Pengantar tentang:

Teknik Sampling dan Koleksi Mikroorganisme

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BRIN
BADAN RISET
DAN INOVASI NASIONAL

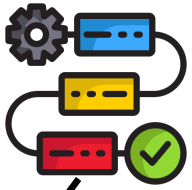
Research Center for Biosystematics and Evolution

EKSPEDISI
BIODIVERSITAS
TERESTRIAL
e-BiTe 2025-2027

Presentation scope

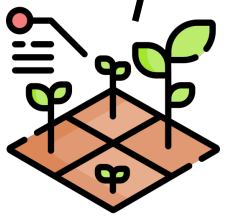
1. General definition of microorganisms

2. The importance of microorganism inventory



3. General sampling workflow

4. Examples of field sampling techniques



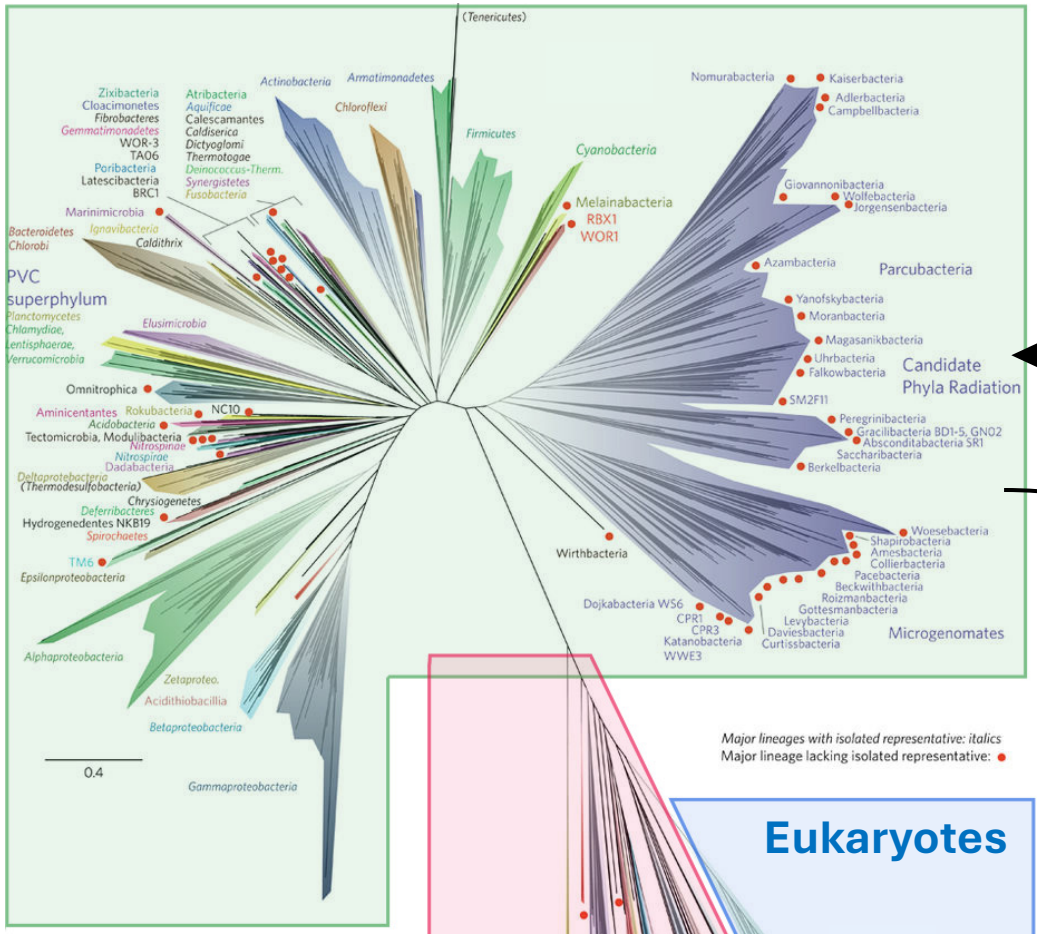
5. Laboratory workflow

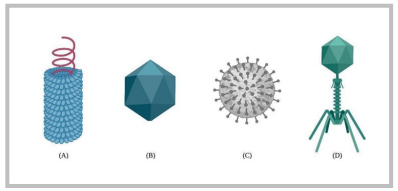
1. General definition of microorganisms

All organisms with very small body size and mostly only observable through microscopes (*e.g.*, light microscopes) or identified using molecular analysis (*e.g.*, DNA, phylogenetic analysis, whole genome sequencing, metabolomics)

Main groupings

- **Prokaryotes, Archaea, and Eukaryotes**
- **Archaea are often included as prokaryotes**
- **Viruses have been debated as microorganisms**





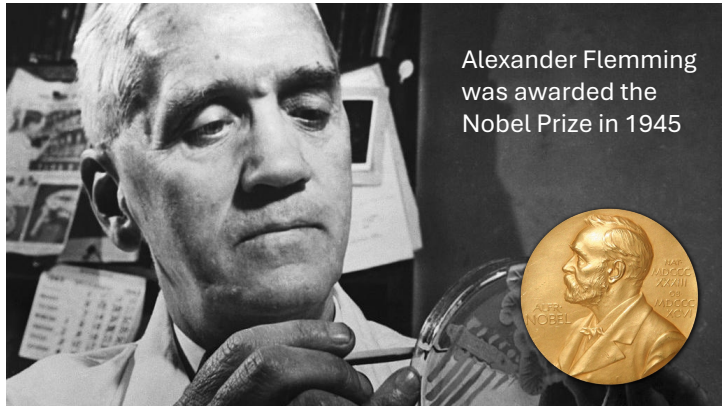
Microorganism sub-themes under e-BiTe program

