Biochemical and Molecular Characterization of collected microbes in relation to virulence and/or their applications

Jocelyn T. Zarate
BIOTECH, UPLB
Proj 4 Forest CANOPI Program

Project 4. Forest Canopy Monerans (Prokaryotes) and Fungi

Program Title: Forest Canopy Observation, Positioning and Investigation (Forest CANOPI) Program: Developing Forest Canopy Science in the Philippines

Proj Leader: Jocelyn T. Zarate, PhD - BIOTECH, UPLB (Flora)
Study leader: Jessica F. Simbahan,PhD–BIOTECH-UPLB (Flora)
Proj Res. Asst. IV Justin Godfred B. Peralta, MsC
Proj Res Asst III Kristelle Anne T. Angeles, BS Biology
Laborer I Joseph D. Manalo

Study leader Y2: Lucille Villegas, PhD – IBS, CAS, UPLB (Fauna)
Study leader Y2: Noel Sabino, PhD- IBS, CAS, UPLB (Fauna)

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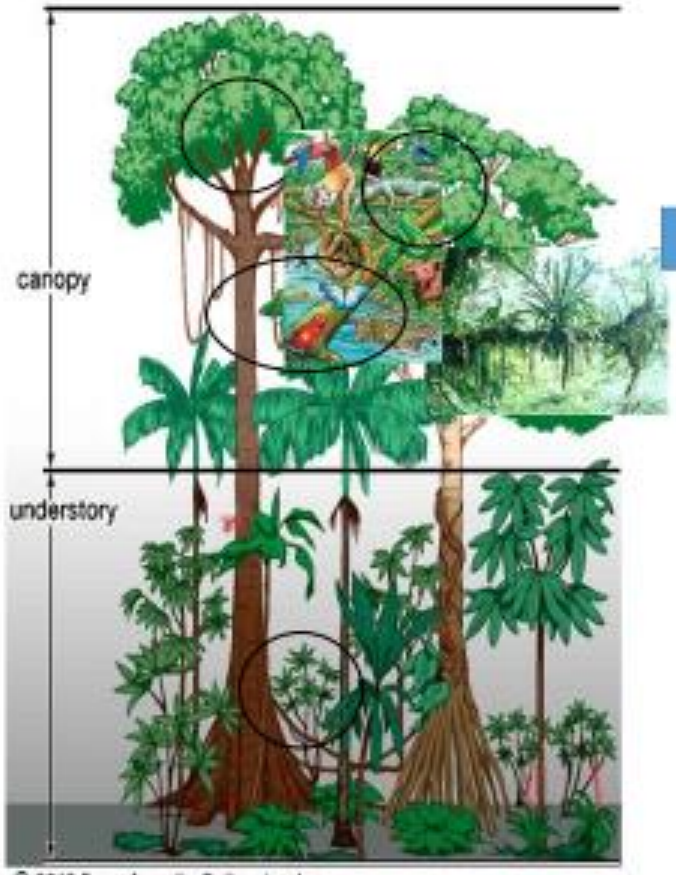
Objectives:

- Assess microbial diversity in flora, soil and fauna associated with forest canopy
- Collect, isolate, purify and preserve microbial strains from the forest canopy
- Determine microbial community diversity as affected by source, landscape and other factors

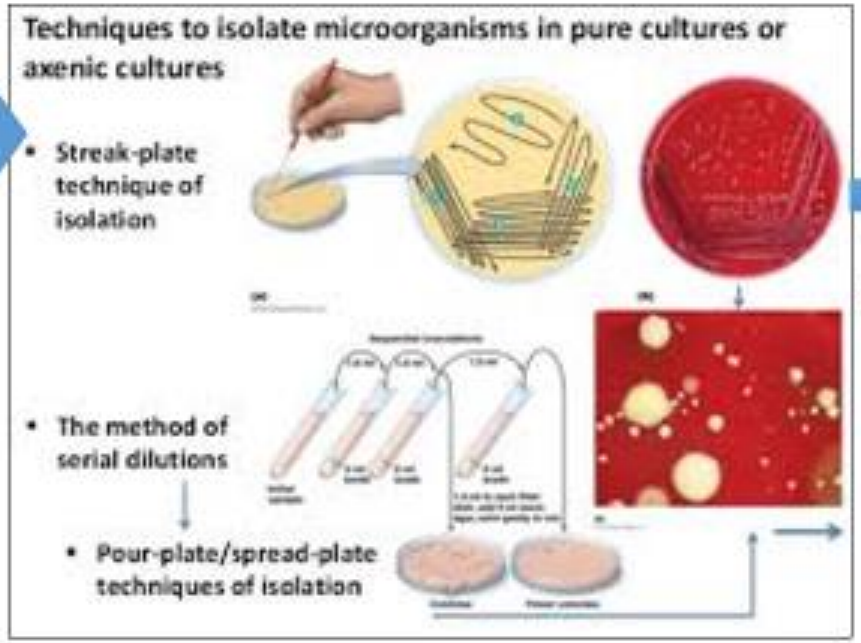
Methods:

- Culture dependent - isolation, culture, identification and preservation of microbial strains (serial dilution and plating)
  - microbial community analysis thru biochemical reaction to growth media (BIOLOG Ecoplate)
- Culture-independent- molecular approach to microbial community analysis (DGGE)

Project 4: Forest Canopy Monerans and Fungi



Culture-dependent Method (Culturing Method)



Mixed Plate Culture

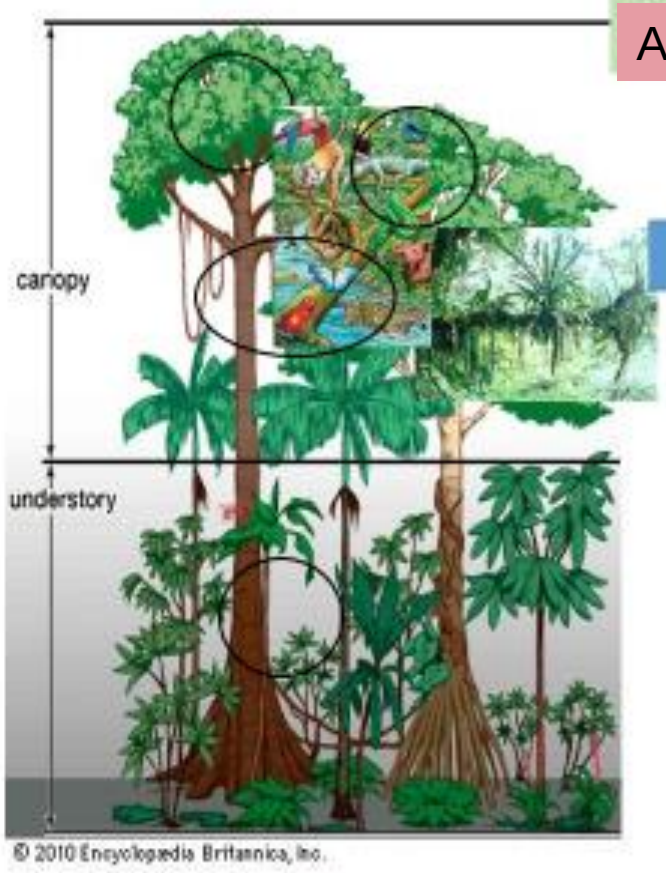
Pure Culture

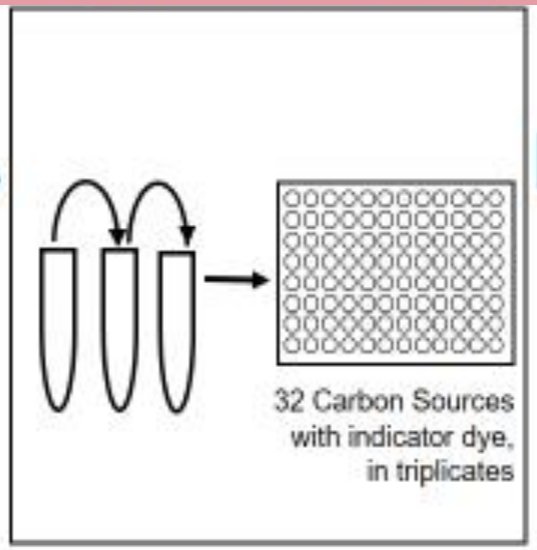
Preserved culture

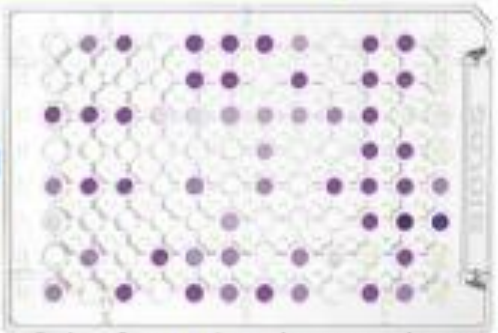
Project 4: Forest Canopy Monerans and Fungi

Culture-dependent Method Microbial community

Assay using BIOLOG Ecoplate

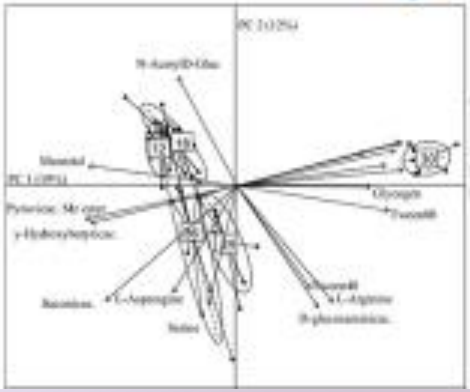






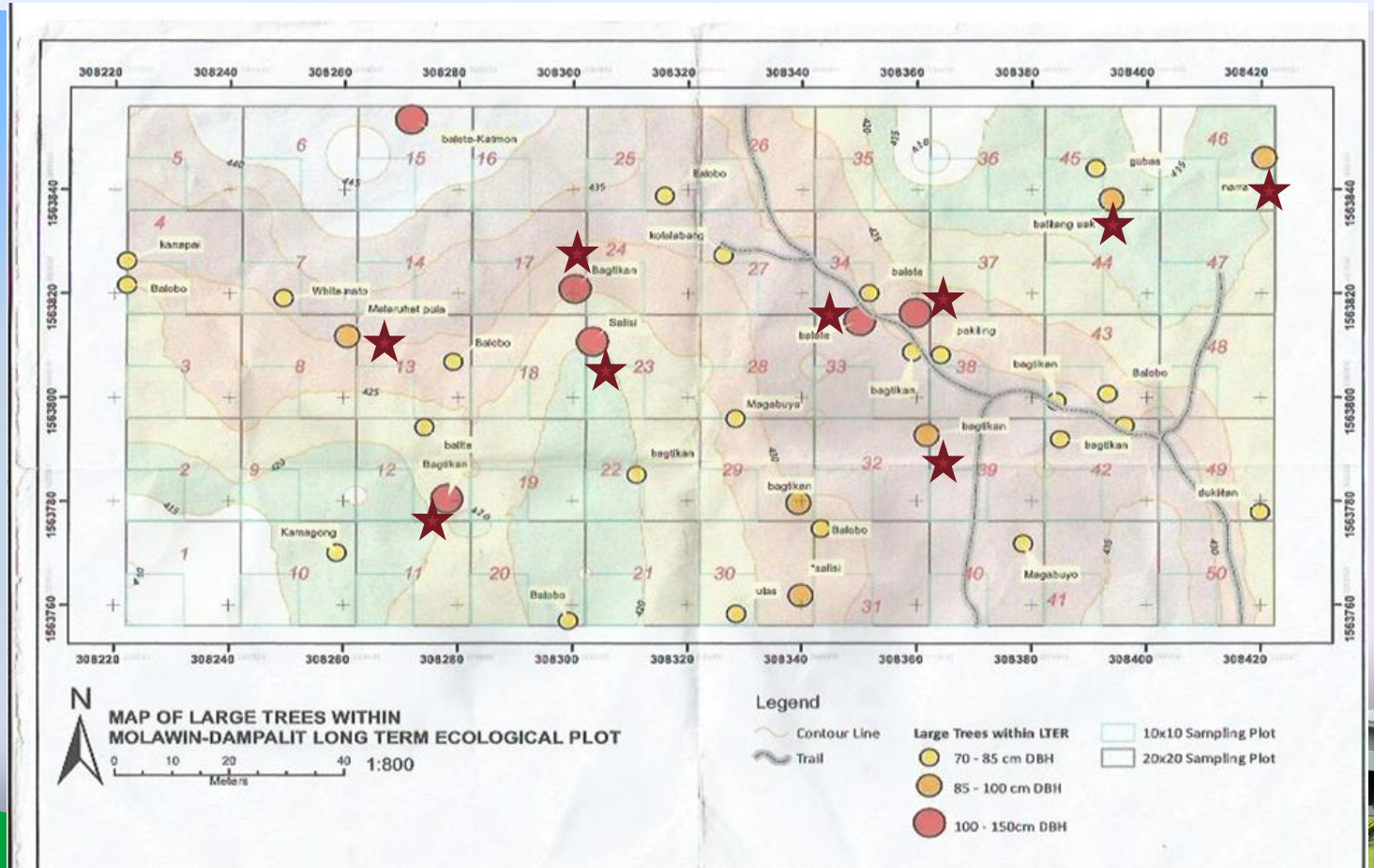
Color formation due to substrate utilization

