreneurs must integrate economic parameters in the conceiving of the research program to secure long-term sustainability of their scientific endeavour. At present, the exercise of conceiving a research program in microbiology is coupled more and more with economic study, cost-benefit evaluation, market assessment and IPR portfolio management. This is not solely true for commercial undertaking but also for non-profit basic research. Managers must have long-term vision and sometimes imagination to foresee the funding of their laboratory.
New laws and international agreements such as the Nagoya Protocol to the Convention on Biological Diversity now govern the functioning of collections that must invest to comply with these new regulations. Initially these laws represented an opportunity to improve the functioning of the collections by forcing them to review the contractual relations with their partners, their suppliers, and their customers.

It was also an occasion to frame their research within a legislative framework and to protect their intellectual property rights and to share them equitably with their partners. But these legal requirements are a costly burden on cultural collections.

The challenge of managing the data is even greater, as the term "big data" implies. The technical challenge of making these huge volumes of data FAIR (Findable, Accessible, Interoperable, Reusable) requires collaboration across collections. The WFCC and the bioinformatics centre World Data Centre for Microorganisms (WDCM) have progressed dramatically. The number of collections currently listed in the CCINFO directory exceeds 800, of which over 100 are WFCC members. The WDCM has created several databases and research tools specially designed for collections and for users including the "GCM" (Global Catalogue of Microorganisms) and the "GCM 2.0", the sequencing programme to fully sequence 10,000 type and reference strains within five years.

All these challenges and resulting mutations cannot be met and realised by isolated culture collections.
To overcome the challenges and effectively fill their basic role in Research and Innovation, culture collections must organise themselves into collaborative networks to master together the flow of information and resources, to improve communication, to cooperate in designing their contractual rules within changing legal environment and have common capacity building programmes to reach excellence set by the latest quality norms.

These are the reasons why the Philippine Network of Microbial Culture Collections was established 25 years ago, and why it remains essential as an infrastructure network for research and innovation in the Philippines and the entire Southeast Asian region.

[1]Biological Resources Centers (BRC) is a concept initiated by the UNESCO Microbial Resources Centers Network (MIRCEN) in the late ‘40ties and actualized in 1999 by the Organization for Economic Co-operation and Development Working Group on BRC. While the emphasis was previously put on the biological resources conserved in specialized facilities, at present a BRC is conceived as a functional unit having all the necessary components to study, preserve and use biological diversity. This functional unit integrates appropriate infrastructure, human, financial and technical resources, skills related to information production, processing and diffusion as well as legal, administrative, management and quality control systems. See OECD (2001) Biological Resource Centres Underpinning the future of Life Sciences and Biotechnology. OECD Science & Information Technology, May 2001, vol. 2001, no.7, pp.1-68 (69 pages).

Dr. Philippe Desmeth is an ex-officio member of the World Federation of Culture Collections (WFCC) Executive Board, having served as its president from September 2010 to July 2017. He is a is bio-engineer and environmental advisor by training. He gained field experience in agro-industrial production and continuing education for farmers in West Africa and Southeast Asia. In 1996, he joined the Belgian Coordinated Collections of Micro-organisms (BCCM) as international cooperation manager. Next to having coordinated EU funded collaborative research projects, at the Belgian Science Policy Office that funds and runs BCCM, he develops standard procedures and equitable cooperation schemes to frame equitable long-lasting collaboration with institutions in developing and developed countries.

Day 2:
October 9, 2021

How Can WDCM Help PNMCC

Dr. Juncai Ma
Director of World Data Center for Microorganisms
Institute of Microbiology, Chinese Academy of Sciences, China

Microbial resources are one of the most important natural resources in the world, which is the scientific basis to support the development of biotechnology and life sciences. WDCM launches the international project Global Catalogue of Microorganisms (GCM) to construct a data management system and a global catalogue to help organize, unveil and explore the data resources of culture collection worldwide. GCM provides a comprehensive view on the microbiological material made accessible online by public collections, and the function of Analyzer of Bio-resources Citation.

Future developments such as "BIG DATA" technology including semantic web or linked data will allow the system to provide more flexible data integration broader data sources. Linking WDCM strain data to broader data sets such as environmental, chemistry and research literature can add value to data mining and targeting microorganisms as potential sources of new drugs or industrial products.

The WDCM will work with Research Infrastructures, Publishers, Research funders, Data holders and individual collections and scientists to ensure data interoperability and provide the environment for enhanced tools for research and development. WDCM is prone to evolve and continue.

In my talk, I will introduce WDCM GCM project and WDCM 10K Type Strain Sequencing Project and how WDCM can help PNMCC.

Dr. Juncai MA received Ph D degree from Department of Bio-resources of Mie University in Japan, now he is the director of The Microbial Resource and Big Data Center in Institute of Microorganisms, Chinese Academy of Science. He is also the director of China National Microbiology Data Center and WFCC-MIRCEN World Data Center of Microorganisms (WDCM), and Board Member of World Federation of Culture Collections (WFCC).

Day 3:
October 16, 2021

Bacteriophage Isolation and Preservation Techniques

Prof. Jaymee R. Encabo
University of the Philippines Los Banos

With the growing interest in bacteriophages as antibacterial agents, reliable methods for bacteriophage isolation and preservation is of crucial importance. In phage biology, the maintenance of a phage collection is essential to preserve phage integrity and to ensure that there will be no alterations during prolonged storage. The aim of this lecture is to provide phage biologists with general procedures to isolate monovalent and polyvalent bacteriophages using single and multiple host enrichment method, and to maintain bacteriophage stocks. The protocols presented are based on our laboratory's experience in bacteriophage research in the previous years. Perspectives for the use of lytic phages to specifically target plant pathogenic bacteria are also briefly presented.

Prof. Jaymee R. Encabo is an Assistant Professor of the Microbiology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños. She obtained her BS degree in Biology, major in Microbiology, *cum laude* in 1998, and her MSc degree in Microbiology in 2007, from UPLB. In 2011, she was the Third Prize Winner in the NAST Talent Search For Young Scientists for her collaborative research work on rice tungro viruses. She has been in the teaching profession for 20 years, and is a five-time recipient of the IBS Outstanding Teacher Award Based on Student Evaluation for Teachers. Her current research works include the dynamics of virus-stress and virus-virus interaction and the use of bacteriophages as biocontrol agents.

Day 4:
October 23, 2021

USA's Center for Chemical Biology and Biotechnology Actinomycetes Collection: Essential Biobank for Drug Discovery during Pandemic and Beyond

Doralyn S. Dalisay, PhD
Department of Biology, College of Liberal Arts, Sciences, and Education Center for Chemical Biology and Biotechnology
University of San Agustin, Iloilo City, Philippines

Antimicrobial resistance (AMR) has become a global public health concern specifically at the height of COVID-19 pandemic due to secondary bacterial pneumonia that progress at initial stage of the infection. It poses an extreme progressive risk particularly against multidrug resistant ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.). The AMR crisis further worsened as the shortage of antibiotics under the drug development pipeline has become apparent, thus the need to identify antibiotics with new chemical entities to fight against multidrug- resistant pathogens.

Actinomycetes produce a wealth of structurally diverse antibiotics, which are essential in medicine due to the emergence of multidrug-resistant pathogens. More importantly, there were 32 antimicrobials reported since the year 2000 originated from actinomycetes, particularly Streptomyces species, comprising 41% of the antimicrobials approved for clinical use. Thus, suggesting the vast potential of this group of bacteria as a rich source of new antimicrobials.

The University of San Agustin's Center for Chemical Biology and Biotechnology (C2B2) have been at the forefront of quelling the danger of multidrug-resistant pathogen infections. For almost four years now, the C2B2 has made it their mission to search the Philippines' marine-environment actinomycetes for novel antibiotics. We isolated biodiversity cultivable Streptomycetes from marine sediments from 21 collection sites in Philippine archipelago. Our biobank assembled more than 3000 diverse Actinomycetes that serves as bioresource not only for antibiotics but also antivirals, anticancer, antimalaria, anti-dengue, and other bioactive molecules to fight against diseases that afflict the lives of Filipinos.

Dr. Doralyn Dalisay is a licensed pharmacist, registered microbiologist and natural products chemist whose expertise and research interests cover microbial, marine invertebrate, and plant natural products for drug discovery. She obtained her PhD in Microbiology at University of New South Wales, Australia and postdoctoral fellowship to specialize on marine natural products chemistry at University of California, San Diego. Dr. Dalisay is a pioneering scientist to work on the biodiversity, biological activities and chemistry of marine sediment-derived actinobacteria from Philippine archipelago, that could one day help to find new antibiotics and potentially address the problem of multidrug resistant pathogens. She has won a number of awards in recognition for her research, publications and patents.

Day 4:
October 23, 2021

Maximizing the Use of Microbial Reference Cultures in a Testing Laboratory

Marlon S.A. Aguinaldo[1], Alxis John C. Movida, Hazelle Marie D. Penaranda

[1]Industrial Technology Development Institute, Department of Science and Technology,Bicutan, Taguig City 1630

Email address: aguinaldo_marlon@yahoo.com

Microbial reference cultures (MRCs) are microbial preparations with sufficiently established characteristics that are traceable to a culture type collection. These are widely used by testing laboratories as part of internal quality control (QC) and applied to various laboratory activities. It is important that the laboratory implements a system in maintaining these MRCs to ensure that the purity, robustness and observed characteristics of the isolates are consistent after successive transfers, and are fit for intended use. As a requirement of the PNS ISO/IEC 17025: 2017 standard, accredited laboratories are required to use MRCs in every step a test method to ensure that results of analysis of samples are accurate and reliable. To support product claims, MRCs are used as challenge microorganisms in performance testing. Furthermore, laboratories use MRCs for their quality assurance (QA) applications, such as, culture media QC, validation and verification of analytical test methods, estimation of uncertainty of measurements and inter – analyst comparison studies.

Marlon S. Aguinaldo is a Senior Science Research Specialist (Technical Manager and Section Head) at the Industrial Technology Development Institute. He has nineteen years of experience in microbiological testing (food, water, cosmetics and other industrial products). He is also a Technical Assessor for ISO/IEC 17025 for microbiological testing. His current research focus is on microbial measurements, development of Proficiency Test materials for microorganisms in food and provision of PT schemes for local microbiology laboratories. He is currently involved as a Project Leader of Biological Metrology for Microorganisms in Food.

Abstracts of Paper Poster Presentations

COMPETING

Epiphytic Microalgal Flora of Laguna de Bay: New Philippine Records, Distributional Patterns and Ecological Notes

Eldrin DLR. Arguelles[1] and Rosario G. Monsalud[1]

[1]*Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, University of the Philippines Los Baños, College, Laguna, Philippines, 4031*

Email: edarguelles@up.edu.ph

INTRODUCTION

Macrophytes are aquatic and amphibious plants found in the littoral zones of running waters (eg. rivers, streams, and the like) and shallow lakes contributing largely to the autochthonous carbon pool and oxygen budget of the aquatic ecosystem. Macrophytes have diverse species of microalgae attached to submerged plant parts which have been shown to be an important productive component of the aquatic ecosystem (Sheldon and Boylen, 1975). Algal epiphytes form a matrix system of several species of microalgae, bacteria and filamentous fungi attached to aquatic macrophytes. These algae are regarded as primary species in different aquatic ecosystem and are involved in maintaining ecological balance among the different groups of macrophytes and aquatic organisms. Several observational and experimental studies on ecological status of bodies of water showed an increase in the population and diversity of algal epiphytes in response to nutrient loading and pollution (Fawzy, 2016). This led to the consideration of these organisms as excellent bioindicators of environmental and water quality alterations because of its sensitivity to external sources of pollution (Fawzy, 2016). Diversity and ecological studies of epiphytic microalgae of different aquatic macrophytes found in rivers and lakes in the Philippines remain poorly understood. To date, no documented taxonomic survey was conducted on these group of microalgae. Thus, additional taxonomic survey of algal epiphytes of different aquatic macrophytes found in these aquatic ecosystems are needed to deepen our understanding of the diversity and ecological roles of these microorganisms. The goal of this investigation is to account the species diversity of algal epiphytes associated to different aquatic macrophytes observed in Laguna de Bay, the largest lake in the Philippines. Also, a brief description of the sampling sites and ecological condition of its existence were documented.

METHODOLOGY

Sampling and Specimen Preparation of Algal Epiphytes

A single preliminary collection of algal epiphytes from submerged aquatic macrophytes was done from the littoral zone of Laguna de Bay (14^{O} 10'- 14^{O} 35' N, 121^{O}-121^{O} 30' E). The plant parts were put into sterile autoclavable plastics filled with water for laboratory examination. A total of 20 aquatic macrophytesamples (each for *Pistia stratiotes*, *Hydrilla verticillata*, *Nymphaea pubescens*, *Eichhornia crassipes* and *Ipomoea aquatica*) were collected and analyzed throughout the study period. Immediately after collection, these samples were washed several times with sterile distilled water. The algal epiphytes from submerged leaves, stems and roots on the collected aquatic macrophytes were set apart from the plant by gently scraping the attached algae on the plant material (Zimba and Hopson, 1997). The collected scraped algal epiphyte was carefully mixed, and a portion of 50 mL was kept for taxonomic enumeration. The mixed algal epiphyte sample was transferred into a sterile beaker and left overnight to allow settling of the scraped algal samples. An aliquot of 45 mL of the liquid specimen was removed in the beaker after the settling period. The residual 5 mL of the collected scraped material was transferred into a sterile drum vial for taxonomic enumeration of algal epiphytes and were preserved using 2-3 drops of Lugol's iodine from the prepared specimens. A small portion (5 mL) of the concentrated scraped epiphytic algal samples was used for the analysis of diatom flora. The scraped samples were chemically digested following the standard procedure for diatom cleaning and slide preparation of Round et al. (1990). Mixture of cleaned diatoms were dried onto glass coverslips and mounted. Three slides were prepared for each aquatic macrophytes sample for microscopic observation and enumeration of diatoms. The preserved algal specimens and diatom slides were kept as voucher specimens at the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines. Water quality parameters were taken at 10:00-13:00 h on each sampling sites. Parameters such as water temperature, pH, and dissolved oxygen were measured using Xplorer GLX (PASCO).