Prokaryotes:
- Bacteria

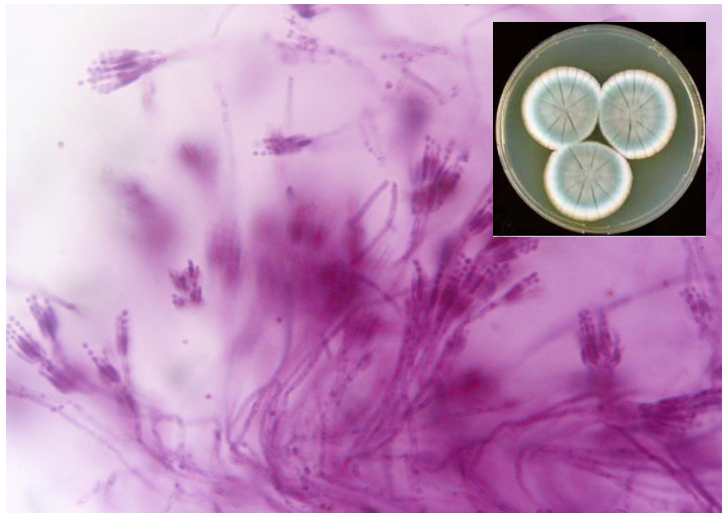
Eukaryotes:
- Microalgae
- Fungi

Eukaryotes also include: Animals, plants, and other protists

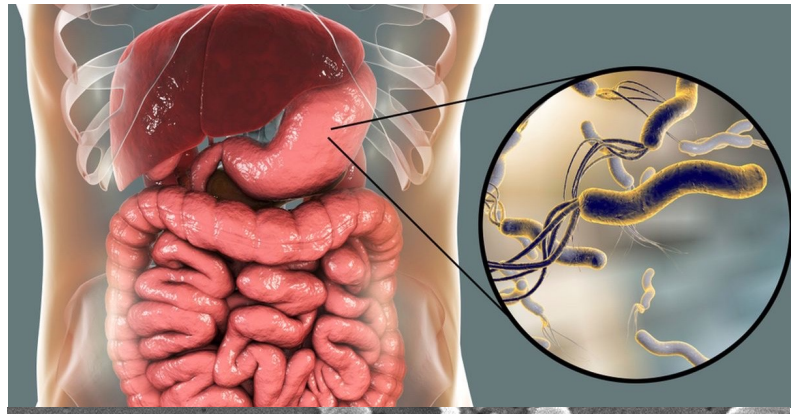
The smallest may make the **biggest** impact!

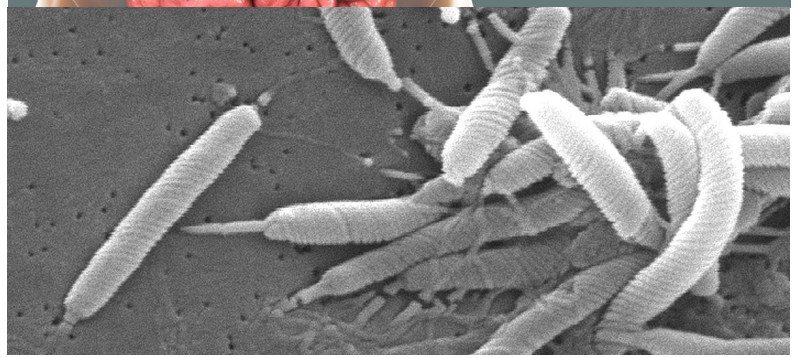


A. Flemming discovered a mould of fungi (*Penicilium* sp.) inhibit the growth of *Staphylococcus aureus*. He 'introduced' the first antibiotic: Penicilin, saving many lives during WW2.



They discovered *Helicobacter pylori,* a bacterium causing gastristis and gastric ulcers, leading to permanent cure of this disease





350 elephants found death near water source contaminated by toxic microalgae (*Microcystis* sp.)

2. The importance of microorganism inventory

A. The biosystematics perspective

Microorganism biodiversity inventories are crucial for providing essential data for:

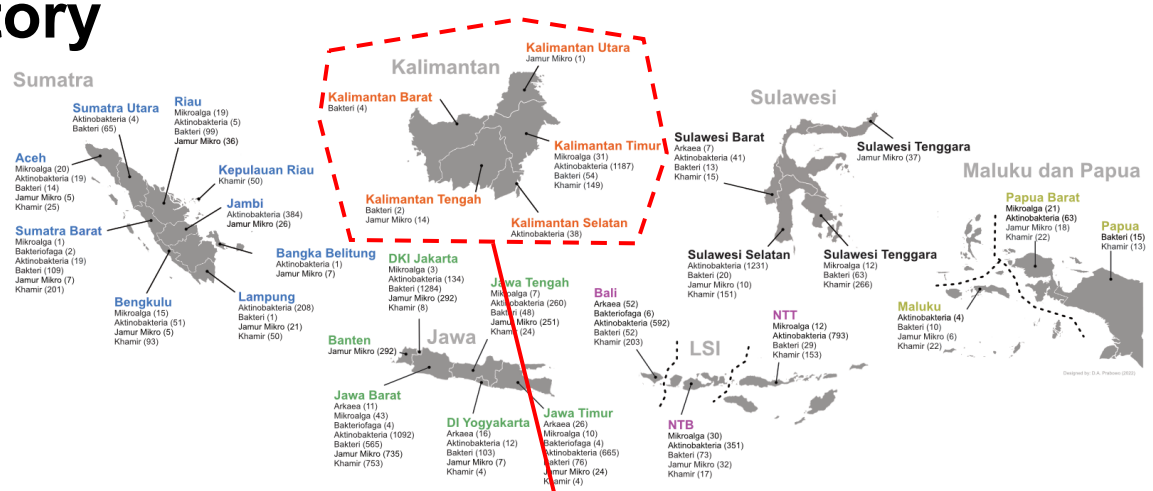
- **discovering new taxa**
- **clarifying evolutionary relationships**
- **refining taxonomic classifications**
- **understanding ecological roles**

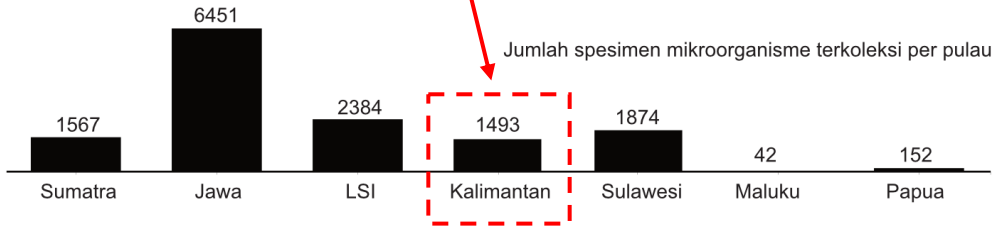
All of which are foundational for conserving biodiversity and advancing biological knowledge.