Statistical analysis through Principal Component Analysis



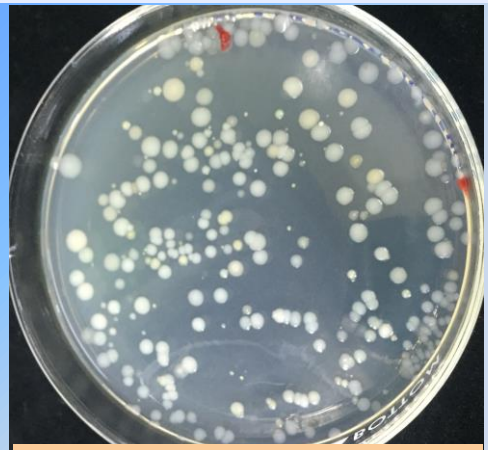
Accomplishments to date (October 25, 2016):

- 1st Sampling: Bark, Lichens and soil from 9 dominant trees (Isolation, Cell count, DGGE)
- 2nd Sampling: additional bark samples (BIOLOG)

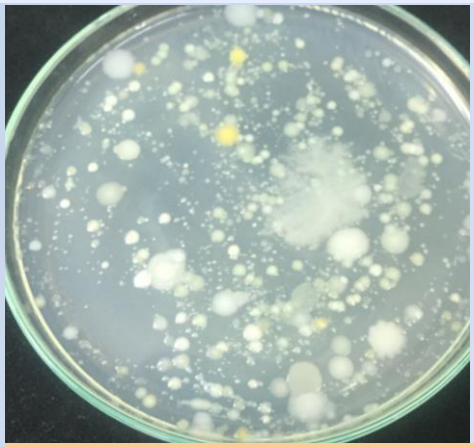


★Trees sampled

Plating, cell count, purification …

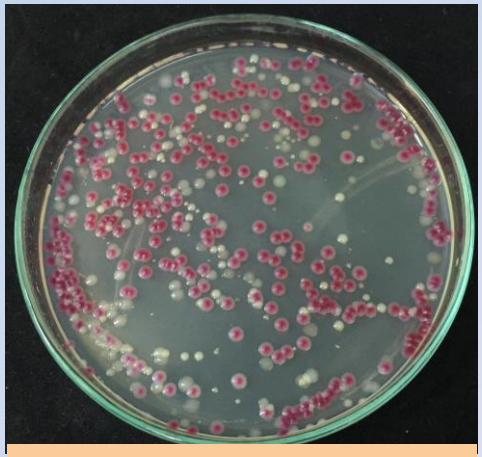


Balilang uak_bark

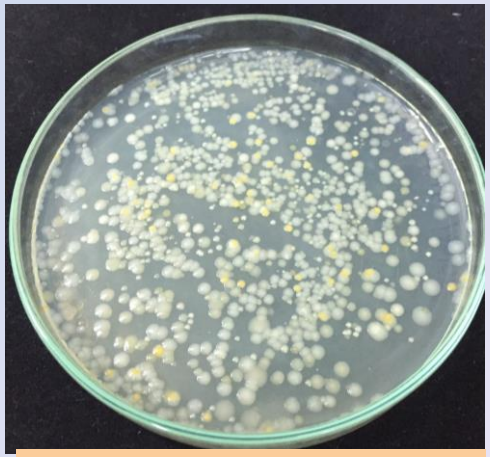


Balilang uak_soil

Salisi_ bark



Salisi_ bark



Bagtikan_bark

Malaruhat pula_ bark

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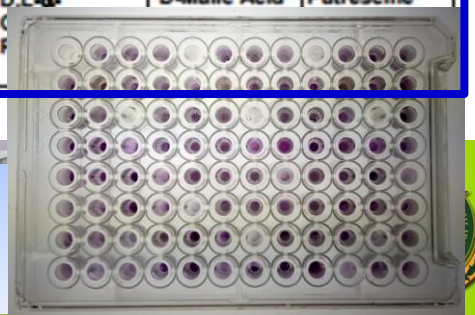
Cultural Microbial community analysis

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

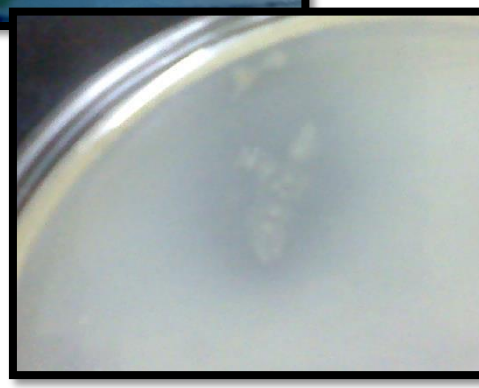