RESULTS AND DISCUSSION

In the Philippines, little is known on the distribution, taxonomy and diversity of microalgae associated with aquatic macrophytes found in marine and freshwater ecosystems. In this study, it is observed that the consortium of epiphyton mats in submerged stem, leaves and roots of the dominant macrophytes are composed mainly of unicellular and filamentous type of the eukaryotic microalgae, bacteria, and filamentous fungi. A total of 65 epiphytic algal taxa from five dominant aquatic macrophyte (*Pistia stratiotes*, *Hydrilla verticillata*, *Nymphaea pubescens*, *Eichhornia crassipes* and *Ipomoea aquatica*) found in

Laguna de Bay were observed in the study. The taxonomic list present 31 taxa belonging to the Chlorophyta, 24 to the Cyanobacteria, 10 to the Bacillariophyta, 5 to the Charophyta and 4 taxa to the division Euglenophyta. The survey reported the occurrence of five rare microalgae – namely, *Cryptoglena skujae* Marin and Melkonian, *Pseudanabaena minima* (G.S. An) Anagnostidis, *Synechococcus nidulans* (Pringsheim) Komárek, *Chroococcus schizodermaticus* West and *Franceia amphitricha* (Lagerheim) Hegewald – for the first time in the Philippines. One species is also reported here for the first time in the Philippines based on recent algal taxonomic nomenclature and this is *Ulnaria ulna* (Nitzsch) Compère that is based on the former name of *Synedra ulna* (Nitzsch) Ehrenberg. Generally, conditions such as a nutrient- rich [high phosphate (0.29 mg/L) and nitrate (5.32 mg/L) concentration] environment, optimum temperature (27-28 ^{O}C) and high light intensity (73.15 klux) were found to be favorable for the existence of algal epiphytes in these aquatic plants. In this study, several algal taxa [such as *Aulacoseira granulata*, *Cyclotella meneghiniana*, *Nitzschia palea*, *Phacus longicauda*, *Lepocinclis acus*, *Trachelomonas armata*, *Oscillatoria tenuis*, *Planktothrix compressa*, *Chroococcus minutus*, *Scenedesmus quadricauda*, and *Chlorella vulgaris*] were identified which can be use as biological indicator for aquatic ecosystem health assessment of Laguna de Bay since these taxa were are known indicator of nutrient pollution in a eutrophic lake ecosystem (Dunn et al., 2008; Effiong & Inyang, 2015). The taxonomic data obtained on this survey served as baseline data in the evaluation of the current ecological status of Laguna de Bay.

CONCLUSION

The taxonomic survey done in this study shows diverse collection of microalgae and cyanobacteria (65 taxa) associated with aquatic macrophytes found in Philippine freshwater ecosystem. Among these taxa, the existence of five epiphytic algal species: *Pseudanabaena minima* (G.S. An) Anagnostidis & Komárek, Planktothrix compressa (Utermöhl), Anagnostidis et Komárek *Synechococcus nidulans* (Pringsheim) Komárek, *Chroococcus schizodermaticus* West, and Franceia amphitricha (Lagerheim) Hegewaldis were described for the first time in the Philippines. The result of this survey suggests that further study on the diversity and ecological interactions of algal epiphytes in relation to other aquatic macrophytes found in both marine and freshwater habitat is needed since only few studies were reported in the Philippines.

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COMPETING

Morphology, Phylogeny and Pigment Composition of Potentially Harmful Marine Microalgae *Karenia* spp. (Kareniaceae, Dinophyceae) cultured from the Philippines

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INTRODUCTION

Marine unarmored dinoflagellates in the family Kareniaceae are harmful microalgal group responsible for economic damages on coastal fisheries worldwide. Of the three major genera (Karenia, Karlodinium and Takayama) in the family, Karenia is the most widely reported genus owing to its high bloom frequency and extent of fisheries damages (Sakamoto et al. 2020; Yñiguez et al. 2020). For example, the red tide of *Karenia mikimotoi* in Fujian province China is considered as most economically devastating bloom ever recorded which incurred >290M USD loss due to the mass mortalities of marine products particularly the cultured abalones (Sakamoto et al. 2020). In the Philippines, blooms of *Karenia mikimotoi* has been reported in Bolinao Pangasinan although no associated fish mortalities was documented (Azanza and Benico, 2015). Nonetheless, occurrence of *Karenia mikimotoi* and other related species in other coastal areas of the countries remains to be less reported and needs to be taxonomically verified since Karenia exhibit high morphological plasticity and their cells could easily be deformed when fixed during routine phytoplankton monitoring.

OBJECTIVES

In this study, morphology, phylogeny and pigment composition of *Karenia* were examined using culture strains originally isolated from Philippine coasts and compared with species isolated from Japan for better understanding of the taxonomy, identification and distribution of the harmful dinoflagellate.

METHODS

Water samples were collected in the coastal waters of Manila Bay and Subic Bay in October 2018. Cultures were established by single-cell isolation from water

samples; and maintained using 1/2 IMK (Wako, Tokyo, Japan), F2 media at 23°C, under a photoperiodic condition of L:D = 12:12 with 40–50 µmol photons $m^{-2}s^{-1}$ light illumination. Cell morphology was observed by a compound microscope Zeiss Axioskop2 equipped with a digital camera Zeiss Axiocam 305 color. The chloroplast was detected by autofluorescence, and nucleus was observed by SYBR Green or DAPI fluorescent staining. For SEM, cells were preserved by 2% osmium tetroxide on a poly-L-lysine coated glass plate, dehydrated via an ethanol series, and dried in a JEOL JCPD-5 critical point dryer. Cells were observed by an SEM Hitachi S-4800. Photosynthetic pigments were extracted from cultures in 95% methanol, and the pigments are analyzed by a HPLC system Shimadzu SPD-M10Avp photodiode array detector (Shimadzu, Kyoto, Japan). Peaks of chlorophylls and carotenoids were identified by referring their retention times and absorption spectra from certified pigment standards . Total DNA was extracted from cultures, purified, and amplified the target gene markers using appropriate primer sets. Phylogenetic positions of established cultures were inferred from the host nucleus-encoded partial LSU rDNA (D1–D6) and ITS region. Phylogenetic trees were constructed by maximum likelihood and Bayesian inference using obtained DNA sequences with those referred from GenBank.

RESULTS

Two species of *Karenia* were identified from cultures established from Philippines. Strain GBMNL526 isolated from Manila Bay was identified as *K. mikimotoi* while strain SUB126 isolated from Subic Bay was identified as *K. papilionacea*. Cells of *K. mikimotoi* were pentagonal, dorsoventrally compressed, measuring 21.9–27.5 µm long and 19.2–26.6 µm wide. The epicone was conical or sometimes hemispherical, lacked an apical carina. The hypocone was hemispherical, bilobed, and the right side was slightly larger than the left side. Nucleus was ellipsoidal and located at left side of the hypocone. Under SEM, the apical structure complex (ASC) was straight with a taller right margin. It was comprised of three rows of AVs; a shorter ridged vesicle a furrow vesicle forming the furrow and knob vesicles. The ASC was long (approx. 12.1 µm in length) with ca. 20 knob vesicles. The ventral ridge, or an intercingular tube-shaped structure, was prominent in the sulcal region. Cells of *K. papilionacea* were butterfly-shaped, dorsoventrally compressed, measuring 24.3–36.3 µm long 25.1–43.2 µm wide. The epicone and hypocone were almost similar in length.. A spherical nucleus was located at left side of the hypocone. Each chloroplast contained a single pyrenoid, which appears conical in some viewing angles . SEM observation of *K. papilionacea* strains showed a short and straight ASC composed of furrowed vesicles and knob vesicles. The ASC measured 6.8–7.4 µm long and possessed 10–11 knob vesicles. Cells possessed a very short ventral ridge.

Phylogenetic analysis inferred from ITS and LSU rDNA sequences showed the well-supported grouping of the *Karenia* clade with maximum support. Molecular identification of both *K. mikimotoi* and *K. papilionacea* was supported

by their clustering to sequences of similar species from other geographic regions. The pigment composition of the two cultures of *Karenia* showed the abundance of carotenoid with molar pigment ratios (carotenoid:Chl a) of 1.34 in *K. mikimoto*i and 0.28 in *K. papilionacea*. Moreover, *K. papilionacea* were dominated by Hex-fuco with 14.90 and 7.10, respectively. It also had a high amount of diadinoxanthin with 8.34 compared to *K. mikimotoi*. But-fucoxanthin was detected in all strains with molar pigment ratios ranging from 0.05 in *K. mikimotoi* to 7.06 in *K. papilionacea.*

DISCUSSION

In this study, two species of *Karenia* were characterized on the basis of their morphology, molecular phylogeny and pigment composition for reliable taxonomic identification. Morphological characters and molecular phylogeny of strain from Manila Bay (MNL526) were similar to the previous description of *Karenia mikimotoi* (Hansen et al. 2000). Morphological characters observed of strain SU126 agree with the earlier description of *K. papilionacea*. However, some morphological variations were observed among cells in the same culture. The typical "butterfly" morphotype, which is wider than long, was commonly observed but cells resembling *K. brevis* and even *K. mikimotoi* (i.e., without apical carina) were observed in culture. In addition, spherical cells were also present. According to Haywood et al. (2004), *K. papilionacea* typically exhibit a highly pronounced hypothecal excavation, which is different from the slightly hypothecal excavation in most *Karenia* species. Previously, two phylotypes of *K. papilionacea* was reported in the Japanese coastal waters, as the original phylotype and phylotype I (Yamaguchi et al. 2016). In their study, these two phylotypes had a high degree of divergence suggesting the possibility of two different cryptic species, and therefore more detailed morphological comparison was recommended. Here, these two phylotypes were cultured, i.e., cultures from Japan belonged to the original haplotype, whereas Subic Bay, Philippines (SU126) to phylotype I. Cell sizes of the strains from the two phylotypes are almost similar and overlapping. Under SEM, there are some minor differences in external ultrastructure of the two phylotypes. The ASC of phylotype I is slightly longer measuring 6.8–7.4 μm longer than that of original phylotype measuring 5.5–6.7 μm long. These results show the difficulty of differentiation between two phylotypes based on morphology.

CONCLUSION

The present study reported two species of *Karenia* from Manila Bay and Subic Bay Zambales; of which, *K. papilionacea* had not been reported before in the country. The two species were identified by detailed morphological characterization, phylogenetic analyses based on LSU and SSU rDNA sequences, and pigment analysis. Since the occurrence of *K. mikimotoi* and *K. papilionacea*,

has been has been extended in two more coastal areas of the Philippines, further research needs to be conducted to better understand the diversity and distribution of these potentially harmful dinoflagellates in other coastal areas in the country.

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COMPETING

Curvularia sp. causes leaf spot to *Citrullus lanatus* (Watermelon)

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INTRODUCTION

Citrullus lannatus (Watermelon) is one of the important fruits in the Philippines that are eaten fresh as dessert. This flowering vine-like plant produced in places with warm climates, is composed of water, small amounts of minerals, protein, fats, carbohydrates, lycopenes, and vitamins, and like other fruit crops, requires proper disease management (Madrid et al., 2014). Growing watermelon has been one of the sources of livelihood of many farmers, moreover, several diseases caused by insect pest, viruses, bacteria, and fungi caused the decline of its yield. Fungal diseases affecting watermelon had been studied in years and several fungal pathogens had been found associated with it. However, in the Philippines, no further information about other diseases that infects this crop has been studied. *Curvularia* sp., a known plant pathogen, was isolated in *C. lanatus* in the present study. According to Liang et al. (2018), *Curvularia* sp. can cause severe or opportunistic diseases in different plant taxa. This genus is considered a threat to different agricultural products and is commonly known for causing leaf spot disease in some crops including Hippeastrum striatum. In a recent study, a species of *Curvularia*, *C. lunata*, was found associated with crown and root disease in watermelon, with a frequency of 1.25% and an appearance of 26.66%. In watermelon, this disease is one of the most prevalent diseases that causes economic loss in the crop worldwide. This disease is characterized by rotting in the crown and root cortex of watermelon that will eventually lead to the yellowing of old leaves and wilting of the whole
shoot (Hussein and Juber, 2014).

OBJECTIVES

This research was done to determine the fungus that cause leaf spot to watermelon. Specifically, this study conducted the following: 1) isolation of the fungus that cause leaf spot to watermelon; 2) identification of the fungal isolates by morphological, molecular and phylogenetic analysis; 3) confirmation of the pathogenicity of the fungal isolates by inoculation test.

METHODOLOGY

Collection of the leaf samples exhibiting leaf spot disease was conducted in Alaminos, Pangasinan in November 2019. Fungal isolates from the leaf samples were grown onto potato dextrose agar (PDA) and incubated at 25°C. Colony morphology were taken from the 7-day culture of the isolates while the micromorphology was observed using the BioBlue Lab microscope. Molecular and phylogenetic analysis were conducted using the ITS DNA barcode by comparing from known ITS sequences from published materials. The sequences were aligned using MAFFT v. 7 and the phylogenetic tree was reconstructed by Maximum Likelihood utilizing the MEGA v. 10. For pathogenicity testing, the isolates were inoculated onto the rind of healthy fruits of watermelon to confirm if the isolates will exhibit symptoms of the disease. Three containers were prepared, each containing three watermelon rind samples, labeled A (unwounded), B (wounded), and C (control). The set-ups were then incubated for 3-4 days under room temperature (25-30°C).

RESULTS

Morphology: The colony of the 7-day old culture of the isolate PUPML_2020227 grown on potato dextrose agar (PDA) at 25°C appeared to be off-white with light olive green color and pale brown at the center; mycelial growths are powdery- cottony with filamentous margin, and slightly elevated (Figure 1A-B). Conidiophores were unbranched, sub-hyaline to pale brown, flexuous to straight and septate (Figure 1C-F). Conidiogenous cells were integrated, terminal and intercalary. The observed conidia were ellipsoidal to obovoid, asymmetrical with hilum, appeared pale brown to brown in color, usually curved at the third cell from the base, and 2-3 distoseptate (Figure 1G-M).

Molecular and Phylogenetic Analysis: The obtained phylogenetic tree by Maximum Likelihood using the ITS gene marker showed that the isolate

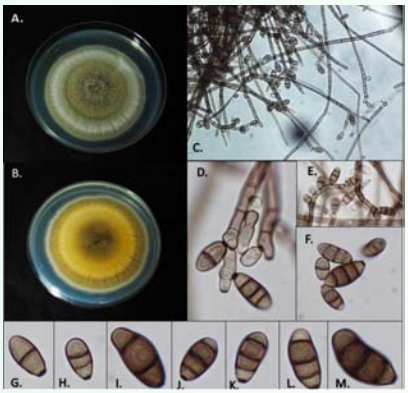

Figure 1. *Curvularia* sp. PUPML_2020227 isolate from watermelon (A) Observed (B) reverse (C)-(E) Conidiophores with an integrated conidiogenous cell producing immature conidia (F)- (M) straight to ovoid septate.

PUPML-2020227 was more closely related with *C. xishuangbannaensis*, *C. soli*, *C. senegalensis* and *C. thailandicum* (Figure 2).

Pathogenicity test: Results showed that the infection of *Curvularia* sp. in watermelon appeared to be white with cloudy texture and abundant in wounded samples (Figure 3A); small, round, scarce with darker color in wounded samples (Figure 3B). Results confirm the Koch's postulate that the fungal isolates exhibit symptom of disease in watermelon fruit samples.