B. The functional / application perspective

A microbial inventory is important as it provides information for:

- **Understanding and Utilizing Functional Diversity**
  - Ecosystem Function
  - Biotechnology and Industry
- **Applications in Food and Health**
  - Food Production
  - Human Health
- **Environmental Monitoring and Management**
  - Environmental Health
  - Threats and risk
  - Biodiversity Conservation

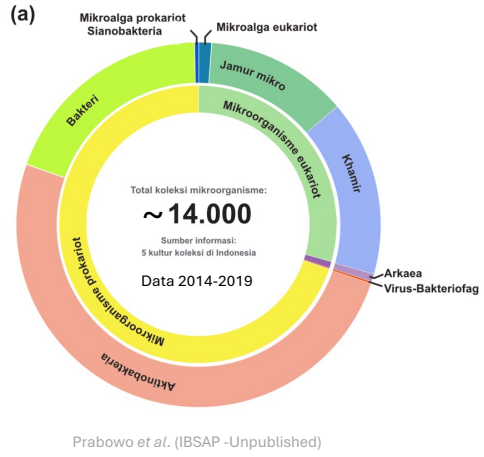




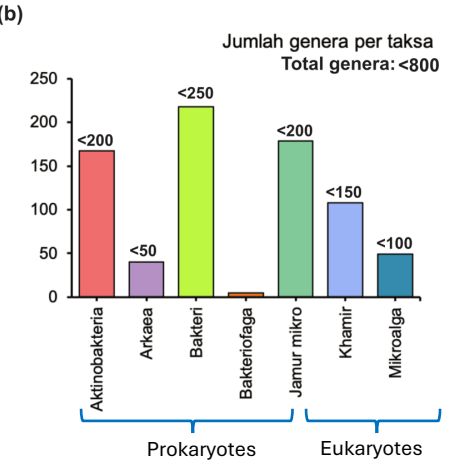
Compared to:

>20,000 (JCM)

>23,000 (KACC)



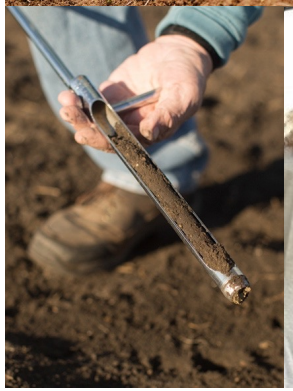
Prabowo *et al.* (IBSAP -Unpublished)



Therefore, biodiversity inventory is required for Indonesia!

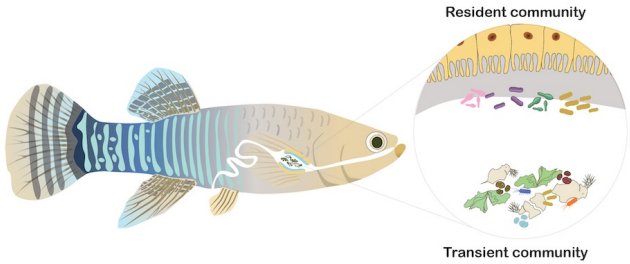
Source of samples for microorganisms

Soil / sediment



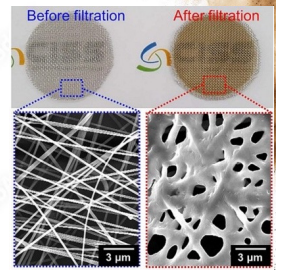
Water

Plants & animals



Rocks / stone wall

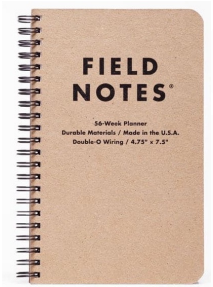
Air



Requires specific sampling technique and strategy!

General materials and tools

Enviornmental Data recording


Sample collection












Others (case by case)


etc.

Schematic workflow

Field

Survey & planning

- Define survey to guide sample location and type
- Choose representative locations
- determine sampling times.

Sampling Design

- Select appropriate sampling methods (random, systematic, or stratified based on research goals and sample type).
- Determine the necessary sample size

Sample Storage and Transportation

- Label sterile containers
- Store samples at appropriate temperatures
- Ship samples to the laboratory promptly

Documentation

- Record essential information: location, time, environmental conditions, photos
- Label each sample: a unique code, date, and collection location.

Laboratory

Sample Reception and Examination

- Receive samples from the field promptly
- Visual Examination: ensure there is no contamination or damage.
- Data Recording: sample code, date and location of collection, sample type etc

Sample Processing in the Laboratory

Depending on the taxa:

- Incubation
- Isolation
- Extraction
- Culture
- Identification

Storage and Disposal of Samples in the Laboratory

- store under appropriate conditions
- Label each container with a unique code
- samples can be disposed if no longer needed

Example 1: Sampling for soil and endophytic microorganisms

Common applied method: Purposive random sampling

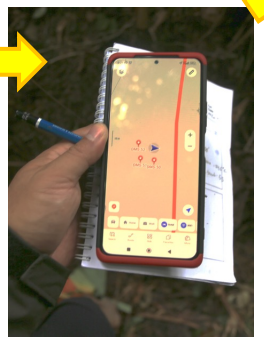
Wild Bananas

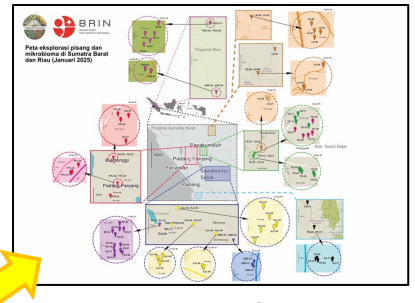
Pseudostem

Cut correctly

Target: endophytic fungi

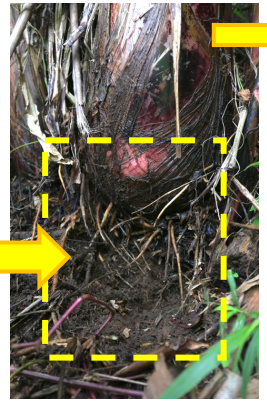
Measure and Document correctly



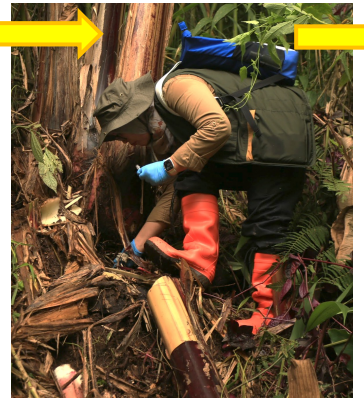


Store at appropriate temperature

Rhizosphere



Dig 10-20 cm deep soil. Take soil and roots.



Label correctly

Add buffer of enrichment media

Embrace new people, work as a team, give support, and learn something new!

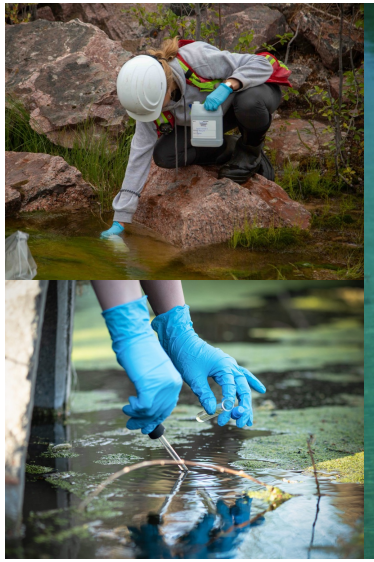
Don’t forget taking meaningful photographs (a lot!) to tell your journey to discovery!