Plating in BIOLOG Ecoplate (triplicate); reading growth at 12 hrs, 24 hrs, and 120 hrs

Cell count ….partial results

Samples	Bark Samples (Ba)	CFU/mL		
		Bacteria	Fungi	Yeast
1st sampling_ Narra (Q46)	1NaQ46Ba	$>10^7$	<10	$2.7x10^5$
1st sampling Balilang uak Q45	1BuQ45Ba	1.9×10^5	3.5×10^3	$1.9x10^6$
1st sampling Balete Q33	1BtQ33Ba	1.6×10^5	4.0×10^2	$2.4x10^6$
1st sampling Bagtikan Q33/32	1BgQ33/32Ba	9.2×10^6	8.1×10^2	$>10^6$
1st sampling Salisi Q23	1SaQ23Ba	6.0×10^7	<10	$>10^7$
1st sampling Bagtikan Q17	1BgQ17Ba	2.1×10^8	<10	$1.2x10^5$
1st sampling Malaruhat pula Q08	1MpQ08Ba	$>10^7$	2.2×10^4	$2.5x10^7$
1st sampling Bagtikan Q12	1BgQ12Ba	$<10^4$	<10	$9.3x10^6$

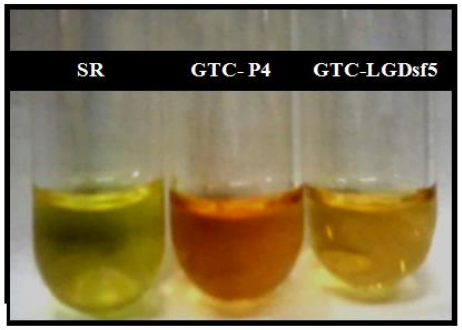
PLANNED METHODOLOGY



Microbial Isolation

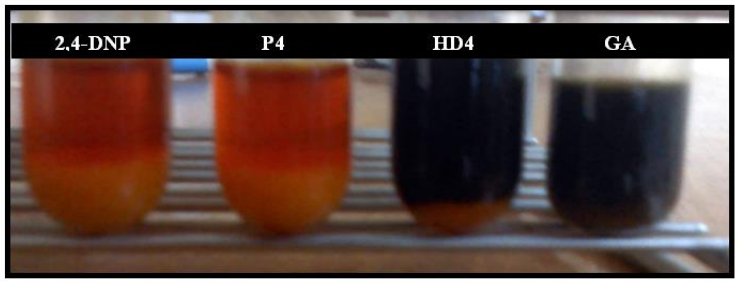
- N-fixing bacteria
- P-solubilizing bacteria