DISCUSSION

The obtained results from the morphological analysis and pathogenicity test confirmed the causal relationship of *Curvularia* sp. to the spot disease in watermelon. In a study conducted by Hussein and Juber (2014), *C. lunata* was found associated with the rotting of crown and root cortex in watermelon, as well as the wilting of the fruit. Other species of *Curvularia* including *C. inaequali*s and *C. spicifera* caused leaf blight and fruit rot on fruits that has the same growth environment as the watermelon. In the study of Ayoubi et al. (2017), symptoms of the disease such as having sunken lesions and white mycelial masses on the fruit surface were observed on strawberry fruits after 9 days of incubation. White pinpoints was also observed on *Gladiolus gandavensis* in the study conducted by Zhang et al. (2021). These white pinpoints that was found to be cause by *C. gladioli* eventually turned into brown lesions and later on develop into oval to circular spots surrounded by yellow halo. In rice crops, leaf spot disease caused by *C. specifera* was also observed, with symptoms of long and wide grayish spots surrounded by brown irregular spot shape (Bawa et al., 2018).

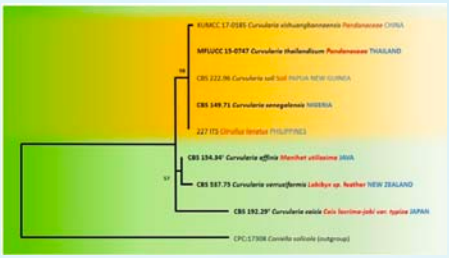

Figure 2. Original tree based on ITS sequences of isolate PUPML_2020227 with the reference sequences, constructed by Maximum Likelihood and Kimura-2 Parameter model. Numerals at the nodes indicate bootstrap percentage derived from 1000 replicates. The ex-type strains are indicated in bold types.

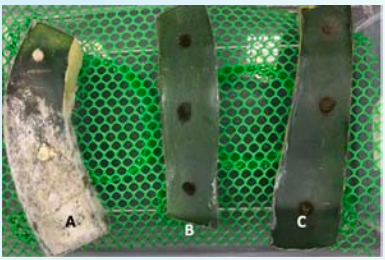

Figure 3. Symptoms of ***Curvularia*** spot disease on watermelon fruit rind after 4 days of inoculation with fungal isolate: A. unwounded; B. wounded watermelon fruit; C. Control

CONCLUSION

This study revealed that the fungus *Curvularia* sp. is responsible for causing spot disease in watermelon. Moreover, multigene analysis is recommended to further delineate the isolate with other *Curvularia* sp.

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COMPETING

Morphological and Molecular Analysis of *Fusarium* species causing Wilt disease in Watermelon *(Citrullus lanatus)*

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INTRODUCTION

Fusarium wilt is one of the common and important disease in the watermelon plant (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). The disease is characterized by darkening of the stem and roots of the plant apparent around the crown and higher taproot. This symptom subsequently causes the withering and drooping of leaves leading to the death of vine or the entire plant. The watermelon *Fusarium* wilt disease is caused by fungal pathogen *Fusarium oxysporum* (Martyn, 2014). This watermelon wilting occurs at the time of fruit maturity, resulting in the entire loss of the crop (Castro et al., 2020).

OBJECTIVES

This study reported *Fusarium* species associated with the wilting of watermelons. Specifically, the study conducted the following: a) isolation of fungi in watermelon plants afflicted by *Fusarium* wilt disease; b) identification of the fungal isolates by morphological, molecular and phylogenetic analysis; and c) verification of the pathogenicity of the isolates obtained by inoculation test.

METHODS

Collection of tissue samples (leaves, stem and fruit) from watermelon plants exhibiting symptoms of *Fusarium* wilt was conducted to a watermelon farm in Alaminos, Pangasinan in November 2019. The causative fungus was isolated from the diseased tissue and grown onto potato dextrose agar (PDA). Morphological characterization was done using the 7-day old cultures observing the colony growth on PDA. The morphology of microscopic features of fungus was observed using the BioBlue Lab microscpe. The ITS barcoding was done to compare the isolates with type-sequences from literature. Multiple alignments of sequences were done using MAFFT v. 7 and phylogenetic tree was constructed

in MEGA v. 10 using Maximum Likelihood (ML) as the statistical method. To establish the pathogenicity of the fungus, the isolates were inoculated onto healthy fruit rinds of watermelon. The rinds were then incubated for 3-4 days under room temperature (25-30°C).

RESULTS

Eight (8) *Fusarium* isolates were obtained from diseased watermelon plants. Based on morphological observation, the isolates displayed a typical morphology of genus *Fusarium* which is the presence of microconidia and macroconidia (Figure 1C, F, & I). Furthermore, the appearance of the colonies grown in the PDA was cottony with abundant aerial mycelia, initially white then gradually turned to orange yellow (both lower and upper surfaces), and these features were observed in all isolates (Figure 1A-H). The pathogenicity test confirmed the association of the *Fusarium* isolates to watermelon. All isolates developed small-medium, round, scarce-moderate infection on the watermelon rinds. The color of fungal growth on the watermelon rinds were brown (Figure 2A) and white (Figure 2B). The ML tree revealed that every isolate is related to a specific *Fusarium* species (Figure 3).

DISCUSSION

Based on morphological features, the isolates were consistent with the morphological characteristics as described by Kee et al. (2020). The present study showed that *Fusarium* species caused disease to watermelon. The findings were similar to the investigation of Hussein and Juber (2014) wherein they found that *Fusarium solani f. sp*. was the causative agent of watermelon crown, root rot, and fruits. Based on a pathogenicity test, unwounded isolates were also affected and produced symptoms, which means, infection can also occur on intact rinds of the watermelon. Furthermore, *Fusarium* species were also observed in other host plants that have similar growth environment to watermelon like lettuce and soybeans. According to Diaz Arias et al. (2011), Fusarium spp. are pathogens that ubiquitously present in soil that cause important soybean diseases such as damping-off, root rot, Fusarium wilt, and sudden death syndrome. Whereas in the study conducted by Mbofung et al. (2007), their results also revealed that *Fusarium oxysporum f. sp. lactucae* is a severe pathogen that causes *Fusarium* wilt in lettuce.

CONCLUSION

The present findings imply that Fusarium species are responsible for causing diseases on *C. lanatus*. However, the constructed phylogenetic tree is not enough for the identification of species since some of the bootstrap values were relatively low. In that case, this study needs to be subjected to multigene barcode

analysis to discriminate the isolates up to species level.

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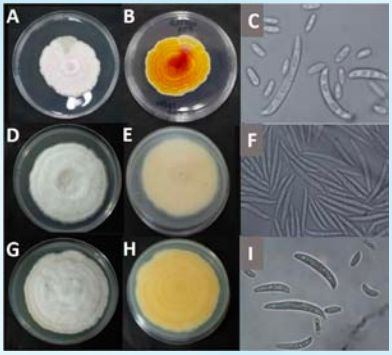

Figure 1. Selected photographs of *Fusarium* spp. Isolated from watermelon. A and B surface and reverse view of the colony of isolate 229; D and E surface and reverse view of the colony of isolate 237; G and H surface and reverse view of the colony of isolate 231. C, F and I macroconidia and microconidia of isolates 229, 237, and 226 respectively.

Figure 2. Pathogenicity test of *Fusarium* sp. isolates on watermelon rinds.

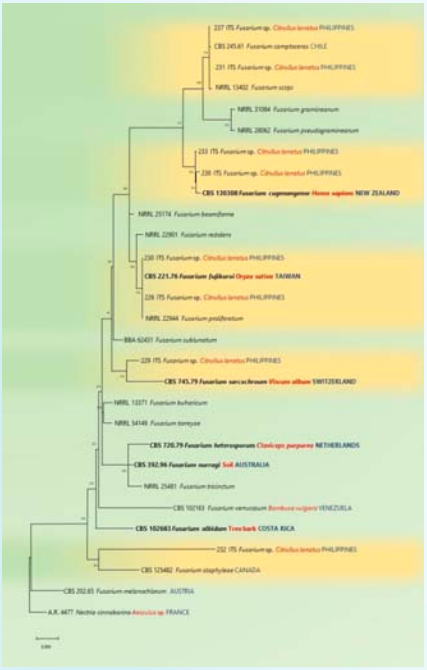

Figure 3. Maximum Likelihood Tree based on the ITS sequences of *Fusarium* sp. isolates and reference strains. Numerals at the nodes indicate bootstrap percentages derived from 1000 replicates. The phylogram is rooted to *Nectria cinnabarina* A.R. 4477. The ex-type strains are indicated in boldface.

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COMPETING

Development of an Automated Image Analysis for the Identification and Classification of Philippine Microalgae Using Convolutional Neural Network

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INTRODUCTION

Microalgae represent a diverse group of microorganisms with approximately 50,000 species identified and classified. This group of heterogeneous photosynthetic microorganisms exists as single cells or as an organized group of cells found in terrestrial, marine, and freshwater habitats acting as primary food and energy source for most of the higher organisms [1]. The taxonomy and classification of microalgae and cyanobacteria are highly dependent on molecular genetic techniques and morpho-taxonomic characterization (e.g., nature of photosynthetic pigment; cell wall characteristics; cellular organization; filament features such as the cell size and cell shape) [1]. However, these methods are considered time-consuming, tedious, and too dependent on the physiological state of algae with low resolution [2]. Generally, it is very difficult for taxonomists to identify several algal species in a specimen. Thus, the use of a microscope scanning system combined with digital image processing and machine learning in establishing a system of automatic identification of algae poses a promising technology [2]. Digital image processing is the application of various computer algorithms to perform image manipulations on digital images. This technology has a wide practical application and is currently being used in the image classification systems of microalgae and cyanobacteria. In the Philippines, studies concerning the use of digital image processing on the classification of Philippine microalgae and cyanobacteria are non-existent. Thus, this study is considered a pioneering research study in the use of digital image processing technology for the identification and classification of local algal isolates. The study aims to use OpenCV and Convolutional Neural Network (TensorFlow) in the development of a digital image identification system of freshwater microalgal species.

METHODOLOGY

Image Acquisition

Images of freshwater algae (*Chlorococcum infusionum*, *Chlorella vulgaris*, *Nostoc commune*, *Leptolyngbya lagerheimii*, *Desmodesmus abundans*, *Acutodesmus dimorphus*, *Oscillatoria proboscidea*, and *Oscillatoria limosa*) were taken under 1000x magnification using a binocular research microscope (Olympus CX31) equipped with Infinity X digital camera. These images were gathered upon request to Mr. Eldrin DLR. Arguelles, Curator of microalgae and cyanobacteria of the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. A total of 400 images were used for both the training and classification phases. Eighty percent of these images were used in training and validating the classification model while the remaining 20% was used for testing the created model in the classification phase. All images under each category were randomly selected to avoid bias in training the model.

Training

The randomly selected images for the training phase were introduced to the convolutional neural network model. The model identifies eight classes each representing the freshwater algal species used in this study. The goal of the whole training phase is to expose and let the model learn from the pre-identified or known images so that it would be able to identify new images. To build the model, it is necessary to first collect and load the datasets. The datasets that would be used for training the model are the resulting images after segmentation. All the images included for the training phase were pre-identified by a microalgae curator and were placed in the same directories to ensure consistency and accuracy.

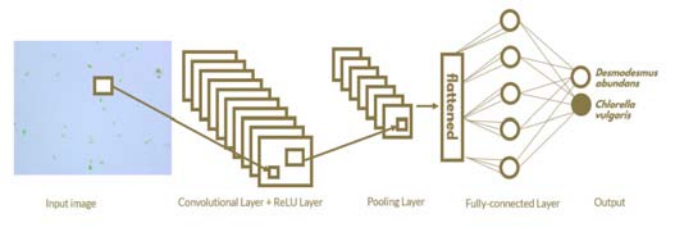

Figure 1. An illustration of convulutional neural network

Classification

The system developed in this study completely automates the recognition and classification of selected microalgae species. Out of the 400 images of microalgae collected and stored in jpg format, a total of 80 unclassified freshwater algae images was used for system testing while the remaining 320 were used earlier to train and validate the model. All images randomly selected for the testing phase were excluded from the set of images used for training the classification model. The classification results were recorded in a confusion matrix to get the system's

accuracy. The result of the recognition and classification system is presented in Table 1. It is observed that most of the test images for each class were properly identified by the system yielding an overall accuracy of 95%.

Table 1. Confusion matrix derived from the given results of the image

Actual\ Predicted	*Acutodesmus dimorphus*	*Chlorella vulgaris*	*Chlorococcum infusionum*	*Desmodesmus abundans*	*Leptolyngbya lagerheimii*	*Nostoc commune*	*Oscillatoria limosa*	*Oscillatoria proboscidea*
Acutodesmus dimorphus	**9**	0	0	1	0	0	0	0
Chlorella vulgaris	1	**8**	0	1	0	0	0	0
Chlorococcum infusionum	0	0	**10**	0	0	0	0	0
Desmodesmus abundans	1	0	0	**9**	0	0	0	0
Leptolyngbya lagerheimii	0	0	0	0	**10**	0	0	0
Nostoc commune	0	0	0	0	0	**10**	0	0
Oscillatoria limosa	0	0	0	0	0	0	**10**	0
Oscillatoria proboscidea	0	0	0	0	0	0	0	**10**

RESULTS AND DISCUSSION

Based on the tabulated results, algal images from the species of *Chlorococcum infusionum*, *Nostoc commune*, *Leptolyngbya lagerheimii*, *Oscillatoria proboscidea* and *Oscillatoria limosa* yielded perfect accuracy results. Most of the misidentification errors were observed only in freshwater algae species with almost similar features (e.g., similar cell color and cellular shape) such as *D. abundans*, *A. dimorphus*, and *C. vulgaris*. Each algal taxa form clusters that can easily be misidentified for the other. The background color on both species may have also contributed to the possible misidentification errors since convolutional neural networks take the data on the whole image as input. On the other hand, *C. infusionum*, even though closely related with *Chlorella vulgaris* (in terms of its shape) has a unique cell color which led the model to yield high accuracy results for *C. infusionum*. This result is similar to those observed in cyanobacterial species, *O. limosa* and *O. proboscidea* where the model easily identified each algal taxa based on the difference in cell color. Differences on cell thickness and diameter of *L. lagerheimii*, *O. limosa* and *O. proboscidea* were also identified and resolved by the developed model. These species exhibited similar filamentous-type (strand-like) characteristic but *L. lagerheimii* is thinner than the other two species. The integration of the developed identification system to a mobile application will allow a more convenient, more flexible, and faster way of recognizing and identifying freshwater algae. It is recommended that future works should focus on creating a database management system in culture collection for all images of Philippine freshwater microalgae species. The proposed system should be accessible via the Internet to encourage further indexing and tagging of new images. Furthermore, online databases can facilitate faster access and retrieval of historical data.