Documentary photographs are often useful for dissemination of results through presentation or publication in popular media.

Example 2: Sampling for microorganisms in water (aquatic environment)

Water sample can be collected:

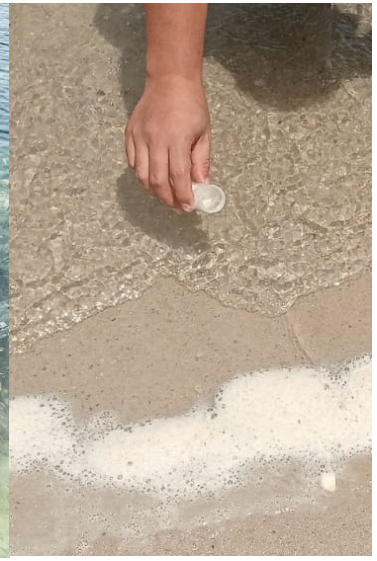
Directly by using bottle or pipette



Using buckets or plankton net (5-20 microns) – ideal for shallow waters

Store in Falcon tubes

Sediments or substrates can be collected in ziplock or tubes

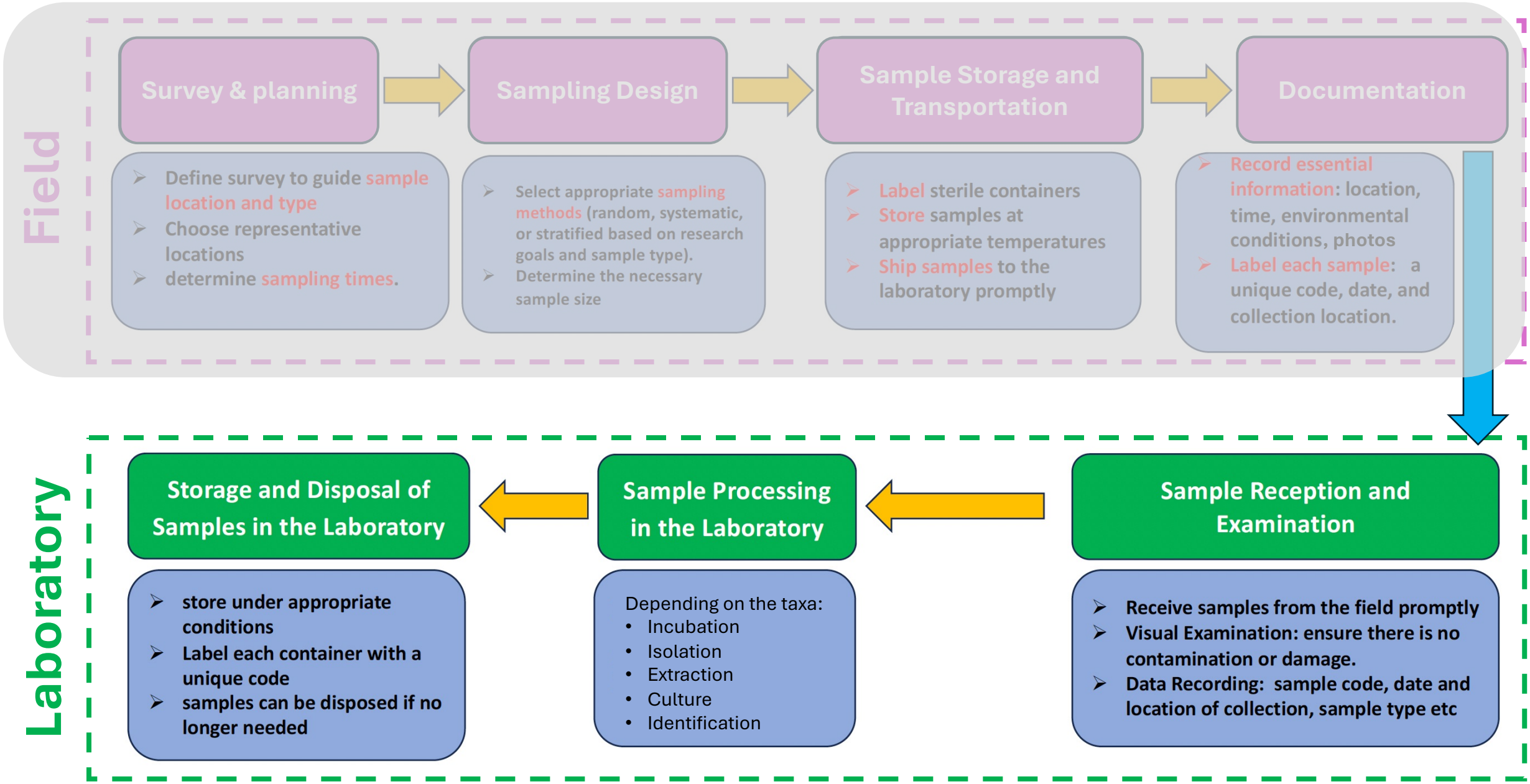


Measure and document

Collected water samples are:

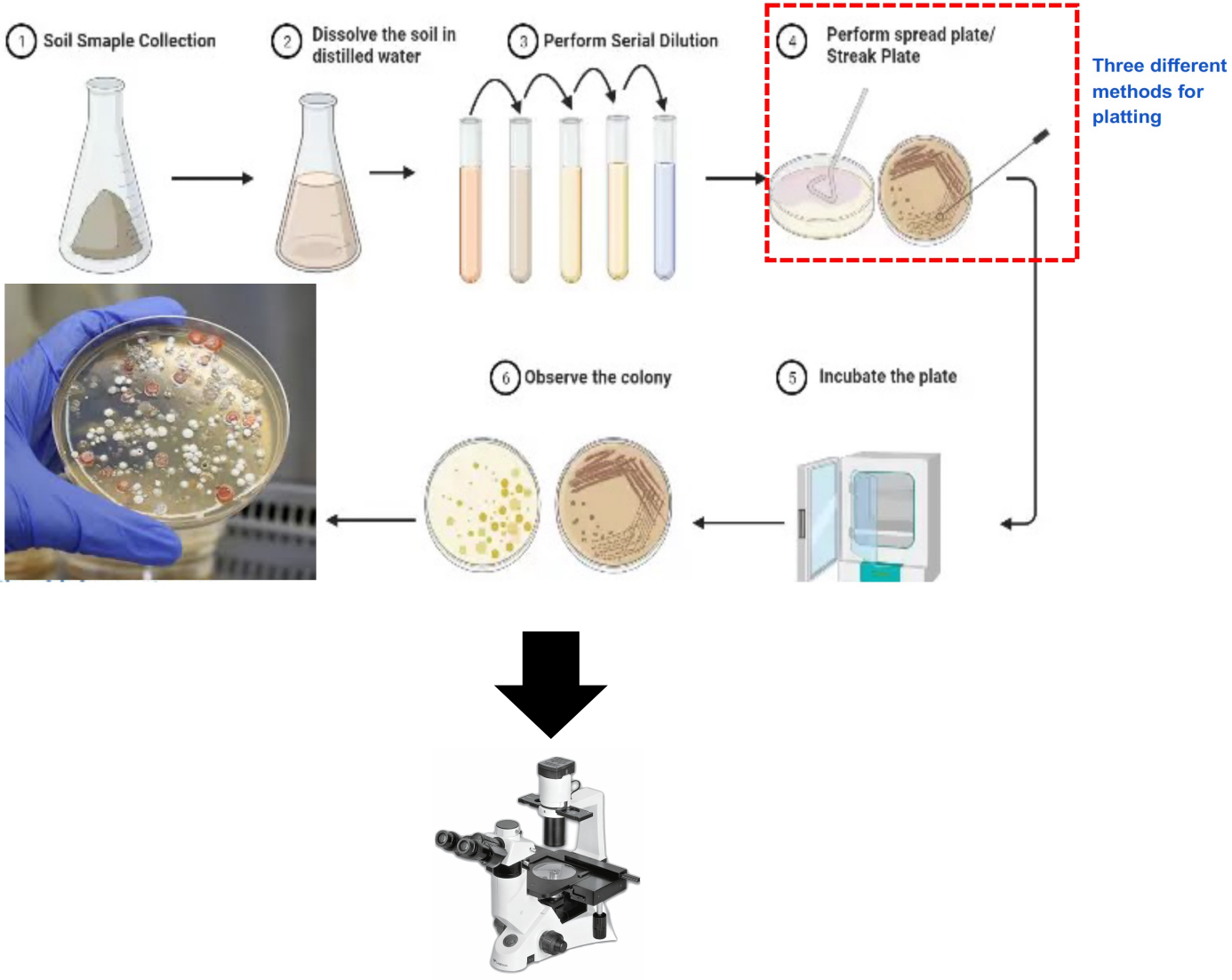
1. **Enriched with growth media to increase the survivalship / number of cells (for isolation and culture)**
2. **Fixed/preserved using lugol, formalin, or glutaraldehyde (*i.e.*, microalgae) for identification and characterization**

Schematic workflow



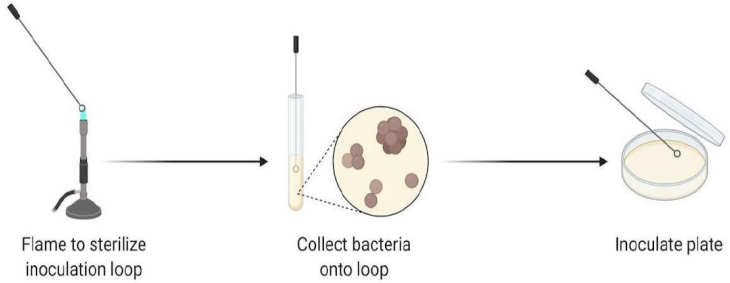
Laboratory workflow for prokaryotes and fungi