IAA Production Assay



GA Production Assay

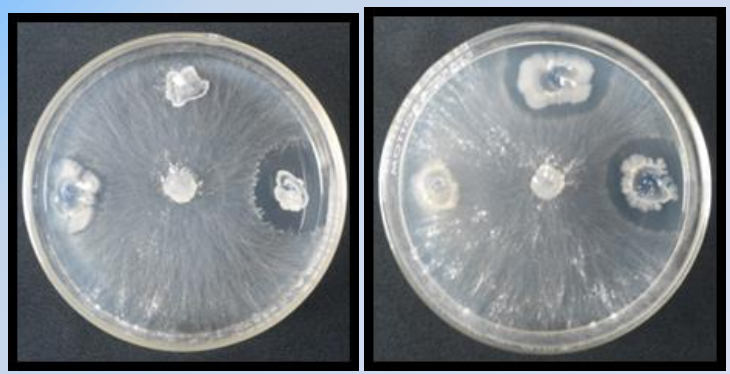
Molecular Identification (16S rRNA)



***In Vivo* Trial**

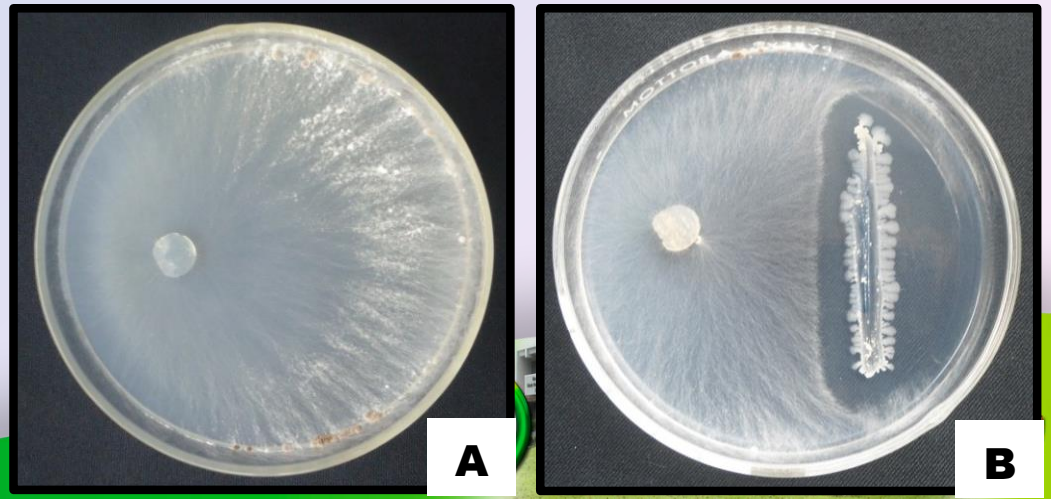
Biocontrol and antimicrobial assays

Antagonism assay



Dual culture assay

***In vivo* (prevention or curative)**



Highlights:

- **Bark, lichens & soil collected from 9 trees differ in their microbial population composition**
- **Spread plating showed intriguing results; few fungal growth in PDA plate in Narra, Salisi and Bagtikan bark implying interaction of trees-microbes**
- **Microbial community plating in BIOLOG ecoplates showed differential color development;**

e.g. 1) at 12 hrs after plating early growth in Tween 40/80 medium (lipase enzyme) but not in all trees; lipase acts on triacylglycerol to glycerol and then to fatty acids; use? degradation of pollutants

e.g. 2) at 12 hrs after plating early growth in well showing presence of asparaginase enzyme; this is required in in food manufacture and has anti-cancer properties; use? Medical and food industry

Future plans:

- Continue plating bark, soil and lichens samples
- Purification and preservation in glycerol stocks and storage in -80^{o}C
- Analyze data from BIOLOG ecoplates
- Preserve cells from individual cells in glycerol stocks
- Assay bark samples for community analysis using Denaturing Gradient Gel Electrophoresis (DGGE)
- Devise enzyme assays to screen/verify microbial cultures for functional diversity

Thank you for your attention!