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COMPETING

Taxonomic, Functional and Resistome Profiles of the Diverse Microbial Communities in Unexplored Karst Forest Soils of Guiuan, Samar Province Using Shotgun Next- Generation Sequencing

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INTRODUCTION

Soil microbial communities (SMCs) play an important role in various biogeochemical processes including decomposition of organic matter, nutrient recycling, remediation of the soil, and regulation of soil fertility in terrestrial ecosystems [1,2]. Recently published records on soil microbial communities have shown that they are highly sensitive to environmental changes [3] related to biotic and abiotic factors which can contribute to the countless individual microbial taxa, as well as viruses that can be found and isolated in a gram of soil [4]. In another perspective, SMCs may somehow contribute to the increasing problem of antimicrobial resistance (AMR) due to both intrinsic and extrinsic resistome, that are capable of horizontal transmission to clinically-associated pathogens [5,6,7]. While soil environments already contain vast amounts of native resistome, anthropogenic activities such as agriculture & waste treatment are also known to enrich the soil resistome [5,7,8].

Karst forests comprise 11.7% of the total land surface of the Philippines. There is limited research regarding this ecosystem since local research is chiefly focused on its economic value—not on biodiversity or the public health threats these unexplored forests may impose [9]. This study is part of the DOST- funded CONKAIGANGAN project which aims to study the biological diversity of unexplored karst forests in the Samar Province. The current study aims to determine the taxonomic, functional, and resistome profile of the microbial communities present within the unexplored karst forest soils in Guiuan, Samar using metagenomic data from shotgun next-generation sequencing. This includes the description of the taxonomic composition of the SMC, while identifying the functional potential of the organism groups present. Furthermore, a resistome profile is also constructed

in order to maximize the use of the metagenomic data received from the Project 3 team—which may consequently supplement the program's objectives. Most importantly, the resistome profile includes the identification of clinically- relevant resistome and resistance gene groups in relation to SMCs.

METHODOLOGY

Taxonomic and Functional Profiling

This research was carried out in part using DOST-ASTI's Computing and Archiving Research Environment (COARE) services. Quality control and resistome profiling were performed in the Saliksik High Performance Computing (HPC) unit. MG-RAST [10] server and WGS pipeline ver. 4.0.3 with the RefSeq database was used for the taxonomic annotation of metagenomic reads. MG-RAST, using the SEED [11] Subsystems and KO [12] database was also used for functional annotation while the Numbers application was used for the visualization of the results. The generated Level 1 SEED subsystems and Level 1 and Level 3 pathways from the KO database were used to describe the functional potential of the SMCs.

Resistome Profiling

A reads-based pipeline (RBP) [13] was devised in order to identify and quantify putative resistomes. Clean metagenome reads were aligned against MEGARes 2.0 [14] using Bowtie 2, 2.4.2 [15]. A Kruskal-Wallis test was used to test if the differences between the abundances between gene groups and sites were significant. Visualizations were produced in RStudio and Excel. Following this, an assembly- based pipeline (ABP) was devised in order to identify putative organisms and the resistomes they contain, respectively. De novo assembly was performed with MEGAHIT 1.2.9 [16]. The output metagenome- assembled genomes (MAGs) from binning with MetaBAT 2.15 [17] were each classified using GTDB-Tk 1.3.0 [18] and screened for AMR genes using ABRicate 1.0.0 [19] using the CARD database's March 2020 update [20]. The resulting AMR genes were verified using the ARGminer web platform [21]. Gephi 0.9.2 [22] was used to construct the undirected & weighted, bipartite/monopartite network graphs.

RESULTS AND DISCUSSION

Taxonomic and Functional Profiling

Proteobacteria was the dominant phylum (G1 - 46.46%, G2 - 44.59%, G3 - 46.4%), followed by Actinobacteria (G1- 22.32%, G2 - 26.22%, G3- 26.35%), while other bacterial phyla were detected in minimal amounts. Members of Proteobacteria, which are abundant in karst soil [23,24,25], are known to be degraders of organic substrates [26] and play a critical role in the cycling of carbon, nitrogen, and sulfur [27]. Actinobacteria are gram-positive bacteria that are commonly found in soil, freshwater, and marine environments—and play

an important role in organic matter turnover and the carbon cycle in soil [28]. Fungi was still detected in very minimal amounts in all three soil samples, with Ascomycota (83.41% - 84.04%) and Basidiomycota (15.39% - 15.88%) being most abundant among the fungi identified. Soil fungi such as these are said to be involved in many environmental processes such as pedogenesis, nutrient cycling, disease suppression, and the process of weathering [29,30].

The functional profile showed abundance of genes that were related to twenty-eight functional subsystems with the most dominant subsystems being Carbohydrates and Clustering-based systems, differing only in the abundances of the sequences. Nine of the twenty-eight subsystems were less than 1% and were annotated and categorized together in Others. Karst soils are described to be a carbon pool [31,32]. In karst environments, the carbon cycle is mainly associated with the weathering of carbonate rocks [31], thus forming carbonic acid, a common agent of rock weathering [33]. All soil samples also exhibited similar top five L3 KEGG metabolic pathways with Glycine, serine, and threonine metabolism having the highest relative abundance value in the metabolic pathways. Following this pathway are the Alanine, aspartate, and glutamate metabolism; Valine, leucine, and isoleucine metabolism; Oxidative phosphorylation; and Purine metabolism pathways. The presence of these pathways may suggest that the soil microbiome present in the karst soil samples possess enzymes needed for different biological processes such as in the biosynthesis and degradation of amino acids, enzymes, cofactors, and lipids.

Resistome Profiling

The reads-based pipeline (RBP) was able to complete analysis of the datasets in 27 minutes on the 88- core HPC machine and 81 minutes on the 4-core home computer. This scalability metric is beneficial for researchers with little to no access to HPCs. The RBP is useful for quick detection and quantification of ultra-low abundance genes. Drugs, metals, and multi-compound resistome types were identified. There were 26 resistome gene groups under drugs, three under metals, and two under multi-compound. A prevalence of Macrolide-Lincosamide-Streptogramin (MLS), Aminoglycoside, and Oxazolidinone resistance was observed. The findings of the current study support a previous claim that such genes can be found in Proteobacteria that have been isolated from unexplored soils [34], similar to some species of

Actinobacteria [35]. The assembly-based pipeline (ABP) produced 35 metagenome-assembled genomes (MAGs), representing individual organisms. The ABP is useful for isolating organisms within the dataset and identifying genes that can be mapped back to each organism [36]. Only 20 out of 35 MAGs hosted resistomes. A total of 35 unique, drug resistance genes organized into 13 gene antibiotic classes, based on CARD nomenclature, were identified—with multidrug resistance comprising 14 out of 35 of those unique genes. Network analysis revealed that three unclassified organisms: Huberarchaeia, Nanosalinia, and Methylomirabilia, served as hosts for approximately 80% of assembly-

screened resistomes—the former two organisms being archaean and the last, a bacterium. Multiple genes conferring resistance to multi-drug, rifamycin, and tetracycline were also observed to be highly co-occurring among the MAGs. In practice, a combination of both types of pipelines is ideal for producing a more complete functional and taxonomic profile.

CONCLUSIONS

The karst soil samples from the Guiuan municipality are composed mostly of members of the domain Bacteria, specifically from the phyla Proteobacteria and Actinobacteria. Predominant SEED subsystems were Carbohydrates, Clustering-based Subsystems, and Amino Acids and Derivatives which may be linked to the members of the previously mentioned dominating phyla. Metabolism was the predominant KEGG Orthology Level 1 functional category, with the most abundant metabolic pathways being those involved with some Amino acid, Purine, and Oxidative phosphorylation.

The resistome profile of the unexplored karst soils obtained from Guiuan, Samar composed mostly of drug resistance. Due to the prevalence of drug resistance genes, information about nearby wastewater treatments, the use of antibiotics, and the activities surrounding the sampling sites may be considered for further research. It is recommended that other factors such as MGEs and virulence factors are profiled alongside the resistome to better understand the pathogenic risks of the SMCs. Future studies could also isolate organisms from environmental samples and identify specific resistance genes (e.g., metal, biocides) for purposes of data accumulation and future bioremediation.

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COMPETING

Pseudopithomyces maydicus causes dark spot disease in tomato (*Solanum lycopersicum*)

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INTRODUCTION

Tomato (*Solanum lycopersicum*) is the 3rd most important vegetable in the Philippines. It is being cultivated throughout the world and has been a profitable source of income. Moreover, plant diseases attribute to the decrease supply of both preharvest and postharvest tomato. The attack of fungi is one of the contributors of the diseases in tomato. Pseudopithomyces species known to be a common cause of diseases of dark spots in various plants including medicinal plants, grasses, cereals, and legumes. It is included in family Didymosphaeriaceae which is normally characterized by brown, thick-walled, 1- septate ascospores and trabeculate pseudoparaphyses, which anastomose above the asci in a gelatinous matrix. Furthermore, several studies suggests that it constitute a potential threat considering its production of toxins which can cause serious skin damage to animals like sheep and goats. Increasing the knowledge about this type of microorganism through conducting of phylogenetic and phenetic analysis will introduce new discoveries which will contribute in the field of mycology.

OBJECTIVES

This research determined the causative fungus that produce dark spots in tomato. Specifically, this study: 1) isolated the causative fungus in the infected region of tomato; 2) identified the fungal isolate by morphological, molecular, and phylogenetic analysis; and 3) verified the pathogenicity of the isolates by inoculation test.

METHODS

A visit to a tomato farm in Alaminos, Pangasinan was made to collect tissue samples (leaves and stems) from plants exhibiting symptom of leaf spot disease in January 2020. The causative fungus was isolated from the infected tomato and grown in potato dextrose agar (PDA). Morphological characterization

was done using the 7-day old culture of the isolates observing the colony growth in PDA and micromorphology of the fungus using BioBlue Lab microscope. For phylogenetic analysis, ITS sequence data was used to compare the isolates from the reference sequences from published journals. Multiple alignments of sequences were done using MAFFT v. 7 and phylogenetic tree was constructed in MEGA v. 10 using Maximum Likelihood as the statistical method. For pathogenicity testing, the isolates were inoculated onto healthy fruits of tomato to confirm if the isolates will exhibit a symptom of disease. Three containers were prepared, each containing three tomato fruits, labeled A (unwounded), B (wounded), and C (control). The leaves were then incubated for 3-4 days under room temperature (25- 30^{o}C).

RESULTS

Morphology. Isolate PUPML-2020243 (Fig. 1); colonies grown on PDA at 25^{o}C appeared to be elevated, powdery texture with filamentous margin reaching (36x36mm in diameter) on 7th day; greyish white center with white outer region; reverse brown to dark brown at center with white outer region. Conidiophores septate, pale brown, smooth, branched, normally packed together. Conidiogenous cells arise perpendicularly from repent hyphae; terminal and indeterminate which disintegrate at maturity to liberate conidia. Conidia (11-20 X 8-10 µm) (mean value 16.48 X 8.5µm), muriform; having mostly 2-3 septa with a transverse septum in the center; dark-brown, deeply pigmented, septate, echinulate, ellipsoidal with obtuse apex, truncate base; characteristically with part of the conidiogenous cell remaining attached as a small pedicel.

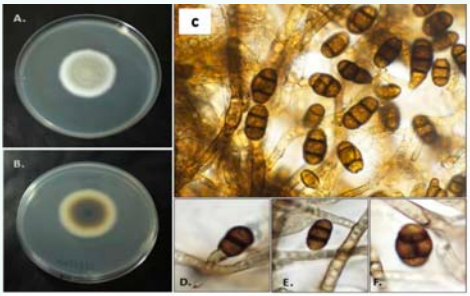

Figure 1. *Pseudopithomyces* sp. PUPML- 2020243 (A)-(B) Observe and reverse of the colony, respectively; (C) Conidiophore bearing immature and mature conidia; (D) developing conidia with a thick medial septum; (E) Immature conidia shape-like a barrel; (F) mature conidia, muriform.

Molecular and phylogenetic analysis. Tree reconstruction based on Maximum Likelihood using the ITS as a gene marker revealed that isolates were closely related to other Pseudopithomyces species, particularly with Pseudopithomyces maydicus with a 95% bootstrap value (Fig. 2).

Pathogenicity Test. Results confirmed the Koch's postulate that the fungal isolate exhibit same symptom of disease in tomato samples (Fig. 3).

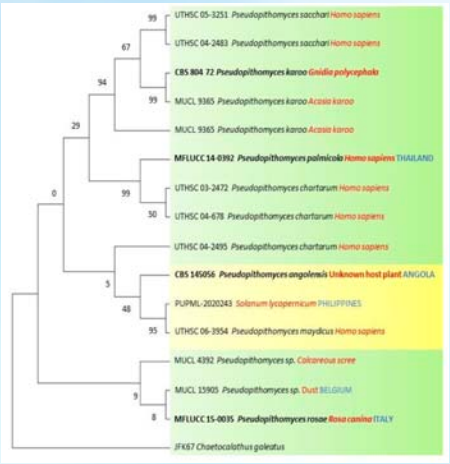

Figure 2. Phylogenetic tree of the PUPML-2020243 grouped with *Pseudopithomyces maydicus*.

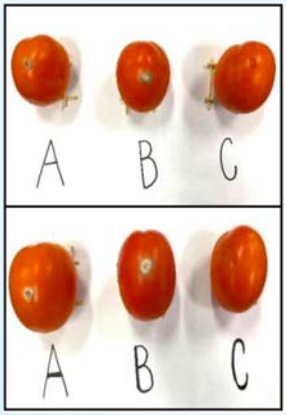

Figure 3. Symptoms of *Pseudopithomyces* disease on tomato fruit after 4 days of inoculation with fungal isolate: A. unwounded; B. wounded tomato fruit; C. Control

DISCUSSION

Based on morphological features, the isolate is consistent with the morphological characters as described by Goh et al. (2020) with *Pseudopithomyces maydicus*. The morphological identification was also congruent to the phylogenetic analysis of its ITS barcode (Goh et al., 2020). However, the use of multigene analysis, which includes the internal transcribed spacer (ITS), and large subunit ribosomal ribonucleic acid (LSU) (Goh et al. 2020; Tennakoon et al. 2016), is necessary for the accurate identification.

The *Pseudopithomyces maydicus* PUPML-2020243 isolate was found to infect the tomato fruit based on the results of the pathogenicity test. The lesion formed by the isolate is comparable to the symptoms of fruit and leaf spot disease caused by *Pseudopithomyces* species.

CONCLUSION

This research showed that the causative fungus of leaf and fruit spots in tomato is a *Pseudopithomyces maydicus*. Moreover, the use of multigene analysis is recommended to confirm the identity of the fungal isolate from this research.