Process for isolating microbes from soil or leaf litter

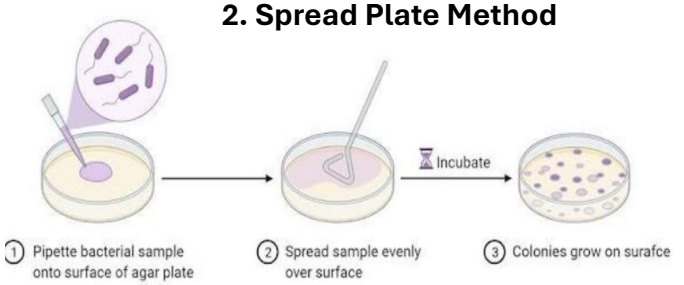


Three different methods for platting

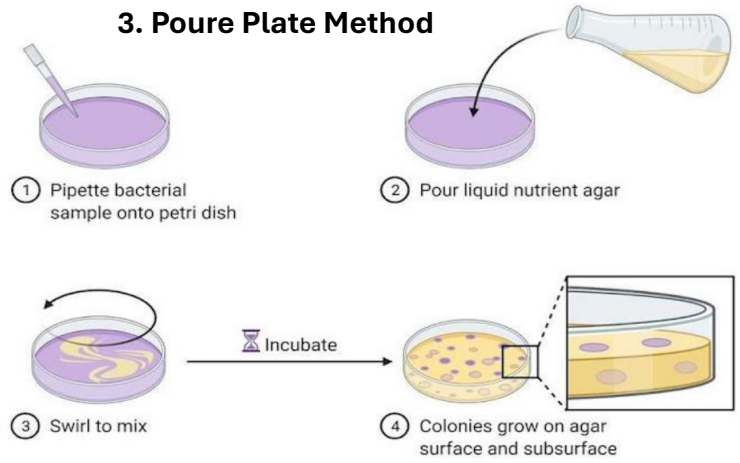
1. Streak Plate Method



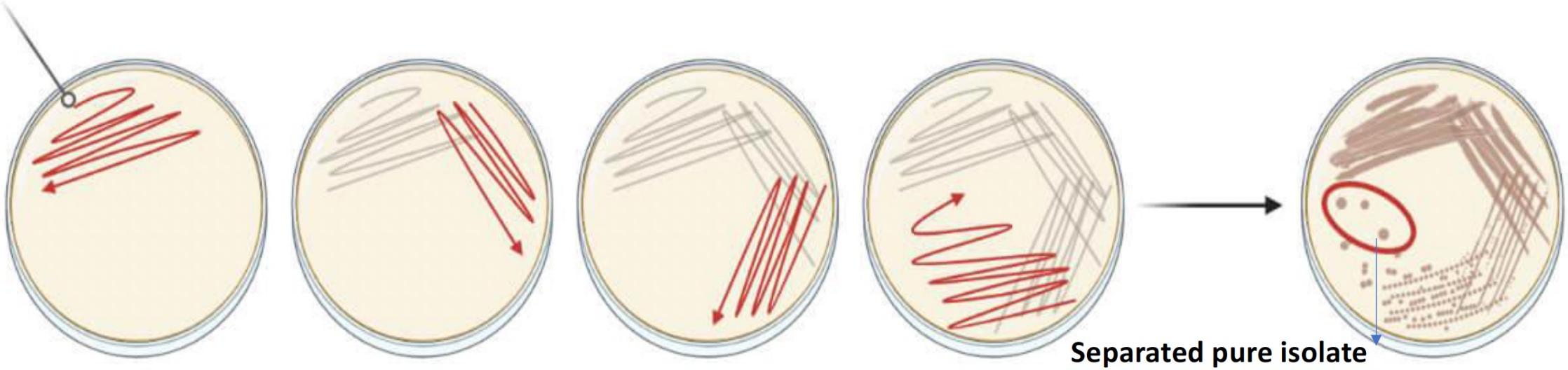
2. Spread Plate Method



3. Poure Plate Method



Inoculation procedure to obtain pure isolates using the quadrant streak method

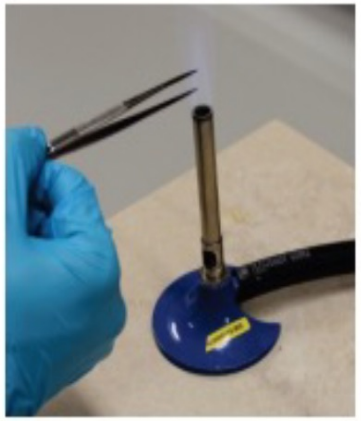


Separated pure isolate

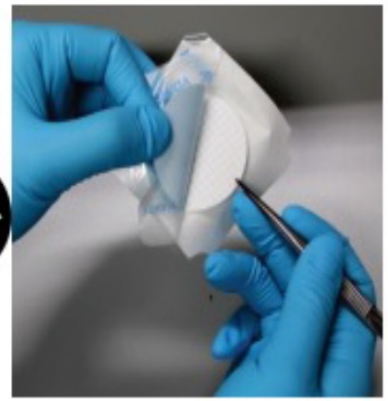
Isolating microbes from water samples using filtration method

(Ideal for obtaining high concentration of cell)

1 Prepare water sample



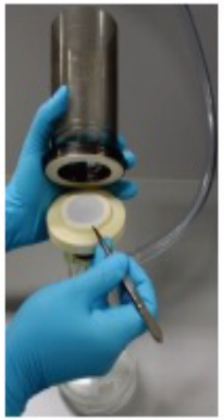
2 Flame-sterilise the forceps



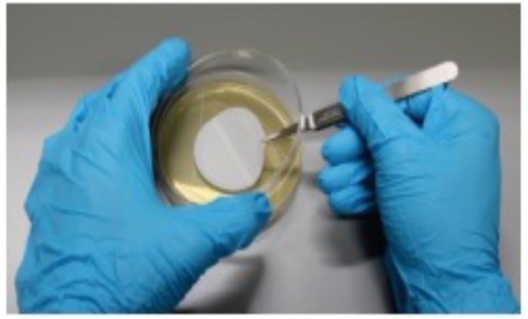
3 take one piece of sterile membrane filter

5 Place the membrane filter in the filtration apparatus

6 transfer water into the funnel



7 Remove the membrane filter using the flame-sterilised forceps

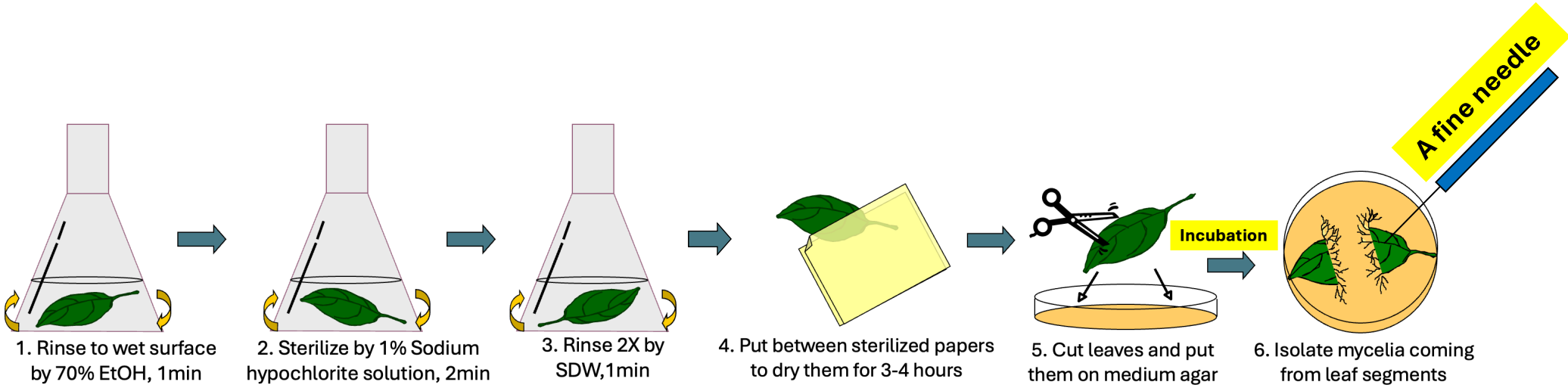


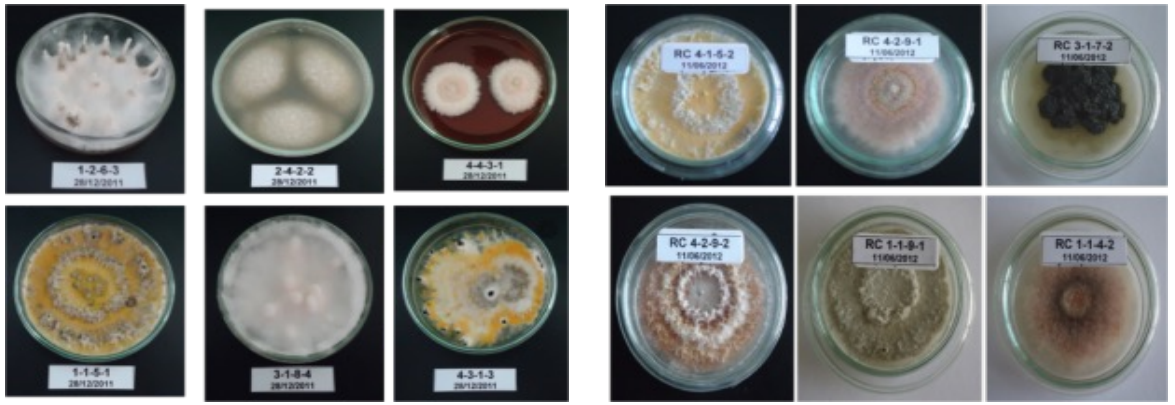
8 Place the membrane filter onto the agar