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COMPETING

Bacterial and Fungal Community Profiling of Forest Over Limestome Ecosystem in Basey, Samar, Philippines Using Shotgun Metagenomic Sequencing

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INTRODUCTION

Forests over limestone, or more popularly known as karst ecosystems, are one of the most ecologically fragile ecosystems in the world. These landscapes are threatened by anthropogenic activities and natural processes. Karst ecosystems are typically thin, coarse, highly erosive and degenerative (He et al., 2008). Nearly 25% of the world's population lives in karst areas (Fleury, 2009; Drew, 2017). In the Philippines, it covers about 30,000 km2 or 10% of the country's total land area, and a large portion of it is not yet studied and protected. Karst soils have excessive amounts of exchangeable calcium that limit the growth of vegetation. The adaptive capability of plants to grow in such an environment, partially or completely comes from symbiotic or associative microorganisms (Li et al., 2018). Most research in karst ecosystems is aimed at their geomorphology, but in the past years, microorganisms have become subject of increasing interest.

Soil microorganisms help in recovering and maintaining the health of ecosystems, particularly Karst ecosystems (Chen et al., 2012). Soil microbial communities (SMC) play a key role in release and retention of soil nutrients and soil fertility. These microorganisms also play an essential role in shaping the biodiversity and functioning of terrestrial ecosystems by driving biogeochemical processes and mediating nutrient turnover (Doran and Zeiss, 2000; Bardgett and van der Putten, 2014). Other uses of soil microbes include production of phytohormones, nutrient cycling, detoxification of contaminated soil, biocontrol of soil-borne phytopathogens, and organic matter decomposition. However, soil microbial processes and microbial resource limitation of a karst ecosystem remain poorly understood (Chen et al., 2018). Soil microorganisms also play a

significant role in soil biogeochemical cycling, but their growth and activities are often limited by resource availability. Studying soil processes that are driven by these microorganisms will help elucidate controls on soil fertility and improve the ability to predict the responses of an ecosystem to global changes. Moreover, baseline data generated from this study may provide valuable insight on this ecosystem and may pave the way in long-term monitoring of microbial diversity. Despite the importance of SMC-plant interactions in regulating the structure and function of karst ecosystems, the diversity of SMC in such a landform remains largely unstudied.

The most studied karst terrains are in China, Europe, and the Yucatan Peninsula in Mexico (Santillán et al., 2021). In this paper, we report for the first time the taxonomic diversity and metabolic functions of bacterial and fungal communities present in forest over limestone ecosystems in the Basey, Samar, Philippines, using metagenomic analysis. Taxonomic and functional profile of SMC in a karst ecosystem will potentially accelerate research on natural microbial communities, thereby promoting the adaptive capacity of host plants to abiotic stresses, such as high calcium stress. Karst forests are more prone to rapid degradation processes as compared to non-karst forests, such as soil loss and reduced water holding capacity, resulting in irreversible changes in vegetation cover (Peng et al., 2013 and Tang et al., 2013). Therefore, understanding the microbial community structure is vital for effective vegetation restoration in karst areas. Data from this study will also be valuable for determining which microbial strains can be used for field application in karst topography.

METHODOLOGY

Taxonomic and Functional Profiling

Raw sequences from Illumina Miseq were processed and analyzed using MG-RAST version 4.0.3 (http://metagenomics.anl.gov/). The data was uploaded as FASTAQ files and the paired-ends were joined. The joined paired-end reads were then subjected to the MG-RAST pipeline analysis (Supplementary Figure S2). The uploaded data were preprocessed using SolexaQA to trim low-quality regions from the FASTQ data. De-replication was then performed using a simple k-mer approach to remove artificial duplicate reads (ADRs). These ADRs were then analyzed using duplicate read inferred sequencing error estimation (DRISEE) to determine the degree of variation among prefix-identical sequences in the template. The sequences were screened using Bowtie so that only reads that do not match the model organisms would proceed to the annotation pipeline. Gene calling was performed using FragGeneScan to predict proteins or protein fragments from de novo sequence data. In order to preserve the relative abundances, QIIME was used to build clusters of proteins at the 90% identity level. A representative for each cluster was then subjected to similarity analysis. Instead of using BLAST, functional identification of representative species was done using sBLAT. Sequence similarity searches were then computed against a protein

database derived from the MD5-based non-redundant protein database (M5NR). By doing so, it's possible to use different databases such as COG, KO, NOG, and SEED Subsystems, without recomputation (Keegan et al., 2016). Sequences were annotated with a representative hit annotation technique, which selected a single, unambiguous annotation for each feature. The RefSeq database was used for taxonomic assignment, while COG, KO, NOG, and SEED Subsystems were used for functional assignment. The maximum E-value was 1e-5, minimum sequence identity was 60 %, and the minimum alignment length was 15 bases. Visualization of the data was done using R-studio, Microsoft Excel, and the visualization tools in MG-RAST.

RESULTS AND DISCUSSION

Taxonomic and Functional Profiling

The numbers of sequences affiliated with each bacterial taxon in karst soil were similar across samples. In all areas, Bacteria is the most dominant domain (98%), followed by Eukarya (0.8-1%). Archaeal (0.9-1%) and unclassified sequences (0.001-0.05%) were also present. After analysis of Domain Bacteria, a total of 28 (1 unclassified) phyla, 51 (9 unclassified) classes, 110 (18 unclassified) orders, 244 (36 unclassified) families, and 596 (34 unclassified) genera, were detected.

Based on the RefSeq database, the number of sequences affiliated with each bacterial taxon in the karst soil is almost similar across all karst soil samples from Basey, Samar. The most dominant taxa are Actinobacteria (B1-38.4%, B2-37.2%, B3-32.5%) and Proteobacteria (B1-34.5%, B2-35.4%, B3-37.8%). In all karst soil samples, there is an abundance of Firmicutes (5.8-6.9%), Acidobacteria (3.6-4.3%), Chloroflexi (3.2-3.4%), Planctomycetes (2.7-2.9%), Cyanobacteria (2.6-2.8%), Verrumicrobia (2.1-2.2%), and Bacteriodetes (2.1-2.4%). Minor groups represented at the phylum level included Cyanobacteria, Chlorobi, Nitrospirae, Aquificae, Candidatus Poribacteria, Chrysiogenetes, Chlamydiae, Deferribacteres, Deinococcus-Thermus, Dictyoglomi, Elusimicrobia, Fusobacteria, Gemmatimonadetes, Lentsphaerae, Spirochaetes, Synergistetes, Tenericutes, and Thermotogae. A small percentage of the sequences were unclassified (0.3%).

As for the fungal groups, a total of 6 (1 unclassified) phyla, 18 (1 unclassified) classes, 42 (1 unclassified) orders, 92 (7 unclassified) families, and 132 genera of fungi, were detected. Based on the RefSeq database, the number of sequences affiliated with each fungal taxon in the karst soil were similar across all samples, with a dominance of Ascomycota (86.2-91.6%), and an abundance of Basidiomycota (8.3-13.6%).

Whole community microbial DNA from the topsoil of a karst forest was sequenced, making this study the first metagenomic survey of this type of habitat in the Philippines. From the sequences in each metagenome, metabolic profiles were constructed using COG, NOG, KO, and SEED subsystem database that

compared homology of functional genes against the database and displayed a category of annotated genes with the metagenomic samples. The predicted protein classification showed that metabolism (51%) and poorly characterized proteins (47-48%) were predominant as per COG and NOG database, respectively. Based on KO, metabolism (60-61%) was most abundant, followed by environmental information processing (17-18%), genetic information processing (16%), cellular processes (4%), human diseases (2%), and organismal systems (0.3%). Functional classification by SEED subsystem database predominance by carbohydrate metabolism (13%) and clustering-based subsystem (13%), which was followed by amino acids and derivatives (10%), protein metabolism (8%), miscellaneous (7%), cofactors, vitamins, prosthetic groups, pigments (6%), respiration (5%), membrane transport (4%), DNA metabolism (4%), RNA metabolism (3%), cell wall and capsule (3%), nucleosides and nucleotides (3%), virulence, disease, and defense (3%), fatty acids, lipids, and isoprenoids (3%), stress response (3%), metabolism of aromatic compounds (2%), nitrogen metabolism (1%), phosphorous metabolism (1%), phages, prophages, transposable elements, plasmids (1%), sulfur metabolism (1%), regulation and cell signaling (1%), potassium metabolism (0.9%), cell division and cell cycle (0.9%), iron acquisition and metabolism (0.7%), motility and chemotaxis (0.6%), secondary metabolism (0.3%), dormancy and sporulation (0.2%), and photosynthesis (0.1%).

CONCLUSIONS

The karst forest in Basey, Samar supports a diverse ecosystem as evidenced by the presence of various bacterial and fungal phyla which perform specialized functions in nutrient cycling. This pioneering study on taxonomic and functional diversity of microbial communities was made possible using Illumina sequencing of the karst soil metagenome. The results highlight the significant link between phylogeny and functional potential. For example, the abundance of Streptomyces in the karst soils can be attributed to their capability of inducing calcification through at least one biomineralization pathway as suggested by the presence of enzymes revealed through sequence comparison with KO database. Furthermore, analysis of the metagenome reveals potential metabolic pathways for the metabolism and cycling of carbon, nitrogen, and sulfur.

Future research may focus on elucidating the interaction of these microorganisms with other organisms, such as plants, in the karst area. Studies on how they could affect the survival of other species in the ecosystem may be done. As the current study provides a baseline for the karst metagenome, comparison with other ecosystems may be worth exploring.

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COMPETING

Bacterial Community Profile of the Soil Rhizosphere and Biotechnological Potential of Putative Actinomycetes Isolated from Forest Over Limestone Ecosystem in Paranas, Samar

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INTRODUCTION

Limestone is a hard, sedimentary rock composed of mainly calcium carbonate. Its natural erosion has given rise to a strongly erratic landscape referred to as "karst". The karst areas are known worldwide to have great biological importance due to its rich endemic floral and faunal species. About 10% of the total land surface in the Philippines is karst area (35,000 km^2) and one of the largest karst formations in the country can be found in the Island of Samar (1). In the biosphere, all organisms depend on microbial activities such as the role of soil bacteria in various biogeochemical cycles, cycling of organic compounds, and ecosystem stabilization that are influenced by the physicochemical properties of the soil. However, majority of the microorganisms present in an ecosystem are not cultivable. Hence, the emergence of culture-independent next-generation sequencing approaches, such as shotgun metagenomics, are necessary to study these microorganisms. Culture-dependent methods, on the other hand, only give a minor portion of the soil microbial community but it is still necessary for the possible applications of cultured isolates. One of the major groups of bacteria that are dominantly present in soils is the actinomycetes. They are known to be the most significant source of antibiotics. Aside from antibiotics production, actinomycetes are also recognized for their metabolic diversity producing a wide array of potential industrial enzymes such as lipases (2). Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are lipid-catalyzing enzymes that catalyze several reactions including lipid hydrolysis, esterification, alcoholysis, aminolysis, peroxidation, epoxidation, and interesterification. Lipases are also important in cosmetics, diagnostics, and detergents as well as in treating oils and fats (3).

Thus, the objectives of the study were to assess the soil quality in the karst ecosystem based on its physicochemical properties, analyze the taxonomic

diversity of the bacterial community from the karst ecosystem using shotgun metagenomics, determine the possible roles of the bacterial community in the karst ecosystem through functional profiling; and screen the biotechnological potential of the putative actinomycetes for lipase production.

METHODS

Three kilograms of soil were purposely collected from each of the three sampling points in Paranas, Samar. One kilogram from each of the soil samples collected were sent to the Agricultural Systems Institute, UPLB, for the analysis of the physicochemical properties of the soil samples. Total genomic DNA was directly extracted from the soil samples using NucleoSpin® Soil kit (Macherey-Nagel) following the manufacturer's protocol with some modifications. The extracted DNA was sent to 1st BASE – Apical Scientific in Malaysia though Noveaulab Asia Corporation for metagenomic shotgun sequencing using Illumina MiSeq platform. The raw reads obtained from the metagenome shotgun sequencing were analyzed following the pipeline as described by Keegan et al. (2016) (4). For the cultivation of actinomycetes, serial dilutions up to 10^{-3} were prepared and spread plated in duplicates onto three different isolation media: Starch Casein Agar (SCA), Starch Nitrate Agar (SNA), and Actinomycete Isolation Agar (AIA). Putative colonies of actinomycetes (i.e., compact, leathery, dry surface, covered with aerial mycelia) were selected for purification using Yeast Malt Extract Agar (YMEA). The qualitative screening of the putative actinomycete isolates for lipase production was done using solid medium selection (5). Then, the ratio of halo diameter and colony diameter was computed to determine the Enzyme Activity Index (EAI) of the isolates. The top 5 putative actinomycete isolates with the highest EAI were selected for the quantification of the lipase activity. For the quantitative screening for lipase, titrimetric method was done (6).

RESULTS AND DISCUSSION

To date, this is the first initiative and successful report related to bacterial community profiling using shotgun metagenomics, and isolation and screening of putative actinomycetes from forest over limestone (karst) ecosystem in Paranas, Samar and in the country. The determination of the physicochemical properties revealed that karst soils were good in quality having neutral pH and high organic matter that needs to be maintained for the environment's long-term sustainability. Moreover, this study also proved that in karst soils, phosphorus limitation is common rather than nitrogen limitation. The soils in karst ecosystem were also non-saline with high amounts of exchangeable calcium and magnesium while the amounts of microelements, especially zinc, indicates that karst soils have high moisture content.

The taxonomic profile of the bacterial community revealed that karst soils in Paranas, Samar was dominated by Proteobacteria, Actinobacteria, Firmicutes,

Acidobacteria, and Planctomycetes having relative abundances of 52.68%, 17.91%, 6.46%, 4.27%, and 3.48%, respectively. Moreover, the dominant genera were Mycobacterium (6.78%), Burkholderia (4.73%), Streptomyces (2.44%), Candidatus (Solibacter) (2.37%), and Bradyrhizobium (2.29%). Functional profiling of the bacterial community revealed that Mycobacterium, Burkholderia, Bradyrhizobium, and Candidatus (Solibacter) were the genera primarily involved in carbon and nitrogen metabolism that are important for the healthy functioning of the ecosystem. Moreover, pathways that were highly rampant in karst areas of Paranas, Samar were ABC transport and two-component pathways. Wherein, antibiotic resistance was linked to ABC transporters being controlled by two-component systems (7). Additionally, one of the prevalent pathways in bacteria of karst soils was aminoacyl-tRNA biosynthesis that also play a role in antibiotic production (8). These pathways might be the mechanisms used by the bacterial community in karst ecosystem. Lastly, the enzyme carbonic anhydrase that catalyzes the chemical reactions for karstification was detected in the most dominant genera, Mycobacterium and Burkholderia.

Putative actinomycetes were isolated from karst soils of Paranas, Samar using enrichment techniques. A total of 86 isolates were obtained in which cultural characterization and Gram staining revealed that the isolates were possibly actinomycetes characterized by being filamentous, branching, Gram-positive rods having various pigmentation. The putative actinomycete isolates were screened for lipase production using solid (Sierra's) medium selection and titrimetric assay. There were 50 out of 86 (58%) isolates that exhibited lipolytic activity detected in Sierra's medium. Isolate P79 had a highest Enzymatic Activity Index (6.40 cm) and lipase activity (44 U/min-ml) in solid medium selection and titrimetric assay, respectively, which was higher than the other reported studies (9, 10).

CONCLUSION

The results of this study uncovered that the karst ecosystem in Paranas, Samar have bacterial communities with diverse metabolic pathways determined through shotgun metagenomics. These afford new insights for further exploring the adaptation of bacteria in this environment and the conservation of karst ecosystem. However, to provide insight into the active members of the community, metatranscriptomic studies are needed. Moreover, a total of 86 putative actinomycetes were isolated from the karst ecosystem. Among them, P79 displayed high levels of lipase production that was higher than the reported studies. Further research that will identify the top lipase-producing isolate as well as optimization of its lipase activity and enzyme purification might lead the way to the potential of this isolate to be a source of lipase for biotechnological application.

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COMPETING

Preliminary identification of *Lasiodiplodia pseudotheobromae* causing tomato (*Solanum lycopersicum*) fruit rot in the Philippines

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INTRODUCTION

Tomato is one of the most profitable crops in the Philippines and is widely cultivated throughout the world. It is the 3rd most important vegetable in the country in terms of volume. However, several fungal species cause diseases on both preharvest and postharvest tomato. Fungi are single-celled or very complex multicellular organisms that are ubiquitous, but they mostly live on the land, mainly in soil or on plant material. They play vital roles in all ecosystems as decomposers, symbionts, and pathogens. One of the many pathogenic fungi includes the *Lasiodiplodia* species. It is one of the common plant pathogens found in tropical and subtropical regions. It belongs to the Botryosphaeriaceae family and is characterized by mature and dark-brown conidia with thick walls and longitudinal striations.

OBJECTIVES

This study aims to report the species associated with the fruit rots in tomato. Hence, the study includes: (a) isolation of Lasiodiplodia species from tomatoes with symptoms of rot; (b) identification of Lasiodiplodia isolates by morphology and by ITS (internal transcribed spacer) gene barcoding and (c) confirm the pathogenicity of the isolates obtained.

METHODS

A visit to a tomato farm in Alaminos, Pangasinan was made to collect tissue samples (leaves and stems) from plants exhibiting symptoms of tomato rot disease in January 2020. The causative fungus was isolated from infected tissues using the single-spore method. The isolated conidia were grown on PDA plates to observe the colony morphology. Meanwhile, the morphology of

the conidiogenous cells and conidia were observed in the 7-day culture of the isolate. The pathogenicity of the isolate was tested on the healthy tomato fruit to fulfill Koch's postulate number 4. Phylogenetic analysis was done using the ITS barcode and compared to the reference sequences of Lasiodiplodia species from published literature. Multiple sequence alignment was done using MEGA X ClustalW and Maximum Likelihood in MEGA X was used for the construction of the phylogenetic tree.

RESULTS

The 7th day colony of PUPML-2020248 grown in PDA is fast growing (85 X 88 mm in diameter), appeared greyish white which turns into black as matures, woolly with abundant aerial mycelium; reverse pale yellow to off- white. Conidiophores (6-15 X 3-4 µm) septate, brown, and occasionally branched. Conidiogenous cells pale brown, smooth, and obpyriform. Young conidia (16-26 X 11-15 µm), colorless, hyaline, unicellular or aseptate, oblong-ellipsoid, and thin-walled; matured conidia (22-28 X 13.3-16 µm), dark-brown, one-septate, ellipsoidal, thick-walled with visible longitudinal striations (Fig. 1).

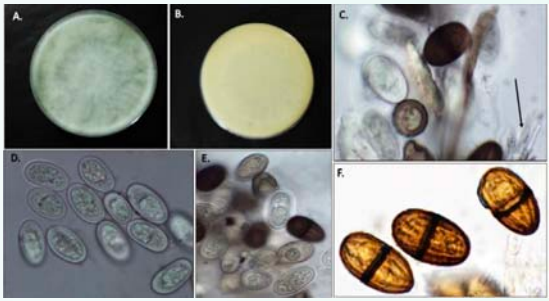

Figure 1. *Lasiodiplodia* sp. PUPML-2020248; 7th day culture (A) Observe (B) Reverse (C) Conidiogenous cells with paraphyses (arrowed) (D) Young conidia having granular, hyaline appearance. (E) developing conidia (F) Mature conidia with visible striations and a thick central septum.

The data from the ITS sequence of the isolate PUPML-2020248 revealed that it is closely related to other Lasiodiplodia species, particularly with *Lasiodiplodia pseudotheobromae* with a bootstrap value of 82% (Fig. 2)

Figure 2. The phylogenetic tree generated from the analysis of ITS sequences of the fungal species together with the reference sequences, constructed by Maximum Likelihood method and Kimura 2- parameter model plus Gamma Distributed with Invariant Sites. Bootstrap values inferred from 1000 replicates are displayed at the nodes. The tree is rooted with Diplodia mutila (CMW 7060). Ex-type cultures are indicated in bold. Isolates are indicated in italics.

The isolate PUPML-2020248, identified as *Lasiodiplodia pseudotheobromae* was inoculated onto fruit of tomato to confirm their pathogenicity (Fig. 3). Three closed containers each containing three healthy tomato fruits, labeled unwounded, wounded (by piercing the skin with disinfected needle) and control were prepared for the isolate. In the pathogenicity test, necrotic lesions were observed on the surface of the tomato fruit. The lesion spread to the whole tomato fruit within 3 to 7 days, resulting in the sogginess of the fruit. Necrotic lesions were observed on wounded tomato fruits, while unwounded fruits and control did not exhibit symptoms.

DISCUSSION

Based on morphological features, the taxon is consistent with the morphological description of L. pseudotheobromae (Dissanayake et al., 2015). The morphological identification was congruent to the phylogenetic analysis of the ITS barcode. However, final identification of the isolate requires a multiple loci sequence analysis which includes the internal transcribed spacer (ITS), β-tubulin (Bt2), and translation elongation factor 1-α (tef1-α) genes (Chen et al., 2021; Bautista-Cruz et al., 2019). The *L. pseudotheobromae* (PUPML-2020248) isolate was found to infect the tomato fruit based on the results of the pathogenicity test. The lesion formed by the isolate is comparable to the symptoms of fruit rot caused by *Lasiodiplodia* species.

Figure 3. Pathogenicity of the *Lasiodiplodia* sp. PUPML-2020248 inoculated on tomato (Solanum lycopersicum) fruit. (a) trial 1. (b) trial 2. (c) trial 3.

CONCLUSIONS

Lasiodiplodia sp. (PUPML-2020248) was identified as *Lasiodiplodia pseudotheobromae* based on the morphological characteristics and phylogenetic analysis using the ITS gene sequence and was responsible for causing diseases on the tomato fruit. However, the phylogenetic tree from ITS gene sequence was insufficient for the identification of species because of the low bootstrap values.

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NON-COMPETING

Screening for Potential Biocontrol Agents Against Banana Fusarium Wilt

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INTRODUCTION

Fusarium wilt of banana (*Musa* spp.), also known as Panama disease, is caused by *Fusarium oxysporum f. sp. cubense* (Foc, E.F. Smith) Snyder and Hansen. To date, it is one of the most deadly fungal diseases of banana that invades the roots then the vascular tissue (xylem), which will cause gradual wilting, progressive yellowing of banana leaves (spreads from leaf margins, and from older leaves to younger leaves), leading to collapse at the petiole, and longitudinal splitting of the outer leaf sheaths in the pseudostem (Yin et al., 2011). In 2006, its appearance in the Philippines was first confirmed in Davao (Daniells, 2011). Recently, 15,700 hectares of the banana plantation had been affected (DA-Davao, 2016).

To increase the pool of potential biocontrol agents against the disease, this study screened potential biocontrol agents against banana Fusarium wilt. Specifically, this study sought to (1) Isolate potential endophytic and rhizospheric bacteria associated with *Musa* sp. cv. Cavendish; (2) Characterize the potential bacterial isolates culturally, morphologically, and biochemically; (3) Determine the anti- fungal activity of bacterial isolates in vitro condition; and (4) Test the plant growth-promoting effects of each potential isolate under nursery conditions.

METHODS

For isolation of putative Endophytic bacteria methodology of Thangavelu & Gopi (2015) was utilized. While for isolation of Rhizospheric bacteria methodology of Gechamba et al (2016) were followed with some modifications. Isolates were maintained in Nutrient agar and morphologically different colonies obtained were sub-cultured on Nutrient Agar slant and Nutrient Agar slant with mineral oil (Thangavelu & Gopi 2015).

Bacterial isolates were first identified using conventional methods. In search of antagonistic bacteria, the Visual agar plate assay of Tan et al., (2015) was followed. The bacterial suspension of isolates was inoculated in the right and left lines 2 cm away from the central line and the width of the upper, middle, and the lower mycelial line were then measured every day up to the 8th day (Tan et al., 2015).

The top seven bacterial isolates characterized preliminarily and showing significant antagonism in vitro against Foc were further identified on the basis of API test results. Identification was performed using API identification kit, API 20E, API 20 NE and API Staph. RS13 was subjected to 16S rRNA analysis for molecular identification.

In the pot assay, Cavendish banana were inoculated with bacterial isolates. All of the experiments were laid out in a randomized block design with ten replications.

RESULTS AND DISCUSSION

Isolation and Characterization of endophytic and rhizospheric bacteria from Musa sp. cv. Cavendish

Isolation of bacteria was carried out from three sources (Musa sp. Cavendish pseudostem, rhizome, and rhizosphere soil). A total of 67 morphologically differentiated colonies were isolated. 70% of which are endophytic bacteria from rhizomes and pseudostem of banana plant and 30% were found from rhizosphere soil. After morphological characterization and Gram staining the 67 colony isolates were resolved to twenty four (24) isolates, of which there are nine (9) endophytic rhizobacteria, seven (7) endophytic pseudostem bacteria, and eight (8) rhizosphere soil bacteria. Most of the isolates have white, small, circular, entire, and raised colonies. Microscopic morphology shows that most of these bacteria are coccus and are either unclustered or clustered. A total of 16 bacteria are identified as Gram negative bacteria and 8 are Gram positive bacteria.

In Vitro Screening of Bacterial Isolates Against Foc

A total of 24 isolates were screened against Foc. At day 2, the width of Foc starts to become visible. At day 4 to day 8, variation in Foc TR4 mycelial width starts to be observed as the mycelia grow nearer to the bacterial isolate lines.
There were statistically significant differences between groups as determined by one-way ANOVA on day 2, day 4, day 5, day 6, day 7, and day at 0.05 level. A Tukey post hoc test revealed that the mycelial width of Foc was statistically significantly inhibited by B. subtilis, RS13, ER03, and ER20 at day 5, 6, 7, and 8 compared to E. coli. There were also statistically significant inhibitions of Foc mycelial width in the presence of ER18 (day 5, 6), ER23 (day 5, 6, 7), ER27 (day 5, 6, 7) and EP03 (day 6,

Furthermore, Tukey post hoc test revealed that there were no significant differences between the antagonistic activity of RS13, ER03, and ER20 at day 4, 5, 6, 7, and 8 compared to B. subtilis. While ER18 (day 4, 5, 6), ER23 (day 4,5,7, 8), and ER27 (day 4,5,7,8) antagonistic activity also showed no significant difference compared to B. subtilis. These showed potential of the isolates as good candidate for biological control agents.

Identification of Potential Antagonistic Bacterial Isolates to Species Level using API

Seven (7) bacterial isolates were identified using their biochemical characteristics through API Tests which shows the characteristics of the isolates to utilize various carbohydrates namely fructose, lactose, maltose, mannitol, mannose, sucrose, trehalose, and xylose. They also have alkaline phosphatase, and capability to utilize various metabolites important for competition against Foc and colonization in the rhizosphere. Among the isolates *Staphylococcus xylosus* (ER03), *Klebsiella ornithinolytic*a (ER18), *Chryseomonas luteola* (ER20), *Pseudomonas fluorescens* (ER23 and ER27), *Kleibsiella pneumoniae* (EP03), and *Staphylococcus sciuri* (RS13) were identified. Further identification reclassified RS13 under Morganella sp. (89%) after 16S rRNA analysis.

In terms of their effects to plant growth, isolates *Staphylococcus xylosus* (ER03), *Chryseomonas luteola* (ER20) and *Staphylococcus sciuri* (RS13) increased significantly the growth of banana plantlets in terms of pseudostem diameter, pseudostem height, and total leaf area compared to the control.

CONCLUSIONS

From the 24 endophytic and rhizospheric bacterial isolates from Musa sp. cv. Cavendish, *Staphylococcus xylosus* (ER03), *Chryseomonas luteola* (ER20) and *Morganella* sp. (RS13) showed robust potential as biocontrol agents against banana Foc. Their characteristics as gram negative bacteria and capability to utilize diverse types of carbohydrates and metabolites are vital for their growth, survival, and efficacy as biocontrol agents in soil and in vivo. Potential isolates also showed their antagonistic activity *in vitro* with significant difference to the negative control and with no significant difference to the commercial biocontrol utilize in the field. These isolates further showed their capacity to induce significant plant-growth in banana plantlets in terms of increasing the pseudostem diameter and height, and the total leaf area of the banana plantlets. These prominent attributes of isolates are important for the eradication of Banana Fusarium wilt and for the survival of banana plants. Thus, these isolates could replenish the pool of effective microbes for suppression of Foc.

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PNMCC President's Report
FY 2021

For year 2021, the PNMCC board embarked on five major projects: 1) 21st year Annual Symposium and General Assembly, 2) 25th year anniversary, 3) Workshop for capacity-building in microbial culture collection, 4) Publication of the Directory of Microbial Strains, and 5) Updating of the PNMCC website.

21st Annual Symposium and General Assembly

Focal Persons: Dr. Ursela T. Bigol and Ms. Dianne L. Dizon

The Board decided to hold the 21st Annual Symposium and General Assembly on four Saturdays of October starting on October 2 via Zoom. This year's theme is: Culture collections in the new normal and beyond. This year's symposium takes into consideration the challenges faced by culture collection amidst the pandemic. Every Saturday has a unique subtheme: Day 1 (October 2, 2021) – Overview of Type Collections, Day 2 (October 9, 2021) – Network and Linkages, Day 3 (October 16, 2021) – Viruses, and Day 4 (October 23, 2021) – Actinomycetes and Microbial References. The symposium features a keynote speech by Dr. Rosario G. Monsalud, the head of the Philippine National Collection of Microorganisms, and nine other lectures to be given by experts in microbiology and culture collections. There are also eleven (11) poster paper presentations which can be viewed in our website, www.pnmcc.org. The official PNMCC newsletter will also be released during the symposium.