9 Place plate in incubator

Isolation of endophytes by a surface-sterilization method

70% EtOH → Sodium hypochlorite solution (1% chlorine) → SDW → Drying

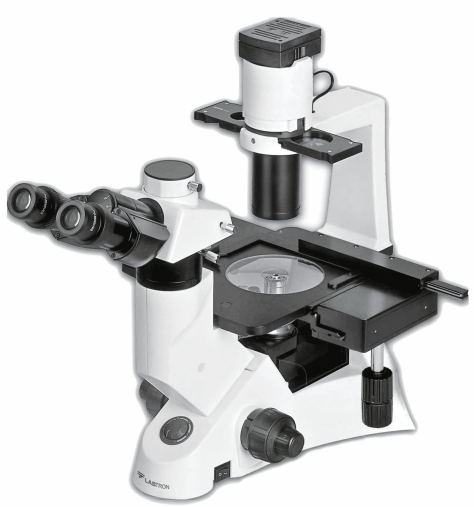




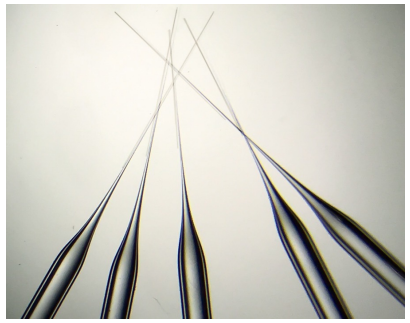
Laboratory procedure for microalgae

Single cell isolation

Inverted microscope



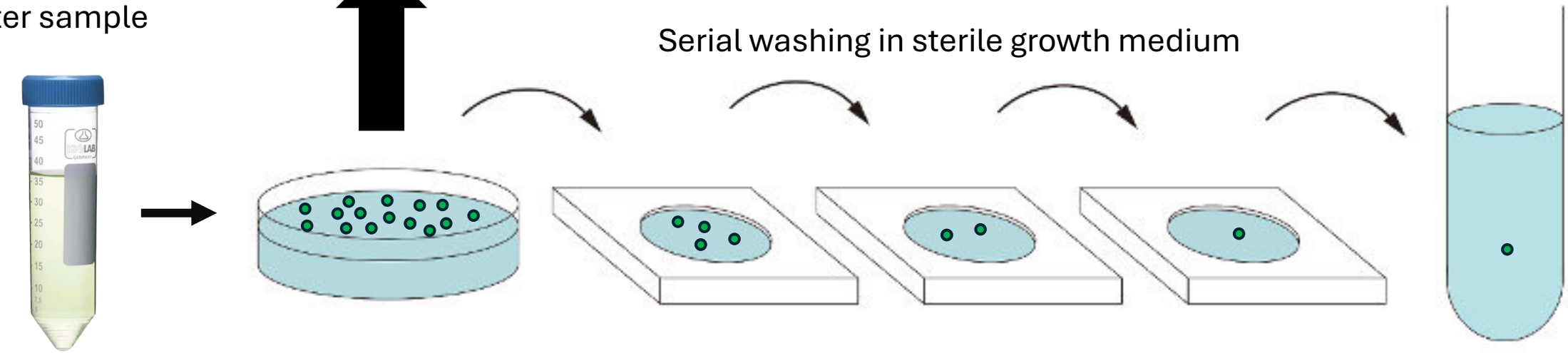
Capillary pipettes



Inoculation in 5-7 ml growth medium under certain light and temperature conditions

Water sample

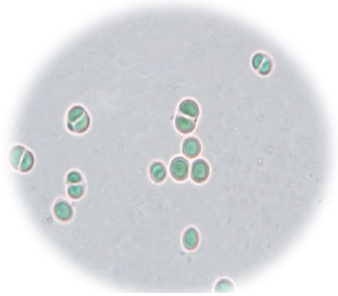
Serial washing in sterile growth medium



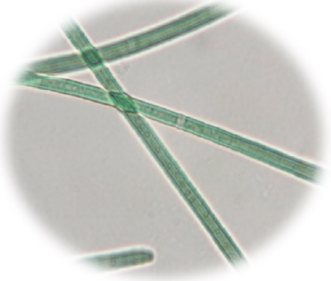
Other methods: Serial dilution, agar platting, automated cell sorting

Microalgae culture at lab for further characterization

Phycology research group - BRIN



Anabaena sp.

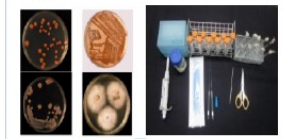


Storing microorganisms isolates / strains

1. Drying method /L-drying ampoule

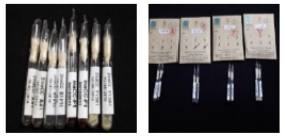
Aktinomisetes, Bakteri, Fungi, Archea, Bakteriophage, Kamir