This year, the PNMCC revitalizes its linkage with the World Federation of Culture Collections (WFCC). On day 2 of the symposium, experts from the WFCC led by the President, Dr. Ipek Kurtboke, will share their expertise and experiences to the participants. We are hoping for more fruitful collaborations with the WFCC in the future.

PNMCC Board Meeting
June 26, 2021

PNMCC @ 25

Focal Persons: Dr. Marilen P. Balolong, Mary Christine Cada and Nik Shawn Tabao

This year, the PNMCC is celebrating its 25th year, having been established in 1996. This is PNMCC's silver anniversary, thus, we have unveiled Pilak: PNMCC@25 (Pilak is Filipino term for silver). With the help of past presidents of PNMCC: Dr. Auxilia T. Siringan, Dr. Rosario G. Monsalud, Dr.

Gina R. Dedeles and the past presidents who are still part of the current PNMCC board, we were able to prepare videos on the history of PNMCC, compiled the list of officers from 2001 up to present, and collected messages from the founders and past presidents of the network. As the PNMCC celebrates its 25th year, we embark on significant projects such as: strengthening our links with our affiliate culture collections, publication of the updated directory of microbial strains of all our affiliate culture collections, and the conduct of the workshop for capacity-building of existing and future culture collections.

Workshop for capacity-building in microbial culture collections
Focal Persons: Dr. Marian P. de Leon and Dr. Esperanza C. Cabrera

In order to build the capacity of those who are interested to establish a new culture collection, the PNMCC will conduct a workshop entitled "Setting Up Microbial Culture Collections: Opportunities and Challenges. This is a 4-day workshop to be held on November 16-19, 2021. The target participants of said workshop are microbiologists in institutions who are planning to establish a culture collection. The workshop will tackle topics related to planning and establishing a culture collection including biorisk management, facility design, biosafety cabinets, waste and biological spill Management, good laboratory practices, preservation methods, ISO 9001 and ISO 17025. The workshop will give the participants an opportunity to assess the needs of their institution and plan for the establishment of their own culture collection. PNMCC will provide continuous technical support to all participants so that more culture collections will be established in the Philippines specially in Visayas and Mindanao.

Publication of the Directory of Microbial Strains
Focal Persons: Eldrin DLR. Arguelles, Dr. Loida R. Medina, Dr. Howell T. Ho, and Dr. Marian De Leon

Meeting with curators of affiliate culture collection *Sept. 6, 2021*

Last September 6, 2021, members of the PNMCC Board had a meeting with curators of our affiliate culture collections: Philippine National Collection of Microorganisms (PNCM), Microbial Culture Collection of the Museum of Natural History (MCC-MNH), UP Natural Sciences Research Institute Culture Collections (UPCC), University of Santo Tomas Research Center for Natural

and Applied Sciences-Collection of Microbial Strains (UST-CMS), Industrial Technology Development Institute Microbial Culture Collection (ITDI-MCC), Unilab Clinical Culture Collection, and Polytechnic University of the Philippines Microbial Culture Collection. In the meeting, it was agreed that all affiliate culture collections shall update their directory of microbial strains so that these can be published by PNMCC. This will make it easier for students, researchers and scientists to locate particular strains of microorganisms that they need for their experiments.

Furthermore, the curators of the different culture collections also agreed to harmonize their procedures in different aspects of culture collection such as procedures for receiving and releasing microbial strains, authentication of the identification of microorganisms and others. These activities will be started this year so that all affiliate culture collections of PNMCC will follow the same protocols.

Updating of website

Focal Persons: Carlo Chris S. Apurillo and Joel C. Cornista

This year, the PNMCC's website went back online at www.pnmcc.org. The website contains important information about the organization, news and updates and dedicated section on important events such as the 21st Annual Symposium. At present, the poster paper presentations and the souvenir program are posted in the website so that the public can view them.

The most important part of the website is the searchable database of the Directory of Microbial Strains. This will help students, researchers, professionals and scientists easily locate strains of microorganisms that are needed for their studies and various applications.

Despite the pandemic, the PNMCC never wavered in its commitment to advance the knowledge, skills and practices in microbial culture collections. On a personal note, I would to thank the members of the board of directors of PNMCC for year 2021 for their support and hard work which enabled us to plan and implement all of these activities.

CARLO CHRIS S. APURILLO, RMT, M.Sc.
President

PNMCC *through the Years*

Date	Theme of Symposium	Workshop
27 Oct 2001	Conserving our Microbial Resources	
26 Oct 2002	Trend, Issues and Concerns in Culture Collection	
18 Oct 2003	Culture Collection 101	Aseptic techniques Culture collection techniques Culture collection techniques Endomycorrhizal fungi
13 Nov 2004	Practical Microbiology	Lactic acid bacteria and their uses (yoghurt-and –cheese-making) Trichoderma for agricultural waste degradation
19 Nov 2005	Microbial Wonders in Special Environments	Simple culture preservation techniques L-drying
18 Nov 2006	Microbes: Friends and Foes	
10 Nov 2007	Microbial Diversity in Mangrove Ecosystems	Specimen viewing of mangrove bacteria and marine fungi
22 Nov 2008	Getting to Know your Hospital Microbes	API/Vitek identification system Antimicrobial assays Specimen viewing of key microbial groups
21 Nov 2009	A Closer Look at Fungal Diversity	Fungal specimen viewing Identification of clinical fungal isolates
13 Nov 2010	Linking Microbial Systematics with Biotechnology	
19 Nov 2011	Inovations in Microbial Biobanking: Biosafety and Biosecurity	Identification of mitosporic fungi

Date	Theme of Symposium	Workshop
24 Nov 2012	Linking Microbial Taxonomy to Education and Industry	Molecular Techniques for Microbial Identification
17 Nov 2013	Marine Fungi: Systematics and Applications	Marine Fungi: Systematics and Applications
4 Oct 2014	The World of Microalgae	The World of Microalgae
3 Oct 2015	Back to Basics: Preparing for K-12	Hands-on Training on Basic Microbiology Techniques
19 Nov 2016	Rapid Detection of Clinically Important Microbes using Molecular Techniques	Rapid Detection of Clinically Important Microbes using Molecular Techniques
11 Nov 2017	Probiotics: Your Healthy Option	Basic Molecular Techniques for Medical Technologists
10 Nov 2018	#TrendingViralDiseases	
9 Nov 2019	Innovations and Challenges in Microbial Culture Collection Curation	Training on Basic Microbial Culture Preservation
3, 10, 17, 24 Oct 2020	3Cs: Culture Collections and COVID-19	

PNMCC OFFICERS

2001

President	Ma. Auxilia T. Siringan
Vice-President	Rosario G. Monsalud
Secretary	Charina Gracia B. Banaay
	Marilen M.. Parungao
Auditor	Sonia SP. Bulaong
PRO	Marian Pulido
Business Manager	Teresita S. Ventura
Adviser	Prof. William L. Fernandez

2002

President	Ma. Auxilia T. Siringan
Vice-President	Rosario G. Monsalud
Secretary	Ms. Vina B. Argayosa
Treasurer	Charina Gracia B. Banaay
Auditor:	Evangeline T. Castillo
PRO:	Mary Ann G. Santos
Business Manager	Marian A. Pulido
Board Members	Teresita S. Ventura
	Belen B. Mercado
Adviser	Prof. William L. Fernandez

2003

President	Ma. Auxilia T. Siringan
Vice-President	Rosario G. Monsalud
Secretary	Vina B. Argayosa
Treasurer	Marilen M. Parungao
	Marie Antonette Ruth V. Guerra
Auditor	Noel G. Sabino
PRO	Manuel V.A. Bravo
Business Manager	Marian Pulido
Board Members:	Milagrosa M. Goss
	Belen B. Mercado
Adviser	Prof. William L. Fernandez

2004

President	Ma. Auxilia T. Siringan
Vice-President	Rosario G. Monsalud
Secretary	Ms. Vina B. Argayosa
Treasurer	Mylele L. Bool
Auditor	Evangeline T. Castillo
PRO	Mary Ann G. Santos
Business Manager	Noel G. Sabino
Board Members	Gina R. Dedeles
	Manuel V.A. Bravo
Adviser	Prof. William L. Fernandez

2005

President	Rosario G. Monsalud
Vice-President	Ma. Auxilia T. Siringan
Secretary	Noel G. Sabino
Treasurer	Mylele L. Bool
Auditor	Gina R. Dedeles
PRO	Elizabeth L. Tenorio
Business Manager	Manuel V.A. Bravo
Board Members	Mary Ann G. Santos
	Vina B. Argayosa
Adviser	Prof. William L. Fernandez

2006

President	Rosario G. Monsalud
Vice-President	Ma. Auxilia T. Siringan
Secretary	Vina B. Argayosa
Treasurer	Noel G. Sabino
Auditor	Gina R. Dedeles
PRO	Elizabeth L. Tenorio
Board Members	Delia dC. Ongtenco
	Mary Ann G. Santos
	Manuel V.A. Bravo
Adviser	Prof. William L. Fernandez

2007

President	Ma. Auxilia T. Siringan
Vice-President	Delia dC. Ontengco
Secretary	Vina B. Argayosa
Treasurer	Rosario G. Monsalud
Auditor	Esperanza C. Cabrera
PRO	Thomas Edison E. dela Cruz
Board Members	Gina R. Dedeles
	Reynaldo Yago
	Manuel V.A. Bravo
Adviser	Prof. William L. Fernandez

2008

President	Delia dC. Ontengco
Vice-President	Rosario G. Monsalud
Secretary	Thomas Edison E. dela Cuz
Treasurer	Irene Papa
Auditor	Esperanza C. Cabrera
Business Manager	Maria Auxilia T. Siringan
PRO	Anthony C. Lee

Board Members	Vina B. Argayosa
	Gina Dedeles
	Marian Pulido
Adviser	Prof. William L. Fernandez

2009

President	Rosario G. Monsalud
Vice-President	Delia dC Ontengco
Secretary	Vina B. Argayosa
Treasurer	Marian A. Pulido
Auditor	Esperanza C. Cabrera
Business Manager	Maria Auxilia T. Siringan
PRO	Anthony C. Lee
Board Members	Thomas Edison E. Dela Cruz
	Gina R. Dedeles
Adviser	Prof. William L. Fernandez

2010

President	Thomas Edison E. dela Cruz
Vice-President	Gina R. Dedeles
Secretary	Paul Richard J. Yulo
Treasurer	Rosario G. Monsalud
Auditor	Esperanza C. Cabrera
Business Manager	Maria Auxilia T. Siringan
PRO	Anthony C. Lee
Board Members	Marilen M. Parungao
	Marian P. De Leon
	Noel G. Sabino

2011

President	Gina R. Dedeles
Vice-President	Marilen M. Parungao-Balolong
Secretary	Paul Rodrigo E. Cordero
Treasurer	Rosario G. Monsalud
Auditor	Joel C. Cornista
Business Manager	Mark A. Ritumalta
PRO	Marian A. Pulido-De Leon
Board Members	Raul V. Destura
	Paul Richard J. Yulo
	Nikki Heherson A. Dagamac

2012

President	Rosario G. Monsalud
Vice-President	Gina R. Dedeles
Secretary	Sittie Aisha B. Macabago
Treasurer	Lydda L. Masangcay

Auditor	Joel C. Cornista
Business Manager	Mark Noe A. Ritumalta
PRO	Marilyn B. Brown
Board Members	Raul V. Destura
	Percival G. Garcia
	Marian A. Pulido-de Leon

2013

President	Gina R. Dedeles
Vice-President	Delia C. Ontengco
Secretary	Thaddeus M. Carvajal
Treasurer	Rosario G. Monsalud
Auditor	Joel C. Cornista
PRO	Marian P. de Leon
Board Members	Marilyn B. Brown
	Evangeline T. Castillo
	Dianne L. Dizon

2014

President	Marian P. de Leon
Vice-President	Thomas Edison E. dela Cruz
Corresponding Secretary	Geraldine B. Dayrit
Recording Secretary	Carlo Chris S. Apurillo
Treasurer	Rosario G. Monsalud
Auditor	Delia DC. Ontengco
Business Manager	Gina R. Dedeles
PRO	Joel C. Cornista
Board Members	Marilyn B. Brown
	Dianne L. Dizon
	Howell T. Ho

2015

President	Joel C. Cornista
Vice-President	Thomas Edison E. dela Cruz
Corresponding Secretary	Geraldine B. Dayrit
Recording Secretary	Mary Christine Cada
Treasurer	Marian P. de Leon
Auditor	Rosario G. Monsalud
Business Manager	Gina R. Dedeles
PRO	Carlo Chris S. Apurillo
Board Members	Marilyn B. Brown
	Delia DC. Ontengco
	Dianne L. Dizon

2016

President	Joel C. Cornista
Vice-President	Marian P. de Leon
Corresponding Secretary	Geraldine B. Dayrit
Recording Secretary	Louella D. Labasbas
Treasurer	Rosario G. Monsalud
Auditor	Delia DC. Ontengco
Business Manager	Gina R. Dedeles
PRO	Carlo Chris S. Apurillo
Board Members	Marilyn B. Brown
	Dianne L. Dizon

2017

President	Geraldine B. Dayrit
Vice-President	Ursela G. Bigol
Corresponding Secretary	Dianne L. Dizon
Recording Secretary	Louella D. Labasbas
Treasurer	Rosario G. Monsalud
Auditor	Delia DC. Ontengco
Business Manager	Joel C. Cornista
PRO	Carlo Chris S. Apurillo
Board Members	Esperanza C. Cabrera
	Gina R. Dedeles
	Marian P. De Leon

2018

President	Ursela P. Guce-Bigol
Vice-President	Marilen P. Balolong
Corresponding Secretary	Dianne L. Dizon
Recording Secretary	Louella D. Labasbas
Treasurer	Marian P. de Leon
Auditor	Delia DC. Ontengco
Business Manager	Joel C. Cornista
PRO	Carlo Chris S. Apurillo
Board Members	Esperanza C. Cabrera
	Gina R. Dedeles
	Eldrin DLR. Arguelles

2019

President	Marilen P. Balolong
Vice-President	Dianne L. Dizon
Corresponding Secretary	Kim Hazel V. Arafiles
Recording Secretary	Kimberly D. Neri
Treasurer	Marian P. de Leon
Auditor	Eldrin DLR. Arguelles
Business Manager	Loida R. Medina
PRO	Carlo Chris S. Apurillo

Board Members	Esperanza C. Cabrera
	Joel C. Cornista
	Delia DC. Ontengco

2020

President	Dianne L. Dizon
Vice-President	Carlo Chris S. Apurillo
Corresponding Secretary	Loida R. Medina
Recording Secretary	Reuel M. Bennett
Treasurer	Marian P. de Leon
Auditor	Eldrin DLR. Arguelles
Business Manager	Ursela P. Bigol
PRO	Nik Shawn Tabao
Board Members	Esperanza C. Cabrera
	Marilen P. Balolong
	Joel C. Cornista

2021

President	Carlo Chris S. Apurillo
Vice-President	Ursela P. Bigol
Corresponding Secretary	Dianne L. Dizon
Recording Secretary	Mary Christine Cada
Treasurer	Marian P. de Leon
Auditor	Eldrin DLR. Arguelles
Business Manager	Joel C. Cornista
PRO	Nik Shawn Tabao
Board Members	Esperanza C. Cabrera
	Marilen P. Balolong
	Howell T. Ho

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Early years of PNMCC

1996 Management Board (L-R): Marilen Parungao, Belen Mercado, Marian Pulido, Prof. William Fernandez, Rosario Monsalud, Auxilia Siringan, Prof. Priscilla Sanchez

1st PNMCC Symposium: *Conserving our Microbial Resources October 27, 2001 UP Diliman, Quezon City*

2nd PNMCC Symposium: *Trends, Issues and Concerns in Culture Collection October 26, 2002*

Photos contributed by: Dr. Maria Auxilia T. Siringan

4th PNMCC Symposium and Workshop
University of Santo Tomas






9th PNMCC Symposium and Workshop
De La Salle University



Philippine Network of Microbial Culture Collections, Inc. (PNMCC)

STATUTES AND BY-LAWS

ARTICLE I
NAME

The name of the organization shall be Philippine Network of Microbial Culture Collections Inc. (PNMCC Inc.).

ARTICLE II
STRUCTURE

The Network was organized through the sponsorship of DOST-PCAS-TRD (currently known as DOST-PCIEERD) and initially composed of the following culture collections: Philippine National Collection of Microorganisms, UPLB, College, Laguna; UP Culture Collection, Natural Sciences Research Institute, UP Diliman, Quezon City; Bacterial Germplasm Collection, International Rice Research Institute, College, Laguna; and Microbial Culture Collection, Museum of Natural History, UPLB, College, Laguna and hereby referred to as the Network.

ARTICLE III
HEADQUARTERS

The official headquarters of the Network shall be at the Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines at Los Banos (UPLB), College, Laguna. A satellite office may be designated by the Board as the need arises.

ARTICLE IV
AFFILIATION

Any institutional microbial culture collection irrespective of size or geographical location within the Philippines may seek affiliation with the PNMCC.

ARTICLE V
OBJECTIVES

The general objectives of the Network shall be:

a.To provide a permanent secretariat for member Philippine microbial culture collections and serve as a central contact point for Philippine scientists and any institution seeking advice and information on microbiological materials and on culture collection-related matters;

b. To establish an effective liaison between individuals and organizations

concerned with culture collections and among the users of the cultures;

c.To collect information on the strains and services offered by the various affiliated microbial culture collections;

d. To publicize the microbial resources and technical expertise within the affiliated microbial culture collections through printed and visual materials for distribution;

e. To lead in the standardization and upgrading of procedures for the isolation, characterization, conservation, distribution of microorganisms and biosafety through trainings and seminars;

f. To maintain and update PNMCC Directory of Strains that include location of, and information about, microorganisms maintained in affiliated microbial culture collections.

ARTICLE VI
ACTIVITIES

The activities of the Network shall include the Annual General Assembly Meeting and Symposium, seminars, trainings, workshops and other relevant activities as deemed necessary by the Board and/or members.

ARTICLE VII
BOARD OF DIRECTORS

Section 1. The Board of Directors hereby, referred to as the "Board", shall be the managing body and shall consist of eleven (11) members as follows: seven (7) officers and three (3) board members elected by the General Assembly and one Recording Secretary appointed by the President. The Board shall promote the objectives of the Network as defined in ARTICLE V hereof.

Section 2. Qualifications. The officers to be elected must be of legal age and members of the Network. The composition of the Board shall preferably reflect the various interests of the membership of the Network.

Section 3. Disqualification. No member convicted by final judgment of an offense punishable by imprisonment for a period exceeding six (6) years or a violation of the Corporation Code of the Philippines committed within (5) years prior to the date of his/her election or appointment, shall qualify as an officer.

Section 4. Composition of the Board. The Board shall consist of the following: President, Vice-President, Corresponding Secretary, Recording Secretary, Treasurer, Auditor, Business Manager and Public Relations Officer (PRO) and three board members. They shall be elected by the general membership during

the General Assembly Meeting except the Recording Secretary who shall be appointed by the President. The positions shall be determined by the newly elected Board members.

In the case of the Vice-President, he/she becomes the President for the following year.

Section 5. Election. Members of the Board shall be elected by plurality vote of members present during the Annual General Assembly Meeting.

Section 6. Term of Office. The word "term" refers to the time elapsing between successive regular General Assembly Meetings. All officers of the Network shall hold office for one term or until their successors are duly elected/appointed.

Section 7. Vacancies. Vacancies, which may occur in-between meetings of the General Assembly, may be filled from the general membership at the discretion of the remaining members of the Board through a resolution.

ARTICLE VIII
FUNCTIONS AND POWERS OF THE BOARD

Section 1. President. The President shall be the Chief Executive Officer of the Network. He/she shall preside at the meetings of the General Assembly of the Network and at the meetings of the Board.

The President shall execute all resolutions of the Board. He/she shall direct and oversee the activities of the Network. He/she shall present to the general membership a complete report of the activities and operations of the Network at the close of the fiscal year.

Section 2. Vice-President. The Vice-President shall, in the absence of the President, perform the duties and exercise the powers of the President, and shall perform duties that may be assigned by the Board.

Section 3. Corresponding Secretary. The Corresponding Secretary shall give all notices required by these by-laws. The Secretary shall keep the seal of the Network and affix such seal to any paper or document requiring the same. He/she shall update the members' register and the correspondence files of the Network. He/she shall also perform all other duties as may be assigned by the Board.

Section 4. Recording Secretary. The President shall appoint a Recording Secretary with the following functions: to record and keep the minutes of the meetings of the Board and the General Assembly; to keep all the records, documents, and inventories of the properties of the Network; to assist the Corresponding Secretary in keeping an updated roster of members; and to co-chair with the Corresponding

Secretary in the Secretariat and Registration Committees of the Network's activities.

Section 5. Treasurer. The Treasurer shall be responsible for the administration of the finances of the Network. He/she shall be authorized to receive monies in behalf of the Network and to pay such debts as may be incurred in the work of the Network from time to time. He/she shall keep and have charge of the books of accounts. He/she shall furnish and submit a financial report to the auditor a month after the last activity prior to submission to an external auditor commissioned by the Board. He/she shall present an audited balance sheet to the General Assembly during its annual meeting. The Treasurer shall keep all monies and other valuables of the Network in banks as designated by the Board. He/she shall also perform other duties as may be assigned by the Board.

Section 6. Auditor. The Auditor shall audit the finances and shall furnish an audited balance sheet to the Treasurer within a month after its receipt. He/she shall also perform other duties and work as may be assigned by the Board.

Section 7. Public Relations Officer (PRO). The PRO shall be generally responsible for the maintenance of effective liaison between the Board and the members of the Network. He/she shall be in charge of the promotion of the activities of the Network. He/she shall take charge of the publication of the Network's newsletters, bulletins and other similar information materials. He/she shall also perform other duties as may be assigned by the Board.

Section 8. Business Manager. The Business Manager shall contact prospective subscribers and advertisers for the Network's publication. He/she shall coordinate fund-raising activities of the Network. He/she shall also perform other duties as may be assigned by the Board.

Section 9. Board Members. They shall perform duties assigned by the Board.

ARTICLE IX
SIGNATURES AND CERTIFICATION

Section 1. Contracts and Legal Documents. Contracts and legal documents, other than those involved in the payment of accounts or other disbursement of funds, shall be signed by the President and another elected member, after approval of the Board.

Section 2. Checks. Payment of accounts or other disbursement of Network funds shall bear the signature of the Treasurer and countersigned by the President.

ARTICLE X
MEETINGS

Section 1. General Assembly Meeting. The General Assembly Meeting shall be held annually preferably in November at a time and place determined by the Board. The President and Treasurer shall present their annual and financial reports, respectively, to the members. The election of the Board shall also be held during this meeting.

Section 2. Special Meetings of the Members. Special meetings of the members shall be called, as the need arises, by the Board or the President or upon petition of 1/3 of the general membership.

Section 3. Regular Board Meetings. The Board shall hold quarterly meetings at the time and place determined by the Board.

Section 4. Special Board Meetings. The Board shall hold special meetings as needed at the time and place determined by the Board.

Section 5. Notices. Notices of the time and place of the General Assembly meeting shall be given through email, PNMCC website and/or social media platforms, at least three months before the date set for such a meeting. Notices for special meetings shall be given at least two (2) weeks before the date of the meeting. The notice of every special meeting shall state briefly the purpose or purposes of the meeting

ARTICLE XI
MEMBERSHIP

Section 1. Types of Membership and Qualifications. Membership in the PNMCC shall be of three types: ordinary, affiliate, and sustaining. Ordinary membership shall be open to any individual interested in culture collections. Membership status may be upgraded from regular to life membership. Regular members (for at least one year) are allowed to apply for life membership and pay a corresponding fee. Affiliate membership shall be open to culture collections, to be represented by an individual, usually the curator or equivalent. Admission as affiliate members shall be determined by the Board. Sustaining membership shall be open to individuals or organizations who espouse the cause of the Network. Sustaining members shall have the rights and privileges of the Network, except the rights to vote and to hold office.

Membership shall be on a national basis. There shall be no restriction on

the number of members from any one institution or organization.

Section 2. Subscriptions and Donations. The subscription for membership to the Network shall be determined from time to time by the General Assembly of the Network, on advice from the Board. A minimum subscription rate shall be defined for any particular period. Members may elect to subscribe above the minimum rate according to their individual means.

The Network may receive donations, which shall be forwarded to the Treasurer of the Network.

Section 3. Rights and Privileges of Members. All members shall have the following rights and privileges.

a. Participation in the affairs of the Network, except where otherwise stated in the Statutes and By-Laws;
b. Right to vote on all matters relating to the affairs of the Network;
c. Eligibility to any elective or appointive office of the Network;
d. Participation in all the General Assembly and special meetings of the members;
e. Availment of the newsletter and publications of the Network. These shall be received either gratis or at a privileged rate to be determined by the financial status of the Network;
f. Examination of all the records or books of the Network during business hours.

Section 4. Duties and Responsibilities of the Members. A member shall have the following duties and responsibilities.

a. Compliance with the Statutes and By-laws, rules, and regulations promulgated by the Network;
b. Attendance to all meetings of the Network;
c. Payment of membership dues and other assessments of the Network;
d. Refraining from using his/her connection with the Network to further the interest of his/her, or any other, organization except as provided for in the Statutes.

Section 5. Application for Membership and Notices of Resignation. Applications for membership or notices of resignation shall be made in writing to the Corresponding Secretary of the Network. Applications for membership shall be accompanied by information provided on a form prescribed by the Network and obtainable from the office of the Secretary of the Network.

Section 6. Suspension, Expulsion, and Termination. Suspension, expulsion, and termination of membership shall be in accordance with the rules and regulations of the Network.

Any member of the Network may file charges against a member by filing a written complaint with the Secretary of the Network. The Board shall call a special meeting to consider the charges. The affirmative vote of all the board members shall be necessary to suspend a member. Where the penalty is expulsion, the affirmative vote of the majority of the general membership shall be required.

ARTICLE XII
APPEALS

Appeals against any decisions of the Board or Network may be made in writing to the Secretary by a minimum of twenty-five percent of the general membership. Recommendations on such appeals shall be made by the Board to the General Assembly.

ARTICLE XIII
COMMITTEES

The Board shall establish such committees as may be requested from time to time by the General Assembly. The Board shall have the power to determine the terms of reference of such committees unless otherwise defined by the General Assembly, and shall have the power to dissolve any committees when, in the opinion of the Board, the task of the committee has been accomplished or if the committee has ceased to function effectively. In the latter case, the Board may, at its discretion, reconstitute the committee and report its action to the next meeting of the General Assembly.

ARTICLE XIV
FUND

Section 1. Funds. The funds of the network shall be derived from membership fees, annual dues, registration fees, sponsorships, advertisements and donations.

Section 2. Disbursements. Withdrawal from the funds of the Network, whether by check or any other instruments shall be signed by the Treasurer and countersigned by the President as stated in ARTICLE IX, Section 2. If necessary, the Board may designate other signatories.

Section 3. Fiscal Year. The fiscal year of the Network shall be from January to December of the following year.

ARTICLE XV
CORPORATE SEAL

The corporate seal of the Network shall be in such form and design as may be determined by the Board.

ARTICLE XVI
AMENDMENTS TO THE STATUTES AND BY-LAWS

These Statutes and By-laws, or any provision thereof, may be amended or repealed by a majority vote of the general membership at any regular or special meeting duly held for the purpose.

PNMCC Affiliate Culture Collections

Philippine National Collection of Microorganisms (PNCM)
PNMCC Headquarters
National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines, Los Baños, Laguna
Represented by: **Dr. Rosario G. Monsalud**

UP Natural Sciences Research Institute Culture Collection (UPCC)
Natural Sciences Research Institute, Miranda Hall
University of the Philippines, Diliman, Quezon City
Represented by: **Dr. Ma. Auxilia T. Siringan**

Microbial Culture Collection of the Museum of Natural History (MCC-MNH)
University of the Philippines, Los Baños, Laguna
Represented by: **Dr. Marian P. De Leon**

Industrial Technology Development Institute Microbial Culture Collection (ITDI-MCC)
DOST Compound, Taguig, Metro Manila
Represented by: **Dr. Ursela P. Guce-Bigol**

UST Research Center for the Natural and Applied Sciences – Collection of Microbial Strains (UST-CMS)
University of Santo Tomas, España, Manila
Represented by: **Dr. Kim Hazel Arafiles**

UNILAB Clinical Culture Collection
66 United St., Mandaluyong City
Represented by: **Dr. Cleofe Calanasan**

Polytechnic University of the Philippines Microbial Culture Collections
Represented by: **Dr. Gary A. Lirio**

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- Protein Purification and Crystallography
- Hematology Kits
- General Molecular Biology Buffers
- Buffers for NA Electrophoresis
- Buffers for Protein Electrophoresis
- Molecular Biology Growth Media
- DNA Polymerase
- HiPer Teaching Kits
- Latex Agglutination Kits

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