Preparation of culture



L-drying process

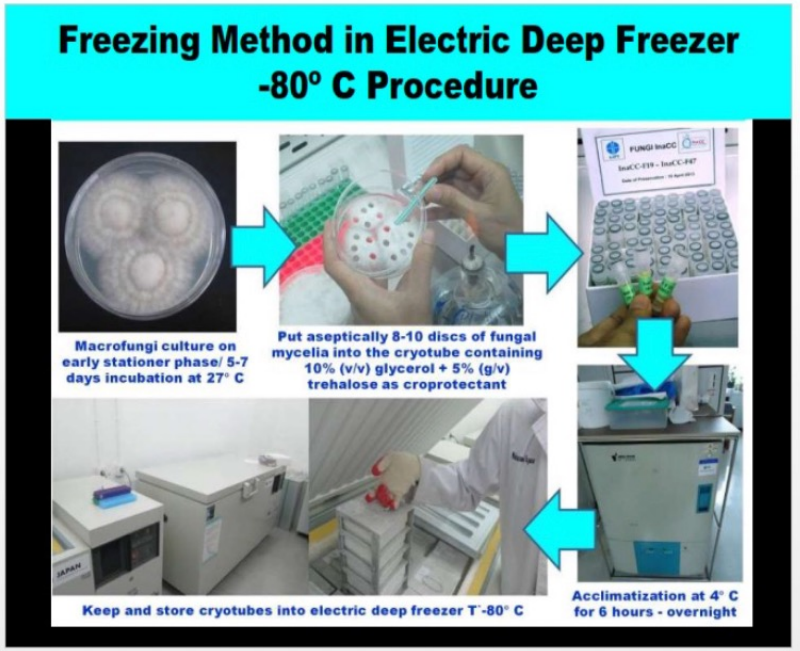
Ampoule after process



Ampoule preserved in 4º C

2. Cryopreservation (Electric Deep freezer (-80ºC)

Aktinomisetes, Bakteri, Fungi, Archea, Bakteriophage, Kamir, mikroalga



3. Active culture (Serial Transfer)

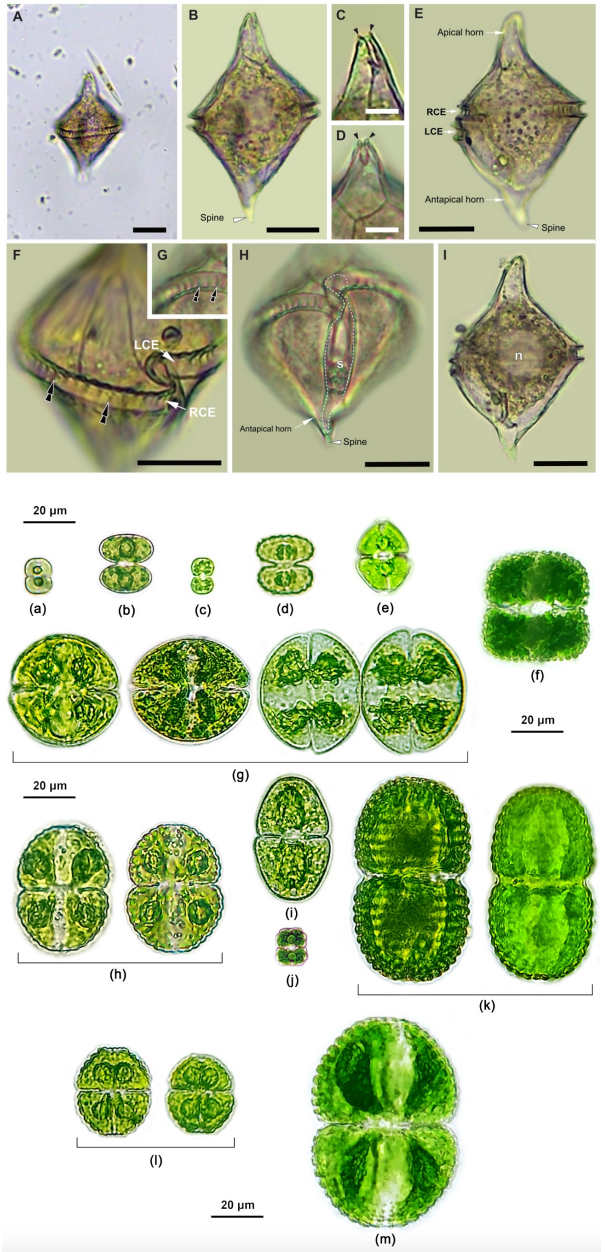
Fungi, Mikroalga

All activities must be conducted in sterile room

A general guideline for identifying microorganisms

Identification methods	Criteria	Methodology
Phenotypic identification	The morphology of the colony(ies)	Visual observation / macroscopic observation
		Pigmentation
	Cell morphologyl	Microscopic observation
		Gram-coloring
		Specific coloring
Biochemical identification	Biochemical testing	Carbohydrate fermentation test
		Enzymatic test
		Metabolites profiling
	Growth testing	Growth under different media
		Growth condition/performance
Genotypic identification	Polymerase Chain Reaction (PCR)	DNA amplification
		Real-Time PCR (qPCR)
	DNA Sequencing	16S rRNA, 18S rRNA, D1D2, ITS
		Whole Genome Sequencing (WGS)
	Molecular Fingerprinting	Restriction Fragment Length Polymorphism (RFLP)
		Random Amplified Polymorphic DNA (RAPD)
Serological identification	ELISA (Enzyme-Linked Immunosorbent Assay)	
	Western Blotting	
	Aglutinasi	
Mass Spectrometry	MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry)	

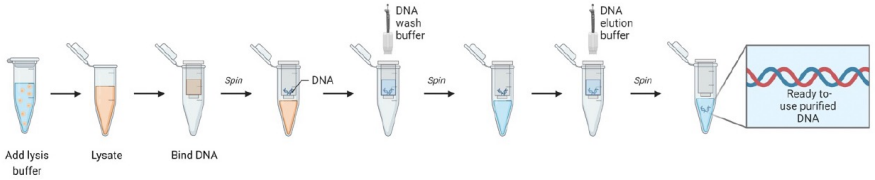
Mostly applies only for microalgae

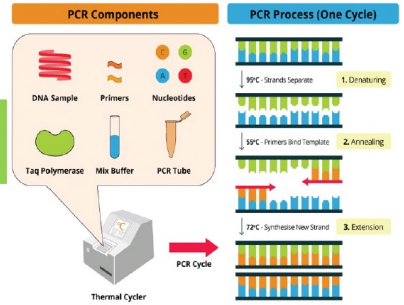


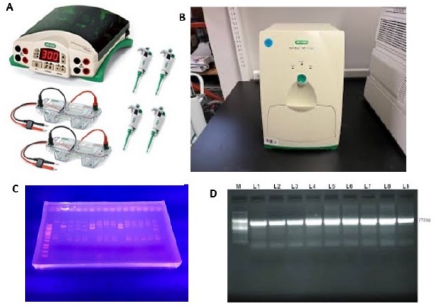
Adam et al. (2025)

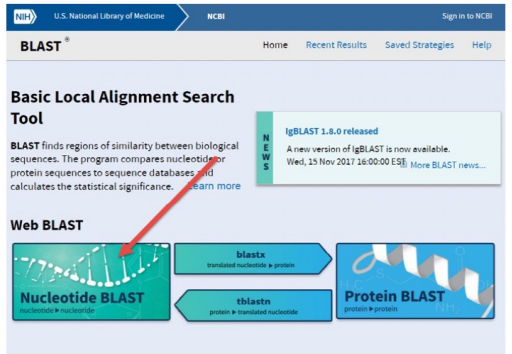
Molecular identification of microorganisms

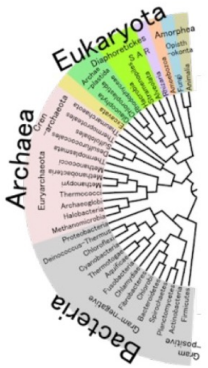
1. Cell Culture
2. DNA Isolation
3. PCR (polymerase chain reaction)
4. Visualization of PCR Product
5. DNA Sequencing
6. Matching nucleotide gene data with a database through BLAST

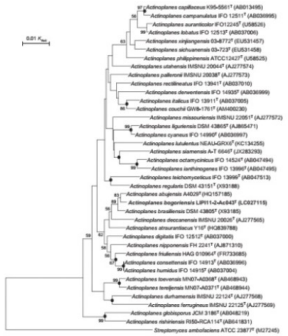












Once, the identity of the isolates is confirmed, the depository collection can be made according to the requirements of Indonesian Culture Collection (InaCC), Direktorat Pengelolaan Koleksi Ilmiah (DPKI-BRIN) and the respective taxonomic regulation when describing a new microorganism species.

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Thank You